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JASN Abstract Supplement

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TH-OR001

Cardiac Dysfunction in *Pkd1*-Deficient Mice and Phenotype Rescue by Galectin-3 Knockout Bruno E. Balbo,[1] Andressa Godoy Amaral,[1] Jonathan Mackowiak Fonseca,[1] Isac De Castro,[1] Vera Mc Salemi,[1] Leandro Eziquiel Souza,[1] Fernando Santos,[1] Maria Irigoyen,[1] Feng Qian,[2] Roger Chammas,[1] Luiz F. Onuchic.[1] *[1]Univ of São Paulo, Brazil; [2]Univ of Maryland.*

Background: Myocardial abnormalities stand out among ADPKD cardiovascular manifestations.

Methods: To elucidate their pathogenesis, we analyzed the cardiac phenotype in distinct models of *Pkd1*-deficiency. We evaluated *Pkd1*$^{cond/cond}$:*Nestin*^{cre} (CY) cystic, hypertensive mice at 20-24 weeks of age, and *Pkd1*$^{+/-}$ (HT) noncystic mice at 10-13 weeks, a model of haploinsufficiency.

Results: Echocardiographic analyses in CY and HT mice revealed decreased left ventricle ejection fraction (LVEF), indicating systolic dysfunction, as well as E/A ratios and deceleration times consistent with diastolic dysfunction in CYs and a similar trend in HTs, when compared to *Pkd1*$^{cond/cond}$ (noncystic; NC) and *Pkd1*$^{+/+}$ (wild type, WT) controls. Speckle-tracking echocardiography showed reduced cardiac deformability in both models. CY and HT hearts presented higher apoptotic rates (TUNEL) and mild fibrosis (Sirius red), without TGFß1 increased expression. In this scenario, we investigated galectin-3 (Gal-3) as a potential modifier of the ADPKD cardiac phenotype. Double mutants *Pkd1*$^{cond/cond}$:*Nestin*^{cre};*Lgals3*$^{-/-}$ (CYG-) and *Pkd1*$^{+/-}$;*Lgals3*$^{-/-}$ (HTG-) displayed improved systolic and deformability parameters compared to single mutants, while such values did not differ from NCs and WTs. HTG-s presented a trend of improvement in diastolic function. CYG- and HTG- hearts showed decreased apoptosis and fibrosis, reaching NC and WT baselines. Western blot analyses revealed higher Gal-3 expression in CY than NC hearts. CYG- and HTG- animals showed no difference in BUN compared to CYs and HTs, but displayed trends of increased fractional excretion of Na^+, Cl^- and K^+. We also employed a more severe cystic model, homozygous for an allele that hinders polycystin-1 cleavage (*Pkd1*$^{V/V}$; VV), to show that *Pkd1*$^{V/V}$;*Lgals3*$^{-/-}$ mice present longer survival than VVs.

Conclusions: Our findings demonstrate myocardial dysfunction in different *Pkd1*-deficient models, reproducing human ADPKD, and reveals that absence of Gal-3 expression significantly rescues this phenotype.

Funding: Government Support - Non-U.S.

TH-OR002

Deletion of Heart *Pkd1* Promotes Cardiac Remodeling via Smyd2 and NF-κB Cross-Talk Changlin Li, Lucy Fan, James P. Calvet, Xiaogang Li. *Medicine, The Univ of Kansas Medical Center, Kansas City, KS.*

Background: ADPKD associated cardiovascular complications have been suggested to result from renal cyst growth induced cardiovascular hypertension, which occurs in patients at an earlier age than in the general population. However, the fact that cardiac hypertrophy also occurs in young ADPKD patients with normal blood pressure and normal renalfunction suggests that cardiac dysfunction in ADPKD does not develop solely in response to hypertension and renal failure, and additional genetic or environmental factors may be required. In this study, we investigated the direct involvement of heart *Pkd1* in regulating cardiac complications.

Methods: To understand the role of *Pkd1* in the heart, we generated a heart specific Pkd1 conditional knockout (*Pkd1-hCKO*) mouse via the deletion of heart *Pkd1* in cardiomyocytes in six-week-old *Pkd1*$^{flox/flox}$:αMHC-MerCreMer mice induced by tamoxifen for five days. The *Pkd1*-hCKO mice were further analyzed at 6, 12, and 18 weeks after tamoxifen induction.

Results: We found that heart specific knockout of *Pkd1* resulted in heart hypertrophy as early as 6 weeks after tamoxifen treatment characterized by increase of 1) the heart weight (HW)/body weight (BW) ratios; as well as the expression of 2) the hypertrophic markers, atrial natriuretic factor (ANF) and β-myosin heavy chain (β-MHC); 3) the cardiac hypertrophy related cytokines, TNF-α, MCP-1, and IL-6; and 4) Smyd2, a histone methyltransferase in *Pkd1*-hCKO cardiomyocytes. Deletion of heart Smyd2 blunted cardiac hypertrophy induced by knockout of heart *Pkd1* as shown by decreased HW/BW ratios in those mice and decreased expression of ANP and β-MHC as well as TNF-α, MCP-1, and IL-6 in *Pkd1* and Smyd2 double knockout cardiomyocytes. In addition, we found that Smyd2 interacted with the NF-κB p65 subunit in *Pkd1*-hCKO cardiomyocytes but not in *Pkd1* wild type cardiomyocytes and that this interaction resulted in the methylation of p65 and activation of NF-κB leading to increased cardiac hypertrophic cytokine expression in *Pkd1*-hCKO cardiomyocytes.

Conclusions: Heart specific deletion of polycystin-1 directly promotes cardiac hypertrophy mediated by cross-talk between the Smyd2 and NF-κB signaling pathways.

Funding: NIDDK Support

TH-OR003

mTORC1 Regulates Polycystin-1 Expression Levels Possibly Explaining Renal Cystogenesis in *Tsc1* Mutant Mice Monika Pema, Marco Chiaravalli, Luca Drusian, Alessandra Boletta. *Div of Genetics and Cell Biology, DIBIT-San Raffaele Scientific Inst, Milan, Italy.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disorder characterized by massive bilateral renal cyst formation. ADPKD results from mutation in the *PKD1* or *PKD2* genes, encoding the Polycystin-1 (PC-1) and 2 (PC-2) proteins respectively. Several studies have implicated a crosstalk between the *PKD* genes and the genes mutated in a genetic disorder called Tuberous Sclerosis Complex (TSC). mTOR is upregulated in PKD and rapamycin slows cyst expansion, PC-1 can inhibit mTORC1 and finally renal inactivation of the *Tsc* genes causes cysts. Thus upregulation of mTORC1 downstream of *PKD1* mutations is viewed as an important driver of cystogenesis. Here we show that this model should be revisited.

Methods: Inactivation of *Pkd1* and *Tsc1* genes in the kidney using the same KspCre recombinase results in different phenotypes.

Results: Whereas both *Pkd1* and *Tsc1* inactivation result in mTORC1 upregulation in the kidneys, *Pkd1* mutant mice develop a more aggressive cystic phenotype leading to death by P12, while *Tsc1* mutants display a milder and delayed phenotype with cysts arising at P20 and death by P80. Interestingly, we found a strong downregulation of PC-1 in *Tsc1*-/- MEFs compared to wild type controls, which was reverted by rapamycin. Thus, our data indicate that mTORC1 can regulate the expression levels of PC-1. This process does not act on the rate of protein degradation, but it requires protein neo-synthesis. Lowering PC-1 to 50% exacerbates cystogenesis in *Tsc1* mutants, while a brief pulse of rapamycin overcomes a critical time-window in which PC-1 is essential leading to a long-lasting rescue.

Conclusions: Thus, we show that mTORC1 upregulation *per se* is not sufficient to drive cystogenesis, but that this is due to downregulation of PC-1 in *Tsc* mutants. Our data might provide a mechanistic explanation for the severe renal cystogenesis observed in the *PKD/TSC* contiguous genes syndrome opening new perspectives for the use of rapamycin analogues in this syndrome or in ADPKD caused by hypomorohic or mild *PKD1* mutations.

TH-OR004

Ciliary Polycystin Complex Traffics Through the Golgi *Via* a Rabep1/GGA1/Arl3-Dependent Mechanism Feng Qian,[1] Hyunho Kim,[1] Hangxue Xu,[1] Qin None Yao,[1] Weizhe Li,[1] Qiong Huang,[1] Patricia Outeda,[1] Marco Chiaravalli,[2] Alessandra Boletta,[2] Gregory G. Germino,[3] Terry J. Watnick.[1] *[1]Medicine, Univ of Maryland School of Medicine, Baltimore, MD; [2]San Raffaele Scientific Inst, Milano, Italy; [3]NIDDK, Bethesda, MD.*

Background: Primary cilia play a critical role in autosomal dominant polycystic kidney disease. We have previously presented that their gene products polycystin-1 and -2 must interact and form a complex (the polycystin complex) to traffic through the Golgi for subsequent ciliary targeting. The purpose of the study is to identify the molecules that mediate ciliary trafficking of the polycystin complex.

Methods: We performed a yeast two-hybrid screen for polycystin-1 interacting proteins with the C-terminal tail as bait and examined their role in mediating polycystin complex ciliary trafficking in renal epithelial cells.

Results: Thirteen unique cDNA clones were identified that specifically interact with the polycystin-1 C-terminus. One of these clones corresponded to amino acids 465 to 799 of Rabep1, an effector of multiple Rab GTPases involved in various steps of intracellular vesicular trafficking. The Rabep1 binding requires the intact coiled-coil domain of polycystin-1, which is outside of the previously reported ciliary targeting sequence. Rabep1 recruits the polycystin complex to GGA1 and Arl3 at TGN for ciliary trafficking. We find that GGA1, previously shown to be important for protein sorting and vesicle budding for endosomal proteins, can function as an Arl3 effector and mediate sorting and targeting of the polycystin complex to the cilium. We find that GGA1/Arl3 recruitment and ciliary trafficking of the polycystin complex require GPS cleavage of polycystin-1 *in vivo*, and are disrupted by the *PKD1* mutation in the REJ domain that impairs the GPS cleavage. Importantly, GGA1/Arl3 recruitment and ciliary trafficking of the mutant polycystin complex can be restored upon rescuing GPS cleavage.

Conclusions: We show that ciliary polycystin complex traffics through the Golgi *via* a Rabep1/GGA1/Arl3-dependent mechanism. Our study provides novel insights into the ciliary trafficking mechanism of transmembrane proteins and has important implications for ADPKD.

Funding: NIDDK Support

TH-OR005

Arl13b and the Exocyst Are Necessary for Ciliogenesis and Interact Synergistically Soo Young Choi,[1] Cecília Seixas,[2] Nicole L. Umberger,[3] Xiaofeng Zuo,[1] Tamara Caspary,[3] Duarte C. Barral,[2] Joshua H. Lipschutz.[1,4] *[1]Depts of Medicine, Univ of Pennsylvania, Philadelphia, PA; [2]CEDOC, Univ Nova de Lisboa, Lisboa, Portugal; [3]Human Genetics, Emory Univ, Atlanta, GA; [4]Depts of Medicine, Philadelphia VAMC, Philadelphia, PA.*

Background: Arl13b belongs to the ADP-ribosylation factor family of small GTPases of the RAS superfamily, and is important for primary ciliogenesis. We previously showed that the eight-protein exocyst complex is necessary for ciliogenesis, and most likely acts by targeting and docking vesicles carrying ciliary proteins. Notably, mutations in Arl13b and exocyst subunit Exo84 were found in patients with Joubert Syndrome, a nephronophthisis form of polycystic kidney disease (PKD).

Methods: To investigate an interaction between Arl13b and the exocyst *in vitro*, we performed immunoprecipitation, immunoblotting, and *in vitro* transcription and translation. To investigate if arl13b and the exocyst interact *in vivo*, we used two different model systems, antisense morpholinos to knock down *arl13b* and exocyst subunit *sec10* in zebrafish, and Cre-Lox to knock out Arl13b in mice.

Results: Arl13b interacts directly with the exocyst subunit Sec8. Sec8 is a *bona fide* effector of Arl13b, as it binds only to the active form of Arl13b. Kidneys from Arl13b deficient mice demonstrated cysts in all segments of the nephron, along with decreased ciliogenesis. The observed MAPK activation suggests that excessive cell proliferation is involved in the cystic phenotype. We previously showed that depletion of *sec10* in zebrafish phenocopies *arl13b* (aka *scorpion*) knockdown with ciliary mutant phenotypes, including: curly tail up and left-right patterning defects. We show here a synergistic genetic interaction between *arl13b* and s*ec10* in zebrafish, suggesting that *arl13b* and the exocyst function in the same pathway.

Conclusions: Our study demonstrates that the exocyst is a novel effector of Arl13b, that these proteins likely function in the same pathway, that both are required for ciliogenesis, and that this may have important implications for PKD patients.

Funding: NIDDK Support, Veterans Affairs Support

TH-OR006

Ciliary Trafficking of Polycystin-1 Is Regulated by Polycystin-2 and Other Factors Xuefeng Su, Gang Yao, Maoqing Wu, Azadeh Tabari, Jing Zhou.

Background: Polycystin-1 (PC1) is the product of the *PKD1* gene that is mutated in over 85% cases of autosomal dominant polycystic kidney disease (ADPKD). Multiple lines of evidence suggests an essential role of ciliary PC1 in the disease process. About one third of the *PKD1* mutations are missense mutations which likely produce full-length proteins that fail to be targeted to their normal functional sites, yet the regulation of PC1 trafficking remains poorly understood. We have recently shown that the Bardet-Biedl Syndrome (BBS) gene products BBS1 and BBS3 regulates the ciliary localization of PC1 and that the C-terminal cytoplasmic domain of PC1 is essential for its targeting to the primary cilia (Hum Mol Genet 2014).

Methods: A set of deletion/mutation in PC1 and chimeric constructs with different PC1 C-terminal motifs were made. They were transfected alone or together with other molecules into IMCD3 cells, and the ciliary trafficking ability of these constructs was evaluated by immunostaining. The role of other genes in this process was also evaluated by depletion of respective proteins by lentiviral shRNAs. Western blot and co-immunoprecipitation were performed to analyze the PC1 protein complex forming ability and GPS cleavage.

Results: Here we report a systemic investigation of cis elements modulating the ciliary trafficking of PC1. We show that a coiled-coil (CC) motif in the cytoplasmic tail (CTT) of PC1 is required for efficient targeting of full-length polycstin-1 to cilia, while the previously identified ciliary targeting VxPx motif critical for several ciliary proteins is dispensable. Besides the CC motif, we also identified the presence of other motif/sequences in CTT that is required for PC1 ciliary trafficking. Knockdown of small GTPases previously shown important for the ciliary localization of rhodopsin has no effect on the full-length PC1 trafficking to cilia. While PC1 remains to traffic to the primary cilia in cells depleted of PC2, expression of PC2 promoted the ciliary trafficking of PC1 and several PC1 mutants. Co-expression of PC1 and PC2 lead to the increased ciliary expression of both proteins. The presence of intact PC2 C-tail but not its channel activity or ciliary targeting sequence is required for this process. The effect of PC2 on the ciliary trafficking of PC1 correlates with its ability to form a complex with PC1. GPS cleavage mutants may or may not affect PC1 trafficking to the primary cilia.

Conclusions: The ciliary trafficking of PC1 is highly regulated by motifs in its primary structure, interactions with PC2, but not cleavage at its GPS site.

Funding: NIDDK Support

TH-OR007

Pkd2 Is Essential for Intraciliary Calcium Signaling In Vivo Zhaoxia Sun,[1] Shiaulou Yuan,[2] Lu Zhao,[1] Martina Brueckner.[2] *[1]Genetics, Yale Univ School of Medicine, New Haven, CT; [2]Pediatrics, Yale Univ School of Medicine, New Haven, CT.*

Background: Mutations in *PKD1* or *PKD2* are responsible for autosomal dominant polycystic kidney disease. PKD2 is a six transmembrane protein that can complex with PKD1 to form a voltage-sensitive, non-selective cation channel. In cultured renal epithelial cells, mechanical stress on the cilium results in a rise in cytosolic calcium that depends on both PKD1 and PKD2. PKD2 is also essential for vertebrate left-right development, which is initiated in the left-right organizer (LRO) by cilia-driven directional fluid flow. However, the exact role of polycystins in ciliary and cellular signaling remains unclear.

Methods: In this study, we utilized multiple compartment-targeted genetically encoded calcium indicators (GECIs) to measure calcium dynamics in the cilium and cytosol in cultured renal epithelial cells and at the LRO in developing zebrafish embryos.

Results: In cultured renal epithelial cells at resting state, the concentration of free calcium in the cilium is slightly higher than that in the cytosol. Strikingly, intra-ciliary calcium levels increase in response to triptolide, a small molecule agonist of Pkd2. Further, this response is dependent on extracellular calcium and there is a temporal delay between intra-ciliary and cytosolic calcium elevations. Our study in intact zebrafish embryos show that (1) Dynamic asymmetric intraciliary calcium waves at the LRO begin with the onset of cilia motility, preceding all other known molecular LR asymmetries; (2) Pkd2 initiates intraciliary calcium waves to direct LR development; and (3) intraciliary calcium signaling is specifically required for establishing LR asymmetry.

Conclusions: Our results suggest that the cilium can be an initiating source for intracellular calcium signaling and that triptolide activates the Polycystin channel on cilia in differentiated renal epithelial cells. Results from zebrafish place Pkd2 directly at mechanosensation at cilia in the LRO to initiate intraciliary calcium oscillations and further cytosolic calcium. Together, these results suggest that the cilium is a distinct calcium signaling compartment that are functionally significant in vivo.

Funding: NIDDK Support, Other NIH Support - NHLBI; NICHD, Private Foundation Support

TH-OR008

Pharmacology of a Novel, Highly Selective Aldosterone Synthase Inhibitor in Non-Human Primates Steven M. Weldon,[1] Nicholas F. Brown,[1] Jeremy G. Richman,[1] Matthew A. Cerny,[1] Derek Cogan,[1] Xin Guo,[1] Glenn A. Reinhart.[1] *[1]CardioMetabolic Diseases; [2]Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT.*

Background: Aldosterone (ALDO) signaling modulates pathology in metabolic diseases including diabetic nephropathy (DN). An ideal, selective aldosterone synthase (CYP11B2) inhibitor (ASI) will reduce ALDO levels in patients and minimize deleterious effects mediated via MR- and non-MR pathways without altering cortisol (CORT). However, discovery of selective ASI molecules is challenging due to 93% identity of AS with cortisol synthase (CYP11B1) and the utility of preclinical rodent models that are limited by low homology. Therefore we developed an acute conscious cynomolgus (CYNO) monkey model to define the in vivo selectivity of ASI molecules including the novel molecule, BI689648.

Methods: Conscious, non-chaired CYNOs received vehicle (n=23), FAD286 (n=9), LCI699 (n=36) or BI 689648 (n=26) at doses of (0.003-10 mg/kg) via NG gavage (po) or iv (lightly ketamine sedated) prior to ACTH-challenge. Maximal ACTH-induced ALDO and CORT production occurred within 15 min at which time blood was collected for plasma concentration of ALDO, CORT and compound. Steroid levels for individual ASI-treated CYNOs were expressed relative to mean values for the vehicle-treated group and plotted against plasma [compound]. This allowed aggregation of data from multiple studies to derive in vivo EC_{50} values for ALDO and CORT by curve-fitting. CYNOs were used for multiple experiments allowing 2 week washouts.

Results: Baseline ALDO was 139±17 pg/ml and CORT was 50±2 ng/ml (n=82; complete study cohort). Both ALDO and CORT increased ~4-fold at 15 min post-ACTH. Calculated EC_{50} ALDO/CORT were: FAD286=13/140 nM (~11-fold selective), LCI699=3.5/920 nM (~260-fold), BI 689648=1.5/>11000 (>7000-fold).

Conclusions: The cyno ACTH-challenge model differentiates highly selective inhibitors from those with moderate (LCI699) or low (FAD) selectivity. BI 689648 was identified as the first example of a potent ASI in CYNO with high selectivity versus CORT representing a major improvement toward the discovery of safe and effective ASIs for clinical use including DN.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim Pharm Inc.

TH-OR009

Heparanase-2 Is an Inhibitor of Heparanase-1, Stabilizes Endothelial Glycocalyx, and Prevents Albuminuria in Zebrafish and Diabetic Mice Hermann G. Haller,[1,2] Anna Bertram,[1] Putri Andina Agustian,[1] Torsten Kirsch,[1] Mario Schiffer,[1,2] Jan Menne.[1] *[1]Department of Nephrology, Medizinische Hochschule Hannover, Hannover, Germany; [2]Mount Desert Biological Laboratory, Bar Harbor, ME.*

Background: Heparanase-1 activity is implicated in endothelial dysfunction. Heparanase-2, a homologue of heparanase-1, associates physically with heparanase-1 and inhibits heparanase enzymatic activity. Using a zebrafish model of glomerular albuminuria we have tested the hypothesis that heparanase-2 is in equilibrium with heparanase-1 and prevents heparanase-1 induced downregulation of glycocalyx and endothelial cell dysfunction.

Methods: Heparanase-2 was down-regulated by morpholino in transgenic zebrafish Tg(l-fabp:DBP:EGFP) and proteinuria asessed by the Fabp-eye-assay. Glomerular barrier was analyzed by immunohistochemistry and electron microscopy. STZ -treated mice received either sham or rivaroxaban. Urinary albumin was measured by ELISA. Immunohistochemistry was performed on cryostat or on paraffin sections. Gene and protein expression was analyzed by real-time qPCR and western blot analysis.

Results: Downregulation of heparanase-2 induced edema, endothelial cell swelling and massive proteinuria in the zebrafish. Concomitant inhibition of both heparanases abolished this effect. Rivaroxaban prevented diabetes-induced albuminuria in the mosue and increased heparanase-2 expression in the glomeruli. Overexpression of heparanase-2 in diabetic mice prevented the development of proteinuria.

Conclusions: Heparanse-2 stabilizes the endothelial glycocalyx and prevents heparanase-1 induced endothelial damage and dysfunction. Both enzymes seem to be necessary for balanced regulation of the endothelial glycocalyx. Induction of heparanase-2 may be a therapeutic strategy in diabetic nephropathy and other glomerulopathies.

Funding: Government Support - Non-U.S.

TH-OR010

Activation of Aryl Hydrocarbon Receptor by Indoxyl Sulfate, a Uremic Toxin, Is Critically Involved in Vascular Inflammation Shunsuke Ito,[1,2] Takeo Edamatsu,[2] Yoshiharu Itoh,[2] Masayuki Yoshida.[1] *[1]Life Science and Bioethics, Tokyo Medical and Dental Univ, Tokyo, Japan; [2]Kureha Corporation, Tokyo, Japan.*

Background: Indoxyl sulfate is associated with cardiovascular disease in patients with chronic kidney disease (CKD). Aryl hydrocarbon receptor (AhR) is a ligand-inducible transcription factor known to mediate the toxic effects of dioxins and uremic toxin such as indoxyl sulfate. Here, we investigated potential roles of AhR in indoxyl sulfate-induced leukocyte-endothelial interaction.

Methods: Endothelial cell-specific AhR deletion (eAhR KO) mice were generated utilizing the Cre-LoxP conditional knockout strategy. Indoxyl sulfate was administrated subcutaneously by osmotic pump for 2 weeks, followed by intraperitoneal injection of 2

μg of tumor necrosis factor-α (TNF-α). Leukocyte recruitment in the femoral artery was evaluated by intravital microscopy system. Human umbilical vein endothelial cells were transfected with siRNA of AhR (siAhR) and treated with indoxyl sulfate for 20 hours, followed by stimulation with TNF-α.

Results: Intravital microscopic analysis in control mice revealed that indoxyl sulfate dramatically enhanced TNF-α-induced leukocyte adhesion to the vascular wall, however, in eAhR KO mice, indoxyl sulfate did not increase the number of adherent leukocytes. *In vitro* studies demonstrated that AhR mediated indoxyl sulfate-enhanced leukocyte adhesion and expression of adhesion molecule such as E-selectin. Though indoxyl sulfate enhanced activation of JNK and NF-κB, siAhR did not suppress their induction. Luciferase assay revealed that the promoter region of E-selectin, corresponding to -153 to -146 bps, was essential for the effect of indoxyl sulfate mediated by AhR. Because the indoxyl sulfate response region is similar to AP-1 response element and cAMP response element (CRE), we investigated which transcription factors were involved in indoxyl sulfate. AP-1 but not CRE was activated by indoxyl sulfate, which is suppressed by siAhR.

Conclusions: AhR mediates indoxyl sulfate-enhanced vascular inflammation through activation of AP-1 transcriptional factor. These results provide a novel mechanistic insight into the CKD-related vascular inflammation.

TH-OR011

Low-Energy Shockwave Treatment Preserves the Kidney Microvasculature in Swine Renovascular Disease

Xin Zhang,[1] James D. Krier,[1] Carolina Amador Carrascal,[3] James F. Greenleaf,[3] Behzad Ebrahimi,[1] Amir Lerman,[2] Lilach O. Lerman.[1,2] *[1]Divs of Nephrology and Hypertension, Mayo Clinic; [2]Div of Cardiovascular Diseases, Mayo Clinic; [3]Dept of Physiology and Biomedical Engineering, Mayo Clinic.*

Background: Atherosclerotic renal artery stenosis (ARAS), an increasing cause of renal dysfunction, is characterized by micro-vascular rarefaction. Low-energy shockwave (SW) therapy is noninvasive, stimulates angiogenesis, and has been shown to improve cardiac function in coronary artery disease. We hypothesized that low-energy SW would alleviate renal dysfunction in ARAS by restoring angiogenesis.

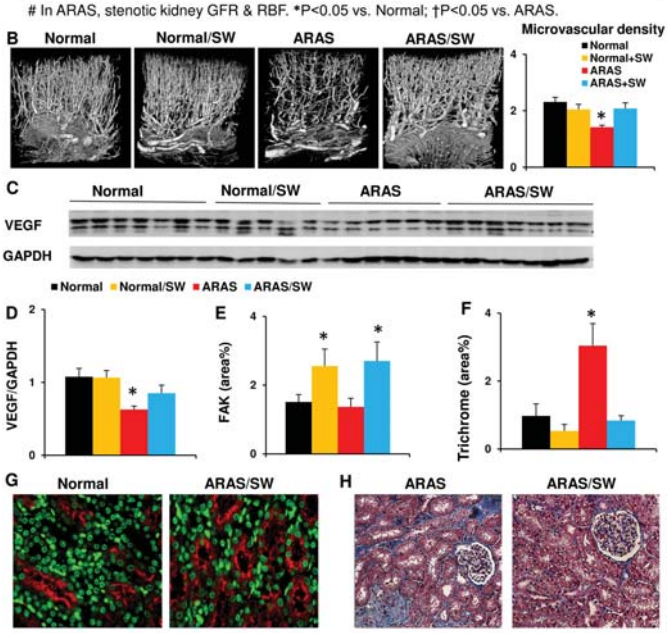
Methods: Domestic pigs were randomized to control or unilateral ARAS, treated with low-energy SW or sham (n=7 each group), bi-weekly for 3 consecutive weeks, starting after 3 weeks ARAS. Blood pressure, urinary protein, single kidney renal blood flow (RBF), glomerular filtration rate (GFR), and microvascular structure were assessed 4 weeks after completion of treatment. The expression of vascular endothelial growth factor (VEGF) and the mechanotransducer focal adhesion kinase (FAK) were determined by western blotting and staining, respectively.

Results: SW attenuated (albeit not normalized) blood pressure in ARAS, alleviated urinary protein excretion, improved stenotic kidney RBF and GFR, and blunted fibrosis (trichrome). This was associated with restored VEGF and elevated proximal tubular FAK expression, as well as normalized microvascular density.

A

	Normal	Normal/SW	ARAS	ARAS/SW
Mean arterial pressure (mmHg)	95.9±5.4	94.1±7.9	142.2±16.5*	119.0±12.8 *†
Urinary protein (mg/dl)	18.6±2.4	23.6±4.3	31.6±6.4 *	24.5±4.7*†
Single-kidney GFR# (ml/min)	70.7±6.1	73.2±7.0	51.2±3.9 *	71.5±10.1*†
Single-kidney RBF# (ml/min)	494.3±53.2	545.8±79.0	335.0±49.9 *	426.8±74.9*†

In ARAS, stenotic kidney GFR & RBF. *P<0.05 vs. Normal; †P<0.05 vs. ARAS.



B: Representative micro-CT images of kidney microcirculation and quantification. C and D: VEGF expression. E, G: FAK expression (red FAK, green nuclei). F, H: Fibrosis. * P<0.05 vs. Normal.

Conclusions: Low-energy SW protects the kidney by preserving its microcirculation, and may attenuate renovascular hypertension. SW may thus serve as a novel therapeutic intervention in the management of renovascular disease, and possibly in other forms of renal dysfunction.

Funding: Other NIH Support - HL085307, DK73608, HL121561, and HL77131

TH-OR012

Glomerular Filtration Rate in the Contralateral Kidney Falls After Successful Revascularization of Stenotic Kidney

Sandra Herrmann, Ahmed Saad, Lilach O. Lerman, Stephen C. Textor. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Overall glomerular filtration rate(GFR) in patients with atherosclerotic renal artery stenosis(ARAS) rarely increases after stenting despite technical success. Occlusive vascular disease is known to cause atrophy of the stenotic kidney(STK) and leads to increased volume of the contralateral kidney(CLK). We sought to examine changes in individual kidney blood flow, oxygenation and GFR before and after unilateral renal revascularization focusing on changes in the CLK.

Methods: Inpatient studies during fixed Na+ intake and ACE/ARB Rx were performed in unilateral ARAS with more than 60% occlusion(US velocities >250cm/s) before and 3 months after stent revascularization(n=10) or medical therapy alone(n=20). Renal blood flow was measured with multidetector CT and GFR by iothalamate clearance, individual kidney GFR was assigned by its percentage of renal blood flow(RBF). Tissue deoxyhemoglobin levels(R2*) were measured by blood oxygen level-dependent MRI at 3T.

Results: Baseline, RBF, GFR and tissue oxygenation(R2*) were reduced in the STK compared to the CLK. Total iothalamate GFR did not change in ARAS patients 3 months after either revascularization or medical therapy alone. STK RBF and tissue oxygenation increased 3 months after stenting, and STK GFR remained unchanged, whereas CLK GFR decreased(p=0.03).No such changes were evident in the medically treated group, in which at 3 months STK RBF and GFR were lower than in the stented group STK(p=0.01 and p=0.03 respectively).

Table : BOLD MRI and Multidetector CT Measurements of Renal Parameters before and after Revascularization or Medical Therapy Alone

Single Kidney	Baseline		3 months	
	Stent STK (N=10)	Stent CLK (N=10)	Stent STK (N=10)	Stent CLK (N=10)
Single Kidney R2*, 1/sec	24.7±6.2	20.7±2.1§	22.4±4.1*	21.5±2
Single kidney blood flow, mL/min	253±123.3	377.9±158.9§	320.8±175.5*	327±206.6
Single kidney GFR, mL/min	30.5±17.7	43.4±19.7§	34.3±15.8	36.6±19.5*
Patient Total Iothalamate Cl, mL/min	74±34.8		70.9±32.1	

- Data are mean and ± SD. STK indicates Stenotic Kidney; CLK, contra-lateral kidney; BOLD, blood oxygen level-depended; CT, computed tomography; GFR, glomerular filtration rate.
- §P<0.05 CLK vs STK within the same group
- *P<0.05 at 3 months vs baseline

Conclusions: Stenting increased RBF and reduced hypoxia in the STK, but failed to change GFR. Our results demonstrate that decreased compensatory GFR from the CLK contributes to the lack of improvement in total GFR after stenting. These data suggest that hypoxia within the STK may participate in crosstalk leading to the compensatory hypertrophy in the CLK.

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TH-OR013

Serum FGF21 Is a Novel Biomarker Associated with Endothelial Dysfunction and Aortic Calcification in Chronic Kidney Disease

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Background: Insulin resistance is present even in the earliest stage of renal dysfunction in patients with chronic kidney diseases (CKD). Fibroblast growth factor (FGF) 21 is a member of the endocrine FGF subfamily and a hepatic hormone involved in the regulation of glucose and lipid metabolism. The purpose of this study was to identify the relationship between the serum FGF21 levels and the vascular disorders in CKD patients.

Methods: We assessed the relationships between the serum FGF21 levels measured by ELISA and vascular parameters, including the flow-mediated dilatation (FMD), intima-media thickness (IMT), ankle-brachial pulse wave velocity (baPWV) and aortic calcification index (ACI) in 200 CKD patients (median age, 56 years; 62% male; 55% had glomerulonephritis).

Results: The serum FGF21 level in the CKD patients with hypertension, diabetes and dyslipidemia was significantly higher than that in the patients without such conditions. The FGF21 level gradually increased along with the CKD stage ($p < 0.0001$). Regarding vascular parameters, the FGF21 level was positively correlated with the baPWV, maxIMT and ACI, and was negatively correlated with the FMD. There were significant increases in the FGF21 level in the patients with a FMD < 6 %, baPWV ≥ 1400 cm/sec, maxIMT ≥ 1.1 mm and ACI > 0%. In a multivariate analysis, the FGF21 level was a significant determinant of endothelial dysfunction, as assessed by a FMD < 6 %, and vascular calcification, indicated

by a value of ACI> 0%, even after adjustment for age, gender, body mass index, smoking, blood pressure, the lipid and glucose profile, the estimated GFR, proteinuria, the phosphate and FGF23 (phosphatonin).

Conclusions: The data indicate that the serum FGF21 level is significantly associated with vascular disorders, including endothelial dysfunction and vascular calcification, independent of the glucose and lipid profiles. The serum FGF21 level may serve as a novel biomarker of vascular dysfunction in CKD patients.

Funding: Government Support - Non-U.S.

TH-OR014

Enhancing Akt Signaling in the Endothelium Restores Defective Angiogenesis in Kidney After Injury Takahide Aburatani,[1] Giovanni Ligresti,[1,2] Ying Zheng,[1] Alfred Aplin,[1] Roberto F. Nicosia,[1,3] Jeremy Stuart Duffield.[1,2] *[1]Depts of Medicine & Pathology & Bioengineering, Univ of Washington, Seattle, WA; [2]Biogen Idec, Cambridge, MA; [3]VAPSHCS, Seattle, WA.*

Background: Acute kidney injury and CKD are characterized by progressive loss of kidney peritubular capillaries, which leads to deterioration of renal function. While many vascular beds can regenerate following tissue injury, the renal microvasculature fails to regenerate. This distinctive property of the kidney microvasculature justifies investigation of organ-specific regulatory mechanisms that can be selectively targeted to promote kidney stable neoangiogenesis. Functional characterization of kidney microvascular endothelial cells (KMVEC) will shed light on the unique properties of this vascular bed and may lead to new therapies to ameliorate renal function in pathologic conditions.

Results: Isolated KMVEC failed to form angiogenic sprouts in collagen invasion assays. Protein analysis of KMVEC showed that in response to VEGF stimulation, downstream signaling via Akt phosphorylation was severely attenuated. Similarly, kidney explants cultured in collagen failed to generate new vessels whereas control cultures of aortic, venous, lung or adipose tissue explants produced angiogenic outgrowths. Akt signaling plays a critical role during angiogenesis and is inhibited by the phosphatase PTEN. Pharmacologic inhibition of PTEN promoted angiogenic sprouting in cultures of KMVEC. PTEN inhibition also enhanced kidney revascularization *in vivo* following ischemia re-perfusion injury; diseased mice treated with the PTEN inhibitor showed higher vessel density in kidney compared to control mice.

Conclusions: KMVEC have limited angiogenic potential in part due to impaired Akt activation following VEGF stimulation. The PTEN inhibitor bpV restored the in vitro sprouting properties of isolated KMVEC and the angiogenic response of the kidney microvasculature in an ischemia re-perfusion injury model. These findings suggest pharmacologic targeting of Akt function may be beneficial for the treatment of kidney diseases associated with inadequate neovascularization.

TH-OR015

Effects of Linagliptin on Early Alterations of Renal Endothelial Function in Patients with Type-2 Diabetes Roland E. Schmieder, Christian Ott, Iris Kistner, Stefanie Friedrich. *Nephrology and Hypertension, Friedrich-Alexander-Univ Erlangen-Nürnberg, Univ Hospital, Erlangen, Germany.*

Background: Animal experiments and human studies indicate an increased nitric oxide (NO) activity and endothelial NO synthase (NOS) expression in type-2 diabetes. The exaggerated NO production, compensatory to the increased oxidative stress in diabetes, lead to early hemodynamic changes characterized by hyperfiltration, hyperperfusion and increased vascular permeability.

Methods: In this randomized, double-blind, investigator-initiated trial, 62 patients (57±9.3 years) with type-2 diabetes were randomly assigned to linagliptin 10mg (n=30) or placebo (n=32) for a 6 weeks treatment period. Endothelial function of the renal vasculature was assessed by constant-infusion input-clearance technique with p-aminohippurate and inulin, as well as urinary albumin creatinine ratio (UACR), both assessed before and after blockade of NOS with systemic infusion of N^G*-monomethyl-L-arginine (L-NMMA*).

Results: Treatment with linagliptin for 6 weeks reduced fasting (137±26 versus 129±30 mg/ml, p=0.072), postprandial (171±44 versus 160±45 mg/ml, p=0.076) blood glucose and HbA1c (6.98±0.7 versus 6.86±0.8 %, p=0.089), whereas not change occurred in placebo. Renal plasma flow (RPF) and glomerular filtration rate (GFR) did not change after linagliptin and placebo, without any difference between the groups. After 6 weeks of treatment the absolute [percental] change in RPF due to L-NMMA (primary objective), was lower (-46.8±34 [-7.61±5.3] ml/min [%] than in the placebo group (-65.1±36 [-10.4±5.6] ml/min [%], p=0.045 (0.046)), indicating a lower basal NO activity after treatment with linagliptin.Consistently, after 6 weeks of treatment UACR due to L-NMMA did not clearly change in linagliptin group (22.2 (11.6–40.3) versus 28.2 (16.5–50.3) mg/g, p=0.061), but increased significantly in the placebo group (13.5 (8.6–25.9) versus 20.9 (15.9–33.5) mg/g, p<0.001), pointing to upregulation of NO activity in the untreated placebo group.

Conclusions: Thus, our data suggest that linagliptin normalizes increased renal endothelial function, and hence NOS-dependency of vascular tone, in patients with type-2 diabetes.

TH-OR016

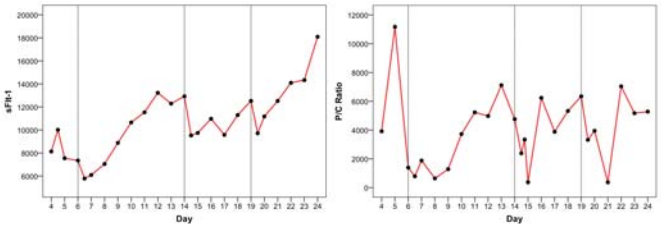
Extracorporeal Removal of sFlt1 in Preeclampsia: A Pilot Trial Ravi I. Thadhani,[1] Henning Hagmann,[2] Wiebke Schaarschmidt,[4] Tuelay Kisner,[2] S. Ananth Karumanchi,[3] Tom H. Lindner,[4] Alexander Fridman,[2] Bernhard Roth,[2] Peter Mallmann,[2] Holger Stepan,[4] Thomas Benzing.[2] *[1]MGH Nephrology, Boston, MA; [2]Univ of Cologne, Cologne, Germany; [3]BIDMC, Boston, MA; [4]Univ of Leipzig, Leipzig, Germany.*

Background: Soluble Flt1 is a potential therapeutic target to treat severe preterm preeclampsia. We previously reported that therapeutic apheresis with a whole blood dextran sulfate column reduces blood levels of sFlt1 and proteinuria in 8 women with severe preterm preeclampsia, and may prolong pregnancy (Circulation 2011). We now report treatment of 10 patients using a plasma specific dextran sulfate (PSDS) column.

Methods: Six patients with severe preterm preeclampsia (mean gestational age 30 weeks) underwent one PSDS apheresis treatment (duration 45-120 minutes), three were treated twice, and one was treated three times.

Results: Mean starting sFlt1 concentration was 17,447 pg/mL (range 7,546-35,301pg/ml), and the mean reduction within ~4 hours following apheresis was 18.6% (range 7.5-31.8%). Mean starting Protein:Creatinine (P/C) ratio was 6.2 g/g (range 0.4-19.3g/g), and the mean reduction within 12 hours following apheresis was 42.4% (range 87.9% reduction-19.2 increase). Among women treated 2-3 times, pregnancy continued for 10 days (range 7-18 days). Changes in sFlt1 and P/C ratio for the patient treated three times (dashed lines represent treatment days) is shown (Figure). The most common side effect during treatment was a reduction in blood pressure (10-20 mmHg), which was managed by withholding anti-hypertensive therapies, saline prehydration, and a reduction blood flow. There were no adverse effects to the fetus, and in women treated 2-3 times, fetal growth occured in-utero.

Conclusions: Therapeutic apheresis reduces sFlt1 and proteinuria in severe preterm preeclampsia, and may prolong pregnancy. A randomized phase III trial is now warranted.



Funding: Pharmaceutical Company Support - Kaneka

TH-OR017

Sphingosine-1-Phosphate Regulates Kidney Vascular Development Yan Hu,[1,2] Maria Luisa S. Sequeira Lopez.[1] *[1]Dept of Pediatrics, Univ of Virginia, Charlottesville, VA; [2]Dept of Biology, Univ of Virginia, Charlottesville, VA.*

Background: Sphingosine 1-phosphate receptor 1 (S1P1) is one of the five G-protein coupled receptors activated by Sphingosine 1-phosphate (S1P), which is a crucial sphingolipid in many biological processes, including vascular development. Deletion of S1P1 in mice results in embryonic (E) lethality at E12.5-14.5 due to failure of migration and/or differentiation of vascular smooth muscle cells (vSMCs) and pericytes and S1P1 functions autonomously in endothelial cells (ECs) as an inhibitor of angiogenesis. However, due to the early lethality, the role of S1P and S1P1 in kidney vascular development has not been determined. This study was designed to test the hypothesis that S1P and S1P1 regulate the development of the renal vasculature.

Methods: 1.Using *EC-SCL-Cre^{ERT}* mice that specifically express tamoxifen inducible Cre in EC progenitors crossed to *S1P1$^{fl/fl}$* and Rosa-LacZ reporter mice, we generated mice with timed deletion of *S1P1* in ECs during kidney development (*EC-S1P1KO*). 2.Histological stainings, including X-gal reaction and immunohistochemistry for markers of ECs and mural cells. 3.Cross-transplantation of E12.5 kidneys from *EC-S1P1KO* and control mice under the kidney capsule of adult WT mice. 4. Embryonic kidney cultures treated with S1P and S1P1 antagonists.

Results: 1. *EC-S1P1KO* embryos develop severe edema, hemorrhages or die around E14.5 to E16.5. 2. *EC-S1P1KO* embryos show kidney vascular abnormalities including dilation of arteries, veins and glomerular capillaries, disruption of vSMC coating of arteries and arterioles and absence of lymphatic endothelium. 3. The transplanted kidneys of *EC-S1P1KO* mice also develop vascular alterations revealing the intrinsic requirement of S1P1 for renal vascular development. 4. The embryonic kidney culture experiments show improved capillary development when exposed to S1P.

Conclusions: These studies strongly suggest that the S1P-S1P1 signaling pathway is an essential regulator of kidney vascular development.

Funding: NIDDK Support

TH-OR018

Prior Ultrasound Reduces Murine Acute Kidney Injury by Modulating Splenocyte Function Joseph C. Gigliotti,[1] Liping Huang,[1] Amandeep Bajwa,[1] Eric Mace,[1] Hong Ye,[1] Diane L. Rosin,[2] Kambiz Kalantari,[1] John Hossack,[3] Mark D. Okusa.[1] *[1]Nephrology & CIIR, Univ of Virginia; [2]Pharmacology, Univ of Virginia; [3]Biomedical Engineering, Univ of Virginia, Charlottesville, VA.*

Background: We have shown that prior (24-48hr) exposure to a modified ultrasound (US) regimen prevents kidney ischemia-reperfusion injury (IRI). Our data suggest that US modulates IRI via the splenic cholinergic anti-inflammatory pathway (CAP) and the α7 nicotinic acetylcholine receptor (α7nAChR). However, it is unclear how US stimulates the splenic CAP and whether it is efficacious in other forms of acute kidney injury (AKI).

Methods: Male C57BL/6 mice were lightly anesthetized and exposed to our modified US regimen (f=14MHz, MI=1.2) 24 hr prior to IRI or cecal ligation and puncture-induced sepsis (CLP). Chemical splenic sympathectomy was performed via splenic injections of 6-hydroxydopamine (6OHDA) 14d prior to US/IRI. Chimeric mice were generated via transfer of wild-type or $\alpha7nAChR^{-/-}$ bone marrow (BM) into irradiated mice 10wks prior to US/IRI. Splenocytes were isolated from US- or sham-treated mice 24hrs after US and then stimulated *ex vivo* with LPS or injected (i.v.) into naïve mice undergoing IRI 24hrs later.

Results: Similar to IRI, prior US reduced septic AKI as compared to sham+CLP (plasma creatinine 0.4±0.08 versus 0.8±0.12mg/dL, P=0.03). Splenic 6OHDA removed US protection from IRI, with reduced function (1.7±0.05mg/dL) and acute tubular necrosis compared to vehicle-injected mice (0.3±0.06, P<0.001). US treatment was protective only in chimeric mice with WT BM regardless of the parenchymal genotype (P<0.001). LPS-induced IL-6 release was greater in splenocytes from US-treated mice than controls (P<0.05); however IRI was significantly reduced when US-treated splenocytes (but not sham treated) were transferred to naïve mice 24hrs prior to surgery (0.4±0.2 versus 1.6±0.2mg/dL, P<0.001).

Conclusions: US reduces AKI in different animal models and is dependent upon the splenic nerve and hematopoietic α7nAChRs. US-treated splenocytes are more responsive to inflammatory stimuli and are capable of modulating IRI in vivo, supporting our ongoing hypothesis that US has therapeutic potential for AKI and other inflammatory conditions.

Funding: NIDDK Support

TH-OR019

Coupling Pulsed Focused Ultrasound with Mesenchymal Stem Cells Prevents and Rescues Established Acute Kidney Injury Scott R. Burks,[1] Saejeong Kim,[1] Jonathan Street,[2] Peter S.T. Yuen,[2] Robert A. Star,[2] Joseph Frank.[1] *[1]Radiology and Imaging Sciences, NIH, Bethesda, MD; [2]NIDDK, Bethesda, MD.*

Background: Pulsed focused ultrasound (pFUS) creates a "molecular zip code" that enhances homing of i.v. infused mesenchymal stem cells (MSC) to murine kidneys. We examined pFUS to enhance homing to kidneys during cisplatin-induced acute kidney injury (AKI). We investigated pFUS-enhanced MSC homing during early AKI; and delayed administration of pFUS and MSC until injury was clinically obvious to attempt rescue of renal function.

Methods: C3H mice received cisplatin (15 mg/kg ip), kidney pFUS (8 MPa; 5% duty cycle) and/or MSC (10^6 cells iv). The study included groups that had cisplatin only, cisplatin+pFUS, cisplatin+MSC, cisplatin+pFUS+MSC, and normal mice. For early treatment, mice received cisplatin at 0 hr and pFUS/MSC at 24hr. Renal function (BUN; SCr), apoptosis, and Ki67 expression were measured at 96 hr. For established disease, mice received cisplatin at 0 hr and MSC/pFUS at 72 hr. BUN/SCr were measured at 4 and 6 d, and 7-day survival was measured.

Results: pFUS enhanced MSC homing 2-3 fold at 24 or 96 hr. MSC alone at 24 hr modestly protected against AKI (reduced BUN ~40%). pFUS alone had no effect on AKI, but pFUS+MSC better protected than MSC alone (reduced BUN ~65%, SCr ~80%, apoptosis ~60%, and increased Ki67). To examine potential intervention during established AKI, treatment was delayed until 72 hr. Only pFUS+MSC improved BUN at day 4 and 6. MSC alone (no pFUS) significantly improved survival through 7 days (57% compared to 14%), but pFUS-enhanced MSC homing yielded additional significant increases in the survival (93%).

Conclusions: pFUS+MSC during early AKI further improves MSC protection against AKI. MSC alone improved survival when administered during clinically-obvious AKI (day 3) but survival further improved with pFUS+MSC. Therapies have largely failed for established AKI that requires treatment after renal function declines. Therefore, MSC with pFUS represent a novel potential therapeutic option to treat established AKI.

Funding: NIDDK Support, Other NIH Support - NIH Clinical Center; National Institute of Biomedical Imaging and Bioengineering

TH-OR020

Glycogen Synthase Kinase 3β (GSK 3β) Inhibition Promotes Macrophage Polarization and Reduces Inflammation and Acute and Chronic Damage following Acute Kidney Injury (AKI) Chunming Jiang,[1,2] Evelyn Tolbert, Weiwei Xu,[1] Hui Bao,[1] Rujun Gong,[1] Lance D. Dworkin.[1] *[1]Nephrology, Medicine, Brown Univ, Rhode Island Hospital, Providence, RI; [2]Nephrology, Drum Tower Hospital, Nanjing Univ, Nanjing, Jiangsu, China.*

Background: Different macrophage (Mø) phenotypes play distinct roles during kidney injury and recovery. Classically activated Mø (M1) promote inflammation and injury, while alternatively activated Mø (M2) help resolve inflammation and facilitate recovery. Although inhibition of GSK 3β has been shown to protect the kidney and promote recovery from AKI, the underlining mechanisms are not fully explored. We hypothesized that specific inhibition of GSK 3β by TDZD-8 might reduce kidney inflammation and injury in AKI by regulating Mø recruitment and polarization.

Methods: C57BL/6 mice were randomly divided into four groups; sham, FA, TDZD-PRE (30 minutes prior to FA), and TDZD-POST (36 hours after FA). Blood and kidney tissue were examined at multiple time points after FA.

Results: Kidney function was improved and morphologic evidence of injury decreased in both treated groups. Mechanistically in mice given FA, TDZD reduced Mø infiltration into the kidney (FA 25.1 ±2.2: TDZD-PRE 9.7±2.1: TDZD-POST 15.4±2.7; # per × 200 field), and markedly shifted Mø phenotype. The ratio of M1/M2 was in FA 2.5±0.6, TDZD-PRE 1.1±0.13, and TDZD-POST 0.2 ±0.1. *In vitro*, mouse bone marrow derived Mø stimulated with LPS underwent a similar shift from M1 to M2 with GSK 3β inhibition, as determined by increased expression of CD206 and arginase I, decreased the expression of iNOS and IL-1 β, and confirmed by flow cytometry. That the phenotypic change resulted from inhibition of GSK was confirmed by specific knockdown of GSK 3β, which also shifted cultured Mø from M1to M2.

Conclusions: Systemic administration of a small molecule GSK 3β inhibitor given both before and 36 hours after onset of AKI attenuated renal inflammation. Kidney Mø infiltration was reduced and Mø switching from pro-inflammatory M1 to anti-inflammatory M2 phenotype was promoted. This was associated with preserved and more rapid recover of renal function, reduced morphologic evidence of acute injury, and less eventual fibrosis.

Funding: Clinical Revenue Support

TH-OR021

KIM-1 Interacts with p85 and Modulates the Proximal Tubule Cell Inflammatory Response through Regulation of NFκB Craig R. Brooks,[1] Li Yang,[1,2] Venkata Sabbisetti,[1] Takaharu Ichimura,[1] Li-Li Hsiao,[1] Joseph V. Bonventre.[1] *[1]Renal Div, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Peking Univ First Hospital, Beijing, China.*

Background: Kidney injury molecule 1 (KIM-1) is a type 1 transmembrane protein which we identified as the most upregulated proximal tubule cell (PTC) protein during acute kidney injury (AKI) and is expressed in chronic kidney disease. Functionally, KIM-1 acts as a phosphatidylserine and scavenger receptor and is the first non-myeloid receptor identified that transforms epithelial cells into phagocytes.

Methods: The function of KIM-1-mediated phagocytosis in AKI was studied *in vivo* by inducing ischemia reperfusion injury in mice expressing wild-type KIM-1 or mutant KIM-1 lacking the mucin domain, $KIM\text{-}1^{\Delta mucin}$, where phagocytosis is markedly impaired. The role of KIM-1-mediated phagocytosis in inflammation was examined *in vitro* by analysis of cytokine secretion by ELISA and activation of NFκB using a luciferase reporter. RNA microarray and Ingenuity pathway analysis were performed to identify potential immuno-modulators regulated by KIM-1. KIM-1 phosphorylation and interaction with p85 was analyzed by immunoprecipitation.

Results: Ablation of the KIM-1 mucin domain significantly reduced PTC phagocytosis *in vivo* and *in vitro*. Decreased KIM-1-mediated phagocytosis *in vivo* led to accumulation of apoptotic cells, increased immune cell infiltration and a secondary worsening of kidney injury. Primary PTCs from $KIM\text{-}1^{\Delta mucin}$ mice released more pro-inflammatory cytokines when stimulated with LPS or TNFα, when compared to wild-type PTCs. Pathway analysis of microarray data revealed that NFκB transcriptional targets were modulated by KIM-1 expression. Wild-type, but not mutant, KIM-1 expression reduced p-NFκB levels and NFκB activity, which was further decreased with phagocytosis. Mechanistically, wild-type KIM-1 co-precipitated with p85 in a tyrosine-phosphorylation dependent manner. Inhibition of the p85/PI3K pathway negated the effects of KIM-1 expression on NFκB phosphorylation.

Conclusions: We have linked the process of KIM-1-mediated phagocytosis to modulation of PTC inflammatory response through KIM-1 interaction with p85 and regulation of NFκB.

Funding: NIDDK Support

TH-OR022

Fibroblast-Specific β-Catenin Signaling Dictates Tubular Injury and Repair After Acute Kidney Injury Dong Zhou,[1] Roderick J. Tan,[2] Haiyan Fu,[1] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

Background: Wnt/β-catenin signaling plays a crucial role in regulating kidney development and tissue homeostasis. We previously demonstrated that tubule-specific ablation of β-catenin aggravates acute kidney injury (AKI) after either ischemic or toxic insults, suggesting a beneficial, cyto-protective activity of Wnt/β-catenin. However, whether β-catenin activation in interstitial fibroblasts plays any role in kidney injury/repair after AKI remains ambiguous.

Methods: To address this issue, we generated conditional knockout mice in which β-catenin was specifically ablated in interstitial fibroblasts by mating β-catenin-floxed mice with $Gli1\text{-}CreER^{T2}$ mice. Mice with fibroblast-specific deletion of β-catenin were subjected to renal ischemia/reperfusion injury (IRI).

Results: Surprisingly, we found that specific ablation of β-catenin in interstitial fibroblasts significantly preserved renal function after IRI at 1 day, comparing with control littermates. Both serum creatinine level and morphologic injury were significantly ameliorated in mice with fibroblast-specific deletion of β-catenin. Consistently, apoptosis was markedly reduced in the kidneys of the knockout mice, which was accompanied by decreased expression of p53, FasL, Bax, and upregulation of phosphorylated Akt. Furthermore, NF-κB signaling was significantly suppressed in β-catenin-knockout mice. Interestingly, fibroblast-specific ablation of β-catenin resulted in an increased expression of hepatocyte growth factor (HGF) after AKI. Accordingly, HGF receptor, c-met, was activated

with increased tyrosine phosphorylation in renal tubules of fibroblast-specific β-catenin knockout mice. Consistently, kidneys with fibroblast-specific deletion of β-catenin displayed reduced myofibroblasts activation and renal fibrosis at 10 days after IRI.

Conclusions: Taken together, these results suggest that in sharp contrast to tubular β-catenin, fibroblast β-catenin activation after AKI is detrimental by attenuating HGF/c-met signaling.

Funding: NIDDK Support

TH-OR023

mTOR Mediates Autophagy Impairment in Telomerase Deficient Mice and Leads to Delayed Renal Recovery after Ischemia Reperfusion (I/R) Injury

Huifang Cheng, Raymond C. Harris. *Nephrology/Medicine, Vanderbilt Univ Medical School, Nashville, TN.*

Background: mTOR is a ubiquitous kinase that controls many different cellular processes and may play important roles in renal disease and aging.

Methods: To understand the role of mTOR in the increased susceptibility to AKI seen with aging, we induced renal I/R in fourth generation telomerase deficient mice (either TerC or TerT deletion), using wild type (Wt) mice as controls. mTOR activation was monitored at day 0, 1, 3, 5, 7 and 14 post I/R. We also investigated the effect of mTORC1 inhibition on hypoxia-induced injury in primary cultured renal tubular epithelial cells (TECs).

Results: Telomerase deficient mice had delayed recovery from I/R injury. Compared to Wt, following I/R injury both TerC / TerT KO mice had greater increases in phosphorylation of mTOR and its downstream signaling targets (4E-BP1 and p70 S6 Kinase) as well as P16, a cell-cycle regulator. I/R-induced markers of epithelial dedifferentiation (CD133, Vimentin, Pax2) and proliferation (Ki67) were markedly blunted in TerC/TerT KO mice.TerC/TerT KO mice also demonstrated impaired autophagy in response to I/R, as indicated by reduced and delayed autophagosome formation and conversion of LC3 I to II, along with prolonged accumulation of P62. Increased mTOR expression and impaired autophagy in response to transient hypoxia was further confirmed in primary cultures of TECs with TerC deletion. The mTOC1 inhibitor, rapamycin, restored the autophagic response in TECs with TerC deletion to that of Wt. However, rapamycin did not significantly alter either upregulation of p16 or impaired proliferation in TerC deficient TECs in response to the hypoxic insult.

Conclusions: These studies indicate that mice with telomerase deficiency have delayed recovery from I/R due both to impairment of autophagy mediated by increased mTOR signaling and activation of the p16 senescence pathway.

Funding: NIDDK Support, Veterans Affairs Support

TH-OR024

Hepcidin Mediates Protection in Renal Ischemia Reperfusion Injury Through Modulation of Systemic Iron Homeostasis

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Background: Iron-mediated oxidative stress has been implicated in the pathogenesis of renal ischemia reperfusion injury (IRI). Hepcidin exerts a precise control over systemic iron status by inducing ferroportin degradation, which results in reticuloendothelial iron sequestration and hypoferremia. We hypothesized that hepcidin treatment would mitigate IRI by decreasing iron availability and ROS-mediated cell death.

Methods: Mice (C57Bl/6 (WT) and hepcidin knock out (*Hamp*^{KO}) were treated with saline or 50 μg of hepcidin i.p, 24 hours prior to bilateral renal IRI (24 min in *Hamp*^{KO} and 26 min in WT). Outcomes (renal function, injury markers, histopathology and inflammation) were examined after 24 hours of reperfusion.

Results: In WT mice, IRI induced a significant increase in serum (100.19±19.8 μg/dl) and kidney non-heme iron levels (109.3±27.7 μg/g). In comparison, hepcidin treatment induced sequestration of iron in the spleen and liver and prevented IRI-associated increase in serum (50.91±13.39 μg/dl; p=0.0017 versus WT-IRI) and kidney non-heme iron (48.2±32.3 μg/g; p=0.018 versus WT-IRI). Kidney function was significantly better (p=0.001) in hepcidin-treated (P_{Cr} 0.9 ± 0.50) than saline-treated WT mice (P_{Cr} 3.1± 0.2) and this was accompanied by less acute tubular necrosis and reduced infiltration of immune cells. Hepcidin treatment decreased kidney ferroportin expression and induced the expression of cytoprotectant, H-ferritin. This was associated with less ROS and tubular epithelial apoptosis, as measured by 4-hydoxynonenal and TUNEL reactivity respectively. Hepcidin deficiency increased susceptibility to IRI as mild IRI (24 min) induced severe renal injury in *Hamp*^{KO} mice compared to WT controls (P_{Cr} 2.7± 0.6 versus WT 0.3± 0.07, p=0.001). Hepcidin supplementation restored a near complete protection in the *Hamp*^{KO} mice (P_{Cr} 0.5±0.2), with striking preservation of kidney architecture.

Conclusions: Our results demonstrate a protective role of hepcidin in IRI achieved through its effects on iron metabolism in the kidney as well as in distinct organs.

TH-OR025

Tubular Repair following Acute Kidney Injury Occurs through Clonal Expansion of a Preexisting Population of Tubular Progenitors in Mouse

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Background: Although renal progenitors (RPC) are well characterized in human, their role in kidney regeneration is still debated due to the lack of a suitable model of RPC lineage tracing in mouse. We propose to use Pax2 as a marker to perform RPC fate mapping and demonstrate RPC involvement in tubular regeneration following acute kidney injury (AKI).

Methods: To track RPC, we used Pax2-rtTA/tetO-cre/ROSA26-Confetti (Pax2/Confetti) inducible mice in which YFP, RFP or CYP is randomly expressed under Pax2 promoter. Pax8-rtTA/tetO-cre/ROSA26-Confetti (Pax8/Confetti) mice were used to track all tubular cells. To visualize cell-cycle progression, we used Pax2- or Pax8-rtTA/tetO-cre/ROSA26-FUCCI2 (Pax2/FUCCI2 and Pax8/FUCCI2) mice in which the promoter drives expression of mCherry or mVenus in cells in G1 or S/G2/M phase, respectively. To induce AKI, mice underwent unilateral ischemia for 35 minutes, followed by a 30 day reperfusion period (ischemia/reperfusion injury, IRI).

Results: In healthy Pax2/Confetti mice, Pax2+ cells form a subset of parietal epithelial cells of the Bowman's capsule and scattered cells in tubular structures. We observed a slow expansion of Pax2+ cells over time in physiologic conditions. After IRI, the percentage of Pax2+ cells increased in the ischemic versus contralateral kidney, suggesting that this population was amplified after IRI Indeed, the Pax2+ cells formed series of 3 to 11 cells labeled with an identical fluorochrome, indicating the clonal origin of the series. Observations of Pax2/FUCCI2 mice following IRI confirm that the RPC proliferate. Moreover, analysis of Pax8/Confetti mice after IRI excludes involvement of fully-differentiated tubular cells in the regeneration process and Pax8-FUCCI2 mice show that only a small population of cells undergo cell division while the rest of the tubular cells undergo hypertrophy as shown by their arrest in S/G2/M phase.

Conclusions: Pax2+ cells correspond to a preexisting population of tubular progenitors that clonally expands following AKI and represent the driver of tubular regeneration after IRI.

TH-OR026

Activated CD47 Contributes to Renal Ischemia Reperfusion Injury by Simultaneously Limiting Self-Renewal Genes and Autophagy

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Background: Renal ischemia reperfusion injury (IRI) is a frequent cause of acute kidney injury and promotes the development of chronic kidney disease. The basis for faulty repair following IRI remains unclear. Recently we reported that the matricellular protein thrombospondin-1 (TSP1), and its high affinity receptor CD47 are induced in renal IRI. However, the role of this receptor-ligand pair in regulating renal recovery is unknown.

Methods: Age matched male wild type (WT) and CD47$^{-/-}$ mice were challenged with 20 minutes bilateral renal ischemia and variable reperfusion time points (1, 3 and 7 days). All animals underwent assessment of renal function and biomolecular analysis. Human, and murine WT and CD47$^{-/-}$ rTEC were studied *in vitro.*

Results: Mice lacking CD47 were resistant to renal IRI with decreased urea and creatinine, and ameliorated histological changes at 1, 3 and 7 days following reperfusion compared to WT animals. CD47$^{-/-}$ mice subjected to renal IRI demonstrated return of renal function compared to ongoing impairment in renal function in WT controls after 7 days. CD47$^{-/-}$ mice, at both baseline and post-IRI, displayed upregulation of key self-renewal genes including cellular homolog of the v-myconcogene (cMyc), Klf4, Oct4, and Sox2, as well as autophagy markers Atg5, Atg7, Beclin-1. In contrast, WT mice demonstrated a negligible self-renewal gene expression and autophagic signaling at all time points post-IRI. RTEC from CD47$^{-/-}$ mice displayed basal and hypoxic upregulation of self-renewal genes and autophagy markers that correlated with enhanced proliferative capacity. siRNA suppression of cMyc down-regulated autophagy markers in RTEC. Conversely, treatment with a CD47 antagonist antibody, to block TSP1-CD47 signaling, or morpholino oligonucleotide increased cMyc expression and promoted cell proliferation.

Conclusions: CD47 is a proximate promoter of renal IRI and inhibits rTEC recovery through inhibition of self-renewal and cMyc-mediated inhibition of autophagy. Functional studies suggest that CD47 is a clinical target to restore renal function following injury.

Funding: Other NIH Support - O'Brien Kidney Pilot award P30 DK079307, Private Foundation Support

TH-OR027

ADAM17 Promotes Kidney Fibrosis after Severe Ischemia-Reperfusion Injury

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Background: ADAM17 expression has been found upregulated in ischemia induced fibrotic kidney lesions in humans and in kidneys of an angiotensin II-induced CKD mouse model. Its substrates, including EGFR ligands and TNFα, have been linked to maladaptive repair. We hypothesize that ADAM17 promotes injury and fibrosis in AKI and CKD.

Methods: We used ADAM17 hypomorph mice (A17ex/ex, very low ADAM17 expression in all cells) and a mouse model with tamoxifen-inducible proximal tubular-specific ADAM17 knockout (SLC34a1-Cre-ERt2/ADAM17flox/flox). Mice were subjected to severe bilateral ischemia reperfusion injury.

Results: ADAM17ex/ex mice show similar initial tubular damage on day 1 after injury but significantly less damage on day 5 after injury, as compared with wt mice. Kidney mRNA expression of ADAM17, EGFRs, EGF ligands and TNFα is significantly increased by injury over days 1-21 in wt mice. ADAM17 protein is strongly upregulated in wt mice as compared to sham controls, but ADAM17ex/ex mice lack detectable upregulation of ADAM17 protein after injury. Thus, ADAM17ex/ex mice show reduced TNFα and EGF ligand cleavage in vivo after injury, reduced activation of EGFR on days 2 and 3 after injury and a delay in proliferation of tubular cells after AKI, as compared to wt mice. ADAM17ex/ex mice show also reduced ingress of macrophages 5 pays post-injury. Interestingly, at later time points ADAM17ex/ex mice are strongly protected against fibrosis. As compared to wt, ADAM17ex/ex mice have lower mRNA induction of fibrosis markers (Tgfb, Pdgfr2, Acta2) 21 days after injury. On the protein level, they show significantly decreased production of the profibrotic markers fibronectin and αSMA at day 21 after injury, resulting in less interstitial fibrosis as detected by Masson stain. SLC34a1-Cre-ERt2/ADAM17flox/flox mice show similar acute injury, but significantly less induction of TGFα and reduced production of profibrotic myofibroblast markers αSMA and fibronectin, as compared to control littermates.

Conclusions: The above results reveal that ADAM17 has a critical role, in particular in proximal tubule, in kidney injury and repair and is a potential drug target in AKI and fibrosis.

Funding: NIDDK Support

TH-OR028

A Genome-Wide Association Study to Identify Single Nucleotide Polymorphisms Conferring Risk for Acute Kidney Injury

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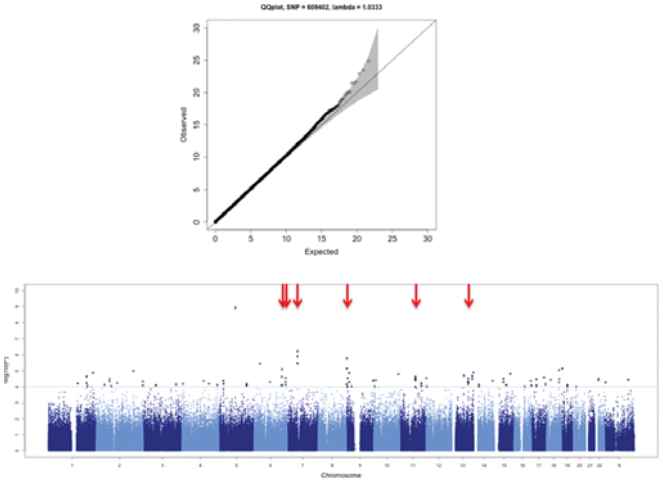
Background: Acute kidney injury (AKI) is a common and severe complication of multiple hospital settings. Identifying patients at heightened genetic risk for AKI may uncover novel pathways for therapeutic interventions. We performed a genome-wide association study (GWAS) in patients at risk for AKI to evaluate for single nucleotide polymorphisms (SNP) associated with the development of AKI.

Methods: 833 adult cases, defined by a rise in creatinine of 0.3mg/dL or 50% from baseline for at least 2 days, and 730 controls were selected from surgical (TRIBE-AKI) and medical (VALID ICU) ICU cohorts. Genotyping was performed with Illumina BeadChips with a total of 992,895 SNPs.

Results: After quality control, 773 (93%) cases and 684 (94%) controls were genotyped for 609,402 (61%) SNPs. 112 SNPs differed between cases and control with a p-value below a pre-specified cut-off of 10^{-4}. Six SNP clusters were identified containing at least 3 positive SNPs, on chromosomes 6 (2 clusters), 7, 9, 11 and 13 (**Figure 1**, red arrows).



While one cluster on chromosome 6 and that on 13 conferred an increased risk (OR range 1.35-1.73), the others were protective (OR range 0.27-0.67). On ontology analysis, significant SNPs were enriched in pathways for intracellular signaling, phosphate metabolism and amino acid phosphorylation. Further analysis on pre-specified sub-groups including patients with CKD and those with mild or severe AKI identified clusters for multiple sub-groups on chromosomes 4 and 14.

Conclusions: GWAS analysis of patients at risk for AKI undergoing cardiac surgery or admitted to the ICU identified multiple clusters of SNPs significantly associated with AKI. Further research is required to confirm these findings, identify candidate genes and characterize their function and role in AKI.

Funding: Other NIH Support - NHLBI

TH-OR029

The Effect of Early Intervention with Renal Replacement Therapy Guiding by Plasma Neutrophil Gelatinase Associated Lipocalin and the Outcome of Acute Kidney Injury (the EARLYRRT Trial): A Randomized Controlled Trial

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Background: Acute Kidney Injury (AKI) is a major complication of patients admitted to the intensive care unit (ICU). Unfortunately, there have been no successful intervention trials. Early initiation of renal replacement therapy (RRT) might provide the benefit to AKI patients in various aspects including controlling fluid balance, prevention of acid-base disorder, and finally improvement in patient survival.

Methods: This prospective RCT was conducted at ICU from 2013 to 2014. The study comprised an intervention trial and a triage trial running serially. Initially, we used novel AKI biomarker, plasma NGAL (pNGAL) to triage between severe AKI group (pNGAL ≥ 400 ng/ml) and less severe AKI group (pNGAL < 400 ng/ml). Only patients who had severe AKI (pNGAL ≥ 400 ng/ml) were randomized into two groups: *Early RRT group* and *Standard RRT group*. While patients who had pNGAL lower than 400 ng/ml were constituted as the *control group*. The primary outcome was mortality and the secondary outcome was ventilator-free days, ICU-free days and dialysis dependence on day 28.

Results: Of the 60 enrolled patients, 40 were randomly assigned to *Early RRT group*, and *Standard RRT group*. The baseline characteristics were comparable. At day 28, 10(50%) deaths had occurred in the *Early RRT group* and 9(45%) deaths in the *Standard RRT group* (P=0.96). While ventilator-free day and ICU-free day for the *Early RRT group* and the *Standard RRT group* were 21.0(8.9) versus 9.0(11.6) days, P=0.03 and 19.0(7.7) versus 10.4(11.4) days, P=0.38, respectively. No patient in the *Early RRT group* was dialysis dependence while 54.6% in the *Standard RRT group* were dialysis dependence, P=0.01.

Conclusions: EARLYRRT was the first RCT to test the role of pNGAL in guiding of RRT intervention and to proof the benefit of early RRT in severe AKI. The preliminary result showed that early RRT did not reduce mortality rate but could increase ventilator-free day and dialysis independence at 28 days.

Funding: Private Foundation Support

TH-OR030

A Multi-Center, Randomized, Control Trial to Assess the Safety and Efficacy of a Selective Cytopheretic Device (SCD) in Patients with Acute Kidney Injury (AKI)

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Background: Acute kidney injury (AKI) is a highly lethal condition in critically ill patients. Previous clinical studies have demonstrated the safety and efficacy of the Selective Cytopheretic Device (SCD) in the treatment of AKI requiring continuous renal replacement therapy in the intensive care unit (ICU).

Methods: We performed a randomized, controlled, multi-center trial of 134 ICU patients with AKI, 69 of whom received continuous renal replacement therapy (CRRT) alone and 65 of whom received SCD therapy.

Results: No significant difference in 60-day mortality was observed between the treated (27/69; 39%) and control patients (21/59; 36%, with 6 patients lost to follow up). Of the 19 SCD subjects (CRRT+SCD) and 31 control subjects (CRRT alone) who maintained a post-filter ionized calcium level in the protocol's recommended range (riCa), ≤ 0.4mmol/L for greater or equal to 90% of the therapy time, 60-day mortality was 16% (3/19) in the SCD group compared to 41% (11/27) in the CRRT alone group (p = 0.07). In the subset of patients who received the prescribed level of iCa, the secondary endpoint of dialysis dependency showed a statistically significant difference between the SCD group versus control with 0% (0/16) and 25 %(4/16), respectively (p=0.03). When the riCa treated subgroups were compared for a composite index of 60 day mortality and dialysis dependency, the percentage of SCD treated subjects was 19% versus 58% in the control subjects (p<.007). The incidence of serious adverse events did not differ between the treated (45/69; 65%) and control groups (40/65; 63%; p=0.86).

Conclusions: Attenuating the inflammatory response to AKI with SCD therapy may improve mortality in a tightly controlled regional hypocalcemic environment.

Funding: Pharmaceutical Company Support - CytoPherx, Inc.

TH-OR031

Remote Ischemic Preconditioning May Reduce Acute Kidney and Myocardial Injury in Children Undergoing Cardiac Surgery

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Background: Remote ischemic preconditioning (RPC) is a noninvasive intervention that protects remote organs from subsequent ischemia-reperfusion injury and may reduce the risk of clinical acute kidney injury (AKI) and acute myocardial injury (AMI).

Methods: We conducted a double-blind randomized clinical trial of RPC versus no treatment among 84 children undergoing cardiac surgery with cardiopulmonary bypass for congenital heart disease (ClinicalTrials.gov Identifier NCT01260259). We elicited

RPC by inflating a blood pressure cuff to 15 mmHg above systolic blood pressure over a lower extremity for 5 minutes and then deflated it for 5 minutes; this procedure was repeated up to 4 times before the start of surgery. Control children had a non-inflated cuff placed over a lower extremity for the same time period. We collected plasma biosamples at baseline and then 6, 12, 24, 48, and 72 hours postoperatively. Our primary aims were to determine whether RPC reduced AKI and AMI. We defined AKI by a 50% increase in plasma creatinine concentration and examined AMI by comparing mean troponin I (TnI) after RPC versus control.

Results: A total of 84 children underwent randomization (mean age 3.7 years; range 3 days-18 years). The incidences of postoperative AKI in the RPC and control groups were 35.6% and 51.3%, respectively; relative risk 0.69; 95% confidence interval (CI) 0.42, 1.14, p=0.15. Mean TnI was 27.3% lower after RPC compared to control (CI -72.5%, 17.9%, p=0.24).

Conclusions: This study demonstrates proof of concept that RPC may lower rates of AKI and AMI in children undergoing cardiac surgery, although results cannot exclude the null hypothesis. While previous groups studying RPC in children undergoing cardiac surgery have demonstrated RPC decreases AMI, only one group has studied AKI and concluded RPC does not decrease AKI. Our study is the first to demonstrate a potentially large protective effect of RPC on kidney injury in children and large clinical trials will be required to confirm or refute the findings.

Funding: NIDDK Support, Private Foundation Support

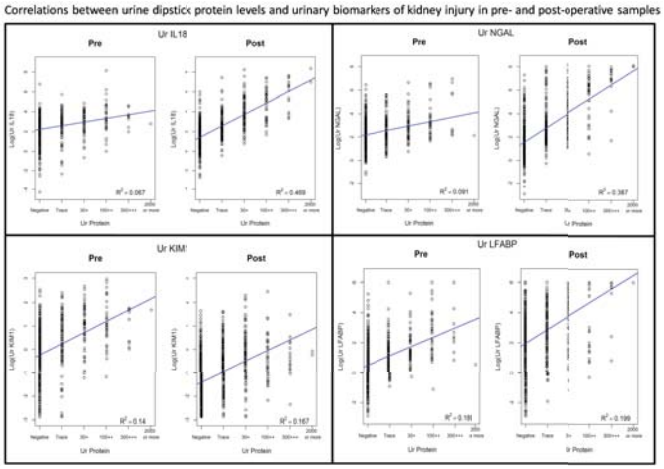
TH-OR032

Association between Urinary Kidney Injury Biomarker Concentrations and Urine Dipstick Findings Steven G. Coca, Allison Meisner, Uptal D. Patel, Jay L. Koyner, Amit X. Garg, Charles L. Edelstein, Michael Shlipak, Chirag R. Parikh. *TRIBE-AKI Consortium.*

Background: Concentrations of urinary biomarkers of kidney injury may be influenced by other factors besides renal tubular damage, such as hematuria or pyuria. We investigated urine dipstick factors that are associated with urinary biomarker levels.

Methods: We examined 714 adults who underwent cardiac surgery who did not experience post-op clinical AKI (peak serum creatinine change of ≥20% were excluded). We examined the association between urinalysis findings and the concentrations of 4 urinary biomarkers: NGAL, IL-18, KIM-1, and L-FABP at two time points- before surgery and immediately after surgery.

Results: Age, sex, and storage time had minimal associations with urinary biomarker concentrations. The presence of leukocyte esterase and nitrites on urinalyses were modestly associated with increased urinary NGAL (R^2 0.16, p<0.001 and 0.07, p<0.001, respectively) in pre-op urine samples. Hematuria weakly associated with increased levels of all 4 biomarkers on pre-op samples (R^2 0.02 to 0.04, p <0.001 for all), but was more strongly associated with IL-18, NGAL and L-FABP in post-operative samples (R^2 0.13, 0.21, and 0.13, respectively). Dipstick proteinuria concentrations correlated with levels of all 4 urinary biomarkers in pre-and post-operative samples



(R^2 between 0.05 and 0.14 in pre-op and between 0.10 and 0.34 in post-op samples).

Conclusions: Several findings from urine dipstick testing are positively associated with urinary biomarker concentrations in patients who underwent cardiac surgery. These associations were seen on both pre-operative and post-operative samples in patients without clinical AKI, which suggests the associations are not fully explained by clinical kidney injury. Future studies should consider these factors when assessing the independent prognostic and diagnostic performance of the biomarkers.

Funding: Other NIH Support - NHLBI

TH-OR033

Furosemide Stress Test Is Better Than Biochemical Biomarkers for the Prediction of AKI Severity, Progression and Mortality Jay L. Koyner, Danielle Davison, Ermira Brasha-Mitchell, Divya M. Chalikonda, John M. Arthur, Andrew Shaw, James A. Tumlin, Sharon A. Trevino, Michael R. Bennett, Paul L. Kimmel, Michael Seneff, Lakhmir S. Chawla. *Furosemide Stress Test Investigators.*

Background: A furosemide stress test (FST)(1.0 or 1.5 mg/kg based on prior diuretic exposure) in the setting of early AKI has been previously demonstrated to predict progression to Stage 3 AKI.

Methods: We measured several biomarkers of AKI in 77 subjects with at least AKIN Stage 1 who received a FST. We compared the ability of FST and biomarkers to predict progression to Stage 3 AKI(n=25, 32.4%), receipt of renal replacement therapy (RRT) (n=11, 14.2%) and inpatient mortality (n=16, 20.7%). We compared the area under the ROC (AUC) of FST to biomarkers alone.

Results:

Biomarker	AUC(SE) Progression to Stage 3 AKI	AUC(SE) Inpatient Mortality	AUC(SE) Receipt of RRT
FST	0.87(0.09)†	0.70(0.09)†	0.86(0.08)†
U NGAL	0.65(0.06)†‡	0.66(0.08)	0.50(0.08)‡
U IL-18	0.65(0.07)†‡	0.57(0.09)	0.61(0.07)‡
U KIM-1	0.63(0.06)‡	0.68(0.07)†	0.61(0.10)‡
Uromodulin	0.54(0.07)‡	0.52(0.08)	0.55(0.11)
U TIMP2 *IGFBP7	0.69(0.08)†‡	0.64(0.11)	0.62(0.13)
U ACR	0.56(0.07)‡	0.47(0.09)	0.67(0.09)
Fractional Excretion of Sodium	0.51(0.07)‡	0.41(0.08)‡	0.64(0.09)
Plasma NGAL	0.75(0.08)†‡	0.43(0.11)	0.52(0.13)
† p<0.05 biomarker to predict endpoint ‡p<0.05 for ΔAUC FST vs. biomarker; U=Urine			

Combining FST with individual biomarkers using logistic regression did not significantly improve its risk stratification (ΔAUC) for progression to Stage 3, receipt of RRT or inpatient mortality (p>0.10 for all). When FST was assessed in patients with increased pre-FST biomarker levels, the performance improved; AUC 0.91(0.06),p<0.001 for receipt of RRT (n=44; urine NGAL>150ng/ml) and 0.90(0.06),p<0.001 (n=32; TIMP2*IGFBP7 >0.3) for Stage 3 progression.

Conclusions: FST, a functional biomarker of AKI, outperformed biochemical biomarkers for the prediction of AKI progression, RRT and mortality. Using FST in patients with increased biomarker levels improved risk stratification. The utility of FST and biomarkers must be tested in randomized trials.

Funding: NIDDK Support

TH-OR034

Transient Tissue Hypoxia without Acute Kidney Injury Immediately and 3 Months after Complex CT Imaging and Renal Revascularization for Atherosclerotic Renovascular Disease Ahmed Saad, Sandra Herrmann, James Glockner, Michael A. Mckusick, Lilach O. Lerman, Stephen C. Textor. *Mayo Clinic.*

Background: Renal angiography and contrast-induced nephrotoxicity (CIN) are thought to pose a risk for older subjects with atherosclerotic renovascular disease (ARVD), particularly during renin-angiotensin system (RAAS) blockade, although specific pathways and degree of injury are poorly understood. We aimed to study the evolution of tissue oxygenation and markers of acute kidney injury (AKI) during early and subsequent months after renal imaging (CT angiography followed immediately by intra-arterial angiography and renal artery stenting).

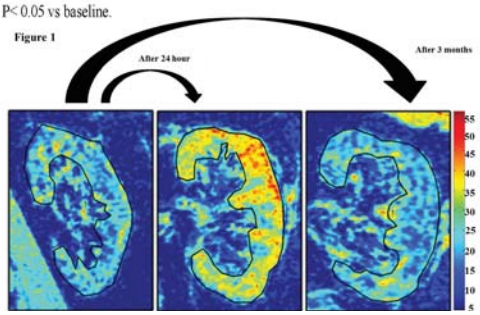
Methods: Inpatient studies were performed in patients with severe ARVD (Doppler Peak Systolic velocity = 318±100cm/sec), during 150 mEq Na+ intake and ACE/ARB Rx. Tissue deoxyhemoglobin levels (R2*) and fractional kidney hypoxia (% of Coronal area with R2*>20/s) of both kidneys were measured by BOLD-MRI at 3T. Blood samples of NGAL, KIM-1, TNF α and creatinine (Cr) were obtained before, 24 hour after and 3 months after imaging with contrast (Iohexol and iodixanol) (500mgI/kg).

Results: BOLD imaging 24 hours after CT imaging/stenting demonstrated substantial cortical hypoxia and elevated fractional kidney hypoxia (p < 0.05)Table. These changes were reversed to baseline after 3 months. Despite early changes in BOLD, no clinical episodes of CIN / AKI were identified and blood levels of NGAL, KIM-1, TNF α and Cr did not change; Serum Cr fell after 3 months for the group (p< 0.05)

Table. N=12	Baseline	After 24 hour	After 3 months
Age		72.3 ± 6.2	
Creatinine mg/dl	1.83 ± 0.49	1.7 ± 0.45	1.47 ± 0.4*
eGFR mL/min/1.73 m²	34.7 ± 11.8	36.9 ± 13.3*	45 ± 13.1 *
Total RBF (ml/min)	219.9 ± 103.8		255.4 ± 122.9
Cortical perfusion (ml/min/mL tissue)	2.5 ± 0.8		2.8 ± 1.1
Cortical R2* (1/s)	20.4 ± 4.3	24.6 ± 6.8*	18.4 ± 6.7
Fractional hypoxia(R2*> 20/s)	53.3 ± 22.1	69.4 ± 26.1*	42.4 ± 17.5
KIM-1 (ng/ml)	0.26 ± 0.2	0.25 0.2	0.24 ± 0.19
NGAL (ng/ml)	264.7 ± 77.4	243.1 ± 65.4	269.6 ± 86.1
TNF-α (pg/ml)	16.6 ± 7.3	16.7 ± 7.2	15.2 ± 13.2

(Mean ± SD) * P< 0.05 vs baseline.

Figure 1



Conclusions: Our results demonstrate widespread tissue hypoxia 24 hours after contrast imaging and renal stenting in patients with ARVD, under standardized conditions. Importantly, these changes were not associated with AKI based on circulating injury markers suggesting renal adaptation to transient hypoxia. These events were followed by improved blood flow and GFR 3 months later.

TH-OR035

Association between In-Hospital Furosemide Exposure and the Incidence of In-Hospital Acute Kidney Injury Joshua Taylor Swan,[1,2,3] Harlan Sparrow,[1] Beverly A. Shirkey,[2] Carol M. Ashton,[1,2,4] Wadi N. Suki,[1,4,5] David Putney,[1] Nelda P. Wray,[1,2,4] A. Osama Gaber.[1,2,4] *[1]Houston Methodist Hospital; [2]Houston Methodist Research Inst; [3]Texas Southern Univ; [4]Weil Cornell Medical College; [5]Baylor College of Medicine.*

Background: Furosemide (FUR) is commonly used in hospitalized patients who have a high risk of developing acute kidney injury (AKI) and may cause AKI through a hemodynamic-mediated mechanism. This study describes the association between FUR and incident AKI in hospitalized patients.

Methods: All first hospital admissions to a tertiary academic medical center in 2012 were included. Patients <18 years, those with preexisting AKI, stage V CKD, or dialysis on admission, and those with a maximum serum creatinine (SCr) <=0.4 mg/dL were excluded. Also, patients lacking 2 SCr values within a 72-hour period were excluded. In-hospital AKI was defined as an increase in SCr by >=0.3 mg/dL or >=50% or a decrease in eGFR by >=25% over a 72-hour interval. Billing claims and ICD9 codes were used to determine FUR use and comorbidities, respectively. We tested the association between FUR and AKI with chi-squared.

Results: This study included 13,951 unique patients, of whom 52% were female, 64% were Caucasian, and 2,568 (18%) developed AKI. FUR was used in 3,599 (26%) patients during the period for AKI determination and was associated with AKI (% AKI, 29.7% with FUR versus 14.5% without FUR, relative risk [RR]=2.1, P<0.001). This association was modified by comorbidity status.

Comorbidity	No. (%) with comorbidity	Comorbidity present				Comorbidity absent			
		% AKI with FUR	% AKI without FUR	RR	P	% AKI with FUR	% AKI without FUR	RR	P
CHF	2345 (17%)	37	38	1.0	NS	24	13	1.9	<.001
CKD	1305 (9%)	52	42	1.2	<.001	25	13	2.0	<.001
Cirrhosis	725 (5%)	32	24	1.3	<0.05	30	14	2.1	<.001
ASCVD	2462 (18%)	37	24	1.6	<.001	27	13	2.1	<.001
HTN	7706 (55%)	27	15	1.8	<.001	34	14	2.5	<.001
Diabetes	3664 (26%)	36	21	1.8	<.001	26	13	2.1	<.001

Conclusions: FUR was commonly used in hospitalized patients and was associated with an increased risk of in-hospital AKI in all subgroups analyzed, both with and without comorbidities, except for patients with CHF. Further research is needed to determine if this association is causal or induced by confounders.

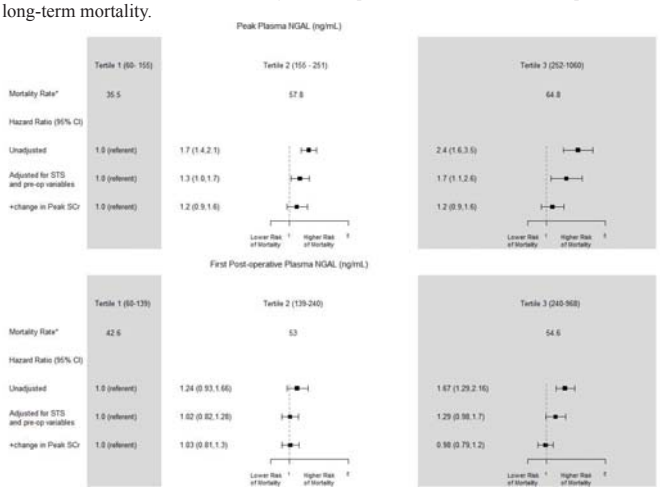
TH-OR036

Peri-Cardiac Surgery Levels of Plasma Neutrophil Gelatinase-Associated Lipocalin Are Not Associated with Long-Term Mortality Independent of Serum Creatinine Levels Dennis Moledina, Chirag R. Parikh, Amit X. Garg, Heather Thiessen Philbrook, Jay L. Koyner, Uptal D. Patel, Prasad Devarajan, Michael Shlipak, Steven G. Coca. *TRIBE-AKI Consortium.*

Background: Higher levels of plasma neutrophil gelatinase-associated lipocalin (pNGAL) are an early marker of acute kidney injury and are associated with increased risk of short-term adverse outcomes. The independent association between pNGAL and long-term mortality is unknown.

Methods: We examined the association between perioperative pNGAL concentrations and 3-year all-cause mortality in a six center, prospective long-term follow-up study of 1191 adults who underwent cardiac surgery between 2007 and 2009. We measured pNGAL both before surgery and on each of the three days after surgery.

Results: During a median follow-up of 3.0 years, 139 participants died (50/1000 person-years). After adjustment for pre- and intra-operative variables, including pre-operative NGAL levels, the first post-operative and peak post-operative NGAL were independently associated with 3-year mortality risk (adjusted HR 1.3, 95% CI 1.0-1.7 and adjusted HR 1.8, 95% CI 1.2-2.7, respectively). However, after adjustment for peri-operative changes in serum creatinine, there was no longer an independent association between pNGAL and long-term mortality.



Conclusions: Peri-operative pNGAL levels are associated with long-term mortality, but not independent of changes in the peri-operative serum creatinine concentration. The findings suggest plasma NGAL measurements may not be clinically useful or cost effective for prognostication beyond testing available in routine care to assess peri-operative kidney injury.

Funding: Other NIH Support - NHLBI

TH-OR037

Intermittent Hemodialysis in Acute Kidney Injury: Results from the VA/NIH ATN Trial Rowena B. Delos Santos,[1] Tingting Li,[1] Paul M. Palevsky,[2] Anitha Vijayan.[1] *[1]Renal Div, Washington Univ in St. Louis, St. Louis, MO; [2]Renal Section, VA Pittsburgh Healthcare System, Pittsburgh, PA.*

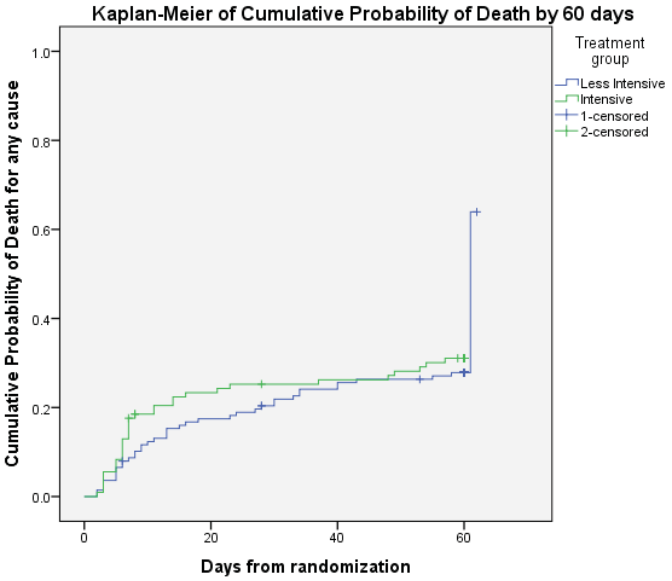
Background: Critically ill patients with AKI were randomized to receive intensive (I) or less-intensive (L-I) renal replacement therapy (RRT) in the VA/NIH Acute Renal Failure Trial Network (ATN) study. Modality of RRT was determined by CV-SOFA score. Hemodynamically stable subjects (CV-SOFA 0-2) received IHD 6X/wk (I) or 3X/wk (L-I). In this *post-hoc* analysis we assessed renal recovery (RR) and 60-day mortality in the 246 subjects who only received intermittent HD (IHD).

Methods: Categorical variables were expressed as proportions and continuous variables as mean±SD. Kaplan-Meier method was used to evaluate survival.

Results: Baseline data including age, sex, race, SCr, cause of AKI and APACHEII scores were not statistically different between the groups. Table 1 shows additional results.

	I IHD (N=108)	L-I IHD (N=138)	P Value
Mean pre-tx BUN(mg/dL)	44.4±27	68.1±31	<0.001
Initial Kt/Vurea	1.31±0.4	1.29±0.3	NS
Subsequent Kt/Vurea	1.33±0.4	1.32±0.3	NS
Mean pre-tx MAP(mmHg)	87.4±17	75.8±35	<0.001
Mean change in MAP during HD(mmHg)	-10.46±11.5	-9.89±13.1	NS
Net fluid removal per HD(L)	1.7±1.3	2.1±1.4	<0.001
Renal recovery	32%	43%	NS
Mortality	31%	29%	NS

Overall mortality was 29.5% and was not different between the 2 groups.



Conclusions: This is the largest study evaluating role of IHD in ICU patients with AKI. IHD 3Xwk required higher UF. Intradialytic hypotension (change in MAP) was not different. There was no difference in 60-day mortality and RR. Overall mortality was lower than typically observed in a critically ill population with AKI, most likely as a result of the selection criteria used in our analysis. There is no benefit to providing IHD more frequently than 3X/wk in hemodynamically stable critically ill AKI patients.

Funding: NIDDK Support, Veterans Affairs Support

TH-OR038

Recellularization of Vascular and Tubular Compartments of Rat Kidney Scaffolds with Embryonic Stem Cells Andrea Remuzzi,[3] Barbara Bonandrini,[1] Marina Figliuzzi,[1] Sara Silvani,[1] Ariela Benigni,[1] Giuseppe Remuzzi.[1,2] *[1]IRCCS Mario Negri Inst, Bergamo; [2]Ospedale Giovanni XXIII, Bergamo; [3]Univ of Bergamo, Dalmine, Italy.*

Background: The recontruction of the kidney is more challenging than regeneration of other tissues because of the complexity of kidney structures. We previusly showed that infusion of embryonic stem (mES) cells in the renal artery of acellular kidney scaffolds allowed repopulation of vascular tree and glomerular capillaries. This study aimed to develop experimental techniques to repopulate the tubular component.

Methods: As previously reported (Bonandrini et al. 2014) a complete decellularization of rat kidneys was achieved to produce extracellular matrix (ECM) scaffolds. There we seeded Kidney rat scaffolds with 15x10^6 mES cells through renal artery at 1 ml/min for 9 min. Then, 15x10^6 mES cells were infused through the ureter at 1 ml/min for 9 min while negative pressure of -50 mmHg was applied, in the perfusion chamber, outside of the scaffold. Scaffolds were then maintained in perfusion culture at 0.4 ml/min for 3 days.

Results: Immunofluorescence and H&E staining showed that mES cells infused in renal artery were uniformly distributed in the vascular network and in glomerular capillaries and expressed endothelial markers, while cells infused in the ureter reached tubular compartment (Figure 1). Cell delivery at proximal tubular level improved when scaffolds were maintained in seeding chamber under a vacuum to generate a pressure gradient across ECM.

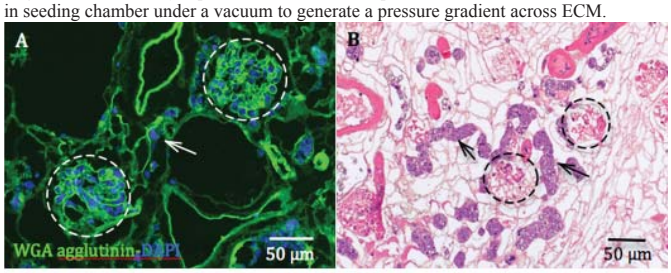


Figure 1. Immunofluorescence (A) and H&E (B) staining of recellularized kidney showing recellularized glomeruli (circle) and tubuli (arrows).

Conclusions: Our results demostrate that acellular kidney scaffolds can be cellularized not only at vascular level but also in the tubular compartment up to proximal tubules. Future studies to obtain more uniform and complete cellularization may open the way to recreate whole kidney in laboratory. *ERC grant RESET-268632 from EU supported this research.*

Funding: Government Support - Non-U.S.

TH-OR039

Long-Term Water Transport and Barrier Function of Proximal Tubule Cells Cultured Under Apical Shear Flow Conditions Paul R. Brakeman,[1] Peter Soler,[4] Nicholas J. Ferrell,[3] William Fissell,[3] Shuvo Roy.[2] *[1]Pediatrics, UCSF, San Francisco, CA; [2]Bioengineering & Therapeutic Sciences, UCSF, San Francisco, CA; [3]Medicine, Vanderbilt Univ School of Medicine, Nashville, TN; [4]Dept of Chemical & Biomolecular Engineering, UC Berkeley, Berkeley, CA.*

Background: Development of a bioartificial kidney (BAK) comprising a hemofilter and a proximal tubule cell bioreactor for treatment of chronic kidney disease requires long-term survival of proximal tubule cells under shear flow conditions. Previous work has only characterized transport properties of proximal tubules cells for 2-3 weeks in perfusion culture. We investigated fluid transport characteristics of proximal tubule cells in a shear flow bioreactor in long-term culture for >60 days.

Methods: LLC-PK1 cells were cultured on polycarbonate filter supports for 14 days and then exposed to apical shear stress. Cells were perfused with media containing urea (40 mg/dL) and creatinine (10 mg/dL) on the apical side and media alone was used on the basal side. The change in volume of the compartments and concentrations of sodium, potassium, phosphorus, urea and creatinine were measured.

Results: Water transport for proximal tubule cells under shear flow conditions increased significantly from 34+/-10 ul/cm^2/day on day 7 of low shear flow (0.2 dyn/cm^2) to 119+/-12 ul/cm^2/day on day 63 (p=.002) with high shear flow (2 dyn/cm^2) and was stable for 14 days at high shear flow from days 49 to 63. We characterized the barrier function of the cells by looking at the leakage of creatinine. On day 63, the leakage of creatinine was 0.012+/-0.009 mg/cm^2/day and did not differ significantly from the leakage of creatinine on day 7.

Conclusions: Proximal tubule cells can maintain barrier function and water transport under shear flow conditions for >60 days. Water transport increased with increased shear flow and time in culture.

Funding: NIDDK Support, Private Foundation Support

TH-OR040

Functional Organic Cation Transporters in Human Proximal Tubule Epithelial Cells Cultured on Hollow Fiber Membranes Jitske Jansen,[1,2,3] Ilaria Ester De Napoli,[4] Carolien M.S. Schophuizen,[1,2,3] Martijn J. Wilmer,[1] Jack F. Wetzels,[5] Lambertus P.W.J. Van den Heuvel,[3,6] Joost Hoenderop,[2] Dimitrios Stamatialis,[4] Rosalinde Masereeuw.[1] *[1]Pharmacology and Toxicology, RadboudUMC, Nijmegen, Netherlands; [2]Physiology, RadboudUMC, Nijmegen, Netherlands; [3]Pediatrics, RadboudUMC, Nijmegen, Netherlands; [4]Biomaterials Science and Technology, Biomedical Technology and Technical Medicine Faculty of Science and Technology, Enschede, Netherlands; [5]Nephrology, RadboudUMC, Nijmegen, Netherlands; [6]Pediatrics, Catholic Univ Leuven, Leuven, Belgium.*

Background: The bioartificial kidney (BAK) aims at developing living membranes for human renal epithelial cells-aided removal of protein-bound uremic toxins. Here, human conditionally immortalized proximal tubule epithelial cell (ciPTEC) monolayer functionality when cultured on polyethersulfone hollow fiber membranes (HFM) was evaluated.

Methods: Cell-free permeability of H_2O, IgG and albumin was studied in HFM coated with L-DOPA (2 mg/ml) and collagen IV (25 µg/ml). The the tight junction protein zonula occludens-1 (ZO-1) was examined by immunostaining in matured ciPTEC on HFM. Monolayer functionality was determined by real-time ASP^+ uptake (10 µM), a known substrate of Organic Cation Transporter 2 (OCT2), in the presence or absence of cimetidine (100 µM). Uptake was measured under a constant flow of 6 ml.h^{-1} by confocal microscopy.

Results: The H_2O permeability was not significantly different in coated HFM (17.1 ± 1.1 L.m^2.h^{-1}.mbar^{-1} *versus* uncoated 17.1 ± 0.4 L.m^2.h^{-1}.mbar^{-1}, n=6). The sievingcoefficient for IgG (0.91 ± 0.02, n=3) and albumin (0.94 ± 0.05, n=3) close to 1.00 indicated free passage of both compounds. After 7 days of culturing at 37°C, ciPTEC developed a tight monolayer with abundant ZO-1 expression along the tight junctions. Intracellular ASP^+ uptake was detectable within 1 min of perfusion, which was significantly inhibited for 61 ± 10% by cimetidine, as measured over 13 min (n=4; p<0.05).

Conclusions: This study reports, for the first time, active OCT2 transport in proximal tubule cell monolayers on HFM. We expect that this living membrane may positively contribute to the excretion of uremic toxins by ciPTEC in BAK.

Funding: Government Support - Non-U.S.

TH-OR041

A Microphysiological 3D Model of the Renal Proximal Tubule Demonstrates Reabsorptive Function Else M. Frohlich,[1] Hyoungshin Park,[1] Jose L. Alonso,[2] M. Amin Arnaout,[2] Joseph L. Charest.[1] *[1]Biomedical Engineering, Draper Laboratory, Cambridge, MA; [2]Div of Nephrology, Massachusetts General Hospital, Charlestown, MA.*

Background: Re-absorption occurs in the proximal tubule as solutes and water are transported from the tubule lumen to the blood stream. Since the re-absorption of fluids and solutes in the kidney constitutes transport through epithelial and vascular components, models of reabsorptive barriers, such as the proximal tubule, should provide both phenotypes to more accurately replicate the physiological barrier architecture.

Methods: In this work we demonstrate a co-culture orientation of primary human renal proximal tubule epithelial cells (hRPTEC) and human microvascular endothelial cells (hMVEC) in a microfluidic model. hRPTEC tissue layers were characterized by

immunofluorescent protein labeling, gene expression, and barrier function quantification techniques. Re-absorptive function was investigated by quantifying molecular uptake and sodium transport across the tissue.

Results: hRPTEC tissue layers were found to have enhanced tissue structure, extended viability, higher proliferation rates, and increased levels of mitochondrial activity in co-culture conditions of the model. hRPTEC tissue also expressed transport proteins and barrier characteristics, demonstrated glucose uptake, and showed decreased sodium re-absorption in response to a drug.

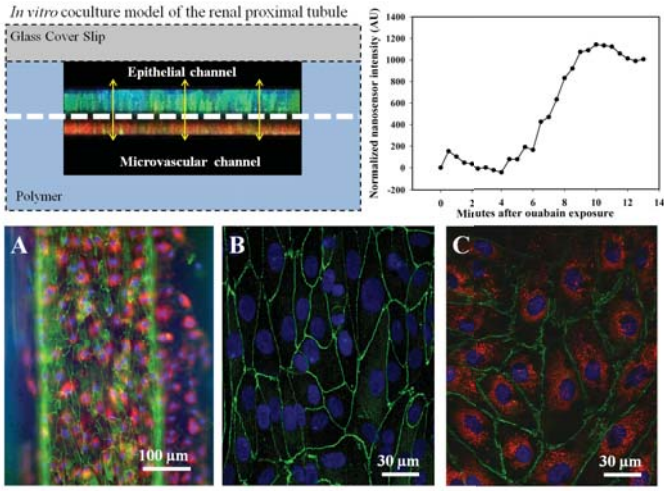


Figure 1. hRPTEC and hMVEC tissue layers are cultured on either side of a membrane in our microfluidic model to mimic the *in vivo* architecture of the renal re-absorption barrier. hRPTEC TJs are labeled (green) and hMVEC tissue layers are labeled for Von Willebrand factor (red). Epifluorescent microscopy shows the coculture of tissue layers in the model (A) and confocal images show well-developed hRTPEC (B) and hMVEC (C) tissue in each channel. The re-absorption of water and solutes can be studied across the tissue layers. Sodium re-absorption decreases across the tissue layers in response to a Na^+/K^+ ATP-ase pump inhibitor drug, evidenced by an increase of sodium-sensitive nanosensor signal in our model (graph).

Conclusions: The physiological microenvironment of our model enhanced tissue structure formation *in vitro* and mimicked *in vivo* architecture and function of the proximal tubule. Our model allows observation of tissue structure development, direct quantification of kidney-specific transport mechanisms and a method to quantify functional change to kidney tissue due to injury, kidney active compounds, or toxic insult.

TH-OR042

Development of a Bioengineered Human Kidney Microphysiological System for Assessment of Drug Transport and Toxicity Alenka Jaklic,[1] Elijah Weber,[1] Danny D. Shen,[1] Jonathan Himmelfarb,[2] Edward J. Kelly.[1] *[1]Pharmaceutics, Univ of Washington, Seattle, WA; [2]Kidney Research Inst, Univ of Washington, Seattle, WA.*

Background: There is a critical need to model the human kidney in order to improve our understanding of drug efficacy and toxicity, especially during drug development. The proximal tubular epithelial cells (PTEC) play a central role in the renal excretion of endogenous toxins and drugs, but to date conventional cultures of human PTECs fail to fully recapitulate their physiological functions in vivo.

Methods: We have developed and assessed the functionality of a 3D culture model of human PTECs in a microfluidic device (microphysiological system or MPS) from Nortis Inc., which allows luminal or basolateral perfusion.

Results: Human PTECs in the Nortis MPS platform expressed sodium-glucose transporter 2 (SGLT2) and did not express kidney injury marker (KIM-1). The exact opposite was observed in 2D culture, which confirms that in the MPS, PTECs are able to maintain its constitutive phenotype. In response to lowering of perfusate pH (7.4 to 6.9), PTECs in 3D culture increased production and secretion of ammonia, i.e., ammoniagenesis. Trans-epithelial transport of organic solutes by PTECs was assessed by measuring secretion of markers for OCT2 (creatinine) and OAT1/3 (p-aminohippurate, PAH). The appearance of markers in the lumenal effluent was reduced in the presence of their respective transport inhibitor. In addition, PAH secretion is reduced in the presence of indoxyl sulfate, confirming competition for the same OAT-mediated transport across PTECs. In ongoing studies with cisplatin and gentamicin, human PTECs in 3D culture showed altered morphology after timed exposure to these prototypical nephrotoxic compounds.

Conclusions: It is possible to recapitulate a functional proximal tubular epithelium ex vivo in an MPS. This system more accurately reflects renal physiology, and demonstrates renal handling of xenobiotics as well as biological response to renal toxins.

Funding: Other NIH Support - UH2TR000504

TH-OR043

Nine-Day Filtration by an Implantable Hemofilter Clark David Kensinger,[1] Seth J. Karp,[1] Joseph J. Groszek,[2] Mark S. Goodin,[3] Rishi Kant,[4] Torin Yeager,[4] Shuvo Roy,[4] William Fissell.[2] *[1]Surgical Sciences, Vanderbilt Univ, Nashville, TN; [2]Nephrology and Hypertension, Vanderbilt Univ, Nashville, TN; [3]Simutech Group, Hudson, OH; [4]Bioengineering and Therapeutic Sciences, Univ of California, San Francisco, CA.*

Background: Patients with end-stage renal disease (ESRD) have high mortality and morbidity on dialysis. ESRD patients could experience better quality of life if implanted with an artificial kidney. A new ultrathin membrane with highly controlled pores made by sacrificial silicon oxide techniques allows size selective sieving in vivo. In a canine model, we have demonstrated protein permselectivity through an implanted high-efficiency hemofiltration membrane over 9 days.

Methods: The device comprised a single channel blood conduit with parallel-plate hemofiltration membranes. *In vivo,* the device blood conduit was connected via PTFE grafts to the common iliac artery and vein. Filtrate was collected in two implanted reservoirs. The animal was housed without restriction and received thromboembolic prophylactic doses of enoxaparin (0.5mg/kg) once a day. After 9 days, filtrate was sampled from the reservoirs. Albumin concentration in filtrate was measured by colorimetric assay. Filtration rates were estimated by indicator dilution.

Results: Effluent sampled on day 9 had low albumin sieving coefficients (bag 1: 0.13, and bag 2: 0.24). Ficoll sieving coefficients at equivalent Stokes radius were 0.14 and 0.19 respectively. *In vivo* hydraulic permeability was consistent with *in vitro* measurements after correction for plasma oncotic pressure.

Conclusions: A high efficiency hemofiltration device is capable of filtration over 9 days in a canine surgical model. Albumin sieving coefficients and hydraulic permeability *in vivo* match preoperative in vitro values. This demonstrates sustained in vivo filtration by silicon nanopore membranes without fouling or thrombosis.

Funding: Other NIH Support - NIH NIBIB 1R01EB014315

TH-OR044

Evaluation of Next-Generation Silicon Nanopore Membranes Optimized for Diffusive Clearance Steven Kim,[1,2] Charles Blaha,[2] Zohora Iqbal,[2] Clarence Chow,[2] Rishi Kant,[2] Benjamin Chui,[3] Jaehyun Park,[2] Ken Goldman,[4] Eun Jung Kim,[2] William Fissell,[5] Shuvo Roy.[2] *[1]Nephrology, UCSF; [2]Bioengineering, UCSF; [3]Ben Chui Consulting; [4]H-Cubed; [5]Nephrology, Vanderbilt Univ.*

Background: Silicon nanopore membranes (HF-SNM) designed for hemofiltration have demonstrated remarkable permeability and selectivity. However, diffusive clearance was hindered by their thickness. Here we report hemodialysis-SNM (HD-SNM) with enhanced diffusive clearance.

Methods: A new MEMS (microelectromechanical systems) fabrication protocol utilizing nested etch-back techniques was used to decrease the effective SNM thickness (HD-SNM 100 µm versus HF-SNM 400 µm). Diffusive clearances of polyethylene glycol coated HD-SNM and HF-SNM with sub-10 nm pore sizes were tested in a parallel plate flow cell. PBS with Cr 10 mg/dL, BUN 90 mg/dL, and albumin 3 g/dL was recirculated (45ml), while dialysate (160 mEq NaCl) was recirculated in a counter-current fashion. At Q_d=Q_b=10 ml/min and zero transmembrane pressure (TMP) clearance was independent of flow rate. Solute clearance (K) was calculated by fitting concentrations measured at 0, 2, 4 hrs (n=3) to an exponential decay function: $C(t)=C_i e^{-Kt/V}$. C(t): conc at time t ,C_i: initial conc, V: volume. Filtration was tested in water and fetal bovine serum at various TMP (1, 2, 4 psi) using cross flow velocities at 0.1, 0.5 and 3 ml/min. Platelet adhesion and activation were evaluated by immunohistochemistry (IHC) and scanning electron microscopy (SEM) after flowing human blood for 2 hrs at 2 ml/min.

Results: HD-SNM had an ~3 fold improvement in K, consistent with mathematical models. Creatinine, BUN and phosphorus clearances were 280.8±20.8, 380.0±18.3, 231.1±7.6ml/min/m^2 (HD-SNM) and 85.5±10.6, 135.3±22.9, 75.5±12.8 ml/min/m^2 (HF-SNM), respectively. HD-SNM maintained mechanical integrity at over 200mmHg. The HD-SNM also showed comparable filtration rates (71.5 ± 21.3 ml/hr/mmHg/m^2) and selectivity to HF-SNM. IHC for CD62 and SEM images showed similar levels of platelet activation and adhesion.

Conclusions: These preliminary studies demonstrate significant improvement in diffusive clearance with the HD-SNM while still maintaining mechanical robustness, selectivity, permeability and hemocompatibility.

Funding: Other NIH Support - NIH NIBIB, Private Foundation Support

TH-OR045

Slit Nanotopography on Silicon Nanopore Membranes Resists Protein Deposition and Cell Attachment Eun Jung Kim,[1] William Fissell,[2] Tejal Ashwin Desai,[1] Shuvo Roy.[1] *[1]Bioengineering and Therapeutic Sciences, Univ of California, San Francisco, San Francisco, CA; [2]Nephrology and Hypertension, Vanderbilt Univ and Medical Center, Nashville, TN.*

Background: Silicon Nanopore Membranes (SNM) with compact geometry and uniform pore size distribution are under development for the hemofiltration unit in an implantable bioartificial kidney.[1] Key concerns for long-term membrane function are centered on protein deposition and cell attachment that can result in surface fouling and thrombotic occlusion.[2] In this study, weinvestigated the influence of surface coatings and nanotopography on protein deposition and cell growth on SNM substrates.

Methods: SNM substrates consisting a 6 x 6 mm slit-array patterned hemofiltration region areain the center surrounded by a 2 mm unpatterned, smoothborder, were modified by physically adsorbing either collagen type I (Col I-SNM) or covalently immobilizing RGD peptide (RGD-SNM).Atomic forcemicroscopy (AFM)was used to characterize the roughness of modified SNM surfaces.The propensity of protein adsorption onSNM surfaceswas evaluated using fluorescein isothiocyanate labeled bovine serum albumin (*FITC-BSA)*. Human umbilical vein endothelial cells growth on both modified and unmodified (Control) SNM were analyzed using immunohistochemistry.

Results: The surface roughness (RMS) of RGD-SNM (12.5nm) wasgreater than that of Col I-SNM (7.8 nm)and Control(6 nm). In unpatterned regions, FITC-BSA adsorbed strongly to the Col I as well as RGD, and RGD-SNM was found to significantly enhance cell growth (1500 % on day 7) compared to Col I-SNM (120%) and Control (100 %). In patterned area of all modified SNMs, however, FITC-BSA protein adsorption and cell growth are strongly attenuated (below 10 % on day 7). In addition, significant actin impairmentand cell detachment were observed on the patterned regions.

Conclusions: These results suggest that RGD is superior to Col I coatings for cell attachment. However, protein deposition and cell attachment on the slit-array region was significantly attenuated despite favorable coatings. This work will inform the development of SNM-based hemofiltration unit.

Funding: Other NIH Support - NIBIB, Private Foundation Support

TH-OR046

Water Permeable Nanoporous Membrane Device for Implantable Artificial Kidney Yoshihiko Kanno,[3] Naoya To,[1] Shinya Morita,[2] Ippei Sanada,[1] Norihisa Miki,[1] Hikaru Itoh.[1] *[1]Faculty of Science and Technology, Keio Univ; [2]Dept of Urology, Keio Univ; [3]Dept of Nephrology, Tokyo Medical Univ.*

Background: In the previous meeting, we have reported a microdialyzer containing nanoporous polyethersulfone (PES) membranes, which could remove urea nitrogen and electrolytes from the dissolved solution and cattle blood. Implantable dialysis system will improve patients' quality of life, which requires a sufficiently small and no-dialysate dialysis device. As a second step for future clinical use, we investigated whether this membrane device had water permeability in vitro and in vivo.

Methods: We developed water-permeable and robust nanoporous membrane from a casting solution of polyether sulfone (PES), polyethylene glycol (PEG), and Dimethylacetamide (DMAc) using a wet phase inversion method. The newly developed composition achieved high water permeability even with a high ratio of PES in in vitro experiments using a cow whole blood. The 24x24x16 cubic dialysis device that stacked 11 micro-channel titan layers and 10 membranes (14.1% PES, 11.8%PEG, 74.1% DMAc) was connected to SD rats. The rat artery and vein are connected to the inlet and outlet of the device. The blood pressure was monitored during the experiment. Then rat kidneys were removed and saline was infused.

Results: After the overload of saline, aqueous filtrate was successfully collected which was affected by blood pressure. Low-molecular-weight electrolytes in the blood was removed with the filtrate while proteins, such as albumin, was maintained in the blood. No leakage was observed and the filtrate was successfully collected. The filtrate was found to increase after the addition of saline, which implies that the filtrate amount depend on the blood pressure. We also investigated the composition of blood before and after the device. The concentration of urea nitrogen and potassium were decreased (31 to 27 mg/dl, 5.1 to 4.6 mEq/L) while that of albumin was maintained (2.4 to 2.3 g/dl).

Conclusions: Our water permeable nanoporous membrane device allowed water and low-molecular-weight molecules to permeate through, which may satisfy the requirements for the dialysis membranes for the future implantable artificial kidney.

Funding: Private Foundation Support

TH-OR047

Extracorporeal Mesenchymal Stromal Cell Therapy for Acute Kidney Injury Biju Parekkadan. *Surgery (Bioengineering), Harvard Medical School, Massachusetts General Hospital, Boston, MA.*

Background: Human mesenchymal stromal cells (MSCs) metabolize and secrete anti-inflammatory and regenerative factors that can be of systemic benefit in the setting of injury to the kidney. When transplanted, MSCs are limited in dose and rapidly cleared by the body, therefore prohibiting controlled exposure to this cell therapy. We have developed a bioreactor technology to maintain MSC viability at high fidelity and continuously delivers secreted factors into the blood stream in a clinical setting. MSCs were integrated into hollow-fiber bioreactor devices whereby the cells, separated by a permeable membrane, can directly and dynamically provide systemic therapy without entering the body. We present a human scale prototype of the technology that has shown sustained cell viability and function throughout clinical-grade manufacturing and large animal efficacy testing in preparation for a Phase I human trial set to begin in 2015.

Methods: A canine study of unilateral nephrectomy and a 90 minute, warm ischemic clamp of the remaining kidney was used to induce acute kidney injury (AKI). The bioreactor or control arms were tested 24 hours after ischemia and used in continuous operation for a 12 hour dose. Animals were monitored for survival with daily sampling of serum and urine with a 7-day study endpoint.

Results: This presentation will report encouraging results from in vivo therapeutic trials in a large animal (canine) model of ischemic acute kidney injury (AKI) whereby 91% of animals survived with extracorporeal MSC therapy compared to 50-60% in control arms. The biological response of extracorporeal MSC therapy in serum and tissue analysis revealed an anti-inflammatory effect at the prescribed dose. Pharmacological analysis of this bioreactor technology in vivo allowed for an unprecedented look at MSC function during product use and verified potency that is unattainable by conventional intravascular delivery of MSCs.

Conclusions: We expect that a combined approach ach to optimize MSC therapy that employs this novel approach to optimize the pharmacological influence of MSCs will enable the translation of this stem cell product to humans for AKI and other critical organ dysfunction syndromes.

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

TH-OR048

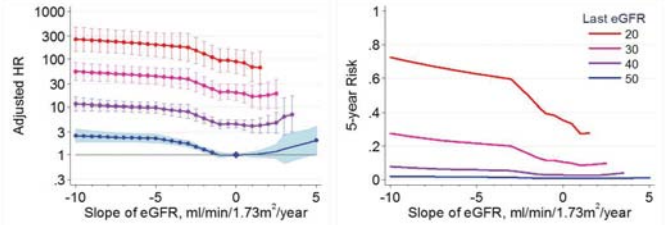
eGFR Level and Its Past Trajectory for Risk of Progression to End-Stage Renal Disease: Meta-Analysis of 22 Cohorts in the CKD Prognosis Consortium Csaba P. Kovesdy, Josef Coresh, Shoshana Ballew, Mark Woodward, David M. Naimark, Joseph V. Nally, Adeera Levin, Dietrich Rothenbacher, Benedicte Stengel, Kunitoshi Iseki, Kunihiro Matsushita, Andrew S. Levey. *CKD Prognosis Consortium.*

Background: The level of eGFR is a potent predictor of risk for progression to end-stage renal disease (ESRD). In practice, rate of progression is inferred from the past trajectory (slope) of eGFR decline. It is unclear how much prognostic information is provided by the current eGFR versus the past slope.

Methods: Slopes were estimated from all eGFR values in a 3-year baseline period of 13 CKD and 9 general/high-risk cohorts. We modeled the hazard ratios (HRs) of subsequent ESRD as a spline function of eGFR slopes after adjusting for age, sex, race, last eGFR, and co-morbid conditions compared to no eGFR decline. We used random effects meta-analyses to combine results across studies, stratified by type of cohort. We calculated the absolute risk of ESRD at 5 years after the last eGFR period using the weighted average baseline risk.

Results: 1,080,221participants experienced 5,159 ESRD events during a mean follow-up period of 2.0 years after the baseline period. In CKD cohorts the last eGFR was associated with a stronger HR than past decline (Figure, left panel), but both contributed substantially to the absolute risk of ESRD (Figure, right panel). Similar results were observed in the general/high risk cohorts. Results were similar when using different baseline periods for slope assessment and when adjusting for last albuminuria.

Figure. Adjusted hazard ratio and absolute risk of ESRD, vs. slope of eGFR



Conclusions: Risk of progression to ESRD is primarily associated with the current eGFR, but past slopes of eGFR decline can add further detail to risk assessment.

Funding: NIDDK Support

TH-OR049

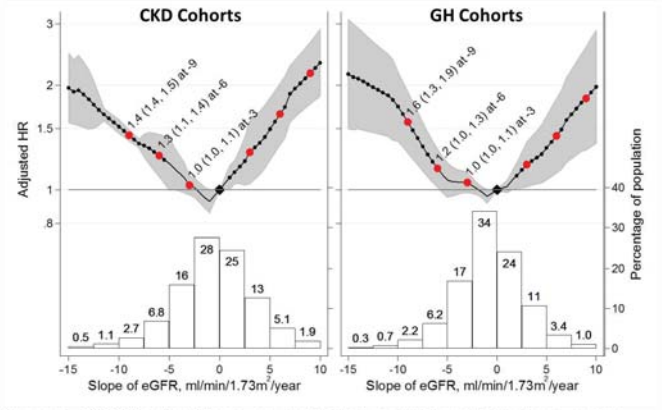
Prior Change in Estimated Glomerular Filtration Rate and the Subsequent Risk of All-Cause Mortality David M. Naimark, M. Grams, Kunihiro Matsushita, Corri Black, Lesley Inker, Areef Ishani, Sun Ha Jee, Akihiko Kitamura, Janice P. Lea, Joseph V. Nally, Carmen A. Peralta, Dietrich Rothenbacher, Seungho Ryu, Marcello Tonelli, Hiroshi Yatsuya, Ron T. Gansevoort, David G. Warnock, Mark Woodward. *CKD Prognosis Consortium.*

Background: A single determination of the estimated glomerular filtration rate (eGFR) is a strong predictor of subsequent all-cause mortality (ACM) risk. Whether an antecedent change in eGFR adds additional prognostic information regarding ACM risk is uncertain.

Methods: We conducted a meta-analysis of 22 general population and cardiovascular high-risk (GH) and 12 chronic kidney disease (CKD) cohorts in which eGFR slope could be determined during a 3-year antecedent period. Hazard ratios (HRs) for ACM associated with eGFR slope, after adjustment for various factors including last recorded eGFR, were estimated in a subsequent observation period.

Results: Among 1,277,202 subjects, 102,484 (8%) died over a mean observation time of 3.2 years after the initial 3-year antecedent period. A U-shaped relationship between ACM risk and eGFR slope was observed (Figure). For example, compared to stable eGFR, a decline of 9 ml/min/1.73m^2/year over the baseline period was associated with a subsequent adjusted hazard ratio of 1.6 (CI: 1.3-2.0) among GH and 1.4 (CI: 1.4 -1.5) among CKD cohorts, respectively. Sensitivity analyses, within strata of eGFR variability (root mean square error), after exclusion of subjects who lost weight during the baseline period, and after restriction to cardiovascular mortality, yielded qualitatively similar results.

Conclusions: Prior slope of the estimated glomerular filtration rate, whether positive or negative, provides additional prognostic information beyond that which can be obtained from a single measurement.



Top panels – ACM HRs adjusted for age, sex, race (black *vs.* non-black), systolic blood pressure, total cholesterol, diabetes, history of CVD, and baseline (last) eGFR. Bottom panels – distributions of antecedent eGFR slope frequencies among the GH and CKD cohorts.

Funding: NIDDK Support

TH-OR050

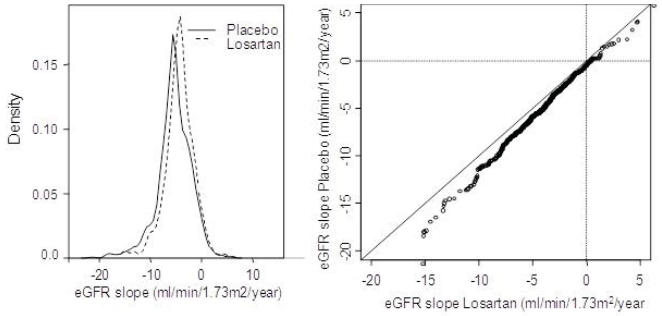
Slope of Glomerular Filtration Rate Decline as Clinical Trial Endpoint Misghina Tekeste Weldegiorgis,[1] Dick de Zeeuw,[1] Jamie P. Dwyer,[2] Tom Greene,[3] Hiddo Jan Lambers Heerspink.[1] *[1]Univ Medical Center Groningen, Netherlands; [2]Vanderbilt Univ School of Medicine; [3]Univ of Utah.*

Background: In some trials of chronic kidney disease (MDRD, AASK, DETAIL), the slope of GFR decline is used as efficacy endpoint. This endpoint provides great statistical power but only if the treatment effect does not depend on the underlying rate of GFR decline. In the MDRD trial it was shown that if this assumption is violated, statistical power is compromised as the lack of treatment effect in a subgroup (e.g. slow progressors) dilutes the overall treatment effect (*Greene Biometrics 2001*). We tested whether this also holds true for a trial testing a drug intervention, Angiotensin Receptor Blockers (ARB).

Methods: We used RENAAL and IDNT data testing the effect of losartan (RENAAL) and irbesartan (IDNT). We used linear mixed models to calculate the eGFR slope from baseline to month 3, and from month 3 until month 48 to exclude the acute effects of ARBs.

Results: In RENAAL, the decline in eGFR at 3 months was larger in the losartan versus placebo group (2.4 versus 1.3 ml/min/1.73m^2; p<0.01). After 3 months, the mean eGFR slope was smaller with losartan versus placebo (-4.4 versus -5.3 ml/min/1.73m^2/year; p<0.01). The variability in eGFR decline was also smaller with losartan (variance 8.5 versus 11.0; p<0.05). The attenuation of both the mean and variability of the slope suggests larger treatment effects in those with higher eGFR decline (Figure 1). Results were similar in IDNT.

Conclusions: The treatment effects of ARBs are larger in those with a faster rate of eGFR decline. The absence of treatment effect in slow progressors dilutes the greater treatment effect in fast progressors if eGFR slope is used as endpoint. These findings should be taken into account when considering eGFR slope as trial endpoint. Figure 1: Distribution of eGFR slopes (left) and QQ plot of eGFR slopes (right)



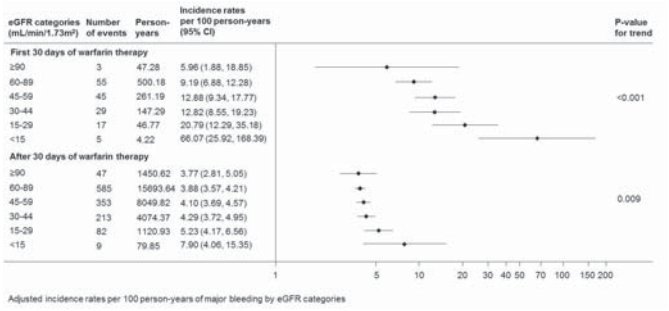
TH-OR051

The Association between Kidney Function and Major Bleeding in People with Atrial Fibrillation Initiating Warfarin Therapy Min Jun,[1] Matthew T. James,[1] Braden J. Manns,[1] Robert R. Quinn,[1] Pietro Ravani,[1] Marcello Tonelli,[2] Vlado Perkovic,[3] Wolfgang C. Winkelmayer,[4] Zhihai Ma,[1] Brenda Hemmelgarn.[1] *[1]U of Calgary, Canada; [2]U of Alberta, Canada; [3]George Inst, Australia; [4]Stanford Uni.*

Background: Limited safety data exist on warfarin in people with atrial fibrillation (AF) and reduced kidney function. The objective was to determine rates of major bleeding by level of kidney function in people with AF initiating warfarin.

Methods: We identified adults aged≥66years in Alberta, Canada, with AF who started warfarin therapy between 2003-2010. Patients were grouped into 6 eGFR categories:≥90, 60-89, 45-59, 30-44, 15-29, <15ml/min/1.73m^2; those with ESRD(dialysis or renal transplant) at baseline were excluded. The outcome assessed was a hospitalization or emergency department visit for major bleeding(intracerebral, upper or lower gastrointestinal, or other).

Results: Of 12403 patients, 45% had an eGFR<60mL/min/1.73m^2. Overall, 1443(11.6%) experienced a major bleeding episode over a median follow-up of 2.1(IQR:1.0-3.8) years. During the first 30 days of warfarin, adjusted rates of major bleeding were higher at lower eGFR(p for trend<0.001;).



eGFR categories (mL/min/1.73m²)	Number of events	Person-years	Incidence rates per 100 person-years (95% CI)	P-value for trend
First 30 days of warfarin therapy				
≥90	3	47.28	5.96 (1.88, 18.85)	
60-89	55	500.18	9.19 (6.88, 12.28)	
45-59	45	261.19	12.88 (9.34, 17.77)	<0.001
30-44	29	147.29	12.82 (8.55, 19.23)	
15-29	17	46.77	20.79 (12.29, 35.18)	
<15	5	4.22	66.07 (25.92, 168.39)	
After 30 days of warfarin therapy				
≥90	47	1450.62	3.77 (2.81, 5.05)	
60-89	585	15693.64	3.88 (3.57, 4.21)	
45-59	353	8049.82	4.10 (3.69, 4.57)	0.009
30-44	213	4074.37	4.29 (3.72, 4.95)	
15-29	82	1120.93	5.23 (4.17, 6.56)	
<15	9	79.85	7.90 (4.06, 15.35)	

Adjusted incidence rates per 100 person-years of major bleeding by eGFR categories

Adjusted bleeding rates per 100 person-years were 66.0 (95%CI:25.9-168.3) in patients with eGFR<15ml/min/1.73m^2 compared to 5.9 (95%CI:1.8-18.8) among those with eGFR>90ml/min/1.73m^2 (IRR 11.0, 95%CI:2.5-48.9). Across all eGFR categories, adjusted bleeding rates were consistently higher in the first 30 days of warfarin therapy than the remainder of follow-up.

Conclusions: Reduced kidney function was strongly associated with the risk of major bleeding in older adults with AF initiating warfarin, with excess risks from reduced eGFR most pronounced in the first 30 days. Our results support the need for careful assessment of bleeding risk in people with CKD and AF being considered for warfarin therapy, especially in the first 30 days of treatment.

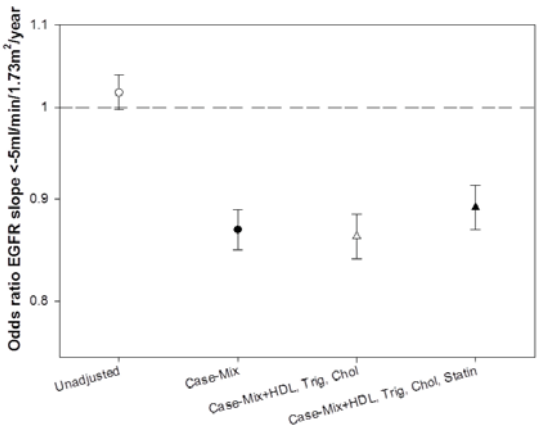
TH-OR052

Association of Baseline Use of Niacin with Progression of Chronic Kidney Disease in over 3 Million U.S. Veterans Elani Streja,[1] Kamyar Kalantar-Zadeh,[1] Hamid Moradi,[1] Miklos Zsolt Molnar,[2] Jun Ling Lu,[2] Csaba P. Kovesdy.[2] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Memphis VAMC, Memphis, TN.*

Background: It has been suggested that dyslipidemia can contribute to deterioration of kidney function. Niacin has been used to manage elevated triglycerides and low HDL levels. We hypothesize that niacin also reduces the progression of renal function decline in a cohort of U.S. Veterans.

Methods: In a cohort of 3.3 million U.S. Veterans with normal baseline eGFR in 2005-2006 we examined the association of baseline use of niacin with slopes of eGFR over median follow up of 7.7 years (6.0, 8.4). The referent group was patients who never used niacin throughout the cohort period. Associations were examined in crude and adjusted logistic regression models (for slopes <-5ml/min/1.73m^2/year), with adjustments for demographics, comorbidities, HDL, triglycerides, total cholesterol, and use of statins.

Results: Patients were 60±14 years old, 6% female, 17% African-American, and 23% diabetic with a mean baseline eGFR 84±15 mL/min/1.73m^2. In the total cohort, 9.3% of patients had a rapid kidney function decline with an eGFR slope of <-5 mL/min/1.73m^2/year. In logistic regression models after adjustment for case-mix covariates including BMI and cardiovascular and other comorbidities, baseline use of niacin was associated with lower odds of rapid kidney function. Associations remained significant after further adjustments for HDL, triglycerides total cholesterol, and use of statins.



Conclusions: Baseline use of niacin is associated with slower progression of CKD. Clinical trials evaluating the effect of niacin on progression of CKD are warranted.

Funding: NIDDK Support

TH-OR053

25-Year Kidney Function Trajectories Associated with *APOL1* High-Risk Variants: The ARIC Study

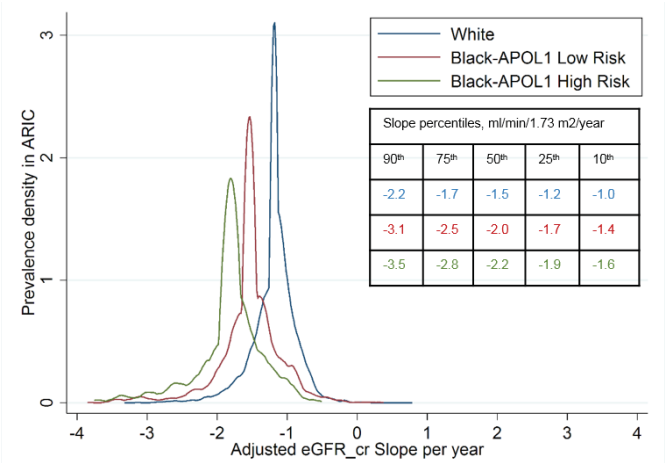
M. Grams, Adrienne Tin, C. Rebholz, Yuan Chen, Wen Hong Linda Kao, Josef Coresh. *Johns Hopkins Univ.*

Background: In populations with preexisting CKD, *APOL1* high-risk status associates with faster eGFR decline. Kidney function trajectories associated with *APOL1* high-risk status in populations without kidney disease have not been characterized.

Methods: Participants from the ARIC study, a community-based prospective cohort followed from 1987-1989 (baseline) until 2011-2013, were followed for yearly change in eGFR, based on study visit-measured creatinine (visit 1, 2, 4, and 5) and an imputed value of 15 ml/min/1.73 m^2 at incident ESRD (via linkage with USRDS). Mixed models with random intercept and slope were adjusted for race/*APOL1* high-risk status, age, sex, as well as baseline hypertension, diabetes, coronary heart disease, HDL cholesterol, and BMI.

Results: Among 12,902 participants (75.3% Caucasian, 21.5% African-American (AA) *APOL1* low-risk, and 3.2% AA *APOL1* high-risk), African Americans in both risk groups were younger, more often female, and had a higher prevalence of hypertension and diabetes at baseline. The annual change in eGFR differed by race/*APOL1* group: median, 75th, and 90th percentiles were -1.5, -1.7, and -2.2 ml/min/1.73 m^2 per year in Caucasians, -2.0, -2.5, and -3.1 ml/min/1.73 m^2 per year in AA *APOL1* low risk, and -2.2, -2.8, and -3.5 ml/min/1.73 m^2 in AA *APOL1* high risk. Applying the full distribution of slopes to the study population, the 25-year probabilities of eGFR <45 ml/min/1.73 m^2 among Caucasian, AA *APOL1* low-risk, and AA *APOL1* high-risk populations were 18.5%, 20.3%, and 30.5%, respectively.

Conclusions: In this population-based cohort, the 25-year slopes of eGFR varied by race and *APOL1* status, with a greater skew toward faster decline in African Americans, particularly those with *APOL1* high-risk status. All race/*APOL1* risk groups showed a wide heterogeneity in eGFR slopes, suggesting caution when making individualized predictions.



Funding: NIDDK Support

TH-OR054

Different Biomarker Profiles Identify Chronic Kidney Disease Patients of Different Etiologies at the Highest Risk of Mortality before Progression to Dialysis Dependence

David Langsford,[1] Ognjenka Djurdjev,[2,3] Mila Tang,[1,3] Adeera Levin.[1,3] *[1]Div of Nephrology, Univ of British Columbia; [2]BC Renal Agency; [3]On Behalf of CAN PREDDICT Steering Committee.*

Background: No model exists for predicting mortality in chronic kidney disease (CKD) patients despite the high risk for mortality in later stages of CKD. Competing risks for death and dialysis dependence vary according to CKD etiology. Newer biomarkers (BM) associated with specific pathological processes could identify CKD patients at risk of death before dialysis (DBD).

Methods: A subset of a pan-Canadian prospective cohort of 2544 referred CKD patients from 25 diversely located nephrology centers were examined for predictive utility of different panels of BM: troponin I, pro-brain natriuretic peptide (NT-proBNP), fibroblast growth factor 23 (FGF23), high sensitivity C-reactive protein (hsCRP) and asymmetric dimethylarginine (ADMA). We assessed all cause mortality before reaching dialysis and dialysis dependence at 3 yrs in 3 groups of CKD patients: those with diabetes (DM) n=672, glomerulonephritis (GN) n=275 and polycystic kidney/tubulointerstitial disease (PCKD/PI) n=222.

Results: The mean age (yrs) DM, GN, PCKD/PI was: 68, 58, 63. The mean eGFR (ml/min/1.73m^2) DM, GN, PCKD/PI was: 27.6, 27.3, 27.1. Overall 173 (14.8%) died: most in DM n=124 (18.3%), then PCKD/PI=28 (12.6%) and GN=21 (7.6%). Different panels of BM were associated with DBD in each group: DM with NT-proBNP and ADMA, GN with FGF23 and troponin and PCKD/PI with ADMA and hsCRP.

Parameter	Diabetic Nephropathy HR	95% C.I.		P value	GN HR	95% C.I.		P value	PCKD/PI HR	95% C.I.		P value
Age, per 5 yrs	1.321	1.188	1.469	<.0001	1.214	1.006	1.464	0.0434	1.672	1.292	2.163	<.0001
CVD history (Reference=No CVD)												
Ischemic	1.977	1.110	3.522	0.0207					3.769	1.360	10.446	0.0108
CHF	3.267	1.708	6.248	0.0003					0.897	0.115	7.022	0.9179
Ischemic+CHF	3.209	1.853	5.559	<.0001					3.197	1.036	9.869	0.0432
Albumin per 1 g/L	0.915	0.878	0.952	<.0001								
Hemoglobin per 1 g/L									0.961	0.936	0.988	0.0042
Cystatin C per 100 ng/mL	1.059	1.025	1.095	0.0006								
ADMA per 0.1 µM	1.300	1.098	1.540	0.0024					1.328	1.187	1.485	<.0001
logProBNP pg/mL	1.396	1.178	1.654	0.0001								
Troponin I (> LDL)					3.562	1.334	9.513	0.0113				
log TGFβ1 pg/mL					0.271	0.080	0.923	0.0368				
log FGF-23 RU/mL					2.065	1.100	3.878	0.0241				
log CRP mg/L									1.864	1.284	2.705	0.001

Conclusions: The risk of DBD is high, particularly in DM. BM can be used to identify patients at higher risk of DBD. BM profile differs between distinct CKD etiologies consistent with different pathological processes which may underlie DBD. Future studies should validate these findings in other cohorts and then test interventions aimed at addressing pathological processes.

Funding: Pharmaceutical Company Support - Ortho Biotech Canada

TH-OR055

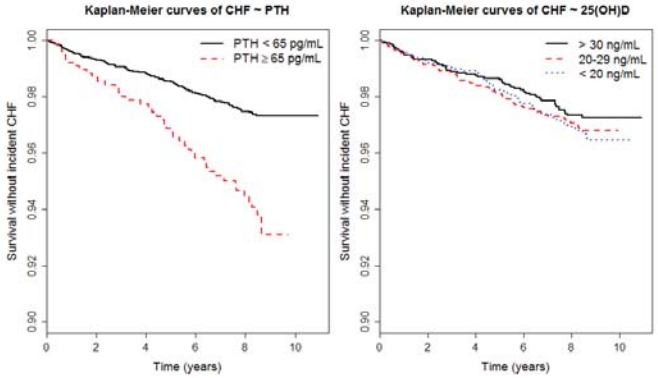
Parathyroid Hormone and 25-Hydroxyvitamin D Concentrations and Risk of Incident Heart Failure: The MESA Study

Leila R. Zelnick,[1] Ian H. de Boer,[1] Cassianne Robinson-Cohen,[1] Andrew N. Hoofnagle,[1] Joachim H. Ix,[2] Abigail Shoben,[4] Carmen A. Peralta,[5] David Siscovick,[1] Bryan R. Kestenbaum,[1] Nisha Bansal.[1] *[1]Univ of Washington; [2]Univ of California, San Diego; [3]Johns Hopkins Univ; [4]Ohio State Univ; [5]Univ of California, San Francisco.*

Background: Heart failure (HF) is common and associated with high mortality. Parathyroid hormone (PTH) and insufficient 25-hydroxyvitamin D (25[OH]D) may promote increased left ventricular mass (LVM) and clinical HF.

Methods: We tested associations of baseline PTH and mean annual 25(OH)D concentrations with incident HF events among 6,459 participants, and with LVM among 4,763 participants without prevalent cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis (MESA). HF events were physician-adjudicated. LVM was measured by cardiac MRI at baseline, concurrent with PTH and 25(OH)D. Cox proportional hazard and linear regression models were used to test associations of PTH and 25(OH)D with incident HF and LVM, respectively.

Results: Mean age was 62 years and 53% were female. There were 180 incident HF events over a median (IQR) follow-up time of 8.5 (7.7-8.6) years. Participants with higher PTH and lower 25(OH)D had higher unadjusted rates of incident HF.



Compared with participants with PTH<65 pg/mL, PTH>65 pg/mL was associated with a 50% greater risk of incident HF (95% CI: 3-219%) and a 5.3 gram higher left ventricular mass (95% CI: 2.6,7.9) in models adjusted for demographics, physical exam measures, comorbidity, kidney function and other mineral metabolism markers. In contrast, there was no association of 25(OH)D with risk of incident HF or elevated LVM.

Conclusions: In a racially/ethnically diverse population without prevalent cardiovascular disease, higher serum PTH concentration was associated with increased LVM and increased risk of incident HF. PTH excess may be a modifiable risk factor for HF.

TH-OR056

Diet and Major Renal Outcomes: The NIH-AARP Diet and Health Study

Andrew Smyth,[1,2,3] Michelle Canavan,[1] Matthew D. Griffin,[2] Martin O'donnell.[1] *[1]HRB Clinical Research Facility Galway, National Univ of Ireland Galway, Galway, Ireland; [2]Nephrology, Galway Univ Hospitals, Galway, Ireland; [3]Nephrology, McMaster Univ, Hamilton, ON, Canada.*

Background: Chronic kidney disease (CKD) affects 14% of the U.S. population and >400,000 people require dialysis. These patients are at increased risk of morbidity and mortality. Diet modification may be a low-cost, simple intervention that could reduce the burden of CKD. To evaluate the association between diet quality, sodium and potassium intake and major renal outcomes (death from renal cause or dialysis).

Methods: Participants from the National Institutes of Health-AARP Diet and Health Study completed a food frequency questionnaire, used to assess diet quality (Healthy Eating

Index (HEI), Alternate Healthy Eating Index (AHEI), Mediterranean Diet Score (MDS) and Recommended Food Score (RFS)), sodium and potassium intake. Participants on dialysis at baseline were excluded. Multivariable adjusted competing risks regression was used to calculate sub-hazard ratios (sHR) for a composite outcome of renal death (based on ICD coding) and self-reported dialysis, with non-renal death as the competing event.

Results: Of 544,635 included participants, 1,879 died from renal disease, 963 reported dialysis and the composite occurred in 2,757. Compared to the lowest quality diet (quintile 1), the highest quintile of HEI (sHR 0.83 (95% CI 0.72-0.98)), AHEI (sHR 0.77 (95% CI 0.68-0.88)) and MDS (sHR 0.84 (95% CI 0.71-0.99)) were associated with a reduced hazard of the composite. There was no significant association with RFS. The highest quintile of sodium:potassium ratio (sHR 1.19 (95% CI 1.05-1.36 versus lowest quintile)) was associated with an increased hazard.

Conclusions: Our findings support an association between healthy dietary patterns, including a diet lower in sodium and higher in potassium intake, and reduced risk of major renal outcomes.

Funding: Other NIH Support - Based on dataset held by NIH but no support provided by the NIH for this analysis

TH-OR057

Sickle Cell Trait and Chronic Kidney Disease and Albuminuria in African-Americans V. K. Derebail,[1,5] R. Naik,[2,5] M. Grams,[2,5] Nora Franceschini,[1,5] Bessie A. Young,[3,5] A. V. Kshirsagar,[1,5] James G. Wilson,[4,5] Alex Reiner.[3,5] *[1]UNC; [2]Johns Hopkins; [3]Univ of Washington; [4]Univ of Mississippi; [5]On Behalf of SCT-CKD Working Group.*

Background: Renal abnormalities are common among individuals with sickle cell trait (SCT) but historically considered benign. Large studies in well-characterized population-based cohorts investigating the relationship between SCT and chronic kidney disease (CKD) have not yet been performed.

Methods: We examined the association of SCT and CKD among self-identified African-Americans from five large population cohorts: the Atherosclerosis Risk in Communities Study (ARIC), Jackson Heart Study (JHS), Coronary Artery Risk Development in Young Adults (CARDIA), Multi-Ethnic Study of Atherosclerosis (MESA), and Women's Health Initiative (WHI). Exposure status was determined by custom genotyping, whole exome sequencing or genetic imputation. Primary outcomes were prevalent and incident CKD (eGFR<60ml/min/1.73m^2), prevalent albuminuria (spot urine albumin-creatinine ratio >30mg/g or albumin excretion rate >30mg/24 hours), and rapid decline in eGFR (decrease of >3ml/min/1.73m^2 per year). Effect sizes were calculated separately for each cohort and subsequently meta-analyzed using a random effects model.

Results: A total of 15,959 African-Americans (7.9% with SCT) were included from the five cohorts. Final regression models adjusted for age, sex, clinic/region, African genetic ancestry, diabetes and hypertension. In pooled meta-analysis, individuals with SCT had increased risk of prevalent CKD (odds ratio 1.56, p<0.001), incident CKD (odds ratio 1.78, p<0.001), and rapid decline in eGFR (odds ratio 1.30, p=0.013) compared to non-carriers. SCT was also associated with albuminuria (odds ratio 1.85, p<0.001). Similar associations were noted in each individual cohort. No interaction between SCT and diabetes or hypertension was noted. *APOL1* status (available in ARIC and JHS) did not influence the association between SCT and CKD or albuminuria.

Conclusions: Sickle cell trait is consistently and strongly associated with an increased risk of CKD, rapid eGFR decline, and albuminuria. Our findings demonstrate that sickle cell trait may contribute to the higher risk of kidney disease in African-Americans.

Funding: NIDDK Support, Other NIH Support - NHLBI, NIA, NIMHD, NCRR, NHGRI

TH-OR058

The *UMOD* Locus Shows Signature of Pathogen Adaptation through Human Evolution Olivier Devuyst,[1] Linda Pattini,[2] Guido Barbujani,[3] Luca Rampoldi.[4] *[1]Physiology, UZH, Zurich, Switzerland; [2]Politecnico di Milano, Milan, Italy; [3]Univ of Ferrara, Ferrara, Italy; [4]San Raffaele Scientific Inst, Milan, Italy.*

Background: Variants in the *UMOD* gene encoding uromodulin have been associated with the risk of developing hypertension and CKD in the general population. Lead variants from association studies map in the same linkage disequilibrium block in the *UMOD* promoter. The *UMOD* risk variants directly increase uromodulin expression *in vitro* and *in vivo*, causing salt-sensitive hypertension and renal lesions. The unusually high frequency of *UMOD* risk variants (about 80% in Europeans), combined with strong biological activity, suggested the action of some sort of selective pressure.

Methods: In order to test this hypothesis, we investigated the frequency of the allelic variants of the lead SNP rs4293393 of *UMOD* in modern and ancient human genomes and in available non-human primate genomes.

Results: The risk T allele had high allelic frequency in nearly all the populations present in Human Genome Diversity Project (HGDP), with few exceptions in populations from equatorial Africa and South America. The major, T allele was the one present in macaque, gorilla, orang-utan and gibbon genomes, suggesting that it is the ancestral one. Noteworthy, the rare, protective C variant was identified in the high-coverage genomes of Denisovans and Neandertals, from whom present-day humans diverged 550,000 to 750,000 years ago. We also correlated patterns of allele frequencies of a large sample of SNPs from the 52 human populations in HGDP to a set of environmental variables including climate, diet regimen and pathogens. Interestingly, the *UMOD* locus was significantly correlated with pathogen diversity (bacteria and helminths), but not with diet nor climatic conditions.

Conclusions: These data suggest pathogen-driven selection in the *UMOD* locus. Since uromodulin is known to interact with uropathogen bacteria and to modulate innate immunity, we speculate that *UMOD* variants associated with increased uromodulin expression and urinary excretion would confer selective advantage by enhancing antimicrobial and immunoregulatory activity. In turn, that might account for the current high frequencies of the *UMOD* risk allele.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-OR059

A Novel Locus for Blood Pressure Identified in GWAS of U.S. Hispanics Nora Franceschini,[1] Mariaelisa Graff,[1] Christina Wassel,[2] Myriam Fornage,[3] Ran Tao,[1] Cara Carty,[4] Stephanie Rosse,[4] Girish N. Nadkarni,[5] Eli A. Stahl,[5] Erwin P. Bottinger,[5] Charles Kooperberg.[4] *[1]Univ of North Carolina, Chapel Hill, NC; [2]Univ of Pittsburgh, Pittsburgh, PA; [3]Univ of Texas Health Sciences Center, Houston, TX; [4]Fred Hutchison Research Cancer Center, Seattle, WA; [5]Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Genome wide association studies identified over 55 loci for blood pressure (BP) and hypertension risk in populations. These studies did not include Hispanics, the fastest growing U.S minority who also has low rates of hypertension control. By leveraging the population admixture in Hispanics, we sought to identify novel loci for BP traits.

Methods: We tested the association of 161,098 metabochip array single nucleotide polymorphism (SNPs) with BP traits using genotyped or 1000 Genomes imputed data from three Hispanic studies (19,744 participants, aged 18 or older). We added 10 and 5 mmHg to systolic and diastolic BP for individuals using BP medications. Each study performed linear regression or mixed models assuming an additive genetic model, adjusted for age, sex, body mass index and population stratification. Results were combined using meta-analysis. A p-value <2.8 x 10^{-7} was considered significant based on multiple testing.

Results: We identified associations of SNPs with BP traits in three loci, including one novel region. An intronic variant at the *KCNK3* gene (rs2586886, minor allele frequency=0.32) was significantly associated with increased diastolic BP (p=5.8 x 10^{-9}). The encoded protein is a potassium channel associated with primary aldosteronism in double knockout mice of the *kcnk3/kcnk9* genes. Additional significantly associated SNPs were located at *FGF5* (systolic and diastolic BPs, p=1.8 x 10^{-10}) and *SHB2B3* (diastolic BP, p=1.2 x 10^{-8}), both known BP loci. At the *FGF5* locus, there was evidence for two independent signals.

Conclusions: We identified a novel locus for BP in Hispanics which has strong biological support, replicated two known BP loci and provided evidence for independent signals at *FGF5*. Our findings support the advantages of using diverse ancestry for gene discovery of BP traits.

Funding: Other NIH Support - 1R01HL118305-01A1; U01 HG007416-01

TH-OR060

Functional Annotation of SNPs Associated with Chronic Kidney Disease Yi-An Ko, Nora Ledo, Pazit Beckerman, Katalin Susztak. *Renal Electrolyte and Hypertension Div, Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA.*

Background: Genetic variants associated with chronic kidney disease (CKD) are localized to the non-coding regions of the genome. We hypothesize CKD associated SNPs localize to kidney specific gene regulatory regions (promoters, enhancers), alter transcription factor binding sites (TFBSs), and influence target transcript expression. Cell type specific gene regulatory regions can be identified by specific histone tail modifications. In addition, these regions show increased interspecies sequence conservation.

Methods: 1. To identify regulatory elements in human kidney, we performed chromatin immunoprecipitation and whole genome sequencing using histone tail modification antibodies (including H3K4me1, H3K4me3, H3K36me3, H3K27ac, and H3K27me3). 2. We develop an algorithm, HST-rank, to rank sequences by histone tail aided regulatory region annotation, sequence conservation, and TFBSs. 3. To validate regulatory elements, we perform lineage tracing by cloning regulatory sequences to zebrafish with EGFP tagged pronephos. 4. To identify genes regulated by specific genetic variation, we generate an atlas of kidney expression quantitative trait loci. We correlate genetic polymorphism with gene expression based on RNAseq of human kidneys.

Results: 1. We generated the first human kidney gene regulatory maps. These maps identify promoters, enhancers and transcribed regions, and can predict outcome after transcription factor binding. 2. Using HST-rank, we found targets including rs1010117, which is located near the cystatin gene family cluster on chromosome 20. It locates at kidney specific enhancers, shows open chromatin structure properties, and displays sequence conservation. 3. We identified target genes regulated by genetic variation using *cis*-eQTL. Target sequences and genes are validated in zebrafish.

Conclusions: CKD SNPs are enriched in the kidney specific regulatory regions, especially in the enhancers. We develop HST-rank to prioritize variants found in GWAS and examine predicted regions in a living organism. We identified and tested kidney specific gene targets in zebrafish kidney reporter lines.

Funding: Other NIH Support - NIH Roadmap Epigenomics Program 5R01DK087635-02

TH-OR061

Rare Mutations Associating with Serum Creatinine and Chronic Kidney Disease Runolfur Palsson,[1,2] Olafur S. Indridason,[1] Gardar Sveinbjornsson,[3] Evgenia K. Mikaelsdottir,[3] Hilma Hólm,[3] Daniel Gudbjartsson.[3] *[1]Div of Nephrology, Landspitali - The National Univ Hospital of Iceland; [2]Faculty of Medicine, Univ of Iceland; [3]DeCODE genetics, Reykjavik, Iceland.*

Background: Chronic kidney disease (CKD) is a complex disorder with a strong genetic component. While a number of common sequence variants have been found to associate with serum creatinine (SCr), estimated glomerular filtration rate (eGFR) and/or CKD, these variants explain less than 1% of the variation in SCr and CKD. The objective of this study was to find rare sequence variants that associate with SCr and CKD.

Methods: Sequence variants identified by whole-genome sequencing of 2,230 Icelanders were imputed into 81,656 chip-typed individuals and 112,630 relatives of genotyped subjects. The subjects were older than 18 years and had a median of 4 (range, 1-645) SCr measurements available. A genome-wide association study was conducted on this sample of 194,286 subjects, testing 24 million single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) for association with SCr. Novel variants associating with SCr were tested for association with CKD defined as eGFR <60 mL/min/1.73 m^2 for more than 3 months duration.

Results: In addition to replicating 15 established loci, we discovered missense and loss of function variants associating with SCr in 3 solute carriers (SLC25A45, SLC47A1, SLC6A19) and 2 E3 ubiquitin ligases (RNF128, RNF186). All these 5 variants are coding variants, 4 of which are rare (minor allele frequency <2%). We tested the 5 variants for association with CKD in a sample of 15,594 cases and 291,428 controls from Iceland. Three of the variants also associated with CKD (P <0.05/5 = 0.01). These rare variants have a greater impact on SCr than previously reported common variants, with a maximum effect of 0.069 SD.

Conclusions: Our findings have revealed novel rare sequence variants that associate with SCr and CKD. All of the variants are predicted to alter an encoded protein, potentially affecting kidney function or creatinine synthesis and excretion.

Funding: Pharmaceutical Company Support - deCODE genetics

TH-OR062

GWAS Results in the PediGFR Consortium Identifies Six Genomic Loci Associated with GFR and Proteinuria Matthias Wuttke,[1] Craig S. Wong,[2] Elke Wuehl,[3] Bradley A. Warady,[2] Anke Doyon,[3] Betul B.S. Sozeri,[4] Daniela Kracht,[5] Anette Melk,[5] Uwe Querfeld,[6] Tom D. Blydt-Hansen,[7] Susan L. Furth,[2] Franz S. Schaefer,[3] Anna Kottgen.[1] *[1]Univ Medical Center, Freiburg, Germany; [2]Univ of New Mexico Children's Hospital, Albuquerque; [3]Univ Medical Center, Heidelberg, Germany; [4]Ege Univ Faculty of Medicine, Izmir, Turkey; [5]Medizinische Hochschule, Hannover, Germany; [6]Charite Univ Hospital, Berlin, Germany; [7]Univ of Manitoba, Canada.*

Background: The international PediGFR Consortium is aimed at the identification of genetic factors associated with kidney function decline in children with chronic kidney disease (CKD). We performed genome-wide association studies (GWAS) to find genetic variants associated with eGFR and proteinuria in children with CKD using cross-sectional data at the PediGFR enrollment visit.

Methods: The Illumina Omni2.5 chip was used to genotype ~2.5 million SNP markers among 1450 participants of the CKiD, ESCAPE and 4C studies. The data was cleaned and divided by parent study and ancestry into six separate study populations, followed by imputation using 1000 Genomes Project reference data, resulting in ~10 million high-quality SNPs per patient. We performed GWAS for GFR estimated from serum creatinine, as well as an analysis of the presence of proteinuria (defined as UACR <0.3 g/g or UPCR <0.5 g/g) among children with CKD. A fixed-effects inverse-variants weighted meta-analysis was used to combine the association results.

Results: After filtering for minor allele frequency >5%, one SNP was associated with eGFR with $p<10^{-6}$ near *NYAP2* and 5 SNPs associated with proteinuria at $p<10^{-5}$ in or near *ARSB*, *FAM151B*, *SAMD3*, *MIR4493* and *RSL24D1*. Findings were consistent in the individual subgroups and for association with alternative measures of GFR. In addition, we detected association signals at previously published kidney disease risk loci from population-based studies such as for *SHROOM3* (p-value=$4.0*10^{-5}$ for the best SNP in the PediGFR data).

Conclusions: We identified 6 potential new loci associated with eGFR and proteinuria in children with CKD. Because of the lack of suitable replication cohorts, external experimental validation of our findings is currently sought.

Funding: NIDDK Support

TH-OR063

Characterization of *HLA-DQA1* and *PLA2R1* Risk Alleles across the Spectrum of Chronic Kidney Disease Etiologies Peggy Sekula,[1] Yong Li,[1] Matthias Wuttke,[1] Robert Kleta,[3] Florian Kronenberg,[4] Kai-Uwe Eckardt,[2] Anna Kottgen.[1] *[1]Univ of Freiburg, Germany; [2]Univ of Erlangen, Germany; [3]Univ College London, United Kingdom; [4]Innsbruck Medical Univ, Austria.*

Background: Genetic variants in *HLA-DQA1* and *PLA2R1* show strong and reproducible associations with membranous nephropathy (MN). We aimed to investigate if the conferred risk extends to additional etiologies of chronic kidney disease (CKD).

Methods: We genotyped rs17830558 in *PLA2R1* and rs9272729 in *HLA-DQA1* in 4,960 patients with CKD from different etiologies enrolled in the German Chronic Kidney Disease (GCKD) study. These patients of European ancestry had estimated glomerular filtration rate (GFR) of 30-60 ml/min/1.73m^2 or eGFR >60 and albumin to creatinine ratio >300 mg/g. Genotypes of 379 European ancestry samples from the 1000 Genomes project were used for comparison.

Results: After correction for multiple testing, both variants were significantly (p<2.8E-03) associated with biopsy-proven MN; odds ratios were comparable to previous estimates. While the association between rs17830558 in *PLA2R1* was specific to MN among 18 CKD etiologies studied, rs9272729 in *HLA-DQA1* conferred susceptibility to additional CKD etiologies, including nephropathy in type I diabetes, FSGS, and lupus nephritis, but not type II diabetes or IgA nephropathy.

odds ratio (95% confidence interval, p-value [*significant])	rs17830558 (*PLA2R1*) coded allele frequency T: 0.46	rs9272729 (*HLA-DQA1*) coded allele frequency A: 0.12
MN, n=137	1.63 (1.23-2.15, 5E-04)*	6.86 (4.55-10.3, 6E-24)*
type I diabetic nephropathy, n=89	0.76 (0.55-1.05, 9E-02)	2.69 (1.67-4.34, 7E-05)*
type II diabetic nephropathy, n=649	0.89 (0.74-1.07, 2E-01)	1.38 (1.02-1.87, 3E-02)
focal-segmental glomerulosclerosis, n=141	0.86 (0.66-1.13, 3E-01)	2.39 (1.58-3.60, 4E-05)*
lupus nephritis, n=125	1.14 (0.87-1.49, 3E-01)	3.23 (2.13-4.89, 3E-08)*
IgA nephropathy, n=362	1.03 (0.84-1.25, 8E-01)	1.17 (0.83-1.64, 4E-01)

Conclusions: MN risk variants in *HLA-DQA1* also show associations with additional CKD etiologies, particularly autoimmune diseases, while variants in *PLA2R1* are specific to MN. Our results strengthen the concept of a shared genetic component of some but not all autoimmune kidney diseases.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-OR064

Genome Wide Exome Array Analysis Identifies *HLA-DQA1* and *PLCG2* as Risk Loci for Childhood Onset Steroid Sensitive Nephrotic Syndrome Rasheed A. Gbadegesin, Adebowale A. Adeyemo, Nicholas J. Webb, Larry A. Greenbaum, Asiri S. Abeyagunawardena, Shenal Thalgahagoda, Debbie S. Gipson, Arundhati S. Kale, Tarak Srivastava, Jen-Jar Lin, Deepa H. Chand, Tracy E. Hunley, Patrick D. Brophy, Arvind Bagga, Michelle N. Rheault, Elizabeth C. Abraham, Halima S. Janjua, Abiodun A. Omoloja, Gina-Marie Barletta, William E. Smoyer, Gentzon Hall, Shashi K. Nagaraj, Delbert R. Wigfall, John W. Foreman, Michelle P. Winn. *Pediatrics, Medicine and DIMP, Duke Univ, Durham, NC.*

Background: Steroid sensitive nephrotic syndrome (SSNS) accounts for over 80% of cases of nephrotic syndrome in childhood. However, its etiology and pathogenesis remain obscure. Hypothesizing that coding variation may underlie SSNS risk; we have conducted an exome array based genome wide association study of SSNS.

Methods: We enrolled a discovery set of 363 subjects (214 South Asian children with SSNS and 149 controls) and genotyped them using the Illumina HumanExome Beadchip. SNPs at or near genome wide significance ($p < 2.5 X10^{-6}$, the Bonferroni adjusted p value for the number of genes in the human genome) were genotyped in subjects of White European ancestry.

Results: Four common SNPs in *HLA-DQA1 and HLA-DQB1* (rs1129740, rs9273349, rs1071630, rs1140343) were associated with SSNS with genome wide significance: OR 2.11 (SE 0.16), $p=1.19 X 10^{-6}$. Two of these SNPs, the missense variants C34Y (rs1129740) and F41S (rs1071630) in *HLA-DQA1*, were replicated in an independent cohort of children with SSNS of White European ancestry (100 cases and up to 589 controls) with a $p= 1.83 X10^{-17}$. Rare variant gene set based analysis demonstrated that missense variants in PLCG2 were most strongly associated with SSNS ($p=7.825X10^{-5}$).The protein encoded by *PLCG2* is involved in adaptive immunity, and is involved in B cell receptor signaling and T cell receptor signal transduction.

Conclusions: This is the first genome-wide study to identify *HLA-DQA1* and *PLCG2* missense coding variants as risk factors for SSNS. The finding of a MHC class II locus underlying SSNS risk points to a major role for immune response in the pathogenesis of SSNS.

Funding: NIDDK Support, Private Foundation Support

TH-OR065

Targeted Sequencing of MHC Region and Immune Related Genes in Han Chinese Identifies Independent Variants Associated with Lupus Nephritis Ricong Xu,[1,2] Qibin Li,[3] Yingrui Li,[3] Zhijian Li,[1,2] Peiran Yin,[1,2] Xueqing Yu.[1,2] *[1]Dept of Nephrology, The First Affiliated Hospital, Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Guangdong Provincial Key Laboratory of Nephrology, Ministry of Health, Guangzhou, Guangdong, China; [3]BGI-Shenzhen, Shenzhen, China.*

Background: Lupus nephritis (LN) is a common complication (15-55%) and fatal target-organ damage of SLE. Previous genetic studies had identified numerous variants for SLE, however, the genetic background of LN is less well known. In this study, we aim to

identify novel variants especially low frequency and functional variants in MHC region and immune related genes that contribute to the pathogenesis of LN using targeted sequencing.

Methods: We employed targeted capture and next generation sequencing (NGS) of the whole MHC region and the protein-coding sequence of 697 known immune related genes for 1331 patients with biopsy-proven LN and 1296 controls. Read mapping, SNP detection, genotype calling, and SNP annotation were performed using public software and database. The canonical HLA alleles were typed using sequencing data. After stringent quality control, we performed single marker association tests for common variants, alleles and amino acid of HLA genes and various burden tests for rare variants. Top associations will be validated in an independent cohort comprised of 2000 samples by amplicon sequencing (Warfergene).

Results: 24 SNPs (5 missense, 3 low frequency) within 20 immune related genes in non-MHC region showed significant associations with LN, including 7 SLE GWAS genes (TNFAIP3, BANK1, STAT4, GTF2IRD1, SLC15A4, SELE, NFKBIA) and 13 newly identified candidate genes, most of them were involved in the IFN-α signaling pathway, cell apoptosis, lymphocytes proliferation and adhesion. We also found 9 independent signals in MHC region showed significant associations, including one low frequency missense variant within a newly identified gene.

Conclusions: The results of study show that targeted sequencing is an efficient approach to identify gene mutations, including low frequency and novel variants for LN. Previously reported variants in SLE are also successfully verified in this study.

TH-OR066

Identification and Validation of New Risk Genes in European-American Population by Exome-Sequencing and RNAi-Based Mouse Model for Focal Segmental Glomerulosclerosis Haiyang Yu,[1] Cheryl Ann Winkler,[2] Jeffrey B. Kopp,[3] Andrey S. Shaw.[1] *[1]Pathology and Immunology, HHMI, Washington Univ School of Medicine, St. Louis, MO; [2]Molecular Genetic Epidemiology Studies Section, National Cancer Inst, Frederick, MD; [3]Kidney Disease Section, NIDDK, National Inst of Health, Bethesda, MD.*

Background: The evidence that Focal Segmental Glomerulosclerosis (FSGS) is a genetic disease has been accumulated in the past decade. However, the genetic causes of sporadic FSGS are still unknown. We identified rare variants that may contribute to the pathogenesis of FSGS by exome-sequencing in European American population. The top candidate genes were further validated by an RNAi-based FSGS mouse model.

Methods: We sequenced 3000 genes in 178 FSGS patients and 384 controls. Common variants were analyzed by Fisher's exact test. Rare variants were analyzed by burden test, varable threshold test and C-alpha test. The top 4 candidate genes were knocked down in podocytes in vivo by a doxycycline-inducible RNAi mouse model. The Albumin/creatinine ratio was calculated for each urine samples to examine the proteinuria. High Albumin/creatinine ratio is used as an early sign of FSGS. The kidneys of proteinuric mice were subjected to histological and electron microscopic analysis.

Results: We identified rare variants in WNK4, ARHGEF17, DLG5, and several other genes that were significantly enriched in the patient population. Inducible podocyte specific RNAi mice were generated to validate whether the loss of function of these genes contribute to FSGS. We observed a proteinuric phenotype and foot process effacement in WNK4 and ARHGEF17 RNAi mice, but not DLG5 RNAi mice. We also identified a SNP in APOL1 gene in EA, rs73885319 (p. S342G) (OR=23.8, P<0.003), which belongs to the G1 allele.

Conclusions: Using exome-sequencing technology, we identified new risk genes and alleles for FSGS. We have validated that Arhgef17 and Wnk4 could contribute to the pathogenesis of FSGS by an inducible RNAi mouse model.

TH-OR067

Missense Mutation of the RhoGTPase Regulator FSGS11 Is a Cause of Autosomal Dominant FSGS Gentzon Hall,[1,2] Rasheed A. Gbadegesin,[1,2] Andrew F. Malone,[1,2] Paul J. Phelan,[1,2] Alison Homstad,[2] Guanghong Wu,[2] Thomas Lindsey,[4] Michelle P. Winn.[1,2] *[1]Nephrology, Duke Univ, Durham, NC; [2]Duke Molecular Physiology Inst, Duke Univ, Durham, NC.*

Background: FSGS is a disorder characterized by focal scarring of the glomerular capillary tuft, podocyte injury, nephrotic syndrome and rapid progression to ESKD. While idiopathic forms of the disorder predominate, insights into the molecular and genetic causes of inherited forms of FSGS have greatly enhanced our understanding of the pathogenic mechanisms of FSGS. We report the discovery of a novel heterozygous missense mutation of the RhoGTPase regulatory protein FSGS11 as the cause of autosomal dominant (AD) FSGS in a Northern European kindred from Canada (Family DUK6516).

Methods: We performed whole-exome sequencing (WES) on two affected members of Family DUK6516. All identified novel variants were confirmed by Sanger sequencing. Complimentary molecular genetic analyses were performed in conditionally immortalized human podocytes (CIHP) to evaluate the effects of gene knockdown on podocyte cytoskeletal dynamics and motility.

Results: We identified a novel heterozygous missense mutation (T369I) within a critical N-terminal protein:protein interaction domain of FSGS11 as the cause of AD FSGS in Family DUK6516. The mutation segregates with the disease in the family and was not found in 1600 control chromosomes. The change is conserved in evolution and is considered damaging by *in silico* simulation. We confirm the expression of FSGS11 in whole kidney and podocytes by RT-PCR and immunoblot analyses. We hypothesize that the T369I is a loss-of-function mutation and may cause FSGS by dysregulation of podocyte motility. To confirm this, we performed targeted gene knockdown of FSGS11 in CIHPs and demonstrated significantly impaired podocyte motility.

Conclusions: In summary, we report the identification of a novel heterozygous missense variant of the RhoGTPase regulatory protein FSGS11 as a new cause of AD FSGS in a Northern European kindred. FSGS11 is expressed in the kidney and podocytes and targeted gene knockdown significantly impaired basal podocyte motility *in vitro*.

Funding: NIDDK Support

TH-OR068

Complement Activation Patterns in Atypical Hemolytic Uremic Syndrome (aHUS) During Acute Phase and in Remission Lambertus P.W.J. Van den Heuvel,[1,6,7] Elena Volokhina,[1] Thea J. Van der Velden,[1] Dineke Westra,[1] Nicole Van de Kar,[1] Tom Eirik Mollnes.[2,3,4,5] *[1]Dept of Pediatric Nephrology, Radboud Univ Medical Center, Nijmegen, Netherlands; [2]Dept of Immunology, Oslo Univ Hospital, Norway; [3]K.G. Jebsen IRC, Univ of Oslo, Norway; [4]Research Laboratory, Nordland Hospital, Bodø, Norway; [5]Faculty of Health Sciences, Univ of Tromsø, Norway; [6]Dept of Laboratory Medicine, Radboud Univ Medical Center, Nijmegen, Netherlands; [7]Dept of Pediatrics, Univ Hospitals Leuven, Belgium.*

Background: Pathogenesis of aHUS is associated with (genetic) defects in the alternative complement pathway. Nevertheless, comprehensive evidence that the complement system in aHUS patients is more prone to activation is still lacking. Therefore, we performed a thorough analysis of complement activation both during the acute phase and in remission of this disease.

Methods: Complement activation patterns of the aHUS patients in acute phase (n=6) and in remission (n=11) were compared to those of healthy controls (n=19). Background levels of complement activation products C3b/c, C3bBbP and TCC were measured in EDTA plasma using ELISA. Efficiencies of the C3b/c, C3bBbP and TCC generation in serum during spontaneous activation in the fluid phase, as well as after triggering by zymosan, were analyzed using the same assays.

Results: Patients with acute aHUS showed elevated levels of C3b/c (P<0.01), C3bBbP (P<0.0001) and TCC (P<0.0001) in EDTA plasma, while values of patients in remission were normal. In a single aHUS patient with CFB mutation we showed normalization of complement activation during aHUS recovery. Serum samples from patients in remission showed normal *in vitro* patterns of complement activation both spontaneously and triggered by zymosan, and the kinetics of complement activation in the fluid phase were similar as for the healthy controls.

Conclusions: Our data indicate that aHUS patients showed significantly increased complement activation during acute phase of the disease but not in remission. The data have implications for interpretation of the complement analyses in aHUS patients and may have an impact on monitoring of these patients, particularly when using complement inhibition therapy.

Funding: Private Foundation Support

TH-OR069

Identification of Distinct Dominant and Subdominant Humoral Epitopes within PLA2R1 in Primary Membranous Nephropathy Laurence H. Beck, Dana Sandor, Hong Ma, David J. Salant. *Medicine, Renal Section, Boston Univ Medical Center, Boston, MA.*

Background: Autoantibodies to the M-type phospholipase A2 receptor 1 (PLA2R1) are found in up to 80% of patients with primary membranous nephropathy (MN). PLA2R1 is a large transmembrane glycoprotein, consisting of an N-terminal cysteine-rich domain (CysR), a fibronectin-like type II domain, and eight C-type lectin-like domains (CTLD1-8) in its extracellular region. We sought to determine which domains contained epitopes reactive with autoantibodies in primary MN.

Methods: The following tagged constructs derived from human PLA2R1 were expressed in HEK293 cells: CysR, CTLD1, CTLD2, CTLD3, CTLD1-3, CTLD4-8, CysR-CTLD3, and CysR-CTLD8. Reactivity was assessed by Western blotting with human MN and control sera. Domain-specific antibodies were affinity purified and their specificity demonstrated by Western blotting.

Results: All constructs could be expressed in HEK293 cells, although the individual CTLD1, 2 and 3 domains were less efficiently expressed. 100% of MN sera (35/35) reacted with CysR alone, as well as with CysR-CTLD8 and with the full-length molecule. Approximately half the sera (17/35 and 19/35) reacted with CTLD1 or CTLD1-3, respectively. There was no reactivity detected with CTLD3 alone, and only 3% (1/35) reacted with CTLD2. 29/35 (83%) demonstrated reactivity with epitopes in the more C-terminal (CTLD4-8) region of the molecule. For an individual patient, reactivities to specific PLA2R1 domains appeared to disappear and reappear in unison during remission and relapse, respectively. There was no correlation noted between duration of disease and number of humoral epitopes recognized. Autoantibodies affinity purified on either a CysR or a CTLD1 column specifically recognized the domain against which they were purified.

Conclusions: There appears to be a dominant humoral epitope located in the CysR domain of human PLA2R1 that is recognized by 100% of patient samples. Additional (subdominant) epitopes exist within the CTLD4-8 region, in CTLD1, and in CTLD2. There was no evidence for progressive epitope spreading found in these samples collected after patients had presented to clinical attention.

Funding: NIDDK Support

TH-OR070

Phospholipase A_2 Receptor Autoantibodies and Renal Function in Patients with Primary Membranous Nephropathy Elion Hoxha,[1] Sigrid Harendza,[1] Hans O. Pinnschmidt,[2] Ulf Panzer,[1] Rolf A. Stahl.[1] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Institut für Medizinische Biometrie & Epidemiologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Background: Loss of renal function in patients with membranous nephropathy (MN) cannot be reliably predicted by laboratory or clinical markers at the time of diagnosis. Phospholipase A_2 receptor autoantibodies (PLA_2R-Ab) have been shown to be associated with changes in proteinuria. Their role in the development of renal function, however, is unclear.

Methods: In this prospective study we analysed the role of PLA_2R-Ab in predicting the decline of renal function in 118 consecutive PLA_2R-Ab positive patients with biopsy proven MN. The clinical endpoint was defined as increase of serum creatinine (Scr) by ≥25% and reaching a Scr ≥1.3 mg/dl.

Results: Patientswere divided in tertiles according to their PLA_2R-Ab at the time of inclusion in the study, which was 1.0±1.4 months after diagnosis of MN by renal biopsy. PLA_2R-Ab of patients in tertile 1 (low) ranged between 20-86 U/ml; in tertile 2 (medium) between 87-201 U/ml, and in tertile 3 (high) above 202 U/ml. At study start, serum creatinine levels and histologic changes (interstitial fibrosis, glomerular lesions, nephrosclerosis) were not different between patients of all three tertiles. Patients in tertile 3 had higher proteinuria than patients of tertile 1 (9.2±5.5g/24h versus 7.4±5.2g/24h; p=0.14). The clinical endpoint was reached in 69% of patients with high PLA_2R-Ab (tertile 3) but only 25% of patients with low PLA_2R-Ab (tertile 1). Patients with high PLA_2R-Ab reached the endpoint significantly faster than patients with low PLA_2R-Ab (17.7 months versus 30.9 months; p<0.001). A multivariate cox regression analysis using age, sex, total IgG PLA_2R-Ab, IgG4 PLA_2R-Ab, proteinuria, serum albumin, Scr, time between renal biopsy and measurement of PLA_2R-Ab, morphologic glomerular lesions, blood pressure and use of immunosuppressive treatment as explanatory variables showed that high PLA_2R-Ab are an independent predictor for progressive loss of renal function.

Conclusions: High PLA_2R-Ab are associated with progressive loss of renal function in patients with primary MN.

TH-OR071

Identification of Thrombospondin Type 1 Domain Containing 7A as a Novel Antigen in Idiopathic Membranous Nephropathy Nicola M. Tomas,[1] Laurence H. Beck,[2] Catherine Meyer-Schwesinger,[1] Barbara Seitz-Polski,[3] Hong Ma,[2] Gunther Zahner,[1] Guillaume Dolla,[3] Elion Hoxha,[1] Udo Martin Helmchen,[1] Michael Merchant,[4] Jon B. Klein,[4] David J. Salant,[2] Rolf A. Stahl,[1] Gerard J. Lambeau.[3] *[1]Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Boston Univ School of Medicine, Boston; [3]Inst of Molecular and Cellular Pharmacology, Valbonne, France; [4]Univ of Louisville, Louisville.*

Background: Idiopathic membranous nephropathy (iMN) is an autoimmune disease associated with autoantibodies against the phospholipase A2 receptor (PLA2R1) in approximately 70% of cases. Antigenic targets in the remaining cases have remained unidentified.

Methods: We screened sera from patients with iMN, other glomerular diseases, and healthy donors for antibodies against native glomerular proteins by Western blotting. We partially purified a potential new antigen, identified this protein via mass spectrometry, and validated the results with recombinant protein expression, immunoprecipitation and immunohistochemistry.

Results: Sera from 5 out of 35 patients with anti-PLA2R1 negative iMN recognized a glomerular protein of 250 kDa. None of the sera from iMN patients seropositive for anti-PLA2R1 (n=60), from patients with other glomerular diseases (n=76), and from healthy donors (n=44) were reactive against this antigen. While clearly different from PLA2R1, this novel antigen was found to share similar biochemical features, i.e. N-glycosylation, membranous location, and reactivity with sera only under non-reducing conditions. Mass spectrometry identified this new antigen as thrombospondin type 1 domain containing 7A (THSD7A). All reactive sera recognized recombinant THSD7A and immunoprecipitated THSD7A from glomerular lysates. Moreover, immunohistologic analyses of patient biopsies localized THSD7A to podocytes and IgG eluted from one of these biopsies was specific for THSD7A.

Conclusions: We have identified THSD7A as a novel target antigen in iMN, with 5 out of 95 iMN patients having circulating autoantibodies to THSD7A and not PLA2R1. This suggests that anti-THSD7A positive patients represent a distinct subgroup of iMN and that anti-THSD7A could be an additional biomarker for diagnosis and monitoring.

TH-OR072

Renal PLA_2R in Hepatitis B Virus-Associated Membranous Nephropathy Qionghong Xie, Yan Li, Chuanming Hao. *Div of Nephrology, Huashan Hospital, Fudan Univ.*

Background: Circulating anti-phospholipase A2 receptor antibody (PLA_2R-Ab) is reported to be a valuable biomarker for idiopathic membranous nephropathy (iMN). Patients with PLA_2R-antibody also exhibit strong positive staining for renal PLA_2R. The present study examined renal PLA2R expression in idiopathic and secondary MNs.

Methods: Patients with biopsy-proven MN, including iMN, hepatitis B virus-associated MN (HBV-MN) and type V lupus nephritis, were enrolled in the study. Circulating PLA_2R-Ab was detected by indirect immunofluorescence (IF), and renal PLA_2R was examined using a specific anti-PLA_2R antibody. For IF staining of paraffin-embedded sections, two antigen retrieval methods were used: autoclave heating at 120°C or microwave heating. Renal PLA_2R positivity was determined according to the microwave heating method.

Results: PLA_2R was detected along the capillary loop in 84% (86/102) of renal biopsies from patients with iMN but not in patients with any other primary glomerular diseases (n=40); In contrast, only 1 out of 38 (2.6%) of type V lupus nephritis patients showed renal PLA2R(+). Interestingly, in HBV-MN, 64% (25/39) showed PLA_2R(+). In PLA_2R(+) HBV-MN, renal PLA_2R staining overlaps with HBsAg and IgG_4 along the capillary loop. Importantly, in 6 HBV-MN patients with serum available, all (6/6) showed serum PLA2R-Ab(+). In the PLA_2R(-) kidneys, renal PLA_2R became detectable after antigen retrieval by autoclave heating, but exhibited a different pattern consistent with whole cell body expression in the podocyte. Serum PLA_2R-Ab was closely associated with renal PLA_2R positivity. Of the 41 iMN cases with both serum PLA_2R-Ab and renal biopsy PLA_2R data available, 24 were serum-positive for PLA_2R-Ab (59%). All of the serum PLA_2R-Ab(+) cases showed renal PLA_2R(+), while 9 of the 17 serum PLA_2R-Ab(-) cases also exhibited renal PLA_2R(+). However, all of the renal PLA_2R(-) patients were also negative for serum PLA_2R-Ab.

Conclusions: Renal PLA_2R(+) was common in iMN and HBV-MN, but rare in lupus-associated MN. Further studies examining the implication of this association of PLA_2R positivity and HBV-MN may shed light on mechanism underlying the pathogenesis of iMN or HBV-MN.

TH-OR073

Reassessment of suPAR in Kidney Disease Joann M. Spinale,[1] Laura H. Mariani,[2,3] Robert J. Weyant,[2] Peter X.K. Song,[4] Deepak Nihalani,[5] Lawrence B. Holzman.[5] *[1]Nephrology, Children's Hospital of Philadelphia, Philadelphia, PA; [2]Arbor Research for Collaborative for Health, Ann Arbor, MI; [3]Nephrology, Univ of Michigan School of Medicine, Ann Arbor, MI; [4]Biostatistics, Univ of Michigan School of Medicine, Ann Arbor, MI; [5]Renal-Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia, PA.*

Background: Soluble urokinase receptor (suPAR) might be a causative circulating factor for FSGS and it might serve as an FSGS biomarker. We undertook validation of these conclusions in both mouse models and in human subjects.

Methods: In wild type mice, we performed intravenous injection of recombinant suPAR and observed mice for 24 h. We also created an inducible transgenic mouse model that maintained serum suPAR concentration at least four-fold above baseline for 6 weeks. Further, we studied 241 glomerular disease patients from the prospective, longitudinal multi-center observational NEPTUNE cohort: 95 patients had FSGS, 62 had minimal change disease (MCD), 52 had membranous nephropathy (MN) and 32 had IgA nephropathy (IGAN).

Results: Mice treated acutely or with prolonged exposure to suPAR did not develop proteinuria. In NEPTUNE cohorts, serum suPAR concentration at baseline was inversely correlated with eGFR (ρ = -0.57, P≤0.01) and urine suPAR/creatinine ratio was positively correlated with urine protein to creatinine ratio (ρ = -0.38, P ≤0.01). After adjusting for eGFR and urine protein, neither serum nor urine suPAR level were independent predictors of FSGS histopathology. Using longitudinal data, a multivariable mixed-effects model was fit to evaluate the association of change in serum suPAR concentration from baseline with eGFR. After adjusting for baseline suPAR concentration, age, sex, proteinuria and time, change in suPAR from baseline was associated with eGFR (p= 0.004), but this association was not different for FSGS patients as compared to those with MCD, MN or IgAN (interaction p-values 0.62, 0.81 and 0.30, respectively).

Conclusions: These results do not support a pathological role for suPAR in FSGS or suPAR's value as a biomarker of FSGS.

Funding: NIDDK Support, Other NIH Support - Financial support for this work provided by the NIDDK Diabetic Complications Consortium (DiaComp, www.diacomp.org), grant DK076169 to LBH and by support to DN (DK087956) and to JS (DK007378). The Nephrotic Syndrome Study Network Consortium (NEPTUNE); U54-DK-083912, is a part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN), supported through a collaboration between the Office of Rare Diseases Research (ORDR), NCATS, and the National Institute of Diabetes, Digestive, and Kidney Diseases, Private Foundation Support

TH-OR074

B7-1/CD80 Is Not a Reliable Immunophenotypical Marker of Focal Segmental Glomerulosclerosis, Membranous Glomerulonephritis, and Diabetic Nephropathy Zoltan G. Laszik,[1] Dejan Dobi,[1] Flavio Vincenti.[2] *[1]Dept of Pathology, Univ of California San Francisco, San Francisco, CA; [2]Transplant Service, Univ of California San Francisco, San Francisco, CA.*

Background: Induced expression of B7-1/CD80 in the podocytes has been recently reported in various glomerular diseases, including focal segmental glomerulosclerosis (FSGS), recurrent FSGS, membranous glomerulonephritis (MN), and diabetic nephropathy (DNP). To survey the potential of B7-1/CD80 podocyte expression as a diagnostic marker and possible therapeutic target we evaluated the immunohistochemical expression of B7-1/CD80 in native and transplant kidney biopsies with FSGS, MN, DNP, and new onset diabetes after transplantation (NODAT).

Methods: Consecutive renal biopsies were selected for the following study groups: FSGS (n=10), early post-transplant recurrent FSGS (n=10), MN (phospholipase A2 [PLA2] positive, n=5), MN (PLA2 negative, n=5), DNP (classes 1, 2b, and 3) (n=15), NODAT (6 month post-transplant protocol biopsies with normal morphology, n=10), and controls (6

month post-transplant protocol biopsies with normal morphology from patients with no diabetes [n=10], and minimal change disease [MCD] [n=5]). Double immunostains were performed for B7-1/CD80 and the podocyte marker synaptopodin on formalin-fixed paraffin-embedded and frozen tissues with standard immunoperoxidase and immunofluorescent methodology, respectively. Cutaneous T cell lymphoma served as method control for the B7-1/CD80 stain.

Results: One native FSGS biopsy showed weak focal podocyte staining with B7-1/CD80; all of the MN biopsies revealed strong B7-1/CD80 granular staining along the periphery of the glomerular capillary loops, however, the signal did not show apparent co-expression with synaptopodin. The rest of the biopsies showed no detectable B7-1/CD80 glomerular signal.

Conclusions: B7-1/CD80 immunostain has a limited value as an immunophenotypical marker in routine biopsy specimens with FSGS, recurrent FSGS, DNP, and NODAT. The B7-1/CD80 signal in MN might be attributed to nonspecific cross reactivity of the B7-1/CD80 antibody with immune complexes; alternatively, the signal can also reflect B7-1/CD80 accumulation in the immune deposits.

Funding: Clinical Revenue Support

TH-OR075

Biomarkers of Disease Pathobiology in Patients with Glomerulonephritis in the NEPTUNE Cohort Heather N. Reich,[1,6] Viji Nair,[2] Wenjun Ju,[2] David Cherney,[1] Marie C. Hogan,[3,6] John C. Lieske,[3,6] Sharon G. Adler,[4,6] Daniel C. Cattran,[1,6] Michelle A. Hladunewich,[1,6] James W. Scholey,[1] Peter J. Nelson,[5,6] Matthias Kretzler.[2,6] *[1]UHN, Univ of Toronto; [2]Univ of Michigan; [3]Mayo Clinic; [4]UCLA; [5]Univ Washington; [6]NEPTUNE Consortium.*

Background: Cytokines/chemokines (CC) contribute to disease immunopathogenesis and to local modulation of tissue injury and extracellular matrix (ECM) turnover in GN. Associations between urine and tissue expression of these mediators in patients with GN could identify non-invasive biomarkers of molecular and clinical response and facilitate development of novel personalized treatment strategies. The NEPTUNE cohort integrates multidimensional molecular and clinical data in patients with proteinuric GN, including biosamples obtained at the time of diagnostic kidney biopsy. We evaluated if urine expression of CC and mediators of ECM turnover reflects renal mRNA expression.

Methods: We used a multiplex platform to measure CC and MMP/TIMPs in serum and urine of 74 patients undergoing kidney biopsy. Results were correlated with microdissected renal biopsy microarray mRNA expression profiles. Serum and urine measures were performed in duplicate; analytes with <40% within-individual variation in >70% of samples were included. Urine analytes were adjusted for creatinine. Spearman correlation coefficients were calculated and FDR-adjusted pvalue <0.05 was considered significant.

Results: The urine expression of 10 CC/ECM proteins was highly correlated with tubulo-interstitial mRNA expression. These included inflammatory cytokines IL8 and MCP1, and mediators of ECM turnover MMP2 and TIMP1 (pvalue <0.05). Serum expression of most of the CC did not correlate with tissue expression, while expression of serum ECM turnover mediators paralleled tissue mRNA levels.

Conclusions: Non-invasive measures of a urine CC/ECM protein panel can be used to evaluate tissue molecular responses in GN. The source of these proteins –systemic or renal– requires further study. Use of a CC/ECM protein panel could allow design of targeted molecular therapies for proteinuric GN. Serial measurement of the analytes in the NEPTUNE cohort will permit longitudinal evaluation and correlation with clinical outcomes.

Funding: NIDDK Support, Other NIH Support - U-54-DK083912

TH-OR076

TNFSF13 Has a Key Role in Both the Susceptibility and Progression of IgA Nephropathy Seung Seok Han,[1] Nara Shin,[1] Seung Hee Yang,[2] Hajeong Lee,[1] Jung Pyo Lee,[3] Jae Hyun Chang,[4] Kwon Wook Joo,[1] Yon Su Kim,[1] Dong Ki Kim.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Kidney Research Inst, Seoul National Univ, Seoul, Korea; [3]Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [4]Internal Medicine, Gachon Univ of Medicine and Science, Incheon, Korea.*

Background: Tumor necrosis factor superfamily 13 (TNFSF13, also known as APRIL) has been found as a susceptibility gene in the genome-wide association study of IgA nephropathy. However, this issue is not fully established at least in Korean subjects, and the role of TNFSF13 in the progression of IgA nephropathy remains unresolved.

Methods: 634 patients with IgA nephropathy were recruited from major 4 hospitals. Two SNPs of TNFSF13 gene (rs3803800 and rs11552708) were evaluated and compared with the data on 1068 healthy controls. Furthermore, plasma TNFSF13 level at the time of diagnosis was measured using ELISA and compared with the data on 66 healthy volunteers. The times to end-stage renal disease or doubling serum creatinine were used as primary outcomes and the odds ratios (ORs) for outcomes were calculated according to the genetic polymorphisms or the quartiles of plasma TNFSF13 level.

Results: For rs3803800, the susceptibility of IgA nephropathy increased in AA homozygotes [OR, 1.4 (1.03–1.94)]. The serum IgA levels in patients with AA homozygotes were significantly higher than patients with AG or GG genotypes (Ps<0.05). For rs11552708, there was neither correlation with disease susceptibility nor the difference in serum IgA levels. Neither of two SNPs was associated with the risk of end-stage renal disease or doubling creatinine. However, plasma TNFSF13 levels had significant correlations with both outcomes: the 4th quartile had greater ORs for end-stage renal disease [8.9 (1.08–74.32)] and doubling of serum creatinine [4.6 (1.20–17.44)] compared with the 1st quartile. Patients with IgA nephropathy had greater plasma TNFSF13 levels compared with healthy volunteers (P<0.001).

Conclusions: The present study first demonstrates the relationships of TNFSF13 with both the susceptibility and progression of IgA nephropathy.

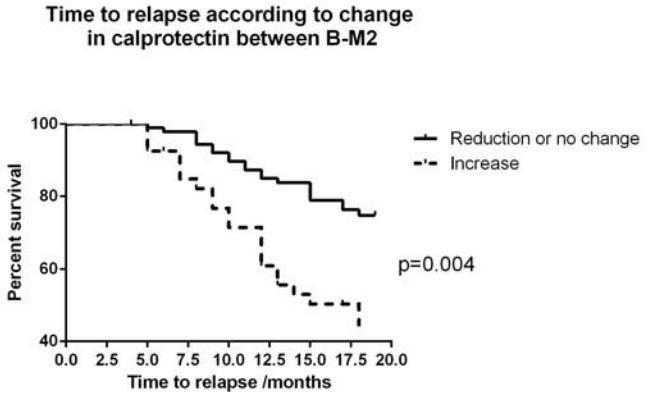
TH-OR077

Serum Calprotectin and Disease Relapse in ANCA-Associated Vasculitis Alan D. Salama,[1] Ruth J. Pepper,[1] Peter A. Merkel,[2,3] Juliana Bordignon Draibe.[1] *[1]Centre for Nephrology, UCL, United Kingdom; [2]Rheumatology, Univ Pennsylvania; [3]RAVE Investigators, RAVE-ITN Research Group.*

Background: In ANCA-associated vasculitis (AAV) disease relapses remain common but there are no reliable means to predict them. We previously showed serum calprotectin was elevated during active AAV and patients in the NORAM trial on treatment who relapsed had higher levels than non relapsers. We set out to validate calprotectin as a biomarker of relapse using samples from the Rituximab in ANCA-associated Vasculitis (RAVE) study.

Methods: Serum was obtained from 182 subjects from the RAVE trial at baseline (B), 1 month (m1), and 2 months (m2) following entry and evaluated by ELISA. Absolute calprotectin levels and changes from B to m1 and m2 were calculated and stratified according to disease relapse at 18 months.

Results: Serum levels of calprotectin were similar between relapsers (R) and non-relapsers (NR) at B and m1, but at m2, levels were significantly higher in relapsers (R median 4750 ng/ml, range 1364-26071 versus NR 3769 ng/ml, 1020-9964; p=0.04). There was no correlation between calprotectin and ANCA titer or CRP, and only weak correlation with white cell count (r=0.27, 0.3, 0.38, at B, m1, and m2 respectively, all p<0.0003). The percentage reduction in levels between m1 and m2 were significantly lower in R patients compared to NR patients (R +9.7% versus NR -9.8%; p=0.035) confirming that R patients failed to suppress calprotectin to the same extent as NR. Time to relapse was significantly faster in those patients with an increase in calprotectin between B and m2 or between m1 and m2, when compared to those in whom levels showed no change or reduction(p=0.0004 and p=0.04 respectively)



Conclusions: Failure to suppress calprotectin following induction AAV treatment is associated with greater and faster rates of disease relapse, now confirmed in two independent cohorts.

Funding: Other NIH Support - Immune Tolerance Network, Private Foundation Support

TH-OR078

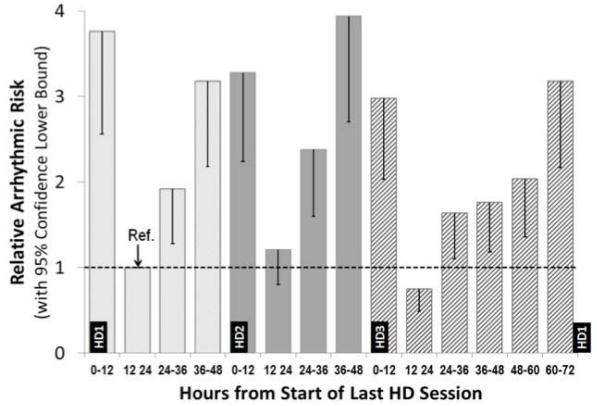
Timing of Arrhythmias with Dialysis Schedule: Preliminary Results of the Monitoring in Dialysis (MiD) Study Prabir Roy-Chaudhury,[1] Alexandru Ionel Costea,[1] James A. Tumlin,[2] Amber S. Podoll,[3] Don E. Williamson,[4] Suresh Chandra Tiwari,[5] Vikranth Reddy,[6] David M. Charytan.[7] *[1]U of Cincinnati and VAMC, Cincinnati; [2]U of Tennessee; [3]U of Texas; [4]Nephrology Associates, Augusta, GA; [5]Fortis Inst, New Delhi, India; [6]Care Hospital, Hyderabad, India; [7]Harvard U.*

Background: ESRD patients are at increased risk of arrhythmias and sudden cardiac death, but little is known about arrhythmia type, prevalence or relationship to dialysis schedule.

Methods: MiD is a prospective, multi-center study to characterize arrhythmias in 3x weekly hemodialysis (HD) patients over a 6-month period using an implantable cardiac monitoring device, Medtronic Reveal® XT. Recorded ECGs were centrally adjudicated, and the time of arrhythmia onset relative to each HD session was determined. Clinically significant arrhythmias (CSA) were defined as bradycardia ≤40 bpm for ≥6 seconds (s), asystole ≥3 s, sustained ventricular tachycardia ≥130 bpm for ≥30 s, and symptomatic arrhythmias.

Results: 45 implanted subjects were followed for a mean of 4.9 months [46% with prior history of arrhythmias]. 605 CSA occurred in 26 subjects (57.8%), a rate of 2.8 events per patient-month (ppm). 6,532 confirmed arrhythmias occurred in 39 subjects (86.7%), a rate of 27.2 events ppm. Arrhythmic risk (Figure) peaks during the 12 hour interval beginning with each HD session (especially after the end of dialysis), decreases for the next 12 hours, and then gradually increases over the remainder of the interdialytic interval; this pattern holds for sinus tachycardia, atrial and ventricular arrhythmias, but not for bradycardia.

Conclusions: Arrhythmias are frequent during HD and strongly associated with the HD schedule. Identification of HD session parameters that result in the CSA in particular, could potentially allow us to modify HD induced stressors and so reduce arrhythmias and improve patient safety in ESRD patients.



Funding: Pharmaceutical Company Support - Medtronic

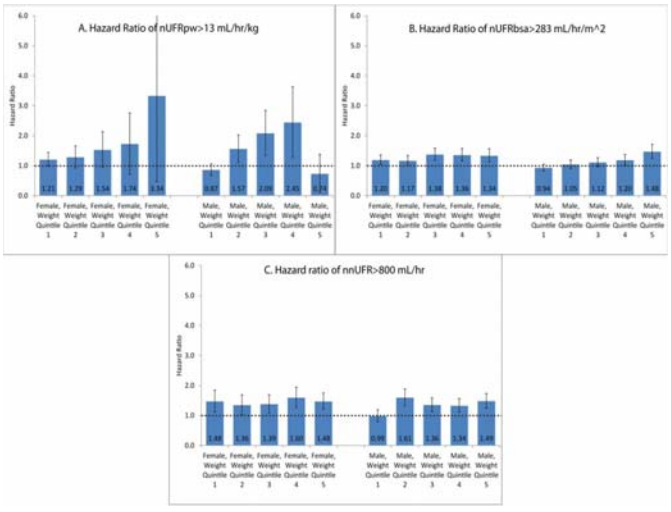
TH-OR079

Body Size and Gender Dependent Differences in Mortality Risks Associated with Ultrafiltration Rates John W. Larkin,[1] Sheetal Chaudhuri,[1] Len A. Usvyat,[1] Paul Balter,[1] Peter Kotanko,[2] Franklin W. Maddux,[1] Eduardo K. Lacson.[1] *[1]Fresenius Medical Care North America (FMCNA); [2]Renal Research Inst.*

Background: Hemodialysis (HD) ultrafiltration rates (UFR)s normalized by body weight ($nUFR_{pw}$) >13mL/kg/hr have been suggested to be associated with poor survival by the CMS TEP, yet it is not known if $nUFR_{pw}$ is a one-size fits all quality metric in a population that varies in body size. We studied whether using $nUFR_{pw}$, UFR normalized by body surface area ($nUFR_{bsa}$), or non-normalized UFR (nnUFR) is associated with differing relative risks of death for gender and body size strata.

Methods: From Jan 1, 2010 to Jan 31, 2013, we studied all incident HD (iHD) Pts at FMCNA clinics. Mortality was tracked until May 1, 2013. Inclusion criteria were: 1) survived the first 90 days of HD and 2) no residual renal function (Kru<0.1mL/min) upon starting HD. Pts were dichotomized into groups of average UFR in the first 90 days as nnUFR >800mL/hr, $nUFR_{pw}$ >13mL/kg/hr and $nUFR_{bsa}$ >283mL/hr/m^2 versus groups below those thresholds. Pts were stratified by gender and quintiles of post HD weights. Cox proportional hazard models were used to assess survival after the first 90 days of HD.

Results: The cohort of 49,617 iHD Pts had mean age of 62.8±15.0 years, 54% male, 64% white, 16% Hispanic, 63% diabetic, mean albumin 3.56±0.45g/dL, and median survival follow-up of 304 (range 1-1,126) days. Risks for mortality varied greatly across different genders and weight quintiles in Pts above pre-defined thresholds of nnUFR, $nUFR_{pw}$, and $nUFR_{bsa}$.



Conclusions: Normalized and non-normalized UFRs are not one-size fits all metrics that uniformly predict survival irrespective of body size or gender. Recommendations to use UFR metrics as performance measures are premature and clinically inappropriate. More studies are needed to define and refine its utility as a population measure; individualized Pt management is prudent at this time.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

TH-OR080

Serum Filtration Markers to Estimate Native Kidney Urea Clearance (KrU) in Hemodialysis Patients Tariq Shafi,[1] Andrew S. Levey,[2] Friedo W. Dekker,[3] Lesley Inker,[2] Raymond T. Krediet,[4] Wieneke Michels,[4] Tiny Hoekstra,[3] Josef Coresh.[1] *[1]Johns Hopkins Univ; [2]Tufts Medical Center; [3]Leiden Univ Medical Center; [4]Academic Medical Center.*

Background: Residual kidney function can contribute substantially to solute clearance in hemodialysis patients but cannot be assessed without urine collection limiting its incorporation in dialysis dose. We hypothesized that serum filtration markers that are either not removed by dialysis [serum β-trace protein (BTP)] or partially removed [cystatin C (CYS), β2-microglobulin (B2M)], might allow estimation of KrU (eKrU) without urine collection.

Methods: We measured serum markers in the hemodialysis participants of the Netherlands Cooperative Study on the Adequacy of Dialysis (N=722) where KrU was measured (mKrU) by 24-hour inter-dialytic urine collection. We developed eKrU equations using linear regression in a training sub-sample (N=469) and then validated them in the remaining participants (N=253). We assessed the equations' performance by calculating median bias (mKrU-eKrU), precision (interquartile range of bias) and accuracy (eKrU within ±2ml/minof mKrU).

Results: Mean age of the participants was 61 years, 60% were male and 88% were white. mKrU was 2.8±2.1 ml/min. All eKrU models included sex and urine volume categorized at 250 ml/day (p<0.01 for both). In the validation dataset, all equations had a low bias. Precision and accuracy were higher with equations including BTP and B2M. Equation containing both BTP and B2M had the best performance.

Performance of eKrU Equations in the Validation Data						
	Urea	**Creatinine**	**BTP**	**B2M**	**CYS**	**BTP B2M**
Bias, ml/min	0.2 (0, 0.3)	0.1 (0, 0.2)	0.1 (0, 0.2)**	0.1 (-0.1, 0.2)**	0.1 (-0.1, 0.2)	0 (-0.1, 0.1)**
Precision, ml/min	1.7 (1.3 2.1)	1.6 (1.1, 2.1)	1.4 (1.2, 1.7)	1.2 (1.0, 1.5)	1.6 (1.2, 2.0)	1.1 (0.9, 1.3)
Accuracy, %	83 (78, 87)	81 (76, 86)	90 (86, 94)***	90 (86, 94)***	86 (81, 90)**	91 (87, 94)***
Compared with creatinine: *p<0.05; **p<0.01; ***p<0.001						

Conclusions: Serum filtration markers have the potential to allow estimation of eKrU in dialysis patients without urine collection.

Funding: NIDDK Support

TH-OR081

Low Predialysis Serum Sodium Modifies the Effect of Hemodialysis (HD) Frequency on Left Ventricular Mass: The Frequent Hemodialysis Network (FHN) Trial Jochen G. Raimann,[1] Christopher T. Chan,[2] John T. Daugirdas,[3] Thomas A. Depner,[4] Frank A. Gotch,[1] Tom Greene,[5] George A. Kaysen,[6] Alan S. Kliger,[7] Peter Kotanko,[1] Robert M. Lindsay,[8] Michael V. Rocco,[9] Glenn M. Chertow,[10] Nathan W. Levin,[1] The FHN Trial Group.[11] *[1]Renal Research Inst; [2]Univ Health Network Toronto; [3]Univ of Illinois College of Medicine; [4]Univ of California Davis; [5]Univ of Utah; [6]UC Davis, Nephrology; [7]Yale New Haven Health System; [8]London Health Sciences Centre; [9]Wake Forest School of Medicine; [10]Stanford Univ School of Medicine; [11]NIDDK, NIH.*

Background: The FHN Trials were designed to compare HD six versus three times per week. More frequent in-center HD significantly reduced left ventricular mass (LVM), an effect that was more pronounced in subjects with low baseline urine volumes. More frequent HD also reduced measures of interdialytic extracellular fluid volume and fluid overload. Patients receiving HD with low predialysis serum sodium concentrations (SNa) are at increased risk of mortality and cardiovascular events. We hypothesized that patients with lower SNa would have a more pronounced benefit of more frequent HD on LVM compared to patients with higher SNa.

Methods: The randomized treatment effect of frequency on LVM was compared in subgroups with baseline SNa≤138 versus >138 mEq/L. The interaction of treatment effect with SNa was tested using a mixed model adjusted for age, diabetes, facility and baseline LVM. Data presented as mean and corresponding 95% CI.

Results: In the in-center Daily Trial, the effect of frequent HD on LVM reduction was more pronounced in patients with lower baseline SNa (**Table**; Interaction P=0.03). In the at home Nocturnal Trial, change in LVM was nominally higher in patients with lower predialysis SNa, but the interaction was not statistically significant (**Table**; Interaction P=0.49).

Conclusions: The effect of frequent HD on LVM is more pronounced in patients with lower pre-dialysis SNa.

	Serum Sodium Concentration (SNa^+) [mEq/L]	LVM reduction (Month 12 – Baseline value)
Daily	≤ 138 mEq/L	**-27.98 (-40.54 to -15.41)**
	> 138 mEq/L	-1.97 (-15.45 to 11.50)
Nocturnal	≤ 138 mEq/L	-14.70 (-32.92 to 3.52)
	> 138 mEq/L	-7.54 (-24.77 to 9.68)

Funding: NIDDK Support

TH-OR082

FSP-1/ROCK1 Signaling Contributes to Diabetes-Induced Neointima Formation in Arteriovenous Graft Jinlong Luo,[1] Ming Liang,[1] Farhad R. Danesh,[2] William E. Mitch,[1] Jizhong Cheng.[1] *[1]Nephrology Div, Baylor College of Medicine, Houston, TX; [2]Emergency Medicine, Univ of Texas MD Anderson Cancer Center, Houston, TX.*

Background: Diabetes-induced endothelial damage could be the initial event leading to arteriovenous graft (AVG) failure. We hypothesized that increased expression of fibroblast specific protein 1 (FSP-1) or ROCk1 in endothelial cells (ECs) triggers diabetes-induced AVG failure.

Methods: Diabetes was induced with streptozotocin (STZ). AVGs were created in wild type (WT), ROCK1 KO, and FSP-1 KO mice with or without diabetes. The underlying mechanism of endothelial damage in diabetes was investigated.

Results: We found that diabetes induces neointima formation in AVGs and this was accompanied by impaired endothelial barrier function and and increased inflammatory cells accumulation. Diabetes also led to significantly increased FSP-1 and ROCK1 expression in AVGs of diabetic mice compared to the expression in control mice. ECs treated with FSP-1 led to ROCK1 activation. FSP-1 treatment or expression of constitutive active ROCK1 decreased expression of junction molecules. Increased FSP-1 expression also induced endothelial permeability and trans-endothelial migration of inflammatory cells. These responses were suppressed by treatment of the ROCK1 inhibitor or by expression of dominant negative ROCK1 vector. Thus, FSP-1-induced endothelial dysfunction can be mediated through ROCK1 activation. To evaluate the function of ROCK1 or FSP-1 *in vivo*, AVGs were created in ROCK1 KO or FSP-1 KO mice. We found that a deficiency of FSP-1 decreased diabetes-induced ROCK1 expression and activation in the endothelium of AVGs. Indeed, FSP-1 KO exhibited decreased infiltration of inflammatory cells and suppressed neointima formation despite diabetes. Finally, ROCK1 KO improved endothelial barrier function and decreased neointima formation in AVGs.

Conclusions: Thus, diabetes induces endothelial dysfunction through activation of FSP-1/ROCK1 signaling. This response increases endothelial permeability which enhances infiltration of inflammatory cells, triggering neointima formation and AVG failure. Blocking FSP-1/ROCK1 could become a therapeutic target for suppressing AVG failure.

Funding: NIDDK Support

TH-OR083

In Vivo Nanoparticle Imaging of Dysfunctional Endothelium Predicts the Development of Inflow Stenosis Jie Cui,[1,2] Chase Kessinger,[2] Jason McCarthy,[3] David E. Sosnovik,[2] Ravi I. Thadhani,[1] Farouc Amin Jaffer.[2] *[1]Nephrology Dept, MGH; [2]Div of Cardiology, MGH; [3]Center for System Biology, Massachusetts General Hospital.*

Background: Inflow stenosis is one of the most common causes of arteriovenous fistula (AVF) failure and is driven by inflammatory endothelium dysfunction after AVF creation. *In vivo* assessment of dysfunctional endothelium following AVF creation could provide new insights into subsequent neointimal hyperplasia (NH) and inflow stenosis.

Methods: AVF were created in C57Bl/6 mice by carotid artery and jugular vein anastomosis. On day 13 post-AVF creation, mice were injected with 10mg/kg of the fluorescent-magnetic nanosensor CLIO-VT680, which targets inflamed endothelial cells. On day 14, *en face* confocal microscopy (n=3), intravital microscopy (IVM) (n=5), *ex vivo* magnetic resonance imaging (MRI) and transmission electron microscopy (TEM) were performed. A subgroup of mice was kept alive after day 14 IVM imaging and then sacrificed on day 42 post-AVF creation. Image analysis was performed using Image J software.

Results: Confocal microscopy imaging demonstrated the deposition of CLIO-VT680 in day 14 AVF. *In vivo* arterial inflammation signals of day 14 AVF by IVM correlated negatively with the distance from the anastomosis (r=-0.98, p<0.0001). TEM of day 14 AVF revealed that most of the CLIO-VT680 deposited in the endothelium and the space between endothelium and internal elastin. The degree of day 14 endothelial dysfunction imaged by CLIO-V680 predicted day 42 arterial NH.

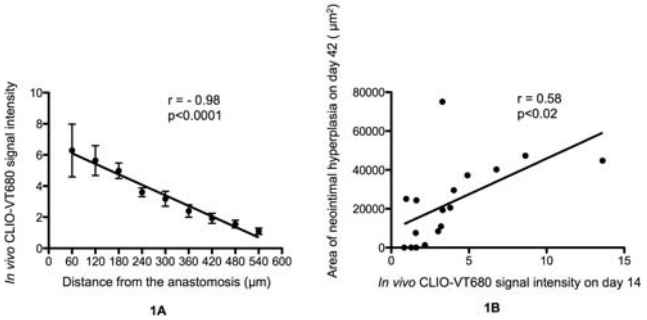


Figure 1:A. On day 14, *in vivo* CLIO-VT680 signal intensity measured by IVM showed a strong negative correlation with the distance away from the anastomosis. B. Day 14 *in vivo* CLIO-VT680 signal intensity can predict the area of neointimal hyperplasia on day 42.

CLIO-VT680 signal of day 14 AVF was detectable by *ex vivo* MRI.

Conclusions: Endothelial dysfunction post-AVF creation can be detected and quantified *in vivo* using high-resolution nanoparticle imaging, and the *in vivo* endothelial signal predicts subsequent inflow neointimal formation. This imaging strategy could be used to test different therapeutic interventions aiming to decrease inflow stenosis.

TH-OR084

Validation of Criteria Diagnosing Catheter-Related Bloodstream Infections in Hemodialysis Patients Friederike S. Quittnat Pelletier,[1,3] Mohammad Z.H. Joarder,[1] Alyssa Loughborough,[1,2] Susan Poutanen,[1,2,3] Charmaine E. Lok.[1,3] *[1]Univ Health Network; [2]Mount Sinai Hospital; [3]Univ of Toronto, ON, Canada.*

Background: Most hemodialysis (HD) units diagnose HD catheter-related bloodstream infections (CRBSI) by obtaining blood cultures (BC) from the HD bloodline (circuit) concurrent with clinical exclusion of other sources of infections. Guidelines from the Infectious Diseases Society of America (IDSA, 2009) recommend making the diagnosis of CRBSI by cultivating the same organism from a peripheral vein and from the catheter hub, meeting criteria for differential time to positivity (DTTP) >120 minutes. These criteria for CRBSI were derived from patient data reflecting practices with indwelling catheters used for drug administration; they have not been validated in HD patients who develop symptoms suspicious for CRBSI during hemodialysis.

Methods: Four sets of BC (peripheral vein, both catheter hubs and HD bloodline) were obtained from adult patients who were suspected of having a CRBSI. CRBSI was determined by an independent hemodialysis infection control committee. Sensitivity, specificity and accuracy of each BC type were calculated. DTTP of peripheral vein BC and HD bloodline BC compared to catheter hub BC was determined.

Results: 100 blood sample sets from patients with suspected CRBSI were obtained. Bacteremia was found in 27% of these patients and these BC consistently grew the same bacteria from all culture sites. Highest sensitivity, specificity and accuracy of BC results were found in samples from the dialysis bloodline and the venous catheter hub (93%, 98%, 96%, respectively), while BC from the peripheral vein combined with the arterial hub were the least sensitive, specific or accurate (88%, 89%, 88%, respectively). DTTP <120 minutes was found in 65-76% of samples.

Conclusions: BC results are more sensitive, specific and accurate if taken from the dialysis bloodline and a catheter hub, rather than from a peripheral vein. DTTP >120 minutes usually used to diagnose CRBSI is seen in less than one third of the compared BC and does not add to the accuracy of CRBSI diagnosis.

Funding: Private Foundation Support

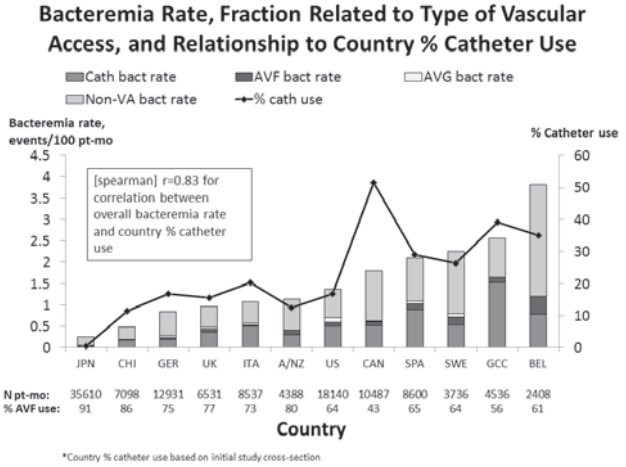
TH-OR085

International Differences in Bacteremia Rates in Hemodialysis Patients in the DOPPS: Strong Relationship with Catheter Use Hugh C. Rayner,[1] Lindsay Zepel,[2] Michel Y. Jadoul,[3] Charmaine E. Lok,[4] David C. Mendelssohn,[5] Jeffrey Perl,[6] Richard J. Fluck,[7] Lawrence M. Spergel,[8] Brenda W. Gillespie,[2,9] Ayman Karkar,[11] Hideki Kawanishi,[10] Brian Bieber,[2] Friedrich K. Port,[2] Bruce M. Robinson,[2,9] Ronald L. Pisoni.[2] *[1]Heart of En. NHS Found.Trust; [2]ARCH; [3]Cliniques U. St. Luc; [4]Toronto Gen. Hos.; [5]Humber River Reg. Hos.; [6]St. Michael's Hos.; [7]Derby Hos. NHS Found.Trust; [8]Dial. Mgmt. Med. Group; [9]UM; [10]Tsuchiya Gen. Hos.; [11]Kanoo Kid. Cent.*

Background: To inform efforts to reduce infection in HD patients (pts), we compared bacteremia (BA) rates in 18 DOPPS countries.

Methods: Monthly prospective data from 2012-2014 for infections for which blood cultures were consistent with bacteremia (BA). Relation of BA to pt's vascular access (VA) was locally attributed. Data were from representative random samples of HD pts in A/NZ, JPN, EUR, CHI, Gulf Cooperation Council (GCC), CAN, and the U.S. Calculation of BA events was not feasible with data from U.S. LDOs. BA rates were **not** calculated according to the time using a particular VA.

Results: Total BA rates varied >10 fold from 0.25/100 pt-months in JPN to 3.8 in BEL. Non-VA-BA rates were higher than VA-BA in most countries. Total BA rates strongly correlated with country % catheters (CATH) at baseline (r=0.83). Total BA attributed to CATH-BA varied from 11% in JPN to 59% in GCC (median ~36%). Even though AV fistula (AVF) use was greater than CATH use in nearly all countries, CATH-BA rates were considerably greater than AVF-BA rates in all countries. Similar results were seen when restricted to only the first 4 months of the baseline period for all pts. Restricting to CAN, ITA, SPA, GER, UK, and U.S., CATH-BA rates were considerably higher than AVF-BA rates in both pts 18-74 yrs and >74 yrs.



Conclusions: Total BA rates are strongly correlated with country % CATH use. The variation in BA rates not attributed to VA requires further investigation.

Funding: Pharmaceutical Company Support - The DOPPS program is supported by grants to Arbor Research from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, Ltd. Additional support for specific projects is provided in Canada by Amgen, BHC Medical, Janssen, Takeda, Kidney Foundation of Canada (for logistics support); in Germany by Hexal, DGfN, Shire, WiNe Institute; for PDOPPS in Japan by the Japanese Society for Peritoneal Dialysis; for PDOPPS by Fresenius Medical Care

TH-OR086

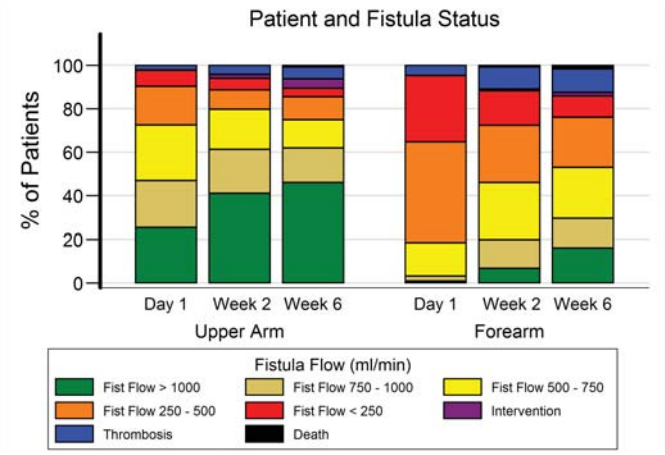
Preoperative and Postoperative Vascular Ultrasound in the Hemodialysis Fistula Maturation (HFM) Cohort Study

Michelle L. Robbin, Tom Greene, The HFM Study Group. *NIDDK, NIH.*

Background: Vascular changes resulting from arteriovenous fistula (AVF) creation for hemodialysis access have not been well characterized, in part due to the lack of use of standardized imaging.

Methods: Preoperative (preop) mapping ultrasound (US) and postoperative (postop) AVF USs were performed by centrally trained sonographers at 7 HFM Study sites using a standardized protocol. AVF diameters and blood flow rates were assessed at ~1 day, 2 weeks and 6 weeks after AVF creation. Images were read by HFM Core radiologists blinded to clinical information, using standardized criteria. This report summarizes findings in the first 582 subjects out of a total 602 enrolled.

Results: There were 444 subjects (76%) with upper arm (UA) AVFs and 138 (24%) with forearm (FA) AVFs. Median (25^{th}-75^{th} percentile) preop artery and vein, and postop AVF diameters are shown in the table. The figure shows deaths, thromboses, and postop AVF blood flow. Individual trajectories were highly diverse. KDOQI physiologic maturation criteria of flow ≥ 600 ml/min and vein diameter ≥ 0.6 cm were met at 1 day, 2 and 6 weeks postop by 16.3%, 42.1%, and 53.9% of UA fistulas and by 0%, 8.9%, and 17.5% of FA fistulas, respectively. Logistic models predicting joint satisfaction of both KDOQI criteria at 6 weeks from AVF diameters and flows, measured respectively at 1 day or 2 weeks postop, had C statistics of 0.84 and 0.89.



	Preop Diameter (cm)		Postop AVF Diameter (cm)		
Location	Artery	Vein	1 day	2 weeks	6 weeks
Upper Arm	0.42 (0.36-0.48)	0.31 (0.24-0.38)	0.50 (0.43-0.57)	0.58 (0.51-0.68)	0.63 (0.52-0.75)
Forearm	0.23 (0.20-0.26)	0.26 (0.18-0.32)	0.39 (0.32-0.44)	0.46 (0.38-0.52)	0.50 (0.40-0.56)

Conclusions: The extent and timing of AVF diameter and flow increases in the first six weeks are highly heterogeneous. US diameter and flow measurement at 1 day and 2 weeks is highly predictive of 6 week values and may have clinical utility.

Funding: NIDDK Support

TH-OR087

Demographic, Clinical, and Anatomic Factors Correlate with Physiologic Adaptation following Fistula Placement: The Hemodialysis Fistula Maturation (HFM) Cohort Study

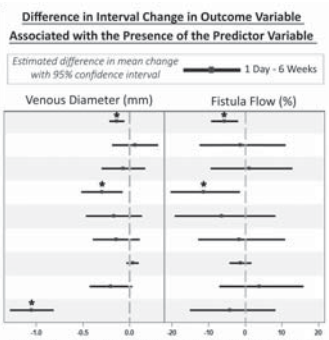
Scott A. Berceli, Tom Greene, The HFM Study Group. *NIDDK, NIH.*

Background: Demographic variables associated with AVF maturation are well established but the physiologic mechanisms that lead to this maturation success or failure are unknown.

Methods: Using a standardized protocol, duplex ultrasound was performed at 1 day, 2 weeks, and 6 weeks to map the temporal changes in flow rate and vein diameter following AVF creation (n=582 subjects). Mixed effects regression analyses with random surgeon effects, controlled for fistula location, were performed to examine the relationships between patient demographic/clinical/anatomic factors and both absolute and interval adaptation differences in diameter and flow.

Results: Greater age, female sex, black race, diabetes, history of coronary artery disease, and AVF transposition were associated with reduced vein diameter at 6 weeks, versus those without these characteristics (Table). Older age, female sex, black race, diabetes, peripheral and coronary artery disease, and smaller anastomotic lengths were associated with reduced AVF flow at 6 weeks. Examining the temporal changes in these parameters between 1 day and 6 weeks (Figure) also revealed differences in vein adaptation between patient subgroups. Patients with increasing age, diabetes, and AVF transposition demonstrated compromised venous dilation. Diabetes and increasing age were associated with progressively diminished flow augmentation over that period.

	6 Week Outcome Variable			
	Venous Diameter (mm)		Fistula Flow (%)	
Predictor Variable	*Diff. in Outcome*	*P Value*	*Diff. in Outcome*	*P Value*
Age (10 years)	-0.16	**.002**	-9.22	**.000**
Female	-0.13	.423	-14.9	**.011**
Black	-0.55	**.000**	-8.01	.126
Diabetes	-0.42	**.002**	-19.0	**.000**
PAD	-0.07	.716	-18.6	**.005**
CAD	-0.30	**.049**	-16.3	**.003**
Length Anastomosis (mm)	0.07	.106	4.17	**.028**
Dialysis Dependent	-0.05	.744	1.01	.851
Transposed Vein	-0.58	**.000**	-4.05	.492



Conclusions: AVF diameter and flow are associated with multiple demographic and clinical factors. Impaired vein dilation and reduced capacity for flow augmentation contributed to moderated diameters and flows among older and diabetic patients, and to smaller diameters among patients with transposed vein fistulas. This understanding of the physiologic mechanisms that are associated with maladaptive remodeling provides needed granularity to clinical maturation failure.

Funding: NIDDK Support

TH-OR088

Effect of Dietary Sodium Restriction on Human Urinary Metabolomic Profiles

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Background: Metabolomics is a relatively new field of "-omics" research, focusing on high-throughput identification of small molecular weight metabolites. Diet has both acute and chronic effects on metabolic profiles; however, alterations in response to dietary sodium restriction (DSR) are completely unknown. The goal of this study was to explore changes in urine metabolites in response to DSR, as well as their association with previously reported improvements in vascular function with DSR.

Methods: Using stored urine samples from a 10 week randomized, placebo-controlled cross-over study of DSR in 17 middle-aged/older adults (6M/11F; 62±2 yrs, mean±s.e.) with moderately elevated SBP (130-159 mmHg) and otherwise healthy, we performed a liquid chromatography–mass spectrometry (LC/MS)-based analysis of 289 metabolites. We identified metabolites that were significantly altered between the normal (153±7 mmol/day) and low sodium condition (70±7 mmol/day), as well as their association with responsiveness to previously reported improvements in vascular endothelial function (brachial artery flow-mediated dilation [FMD_{BA}]) and large elastic artery stiffness (aortic pulse-wave velocity [aPWV]).

Results: 13 metabolites were significantly altered (12 up- and 1 down-regulated) during the low sodium condition, and 10 of these exceeded our pre-specified threshold of a clinically significant change of >40%. These metabolites were involved in biological pathways broadly related to cardiovascular risk, nitric oxide production, oxidative stress, osmotic regulation, and metabolism. Furthermore, 2 of these metabolites (serine and D-gluconate) were independently associated with previously reported improvements in the primary vascular outcomes of FMD_{BA} and/or aPWV.

Conclusions: This proof of concept study provides the first evidence that DSR is a stimulus that induces significant changes in urinary metabolomic profiles, some of which are related to improved vascular function with reduced dietary sodium. Larger follow-up studies will be required to confirm and further elucidate pathways that are altered in response to DSR.

Funding: Other NIH Support - AG013038, AG006537, AG033994, TR000154

TH-OR089

A Plasma Proteomic Classifier to Predict Transition of Albuminuria Stage in Subjects with Hypertension Michelle Pena,[1] Georg Heinze,[3] Peter Rossing,[4] Dick de Zeeuw,[1] Hiddo Jan Lambers Heerspink,[1] Joachim Jankowski.[2] *[1]Univ Medical Center Groningen, Groningen, Netherlands; [2]Univ Hospital RWTH, Inst for Cardiovascular Research, Aachen, Germany; [3]Medical Univ of Vienna, Vienna, Austria; [4]Steno Diabetes Center, Gentofte, Denmark.*

Background: Development of micro- or macroalbuminuria is a hallmark of progression of nephropathy in various diseases including hypertension. Early detection of patients with hypertension at risk for micro- or macroalbuminuria may facilitate prevention and treatment of renal diseases. We aimed to develop a plasma proteomic classifier specific to hypertension that could predict the development of micro- or macroalbuminuria.

Methods: Patients with hypertension (n=125) were selected for this case-control study from the PREVEND cohort. Cases transitioned in albuminuria stage from normo- to microalbuminuria or micro- to macroalbuminuria during 2.7-years. Controls, matched for age, gender, and baseline albuminuria stage, did not transition. Plasma proteomic profiles were measured by LC-electrospray-trap mass-spectrometry and a classifier was developed and cross-validated for prediction of transition to micro- or macroalbuminuria. Improvement in risk prediction was tested on top of a baseline model of albuminuria, eGFR, and renin-angiotensin-system intervention (RASi) use. The external validity of the classifier was tested in a type 2 diabetes cohort (n=82).

Results: The proteomic classifier was independently associated with transition in albuminuria (Odds ratio 1.26, 95% CI=1.11-1.42, p<0.01). The classifier improved risk prediction for transition in albuminuria on top of the baseline model (C-index from 0.68 to 0.78; p<0.01; IDI 0.061 p=0.034). The association was similar in case-control pairs with RASi use (C-index from 0.71 to 0.78) or without RASi use (C-index from 0.69 to 0.81). The peptides included in the classifier could not be detected in a type 2 diabetes cohort suggesting disease specific peptides.

Conclusions: A hypertension-specific plasma proteomic classifiers was developed and validated that could predict the transition of albuminuria stage beyond established renal risk markers. These results have to be validated in a prospective cohort.

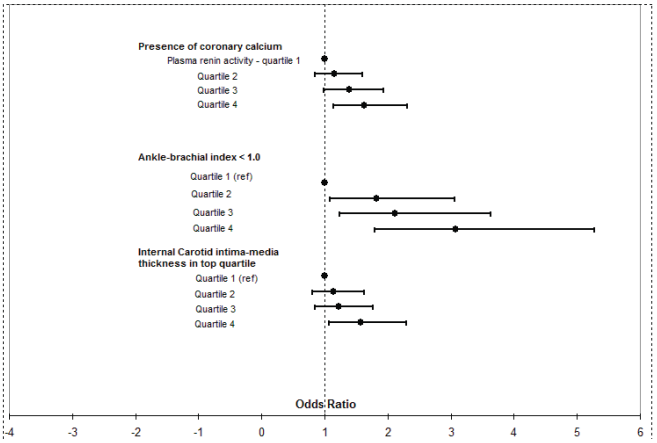
TH-OR090

Association of Plasma Renin with Subclinical Cardiovascular Disease in a Population-Based Cohort: The Multi-Ethnic Study of Atherosclerosis Dena E. Rifkin,[1,2] Joseph A. Abdelmalek,[1,2] Nancy Jenny,[3] Robyn L. Mcclelland,[4] Matthew Jay Budoff,[5] Joachim H. Ix,[1,2] Matthew Allison.[1,2] *[1]UC San Diego; [2]VA San Diego; [3]Univ of Vermont; [4]Univ of Washington, Seattle; [5]UCLA.*

Background: Several studies have demonstrated an association with renin and cardiovascular disease. Many experimental studies support a link between RAAS activation and atherosclerosis, as both renin and aldosterone are known to be profibrotic. We hypothesized that plasma renin activity (PRA) and aldosterone would be associated with markers of subclinical CVD, independent of traditional cardiovascular risk factors in a multi-ethnic population without clinical CVD.

Methods: A cross sectional analysis of 1800 individuals from the Multi-Ethnic Study of Atherosclerosis (MESA) study was performed. PRA and aldosterone were measured, in addition to quantification of coronary artery calcification (CAC), internal carotid artery intima-media thickness (IMT), and ankle-brachial index (ABI).

Results: The mean age was 64.5 years, and 50.3% were male. Twenty percent of the study population was African American, 26% Hispanic, 13.1% Asian and 40.8% White. After multivariable adjustment including demographic, cardiovascular, and kidney markers, increasing quartiles of PRA were associated with a more than 50% higher odds of CAC presence or high cIMT and a tripling of the odds of having a low ABI in the highest renin quartile.



Race did not modify associations of PRA with either CAC or IMT. Aldosterone was not independently associated with subclinical atherosclerosis, and adjustment for aldosterone did not affect these estimates.

Conclusions: In a large multi-ethnic cohort, renin was independently associated with greater prevalence of subclinical atherosclerosis in multiple vascular beds. This finding was independent of demographic factors as well as aldosterone levels.

TH-OR091

Association of Body Fluid Composition with Resistant Hypertension in Patients with Chronic Kidney Disease Toshiyuki Aoki,[1] Yasushi Ohashi,[1] Reibin Tai,[1] Sonoo Mizuiri,[2] Yoshihide Tanaka,[1] Atsushi Aikawa,[1] Ken Sakai.[1] *[1]Nephrology, School of Medicine, Faculty of Medicine, Ohta-ku, Tokyo, Japan; [2]Nephrology, Ichiyokai Harada Hospital, Hiroshima-shi, Japan.*

Background: Treatment-resistant hypertension remains a common clinical issue in patients with chronic kidney disease (CKD). Our objective is to investigate the association of body fluid composition with treatment-resistant hypertension in patient with CKD.

Methods: Using bioimpedance analysis (BIA), body fluid composition was measured in 149 patients with CKD from 2005 to 2009, who were followed until August 2013. The ratio of extracellular water measured by BIA (ECW_{BIA}) to total body water calculated using the Watson formula (TBW_{Watson}) was used as an indicator of volume excess. Treatment-resistant hypertension was defined as an office BP of ≥130/80 mmHg, despite receiving ≥3 antihypertensives including diuretics, or ≥4 drugs usage. Main outcomes were adverse renal outcomes, as defined by a decline of 50% or more from baseline glomerular filtration rate or initiation of renal replacement therapy and cardiovascular events.

Results: Treatment-resistant hypertension was present in 37 patients (24.8%). Patients with treatment-resistant hypertension were more likely to be older and male and have diabetes mellitus, higher body mass index (BMI), lower eGFR and serum albumin, higher triglyceride and UPCR levels, and volume excess ($p < 0.05$). Compared with patients with no treatment-resistant hypertension during a median 4.9-year follow-up, those with treatment-resistant hypertension had worse adverse renal outcomes (21.3 versus 6.1 per 100 patient-years, $P < 0.001$) and cardiovascular events (5.0 versus 1.5 per 100 patient-years, $P = 0.009$) by Kaplan–Meier survival analysis. In multivariate analysis, body mass index and volume excess remained independently associated with treatment-resistant hypertension. The best cut-off value of ECW_{BIA}/TBW_{Watson} ratios for Treatment-resistant hypertension was 39.9%.

Conclusions: Treatment-resistant hypertension is associated with fat content and volume excess, which exhibits adverse renal outcomes and cardiovascular events. These findings emphasize the importance of adequate weight and volume status.

TH-OR092

Elevated Endogenous Ouabain and Reduced Excretion of Uric Acid after Acute Salt Load in Hypertensive Patients Chiara Lanzani,[1] Lorena Citterio,[1] Elena Brioni,[1] Marco Simonini,[1] Chiara Maggioni,[1] Elisabetta Messaggio,[1] John Hamlyn,[2] Paolo Manunta.[1] *[1]San Raffaele Scientific Inst, Milan, Italy; [2]Univ of Maryland, Baltimore.*

Background: The independent functional role of serum uric acid (UA) in enhancing cardio-renal risk is controversial. The permeability glycoprotein (PGP) encoded by the ATP-binding cassette B1 gene (ABCB1), extrudes UA in the kidney, and is associated with chronic kidney disease. Endogenous ouabain (EO), an adrenal steroid hormone, and UA are increased among hypertensives and both are associated with increased cardiovascular risk. The present study examined the relationship between baseline EO and the renal excretion of UA in hypertensives after an acute Na load.

Methods: Plasma EO, and the fractional excretion of Na+ and UA were determined in 222 naïve hypertensives (age 52.3±10.6 years, BMI 25.7±2.99 Kg/m^2) after 2 hour of 0.9% NaCl infusion.

Results: Elevated plasma EO was associated with higher systolic and diastolic BP**.** Genotype analysis for EO levels and UA were significant for the ABCB1 rs1045642, with the minor allele tracking with higher EO (T/T 184.7 (147–272) versus C/C+C/T 211.1 (193–366) pmol/l, p=0.033). FEUA after the NaCl load was lower in T/T carriers (7.21±0.34) versus the CC+CT variants (8.4±3.95 p=0.046). A direct (b=3.57) linear relationship (p=0.0004) was present between baseline plasma EO and FEUA after salt loading.

Conclusions: EO and urinary UA excretion are strictly related, likely via the same (PGP) tubular transporter. Our results imply that UA is a marker for high EO and that EO is the functional effector of UA risk.

TH-OR093

Effect of Arteriolar Hyalinosis on the Blood Pressure-Dependent Proteinuria in Non-Nephrotic Chronic Kidney Disease Ryo Zamami,[1] Kentaro Kohagura,[1] Yusuke Ohya,[1] Kunitoshi Iseki.[2] *[1]Cardiovascular Medicine, Nephrology and Neurology, Univ of the Ryukyus, Nishihara-cho, Okinawa, Japan; [2]Dialysis Unit, Univ Hospital of the Ryukyus, Nishihara-cho, Okinawa, Japan.*

Background: It has been suggested that afferent arteriolar hyalinosis was associated with disturbed autoregulation of glomerular hemodyanamics. However, effect of afferent arteriolar hyalinosis on a relationship between blood pressure (BP) and proteinuria is unknown in chronic kidney disease (CKD) patients.

Methods: A total of 208 consecutive patients who underwent renal biopsy at our department between 2003 and 2007 were considered for the study. We excluded patients receiving renin angiotensin inhibitors, or steroids or calcineurin inhibitors, and those with diseases such as vasculitis, which cause morphological changes to renal arterioles. We then selected non-nephrotic CKD patients defined as serum albumin equal or more than 3 g/gCr, leaving us with 103 patients (48 men and 55 women) for analysis.Arteriolar hyalinosis

were assessed by semi quantitative grading for arterioles. We compared the relationship between systolic BP category (<120, 120-140, >140) and urine protein (g/gCr) the patients with or without arteriolar hyalinosis.

Results: The mean ± standard deviation values for patients' age, BP, estimated glomerular filtration rate (eGFR), and urine protein were as follows: 39± 18 years, 126 ± 20/75 ± 12 mmHg, and 86 ± 38 ml/min/1.73 m^2, and 1.2±1.1 g/gCr, respectively. Either, systolic BP or mean arteriolar hyalinosis index was significantly correlated with log-transformed urine protein (r=0.27, p=0.006; r=0.35, P=0.0003), respectively. In the patients with hyalinosis (n=53), proteinuria (g/gCr) was significantly increased as systolic BP category was increasing (1.0, 1.2, 2.1, ANOVA p=0.03). In contrast, there was no significant association between systolic BP category and urine protein in the patients without hyalinosis (n=50).

Conclusions: These results indicated that renal arteriolar hyalinosis might potentiate the susceptibility of glomerular damage by BP in non-nephrotic CKD patients. Dysregulation of afferent arteriolar resistance via arteriolar sclerosis may be involved in hypertensive renal damage.

TH-OR094

Long-Term Intake of Animal Flesh and Risk of Developing Hypertension in Three Prospective Cohorts

Lea Borgi, John P. Forman. *Nephrology, Brigham and Women's Hospital, Boston, MA.*

Background: Prospective data are scarce on the relation of red meat, seafood, and poultry consumption with hypertension risk. Although red and processed meats are generally considered to have adverse cardiovascular consequences, seafood is believed to be protective and poultry's effect is controversial.

Methods: We prospectively examined the independent association of animal flesh intake with incident hypertension in three large longitudinal cohort studies of originally non-hypertensive individuals: Nurses' Health Study I (NHS1, n=62,273, aged 38-63 years in 1984), Nurses' Health Study II (NHS2, n=88,831, aged 27-44 years in 1991), and Health Professionals Follow-up Study (HPFS, n =37,414, aged 40-75 years in 1986). Information about diet (using a validated food frequency questionnaire), behaviors, and health status was updated biennially. We used multivariable Cox proportional hazards regression to study the associations of different types of flesh (red meat, processed meat, seafood, and poultry) with the risk of developing hypertension while controlling for numerous other hypertension risk factors. We then used random effects meta-analysis to derive pooled estimates of effect.

Results: Compared with participants whose consumption was <1 serving/month, the pooled hazard ratios (HR) among those whose intake was ≥1 serving/day were 1.31 (95% CI: 1.23-1.39) for total meat (a combination of processed and red meat), 1.06 (0.98-1.14) for seafood, and 1.22(1.11-1.33) for poultry. Seafood was associated with an increased risk of hypertension in NHS2 (HR= 1.15, p-trend<0.001) and HPFS (HR= 1.18, p-trend<0.001), but not in NHS1. In substitution analyses, replacing one type of flesh with another (eg, replacing red meat with seafood) did not increase or decrease risk of hypertension. Consumption of any animal flesh ≥1 serving/day was associated with an important increase in the risk of hypertension (pooled HR= 1.29[1.15-1.45]).

Conclusions: Our results suggest that long-term intake of animal flesh is prospectively and independently associated with an increased risk of developing hypertension. Furthermore, in addition to meat, poultry intake was associated with increased risk, and seafood was not found to be protective.

TH-OR095

Elevated Blood Pressure Index and Decreased Nocturnal Dip Are Risk Factors for Cognitive Dysfunction in Children and Young Adults

Nina Laney,[1] Stephen R. Hooper,[2] Jerilynn Radcliffe,[1] Ji Young Kim,[1] Rebecca Ruebner,[1] Erum A. Hartung,[1] Divya Ganeshmurthy Moodalbail,[3] Susan L. Furth.[1] *[1]Children's Hospital of Philadelphia; [2]Univ of North Carolina; [3]Nemours/A.I. duPont Hospital for Children.*

Background: Hypertension (HTN) is a known risk factor for cognitive dysfunction in adults, yet there are few studies examining the same in children and young adults. Thus, we aim to elucidate the relationship between HTN and cognitive function in a younger population.

Methods: Cross-sectional study of 104 subjects, ages 8-25, including 65 with chronic kidney disease (CKD) [mean(SD) age 15.7(3.9) yrs, eGFR 48.6(23.7) mL/min/1.73 m^2, 16 with transplant, 2 on dialysis] and 39 healthy controls [mean(SD) age 15.1(3.7) yrs, eGFR 103.1(20.3) mL/min/1.73 m^2]. Each completed 24 hour ambulatory blood pressure measurement (ABPM) and a neurocognitive battery consisting of measures of Language, Attention, Inhibitory Control, Problem Solving, Set Shifting, Visuospatial, Memory (Verbal and Visual), Working Memory (Verbal and Visual), and Executive Function. Neurocognitive results were converted to z-scores based on published norms. ABPM data yielded measures of systolic and diastolic means, percent dipping, load, and index, using 95th percentile limits based on age, height, and gender.

Results: After controlling for CKD versus control, race, and maternal education, multiple regression analysis showed significant associations with ABPM measures in Verbal Memory and Set Shifting. Systolic and diastolic index were negatively associated with performance in the Verbal Memory domain, with a decrease of 0.66 (P=0.002) and 0.53 (P=0.006) in z-scores for every 0.1 point increase in systolic and diastolic index, respectively. Systolic dipping was negatively associated with Set Shifting, with a decrease of 0.03 in z-score (P = 0.04) for every 1% decrease in systolic dipping.

Conclusions: Children and young adults with increased systolic and diastolic indices and decreased systolic dipping on ABPM show clinically significant deficits in Verbal Memory and Set Shifting, regardless of disease, race, and maternal education. Early identification and treatment of HTN may be effective in preserving cognitive function.

Funding: Other U.S. Government Support

TH-OR096

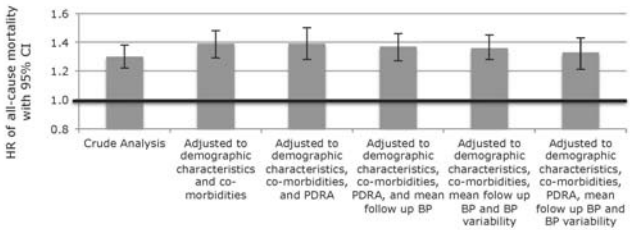
Examining Association of Medical Non-Adherence with All-Cause Mortality in Incident Hypertensive U.S. Veterans

Elvira Gosmanova,[1] Jun Ling Lu,[1] Elani Streja,[2] William C. Cushman,[3] Miklos Zsolt Molnar,[1] Kamyar Kalantar-Zadeh,[2] Csaba P. Kovesdy.[3,1] *[1]Univ of Tennessee; [2]Univ of California Irvine; [3]Memphis VAMC.*

Background: Non-adherence to antihypertensive drugs (AHD) is associated with adverse outcomes, but mediators of this relationship are poorly understood. We examined the effect of ICD-9 code for medical treatment non-adherence (V15.81) on survival in a cohort of hypertensive U.S. veterans and explored possible mediators of this association.

Methods: Historical prospective cohort study involving 311,622 newly treated hypertensive individuals (mean age 53.8 years, 90.9% males, 20.3% black, 7.1% with V15.81). Associations between V15.81 assigned prior to treatment with AHDs, and all-cause mortality were examined in Cox models adjusted for demographics and co-morbidities. Effect mediation was examined by additional adjustment for follow-up adherence to AHD through pharmacy dispensation records analysis (PDRA), mean follow-up BP, and follow-up BP variability (measured as the median absolute deviation from the mean).

Results: 27,384 patients (8.9%) died (mortality rate 23.1 [22.8-23.4]/1000 patient-years) during a median follow up of 3.8 years. Patients with V15.81 had higher all-cause mortality and this association remained unchanged after serial adjustments (Figure), even though PDRA, mean BP, and BP variability were independently associated with higher mortality.



Conclusions: Assignment of V15.81 prior to AHD therapy was associated with increased all-cause mortality in incident hypertensive U.S. veterans, independent of AHD adherence or follow up BP. Non-pharmacologic aspects of adherence may explain these associations. Non-adherence is a complex condition and further research is needed to understand what modifiable patient characteristics are prone to interventions that could improve outcomes in hypertensive individuals.

Funding: NIDDK Support, Veterans Affairs Support

TH-OR097

Renal and Heart Function in Essential Hypertension

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Background: The relationship between heart and renal function is a precarious matter that has been investigated through the years. Lately many studies suggest that renal injury is associated with early stage heart dysfunction. The aim of the present study is to investigate the grade of renal deterioration according to heart function in non diabetic essential, never treated, hypertensive patients.

Methods: We studied 7970 consecutive patients (4419 males, mean age 53 years) with uncomplicated essential hypertension and the correlation between cardiac function using the Tei index (defined as the sum of the isovolumetric relaxation and contraction time divided by the ejection time), transmitral blood flow velocities ratio (E/A), ejection fraction (EF) and kidney injury [eGFR(ml/min), ACR (urine albumin-creatinine ratio)]. Patients were grouped by ACR (ACR>30 and ACR<30) and eGFR (eGFR>90, eGFR 90-60, eGFR<60).

Results: The model was adjusted for age, gender, blood pressure, lipid profile and smoking habit. EF was decreased with renal function deterioration as did the E/A ratio, while the Tei index increased (Table 1). Furthermore EF has higher normal levels in patients with ACR <30 as did the E/A ratio while Tei index present lower levels (Table 1).

	eGFR >90 n: 1926	eGFR 90-60 n: 4693	eGFR <60 n: 1351				F value	p-value
EF	67.3±4.6	66.1±5.2	63.6±5.2				199.5	<0.0001
E/A	1.094±0.207	0.978±0.167	0.859±0.148				723.9	<0.0001
Tei index	0.561±0.063	0.584±0.068	0.627±0.080				363.3	<0.0001

		ACR >30 n:2210	ACR <30 n:5760	t-test		p-value
	EF	63.1±5.9	67.0±4.8	30.1	-	<0.0001
	E/A	0.880±0.159	1.027±0.185	32.8		<0.0001
	Tei index	0.623±0.029	0.571±0.064	29.9		<0.0001

Conclusions: We found that the Tei index and E/A was correlated with eGFR, and ACR. It seems that systolic and diastolic heart function indices can be used not only for the evaluation of the global myocardial performance of hypertensive patients but also for the assessment of the risk of kidney damage.

TH-OR098

Long Term Cholecalciferol Supplementation in Hemodialysis Patients: Effects on Mineral Metabolism, Inflammation, and Cardiac Parameters Patrícia Matias,[1,2,3] Cristina Jorge,[1,2,3] Ana Azevedo,[2,3] Ivo Laranjinha,[2,3] David Navarro,[1,3] Ana Carina Ferreira,[2,3] Tiago Amaral,[2,3] Marco Mendes,[1,3] Ines Aires,[1,2,3] Célia Gil,[1,2,3] Manuel A. Ferreira.[1,2,3] *[1]Dialysis Clinic, Nephrocare - Vila Franca de Xira, Vila Franca de Xira, Portugal; [2]Dialysis Clinic, Dialverca, Forte da Casa, Portugal; [3]NIDAN, Lisboa, Portugal.*

Background: Vitamin D deficiency is highly prevalent in chronic kidney disease. The aim of this 5-year prospective study was to evaluate the effects of long term oral cholecalciferol supplementation on mineral metabolism, inflammation and cardiac parameters in chronic hemodialysis (HD) patients.

Methods: Serum levels of 25-hydroxyvitamin D [25(OH)D], circulating bone metabolism, inflammation parameters, pulse pressure (PP) and left ventricular mass index (LVMI) were evaluated before and after supplementation. Baseline 25(OH)D levels were measured twice (end of winter and of summer, respectively). Therapy with active vitamin D and darbepoietin was evaluated. The study included 97 HD patients that completed 60 months of supplementation, 40.6% female, with a mean age of 63.19±14.14 and with a median HD time, at baseline, of 25 months. Cholecalciferol was given after dialysis according to 25(OH)D levels in the first 6 months (mean dose of 21.000 IU/week) and afterwards 8.000 IU/week.

Results: There was a significant increase in serum 25(OH)D levels after supplementation (23.13±13.16 *versus* 44.13±11.92; p<0.001). On the opposite, serum calcium (p=0.02), phosphorus (p=0.018) and iPTH (p=0.03) were decreased. A reduction in the dose and number of patients treated with active vitamin D (p<0.001) and darbepoietin was also observed (p=0.02), with no modification of hemoglobin values. Serum albumin increased (p<0.001) and C-reactive protein decreased (p=0.01) during the study. PP (p=0.007) and LVMI (p=0.02) were significantly reduced at the end of supplementation.

Conclusions: Long term oral cholecalciferol supplementation in HD patients appears to be an easy and cost-effective therapeutic measure. It allows correction of vitamin D deficiency, better control of mineral metabolism with less use of active vitamin D, attenuation of inflammation, reduced dosing of erythropoiesis stimulating agents, and possibly, improvement of cardiac dysfunction.

TH-OR099

Proteinuria Increases Phosphatemia By Altering Tubular Handling of Phosphate Pierre-Yves F. Martin,[1] Sophie M. De Seigneux,[1] Marie Courbebaisse,[2] Joseph M. Rutkowski,[4] Alexandra Wilhelm-Bals,[1] Marie Metzger,[2] Dominique Prie,[2] Carsten A. Wagner,[3] Benedicte Stengel,[2] Philipp Scherer,[4] Eric Feraille,[1] Pascal Houillier,[2] *[1]Univ Hospital of Geneva, Switzerland; [2]Hopital Européen Georges Pompidou, France; [3]Univ of Zurich, Switzerland; [4]UT Southwestern Medical Center.*

Background: Proteinuria and elevated phosphatemia are cardiovascular risk factors independent of glomerular filtration rate (GFR). We hypothesized that proteinuria modifies tubular handling of phosphate.

Methods: We studied phosphate handling in nephrotic children and in a cohort of 1738 CKD patients. To understand the molecular mechanisms, we induced glomerular proteinuria in two animal models: a toxic and a genetic model of glomerular lesions. OK and mDCT cells were used for in vitro studies.

Results: Plasma FGF23 and phosphate levels increased whereas fractional excretion of phosphate decreased during proteinuric phase as compared to remission phase in nephrotic children. Cross-sectional analysis of a cohort of 1738 CKD patients showed that 300mg/24h albuminuria or higher is predictive of higher phosphate levels, independent of GFR and other factors. Albuminuric patients also displayed higher plasma FGF23 and PTH levels. Rats with puromycin aminonucleoside induced proteinuria displayed elevated FGF23, but higher renal NaPi-IIa and lower Klotho proteins expression associated with decreased phosphorylation of FRS2α, a major FGF23 receptor substrate. In phosphate loaded rats, proteinuria impaired NaPi-IIa apical retrieval in proximal tubular cells. These findings were confirmed in transgenic mice that develop proteinuria resulting from podocyte depletion. In vitro, albumin did not directly alter phosphate uptake in proximal OK cells, whereas it downregulated Klotho in distal tubular cells.

Conclusions: We show that proteinuria increases phosphatemia independent of GFR and despite elevated FGF23 in children and adults CKD patients. Experimentally, we observed that proteinuria increased proximal tubule NaPi-IIa expression and is associated with downregulation of Klotho which confers FGF23 resistance. Proteinuria thus induced elevation of both FGF23 and phosphate, potentially contributing to cardiovascular disease.

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TH-OR100

PAI-1 Regulates FGF-23 Metabolism in Plasma Aaron T. Place,[1,2] Mesut Eren,[1] Toshio Miyata,[3] Douglas E. Vaughan.[1,2] *[1]Dept of Medicine, Northwestern Univ, Chicago, IL; [2]Feinberg Cardiovascular Research Inst, Northwestern Univ, Chicago, IL; [3]United Centers for Advanced Research and Translational Medicine, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan.*

Background: Elevated blood levels of fibroblast growth factor-23 (FGF-23) are present in a variety of human diseases and conditions including chronic kidney disease, autosomal dominant hypophosphatemic rickets, and tumor-induced osteomalacia. Furthermore, increased levels of FGF-23 are independently associated with mortality in patients undergoing dialysis. Clearly, methods to reduce the elevated FGF-23 levels in these settings offer novel therapeutic potential.

Methods: We investigated the effects of plasminogen activator inhibitor type-1 (PAI-1) activity on plasma levels of FGF-23 in transgenic mice that overexpress a stable form of human PAI-1 and in *klotho* mice that were treated with a novel small molecule PAI-1 inhibitor, TM5441. We also investigated how plasminogen activators (PAs) affect FGF-23 using recombinant human FGF-23, tissue-type (t-PA) and urokinase (uPA) PAs and PAI-1.

Results: We observed that the PAI-1 transgenic mice exhibit 3.5-fold elevated plasma levels of FGF-23 compared to littermate controls. Additionally, pharmacologic inhibition of PAI-1 activity with TM5441 reduced plasma levels of FGF-23 by over 60% (p<0.01) in *klotho* mice, which also display elevated PAI-1 levels compared to WT littermates. The mechanism through which PAI-1 contributes to increased FGF-23 levels appears to involve PA-dependent proteolysis, as both t-PA and uPA were observed to cleave FGF-23 *in vitro*. Furthermore, inhibition of t-PA or uPA by PAI-1 prevented the proteolysis of FGF-23, which was then restored by pretreatment of PAI-1 with TM5441.

Conclusions: Taken together, these results suggest that t-PA and uPA play a critical role in the proteolytic clearance of circulating FGF-23. PAI-1, the primary inhibitor of PAs in plasma, is a key regulator of this process. Furthermore, selective inhibition of PAI-1 offers a novel and direct therapeutic approach to reduce the high levels of FGF-23 seen in many human diseases and conditions.

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TH-OR101

Rapid Minute-to-Minute Regulation of Plasma Fibroblast Growth Factor 23 – A Key Role of the Kidney Maria Lerche Mace,[1] Eva Gravesen,[2] Jacob Hofman-Bang,[2] Klaus Olgaard,[2] Ewa Lewin.[1,2] *[1]Nephrology, Herlev Hospital, Univ of Copenhagen, Denmark; [2]Nephrology, Rigshospitalet, Univ of Copenhagen, Denmark.*

Background: Chronic uremia is characterized by high circulating levels of fibroblast growth factor 23 (FGF23), yet the mechanisms behind are not completely clarified. Recently, studies reported a rise in FGF23 during acute kidney failure. The aim of the present study was to examine the impact of the kidney on the very early minute-to-minute regulation of FGF23, along with the importance of PTH and the FGF receptor 1 (FGFR1) signaling pathway.

Methods: Adult male Wistar rats were randomized to either acute bilateral or unilateral nephrectomy (NX, UNX), parathyroidectomy (PTX) or sham surgery. The FGFR1 signaling pathway was blocked by pharmacological inhibitor of FGFR1 (PD173074) prior surgery. Furthermore, to examine FGF23 kinetics an iv bolus of 0.1 µg of recombinant human FGF23 (LC lab) was administered after suppression of endogenous FGF23 by PD173074. Intact FGF23 levels were measured using the intact FGF23 Kainos assay.

Results: NX resulted in an immediate, within 15 minutes, increase in FGF23 levels from 112±18 to 267±26 pg/ml (p< 0.001), and then the following levels remained stable. UNX generated as well a prompt rise in FGF23 to 147±20 pg/ml, reaching a level in between NX and sham group, the latter demonstrating stable FGF23 levels. NX and PTX+NX showed same FGF23 levels. PD173074 suppressed FGF23 levels to 36±8 pg/ml, which similarly increased by 134±34% in PD173074 and 155±30% in vehicle groups after NX (ns). The half-life of rhFGF23 was significantly prolonged from 5.4 to 14.5 min in anephric rats (p<0.01) independently of FGFR1 inhibition.

Conclusions: The present results clearly demonstrate the importance of the kidney in the very rapid regulation of FGF23, as acute total nephrectomy immediately, within minutes, resulted in a more than 200% rise in FGF23 levels. This effect was independent of PTH and a similar increase was demonstrated, too, after inhibition of the FGFR1 signaling pathway. The half-life of FGF23 was significantly prolonged in anephric rats. A principal role of the kidney in the rapid regulation and rapid clearance of FGF23 is shown.

Funding: Government Support - Non-U.S.

TH-OR102

Inflammation and Iron Deficiency Stimulate FGF23 Production
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Background: Iron deficiency and inflammation are common in CKD, and associated with elevated circulating levels of fibroblast growth factor-23 (FGF23). We tested the hypothesis that iron deficiency and inflammation regulate FGF23 production.

Methods: To investigate the effects of inflammation on FGF23 production, wild-type (WT) mice received a single injection of heat-killed Brucella abortus (BA), interleukin-1B (IL1B) or saline (Ctr). To test the effects of iron deficiency, WT mice were fed low-iron or normal diets for 3 weeks.

Results: Inflammation decreased serum iron levels 6 hours after injection of both BA and IL1B mice compared to Ctr mice. In BA mice, inflammation was accompanied by increased FGF23 mRNA expression in bone and a 9-fold increase in serum C-terminal FGF23 levels (cFGF23, which detects both intact FGF23 and its inactive C-terminal cleavage fragments). IL1B-injected mice demonstrated a comparable 3.5-fold and 8-fold increase in cFGF23 at 3 and 6 hours post-injection, respectively. No acute changes in intact FGF23 (iFGF23) levels were observed during the early post-injection period in either model, suggesting that FGF23 production and cleavage initially increase in parallel in response to inflammation. By 4 days post-injection, serum iFGF23 levels increased by 1.4-fold in IL1B mice compared to Ctr. Consistent with increased bioactive FGF23, mRNA expression of renal Cyp24a1 increased 4–fold, Cyp27b1 decreased 3-fold, and Npt2a decreased 2-fold, resulting in increased phosphate excretion. Sustained iron deficiency induced by a low iron diet mimicked the effects of inflammation and resulted in a 2.5-fold increase in cFGF23, a 3-fold increase in osseous FGF23 mRNA expression, and a 2-fold increase in iFGF23.

Conclusions: Inflammation and iron deficiency stimulate FGF23 production but secretion of the intact protein is overridden initially by a simultaneous increase in proteolytic cleavage of FGF23. Chronic inflammation and sustained iron deficiency, however, lead to increased intact FGF23. These data suggest that iron deficiency and inflammation may contribute to elevated FGF23 levels in CKD.

TH-OR103

FGF23 Targets the Heart by Activating FGFR4
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Background: Serum FGF23 levels are elevated in patients with chronic kidney disease (CKD) and are independently associated with increased rates of left ventricular hypertrophy (LVH). A recent translational study from our group demonstrated that FGF23 is a causal factor in the pathogenesis of LVH: we reported that FGF23 induces hypertrophy of cardiac myocytes via FGF receptor (FGFR)-dependent signaling and independent of klotho, which is the FGF23 co-receptor in the kidney. Different from klotho expressing cell types, FGF23 does not stimulate Ras/MAPK signaling in cardiac myocytes, but activates the PLCγ/calcineurin/NFAT axis, a potent pro-hypertrophic signaling pathway in the heart. Mammals express four different FGFR isoforms (FGFR1-4), and we currently focus on the identification of the FGFR isoform that mediates FGF23's cardiac effects. Our preliminary work in HEK293 cells that lack klotho indicates that of the four ectopically expressed FGFR isoforms, only FGFR4 can bind and activate PLCγ upon FGF23 stimulation.

Methods: To determine if FGFR4 activation in cardiac myocytes is required for FGF23-induced hypertrophy, we studied constitutive FGFR4 knockout (-/-) mice. Cardiac myocytes were isolated from newborn mice, treated with recombinant FGF23 for 48 hours and analyzed for hypertrophic cell growth. Serum FGF23 levels were elevated in adult FGFR4-/- mice and wild type littermates via administration of a high phosphate (2%) diet for 3 months followed by histological and serological analyses.

Results: Isolated cardiac myocytes from FGFR4-/- mice are protected from FGF23-induced hypertrophic growth. High phosphate diet causes a significant increase in serum phosphate and FGF23 in wild type and FGFR4-/- mice, without inducing kidney injury. However, only wild type but not FGFR4-/- mice develop LVH.

Conclusions: The FGF23-induced hypertrophic growth of cardiac myocytes requires FGFR4 and mice with elevated FGF23 lacking FGFR4 are protected from LVH. Pharmacologically blocking FGF23-mediated FGFR4 activation in the heart could represent a novel approach to reduce cardiac damage in CKD.

Funding: NIDDK Support

TH-OR104

Pharmacologic FGFR4 Blockade Attenuates LVH in a Rodent Model of CKD
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Background: Left ventricular hypertrophy (LVH) is an important mechanism of cardiovascular disease in chronic kidney disease (CKD) and affects up to 90% of patients by the time they reach dialysis. Serum levels of fibroblast growth factor (FGF) 23 continuously rise as patients progress to renal failure. Human studies demonstrated a dose-dependent association between FGF23 levels, increased prevalence of LVH and greater risk of mortality among CKD patients. In a recent study we showed that increased FGF23 induces LVH thereby repositioning FGF23 from biomarker of risk to mechanism of disease.This cardiac effect is mediated by FGF receptors (FGFR) but does not require klotho, the co-receptor in "classic" FGF23 target organs like the kidney. Our preliminary analyses indicate that of the four FGFR isoforms, FGF23 can directly activate FGFR4 in the absence of klotho.

Methods: To determine if FGFR4 activation in cardiac myocytes is required for FGF23-induced LVH, we used an isoform-specific anti-FGFR4 blocking antibody that is currently in clinical cancer trials. Neonatal rat ventricular myocytes were co-treated with recombinant FGF23 and anti-FGFR4 and analyzed for hypertrophic growth. Anti-FGFR4 was administered intraperitoneally to the 5/6 nephrectomy rat model of CKD every three days for two weeks, starting one hour after surgery, and serum, heart and kidney were isolated for molecular and histological analyses.

Results: Anti-FGFR4 treatment inhibits FGF23-induced hypertrophic growth of cardiac myocytes in vitro, and protects 5/6 nephrectomized rats from developing LVH without reducing the animals' elevated blood pressure and FGF23 levels or improving kidney function.

Conclusions: The development of LVH in the 5/6 nephrectomy rat model with elevated serum FGF23 requires FGFR4 activation. FGFR4 blockade might serve as a novel pharmacological intervention for LVH in patients with CKD.

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TH-OR105

Association of Bioavailable Vitamin D, Bone Mineral Density and Markers of Mineral Metabolism in Adults with Nephrotic Syndrome
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Background: Blood levels of 25-hydroxyvitamin D [25(OH) D] are reduced in patients with nephrotic syndrome (NS). The relationship between reduction in vitamin D and bone health and abnormalities in mineral metabolism in NS is unclear. We hypothesized that alterations in bioavailable vitamin D levels might be linked to these abnormalities in NS.

Methods: We measured ciculating levels of total 25(OH)D, vitamin D binding protein (DBP), serum albumin, iPTH and fibroblast growth factor-23 (FGF-23) in 43 adults with sporadic idiopathic NS and 40 healthy controls. Free and bioavailable 25(OH)D were calculated from previously validated formulae. Bone mineral density (BMD) was measured at left neck of femur by DEXA.

Results: Compared to healthy controls, total, free and bioavailable 25(OH)D levels were significantly reduced in patients with NS. Among the nephrotic patients, bioavailable 25(OH)D was positively correlated with BMD ($r = 0.345$; $p = 0.024$), whereas total 25(OH) D ($r = 0.072$; $p = 0.65$) and free 25(OH)D levels ($r = 0.18$; $p = 0.25$) were not. Bioavailable 25(OH)D levels showed a strong inverse correlation with iPTH on univariate ($r = -0.428$; $p = 0.004$) and multivariate ($b = 0.42$, $p<0.001$) analyses. FGF -23 correlated with iPTH but not with calcium and phosphorus or vitamin D.

Conclusions: We conclude that bioavailable 25(OH)D is a better measure of vitamin D status with respect of BMD and mineral metabolism in patients of nephrotic syndrome.

Funding: Government Support - Non-U.S.

TH-OR106

Association of Bone Turnover with Oxidized PTH, but Not 'Intact' PTH or Non-Oxidized PTH, in Patients Undergoing Hemodialysis (HD) Treatment
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Background: Oxidized PTH (oxPTH) does not stimulate the PTH/PTHrp receptor, in contrast to non-oxidized PTH (n-oxPTH). PTH oxidation has been ignored in the development of PTH assays: the current intact PTH (iPTH) assays recognize both n-oxPTH and oxPTH. Our recently developed assay separately measures oxPTH and n-oxPTH. Here we analyze the relation of iPTH, oxPTH and n-oxPTH with bone morphology parameters in HD patients.

Methods: Bone histomorphometry data and blood samples for serum biochemistry were available from 51 HD patients. Mean (± SD) patients' age was 55±14 yr, and dialysis vintage 66±51 mo.

Results: Mean serum iPTH was 695±717, n-oxPTH 97±100, and oxPTH 598±628 pg/mL. Mean serum Ca was 8.4±2.7 mg/dL, phosphorus 6.0±2.1 mg/dL, 25OH vitD 54±28 ng/mL, and FGF23 9578±16483 pg/mL. Using Pearson's correlation analyses there was a strong relationship between iPTH and oxPTH ($r=0.998$; $p<0.0001$). The relationship was much weaker between iPTH and n-oxPTH ($r=0.861$; $p<0.0020$). Multivariate linear regression analysis revealed a positive correlation of mineral apposition rate with oxPTH ($\beta=0.725$, $p=0.006$) but a negative correlation with n-oxPTH ($\beta=-0.661$, $p=0.020$), and no correlation with iPTH (p=NS). Similarly, there was a positive correlation of bone formation rate with oxPTH ($\beta=0.816$, $p=0.002$), a negative correlation with n-oxPTH ($\beta=-0.714$, $p=0.010$), and no correlation with iPTH (p=NS). In addition to these dynamic parameters, there were similar negative and positive correlations of oxPTH and n-oxPTH, respectively, with the static parameters osteoblast surface and osteoclast surface. None of the 3 PTH species was associated with mineralization lag time, bone volume, or fibrosis volume.

Conclusions: Our study in HD patients unexpectedly showed positive correlations of dynamic and static bone activity parameters with oxPTH, but not with n-oxPTH. These findings either could indicate a direct stimulation of bone turnover by oxPTH under conditions of secondary hyperparathyroidism or be merely a reflection of enhanced bone turnover by oxidative stress.

TH-OR107

Increased Bone Density in Mice Lacking the Proton Receptor OGR1 Is due to Increased Osteoblast Activity Nancy S. Krieger, Min Ho Kim, David A. Bushinsky. *Medicine, Univ of Rochester, Rochester, NY.*

Background: Chronic metabolic acidosis (MET) stimulates net calcium (Ca) efflux from bone by decreasing osteoblastic (OB) bone formation and increasing osteoclastic (OC) bone resorption. OBs express the H^+-sensing G-protein coupled receptor, OGR1, which appears necessary for MET-induced bone resorption. Mice with a genetic null mutation in OGR1 (KO) have increased bone density compared to wild type (WT) which may be due to increased OB bone formation and/or decreased OC bone resorption. Here we test the hypothesis that the increased bone density in KO is caused by increased OB activity due to an inability of these KO to respond to the large endogenous H^+ production generated during rapid growth of the mice.

Methods: Femurs were dissected from 8 wk old KO and WT mice and RNA isolated for real time PCR (QPCR). OBs were isolated from neonatal KO and WT calvariae. RNA was obtained at confluence for baseline gene expression by QPCR and then after culture for 2 wks in mineralization medium. RNA expression was normalized to RPL13a.

Results: There is no difference in gross phenotype of KO compared to WT; however, both μCT and histology demonstrate an increase in bone volume, trabecular number and trabecular thickness in KO compared to WT (all $p<0.05$). Bone formation rate and OC number are also increased (both $p<0.05$). Femur mRNA from 8 wk old KO mice have increased OB runx2 expression compared to WT (3.0±0.9 versus 1.8±0.3, $p<0.05$), with no difference in OC TRAP (3.2±0.6 versus 2.5±0.5, p=ns) or cathepsin K (2.5±0.6 versus 3.3±0.7, p=ns) levels. Primary KO calvarial OBs have increased expression of runx2 (1.6±0.2 versus 1.1±0.2), osteocalcin (1.8±0.3 versus 1.0±0.1), collagen 1a1 (3.0±0.7 versus 1.2±0.2) and alkaline phosphatase (6.7±2.2 versus 2.0±0.6) at baseline compared to WT (all $p<0.05$). After 2 weeks in mineralization medium, KO OBs have elevated runx2 (5.2±1.3 versus 1.1±0.4), collagen 1a1 (4.3±0.9 versus 0.7±0.2) and osterix (2.5±1.2 versus 0.4±0.2) compared to WT (all $p<0.05$).

Conclusions: OGR1 KO mice have a consistent increase in markers of OB activity. Lack of OGR1 in KO mice results in OBs unable to respond to the large amount of H^+ generated by these rapidly growing mice and may account for their increased bone density.

Funding: Private Foundation Support

TH-OR108

Calcineurin Homologous Protein 1 Regulates the Renal Na-K-2Cl-Cotransporter Kerim Mutig,[1] Christin Dathe,[1] Alexander Paliege,[1] Vera Jankowski,[2] Shigeo Wakabayashi,[3] Takashi Hisamitsu,[3] Sebastian Bachmann.[1] *[1]Dept of Anatomy, Charité Universitaetsmedizin Berlin, Germany; [2]Dept of Nephrology, Charité Universitaetsmedizin Berlin, Germany; [3]Dept of Molecular Physiology, National Cerebral and Cardiovascular Center Research Inst, Osaka, Japan.*

Background: Activity of the thick ascending limb (TAL) Na-K-2Cl-cotransporter (NKCC2) is facilitated by phosphorylation. Our recent data suggest that calcineurin is involved in dephosphorylation and deactivation of NKCC2. The activity of the phosphatase can be modulated by members of the calcineurin homologous protein family (CHP). CHP1 has been implicated in the regulation of several membrane proteins either through direct interactions or via inhibition of calcineurin. This study focuses on the role of CHP1 in the regulation of NKCC2.

Methods: CHP1 was localized along the nephron using immunofluorescence. Protein-protein interactions were studied by co-immunoprecipitation (co-IP) and GST pulldown assay. Effects of CHP1 on NKCC2 phosphorylation have been evaluated in cell culture. To evaluate the role of CHP1 during vasopressin (AVP)-induced NKCC2 activation the AVP analogue desmopressin (dDAVP) has been acutely administered to AVP-deficient rats.

Results: CHP1 and NKCC2 were closely co-localized in the apical compartment of TAL cells and interacted by co-IP. GST pull down assay with recombinant NKCC2 C-terminus and N-terminal NKCC2 mutants mimicking its (de)phosphorylation at functionally relevant residues (T96, T101, T114, and S126) suggested that CHP1 preferentially interacts with the phosphorylated N-terminal tail of NKCC2. Overexpression of CHP1 substantially increased phosphorylation of endogenous NKCC2. The role of CHP1 during vasopressin (AVP)-induced NKCC2 activation was studied in AVP-deficient Brattleboro rats. Short-term stimulation of the AVP/V2 receptor axis in these rats resulted in a marked increase of NKCC2 phosphorylation which was paralleled by stronger interaction of the transporter with CHP1 and concomitant, weaker association between NKCC2 and calcineurin.

Conclusions: In sum, our data provide evidence for a permissive role of CHP1 in NKCC2 phosphorylation at baseline and during AVP-induced activation of the transporter.

TH-OR109

Identification of DNPEP, a Cytosolic Aminopeptidase That Cleaves SPAK in the Kidney Nicolas Markadieu,[1] Paul A. Welling,[2] Eric J. Delpire.[1] *[1]Dept of Anesthesiology, Vanderbilt Univ, Nashville, TN; [2]Dept of Physiology, Univ of Maryland, Baltimore, MD.*

Background: In the kidney, SPAK and OSR1 phosphorylate and activate the sodium chloride cotransporters NKCC2 and NCC. Although SPAK and OSR1 share high sequence homology, the molecular size of SPAK is larger than the size of OSR1, due to the presence of an N-terminal proline-alanine rich domain. In the mouse kidney, SPAK fragments have been detected and shown to be inhibitory to the activity of NKCC2. It has been hypothesized that those fragments originate from alternative translation start sites, but their precise origin is unknown. Here, we demonstrate that kidney possesses a protease cleaving SPAK but not OSR1.

Methods: Recombinant fusion proteins were purified from bacteria. Proteolytic cleavage was monitored by Western blotting. Protease was identified by ion exchange and size exclusion chromatography combined with mass spectrometry (MS). Identification of sites of cleavage was done by MS. Inhibitory effect of SPAK fragments was studied in *Xenopus laevis* oocytes.

Results: Lysates prepared from mouse kidney possess protease activity that leads to the cleavage of SPAK into multiple fragments. Cleavage was not prevented by widely used protease-, proteasome-, aminopeptidases (bestatin)-inhibitors. Proteolytic activity was inhibited by 1-10 mM DTT, 5-20 mM EDTA and by 5 mM phenanthroline. These results point to the action of a Zn^{2+} metalloprotease. Active fractions collected from gel filtration indicated that the protease molecular weight was as large as thyroglobulin. Cation exchange chromatography combined with MS provided a list of 44 proteases, including seven Zn^{2+} metalloproteases. Among those, DNPEP caught our attention as it has a molecular weight of 624 kDa and is sensitive to DTT, EDTA, phenanthroline, but not bestatin. DNPEP was cloned from mouse kidney and recombinant DNPEP produced the same pattern of degradation. By mass spectrometry, we identified the cleavage sites within SPAK. Corresponding SPAK fragments were constructed and demonstrated to be inhibitory to NKCC2.

Conclusions: Our data indicate that DNPEP specifically cleaves SPAK in kidney to give rise to fragments which are inhibitory to NKCC2.

Funding: NIDDK Support

TH-OR110

Functional Characterization of an Alternative Splice Variant of the Thiazide-Sensitive NaCl Cotransporter Sabina K. Jelen, Omar Tutakhel, Marco Valdez Flores, Joost Hoenderop, René J. Bindels. *Dept of Physiology, Radboud Univ Medical Centre, Nijmegen, Netherlands.*

Background: The importance of the thiazide-sensitive Na^+-Cl^- cotransporter (NCC) in renal electrolyte transport and blood pressure regulation has been well documented. Recently, a new phosphorylation site at serine 811 (pS811) in the carboxyl (C)-terminal domain of NCC was identified. This phosphorylation site is present only in the longest NCC splice variant (NCC-Sv), up to now an isoform poorly characterized. The aim of the present study was to establish localization of NCC-Sv in the human kidney as well as to determine its functional properties.

Methods: Immunohistological analysis was performed on kidney biopsies of healthy human subjects. Urinary exosomes were isolated and probed for protein abundance in Western blot analysis. Functional properties of NCC-Sv was evaluated by $^{22}Na^+$ influx measurements using the *Xenopus laevis* expression system.

Results: Immunohistological analysis of NCC-Sv in the human kidney showed a robust expression at the apical site of the distal convoluted tubule. Similarly, the NCC-Sv protein was detected in human urinary exosomes. We also demonstrated that acute water loading in human subjects significantly reduces abundance of NCC-Sv in urinary exosomes. Thiazide-sensitive $^{22}Na^+$ transport of NCC-Sv was significantly increased in comparison to the wild type NCC (NCC-Wt). The mutant mimicking a constitutively active phosphorylation site at the residue 811 (S811D) presented an additional increase of Na^+ transport in comparison to unmutated NCC-Sv. Consequently, inactive mutant (S811A) prevented the enhanced response.

Conclusions: The present study for the first time demonstrates a vast abundance and dynamic regulation of the alternative isoform of NCC protein in the human kidney, potentially controlled by additional phosphorylation pathway. Previously underrepresented NCC-Sv may constitute a unique route of Na^+ reabsorption and could play an important role in blood pressure regulation. Analysis of samples from different human pathological states as well as various *in vitro* models could aid in determining the precise regulation and function of NCC-Sv.

TH-OR111

KCNJ10 (Kir4.1) Determines the Apical Na-Cl Expression in the Distal Convoluted Tubule (DCT) WenHui Wang. *Pharmacology, New York Medical College, Valhalla, NY.*

Background: The renal phenotype induced by loss-of-function mutations of Kcnj10 is characterized by salt wasting, hypomagnesemia, metabolic alkalosis and hypokalemia. However, the mechanism by which Kir.4.1 mutations cause the tubulopathy is not completely understood.

Methods: Because homozygous *Kcnj10*-/- mice were not able to survive more than two weeks after birth, it prevents studying renal phenotype of adult Kcnj10 knockout mice. Thus, the present study was carried out in p7-p10 neonatal littermates to explore the role of Kcnj10 in regulating the membrane transport in DCT.

Results: Immunostaining demonstrated Kcnj10 and Kcnj16 were expressed in the basolateral membrane of DCT. The patch-clamp study detected a 40 pS K channel, a heterotetramer of Kcnj10 and 16, in the basolateral membrane of the early DCT (DCT1) of p8-p10 wild type *Kcnj*10+/+ mice (WT). The probability of finding the 40 pS K channel was decreased in Heterozygous *Kcnj10*+/- (het) and was zero in homozygous *Kcnj*10-/- (knockout) mice. Moreover, the disruption of Kcnj10 almost completely eliminated the basolateral K conductance and decreased the negativity of the cell membrane potential in DCT1. Moreover, the lack of Kcnj10 also decreased the basolateral Cl conductance and inhibited the expression of Ste20-prorin-alanine-rich protein kinase (SPAK). Immunostaining image showed that NCC was highly expressed in the apical membrane

of DCT of WT but the staining of NCC was fait in the apical membrane and diffused into the intracellular space of knockout mice. Western blot further demonstrated that NCC expression was low in het mice but it was the lowest in knockout mice.

Conclusions: We conclude that Kcnj10 plays a dominant role in determining the basolateral K conductance and membrane potential of DCT1 and that the basolateral K channel activity in the DCT determines the apical NCC expression possibly through SPAK-dependent mechanism.

Funding: NIDDK Support, Other NIH Support - Heart and Lung

TH-OR112

Increased Calcium-Binding Protein 39 (Cab39) Expression in Renal Tubule Recuses the Gitelman-Like Phenotype in Wnk4 Knockout Mice

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Background: Recently, it was shown that an ubiquitously expressed Cab39 protein could also stimulate Na-(K)-(2)Cl cotransporter [N(K)CC] through activating SPAK/OSR1 kinases mimicking WNK1/4 kinases *in vitro study.*To uncover the physiological role of Cab39 on the regulation of SPAK/OSR1 and N(K)CC *in vivo*, especially in the renal tubules.

Methods: We generated and analyzed both the kidney tubule-specific cadherin gene promoter driven flag-tagged mouse Cab39 (KSP-Flag-mCab39) transgenic (Tg) and WNK4 knockout mice. At age of 10-12 weeks fed with normal rodent chaw, phenotype including blood pressure as well as serum and urine electrolytes was measured in WT, Cab39 Tg, Wnk4 knockout and Cab39 TgxWnk4 knockout transgenic mice. The expression of WNK1/4, Cab39, SPAK/OSR1 and N(K)CC was evaluated by western blotting and immunofluorescence stain.

Results: Offspring from Cab39 Tg mice with mildly overexpressed abundance of flag-Cab39 (25% ±6%) were phenotypically normal but a slightly increased p-SPAK/OSR1, p-NKCC2 and p-NCC in the kidneys was found. A significantly elevated blood pressure ($p<0.05$) and slightly hyperkalemia ($p=0.16$) but a resistance of p-SPAK/OSR1, p-NKCC2 and p-NCC suppression were observed in the Cab39 Tg mice with high NaCl intake. Wnk4 knockout mice manifested Gitelman-like syndrome (with hypotensionm hypokalemia, hypomagnesemia and hypocalciuria) with significantly reduced abundance of phosphorylated Spak, Osr1 and Ncc ($p<0.05$). The phenotype and abundance of the p-SPAK/OSR1 and p-NCC in WNK4 knockout mice was partially corrected after crossing with Cab39 Tg mice.

Conclusions: Augmented Cab39 expression in renal tubule may lead to salt-sensitive hypertension through activating SPAK/OSR1-N(K)CC signaling. Reduced WNK4 stimulation of SPAK/OSR1-NCC phosphorylation signaling could be rescued by Cab39 overexpression.

Funding: Government Support - Non-U.S.

TH-OR113

Renal Epithelial-Specific Disruption of Cullin 3 Causes Salt-Wasting, Polyuria, and Renal Fibrosis

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Background: Familial Hyperkalemic Hypertension (FHHt) is caused by mutations in genes that regulate activity of the renal sodium chloride cotransporter (NCC), including the kinases WNK1 and WNK4, and Cullin3 (Cul3) and KLHL3, members of a ring ubiquitin ligase complex.

Methods: To examine the physiological roles of Cul3 and gain insight as to how Cul3 mutations might cause FHHt, we generated doxycycline-inducible kidney-specific $Cul3^{-/-}$ mice using the Pax8-LC1 system.

Results: Western blots showed nearly complete absence of Cul3 in $Cul3^{-/-}$ kidneys. Expression levels of WNK4 were dramatically elevated in $Cul3^{-/-}$ mice, while expression of total and phospho-NCC, KLHL3, WNK1, and WNK3 were only modestly elevated. Surprisingly the effects of Cul3 disruption on renal function were the opposite of those predicted by western blotting. $Cul3^{-/-}$ mice displayed volume contraction, hypochloremia, metabolic alkalosis, elevated plasma aldosterone, and a trend toward hypokalemia. Blood pressure, measured by radiotelemetry, was similar in wild type and $Cul3^{-/-}$ mice on normal NaCl diet, but during NaCl restriction, $Cul3^{-/-}$ mice displayed a progressive reduction in blood pressure; at 9 days, their 24h mean blood pressure was 18 mmHg lower. $Cul3^{-/-}$ mice displayed profound polyuria (5-fold higher urine volume than wild type), and salt, potassium and calcium wasting. Expression levels of p-NKCC2 were reduced in the medulla, while AQP2 expression was almost absent in $Cul3^{-/-}$ mice, providing mechanisms for the observed salt, potassium and calcium wasting, and for the polyuria. Histological analysis revealed the rapid onset of tubulointerstitial inflammation and fibrosis, with signs of inflammation including neutrophil and white cell casts. $Cul3^{-/-}$ mice also displayed elevated plasma creatinine and BUN, and proteinuria. Expression levels of the cell cycle regulator Cyclin E, a target of Cul3 that plays a role in the development of fibrosis, were also elevated.

Conclusions: In conclusion, these data suggest that loss of Cul3 activity along the nephron does not mimic FHHt, but leads to salt wasting and polyuria via effects on NKCC2 and AQP2, and also causes kidney injury.

Funding: NIDDK Support

TH-OR114

Distal Nephron Remodeling Occurs in Response to a Loss of Thiazide-Sensitive Sodium-Chloride Channel Phosphorylation

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Background: Salt balance is maintained in the absence of the renal thiazide-sensitive sodium-chloride cotransporter (NCC) by poorly defined adaptive mechanisms, which may limit diuretic efficacy. Here, we identify nephron remodeling processes in SPAK null mice, which are unable to phospho-activate NCC and exhibit a salt-wasting nephropathy similar to Gitelman Syndrome.

Methods: Global transcriptional profiling, combined with biochemical and physiological phenotyping, identified the gene expression signature of the adaptive response in the SPAK KO mice, and revealed how it establishes a new adaptive physiology.

Results: We found salt reabsorption pathways are created by the coordinated induction of a multi-gene transport system in Pendrin(+)-intercalated cells (PPIC), involving solute carriers (pendrin, *Slc26a4*; NDCBE, *Slc4a8*; AE4, *Slc4a9*), carbonic anhydrase 2 and 15, and V-type H^+-ATPase subunits, and activation of ENaC in principal cells (PC). The gene profile was also enriched in Notch signaling factors that contribute to nephrogensis in the developing kidney, prompting us to examine morphogenesis of the adult distal nephron. Analytical confocal microscopy and morphometric measurements revealed that activation of the transport pathways coincides with a remodeling process, which replaces acid-secreting α-intercalated cells with PPIC, and expands the CNT with an increased number of PC to replace the dystrophic DCT. Up-regulation of the Notch receptor ligand, Jagged 1 (Jag-1), and down-regulation of Jag-2 was accompanied by an increase in a key Notch-activated transcriptional factor, Hes1, in PC of the CNT, suggesting that Notch signaling is activated to drive a program of PC differentiation and proliferation.

Conclusions: Nephrogenic cell fate determinants are reactivated in the adult kidney to remodel the distal nephron in response to NCC inhibition. Activation of adaptive salt reabsorption pathways are explained by the CNT remodeling processes that replaces α-IC with PPIC and increases the number of PC.

Funding: NIDDK Support

TH-OR115

MicroRNA-27 Is Upregulated by Aldosterone in the Kidney Distal Nephron Where It Facilitates the Regulation of ENaC-Mediated Sodium Transport

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Background: The epithelial sodium channel (ENaC) is a major determinant of sodium (Na^+) reabsorption in the kidney. We recently reported that aldosterone (aldo) modifies expression of microRNAs (miRs) to alter target gene expression and regulate Na^+ transport in principal cells of the cortical collecting duct (CCD) (JASN, in press). Here we investigated two miR clusters that are significantly upregulated by aldo, and are members of the miR-23~24~27 family.

Methods: Experiments were carried out in cultured mCCD-cl1 cells or from CCD cells isolated from mouse kidney using a magnetic bead/lectin conjugation procedure.

Results: Aldo stimulation (50nM, 24hr) of mCCD cells resulted in >1.7 fold increase in miR-23~24~27 family members as determined by microarray and qRT-PCR. In isolated ex-vivo CCD cells from aldosterone stimulated mouse kidneys these miRs were significantly increased (1.6-3.5 fold). Overexpression of the miRs, using mimics, increased ENaC-mediated Na^+ transport in the mCCD cell line (44±5.9%, n=8, $p<0.002$), without aldo stimulation. Inhibition of miR family members using locked nucleic acid inhibitors, blunted aldosterone stimulation. Of the family members a change in miR-27 expression was the most effective in altering ENaC-mediated Na^+ transport. To establish the role of miR-27 in aldo regulation, we depleted the expression of the miR processing enzyme Dicer1 in mCCD cells. As we recently reported, Dicer1 depletion prevented stimulation of mCCD cells by aldo (7±20% stimulation compared to 96±15% in controls n=6). We next overexpressed miR-27 in the Dicer1 KD cells, so that only mature miR-27 was present. MiR-27 overexpression partially restored the aldo stimulation (74±30%, n=5), comapred to control KD cells.

Conclusions: The results support our recent findings that miRs are essential components of the aldo signaling cascade that regulate Na^+ homeostasis, and further implicate miR-27 as an essential miR in this new signaling pathway.

Funding: NIDDK Support, Private Foundation Support

TH-OR116

The PIM-3 Kinase as a New Player in the Aldosterone-Regulated Renal Salt Handling

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Background: The kidneys play a central role in blood pressure regulation as defaults in maintaining salt balance can result in the development of hypertension, the most common disease in the human population. The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in salt handling. Aldosterone is the key hormone in the control of sodium balance, blood volume and blood pressure, acting in the aldosterone-sensitive distal nephron (ASDN) and stimulating a complex transcriptional, translational and cellular program.

Methods: We have carried out a gene expression profiling in a mouse cortical collecting duct cell model (mpkCCD), stimulated or not by aldosterone, and identified the PIM-3 Ser/Thr kinase as a novel aldosterone-induced protein. PIM kinases (PIM-1, -2 and -3), and

in particular PIM-3, are overexpressed in different tumors. PIM-3, the only PIM kinase family member expressed in the kidney, has similar substrate specificities to other Ser/Thr kinases (e.g. SGK1, PKB) but its role in the kidney is largely unknown. Here we studied a possible new role of PIM-3 in the regulation of renal salt handling, both *in vitro* ($mCCD_{cl1}$ cells) and *in vivo* (PIM-3 KO mice).

Results: In $mCCD_{cl1}$ cells, we confirmed that PIM-3 expression is stimulated by aldosterone and observed that shRNA-based suppression of PIM-3 reduces basal and aldosterone-induced sodium currents as well as the activation of the aldosterone-mineralocorticoid receptor pathway. In PIM-3 KO mice, we found increased circulating renin activity and elevated plasma aldosterone levels compared to control littermates. Moreover, preliminary measurements of metabolic parameters indicate that sodium handling is impaired in PIM-3 KO mice.

Conclusions: In conclusion, our data suggest a possible novel function of the PIM-3 kinase and its potentially important role in the control of sodium homeostasis and blood pressure.

TH-OR117

The BKα/β1 Channel Localizes to Cilia of Principal Cells (PC) in Cortical Collecting Duct (CCD) Rolando Carrisoza-Gaytan,[1] Carlos Schreck,[1] Lijun Wang,[2] WenHui Wang,[2] Lisa M. Satlin.[1] *[1]Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Pharmacology, New York Medical College, Valhalla, NY.*

Background: Conducting BK channels, present in PC and intercalated cells (IC) of the CCD, mediate Ca^{2+}-dependent iberiotoxin (IbTX)-sensitive flow-induced K secretion (FIKS). PC, which possess a single apical cilium which responds to manipulation with an increase in cell Ca^{2+} concentration, are considered to mediate transepithelial Na absorption and K secretion. However, immunodetectable BKα has been consistently identified along the apical membrane of acid-base transporting IC, which are decorated with apical microvilli and microplicae, and not PC, raising uncertainty as to the identity of the cells that mediate FIKS. Based on the localization of mechanosensitive Ca^{2+} channels (polycystin-2) to cilia, we hypothesized that BKα localizes to the cilia of PC and thus mediates FIKS.

Methods: Microperfused CCDs from NZW rabbits fed control, low, or high K (HK) diet x 5 d were fixed for immunolabeling for BKα, β1 or β4 subunits, α-tubulin, or AQP2, and visualized by confocal microscopy. Net transport (Jx) of Na and K was measured in microperfused control or deciliated (with 1 mM dibucaine) CCDs. Single channel recordings were obtained from cell-attached patches of cilia or apical membrane of mpkCCD cells stably-transfected with Ssr3::GFP.

Results: Immunodetectable BKα colocalizes with β1 in cilia and apical membrane of PC and microvilli of IC, but not β4, which is distributed diffusely throughout the cytoplasm of all CCD cells. A HK diet enhances BKα labeling in apical membrane of IC but not cilia in AQP2 positive cells. Pharmacological deciliation of CCD leads to no apparent change in FIKS but tends to reduce JNa (77.8+2.6 to 11.6+29.1 pmol/min.mm; n=3, p=0.07). K channels with conductance and kinetics typical for the BK channel are present in cilia.

Conclusions: Immunodetectable BKα/β1 is present on cilia of PC and may represent the functional channel detected by patch clamp analysis. Given that deciliation of CCD PC does not affect FIKS, we speculate that the cilia BK channel participates in maintaining a favorable membrane potential for sustained Ca^{2+} entry into cilia.

Funding: NIDDK Support

TH-OR118

In Vivo Measurement of Albumin Concentration Gradients within the Glomerular Capillary Wall Bingshu Wang,[1] Ruben M. Sandoval,[2] Bruce A. Molitoris,[2] Don Mitchell Wilkes,[1] William Fissell.[3] *[1]Electrical Engineering, Vanderbilt Univ, Nashville, TN; [2]Div of Nephrology, Indiana Univ, Indianapolis, IN; [3]Nephrology and Hypertension, Vanderbilt Univ, Nashville, TN.*

Background: Proteinuria is the single strongest risk factor predicting end stage renal disease and is mechanistically important in tubulointerstitial scarring. There is still significant uncertainty regarding the mechanism by which the glomerular capillary wall (GCW) retains proteins. Imaging studies of frozen tissue as well as transport theory do not support a role for the podocyte as molecular sieve, yet foot process effacement invariably accompanies significant proteinuria. We present the first measurements of solute concentration profiles within the GCW in living animals.

Methods: Glomeruli of 20 week old Fromter strain and 11 week old Simonsen strain Munich-Wistar rats were imaged using two-photon intravital microscopy with FITC-labelled low molecular weight dextran (LMWD) and Texas Red labeled albumin. Lateral resolving power for the confocal microscope is estimated to be 220 nm sampled at a spatial frequency of 62 nm. Regions of interest with capillary lumen, Bowman's space and podocyte foot processes were identified. A computer algorithm selected a contour corresponding to the glomerular basement membrane (GBM) based on the locations of the foot processes in the LMWD image. The algorithm then selected transects perpendicular to the GBM contour and recorded the brightness of the albumin signal along each transect from lumen to Bowman's space. For each region of interest, transect solute profiles were aligned to the GBM contour and averaged.

Results: Both LMWD and albumin concentrations decrease monotonically from capillary lumen to Bowman's space. There is no evidence for a concentration polarization of albumin within the GBM as would be expected if the podocyte slit diaphragm were the most stringent filter in the GCW.

Conclusions: This finding is consistent with inferences from multiple other studies that suggest a significant role for the basement membrane in protein retention. Further mechanistic insight regarding protein retention by the GCW is needed.

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TH-OR119

Genetic Deletion of Endothelial Hyaluronan Synthase 2 Results in Glomerular Injury and Albuminuria Bernard van den Berg,[1,3] Margien G.S. Boels,[1] Cristina Avramut,[1,2] Erik Jansen,[3] Sascha Meldner,[4] Martijn Dane,[1] Johan Van der Vlag,[5] Hans Vink,[6] Abraham J. Koster,[2] Anton Jan Van Zonneveld,[1] Hermann-Josef Groene,[4] Eelco de Koning,[1,3] Ton J. Rabelink.[1] *[1]Nephrology, LUMC, Leiden, Netherlands; [2]Molecular Cell Biology, LUMC, Leiden, Netherlands; [3]De Koning Group, Hubrecht Inst for Developmental Biology and Stem Cell Research, Utrecht, Netherlands; [4]Cellular and Molecular Pathology, DKFZ, Heidelberg, Germany; [5]Nephrology, RUNMC, Nijmegen, Netherlands; [6]Physiology, MUMC, Maastricht, Netherlands.*

Background: We tested the hypothesis that the glomerular endothelial glycocalyx constitutes a primary barrier against albumin filtration and development of renal injury. To this end we constructed mice that have an inducible deletion of endothelial hyaluronan synthase 2 (has2). This enzyme produces the polysaccharide hyaluronan, a major constituent of the glycocalyx, in particular with respect to its physical dimensions and structure.

Methods: 8-Week old B6.cdh5-creERT2.has2$^{f/f}$.R26R-tdTom (has2-cKO) mice were injected i.p., 5x with 2mg/0.2mL tamoxifen (ctrl, B6.cdh5-creERT2.R26R-tdTom). Tail-cuff DBP and SBP measurements, 24hrs urine and organs were collected at 2-, 4- and 8wks after induction for analysis.

Results: Over the 8 week period following induction, has2-cKO mice developed systemic edema and increased urinary albumin excretion (ACR of 58±8 versus ctrl 36±2, p=0.036). DBP and SBP did not change. At 4 weeks the histology showed glomerular capillary hypertrophy (1194±77 versus 795±55μm^2, p<0.01) and diffuse mesangial expansion (2200±75 versus 888±77μm^2, p<0.01). This was associated with focal mesangiolysis and the formation of micro aneurisms. At 8 weeks these lesions progressed to diffuse glomerular capillary rarefaction (capillary area 531±26 versus 834±47μm^2, p<0.01) and was accompanied by focal loss of podocyte foot processes, without signs of tubular damage.

Conclusions: Loss of hyaluronan from the glomerular endothelial glycocalyx results in destabilization of glomerular capillary structure and albuminuria. Financial Support by the Dutch Kidney Foundation (grants C08.2265 and C09.03).

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TH-OR120

The Absence of Albumin Improves Kidney Disease in Alport Mice George Jarad, Jeffrey H. Miner. *Medicine/Renal, Washington Univ School of Medicine, St. Louis, MO.*

Background: Alport syndrome is an inherited glomerular disease that leads to ESRD. Alport syndrome is the result of the absence of normal collagen IV in the glomerular basement membrane (GBM). Previous investigations in Alport mice suggested that increased albumin permeability is an early phenomenon preceding the development of severe GBM, podocyte, and tubulointerstitial abnormalities. Whether filtered albumin contributes to kidney disease progression and whether reducing albuminuria can improve outcomes are still open questions.

Methods: To investigate the role albuminuria plays in Alport syndrome, we generated an albumin null (*Alb-/-*) mouse, then generated a mouse model of Alport syndrome that lacks any circulating or filtered albumin (*Col4a3-/-;Alb-/- mice*). *Alb-/-* mice are viable and fertile. Despite the lack albumin, reduced total serum protein and associated dyslipidemia, *Alb-/-* mice do not develop edema or ascites and their blood pressures are within normal range. *Alb-/-* mice have normal kidneys and BUN and creatinine. *Alb+/-* mice show significant reduction in serum albumin (~50%) and the same level of dyslipidemia as *Alb-/-* mice.

Results: *Col4a3-/-;Alb-/-* mice are viable and indistinguishable from their *Col4a3-/-; Alb+/-* littermates at birth. Although they do eventually succumb to kidney disease, their survival is improved compared to Alport mice with albumin (>1 year compared to 6.5-8.5 months). In the absence of albumin, there is some improvement in tubulointerstitial injury, but more significantly, there is also significant improvement in the glomerular phenotype. The improvement includes reductions in glomerulosclerosis, GBM thickening, podocyte foot process effacement, deposition of laminin alpha2 in the GBM, and better preservation of slit diaphragm components. These improvements are not secondary to lower blood pressure in the double mutant mice or to lipid abnormalities.

Conclusions: These early results lead us to conclude that the absence of albumin attenuates kidney injury in Alport syndrome, however, contrary to current dogma, the primary site of albumin-induced injury appears to be the glomerulus, especially podocytes, not tubular cells. The mechanism for this improvement is under further investigation.

Funding: NIDDK Support, Private Foundation Support

TH-OR121

Tubule-Specific ß-Catenin Signaling Contributes to Glomerular Injuries Roderick J. Tan,[1] Dong Zhou,[2] Liangxiang Xiao,[2] Robert Powers,[3] Youhua Liu.[2] *[1]Medicine, Univ of Pittsburgh, Pittsburgh, PA; [2]Pathology, Univ of Pittsburgh, Pittsburgh, PA; [3]Obstetrics, Gynecology and Reproductive Sciences, Univ of Pittsburgh, Pittsburgh, PA.*

Background: Wnt/ß-catenin is an evolutionarily conserved signaling pathway important in kidney development. Although quiescent in adults, this pathway is re-activated in disease and contributes to kidney pathology. Previous evidence has shown that activation of glomerular ß-catenin expression causes glomerular injury and proteinuria, but whether tubule-specific ß-catenin activation contributes to these injuries was unknown.

Methods: We crossed ß-catenin floxed mice with mice expressing Cre under the tubule-specific Ksp promoter, resulting in tubule-specific knockouts for ß-catenin (Ksp-ß-catenin). To induce proteinuria, we exposed these mice to angiotensin II (1.5 mg/kg/day), or to adriamycin (14 mg/kg). Urinary albumin-to-creatinine ratios were determined. Wilms tumor 1 (WT1) was detected with western blot and immunofluorescence. Blood pressure was measured with tail volume-pressure recordings.

Results: All mice developed albuminuria, but Ksp-ß-catenin mice had significantly less albuminuria compared to control littermates. We obtained similar results with adriamycin, demonstrating that this effect was generalizable to proteinuric diseases. The albuminuria was not accompanied by increases in low-molecular weight proteins, suggesting that excretion originated from glomeruli rather than the tubules. In support of this, we found that angiotensin-treated control littermates expressed less WT1 compared with Ksp-ß-catenin mice. As WT1 is critical for maintenance of podocyte differentiation and function, this suggests that tubule-derived ß-catenin adversely affects podocytes during disease. Another interesting finding was that Ksp-ß-catenin mice were protected from angiotensin-induced increases in blood pressure.

Conclusions: Tubule-specific ß-catenin activation plays a key role in the development of glomerular injuries, primarily through a reduction in WT1 expression and an increase in systemic blood pressure. Our studies suggest a novel ß-catenin-mediated tubular-glomerular crosstalk in the pathogenesis of proteinuric kidney diseases.

Funding: NIDDK Support, Private Foundation Support

TH-OR122

Endoplasmic Reticulum Stress Is a New Therapeutic Target in Proteinuric Chronic Kidney Diseases Khalil El Karoui,[1] Amandine Viau,[1] Clement Nguyen,[1] Martine Burtin,[1] Laurence Heidet,[2] Geraldine Mollet,[2] Frank Bienaime,[1] Morgan Gallazzini,[1] Fabiola Terzi.[1] *[1]INSERM U1151 - Hôpital Necker Enfants Malades, Paris, France; [2]INSERM U1163 - Hôpital Necker Enfants Malades, Paris, France.*

Background: Proteinuria is a critical player of CKD progression. Proteinuria may accelerate the development of tubulo-interstitial lesions by favoring the production of several molecules, such as endothelin-1, MCP-1, RANTES or complement components, in tubular cells. However, none of these pathways has led so far to new treatments in humans. Our aim was to identify a new therapeutic strategy to slow down proteinuric CKD progression.

Methods: We combined genetic and pharmacologic models of proteinuria with in vitro studies in which tubular cells were exposed to albumin or ER stress inductors. Pharmacological inhibitors were used. Human biopsies from CKD patients were studied.

Results: We demonstrated that ER stress and unfolded protein response (UPR) activation in tubular cells is a hallmark of proteinuric diseases. Mechanistically, we showed that extracellular albumin directly induced UPR in tubular cells in a calcium-dependent manner. We also identified a novel pathway in which proteinuria-induced UPR regulates the expression Lipocalin2 (Lcn2), a known mediator of CKD progression, via the activating transcription factor 4. These results were not restricted to mice, as we identified Lcn2 activation in proteinuric patients. Notably, we demonstrated Lcn2 is involved in the deleterious effect of proteinuria. In fact, Lcn2 gene invalidation protected proteinuric mice from CKD progression and mortality by decreasing ER-induced apoptosis. More importantly, we demonstrated that this pathway can be pharmacologically inhibited by phenylbutyrate, a drug already used in humans, which has a chaperone activity: inhibition of ER stress reduced tubular lesions, renal dysfunction, and Lcn2 activation in proteinuric mice. Interestingly, a pilot study revealed that phenylbutyrate might decrease urinary Lcn2 in proteinuric patients.

Conclusions: In conclusions, our study uncovers a novel molecular pathway of proteinuria and identified ER stress inhibitors as a novel therapeutic strategy for the maintenance of tubular structures in proteinuric CKD.

Funding: Government Support - Non-U.S.

TH-OR123

Injury to Glomerular Permeability Barrier by Cardiotrophin-Like Cytokine Factor 1 (CLCF1) or Focal Segmental Glomerulosclerosis (FSGS) Serum Is Mediated by JAK2 and STAT3 Activation Mukut Sharma,[1] Jianping Zhou,[1] Tarak Srivastava,[2] Ellen T. McCarthy,[3] Ram Sharma,[1] Virginia J. Savin,[1] Jean-Francois Gauchat.[4] *[1]Nephrology Research, Kansas City VAMC, Kansas City, MO; [2]Pedicatric Nephrology, Children's Mercy Hospital, Kansas City, MO; [3]Kidney Inst, Univ of Kansas Medical Center, Kansas City, KS, United States Minor Outlying Islands; [4]Pharmacology, Univ of Montreal, Montreal, Canada.*

Background: We have shown that CLCF1 is present in plasma from patients with recurrent FSGS. CLCF1 is an IL6 family cytokine that activates the JAK/STAT pathway and is believed to be secreted as a heterodimer with cytokine receptor-like factor 1 (CRLF1). Recombinant CLCF1 increases albumin permeability (P_{alb}) while the heterodimer CLCF1-CLF1 or CRLF1 alone blocks this effect. JAK2 and STAT3 are dominant isoforms in glomeruli and podocytes. We hypothesized that inhibitors of JAK/STAT pathway would also block the effect of CLCF1 on P_{alb}.

Methods: We incubated isolated rat glomeruli with rCLCF1 (10 ng/mL) or FSGS sera (n=6, 2% vol/vol). We included anti-CLCF1 mAb (0.5-50 mkg/mL) in some conditions. In separate experiments, we pre-treated glomeruli with JAK2 inhibitor BMS911543 (5nM) or STAT3 inhibitor STATTIC (0.001-10 mkM) followed by addition of rCLCF1 or FSGS serum. Change in albumin permeability (P_{alb}) was detemined using an in vitro assay.

Results: BMS911543 blocked the effect of both rCLCF1 and the effect of each FSGS serum ($p<0.001$). STATTIC also blocked the effect of both CLCF1 and of FSGS serum. This effect was dose dependent with maximal effect at 100nM ($p<0.001$ in each case). Anti-CLCF1 antibody blocked the CLCF1 or FSGS serum-induced increase in P_{alb} in a dose-dependent manner ($p<0.001$ at 5 mkg/mL antibody).

Conclusions: We conclude that activation of JAK2/STAT3 by CLCF1 or FSGS sera is required for the observed loss of glomerular permeability barrier. Small molecule JAK2 and STAT3 inhibitors have recently become available and are being tested in clinical trials to treat other diseases. Repurposing orally available JAK2 and STAT3 inhibitors may provide effective intervention to treat glomerular dysfunction and proteinuria in FSGS as well as other diseases.

Funding: Veterans Affairs Support, Private Foundation Support

TH-OR124

Neutrophil and Macrophage Extracellular Traps and Extracellular Myeloperoxidase Are Prominent in Human Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis Kim M. O'Sullivan,[1] Shaun A. Summers,[1,2] Poh-Yi Gan,[1] Sharon Lee Ford,[1,2] A. Richard Kitching,[1,2] Peter G. Kerr,[1,2] Stephen R. Holdsworth.[1,2] *[1]Dept of Medicine, Monash Univ, Melbourne, Victoria, Australia; [2]Dept of Nephrology, Monash Health, Melbourne, Victoria, Australia.*

Background: Myeloperoxidase (MPO) is the major autoantigenic target in MPO ANCA associated Vasculitis (AAV) but is potentially also a mediator of glomerular injury.

Methods: To assess the participation of MPO in the glomerular lesions of glomerulonephritis (GN) in MPO AAV patients, 48 renal biopsies were examined by confocal microscopy for MPO positive infiltrating cells, the extent of extracellular MPO and its association with either neutrophil extracellular traps (NETs) or macrophage extracellular traps (METs). These were correlated with clinical and histological parameters. Control biopsies included 10 patients within minimal renal disease (MRD) and 11 with anti PR3 AAV.

Results: MPO was rarely detected in MRD controls but was prominent in all cases of MPO AAV. The most abundant MPO positive cells were a sub population (31.7%) of CD68+ macrophages then MPO+CD15+ neutrophils. The total number of glomerular MPO+ cells correlated with decreased eGFR at the time of biopsy, ($r=-0.3$, $P=0.04$) as did the interstitial presence of MPO+ macrophages and neutrophils ($r=-0.34$, $P=0.02$, and $r=-0.39$, $P=0.01$). Extracellular MPO comprised 24.7% of total renal MPO. NETs were found in the glomeruli and interstitium of 63% of patient biopsies and patients with NETS had significantly more total and extracellular MPO. Seventy five percent of NET+ biopsies had NETs present in over 50% of their glomeruli. Co-localisation studies suggest neutrophils are the major source of extracellular MPO ($P=0.0006$). MPO+CD68 macrophages also demonstrated MPO in extracellular traps (METs). Control PR3 AAV biopsies showed similar extent and cell associations of MPO as seen in MPO AAV biopsies.

Conclusions: This study demonstrates the prominent participation of MPO in AAV. In MPO-AAV, most renal MPO is contained in macrophages the predominant leukocyte in this disease. MPO is also prominent extracellularly, alone and as NETS. We now report MPO containing METs contribute to MPO participation in this disease.

Funding: Government Support - Non-U.S.

TH-OR125

Neutrophil-Extracellular Traps Cause ANCA-Induced Glomerulonephritis in a Complement-Dependent Mannor Adrian Schreiber,[1,2] Ralph Kettritz,[1,2] Astrid Bergmann.[1] *[1]Experimental and Clinical Research Center (ECRC) at the MDC Berlin, Charité, Berlin, Germany; [2]Dept of Nephrology and Intensive Care Medicine, Campus Virchow Clinic, Medical Faculty of the Charité, Berlin, Germany.*

Background: ANCA are found in patients with necrotizing crescentic glomerulonephritis (NCGN). Numerous experiments showed that ANCA activate several neutrophil functions in vitro, including respiratory burst, degranulation, complement activation, and neutrophil

extracellular trap (NET) formation. Using an ANCA mouse model, we established neutrophil serine proteases and the alternative complement pathway as a disease inducer, whereas NADPH oxidase-dependent ROS were protective. We now hypothesized that NETs are a mediator of ANCA-induced NCGN.

Results: By immunofluorescence, primed human neutrophils challenged with control IgG generated small NET amounts after 3 h (9.0 ± 1.1% NET-positive cells), which was upregulated in response to MPO-ANCA IgG (21.4 ± 3.4%) and PR3-ANCA IgG (28.4 ± 6.7%, n=5, $p<0.05$), respectively. Monoclonal Abs to PR3 and MPO recapitulated NET formation (not shown). The highly specific PI3Kγ inhibitor AS505240 (0.5 μM) significantly reduced anti-MPO mAb induced NET formation indicating PI3Kγ as an important signaling pathway in this process (34.0 ± 2.4 % versus 11.0 ± 1.2%). When ANCA-induced NETs were harvested and incubated with normal human plasma, complement activation occurred as demonstrated by NET staining for C3d and ELISA (data not shown). To assess the role of NETs in vivo, we used a murine model where anti-MPO IgG, generated in murine MPO-immunized MPO-deficient mice, was transferred to LPS-challenged wild-type mice. Mice received either buffer or rec DNAse I (twice daily 10mg/g BW iv, n=6 in each group). After 6 days, disease was less severe in DNAse I-treated animals as assessed by urine pathology (not shown) and renal histology (22.3 ± 3.3 % crescents in controls 5.7 ± 2.9 % with DNAse I).

Conclusions: Our findings indicate that PI3Kγ-mediated NET generation by neutrophils contributed to ANCA-induced NCGN. Complement activation could provide a mechanistic link between NETs and tissue damage. In addition, PI3Kγ, NET formation and complement are treatment targets to abrogate disease.

Funding: Government Support - Non-U.S.

TH-OR126

Fanconi Syndrome Associated Light Chains Trigger Aberrations of Endolysosomal Compartments and Apical Dedifferentiation in Proximal Tubule Cells Alessandro Luciani,[1] Claudia Raggi,[2] Jenny A. Kuerth,[1] Vincent Javaugue,[3] Christophe Sirac,[3] Olivier Devuyst.[1] *[1]Physiology, UZH, Zurich, Switzerland; [2]UCL Nephrology, Brussels, Belgium; [3]CNRS UMR 6101, Limoges, France; [4]CHU Jean Bernard, Poitiers, France.*

Background: Generalized dysfunction of the proximal tubule (renal Fanconi syndrome) can occur when specific monoclonal immunoglobulin light chains (LCs) accumulate within the endolysosomal system of tubular cells and form intracellular crystals. The early molecular events involved in proximal tubule (PT) dysfunction remain poorly understood.

Methods: We characterized transgenic mice expressing human k-LC associated Fanconi syndrome (k-CHEB) and analyzed endocytic uptake, lysosome function, and differentiation and proliferation markers using primary cultures of PT cells derived from these transgenic and control mice.

Results: Metabolic studies revealed that k-CHEB mice show progressive manifestations of renal Fanconi syndrome, before structural damage and renal failure. These changes are related to decreased expression of specific apical transporters and receptors (megalin/cubilin) and to increased dedifferentiation (ZONAB transcription factor) and proliferation (PCNA and CyclinD1) rates. Exposure of PT cells to low concentration (25μg/mL) of k-CHEB-LC resulted in perinuclear positioning of enlarged and dysfunctional lysosomes with impaired clearance of autophagosomes containing ubiquitinated proteins and damaged mitochondria. These changes led to excessive production of reactive oxygen species (ROS) and increased tyrosine phosphorylation of ZO-1, disrupting the integrity of tight junctions and promoting the nuclear translocation of ZONAB responsible for proliferation and dedifferentiation of the cells. These changes were dramatically reduced in PT cells exposed to LCs lacking crystal formation in lysosomes.

Conclusions: These findings reveal that accumulation of specific LCs within PT cells impairs the autophagy-lysosome pathway and activates a chain of event promoting dedifferentiation and dysfunction of the cells. The characterization of these early events opens new perspectives to prevent the progression of LC-induced renal Fanconi syndrome.

Funding: Government Support - Non-U.S.

TH-OR127

Genetic Activation of Hypoxia-Signaling Restores Erythropoietin Synthesis in Renal Myofibroblasts Tomokazu Souma,[1,2,3] Masahiro Nezu,[1,2] Sadayoshi Ito,[2] Norio Suzuki,[1] Masayuki Yamamoto.[1] *[1]Medical Biochemistry, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan; [2]Medicine, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan; [3]Medicine, Nephrology, Northwestern Univ, Chicago, IL; [4]United Centers for Advanced Research and Translational Medicine, Tohoku Univ Graduate School of Medicine, Sendai, Miyagi, Japan.*

Background: The conversion of renal erythropoietin (Epo)-producing cells (REPs) to myofibroblasts is a major cause of fibrosis and anemia. It is characterized that prolyl-hydroxylase domain–containing proteins (PHDs) directly sense oxygen tension and regulate Hypoxia-inducible factor (HIF)-mediated Epo synthesis in REPs. However, the role of PHD-HIF pathways in renal myofibroblasts and Epo insufficiency has remained elusive.

Methods: To analyze PHD-HIF pathway in REPs, we generated single and combinational deletions of 3 isoforms of PHDs (PHD1-3) selectively in Epo-producing cells using Cre-lox approach in mice. We utilized unilateral ureteral obstruction (UUO) as a model for the myofibroblast conversion of REPs.

Results: Deletions of PHD2 alone or in combination with PHD1 and/or PHD3 induced polythythemia by HIF-2α-dependent Epo production from REPs. Epo production from injured kidneys in these conditional knockout mice were significantly higher than control littermates after UUO (% Epo repression [deleted alleles]; 93% [control littermates], 54% [Phd2], 51% [Phd2 and Phd3], 50% [Phd1and Phd2], 31% [Phd1, Phd2, and Phd3]). Intriguingly, combined deletions of PHD1 and PHD3 did not result in polythythemia in normal but prevented the loss of Epo-production in UUO (% repression; 69%). Furthermore, PHD-deficient REPs showed resistance to lipopolysaccharide-induced Epo repression, suggesting augmented HIF signal counterbalanced the NFκB signaling.

Conclusions: Our findings demonstrate that attenuated HIF signaling in myofibroblast-transformed REPs is a major cause of renal anemia and augmenting HIF signaling by inhibiting PHDs is an attractive therapeutic strategy for renal anemia by re-activating Epo synthesis from damaged and transformed REPs.

Funding: Government Support - Non-U.S.

TH-OR128

Pathogenic Form of suPAR in FSGS Sanja Sever,[1] Jochen Reiser.[2] *[1]Nephrology, MGH, Boston, MA; [2]Medicine, Rush Univ, Chicago, IL.*

Background: Soluble urokinase plasminogen receptor (suPAR) has been implicated in the pathogenesis of focal segmental glomerulosclerosis (FSGS) through activation of a_vb_3 integrin on podocytes. suPAR exists in different forms with different degree of glycosylation. Total suPAR serum levels as measured by commercial ELISA can be elevated through reduced GFR or in some other disease conditions including inflammation and certain cancers.

Methods: To identify pathologic effects of various suPAR forms on podocytes, we employed a novel, high throughput cellular assay whereby we quantitatively assessed a_vb_3 integrin activation on podocytes in response to different suPAR levels in serums of patients suffering from FSGS, sepsis and end-stage renal disease (ESRD).

Results: suPAR containing FSGS patient sera but not sepsis or general ESRD sera strongly activated podocyte a_vb_3 integrin. We next wondered if specific fragments of suPAR are particularly strong transmitters of podocyte injury. Biochemical analysis revealed a soluble D2-D3 fragment of suPAR in the blood and urine of FSGS patients but not in healthy individuals. Functionally, low concentration of this fragment potently activates a_vb_3 integrin in cultured human podocytes and also produced nephrinuria and albuminuria in mice. Additional cellular studies revealed a circulating suPAR feed-forward loop in which activation of a_vb_3 integrin on podocytes lead to proteolysis of uPAR from the podocyte membrane thereby generating high local concentration of D2-D3 fragment.

Conclusions: Our study unravels a pathological fragment of suPAR in human FSGS and describes a cooperative injury pathway between systemic and podocyte-proteolyzed suPAR as potent disease mediators.

Funding: NIDDK Support

TH-OR129

Podocyte Autophagy Is Down Regulated by Cardiotrophin-Like Cytokine Factor-1 (CLCF1), a Permeability Factor in Focal Segmental Glomerulosclerosis (FSGS) Mukut Sharma,[1] Jianping Zhou,[1] Alok De,[1] Tarak Srivastava,[3] Ellen T. McCarthy,[2] Ram Sharma,[1] Jean-Francois Gauchat,[4] Virginia J. Savin.[1] *[1]Nephrology, Kansas City VAMC, Kansas City, Mo; [2]Kidney Inst, Univ of Kansas Medical Center, Kkansas City, KS; [3]Nephrology, Children's Mercy Hospital, Kansas City, MO; [4]Pharmacology, Univ of Montreal, Montreal, Canada.*

Background: We have detected CLCF1 in FSGS plasma and proposed that it is a FSGS permeability factor. CLCF1, a member of the IL6 family, activates the JAK/STAT pathway but its role in maintaining podocyte integrity is unknown. Podocyte autophagy is required for cellular repair and survival. Disruption of constitutive autophagy is associated with proteinuria, loss of podocytes, and glomerular sclerosis. We hypothesized that CLCF1 modulates podocyte autophagy and adversely affects cell survival.

Methods: We treated immortalized mouse podocytes with recombinant human CLCF1 (rhCLCF1) for up to 24 hours. We added rapamycin, an inducer of autophagy, to some incubations. We used Western blotting to analyze cell lysates for elements of autophagy pathway (microtubule-associated protein 1 light chain (LC3 aka Atg8), beclin, Atg3, Atg5 and Atg7), and measured phosphorylation of Akt and ERK1/ERK2 to define signaling pathways. We also observed effects of CLCF1 on podocyte morphology and LC3 expression and intracellular distribution using microscopy.

Results: Podocytes express LC3-A and LC3-B with different intracellular distribution. Incubation of podocytes with CLCF1 (10 ng/mL) decreased the expression of LC3A and B with maximal effect at 4-6 hours ($P<0.001$). CLCF1 also attenuated rapamycin-induced up-regulation of autophagy as indicated by decreased LC3 expression. CLCF1 did not down-regulate beclin, Atg3, Atg5 or Atg7 appreciably. Additionally, CLCF1 also down-regulated the phosphorylation of Akt and ERK1/ERK2 and decreased the number of vacuolar structures.

Conclusions: We interpret these novel findings as evidence that CLCF1 down-regulates autophagy by a STAT3-mediated effect on LC3. Thus, CLCF1 may impair autophagy putting podocytes at risk. We propose that small STAT3 blocker(s) may be valuable in protecting podocyte function and structure in diseases such as FSGS.

Funding: Veterans Affairs Support, Private Foundation Support

TH-OR130

Identification of Novel Gene Products that Regulate Glomerular Filtration Barrier Function Davide Pietro Cina,[1,2] Chengjin Li,[3] Jason Moffat,[4] Susan E. Quaggin.[1,2] *[1]Feinberg Cardiovascular Research Inst, Northwestern Univ, Chicago, IL; [2]Div of Nephrology, Northwestern Univ, Chicago, IL; [3]Lunenfeld-Tanenbaum Research Inst, Mount Sinai Hospital, Toronto, ON, Canada; [4]Donnelly Centre for Cellular and Biomedical Research, Univ of Toronto, Toronto, ON, Canada.*

Background: Podocyte function is linked to the complex organization of its cytoskeleton and adhesion to the glomerular basement membrane. Disruption of genes involved in these processes results in proteinuria and glomerular injury. To identify novel genes that regulate podocyte function, we designed a genome-wide screen for genes involved in podocyte adhesion to both fibronectin, an integrin mediated pathway, and sFLT1/Fc, a novel heparin sensitive pathway, using a pooled shRNA library.

Methods: A pool of shRNA knock-down podocytes was generated using this library and plated on both substrates for one hour followed by separation of adherent and non-adherent fractions, and deconvolution of the hairpins present in each fraction by sequencing. Following deconvolution, we developed a highly stringent method for statistical analysis and 'hit' determination, and performed a small-scale validation of several top candidates.

Results: We identified 214 hairpins that increased podocyte adhesion to fibronectin and 237 hairpins that increased adhesion to sFLT1/Fc. We also identified 183 hairpins that decreased adhesion to fibronectin and 243 hairpins that decreased adhesion to sFLT1/Fc. Hairpins against *DPH1*, *DPH2*, *DPH3* and *DPH4* were in the top hits for increased adhesion to both substrates. Stable cell lines for these hairpins showed knock-down of gene expression by qPCR and a statistically significant increase in adhesion to both substrates. Stable overexpression of *DPH2* in human podocytes resulted in decreased adhesion to both substrates.

Conclusions: We have developed a novel screen for identifying candidate genes involved in regulating podocyte adhesion. Future work will include a large-scale validation of hits, and follow-up of select candidates. This approach will yield a list of genes that broadens our understanding of podocyte biology and conditions that perturb this system resulting in kidney disease.

Funding: Government Support - Non-U.S.

TH-OR131

Regulation of Fluid-Phase Uptake in Podocytes by Albumin-Associated Lipids Jun-Jae Chung,[1] Tobias B. Huber,[1] George Jarad,[2] Bjorn Hartleben,[1] Jeffrey H. Miner,[2] Andrey S. Shaw.[1,3] *[1]Pathology and Immunology, Washington Univ School of Medicine, Saint Louis, MO; [2]Medicine, Renal Div, Washington Univ School of Medicine, Saint Louis, MO; [3]Howard Hughes Medical Inst, Washington Univ School of Medicine, Saint Louis, MO.*

Background: The mechanisms by which the glomerular filter is maintained and prevented from clogging is unclear. We postulated that fluid-phase uptake by podocytes might play a role in maintaining the permeability of the filtration barrier.

Methods: Using fluorescently labeled fluid-phase tracers, we examined whether podocytes are capable of fluid-phase uptake in vivo or in culture. Podocytes were treated with serum albumin and various lipids to determine the regulation of fluid-phase uptake. Finally, shRNA-mediated knockdown and chemical inhibition of lipid-binding receptors and downstream signalling molecules were used to elucidate the signaling pathway mediating fluid-phase uptake in podocytes.

Results: Using fluid-phase tracers, we showed that podocytes actively internalize fluid from the serum and that the rate of internalization is enhanced when the filtration barrier is disrupted. In vitro experiments demonstrated that lipids associated with serum albumin stimulated macropinocytosis in podocytes. This process was specific to podocytes as known stimuli that induce macropinocytosis in other cells had no effect on podocytes, while serum lipids did not stimulate macropinocytosis in other cells. A candidate lipid approach showed that free fatty acids stimulate macropinocytosis through G protein-coupled receptors, FFAR1, FFAR2, and FFAR3 and the Gbeta/Ggamma complex.

Conclusions: Our observations suggest that podocytes sense the disruption of the filtration barrier via free fatty acids bound to albumin and respond to the increased protein by enhancing fluid-phase uptake. Dysregulation of this process could play and important role in the development of podocyte disease.

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TH-OR132

MiR-193a Regulates the Transdifferentiation of Human Parietal Epithelial Cells Toward a Podocyte Phenotype Catherine Meyer-Schwesinger,[1] Sebastian Guhr,[1] Tobias N. Meyer,[3] Marlies Sachs,[1] Rolf A. Stahl,[1] Moin Saleem,[4] Dontscho Kerjaschki.[2] *[1]Dept of Internal Medicine, Nephrology, Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Dept of Clinical Pathology, Medical Univ of Vienna, Vienna, Austria; [3]Dept of Internal Medicine, Nephrology, Univ Affiliated Asklepios Clinic Hamburg Barmbek, Hamburg, Germany; [4]Childrens Renal Unit, Bristol Royal Hospital for Children, Bristol, United Kingdom.*

Background: Overexpression of microRNA-193a (miR-193a) in mice induces FSGS through down-regulation of WT1, a transcription factor and master regulator of podocyte differentiation. In in situ hybridizations of normal human renal biopsies miR-193a was detected in parietal epithelial cells (PEC), but not in podocytes. Here we investigated, whether knockdown of miR-193a induced the differentiation of PECs towards a podocyte phenotype in vitro and in vivo.

Methods: We established an immortalized human PEC (hPEC) line from naïve Bowman's capsule cells isolated by mechanical micro-dissection. MiR-193a was stably knocked down in culture. In mice, miR193a was inhibited or over expressed. The expression of PEC and podocyte markers was investigated by qPCR, IF and WB.

Results: Cultured hPECs expressed high levels of PEC proteins such as Claudin-1, UCH-L1 and Pax-8 and of miR-193a, whereas they expressed low levels of podocyte markers. Following knock-down of miR-193a, hPECs adopted a podocyte-like morphology and expressed high levels of the podocyte proteins WT1, Podocalyxin, Synaptopodin, Nephrin and a-Actinin-4. We could also observe an upregulation of the podocyte marker Synaptopodin and WT1 upon inhibition of miR-193a in mice indicating at least a partial transdifferentiation *in vivo*. On the other hand, overexpression of miR-193a *in vivo* resulted in the upregulation of PEC and the loss of podocyte markers in isolated glomeruli.

Conclusions: Taken together, we successfully established a human PEC line and demonstrate that miR-193a acts as a master switch in regulating the expression of PEC and podocyte markers. Glomerular epithelial cells with high levels of miR-193a adopt a PEC phenotype whereas glomerular epithelial cells with low levels of miR-193a adopt a podocyte phenotype.

TH-OR133

The Role of Mesangial Cell-Derived CTGF in Anti-Glomerular Basement Membrane Nephritis Naohiro Toda,[1] Hideki Yokoi,[1] Masato Kasahara,[2] Kiyoshi Mori,[3] Takashige Kuwabara,[1] Hirotaka Imamaki,[1] Akira Sugawara,[4] Taiji Matsusaka,[5] Kazuwa Nakao,[3] Motoko Yanagita,[1] Masashi Mukoyama.[1,6] *[1]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [2]Inst for Advancement of Clinical and Translational Science, Kyoto Univ Hospital, Kyoto, Japan; [3]Medical Innovation Center, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [4]Div of Nephrology, Osaka Red Cross Hospital, Osaka, Japan; [5]Dept of Internal Medicine, Tokai Univ School of Medicine, Kanagawa, Japan; [6]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan.*

Background: Connective tissue growth factor (CTGF/CCN2) regulates signaling of other growth factors and promotes fibrosis. We previously showed that systemic deletion of CTGF ameliorates proteinuria and glomerular injury in anti-glomerular basement membrane (GBM) nephritis. To explore which glomerular cells producing CTGF are most important, we generate podocyte-specific CTGF knockout mice (Pod-CTGF cKO) and mesangial cell-CTGF knockout mice (Mes-CTGF cKO).

Methods: CTGF floxed mice were crossed with Nephrin-Cre mice to create Pod-CTGF cKO or PDGFRα-Cre mice for Mes-CTGF cKO. We evoked anti-GBM nephritis at 8 wksof age and investigated glomerular injury after 28 days. *In vitro*, Mes13 cells were used to examine the effects of CTGF deletion by siRNA transfection on MCP-1 expression.

Results: Pod-CTGF cKO and Mes-CTGF cKO showed normal renal structure. After induction of anti-GBM nephritis, severe proteinuria and glomerular injury were developed in control mice. Pod-CTGF cKO showed no improvement of glomerular injury or proteinuria. In contrast, Mes-CTGF cKO exhibited reduced proteinuria by 30% with ameliorated histological changes. The number of Mac2-positive cells (7 versus 2.4 cells/glomeruli) and expressions of TGF-β1 and F4/80 in glomeruli were reduced in Mes-CTGF cKO by 70% but not in Pod-CTGF cKO. TGF-β1-stimulated MCP1 expression was downregulated by reduction of CTGF in MES13 cells.

Conclusions: These results indicate that CTGF produced by mesangial cells plays a role in macrophage infiltration, suggesting that inhibition of CTGF/MCP1 axis could be a therapeutic target for anti-GBM nephritis.

TH-OR134

Crosstalk of mTOR Signaling and Autophagy Tillmann Bork,[1] Philipp Lee,[1] Bjorn Hartleben,[1,2] Tobias B. Huber.[1] *[1]Renal Div, Univ Hospital Freiburg, Freiburg, Germany; [2]Inst of Pathology, Univ Hospital Hamburg-Eppendorf, Hamburg, Germany.*

Background: Autophagy emerged as a key mechanism to eliminate unwanted cytoplasmic materials, thereby preventing cellular damage and stress to safeguard long-lived podocytes. The serine/threonine kinase mTOR (mammalian target of rapamycin) is a central regulator of cell growth and metabolism and usually inhibits autophagy. Podocytes however show high levels of basal autophagy in the presence of mTOR activation suggesting and unexpected uncoupling of mTOR and autophagy in podocytes. We did set up complementary mouse models to study the functional interplay of mTOR signaling and autophagy in podocytes.

Methods: Autophagy levels were monitored *in vivo* by crossing *GPF-LC3* reporter mice to models of mTOR hyperactivation (*Tsc1* PcKO) and mTOR loss of function (*Raptor* PcKO). In addition, podocyte-specific *Raptor* and *Tsc1* KO mice were crossed to a *Tomato/eGFP* reporter line to efficiently isolate podocytes for primary cell culture studies.

Results: In contrast to other known cellular systems podocytes did exhibit high basal autophagy rates independently of the mTOR activation status. Strikingly, there was no difference in LC3 conversion in vivo and no difference of the GFP-LC3 signal between *Raptor* and *Tsc1* PcKO mice. Unexpectedly, Beclin 1 expression was inversely regulated by mTOR activation. Isolated primary podocytes showed typical features of mTOR activation (proliferation, hypertrophy) and mTOR suppression, respectively, but autophagy

remained unaffected even in case of starvation suggesting that mTOR signaling influences the repertoire of expressed autophagy-related proteins but did not regulate the autophagy-response towards metabolic stimuli.

Conclusions: mTOR and autophagy are key regulators of podocyte function and maintenance. Our data highlight a specific mTOR-autophagy regulatory cascade, which allows to operate mTOR activity and high basal autophagy rates simultaneously in podocytes.

TH-OR135

Investigating a Role for the ShcA Adaptor Protein in Kidney Podocytes Claire E. Martin,[1] Kelly A. Petersen,[1] Vera Eremina,[2] Susan E. Quaggin,[2,3] Tomoko Takano,[4] Nina Jones.[1] *[1]Univ of Guelph, Guelph, ON, Canada; [2]The Lunenfeld-Tanenbaum Research Inst, Mount Sinai Hospital, Toronto, ON, Canada; [3]Feinberg Cardiovascular Research Inst and Div of Nephrology and Hypertension, Northwestern Univ, Chicago, IL; [4]McGill Univ, Montreal, QC, Canada.*

Background: The transmembrane protein nephrin is a key component of the kidney glomerular slit diaphragm that is required for normal morphology and permselectivity of podocytes. It has recently been reported that tyrosine phosphorylation of the cytoplasmic tail of nephrin facilitates recruitment of several signaling proteins that regulate podocyte function. The ShcA adaptor protein's importance within a broad range of cell types is well established and has been attributed to its ability to bind and mediate signaling between a diverse group of membrane receptors and cytoplasmic proteins. However, its role within the podocyte to this point remains under-investigated.

Methods: Multiple approaches were used to identify ShcA as a nephrin binding partner, to map this interaction and to evaluate ShcA's effect on nephrin signaling. Cre-loxP technology was used to generate podocyte-specific knockout of ShcA in mice.

Results: We demonstrate that the adaptor protein ShcA is differentially expressed in podocytes and binds phosphorylated nephrin via its SH2 domain. This interaction can be mapped to three conserved phosphotyrosine-based docking sites on nephrin. Loss of ShcA expression specifically within developing mouse podocytes leads to reduced barrier function and disruption of podocyte ultrastructure by 6-8 weeks of age. Furthermore, in mature kidneys and cultured podocytes, response to PAN-induced injury is associated with increased expression of ShcA, which correlates with activation of the stress-activated p38 and JNK MAP kinases. Interestingly, we found that transactivation of AP-1, which occurs downstream of nephrin as well as p38 and JNK, is attenuated by ShcA, and this effect is reversed by mutation of the ShcA SH2 domain.

Conclusions: Together these findings suggest a protective effect of ShcA in nephrin-dependent signalling, that is required during both podocyte maturation and response to injury.

Funding: Government Support - Non-U.S.

TH-OR136

Genetic Deletion of Myo1c in Podocytes Protects Them From Glomerular Injury Inducing Agents Ehtesham Arif, Yogendra Singh Rathore, Lawrence B. Holzman, Deepak Nihalani. *Medicine, Univ of Pennsylvania, Philadelphia, PA.*

Background: Glomerular diseases including FSGS and nephrotic syndrome that induce podocyte effacement are leading causes of kidney failure. Podocyte proteins Nephrin and Neph1 are major components of the slit diaphragm and it is now well documented that the organization of Nephrin and Neph1 at the membrane is lost in various glomerular disorders, which may contribute towards the development of disease pathology. Our recent observations suggest that Myo1c is directly involved in turnover of these proteins at the podocyte cell membrane.

Methods: To further understand the function of Myo1c in podocytes, we generated podocyte specific Myo1c knockout mice in different genetic backgrounds.

Results: Myo1c variants on all genetic backgrounds tested were born with normal Mendelian frequency and when aged to at least 6 months showed no evidence of proteinuria. However, podocyte-specific Myo1c null mice bred to an adriamycin-sensitive (C57BL/6**N**) background showed remarkable resistance to adriamycin-induced glomerular injury; they did not develop proteinuria as compared to the control mice. To gain insight into the mechanism of this protection the kidney sections were analyzed for change in expression levels of Neph1 and Nephrin in response to injury. Remarkably, Nephrin and Neph1 were maintained at the podocyte intercellular junction following injury, indicating that loss of Myo1c prevented loss of these proteins in response to injury. Additionally, adriamycin-induced expression of autophagy marker LC3 was diminished in Myo1c depleted glomeruli suggesting that loss of Myo1c prevents autophagy induction in response to injury.

Conclusions: Several published studies indicate that Myo1c is involved in cellular trafficking ranging from compensatory endocytosis to recycling. Therefore, we hypothesize that loss of Myo1c in podocytes affects cellular trafficking events including autophagy that may prevent injury-induced loss of Nephrin and Neph1. Under these conditions podocytes fail to generate an appropriate injury response, and are thus protected from injury.

Funding: NIDDK Support, Private Foundation Support

TH-OR137

The Glomerular Filter as an Electrical Powerhouse: How Much Voltage Is Required to Prevent Proteinuria? Turgay Saritas,[1] Sandra Uhlig,[1] Ralf Hausmann,[2] Jürgen Floege,[1] Marcus J. Moeller.[1] *[1]Nephrology and Clinical Immunology, Univ Hospital RWTH Aachen, Aachen, North Rhine-Westphalia, Germany; [2]Molecular Pharmacology, Univ Hospital RWTH Aachen, Aachen, North Rhine-Westphalia, Germany.*

Background: It is still incompletely understood how the glomerular filter functions and why it never clogs. Recently, the electrokinetic model for glomerular filtration has been proposed: Streaming potentials generate an electrical field across the filtering capillaries, which in turn repel the negatively charged plasma proteins from entering the glomerular filter. From mathematical considerations, it was argued against this concept was that an electrical field of approx. 1600 Volts/m (as measured by micropuncture in Necturus *in vivo*) may not be sufficient to prevent albumin from passing the across the glomerular filter. Therefore, we have determined the electrophoretic mobility of albumin experimentally in isolated perfused kidney (IPK) of a mammalian (rat) kidney in this study.

Methods: IPK were fixed with glutaraldehyde to block tubular or other cellular artifacts while preserving the electrical characteristics of the glomerular filter. An electrical field was applied externally to IPK via electrodes. The electrokinetic model requires that already relatively weak electrical fields (3.5 Volts, i.e. approximately 700 Volts/m) interfere with the endogenous electrical field of the glomerular filter resulting in significant increases of the albumin sieving coefficient. In contrast, mathematical modeling of the traditional pore model predicts that 100x more voltage is needed. As control, no current or 3.5 Volts high frequency alternating current (AC, 41 kHz) were used.

Results: Albumin sieving coefficient increased significantly when applying 3.5 Volts DC but not when applying AC or no current.

Conclusions: The results show that already relatively weak extracellular streaming potentials influence the passage of albumin across the glomerular filter supporting the electrokinetic model.

Funding: Private Foundation Support, Clinical Revenue Support

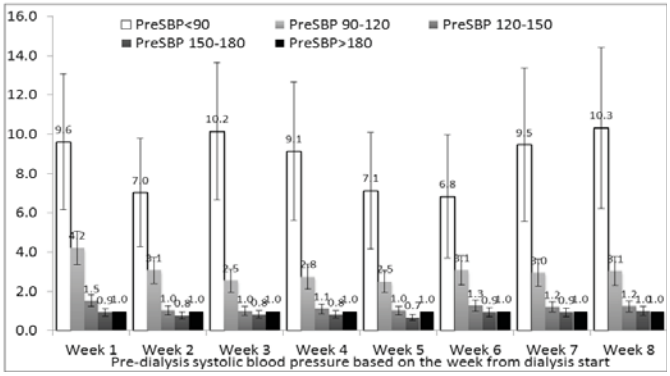
TH-OR138

Lower Systolic Blood Pressures Are Consistently Associated with Poorer Outcomes in Incident Hemodialysis Patients in the First 8 Weeks of Treatment Dugan Maddux,[1] Len A. Usvyat,[1,3] Jeroen Kooman,[2] Frank van der Sande,[2] Kevin Chan,[1] Peter Kotanko,[3] Franklin W. Maddux,[1] John W. Larkin.[1] *[1]FMCNA, Waltham, MA; [2]Dept of Internal Medicine, Univ Hospital Maastricht, Maastricht, Netherlands; [3]RRI, NY, NY.*

Background: Hypertension management is recommended in CKD to reduce cardiovascular risk; however, low pre-dialysis systolic BP (preSBP) has been paradoxically reported to associate with poor outcomes among prevalent hemodialysis (HD) patients. We investigated if this association also exists among incident dialysis patients.

Methods: Incident HD patients in FMCNA who initiated dialysis between Jan 1, 2004 and Dec 31, 2010 and had >=1 preSBP measurement were included. We computed mean preSBP weekly in patients up to week 8 from the start of dialysis. The primary outcome was 120 day survival from the start of each of the 8 weeks. Patients were stratified into 5 groups by preSBP and analyzed by <90, 90-120, 120-150, 150-180, and >180mmHg. Cox models adjusted for age, gender, and race were used to relate preSBP level to outcomes.

Results: 47,315 patients were included in the analysis (mean age 62.3+/-12.2, 57% male, 66% White, 28% Black, 60% diabetic). Mean preSBP was 149.1 in week 1 and 150.0 mmHg in week 8. 88% of patients were alive and dialyzing in week 8 (N=41,546). Mortality risk was consistently high in preSBP<90mmHg group ranging from HR=6.8 [CI 4.0-10.0] (week 6) to 10.3 [6.0 -14.0] (week 8). Groups with preSBP 90-120 and 120-150 mmHg were also higher than the reference group of preSBP>180 mmHg. The hazard ratios did not change significantly between weeks.



Conclusions: PreSBP <90-120 mmHg in the first months of dialysis was associated with high mortality. At any point during the first 2 months of HD a mean preSBP <90 mmHg was associated with increased mortality risk. Moderately low preSBP between 90 and 120 mmHg was also associated with increased mortality risk.

TH-OR139

The Safety and Efficacy of Mineralocorticoid Receptor Antagonists in Patients Who Require Dialysis: A Systematic Review and Meta-Analysis Kevin Quach, Lyubov O. Lytvyn, Colin Baigent, Joe A. Bueti, Amit X. Garg, Carmel M. Hawley, Richard Haynes, Braden J. Manns, Vlado Perkovic, Christian G. Rabbat, Ron Wald, Michael Walsh. *McMaster Univ/Population Health Research Inst.*

Background: Mineralocorticoid receptor antagonists (MRAs) prevent cardiovascular events in patients with heart failure who do not require dialysis. Whether MRAs are effective and safe in dialysis patients is uncertain. We conducted a systematic review and meta-analysis of randomized controlled trials testing the effect of MRAs in dialysis patients.

Methods: We identified relevant randomized controlled trials from electronic databases, bibliographies, trial registries, and by contacting experts. Eligible trials randomized patients on chronic dialysis between a MRA (spironolactone or eplerenone) and control and reported at least one outcome of interest: cardiovascular death, all cause death, hyperkalemia, or blood pressure. Data was abstracted in parallel by two reviewers and analyzed using random effects models.

Results: Of 1006 screened citations, seven relevant trials including 729 patients were included. The relative risk (RR) of cardiovascular death (MRA versus control) was 0.34 (95% confidence interval [CI] 0.15 to 0.75]) and the RR of all-cause death was 0.40 (95% CI 0.23 to 0.69). However, mortality benefits were not observed in sensitivity analyses that explored assumptions about losses to follow-up. Additionally, the RR of hyperkalemia was 3.14 (95% CI 1.08 to 9.17) suggesting potential harm. Sensitivity analyses suggested the risk of hyperkalemia may be underestimated. Overall, there were insufficient data to robustly estimate any of the effects of MRA treatment in dialysis patients.

Conclusions: Treatment of dialysis patients with MRAs is promising but further large, high quality trials are required to definitively assess the benefit-risk balance.

TH-OR140

Comparative Effectiveness of Renin-Angiotensin System Antagonists in Chronic Dialysis Patients Theresa I. Shireman,[1] James B. Wetmore,[2] Edward F. Ellerbeck,[1] Jonathan D. Mahnken,[1] Milind A. Phadnis.[1] *[1]Univ of Kansas School of Medicine, Kansas City, KS; [2]Hennepin County Medical Center, Minneapolis, MN.*

Background: Renin-angiotensin system antagonists are known to significantly reduce cardiovascular events and mortality in the ESRD setting, but their inherent characteristics suggest they may have different within class pharmacodynamic effects. The purpose of this study was to compare outcomes between chronic dialysis patients with hypertension who were treated singularly with either ACEs or ARBs.

Methods: We linked Medicaid pharmacy claims, United States Renal Data System (USRDS) core files, and Medicare inpatient and outpatient claims. The cohort included hypertensive Medicare-Medicaid eligible ESRD patients initiating dialysis. We followed the new user cohorts from their 1st day on either an ACE or an ARB until they incurred an outcome event (all-cause mortality (ACM) and combined cardiovascular hospitalization or death (CV-endpoint)) or were no longer observable. Patients who changed from an ACE to ARB or vice versa were excluded. Covariate adjustments included demographics, vintage, functional measures, and comorbidity. We used Cox proportion hazards models to compare the effect of ACE versus ARB use on outcomes.

Results: ACM models were based on 8,246 ACE and 3,012 ARB new users: CV-endpoint models included 6,560 ACE and 2,609 ARB new users. After adjustment for several small but significant differences in baseline characteristics between groups, ACE users had higher hazard ratios for ACM (AHR = 1.21, 99% CI 1.10-1.34) and CV-endpoints (AHR = 1.11, 99% CI 1.02-1.22). When new users were limited to persons with drug initiation early in the observation window (first 3 months), findings were comparable for ACM (AHR = 1.22, 99% CI 1.05-1.42) but the association with CV-endpoint was no longer significant (AHR = 1.12, 99% CI 0.99-1.27).

Conclusions: In this observational study, we demonstrated that ACE versus ARB users face an increased risk for mortality and possibly an increased risk for CV-endpoints. Validation of these results in a rigorous clinical trial is warranted given their widespread use in the ESRD community.

Funding: NIDDK Support

TH-OR141

Comparative Effectiveness of Cardioselective and Non-Selective β-Adrenergic Blockers in Chronic Dialysis Patients Theresa I. Shireman,[1] James B. Wetmore,[2] Edward F. Ellerbeck,[1] Milind A. Phadnis,[1] Jonathan D. Mahnken.[1] *[1]Univ of Kansas School of Medicine, Kansas City, KS; [2]Hennepin County Medical Center, Minneapolis, MN.*

Background: Although β-adrenergic blockers reduce cardiovascular events and mortality in ESRD patients, it is unknown if there significant within class cardiovascular outcomes between agents with β_1 or cardio-selectivity and differential alpha blockade and vasodilatory effects (cardioselective and non-cardioselective agents).

Methods: We linked Medicaid pharmacy claims, United States Renal Data System (USRDS) core files, and Medicare Parts A and B claims to create a cohort of hypertensive Medicare-Medicaid eligible patients initiating dialysis from 2000-05. We followed new users of either a cardioselective (metoprolol or atenolol) or non-selective (carvedilol or labetolol) β- blocker from their 1st day on medication until they incurred an outcome event (all-cause mortality (ACM) and combined cardiovascular hospitalization or death (CV-endpoint)) or were censored. We used Cox proportion hazards models to compare the effect of cardioselective or non-selective β-blockers use on ACM and CV-endpoint, adjusting demographics, vintage, functional measures, and comorbidities.

Results: ACM models were based on 9,196 cardioselective and 2,650 non-selective β-blocker new users: CV-endpoint models included 7,386 cardioselective and 1,978 non-selective β-blocker new users. After adjustment for differences in baseline characteristics between groups, cardioselective β-blocker users had lower hazard ratios for ACM (AHR = 0.79, 99% CI 0.72-0.87) and CV-endpoint (AHR = 0.80, 99% CI 0.73-0.89). When new users were limited to persons with drug initiation in the first 3 months, findings were comparable: ACM (AHR = 0.84, 99% CI 0.72-0.97) and CV-endpoint (AHR = 0.86, 99% CI 0.75-0.99). Results also did not vary when heart failure was included in an interaction term with drug selection.

Conclusions: Chronic dialysis patients who received cardioselective β-blocker had a lower risk for mortality and CV-endpoints when compared to their peers who received non-selective β-blockers. Validation of these results in a rigorous clinical trial is warranted given their widespread use in dialysis patients.

Funding: NIDDK Support

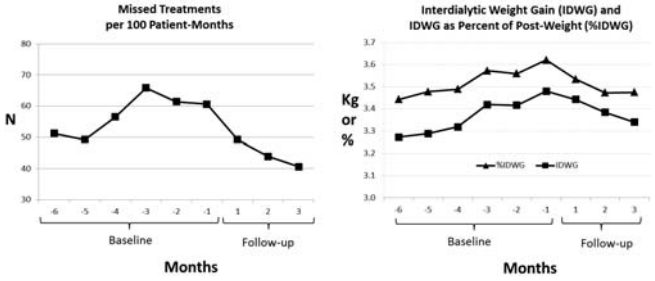
TH-OR142

A Social Worker Initiated Program to Reduce Fluid Overload in Hemodialysis Patients Stephanie Johnstone, Nien-Chen Li, Franklin W. Maddux, Amy R. Weissman-Hunt, Jessica Demaline, Eduardo K. Lacson. *Fresenius Medical Care, North America, Waltham, MA.*

Background: A social worker (SW) initiated intensive program was implemented as a quality improvement (QI) initiative to address problematic hemodialysis (HD) patient compliance. We report preliminary results of the intervention for patients with worsening fluid overload.

Methods: The cohort included 246 patients completing ≥6 of the 8 session intervention (1 session every 1-2 weeks) from 167 FMCNA centers between 2/1/13 and 01/31/14, with at least a 6-month baseline and 3-month follow-up period. The intervention included patient-empowerment education and cognitive/behavioral counseling designed to address potential root causes of fluid non-compliance. Baseline versus follow-up interdialytic weight gain (IDWG; also as %IDWG of post-weight), rates of missed treatments, and hospitalization for fluid overload were tracked.

Results: The patients' mean age was 54 years, with 56% males; 69% white, 29% black; 67% had DM, 23% CAD, and 47% CHF. During baseline, at least 1 missed treatment was noted in 46%. While all-cause hospitalization rates were unchanged, baseline versus follow-up hospitalizations due to fluid overload decreased from 1.7 to 0.8 episodes per 100 patient-months. Missed treatment rate was 57 versus 45 per 100 patient-months while period mean %IDWG was unchanged at ~3.5%. However, slopes for both were increasing at baseline then slopes declined during follow-up.



Conclusions: Preliminary results indicated that the intensive SW-initiated program reduced fluid overload-related hospitalizations in the short term (3 months). Furthermore, trends for contributory non-adherent behavior such as %IDWG and missed treatments improved, which may help sustain the favorable results over the long term. This QI study is ongoing and updated outcomes will subsequently be reported.

TH-OR143

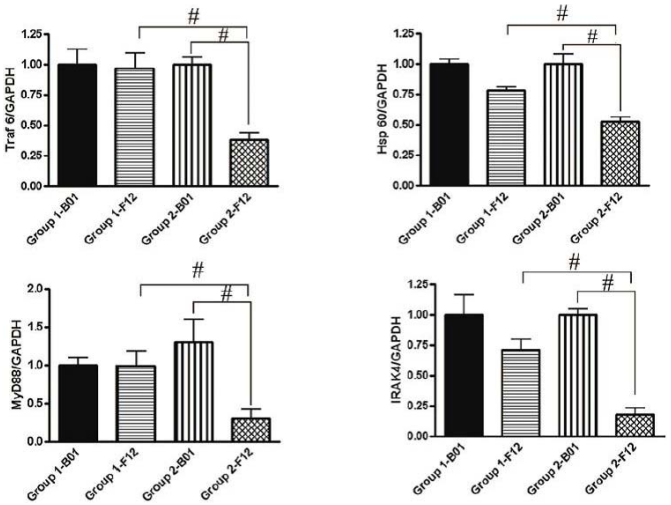
The Role of Cardiomyocyte Apoptosis in the Pathogenesis of Left Ventricular Hypertrophy (LVH): Results from the Frequent Hemodialysis Network (FHN) Christopher T. Chan, Michael V. Rocco, Glenn M. Chertow, George A. Kaysen, Nathan W. Levin, Brett Larive, Gerald J. Beck, Alan S. Kliger. *NIDDK - FHN Cardiac Biomarker Group.*

Background: Cardiomyocyte apoptosis (CA) is a novel cardiac biomarker. We hypothesize that extent of CA is responsible in part for the response of LV mass (LVM) with frequent hemodialysis in end-stage renal disease (ESRD) and that this effect is transmitted by a factor found in plasma.

Methods: Neonatal cardiomyoytes were cultured with plasma from responders (LVM reduced by a min of 10%) and progressors (LVM increased by a min of 10%) among randomized patients in FHN trials. The primary aim was to ascertain the effect of plasma on CA.

Results: Among the 332 patients randomized, there were 105 responders and 63 progressors. Selected baseline and clinical variables are shown in Table. LVM changed by -33.9 (-37.9,-29.8) g in responders and +24.1 (19.0,29.2) g in progressors, p<0.0001. CA (Anexin V) was similar at baseline but significantly higher at 12 months after exposure

to plasma from progressors versus responders (1.15 ± 0.19 versus 0.73 ± 0.09, p=0.02). Expressions of Innate immunity pathway remained elevated in progressors (group1) and fell in responders (group2) (Figure).



Variables	Responders (N=105) (69M)	Progressors (N=63) (34M)
Age (yrs)	51.2±14.0	50.5±11.6
ESRD vintage (yrs) (10^{th},90^{th}%tile)	3.3 (0.3,16.7)	2.8 (0.2,12.6)
Change in weekly volume removal rate (ml/kg/h)	-1054±356	-529±452
Change in pre-dialysis systolic BP (mmHg)	-11.4±1.8	4.1±2.3*
Change in pre-dialysis phosphate (mmol/L)	-0.72±0.17	-0.23±0.21*

*p<0.05

Conclusions: CA was associated with progression of LVM in ESRD. Cardiomyocyte death and remodelling with fibroblast-like cells may play a pivotal role in LVH progression. Both may be affected by a factor found in plasma at concentrations that vary depending on the frequency of HD.

Funding: NIDDK Support

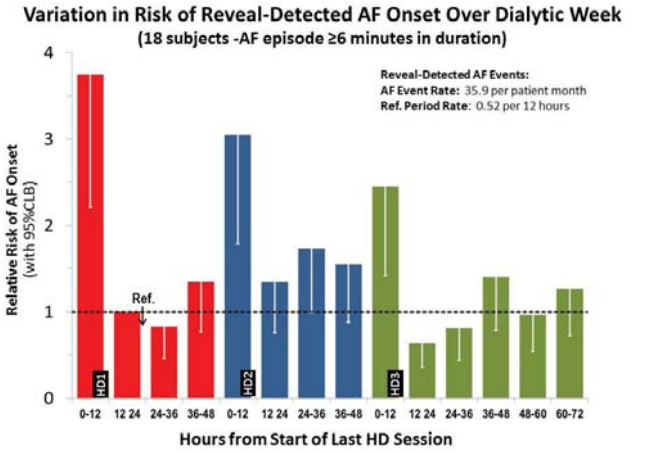
TH-OR144

Frequency and Distribution of Dialysis-Associated Atrial Fibrillation: Results of MiD Study James A. Tumlin,[1] David M. Charytan,[2] Don E. Williamson,[3] Amber S. Podoll,[4] Suresh Chandra Tiwari,[5] Prabir Roy-Chaudhury,[6] Vikranth Reddy.[7] *[1]Internal Medicine Renal Div, Univ of Tennessee College of Medicine, Chattanooga, TN; [2]Internal Medicine Renal Div, Harvard Brigham and Women's Hospital, Boston, MA; [3]Nephrology Associates, Augusta, GA; [4]Internal Medicine Renal Div, Univ of Texas Houston, Houston, TX; [5]Transplantation, Fortis Inst of Renal Sciences and Transplantation, Vasant Kunj, New Delhi, India; [6]Internal Medicine Renal Div, Univ of Cincinnati, Cincinnati, OH; [7]Care Hospital, Hyderabad, India.*

Background: End-stage renal disease (ESRD) patients are at increased risk for atrial arrhythmias. While previous studies report atrial fibrillation (AF) rates of up to 40%, the link between the hemodialysis (HD) cycle and AF is unknown. Therefore, we conducted a prospective, multi-center, 6-month observational study characterizing the incidence of AF in ESRD patients.

Methods: MiD is a prospective multi-center study designed to characterize the frequency of AF in 45 ESRD patients implanted with the Medtronic Reveal® XT device. The Reveal device detects AF events based on incoherence in adjacent R-R intervals. Reveal device data was centrally adjudicated weekly for 6 months of follow up.

Results: 45 subjects were implanted and followed for a mean of 4.9 months. A total of 1231 AF episodes with ≥6 min duration were detected in 18 of 45 (40.0%) subjects. This corresponds to an overall AF rate of 5.6 events per patient month (ppm), while patients with AF > 6 minutes duration had 14.1 ppm.



The rate of AF events was 3.5 fold higher in the 12 hours following dialysis (Figure). Only 1 of 18 had any prior history of AF.

Conclusions: Atrial fibrillation is seen in up to 40% of ESRD patients. The dialysis procedure may enhance AF rates with the greatest incidence occurring during the first 12 hours after the initiation of dialysis.

Funding: Pharmaceutical Company Support - Medtronic Corporation; Dialysis Corporation Inc., Private Foundation Support, Clinical Revenue Support

TH-OR145

Greater Frequency of Clinically Significant Bradycardia Than Ventricular Tachycardia in Hemodialysis Patients: Preliminary Results of the Monitoring in Dialysis (MiD) Study David M. Charytan,[1] Bruce A. Koplan,[1] Amber S. Podoll,[2] Vikranth Reddy,[3] Prabir Roy-Chaudhury,[4] Suresh Chandra Tiwari,[5] James A. Tumlin,[6] Don E. Williamson.[7] *[1]Harvard U; [2]U of Texas; [3]Care Hospital, Hyderabad, India; [4]U of Cincinnati; [5]Fortis Inst, New Delhi, India; [6]U of Tennessee; [7]Nephrology Associates, Augusta, GA.*

Background: End-stage renal disease patients are at increased risk of arrhythmias and sudden cardiac death (SCD), but little is known about arrhythmia type, prevalence or relationship to dialysis schedule.

Methods: MiD is a prospective, multi-center study to characterize arrhythmias occurring in 3x weekly hemodialysis (HD) patients using an implantable cardiac monitoring device, Medtronic Reveal® XT. ECGs recorded during the first 6 months of monitoring were centrally adjudicated. Reveal-detected arrhythmic event data were collected for an additional 6 months thereafter without adjudication. Clinically significant arrhythmias (CSA) include bradycardia ≤40 bpm for ≥6 seconds (s), asystole ≥3 s, or sustained ventricular tachycardia (SVT) ≥130 bpm for ≥30 s are limited to the main 6-month study period.

Results: 45 implanted subjects were followed for a mean of 6.4 months (0.6-12.0) [mean age: 56 (27-76) years; male: 64%; history of cardiac arrhythmias: 46%]. The overall rate of bradycardia was 10.2 [95% CI: 3.1-34.1] events per patient month (ppm) for all subjects, and 28.7 [95% CI: 10.1-81.7] events ppm for 16 subjects with ≥1 bradycardia episode. Average time to first episode of bradycardia or asystole was 62 days after Reveal XT implant. There was no apparent association of bradycardia with the timing of dialysis. Among 605 CSA, 74.9% were bradycardia or asystole events. In contrast, only 1 episode was SVT (0.2%). To date, 4 subjects received a pacemaker; 3 subjects had no prior history bradycardia, and 1 subject had a prior history.

Conclusions: Clinically significant bradycardia is common in previously asymptomatic HD patients and occurs at a much higher frequency than VT. Our data suggests that bradycardia converting to asystole may be the dominant terminal arrhythmia compared to VT in HD patients. This finding has important implications for the prevention of SCD in HD patients.

Funding: Pharmaceutical Company Support - Medtronic

TH-OR146

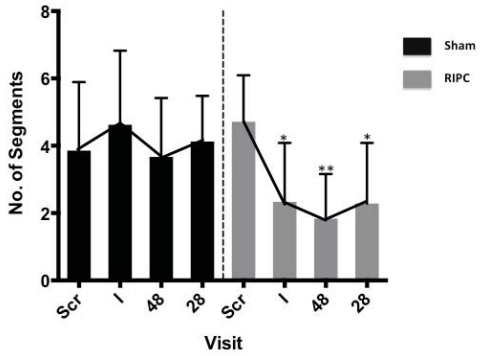
Remote Ischaemic Preconditioning in Haemodialysis: An Initial Randomised Controlled Trial Lisa E. Crowley,[1] Aghogho Odudu,[3] Chris W. McIntyre.[2] *[1]Royal Derby Hospital, United Kingdom; [2]Univ of Nottingham, United Kingdom; [3]Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom.*

Background: Haemodialysis results in a recurrent segmental ischaemic cardiac injury which helps to drive the excess cardiovascular morbidity in HD patients.Previous exposure to an ischemic insult (even if delivered remotely) has been shown to be cardioprotective in numerous experimental and clinical studies.We investigated if application of Remote Ischaemic Preconditioning (RIPC) prior to HD provided protection against dialysis induced cardiac injury.

Methods: 20 patients receiving hospital HD were recruited.Presence of dialysis induced myocardial stunning was confirmed with echocardiography before randomisation into 2 groups.One group received a single RIPC stimulus administered via a BP cuff inflated to 200mmHg in the lower limb.The other received Sham-RIPC with the cuff inflated to

40mmHg.All patients were studied at intervention,48 hours and 28 days post RIPC.Stunning was identified using pre and peak stress echo analysed using 2D speckle tracking.A stunned segment was defined as undergoing a 30% reduction in longitudinal strain.

Results: At baseline the number of stunned segments was 4.7±1.3 versus 3.8±2.0 (RIPC and sham respectively).Following administration of RIPC the number of affected segments fell significantly in the intervention arm.This reduction was sustained at both 48hrs and 28 days.No change was observed in the sham group.The largest reduction was at 48hrs in comparison with screening (1.83±1.3 versus 4.70±1.3 p=0.002).The difference in pre and peak global longitudinal strain present in the RIPC group at screening (15.98±3 versus 13.04±3.4) was abolished at subsequent visits.Levels of HD induced circulatory stress were lower in the sham group (as judged by UF volume or intradialytic reduction in BP).



Conclusions: A single RIPC stimulus administered prior to conventional HD provides relative protection against HD induced cardiac injury for up to 28 days.

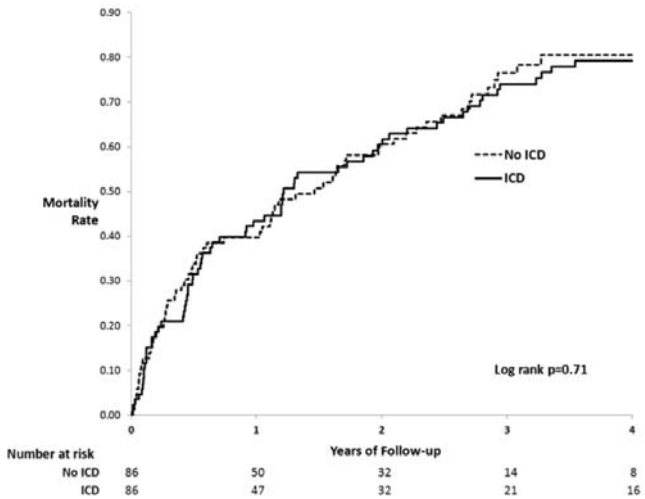
TH-OR147

Primary Prevention Implantable Cardioverter Defibrillators in Dialysis Patients: A Matched Cohort Study Patrick H. Pun,[1] Anne Hellkamp,[1] Gillian Sanders,[1] John Paul Middleton,[1] Stephen Hammill,[3] Hussein Al-Khalidi,[1] Lesley H. Curtis,[1] Gregg C. Fonarow,[2] Sana M. Al-Khatib.[1] *[1]Duke Univ Medical Center, Durham, NC; [2]UCLA Medical Center, Los Angeles, CA; [3]Mayo Clinic, Rochester, MN.*

Background: Sudden cardiac death is the leading cause of death among dialysis patients, but the benefit of primary prevention implantable cardioverter defibrillators (ICD) in this population is uncertain. We conducted this investigation to compare the mortality of dialysis patients receiving a primary prevention ICD with matched controls.

Methods: We used data from National Cardiovascular Data Registry's ICD Registry to select dialysis patients who received a primary prevention ICD, and the Get With The Guidelines-Heart Failure Registry to select a comparator cohort. We matched ICD recipients and no-ICD patients using propensity score techniques to reduce confounding, and survival was compared between groups.

Results: We identified 108 dialysis patients receiving primary prevention ICDs and 195 comparable dialysis patients without ICDs. One year (3-year) mortality was 42%(69%) in the ICD registry cohort compared to 38%(76%) in controls. There was no significant survival advantage associated with ICD (HR=0.87, 95%CI 0.66-1.13, p=0.29). After propensity matching, our analysis included 86 ICD patients and 86 matched controls. Comparing the propensity-matched cohorts, 1 year(3 years) mortality was 43% (74%) in the ICD cohort and 40% (77%) in controls; there was no significant difference in mortality outcome between groups (HR=0.94, 95% CI 0.67-1.31, p=0.71).



Conclusions: We did not observe a significant association between primary prevention ICDs and reduced mortality among ESKD patients receiving dialysis. Consideration of the potential risks and benefits of ICD implantation in these patients should be undertaken while awaiting the results of definitive clinical trials.

Funding: Other NIH Support - 1R01-HL093071-01A1

TH-OR148

Effect of Maternal Obesity in Offspring Predisposition to Chronic Kidney Disease in Rats Sarah J. Glastras,[1] Hui Chen,[2] Carol A. Pollock,[1] Sonia Saad.[1] *[1]Renal Medicine, Kolling Inst, Univ of Sydney, NSW, Australia; [2]School of Biomedical Sciences, Univ of Technology, Sydney, NSW, Australia.*

Background: Exposure to excess nutrition *in utero* and in early postnatal life is associated with adverse consequences for the offspring including diabetes, hypertension, dyslipidemia, cardiovascular disease and CKD. We aimed to determine whether maternal obesity predisposes the offspring to CKD.

Methods: Dams were fed normal or high-fat diet (HFD; 20 kJ/g)) 6 weeks before gestation until pups weaned, and their offspring's kidneys examined at Day 20 (weaning), Week 9 (adolescence) or Week 13 (adulthood). Animals were exposed to a normal or HFD from weaning. The pups' anthropometric measures and hormonal levels were recorded. Renal structure was assessed using PAS and Masson's trichrome staining. Gene expression of profibrotic factors (TGF-β, CTGF), proinflammatory cytokines (TNF, IL-6 and MCP-1), and metabolic markers (FAS, PPARα, SREBP) was measured by RT-PCR. Western blotting and immunohistochemistry was utilized to confirm these changes.

Results: Offspring from obese dams displayed increased fat mass, body weight, blood triglycerides levels, and glucose intolerance compared with those from lean rats in adolescence (week 9). Hormonal levels of leptin, ghrelin, GLP-1, and glucagon were increased in the offspring of obese mothers compared to control animals at Week 9 and Week 13. This is associated with increased renal inflammatory markers (MCP1 and TGFβ), increased fibrotic markers (fibronectin and collagen) and increased oxidative stress markers (iNOS and SOD activity). In addition the obese offspring have lipid droplets in their kidneys, and signs of tubulointerstitial injury which foreshadow the development of CKD. Interestingly, those markers were largely normalized when examined in adulthood when they were glucose intolerant at week 13.

Conclusions: Maternal HFD causes metabolic and hormonal derangement associated with altered fibrotic, inflammatory and oxidative stress markers within the kidneys of rat offspring at adolescence but not sustained to adult life. This suggests that maternal obesity may predispose the offspring to further renal damage in the setting of other insults such as diabetes.

Funding: Government Support - Non-U.S.

TH-OR149

Tubular Dysfunction Results in Altered Nucleoside Metabolism in Diabetic Nephropathy Anna V. Mathew, Farsad Afshinnia, Jaeman Byun, Pradeep Kayampilly, Frank C. Brosius, Subramaniam Pennathur. *Dept of Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: Our recent work demonstrated that plasma nucleosides pseudouridine (PU) and dimethyl guanosine (DMG) predict type 2 diabetic nephropathy (DN) progression, but the mechanism underlying this increase is not well-understood.

Methods: In order to study the metabolism and flux of the nucleosides *in vivo* systematically, we developed a targeted quantitative analysis of nucleosides by liquid chromatography/ tandem mass spectrometry (LC/MS). Mice which exhibit characteristic pathological features of DN (BKS db/db) and control (BKS db+) were used to examine the static and dynamic changes in nucleoside metabolism in DN. We performed *in vivo* metabolic flux analysis (MFA) by LC/MS following intraperitoneal administration of isotopically labeled substrates ^{13}C Uracil (precursor of PU) and ^{13}C hypoxanthine (precursor of DMG and allantoin) to 24-week old mice.

Results: MFA revealed statistically significant elevations in label incorporation into PU and hypoxanthine (HX) in plasma with corresponding decrease in urinary labeling in diabetic mice compared with controls. In contrast, label incorporation of uracil, HX, PU, DMG and allantoin were unchanged in diabetic renal cortex, liver and muscle suggesting that renal and tissue metabolism were not the source of the altered nucleosides. The elevated plasma levels and diminished urinary excretion strongly implicate altered tubular handling of nucleosides in DN. In order to determine if the altered nucleoside handling was specific to DN, we measured nucleosides in baseline samples of subjects with stages 3 and 4 chronic kidney disease (CKD) from the CPROBE cohort (16 DN and 24 non-diabetic subjects). After adjusting for serum creatinine, plasma PU, DMG and allantoin, and urinary allantoin and HX were significantly associated with DN. Levels of plasma PU and DMG predicted renal progression in one year (defined as 20% decrease in eGFR or 1.5 fold increase in proteinuria) only in DN, but not in non-diabetic CKD.

Conclusions: Taken together, these results highlight previously unrecognized unique role for altered nucleoside metabolism and handling in the pathogenesis of DN.

Funding: NIDDK Support

TH-OR150

G Protein Coupled Receptor TGR5 Agonist Prevents Kidney Disease in Mice with Diet Induced Obesity Xiaoxin Wang,[1] Yuhuan Luo,[1] Cherelle Parker,[1] Luciano Adorini,[3] Michal Herman-Edelstein,[2] Moshe Levi.[1] *[1]Medicine, Univ of Colorado Denver; [2]Univ of Tel Aviv; [3]Intercept Pharmaceuticals.*

Background: Obesity-related glomerulopathy (ORG) is becoming one of the leading causes of kidney disease. In addition to life style changes and/or bariatric surgery additional treatment modalities are needed to prevent ORG.

Methods: In recent studies in kidney biopsies from subjects with ORG we have determined that there is decreased expression of the G Protein Coupled Receptor TGR5. In the present study we therefore determined the role of the G Protein Coupled Receptor TGR5 in modulation of kidney disease in a mouse model of diet induced obesity and insulin resistance.

Results: Treatment of mice with the selective TGR5 agonist INT-777 decreased urinary albumin (73±8 in HF versus 26±9 in HF+INT-777, $p<0.05$), podocyte injury, mesangial expansion, tubulointerstitial fibrosis, and CD68 macrophages in the kidney. These beneficial effects of INT-777 were associated with significant increases in p-AMPK (12±1.2 in HF versus 19±1.3 in HF+INT-777, $p<0.01$), PGC-1α (37±0.8 in HF versus 50±4.1 in HF+INT-777, $p<0.05$), and Sirt3 (85±2.1 in HF versus 142±15 in HF+INT-777, $p<0.01$) protein abundance. At the same time there was an increase in MnSOD (94±3.4 in HF versus 105±2.4 in HF+INT-777, $p<0.05$) protein abundance. We also performed studies in cultured human podocytes to determine direct renal specific effects of TGR5 activation. Treatment with INT-777 prevented glucose induced podocyte apoptosis and increased podocyte SIRT1, p-AMPK, and PGC-1α protein abundance. These effects were associated with increased mitochondrial number, decreased mitochondrial ROS production, and increased SOD2 gene expression. In addition, INT-777 decreased SREBP-1 protein and increased PPARα, LCAD, and CPT-1b expression, resulting in decreased lipid accumulation in podocytes.

Conclusions: These studies indicate a novel role for TGR5 in inducing energy metabolism, mitochondrial biogenesis, and fatty acid oxidation which result in prevention of oxidative stress and lipid accumulation and firmly establish an important role for the GPCR TGR5 in preventing kidney disease in obesity.

Funding: NIDDK Support, Veterans Affairs Support

TH-OR151

Glomerular microRNA-26a Expression Correlates with eGFR in Diabetic Nephropathy and Its Regulation in Podocytes Kenichi Koga,[1] Hideki Yokoi,[1] Kiyoshi Mori,[2] Masato Kasahara,[3] Takashige Kuwabara,[1] Moin Saleem,[6] Akira Sugawara,[4] Kazuwa Nakao,[2] Motoko Yanagita,[1] Masashi Mukoyama.[5] *[1]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [2]Medical Innovation Center, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [3]Inst for Advancement of Clinical and Translational Science, Kyoto Univ Hospital, Kyoto, Japan; [4]Dept of Nephrology, Osaka Red Cross Hospital, Osaka, Japan; [5]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan; [6]Academic and Children's Renal Unit, Univ of Bristol, United Kingdom.*

Background: microRNAs are small non-coding RNAs which negatively regulate target genes. We reported that microRNA-26a (miR-26a) directly inhibits connective tissue growth factor (CTGF/CCN2) expression and attenuates TGF-β/SMAD signaling in cultured human podocytes. The aim of this study is to investigate the relation between glomerular miR-26a expression in human diabetic nephropathy (DN) and renal function, and to evaluate its regulation in podocytes.

Methods: We analyzed miR-26a expression in microdissected glomeruli from human biopsy samples of 11 subjects with DN. We transfectedmiR-26a mimic and inhibitor into cultured human podocytes and analyzed expression of the host genes of miR-26a, small C-terminal domain phosphatases 2 and 3 (SCP2/3). We also examined miR-26a and SCP2/3 expressions in glomeruli of streptozotocin (STZ)-induced diabetic mice at 3 weeks after induction of diabetes.

Results: Glomerular miR-26a expression in human DN correlated positively with eGFR ($p < 0.05$, $r = 0.659$). SCP2/3 expressionswere downregulated by miR-26a mimic and upregulated by its inhibitor. SCP2/3 siRNA attenuated TGFβ1-induced ECM accumulation. STZ mice exhibited lower miR-26a and higher CTGF and SCP2/3 expressions in glomeruli than control mice.

Conclusions: We showed that miR-26a expression was lower in glomeruli of both the advanced phase of human DN and the early phase of a mouse model of DN, suggesting that downregulated miR-26a expression together with upregulated SCP2/3 can be involved in the pathophysiology of DN through the dysregulation of CTGF and TGF-β/SMAD signaling.

TH-OR152

Overexpression of Transcription Factor FOXC2 Induces Dedifferentiation and Increased Motility of Cultured Human Podocytes Sanna H. Lehtonen,[1] Naoyuki Miura,[2] Moin Saleem,[3] Neeta Datta.[1] *[1]Haartman Inst, Dept of Pathology, Univ of Helsinki, Helsinki, Finland; [2]Dept of Biochemistry, Hamamatsu Univ School of Medicine, Hamamatsu, Japan; [3]Academic and Children's Renal Unit, Univ of Bristol, Bristol, United Kingdom.*

Background: The molecular mechanisms leading to the development of diabetic nephropathy remain poorly understood, but podocyte injury is known to be involved. We hypothesized that transcription factor FOXC2, known to associate with obesity, insulin resistance and type 2 diabetes mellitus, could play a role in the pathophysiology of diabetic nephropathy.

Methods: We analyzed by immunostaining the localization of FoxC2 in the glomeruli of obese Zucker rats, and overexpressed FOXC2 in cultured human podocytes by lentiviral transduction. The effects on podocytes were studied by immunostaining and quantitative Western blotting, and the motility of podocytes was assessed by the wound healing (scratch) assay.

Results: We observed increased expression and nuclear concentration of FoxC2 in podocytes of obese Zucker rats that are insulin resistant and proteinuric. Overexpression of FOXC2 in differentiated human podocytes *in vitro* led to increased nuclear expression of FOXC2 and dedifferentiation of podocytes associated with a change of cellular morphology. This was accompanied by upregulation of key mesenchymal markers including vimentin, alpha-smooth muscle actin and active beta-catenin, re-organization of the actin cytoskeleton and disrupted localization of the tight junction protein ZO1. FOXC2 overexpressing podocytes also showed increased motility.

Conclusions: The data indicate that the expression and localization of FOXC2 in podocytes needs to be tightly regulated, and that its overexpression induces a chain of cellular events leading to podocyte dysfunction. These changes may lead to podocyte detachment and depletion ultimately contributing to albuminuria and diabetic nephropathy.

Funding: Private Foundation Support

TH-OR153

Attenuated Cholesterol Efflux Causes Podocyte Cholesterol Accumulation and Apoptosis in Diabetic Kidney Disease Christopher E. Pedigo,[1,5] Farah Leclercq,[1,5] Mayrin Correa-Medina,[1,5] Alla Mitrofanova,[1,4,5] Armando Mendez,[3] Robert G. Nelson,[2] George William Burke,[4] Alessia Fornoni,[1,3,5] Sandra M. Merscher.[1,3,5] *[1]Div of Nephrology and Hypertension, Univ of Miami, Miami, FL; [2]NIDDK, Phoenix, AZ; [3]Diabetes Research Inst, Univ of Miami, Miami, FL; [4]Dept of Surgery, Univ of Miami, Miami, FL; [5]Katz Family Drug Discovery Center, Univ of Miami, Miami, FL.*

Background: Diabetic Kidney Disease (DKD) is the most common cause of ESRD and serum Tumor Necrosis Factor alpha (TNFα) levels correlate with the development and progression of DKD. In DKD decreased podocyte number and glomerular cholesterol accumulation are associated with albuminuria. We hypothesized that TNFα causes lipid dependent podocyte apoptosis in DKD.

Methods: Caspase 3 activity was determined in human podocytes. ^{3}H-cholesterol was used for efflux experiments and cyclodextrin to deplete cells of cholesterol. TNFα inhibition was achieved using Infliximab.

Results: Human podocytes cultured in the presence of sera from patients with T2D and progressive DKD (ΔGFR of -97.39±8.2, n=15) revealed significant reduction in ABCA1 (ATP Binding Cassette A1) mRNA expression compared to podocytes cultured in sera from patients with non-progressive DKD (ΔGFR of 40.62±8.6, n=16). TNFα treatment reduced ABCA1 mRNA and protein expression ($p<0.05$) but did not affect mRNA expression of other cholesterol metabolism genes (HMGCR, LDLR and SOAT1). TNFα treatment reduced ABCA1 mediated cholesterol efflux ($p<0.01$) in association with increased cholesterol content ($p<0.05$), reduced esterified cholesterol($p<0.05$) and reduced SOAT1 activity ($p<0.05$). TNFα treatment also increased caspase 3 activity ($p<0.05$) that was prevented by ABCA1 overexpression or cholesterol depletion ($p<0.05$). TNFα inhibition with Infliximab decreased albuminuria in BTBR*ob*/ob mice (T2D and DKD).

Conclusions: ABCA1 expression is reduced in DKD sera treated podocytes. TNFα attenuates ABCA1 mediated reverse cholesterol transport in podocytes leading to cholesterol accumulation and apoptosis. Our data suggest that treatments targeting the TNFα-ABCA1 axis in podocytes may inhibit the development of DKD.

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TH-OR154

Podocyte-Specific Nox4 Deletion Attenuates Albuminuria in Diabetic Nephropathy Jay Chandra Jha,[1,2] Stephen P. Gray,[1] Mark E. Cooper,[1,2] Harald H. Schmidt,[3] Karin Jandeleit-Dahm.[1,2] *[1]Diabetic Complication Div (Diabetes and Kidney Disease Laboratory), Baker IDI Heart and Diabetes Inst, Melbourne, VIC, Australia; [2]Medicine, Monash Univ, Melbourne, VIC, Australia; [3]Dept of Pharmacology Cardiovascular Research Inst, Faculty of Medicine Health & Life Sciences Maastricht Univ, Maastricht, Netherlands.*

Background: Chronic kidney disease is a major microvascular complication of diabetes; however the underlying causes remain unclear. NADPH oxidase and in particular Nox4 derived reactive oxygen species (ROS) in the kidney play a crucial role in the development and progression of diabetic nephropathy. Albuminuria is a key feature of

diabetic nephropathy and podocyte injury leads to the development of albuminuria in diabetes. We examined the effect of podocyte specific NADPH oxidase Nox4 deletion in diabetic nephropathy (DN) using a podocyte specific Nox4 deficient mice.

Methods: Podocyte-specific Nox4 KO (podNox4KO) and floxedNox4 mice were rendered diabetic via streptozotocin injection and followed for 10 and 20 weeks. Urine samples were collected for the assessment of renal function. After 10 and 20 weeks of diabetes animals were culled and kidneys were removed for assessment of structural damage, as well as for the assessment of gene and protein expression of extracellular matrix (ECM), pro-fibrotic and pro-inflammatory markers.

Results: Podocyte-specific Nox4 KO mice were generated. Podocyte specific Nox4 deletion in diabetic podNox4KO mice significantly attenuated the diabetes induced increase in albuminuria compared to the diabetic floxedNox4 mice. However, no significant changes in renal structure as well as glomerular gene expression of profibrotic and proinflammatory markers were observed in diabetic podNox4KO when compared with diabetic floxedNox4 mice. In contrast, the gene expression of nephrin was down regulated in diabetic floxedNox4 mice and was restored to normal in diabetic podNox4KO mice.

Conclusions: Collectively, these results identify Nox4 is a key source of ROS in podocytes and may mediate podocyte injury in diabetes leading to the development of albuminuria.

Funding: Government Support - Non-U.S.

TH-OR155

Unraveling the Mechanism of Action of Glucocorticoids in Glomerulonephritis Christoph Kuppe,[1] Claudia R.C. van Roeyen,[1] Antonio Sechi,[2] Tammo Ostendorf,[1] Bart Smeets,[1] Jürgen Floege,[1] Hermann-Josef Groene,[3] Marcus J. Moeller.[1] *[1]Dep. of Internal Medicine II, Div. of Nephrology and Clinical Immunology, RWTH Aachen, Aachen, Germany; [2]Dep. of Cell Biology, RWTH Aachen Univ, Aachen, Germany; [3]Dep. of Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Heidelberg, Germany.*

Background: Glucocorticoids are commonly used for the treatment of glomerulonephritis. Particularly in the most severe form, crescentic glomerulonephritis (CGN), high-dose steroids are first-line therapy. To date, the mechanisms or target cells of glucocorticoid actions remain incompletely understood. The present study investigated whether glucocorticoids – beside their immunosuppressive effects - directly act on intrinsic glomerular epithelial cells in CGN.

Methods: For this purpose, the glucocorticoid receptor was inactivated specifically in all kidney epithelial cells in Pax8-Cre/GR$^{fl/fl}$ mice, including podocytes and parietal epithelial cells (PECs).

Results: GR inactivation did not impair renal development or function up to 12 months of age. Next, the nephrotoxic serum nephritis model (NTS) was induced in knockout and wild-type control mice. CGN was attenuated by high-dose prednisolone, as expected. Surprisingly, disease activity was also attenuated in renal epithelial GR knockout animals. To verify this, animals were treated with the GR antagonist mifepristone alone. CGN was attenuated more potently than when using prednisolone without obvious effects on the immune system. These findings were verified in a second, non-immunological model (Alport-mouse), where again progression of glomerulosclerosis by activated PECs was attenuated more efficiently by mifepristone than by high-dose prednisolone treatment. Direct actions of steroids on intrinsic renal cells were also verified *in vitro* using primary parietal epithelial cells.

Conclusions: In summary, glucocorticoids exert direct effects on activated epithelial cells in CGN, which appear to be therapeutically more important than immunosuppressive effects. Strikingly, genetic or pharmacological GR inactivation in glomerular epithelial cells is at least as effective as GR stimulation using high-dose steroids. Thus, we propose GR antagonism as novel therapeutic concept.

Funding: Private Foundation Support

TH-OR156

The Kidney Regulates Granulopoiesis and Neutrophil Homeostasis via Tamm-Horsfall Protein–Dependent Epithelial Activation of the IL-23/IL-17 Axis Radmila Micanovic,[1] Edward F. Srour,[1] Pierre C. Dagher,[1] Brahmananda Reddy Chitteti,[1] Shehnaz Khan,[1] Takashi Hato,[1] Tarek M. El-Achkar.[1,2] *[1]Indiana Univ School of Medicine; [2]Indianapolis VA Medical Center.*

Background: Tamm-Horsfall Protein (THP) is a glycoprotein uniquely expressed in the kidney and excreted as one of the most abundant proteins in the urine. We recently showed that THP is also targeted to the interstitium, and plays a role in tubular cross-talk during kidney injury. In this study, we investigate the modulatory role of THP on the immune system.

Methods: We used THP knockout mice and wild type background strains as previously described. Various experimental methodologies and interventions were used as described in results.

Results: Using immuno-histochemistry and flow cytometry in THP-/- and THP+/+ mice, we show that THP deficiency caused significant depletion of resident macrophages in the kidney but not in other organs such as the liver. This was associated with systemic neutrophelia, and stimulation of granulopoesis in the bone marrow. By means of an ELISA multiplex assay, we confirm that THP-/- kidneys are a unique source of myeolopoeitic growth factors, and differentially activate the IL-23/IL-17 axis, which is known to stimulate granulopoiesis. Indeed, neutralization of IL-17 in THP-/-mice reversed the neutrophelia. To determine the source of IL-23, we used real-time PCR on RNA extracted from S3 segments, thick ascending limbs, macrophages and lymphocytes (isolated with laser micro-dissection and FACS, respectively). Surprisingly, S3 segments were the major source of IL-23 in THP-/- kidneys. Furthermore, macrophage depletion in WT mice using clodronate did not increase the levels of IL-23 in the kidneys, suggesting that the effect of THP deficiency on IL-23 is independent from macrophage depletion.

Conclusions: In conclusion, we show that THP is essential to maintaining resident renal macrophages, and that THP deficiency stimulates proximal epithelial activation of the IL-23/IL-17 axis and systemic neutrophelia. Our findings provide novel insights on the immune-modulatory role of THP and its importance in defining how the kidney can regulate granulopoiesis and systemic neutrophil homeostasis.

Funding: Veterans Affairs Support

TH-OR157

The Lymphotoxin β Receptor Is a Therapeutic Target in Renal Inflammation Stephan Segerer,[2] Gitta Seleznik,[1] Harald Seeger,[2] Kai Fu,[3] Maja Lindenmeyer,[2] Marcus J. Moeller,[4] Judith Bauer,[5] Mathias Heikenwaelder,[5] Jeffrey L. Browning.[6,3] *[1]Div of Visceral Surgery, Univ Hospital, Zurich, Switzerland; [2]Div of Nephrology, Univ Hospital, Zurich, Switzerland; [3]Dept of Immunobiology, Biogen Idec, Cambridge, MA; [4]Dept of Nephrology and Clinical Immunology, Rheinisch-Westfälische Technische Hochschule (RWTH) Univ Hospital Aachen, Aachen, Germany; [5]Inst of Virology, TUM, Munich, Germany; [6]Dept of Microbiology and Section of Rheumatology, Boston Univ School of Medicine, Boston, MA.*

Background: Accumulation of inflammatory cells in the tubulointerstitium, at times with a defined microarchitecture, is a hallmark of chronic glomerulonephritis (GN). Lymphotoxin β receptor (LTβR) is important for the formation of lymphoid tissue. We hypothesized that LTβR signaling plays a role in chronic renal inflammation.

Methods: LTs, LTβR, and regulated genes were evaluated by microarray analysis and confirmed by real-time RT-PCR in renal biopsies (n=53). LTβ protein was localized by immunohistochemistry in 48 biopsies from patients with the most common forms of GN. Regulation of LTs and response to LTβR signaling was tested in human mesangial cells, tubular epithelial cells and mouse parietal epithelial cells in-vitro. LTβR signaling was blocked in two mouse models of GN.

Results: Renal biopsies with GN displayed increased levels of LTβ mRNA and protein. LTβ was localized to interstitial lymphocytes, and tubular epithelial cells. Human mesangial and tubular epithelial cells expressed both LTα and LTβ RNA upon stimulation with TNF-α in vitro, and expressed chemokines in response to LTβR signaling. In an anti-glomerular basement membrane antibody induced model of GN, the blockade of LTβR signaling reduced crescent formation and parietal epithelial cells responded to LTβR signaling with CCL2 induction. In a lupus model, LTβR blockade improved renal function without reduction in glomerular immune complex deposition.

Conclusions: Preclinical mouse models and human data strongly suggest that LTβR signaling is involved in renal injury and is a new therapeutic target in renal diseases, mediating a novel pathway in parietal epithelial cell activation with crescent formation.

Funding: Government Support - Non-U.S.

TH-OR158

Milk Fat Globule-Epidermal Growth Factor-8 Limits Tissue Damage Through Inflammasome Inhibition in Renal Obstruction Jean-Francois Cailhier, Marie-Joelle Brissette, Patrick Laplante. *Medicine, CRCHUM, Univ de Montreal, Montreal, QC, Canada.*

Background: Renal diseases are characterized by tubulointerstitial fibrosis due to the loss of renal parenchymal cells. During renal inflammation, macrophages induced apoptosis of resident cells leading to renal fibrosis. We described that milk fat globule-epidermal growth factor 8 (MFG-E8) released by apoptotic renal cells can modulate the renal microenvironment and reprogram macrophages. In this study, we investigated the role of MFG-E8 on the modulation of inflammasome activation, tissue damage and fibrosis in a renal obstruction model.

Methods: C57BL/6 WT or MFG-E8 KO mice underwent unilateral ureteral ligation for 3, 7 and 14 days. MFG-E8 (30μg/kg) was administered intra-peritoneally 1 day before and every 3 days subsequently. In order to evaluate the role of MFG-E8 on macrophage reprogramming, inflammasome activation and renal damage, we adoptively transferred MFG-E8-treated macrophages to MFG-E8 KO mice prior to the renal obstruction. At the end of experiments, kidneys were harvested and were stained for PAS and Sirius Red to evaluate renal damage and fibrosis. Kidneys were also harvested for immunoblotting against inflammasome components (NLRP3, ASC, Caspase-1, IL-1β and IL-17).

Results: MFG-E8 reduced kidney damage and fibrosis compared to control, whereas its absence in the MFG-E8 KO mice was associated with worse disease. Moreover, MFG-E8 administration was associated with a decreased activation of inflammasome in the kidney. Furthermore, administration of ex vivo MFG-E8-stimulated macrophages prior to obstruction conferred renal protection against kidney damage by reducing inflammasome activation and renal injury.

Conclusions: MFG-E8 reduced renal damage through decreased activation of inflammasome. Moreover, administration of ex vivo MFG-E8-stimulated macrophages was sufficient to attenuate renal inflammasome and renal damage suggesting that MFG-E8 present in the renal microenvironment is crucial for macrophage reprogramming. These data identified MFG-E8 as a novel target for the regulation of renal inflammation and chronic kidney disease progression.

Funding: Private Foundation Support

TH-OR159

Myeloid-Derived tPA Promotes Macrophage Motility through FAK, Rac1, and NF-κB Pathway Ling Lin, Kebin Hu. *Medicine, Penn State Univ College of Medicine, Hershey, PA.*

Background: Macrophage accumulation is one of the hallmarks of progressive kidney disease. Tissue-type plasminogen activator (tPA) is known to promote macrophage infiltration and renal inflammation during chronic kidney injury. However, the underlying mechanism remains largely unknown.

Methods: We examined the role of tPA in macrophage motility in vivo by tracking fluorescence-labeled bone marrow-derived macrophages, generated bone marrow chimeric mice to determine the source of endogenous tPA that promotes renal inflammation and macrophage infiltration, and investigated the underlying signaling mechanisms.

Results: We found that tPA-deficient mice had markedly fewer infiltrating fluorescence-labeled macrophages than the wild-type mice. Experiments in bone marrow chimeric mice further demonstrated that myeloid cells are the main source of endogenous tPA that promotes macrophage migration. In vitro studies showed that tPA promoted macrophage motility through its CD11b-mediated protease-independent function; and FAK, Rac-1 and NF-κB were indispensable to tPA-induced macrophage migration as either infection of FAK dominant-negative adenovirus or treatment with a Rac-1-specific inhibitor or NF-κB inhibitor abolished the effect of tPA. Moreover, ectopic FAK mimicked tPA and induced macrophage motility. tPA also activated migratory signaling in vivo. The accumulation of phospho-FAK-positive CD11b-macrophages in the obstructed kidneys from wild-type mice was clearly attenuated in tPA knockout mice, which also displayed lower Rac-1 activity than their wild-type counterparts.

Conclusions: Therefore, our results indicate that myeloid-derived tPA promotes macrophage migration through a novel signaling cascade involving FAK, Rac-1 and NF-κB.

Funding: Private Foundation Support

TH-OR160

Lysosomal Transporter Cystinosin Alters Macrophage Activation and Promotes Renal Fibrosis Daryl M. Okamura, Nadia Bahrami. *Center for Developmental Biology & ReGenerative Medicine, Seattle Children's Research Inst, Seattle, WA.*

Background: Nephropathic cystinosis has traditionally been considered a primary disease of renal tubules induced by the toxic effects of lysosomal cystine accumulation, which leads to renal Fanconi syndrome and eventual tubular apoptosis. However, recent studies demonstrate that the cystinosin-deficient (CTNS-/-) macrophage (mphi) plays a critical role in progressive nephron loss.

Methods: The present study was designed to investigate the role of Ctns in mphi function in nephropathic cystinosis and during kidney injury.

Results: In order to investigate Ctns-/- mphi behavior, we performed FACS analysis on mphi subpopulations in nephropathic cystinotic kidneys at mild (6m) to moderate-severe fibrosis (12m). We found that the number of M1-type mphi ($Ly6c^{med/hi}$) almost doubled between 4 to 6 months and suggests that these cells may initiate an early pro-inflammatory response. During the progression from mild to moderate-severe fibrosis (12m) there was a significant increase in the M1 subset by 120% and is the predominant subpopulation within the cystinotic kidney. These results suggest that M1 mphi and phagocytic dendritic cells are important in the progression of injury in nephropathic cystinosis. We examined proinflammatory and fibrogenic gene expression with M1/M2 activation in bone marrow derived macrophages (BMDM). At baseline, Ctns-/- BMDM have a 43-fold increase in iNOS expression (P=0.03) and with M1 activation, there is a 6.3-fold increase in IL6 expression (P=0.001) compared to Ctns+/+ BMDM. With M2 activation, there was a nearly 6-fold reduction in IL4 receptor and a 2.2-fold reduction in IL10 expression. We further examined the role of Ctns in mphi phagocytosis and chemotaxis. We found that Ctns-/- mphi are hyperphagocytic and do not respond appropriately to chemotactic stimuli. Using a model of chronic kidney injury, we found a significant increase (63%) in Ctns-/- mphi with more severe fibrosis (increased 19%) in UUO kidneys compared to wild-type mice.

Conclusions: These data suggest that the lysosomal transporter cystinosin may play an important role not only in nephropathic cystinosis but in chronic kidney disease progression as well.

Funding: Private Foundation Support

TH-OR161

A Small Molecule Inhibitor of Apoptosis Signal-Regulating Kinase 1 (ASK1) Reduces Key Pathological Processes Related to CKD John T. Liles,[1] Haichun Yang,[2] Agnes B. Fogo,[2] David G. Breckenridge.[1] *[1]Gilead Sciences, Inc; [2]Dept of Pathology, Microbiology and Immunology, Vanderbilt Univ Medical Center.*

Background: Oxidative stress (OS) is elevated in patients with chronic kidney disease (CKD) and is a major driver of disease progression. ASK1 is a critical signaling node through which OS promotes inflammation, apoptosis and fibrosis via downstream activation of the MAPK kinases p38 and JNK.

Methods: To validate ASK1 as a therapeutic target in CKD, we evaluated the effects of a potent and selective small molecule inhibitor of ASK1 (GS-444217) in rodent models of acute and chronic kidney disease.

Results: In an acute renal I/R rat model (30 min of ischemia followed by 24 hr reperfusion), prophylactic treatment with GS-444217 (30 mg/kg, p.o.) significantly reduced elevations in serum creatinine (1.76 ±0.4 [SEM] versus 0.85 ±0.2 mg/dl) and BUN (100 ±12 versus 58 ±8 mg/dl), and decreased renal apoptosis (61 ±8 versus 39 ±8; # TUNEL cells) and tubular necrosis (pathology score of 3.7 ±0.3 versus 2.7 ±0.2). Unilateral ureteral obstruction (UUO) for 7 days in rats caused significant increases in renal ASK1 activation, fibrosis, and apoptosis compared to sham animals. GS-444217 (30 mg/kg b.i.d., p.o, 7 days) blocked activation of ASK1 pathway, reduced fibrosis by 88% as measured by picrosirius red staining, and reduced apoptosis by 79% as measured by TUNEL. Rats given deoxycorticosterone acetate (DOCA)-salt for 2 weeks had progressive increases in blood pressure and urinary albumin-to-creatinine ratio (ACR). GS-444217 reduced ACR (2.4 ±0.5 versus 0.3 ±0.1 mg/mg)and decreased both urinary KIM-1 and 8-OH-dG by 50% without impacting systemic blood pressure. 5/6 nephrectomized (5/6 Nx) rats treated orally with GS-444217 for 4 weeks (starting 8 weeks after 5/6 Nx) had decreased glomerulosclerosis (pathology score 1.21 ± 0.12 versus 0.7 ± 0.1) and ACR (152±21 versus 52 ±9mg/mg) compared to vehicle-treated 5/6 Nx.

Conclusions: Results from several *in vivo* studies demonstrate that selective inhibition of ASK1 decreases renal injury, tubulointerstitial fibrosis, glomerulosclerosis, and proteinuria, and improves renal function. The data provide compelling evidence for ASK1 as a therapeutic target in kidney disease.

Funding: Pharmaceutical Company Support - Gilead Sciences, Inc

TH-OR162

Loss of *Sec63* and *Xbp1* Caused Renal Interstitial Inflammation and Fibrosis Yasunobu Ishikawa,[1] Sorin V. Fedeles,[1] Ming Ma,[1] Rachel Gallagher,[1] Stefan Somlo.[1,2] *[1]Internal Medicine, Yale Univ School of Medicine, New Haven, CT; [2]Genetics, Yale Univ School of Medicine, New Haven, CT.*

Background: *SEC63* is one of genes linked with the occurrence of autosomal dominant polycystic liver disease. The product of *SEC63*, SEC63p is a protein associated with the SEC61 translocon and involved in co-translational protein translocation and folding. In the presence of endoplasmic reticulum (ER) stress, a housekeeping mechanism called the unfolded protein response (UPR) is turned on in order to mitigate stress. The UPR is mediated by three major stress sensors, IRE1α, PERK and ATF6. Phosphorylation of IRE1α leads to spliced XBP1 which is a transcription factor that activates transcription of chaperones and proteins involved in ER-associated degradation. In the current study we investigated the effect of Xbp1 inactivation in the kidney in the presence and absence of Sec63.

Methods: We used combinatorial genetics to generate WT, SKO (*$Sec63^{fl/fl}$;$Pax8^{rtta}$;TetOcre*) and DKO (*$Sec63^{fl/fl}$;$Xbp1^{fl/fl}$;$Pax8^{rtta}$;TetOcre*) mice. All mice were induced with 2 mg/mL doxycycline (DOX) in drinking water for 2 weeks beginning at P28. We investigated the kidney phenotype and UPR activation pathways 1, 2, 3 and 6 weeks kidney after the start of DOX induction.

Results: Kidney weight to body weight ratio of DKO mice 1 and 2 weeks after the start of induction was higher than those of WT and SKO mice due to the accumulation of inflammatory cells, while the WT and SKO kidneys were normal by histology. The interstitial inflammatory infiltrate in the DKO kidneys included lymphocytes, plasma cells, macrophages and fibroblasts. Few cysts were also observed. Gene expressions of Mcp-1, F4/80 and Pai-1 were elevated 1 week after the start of induction, while Tgf-β and Fsp-1 were increased 2 weeks after the start of induction. Notably, dual loss of *Sec63* and *Xbp1* lead to a complete activation of the Ire1α pathway while Perk and Atf6 did not exhibit signs of activation compared to WT and SKO kidneys.

Conclusions: Activated IRE1α is an important driver of renal interstitial inflammation and fibrosis like chronic kidney disease (CKD) in the absence of *Sec63*/*Xbp1*. The *Sec63*/*Xbp1* DKO mice may represent a useful genetic model of CKD.

TH-OR163

De Novo Synthesis of Acetylcholine by Podocytes Is a Key Mechanism for Kidney Self-Defense against Progressive Kidney Injury Gabriela E. Garcia,[1] Luan D. Truong,[2] Jessica Helen Trostel,[1] Richard J. Johnson,[1] Lili Feng.[3] *[1]Medicine, Univ of Colorado Denver, Aurora, CO; [2]Pathology, The Methodist Hospital, Houston, TX; [3]Medicine, Baylor Collage of Medicine, Houston, TX.*

Background: There is a communication between the immune and neuroendocrine systems. Acetylcholine (ACh), the principal vagal neurotransmitter, is expressed in non-neuronal cells and inhibits proinflammatory cytokines production.

Methods: Using ChAT(BAC)-EGFP transgenic mice, which express enhanced green fluorescent protein (EGFP) under the control of transcriptional regulatory elements for choline acetyltransferase (ChAT), the sole enzyme that produces ACh, we investigated the role of kidney derived ACh in kidney injury in the cytokine-dependent anti-glomerular basement membrane glomerulonephritis (anti-GBM GN).

Results: ChAT is expressed in the kidney, during GN ChAT expression is induced in the glomeruli, mainly in podocytes. Mice with less kidney injury expressed less podocytes ChAT. In contrast, higher podocytes ChAT expression was observed in mice with more kidney injury, suggesting that increased ChAT is a counter-regulatory response to inflammation. Importantly, nephritic kidneys from ChAT mice showed less kidney damage compared to WT mice. Glomerular proliferation, fibrinoid necrosis, crescent formation, tubulointerstitial injury and macrophages (MΦ) infiltration were significantly lower in ChAT mice. Similarly, collagen (Col) III and Col IV deposition were decreased in nephritic kidneys from ChAT mice compared with WT mice. To investigate if ACh could arrest established GN, anti-GBM GN was induced in WKY rats to generate a progressive model of kidney injury. Treatment with donepezil (DPZ), that increases ChAT promoter activity and ChAT protein amount and inhibits acetylcholinesterase, was delayed until day

3 or day 6 after the induction of GN and rats were euthanized at day 25. In rats treated with DPZ, glomerular proliferation, necrotizing lesion, MΦ infiltration and Col deposition were significantly reduced compared with the control group.

Conclusions: These findings suggest that de novo synthesis of podocytes ACh protects from excessive inflammation preventing progressive kidney fibrosis.

Funding: NIDDK Support

TH-OR164

Transcriptome-Based Cluster Analysis Identifies TNF-Subgroup in Focal and Segmental Glomerulosclerosis Sebastian Martini,[1] Viji Nair,[1] Felix H. Eichinger,[1] Heather N. Reich,[2] Matthias Kretzler.[1] *[1]Internal Medicine, Div of Nephrology, Univ of Michigan, Ann Arbor, MI; [2]Div of Nephrology, Univ Health Network, Toronto General Hospital, Toronto, ON, Canada.*

Background: The histopathology-based taxonomy of FSGS and MCD fails to capture the molecular basis of these diseases and does not adequately predict response to therapy. Novel treatment strategies like anti-TNF-alpha drugs (FONT trial, Trachtman, *Am J Kidney Dis. 2010*) led to remission induction in only ~25% of participating FSGS patients. We here describe a strategy that could potentially predict treatment response using transcriptomic profiling.

Methods: Transcriptomic profiles from microdissected clinically indicated renal biopsies were generated from 49 study participants with FSGS or MCD of the NEPTUNE study using the Affymetrix 2.1 ST platform. Gene expression levels were analyzed by hierarchical clustering via Arraytrack. The functional context was defined using Ingenuity pathway analysis tools and the Connectivity Map project (CMAP) database that comprises transcriptomic profiling data from >6,100 treatment control pairs and >1,300 pertubagens.

Results: Unsupervised hierarchical clustering grouped subjects into 3 distinct molecular subgroups with significant differences in the clinical outcomes: proteinuria remission (25 remission cases in cluster 1+2 versus none in cluster 3) and eGFR at baseline (cluster 1/2/3: 108/79/42 mL/min/m^2). Functional analysis of the transcripts differentially regulated between subgroups identified a transcriptional network centered on TNF-alpha whose transcripts were upregulated in cluster 3 compared to clusters 1+2. This upregulation of TNF-associated genes was confirmed in an independent cohort of 31 patients with FSGS or MCD and was concordant with the CMAP findings that predicted a reversal of expression signatures in cluster 3 with therapeutics targeted against TNF alpha.

Conclusions: Developing a molecular taxonomy for primary proteinuric kidney disease should rationalize clinical trial design and identify patients most likely to benefit from intervention for enrollment. Supported by NIH grant to NEPTUNE: U-54-DK-083912.

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TH-OR165

Apolipoprotein L1 Gene Variants in Deceased Organ Donors Are Associated with Renal Allograft Failure Barry I. Freedman,[1] Bruce A. Julian,[2] Stephen O. Pastan,[3] Ajay K. Israni,[4] Allan D. Kirk,[5] Robert S. Gaston,[2] Robert J. Stratta,[1] Sumit Mohan,[6] Patricia L. Adams,[1] Amudha Palanisamy,[1] Amber M. Reeves-Daniel,[1] Jasmin Divers.[1] *[1]Wake Forest School of Medicine; [2]Univ of Alabama at Birmingham School of Medicine; [3]Emory Univ School of Medicine; [4]Hennepin County Medical Center, Univ of Minnesota; [5]Duke Univ School of Medicine; [6]Columbia Univ College of Phaysicians and Surgeons.*

Background: Apolipoprotein L1 gene (APOL1) nephropathy variants in African American deceased kidney donors were associated with shorter renal allograft survival in a single-center report from North Carolina. Replication analyses were performed in a larger cohort.

Methods: APOL1 G1 and G2 nephropathy variants were genotyped in newly accrued DNA samples from African American deceased donors of kidneys recovered and/or transplanted in Alabama and North Carolina. Data from the Scientific Registry of Transplant Recipients (SRTR) was utilized to link allograft outcomes and APOL1 genotypes (recessive model) in subsequent kidney transplants performed at 55 centers across the country, adjusting for age, sex and ancestry of recipients, HLA match, cold ischemia time, panel reactive antibody levels, and standard-criteria versus expanded-criteria donor.

Results: For 221 transplantations from kidneys recovered in Alabama, allograft failure was significantly associated with APOL1 genotype (odds ratio [OR] 2.87; p=0.04). For the 675 kidneys transplanted from donors at both centers, APOL1 genotype (OR 1.83; p=0.03), African American recipient ancestry (OR 1.72; p=0.01), and expanded-criteria donation (OR 1.79; p=0.04) were associated with allograft failure. Allograft survival was significantly shorter in recipients of two-APOL1-nephropathy-variant kidneys (hazard ratio 2.26; p=0.001).

Conclusions: Kidneys from African American deceased donors with two APOL1 nephropathy variants are reproducibly associated with a higher risk for allograft failure after transplantation. These findings warrant consideration of rapid genotyping of deceased African American kidney donors for APOL1 G1 and G2 variants at the time of organ recovery and incorporation of the results into allocation and informed-consent processes.

Funding: NIDDK Support

TH-OR166

Variation in the Multidrug Resistance Protein 1 Gene (*ABCB1*) in African American Deceased Organ Donors Is Associated with Renal Allograft Failure Jun Ma,[1] Jasmin Divers,[1] Nicholette D. Palmer,[1] Bruce A. Julian,[2] Stephen O. Pastan,[3] Ajay K. Israni,[4] Robert S. Gaston,[2] Lijun Ma,[1] Robert J. Stratta,[1] Amudha Palanisamy,[1] Amber M. Reeves-Daniel,[1] Barry I. Freedman.[1] *[1]Wake Forest Sch Med; [2]Univ of Alabama at Birmingham Sch Med; [3]Emory Univ Sch Med; [4]Hennepin County Med Cen, Univ of Minnesota.*

Background: ABCB1 and caveolin 1 (CAV1) gene variants in deceased organ donors of European ancestry are associated with allograft failure after kidney transplantation.

Methods: To further analyze this effect in African Americans (AAs), 39 haplotype-tagging single nucleotide polymorphisms (SNPs) spanning ABCB1 and 15 tag SNPs spanning CAV1 were genotyped in 368 AA deceased organ donors; 679 resultant kidney transplants were assessed. Tests for genetic association were performed with time to allograft failure in the Scientific Registry of Transplant Recipients (SRTR) adjusting for recipient age, sex and ethnicity, cold ischemia time (CIT), panel reactive antibodies (PRA), HLA mismatch, standard- versus expanded-criteria donation, and apolipoprotein L1 gene variants.

Results: AA deceased kidney donors were 60.3% male and 82.3% standard-criteria donors, with mean±SD (median) age 34.9±16.8 (36) years, terminal serum creatinine 1.25±0.7 (1.1) mg/dl, CIT 23.5±11.2 (22) hours, HLA mismatch 4.3±1.4 (5), and recipient PRA 23.9±32.7 (5.5)%. In fully adjusted models, four ABCB1 SNPs were significantly associated with time to renal allograft failure: rs1055302 (hazard ratio±SE 1.32±0.08; p=0.0007, additive model), rs17064 (HR±SE 2.51±0.25; p=0.0002 recessive), rs17209837 (HR±SE 1.29±0.08; p=0.0021 additive), and rs3789246 (HR±SE 0.54±0.22; p=0.005 recessive); two ABCB1 SNPs were weakly associated: rs6949448 and rs956825 (both p<0.03, recessive). In contrast, CAV1 SNPs were not significantly associated with renal allograft failure.

Conclusions: Genetic variation in ABCB1 in deceased organ donors of European and African ancestry is reproducibly associated with allograft failure after kidney transplantation. Because ABCB1 mediates cellular transport of calcineurin inhibitors, alterations in drug metabolism may be involved. Pharmaco-genetic profiling based on ABCB1 should be evaluated to improve outcomes in kidney transplantation.

Funding: NIDDK Support

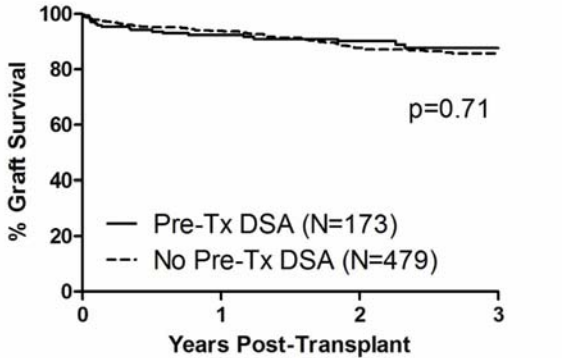
TH-OR167

Impact of Pre Transplant Donor Specific Antibodies in the Setting of a Negative Cell Based Flow Cytometry Cross Match on Graft Outcomes Oluwafisayo O. Adebiyi, Jane Gralla, Alexander C. Wiseman, James E. Cooper. *Div of Renal Disease and Hypertension, Univ of Colorado, Aurora, CO.*

Background: Presence of antibodies to donor specific HLA antigens (DSA) detected by single antigen bead (SAB) analysis prior to kidney transplant(Tx) has been associated with inferior graft outcomes. However, it is unclear if low-level DSA leads to a higher risk of rejection or late graft dysfunction. Most studies, limited by small sample size, have not considered flow cytometry cross match(FCXM) results or desensitization therapy in the analysis.

Methods: 652 recipients of a kidney or kidney/pancreas Tx with a negative pre-Tx FCXM(-FCXM) between September 2007 and August 2012 at our center without desensitization therapy were analyzed. All patients underwent cell-based FCXM and SAB analysis on current and historic sera prior to transplantation. Patients were placed on triple immunosuppression. Graft outcomes were compared for patients with (DSA+) and without (DSA-) pre-Tx DSA.

Results: Of 652 patients with -FCXM, 173 had DSA (MFI ≥ 500) detected prior to Tx by SAB analysis. Acute rejection (AR) rates at one year were similar in DSA+ versus DSA- groups, (14.2% versus 11.6% respectively, p=0.349) With a median follow up period of 2.3 years, mean GFR, mean proteinuria, 3 year graft survival by Kaplan-Meier estimate were similar in DSA+ and DSA-patients (p= 0.71) and remained similar irrespective of the strength of DSA. Effect of pre-Tx DSA on graft outcome remained non-significant with multivariate analysis.



Conclusions: To our knowledge, this is the largest series characterizing graft outcomes of patients with pre-Tx +DSA/-FCXM. Presence of pre-Tx DSA in the setting of -FCXM was not associated with poorer graft outcomes. These data suggest that DSA detected by SAB alone may confer minimal immunologic risk and does not require preemptive B-cell/antibody-targeted therapies.

TH-OR168

Persistence or De Novo Occurrence of C1q-Binding Donor Specific Antibodies Strongly Predict Future Graft Loss in Presensitized High-Risk Kidney Transplant Recipients

Sebastian Markus Schaefer,[1] Caner Süsal,[2] Gerhard Opelz,[2] Luis Eduardo Becker,[1] Stefanie Sickmüller,[1] Ruediger Waldherr,[3] Stephan Macher-Goeppinger,[3] Martin G. Zeier,[1] Christian Morath.[1]
[1]Nephrology, Univ of Heidelberg, Heidelberg, Germany; [2]Transplantation Immunology, Univ of Heidelberg, Heidelberg, Germany; [3]Pathology, Univ of Heidelberg, Heidelberg, Germany.

Background: Certain subgroups of presensitized patients are at a higher risk for early adverse events post transplant (tx).

Methods: We studied the impact of pre- and post-tx presence of HLA antibodies on subsequent occurrence of antibody-mediated rejection (AMR) episodes and graft loss in a unique cohort of 80 presensitized high-risk kidney tx recipients. All patients received desensitization by apheresis and anti-CD20 therapy, and were transplanted until December 2011. Follow-up was until September 2013. Single Antigen Bead (SAB) assay-detected DSA with or without C1q-binding capability were measured a) pre-tx, and b) post-tx, either before the occurrence of an AMR episode, before graft loss, or at year one.

Results: The rates of AMRs and graft loss were not significantly different in patients with or without C1q-binding capability of their pre-tx DSA. This was attributable to the post-tx loss of pre-tx C1q-DSA in 11 of 13 patients who showed only one graft loss during the further course. In contrast, post-tx presence of C1q-DSA was associated with a significantly higher rate of AMRs (compared to the absence of C1q-DSA or the overall absence of DSA: 86% versus 33% versus 0%, p<0.001) and graft loss from rejection (86% versus 3% versus 3%, p<0.001). Patients with post-tx C1q-DSA had either persistent (N=1), de novo (N=6) or persistent and de novo (N=1) C1q-DSA. Patients with persistent C1q-DSA (N=2) had higher pre-tx SAB IgG-DSA mean fluorescence intensity (MFI) values as compared to C1q-DSA that were lost post-tx (24.867-28.435 MFI versus 1.098-13.317 MFI). De novo DSA (N=6) occurred primarily in patients with insufficient immunosuppression in the early post-tx phase.

Conclusions: In this unique cohort of presensitized kidney tx recipients we found that persistent and de novo C1q-DSA in the early post-tx phase were strongly associated with AMR episodes and consecutive graft loss.

Funding: Private Foundation Support

TH-OR169

A Novel Multiplex Approach to Define Peripheral Blood HLA-Specific B-Cell Subsets in Clinical Transplantation

Ahmed Akl, Anat R. Tambur, Mohammed Javeed Ansari. *CTC, Northwestern Univ, Chicago, IL.*

Background: Naïve B cells have been associated with long term allograft survival. Memory B cells have been linked to poor allograft survival. Role of HLA-specific B cells in chronic allograft rejection is still unknown. Quantifying HLA-specific B cells (donor specific or not) might be of importance for better understanding of the mechanisms of graft lesions, for an earlier diagnosis of chronic rejection, and eventually to guide specific B cell-targeted therapy. In here, we describe our method to quantify, characterize and correlate the phenotype of HLA-specific B cells (HSB) in kidney transplant recipients (TR).

Methods: PBMC from TR [n=8; 4 patients with good graft outcome (GO), the others with poor outcome (PO)] and healthy volunteers (HV) [n=10] were incubated with HLA single antigen beads (SAB) for class I and II. HLA Bead-B-cell Rosette (BBR) frequency and specificity were analyzed by flow cytometry. HSB polyreactivity was defined by the percentage of total SAB forming BBR. TR serum was tested for HLA antibodies using SAB. Phenotype of total B cells and HSB was determined by CD27, CD38 and IgD expression.

Results: Significantly higher frequency of HSB were identified in TR compared to HV. HSB were in higher frequency (HLA class I: 1.86% versus 0.47%; class II: 8% versus 0.7% ; p<0.05), had a wide breadth of polyreactivity (HLA class I: 76.97% versus 13.1%; class II: 91.9% versus 13.69%; p<0.05) among PO compared with GO. Not all HSB were associated with IgG HLA antibodies. Majority of HSB and total B cells were in the naive compartment in the GO while in the PO HSB were among the memory, pregerminal and centroblasts phenotypes.

Conclusions: Our novel approach enables identification and characterization of HLA-class I and II specific B cells. HSB memory phenotype correlated with poor graft outcome. Further functional and gene expression analysis of HSB will be done.

Figure 1: A) Significant difference between the frequency of B cell rosettes identified in transplant recipients compared with healthy volunteers (HLA class I p=0.024; class II P<0.05). Anti-HLA class II B cell recognition pattern differences at 12 months PT between transplant recipient with good (B) and in contrast poor (C) graft outcome 2 years later (3 years PT). dark dots represent the HSB, orange dots represent the HLA beads.

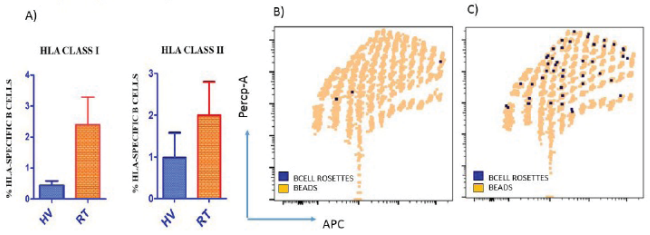
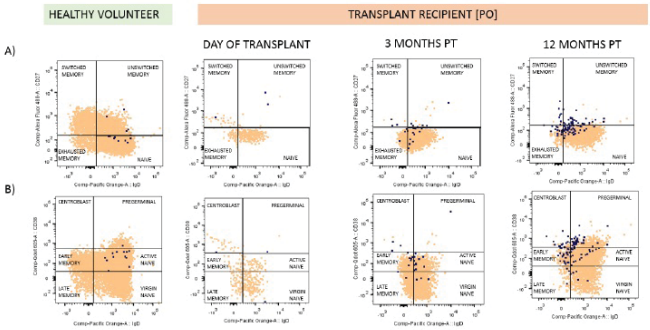


Figure2: A) CD27/IgD classification of B cells shows HSB (in dark dots) expansion and deviation toward unswitched, switched and exhausted memory phenotypes within total B cells (in orange dots). B) CD38/IgD classification of B cells shows HSB (in dark dots) expansion and distribution among active naïve, pregerminal, centroblasts, early memory phenotypes within total B (in orange dots).



TH-OR170

Urine Kidney Injury Biomarkers and Risk of Cardiovascular Events, Death and End-Stage Renal Disease among Prevalent Kidney Transplant Recipients: The FAVORIT Trial

Nisha Bansal,[1] Daniel E. Weiner,[2] Myra A. Carpenter,[3] Andrew S. Levey,[2] Marc A. Pfeffer,[4] Meyeon Park,[5] Kathleen D. Liu,[5] John W. Kusek,[6] Chi-Yuan Hsu.[5] *[1]UW; [2]Tufts; [3]UNC; [4]BWH; [5]UCSF; [6]NIH.*

Background: Kidney transplant recipients (KTR) are at high risk for cardiovascular (CVD) and kidney failure. Urine injury biomarkers are associated with poor outcomes in native kidney disease. It is unknown whether the same associations are true among KTR.

Methods: We conducted a post hoc analysis of FAVORIT, a multicenter, trial of KTR examining the effect of B-vitamin therapy on CVD and death. We used a case-cohort design, selecting 737 participants with adjudicated CVD events, ESRD or death and a random sub-cohort of 489 participants. CVD was defined by CVD death, myocardial infarction, and stroke. Urine neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule (KIM)-1, interleukin (IL)-18 and liver-type fatty acid binding protein (L-FABP) were measured in spot urine samples at baseline and standardized to urine creatinine concentration. Weighted Cox models were used to examine the association of each biomarker with each outcome.

Results: Among the 489 participants in the random sub-cohort, mean age was 51.4(±9) years, 39% were women, 18% were Black and median graft vintage was 3.8(1.7, 7.0)years. Mean systolic blood pressure was 136(±20)mm Hg, 37% had diabetes, mean eGFR was 46(±18)ml/min/1.73 m2 and median ACR was 24.5(9.5, 104.7)mg/g. 281 adjudicated CVD events, 359 deaths and 257 cases of ESRD were identified. Higher NGAL was associated with greater risk of CVD, death and ESRD (Table). Higher KIM-1 and IL-18 were associated with greater risk of death (Table).

Conclusions: Among KTR, elevations in urine NGAL, KIM-1 and IL-18 are associated with death and elevation in NGAL is associated with CVD and ESRD, even with adjustment for ACR. Urine biomarkers may help identify KTR at high-risk for poor outcomes.

Urine biomarker / urine creatinine	Median (IQR) level in random cohort	Cardiovascular Events HR (95%) CI (per log increase)*	All-cause Death HR (95%) CI (per log increase)*	ESRD HR (95%) CI (per log increase)*
NGAL	0.2 (0.1, 0.5) mg/dL	1.24 (1.06-1.45)**	1.44 (1.26-1.65)**	1.40 (1.16-1.68)**
KIM-1	7.3 (3.8, 12.8) mg/dL	1.14 (0.91-1.43)	1.29 (1.03-1.61)**	0.93 (0.71-1.22)
IL-18	0.3 (0.1, 0.7) mg/dL	1.03 (0.86-1.23)	1.25 (1.04-1.49)**	1.15 (0.93-1.43)
L-FABP	0.1 (0.0, 0.2) mg/dL	1.00 (0.83-1.20)	1.12 (0.94-1.35)	1.07 (0.85-1.35)

*Adjusted for demographics, treatment, country, history of CVD, diabetes, smoking, graft vintage, donor, blood pressure, lipids, BMI, eGFR, urine ACR
**p<0.05

Funding: NIDDK Support

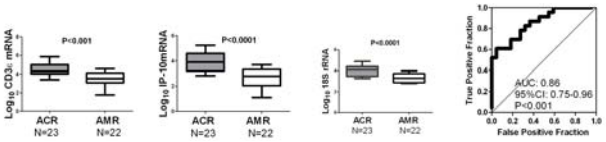
TH-OR171

Distinguishing Acute Antibody-Mediated Rejection (AMR) From T Cell-Mediated Acute Cellular Rejection (ACR) with the 3-Gene Urinary Cell Signature Thangamani Muthukumar, Darshana Dadhania, Ruchuang Ding, Catherine Snopkowski, Carol Y. Li, John R. Lee, Hua Yang, Vijay K. Sharma, Manikkam Suthanthiran. *Cornell Univ.*

Background: Noninvasive diagnosis of ACR and AMR would represent a major clinical progress. We recently reported that a 3-gene signature of 18S rRNA normalized measures of CD3ε mRNA, IP-10 mRNA and 18S rRNA in urinary cells discriminates between biopsies showing ACR and those not showing rejection (Suthanthiran M et al. N Engl J Med 2013). Despite mRNA profiling of 4300 urine specimens from 485 kidney graft recipients prospectively enrolled in the CTOT-4 study, there were insufficient cases of AMR (9 patients) to determine whether the signature distinguishes ACR from AMR. We have addressed this issue by profiling 45 urine specimens collected from 45 kidney allograft recipients from an independent study cohort.

Methods: Biopsy-matched urine samples were collected from 22 patients with AMR and 23 patients with ACR prior to any anti-rejection therapy. We measured absolute mRNA copies from the urinary cells by RT-PCR assay.

Results: CD3ε mRNA, IP-10 mRNA and 18S rRNA (Fig.1A-C) were higher in patients with ACR compared to patients with AMR. The 3-gene signature accurately discriminated ACR from AMR (Fig.1D).



Additionally, we found that the 3-gene signature distinguishes not only ACR from biopsies showing acute tissue injury (ATI, n=29 patients) due to non-immunological causes, but also AMR from the ATI biopsies (AUC 0.97 [95%CI 0.93-1.00] P<0.0001 and 0.72 [95%CI 0.56-0.87] P<0.01, respectively).

Conclusions: Our findings that the 3-gene signature of 18S rRNA normalized measures of CD3ε mRNA, IP-10 mRNA and 18S rRNA in urinary cells accurately discriminates ACR from AMR, ACR from acute graft injury and AMR from acute graft injury demonstrate the feasibility of noninvasive diagnosis of ACR, AMR or acute graft injury due to non-immunological causes.

Funding: NIDDK Support

TH-OR172

Soluble CASK, a New Factor Implicated in the Recurrence of FSGS After Renal Transplantation Séverine Beaudreuil,[12] Xiaomeng Zhang,[2] Ye Fan,[2] H. Francois,[12] L. Lecru,[2] Jean Jacques Candelier,[2] Bernard Charpentier,[12] Aime Vazquez,[2] Hans Kristian Lorenzo,[12] A. Durrbach.[12] *[1]Nephrology Dialysis Transplantation IFRNT, Univ Hospital Paris Sud, Le Kremlin Bicetre, France; [2]INSERM U1014, Villejuif, France.*

Background: Focal segmental glomerulosclerosis (FSGS) account for 20% of all cases of nephrotic syndrome, both in children and adults. Its recurrence after renal transplantation has suggested the presence of a soluble factor of permeability (sFP) which has not be clearly characterized. In case of recurrent FSGS, the reduction of proteinuria with immunoadsorption on protein A columns (IA) suggest that the sFP can bind to this columns and cad be eluted from them. In order to characterize the sFP, we have analyze the elute from these columns.

Methods: Elutes of IA used for the treatment of recurrent FSGS have been compared to elutes of IA used for the treatment of antibody mediated autoimmune disease on SDS page and different proteins (bands) have been analyze by mass spectrometry. We have identified one molecule cask (calcium/calmodulin-dependent serine-threonine kinase). Recombinant CASK has been produced in E.coli (recCASK) to test its effect in vitro on podocyte culture and in vivo in mice.

Results: We have observed a protein of 85kDa which has been identifed as a serine Threonine Kinase, CASK to be eluted from IA of patients with recurrent FSGS. CASK can be immunoprecipited in sera of those patients but not in healthy donor or patients having a diabetes nephropathy. recCASK impairs the morphology of podocytes in vitro with a redistribution of actin stress fibers, ZO-1, synaptopodin, vinculin and induces albumin permeability of a podocyte monolayer in a transwell assay. The intravenous injection of cask induces proteinuria in mice and electron microscopy analysis of kidneys shows podocyte foot process effacement in treated animal with CASK.

Conclusions: A new soluble form of CASK has been detected in sera from patients with recurrent FSGS. Our preliminary data suggest that CASK would be implicated in the pathogenesis of recurrent FSGS after renal transplantation.

TH-OR173

The Double-Edged Sword of Therapeutic Tacrolimus Levels in African-American Kidney Transplant Recipients David J. Taber,[1] Titte Srinivas,[2] Mulugeta Gebregziabher,[3] Kenneth Chavin,[1] Prabhakar Baliga,[1] Leonard Egede.[4] *[1]Transplant Surgery, MUSC; [2]Transplant Nephrology, MUSC; [3]Public Health Sciences, MUSC; [4]Center for Health Disparities Research, MUSC, Charleston, SC.*

Background: African-Americans (AA) may require higher doses of tacrolimus (FK) to achieve therapeutic levels. We examined racial differences in the concentration effect relationships with FK as a potential contributor to disparities in outcomes.

Methods: Retrospective longitudinal cohort study of solitary adult kidney transplant (KTX) with >3 mo graft survival transplanted between 2005-13 with the aim of determining the influence of therapeutic FK levels (≥8 ng/mL) on clinical outcomes across recipient race (AA versus non-AA). We included only those that received FK/MMF-based therapy.

Results: 1,083 patients were included (570 AA, 513 non-AA). Mean FK levels are displayed in Figure 1. AAs were 1.7 times less likely to achieve a mean therapeutic FK level (≥8 ng/mL) during the 1st year post-KTX (35% versus 21%, p<0.001). AAs that did not achieve FK ≥8 ng/mL were twice as likely to have acute rejection (19% versus 8%, p=0.016, Figure 2) and 10 times more likely to have antibody mediated rejection ([AMR] 8.6% versus 0.9%, p=0.007) compared to non-AAs. Rejection (10% versus 7%, p=0.122) and AMR (3.8% versus 2.0%, p=0.131) rates were similar between AAs and non-AAs that had FK levels ≥8 ng/mL. AAs that achieved therapeutic levels had significantly higher rates of de novo IF/TA (18% versus 11%, p=0.006, Figure 3); conversly, AA and non-AAs with low FK levels had similar rates of IF/TA (26% versus 22%, p=0.344).

Conclusions: In contrast to non-AAs, AAs that achieve therapeutic FK levels have substantially lower rejection rates. AAs achieving therapeutic FK levels are prone to developing IF/TA, unlike non-AAs. These findings may reflect modifiable time dependent racial differences in the concentration effect relationship with FK in KTX recipients.

Figure 1. Mean FK Levels for the Four Comparison Groups

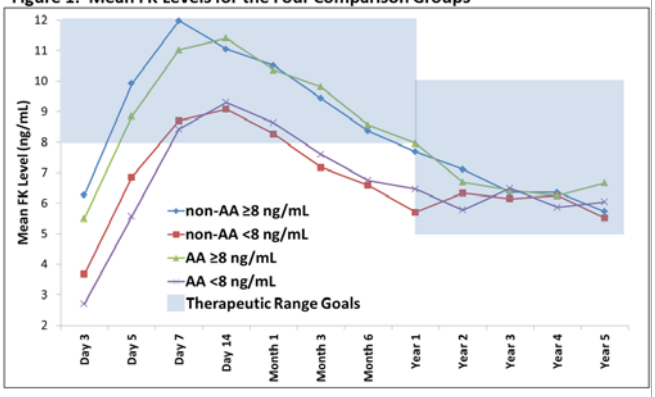


Figure 2. Impact of FK Levels on Acute Rejection Based on Race

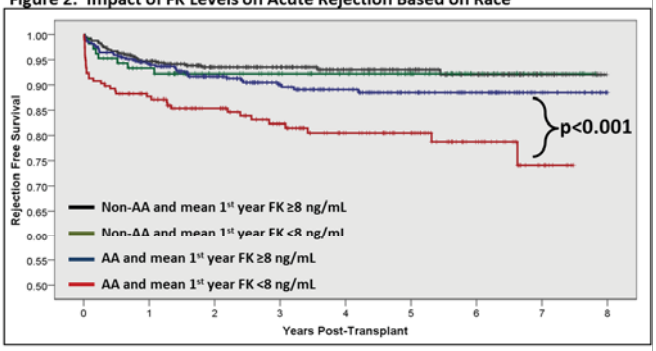
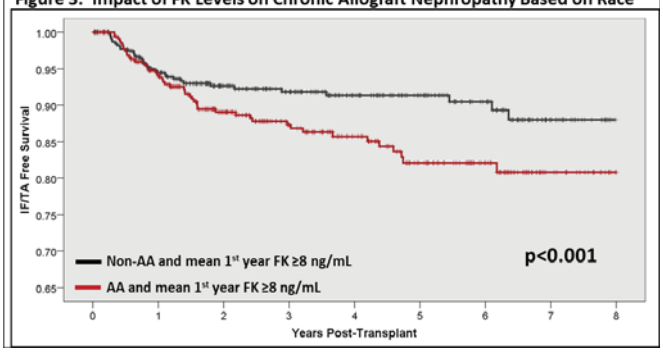


Figure 3. Impact of FK Levels on Chronic Allograft Nephropathy Based on Race



Funding: NIDDK Support

TH-OR174

Epithelial to Mesenchymal Transition Markers in Kidney Transplantation Recipients: The CERTITEM Trial Alexandre Hertig,[1] Lionel Rostaing,[2] Dany Anglicheau,[2] Pierre Merville,[2] Bruno Moulin,[2] Marc Hazzan,[2] Guy Touchard,[2] Fabienne Di Giambattista,[3] Eric Rondeau.[2] *[1]Urgences Nephrologiques et Transplantation Renale, APHP, Hopital Tenon, Paris, France; [2]For the Certitem Study Group; [3]Novartis Pharma SAS.*

Background: We conducted a randomized trial to determine whether epithelial to mesenchymal transition (EMT) markers would help to identify kidney transplant (tx) recipients at such a high risk of graft fibrogenesis that they would benefit from an early withdrawal of calcineurin inhibitor (CNI).

Methods: Initial treatment consisted in CsA, mycophenolate sodium (MPS), corticosteroids and basiliximab. We measured by immunohistochemistry expression of vimentin and beta-catenin translocation in tubular cells, on a first biopsy performed at 3 months post-tx. A biopsy in which ≥10% of tubules expressed mesenchymal markers defined EMT+ patients (pts). We randomly assigned both EMT+ (n=75) and EMT- (n=119) pts to either continue CsA+full dose MPS (n=98), or to withdraw CsA and to start on everolimus+low dose MPS (EVL/CNI-free) (n=96).

Results: Study objective was to evaluate if the progression of interstitial fibrosis (IF) between the first and a second surveillance biopsy performed at 12 months post-Tx would be attenuated in EMT+ pts converted to a CNI-free regimen, when compared to those maintained on CsA. The primary endpoint (PE) was the progression of IF and tubular atrophy (TA) Banff score from M3 to M12 (ΔIF/TA>1). In the intent-to-treat analysis the PE occurred in 16 of the 31 EMT+ pts maintained on CsA and in 12 of the 26 EMT+ pts converted to EVL (p=0.68). Biopsy proven acute rejection (BPAR) occurred more frequently in the EVL group (25.0 *versus* 5.1%, p<0.001). Of these, subclinical BPAR was diagnosed on M12 biopsy in 10.4% of the EVL/CNI-free pts and 2.0% of the CsA pts (p=0.015). Independent associated factors of BPAR was a MPS dose inferior to the recommended one during >28 consecutive days, and a trough level of EVL < 7.0 ng/mL.

Conclusions: An early CNI withdrawal with a switch from CsA to EVL does not prevent IF progression in patients at high fibrogenic risk and carries out a significant risk of rejection.

Funding: Pharmaceutical Company Support - NOVARTIS

TH-OR175

Anti CD40 Autoantibodies Functionally Interact with suPAR to Predict and Cause FSGS Recurrence in Kidney Transplantation Minnie Sarwal,[1] Tara Sigdel,[1] Changli Wei,[2] Alessia Fornoni,[3] Dany Anglicheau,[4] Nada Alachkar,[5] Jochen Reiser.[2] *[1]CPMC, Research Inst, San Francisco, CA; [2]Medicine, Rush Univ, Chicago, IL; [3]Nephrology, Univ of Miami, Miami, FL; [4]Necker Hospital, Paris, France; [5]Medicine, Johns Hopkins, Baltimore, MD.*

Background: Recurrence of focal segmental glomerulosclerosis (rFSGS) after kidney transplantation is a significant cause of early and accelerated graft loss that likely involves circulating factor. Immuneadsorption can alleviate renal dysfunction and suggests that circulating antibodies (Ab) are likely implicated in disease pathogenesis as well.

Methods: To evaluate novel, correlative, pathogenic Ab in rFSGS, we processed 141 unique serum samples from patients with and without primary rFSGS (n=64) and 34 non-FSGS control, transplanted at five (U.S. and EU) hospitals. 9000 antigens were screened in pre-transplant sera by protein arrays and 10 Ab targeting glomerular antigens were selected for ELISA validation.

Results: A panel of 7 Ab (CD40, PTPRO, CGB-5, FAS, P2RY11, SNRPB2 and APOL2) could predict post-transplant FSGS recurrence with 92% accuracy. Pre- transplant elevation of anti-CD40 Ab levels alone had maximal impact (78% accuracy) on the identification of rFSGS risk after transplantation. Epitope mapping of CD40 with customized peptide arrays and rFSGS sera demonstrated altered immunogenicity of the extracellular CD40 domain in rFSGS. Anti-CD40 Ab purified from rFSGS patients were uniquely pathogenic in human podocyte cultures; injection of patient-derived anti-CD40 Ab resulted in proteinuria in a rodent mode but not when blocking CD40 using a monoclonal blocking Ab. The kidney pathogenic effects were further enhanced in combination with suPAR.

Conclusions: In conclusion, a novel panel of 7 Ab can identify primary FSGS patients at high risk of recurrence prior to transplantation. In particular, anti-CD40 Ab together with suPAR were most pathogenic in mice., allowing for customized therapies and improved patient selection for transplant. Intra-renal CD40 is an important axis of disease pathogenesis, and human trials of anti-CD40 therapies are warranted to evaluate their efficacy in preventing rFSGS and improving graft survival.

Funding: Private Foundation Support

TH-OR176

Salt Reduces Regulatory T Cells and Accelerates Allograft Rejection Kassem Safa,[1,2] Shunsuke Ohori,[1] Ciara N. Magee,[1] Anil K. Chandraker,[1] Leonardo V. Riella.[1] *[1]Renal Div/Transplantation Research Center, Brigham and Women's Hospital, Boston, MA; [2]Renal Div, Massachusetts General Hospital, Boston, MA.*

Background: Recent reports suggest that high-salt diet (HSD) might exacerbate autoimmunity in mice. Whether HSD is deleterious in transplantation is unknown. Herein, we examined the effect of NaCl in the alloimmune response, using mixed-lymphocyte cultures and a murine model of solid organ transplantation.

Methods: *In vitro*, B6.Foxp3.GFP mice were sensitized with BALB/c skin grafts. 2 weeks later, $CD4^+Foxp3.FGP^-$ cells were isolated and stimulated with irradiated BALB/c $CD3^-$ cells in the presence of increments of NaCl concentrations ([NaCl] 0-40 mM). Proliferation was then measured by thymidine incorporation. *In vivo*, B6 mice were fed either HSD or normal diet (NSD) and received bm12 heart transplants (single MHC II mismatch).

Results: *In vitro*, proliferation of $CD4^+Foxp3^-$ cells augmented with [NaCl] increase from 0 to 40 mM (7355 versus 18588 CPM, p=0.004). *In* vivo, feeding mice HSD accelerated allograft rejection compared with NSD (median survival time 43 versus >56 days, p=0.04). Serum sodium and mean blood pressure were similar between groups (Na 149.5 versus 150.4 mEq/L, p=0.8 and MBP 63.43 versus 65.42 mmHg, p=0.57). At 25 days after transplantation, HSD fed mice had decreased draining Lymph nodes (LN) Tregs ($CD4^+Foxp3^+$) versus NSD (6.1 versus 15.4%, p=0.01); also Treg proliferation (measured by $\%Ki67^+$) was reduced in LN and spleens of mice fed HSD versus NSD (14.44 versus 20.17%, p=0.04 and 21.32 versus 44.97%, p=0.02, respectively). In addition, HSD resulted in higher $CD4^+$ eff/Treg ratio versus NSD (2.57 versus 1.54, p=0.02). As sodium has been shown to activate the SGK1 kinase in immune cells, we performed the *in vitro* and *in vivo* experiments using $CD4^{Cre}SGK1^{fl/fl}$ B6 mice. Indeed, the increased proliferation and reduced Tregs were abrogated in the absence of SGK1 on CD4 cells, implicating SGK1 as a mediator of the observed effects.

Conclusions: In summary, we show for the first time a deleterious effect of NaCl in transplantation by affecting the regulatory balance of T cells and precipitating rejection. This effect seems to be partially mediated by SGK-1 activation.

TH-OR177

Small Molecule Agonists of CD11b/CD18 Reduce Leukocyte Activation and Recruitment to Promote Kidney Allograft Survival Samia Khan,[1] Mohd Hafeez Faridi,[1] Hatem A. Elshabrawy,[1] James George,[2] Anupam Agarwal,[2] Vineet Gupta.[1] *[1]Internal Medicine, Rush Medical Center, Chicago, IL; [2]George M. O'Brien Kidney Research Center, The Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Allograft rejection in kidney transplantation is associated with leukocyte infiltration, including myeloid cells expressing β2 integrin CD11b/CD18. Previously, we reported that activation of CD11b/CD18 by a small molecule compound, leukadherin-1 (LA1), increases leukocyte cell adhesion to the inflamed endothelium, prevents transmigration and inhibits leukocyte tissue recruitment resulting in improved kidney function in a murine nephritis model.

Methods: Here, we test whether LA1 mediated activation of CD11b/CD18 enhances kidney allograft survival in a mouse model of fully MHC-mismatched orthotopic kidney transplant, where C57BL/6J (H-2b) recipients received a kidney allograft from Balb/c mice (H-2d). Isograft control recipients received a kidney from a littermate. Control isograft and allograft recipients were treated daily with cyclosporine (CsA) only for 2 weeks, while the test group received standard CsA therapy and daily LA1 injections during week 1 and alternate days during weeks 2-8. Renal allograft rejection was considered when mice displayed signs of ill health and high serum creatinine levels.

Results: LA1 treatment reduced interstitial leukocyte infiltration in the allograft, neointimal hyperplasia and glomerular damage and prolonged graft survival from 48.5% (CsA only) to 100% (CsA and LA1) on day 60. At week 8, CsA treated controls had serum creatinine values of 0.5 mg/dL while LA1 and CsA treated mice had baseline values of 0.2 mg/dL. Furthermore, combination therapy reduced macrophage infiltration and increased the frequency of FoxP3+ Tregs in the allograft.

Conclusions: LA1 treatment enhances the survival and the function of the allograft by reducing inflammatory cell infiltration and increasing the number of Tregs resulting in an overall immunosuppresive effect of LA1 administration. These findings indicate a role for CD11b/CD18 in the control of leukocyte migration to the transplanted kidney and identify leukadherins as therapeutic agents for renal transplantation.

Funding: NIDDK Support

TH-OR178

MCMV Infection and Reactivation in Transplantation Tolerance Lei Zhang,[1] Xiaomin Zhang,[2] Zheng Jenny Zhang,[2] Mary Hummel,[2] Xun-Rong Luo.[1,2] *[1]Medicine, Northwestern Univ Feinberg School of Medicine; [2]Surgery, Northwestern Univ Feinberg School of Medicine.*

Background: Experimental tolerance is meeting initial success in clinical transplantation. CMV is a ubiquitous virus that presents risks for reactivation or de novo infection in recipients. Limited information is available on how CMV infection may affect transplant tolerance, or how donor-specific tolerance may influence CMV reactivation.

Methods: To determine the effect of MCMV infection on transplant tolerance, we used a BALB/c to B6 islet transplant model. Transplant tolerance was induced using infusions of donor splenocytes (SPs) chemically fixed with ethylene carbodiimide (ECDI). MCMV infection was given to B6 mice by injection of 10^9 PFU of Δm157 MCMV.

Results: We found that acute Δm157 infection impaired tolerance induction, leading to 80% of recipients acutely rejecting the islet grafts. Anergy of donor-specific T cells induced by ECDI-SP tolerance therapy was reverted by acute Δm157 infection. Similarly, acute Δm157 infection during tolerance maintenance led to reversal of tolerance and precipitation of graft rejection in 30% tolerant recipients. Surprisingly, recipients latently infected with MCMV were also less susceptible to tolerance induction, with 50% recipients rejecting the islet grafts. To examine the effect of donor-specific transplant tolerance on MCMV reactivation, BALB/c kidneys latently infected with MCMV were generated by infecting 3-5 wk old mice with of $5x10^5$ PFU of MCMV and transplanted in B6 recipients 90 days later. Transplanting in non-tolerant recipients led to significant up-regulation of the IE-3 gene from the latently infected kidneys, whereas transplanting in tolerant recipients significantly suppressed this up-regulation.

Conclusions: In conclusion, CMV infection significantly impairs tolerance efficacy, and the presence of donor-specific tolerance effectively suppresses IE gene expression and possibly CMV reactivation. The interaction between CMV infection and tolerance thus warrants future studies, and will likely provide critical information for adequately approaching CMV infections in clinical tolerance protocols.

Funding: NIDDK Support

TH-OR179

Molecular and Immunological Phenotype of CNI Minimization Strategies Using an Investigational Proteasome Inhibitor, Ixazomib Citrate

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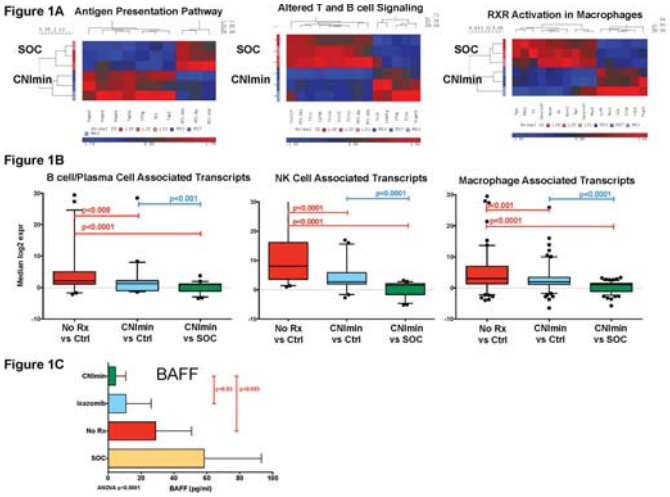
Background: CNI nephrotoxicity is a frequent complication kidney transplantation. We sought to determine whether ixazomib (IX), an investigational proteasome inhibitor, can mitigate antibody mediated rejection (ABMR) while minimizing CNI toxicity in a sensitized rat model.

Methods: ABMR was induced in Lewis (RT1[l]) recipients sensitized with donor-specific blood transfusion from Brown Norway (RT1[n]) rats 3 weeks prior to kidney allograft transplantation. Treatment groups included:control, no treatment (NoRx), Standard of Care (SOC, 10 mg/kg/d CsA) ixazomib alone (IX) or CNI minimization (CNImin, IX + 5mg/kg/d CsA).

Results: Comparing CNImin to SOC by microarray using kidney RNA isolated at 1wk post transplant, highly significant pathways included antigen processing, T and B signaling and the retinoic acid pathway in macrophages. Treatment was associated with up regulation of MHC class I, IL-1A, LBP and MSR1, with down regulation of MHC class II, CD79 and TLRs, Figure 1A. Transcript lists of differentially expressed genes (DEG) for B cell/plasma cells, NK cells and macrophages were developed for the rat model. Median log2 fold expression differences for NoRx were up regulated, Figure1B. Ixazomib treatment alone reduced median DEG, comparison of SOC to CNImin had a median of 0, indicating these treatments have comparable reductions in molecular signature of ABMR. Compared to SOC, BAFF levels measured by ELISA in IX and CNImin groups were significantly reduced, Figure 1C, indicating ixazomib improved the immunological phenotype.

Conclusions: A CNI minimization strategy using ixazomib has an immunological signature similar to full dose CNI SOC, while reducing serum BAFF, suggesting that the protease inhibitor ixazomib may have a role in preventing ABMR.



Funding: NIDDK Support, Pharmaceutical Company Support - Millenium - Takeda

TH-OR180

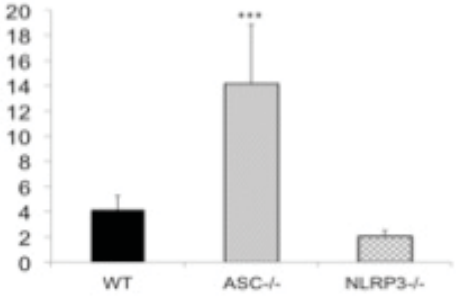
Enhanced Engraftment of Hematopoietic Stem Cells for Solid Organ Transplant Tolerance

Reza Elahimehr, Sashi Kasimsetty, Alana Shigeoka, Dianne B. McKay. *UC San Diego.*

Background: Engraftment of donor-derived hematopoietic stem cells (HSCs) is essential for successful bone marrow transplantation, and ground breaking clinical trials demonstrated that successful HSC engraftment also allows for induction of tolerance to solid organ allografts. Experimental evidence suggests that inducing chimerism promotes central T cell tolerance and allows for acceptance of an allograft without need for immunosuppression. A logical approach to enhance HSC engraftment and differentiation into tolerogenic APCs involves blockade of primitive receptors that are the triggers of cellular activation and stress pattern recognition receptors (PRRs). The NLRP3 inflammasome family of these receptors is highly expressed in HSCs, and HSCs are activated by specific signals delivered through these PRRs, resulting in their expansion and migration in inflammatory states.

Methods: BALB/c mice were preconditioned (sublethal irradiation, anti-CD8 and anti-MR-1Ab) and reconstituted with HSCs isolated from either WT (C57BL/6) mice or from mice deficient in key inflammasome proteins, ASC-/- or NLRP3-/- mice (all on B6 background). Engraftment of HSCs in BALB/c recipient mice was detected by FACS (staining for H-2b+ cells) six weeks after transplantation.

Results: Disruption of ASC, resulted in significantly enhanced HSC engraftment ($p<0.005$). Interestingly, deletion of NLRP3 had no effect on HSC engraftment, suggesting important differences in the role of these inflammasome proteins in HSC function.



Conclusions: Possible conclusion of our data is the absence of ASC confers enhanced HSC engraftment due to a direct effect on DC maturation and antigen presentation, essentially inducing a tolerizing DC phenotype. Ongoing studies in our laboratory are now focused on the mechanisms by which a deficiency in this central inflammasome component enhances engraftment of HSCs and promotes allograft tolerance.

TH-OR181

Potent Beneficial Effects of HDAC11 Targeting on Foxp3+ Treg-Dependent Allograft Survival

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Background: There is now intense interest in harnessing Foxp3+ Treg cells in transplantation. The most pedestrian approach being proposed is to harvest Tregs from a potential recipient, expand them in vitro, and infuse them soon after transplantation. This approach is ultra-expensive, has risks of the infused cells becoming pathogenic given their limited purity, and is likely only a one-off shot of cells that survive 7-10 days. By contrast, pharmacologic modulation of Treg function has the advantages of being able be titrated and given as desired, including potentially long-term. Some but not other HDAC inhibitors can promote Treg production and suppressive functions, depending upon the isoforms being targeted. Hence, there is considerable interest in isoform-selective targeting strategies.

Methods: Histone/protein deacetylase-11 (HDAC11) is the sole class IV HDAC enzyme and was first described in 2002. We report data from mice with constitutive HDAC11 deletion, as well as from mice with conditional deletion of HDAC11 just within Foxp3+ Treg cells.

Results: HDAC11 deletion had no effect on overall health or development, including that of host immune cells. Compared to WT controls, Foxp3+ Tregs lacking HDAC11 showed increased suppressive function in vitro, along with increased expression of Foxp3 ($p<0.05$) and TGF-beta ($p<0.0005$), but not of IL-10 or CTLA-4 (both $p>0.05$). HDAC11-/- Likewise, compared to WT recipients, C57BL/6 recipients of fully MHC-mismatched BALB/c cardiac allografts showed prolonged allograft survival ($p<0.01$), and this effect was matched by longterm allograft survival in mice with conditional deletion of HDAC11 just within Treg cells ($p<0.01$).

Conclusions: Very little is known of the functions of HDAC11. A sole previous report linked HDAC11 with regulation of IL-10 expression in antigen-presenting cells. However, the current data indicate that HDAC11 has more complex, IL-10-independent roles in regulation of adaptive immune responses, especially with regard to functions of Foxp3+ Treg cells. These data are powerful stimuli for our development and testing of selective HDAC11 small molecule inhibitors, and may provide new approaches post-transplantation or for therapy of autoimmune diseases.

TH-OR182

Inducible Knockdown of SHROOM3 Reduces Renal Fibrosis in Mice

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Background: From the Genomics of Chronic Allograft Rejection (GoCAR) cohort, we previously identified SHROOM3 as a candidate gene whose expression in the allograft precedes and correlates with the histological development of renal fibrosis and GFR-decline by 12-months post-transplant.

Methods: To study the mechanism of facilitation of renal fibrosis by SHROOM3, we developed a murine model of inducible shRNA-mediated SHROOM3 knockdown. Here, RTTA-elements were linked to the universal ROSAm26 promoter for RTTA expression in all cell-types. After invitro selection, a shRNA hairpin on a mir30 backbone was linked to doxycycline-RTTA-responsive elements and positioned 3' to the Collagen-1 gene. Doxycycline (DOX) feeding of these animals confirmed SHROOM3 knockdown (~75%) at 3-weeks by RT-PCR and western blot (WB). We performed unilateral ureteric obstruction

surgery (UUO) on 8-10 week old animals after 3-weeks of DOX-feeding (n=5). Mice were sacrificed at 10-days post UUO. Littermates that were not fed with DOX were used as controls. Results were analyzed quantitatively by the unpaired t-test.

Results: We observed an increased SHROOM3 expression in UUO-kidneys compared to controls by RT-PCR in DOX-fed and non-DOX animals; however knockdown was still demonstrable. Among UUO-kidneys, COL1A1 expression was significantly inhibited in DOX-fed animals compared to controls. Kidney lysates of UUO-kidneys showed reduction in phosphorylated-SMAD3 in DOX-fed animals compared to controls by WB. Interstitial fibrosis (by picrosirius red staining) was significantly reduced in DOX-fed UUO kidneys. Type-I Collagen staining was also significantly reduced in UUO-kidneys of DOX-fed animals by immunofluorescence.

Conclusions: In summary, we demonstrate in a murine model that knockdown of SHROOM3 inhibits canonical TGFB1 signaling, COL1A1-production, and ameliorates renal interstitial fibrosis. This further validates our translational finding that SHROOM3 may be a therapeutic target in chronic allograft nephropathy and chronic kidney disease.

Funding: NIDDK Support, Other NIH Support - NIAID, Private Foundation Support

TH-OR183

The H_2S System during Cooling and Rewarming in the Kidney of Hibernators: From Hibernation to Kidney Transplantation

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Background: Hypothermic preservation is widely used to protect kidney and other solid organs during transplantation. However, hypothermic storage induces cold ischemic injury and is aggravated during reperfusion. Interestingly, hibernating animals have shown to possess a natural protection against kidney injury induced by cooling-rewarming (torpor-arousal). Unlike cold ischemic and reperfusion injury in human kidneys during transplantation, the repetitive stress of cooling-rewarming in hibernators does not seem to cause irreversible kidney injury. Therefore, this study aimed at unraveling possible mechanisms that confer tolerance and protection of the kidney during the physiological extremes in hibernating animals.

Methods: Syrian hamsters (*Mesocricetus auratus*) were housed in cages in a climate controlled chamber at 5°C under dim red light to induce torpor. Movement of all animals was continuously monitored with passive infrared detectors. Periods with >24h of inactivity were considered to be torpid phases. Arousal occurred naturally without outside stimuli or changes in ambient temperature. Subsequently, animals were sacrificed 24h after torpor entrance (torpor early; $n = 5$), ≥72h after torpor entrance (torpor late; $n = 5$), 2h after the onset of arousal (early arousal; $n = 8$) and ≥9h after the onset of arousal (late arousal; $n = 8$). Blood samples were taken and kidney of the hamsters were obtained. Summer euthermic hamsters ($n = 7$) served as controls.

Results: Levels of endogenous H_2S were significantly increased during torpor and restored to euthermic level during arousal. Also, CBS expression was upregulated during torpor and normalized upon arousal. Further, there was no significant difference in ROS production during torpor and arousal phases.

Conclusions: The tight regulation of renal H_2S, CBS and HSP-70 during hibernation suggests an important role of the H_2S system in normalizing renal ROS production during cooling and rewarming. Therefore, understanding the mechanisms of hibernation may lead to development of pharmacological strategies which will protect kidneys during hypothermic storage and reperfusion in transplantation.

TH-OR184

Environment and Graft Interaction on Renocardiac Injury in Renal Transplantation

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Background: Renal transplantation from expanded–criteria donors increases mortality and graft failure. Preemptive transplantation has been associated with an allograft survival advantage. Uremic state in CKD patients receiving a kidney allograft could also influence transplantation outcome.The aim of our study was to investigate the interaction between graft and environment on renocardiac injury in renal transplantation.

Methods: CKD and proteinuria developed in male Lewis rats after bilateral ablation of 2/3 of kidney mass. When proteinuria exceeded 200 mg/d, L-NNA (20 mg/L water) and 6% salt diet were switched to normal water and chow. Control rats were age-matched. Orthotopic transplantations (TX, 30 min cold- and 40 min warm ischemia time) were performed: healthy kidney to healthy rat (HH); CKD kidney to healthy rat (CH); healthy kidney to CKD rat (HC); CKD kidney to CKD rat (CC). Contralateral kidney was removed 10-14 days after transplantation. Proteinuria (UpV) was measured 3 and 5 wk after TX. At wk 6, we evaluated glomerular filtration rate (GFR: inulin); renal plasma flow (RPF: PAH), mean arterial pressure (MAP) and left ventricular mass (LVM) of every recipient.

Results: Diseased kidneys performed better in a healthy environment (CH) shown as reduced UpV at wk 3 (25±5 versus 60±17 mg/24h, P<0.01) and wk 5 (51±30 versus 102±32, P<0.01) in comparison to CC. At 6 wk after TX, MAP and LVM were increased in CH versus HH (162±25 versus 110±4 mmHg, P<0.01 and 0.32±0.04 versus 0.24±0.01 mg/100g,P<0.01). In HC MAP was normalized but LVM remained unchanged compared to CC (111±7 versus 147±13 mmHg, P<0.01 and 0.28±0.07 versus 0.32±0.06 mg/100g, NS). Plasma creatinine, GFR and RPF of healthy (HH versus HC) and CKD (CH versus CC) kidneys were not influenced by environment.

Conclusions: Healthy environment improved proteinuria of diseased kidneys. Diseased renal grafts increased MAP and LVM, but pre-existent left ventricular hypertrophy was not reversed by a healthy renal graft. In conclusion, our results demonstrate that as expected blood pressure follows the kidney. Moreover, a healthy environment can reduce proteinuria, but transplantation of a healthy kidney is not able to reverse cardiac hypertrophy despite normalization of hypertension.

FR-OR001

Heme Oxygenase-1 (HO-1) Expression Regulates Trafficking of Myeloid Cells in Acute Kidney Injury (AKI)

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Background: Ischemia reperfusion injury (IRI), a major cause of AKI, is mediated by a complex cascade of immunological events secondary to oxidative injury to renal epithelial cells. Induction of HO-1 expression is a protective response to IRI. We tested the hypothesis that HO-1 ameliorates renal IRI by regulating the recruitment, infiltration and phenotypic differentiation of myeloid-derived immune cells.

Methods: Age-matched male wild-type (WT), HO-1 knockout (HO-1$^{-/-}$), and HO-1$^{-/-}$ mice expressing the human HO-1 gene (HBAC) underwent bilateral renal ischemia for 10 minutes. Kidneys were harvested after 1 or 7 days of reperfusion.

Results: Consistent with previous findings,IRI resulted in significantly elevated mortality (50% in HO-1$^{-/-}$ versus 0% in WT or HBAC) and serum creatinine (1.0±0.3 mg/dL in HO-1$^{-/-}$ versus 0.16±0.05 mg/dL in WT, n=4-8/group, P<0.05, day 1) only in HO-1$^{-/-}$ mice. Relative to sham controls, cast formation and brush border loss were only observed in HO-1$^{-/-}$ kidneys. FACS analysis revealed significantly increased inflammatory macrophages (P<0.01) and neutrophils (P<0.05) in HO-1$^{-/-}$ mice subjected to IRI, while resident renal mononuclear cells (RMNC) were proportionally decreased in HO-1$^{-/-}$ mice. Using FACS analysis, RMNCs were confirmed to phenotypically resemble dendritic cells (CD11b^{int} F4/80^{hi}). Syngeneic kidney transplant experiments utilizing GFP^{+} donor kidneys (25 minutes ischemia) and WT GFP^{-} recipients confirmed increased migration of the RMNC population from HO-1$^{-/-}$ donor kidneys to the primary lymphoid organs compared to controls with WT grafts. This effect on RMNC migration was corroborated in Mye-HO-1$^{-/-}$ mice (myeloid-specific HO-1 deficiency) subjected to 25 minutes of ischemia, confirming the importance of HO-1 expression in myeloid cells.

Conclusions: These results highlight an important role for HO-1 in modulating the trafficking of myeloid cells in acute kidney injury, which may represent a key pathway for therapeutic intervention.

Funding: NIDDK Support

FR-OR002

Retinoic Acid Signaling Promotes Repair by Altering Renal Macrophage Responses after Acute Kidney Injury

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Background: Retinoic acid (RA) signaling is activated in proximal tubular epithelial cells (PTECs) after gentamicin-induced acute kidney injury (AKI) in zebrafish larvae and inhibition of RA signaling impairs normal repair in this model. However the regulation and role of RA signaling in mammalian kidneys after AKI is unknown. In addition, while renal mononuclear phagocyte (MNP) polarization towards a pro-regenerative M2 phenotype plays an important role in tissue repair after AKI, the signals regulating MNP phenotypes post-AKI are unclear.

Methods: We used ischemia/reperfusion AKI (IR-AKI) in mice with the RARE-Hsp68-LacZ transgenic reporter to assess the regulation of RA signaling post AKI. FACS and MACS sorting were used to isolate PTECs and renal MNPs, respectively. The RA antagonist BMS493 and agonist all-trans RA (ATRA) were used to evaluate functional effects of RA signaling, as well *PEPCK CRE; R26-LSL-RARa430X* mice (PTEC DN-RAR) to inhibit RA signaling in PTECs.

Results: RA signaling is activated 12-72 hours after IR-AKI in injured PTECs. Inhibition of RA signaling increases PTEC injury and long-term fibrosis, and decreases renal MNP M2 polarization 3 days after IR-AKI. Conversely, activation of RA signaling with ATRA reduces PTEC injury and fibrosis, and reduces M1 MNP polarization post AKI. To study the role of PTEC RA signaling in mediating these effects, we evaluated IR-AKI in PTEC DN-RAR mice. These mice have increased PTEC injury and decreased repair as well as decreased M2 renal MNP polarization post IR-AKI.

Conclusions: These studies suggest that activation of RA signaling in PTECs enhances post-AKI repair by promoting renal MNP polarization towards a pro-regenerative M2 phenotype. This demonstrates a novel mechanism whereby reactivation of an embryonic signaling pathway that is conserved in zebrafish and mouse kidneys drives repair post AKI, and suggests that RA-dependent signaling in PTECs regulates renal MNP phenotypes post-AKI.

Funding: NIDDK Support

FR-OR003

The Sentinel Role of Renal Dendritic Cells in Systemic Infection Karim Yatim, Martin H. Oberbarnscheidt. *T.E. Starzl Transplantation Inst, Univ of Pittsburgh, Pittsburgh, PA.*

Background: Dendritic cells (DC) in barrier organs such as the lung and intestine have been shown to sample intraluminal compartments by extending cellular processes across epithelial layers. The kidney is a non-barrier, highly vascularized organ with an extensive interstitial DC network that can take up intravascular, non-filterable antigen (Ag). Here, we hypothesized that renal DCs sense Ag by extending cellular processes into the blood vessel lumen, playing a sentinel role in sensing intravascular pathogens in systemic infection.

Methods: Native kidneys of WT or fluorescent reporter mice were analyzed by flow cytometry and two photon intravital microscopy (2PIM) using an E. coli systemic infection model.

Results: Flow cytometric analysis of renal DC revealed that the majority of the DC (lineage$^-$(CD19, CD90, NK1.1, Ly-6G) CD11c$^+$MHCII$^+$) were monocyte-derived DC (mono-DCs:CD11b$^+$), with less than 15% displaying a classical DC phenotype (CD11b$^-$). Monocyte-derived DC are comprised of two subsets, F4/80$^+$ (60%) and F4/80$^-$ (40%).2PIM performed on double reporter mouse kidneys (CX3CR1-GFP = monocytic-lineage cells; CD11c-YFP = DCs), where mono-DCs express GFP and YFP, revealed a cellular network of mono-DCs. This network encased renal post-capillary venules with 30% of the cells extending processes into the venular lumina. 2PIM of mouse kidneys demonstrated that mono-DC processes caused intravascular filling defects and captured intravascularly retained, anti-CD11c-coated beads. Anti-CD11b antibody injected i.v. labeled 80% of renal mono-DCs (flow cytometry). Systemic administration of antigen-coated beads or fluorescent E. coli was followed by mono-DC uptake of beads or bacteria from the intravascular space within 2 hours (2PIM and flow cytometry).

Conclusions: We identified mono-DC as the major renal DC subset. Our data demonstrate access of renal mono-DC to the intravascular compartment via cellular processes that allow sampling and uptake of blood borne antigen. These findings could have important implications for the initiation of adaptive immunity as well as local regulation of kidney function in the setting of systemic infection. Further studies will investigate the consequences of Ag-uptake by mono-DC.

Funding: Private Foundation Support

FR-OR004

Transcriptional Regulation of Th17 Cell Differentiation in Ischemia-Reperfusion Injury Hajeong Lee, Seung Hee Yang, Ji In Park, Kyung Don Yoo, Seung Seok Han, Dong Ki Kim, Yon Su Kim. *Seoul National Univ Hospital, Seoul, Korea.*

Background: Although Th17 cells are crucial for the pathogenesis of acute kidney injury (AKI), the precise molecular pathways involved are unknown. STAT3 is essential for the initiation of the transcription that mediates Th17 differentiation. Cln three requiring 9 (Ctr9) is a negative regulator of STAT3. However, crosstalk between STAT3 and Ctr9 in AKI has not been investigated.

Methods: We evaluated the role of STAT3/Th17 pathway focusing on Ctr9 in bilateral renal ischemia/reperfusion injury (IRI) model. Mice with a STAT3 deletion in T cells (STAT3 knock-out [KO]) were generated by crossing mice with the floxed STAT3 allele with mice expressing Cre under the control of the Lck promoter 17. We used caffeic acid 3,4-dihydroxy-phenethyl ester (CADPE) as JAK2/STAT3 blockade.

Results: The renal injury with macrophage, neutrophil and T-cell infiltrations were improved in both STAT3 KO and CADPE-treated wild type (WT), compared to WT mice at day 2. Intra-renal mRNA expressions of Th17 related cytokines were up-regulated in WT, however, suppressed in STAT3-KO and CADPE-treated WT mice. In contrast, Ctr9 expression was attenuated in WT and accentuated in STAT3-KO and CADPE-treated WT mice. These findings were reproducible in the intra-renal lymphocyte, which monocyte and macrophage frequency was increased in WT and decreased in STAT3-KO and CADPE-treated WT mice. In vitro study using human tubular epithelial cell (TEC) cultured on hypoxic condition showed that STAT3, RORγt, and IL-17R mRNA expressions were increased, and decreased by CADPE. When rIL-17 was applied, STAT3 dependent Th17 pathway was activated with increase of IL-6 and IL-18 secretion, however CADPE inhibited these activations. To regulate STAT3/Th17 pathway transcriptionally, we suppressed Ctr9 expression in intrarenal lymphocytes, and STAT3, RORγt and IL-17 expressions were increased in co-cultured mouse TECs, resulting in IL-17 production, which coincided with an increase in CD4$^+$IL-17$^+$ T cells.

Conclusions: In conclusion, STAT3/Th17 pathway plays a critical role in IRI model. As a negative regulator of the STAT3-dependent Th17 pathway, Ctr9 might be a novel therapeutic target for the treatment of IRI.

FR-OR005

Th-17 Cell Activation in Response to High Salt following Acute Kidney Injury Is Associated with Progressive Fibrosis and Attenuated By AT-1 Antagonism Purvi Mehrotra, David P. Basile, Jaymin B. Patel, Carlie M. Ivancic. *Cellular and Intergrative physiology, Indiana School of Medicine, Indianapolis, IN.*

Background: Exposure of rats to elevated dietary sodium (4% NaCl) following recovery renal ischemia reperfusion accelerates the transition of AKI to CKD. Lymphocyte inhibition with mycophenolate mofetil attenuated the development of hypertension, fibrosis and proteinuria of injury post-AKI provided a high salt diet (Pechman et al Am J Physiol 294:R1234, 2008). We hypothesized that high salt diet will trigger lymphocyte activity in post-ischemic rat kidneys to exacerbate renal inflammation and fibrosis.

Methods: Male SD rats on 0.4% NaCl diet were subjected to left unilateral I/R for 40 minutes and allowed to recover for 5 weeks, which resulted in recovery of renal structure with minimal secondary damage and a mild elevation of CD4+ T-cells relative to sham. Surgical removal of the contralateral kidney at 5 weeks and exposure to 4% dietary NaCl for an additional 4 weeks induced chronic kidney disease as indicated by rapid proteinuria and significant interstitial fibrosis.

Results: Activated T cells were increased in kidney by 3 fold after 4 weeks of 4% dietary salt exposure relative to kidneys from post AKI rats on standard salt diet. Further analysis determined that the T-cells were positive for TNF-α and IL-17, indicative of Th-17 cells, while a small percentage was positive Th-1 or Th-2 cells. Because progression following AKI is associated with enhanced sensitivity to Ang II activity (Am J Physiol 302:F1494, 2012) and Ang II activity may influence lymphocyte activation, injured rats were given Losartan (30 mg•kg^{-1}•day^{-1} in drinking water) during the high salt phase. Losartan treatment reduced the number of renal Th-17 cells by 75 % ($P<0.05$) attenuated the development of interstitial fibrosis (80%, $P< 0.05$) and reduced level of proteinuria. In vitro studies in AKI primed CD4+ T cells indicated Ang II stimulation enhanced, and Losartan inhibited IL-17 secretion.

Conclusions: Taken together, these data suggest that dietary sodium modulates immune cell activity in post ischemic recovered kidneys, due in part to the activity of the local RAS, and participating in the AKI to CKD transition.

Funding: NIDDK Support

FR-OR006

T Lymphocyte Specific Nuclear Factor Erythroid-Derived 2-Like 2 (Nrf2) Activation Protects from Acute Kidney Injury in Mice Sanjeev Noel,[1] Maria Noel Martina Lingua,[2] Samatha Bandapalle,[1] Sekhar P. Reddy,[3] Lorraine C. Racusen,[2] Abdel Hamad,[2] Hamid Rabb.[1] *[1]Dept of Medicine; [2]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD; [3]Dept of Pediatrics, Univ of Illinois, Chicago, IL.*

Background: T lymphocytes play an important role in ischemic acute kidney injury (AKI), as does the transcription factor Nrf2 that regulates antioxidant and stress genes. Nrf2 is thought to work in kidney resident epithelial cells, however, it could be important in T cell function. We therefore hypothesized that T cell Nrf2 activation mediates AKI.

Methods: T cell specific Keap1-deficient (T cell-Keap1-KO) mice were generated using Cre-LoxP mechanism and its effect on Nrf2 activity was assessed by measuring Nqo1 and HO-1 mRNA levels. AKI was induced by bilateral ischemia reperfusion and serum creatinine (SCr) measured to assess kidney function. Post-ischemic kidneys were assessed for histologic changes, pro-inflammatory cytokines, infiltrating immune cells and intracellular cytokine production. Adoptive transfer of T cells from T cell-Keap1-KO mice into WT mice was also performed.

Results: T cell specific augmentation of Nrf2 resulted in significant increase in baseline Nqo1 ($p\leq0.01$) and HO-1 ($p=0.05$) expression in T cell-Keap1-KO mice. T cell-Keap1-KO mice had significantly lower SCr (0.53±0.20 versus 1.6±0.26 mg/dL, $p=0.01$) and fewer necrotic tubules in cortex ($p=0.001$) and outer medulla ($p=0.05$) 24h post ischemia as compared to Keap1 floxed control mice. T cell-Keap1-KO mice kidneys revealed a high frequency of CD25+Foxp3+ Treg (4.1%±0.4 versus 2.8%±0.7, $p=0.01$) and decreased frequency of CD11bCD11c (14.4%±2.2 versus 21.2%±3.5, $p=0.01$) and F4/80 (9.8%±2.6 versus 17.2%±2.8, $p=0.01$) positive cells among CD45+ cells at baseline. Intracellular TNFα, IFNγ and IL17 were significantly lower in CD4, CD8 and double negative T cells in KO mice. Adoptive transfer of T cells from T cell-Keap1-KO mice significantly improved kidney function ($p=0.02$) and survival ($\chi2\leq0.01$) in WT mice.

Conclusions: These data demonstrate that T cell specific Nrf2 augmentation protects from IR induced AKI. This provides novel converging mechanistic information on T cells and Nrf2 function in AKI.

Funding: NIDDK Support

FR-OR007

Role of IKK2 Mediated NF-Kappa B Activation in CD4 Positive Lymphocytes in Ischemia Reperfusion Injury Model Linlin Guo, Hannah Heejung Lee, Hans-Joachim Paust, Gunther Zahner, Friedrich Thaiss. *MED III, Univ Hospital, Hamburg, Germany.*

Background: We and others have previously shown a causal relation between the leukocyte activation and infiltration into kidney as well as NFκB activation after ischemia reperfusion injury (IRI). Therefore, we hypothesized that T cell specific NFκB inhibition may have effects on IRI and tested this idea by ablating IKK2 expression in CD4+ lymphocytes in IRI model.

Methods: Briefly, IRI was induced in 8 to 10 weeks old male CD4Cre and CD4-Cre x IKK2-fL/fL [CD4IKK2] mice by placing atraumatic microvascular clamp on both renal pedicles for 75 minutes. Tissue was collected at 12h and 7d. Renal function, kidney morphology, RT-PCR and FACS were examined.

Results: The results demonstrated improved renal function after IRI in CD4IKK2 mice at 12h (BUN 12: 85±6 versus 115±5 mg/dL; $p< 0.05$), while no significant change at 7d (BUN 7d: 35±3 versus 38±5 mg/dL).CD4+ T cells infiltration in kidney after IRI was significantly increased in CD4IKK2 mice at 7d while not at early point when compared with CD4Cre mice (12h: 41±5 versus 39±3 %CD3cells and 7d: 55±7 versus 41±3 %CD3cells; $p< 0.05$). More remarkably Tregs in kidney were significantly reduced in CD4IKK2 when compared with CD4Cre mice (12h: 6±2 versus 11±3 %CD4 cells; $p< 0.05$ and 7d: 7±1 versus 15±2 %CD4cells; $p< 0.05$). Spleen Tregs were unchanged in CD4Cre mice after

IRI when compared with sham operated animals (9±2 versus11±3 %CD4cells), however, significantly increased in CD4IKK2 mice from 12h to 7d after IRI (1±2 versus 13±3 %CD4 cells; p<0.01).The inflammatory mediator IL6 and chemokines CCL2 and CCL20 were significantly increased (p<0.05) after renal IRI in CD4IKK2 when compared with CD4Cre mice.

Conclusions: It has been proven that T cells play a central role in mediating IRI. Our results extend recent findings (Kim et al. JASN, 2013) that expansion of Tregs in kidney after IRI is critically dependent on IKK2 and Tregs in spleen and kidney are differentially regulated. The pathway of IL6 driven CCL20 expression in the recruitment of CCR6-positive Th17 cells in CD4IKK2 mice after IRI will be further evaluated. Our findings caution against the therapeutic use of systemic application of IKK2 inhibitors in the inflammatory kidney injury.

Funding: Government Support - Non-U.S.

FR-OR008

IL-233 Fusion Cytokine - A Novel Therapeutic Approach for Acute Kidney Injury Marta Stremska,[1] Liping Huang,[1] Amandeep Bajwa,[1] Diane L. Rosin,[1,2] Mark D. Okusa,[1] Rahul Sharma.[1] *[1]Div of Nephrology, Univ of Virginia, Charlottesville, VA; [2]Dept of Pharmacology, Univ of Virginia.*

Background: The Foxp3$^+$ regulatory T cells (Tregs) are important for suppressing inflammation, which is a common mechanism in acute kidney injury (AKI). Based on the dependence of Tregs on IL-2 and our finding that Tregs constitutively express IL-33 receptor (ST2), we designed a novel fusion cytokine IL-233, comprised of two pleiotropic interleukins: IL-2 and IL-33. ST2 is also expressed on T helper 2 (Th2) cells, mast cells and innate lymphoid type 2 cells (ILC2).

Methods: The cytokines were expressed in *E.coli* and purified. We pretreated 6-8 week old C57BL/6 male mice with different doses of cytokines (ip) administered 48 hrs prior to 26 min of bilateral ischemia and 24 hours of reperfusion. Adoptive transfers of purified Tregs (iv) were performed. Plasma creatinine and kidney morphology in H&E stained sections were assessed. Flow cytometry and immunofluorescent microscopy were used to phenotype the immune cells.

Results: Pretreatment with low dose IL-233 protected kidneys from ischemia reperfusion injury (IRI). Acute tubular necrosis score, plasma creatinine and infiltrating neutrophils numbers were significantly lower in the IL-233-treated kidneys, as compared to IL-2 or IL-33 injected separately or in combination. Our findings show that IL-233 treatment first increases the splenic reservoir of Tregs, of which especially the ST2$^+$ proportion, is then mobilized into the blood. Additionally, we observed an increase in Th2 cytokine production in the spleen and decreased inflammatory cytokines IFN-γ and TNF-α, indicating a shift towards a protective Th2 response. Adoptive transfer studies revealed that recipient mice injected with Tregs isolated from IL-233 pre-treated mice had significantly higher proportion of ST2$^+$ Tregs in the kidney and had better protection from AKI compared to those injected with either saline or Tregs from saline-treated mice.

Conclusions: Pretreatment with IL-233 protects against AKI. The putative mechanism of action is through targeting Treg homeostasis and stimulating the anti-inflammatory response of Th2 and the ILC2 cells. Thus, our novel fusion cytokine bears therapeutic potential.

Funding: NIDDK Support, Private Foundation Support

FR-OR009

Kidney Derived Endothelial Progenitors Play a Critical Role during Development and Kidney Injury Sunder Sims-Lucas, Natasha M. Rogers, Christopher Cain Rymer. *Univ of Pittsburgh, Pittsburgh, PA.*

Background: Acute kidney injury leads to the increased risk of morbidity and mortality in hospitalized patients. Acute kidney failure is particularly prevalent amongst subpopulations that are already susceptible, including patients with chronic kidney disease, and kidney transplant recipients. The microvasculature of the kidney is an important vascular network that is critical for maintaining normal tissue homestasis during baseline conditions. We have recently determined that the endothelium of the peritubular capillaries (critical for normal homeostasis) is derived from inherent kidney progenitors. Thus, we hypothesize that abnormal formation of the renal microvasculature leaves kidneys susceptible to ischemic injury.

Methods: To specifically target the kidney-derived peritubular capillary endothelium, we conditionally deleted Flk1 (critical for endothelial development) with the Foxd1EGFPcre line (kidney specific). We utilized sophisticated 3D reconstruction to determine the effects upon vascular growth. We interrogated the location and initiation of embryonic blood flow using in utero intracardiac microinjection. To test postnatal vessel phenotypes, we performed fluorescent microangiography. Furthermore, post-natal mice were subjected to ischemic-reperfusion injury, and pre- and post- kidney perfusion was monitored using laser Doppler imaging.

Results: Deletion of the kidney-derived endothelium produced mutant kidneys with severe kidney medullary hypoplasia, and dilated remnant Flk1-positive peritubular capillaries with normal appearing glomerular capillaries. There also appears to be less blood flow into the developing kidney of the mutants compared to the controls. Our microangiography reveals that the mutant post-natal vessels also appear dilated, suggesting they are susceptible to injury. Further to this, the ischemic injury model revealed that there was reduced reperfusion 24 hours after the ischemic episode, and increased kidney tubular damage in the mutants compared to controls.

Conclusions: In conclusion, kidney derived endothelial cells are critical for normal kidney and vascular development, and deletion of these progenitors leaves kidneys susceptible to ischemic injury.

Funding: NIDDK Support

FR-OR010

Human Endothelial Progenitor Cells Protect against Acute Kidney Injury: Role of Exosomes and miR-486 Jose L. Vinas, Dylan Burger, Alex Gutsol, Pearl Ann Campbell, David Allan, Kevin D. Burns. *Nephrology, Medicine, Kidney Research Centre, OHRI, Univ of Ottawa, Ottawa, ON, Canada.*

Background: In preliminary data, we have shown that human cord blood endothelial-colony forming cells (ECFCs) are protective in a mouse model of ischemia-reperfusion (I/R) acute kidney injury (AKI). Extracellular vesicles (EVs) diminish apoptosis by horizontal miRNA transfer. Since two main classes of EVs exist with distinct properties (exosomes and microparticles), we determined the miRNA profile of ECFC-derived EVs and their role in ischemic injury.

Methods: Mice subjected to kidney I/R were injected with ECFCs at reperfusion. Cultured human umbilical vein endothelial cells (HUVECs) were treated with EVs and rendered hypoxic for 24 hr, followed by reoxygenation (H/R). In both models, apoptosis was assessed by expression of caspase-3, PTEN and AKT phosphorylation. The expression profiles of miRNAs isolated from exosomes and microparticles were determined by next-generation sequencing, followed by bioinformatic pathway analyses.

Results: In mice with I/R AKI, ECFCs blocked increases in renal caspase-3 activity and expression (P<0.05 versus I/R alone, n=6). In the cell model, H/R increased caspase-3 activity and expression, effects that were inhibited by incubation with ECFC-conditioned medium (CM) or ECFC-derived exosomes (P<0.001 versus H/R alone, n=3). By contrast, treatment with CM without EVs or microparticles had no effect on apoptosis. ECFC exosomes were highly enriched in miR-486 (288-fold increase versus microparticles, 30-fold increase versus ECFC lysate). In mice with I/R AKI, ECFCs significantly increased kidney miR-486 levels, associated with decreases in PTEN expression and increased AKT phosphorylation (P<0.05 versus I/R alone; n=6). In HUVECs subjected to H/R, ECFC exosomes decreased PTEN expression, associated with an increase in AKT phosphorylation (P<0.001 versus H/R alone; n=3).

Conclusions: These data show that the protective effects of ECFCs are mediated by exosomes activating the pro-survival AKT pathway, possibly due to horizontal transfer of antiapoptotic miR-486 and subsequent PTEN inhibition. The results suggest that exosomes enriched in miR-486 could represent a therapeutic tool in human I/R AKI.

Funding: Private Foundation Support, Clinical Revenue Support

FR-OR011

Exploring the Pathophysiological Functions of Resident Fibroblasts in the Kidney Jin Nakamura, Akiko Oguchi, Ryo Yamada, Motoko Yanagita. *Nephrology, Kyoto Univ, Kyoto, Japan.*

Background: We previously reported that resident fibroblasts in the kidney cortex and outer medulla are myelin protein zero-Cre (*P0-Cre*) lineage-labeled cells of extra-renal origin, and that some of them are erythropoietin (EPO) producing cells in the healthy kidney. In UUO kidney, *P0-Cre* lineage-labeled fibroblasts transdifferentiate into myofibroblasts and predominantly contribute to fibrosis, with concomitant loss of EPO production. In this study, we further investigated the pathophysiological function of fibroblasts and the crosstalk between the fibroblasts and tubular epithelial cells.

Methods: To analyze the function of fibroblasts in the kidney, we utilized *P0-Cre* inducible simian diphtheria toxin receptor (DTR) transgenic mice (*P0-Cre:iDTR* mice) in which Cre-mediated excision of a STOP cassette renders *P0-Cre* linage-labeled fibroblasts sensitive to diphtheria toxin (DT). The binding of DT to DTR halts protein synthesis within the cells.

Results: First we confirmed that DT administration ablated the expression of fibroblast markers in the kidney, indicating the effective cessation of protein synthesis in *P0-Cre* linage-labeled fibroblasts. Simultaneously, the expression of tubular injury markers, as well as the proliferation of proximal tubule cells was observed. In UUO kidney, DT administration enhanced the expression of tubular injury markers. Unlike the results of healthy kidney, DT administration significantly attenuated tubular proliferation, mainly in proximal tubules. To search for the fibroblasts-derived molecules supporting tubular epithelial cells in healthy condition, we performed microarray analysis and identified several signaling pathways down-regulated in DT-administered kidney, suggesting resident fibroblasts triggering corresponding signals in tubular epithelial cells.

Conclusions: Our results indicate that resident fibroblasts inhibit tubular proliferation and injury in healthy kidney, while support the repair of injured tubule by promoting tubular proliferation in diseased kidney. These results indicate the possible interactions between the fibroblasts and tubular epithelial cells, and the signaling pahtways responsible for the interaction may be of therapeutic implication.

FR-OR012

Cell Autonomous and Non-Cell Autonomous Responses Mediated By Angiogenin During Kidney Injury Nicolas Pallet, Iadh Mami, Eric Thervet. *Hopital Europeen Georges Pompidou.*

Background: Endoplasmic Reticulum (ER) stress contributes to kidney disease and aging. The Unfolded Protein Response (UPR) helps to adapt to the changing environment and reestablish normal ER function. Angiogenin (ANG) is a stress-activated ribonuclease that cleaves tRNA and produces stress-induced tRNA fragments (tiRNA), which inhibit translation. Whether ANG is integrated in the UPR is currently unknown.

Methods: We combined in vitro, in vivo and human models of kidney injury to explore the molecular basis whereby ANG is regulated by the UPR, and to characterize how it promotes cellular adaptation during ER stress.

Results: In mice models of ischemic injury and cyclosporine nephrotoxicity, and in human kidney biopsies, ANG expression was upregulated in the tubules, and this expression occured with ER stress. In human renal epithelial cells in culture (HREC), IRE1a, and the transcription factors sXBP1 and p65 induced ANG expression, and also IL6. We showed that ANG, like IL6, was conventionnally secreted. Secreted ANG primed non-stressed HREC to activate the UPR, but without apoptosis, indicating that ANG may induce non-stressed HREC precontionning. In addition, ANG activated macrophages to produce IL6, IL8 and TNFa. Looking at cell intrinsic properties of ANG during early ER stress, we have found that the inhibition of expression of ANG increased HREC apoptosis, and these adaptive effects of ANG were mediated by a reduction in protein synthesis, which alleviated ER stress. The inhibitory effects of ANG on protein translation were mediated by the production of tiRNA, which interfere with translation initiation. Validation of these findings in mice knocked-out for ANG are ongoing. Finally, we measured the urinary concentrations of ANG in 110 consecutive patients referred for a kidney biopsy, and we demonstrated that the urinary concentrations of ANG were positively correlated with renal function and the intensity of tissue injury.

Conclusions: ANG is a regulator of the stress response integrated to the UPR during kidney injury, which acts in a cell intrinsic mode by inhibiting translation, and in a cell extrinsic mode by inducing pre-emptive responses in neighbouring cells. ANG is a non invasive marker of kidney tissue injury.

FR-OR013

Podocytes Proliferation Is Detected in Rat and Human Nephoritis

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Background: Podocyte depletion is a pivotal pathogenesis for many types of glomerulonephritis. It has been found recently that podocytes is able to replicate in certain glomerular diseases, such as idiopathic collapsing glomerulopathy and HIV-associated nephropathy. But in most immune complex mediating glomerulonephritis, the question whether podocytes can proliferate and replenish its lost after injury is still undefined. In this study, we used a retrovirus- enhanced green fluorescent protein (pMSCV-EGFP) tracing technology to observe direct evidence of podocyte proliferation in two type rat nephropathy models, cytotoxic nephrosis induced by adriamycin and immune-mediated injury of passive Heymann nephritis (PHN) and anti-Thy1.1 nephritis.

Methods: It is a new technology developed recently to investigate cells proliferation by infecting of retrovirus in animal experiment. Retrovirus is positive-strand RNA viruses which can integrate into the host DNA only during the host cells division. The proliferated podocytes in rat glomeruli were captured by detecting EGFP as well as BrdU via immunohistochemistry staining.Moreover, PCNA immunohistochemistry staining was used in human renal biopsy tissue and Western blot was used in cultured mice podocytes to detect cell cyclin kinase as well as Wnt/β-catenin molecules.

Results: The clear evidence of positive for EGFP and BrdU were found in glomeruli of rat nephropathy. It confirmed that podocytes do proliferate in animal cytotoxic nephropathy and in immune complex mediating glomerulonephritis. Meanwhile, podocytes positive for PCNA and WT1 double staining were detected by immunohistochemistry in human renal biopsy tissue of diverse nephritis. In vitro studies showed that the up-regulation of cyclinB1, cdc2, and PCNA were observed in cultured podocytes after treatment of inflammatory stimulation, and these effects were mediated via the activation of Wnt/β-catenin.

Conclusions: These results indicate that podocytes have a capability of proliferation in rat and human common immune mediating glomerulonephritis, which was regulated by activation of cyclin kinases via the mediating of Wnt signaling.

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FR-OR014

Krüppel-Like Factor 6 Protects the Podocyte from Mitochondrial Injury under Cell Stress

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Background: Krüppel-like factors (KLFs) play a critical role in podocyte biology. As compared to other KLFs, Krüppel-like factor 6 (KLF6), a zinc-finger DNA-binding transcription factor, expression was reduced in HIV-1 infected podocytes, HIV-1 transgenic mice, and in human biopsies with HIVAN. Podocyte-specific loss of *Klf6* (*Cre*$^{+}$ *podocin Klf6*$^{flox/flox}$) in mice resulted in significant albuminuria, podocyte effacement, and FSGS with adriamycin (ADR) treatment. ADR-treated *Cre*$^{+}$ *podocin Klf6*$^{flox/flox}$mice exhibited dysmorphic mitochondria with a reduction in mitochondrial transcripts in the podocytes. Promoter analysis also showed putative binding sites for KLF6 exist on cytochrome c oxidase assembly gene (*SCO2*).

Methods: Cultured human podocytes (HP) were treated with and without ADR (0.4μg/μl) for 6, 12, 18, and 24 hours. Stable knockdown for *KLF6* and *SCO2* (shRNA) was performed in HP and mitochondrial injury and apoptosis was determined.

Results: KLF6 expression increased within 6 hours of ADR treatment and shRNA-*KLF6* reduced the mitochondrial membrane potential as compared to shRNA-EV (control). In ADR-treated HP, shRNA-*KLF6* increased mitochondrial fragmentation with reduced SCO2 expression and ATP levels. Also, ADR-treated shRNA-*KLF6* increased cleaved-caspase 3, 9, and cytosolic cytochrome c expression and decreased pro-caspase 3 expression. KLF6 regulation of mitochondrial function was determined by identifying KLF6 transcriptional binding sites on *SCO2* using ChIP assay. ADR-treated *Podocin-Cre Klf6*$^{flox/flox}$ mice exhibited a podocyte-specific reduction in *Sco2* expression as compared to ADR-treated *Podocin-Cre Klf6*$^{+/+}$ mice. *Sco2* expression was also reduced in HIV-1 transgenic mice as compared to wild-type mice. Also, shRNA-*SCO2* increased cytosolic cytochrome c, cleaved-caspase 3 and 9 levels. Finally, immunostaining revealed a reduction in podocyte-specific KLF6 expression in human biopsies with FSGS as compared to healthy donor nephrectomies.

Conclusions: These findings suggest that *KLF6* is an early inducible injury response gene, critical to the maintenance of mitochondrial function and preventing the activation of the intrinsic apoptotic pathway upon stress to the podocyte.

Funding: NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic Inc.

FR-OR015

Suppression of Acetyl-CoA Carboxylase 2 Rescues Kidney Proximal Tubule Epithelial Cells from Palmitic Acid-Induced Lipotoxicity via Autophagy

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Background: Acetyl-CoA carboxylase b (ACC2) enzyme plays a crucial role in the fatty acid (FA) metabolism. Autophagy is known to control the cellular energy balance. In the present study we aim to investigate the effects of ACC2 downregulation on palmitic acid (PA) induced lipotoxicity in human proximal tubular cells and the putative role on autophagy in this process.

Methods: ACC2 expression was suppressed by shRNA transfecion. The lipid deposition was accessed by oil-red O staining. The cell viability was analyzed by MTT. Immunoblot and immunostaining were used to characterize autophagic proteins expression. The autophagy level in vivo was characterized by immunohistochemistry.

Results: Autophagy level was dramatically higher in high fat fed rats compared to controls. PA induced autophagy in HK-2 cells evidenced by decreased P62 expression. More interestingly, the knockdown of ACC2, representing an accelerated b-oxidation rate, reduced autophagy. Further, the ACC2 knockdown ameliorated the PA-induced lipid accumulation (not shown) and cell viability defects.

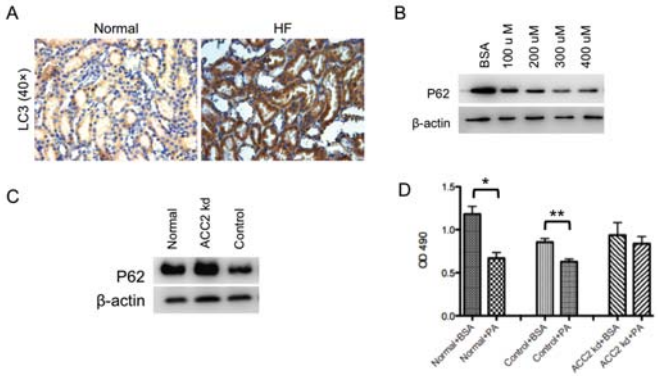

Fig.1 Role of ACC2 knockdown on autophagy in PA stimulated HK-2 cells. A) Autophagy level was dramatically increased in high fat fed rats (HF) compared to normal rats. B) Autophagy was induced in HK-2 cells after stimulated by PA for 2h. C) ACC2 knockdown decreased autophagy level in HK-2 cells. D) ACC2 knockdown attenuated PA induced proliferation defects of HK-2 cells.

Conclusions: Taking together, it was proved that PA was able to induce autophagic flux both in vivo and in vitro and the knockdown of ACC2 reduced PA-induced autophagy and thus protects cells from PA-induced lipotoxicity with attenuated lipid accumulation and rescued cell viability. Thus we proposed a novel autophagy-involved mechanism of PA-induced renal lipotoxicity and provided potential therapeutic strategy by modulating lipid b-oxidation for DN.

Funding: Government Support - Non-U.S.

FR-OR016

Long-Chain Saturated Fatty Acid-Induced Endoplasmic Reticulum Stress Suppresses Erythropoietin Production in Kidney

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Background: Renal anemia is caused by derangement of EPO production in renal EPO-producing (REP) cells. Long-chain saturated fatty acid (palmitate) induces endoplasmic reticulum (ER) stress, which contributes to kidney cell damages. Thus, we evaluated the effect of palmitate on EPO production via the ER stress pathway.

Methods: C57/BL6 or inherited super-anemic mice (ISAM), in which the renal EPO gene was replaced with GFP, were daily injected with palmitate (BSA-conjugated) or vehicle for 11 days. Free fatty acids, expression of EPO and other hypoxia inducible factor (HIF) target genes (VEGF and Glut-1), or ER stress state in kidney were measured. Alteration of EPO production in REP cells was estimated by GFP detection in ISAM mice. We also stimulated cultured EPO-producing cells (HepG2) with palmitate in various doses and durations and/or under hypoxic condition (1%O_2 or chemical inducer of HIF-1 [$CoCl_2$]).

Results: Palmitate suppressed EPO mRNA and protein in HepG2 and the kidney of C57/BL6 mice ($p<0.05$). Particularly in hypoxic condition, the EPO suppression by

palmitate was conversely associated with activation of ER stress signal (ATF4 and XBP-1). Interestingly, we identified a novel ATF4 binding site (TGACCTCT) near HIF binding site at the 3'-enhancer region of EPO gene. ATF4 upregulation selectively suppressed the HIF-dependent-enhancer activity of EPO gene but not the other HIF target genes. In ISAM mice, palmitate significantly reduced renal EPO production and REP cell number at cortico-medullary junction as compared with the control, respectively.

Conclusions: EPO production by REP cells was suppressed by palmitate especially under hypoxic condition via the ER stress pathway. The link between ER stress, dyslipidemia and hypoxia in CKD may contribute to development and progression of anemia.

FR-OR017

Altered Autophagy Flux Contributes to Kidney Aging via Mitochondrial Dysfunction Takeshi Yamamoto,[1] Yoshitsugu Takabatake,[1] Tomonori Kimura,[1] Atsushi Takahashi,[1] Tomoko Namba,[1] Jun Matsuda,[1] Fumio Niimura,[2] Taiji Matsusaka,[3] Motoko Yanagita,[4] Hiromi Rakugi,[1] Yoshitaka Isaka.[1] *[1]Dept of Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Dept of Pediatrics, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan; [3]Inst of Medical Science and Dept of Internal Medicine, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan; [4]Dept of Nephrology, Graduate School of Medicine, Kyoto Univ, Kyoto, Japan.*

Background: Autophagy is essential for cellar homeostasis through the quality control of proteins and organelles. Controversy exists whether the decline of autophagic activity is involved in aging process. We have previously demonstrated that autophagy maintains proximal tubule cell homeostasis and protects against ischemic and nephrotoxic injury.

Methods: We extended this prior study to longer observation period to elucidate the association of autophagy and kidney aging.

Results: Temporal cessation of autophagy in recently generated tamoxifen-inducible proximal tubule-specific Atg5-knockout mice exhibited increased accumulation of p62/SQSTM1- and ubiquitin-positive aggregates in the aged kidneys, but not in younger kidney, indicating that basal autophagic activity is high in aged kidney. Younger GFP-MAP1LC3 transgenic mice showed a drastic increase of GFP-positive puncta in proximal tubules in response to starvation, whereas aged GFP-MAP1LC3 mice did not, suggesting that autophagic flux is blunted with aging. Proximal tubule-specific Atg5-knockout mice at 24 months of age exhibited a significant deterioration in kidney function and fibrosis. Furthermore they revealed deformation and dysfunction of mitochondria and mitochondrial DNA abnormalities in the proximal tubules.

Conclusions: These results suggest that age-dependent high basal autophagy plays a crucial role in counteracting kidney aging through mitochondrial quality control but that lack of upregulation of autophagy flux in response to metabolic stress with age may be associated with age-related kidney diseases.

Funding: Government Support - Non-U.S.

FR-OR018

A Lifetime Health Outcomes Model in Moderate to Severe Chronic Kidney Disease Based on the Study of Heart and Renal Protection (SHARP) Borislava N. Mihaylova, Iryna Schlackow. *On Behalf of the SHARP Collaborative Group, Nuffield Dept of Population Health, Univ of Oxford, Oxford, United Kingdom.*

Background: Chronic kidney disease (CKD) increases the risk of cardiovascular disease (CVD) and CVD may contribute to kidney disease progression. A model taking into account this interdependence is developed to evaluate lifetime health outcomes of patients with moderate-to-severe CKD.

Methods: The individual participant data of SHARP (9,270 participants, 5 years' mean follow-up) were used to develop CKD risk equations predicting progression through CKD stages, and CVD risk equations predicting major vascular events and vascular mortality. These were combined into a lifetime Markov SHARP CKD-CVD model. A range of socioeconomic, CKD and CVD risk factors together with annually updated age, CKD duration, CKD stage and nonfatal vascular events were used to estimate disease risks. The model was validated within subgroups of participants in SHARP as well as using external clinical trial and observational CKD patient data.

Results: Cardiovascular events, CKD stage and age were the major determinants of CVD risks and CKD progression and, consequently, of patient survival (Table). Model predictions of rates of cardiovascular events and progression of CKD corresponded well to observed rates in different subgroups of SHARP participants and in the external CKD cohorts.

CKD stage at baseline	Age at baseline (years)				
	<50	50-59	60-69	70-79	≥80
	Predicted survival (years), in the absence of any lipid-lowering therapy				
Stage 1-3 (predomintantly 3B)	23.8	18.3	13.5	9.6	6.7
Stage 4	22.3	16.2	11.8	8.4	5.7
Stage 5, not on renal replacement therapy	20.6	13.3	9.2	6.4	4.6
On dialysis	19.4	13.1	8.7	6.1	4.3
	Rediction in survival (years) associated with a nonfatal major atherosclerotic event				
Stage 1-3 (predominantly 3B)	2.7	2.3	1.6	1.0	0.5
Stage 4	2.5	2.2	1.8	1.1	0.6
Stage 5, not on renal replacement therapy	2.0	1.6	1.4	0.9	0.5
On dialysis	1.5	1.1	0.9	0.6	0.5

Conclusions: The SHARP CKD-CVD model performs well and will be made available for simulating lifetime health outcomes in moderate-to-severe CKD.

Funding: Pharmaceutical Company Support - The SHARP study was funded mainly by Merck/Schering-Plough Pharmaceuticals (North Wales, PA, USA), Government Support - Non-U.S.

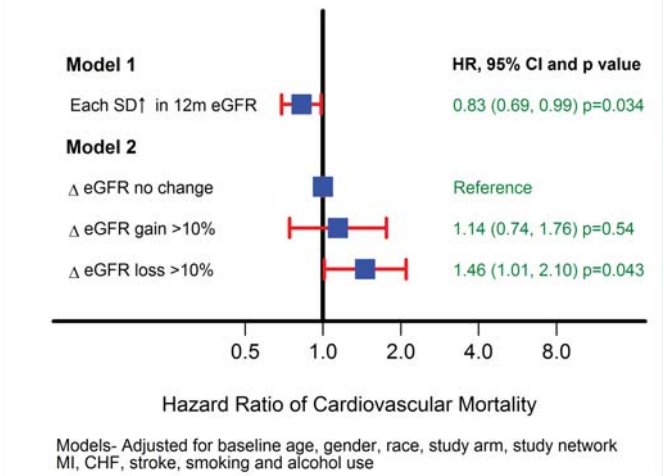
FR-OR019

Early GFR Decline as a Predictor of Cardiovascular Mortality in Non-CKD Diabetics Srini Beddhu,[1,2] G. Wei,[2] E. Constantz,[2] Xiaorui Chen,[2] R. Boucher,[2] Ajay Giri,[2] Debra Lynn Simmons,[2] Molly B. Conroy,[3] Jane J. Lee,[3] Tom Greene.[2] *[1]SLC VAMC; [2]Univ Utah; [3]Univ of Pitt.*

Background: Early GFR decline in non-CKD diabetics might reflect systemic microvascular disease and predict cardiovascular mortality (CVM).

Methods: We examined this hypothesis in a secondary analysis of the ACCORD study (a RCT conducted by NHLBI to examine the effects of glycemic control, BP control and fibrates on CV outcomes in type 2 DM). The cohort for this analysis consisted of those with a). baseline and 12 m CKD-EPI eGFR and b). 12 m eGFR ≥ 60 ml/min/1.73 m^2. Based on the baseline and 12m eGFR, ΔeGFR groups (no change: -10% to +10%, >10% loss: ΔeGFR <-10% and >10% gain: ΔeGFR>+10%) were defined. Separate Cox regression models were used to relate ΔeGFR groups and 12 m eGFR with CVM.

Results: 7574 diabetics without CKD at 12m were included. The mean age was 62.8 ± 6.2 yrs, 62.6% were men, 61.9% were Caucasian. The mean ± SD 12m eGFR was 86.3 ± 14.2 ml/min/1.73 m2. The mean ΔeGFR in the no change, ΔeGFR loss and ΔeGFR gain groups were -1.2 ±3.8, -17.7±7.5, 15.7±7.8 ml/min/1.73 m2, respectively. There were 159 CVM events over 30070 patient-yrs of follow-up.



Each SD ↑ in 12m eGFR was strongly associated with lower hazard of CVM (Fig). Compared to the group ΔeGFR no change group, ΔeGFR loss > 10% group had higher hazard of CVM (Fig). ΔeGFR and 12 m eGFR were strongly correlated (r =0.53, p <0.001) and when both were included in the same model, neither had significant association with CVM.

Conclusions: Both current eGFR and loss of eGFR in the preceding 12 m in non-CKD diabetics predict CVM. Early decline in eGFR might be a marker of cardiovascular disease in diabetics without CKD.

Funding: NIDDK Support

FR-OR020

Risk of Complications in Hospitalized Patients with Chronic Kidney Disease

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Background: Complications that arise during hospitalization are associated with prolonged stay, increased disability, death; many of these complications are preventable. Patients with CKD may be at high risk of in-hospital complications. Objective: Determine the association of CKD and the risk of in-hospital complications.

Methods: All adults (age >= 18) in Alberta, Canada with a hospital admission between March 31, 2003 to April 1, 2008 were included. CKD was defined using outpatient proteinuria and eGFR (CKD-EPI). Other comorbid conditions were identified using validated algorithms. In-hospital complications were identified and categorized by the type 2 flag (Hospital Acquired Diagnoses; ICD-10 CA). Multiple logistic regression assessed the independent association between CKD and the risk of developing any in-hospital complication.

Results: Of 536,549 hospitalization, 8.5% involved patients with CKD. Subjects with CKD were older (72, SD: 15) and more likely to be female than those without CKD. Disease of circulatory system was the most common reason for admission (20.8%), diabetes was the most common co-morbid condition (23.6%), and the unadjusted proportion of patients with >=1 HAD was 7.3% and 13% for those without and with CKD, respectively. In a fully adjusted analysis the OR of HAD in patients with CKD (reference: no CKD) was 1.22(95% CI: 1.18 – 1.26). A graded association with severity of CKD was observed

		Adjusted OR	95% CI
CKD (Ref: no CKD)		1.22	1.18 – 1.26
eGFR (ml/min/1.73/m^2)	> 60	Ref	
	45 - 59	1.14	1.09 – 1.19
	30 - 44	1.25	1.18 – 1.33
	15 - 29	1.48	1.35 – 1.62
Proteinuria None	None	Ref	
	Moderate	1.22	1.14 – 1.30
	Heavy	1.37	1.27 – 1.49

Conclusions: CKD and its severity are associated with an increased risk of in-hospital complications. Our findings can be used to guide preventive strategies aimed at reducing complications in vulnerable hospitalized patients.

FR-OR021

Infection Is a Risk Factor for Faster Progression to Renal Replacement Therapy and Death in Chronic Kidney Disease

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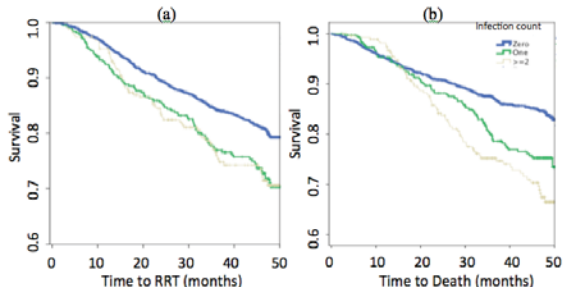
Background: Chronic Kidney Disease (CKD) affects 10% of adults and is associated with increased risk of infection (Inf). In patients undergoing renal replacement therapy (RRT), Inf is an established and increasing cause of death. However, the role of Inf in non dialysis CKD patients remains understudied. We aim to establish if Inf is a risk factor for faster progression to RRT and mortality in CKD patients.

Methods: CanPREDDICT is an observational pan-Canadian cohort of prospectively followed up CKD outpatients. Baselines characteristics were recorded at initial visit and patients followed up six monthly. Inf were defined by use of antibiotics, categorised by anatomical location and counted for each six monthly interval. Endpoints were death and commencing RRT.

Results: The analysis cohort consisted of 2529 patients (mean follow up 35.3 months); median age was 70.6 years, males (62.5%) and Caucasians (88.7%). 399 patients (15%) died and RRT was started in 464 patients (18%). 30% had an Inf. Those with Infs were more likely to be male (p<0.001), have diabetes (p= 0.01) and cardiovascular disease (p< 0.001), lower eGFR (p=0.019) and higher albumin (p< 0.001) and C reactive protein (p= 0.003) as compared to those with no Inf; no statistical difference in age (p=0.71) or urine albumin/creatinine (p=0.89) were seen. Patients with Inf were more likely to die (No Inf= 13.2%, 1 Inf= 20.8%, ≥2 Inf= 26.4%, p< 0.001) and undergo RRT (No Inf= 16.2%, 1 Inf= 24.5%, ≥2 Inf=24.3%, p< 0.001).

Kaplan-Meier curves a) time to RRT and b) time to mortality based on number of infections.
p< 0.001 by Log Rank for both curves



Conclusions: We demonstrate that the presence of any Inf in this CKD cohort is a risk factor for increased mortality and faster RRT progression. Reasons for this association is the focus of future study.

Funding: Pharmaceutical Company Support - Ortho biotech Canada

FR-OR022

Incident Atrial Fibrillation and Risk of End-Stage Renal Disease in Chronic Kidney Disease: The CRIC Study

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Background: Atrial fibrillation (AF) frequently occurs in patients with chronic kidney disease (CKD) and may be associated with adverse kidney outcomes. We examined the association of incident AF with the risk of end-stage renal disease (ESRD) among adults with CKD.

Methods: We studied participants in the prospective Chronic Renal Insufficiency Cohort (CRIC) Study between 2003-2008 without AF at baseline. Incident AF was identified by study visit ECGs, self-report and hospital discharge diagnostic codes, with confirmation by physician adjudication of medical records. ESRD through 2012 was ascertained by self-report, medical records and linkage to the U.S. Renal Data System. Multivariable Cox regression models were used to study the association of time-updated incident AF with subsequent risk of ESRD.

Results: Among 3,091 participants, 172 (5.6%) developed confirmed incident AF. Participants who developed incident AF were more likely to be older, white, have a history of hypertension, cardiovascular disease and have more advanced CKD at baseline. During a mean follow-up of 5.9 years, 43 cases of ESRD occurred after development of incident AF (4.23 per 100 p-y) compared with 581 cases in those without incident AF (3.54 per 100 p-y). After adjustment for potential confounders, incident AF was associated with a threefold increase in rate of ESRD (**Table**) and this association was consistently strong across important subgroups (**Table**).

Conclusions: Incident AF independently increases the risk of developing ESRD in adults with CKD. Further study is needed to identify potentially modifiable pathways through which AF leads to a higher risk of progression to ESRD.

Table. Association between incident atrial fibrillation and progression to ESRD in the CRIC study (N=3,091)

	Hazard Ratio (95% CI)
Unadjusted Model	3.40 (2.48, 4.65)
Fully adjusted Model*	3.29 (2.36, 4.57)
Age group, yr*	
<60	3.10 (1.84, 5.21)
≥60	3.50 (2.19, 5.58)
Gender*	
Men	3.54 (2.30, 5.44)
Women	3.53 (1.98, 6.30)
Race*	
White/European	6.68 (3.97, 11.23)
Black/African American	2.11 (1.18, 3.79)
Diabetes status*	
Yes	2.00 (1.27, 3.15)
No	9.97 (5.89, 16.86)
Estimated GFR category at baseline,* ml/min/1.73 m^2	
<45	2.50 (1.74, 3.61)
≥45	3.89 (1.64, 9.23)

*Adjusted for age, sex, race, clinical site, baseline eGFR, urine ACR, tobacco use, diabetes, hypertension, history of MI, history of HF, ACE/ARB use, diuretics, systolic blood pressure BMI, hemoglobin

Funding: NIDDK Support

FR-OR023

Chronic Kidney Disease and 10-Year Risk of Cardiovascular Death: An Evaluation of European Cardiovascular Prevention Guidelines in a Community-Based Cohort

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Background: The updated European Society of Cardiology prevention guidelines recommend that subjects with chronic kidney disease should be considered at high or very high 10-year risk of future cardiovascular death regardless of age, sex, or other risk factors. As data is limited to support this recommendation we aimed to investigate if moderate, or severe chronic kidney disease is associated with a high, or very high 10-year risk of cardiovascular death, respectively.

Methods: We included 295 191 [46% women] individuals between the age of 40 and 65 years in accordance with current European Society of Cardiology guidelines who underwent health check-ups in primary health care in Stockholm, Sweden between 1985 and 1996. Moderate and severe chronic kidney disease was defined as an estimated glomerular filtration rate 30 to 60 ml/min/1.73 m^2, and 15 to 30 ml/min/1.73 m^2, respectively; high risk, or very high risk of cardiovascular death was defined as 5 to 10%, or > 10% absolute 10-year risk of cardiovascular death, respectively, in accordance with guidelines.

Results: In total there were 4290 cardiovascular deaths within 10 years. Individuals with moderate chronic kidney disease had a 3.91 % absolute 10-year risk of cardiovascular death (95% confidende interval (CI) 3.46-4.36), while participants with severe chronic kidney disease had 14.0% risk (95% CI 7.86-20.2). Women had substantially lower

absolute risks than men, and the absolute risks were substantially lower in younger than in older age groups. Severe chronic kidney disease was associated with a very high risk of cardiovascular death in both men and women older than 50 years.

Conclusions: Our data suggest that risk estimation of future cardiovascular death solely based on the European Society of Cardiology risk categories overestimate the true absolute risk in individuals with moderate chronic kidney disease, particularly in women or in individuals < 60 years of age.

FR-OR024

Weight Loss after Bariatric Surgery Is Associated with Increased eGFR and Decreased Albuminuria Alex R. Chang,[1] Robert M. Perkins,[1] G. Craig Wood,[2] M. Grams.[3] *[1]Nephrology, Geisinger Health System, Danville, PA; [2]Geisinger Obesity Inst, Geisinger Health System, Danville, PA; [3]Nephrology, Johns Hopkins Univ, Baltimore, MD.*

Background: Bariatric surgery is highly effective at inducing rapid weight loss in patients with morbid obesity, who are at markedly increased risk for end-stage renal disease. The effect of bariatric surgery-induced weight loss on kidney function is unknown.

Methods: Using the Geisinger Clinic Bariatric Surgery Research cohort, we examined the association between weight loss after bariatric surgery and estimated glomerular filtration rate (eGFR) calculated by the CKD-EPI equation. The association between weight loss and resolution of albuminuria was examined in the subset of patients with baseline albuminuria (albumin/creatinine ratio >=30mg/g) and subsequent ACR quantification. Mixed effects models were used, adjusted for time and baseline weight, allowing intercepts and slopes to vary for each individual.

Results: A total of 3134 participants were followed for a median of 2.4 years (interquartile range 0.9 to 4.2). Mean age was 47.2, 36.6% had diabetes, and 6.4% had eGFR <60 ml/min/1.73m^2. One year after bariatric surgery, mean weight decreased from 130.1 to 90.9 kg, mean BMI decreased from 46.8 to 32.6 kg/m^2, and mean eGFR increased from 94.8 to 99.1 ml/min/1.73m^2. Every 5 kg of weight loss was associated with a 0.50 ml/min/1.73m^2 (95% CI: 0.42 to 0.57; p<0.001) increase in eGFR. The association between weight loss and increased eGFR was stronger in patients with eGFR <60ml/min/1.73m^2: 0.89 ml/min/1.73m^2 per 5kg decrease (95% CI: 0.63 to 1.15; p<0.001) compared to 0.46 (0.39 to 0.54; p<0.001) for those with eGFR ≥60 ml/min/1.73m^2 (p<0.001 for interaction). In the subset of 108 patients with baseline albuminuria, weight loss was associated with resolution of albuminuria (odds ratio 2.03 per 5kg weight loss, 95% CI: 1.06 to 3.87; p=0.03).

Conclusions: Weight loss after bariatric surgery is associated with increased eGFR and resolution of albuminuria. While these findings suggest potential benefits to the kidney, additional studies using measured GFR or alternative filtration markers to confirm these findings are needed.

FR-OR025

Uromodulin Excretion Predicts Renal Disease Progression in Children with Chronic Kidney Disease Elke Wuehl,[1,2] Anke Doyon,[1,2] Aysun Karabay Bayazit,[2] Nur Canpolat,[2] Ali Duzova,[2] Daniela Kracht,[2] Betul B.S. Sozeri,[2] Mieczyslaw Litwin,[2] Matthias Wuttke,[2] Anna Kottgen,[2] Marietta Kirchner,[2] Olivier Devuyst,[2] Franz S. Schaefer.[1] *[1]Center for Pediatrics and Adolescent Medicine, Heidelberg Univ, Germany; [2]ESCAPE/4C Study Consortium.*

Background: Uromodulin (Tamm-Horsfall protein) has recently received attention as a potential biomarker of CKD progression. A common *UMOD* gene variant is linked to GFR and CKD in the general population. Here, we sought to explore the determinants of uromodulin excretion in children with CKD and its predictive value for renal disease progression.

Methods: Uromodulin concentration was measured in spot urine samples of 770 children with CKD from the 4C Study and ESCAPE Trial cohorts (age 12.4 (4-20) years, eGFR 33 (10-75) ml/min/1.73 m^2). The single nucleotide polymorphism rs4293393 was assessed as part of a genome-wide SNP screening (Illumina Omni 2.5M chips). Mixed linear modeling was performed to identify predictors of uromodulin concentrations, and extended proportional hazard analysis to identify factors affecting the risk of CKD progression. Renal 'death' was defined by eGFR loss >50% from baseline, eGFR <10 or start of renal replacement therapy.

Results: Uromodulin excretion indexed for eGFR was higher in girls (p<0.0001), and decreased with declining eGFR (p<0.0001) and with each copy of the minor *UMOD* allele (median (IQR) TT: 0.25 (0.23); CT: 0.21 (0.22), CC: 0.19 (0.22); p<0.05). Moreover, uromodulin excretion differed significantly among renal diagnosis groups (p<0.005), with highest levels in children with tubulointerstitial nephropathies (0.35 (0.34)) and lowest levels in glomerulopathies (0.18 (0.20)). The renal endpoint was reached by 31.9% of patients with uromodulin excretion in the lower, as compared to 66.5% in the upper distribution quartile (p<0.0001). The risk of renal 'death' increased by 5.3% per 0.1 increase of the uromodulin index (HR 1.053, 95%CI 1.021-1.1086, p=0.001) independently of gender, age, proteinuria, genotype, and renal diagnosis.

Conclusions: In children with CKD uromodulin excretion is independently affected by genotype, gender, renal disease type and renal function. Uromodulin excretion is an independent predictor of renal disease progression.

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

FR-OR026

Children and Young Adults with Chronic Kidney Disease Have Neurocognitive Deficits Compared to Healthy Controls Rebecca Ruebner,[1] Nina Laney,[1] Ji Young Kim,[1] Stephen R. Hooper,[3] Jerilynn Radcliffe,[2] Susan L. Furth.[1] *[1]Pediatrics, Children's Hosp of Phila; [2]Psychology, Children's Hosp of Phila; [3]Psychiatry, Univ North Carolina.*

Background: Neurocognitive deficits have been reported in children and adults with CKD.

Methods: As part of the Neurocognitive Assessment and Magnetic Resonance Imaging Analysis of Children and Young Adults with Chronic Kidney Disease (NiCK) Study, we assessed neurocognitive (NC) function in subjects aged 8-25 yrs with CKD Stages 2-5, compared to age matched healthy controls. A battery of NC measures was administered. Relevant tests were grouped into 11 NC domains. Results of NC tests were converted to age-normalized Z-scores. For domains defined by multiple tests, median Z-score of tests in that domain was used. Each NC domain was analyzed in linear regression with test Z-score as the dependent variable and CKD versus control as the main explanatory variable, adjusted for age, race, and maternal education level.

Results: There were 76 CKD subjects (median eGFR 49.3) and 50 controls (median eGFR 95.7 ml/min/1.73 m2). 20 CKD subjects had a transplant; 16 were previously or currently on dialysis. Significantly poorer performance was seen in CKD subjects versus controls in language, visual spatial, short-term memory, working memory, and inhibitory control; no differences were seen in attention regulation, set-shifting, or global ratings of executive functions.

Cognitive domain	Group difference in Z-score (control vs CKD)*	p value
Language	0.355	<0.001
Visual spatial	0.529	<0.001
Verbal short-term memory	0.882	<0.001
Visual short-term memory	0.414	0.036
Verbal working memory	0.711	<0.001
Visual working memory	0.377	0.05
Inhibitory control	0.239	0.039
Attention regulation	0.216	0.09
Set-shift	0.194	0.23
Problem solving	0.136	0.25
Global executive composite	0.383	0.099

*adjusted for age, race, maternal education

Conclusions: Children and young adults with CKD have significantly lower NC abilities, particularly in verbal memory, verbal working memory, and visual spatial domains. Recognition of these areas could influence how health information is delivered to patients with CKD. Future analyses will focus on correlating structural and functional brain imaging studies with NC performance in CKD.

Funding: Other U.S. Government Support

FR-OR027

Association between Body Mass Index and ESRD Outcomes in U.S. Children Elaine Ku, David V. Glidden, Barbara A. Grimes, Chi-Yuan Hsu, Anthony A. Portale, Kirsten L. Johansen. *UCSF.*

Background: Obesity in adult hemodialysis patients associates with decreased mortality, but in children with ESRD, the association of obesity with survival and transplantation has not been examined in large contemporary cohorts. Obesity may lower the probability of receiving a living-donor transplant, as potential related donors may also be obese and therefore excluded from donation. We examined the association between BMI at initiation of RRT and all-cause mortality and odds of receiving a living-donor transplant.

Methods: We analyzed USRDS data on 11,123 children ages 2-18 who developed ESRD between 1995 and 2011 followed through June 2012. BMI at incident ESRD was expressed as age-standardized z-scores and categorized as underweight (z-score < -2), normal weight (z-score -2 to 2), or overweight (z-score >2). We used Cox models to determine the association between BMI category and death, and logistic regression to determine the association between BMI category and odds of receiving any living versus deceased donor transplant in those who received a transplant within 18 months of ESRD onset (adequate time frame for transplant workup and waitlist time accrual). Models were adjusted for demographic factors, cause of ESRD, initial treatment modality (dialysis versus transplant), and calendar year.

Results: Prevalence of overweight was 10.9% and underweight 6.6% at incident ESRD. There were 1350 deaths over 81,141 person-years of follow-up, with median follow-up of 7.0 years. In multivariable analysis, risk of death was higher in overweight (hazard ratio [HR] 1.48 [95% CI 1.26-1.73]) and underweight children (HR 1.42 [95% CI 1.18-1.71]) relative to normal weight children. 5,953 patients received a kidney transplant within 18 months of ESRD onset, of which 55.6% were from living donors. Those who were overweight were less likely to receive a living versus deceased-donor transplant (odds ratio 0.72 [95% CI 0.60-0.87], p=0.001).

Conclusions: Both extremes of BMI were associated with higher risk of death in children, which differs from observations in adult hemodialysis patients. Being overweight may be a barrier to living-donor transplantation in children and its association with higher mortality warrants further investigation.

Funding: NIDDK Support, Private Foundation Support

FR-OR028

Does Tubular Secretion of Creatinine Increase Proportionally as Renal Function Declines? Xuehan Zhang,[1,2] Yen Chung Lin,[3] Charles E. McCulloch,[2] Isabel Elaine Allen,[2] Nisha Bansal,[4] Alan S. Go,[2,5] Chi-Yuan Hsu.[2,5] *[1]Peking Union Medical College Hospital; [2]Univ of California-San Francisco; [3]Taipei Medical Univ Hospital; [4]Univ of Washington; [5]Kaiser Permanente Northern California.*

Background: Numerous textbooks and review articles state that as renal function declines, the proportion of creatinine that is cleared by tubular secretion increases. This is identified as a major weakness of using creatinine clearance(CrCl) to estimate renal function. However, this is based on the assumption that measured glomerular filtration rate (GFR) equals true renal function and does not take into consideration errors in measured GFR (and CrCl) due to short-term biological variability or test imprecisions.

Methods: We analyzed cross-sectional data from 1342 Chronic Renal Insufficiency Cohort (CRIC) Study participants who underwent measurement of glomerular filtration rate by iothalamate clearance (iGFR) and of CrCl by 24-hour urine collection. We examined the CrCl/iGFR ratio classified by categories of iGFR and also by categories of CrCl.

Results: Overall, mean CrCl to iGFR ratio was 1.13. CrCl/iGFR ratio was higher at lower iGFR levels(Table). However, in the same patients, the ratio of CrCl/iGFR was *lower* at lower CrCl levels(Table).

iGFR (ml/min/1.73m²)	<30 (n=250)	30-45 (n=400)	45-60 (n=362)	≥60 (n=330)
median CrCl/iGFR ratio	1.27	1.13	1.07	0.99
CrCl (ml/min/1.73m²)	<30 (n=244)	30-45 (n=341)	45-60 (n=333)	≥60 (n=424)
median CrCl/iGFR ratio	0.78	1.04	1.12	1.21

We were able to replicate these trends in a simulation exercise in which there was no variation in the ratio of CrCl/iGFR with true renal function. Our simulation generated realistic true renal function values with measured iGFR differing from true renal function by only measurement error and CrCl differing from true renal function by a factor of 1.13 and measurement error.

Conclusions: Observations that the CrCl/iGFR ratio increases with lower iGFR can be entirely explained by measurement errors (in both CrCl and iGFR) without the need to postulate that progression of chronic kidney disease leads to actual physiological increase in tubular secretion of creatinine.

FR-OR029

Serum Filtration Markers to Estimate Residual Kidney Function (RKF) without Urine Collection in Dialysis Patients Tariq Shafi,[1] Andrew S. Levey,[2] Friedo W. Dekker,[4] Lesley Inker,[2] Raymond T. Krediet,[5] George J. Schwartz,[3] Wieneke Michels,[5] Tiny Hoekstra,[4] Josef Coresh.[1] *[1]Johns Hopkins Univ; [2]Tufts Medical Center; [3]Univ of Rochester; [4]Leiden Univ Medical Center; [5]Academic Medical Center.*

Background: RKF is a significant contributor to solute clearance but cannot be assessed without urine collection. We hypothesized that filtration markers that are either not removed by dialysis [β-trace protein (BTP)] or partially removed [cystatin C (CYS), β2-microglobulin (B2M)], might allow RKF estimation (eRKF) without urine collection.

Methods: We developed eRKF equations in a derivation cohort (N=74) of dialysis patients in Baltimore with glomerular filtration rate measured by iohexol clearance (mRKF). We validated the equations in the Netherlands Cooperative Study on the Adequacy of Dialysis (NEOCSAD; N=994; mRKF=24-hour urine urea+creatinine clearance). We assessed equations' performance by calculating median bias (mRKF-eRKF), precision (bias' interquartile range) and accuracy (eRKF within ±2ml/min/1.73m² of mRKF).

Results: In the derivation and validation cohorts, mean age was 53 and 61 years; 62 and 60% were male; 51 and 88% were white; and mRKF was 3.0±3.2 and 3.7±2.7 ml/min/1.73m², respectively. Age, sex and race were not included in eRKF models (p≥0.01). Bias was lower in HD compared with PD patients whereas precision was similar. BTP, B2M, CYS and combination improved RKF estimation compared with urea or creatinine.

Performance (95% CI) in NECOSAD						
HD(N=723)	**Urea**	**Creatinine**	**BTP**	**B2M**	**CYS**	**BTP B2M CYS**
Bias, ml/min	1.2 (0.9, 1.4)	0.6 (0.5, 0.8)	0.5 (0.4, 0.7)*	0.9 (0.8, 1)	0.5 (0.3, 0.6)***	0.6 (0.5, 0.7)***
Precision, ml/min	3.1 (2.9, 3.4)	2.5 (2.3, 2.8)	2.1 (1.9, 2.3)	1.9 (1.8, 2)	2.3 (2.1, 2.5)	1.8 (1.7, 2)
Accuracy,%	65	70	74**	72	74**	78***
PD(N=271)						
Bias, ml/min	1.3 (1, 1.6)	1.3 (1, 1.6)	1.2 (1, 1.5)*	1 (0.8, 1.2)	0.9 (0.7, 1.2)***	1.1 (0.9, 1.3)*
Precision, ml/min	3.6 (2.9, 4.2)	2.7 (2.3, 3.1)	2.3 (1.9, 2.7)	2 (1.7, 2.2)	2.5 (2.1, 2.9)	2 (1.7, 2.3)
Accuracy,%	59	65	66	71*	70*	72**
*p<0.05 **p<0.01 ***p<0.001 (vs. Creatinine)						

Conclusions: Serum filtration markers may allow RKF estimation in dialysis patients without cumbersome urine collections.

Funding: NIDDK Support

FR-OR030

Independent Predictors of Uromodulin Excretion in a Large Population Survey: The CARTaGENE Study Catherine Delmas-Frenette,[1] Stephan Troyanov,[1] Philip Awadalla,[2] Olivier Devuyst,[3] Francois Madore.[1] *[1]Dept of Nephrology, Hopital du Sacre-Cœur de Montreal, Montreal, QC, Canada; [2]Medical and Population Genomics Laboratory, Univ of Montreal, Montreal, Qc, Canada; [3]Inst of Physiology, Univ of Zurich, Zurich, Switzerland.*

Background: Single nucleotide polymorphisms near the *UMOD* gene have been associated with uromodulin excretion and CKD risk. However, the impact of non-genetic factors on uromodulin excretion remains ill-defined.

Methods: A sample of 946 participants from the CARTaGENE survey was genotyped for rs4293393. Urinary uromodulin levels were measured using an ELISA method and expressed as a creatinine ratio. We tested associations between uromodulin and several determinants (gender, BMI, smoking status, diabetes status, mean arterial pressure, GFR [using the CKD-EPI equation], serum HDL cholesterol levels and the fractional excretion [FE] of sodium and uric acid) using univariate and multivariate linear regression after log transformation of uromodulin measurements.

Results: The population studied was 54±9 years old, composed of 51% women and had a mean GFR of 90±14 ml/min/1.73m². The CC/CT genotypes (rs4293393) were present in 32% of participants and were associated with lower urinary uromodulin levels (CT/CC: 21, IQR 10-34 *versus* TT: 27, IQR 12-44 mg/g creatinine, p<0.001). **Table 1** shows predictors of urinary uromodulin:

Variable	Univariate		Multivariate	
	Standardized B	p-value	Standardized B	p-value
Gender (men)	-0.122	<0.001	-0.018	0.621
Current smoking	0.003	0.067	0.055	0.098
BMI (body mass index)	-0.085	0.009	-0.012	0.737
HDL-Cholesterol	0.139	<0.001	0.035	0.354
Diabetes mellitus	0.085	0.009	0.048	0.134
Mean arterial pressure	-0.072	0.026	-0.013	0.713
GFR (CKD-EPI)	0.108	0.001	0.126	<0.001
rs4293393 CT/CC	-0.100	0.002	-0.107	0.001
Fe Na	0.158	<0.001	0.097	0.005
Fe Uric acid	0.038	0.223	0.263	<0.001

Conclusions: This study shows that several factors impact uromodulin excretion. In addition to genetic polymorphism (CT/CC at rs4293393), a lower GFR and lower fractional excretion of sodium and uric acid were independently associated with lower uromodulin excretion. Further studies are warranted to elucidate the mechanisms underlying these associations.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR031

Precise Estimation of Glomerular Filtration Rate from Multiple Blood Biomarkers Josef Coresh,[1] Lesley Inker,[2] Andrew S. Levey.[2] *[1]Epidemiology, Johns Hopkins Univ, Baltimore, MD; [2]Biomedical, Tufts Univ, Boston, MA; [3]Medicine, Tufts Univ, Boston, MA.*

Background: Precisionof estimated GFR (eGFR) is improved by adding serum cystatin C (Scys) to serum creatinine (Scr) and demographics (Inker et al. NEJM 2012). It is unclear if adding other markers in a panel (peGFR) can improve precision to a level comparable to direct measurement of GFR (mGFR).

Methods: Study Population: 200 individuals with mGFR by urinary clearance of I-125 Iothalamate at the AASK F48 (48 month follow-up visit) with mGFRs at F42 and F54 visits within 25% of mGFR at F48. **Laboratory Methods:** Untargeted, GCMS and LCMS-based metabolomic quantification of serum (Metabolon, Inc.). **Data Analysis Methods:** On the log scale, metabolites were ranked by correlation with average mGFR (at F42, F48 and F54). peGFR constructed by stepwise regression excluding creatinine. Precision of eGFR estimate evaluated using root-mean square error (RMSE) and the percent of errors greater than 30%, 20% and 10% from average mGFR (e.g. 1-P30).

Results: Average mGFR was highly correlated with many metabolites (195 negative and 61 positive at $p<10^{-3}$; 12 better than Scr r<-0.7, $p<10^{-16}$; n=188 with data on 780 analytes). peGFR based on the top 5 (excluding creatinine) showed excellent precision and low error rates. Jaffe assay was superior to metabolite discovery assay (M513 1-P30 16.0% versus 28.5%) but both required demographics for good performance while peGFR using the top 5 or 10 metabolites did not.

Table: Precision (RMSE) and Proportion with Errors (1-**P30** = >**30**% between eGFR and mGFR)				
	eGFR without age & sex		eGFR with age & sex	
Analytes in the estimate:	RMSE	1-P30	RMSE	1-P30
Creatinine (M513)	0.29	29.8%	0.26	23.9%
Creatinine (Jaffe)	0.23	17.0%	0.19	8.5%
Top 5 Metabolites (excl. creatinine)	**0.14**	**3.2%**	**0.14**	**2.1%**
Creatinine + Cystatin C	0.16	5.8%	0.14	4.3%
Creatinine + Cystatin C +top5 Metabolites	0.14	1.6%	0.13	1.1%

Conclusions: A panel of markers from a single blood draw can yield precision comparable or better than a single mGFR (RMSE ~0.14, 1-P30~6.9%; Kwong et al. 2010 in AASK). Targeted assays and further validation are ongoing to study clinical implications. [Patents pending by the authors and Metabolon].

Funding: NIDDK Support, Private Foundation Support

FR-OR032

Estimated GFR Is Biased by Non-Traditional Cardiovascular Risk Factors

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Background: Estimated GFR (eGFR) based on cystatin C or creatinine performs equally well in estimating measured GFR, but associates differently with CVD and mortality. Non-GFR related components associated with creatinine or cystatin C may explain this paradox. We investigated the non-GFR related associations between eGFR and two groups of non-traditional CVD risk factors; the L-arginine/dimethylarginine metabolism and insulin resistance (IR).

Methods: GFR was measured with iohexol-clearance in a cross-sectional study of 1627 persons from the general population without CVD or chronic kidney disease.

Results: ADMA, SDMA, the L-arginine/ADMA ratio and IR were associated with eGFRcre after accounting for mGFR. eGFRcys had a similar residual association with SDMA, a borderline significant association with ADMA in the opposite direction, and a stronger residual association with IR compared to eGFRcre.

Generalized estimating equations showing associations between risk factors and eGFR after accounting for mGFR[a]

		Adjusted[d]	
Dependent variable	**Independent (per SD increase)**	**Estimate[b] (ml/min/1.73m)**	**P[c]**
eGFRcre	L-arginine	-0.7	0.03
	ADMA	1.6	<0.001
	L-arg/ADMA ratio	-1.8	<0.001
	SDMA	2.1	<0.001
	Insulin	-0.1	0.8
	HOMA-IR	-0.8	0.04
eGFRcys			
	L-arginine	-0.4	0.1
	ADMA	-0.6	0.06
	L-arg/ADMA ratio	0.0	1.0
	SDMA	1.3	<0.001
	Insulin	-1.0	0.01
	HOMA-IR	-1.4	<0.001

[a]GEE with eGFR and mGFR as stacked dependent variables regressed on each independent variable to compare the difference in eGFR and mGFR regression coefficients. [b]Difference between eGFR and mGFR estimates. [c]Significance determined by the interaction between each risk factor and eGFR relative to mGFR. [d]adjusted for age, sex, ACE-i, ARB, BMI, BP, chol., gluc.,smoking and ACR.

Conclusions: Non-traditional CVD risk factors are usually not measured in epidemiological studies of eGFR. Thus, adjustment cannot be performed to reduce non-GFR related bias of these risk factors. Our findings indicate that both eGFRcre and eGFRcys are influenced by non-traditional risk factors, and this may bias risk prediction in longitudinal studies.

Funding: Government Support - Non-U.S.

FR-OR033

Change in Measured GFR Does Not Outperform Change in Estimated GFR in Predicting Adverse Outcomes in CKD: Results from the CRIC Study

Elaine Ku, Dawei Xie, Michael Shlipak, Amanda Hyre Anderson, Jing Chen, Alan S. Go, Jiang He, Edward J. Horwitz, Mahboob Rahman, Ana C. Ricardo, James H. Sondheimer, Raymond R. Townsend, Chi-Yuan Hsu. *CRIC Study Group.*

Background: Measured GFR has long been considered the "gold standard" measure of kidney function, but recent studies have shown that measured GFR is not consistently superior to estimated GFR in explaining CKD-related comorbidities. The association between longitudinal changes in measured or estimated GFR and adverse outcomes has not been previously examined.

Methods: We studied 942 CKD patients who had at least 2 of the following determined concurrently: iothalamate GFR (iGFR), estimated GFR by creatinine (eGFRcre, MDRD equation), and estimated GFR by cystatin C (eGFRcys, Stevens 2008). 839 of these patients also had 24-hour urine creatinine clearance (CrCl) measures. We examined the associations between changes in these parameters and risk of ESRD, cardiovascular (CVD) events (MI, HF, CVA, and PAD), and all-cause mortality using univariate Cox models.

Results: Declines in iGFR (as a continuous and dichotomized predictor) were not consistently more strongly associated than declines in eGFR with risk of ESRD or CVD events.

Decline in kidney function measured by	**ESRD** Hazard ratio [HR] (95% CI) for every 5 mL/min/1.73 m^2 decrease over 2 years	C-statistic	**ESRD** HR comparing most negative slope tertile versus all others	C-statistic
iGFR	1.28 (1.20, 1.36)	0.72	2.85 (2.01, 4.03)	0.65
$eGFR_{cre}$	1.54 (1.44, 1.66)	0.76	4.54 (3.16, 6.52)	0.70
$eGFR_{cys}$	1.31 (1.23, 1.40)	0.70	2.09 (1.48, 2.96)	0.61
CrCl	1.03 (1.00, 1.05)	0.60	1.51 (1.06, 2.14)	0.56
	CVD event		**CVD event**	
iGFR	1.12 (1.03, 1.20)	0.59	1.62 (1.10, 2.40)	0.55
$eGFR_{cre}$	1.22 (1.11, 1.36)	0.60	1.91 (1.30, 2.80)	0.59
$eGFR_{cys}$	1.10 (1.02, 1.20)	0.57	1.13 (0.76, 1.70)	0.54
CrCl	1.00 (0.97, 1.03)	0.50	0.90 (0.60, 1.36)	0.51

There was no association between death and change in any of the kidney function measures.

Conclusions: Decline in iGFR is not consistently superior in predicting adverse outcomes in CKD than decline in eGFR (even when earlier generations of estimating equations were used). These results question continued acceptance of iGFR as the "gold standard" measure of kidney function.

Funding: NIDDK Support, Private Foundation Support

FR-OR034

The Association of Maternal Obesity with Infant Congenital Abnormalities of the Kidney and Urinary Tract in Washington State

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Background: Congenital abnormalities of the kidney and urinary tract (CAKUT) are diagnosed in up to 1% of pregnancies and account for 20-30% of the abnormalities identified in the prenatal period. In previous studies, maternal obesity has been associated with congenital malformations in offspring. Our aim was to evaluate the association between maternal obesity and CAKUT in offspring.

Methods: We conducted a population-based, case control study using linked birth-hospital discharge records from Washington State, 2003-2012. We identified 3,221 cases using ICD-9 codes. Controls were defined as births without any classifying ICD-9 codes, matched to cases by year of birth in a 4:1 ratio.

Results: We found no parental factors that altered the OR estimate substantially; thus we reported the crude OR. Compared to controls, mothers giving birth to infants with CUTA were more likely to be obese (OR=1.26, 95% CI=1.14-1.40). We found a significant positive trend between odds of CAKUT in offspring and higher categories of obesity (score test for trend of odds p<0.0001). This association remained significant in offspring with isolated CAKUT (OR=1.20, 95% CI 1.07-1.35). Maternal overweight was not associated with CAKUT in offspring.

Association Between Maternal Weight Category and CAKUT in WA State 2003-2012

	Controls		**Overall CAKUT**		**OR**	**95% CI**	**Renal Anomalies**		**OR**	**95% CI**
	No.	**%**	**No.**	**%**			**No.**	**%**		
Normal BMI	5608	49.8	1271	46.6	1.00	Ref	981	46.1	1.00	Ref
Overweight	3044	27.0	712	26.1	1.03	0.93-1.14	561	26.4	1.05	0.94-1.18
Obese	2603	23.1	745	27.3	1.26	1.14-1.40	583	27.4	1.28	1.14-1.43
Obese Cat I	1493	57.4	419	56.2	1.24	1.09-1.40	327	56.1	1.25	1.09-1.44
Obese Cat II	680	26.1	187	25.1	1.21	1.02-1.44	149	25.6	1.25	1.04-1.51
Obese Cat III	430	16.5	139	18.7	1.43	1.17-1.74	107	18.3	1.42	1.14-1.78

Conclusions: Our results show a robust positive association between maternal obesity and CAKUT in offspring. In addition, we show for the first time an obesity severity effect on the association with CAKUT in offspring. This research provides additional evidence for the public health importance of obesity, particularly as a modifiable risk factor.

Funding: NIDDK Support

FR-OR035

Payer Impact of Anemia Management Among Medicare Insured Dialysis Patients During the Implementation of the ESRD Prospective Payment System (PPS)

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Background: Since the 2011 implementation of the ESRD PPS and the FDA label changes for erythropoietin stimulating agents (ESAs), hemoglobin (Hb) concentrations have declined while red blood cell (RBC) transfusion rates have risen. CMS intended to reduce outpatient dialysis costs; however, whether these reforms reduced anemia management costs or merely shifted them to other payers is uncertain.

Methods: Using 2009-2011 U.S. Medicare ESRD data, including Parts A and B claims, we identified maintenance hemodialysis patients from facilities that initially enrolled 100% into the ESRD PPS. Outpatient (OP) transfusion- and dialysis-related costs were identified and summed at the facility level, with a per patient per month (PPPM) cost calculated. Patterns of ESA and IV iron use, Hb concentrations, and transfusion rates were also analyzed.

Results: Approximately 4,200 facilities were included over the study period; the number of eligible patients per facility, as well as the basic demographics of those included, remained relatively stable, except time on dialysis, which increased slightly. From 2009-2011, the distribution of the median % of patients per facility with Hb < 10 g/dL in a given month shifted upwards (from 9.4% to 18.2%). While the OP transfusion rates remained relatively stable, dialysis facilities saw the mean number of inpatient (IP) transfusions for patients dialyzing at their facility increase by > 20%; the mean PPPM OP transfusion-related ($5-$6) and dialysis-related (~$2,300) costs per facility remained relatively stable over the same time period.

Conclusions: Although on a PPPM basis OP transfusion-related costs to Medicare did not significantly change from 2009 to 2011, the marked increase in IP transfusions, the most common (~80%) site of transfusions, suggests that part of the cost burden of anemia management in dialysis patients has been shifted from dialysis facilities to the hospital IP setting. Further exploration to determine the economic burden to payers when maintenance dialysis patients receive IP RBC transfusions is warranted.

FR-OR036

Evaluation of the Annual Red Blood Cell Transfusion Rate in Patients on Dialysis before and after the Implementation of the New Prospective Payment System (PPS) Allan J. Collins,[1] Scott Jackson,[1] Yi Peng,[1] Anne C. Beaubrun,[2] Keri Monda,[2] Brian D. Bradbury,[2] David T. Gilbertson.[1] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]Amgen, Inc., Thousand Oaks, CA.*

Background: On January 1, 2011, the Centers for Medicare and Medicaid Services implemented a new PPS, bundling payment for injectable drugs for dialysis patients. This change, along with clinical trial-based FDA changes to ESA labeling to increase safety, led to a decline in ESA doses and Hb levels. This study evaluated transfusion event rates before and after PPS implementation.

Methods: We used Medicare ESRD yearly standard analytical files, which allow for the most complete identification of transfusion events, to create yearly cohorts, 2008-2011. Each cohort comprised patients who were dialyzing as of January 1 of the year. Each patient was followed for transfusion events from January 1 until death, kidney transplant, loss to follow-up, or December 31 of the year.

Results: Between 2008 and 2010, annual transfusion rates were 337.3 to 340.5 per 1000 patient-years (PY). Rates increased to 411.0 transfusions per 1000 PY in 2011 (21.8% increase between 2008 and 2011). Rates were consistently higher in PD versus HD and also varied consistently over time within groups defined by age, sex, race, and dialysis vintage.

Conclusions: Concomitant with lower ESA doses and Hb levels, transfusion rates increased after implementation of the ESRD PPS and the label change. Balancing higher risk of adverse events due to high hemoglobin levels against the consequences of increased transfusion rates will be important.

Funding: Pharmaceutical Company Support - Amgen

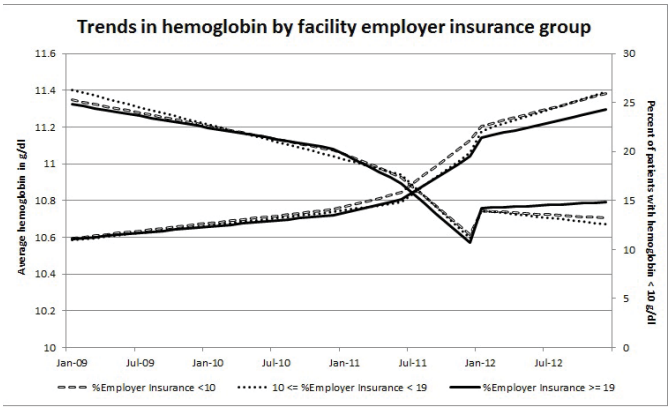
FR-OR037

Changes in Hemoglobin under the Dialysis Prospective Payment System (PPS) by Facility Payer Mix Marc Turenne,[1] Purna Mukhopadhyay,[1] Jeffrey Pearson,[1] Elizabeth L. Cope,[1] Chad M. Cogan,[1] Brenda W. Gillespie.[2] *[1]Arbor Research Collaborative for Health, Ann Arbor; [2]Univ of Michigan, Ann Arbor.*

Background: The Medicare ESRD PPS set fixed payment rates for outpatient dialysis-related services, which increased risk to facilities that payments may not cover the cost of caring for some patients. These financial risks are likely to be greater for independent dialysis facilities, especially those with relatively few privately insured patients for whom payment rates may be higher.

Methods: We used Medicare dialysis claims to examine trends in Hgb values from 77,000+ Medicare dialysis patients in over 530 independent dialysis facilities each month from 2009-2012. We fitted mixed models of average Hgb and the percent of patients with Hgb<10 by facility month, with spline points corresponding to key payment and regulatory changes and adjustment for repeated facility observations, to compare three groups of facilities: low, moderate, or higher levels of employer insurance (EI) among incident patients.

Results: Similar initial trends for all three facility groups in 2009-10 were followed by a greater decline in predicted average Hgb and a greater increase in predicted Hgb<10 in the post-PPS period for facilities with low/moderate EI (p<0.0001).



Similar trends by facility EI group were observed for black and non-black patients. Facilities with low/moderate EI were more likely to be in rural areas (24% and 19%, respectively) than those in the high EI group (10%).

Conclusions: There appear to be emerging differences in Hgb levels in independent facilities based on facility payer mix, which may have a disparate impact on rural populations. In addition to evaluating the overall impact of the PPS and other recent policy and regulatory changes, it is important to consider the role of potential mediating factors such as facility payer mix that could have implications for health disparities.

Funding: Other NIH Support - National Institute on Minority Health and Health Disparities (NIH-NIMHD)

FR-OR038

Decreasing the Cost of Anemia Treatment, in Hemodialysis Patients, Through Splitting of Iron and Darbepoetin Doses Marco Oliveira Mendes,[1] David Navarro,[1] Ana Azevedo,[2] Cristina Jorge,[1] Patrícia Matias,[1] Ana Carina Ferreira,[2] Bruno Costa Pinto,[1] Fernanda Gomes,[1] Célia Gil,[1] Manuel A. Ferreira,[1] Ines Aires.[1] *[1]Nefrologia, Nephrocare de Vila Franca de Xira, Vila Franca de Xira, Lisboa, Portugal; [2]Nefrologia, Dialverca, Alverca, Lisboa, Portugal.*

Background: As erythropoiesis is a continuous process, we hypothesized that smaller doses of darbepoetin alpha (EPO) administered more regularly, could result is less variations of its seric levels and be more "physiologic" and effective. In a prevalent HD population already exposed to iron splitting, we evaluated the hypothetical additive effects of the EPO splitting in "mini-doses".

Methods: A 34-month prospective study was performed in a cohort of 110 pts, with mean age of 67.9±14.2 years, 50.9% female, 36.3% diabetics, with mean HD time of 85.3±49.4 months. In the first 12 months of the study (T1), the usual dose regime for EPO (weekly, every other week or monthly) was unchanged. In the following 22 months (T2), EPO doses were divided into multiples of 10 µg and distributed among the maximum possible of HD sessions. The same membrane (FMC-Cordiax) and dialysis method (on-line hemodiafiltration) was used throughout the study. Paired T-student and Wilcoxon analysis were performed.

Results: After EPO splitting we verified a slight decrease in hemoglobin levels (T1: 11,45±0,61 g/dl versus T2: 11,31±0,62 g/dl; $p < 0.01$), but with a remarkable decrease in EPO consumption (T1: 1,34±1,83 µg/Kg versus T2: 0,8±1,48 µg/Kg; $p < 0.001$) and in the erythropoietin resistance index (ERI) (T1: 5,87±8,90 IU/Kg/week versus T2: 3,5±7,53 IU/Kg/week; $p < 0.001$). We also observed an increase in ferritin (T1: 490±138 µg/L versus T2: 560±149 µg/L;$p < 0.001$) and in CRP levels (T1: 8,91±8,27 mg/L versus T2: 10,87±9,53 mg/L; $p < 0.001$), with the same consumption of iron (T1: 1,62±1,71 mg versus T2: 1,82±1,52 mg; $p = 0.172$). No correlation between ferritin and CRP levels was found.

Conclusions: From these results, we conclude that the splitting of iron and EPO in "mini-doses" is highly effective and allows a significant decrease in the costs of anemia treatment. Larger and randomized studies are needed to exclude that this new strategy further induces inflammation.

FR-OR039

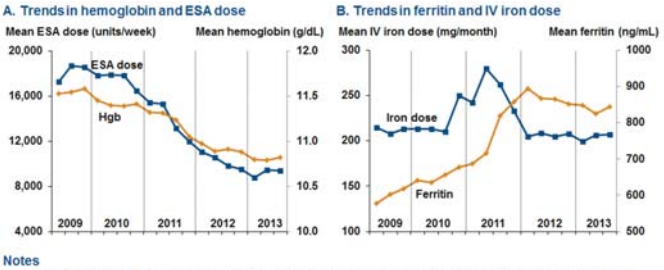
Understanding the Recent Increase in Ferritin Levels in U.S. Dialysis Patients Angelo Karaboyas,[1] Hal Morgenstern,[1,2] Jacqueline G. Nolen,[3] Raymond M. Hakim,[4] Kamyar Kalantar-Zadeh,[5] Philip Zager,[6] Ronald L. Pisoni,[1] Friedrich K. Port,[1] Bruce M. Robinson.[1,2] *[1]Arbor Research Collaborative for Health; [2]U of Michigan; [3]Vifor Pharma; [4]Vanderbilt U; [5]U of Cal-Irvine; [6]U of New Mexico.*

Background: Ferritin levels in U.S. hemodialysis (HD) patients rose dramatically from 2010 to 2012, coincident with changes to reimbursement and ESA prescribing info in 2011. Over this time, ESA dose and hemoglobin (Hgb) decreased; case-mix changed minimally. Clear explanation for the ferritin rise is lacking; here we evaluate possible causes.

Methods: Quarterly trends (2009-13) in Hgb, ESA dose, ferritin, and IV iron dose were summarized from treatment-level data in 8961 Dialysis Outcomes and Practice Patterns Study (DOPPS) patients in 91 U.S. HD facilities.

Results: ESA dose and Hgb declined from 2010 to 2012 (Fig A). IV iron dose rose sharply in Q4 2010, stayed elevated for 1 yr, then returned to 2009-10 levels (Fig B). Ferritin began to rise in 2010 before IV iron dose increased, rose dramatically during 2011, and remained high >1 yr after reduction in IV iron dose. Other findings: (1) Ferritin was not higher due to timing of measurement with respect to IV iron dosing: mean dose over 3 wks before ferritin measurement was 157 versus 137 mg in 2010 versus 2012. (2) DOPPS Practice Monitor data did not show greater iron blocking (e.g., inflammation/infection): mean TSAT and albumin were stable.

Conclusions: The large rise in ferritin after ESA bundling in 2011, sustained into 2013, has received considerable attention because the levels (>800 ng/mL) exceed KDIGO recs and clinical consequences are unclear. Contrary to expectation, rise in IV iron dose was curtailed after 2011 so does not readily explain the persistently high ferritin. High ferritin may reflect retained excess iron due to lower erythropoietic requirements (lower Hgb and ESA dose). To evaluate further, analyses of within-patient changes over time are planned.



Funding: Pharmaceutical Company Support - The DOPPS program is supported by grants to Arbor Research from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, Ltd. Additional support for specific projects is provided in Canada by Amgen, BHC Medical, Janssen, Takeda, Kidney Foundation of Canada (for logistics support); in Germany by Hexal, DGfN, Shire, WiNe Institute; for PDOPPS in Japan by the Japanese Society for Peritoneal Dialysis; for PDOPPS by Fresenius Medical Care

FR-OR040

Longitudinal Changes in Serum Ferritin over 5 Years in 134,090 Incident Dialysis Patients

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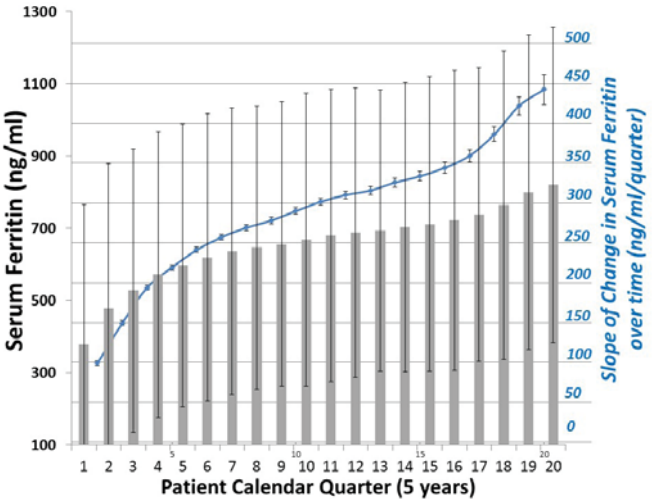
Background: Recent rise in averaged serum ferritin in prevalent dialysis patients in the U.S.A. have been attributed to changes in practice pattern including higher doses of IV iron and/or lower use of ESA. We hypothesized that maintenance dialysis therapy per se may have a bearing on the upward trend of ferritin over time independent of secular trend.

Methods: We examined changes in serum ferritin over up to 5 yrs in 134,090 incident dialysis patients (124,395 HD and 9,527 PD) who initiated dialysis therapy bewteen 2007-2011 in one of the clinics of a large dialysis organization in the U.S.A. We examined mean serum ferritin in each patient quarter (91 days) up to 20 quarters and calculated the rate of change in ferritin from first quarter to the n^{th} quarter using time-varying mixed models.

Results: The 134,090 incident dialysis patients were 61±15 yrs old and included 43% women, 30% African Americans, 14% Hispanics, and 57% diabetics. Mean serum ferritin (±SD) at the start of dialysis (Quarter 1) was 377±388 ng/ml, increasing to 597±391, 655±394, 693±389, and 737±407 ng/ml at the start of Year 2, Year 3, Year 4, and Year 5 of dialysis therapy, respectively. The slope of change in the rate of ferritin change over time continued to show upward trends.



Conclusions: Notwithstanding the current limitation related to medication and their doses, we observed a consistent rise in serum ferritin over time from the start of maintenance dialysis therapy, and this upward movement exhibits no evidence of slowing tendency at any point in time for up to 5 years. Whether the rise in serum ferritin is inherent to dialysis therapy independent of changes in practice pattern warrants additional studies.

Funding: NIDDK Support

FR-OR041

Association between Intravenous Iron Receipt during Infection-Related Hospitalization and Clinical Outcomes among Hemodialysis Patients

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Background: Guidelines for the treatment of anemia of chronic kidney disease have recommended withholding intravenous (IV) iron in the setting of active infection. However, no data specifically supports this recommendation, and there is a critical need for investigation of how clinical outcomes differ according to receipt of IV iron in the setting of infection among hemodialysis patients.

Methods: From the USRDS, we identified 23,306 Medicare-covered adults on in-center hemodialysis who had received IV iron within 14 days of their first hospitalization for bacterial infection in 2010. Using logistic, Cox, and linear regression models in which we adjusted for demographics, comorbidities and the infected organ system, we evaluated the association between continued receipt of IV iron (any dose or duration) versus no receipt of IV iron during the infection-related hospitalization (IRH) and all-cause mortality (within 30 days of admission and in 2010), need for ICU/CCU care, readmission for IRH within 30 days of discharge, and length of hospital stay (log-transformed).

Results: There were 2,518 patients who continued to receive IV iron during their first IRH in 2010. Continued receipt of IV iron was not associated with age, dialysis vintage or comorbidities. There were 2,684 deaths within 30 days of admission and 7,059 deaths in 2010 with a median follow-up time of 173 days (25-75^{th} percentiles: 78-271 days). Continued receipt of IV iron during an IRH was not associated with adverse outcomes in hemodialysis patients (Table).

Outcome	Estimated IV iron association	95% CI
All-cause 30-day mortality	OR 0.91	0.76-1.08
All-cause mortality (2010)	HR 0.94	0.86-1.03
ICU/CCU care	OR 0.92	0.83-1.03
30-day IRH readmission	OR 1.06	0.89-1.25
Length of stay	1.9% fewer median days with continued IV iron receipt	-5.3 to +1.7%

OR = odds ratio, HR = hazard ratio, CI = confidence interval

Conclusions: Our analysis does not support a guideline recommendation to withhold IV iron in the setting of active infection among hemodialysis patients.

Funding: NIDDK Support, Private Foundation Support

FR-OR042

Robust Treatment for Renal Anemia Increase Frataxin Level in Parallel with the Improved Mitochondria Metabolism and Oxidative Stress in ESA-Hyporesponsive Anemic Patients on Maintenance Hemodialysis (MHD)

Yukiko Hasuike,[1] Wataru Fukao,[2] Kazuhiro Toyoda,[2] Satoko Masachika,[2] Yuki Morikami,[1] Tomoko Kimura,[1] Kiyoko Yamamoto,[1] Sayuri Kawada,[1] Kosuke Mizusaki,[1] Soshi Yorifuji,[1] Mai Oue,[1] Shoji Kaibe,[1] Mana Yahiro,[1] Aritoshi Kida,[1] Masayoshi Nanami,[1] Takeshi Nakanishi.[1] *[1]Div of Kidney and Dialysis, Dept of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan; [2]Kidney and Dialysis, Meiwa Hospital, Nishinomiya, Japan.*

Background: In MHD, the disturbance of iron regulation for erythropoiesis could cause hyporesponsiveness to erythropoiesis stimulating agents (ESA). We previously reported the decrease in frataxin, an iron regulator, and a significant association between frataxin and oxidative stress in MHD *[Clin Exp Nephrol 2012]*. The effects of robust treatment using longer-acting ESA on hepcidin level, iron regulation, and redox status were investigated.

Methods: The ESA-hyporesponsive anemic HD patients without iron deficiency (serum ferritin ›250 ng/ml) were recruited (N=20) and administrated darbepoetin-α weekly whose dose was adjusted to increase hemoglobin up to 11.0 g/dl. Factors related to iron regulation (ferritin, frataxin, hepcidin) and markers of oxidative stress (advanced oxidation protein products (AOPP), pentosidine, GSH/GSSG ratio) were investigated during the 6 months period.

Results: During the 6 months period, hepcidin levels were significantly decreased by 25.3% (137.3±19.1 ng/ml to 102.5±9.5 ng/ml, p=0.0061) with a significant reduction of ferritin (median 510 ng/ml to 415 ng/ml, p=0.0032).Frataxin protein were significantly increased compared with that at start (202.9±7.1 ng/gHb, versus 155.5±4.7 ng/gHb, p<0.0001). GSH/GSSG ratio tended to increase (p=0.0825) and both AOPP and pentosidine tended to decrease (p=0.0872, 0.0978).

Conclusions: The robust treatment of renal anemia may increase frataxin and improve mitochondrial iron metabolism in MHD, which could subsequently parallel with the improvement of iron metabolism and oxidative stress. Further studies may be necessary to better elucidate the biological usefulness of ESA.

Funding: Private Foundation Support

FR-OR043

Is Erythropoietin Resistance Index (ERI) Merely a Proxy for Inflammation?

Anirudh Rao,[1] Julie A. Gilg,[1] Fergus J. Caskey.[1,2] *[1]UK Renal Registry, Bristol, United Kingdom; [2]Univ of Bristol, Bristol, United Kingdom.*

Background: Inflammation contributes to EPO resistance with ERI being increasingly used to measure EPO resistance and as a predictor for adverse outcomes. **Aim:** To describe the prevalence of high ERI, to identify factors associated with high ERI and 1 year survival.

Methods: Prevalent dialysis patients submitted to UK Renal Registry (UKRR) on 31st December 2011 were included. ERI was calculated as the weekly weight-adjusted dose of ESA divided by the hemoglobin level. Patients were stratified into 3 groups ERI <5; ERI=5–15, ERI>15 IU/kg/week/g per dl. Statistical analysis was by logistic models and Cox proportional hazards models as appropriate.

Results: Analysis included 6,144 patients from 30 centres for hemodialysis (HD) and 343 patients from 17 centres for peritoneal dialysis (PD) equating to 31% and 11% of prevalent HD and PD patients in UK. In the univariate analysis, dialysis vintage (OR 1.03,p=0.0004) for HD and (OR 1.24,p=0.0007) for PD; ferritin >800 µg/L (OR 1.47,p<.0001) for HD and (OR 4.5,p=.009) for PD; lower albumin (OR 0.92,p=.0001) for HD and (OR 0.90,p=0.002) for PD to name a few were identified as predictors of high ERI.

Table 2 Unadjusted and adjusted 1 year survival, HR and (p-value), for HD & PD patients

	HD (N=5073)			PD(N=343)	
ERI Group	Unadjusted	Adj for age & sex	+ URR, dialysis vintage, ferritin & albumin	Unadjusted	Adj for age & sex
<5	-	-	-	-	-
5-15	1.2 (0.02)	1.3(0.01)	1.2(0.06)	1.2(0.57)	1.7(0.15)
>15	2.4(<.0001)	2.7(<.0001)	2.2(<.0001)	1.9(0.16)	2.6(0.05)

Adjusted stratified analyses, in HD patients to evaluate whether it is the high EPO component of ERI that contributed to the worse outcome, showed that, in patients with an Hb in the 10-12 g/dL range, there was an increasing hazard with increasing EPO dose with nearly three times the risk (HR 2.8, p=0.02) in patients requiring >450 IU/Kg/week versus <50 IU/kg/week.

Conclusions: Despite controlling for ferritin and albumin, high ERI portended mortal outcomes suggesting either residual confounding or as yet unidentified deleterious effect of EPO resistance. Completely disentangling the effect of low Hb and high ESA on mortality is complex. Statistical techniques such as marginal structural modelling might help to better understand cause and effect.

FR-OR044

ESA Hyporesponsiveness Is Associated with Adverse Event in Maintenance Hemodialysis (MHD) Patients, but Not with Iron Storage

Takahiro Kuragano, Takeshi Nakanishi. *Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan.*

Background: It has been reported that hyporesponsiveness to erythropoiesis-stimulating agent (ESA) is associated with adverse events in the maintenance hemodialysis (MHD) patients. However, it has not been well determined whether higher iron storage is associated with the improved response to ESA or better survival.

Methods: In 1086 MHD patients, we measured serum ferritin, Hb, and transferrin saturation (TSAT) levels every 3 month during 2 years. The weekly dose of ESA /Hb (ESA/Hb) level was also calculated as an index of ESA responsiveness index. The associations between ESA/Hb and several adverse events were investigated with the cox proportional hazards model for time-dependent variables.

Results: There was no significant difference in ERI and Hb levels among the patients with serum ferritin levels (< 50, 50 ≤ ferritin < 100, 100 ≤ ferritin < 300, ≥ 300 ng/mL). A significant correlation (p<0.001, R=0.89) between ferritin and Hb was found only in the patients with ferritin levels <50. In LASSO regression analysis, high dose (>50mg/week) of intravenous iron administration, female, low serum albumin, and use of ACE-I were selected as significant predictors of higher ESA/Hb (>280), but serum ferritin and TSAT were not. Patients were divided into 4 groups according to their ESA/Hb and ferritin levels. In time dependent Cox hazard model, the risk for CCVD (p=0.029, HR: 5.28) and hospitalization (p=0.087, 1.98) were significantly higher in patients with high ESA/Hb (>280) and high ferritin (≥100 ng/mL) than those high ESA/Hb (>280) and low ferritin (<100 ng/mL).

Conclusions: In patients with ferritin < 50, Hb was dependent on ferritin, but not in those with ferritin ≥ 50. Although the patients with the hypo-responsiveness to ESA had higher risk for CCVD and hospitalization, ESA/Hb were not related to the iron storage. Among patients with hyporesponsiveness to ESA, higher serum ferritin levels bears higher risk for CCVD and hospitalization. We concluded that excessive iron storage did not necessarily lead to the improvement of the responsiveness to ESA in MHD patients.

FR-OR045

Phosphorus Binding with Ferric Citrate Is Associated with Reduced Hospitalizations and Costs for Cardiac, Gastrointestinal, and Infection-Related Causes

Roger A. Rodby,[1] Robert M. Niecestro,[2] T. Christopher Bond,[3] Mohammed Sika,[4] Jamie P. Dwyer,[4] Julia Lewis,[4] Kausik Umanath.[5] *[1]Rush Univ Medical Center, Chicago, IL; [2]Independent Consultant, Pocono Pines, PA; [3]Covance Market Access Services, Inc, Gaithersburg, MD; [4]Vanderbilt Univ, Nashville, TN; [5]Henry Ford Hospital, Detroit, MI.*

Background: In a Phase III study of 441 ESRD subjects, the iron-based phosphate binder ferric citrate (FC) provided equivalent phosphorus control while increasing and maintaining iron stores compared to an active control (AC) group of sevelamer carbonate and/or calcium acetate. Due to the effect of FC on iron stores, subjects also received significantly less intravenous (IV) iron and erythropoiesis-stimulating agents (ESAs).

Methods: Two blinded physicians (Covance Market Access) examined the records of serious adverse events (SAEs) from the active control period of the trial and mapped the hospitalizations to diagnosis-related groups (DRGs) based on standard billing practices. Medicare 2011 payment rates were applied to these DRG designated admissions, and inflated to 2013 dollars. Costs were cumulated and expressed as mean cost per patient year. This analysis focuses on three areas of morbidity potentially related to use of IV iron and ESAs in this patient population.

Results: FC-treated subjects had lower hospitalization rates than AC-treated subjects in all three system-organ classifications (considered independent categories). Per-patient per-year (PPPY) cost savings were also substantial in each of the three categories: from $896 to $1033 per category.

	Cardiac		Gastrointestinal		Infection	
	AC (n=149)	FC (n=289)	AC (n=149)	FC (n=289)	AC (n=149)	FC (n=289)
Subject-years	140.8	242.2	140.8	242.2	140.8	242.2
Events/100 subject-yrs	9.95	6.19	18.47	10.32	20.60	17.75
Total cost	$257,960	$193,602	$288,607	$249,294	$442,051	$543,608
Cost/subject-yr	$1832	$799	$2050	$1029	$3140	$2244
Savings with FC	$1033 PPPY		$1021 PPPY		$896 PPPY	

Conclusions: FC-treated subjects experienced substantially lower hospitalization rates and costs related to cardiac, gastrointestinal, and infection-related events compared to AC-treated subjects.

Funding: Pharmaceutical Company Support - Keryx Bio[pharmceuticals, NY, NY

FR-OR046

Reduced Hemodialysis Patient Hospital Admissions and Readmissions Associated with Right TraC (RT) Transitions of Care Program

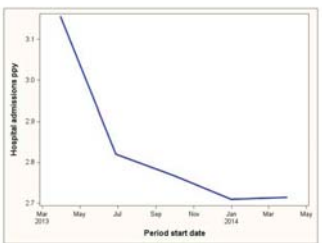
Andrew D. Howard,[2] Rebecca L. Wingard,[1] Kathryn A. McDougall,[1] Billie Axley,[1] Cathleen Okeefe,[1] Janice B. Sitzlar,[1] Sharon Deluca,[1] Fern Parlier,[1] John W. Larkin,[1] Len A. Usvyat,[1] Franklin W. Maddux.[1] *[1]Fresenius, Waltham, MA; [2]Metropolitan Nephrology Associates, Clinton, MD.*

Background: The 30-day readmission rate for hemodialysis (HD) patients (pts) is high at 36%. The Right TraC™ Program was designed to address multiple factors aimed at reducing readmissions.

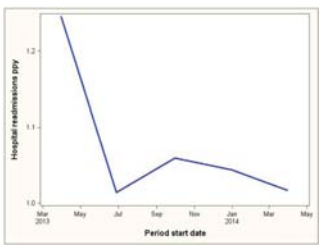
Methods: 28 HD clinics participated in RT. Phase I (began Apr 2013): Staff used admit- and post-hospital checklists including enhanced exchange of pt information, rapid anemia management, oral nutritional supplements, target weight assessment, medication review, and pt counseling for "red flag" symptoms. Phase II (Feb 2014): telephonic case management for 30 days post-discharge. Phase III (Mar 2014): Dialysis Link centralized transitional care information exchange in 9 clinics. Hospital admission and readmission rates per pt year (ppy) for each quarter from April 1, 2013 to March 31, 2014 were computed. Comparisons were made using Poisson regression with log of exposure days as offset.

Results: For the 2542 pts in RT, hospital admissions ppy decreased from 3.2 in Q2 of 2013 to 2.71 in Q1 of 2014, while hospital readmissions ppy decreased from 1.25 to 1.02 during this time. Poisson regression between Q2 of 2013 and Q1 of 2014 showed significant differences between periods (p<0.01). Crude comparison between these periods suggests 75 admissions were avoided. Analysis of matched control clinics based on clinic size, urban/rural setting, and baseline hospital admission and readmission rates showed that RT clinics had a larger decline in admissions (results not shown).

Panel 1. Hospital Admission Rate



Panel 2. Hospital Readmission Rate



Conclusions: The RT Program in its early stages has demonstrated reduced hospital admission and readmission rates. More time and further analysis are needed to demonstrate which components of RT have the most significant impacts on outcomes.

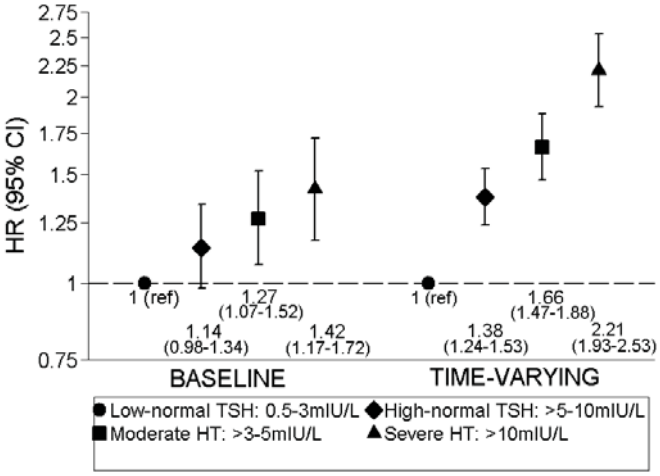
FR-OR047

Hyperthyrotropinemia and Mortality in a National Incident Hemodialysis Cohort Connie Rhee,[1] Steven B. Kim,[1] Bahattin T. Oztan,[1] Jiaxi Wang,[1] Daniel L. Gillen,[1] Rajnish Mehrotra,[2] Steven M. Brunelli,[3] Csaba P. Kovesdy,[4] Gregory Brent,[5] Kamyar Kalantar-Zadeh.[1] *[1]UCI, Irvine, CA; [2]Univ of Washington, Seattle, WA; [3]DaVita Clinical Research, Minneapolis, MN; [4]Memphis VAMC, Memphis, TN; [5]UCLA, Los Angeles, CA.*

Background: Hemodialysis (HD) patients have a higher risk of both hypothyroidism (HT), defined by elevated thyrotropin (TSH), and cardiovascular (CV) mortality. In the general population HT is a CV risk factor, but data examining the HT-mortality association in HD patients are inconsistent.

Methods: We studied a 5-year cohort (1/2007-12/2011) of adult incident HD patients from a large national dialysis organization with ≥1 serum TSH level(s) during their baseline HD quarter. We examined the association between baseline HT versus euthyroidism (TSH >5 versus 0.5-5mIU/L, respectively) with all-cause mortality using case-mix adjusted traditional Cox models. To address variations in thyroid function over time, we examined the association between time-dependent HT versus euthyroidism with mortality using time-dependent Cox models. In parallel analyses, we examined granular categories of thyroid function (low-normal TSH, high-normal TSH, moderate HT, severe HT).

Results: Among 8840 patients, 22% had HT at study entry. In analyses of baseline and time-dependent thyroid function, HT was associated with higher death risk: HR (95%CI) 1.29 (1.12-1.48) and 1.72 (1.57-1.89), respectively. Baseline moderate and severe HT were associated with higher mortality versus low-normal TSH; high-normal TSH trended to higher risk but was not statistically significant (Figure). However, in time-dependent analyses, high-normal TSH, moderate HT, and severe HT were incrementally associated with higher mortality.



Conclusions: HD patients with incrementally higher TSH levels have heightened mortality. Further studies are warranted to determine if the HT-mortality association is ameliorated by thyroid hormone replacement.

Funding: NIDDK Support

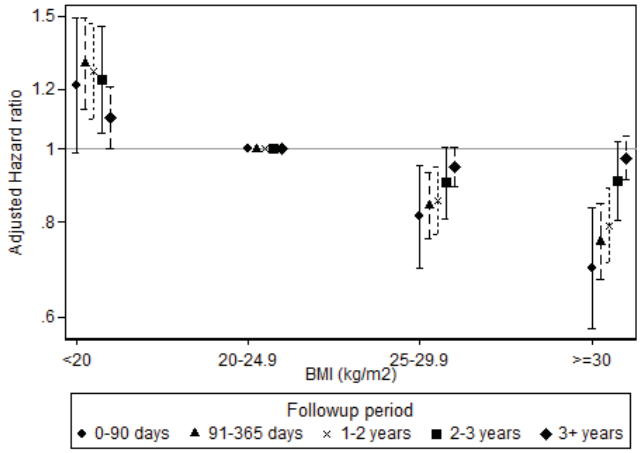
FR-OR048

The BMI/Survival "Paradox" among HD Patients Is Explained By Differential Followup Stephen P. McDonald,[1,2] Mark R. Marshall,[4,5] Kevan Polkinghorne.[1,3] *[1]ANZDATA Registry, Adelaide, SA, Australia; [2]School of Medicine, Univ of Adelaide, Adelaide, SA, Australia; [3]Monash Univ, Melbourne, VIC, Australia; [4]Renal Unit, Middlemore Hospital, Auckland, New Zealand; [5]Baxter Healthcare, Auckland, New Zealand.*

Background: In the general population, BMI in the overweight and obese categories is associated with increased mortality, but reports from the haemodialysis population have shown a survival advantage for haemodialysis patients in these groups. Reports have, however, assumed a constant relationship of BMI with early and with late mortality.

Methods: Using the ANZDATA registry, we examined the relationship of BMI at the start of dialysis with mortality in early and later time periods. Cox models were used to account for confounding by demographic factors and comorbidity. Shared frailty was used to account for "centre effects", and patients were censored at transplantation. Patients who began haemodialysis between 1991 and 2012 (n=33,559) were included.

Results: BMI has progressively increased over the period, with the prevalence of overweght and obesity among those starting HD rising from 25 and 14% respectively in 1991 to 31 and 39% respectively in 2012. In univiariate and multivairiate analyses, there was a substantial survival advantage in the first year among overweight and obese HD patients, but this advantage waned over longer followup periods. Underweight is associated with an increased risk of deaths at all time points of followup.



Interactions were examined with age, comorbidities, year of dialysis start and late referral.

Conclusions: The "paradoxical" association of higher BMI with better survival among HD patient wanes over time, although there is no clear disadvantage seen among this group. These trends in fact are similar to the general population, where the adverse associations of high BMI are mediated by processes such as diabetes and hypertension that take years to become manifest.

Funding: Government Support - Non-U.S.

FR-OR049

A Personalized, Low-Intensity, Easy to Implement, Home Exercise Program Improves Physical Performance in Dialysis Patients: The Exercise Introduction to Enhance Performance in Dialysis (EXCITE) Trial Francesca Mallamaci,[1] Fabio Manfredini,[2] Davide Bolignano,[1] Silvio Bertoli,[2] Piergiorgio Messa,[2] Alessandro Zuccalà,[2] Pasquale Fatuzzo,[2] Francesco Rapisarda,[2] Luigi Lombardi,[2] Adamasco Cupisti,[2] Giorgio Fuiano,[2] Claudia Torino,[1] Rossella Baggetta,[1] Giovanni Tripepi,[1] Luigi Catizone,[2] Carmine Zoccali.[1,2] *[1]CNR-IFC/IBIM & Nephrology and Transplantation Unit, Reggio Calabria, Italy; [2]On Behalf of the EXCITE Working Group (Renal Research and Academic Units in Ferrara, CNR-IFC Reggio Cal, Milan, Imola, Catania, Catanzaro, Pisa ITALY).*

Background: EXCITE (ClinicalTrials.gov NCT01255969) is a multicenter, randomized, controlled clinical trial testing the effectiveness of a low-intensity, easy to implement, home exercise program on physical performance in dialysis patients.

Methods: From a dialysis population of 648 patients, 297 eligible patients were randomized to the exercise group (n=151) or to the control group (n=146).To set and maintain the target walking cadence patients in the active group were instructed to follow the cadence of an inexpensive metronome (Seiko DM50, Seiko LTD, Japan). Patients in the control group maintained their habitual physical activity.Physical performance was assessed by the 6 Minute Walking Test (6 MWT) and the Sit- to- Stand test at baseline and after 6 months. The primary outcome was the change in physical performance at 6 months.

Results: At baseline the two groups were comparable for demographic and clinical characteristics as well as for physical performance. The 6 MWT improved in the exercise group (baseline: 328±96 m versus 6 months: 369±113 m), but remained unmodified in the control group (baseline: 320 ± 107 m, 6 months 324±116 m) and the between groups difference was highly significant (P <0.001). Similarly, the Sit to Stand test improved in the exercise group (from 20.5±5.0 seconds to 18.3±5.1 seconds), while no change was observed in the control group (20.8±6.1 seconds versus 19.7±6.7 seconds) (P between groups = 0.009).

Conclusions: A personalized, low-intensity home exercise program improves physical performance in dialysis patients. The simplicity and adaptability of the program make it suitable to the needs of a high-risk population such as the dialysis population.

FR-OR050

Upper Urinary Tract Cancer, but Not Kidney Cancer, Is Significantly Associated with Chinese Herbal Medicines Containing Aristolochic Acid in Dialysis ESRD Patient Sheng-Wen Niu,[1,2] Ming-Yen Lin,[2,3] Huei-Lan Lee,[4] Shu-An Yang,[2] Li-Tzong Chen,[5] Shang-Jyh Hwang.[2,4] *[1]Graduate Inst of Medicine, College of Medicine, Kaohsiung Medical Univ, Kaohsiung, Taiwan; [2]Div of Nephrology, Dept of Internal Medicine, Kaohsiung Medical Univ Hospital, Kaohsiung Medical Univ, Kaohsiung, Taiwan; [3]Instrument Technology Research Center, National Applied Research Laboratories, Hsinchu, Taiwan; [4]Faculty of Renal Care, College of Medicine, Kaohsiung Medical Univ, Kaohsiung, Taiwan; [5]National Inst of Cancer Research, National Health Research Insts, Tainan, Taiwan.*

Background: Popular uses of Chinese herbs containing aristolochic acid (AA) were noted in Taiwan. It had been reported increasing risk of renal disease and urinary tract cancer. However, there is no data on the development risk of renal cancer. The study is

aimed to identify the possibility of Chinese herbs associated with the risk of renal cancer and upper urinary tract cancer (UTUC) in dialysis patient, through Taiwan National Health Insurance Research Database.

Methods: The study population included incident dialysis patients from 1997 to 2008. Patient who was newly diagnosed renal cancer or UTUC during the period was considered as potential case, and a 1:5 matching with ESRD patient without these two cancers. Chinese herb drugs, including 15 brand names for Mu-Tong, and 15 for Xi-Xin, were identified and traced up to the second year before cancer diagnosis. Conditional logistic regression was used and p-value less than 0.05 was consider as statistical significance.

Results: Totally, there were 159 dialysis patients with renal cancer, 493 with UTUC and corresponding to their age- and sex- matched dialysis control subjects for final analyses. After adjusting demographic factors, comorbidities, use of any Chinese herbal medicine containing Mu-Tong (OR:2.3, 95%CI:1.5-3.5) or Xi-Xin (OR: 1.9, 95%CI:1.3-2.8) was associated with the risk of UTUC only, but no association with renal cancer.

Conclusions: The findings indicate that upper urinary tract had a higher risk to develop malignant lesion by herbal medicines containing Mu-Tong and Xi-Xin, but it has no association with renal cancer in dialysis patient.

Funding: Government Support - Non-U.S.

FR-OR051

Influenza Vaccination Reduced Pneumococcal Disease in Incident Dialysis Patients Jordan T. Powner,[1] Thomas Ryan Gallaher,[1] Rhonda E. Colombo,[1] Stephanie L. Baer,[1,2] Lulu Huber,[1] Mufaddal F. Kheda,[1] N. Stanley Nahman,[1,2] Kristina Weis Kintziger.[1] *[1]Dept of Medicine, Georgia Regents Univ, Augusta, GA; [2]Dept of Medicine, Norwood VAMC, Augusta, GA.*

Background: Pneumococcal disease (PnD) may manifest as local or systemic infection and may be fatal, yet there is limited data on PnD in dialysis patients. Because vaccines against PnD are readily available, we queried the UDRDS to further define the incidence and clinical scope of PnD in ESRD patients.

Methods: All incident dialysis cases from 2005-2008 were included. PnD and the administration of pneumococcal (PnV) or influenza (IFV) vaccines were defined using ICD-9 diagnosis or CPT procedure codes. Pneumococcal septicemia and meningitis were categorized as invasive pneumococcal disease (IPD). Additional demographic and clinical data was obtained from form 2728. Individuals were classified by risk category for PnD according to the CDC's Advisory Committee on Immunization Practices (ACIP) guidelines. Data were analyzed using bivariate analysis.

Results: 355,084 dialysis patients met criteria for analysis. There were 10,188 (2.4%) cases of PnD, including pneumonia (N=9,067), septicemia (N=930), pneumococcal infection not otherwise specified (N=154), and meningitis (N=37). Demographics showed: 55% male, 71% white and 65% > age 65 years. Clinical co-variates conferring the greatest adjusted relative risk (aRR) of PnD included ACIP at-risk category (medium risk: aRR 8.86, 95% CI 7.78-10.08; high risk: aRR 8.83, CI 7.71-10.11), age > 65 (aRR 1.62, CI 1.53-1.72), and starting dialysis with a catheter (RR 1.40, CI 1.27, 1.54). IPD was associated with black race (p=0.003). IFV was documented in 35% of patients, PnV in 13%. IFV was associated with a lower risk of PnD (aRR 0.43, CI 0.40, 0.46), but not IPD (p=0.071). An effect of PnV on the development of PnD was not detected.

Conclusions: PnD occurs in ESRD patients and the ACIP guidelines are predictive. Prior IFV may have benefit in preventing PnD. The lack of detectable impact of PnV in preventing infection in this cohort may be attributable to low vaccination rates. These data suggest that improving both IFV and PnV administration may be of therapeutic benefit in preventing PnD.

FR-OR052

Perceived Facilitators of and Barriers to Home Dialysis Use: A Canadian National Survey of Nephrologists Gihad E. Nesrallah,[1] Lianne Barnieh,[2] Braden J. Manns,[2] Andreas Pierratos,[1] David C. Mendelssohn,[1] Catherine M. Clase,[3] Gordon Guyatt.[3] *[1]Nephrology Program, Humber River Hospital, Toronto, ON, Canada; [2]Nephrology, Foothills Medical Centre, Calgary, AB, Canada; [3]McMaster Univ, Hamilton, Canada.*

Background: Barriers to and facilitators of home dialysis (peritoneal dialysis and hemodialysis) are numerous and complex. In order to better understand these factors in the Canadian context, we developed a de novo survey to measure nephrologists' attitudes and perceptions.

Methods: We used in-depth interviews and rigorous qualitative methods to develop a theoretical framework, which led to an initial item (N=60) pool. We used preference ratings and a Delphi process to reduce and refine candidate items into a 47-item survey, which we administered to all Canadian nephrologists providing dialysis and predialysis care to adult (>18 years) patients.

Results: We received 199 complete and 6 partial responses (response rate 41%). We examined item-level responses and used factor analysis to reduce items into domains. An 11-factor solution explained 67% of the variance across 41 item scores, denoting excellent construct validity. Test-retest reliability was 0.70; Crohnbach's α was 0.60-0.95. Domain-level median scores for barriers were low (12-19; possible range 0 to 100), indicating that respondents were unconcerned about most barriers. The highest median domain scores were for facilitators related to nephrologist training (75), funding for personnel and infrastructure (64), physician reimbursement (42), and offering home dialysis to most patients (38). The highest-ranked items included: nurse and lay caregiver-assisted home dialysis, PD in long-term care facilities, pre-dialysis nurse case-managers, multi-disciplinary modality rounds, public funding of water and electricity for home HD, and mandatory fellowship training in home HD. There was little support for home dialysis targets or a mandatory "PD first" policy.

Conclusions: We identified several potential target interventions to promote appropriate use of home dialysis. We also established the reliability and validity of a barrier assessment tool for potential use in other jurisdictions.

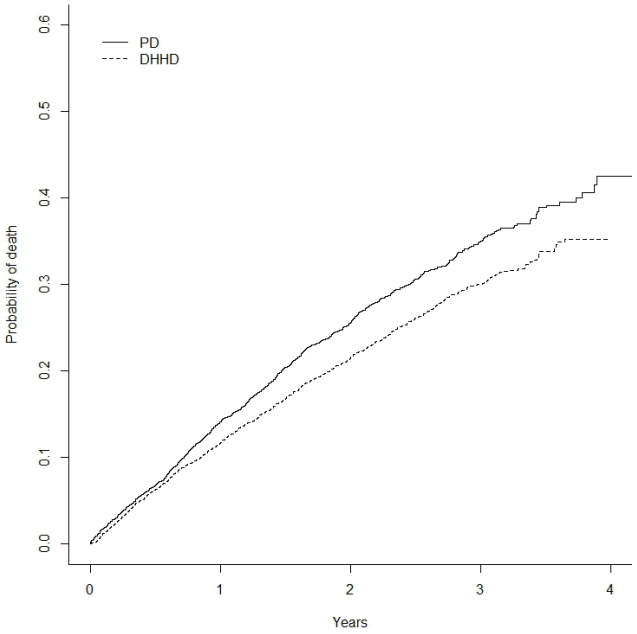
FR-OR053

Lower Risk of Death in Daily Home Hemodialysis versus Peritoneal Dialysis Patients Eric D. Weinhandl,[1] David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]School of Medicine, Univ of Minnesota, Minneapolis, MN.*

Background: Daily home hemodialysis (DHHD) and peritoneal dialysis (PD) have grown in popularity, but direct comparisons between these modalities are lacking. We aimed to compare the risk of death in U.S. patients initiating DHHD or PD.

Methods: We identified new DHHD patients, Jan 1, 2007-Jun 30, 2010, from a registry of NxStage System One users and linked them to United States Renal Data System (USRDS) records. We identified new PD patients from USRDS records. For each DHHD patient, we selected 1 matched PD patient according to the date of home dialysis initiation and a 33-factor propensity score of DHHD initiation. We followed patients from home dialysis initiation to the earlier of death or Dec 31, 2010.

Results: We identified 4460 DHHD and 4460 matched PD patients. Cumulative incidence of death for DHHD versus PD was 11.6% versus 14.0% at 1 yr, 21.4% versus 25.5% at 2 yr, and 30.0 versus 34.9% at 3 yr (figure). From Cox regression, the morality hazard ratio (HR) for DHHD versus PD was 0.84 (95% CI, 0.77-0.91); cause-specific mortality HRs were 0.87 (0.76-1.00) for cardiovascular disease and 0.80 (0.62-1.02) for infection. In patients (n = 3092 per group) initiating home dialysis after >6 mo following ESRD onset, the mortality HR was 0.80 (0.72-0.88). In patients (n = 1368 per group) initiating home dialysis within 6 mo following ESRD onset, the mortality HR was 0.95 (0.80-1.13), but HRs varied during the follow-up interval: 1.05 in year 1, 0.89 in year 2, and 0.80 in years 3-4.



Conclusions: DHHD was associated with lower risk of death than PD. The association was most pronounced in patients initiating home dialysis after >6 mo following ESRD onset and after the first year of follow-up in patients initiating home dialysis within 6 mo following ESRD onset.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

FR-OR054

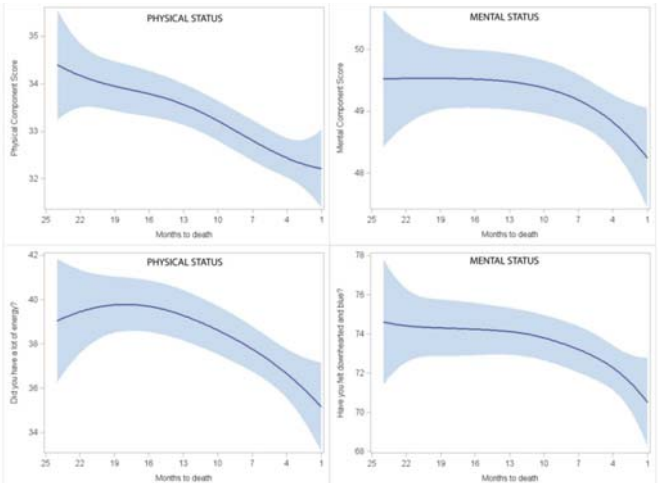
Declines in Hemodialysis Patients' Physical and Mental Component Scores before Death Stephanie Johnstone,[1] Lisa Dombro,[1] Greg S. Garza,[1] Krister Cromm,[1] John W. Larkin,[1] Len A. Usvyat,[1] Eduardo K. Lacson,[1] Peter Kotanko,[2] Jeffrey L. Hymes,[1] Franklin W. Maddux.[1] *[1]Fresenius Medical Care North America (FMCNA); [2]Renal Research Inst.*

Background: Prior studies indicate that patients (Pts) experience a decline in several biomarkers such as blood pressure, body weight, and albumin before their demise (Usvyat, KI). We sought to understand whether hemodialysis (HD) Pts' self-assessment of their Health Related Quality of Life (HRQOL) also declines before death.

Methods: All Pts in FMCNA clinics are invited to complete KDQOL surveys annually. Between Jan 1, 2009 and Dec 31, 2012, we analyzed data on all in-center HD Pts treated up to 24 months before and within 30 days preceding death with at least one KDQOL survey. Cubic spline models were constructed to demonstrate trajectory of key KDQOL parameters prior to death. KDQOL areas studied were: 1) the measure relating to physical

status (physical component score and question 10, "Did you have a lot of energy?") and 2) the measure relating to mental status (mental component score and question 11, "Have you felt downhearted and blue?").

Results: We studied 4,227 Pts who died and completed KDQOL surveys; only a range of 105-314 surveys were performed 30 days before death. Both the physical component and mental component scores declined in Pts before death. While the decline in the physical status score appears more linear, the decline in the mental status score is more pronounced approximately 6 months before death.



Conclusions: Our analysis suggests that HD Pts' self-assessment of their physical and mental status deteriorates before death. More frequent measurements with the KDQOL may be needed to properly assess the decline in physical and mental status of HD Pts before death. Outcome studies are needed to identify the impact of behavioral health interventions on Pt perceived HRQOL status and utilization of care during this end of life period.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

FR-OR055

Skeletal Muscle Cytokine Gene Expression after Resistance Exercise in Chronic Kidney Disease Joao L. Viana,[1] Emma L. Watson,[2] Neil J. Greening,[2] Jonathan Barratt,[2] Alice C. Smith.[2] *[1]School of Sport, Exercise and Health Sciences, Loughborough Univ, United Kingdom; [2]Leicester Kidney Exercise Team, Univ of Leicester, United Kingdom.*

Background: Pro-inflammatory genes are up-regulated in the skeletal muscle of chronic kidney disease (CKD) patients contributing to local and systemic inflammation and facilitating muscle atrophy through reduced protein synthesis and increased protein degradation. In healthy individuals, acute resistance exercise (RE) induces a transient inflammatory response that is required for appropriate muscle regeneration and adaptation, but its effects in CKD are unknown. Therefore, we investigated skeletal muscle cytokine gene expression in response to acute RE before and after 8 weeks of RE training in pre-dialysis CKD.

Methods: 18 patients with CKD3b-4 (mean eGFR 23, range 16-36ml/min/1.73m^2; mean age 63, range 45-77 years) were randomised to RE (3 sets of 10-12 leg extensions at 70% maximum, 3/week for 8 weeks; n=11), or control (usual activity; n=7). Quadriceps muscle volume was measured by ultrasonography at baseline and 8 weeks. Muscle biopsies were obtained at baseline and 8 weeks in controls, and baseline, 24h post first training session and 24h post final training session in exercisers. Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-alpha (TNF-α) mRNA expression were analysed by RT-PCR.

Results: No changes were seen in the controls. In exercisers, 8 weeks of RE training significantly increased quadriceps muscle volume (baseline 153±9 to 8 weeks 168±11cm^3; P=0.009). 24h after the first RE training session IL-6, MCP-1 and TNF-α mRNA expression were all up-regulated from baseline by medians of 22-fold (P<0.001), 20-fold (P<0.001) and 4-fold (P=0.002), respectively. However, no changes from baseline were observed 24h after the last RE training session.

Conclusions: These data reveal a previously unknown heightened and prolonged inflammatory response to unaccustomed RE in the skeletal muscle of CKD patients, which likely contributes to its impaired regenerative capacity. Fortunately, regular RE training appears to normalize this inflammatory response, which probably contributes to the significant muscle hypertrophy observed.

Funding: Private Foundation Support

FR-OR056

Muscle Mass and Function following Renal Transplantation Thomas Dienemann, Shaun Bender, Francis Perry Wilson, Peter P. Reese, Jin Long, Mary B. Leonard. *Perelman School of Medicine at the Univ of Pennsylvania, Philadelphia, PA.*

Background: Advanced CKD is associated with sarcopenia and impaired physical function. These deficits are associated with increased morbidity and mortality. Changes in body composition and muscle function in kidney transplant recipients (KTRs) have not been well characterized.

Methods: 60 incident KTRs (48% male, 44% black race, ages 19 – 60 years) were enrolled and DXA measures of body composition [appendicular lean mass index (ALMI, kg/m^2), fat mass index (FMI, kg/m^2)], quantitative CT measures of calf muscle density (mg/cm^3) and dynamometric measures of calf muscle strength (ft-lbs) were obtained at the time of transplantation, and 6, 12, and 24 months. Results were expressed as sex- and race- specific Z-scores relative to age based on 500 healthy controls. KTRs were treated with glucocorticoids rapidly tapered to 5 mg daily.

Results: At baseline, ALMI, muscle density and muscle strength Z-scores were significantly lower in KTRs (all p<0.001) compared with controls. The baseline strength deficit was minimally attenuated to -0.71 (95% CI -0.98, -0.45, p<0.001) with adjustment for ALMI Z-score; muscle density was not associated with strength. ALMI Z-scores recovered rapidly; however, strength deficits persisted at 24 months. FMI increased progressively over 24 months; the prevalence of obesity increased from 18 to 45%. The low muscle density Z-score at 24 months was partially explained by the greater FMI Z-score [adjusted muscle density Z-score = -0.49 (95% CI -0.77, -0.22, p<0.001)].

Z-Score	Baseline Mean±SD	6 Months Mean±SD	12 Months Mean±SD	24 Months Mean±SD	Change between Baseline & 6 Months	Change between 6 & 24 Months
ALMI	-0.52±1.02***	-0.09±1.00	0.01±1.01	0.02±1.01	p<0.001	p=0.06
Muscle density	-0.94±1.07***	-0.64±1.04***	-0.72±1.05***	-0.71±1.04***	p<0.001	p=0.61
Muscle strength	-0.85±1.02***	-0.43±1.01*	-0.32±1.02*	-0.32±1.01*	p<0.01	p=0.34
FMI	0.02±1.03	0.42±1.03**	0.62±1.04***	0.69±1.05***	p<0.001	p<0.001
* p<0.05; ** p<0.01; *** p<0.001 compared w controls						

Conclusions: Renal transplantation is associated with excess adiposity, increased intramuscular fat, and incomplete recovery of muscle function despite improvements in lean mass.

FR-OR057

N-Terminal pro-B-Type Natriuretic Peptide as a Biomarker for Sarcopenia in Prevalent Hemodialysis Patients Misa Ikeda,[1] Hirokazu Honda,[2] Hiroaki Ogata,[3] Fumihiko Koiwa,[4] Eriko Kinugasa,[3] Kanji Shishido,[5] Takanori Shibata.[1] *[1]Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine, Tokyo, Japan; [2]Div of Nephrology, Dept of Medicine, Showa Univ Koto Toyosu Hospital, Tokyo, Japan; [3]Depts of Internal Medicine, Showa Univ Northern Yokohama Hospital, Yokohama, Japan; [4]Div of Nephrology, Dept of Medicine, Showa Univ Fujigaoka Hospital, Yokohama, Japan; [5]Dialysis Center, Kawasaki Clinic, Kawasaki, Japan.*

Background: N-terminal pro-B-type natriuretic (NT-proBNP) was demonstrated as a risk factor for protein-energy wasting (PEW) in prevalent hemodialysis (HD) patients. The aim of this study was to assess an association between NT-proBNP and loss of muscles mass, sarcopenia, as an important element of PEW in HD patients.

Methods: Two hundred and twenty-six prevalent HD patients (mean age 64 year, men 63%, diabetes mellitus state 30%) were recruited for this prospective study. Blood samples were obtained at baseline measuring high-sensitive (hs) CRP, interleukin (IL)-6, adiponectin (ADN) and NT-proBNP. Cardiac function was estimated by ultrasound cardiography. Nutritional state was assessed by subjective global assessment (SGA), body mass index (BMI), normalized protein catabolic rate, and geriatric nutritional risk index. Dual-energy X-ray absorptiometry was performed at baseline and 1 year later and change in total fat mass (TFM) and total lean body mass (TLBM) were estimated.

Results: NT-proBNP was positively correlated with age, hsCRP, IL-6, ADN, LVDd and inversely correlated with BMI, ejection fraction, TFM and TLBM. NT-proBNP level in SGA-positive patients (median 7,195, range 1,340-63,500 pg/mL) was significantly increased as compared with SGA-negative patients (median 2,535, range 508-78,400 pg/mL). NT-proBNP was independently associated with decreased changes in TLBM, but not with changes in TFM, by the multivariate regression model adjusted with confounders (adjusted r^2 0.16, p=0.0003).

Conclusions: NT-proBNP may be a reliable biomarker for PEW in prevalent HD patients, especially those with sarcopenia.

FR-OR058

Association between Fibroblast Growth Factor-23 and Frailty in Elderly Community-Living Adults: The Cardiovascular Health Study
Tomasz Beben,[1,2] Michel Chonchol,[3] Linda F. Fried,[4] Bryan R. Kestenbaum,[5] Mark J. Sarnak,[6] Michael Shlipak,[7] Ian H. de Boer,[4] Joachim H. Ix,[1,2] Dena E. Rifkin.[1,2] *[1]UCSD; [2]VA San Diego; [3]Univ of Colorado; [4]VA Pittsburgh; [5]Univ of Washington; [6]Tufts Medical Center; [7]UCSF.*

Background: Frailty has been previously associated with low 25-OH vitamin D and high parathyroid hormone (PTH) levels. Fibroblast Growth Factor 23 (FGF23) is related to phosphate and vitamin D metabolism and has been associated with cardiovascular and kidney disease. Whether FGF23 is related to frailty is unknown.

Methods: 2970 individuals from the Cardiovascular Health Study (CHS) at the year 9 (1996/1997) study visit had FGF23 levels measured and were assessed for frailty using previously described criteria of weight loss, weakness, exhaustion, slowness, and decreased physical activity. Frailty was defined as ≥3 and prefrailty as 1 or 2 criteria present. Logistic regression was performed with multivariate adjustment for demographics, CVD risk factors, C reactive protein, and kidney function.

Results: The mean age was 78 years; 40% were male, 83% were Caucasian and 16% were African American. The median FGF23 value was 70.8 RU/mL [IQR 53.6-100.4]. 52% were prefrail and 13% were frail. After multivariate adjustment, a doubling of FGF23 level was associated with 33% higher odds of frailty versus nonfrailty and 15% higher odds of prefrailty (Figure). Sensitivity analysis in 1208 subjects who had phosphorus, PTH, and vitamin D measurements showed that these did not meaningfully alter the FGF23-frailty association.

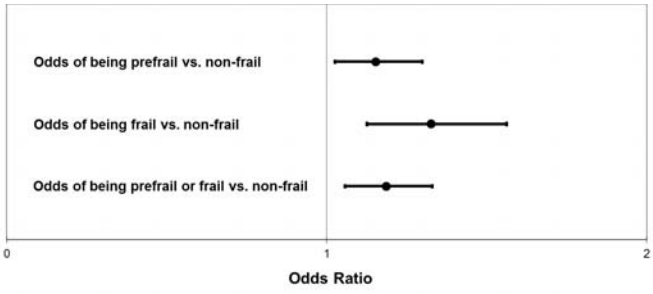


Figure: Odds ratios of prefrailty, frailty, and prefrailty or frailty relative to nonfrailty are presented adusted for age, race, gender, BMI, HTN, diabetes, CHF, CHD, stroke, claudication, CRP, eGFR (cystatin C), and albumin to creatinine ratio.

Conclusions: In a large elderly cohort, higher FGF23 was independently associated with higher odds of frailty and pre-frailty. In the context of previous findings, it is becoming increasingly clear that FGF23 is not only a marker for CVD and mortality, but also for functional outcomes of relevance for geriatric populations.

Funding: Other NIH Support - 5T32HL007261-33; 5R01HL094555; K23 DK091521

FR-OR059

Insulin Resistance in CKD-A New Role for the Ubiquitin System in Muscle
Molly Colleen Tokaz, Yanlan Dong, Liping Zhang, William E. Mitch. *Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Mechanisms of insulin resistance in chronic kidney disease (CKD) are poorly understood. We reported that CKD activates the signal transducer and activator of transcription 3 (Stat3) in muscle and suppresses phosphatidylinositol 3-kinase/p-Akt activities leading to activation of protein degradation by the ubiquitin-proteasome system (UPS; Cell Metab., 2013 and JASN, 2006). Now we find that CKD increases expression of Fbxo40, a muscle-specific, E3 ubiquitin ligase, in mouse muscles. Since Fbxo40 mediates ubiquitination/degradation of IRS1 (Dev Cell., 2011), we examined if p-Stat3 stimulates Fbxo40 to degrade IRS1 in muscles of mice with CKD or fed a high-fat diet (HFD, 58% kcal from fat).

Methods: We created CKD in mice or fed mice a HFD to create diabetes. We studied muscle-specific Stat3 KO mice or mice treated with a Stat3 inhibitor (C188-9; 12.5mg/kg/d). Insulin (ITT) and glucose tolerance (GTT) tests were measured in both groups and in muscles, p-Stat3, Stat3, p-Akt, Fbxo40, IRS1 and GAPDH were measured. Adenovirus expressing constitutively active Stat3 was transfected into C2C12 myotubes to evaluate signaling pathways. We found Stat3 binding sites in the Fbxo40 gene promoter and performed CHIP assays to examine if activated Stat3 can increase Fbxo40.

Results: In muscles of mice with CKD or HFD-induced diabetes, Fbxo40 and p-Stat3 were increased, p-Akt was decreased; ITT and GTT were both lower. Both Stat3 KO in muscles or C188-9 Stat3 inhibition in CKD or HFD fed mice had lower fasting glucose, improved ITT and GTT and decreased Fbxo40 with higher IRS-1 and p-Akt in muscles versus control mice. C2C12 myotubes overexpressing constitutively active Stat3 had increases in p-Stat3 and Fbxo40 but reduced levels of IRS1 and p-Akt; infection of C2C12 myotubes with lentivirus expressing Stat3-shRNA had suppressed Fbxo40 mRNA and protein. CHIP assay revealed Stat3 associated with Fbxo40.

Conclusions: Activation of p-Stat3 stimulated expression of Fbxo40 to degrade IRS1, suppressing p-Akt in muscle. These results provide a new mechanism for the insulin resistance in CKD and diabetes as both disorders cause degradation of IRS1.

Funding: NIDDK Support

FR-OR060

Prevalence, Body Composition and Mortality Associations of Protein-Energy Wasting in Non-CKD and CKD in the U.S.
Srini Beddhu,[1,2] G. Wei,[2] R. Boucher,[2] E. Constantz,[2] Xiaorui Chen,[2] Tom Greene.[2] *[1]SLC VAMC; [2]Univ of Utah SOM.*

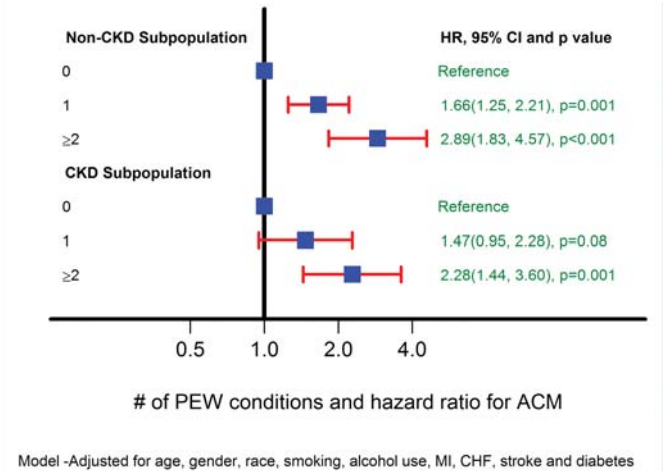
Background: The prevalence and clinical significance of protein-energy wasting (PEW) defined by the International Society of Renal Nutrition and Metabolism (ISRNM) criteria in non-CKD and moderate CKD populations have not been well established.

Methods: 7987 participants in the 1999-2002 NHANES were included. CKD-EPI eGFR < 60 was defined as CKD. Serum chemistry (serum alb < 3.8 g/dl or cholesterol < 100 mg/dl), body weight (BMI < 20 kg/m^2, unintentional wt loss > 10% over 1 yr or body fat% < 10%), muscle mass (mid-arm muscle circumference (MAMC) < 10% of age and gender specific 50th percentile) and dietary protein intake (< 0.6 g/kg/d based on 24h diet recall) were the 4 PEW criteria. Mortality data was through 12/31/2006. The associations of the # of PEW criteria with MAMC were examined in linear regression models and mortality in Cox regression models using survey weights. There were very few with ≥ 3 PEW conditions; hence, these were merged with those with ≥ 2 PEW conditions.

Results: 6.3% had CKD. The mean eGFR were 98 ± 14 and 47 ± 11 in non-CKD and CKD, respectively. Prevalence of the number of PEW conditions and the associations with FFM in non-CKD and CKD are summarized in the table.

#PEW Conditions	Prevelence(%)		β,95% CI for MAMC(cm)*	
	Non-CKD	CKD	Non-CKD	CKD
0	58	48	Ref	Ref
1	32	36	-1.3(-1.6,-1.1), p<0.001	-0.5(-1.1,0.1)
≥2	10	17	-3.7(-4.1, -3.3), p<0.001	-1.5(-2.5,-0.4)

There were 391 and 232 deaths over 41929 and 3527 years of follow-up in non-CKD and CKD, respectively. The associations of # of PEW conditions with mortality in non-CKD and CKD are summarized in the figure.



Model -Adjusted for age, gender, race, smoking, alcohol use, MI, CHF, stroke and diabetes

Conclusions: PEW conditions are highly prevalent and associated with ↓ muscle mass and ↑ mortality in non-CKD and CKD populations in the U.S. Further studies are warranted to determine the etiology and Rx of PEW in the non-CKD and CKD populations.

Funding: NIDDK Support

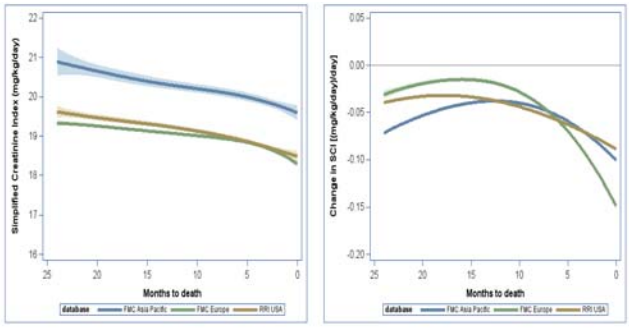
FR-OR061

Creatinine Index Dynamics before Death in Chronic Hemodialysis Patients – Results of an International Study
Daniele Marcelli,[1] Len A. Usvyat,[1] Peter Kotanko,[2] Michael Etter,[1] Gero D. von Gersdorff,[3] Aileen Grassmann,[1] Cristina Marelli,[1] Adrian M. Guinsburg,[1] Laura Scatizzi,[1] Inga Bayh,[1] Yuedong Wang,[4] Frank van der Sande,[5] Jeroen Kooman,[5] Bernard J. Canaud.[1] *[1]Fresenius Medical Care; [2]Renal Research Inst; [3]Univ Hospital Cologne; [4]Univ of California; [5]Maastricht Univ Medical Centre.*

Background: Lean tissue mass (LTM) is predictive of survival in HD patients. In health, LTM estimations are based on creatinine excretion. For anuric patients, Canaud [PLoS One, 2014] derived the Creatinine Index (CI): $CI(mg/kg/day)=16.21+1.12x[1\ if\ male;0\ if\ female]-0.06xage\ (years)-0.08xspKt/V_{urea}+0.009xCr_{preHD}(\mu mol/L)$. Here, CI dynamics in the months prior to death and CI survival predictive value were investigated.

Methods: CI was calculated from the MONitoring Dialysis Outcomes (MONDO) initiative database [Usvyat et al, Blood Purif 2013]. Dynamics for 2 years before death were investigated using partially conditional means E(Y(u) | T>u), fitted using quintic splines, and trajectories of first derivatives [Usvyat et al, Kidney Int 2013]. The relationship to all-cause mortality was studied by adjusted multivariate Cox proportional-hazard models.

Results: CI levels for 13,620 HD patients (22 countries) fell by ~1 mg/kg/day in the 2 years before death.



Trend of CI (left) and of CI first derivative (right) in male HD patients (two years preceding death

CI levels were higher for males, and generally highest in Asia. CI decline accelerated ~10-12 months before death and was virtually identical in all regions (except females in Asia). A CI increase of 1 mg/kg BW/day was associated with a 16.5% lower risk of death (HR=0.835; 95% CI:0.812-0.859, p<0.001).

Conclusions: CI, a surrogate of muscle mass, declines as early as 10-12 months before death in an accelerated fashion. The predictive value of CI was demonstrated by regression analysis. This supports the need for following CI. Future trials should address outcome and physical activity and nutritional intervention effects on CI.

FR-OR062

Histologic versus Clinical Remission in Lupus Nephritis (LN) Ana Malvar,[1] Valeria Gabriela Alberton,[2] Cecilia Recalde,[1] Bernarda Fazzini,[1] Bruno Jorge Lococo,[1] Paola Pirruccio,[1] Brad H. Rovin.[3] *[1]Nephrology, Hospital Fernandez, Buenos Aires, Argentina; [2]Pathology, Hospital Fernandez, Buenos Aires, Argentina; [3]Nephrology, State Univ, Columbus, OH.*

Background: Treatment response in LN is defined as resolution of proteinuria and improvement of GFR. Histology is not considered in LN response. Several studies show discordance between long-term clinical and histologic findings, but only a few have systematically examined the histologic response to induction.To examine this question we reviewed protocol kidney biopsies done after LN induction.

Methods: SLE patients(n=69)were biopsied at first presentation of kidney involvement(Bx1)and again after induction treatment(Bx2,mean intra-biopsy interval 8±2 months).All patients had Class III or IV LN, and were induced with steroids plus mycophenolate or cyclophosphamide. NIH activity(AI)and chronicity(CI)indices were calculated.

Results: Clinical and histologic data are shown in the Table.After induction AI decreased and CI increased (P<0.0001for both). At Bx2, 13 patients (19%) had an AI of 0, but 6(46%)of these still had proteinuria ≥500 mg/d (0.75-2.63g/d).In contrast, of the 30(43%)patients who had proteinuria ≤500 m/d, 15(22%) patients had an AI ≥ 4, 8 (11.6%) had an AI ≥ 5, and 2(3%) had an AI ≥ 6. Of the 30 patients who had proteinuria ≤500 mg/d, 19(63%) had a CI≥4 at Bx2.Ten patients(14%)had a CI of 0 or 1 on Bx1 and developed a CI of≥4 at Bx2.

PATIENTS	Bx1			Bx2		
	AI	CI	PROT(g/d)	AI	CI	PROT(g/d)
All	8,5	2,6	2,9	3,5	4	1,1
responders(prot≤500 mg/d,n:32)	7,9	2,6	2,1	3,1	3,7	0,2
non responders(n:37)	8,9	2,5	3,5	3,8	4,1	1,8

Conclusions: After induction 9% of this cohort achieved histologic remission but still had significant proteinuria, while 22% achieved clinical remission, but still had significant histologic activity(AI≥4). Despite intense therapy several patients rapidly developed histologic chronic kidney disease(CKD)with a Bx2 CI≥4. These data suggest that: 1) a repeat kidney biopsy after induction could stratify patients who need typical prolonged maintenance immunosuppression from those who may do well with a shortened maintenance phase; and 2)early use of anti-fibrotic therapies may be necessary to prevent CKD.

FR-OR063

Repeat Renal Biopsies Help to Tailor Immunosuppression in Lupus Nephritis Angela Pakozdi,[1] Dev Pyne,[1] Michael Sheaff,[3] Ravindra Rajakariar.[2] *[1]Rheumatology, Barts Health NHS Trust, London, United Kingdom; [2]Renal Medicine, Barts Health NHS Trust, London, United Kingdom; [3]Histopathology, Barts Health NHS Trust, London, United Kingdom.*

Background: Lupus nephritis (LN) is the major cause of morbidity and mortality in patients with SLE. The role of repeat kidney biopsies (RB) to guide treatment or to predict outcome and prognosis has been controversial. In this retrospective study we focused on histological characteristics of RBs and aimed to identify any clinical variables useful to predict histological changes.

Methods: In a large single-centre cohort of 257 patients from 1988-2014 with biopsy proven LN, 58 (23%) had two or more biopsies (a total of 68 RBs). LN classes based on glomerular pathology were defined according to the ISN/RPS classification. Clinical and laboratory data were obtained from electronic records of patients.

Results: The median time between initial and RB was 33 months [IQR, 15-84]. Caucasians (n=8) had a lower RB rate of 16% compared to blacks (n=37, 33%; p=0.010). Indication for RB was worsening proteinuria (n=38, 71%; of which 23 had associated rising creatinine, 61%), rise in serum creatinine alone (n=6, 11%) and lack of treatment response (n=9, 17%) defined as <50% reduction in proteinuria. At time of RB, 25 (78%) had raised dsDNA, 33 (73%) had low complements. LN class transition occurred in 31 (48%), most commonly from class II or V to III or IV (n=11, 36%). 6 RB (6.8%) showed inactive lesions either due to FSGS or advanced sclerosing LN. 42 (65%) had a change in their treatment regime. Immunosuppression was more likely to be escalated in case of a class switch (87% versus 38%, p=0.002). The histological transition could not be predicted by any serological or biochemical variables.

Conclusions: Over a 1/3 of our LN patients showed histological transition to a more aggressive class, based on which the majority (87%) had treatment escalation. Histological transition could not be predicted by clinical values. Hence, we conclude that RB remains an important tool to guide management of LN, particularly in those with initial class II or V who flare.

FR-OR064

Outcomes of Maintenance Therapy in Patients with Less Severe Lupus Nephritis Previously Randomized to Receive Either Low Dose Cyclophosphamide versus Oral Mycophenolate Mofetil Manish Rathi,[1] Krishan L. Gupta,[1] Ajay Jaryal,[1] Aman Sharma.[2] *[1]Nephrology; [2]Rheumatology, Postgraduate Inst of Medical Education and Research, Chandigarh, India.*

Background: Management of lupus nephritis is always challenging. No trial has compared induction by oral mycophenolate mofetil (MMF) and low dose intravenous cyclophosphamide (CYC) followed by maintenance with azathioprine (AZA).

Methods: This study followed the previous open label, ongoing prospective, randomized trial comparing the efficacy and safety of low dose CYC (Euro-lupus regimen) and oral MMF in subjects with class III, IV, V, III+V, or IV+V LN. Subjects with crescentic LN, serum creatinine >3mg/dl, neurological or pulmonary lupus, ongoing infection, pregnancy and prior CYC or MMF use were excluded. The dose of MMF was 2–3 gm/day for 6 months, while CYC was administered as six fortnightly infusions of 500 mg each. All subjects also received three intravenous methylprednisolone injections initially followed by oral steroids at 1mg/kg up to 2 months and then tapered to a dose of 5-7.5 mg/day. After completion of induction treatment, all subjects were prescribed AZA (2 mg/kg) with low dose steroid.

Results: A total of 55 subjects completed one year of maintenance treatment with AZA after receiving either MMF (n=28) or CYC (n=27) as induction agent. Baseline characteristics were similar except for a higher 24 hour protein excretion in the CYC group. Forty one of total subjects, 74.54%, (n=22 in CYC and n =19 in MMF, p=ns) achieved remission within mean duration 21.81 weeks in CYC arm was and 19.47 weeks in MMF arm. Five patients had resistant disease (MMF =4, CYC=1), six deaths (MMF=5, CYC=1), four lost to follow up (MMF=1, CYC=4) and one patient deviated from protocol. Four subjects, 3 in MMF and 1 in CYC arm had proteinuric flare during follow up and none had nephritic flare. Main adverse events during maintenance phase were cytopenia, herpes zoster and avascular necrosis of femoral head in one patient each. The treatment cost of MMF was ten times more than the CYC therapy.

Conclusions: One year outcome of maintenance phase with azathioprine were similar in low dose intravenous CYC and oral MMF arm in treatment of less severe lupus nephritis.

FR-OR065

Long Term Follow Up of the Rituxilup Steroid Sparing Regimen in Lupus Nephritis Andrew Porter, Marie B. Condon, Anne Frances Doyle, Megan Griffith, H. Terence Cook, Tom Cairns, Liz Lightstone. *Imperial Lupus Centre, Hammersmith Hospital, London, United Kingdom.*

Background: Lupus nephritis is a relapsing/remitting condition and much of the long term damage relates to oral steroid use. In a single centre cohort study, the Rituxilup regimen (2 doses Methyl-prednisolone 500mg and Rituximab 1g, 2 weeks apart, followed by maintenance Mycophenolate Mofetil alone) was safe, effective treatment for ISN/RPS class II/IV/V lupus nephritis (Ann Rheum Dis 2013) over 1 year follow-up. We now report outcomes over ≥ 5 years.

Methods: Renal outcomes in the 50 patient cohort were assessed. Definitions: Complete Remission (CR)– urine PCR<50mg protein/mmol creatinine + creatinine no > than 15% above baseline; Partial Remission (PR)– uPCR <300mg/mmol + >50% fall from baseline + creatinine no > than 15% above baseline. Renal flare: uPCR>100mg/mmol on 2 consecutive visits + >50% above remission value +/ or creatinine rise >30% above baseline OR biopsy proven relapse. Non Response (NR)– failure to achieve CR or PR.

Results: Out of 50 patients, 1, in CR, lost to f/up early. By May 2014, 49 had median f/up of 78 mths (median range 50-90). There were 4 NR (8%). Of the 45 achieving CR/PR: 22/45 (48.9%) patients never flared. Mean time to flare in the rest was 54 mths (+/- 4.81). At 5 years and latest f/up respectively, 2 (5.1%) and 4(10.1%) had doubled creatinine. At latest f/u, there were 4 (8%) deaths (median age 72yrs (61-76) of whom 2 in ESRF) and 3 (6%) were alive with ESRF; those who died were much older at presentation (median 67yrs (59-73)) versus those who survived (39yrs (18-74)). Importantly, over this long f/up, of 40 patients in whom data available, 29 (72.5%) never required oral steroids, 4 (10%) had courses <4 weeks and just 6 (15%) required long term use, usually as a switch to Eurolupus regimen for flare.

Conclusions: These data suggest the Rituxilup regimen leads to sustained remission and minimal steroid use in a significant proportion of patients. Relapses often related to non-adherence, and usually responded to retreatment, again with no oral steroids. The deaths were in older patients. The planned Rituxilup trial (NCT01773616) will address efficacy and safety in an international multicentre randomised controlled trial.

FR-OR066

Tacrolimus Combined with Corticosteroids versus Modified Ponticelli Regimen in Treatment of Idiopathic Membranous Nephropathy: Randomized Control Trial Raja Ramachandran, Harsha Kumar Hn, Vinod Sharma, Ashwani Kumar, Ashok Kumar Yadav, Ritambhra Nada, Harbir Singh Kohli, Vivekanand Jha, Krishan L. Gupta. *PGIMER, Chandigarh, India.*

Background: Very few studies have evaluated the effectiveness and safety of Tacrolimus (TAC) versus the standard Cyclophosphamide (CYC) based regimen in IMN. So the present study was aimed at comparing the efficacy and safety of TAC with CYC in patients with IMN.

Methods: The study was Randomized, Parallel Group, Active Controlled Trial. Adult IMN patients with persisting nephrotic syndrome after 6 months of ACEi or ARBs or with complications of nephrotic syndrome were randomized to receive Modified Ponticelli (MP) regimen (6-month course of alternating prednisolone and cyclophosphamide) or Tacrolimus (0.1 mg/kg/day for 12 months; trough level 5-10 ng/ml in 1st 6 months and 4-8 ng/ml for next 6 months) with oral prednisolone (0.5 mg/kg/day) for 6 months. Tissue staining for phospholipase A2 receptor (PLA2R) at baseline and serum anti PLA2R antibodies (ELISA, EUROIMMUN, Germany) was done at baseline, 6 and 12 months. Primary end point was achievement of remission (complete and partial) and secondary end points was adverse effects and eGFR in both the study groups.

Results: Study included 61 patients (32 in the CYC arm and 31 in TAC arm). There was no difference in the baseline parameters, PLA2R in the glomeruli and anti PLA2R antibody. Intention-to-treat analysis suggested that remission at the end of 12 months were comparable in both the groups (75% with CYC versus 67% with TAC; p=0.58). Complete and partial remission was seen in 56% and 19% in CYC and 35% and 32% in TAC group respectively. There were no significant differences in the anti PLA2R titers at 6 and 12 months in both groups. CYC treated patients had significantly higher risk of leucopenia and azoospermia/ amenorrhea and those on TAC had significantly higher incidence of reversible nephrotoxicity, gastrointestinal side effects and tremors. There were no significant differences in the incidence of Diabetes mellitus, eGFR and infection in both the groups.

Conclusions: In the management of IMN, both TAC with steroids and CYC regimen have similar efficacy, but with different adverse effect profile.

Funding: Government Support - Non-U.S.

FR-OR067

The Risk of Cardiovascular Events (CVE) Is Greater in Membranous Nephropathy (MN) Than Focal Segmental Glomerulosclerosis (FSGS) Taewoo Lee,[1] V. K. Derebail,[1] A. V. Kshirsagar,[1] Caroline J. Poulton,[1] Susan L. Hogan,[1] Sophia Lionaki,[2] Ronald J. Falk,[1] Heather N. Reich,[3] Patrick H. Nachman.[1] *[1]Univ of North Carolina; [2]Laiko Hospital, Athens, Greece; [3]Univ of Toronto, Canada.*

Background: Patients with MN have a greater risk of venous thromboembolism than patients with FSGS. We hypothesized that the risk of CVE in MN exceeds that in FSGS. We compared the risk of CVE between MN and FSGS adjusting for other risk factors.

Methods: We investigated the incidence of ESKD, all-cause mortality and CVE (defined as acute coronary syndrome, stroke or peripheral artery thrombosis) in inception cohorts of MN and FSGS. Cumulative incidence rates of CVE before reaching ESKD were estimated using competing risk analysis. To determine risk factors, competing risk regression analysis was performed with relevant covariates at baseline, including underlying disease (MN versus FSGS). Severe nephrotic syndrome was defined as proteinuria ≥3.5 g/day and/or hypoalbuminemia <2.5 g/dL and used as a time-dependent variable.

Results: We identified 404 patients with MN [median follow-up 23 m (IQR 9 -50)] and 387 with FSGS [median follow-up 24 m (IQR 10 -37)]. The cohorts were similar with respect to age, sex, diabetes, smoking, previous CVE and baseline eGFR, proteinuria and cholesterol. The FSGS cohort had a higher proportion of blacks and patients with eGFR<45 ml/min/1.73m2, but fewer patients with severe nephrotic syndrome. The estimated cumulative incidence rates of CVE was significantly higher in the MN than FSGS cohort (6.5% versus 3.5% at 3 yrs). Whereas the incidence of CVE and ESKD were similar during the first 2 yrs among MN patients, ESKD was far more frequent than CVE in the FSGS cohort. The table summarizes the multivariable analysis of risk factors of CVE adjusting for demographics, eGFR, diabetes and smoking.

Variables (reference)	Hazard ratio	95% C.I.	*P*-value
MN (vs. FSGS)	2.1	1.1-4.2	0.026
Severe nephrotic syndrome (time-varying)	1.7	1.0-2.9	0.043
Age decades at biopsy (<30 years old)	1.6	1.2-2.0	<0.001
Previous history of CVE	2.6	1.3-5.2	0.005

Conclusions: MN is associated with a disease-specific increased risk for CVE compared to FSGS, independent of severity of nephrotic syndrome, CKD and traditional risk factors.

FR-OR068

Extended NIH Cyclophosphamide Regime Is Associated with Improved Patient Outcomes in ANCA Associated Vasculitis Valeed Ghafoor,[1] Laura Chadwick,[2] Ajay Prabhakar Dhaygude,[1] Michael Venning.[2] *[1]Renal Medicine, Royal Preston Hospital, Preston, United Kingdom; [2]Renal Medicine, Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: Despite vigorous induction immunotherapy, ANCA Associated Vasculitis (AAV) still carries a 50% 5- year relapse rate. Morbidity/mortality are closely linked with immunosuppression exposure; infection being the greatest problem after treatment. Current European League Against Rheumatism recommendation is to treat organ/life-threatening disease with cyclophosphamide by CYCLOPS protocol, 10 doses over 26 weeks. The NIH regime extending Cyp after 6 months with quarterly doses to 30 months total reduces relapses in lupus nephritis[1] and AAV[2]. In this study we compared the outcomes of AAV pts treated with CYCLOPS and extended NIH regimes in NW of England.

Methods: A retrospective study was performed of 90 sequential AAV patients treated with the 2 Cyp regimens. Prospectively collected demographic, and outcomes data were analysed.

Results: Results are presented in Table-1.

	NIH regime	CYCLOPS regime	p value
Patients	33	57	
Average Age	58 (30-82)	60 (18-80)	
Average Follow up months (pt*months)	56(1848)	50(2850)	
PR3 +Ve	63%	52%	
Cumulative Cyp dose (mili-grams)	8807	6951	
Dialysis dependant at diagnosis	30%	33%	
Dialysis dependant at last F/U	12%	14%	0.6
Relapses (relapse/pt*months)	7(0.003)	27(0.009)	0.01
Infections (inf/pt*months)	6(0.003)	12(0.004)	0.44
Deaths	15%	14%	

Use of methyl prednisolone, plasma exchange and maintenance immunotherapy did not differ between groups.

Conclusions: These data appear to support the previous findings that a more intensive Cyp regime may be associated with lower relapse rates with no increase in treatment related complications. A large RCT is required to confirm these findings: addressing the question of whether a more intensive induction regime than CYCLOPS might carry benefit.

References: 1.Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. Boumpas et al. Lancet 1992. Sep26;340. 2.Prolonged treatment with low-dose intravenous pulse cyclophosphamide may reduce rate of relapse in ANCA-associated vasculitis. – DhaygudeA et al. Nephron Clin Pract. 2004; 97(4):c 154-9.

FR-OR069

Can Autologous Stem Cell Transplantation Improve the Prognosis of Primary AL Amyloidosis via Cardiac Effects? Masahiro Kawada, Yoshifumi Ubara, Junichi Hoshino, Koki Mise, Kenmei Takaichi. *Nephrology, Toranomon Hospital, Minato-ku, Tokyo, Japan.*

Background: AL amyloidosis shows a wide spectrum of organ involvement and is known to have a poor long-term prognosis, with cardiac disorders being an adverse prognostic factor. High-dose melphalan combined with autologous stem cell transplantation (HDM/ASCT) has been expected to prolong the survival of these patients, but long-term efficacy has not been demonstrated.

Methods: Between 2004 and 2012, HDM/ASCT was carried out in 18 patients with AL amyloidosis after one or two courses of VAD therapy (vincristine, doxorubicin, and dexamethasone) according to the criteria and regimen of Gono. The long-term outcome and organ responses were investigated in this study according to the classification of Gertz (Am J Hematol, 2005).

Results: The mean follow-up time after HDM/ASCT was 65.8 months. At treatment, these patients had a mean age of 51 years (6 women), mean serum Cre of 0.86 mg/dL, proteinuria of 7.11 g daily, BNP of 181 pg/mL, ejection fraction of 68 %, and left ventricular mass (LVM) of 169.2 g on echocardiography. After treatment, four patients died (3 of amyloid-related causes and 1 of infection), and 14 patients are alive. There was no treatment-related mortality. A renal response occurred in 12 patients (66.7%) and a cardiac response was seen in 77.8%. Hematological complete remission (HCR) was achieved in 14 patients (77.8%), and overall survival (OS) was 83.3% at 5 years. OS was good (100 %) in 14 patients with HCR at 37 months after treatment, while OS was poor (0 %) in 4 patients without HCR. LVM was reduced from 207.1 g to 188.0 g after 2 years in 6 HCR patients with left ventricular hypertrophy (LVH), while LVM was increased from 204.3 g to 231.3 g in 3 patients who had LVH without HCR.

Conclusions: When HCR is achieved after VAD + HDM/ASCT in patients with AL amyloidosis, a good prognosis can be expected due to cardiac improvement.

FR-OR070

Abstract Withdrawn

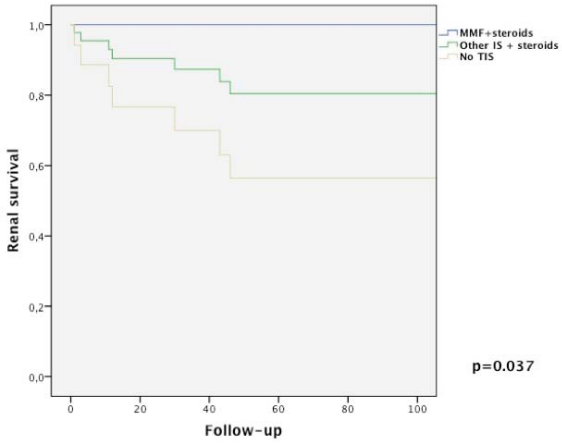
FR-OR071

Mycophenolate Mofetil in C3 Glomerulonephritis Cristina Rabasco, Manuel Praga. *Nephrology, Spanish Nephrology Society's Glomerular Diseases Study Group (GLOSEN), Spain.*

Background: C3 Glomerulonephritis (C3GN) is a recently recognized clinicopathologic entity defined by the finding of dominant deposits of C3 on immunofluorescence. Some patients have treated with eculizumab with variable results, but information about the efficacy of immunosuppresssion is very scarce. We aimed to investigate if immunosuppressive therapy (IST) can influence long-term outcomes in C3GN.

Methods: A multi-center study of the GLOSEN that collected patients with biopsy-proven C3GN. Demographic data, clinical presentation, histological findings, and response to IST were analysed. Outcomes of the study were: response, doubling of serum creatinine or progression to end stage renal disease (ESRD).

Results: Sixty patients were identified (57% male, median age 37yr). Clinical presentations were nephrotic syndrome (50%), nephritic syndrome (33%) and asymptomatic urinary abnormalities (17%). Membranoproliferative GN was found in 74%, the remaining patients showing other histological patterns. Most patients (90%) received ACEI/ARB. 66% patients received IST: corticosteroids (CS) plus mycophenolate mofetil (MMF) in 55%, CS plus immunossupressants other than MMF in 22% and CS only in 22%. There were no differences at baseline between treated and untreated patients. Median follow-up was 48 months. Rate of response was higher among treated patients (70% versus 25%;p=0,001) and the number of patients reaching ESRD was significantly higher in nontreated patients (35% versus 7%;p=0,007). When analyzing the different types of IST, patients who had received MMF had an almost significantly higher rate of responses than patients who received other types of IST and their probability of renal survival was significantly higher than that patients treated with other IST: 100% versus 78% at 50 months(P=0,034)



Conclusions: IST, particularly CS + MMF, is effective in C3GN, improving outcomes in comparison with nontreated patients.

FR-OR072

Ablation of Dendritic Cells Prevents Hypertension in Mice Infused with Angiotensin II plus High Salt Diet Daniel E. Hevia,[1] Carolina E. Prado,[2] Eugenia L. Fuentes,[1] Rodrigo Pacheco,[2] Luis F. Michea.[1] *[1]Centro de Estudios Moleculares de la Célula, Inst Milenio de Inmunología e Inmunoterapia, ICBM, Facultad de Medicina, Univ de Chile, Santiago, Chile; [2]Laboratorio de Neuroinmunología, Fundación Ciencia y Vida, Santiago, Chile.*

Background: High levels of Angiotensin II (AngII) cause hypertension (HT), inflammation, fibrosis and hypertrophy in heart and kidney. Inflammation implies the immunogenic response of Dendritic Cells (DC). Previous studies showed that AngII promoted DCs maturation and proinflamatory cytokine production *in vitro*. Moreover, blocking of DC-T Lymphocyte interaction *in vivo* partially prevented AngII-induced HT. We tested the hypothesis that DCs are necessary for blood pressure increase and tissue damage caused by AngII infusion and high salt diet (AngII+HS).

Methods: WT andCD11c.DOG mice (with selective loss of DCs, $CD11c^{Hi}$ cells) after Diphteria Toxin (DT) administration received AngII+HS treatment during 14 days. Arterial blood pressure and physiological parameters were measured, and at day 14 we characterized markers for: oxidative stress, inflammation, fibrosis and hypertrophy.

Results: Ablation of $CD11c^{Hi}$ cells in CD11c.DOG mice prevented HT and cardiac hypertrophy, as compared to vehicle-treated group (101.2±3.8 mmHg). DT administration to CD11c.DOG mice depleted $CD11c^{Hi}$ cells in ~91.6% from spleen and ~83.7% from kidney. WT mice receiving AngII+HS developed HT irrespective to DT infusion (166.2±5.3 mmHg). qRT-PCR studies in kidney tissues of both strains showed that AngII+HS without DT administration increased expression of NOX2, NOX4, TNF-α, IL-1β, MCP-1, CTGF, TGF-β, Collagen and Fibronectin ($p<0.05$; n=5-7 *versus* vehicle). AngII+HS increased $CD8^{+}IFN\text{-}\gamma^{+}$ and $CD4^{+}IFN\text{-}\gamma^{+}$ and reduced $CD4^{+}Foxp3^{+}$ and $CD4^{+}IL\text{-}10^{+}$ populations in in renal lymph nodes. DCs ablation prevented changes mentioned above.

Conclusions: These results show for the first time that DCs ($CD11c^{Hi}$ cells) are necessary for HT development and tissue damage in response to AngII+HS. The potential role of DCs in the activation of the intrarenal RAAS and/or the modulation of the expression of sodium transporters in nephron is under investigation. Funded by CONICYT/FONDECYT/RegularN°1130550 and 21130762/IMII P09-016-F.

Funding: Government Support - Non-U.S.

FR-OR073

IL-1 Potentiates Sodium Retention in Angiotensin II-Dependent Hypertension Through Nitric-Oxide-Mediated Regulation of Na^{+}-K^{+}-2Cl^{-} Cotransporter Jiandong Zhang, Matthew A. Sparks, Steven D. Crowley. *Div of Nephrology, Duke Univ, Durham, NC.*

Background: The macrophage cytokine interleukin 1 (IL-1) is a key regulator of innate immunity, and chronic angiotensin (Ang) II infusion raises IL-1 levels in the kidney.

Methods: To study the role of IL-1 receptor (IL-1R) activation in Ang II-dependent hypertension, we chronically infused uni-nephrectomized IL-1R-deficient (KO) and wild-type (WT) mice with Ang II for 4 wks.

Results: Baseline mean arterial pressures (MAPs) were similar between groups. By contrast, after the 1st wk of Ang II, KOs had blunted MAP elevations versus WTs (169±6 versus184±3 mm Hg; $p<0.05$). At 4 wks of Ang II, KO kidneys contained more F4/80+ macrophages than WTs (18±1 versus 14±2 per HPF; p=0.03). Among these macrophages, monocytic myeloid-derived suppressor cells (Mo-MDSC) that produce nitric oxide (NO) showed 60% greater accumulation in KO kidneys than in WTs by FACS (p=0.05). In turn, KOs had enhanced renal expression of iNOS mRNA (1.7±0.3 versus 1.0±0.2 au; $p<0.05$) and higher excretion of NO metabolites versus WTs (152±46 versus 60±15 nmol/mg Cr; p=0.05). NO inhibits renal sodium reabsorption, and during the 2nd wk of Ang II when WT and KO BPs separated, only the KOs entered negative Na balance (-16.8±16.8 versus WT 34.8±7.5 µmol/day, p=0.01). To confirm that preserved NO bioavailability in the Ang II-infused KOs led to their lower BP versus WTs, we blocked NO generation in the groups with L-NAME starting on day 7 of Ang II when the WT and KO BPs were 179±3 versus 165±6 mm Hg ($p<0.01$). By day 14, BPs converged (186±5 versus 185±8; p=NS). As NO is known to inhibit NKCC2, we measured NKCC2 activity at day 10 of Ang II by quantitating UNa/UCr 3 hours after an IP injection of saline with or without furosemide. After saline alone, the KOs had a higher UNa/UCr than the WTs (237±38 versus120±14 mmol/mmol; p=0.01) whereas the UNa/UCr converged in response to furosemide (481±103 versus453±60; p=NS). Thus, reduced NKCC2 activity in the KOs protects them from Ang II-induced sodium retention.

Conclusions: In sum, our studies define a novel immune-mediated hypertension mechanism in which stimulation of IL-1 signaling by Ang II promotes renal sodium retention through NO-dependent regulation of NKCC2 activity.

Funding: NIDDK Support, Veterans Affairs Support

FR-OR074

Kidney Specific FKBP12 Knockout Mice Are Protected From Tacrolimus Induced Hypomagnesemia and Hypercalciuria Rebecca A. Lazelle,[1] Belinda H. McCully,[2] Chao-Ling Yang,[1] David H. Ellison.[1] *[1]Div of Nephrology and Hypertension, Oregon Health and Sciences Univ, Portland, OR; [2]Div of Trauma, Critical Care & Acute Care Surgery, Oregon Health and Sciences Univ, Portland, OR.*

Background: The calcineurin inhibitor tacrolimus (FK-506) is widely used to prevent organ transplant rejection, but also causes side effects, including hypomagnesemia and hypercalciuria. Although tacrolimus alters renal calcium and magnesium transport proteins, contributing to these effects, the cellular mechanisms have not been established. Tacrolimus inhibits calcineurin by binding FKBP12. In T-cells this results in immunosuppression. FKBP12 is an endogenous immunophillin, and global FKBP12 knockout is lethal. Thus, we developed an inducible kidney tubule-specific FKBP12 knockout mouse to test whether magnesium and calcium wasting result from disruption of FKBP12 itself or from disruption of calcineurin signaling.

Methods: Using a Doxycycline inducible CRE/LOX system, we deleted FKBP12 from the kidney tubules of adult mice (FKBP12-KO). We characterized these mice at baseline, and after tacrolimus treatment (3 mg/kg tac/veh intraperitoneally for 3 weeks on a high K^{+} diet), using metabolic cages, colorimetric assays and Western blotting.

Results: Doxycycline treatment reduced FKBP12 by >90% in kidney. At baseline, FKBP12-KO mice appeared normal, without signs of magnesium or calcium imbalance. While tacrolimus caused significant hypomagnesemia (veh 1.67 ± 0.01 v tac 1.07 ± 0.01 mg/dL) and hypercalciuria (veh 0.09 ± 0.01 v tac 0.37 ± 0.01 mg/g BW/24 hours) in FKBP12-WT mice, these effects were absent in FKBP12-KO mice (veh 1.57 ± 0.03 v tac 1.73 ± 0.08 mg/dL) and (veh 0.11 ± 0.03 v tac 0.13 ± 0.01 mg/g BW/24 hours)(Data:mean± SEM).

Conclusions: Loss of renal FKBP12 itself does not cause kidney dysfunction. Loss of renal FKBP12, however, protects kidneys from tacrolimus effects on renal magnesium and calcium transport. These data suggest that tacrolimus causes electrolyte imbalance by inhibiting calcineurin in kidney epithelial cells. Since the toxic and therapeutic effects both result from calcineurin inhibition, safer immunosuppressive drugs will likely require tissue specificity.

Funding: NIDDK Support, Private Foundation Support

FR-OR075

Increased Susceptibility to Angiotensin II-Induced Renal Injury and Glomerular Filtration Rate Reduction in Mice with Vascular-Specific EP4 Receptor Deletion Jean-Francois Thibodeau,[1,2] Chet E. Holterman,[2] Chris R. Kennedy.[2] *[1]Dept of Cellular and Molecular Medicine, Univ of Ottawa, Ottawa, ON, Canada; [2]Kidney Research Center, Ottawa Hospital Research Inst, Ottawa, ON, Canada.*

Background: Cyclooxygenase (COX)-inhibition by NSAIDs is contraindicated in CKD patients as it reduces GFR by diminishing renal blood flow. Prostaglandin E2 (PGE2) is vasodilatory and counteracts pressor hormones such as angiotensin II (AngII). PGE2 responsiveness in the renal microcirculation is governed primarily by pre-glomerular E-Prostanoid 4 (EP4) receptors in vascular smooth muscle cells.

Methods: Using a Cre/LoxP approach, we generated mice with inducible vascular smooth muscle cell EP4 receptor deletion ($EP4^{VSMC-/-}$) and subjected them to AngII-induced hypertension by implantation of osmotic mini-pumps (1000ng/kg/min, s.c.).

Results: After 4 weeks of AngII administration, systolic blood pressures were significantly yet similarly elevated in both wild type (WT) and $EP4^{VSMC-/-}$groups (WT, 104±1; AngII, 169±5; $EP4^{VSMC-/-}$, 106±7; $EP4^{VSMC-/-}$ + AngII, 178±5 mmHg) while albuminuria was significantly exacerbated in $EP4^{VSMC-/-}$ mice versus WT mice (WT, 430±70; AngII, 917±284; $EP4^{VSMC-/-}$, 354±54; $EP4^{VSMC-/-}$ + AngII, 2907±420 µg albumin/mg creatinine). AngII-treated $EP4^{VSMC-/-}$ mice but not WT mice exhibited severe renal structural damage characterized by glomerulosclerosis (WT, 28.6±1.2; AngII, 32.0±1.2; $EP4^{VSMC-/-}$, 31.0±1.3; $EP4^{VSMC-/-}$ + AngII, 44.0±2.0 % glomerular area), tubulointerstitial fibrosis and tubular proteinaceous casts. GFR decreased significantly in AngII-treated $EP4^{VSMC-/-}$ mice, but was unaffected in AngII-treated WT mice (WT, 0.26±0.04; AngII, 0.24±0.07; $EP4^{VSMC-/-}$, 0.16±0.05; $EP4^{VSMC-/-}$ + AngII, 0.07±0.02 ml/min). In addition, renal medullary COX-2 mRNA induction in AngII-treated mice was impaired in $EP4^{VSMC-/-}$ mice compared with WT counterparts (WT, 0.9±0.2; AngII, 2.8±0.7; $EP4^{VSMC-/-}$, 1.7±0.7; $EP4^{VSMC-/-}$ + AngII, 0.6±0.2 a.u.).

Conclusions: These results indicate that vascular EP4 receptor activation in a hypertensive context is critical as it buffers AngII's effect on GFR and protects against associated renal structural injury.

Funding: Government Support - Non-U.S.

FR-OR076

Cardiac Hypertrophy in Angiotensin II-Dependent Hypertension: Dominant Effect of Blood Pressure Matthew A. Sparks,[1] Johannes Stegbauer,[2] Steven D. Crowley,[1] Subramaniam Pennathur,[3] Susan B. Gurley,[1] Thomas M. Coffman.[1] *[1]Duke Univ; [2]Heinrich-Heine-Univ Düsseldorf; [3]Univ of Michigan.*

Background: LVH is a common result of end-organ injury in HTN, leading to higher CV risk. ACEi's and ARBs attenuate LVH through actions suggested to depend on direct interruption of AngII signaling via AT_1R in cardiac myocytes, rather than effects to lower BP.

Methods: In order to define the contribution of AT_1R in myocytes to LVH, we generated 2 mouse lines with cell-specific deletion of $AT_{1A}R$ from the heart using a floxed Agtr1a allele. In the 1st line, elimination of $AT_{1A}R$ was achieved with a Cre transgene under control of the Sm22 promoter(Tagln-Cre), which expresses Cre in smooth muscle and myocyte cells.

Results: In Tagln-KO mice, there was loss of $AT_{1A}R$ from cardiac myocytes and VSMCs in aorta, but not in smaller vessels. With chronic AngII infusion, Tagln-KOs develop HTN similar to controls(MAP:157±6 versus 153±6 mmHg, P=NS). In AngII-infused controls, there was oxidative stress in the heart with enhanced nitrated tyrosine, but this was reduced by ~75% in Tagln-KOs($P<0.005$). Despite the loss of $AT_{1A}R$ in myocytes and differences in free radicals, the extent of cardiac hypertrophy was similar in Tagln-KOs and controls(6.9±0.2 versus 6.7±0.2 mg/gm; P=NS). Heart BNP mRNA and cardiac injury were not reduced in Tagln-KOs compared to controls. The phenotype and molecular signature of LVH were not affected by the absence of cardiac $AT_{1A}R$ in Tagln-KOs. The 2nd line in which the $AT_{1A}R$ floxed allele was excised by a Cre cassette "knocked-in" to the Sm22 locus(KISm22-KOs). The KISm22-KOs showed loss of $AT_{1A}R$ from myocytes and VSMCs in large arteries, but in this case $AT_{1A}R$ were also deleted from VSMCs in resistance vessels. Accordingly, AngII-induced HTN was attenuated in KISm22-KOs(MAP: 122±3 versus 145±4 mmHg; P=0.004). Unlike the Tagln-KOs, cardiac hypertrophy was lower(5.5±0.2 versus 7.4±0.6 mg/gm; p=0.01) and BNP mRNA levels was reduced(1.0±0.2 versus 2.6±0.8; $p<0.05$).

Conclusions: In 2 models lacking AT_{1AR} in the heart, the extent of cardiac hypertrophy in AngII HTN followed the BP, rather than $AT_{1A}R$ in myocytes. Suggesting that the magnitude of BP elevation is of primary importance in driving cardiac hypertrophy.

FR-OR077

Kidney Androgen Regulated Protein Controls Baseline Blood Pressure Kamyar A. Zahedi,[1,2] Saeed Alshahrani,[3] Marybeth Brooks,[1,2] Sharon L. Barone,[1,2] Jie Xu,[1,2] Manoocher Soleimani.[1,2,3] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Research Services, Veterans Affairs Medical Center, Cincinnati, OH; [3]Pharmacology and Cell Biophysics, Univ of Cincinnati, Cincinnati, OH.*

Background: The kidney androgen-regulated protein (KAP) is abundantly and exclusively expressed in the proximal tubule. KAP function in proximal tubule remains unknown. KAP interacts with the Na-K-ATPase B subunit through binding with cyclophiline and its over-expression in the proximal tubules of transgenic mice leads to increased 20-HETE generation, resulting in vasoconstriction and systemic hypertension (Circ. 2009). In order to investigate its role in blood pressure (BP) regulation, kidney specific KAP KO mice were generated.

Methods: Proximal tubule specific KAP knockout (KAP-KO) mice were generated by cross breeding of floxed-KAP (CONT) and villin cre mice. Animals were subjected to systemic BP measurement by tail cuff method, and kidney RNA from KO and CONT mice were examined by RNA-SEQ. Northerns and immunofluorescence labeling were performed.

Results: Compared to CONT, KAP-KO mice display significantly reduced systolic BP (145+/-2 versus 108+/-3, $p<0.01$). Placing the animals on high salt diet for 5 days led to rapid normalization of BP in KAP-KO mice without significantly affecting CONT mice. RNA-SEQ analysis revealed that the expression of Cyp4a12a, the main kidney 20-HETE-generating enzyme was hugely up-regulated whereas the Na-K-ATPase B subunit was down-regulated in KAP-KO mice. Northern blot analyses confirmed RNA-SEQ results. Immunofluorescent studies indicated that the expression of NBCe1, the basolateral sodium/bicarbonate cotransporter, is upregulated in KAP-KO mice. The latter suggests the presence of a sodium reabsorption mechanism that may compensate for reduced activity of Na-K-ATPase in the proximal tubules of KAP-KO mice.

Conclusions: Our results suggest that KAP plays an important role in systemic blood pressure homeostasis, likely through regulation of sodium reabsorption in the proximal tubule. Further, the up-regulation of Cyp4a12a, the main 20-HETE-generating enzyme, points to a compensatory vaso-constricting response aimed at keeping the BP within physiologic range.

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FR-OR078

Role of the Natriuretic Peptide GC-A Receptor on Podocytes in Aldosterone-Induced Glomerular Injury Yukiko Kato,[1] Hideki Yokoi,[1] Kiyoshi Mori,[2] Masato Kasahara,[3] Yoshihisa Ogawa,[4] Takashige Kuwabara,[1] Takeshi Tokudome,[5] Ichiro Kishimoto,[6] Akira Sugawara,[4] Taiji Matsusaka,[7] Kazuwa Nakao,[2] Motoko Yanagita,[1] Masashi Mukoyama.[1,8] *[1]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [2]Medical Innovation Center, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [3]Inst for Advancement of Clinical and Translational Science, Kyoto Univ Hospital, Kyoto, Japan; [4]Div of Nephrology, Osaka Red Cross Hospital, Osaka, Japan; [5]Dept of Biochemistry, Research Inst Index, National Cerebral and Cardiovascular Center, Osaka, Japan; [6]Dept of Atherosclerosis and Diabetes, National Cerebral and Cardiovascular Center, Osaka, Japan; [7]Dept of Internal Medicine, Tokai Univ School of Medicine, Kanagawa, Japan; [8]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan.*

Background: Aldosterone is a key molecule in glomerular injury. The natriuretic peptide receptor guanylyl cyclase-A (GC-A) signaling exerts renoprotective effects by eliciting natriuresis and reducing blood pressure. We demonstrated that systemic GC-A knockout (KO) mice with aldosterone and sodium overload exhibited accelerated hypertension with massive proteinuria. In this model, inhibition of p38 MAP kinase reduced blood pressure and ameliorated renal injury. Local function of the GC-A receptor in podocytes, however, remains unknown.

Methods: We generated podocyte-specific GC-A KO mice (pod-GC-A KO). Control and KO mice were uninephrectomized and then treated with high salt orally and aldosterone subcutaneously for 4 weeks.

Results: Pod-GC-A KO mice with aldosterone and high salt showed significantly increased urinary albumin excretion by 13-fold with marginal blood pressure elevation similar to control. Glomerular hypertrophy, mesangial expansion and apoptosis were aggravated in pod-GC-A KO mice. Overexpression of MKK3 in cultured podocytes induced p38 MAPK phosphorylation and BAX mRNA expression. Such increase was inhibited by ANP.

Conclusions: These results suggest that the endogenous natriuretic peptides and GC-A system on podocytes plays renoprotective and anti-apoptotic roles in aldosterone-induced glomerular injury by inhibiting phosphorylation of p38MAPK.

FR-OR079

B Cell Derived IL-4 Induces Proteinuria and Foot Process Effacement Alfred Hyoungju Kim,[1] Shreeram Akilesh,[1] Ania B. Koziell,[2] Sanjay Jain,[1] Jeffrey B. Hodgin,[3] Jeffrey H. Miner,[1] Andrey S. Shaw.[1,4] *[1]Washington Univ School of Medicine, St. Louis, MO; [2]Experimental Immunobiology, King's College, London, United Kingdom; [3]Univ of Michigan, Ann Arbor, MI; [4]Howard Hughes Medical Inst, St. Louis, MO.*

Background: Podocyte foot process effacement is a feature of proteinuria, thought to be a stereotyped response of the podocyte to injury. The stimulus for podocyte injury and foot process effacement is unknown. B cell depletion therapies have demonstrated efficacy in some patients with proteinuria including those with minimal change disease. Since pathogenic antibodies are not causative, we hypothesized that a B cell derived cytokine might be capable of directly inducing podocyte injury and foot process effacement.

Methods: B cell model antigen model hen egg lysozyme (HEL) was biotinylated, complexed to avidin and injected into mice. HEL-specific B cells were adoptively transferred and proteinuria assessed. Cultured podocyte membrane ruffling was assessed with DIC videomicroscopy. IL-4 expression in mice was achieved by hydrodynamically injecting murine IL-4 in the piggyBac vector system. Human kidney biopsies were assessed for phospho-STAT6 by immunohistochemistry.

Results: We identified IL-4 as a B cell derived cytokine capable of stimulating podocyte membrane ruffling. Using a novel model of B cell induced proteinuria, B cells polarized to secrete IL-4 upon activation induced proteinuria without antibody or complement deposition. IL-4 overexpression was sufficient to induce foot process effacement and

proteinuria in mice. Inhibition of IL-4 signaling with a JAK1/3 inhibitor markedly reduced proteinuria in these IL-4 overexpressing mice. A subset of patients with minimal change disease possessed glomerular STAT6 activation.

Conclusions: These findings suggest a potential explanation for the utility of immunosuppression and more targeted anti-B cell therapy with rituximab in the treatment of minimal change disease. These results supporting the role of IL-4 in human nephrotic syndromes and a novel therapeutic target.

Funding: NIDDK Support, Private Foundation Support

FR-OR080

Pathogenic RORγt$^+$ Regulatory T Cells Aggravate Crescentic Glomerulonephritis Malte A. Kluger,[1] Matthias C. Meyer,[1] Michael Luig,[1] Boeren Goerke,[1] Anna Nosko,[1] Claudia Wegscheid,[2] Rolf A. Stahl,[1] Ulf Panzer,[1] Oliver M. Steinmetz.[1] *[1]III. Med. Klinik, Univ Hospital Eppendorf, Hamburg, Germany; [2]Experimentelle Immunologie und Hepatologie, Univ Hospital Eppendorf, Hamburg, Germany.*

Background: Recently, cells co-expressing the regulatory T cell (Treg) transcription factor Foxp3 and the Th17 transcription factor RORγt were identified in mice and humans. It remains unclear whether these cells belong to the newly defined Th17 specific Treg17 cells, represent intermediates during Treg/Th17 transdifferentiation, or constitute a distinct cell lineage. Since nothing is known about their role in inflammatory renal disease, we studied the Nephrotoxic nephritis (NTN) model of crescentic glomerulonephritis.

Methods: Deletion of genes was achieved using Foxp3Cre x RORCfl/fl or STAT3fl/fl mice. NTN was induced and renal histology and function were assessed at day 10. Immune responses were analyzed by FACS, ELISA and Treg suppression assay. Foxp3 and RORγt stability was assessed in Foxp3 Cre x Ai9 fate reporter mice and by cell transfer from Foxp3 and RORγt activity reporters.

Results: Induction of NTN resulted in rapid expansion of RORγt+ Foxp3+ cells. A high percentage produced IL-17 and expressed the chemokine receptor CCR6. RORγt+ Foxp3+ cells were still present in Treg17 deficient Foxp3Cre x Stat3fl/fl mice showing that they are a different Treg subtype. Multiple fate reporter and cell transfer studies revealed that RORγt+ Foxp3+ cells are not Treg/Th17 transdifferentiating cells but rather represent an independent cell lineage. To study their functional role, we generated Foxp3Cre x RORCfl/fl mice lacking RORγt specifically in Foxp3+ cells. Systemic Th17 and Treg responses including in vitro suppressive activity remained unchanged. Importantly, however, NTN was significantly ameliorated in the absence of RORγt+ Foxp3+ cells, revealing a pro-inflammatory role.

Conclusions: In summary we provide evidence that RORγt+ Foxp3+ cells represent a novel and independent pro-inflammatory T-cell lineage distinct from Th17 and Treg17 cells. For the first time, we show that these pathogenic regulatory T cells act as potent mediators of crescentic glomerulonephritis and thus represent a novel therapeutic target.

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FR-OR081

Micromanging Autoimmune Nephritis: Role of miR-17 in Modulating Regulatory T Cell Activity by Targeting Foxp3 Co-Regulators Huang-Yu Yang, Chih-Wei Yang. *Kidney Inst, Dept of Nephrology, Chang Gung Memorial Hospital, Taiwan.*

Background: Regulatory T (Treg) cells play a critical role in maintaining self-tolerance and controlling the magnitude of physiologic immune response. The Treg transcription factor forkhead box P3 (Foxp3) works in concert with other co-regulator molecules including Eos to determine the transcriptional signature and characteristic suppressive phenotype of Treg. A strong correlation between systemic lupus erythematous and deficiencies of Treg has been reported. We were interested in determining whether Treg differentiation and/or suppressive activity are regulated by microRNAs (miRNAs) so that we can modulate the miRNAs to improve lupus nephritis.

Methods: We generated T cell specific miR-17-92 knockout (miR-17-92-/-) mice, followed by induction of pristane nephropathy in miR-17-92 -/- and wild type littermates. By bioinformatics study, possible targets of mir-17-92, related to Treg function was evaluated. Luciferase reporter assay was utilized for verification. Forced expression and knockdown of miRNA in Treg was performed by lentivirus.

Results: mir17-92 knockdown can mitigate pristane nephropathy in mice. Here, we showed for the first time that one miRNA of the miR17-92 cluster, miR-17, regulates the suppression function of Tregs. We identify a gene target of miR-17, Eos, which regulates Tregs through Foxp3-mediated gene suppression. Ectopic expression of miR-17 downmodulates the suppression functions of Tregs and provides Tregs with partial effector activity via de-repression of cytokine genes. In addition, mir17 knockdown improve colitis by enhancing the suppressive function of Treg.

Conclusions: Our studies suggest that miR-17 modulates Treg cell function by targeting Eos, revealing the future therapeutic potential of miR-17 manipulation in lupus nephritis or other autoimmune diseases.

Funding: Government Support - Non-U.S.

FR-OR082

Myeloperoxidase Peptide Based Nasal Tolerance as Treatment for Anti-Neutrophil Cytoplasmic Antibody Associated Glomerulonephritis Stephen R. Holdsworth, Poh-Yi Gan, A. Richard Kitching. *Medicine, Monash Univ, Melbourne, Victoria, Australia.*

Background: Myeloperoxidase (MPO)-ANCA associated glomerulonephritis (GN) is a common form of crescentic GN and current treatments are non-specific and relatively toxic. New less toxic treatments are needed. We have defined the dominant nephritogenic MPO peptide, $MPO_{409-428}$. We assessed the capacity and mechanisms of nasal insufflation of $MPO_{409-428}$ to induce immune tolerance thereby preventing and treating experimental autoimmune anti-MPO ANCA GN.

Methods: MPO autoimmunity and GN was compared between C57BL/6 mice nasally insufflated with either $MPO_{409-428}$ or control $OVA_{323-339}$. Autoimmunity was induced by MPO immunization and GN triggered using a subnephritogenic dose of anti-GBM globulin.

Results: Nasal insufflation of $MPO_{409-428}$ prior to induction of anti-MPO GN reduced renal injury (glomerular segmental necrosis (GSN); 23±13 versus 4±1% of glomeruli affected $p<0.01$) and systemic anti-MPO Tcell recall responses to MPO (ELISPOTS IFN-γ; 222±19 versus 144±19cells $p<0.05$ and IL-17A; 124±48 versus 14±1cells $p<0.05$) compared to control $OVA_{323-339}$. In mice with established MPO autoimmunity, $MPO_{409-428}$ nasal insufflation attenuated GN (GSN; 22±3 versus 9±2% $p<0.01$, reduced glomerular leukocyte accumulation and albuminuria). Both Foxp3+ and Foxp3- CD4+ Tcells from the nasal draining lymph nodes of $MPO_{409-428}$ nasally insufflated mice exhibited greater immunosuppression of anti-MPO Teff cells than Foxp3+ and Foxp3- CD4+ Tcells from nasally insufflated $OVA_{323-339}$ mice, confirming antigen specific immunosuppression. Adoptive transfer of CD4+ Tcells from mice nasally administered with $MPO_{409-428}$ to mice with established MPO autoimmunity attenuated the development of GN (GSN; 23±2 versus 9±1% $p=0.01$) with reduced glomerular leukocyte accumulation and albuminuria compared to mice that did not receive CD4+ cells.

Conclusions: Nasal insufflation with the immunodominant $MPO_{409-428}$ induces Tcell mediated immune tolerance to MPO which can prevent the induction of MPO autoimmunity and treat established disease. This provides proof of concept that peptide based nasal tolerance has therapeutic potential relevant to human MPO-ANCA associated GN.

Funding: Government Support - Non-U.S.

FR-OR083

TLR9 Ligation Enhances Anti-Myeloperoxidase Autoimmunity and Glomerulonephritis Through Dendritic Cell Activation Sharon Lee Ford,[1] Irina Caminschi,[2] A. Richard Kitching,[1] Stephen R. Holdsworth.[1] *[1]Depts of Medicine & Nephrology, Monash Univ, Clayton, VIC, Australia; [2]Burnet Inst of Medical Research, Melbourne, VIC, Australia.*

Background: ANCA associated vasculitis is linked with infections. TLR9 is stimulated by a variety of pathogens and could link infections with anti-myeloperoxidase (MPO) autoimmunity and disease. We hypothesize that dendritic cell (DC) TLR9 ligation promotes anti-MPO autoimmunity and glomerulonephritis (GN).

Methods: Autoimmunity to MPO was induced either by injection of MPO in Freund's adjuvant or by subcutaneous transfer of $1x10^6$ MPO-pulsed DCs. Renal injury was triggered with a sub-nephritogenic dose of anti-glomerular basement membrane globulin, according to published protocols.

Results: After MPO/adjuvant immunisation, compared to WT mice TLR9-/- mice developed less severe glomerulonephritis (proteinuria; WT 4.2±0.3 versus TLR9-/- 1.6±0.5mg/day, $p<0.0005$; abnormal glomeruli; 35±7 versus 13±1%, $p<0.05$). The role of DC derived TLR9 was assessed by generating DCs in vitro, stimulating them with MPO and a TLR9 ligand (CpG oligodendronucleotide [ODN]) or MPO and control (GpC) ODN, then transferring DCs to WT mice and inducing anti-MPO GN. CpG ODN stimulated DCs compared to controls showed enhanced functional (serum urea; Control 12±1 versus CpG 35±9μmol/L, $p<0.05$) and histological (glomerular segmental necrosis Control 12±3 versus CpG 70±8%, $p<0.0001$) injury with increased anti-MPO Th17 responses (MPO-stimulated splenocyte IL-17A; Control 1348±156 versus CpG 677±110pg/mL, $p<0.006$). Th17 responses (IL-17A) and glomerular injury were reduced in MPO/CpG-stimulated TLR9-/- DCs, confirming the specificity of the ligand. The TLR9 ligand's effect was mediated through DEC205 dependent enhanced CD40 expression; injury was significantly reduced following transfer of both DEC205-/- and CD40-/- DCs.

Conclusions: In anti-MPO GN,DC TLR9 ligation promotes renal injury through enhanced DC activation. TLR9 inhibitors represent a potential future therapeutic option in modulating autoimmunity in ANCA associated vasculitis.

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FR-OR084

CXCR3 Guides the Exit of CD4$^+$ Renal T Cells to the Draining Lymph Node in Crescentic GN Christian Franz Krebs, Tobias Koyro, Hans-Joachim Paust, Anna Kaffke, Jan-Eric Turner, Rolf A. Stahl, Ulf Panzer. *III. Medizinische Klinik, Univ Hospital Hamburg-Eppendorf, Hamburg, Germany.*

Background: Leukocyte trafficking throughout the body is essential for the defense against pathogenic infections, but also contributes to tissue damage in autoimmune disease, such as crescentic glomerulonephritis (GN). Chemokines and their receptors have been identified as key regulators of renal leukocyte infiltration under inflammatory conditions. However, hardly anything is known about the egress of leukocytes (lymphocytes) from the kidney to the renal lymph node or the involvement of this process in autoimmunity.

Methods: To address these open questions, we established an experimental system to mark intrarenal leukocytes by using transgenic mice expressing the photoconvertible fluorescent protein "Kaede".

Results: By exposing the kidney of Kaede-transgenic mice to near UV-A light (wavelength λ= 385 nm, dose 11.2 J/cm^2), 30-40% of renal leukocytes were labelled in the kidney by photoconvertion from Kaede-green to Kaede-red. Subsequently leukocyte trafficking to the renal lymph node was investigated by flow cytometry. Under homeostatic conditions renal dendritic cells (CD11b$^+$CD11c$^+$), but no CD4$^+$ T cells, entered the renal lymph node. In contrast, after induction of experimental GN (nephrotoxic nephritis) CD4$^+$ T cells migrated out of the kidney and could be detected in the draining lymph node. Maximum T cell emigration was detected 3 days after photoconversion at day 10 after GN induction. Chemokine receptor expression analysis of Kaede-red emigrating CD4+ T cells in the renal lymph node revealed an enrichment of CXCR3$^+$, but not CCR7$^+$ or CCR6$^+$ cells. In line with this the number of emigrated cells was decreased in CXCR3-deficient mice as compared to nephritic control mice (and CCR7 KO mice), indicating that the egress of T cells from the inflamed kidney to the renal lymph node is an active process that is driven by CXCR3.

Conclusions: We have established a method to study the exit of renal leukocytes out of the kidney. The expression of CXCR3 by renal CD4$^+$ T cells may control their exit, acting with recruitment mechanisms to regulate the accumulation of lymphocytes in the kidney in experimental glomerulonephritis.

Funding: Government Support - Non-U.S.

FR-OR085

Development, and Morphologic Characteriziation of a Mouse Model of Membranous Nephropathy Involving the Human Phospholipase A2 Receptor

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Background: Phospholipase A2 receptor (PLA2R) auto antibodies are associated in 70% of patients developing membranous nephropathy (MN). Since rodents do not express the PLA2R on podocytes we developed human PLA2R knock in mice that specifically express this protein on podocytes.

Methods: The extracellular part of the human PLA2R was fused with a GPI-anchor and cloned into a R26/neo-stop target vector. PLA2R positive ES-cells were injected in blastocysts and implanted in C57Bl6 females. The F1 littermates were tested for germ line transmission of the PLA2R knock in. Breeding with PodoCre mice animals were obtained that specifically express PLA2R on podocytes. These mice are called PodoCre PLA2R and compared with PodoCre-free PLA2R animals as controls. For disease induction, control and PodoCre PLA2R mice were either actively immunized with recombinant human PLA2R or received concentrated human serum from patients with high levels of serum PLA2R antibodies. Histologic assessment of the kidneys was performed after several time intervals following immunization.

Results: PLA2R expression was detected in PodoCre PLA2R but not in the appropriate control mice. PodoCre PLA2R and control mice developed PLA2R antibodies in response to the immunization. PodoCre PLA2R mice developed granular subepithelial immune deposits along the glomerular basement membrane which were positive for mouse IgG. No immune deposits could be detected in control mice. Electron microscopy showed dense deposits located in the subepithelial space exclusively in immunized PodoCre PLA2R mice. PodoCre PLA2R mice which received serum from patients with MN positive for PLA2R antibodies developed granular deposits along the glomerular capillary wall, which were positive for human IgG.

Conclusions: These studies show that the human PLA2R when expressed on mice podocytes, is a target for autoantibodies induced in mice or serves as antigen for transferred human PLA2R antibodies from patients with membranous nephropathy.

FR-OR086

The Immunodominant Epitope in PLA_2R Mediating Idiopathic Membranous Nephropathy Is Located in the CysR-FnII-CTLD1 Region

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Background: Clinical studies have firmly established that over 70% of patients with idiopathic membranous nephropathy (IMN) possess high levels of circulating autoimmune antibodies targeting phospholipase A_2 receptor (PLA_2R) in the glomerular visceral epithelial cells. The level of auto-antibodies correlates with the severity of proteinuria in patients. The epitope is known to be conformational in nature and to require specific disulfide bonds. PLA_2R is a transmembrane receptor containing ten extracellular domains: an N-terminal cysteine rich domain (CysR), a fibronectin type II domain (FnII), and eight repeated C-type lectin like domains (CTLD).

Methods: To determine the location of the antigenic epitope in PLA_2R, we created various truncated extracellular domains and expressed them in HEK 293 cells. These isolated domains were analyzed on the immunoblot by probing with sera derived from IMN patients. The identified epitope region was then tested for its immunodominance using epitope competition assays in the liquid phase. Finally, the identified immunodominant epitope region was verified by comparing side by side with the full length PLA_2R for 74 IMN patient sera recognition.

Results: Our results showed that the anti-PLA_2R antibodies from patients specifically recognize a three domain structure consisting of the CysR, FnII, and the CTLD1 domains under non-reducing conditions. When the three domains were missing, none of the remaining domains were recognized. This three domain structure completely blocked the reactivity of auto-immunue antibodies with the full length PLA_2R protein on the immunoblot.

Conclusions: Our results demonstrate that the immunodominant epitope in PLA_2R mediating IMN is exclusively formed by the CysR, FnII, and CTLD1 domains in the N-terminal region of the receptor.

Funding: Private Foundation Support

FR-OR087

Alternative Pathway Amplifies Complement Activation by Human Anti-PLA2R Antibodies in Membranous Nephropathy

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Background: About 70-80% of patients with primary MN have autoAbs against phospholipase A2 receptor (PLA2R), predominantly but not exclusively of IgG4 subclass. Whether and how anti-PLA2R autoAbs cause glomerular injury remains unknown. C3 and C5b-9 occur in glomerular immune deposits, implicating complement activation as a putative effector mechanism. Purified anti-PLA2R IgG4 can activate the lectin pathway (LP). Here, we investigated how unfractionated anti-PLA2R autoAbs activate complement and the role of the classical (CP) and alternative (AP) pathways.

Methods: We developed a functional assay to evaluate the complement activating ability of serum anti-PLA2R autoAbs from MN patients. Binding of complement components to complexes of human PLA2R antigen and anti-PLA2R Abs was measured after incubation with normal and complement-deficient human sera as complement source, with and without specific inhibitors.

Results: All anti-PLA2R autoAbs activated complement, with deposition of C3, C4, factors B, H and properdin (P). Binding of C3, B, H and P (but not C4) occurred under conditions where only the AP was functional. Deposition of C3 and C4 occurred under conditions where only the CP and LP were active. C3 and C4 deposition was strongly inhibited in C1q-deficient serum (lacking CP) and further reduced in C4-deficient serum (lacking CP and LP). Under physiological conditions with all pathways functional, specific AP inhibitors reduced C3 activation by 4-8 fold.

Conclusions: Anti-PLA2R autoAbs can activate complement via all three pathways. The classical pathway may be activated by anti-PLA2R IgG1/3, which were found in all MN sera. The alternative pathway likely amplifies complement activation initiated by the other pathways to pathologic levels responsible for tissue injury. We provide proof of concept that inhibiting the alternative pathway effectively limits complement activation by anti-PLA2R autoAbs. These findings are relevant for the design of interventions aimed at preventing complement-mediated injury in MN.

Funding: Private Foundation Support

FR-OR088

Glomerular Deposition of Food Antigens in Adult Patients with Membranous Nephropathy

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Background: Cationic bovine serum albumin, a modified food-derived antigen, was reported to be related to childhood membranous nephropathy. We hypothesized that food antigen might play a role in adult membranous nephropathy, as well.

Methods: We detected food antigens, which were major allergic proteins extracted from egg, shrimp, fish, milk, hazelnuts, wheat, peanuts and soybeans in kidney biopsy specimens obtained from 67 patients with membranous nephropathy and 94 controls, through immunofluorescence technique with monoclonal antibodies to these proteins. Double-labeling confocal studies were used to analyze the location of food antigens and IgG. PLA2R1 staining was also performed in partial patients at the same time.

Results: Multiple food antigens were detected simultaneously in patients with idiopathic membranous nephropathies. Food antigens were found in 70.1% patients with IMN, while no food antigens were found in patients with other kidney diseases. Food antigens were detected in most patients with positive PLA2R1 in the biopsy. Food antigens co-localized with IgG in a fine linear and granular pattern along the glomerular capillary walls. However, there were no significant differences in clinical parameters between MN patients with or without glomerular deposition of food antigens.

Conclusions: Multiple food antigens were present in the glomeruli of patients with idiopathic membranous nephropathies. The potential role of some kinds of food antigens or curtain components of foods such as dietary lectins in the development of MN merits considering, both in exploring the pathogenesis of MN and giving a specific treatment to the patients with MN.

Funding: Government Support - Non-U.S.

FR-OR089

A Population-Based Study of the U.S. Population Shows the Majority of Persons Cannot Donate due to Preventable Diseases and Socio-Economic Conditions

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Background: There is a shortage of living kidney donors in the U.S., but there have been no population-based studies examining the potential U.S. donor pool.

Methods: We obtained demographic data and information on potential exclusionary criteria for living donor kidney donation (LKD) from the NHANES Survey for persons 21-70 y. We defined exclusionary critera as: BMI > 35 kg2/m2, history of DM or a Hb A1c >6.5%, >4 alcoholic drinks/day, microalbuminuria >30 mg/g creatinine, eGFR < 80 ml/min/1.73m2, BP >140/90 or history of HTN, non-melanoma skin cancer, congestive heart failure, coronary artery disease, and HIV infection. Social factors included being a non-U.S. citizen and an annual family household income (FHI) <$25,000 (the U.S. poverty level).

Results: 55.2% of the population could not donate due to medical conditions: obesity (15%), hypertension (19.2%), DM (11.5%), and excessive alcohol intake (11.6%). 60.1% of individuals with FHI<$35000 could not donate due to medical conditions versus only 49.3% for FHI > $100,000 (p<0.0001). If one designates FHI below the poverty level and non-U.S. citizenship as exclusionary criteria, 68.5% could not donate. If one excludes smokers and individuals who have shortness of breath walking up an incline, 75.8% could not donate

Characteristic	White	African American	Hispanic	Asian
Obesity	13.8	24.1	15.7	2.1
Hypertension	17.9	30.3	17.6	14.9
Excess alcohol intake	10.9	8.8	18.9	3.9
Diabetes mellitus	9.5	17.6	12.8	11.5
eGFR < 80 ml/min	20.0	17.3	8.0	9.9
Microalbuminuria	5.6	12.9	9.6	8.6
Congestive heart failure	1.8	2.4	1.1	.6
Coronary artery disease	2.1	1.4	1.1	1.1
HIV infection	.2	1.2	.3	0
Total medical exclusion	54.8	63.9	54.2	37.7
Non-US citizen	2.1	5.2	42.6	37.4
Medical exclusion + non-US citizen + below poverty threshold	63.6	78.9	80.1	68.1

Conclusions: Many U.S. citizens are unable to donate a kidney due to preventable health conditions. If one includes financial and social restrictions, the proportion rises substantially. Efforts to improve the general health of the population and providing financial compensation would improve the number of possible kidney donors.

Funding: Clinical Revenue Support

FR-OR090

Kidney Function Evaluation in Prospective Kidney Donors – Performance of Creatinine Clearance versus GFR as Measured by Iothalamate Clearance

Grace Snyder,[1] Jesse D. Schold,[1] Didier A. Mandelbrot,[2] Stuart M. Flechner,[1] Emilio D. Poggio.[1] *[1]Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH; [2]Transplant Nephrology, Univ of Wisconsin, Madison, WI.*

Background: The evaluation process of living kidney donation is dependent on the assessment of kidney function. While glomerular filtration rate(GFR) as measured by a tracer clearance is the gold standard, most institutions rely on 24-hour creatinine clearances(CrCl), and direct comparison of the two measurements in prospective living donors is limited. We therefore compared the 24-hour CrCl to ^{125}I-iothalamate GFR(iGFR) in living donors.

Methods: This is a single center, cross-sectional study of prospective living donors simultaneously undergoing both tests. We compared iGFR and 24-hour CrCl using univariate and multivariable linear regression adjusted for age, gender, body surface area(BSA), and race.

Results: 342 adults with iGFR and 24-hour CrCl measurements were included. Demographics: females:58%; Caucasians:85%; African Americans:9%; mean age 41(SD 11 range 18-72). We compare normalized iGFR and 24-hour CrCl across age groups.

			iGFR (ml/min per 1.73 m2) / 24 hr CrCl (ml/min)		
	Age subgroups (years)	n	25th percentile	Median	75th percentile
Men	18-40	76	100.1 / 95.4	108.5 / 115.0	117.9 / 135.3
	41-55	54	91.8 / 92.8	100.3 / 108.8	106.9 / 124.8
	>55	14	84.1 / 102.6	97.0 / 106.6	106.2 / 118.6
Women	18-40	94	97.8 / 91.8	111.0 / 110.5	120.0 / 122.3
	41-55	84	91.1 / 92.5	103.6 / 104.9	113.0 / 115.3
	>55	20	80.0 / 82.4	91.5 / 90.1	96.5 / 100.0

Multivariable regression showed a sigificant overestimation of GFR by 24-hour CrCl in African Americans versus others (18.5 versus 4.9, p=0.04) and men versus women (15.8 versus 7.5,p=0.01). Age and BSA were not associated with a difference in measurements.

Conclusions: 24 hour CrCl can be a useful measure of GFR in living donor evaluation, especially for women, but additional measures of GFR may be needed in men and African Americans given their significant overestimation of GFR. This issue becomes clinically relevant when assessing donors with GFRs in the lower ranges of normality.

FR-OR091

Long Term Survival in Kidney Donors with Pre Existing Hypertension

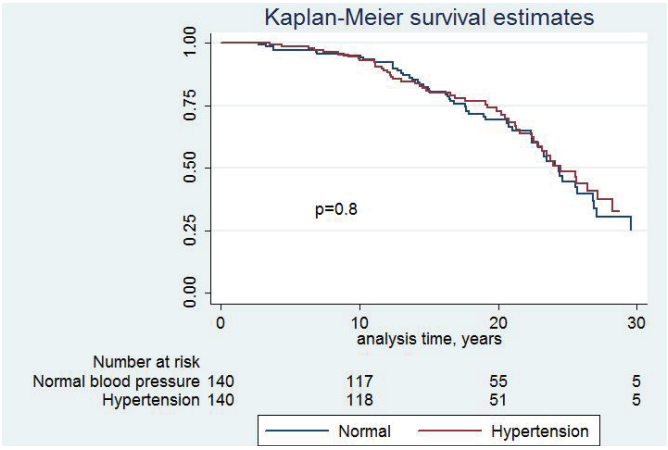
Geir Mjøen, Hallvard Holdaas. *Oslo Univ Hospital, Norway.*

Background: In the general population, even small increases in blood pressure are associated with mortality. However, in otherwise healthy kidney donors, this association is less certain. There is currently a lack of evidence whether pre-existing hypertension is associated with increased long-term mortality.

Methods: All donors in the period 1963-2007 were included. Baseline data were obtained from a national registry and patient files. Hypertension was defined retrospectively as BP> 140/90 or use of medication. Hypertensive and normotensive donors were matched 1:1 for age, gender and time of donation. As a sensitivity analysis we performed cox regression including all donors after multiple imputation adjusted for matching variables and also for BMI and smoking.

Results: Among a cohort of 2269 donors, 140 (6.2 %) had hypertension. Mean age was 57.7 years and 44.3 % were male. During a median follow-up of 16.9 years there were 51 deaths. Mean blood pressure was 147/89 mmHg in donors and 128/80 mmHg in controls. In matched analysis, there were no associations between pre existing hypertension and long-term mortality (p=0.8). Multivariate sensitivity analysis did not change results (HR 1.2, 0.9-1.7, p=0.2).

Conclusions: In otherwise healthy kidney donors, mild hypertension should not be considered a strict contraindication to kidney donation.



Funding: Government Support - Non-U.S.

FR-OR092

Gout in Living Kidney Donors

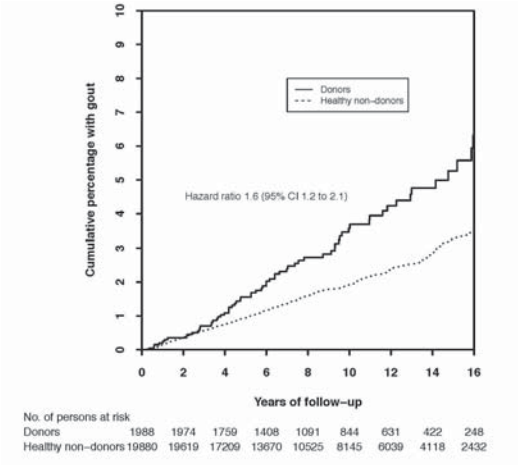
Ngan Lam,[1] Eric Mcarthur,[2] Joseph Kim,[3] G.V. Ramesh Prasad,[3] Krista L. Lentine,[4] Peter P. Reese,[5] Bertram L. Kasiske,[6] Charmaine E. Lok,[3] Amit X. Garg.[1,2] *[1]Western Univ; [2]Inst for Clinical Evaluative Sciences; [3]Univ of Toronto; [4]Saint Louis Univ; [5]Univ of Pennsylvania; [6]Univ of Minnesota.*

Background: To determine whether by donating a kidney, a person increases their risk of gout, as the serum concentration of uric acid rises in donors after nephrectomy.

Methods: We performed a retrospective matched cohort study using large healthcare databases. We included all living kidney donors in the province of Ontario, Canada who donated a kidney between 1992 and 2010. The pre-donation charts were manually reviewed and linked to provincial healthcare databases. Matched non-donors were selected from the healthiest segment of the general population. A total of 1988 donors and 19,880 matched non-donors were followed for a median of 8.4 years (maximum 20.8 years). Median age was 43 years at the time of donation (interquartile range [IQR] 35 to 51) and 52 years at time of last follow-up (IQR 45 to 60). 61% were women. The primary outcome was the time to a diagnosis of gout. The secondary outcome was receipt of medications typically used to treat gout (allopurinol, colchicine).

Results: Donors compared to non-donors were more likely to be diagnosed with gout (67/1988 [3.4%] versus 390/19,880 [2.0%]; 3.5 versus 2.1 events per 1000 person-years; hazard ratio 1.6; 95% confidence interval [CI] 1.2 to 2.1; P<0.001). Similarly, donors compared to non-donors were more likely to receive a prescription for allopurinol or colchicine (3.8% versus 1.3%; odds ratio 3.2; 95% CI 1.5 to 6.7; P=0.002). Results were consistent in multiple additional analyses.

Conclusions: The findings suggest that donating a kidney modestly increases an individual's absolute long-term risk of gout. This information should not deter living donor kidney transplantation, but it can be shared with potential donors and their recipients as part of the informed consent process.



FR-OR093

Number of Glomeruli Estimated from Renal Biopsy and CT Scan of Living Donors Aleksandar Denic,[1] Harini A. Chakkera,[1] Emilio D. Poggio,[2] Prince Singh,[1] Walter Park,[1] Mariam P. Alexander,[1] Walter K. Kremers,[1] Andrew D. Rule.[1] *[1]Mayo Clinic; [2]Cleveland Clinic, OH.*

Background: Nephron endowment is difficult to study in living humans but is thought to be an important determinant of renal health.

Methods: We studied 1281 living kidney donors at 3 different centers (Mayo Clinic in Minnesota and Arizona and Cleveland Clinic). Cortical volumes (mm^3) from both kidneys were obtained from pre-donation CT scans. At the time of surgical implantation of an allograft, a biopsy of the cortex was obtained and sections were analyzed for the numbers of non-sclerotic (NSG) and globally sclerotic (GSG) glomeruli. Weibel and Gomez models were used to calculate NSG and GSG densities (per mm^3) from the biopsy sections.

Results: We estimated the total number of NSG and GSG in each donor by multiplying cortical volume by the glomerular densities, adjusting for 30% biopsy shrinkage. There were a mean 1,378,302 NSG and 19,673 GSG per kidney in 18-19 year olds and this progressively decreased to 751,143 NSG and increased to 170,711 GSG in 70 year olds (P<0.001 for both trends). Reabsorbed glomeruli were estimated from the total number of detected glomeruli in each age group subtracted from that detected in 18-19 year olds (assumed 0 per kidney). The number of reabsorbed glomeruli increased to 481,525 in 70 year olds (P<0.001). The loss of NSG as assessed by the increase in GSG with age on biopsy fails to detect the substantial loss from reabsorbed glomeruli (Figure).

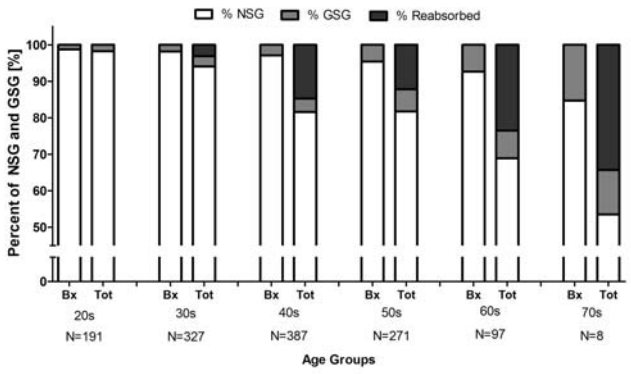


Figure. Percent of NSG and GSG on biopsy sections (Bx) and percent NSG, GSG and reabsorbed glomeruli among total glomeruli per kidney (Tot) by age group.

Analysis found that older age, shorter height, family history of ESRD, lower measured GFR and higher uric acid levels were independently associated with a decreased number of NSG (p<0.05 for all).

Conclusions: Loss of NSG with aging is much more severe than appreciated on biopsy due to the reabsorption of glomeruli. Number of NSG also associates with characteristics with congenital determinants (height and family history of ESRD).

Funding: NIDDK Support

FR-OR094

The Effects of Recipient-Donor Gender Combinations and Body Size on Kidney Allograft Survival Valarie B. Ashby,[1] Alan B. Leichtman,[1] Michael A. Rees,[2] Peter X.K. Song,[1] Mathieu Bray,[1] Richard Eikstadt,[1] Audrey J. Goulding,[1] Wen Wang,[1] John Kalbfleisch.[1] *[1]Univ of Michigan, Ann Arbor, MI; [2]Univ of Toledo Medical Center, Toledo, OH.*

Background: Outcomes for transplants from living non biologically related donors (LNBRD) are of particular interest in kidney paired donation programs (KPDP) where exchanges are arranged among incompatible donor-recipient pairs, and chains are created from bridge, non-directed or altruistic donors. This work examines graft survival as a function of donor and recipient sex and body size.

Methods: SRTR data were used to analyze 222,443 kidney transplant recipients from 1996-2010. Rates of graft failure (HR) over 5 and 10 years were assessed using Cox models for transplants from LNBRD, living biologically related donors (LBRD), and deceased donors (DD). All models were adjusted for donor and recipient age and race/ethnicity, and recipient's primary disease, PRA, HLA mismatch, previous transplant, Hepatitis C, and years of ESRD.

Results: Males comprised 63%, 57% and 61% of LNBRD, LBD, and DD recipients; and 37%, 43% and 60% of the LNBRD, LBD, and DD groups, respectively. For each donor type, male donor to male recipient had superior graft survival compared to all other donor recipient combinations and, in particular, better than female to male. The table shows these differences persist as measured by hazard ratios after adjustment for donor and recipient body size.

Characteristic	Group	Living Not Biological Related Donor (N=28,049)		Living Biological Related Donor (N=57,102)		Deceased Donor (N=137,292)	
		N (%)	HR	N (%)	HR	N (%)	HR
Recipient-Donor Type	Female to Female	4,870 (17%)	0.97	14,729 (26%)	1.10*	22,479 (16%)	0.94*
	Female to Male	5,379 (19%)	1.04	9,577 (17%)	1.02	31,667 (23%)	0.91*
	Male to Female (Ref)	12,908 (46%)	1.00	17,682 (31%)	1.00	33,082 (24%)	1.00
	Male-Male	4,892 (17%)	0.82*	15,114 (27%)	0.88*	50,064 (37%)	0.92*
Recipient BMI	Obese (vs. Not Obese Ref)	7,001 (25%)	1.14*	11,083 (19%)	1.11*	31,576 (23%)	1.03*
Donor BMI	Obese (vs. Not Obese Ref)	5,191 (19%)	1.09*	9,741 (17%)	1.06	31,112 (23%)	1.05*
Donor-recipient weight Ratio	< 0.75	5,059 (18%)	1.15*	5,616 (10%)	1.06	28,014 (20%)	1.15*
	0.75-0.90	5,016 (18%)	1.09	7,404 (13%)	0.98	21,531 (16%)	1.04*
	0.90-1.15 (Ref)	6,520 (23%)	1.00	13,198 (23%)	1.00	32,617 (24%)	1.00
	> 1.15	6,309 (23%)	1.04	14,440 (25%)	0.93*	39,530 (29%)	0.98
	Missing	5,145 (18%)	1.31*	16,444 (29%)	1.20*	15,600 (11%)	1.34*

P<0.05

Conclusions: Male recipients have significantly better graft survival outcomes with a kidney from a male donor. This difference is larger for living donors than differences due to age or HLA mismatch. These results may help guide the practice of inclusion of compatible pairs in KPDP; extensions could lead to a calculator to predict and compare donor-recipient outcomes.

Funding: Other NIH Support - Optimization and Simulation of Kidney Paired Donation Programs (R01-DK093513-01A1)

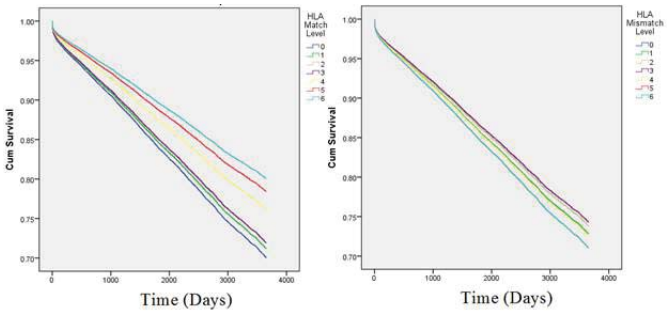
FR-OR095

HLA Match/Mismatch Profiles and Impact on 10 Year Graft Survival: An Analysis of OPTN/UNOS Registry Data Rabi Yacoub, Madhav C. Menon, Girish N. Nadkarni, Etti Deborah Zeldis, Ioannis Konstantinidis, John C. He, Barbara T. Murphy. *Nephrology, Icahn School of Medicine at Mount Sinai.*

Background: Degree of Human Leukocyte Antigen(HLA) mismatch has known impact on allograft survival. The presence of homozygous/heterozygous HLA alleles can cause different degrees of HLA match within the same mismatch degree. There are limited data on HLA match/mismatch combinations impacting graft survival.

Methods: We utilized data from the United Network for Organ Sharing (UNOS) database from 1987-2012. We divided HLA match/mismatch profile into 22 permutations and analyzed graft survival using Cox-regression. We calculated graft loss risk using hazard ratios (HR) adjusted for: recipient demographics and comorbidities, donor demographics, terminal creatinine, ECD, DGF, CIT, and preemptive transplantation. We accounted for differences in HLA match/mismatch terminologies in the database over time by adjusting for transplantation date. Groups with <1% of the final sample size, patients with incomplete data, previous or concomitant organ transplantation were excluded.

Results: Of the 355812 recipients, we had complete data on 46048 recipients (Mean age 47.8, 60.7% male). Analysis revealed a stepwise worse 10-yr graft outcome with progressively unfavorable match/mismatch combinations (P<0.01). Both the degree of matching and mismatching had significantly independent effects on graft loss. However, when added in the same multivariable model, the degree of mismatching lost significance while HLA matches continued to be a significant predictor (P=0.579 and P=0.043, respectively).



Conclusions: Our analysis incorporating newer outcome data highlights the possible separate roles the degrees of HLA match and mismatch play in DDKT graft survival. A novel approach incorporating the HLA match/mismatch profiles in a weighted manner to stratify risk and predict graft survival may be optimal.

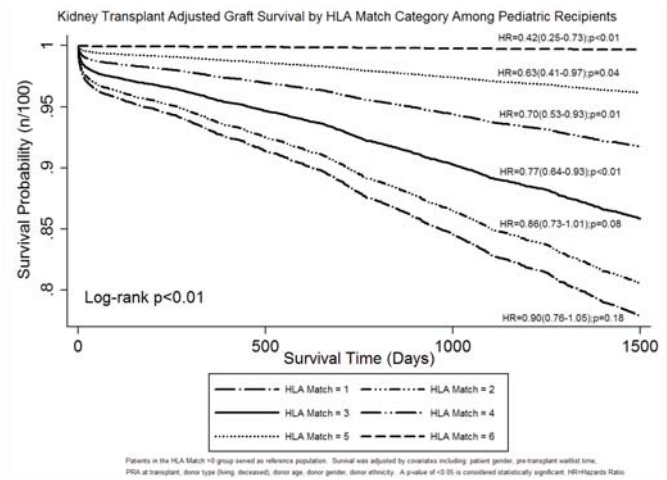
FR-OR096

Better Human Leukocyte Antigen Matching Is Associated with Graft Outcomes Among Pediatric Kidney Transplant Recipients Meera Gupta, Peter Abt, Matthew H. Levine. *Surgery, Div of Transplantation, Univ of Pennsylvania, Philadelphia, PA.*

Background: Kidney transplant(KT) graft survival is superior if received from ideal donors. Given the decline of living donor(LD) availability and the initiation of the Share35 kidney policy, we sought to determine the impact of HLA matching on graft outcomes.

Methods: A retrospective analysis of OPTN data(1988-2009) was conducted examining KT graft survival among pediatric(age<18) recipients based on HLA matching(0-6), Share35 policy status, and donor-type(LD versus Deceased Donor=DD) using Kaplan-Meier statistics and Cox regression analysis.

Results: Of 13,489 first KT pediatric recipients, there was a significant decline in LD availability post-Share35 initiation(-20.17%;p<0.01). Additionally, post-Share35 groups had poorer(0-2 HLA-antigen) matched grafts(64.88%) compared to the pre-Share35 cohort(39.69%;p<0.01). Adjusting for all factors, better HLA-matching(log-rank p<0.01), LD grafts(HR=0.63;p<0.01), and post-Share35 transplantation(HR=0.71;p<0.01) conferred better graft outcomes.



In a secondary analysis of recipients with poorly matched(0-2 antigen) grafts at first KT who underwent second KT, median wait-list time prior to second KT was longer(p<0.01), while second kidney graft survival was shorter(p=0.02). We also determined that better HLA matching at first KT correlated with lower peak PRA level at second KT(-0.14;p<0.01).

Conclusions: Among pediatric KT recipients, improved outcomes exist for better HLA-matched transplants, despite Share35 initiation and decline of LD availability. HLA matching retains its prognostic value in graft outcomes for first and subsequent KTs given its association with PRA level. Finally, patients who are better matched for previous transplants are less sensitized, have shorter waiting times, and longer graft survival times.

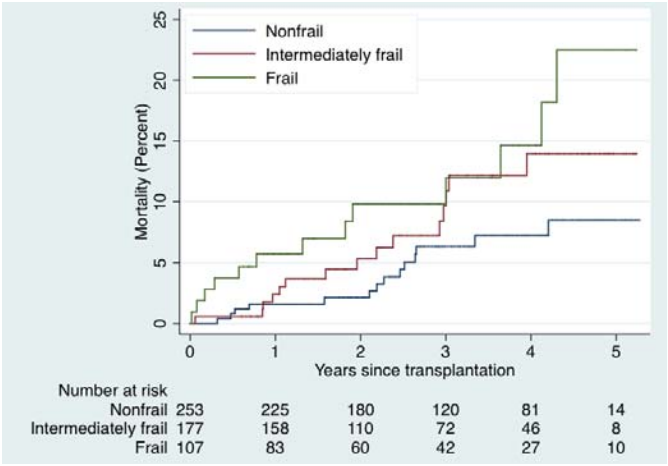
FR-OR097

Frailty as a Novel Predictor of Mortality in Kidney Transplant Recipients of All Ages Mara Mcadams-DeMarco, Andrew Law, Elizabeth King, Babak Orandi, Megan Salter, Nada Alachkar, Niraj Desai, Ravi Varadhan, Jeremy Walston, Dorry L. Segev. *Johns Hopkins.*

Background: We have described strong associations between frailty, a measure of physiologic reserve initially described and validated in geriatrics, and short-term outcomes in kidney transplantation (KT) recipients, including early hospital readmission and delayed graft function. In this study we estimated its association with mortality.

Methods: Frailty was prospectively measured in 537 KT recipients at KT (12/08-8/13). Cox proportional hazards models were adjusted for confounders using a novel approach to improve model efficiency in single-center studies. It is well-known that there are dozens of mortality predictors, but it is impossible to adjust for all of these in single centers studies with relatively small sample sizes (because of imprecision and risk of overfitting). We precisely estimated the coefficients of every confounder using the huge sample size of the SRTR (n=37,858), and fed these back into a single-center model, where frailty was the only coefficient estimated.

Results: Mean age was 53.0 years (SD=14.0), 40.0% were female, 38.9% were African American and 44.9% received live donor transplants; 19.9% were frail and 33.0% were intermediately frail at KT. At 5 years, the survivals were 91.5%, 86.0% and 77.5% for non-frail, intermediately frail and frail KT recipients, respectively. Figure: Mortality by Frailty Status



Frailty was independently associated with a 2.17-fold (95%CI:1.01-4.65, P=0.047) higher death risk with no effect difference between older and younger recipients (P for interaction=0.4).

Conclusions: KT recipients of all ages have a high frailty prevalence, >3-fold higher than community dwelling older adults. In this population, regardless of age, frailty is a strong, independent predictor of mortality, after carefully adjusting for many confounders using a novel statistical approach.

Funding: Other NIH Support - NIA

FR-OR098

The Histologic Staging of Polyoma-BK-Virus Nephropathy: Results from a Multicenter Study Volker Nickeleit,[1] Surya V. Seshan,[2] A. Gasim,[1] Harsharan Kaur Singh.[1] *[1]Div of Nephropathology, The Univ of North Carolina, Chapel Hill, NC; [2]Dept of Pathology, Weill Cornell Medical Center, New York, NY.*

Background: Polyomavirus nephropathy (PVN) is the most important infection in renal allografts (incidence 4% in 2010)and can lead to chronic graft failure in >50% of patients reported in some series. Since PVN is not routinely classified into different disease stages, it is unclear if specific morphologic phenotypes carry prognostic significance. The **AIM** of this multicenter study is to define clinically predictive morphologic PVN disease stages in a large cohort of diverse patient populations.

Methods: 9 U.S. and European centers collected 192 diagnostic index biopsies with PVN from 192 patients; 90% transplanted between 2003-2008 (74% males, 31% blacks). Histologic changes were evaluated at time of initial diagnostic index biopsy (Banff scores; intra-renal PV load-levels (pvl)-percentage of tubular SV40-T staining). Clinical data were collected from time of transplant to 24 months post-index biopsy. Statistical analyses: hierarchical mixed models, stepwise regression, with specific emphasis on racial backgrounds.

Results: Renal function and graft survival: Strongest associations were found with intra-renal pvl and Banff ci interstitial fibrosis scores (minor: <25% and major: >25% fibrosis). Limited associations were seen with presence or absence of intra-nuclear PV-inclusion bodies. Based on these statistics, 3 PVN disease grades were defined: 1 (minimal – pvl <1% and ci <25%), grade 2 (florid – pvl and ci scores other than listed in grades 1 and 3), and grade 3 (advanced – pvl >10% and ci >25%). 24% of cases (46/192) were PVN grade 1, 64% grade 2, and 13% grade 3. PVN grades correlated significantly with: a) time of diagnosis (earliest:median 18 months in grade 1), b) peak serum creatinine level-time of index biopsy (best in grade 1 with stable function and minimal Δ change from baseline readings), 4) PVN resolution (79% in grade 1), and 5) allograft failure (grade 3 with 50%). All p <0.001.

Conclusions: The subclassification of PVN into 3 disease grades (minimal, florid, advanced) helps to predict graft function, graft survival and the resolution of PVN.

FR-OR099

Sirtuin 7 Modulates Renal Acid-Base Homeostasis through Deacetylation of the K^+:Cl^- Cotransporter, KCC4 Zesergio Melo,[1] Armando R. Tovar,[2] Silvia Cruz-Rangel,[1] Adriana P. Mercado,[3] Dongryeol Ryu,[4] Nimbe Torres,[2] Gerardo Gamba,[1] Lilia G. Noriega.[2] *[1]Molecular Physiology Unit, INCMNSZ-IIB-UNAM; [2]Dept of Nutrition Physiology, INCMNSZ; [3]INCICH; [4]Univ of Lausanne.*

Background: Sirtuin 7 (SIRT7) is a NAD+ dependent deacetylase. Its molecular targets and physiological functions are unknown. In silico analysis of 41 recombinant inbred mouse strains kidney transcripts (GeneNetwork database) revealed a positive correlation between SIRT7 and KCC4 expression. The expression of KCC4 in the basolateral membrane of α-intercalated cells is modulated by acid-base status (Melo et al. AJP Renal 2013) and KCC4-KO mice exhibit renal tubular acidosis. We thus analyzed the effect of Sirt7 on KCC4 expression and activity.

Methods: We used *Xenopus laevis* oocytes, HEK293 cells, and a mouse model of metabolic acidosis to assess SIRT7 localization, KCC4 activity and acetylation, and the interaction between the two proteins by co-immunoprecipitation. KCC4 protein expression in kidney was assessed in Sirt7$^{-/-}$ mice. Activity of KCC4 was assessed by measuring the Cl^--dependent, $^{86}Rb^+$ uptake in control and hypotonic conditions.

Results: KCC4 is acetylated and its activity is significantly reduced when *Xenopus laevis* oocytes and HEK cells are incubated with the deacetylase-inhibitor nicotinamide (NAM). Incubation with NAM also decreases KCC4 protein levels. In contrast, KCC4 is deacetylated and its activity is enhanced in the presence of SIRT7 and its co-activator NAD^+, without affecting the protein levels. Moreover, KCC4 and SIRT7 interact and NAD^+ enhances this process in HEK cells. As occurs with KCC4, metabolic acidosis increased the expression levels of Sirt7 and its co-localization with KCC4 at the basolateral plasma membrane of α-intercalated cells. Finally, KCC4 expression levels in the kidney are significantly reduced in SIRT7$^{-/-}$ mice.

Conclusions: Our data demonstrate that SIRT7 colocalize and interacts with KCC4 and up-regulates its activity by deacetylation. This positive effect of Sirt7 is implicated in KCC4 regulation during metabolic acidosis.

Funding: Government Support - Non-U.S.

FR-OR100

The Function of ATP6AP2/PRR in Endosomal Trafficking in the Proximal Tubules of the Mouse Kidney Simon Daniel Gerber,[1,2] Florian Grahammer,[1] Tobias B. Huber,[1] Matias Simons.[1,2] *[1]Univ Medical Center Freiburg, Freiburg, Germany; [2]Imagine Inst, Paris, France.*

Background: The V-ATPase subunit, ATP6AP2 or PRR, is a highly conserved protein that has initially been reported to participate in the renin-angiotensin system. However, our recent data from *Drosophila* suggests a role in endolysosomal function, mTOR signaling and transcriptional regulation of Megalin (Gleixner et al., Cell Reports, in press). In this study, we investigate the function of PRR in endosomal trafficking in the proximal tubules of the murine kidney.

Methods: We used doxycycline-inducible *Pax8-rtTA;TetOCre* and *PRR floxed* mice to permanently delete PRR in the whole nephron except glomeruli in the mouse kidney.

Results: In the kidney, PRR is expressed in intercalated cells of the distal nephron and collecting duct. PRR is also highly expressed in the proximal tubules. The proximal tubules recover filtered proteins by endocytosis and subsequently degrade them in the lysosomes, a process that is dependent on V-ATPases. Time-specific deletion by administering doxycycline to *Pax8-rtTA;TetOCre;PRR floxed* mice ablated PRR in all proximal tubules and distal tubules and the entire collecting duct of the mouse kidney, but not in the glomeruli and interstitium. Ablation of PRR resulted in low molecular weight (LMW) proteinuria and to a high albumin to-creatinine-ratio (ACR) value. Furthermore, enrichment of lysosomal markers (Lamp-2) in proximal tubular cells was observed.

Conclusions: Collectively, these results demonstrate that deletion of PRR in the proximal tubules of the mouse kidney leads to LMW proteinuria and albuminuria as well as the upregulation of lysosomal markers. We suggest that PRR is essential for normal proximal function and may have a role in endosomal trafficking. Follow-up studies are under way to address the role of PRR and the V-ATPase in mTOR signaling and the control of Megalin-mediated endocytosis in mouse proximal cells.

FR-OR101

Renal Peroxiredoxin 6 Interacts with Anion Exchanger 1 and Plays a Novel Role in pH Homeostasis Sara Lynn Sorrell,[1] Zoe J. Golder,[1] D. B. Johnstone,[2] Fiona E. Karet.[1] *[1]Dept of Medical Genetics, Univ of Cambridge, Cambridge, United Kingdom; [2]Dept of Medicine, Section of Nephrology and Kidney Transplant, Temple Univ, Philadelphia, PA.*

Background: Peroxiredoxin 6 (PRDX6) is one of six members of the peroxiredoxin family, which have peroxidase activity and act as antioxidants. PRDX6 is a unique member because 1) it contains only one conserved cysteine residue, the others having two and 2) it has additional phospholipase A_2 activity. A yeast-two-hybrid screen elicited PRDX6 as a potential binding partner of the C-terminal tail of Anion Exchanger 1, also known as Band-3, (AE1(C)). AE1 is a Cl^-/HCO_3^- exchanger basolaterally expressed in renal intercalated cells involved in pH regulation.

Methods: Yeast mating; immunostaining; pulldown and co-IP; mass spectrometry; acid challenge testing.

Results: Human kidney immunostaining showed widespread cellular expression of PRDX6 and basolateral localization in AE1-positive cells. *In vitro and ex vivo* pulldowns and immunoprecipitation assays in human kidney confirmed specificity of the interaction between PRDX6 and AE1. Purified PRDX6 protein exhibits four oxidation states, which we verified by mass spectrometry, and which we showed can also be found in human and mouse kidney protein fractions when separated under non-reducing conditions. Site-directed mutagenesis of the conserved cysteine (47C) in PRDX6 resulted in loss of binding to AE1 and considerable loss of dimerization. We found that *Prdx6*$^{-/-}$ mice have a baseline acidosis and greater Ae1 expression than wild type animals. After acute oral acid challenge, acidosis developed transiently in +/+ animals, and worsened over a similar time period in -/- mice. Concomitantly, Prdx6 protein levels increased while Ae1 remained the same in +/+ mice, but Ae1 expression significantly decreased in *Prdx6*$^{-/-}$ animals, suggesting a requirement for Prdx6 to maintain Ae1 during acute acidosis. H+E staining of kidneys from acidified mice showed widespread cellular vacuolation (a normal response to cellular stress) in wild-type but not knockout animals.

Conclusions: These results indicate a novel role for Prdx6 in pH homeostasis.

Funding: Private Foundation Support

FR-OR102

High Mobility Group Box 1 (HMGB1) Inhibits HCO_3^- Absorption in Medullary Thick Ascending Limb (MTAL) through a Receptor for Advanced Glycation End Products (RAGE)-Dependent Pathway David W. Good, Thampi George, Bruns A. Watts. *U TX Med Branch, Galveston, TX.*

Background: HMGB1 is an endogenous damage-associated molecule that plays a role in mediating kidney dysfunction in sepsis and other inflammatory disorders. HMGB1 is a nuclear protein that is released by cells into the extracellular space in response to infection or injury, where it interacts with Toll-like receptor 4 (TLR4) and other receptors to activate immune and inflammatory responses. We have shown that bacterial LPS inhibits HCO_3^- absorption in the MTAL through activation of a basolateral TLR4-ERK pathway. Here we examined whether HMGB1 could inhibit MTAL HCO_3^- absorption through activation of the same TLR4 pathway.

Results: Adding HMGB1 (1 μg/ml) to the bath decreased HCO_3^- absorption by 25% in isolated, perfused mouse and rat MTALs. In direct contrast to inhibition by bath LPS, the inhibition of HCO_3^- absorption by HMGB1 was preserved in MTALs from TLR4$^{-/-}$ mice and was not affected by ERK inhibitors. Also, the inhibition by HMGB1 was additive to inhibition by LPS. Thus, HMGB1 receptors other than TLR4 were investigated. Immunoblot analysis showed that the cell surface HMGB1 receptor RAGE is expressed in the inner stripe of the outer medulla of mouse and rat kidney. The selective RAGE antagonist FPS-ZM1 eliminated inhibition of HCO_3^- absorption by HMGB1 but had no effect on inhibition by bath LPS. Incubation of MTALs with neutralizing anti-RAGE antibody also eliminated inhibition by HMGB1.

Conclusions: We conclude that HMGB1 inhibits HCO_3^- absorption in the MTAL through a RAGE-dependent pathway that is additive to inhibition by LPS through TLR4. These results provide the first evidence that HMGB1 acts directly to impair the function of renal tubules and that RAGE signaling modulates renal tubule transport. Our studies reveal novel pathophysiological mechanisms for sepsis-induced renal tubule dysfunction, whereby exogenous pathogen-associated molecules and endogenous damage-associated molecules function directly and independently through different receptor signaling pathways to inhibit MTAL HCO_3^- absorption. These effects could impair the ability of the kidneys to correct acidosis that contributes to sepsis pathogenesis.

Funding: NIDDK Support

FR-OR103

Furosemide Stimulates Pronounced H^+ Secretion in the Thick Ascending Limb Pauline I.A. de Bruijn,[1] Nina Himmerkus,[2] Helle A. Praetorius,[1] Markus Bleich,[2] Jens G. Leipziger.[1] *[1]Dept of Biomedicine, Aarhus Univ, Aarhus, Denmark; [2]Inst of Physiology, Christian-Albrechst Univ, Kiel, Germany.*

Background: Furosemide is a loop diuretic that inhibits Na^+ and Cl^- reabsorption in the thick ascending limb (TAL). Besides massive diuresis, furosemide also induces a pronounced urinary acidification. This is commonly explained by the increased Na^+ delivery to the distal tubule, which is believed to stimulate H^+ secretion by the V-type H^+ ATPase in the α-intercalated cells. The direct role of the TAL on urinary acidification has never been investigated directly. We have previously reported that furosemide causes a NHE3-dependent H^+ secretion in isolated perfused medullary TAL and here we wanted to investigate if this H^+ secretion contributes to furosemide-induced urinary acidification in whole animals.

Methods: Mice were anesthetized and the bladders were catheterized. Urinary net acid excretion (NAE) and urinary pH was determined during furosemide administration with or without the NHE3 specific inhibitor #4167.

Results: In control animals, urine pH and NAE remained unaltered for the duration of the experiment. Furosemide administration caused a rapid onset of urinary acidification and reached the maximum H^+ secretion after 20 minutes (3.16 ± 0.83 μM), which was reflected by a significant increase in NAE (ΔNAE 5.05 ± 0.98 μmol/h). When #4167 was given, a reduction in H^+ secretion was observed (i.e. an alkalisation) but this did not cause a change in NAE. When both drugs were given together, the furosemide-induced H^+ secretion and NAE were much attenuated compared to furosemide alone (H^+ peak 1.17 ± 0.64 μM; ΔNAE 2.27 ± 0.84 μmol/h).

Conclusions: We show that inhibition of the apical NHE3 causes a significant drop in furosemide induced H^+ and NAE excretion. These results indicate that furosemide stimulates H^+ secretion in the TAL via the apical NHE3 and revise the current understanding of loop diuretic-induced urinary acidification.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR104

Axial Flow Stimulates Acid Secretion in the Distal Convoluted Tubule
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Background: It is well documented that axial flow regulates Na^+ and HCO_3^- transport in the proximal tubule, but whether flow regulates distal tubule acid/base transport has never been directly measured. Mathematical models have examined flow-dependent transport of Na^+ and K^+ transport in the distal nephron; however, there appears to be no information about the impact of flow on the components of net acid excretion, except indirect evidence that furosemide increased urinary acid secretion.

Methods: We have examined flow-mediated changes in both Na^+ and HCO_3^- absorption along the distal tubule by *in vivo* microperfusion in rat kidney. The net fluid and HCO_3^- absorption was examined at tubular perfusion rates of 5, 10, 15 and 20 nl/min with constant luminal HCO_3^- concentration (25 mM) in the distal tubule.

Results: The fluid absorption (J_v) increased from 0.56±0.1 to 1.1±0.4 (n=8; P>0.05); 1.3± 0. 3 nl/min/mm (n=6; P<0.05) and 1.6±0.3 (n=6; p<0.05); and the HCO_3^- absorption (J_{HCO3}) increased from 26.3±12.2 to 43.4±12.7 (P>0.05); 65.3± 22.6 (p<0.05) and 160.8±16.6 pmole/min/mm (P<0.001) when flow rate increased from 5 to 10, 15 and 20 nl/min respectively. The fractional increase in J_v was 109% and 128%; fractional increase in J_{HCO3} was 65% and 148% when flow rate increased from 5 to 10 and to 15 nl/min, respectively. There is no significant difference between the increments of Jv and J_{HCO3}. However, when the flow rate increased to 20 nl/min, Jv increased 188% but J_{HCO3} increased 510%.

Conclusions: These results indicate that flow stimulates both Na^+ and HCO_3^- absorption in the distal tubule, but that HCO_3^- absorption may be driven quite a bit higher under conditions of very high flow, such as might be seen with loop diuretics. Compared to the proportional flow-induced changes in both Na^+ and HCO_3^- absorption in proximal tubule, the disassociation of flow-stimulated Na^+ and HCO_3^- absorption suggests that different mechanisms may mediate flow-dependent transport in the distal tubule.

Funding: NIDDK Support

FR-OR105

Targeted NBCe1A Deletion Causes Proximal RTA: *Whole nbce1* (*sh_nbce1*) KO versus *nbce1A* KO Mice
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Background: NBCe1 (SLC4A4) transports Na^+ and $nHCO_3^-$ out of the basolateral membrane of the renal proximal tubule. Human, recessive mutations in *SLC4A4* result in a severe proximal renal tubular acidosis (pRTA) with glaucoma and cataracts, but do not cause death. However, the NBCe1 whole-gene knockout mice (*sh_nbce1* KO; A, B and C isoforms, Gary Shull, U Cinc) die within 21d due to systemic defects gut plugging. Interestingly, one patient (Q29X) is only missing the NBCe1A-isoform but still has pRTA and glaucoma.

Methods: To distinguish the biological function of NBCe1A (only isoform in kidney), we generated mice selectively deficient in *nbce1*A using the TALEN approach. We compared non-anaesthetized, blood chemistries (cheek bleeds) of mice using a Nova pHOx Ultra analyzer.

Results: Both *sh_nbce1* and *nbce1A* KO mice are acidotic (blood pH = 7.08 and 7.10; $[HCO_3^-]$=4.9 and 7.2 mM; P_{CO2} = 16.6 and 22.7 mmHg, respectively). WT controls for both genotypes are not acidotic (blood pH = 7.44 and 7.34; $[HCO_3^-]$=17.8 and 12.9 mM; P_{CO2} = 24.7 and 23.3 mmHg, respectively). While *sh_nbce1 KO* mice die by 21d [Gawenis et al, 2007. PMID 17192275], the *nbce1*A KO mice are still alive at 9m, are not obviously runted and are fertile. Both KO mice have elevated Cl^- (131 mM for *sh_nbce1 KO and* 129 mM for *nbce1*A KO v. 114-120 mM for WT). The *nbce1*A KO mice show normal P_{CO2}, P_{O2}, hematocrit, K^+, glucose, and BUN. However, the *sh_nbce1 KO* mice show low P_{CO2}, high P_{O2}, low hematocrit, low K^+, low glucose, and high BUN.

Conclusions: Deletion of the NBCe1 protein in the renal proximal tubule results in a pRTA. If the non-renal isoforms (B,C) are also deleted (*sh_nbce1 KO*), then systemic homeostasis is further compromised. These differences in these two models should highlight which non-renal tissues play roles in systemic acid-base homeostasis. Targeted deletion of *nbce1A (nbce1A KO)*, results in a phenotype which replicates the human SLC4A4 pRTA and glaucoma, allowing aging-related investigation of the NBCe1A role in the kidney and eye. Funding: Mayo Clinic Robert and Arlene Kogod Center on Aging (APC, MFR).

Funding: Private Foundation Support

FR-OR106

Dietary Phosphate Is a Novel Regulator of Glucose and Lipid Metabolism
Maerjianghan Abuduli, Yutaka Taketani, Hirokazu Ohminami, Haruka Ueda, Eiji Takeda. *Dept of Clinical Nutrition, Univ of Tokushima, Tokushima, Japan.*

Background: Recent epidemiological and animal studies have suggested that excess intake of phosphate would be a risk factor for progression of chronic kidney disease and cardiovascular complication. However, the effect of high phosphate (HP) diet intake on the development of metabolic disorder such as obesity and type 2diabetes is not elucidated. In this study, we investigated the effect of dietary phosphate loading on glucose and lipid metabolism in healthy rats.

Methods: Male, Sprague-Dawley rats were divided into three groups and fed experimental diets including 0.2% (LP), 0.6% (CP) or 1.2% (HP) phosphate, respectively. After 4 weeks, serum, urine, liver and brown adipose tissue (BAT) were obtained and subjected to evaluation.

Results: HP group showed the lower plasma levels of insulin and free fatty acid and significantly reduce the visceral fat accumulation than other groups. In addition, the respiratory quotient was decreased in HP group without changes in locomotive activity, which indicated preferential utilization of fat for energy production. HP group rats were also suppressed hepatic lipogenic gene expression including sterol regulatory element binding protein-1c, fatty acid synthase and acetyl-CoA carboxylase, whereas there was no difference in fat oxidation among the groups. On the other hand, uncoupling protein1(UCP1) and peroxisome proliferator-activated receptor gamma coactivator1 alpha were highly expressed in HP group than other groups due to improvement of ectopic fat deposition in BAT.BAT is known to play a critic role in energy expenditure by regulating thermogenesis, which is mediated by its characteristic UCP1 expression. It is suggested that HP diet has a potential role in the increased energy expenditure by upregulation of BAT activation.

Conclusions: Our data indicates that HP diet can negatively regulate lipid synthesis in liver and increased lipid oxidation through effective thermogenesis in BAT, thereby preventing visceral fat accumulation. Dietary phosphate is a novel regulator of glucose and lipid metabolism, although further studies are needed to elucidate the biological importance and underlying mechanisms.

FR-OR107

Metabolic Effects of Different Sources of Dietary Phosphorus
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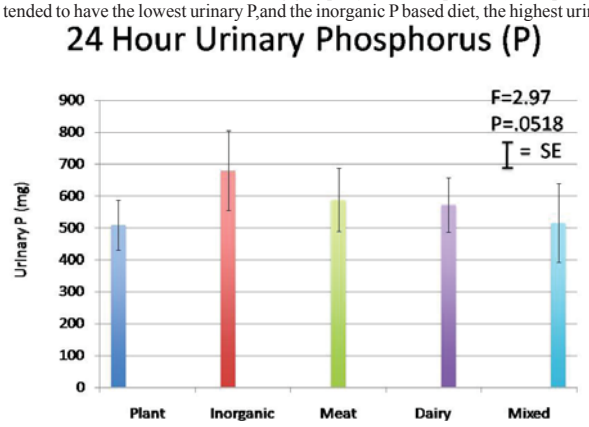
Background: Serum phosphorus (P) is associated with increase vascular calcification and endothelial cell dysfunction, and in chronic kidney disease (CKD) patients, high serum and dietary P are associated with increased mortality. Animal data and some human data suggest that different food sources of dietary P may affect intestinal P absorption. We examined in CKD patients the effect of 5 different dietary sources of P on intestinal P absorption, as determined 24 h urinary P excretion.

Methods: 24 hour urine P was measured in five stage 4 or 5 CKD patients who lived in a metabolic balance ward for a 31 hour study on five occasions separated by two week intervals each. Subject ate the same amount of P on each admission, but the source of dietary P varied in random order from P primarily from plants, inorganic P, meat, dairy foods or mixed with 25 % of P derived from each of these four food sources. For each patient, diets were isocaloric, isonitrogenous and provided about 800 mg calcium per day and 1000 mg P/day.

Results: 24 hour urine P varied among groups by ANOVA (p=.0518). The plant based diet tended to have the lowest urinary P,and the inorganic P based diet, the highest urinary P.



Conclusions: Inorganic P is the most readily intestinal absorbed source of dietary P. The preliminary data from this study suggest that to avoid or reduce high P burden, for a given P intake, dietary inorganic P should be minimized and plant sources of dietary P encouraged. This is the first study to examine the effects of five different food sources of P on intestinal P absorption in CKD patients.

Funding: Other NIH Support - CTSI Scholars Grants, Pharmaceutical Company Support - Genzyme Corporation, Private Foundation Support

FR-OR108

Intracellular Pi Evolution in Pig Muscle during Hemodialysis: An In Vivo Phosphate MR Spectroscopy Study
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Background: Among the 600 to 700 mg of inorganic phosphate (Pi) removed during a 4-hour hemodialysis session, only 10% is extracted from the extracellular space (1). The origin of the other 90% of removed Pi is unknown and two hypothesis have been proposed being either the intracellular compartment or bone. Spalding et al (2) hypothesized that Pi exchanges between intra and extracellular compartments were diffusive, suggesting that the intracellular compartment could be the main source of Pi removed during hemodialysis. We tested this hypothesis during a hemodialysis session, by using Phosphorus (^{31}P)

Magnetic Resonance Spectroscopy (MRS), which is the only tool allowing in vivo and dynamic measurement of the intracellular Pi concentration as well as the other's phosphate metabolites such as Phosphocreatine (PCr) and ATP.

Methods: 3-hour hemodialysis sessions were performed in 6 pigs, after surgical bi-nephrectomy, with a Prismaflex® generator and a M100® dialyser (Hospal). The extra-corporal circulation blood flow was maintained between 100 and 150 mL/min. ^{31}P MRS exams were performed with a 1.5T Siemens Sonata system using a surface coil placed over the gluteal muscle region. ^{31}P MR spectra (TR=10s, TE=0.35ms) were acquired every 2'40" before, during and after dialysis. Blood samples were obtained during the whole examination to measure plasma Pi concentrations.

Results: During the dialysis, the mean PCr/Pi ratio decreased significantly (+6.9%, $p<0.00001$), while the PCr/βATP ratio increased (+22.2%, $p<0.00001$). Plasma Pi concentration felt rapidly within 60 min from 2.30 ± 0.18 mmol/L to 1.65 ± 0.10 mmol/L (-28,08%, p=0.003) then plateaued.

Conclusions: This study demonstrated that intracellular Pi concentration did not decrease in parallel with the extracellular Pi decrease as proposed (2). In contrast, the intracellular Pi increase may reflect a cellular stress induced by hemodialysis and/or a strong intracellular Pi production, using the mitochondrial Pi reserve, to respond to the extracellular decrease.

FR-OR109

Inorganic Phosphate Inhibits Osteoclastogenesis by Modulating miR-223. Implications for Chronic Kidney Disease-Mineral and Bone Disorder Laurent Metzinger, Eleonore Ourouda Mbaya, Loïc Louvet, Valérie Metzinger-Le Meuth, Ziad Massy. *INSERM U1088, INSERM u1088, Amiens, Na, France.*

Background: Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a common complication of CKD, and uremic toxins have been shown to be instrumental in this process. We have previously shown that miR-223 is increased in smooth muscle cells subjected to the uremic toxin inorganic phosphate (Pi), and in the aorta of CKD mice. Here, we aimed to understand the role of miR-223 in osteoclastogenesis, and thus in the course of CKD-MBD.

Methods: RT-qPCR was used to measure the expression of miR-223. Up- and down-regulation of this miRNA was performed using specific pre-miR and antagomiR. We assessed differentiation of monocyte/macrophage precursors using RAW 264.7 cells and PBMC. TRAP activity and bone resorption were measured to assess osteoclast activity.

Results: As previously described, Pi induced a strong decrease of osteoclastogenesis in RAW cells. Concomitantly, miR-223 levels were decreased. An antogomiR directed against miR-223 inhibited osteoclastogenesis, while in contrast overexpression of miR-223 triggered differentiation. Similar results were found when measuring TRAP activity. We show that miR-223 affects the expression of targets Mef2 and RhoB, but also of the Akt signalling pathway, which induces osteoclastogenesis. These results were confirmed by measuring bone resorption activity in human PBMC differentiated into osteoclasts.

Conclusions: We show here an implication of miR-223 in osteoclast differentiation, with possible implications of the role of this miRNA in the course of CKD-MBD. Modulating miR-223 in CKD patients could thus be useful in the management of this complication of CKD.

FR-OR110

Down-Regulation of Renal Type IIa Sodium-Dependent Phosphate Co-Transporter During Lipopolysaccharide-Induced Acute Inflammation Hironori Yamamoto,[1] Shoko Ikeda,[2] Otoki Nakahashi,[2] Yutaka Taketani,[2] Masayuki Iwano,[4] Ken-Ichi Miyamoto,[3] Eiji Takeda.[2] *[1]Health and Nutrition, Jin-ai Univ, Echizen, Fukui, Japan; [2]Clinical Nutrition, Univ of Tokushima, Tokushima, Japan; [3]Molecular Nutrition, Univ of Tokushima, Tokushima, Japan; [4]Nephrology, Univ of Fukui, Fukui, Japan.*

Background: The type IIa sodium-dependent phosphate cotransporter (Npt2a) plays a critical role in reabsorption of inorganic phosphate (Pi) by renal proximal tubular cells. Pi abnormalities during early stages of sepsis have been reported, but the mechanisms regulating Pi homeostasis during acute inflammation are poorly understood. We examined the regulation of Pi metabolism and renal Npt2a expression during lipopolysaccharide (LPS)-induced inflammation in mice.

Methods: C57/BL6J mice were treated with LPS in a dose-dependent manner and were sacrified after 3hrs. Wistar rats with parathyroidectomized (PTX) or sham surgery were purchased from Japan SLC Inc. Plasma Pi, intact parathyroid hormone (iPTH) and intact fibroblast growth factor 23 (iFGF23) levels were measured and renal Npt2a gene expression analysis were performed by western blotting, immunostaining, and quantitative real-time PCR.

Results: Dose-response and time-course studies with LPS showed significant increases of plasma Pi and iPTH levels and renal Pi excretion, while renal calcium excretion was significantly decreased. There was no difference in plasma 1,25-dihydroxyvitamin D levels, but the induction of plasma iFGF 23 levels peaked 3 h after LPS treatment. LPS administration significantly decreased Npt2a protein expression in the brush border membrane (BBM) 3 h after injection, but there was no change in renal Npt2a mRNA levels. Moreover, tumor necrosis factor-α injection also increased plasma iPTH and decreased renal BBM Npt2a expression. Importantly, we revealed that parathyroidectomized rats had impaired renal Pi excretion and BBM Npt2a expression in response to LPS.

Conclusions: These results suggest that the downregulation of Npt2a expression in renal BBM through induction of plasma iPTH levels alter Pi homeostasis during LPS-induced acute inflammation.

Funding: Government Support - Non-U.S.

FR-OR111

Tenapanor Inhibits Phosphorous Absorption, and Protects against Vascular Calcification in Nephrectomized Rats Dominique Charmot, Andrew G. Spencer, Marc Navre, Jason G. Lewis, Christopher Carreras, Jeffrey Jacobs, Noah Bell, Limin He, Desiree Deshpande, Kenji Kozuka, Samantha Koo-Mccoy, Ingrid Langsetmo, Edward E. Dy, Michael R. Leadbetter, Jill N. Kohler, Ziyang Zhong, Eric Daniel Labonte. *Ardelyx Inc., Fremont, CA.*

In chronic kidney disease (CKD), phosphate retention arising from diminished glomerular filtration rate (GFR) is a key early step in a pathological cascade leading to hyperparathyroidism, metabolic bone disease, vascular calcification, and cardiovascular mortality. Tenapanor, a minimally-systemic inhibitor of the intestinal sodium/proton exchanger subtype 3 (NHE3), reduces sodium absorption in humans and is currently being evaluated in patients with CKD and end-stage renal disease on hemodialysis (ESRD-HD), as well as patients with constipation predominant irritable bowel syndrome (IBS-C). We report here that tenapanor also reduces phosphorous (P) absorption from the rodent gut, despite the lack of any direct inhibition of the phosphate transporters PiT1 (SLC20A1) and NaPi2b (SLC34a2). Our studies demonstrate that intestinal NHE3 inhibitors decrease absorption of dietary P in rats as evidenced by 1) increased fecal P excretion (treated 1115 ± 61 μmol/d versus untreated 801 ± 73 μmol/d, mean ± SEM); 2) decreased urinary P excretion (treated 1321 ± 49 μmol/d versus untreated 1523 ± 33 μmol/d), and 3) reduced uptake of orally administered [^{33}P]orthophosphate (AUC reduced by 40% relative to controls). In a rodent model of vascular calcification, tenapanor reduced P absorption, and led to significant decreases in ectopic calcification (aortic P reduced 78% and Ca reduced 76%), serum creatinine levels (treated 0.85 ± 0.05 mg/dl versus untreated 1.27 ± 0.07 mg/dl), circulating phosphaturic hormone fibroblast growth factor levels (FGF)-23 (treated 84 ± 25 ng/ml versus untreated 369 ± 65 ng/ml), and heart mass (reduced by 16%). These results suggest that tenapanor may provide a new approach to P management in renal disease and associated mineral disorders.

FR-OR112

Tenapanor, a Minimally Absorbed NHE3 Inhibitor, Reduces Dietary Phosphorus Absorption in Healthy Volunteers David P. Rosenbaum,[1] Susanne Johansson,[2] Bjorn Carlsson,[2] Andrew G. Spencer,[1] Bergur V. Stefansson,[2] Mikael Knutsson,[2] Jeffrey Jacobs,[1] Dominique Charmot.[1] *[1]Ardelyx, Inc., Fremont, CA; [2]AstraZeneca R&D, Molndal, Sweden.*

Background: Tenapanor (AZD1722) is a first-in-class small-molecule inhibitor of the Na^+/H^+ exchanger isoform 3 (NHE3). Tenapanor was previously shown to reduce intestinal sodium (Na) absorption in healthy adult volunteers. Here, we report that administration of tenapanor also reduced the absorption of dietary phosphorus (P) in these individuals.

Methods: Double-blind, placebo-controlled, clinical pharmacology studies on tenapanor hydrochloride, administered orally for 7 days, were performed to evaluate multiple ascending doses (MAD) of 3, 10, 30 and 100 mg qd (RDX5791-101) and different dosing regimens (DRs) of 30 mg qd, 15, 30 and 60 mg bid, and 30 mg tid (RDX5791-102). P content of 24-hour stool samples was measured daily. Stool and urinary P levels were also evaluated daily in other clinical pharmacology studies (formulation, D5611C00002; and food-effect, D5611C00003).

Results: Tenapanor was well tolerated in all studies and no serious adverse events were reported. The placebo groups in the MAD and DR studies exhibited the same mean daily stool P levels (table). Increases in stool P with tenapanor treatment were 4.3–7.1 mmol/day in the MAD study and 6.0–12.5 mmol/day in the DR study compared with placebo. In the other clinical studies of tenapanor, decreases in urinary P excretion were observed, consistent with observed increases in stool P excretion.

Stool P excretion (mmol/day)

Multiple ascending dose (MAD) study[a]

	Placebo	3 mg qd	10 mg qd	100 mg qd
Mean	16.8	21.1	23.9	23.0
SD	3.4	4.3	4.1	4.7
n	8	8	8	8

Dosing regimen (DR) study

	Placebo	30 mg qd	15 mg bid	30 mg bid	60 mg bid	30 mg tid
Mean	16.8	28.0	29.3	25.4	22.8	27.4
SD	4.6	5.0	11.1	8.8	10.9	8.0
n	21	11	12	12	12	12

[a]30 mg qd cohort not analyzed

Conclusions: Tenapanor reduced the absorption of P from the gut, as shown by increases in stool P excretion and concomitant decreases in urinary P excretion. Tenapanor may provide a new mechanism for the treatment of hyperphosphatemia, with the potential added benefits of reducing pill burden and improving Na overload in patients with end-stage renal disease.

Funding: Pharmaceutical Company Support - Ardelyx; AstraZeneca

FR-OR113

Uromodulin Upregulates the Epithelial Magnesium Channel TRPM6 by Impairing Dynamin II-Dependent Endocytosis

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Background: Uromodulin (UMOD) is the most abundant urinary protein in humans but its physiological function remains unclear. *Umod* knockout mice are characterized by urinary Mg^{2+} loss. Recently, upregulation of UMOD expression was shown in hypomagnesemic mice, suggesting a role of UMOD in renal Mg^{2+} reabsorption. We examined if UMOD regulates the epithelial Mg^{2+} channel TRPM6, which is expressed apically in the early DCT. UMOD expression has been shown in both TAL and DCT. Therefore, UMOD could act either from the urinary space or from intracellularly. We tested the hypothesis that UMOD upregulates TRPM6 from the extracellular side.

Methods: We expressed TRPM6 in HEK293 cells which were treated with purified UMOD. TRPM6 whole-cell current density was analyzed by patch-clamp recording.

Results: Coexpression of UMOD and TRPM6 resulted in higher TRPM6 current density compared to UMOD mutant C150S or control (216 ± 21 versus 56 ± 19 versus 75 ± 9 *p*A/*p*F for UMOD versus mutant versus control; $p<0.001$). UMOD also increased TRPM6 whole-cell current when applied extracellularly. The extracellular effect of UMOD towards TRPM6 was abrogated when UMOD antibody was added to UMOD-containing supernatant (260 ± 48 versus 89 ± 18 *p*A/*p*F for UMOD versus antibody; $p<0.05$). We determined a half-maximal effective concentration (EC_{50}) for purified UMOD treatment in TRPM6-expressing cells of 600 ng/ml. This is approximately 20x lower than UMOD concentration in human urine, underlining the physiological significance. Cells cotransfected with dominant-negative dynamin II (K44A), which impairs constitutive endocytosis, were resistant to the TRPM6-stimulating effect of UMOD (313 ± 34 versus 349 ± 30 *p*A/*p*F for UMOD versus control, *p*= n.s.). All three major domains of UMOD including EGF-like, D8C and zona pellucida domains but not the GPI anchor were required for TRPM6 upregulation.

Conclusions: Extracellular UMOD upregulates the activity of the TRPM6 Mg^{2+} channel by impairing dynamin II-dependent endocytosis. This suggests that UMOD may participate in Mg^{2+} homeostasis by promoting Mg^{2+} reabsorption in the DCT.

Funding: NIDDK Support

FR-OR114

Mutations in CNNM2 Cause Hypomagnesemia and Seizures in Patients due to Impaired Renal Magnesium Handling

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Background: Recently, we have identified mutations in the gene encoding cyclin M2 (CNNM2) in two unrelated families with dominant isolated hypomagnesemia (MIM 607803). Patients suffered from symptoms associated with low serum Mg2+ levels (0.3–0.5 mM) such as tremors, headaches and muscle weakness and also mental disability. The role of CNNM2 in the kidney for the maintenance of serum Mg2+ levels can be traced to the distal convoluted tubule (DCT). In the present study, we aim to elucidate the function of CNNM2 in the kidney.

Methods: By combining a loss-of-function approach in a zebrafish model, functional transport assays in renal cells and immunohistochemical characterisation in kidney sections we provide functional evidence for a key role of CNNM2 in renal Mg2+ handling and also brain development.

Results: Mutations in CNNM2 in five families suffering from hypomagnesemia, seizures, and mental retardation have been identified. To elucidate the physiological role of CNNM2 and explain the pathomechanisms of the disease, we studied CNNM2 function. Using stable Mg2+ isotopes, we demonstrated that CNNM2 increases cellular Mg2+ uptake in human embryonic kidney (HEK293) cells and that this process occurs through regulation of the Mg2+-permeable cation channel TRPM7. In contrast, cells expressing mutated CNNM2 proteins did not show increased Mg2+ uptake. Knockdown of cnnm2 isoforms in zebrafish resulted in disturbed brain development including neurodevelopment impairments, weak touch-evoked escape behaviour and reduced body Mg content, indicative of impaired renal Mg2+ absorption. These phenotypes were rescued by injection of mammalian wild-type Cnnm2 cRNA, whereas mammalian mutant Cnnm2 cRNA did not improve the zebrafish knockdown phenotypes.

Conclusions: CNNM2 is fundamental for renal Mg2+ homeostasis, brain development and neurological functioning. By establishing the loss-of-function zebrafish model for CNNM2 genetic disease, we provide a unique system for testing therapeutic drugs targeting CNNM2 and for monitoring their effects on the kidney phenotype.

Funding: Government Support - Non-U.S.

FR-OR115

Effect of Intravenous Bisphosphonates (IVBP) on Bone Mineral Density (BMD) and Bone Turnover Markers (BTM) Is Reversible in Children

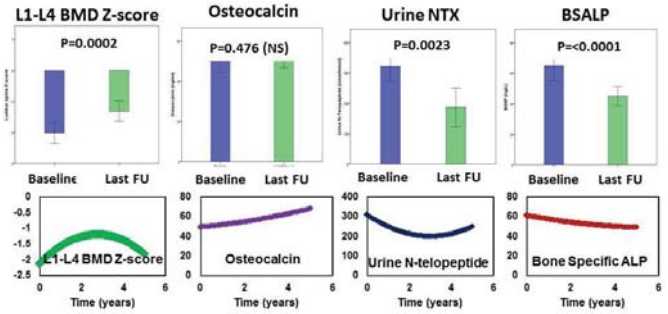
Tarak Srivastava, Naziya Tahseen, Hongying Dai, Uri S. Alon. *Children's Mercy Hospital, Kansas City, MO.*

Background: IVBPs drugs increase BMD and decrease BTM by inhibiting osteoclastic activity. IVBPs are increasingly being used in treatment of osteopenia in children. We previously reported 17 children in whom the effect of IVBP on BMD was sustained for up to 2 years [Pediatr Nephrol 2007;22:282]. The aim of the study was to evaluate if the effects of IVBPs on BMD and BTM is reversible or not in children over a period of 5 years from time of treatment.

Methods: Children treated with 2 doses of IVBP in our "Bone and Mineral Disorder Clinic" between 1997-2010 for osteopenia and had at least >3 DXA and BTM measurements were included. Data collected were age, gender, pubertal status, ambulation, parathyroid hormone (PTH), 25-Vitamin D, BTM [serum osteocalcin (OS), bone specific alkaline phosphatase (BSALP), urine N-telopeptide (NTX)], and L1-L4 BMD Z-score on DXA. Mixed Procedure Model and Quadratic Model were used to analyze the data over 4 time periods: (a) 0-5 yrs (b) 0-1 yrs (c) 1-3 yrs and (d) 3-5 yrs.

Results: 104 children met the criteria. Mean age was 11.2 ± 4.5 yrs, 59% girls, 64% pubertal and 72% ambulatory. The change in BMD and BTM over time and estimated changes/year over the 4 time periods are shown in Table (*p<0.01) and Figure. There was no significant change in PTH and Vitamin D. The effects of IVBP on BTM start to reverse in order of OS, NTX and BSALP, and on Lumbar BMD by 3 yrs.

	0-5 yrs	0-1 yrs	1-3 yrs	3-5 yrs
L1-L4 BMD Z-Score	0.19±0.04*	0.81±0.16*	0.28±0.10*	0.05±0.24
OS	3.4±0.9*	-4.9±6.5	2.7±2.8	1.2±4.2
BSALP	-2.8±0.8*	-12.5±4.5*	-8.7±2.9*	-0.4±2.6
NTX	-25.6±6.3*	-130.0±35.6*	-50.2±20.8*	5.7±30.2



Conclusions: The effects of IVBP drugs on BMD and BTM are not permanent, and start to reverse by 3 years in children; with OS heralding the process.

Funding: Clinical Revenue Support

FR-OR116

miRNome Expression Profiling Identifies Circulating MicroRNAs That Are Differentially Expressed among Type 1 Diabetic Patients with Proteinuria and at Increased Risk of Rapid Renal Function Decline

Marcus G. Pezzolesi,[1,2] Eiichiro Satake,[1,2] Kevin P. Mcdonnell,[1] Adam Smiles,[1] Andrzej S. Krolewski.[1,2] *[1]Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA; [2]Dept of Medicine, Harvard Medical School, Boston, MA.*

Background: MicroRNAs (miRNAs) are key regulators of gene expression that are expressed and stable in a variety of biofluids and thus have the potential to serve as novel biomarkers for the diagnosis of a variety of human diseases. The aim of this study is to begin to understand the miRNA signature that predicts high risk of renal function decline and the progression to end-stage renal disease (ESRD) in patients with Type 1 diabetes (T1D).

Methods: We measured the expression of 1,811 miRNAs in pooled baseline plasma samples from 38 Rapid Progressors who had normal renal function at baseline and subsequently lost renal function at a rate of ≥3.3ml/min/year before reaching ESRD and 40 control subjects with normal renal function and persistent normoalbuminuria despite more than 20 years duration of T1D.

Results: A total of 425 miRNAs were detectable in plasma samples from both study groups. Among these miRNA, we identified 95 (22.4%) with <0.4 or >2.5 fold change between Rapid Progressors and controls and selected the top 20 differentially expressed miRNAs for measurement in individual samples from these two study groups. Using multivariable logistic regression analysis, 5 miRNAs were shown to be significantly increased in Rapid Progressors compared to normoalbuminuric controls. Three additional miRNAs were shown to be significantly increased in subjects with persistent normoalbuminuria relative to Rapid Progressors. Multivariable linear regression analysis revealed that 10 miRNAs were also significantly associated with longitudinal changes in estimated glomerular filtration rate.

Conclusions: These data suggest that miRNAs may be able to distinguish individuals who are at the greatest risk of losing renal function and developing ESRD from those who are protected against these complications. The differentially expressed miRNAs identified in this study represent novel therapeutic targets that may prove useful in inhibiting renal function decline in T1D.

Funding: NIDDK Support

FR-OR117

Identification of New Predictors of Declining Renal Function in Patients with Type 2 Diabetes by Metabolomics Ele Ferrannini,[1] Anna Solini,[1] Giuseppe Penno,[1] Giuseppe Pugliese,[2] Jeff E. Cobb,[3] Regis Perichon.[3] *[1]Univ of Pisa, Italy; [2]"La Sapienza" Univ, Rome, Italy; [3]Metabolon, Durham, NC.*

Background: Renal disease in type 2 diabetes (T2D) is associated with excess morbidity/mortality. While glomerular filtration rate (eGFR) and albuminuria are routine for assessing renal impairment, new biomarkers could improve risk stratification and prediction.

Methods: We performed screening metabolomics in fasting plasma and urine samples from 285 T2D pts (109 women, age 63[10] yrs, median[IQR], fasting glucose 144[47] mg/dL, HbA_{1c} 7.1[1.3]%, eGFR 93[26] ml·min^{-1}·1.73m^2, albumin/creatinine ratio (ACR) 14.3[50.2] mg/g). Non-targeted metabolomic profiling was carried out by gas chromatography or ultra-HPLC coupled with tandem mass-spec. 256 pts were seen again 3 yrs later. Biomarker identification was performed by random forest using an eGFR cutoff of ±60 or normoalbuminuria versus micro/macroalbuminuria (μMA) as endpoints for serum and urine metabolites. Data were analyzed by multivariate logistic regression and ROC Area.

Results: At follow up eGFR had declined by 4[10] ml·min^{-1}·1.73m^2 in the whole cohort ($p<0.0001$), and by 16[9]in patients in the top quartile of eGFR change; the latter was used as endpoint. Significant clinical covariates (female gender, age, fasting glucose, and baseline eGFR) predicted outcome with ROC=0.671. The 5 serum metabolites best correlated with either eGFR<60 or μMA at baseline were tested for their ability to improve clinical prediction. Sum of 3 biomarkers (C-glycosyltryptophan, pseudouridine, and N-acetylthreonine, Bio-Index) raised ROC to 0.739 ($p<0.0001$). Outcome was predicted by the top Bio-Index quartile (OR=5.48 [95%CI=2.23-14.47] versus OR's of 1.73[1.073.21], 1.59[1.09-2.40], 1.49[1.10-2.02] and 2.66[1.61-4.70] for gender, age, glucose, and baseline eGFR, respectively). In the same model, Bio-Index also predicted a change in ACR in the top quartile (+41[135] mg/g) with an OR of 2.82[1.20-7.03] and a ROC of 0.750. Top urine metabolites did not add significant predictivity.

Conclusions: A limited number of circulating intermediates of amino acid and nucleotide pathways carry clinically significant predictivity for deterioration of renal function in T2D pts.

FR-OR118

Plasma Concentrations of Midregional Pro-Atrial Natriuretic Peptide Are Associated with Cardiorenal Function and Predicts All-Cause Mortality and End Stage Renal Disease in Type 1 Diabetes Simone Theilade,[1] Tine Hansen,[1] Jens Peter Goetze,[2] Peter Rossing.[1,3,4] *[1]Steno Diabetes Center, Denmark; [2]Rigshospitalet, Denmark; [3]Aarhus Univ, Denmark; [4]Univ of Copenhagen, Denmark.*

Background: We examine associations between plasma pro-atrial natriuretic peptide (proANP) levels and diabetic complications at baseline and risk of mortality and end stage renal disease (ESRD) during follow-up.

Methods: The observational study included 665 type 1 diabetes patients from a tertiary diabetes ambulatory. Midregional (MR) proANP was measured on a Kryptor platform (Thermo-Fisher, Germany). Complications were defined as micro- (n=168) or macroalbuminuria (n=190) (urinary albumin excretion rate (UAER) 30-299 or ≥300mg/24h), history of cardiovascular disease (CVD) (n=144), cardiac autonomic dysfunction (heart rate variability <11 beats/min) (n=252) or retinopathy (n=523). Adjustments were made for gender, age, systolic blood pressure (SBP), eGFR, UAER, HbA_{1c}, total cholesterol, C-reactive protein, urinary sodium excretion, body mass index, daily insulin dose, antihypertensive treatment and smoking in linear regression and ANCOVA models. Development of ESRD (dialysis, renal transplantation or GFR/eGFR<15 ml/min/1.73m^2) and mortality was traced through national registers.

Results: Patients were (mean±SD) 55±13 years, 293(44%) female, and (median [IQR]) plasma MR-proANP concentration was 73.6[49.2-116.9]pmol/L. In adjusted models, proANP correlated positive with age, SBP, UAER, total cholesterol and CRP; and negatively with eGFR, total daily insulin dose and HbA_{1c} ($p\leq0.05$). Adjusted, MR-proANP levels increased with albuminuria degree and were higher in patients with previous CVD ($p\leq0.043$), but similar in patients with or without autonomic dysfunction or retinopathy ($p\geq0.14$). During follow-up (3.5[3.1-4.0] years), higher MR-proANP predicted ESRD and mortality combined (n=35) adjusted for gender, age, SBP and eGFR (hazard ratio per 1 SD increase in logANP: 2.2(1.4-3.6), p=0.001).

Conclusions: Higher plasma MR-proANP levels were associated with impaired renal function, increased albuminuria, and previous CVD. Moreover, higher proANP levels were associated with increased risk of ESRD and mortality combined.

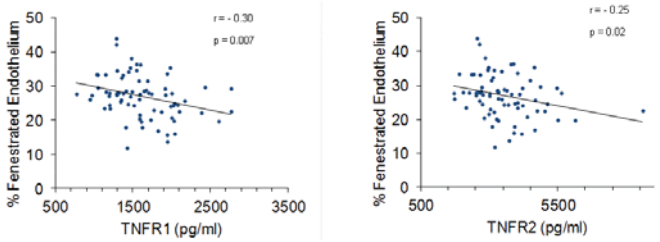
FR-OR119

Circulating Tumor Necrosis Factor Receptors 1 and 2 Correlate with Glomerular Structure in Type 2 Diabetes Gudeta D. Fufaa,[1] Robert G. Nelson,[1] E. Jennifer Weil,[1] William Knowler,[1] Monika A. Niewczas,[2] Andrzej S. Krolewski.[2] *[1]NIH; [2]Harvard.*

Background: Elevated Tumor necrosis factor receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) concentrations may initiate and promote renal function decline leading to kidney failure. We examined the relationships between TNFRs and glomerular structure in American Indians with type 2 diabetes.

Methods: Serum TNFRs were measured at a research examination that included a kidney biopsy and measurement of glomerular filtration rate (GFR) by iothalamate clearance. Associations between clinical characteristics, glomerular structural variables and TNFRs were explored by Spearman correlations. Associations with glomerular structural measurements were also examined by linear regression, adjusted for age, sex, diabetes duration, HbA1c, blood pressure, GFR, and urinary albumin/creatinine ratio (ACR).

Results: Participants (n=83, mean age 46±10 years) had median GFR=130 ml/min(IQR=107-174 ml/min), median ACR=26 mg/g (IQR=12-127 mg/g), median TNFR1=1500 pg/ml (IQR=1205-1960 pg/ml), and TNFR2=3284 pg/ml (IQR=2671-4151 pg/ml). TNFR1 and TNFR2 correlated with each other (r=0.84, p<0.001), with ACR (r=0.36 and 0.37, respectively; p<0.001), and inversely with GFR (r=-0.35, p=0.001; r=-0.28, p=0.010). TNFR1 and TNFR2 correlated inversely with the percentage of endothelial capillary fenestration(r=-0.42 and -0.43; p<0.001; adjusted correlations shown in **Figure**),



filtration surface area (r=-0.27, p=0.013; r=-0.29, p=0.007), and filtration slit frequency (r=-0.24, p=0.029; r=-0.29, p=0.008). TNFRs correlated with fractional mesangial area (r=0.36; 0.38, p<0.001), basement membrane width (r=0.23, p=0.038; r=0.26, p=0.016), and podocyte foot process width (r=0.29, p=0.007; r=0.31, p=0.004). TNFR1 correlated with the number of podocytes per glomerulus (r=-0.23, p=0.04).

Conclusions: Elevated TNFR1 or TNFR2 in early diabetic nephropathy associate with glomerular structural damage.

Funding: NIDDK Support

FR-OR120

Anti-inflammatory and Renoprotective Effects of CCL2 Inhibition with Emapticap Pegol (NOX-E36) in Type 2 Diabetic Patients with Albuminuria Hermann G. Haller,[1] Jan Menne,[1] Dirk Eulberg,[2] Matthias Baumann.[2] *[1]Clinic of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Noxxon Pharma AG, Noxxon Pharma AG, Berlin, Germany.*

Background: Inflammatory mechanisms with increased expression of chemokines such as CCL2/MCP-1 and monocyte infiltration seem to play a major role in the pathogenesis of diabetic nephropathy. Using Emapticap pegol (NOX-E36), a novel mirror-image RNA molecule (Spiegelmer®) that binds and inhibits the pro-inflammatory chemokine CCL2 (MCP-1) we tested the hypothesis that this anti-inflammatory approach exerts a renoprotective and antidiabetic effect in patients with diabetic nephropathy (DN).

Methods: A randomized, double blind, placebo-controlled multi-center phase IIa study in 5 European countries was initiated in 75 DN patients. Eligibility criteria included stable RAS blockade, ACR >100 mg/g and HbA1c from 6.0-10.5%. Emapticap was administered SC at 0.5 mg/kg twice weekly for 12 weeks, followed by a treatment-free phase of 12 weeks. Inflammatory biomarkers were evaluated. For the primary efficacy analysis, patients with major protocol violations, dual RAS blockade and concomitant hematuria and leukocyturia were excluded.

Results: Emapticap pegol was safe and well tolerated and resulted in clear beneficial effects on ACR (-32% at Day 85, P=0.014) and HbA1c (-5% at Day 85, P=0.096) which were maintained even after cessation of dosing (ACR: -39% at Day 141, P=0.010; HbA1c: -7% at Day 113, P=0.036). No change was observed for blood pressure and eGFR. Treatment with emapticap was accompanied by a rapid decrease in the number of peripheral monocytes by approx. 20% and a concomitant decrease in surface marker CCR2. Urinary sTNFR1 was elevated at baseline and decreased during treatment with emapticap.

Conclusions: Treatment with emapticap was safe and efficacious in reducing ACR and HbA1c. The sustained mode of action as well as the absence of hemodynamic changes differentiates emapticap from approved treatment strategies in diabetic nephropathy. The maintenance of beneficial effects after the treatment phase and the concomitant reduction of urinary sTNFR1 suggests that emapticap interferes with the underlying inflammatory pathophysiology in diabetic patients.

Funding: Pharmaceutical Company Support - Noxxon

FR-OR121

Selective Inhibition of Phosphodiesterase Type 5 Reduces Macroalbuminuria in Subjects with Type 2 Diabetes, and Overt Nephropathy Willem H. Scheele,[1] Susan Diamond,[2] Jeremy D. Gale,[3] Valerie Clerin,[1] Nihad Tamimi,[3] Vu Le,[1] Rosalind Jane Walley,[3] Fernando Grover Páez,[4] Meguid El Nahas.[5] *[1]Pfizer, Inc, Cambridge, MA; [2]San Antonio Kidney Disease Center, San Antonio, TX; [3]Pfizer, Ltd, Sandwich, United Kingdom; [4]ICLE, Guadalajara, Mexico; [5]Global Kidney Academy, Sheffield, United Kingdom.*

Background: Diabetic nephropathy (DN) is the leading cause of end stage renal disease in the Western world. Reduced availability of nitric oxide (NO) leading to decreased intracellular level of the NO pathway effector molecule cyclic guanosine monophosphate (cGMP) in the kidney has been implicated in the progression of DN. Pre-clinical studies suggest that elevation of the cGMP intracellular pool through inhibition of the cGMP-hydrolyzing enzyme phosphodiesterase type 5 (PDE5) exerts renoprotective effects in DN.

Methods: To test this hypothesis in humans, the effect on albuminuria of the novel, highly specific, and long-acting PDE5 inhibitor PF-00489791 was assessed in a multinational, multicenter, randomized, double-blind, placebo-controlled, parallel group trial of subjects with Type 2 diabetes and overt nephropathy already receiving ACE$_i$ or ARB background therapy. A total of 256 subjects with an eGFR between 25 and 60mL/min/1.73m^2 and macroalbuminuria defined by a Urinary Albumin Creatinine Ratio (UACR) > 300mg/g were randomly assigned to receive PF-00489791 20mg or placebo in a 3:1 fashion, orally, once daily for 12 weeks.

Results: A significant reduction in UACR (15.7%) was observed in response to the 12-week long treatment with PF-00489791 compared to placebo using the pre-defined primary assessment of efficacy (Bayesian analysis with informative prior). A MMRM sensitivity analysis of the results demonstrated a time-dependent reduction in UACR in subjects treated with PF-00489791 compared to placebo, reaching 21.7% reduction at Week 12 (p=0.003). PF-00489791 20mg was safe and generally well-tolerated in this population. Most common adverse events included headache and upper gastrointestinal events.

Conclusions: In summary, the safety and efficacy profile of PF-00489791 in this study supports further investigation of its potential as a novel therapeutic strategy to improve renal outcome in DN.

Funding: Pharmaceutical Company Support - Pfizer,Inc

FR-OR122

Efficacy and Safety of Liraglutide versus Placebo by eGFR Subgroup in Subjects with Type 2 Diabetes and Moderate Renal Impairment (LIRA-RENAL): A Randomized Trial

David Scott,[1] Guillermo Umpierrez,[2] Stephen Atkin,[3] Stephen Bain,[4] Peter Rossing,[5] Minara Shamkhalova,[6] Heidrun Bosch-Traberg,[7] Annika Syrèn,[7] Melanie Davies.[8] *[1]Diabetes, Clinical Research Development Associates, New York, NY; [2]Emory Univ, Atlanta, GA; [3]Weill Cornell Medical College Qatar, Doha, Qatar; [4]Inst of Life Science, Swansea Univ, Swansea, United Kingdom; [5]Steno Diabetes Center, Gentofte, Denmark; [6]Endocrinology Research Centre, Moscow, Russian Federation; [7]Novo Nordisk A/S, Søborg, Denmark; [8]Univ of Leicester, Leicester, United Kingdom.*

Background: To determine the effects of adding liraglutide 1.8 mg (lira) or placebo (PBO) to existing oral antidiabetic agents and/or insulin therapy to subjects with moderate renal impairment (eGFR 30-59 mL/min/1.73 m^2; MDRD), we evaluated renal function group-dependency (CKD Stages 3a and 3b) on selected efficacy and safety parameters.

Methods: In this 26-week, placebo-controlled, double-blind trial, adults with T2DM and moderate renal impairment, BMI of 20-45 kg/m^2, HbA$_{1c}$ of 7.0-10.0% and on stable diabetes medication were randomized 1:1 to receive either once-daily lira or PBO. The primary endpoint was HbA$_{1c}$ change from baseline (BL) to Week 26.

Results: 277 subjects were exposed to lira or PBO and included in the analysis. Lira exhibited a significant reduction in HbA$_{1c}$ consistent across eGFR subgroups compared to PBO (Table). More GI AEs (mostly nausea and vomiting) were reported in all eGFR lira subgroups compared to PBO. There was no significant treatment effect on UACR in any of the UACR subgroups. No difference was seen in eGFR change from BL (lira -1%; PBO +1%; p=0.3575) for the total population.

Conclusions: In subjects with T2DM and moderate renal impairment, lira showed superior HbA$_{1c}$ reduction across all eGFR subgroups compared to PBO. Overall, there was no difference in the safety profile of lira between eGFR subgroups.

Stage CKD (eGFR Subgroup)	Stage 3 CKD (30-59 mL/min/1.73 m^2)		Stage 3b CKD (30-<45 mL/min/1.73 m^2)		Stage 3a CKD (45-59 mL/min/1.73 m^2)	
Treatment Group	Lira n=140	PBO n=137	Lira n=61	PBO n=59	Lira n=79	PBO n=78
HbA$_{1c}$, BL, mean % (SD)	8.08 (0.79)	8.00 (0.85)	8.09 (0.81)	8.06 (0.92)	8.07 (0.78)	7.95 (0.80)
Change from BL at Week 26, estimated means	-1.05	-0.38	-0.97	-0.40	-1.10	-0.38
Treatment difference, estimated; p-value	-0.66 p<0.0001		-0.57 p=0.0022		-0.72 p<0.0001	
Subgroup by treatment interaction	na		p=0.4897			
AE, % subjects	76.4	68.6	77.0	78.0	75.9	61.5
SAE, % subjects	10.0	10.9	14.8	15.3	6.3	7.7
GI AE, % subjects	35.7	17.5	32.8	20.3	38.0	15.4
Confirmed hypo,% subjects	10.7	16.8	13.1	15.3	8.9	17.9
Albuminuria category	**Normal (A1)** (< 30 mg/g)		**Moderately Increased (A2)** (30-300 mg/g)		**Severely Increased (A3)** (>300 mg/g)	
Treatment group, n	Lira n=49	PBO n=42	Lira n=38	PBO n=42	Lira n=29	PBO n=31
UACR, BL, geo mean (CV)	9.29 (0.82)	10.21 (0.58)	57.05 (0.60)	73.31 (0.73)	1094.6 (0.92)	885.1 (0.89)
Ratio to BL, estimated means	0.90	0.83	0.74	1.02	1.00	1.52
Treatment ratio (lira/PBO), estimated; p-value	1.09 p=0.7298		0.73 p=0.1985		0.66 p=0.1310	
Subgroup by treatment interaction	p=0.7320					

eGFR=estimated glomerular filtration rate; na=not applicable; BL=baseline; AE=adverse event; SAE=serious adverse event; GI=gastrointestinal; hypo=hypoglycemic; UACR= urinary albumin:creatinine ratio; A1=normoalbuminuria; A2=microalbuminuria; A3=macroalbuminuria; CV=coefficient of variation

Clinical trial reg. number: NCT01620489

Funding: Pharmaceutical Company Support - Novo Nordisk A/S

FR-OR123

Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial of Once-Daily ZS-9 for Treatment of Hyperkalemia: Achievement and Maintenance of K$^+$ in Subgroup Analysis of Patients with Significant Renal Impairment and Diabetes

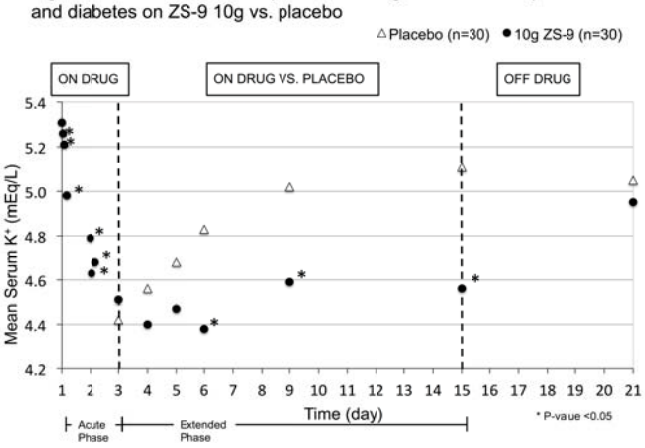
Bhupinder Singh,[1] Henrik S. Rasmussen,[2] Philip T. Lavin,[3] Alex Yang,[4] Wajeh Y. Qunibi.[5] *[1]Apex Research, Riverside, CA; [2]ZS Pharma, Inc., Coppell, TX; [3]Boston Biostatistics Research Foundation, Framingham, MA; [4]Xelay Acumen, Inc., Belmont, CA; [5]Univ of Texas Health & Science Center, San Antonio, TX.*

Background: Hyperkalemia (HK) increases risk of mortality and limits therapy with RAASi in patients (pts) with heart failure, chronic kidney disease, and diabetes mellitus (DM). ZS-9, a nonabsorbed cation exchanger designed to trap K$^+$ in the gut, was evaluated in a subgroup of hyperkalemic pts with significant renal impairment (eGFR<60) and DM from a Phase 3 trial.

Methods: Pts (N=753) with K$^+$ 5.0-6.5 mEq/L were randomized (1:1:1:1:1) to ZS-9 (1.25, 2.5, 5 or 10g) or placebo (PBO) orally 3× daily for 48hr (acute phase). Following this phase, pts with K$^+$ 3.5-5.0 mEq/L (n=542) were randomized 1:1 to the same ZS-9 acute phase dose or PBO 1× daily on Days 3-15 (extended phase). RAASi were maintained during the study. Unpaired t-tests were used to compare K$^+$ in pts treated with ZS-9 versus PBO.

Results: Of 753 pts, 366 (49%) had eGFR<60 and DM. Mean baseline K$^+$ was 5.3 mEq/L. At 48hr, K$^+$ decreased significantly with 2.5g (n=69), 5g (n=78) and 10g (n=64) ZS-9 (-0.45, -0.58, and -0.81 mEq/L, respectively) compared with PBO (n=75, 0.24 mEq/L; p=0.026, p<0.001, p<0.001, respectively). In the extended phase, baseline K$^+$ was similar in the 10g ZS-9 (n=30, 4.5 mEq/L) and PBO groups (n=30, 4.4 mEq/L). Pts on ZS-9 10g maintained normokalemia (4.6 mEq/L at Day 15; p<0.0001), versus PBO (5.1 mEq/L at Day 15). ZS-9 5g showed similar results (p<0.0001).

Figure. Mean K$^+$ over time in patients with significant renal impairment and diabetes on ZS-9 10g vs. placebo



Conclusions: ZS-9 effectively restored and maintained normokalemia and thus allows continued use of renoprotective RAASi in diabetic patients with significant renal impairment with HK.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

FR-OR124

The Risk Pattern for CV Events Differ Between Coronary Heart Disease (CHD) and Stroke in Patients with Type 2 Diabetes and Renal Impairment

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Background: We assessed the J-shape relationship between blood pressure (BP), risk of coronary heart disease (CHD) and stroke in patients with type 2 diabetes (T2D) and renal impairment (RI) treated and followed up in clinical practice.

Methods: 33,356 patients (47% men, age 75±9 years, BMI 29.1±4.8 kg/m^2, diabetes duration 10±8 years) with complete data on serum creatinine and blood pressure (BP) in the Swedish National Diabetes Register (NDR) were followed between 7/1/2005 and 12/31/2007 until 12/31/2011. RI was defined as estimated glomerular filtration <60ml/min/1.73m^2 according to MDRD. BP-values were the mean of all reported values during the follow-up period. The relationships between mean BPs, CV events were examined by time-dependent Cox models, to estimate hazard ratios (HR), adjusting for CV risk factors and medications.

Results: During the follow-up period (median 5.3 years) a total of 11 317 (34%) CVEs occurred, 7704 (23%) patients had CHD and 2284 (6.8%) suffered a stroke. The highest risk of CHD was found with the lowest SBP interval 80-120 mmHg (n=1211, 37%) (HR 2.6, 95% CI 2.2-3.0) and highest prevalence of stroke were found with the highest interval SBP 160-230 mm Hg (n=376, 11.3%) (HR 2.6, 95% CI 2.0-3.4). Similarly, the highest risk of CHD was found in the lowest DBP interval 40-63 mm Hg (1197, 36.1%) (HR 2.0, 95% CI 1.8-2.3) and the highest occurrence of stroke in the highest DBP interval 83-125 mm Hg (n=790, 24%) (HR 2.6, 95% CI 2.0-3.3). Adjustments for presence of albuminuria did not markedly alter the results.

Conclusions: The risk pattern for CV events differ between coronary heart disease (CHD) and stroke. The highest risk of CHD was found with the lowest BP intervals and the highest risk of stroke with the highest BP intervals. This difference in risk patterns is important to keep in mind when assessing overall risk of cardiovascular events in patient populations.

Funding: Government Support - Non-U.S.

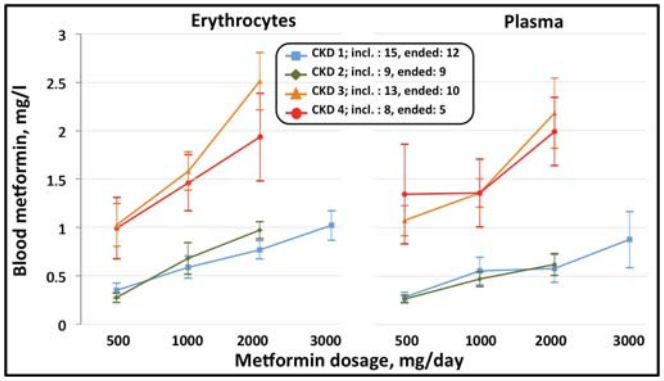
FR-OR125

Metformin Therapy at Different Stages of Chronic Kidney Disease Farshad Kajbaf,[1] Jean-Daniel Lalau,[1] Mohamed Azzoug,[1] Anne-Sophie Lemaire-Hurtel,[2] Marc E. De Broe.[3] *[1]Dept of Endocrinology-Nutrition, Univ Hospital, Amiens, France; [2]Dept of Clinical pharmacology, Univ Hospital, Amiens, France; [3]Laboratory of Pathophysiology, Univ of Antwerp, Belgium.*

Background: We are conducting an open-label pilot study in order to establish whether or not it is feasible to continue metformin therapy in diabetes patients with stable CKD 1-5 not yet in dialysis provided that the dose is adjusted. The objectives are to evaluate metformin levels in plasma and in erythrocytes (providing a better estimation of the risk of accumulation) according to metformin dosage and CKD stage and to demonstrate the absence of hyperlactatemia (≥2.5 mmol/L).

Methods: Inclusion criteria are Type 2 diabetes patients, untreated or treated with metformin and with HbA1c >6.5%, having stable renal function for at least 3 months. All patients undergo 3 one week-blocks of metformin treatment at an increasing dosage, each of which is followed by a one week-wash-out period: 500 mg/day in the evening (E) in phase 1; 1,000 mg/day (500 mg morning (M) and E) in phase 2; 2,000 mg/day (1000 mg M and E) in phase 3. Stage 1 CKD patients further complete a phase 4 with 3,000 mg/day (1000 mg M and 2000 mg E). Metformin levels are assayed in erythrocytes and in plasma 12 hours after the last metformin intake; lactate concentrations are also assayed in CKD 3-5.

Results: The available data are shown in the Fig.1.



The maximal lactate value is 2.7 mmol/L, other values are < 2.1 mmol/L.

Conclusions: 1) as CKD progresses,metformin levels did not increase more in erythrocytes (reflecting the deep-compartment) than in plasma; 2) the rather linear metformin dosage/level relationship forms a solid base to perform a pharmacokinetic study in CKD 3-5 to validate the adjustment of the metformin dosage in moderate to severe CKD; 3) in terms of blood lactate concentrations metformin therapy seems well-tolerated.

Funding: Pharmaceutical Company Support - Merck Serono

FR-OR126

Epithelial-Derived Wnt Ligand Drives Interstitial Fibrosis Through Paracrine Signaling Omar H. Maarouf,[1] Deepika Rangarajan,[2] Jeremy Welborn,[1] Benjamin D. Humphreys.[1] *[1]Medicine, Brigham and Women's Hospital, Boston, MA; [2]Biology, SRM Univ, Chennie, India.*

Background: Injured epithelial cells secrete paracrine factors that regulate interstitial fibrogenesis, but the identity of these factors is poorly defined. We have previously shown that activation of the canonical Wnt signaling pathway in pericytes cell-autonomously drives myofibroblast activation *in vivo*. Here we have investigated whether Wnt ligand derived from tubular epithelial cells is sufficient to drive interstitial fibrosis *in vivo*, and the interaction between Wnt and TGFb pathways in mediating myofibroblast activation.

Methods: We generated a novel mouse strain ($SLC34a^{GCE/+}$; $R26^{Wnt1\text{-}GFP/+}$) in which tamoxifen administration induces constitutive Wnt1 secretion by proximal tubular epithelial cells in adult kidney. We cultured NRK49F cells and primary human dermal fibroblasts (HDF) to investigate the dependence of TGFb-mediated aSMA and matrix protein expression on Wnt signaling using the Wnt pathway inhibitor XAV939, and activator CHIR99021.

Results: Tamoxifen or vehicle was administered to 8-week old $SLC34a^{GCE/+}$; $R26^{Wnt1\text{-}GFP/+}$ mice and groups were sacrificed at 16 or 20 weeks of age. Mice that received tamoxifen constitutively expressed Wnt1 in proximal tubule, and in these mice confocal microscopy demonstrated progressive interstitial aSMA+ myofibroblast accumulation in the absence of any other stimulus. Compared to vehicle-treated controls, we noted increased interstitial proliferation as well as increased aSMA and fibronectin protein levels in tamoxifen-treated mice. By contrast, neither group expressed Kidney Injury Molecule-1, indicating that epithelial damage per se was not driving renal fibrogenesis. *In vitro*, TGFb induced myofibroblast transition (aSMA and fibronectin expression) was inhibited by the canonical Wnt inhibitor XAV939. Canonical Wnt activation by CHIR99021 potentiated TGFb-induced aSMA and fibronectin expression in both NRK49F and HDF cells.

Conclusions: Epithelial-derived Wnt1 alone is sufficient to drive interstitial fibrosis *in vivo* in the absence of an injury stimulus. Wnt ligands cooperate with TGFb to drive pericyte to myofibroblast transition.

FR-OR127

Tubule-Derived Wnts Are Indispensable for Fibroblast Activation and Kidney Fibrosis Dong Zhou,[1] Roderick J. Tan,[2] Haiyan Fu,[1] Liangxiang Xiao,[1] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

Background: Cell-cell communication via Wnt signals plays fundamental roles in regulating embryonic development and kidney homeostasis. Accordingly, dysregulation of Wnts expression is implicated in the development and progression of kidney diseases.

Results: We found that in kidney biopsy specimens from CKD patients with different etiologies, Wnts proteins displayed distinct expression patterns. While Wnt4 was induced in both tubular cells and interstitial fibroblasts, Wnt1 induction was limited to epithelial cells, but not fibroblasts. To investigate the potential roles of Wnts produced by different kidney cells, we generated conditional knockout mouse model in which Wntless (WLs)/Evi, a novel cargo protein specifically dedicated to the secretion of Wnt proteins, was selectively ablated in kidney tubular epithelial cells. Mice with tubule-specific deletion of Wls/Evi did not show overt abnormality. However, blockade of Wnt secretion by deleting Wls/Evi in tubular cells markedly ameliorated renal fibrosis after unilateral ureteral obstruction (UUO), comparing with controls. Consistently, renal expression of the major fibrosis-related genes including collagen, fibronectin and plasminogen activator inhibitor 1 (PAI-1) were significantly inhibited in mice with tubule-specific ablation of Wls/Evi after UUO. Tubular loss of Wls/Evi also resulted in an accelerated activation of myofibroblasts. Interestingly, tubule-specific deletion of β-catenin had no or little effect on the severity of renal fibrosis after UUO, suggesting that blockade of tubule-derived Wnt secretion mainly affects fibroblast activation by a paracrine mechanism. In vitro, incubation of normal rat kidney fibroblast (NRK-49F) with recombinant Wnt proteins promoted their proliferation and extracellular matrix expression and deposition.

Conclusions: These results illustrate that tubule-derived Wnts play an essential role in promoting fibroblast activation and kidney fibrosis via cell-cell communication.

Funding: NIDDK Support

FR-OR128

Loss of Angiopoietin-1 Increases Kidney Fibrosis Krishnapriya Loganathan,[1] Marie Jeansson.[1] *[1]Immunology, Genetics and Pathology, Uppsala Univ, Uppsala, Sweden; [2]Feinberg School of Medicine, Northwestern Univ, Chicago, IL.*

Background: The presence of renal tubulointerstitial fibrosis is predictive of progressive decline in kidney function. It is characterized by an increase in aSMA+ fibroblasts, myofibrosblasts, that produce collagen. Identification of factors that regulate the fibrotic response are excellent candidate targets for treatment of kidney diseases. We previously showed that loss of Angiopoietin-1 (Angpt1) in adult mice predisposes to systemic fibrosis in an ear punch model. Angpt1 is expressed in pericytic lineages and acts through the Tie2 tyrosine-kinase receptor expressed on adjacent endothelium. Here, we test the hypothesis that loss of Angpt1-Tie2 signaling in renal fibrosis will result in a more aggressive fibrotic response.

Methods: To better understand the origin of fibroblast populations in renal fibrosis, we first performed lineage tagging experiments using Z/EG reporter mice that were bred to 4 independent Cre-driver strains and subjected to UUO. These Cre strains mark the tubular epithelium, renal pericytes, renal endothelium and macrophages, respectively (Pax8-rtTA/tetOCre, Pdgfrb-Cre, Tie2-Cre and LysM-Cre). Secondly, we performed UUO on Angpt1 conditional knockout mice and studied gene expression changes at different time points.

Results: Kidneys were analyzed and co-stained for the lineage tag (EGFP+) and aSMA+, 10 days post-UUO. We found that endothelial cells and pericytes each contributed to about about 15% of myofibroblast. Tubular epithelium and macrophages contributed minimally. UUO in conditional Angpt1 knockout mice show an increased expression of fibrosis markers compared to control mice subjected to UUO. For example, there is a significant upregulation of Fibronectin 3 days after UUO in Angpt1 knockout mice compared to controls.

Conclusions: Our results show that several lineages, including endothelial cells, contribute to the myofibroblast population in fibrosis. In addition, our results suggest that loss of Angpt1-Tie2 signaling increases fibrosis as seen by the increased expression of fibrosis markers. Ongoing work is designed to elucidate the mechanism(s).

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR129

FGFR2 Signaling Promotes Kidney Fibroblast Activation and Kidney Fibrosis Zhuo Xu, Weichun He, Junwei Yang, Chunsun Dai. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

Background: Fibroblast growth factors (FGFs) are heparin-bindingproteins involved in a variety of biological processes, including cell proliferation, differentiation, survival and angiogenesis. However, the role and mechanisms of FGFs/FGFR2 signaling in kidney fibroblast activation and kidney fibrosis need further investigation.

Methods: Mice with fibroblast specific FGFR2 deletion was created with Cre-LoxP system. Kidney fibrosis was induced by unilateral ureter obstruction (UUO) in mice.

Results: In this study, in the kidneys from mice with UUO nephropathy, the mRNA expression level for FGFs including FGF7, FGF10, FGF11, FGF12, FGF13, FGF16, and FGF22were significantly up-regulated. To further explore the role of FGF signaling in kidney fibroblast activation and fibrosis, a mouse model with fibroblast specific FGFR2 gene disruption was generated by using Cre-LoxP system. The knockouts were born normal and no obvious kidney dysfunction or histological abnormality was found within 2 months after birth. Kidney ischemia/reperfusion model was created in the knockouts and their control littermates and kidney tissue was harvested at 4 weeks after surgery. Kidney interstitial fibrosis was induced in the control littermates, while in the knockouts, total collagen deposition, fibronectin and a-SMA expression, interstitial inflammatory cell infiltration were all decreased compared to those in the control littermates. In addition, Ki67 positive interstitial cell number was also less in the knockout kidneys at 4 weeks after surgery. Phosphorylated Erk1, 2, a downstream signaling molecule for FGFR, was also downregulated in the knockout kidneys at 2 and 4 weeks after IRI compared to those in the control littermates.

Conclusions: Taken together, these results suggest that FGFs/FGFR2 signaling promotes the proliferation and activation of kidney fibroblasts and contributes to the development of interstitial fibrosis.

Funding: Government Support - Non-U.S.

FR-OR130

Role of Platelet-Derived Growth Factor DD in Healthy Kidneys and Renal Fibrosis Eva Miriam Buhl,[1] Sonja Djudjaj,[2] Barbara Mara Klinkhammer,[2] Janka Babickova,[1] Jürgen Floege,[1] Peter Boor.[1,2] *[1]Nephrology, RWTH Univ, Aachen, Germany; [2]Pathology, RWTH Univ, Aachen, Germany.*

Background: Platelet-Derived Growth Factors (PDGF) play a role in various renal diseases. The PDGF-DD isoform is the only specific PDGF receptor β ligand and mediates mesangial cell proliferation. Our previous data in progressive mesangioproliferative glomerulonephritis in rats also suggested a role in interstitial fibrosis.

Methods: To address the functional role of PDGF-DD we have generated PDGF-DD-/- mice, which also serve as reporter mice for PDGF-DD (using LacZ). We analysed the expression pattern of PDGF-DD in healthy and diseased kidney as well as the consequence of PDGF-DD deficiency. We compared PDGF-DD-/- mice and their wild-type (WT) littermates in models of renal fibrosis (unilateral ureteral obstruction – UUO and unilateral ischemia/reperfusion - I/R).

Results: PDGF-DD-/- mice (on both Bl6 and mixed Bl6/Sv129 backgrounds) were viable and showed no obvious spontaneous phenotype until the age of 50 weeks. Expression of PDGF-DD in healthy kidneys was observed in renal mesenchymal cells including the mesangial cells, fibroblasts and vascular smooth muscle cells. During renal fibrosis PDGF-DD and its receptor PDGFR-β were markedly upregulated in both human and murine tissues. The expanding and activated mesenchymal cells in fibrosis retained their PDGF-DD expression. In addition, PDGF-DD was expressed *de novo* in injured renal tubular cells. The PDGFR-β receptor was found on mesenchymal cells in both healthy and fibrotic kidneys and was highly upregulated during fibrosis. *In vitro,* PDGF-DD induced proliferation and expression of proinflammatory cytokines in fibroblasts. Compared to wild-type littermates, PDGF-DD-/- mice had significantly reduced indices of renal fibrosis in UUO on day 10 and following I/R on day 21.

Conclusions: PDGF-DD was strongly upregulated in kidney injury and scarring and its genetic deletion significantly attenuated renal interstitial fibrosis. PDGF-DD might represent a novel therapeutic target in kidney fibrosis.

Funding: Government Support - Non-U.S.

FR-OR131

Blocking SIRT 1/2 Inhibits Renal Interstitial Fibroblast Activation and Attenuates Renal Interstitial Fibrosis in Obstructive Nephropathy Shougang Zhuang. *Dept of Medicine, Alpert Medical School and Rhode Island Hospital, Providence, RI.*

Background: Our recent studies revealed that blocking class I/II histone deacetylases (HDACs) inhibits renal interstitial fibroblast activation and proliferation and alleviates development of renal fibrosis. However, the effect of class III HDACs, in particular, Sirtuin 1 and 2 (SIRT1 and SIRT2) inhibition on renal fibrogenesis remains elusive.

Methods: Here, we examined the effect of SIRT1/2 inhibition on the activation of renal interstitial fibroblas and development of renal fibrosis in a murine model of unilateral ureteral nephropathy.

Results: Our ressults showed that both SIRT1 and SIRT2 were expressed in cultured renal interstitial fibroblasts (NRK-49F). Exposure of NRK-49F to Sirtinol, a selective inhibitor for SIRT1/2, or EX527, an inhibitor for SIRT1, resulted in reduced expression of fibroblast activation markers (α-smooth muscle actin, fibronectin and collagen I) as well as proliferation markers (proliferating cell nuclear antigen, cyclin D1, cyclin E) in dose and time dependent manners. Treatment with a SIRT2 inhibitor, AGK2, also dose- and time-dependently inhibited renal fibroblast activation and to a less extent, proliferation. Furthermore, silencing of either SIRT1 or SIRT 2 by siRNA exhibited similar inhibitory effects. In a mouse model of obstructive nephropathy, administration of Sirtinol attenuated deposition of collagen fibrils as well as reduced expression of α-smooth muscle actin, collagen I and fibronectin in the injured kidney. SIRT1/2 inhibition-mediated anti-fibrotic effects are associated with dephosphorylation of epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor-β (PDGFR-β) and STAT3.

Conclusions: Thus, SIRT1/2 activity may contribute to renal fibroblast activation and proliferation as well as renal fibrogenesis through activation of at least EGFR and PDGFRβ signaling. Blocking SIRT1/2 activation may have therapeutic potential for the treatment of chronic kidney disease.

Funding: NIDDK Support

FR-OR132

Tenascin-C Plays an Important Role in Kidney Fibrosis Qionghong Xie, Min Zhang, Shaojun Liu, Chuanming Hao. *Div of Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China.*

Background: Tenascin-C (TNC) is a glycoprotein expressed in the extracellular matrix of various tissues during development, disease or injury repairing. The present study examined the role of tenascin C in renal fibrosis.

Methods: A renal medullary interstitial cell (RMIC) specific TNC promoter driven inducible CreER2 knock-in mouse with an EGFP reporter was generated (TNCCreER). UUO and ischemic perfusion injury (IRI) models were used to induce renal fibrosis. TNCCreER$^{+/+}$ (TNC knockout) was used to examine the role of TNC in renal fibrosis. To examine whether RMIC contribute to fibrosis, a cell linage chasing study was conducted. Recombination was induced in TNC-CreER2$^{+/-}$/Rosa-lacZ$^{+/-}$ mice by tamoxifen (1.5mg/d by ip for 7 days) at least three weeks before the fibrotic challenge.

Results: In normal kidney, TNC is expressed in RMICs. In fibrotic kidney after IRI (14 days) or UUO (7 days), the expression of TNC was markedly induced in renal cortex. The GFP (+) TNC expressing cells accounted for 42.4% of all the interstitial cells in fibrotic regions. Among these GFP (+) TNC expressing cells, 40% also expressed α-SMA, and none of the GFP(+) cells expressed macrophage marker F4/80. Among the renal interstitial cells in fibrotic regions, 25.3% were F4/80(+) cells while 32.3% were GFP(-)F4/80(-) cells. To examine whether the induced TNC contributes to the fibrosis, TNC$^{-/-}$ mice was used and TNC$^{-/-}$ kidney showed less fibrosis by Masson staining, fewer S100A4(+) cells (26.7 versus 36.3/HPF in UUO7d) and less F4/80(+) areas (5.4% versus 3.8% in UUO7d) than wild-type kidney. TNC deletion is also associated with reduced collagen Iα, PAI-1 and fibronectin mRNAs. To further examine whether the RMICs that normally express TNC contribute to TNC(+) cells in the fibrotic region, we did a cell-linage chasing study using a TNCCreER/ROSA26R mouse. The results show that the recombination reporter remained in papillary and was not observed in the fibrotic area where TNC expression was observed.

Conclusions: During fibrotic process in the kidney, tenascin C is induced in cells that may trans-differentiate to α-SMA(+) myofibroblasts. TNC plays an important role in renal fibrosis and TNC deficiency alleviates renal fibrosis induced by IRI or UUO.

FR-OR133

Role for Rictor/mTORC2 Signaling in Mediating TGFβ1-Induced Fibroblast Activation and Kidney Fibrosis Jianzhong Li, Weichun He, Junwei Yang, Chunsun Dai. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

Background: The Mammalian target of rapamycin (mTOR) is recently identified with two structurally distinct multiprotein complexes. MTORC1 has been implicated in the pathogenesis of several types of kidney disease. Our previous study demonstrated that Rictor/mTORC2 protects against cisplatin-induced tubular cell death and acute kidney injury. However, the role and mechanisms for mTORC2 in TGFβ1-induced fibroblast activation and kidney fibrosis have not been reported.

Methods: Rat kidney interstitial fibroblasts (NRK-49F) were stimulated with TGFβ1 and kidney fibrosis was induced by unilateral ureter obstruction (UUO) in mice.

Results: Here, we found that TGFβ1 could activate Rictor/mTORC2 signaling in cultured NRK-49F cells, a rat kidney fibroblast cell line, with a time-dependent manner. Blocking Rictor/mTORC2 signaling with either Rictor, Akt1 or Akt2 small interfering RNA transfection markedly inhibited TGFβ1-induced fibronection and a-SMA expression. In addition, Western blotting or immuno-staining results showed that Rictor/mTORC2 signaling was activated in kidney interstitial myofibroblasts from mice with unilateral ureter obstruction (UUO) nephropathy and from patients with various types of kidney disease including diabetic nephropathy, IgA nephropathy, lupus nephritis as well as membranous nephropathy. To further investigate the role of Rictor/mTORC2 signaling in fibroblast activation and kidney fibrosis, a mouse model with fibroblast-specific deletion of Rictor was generated with Cre/LoxP system. The knockouts were born normal and no obvious kidney dysfunction or kidney morphologic abnormality was found within 5 months after birth. However, kidney interstitial fibrosis and inflammatory cell infiltration were markedly diminished in the knockouts at 1 and 2 weeks after UUO compared to those in their control littermates.

Conclusions: Together, these results suggest that Rictor/mTORC2 signaling activation mediates TGFb1-induced fibroblast activation and contributes to the development of kidney fibrosis, possibly providing a therapeutic target for chronic kidney diseases.

Funding: Government Support - Non-U.S.

FR-OR134

GLI2 Antagonism Is a Novel Antifibrotic Strategy by Inducing Myofibroblast-Specific Cell Cycle Arrest Rafael Kramann, Susanne V. Fleig, Benjamin D. Humphreys. *Medicine, Brigham and Women´s Hospital, Boston, MA.*

Background: The Hedgehog effectors Gli1 and Gli2 are expressed in kidney pericytes as well as activated myofibroblasts during fibrosis. Whether these transcription factors might serve as therapeutic targets in chronic kidney disease (CKD) is unknown. Recently arsenic was described as an inhibitor of Hedgehog signaling, though the mechanism is unknown.

Methods: We utilized Gli1 knockout mice, in vitro drug binding, RNAi and overexpression studies to dissect the roles of Gli1 versus Gli2 in myofibroblast activation. We tested the antifibrotic efficacy of Darinaparsin, an arsenical, and GANT61, a small molecule Gli inhibitor, in two independent CKD models in mice. These drugs were given in a therapeutic protocol, ie, after the onset of fibrosis.

Results: Surprisingly, knockout of Gli1 had no effect on fibrosis compared to WT mice (n = 15 mice per group). Therefore we turned our attention to Gli2. Knockdown of Gli2 in vitro reduced Gli1 and triggered G0/G1 cell-cycle arrest, whereas knockdown of Gli1 had no effect, consistent with our in vivo results. We found that darinaparsin directly binds to Gli2, and it mimicked the effects of Gli2 knockdown. Conversely, retroviral overexpression of Gli2 increased Gli1 expression, drove cell proliferation and rescued the cell cycle effect of darinaparsin. Gli2 knockout is embryonic lethal, but when administered two days after UUO, darinaparsin potently inhibited renal Gli1 and 2 expression and reduced renal fibrosis through upregulation of p21 and induction of a myofibroblast specific G0/G1 cell-cycle arrest. There was no effect on epithelial cell cycle. Darinaparsin also improved GFR in a four week severe bilateral IRI CKD model when administered beginning one week after IRI. Importantly, the small molecule GANT61 also reduced Gli1 and Gli2 protein levels and blocked renal fibrosis following UUO in a therapeutic model.

Conclusions: Gli2 is the critical Gli effector that regulates myofibroblast cell cycle progression whereas Gli1 is dispensable. Our data provide the first conclusive evidence that targeting Gli2 is a novel and powerful antifibrotic therapeutic strategy to slow CKD progression.

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

FR-OR135

Notch1, but Not Notch2, Regulates Podocyte Dedifferentiation in Glomerulosclerosis via Snail1 Mariya T. Sweetwyne, Katalin Susztak. *Renal-Electrolyte and Hypertension, Univ of Pennsylvania, Philadelphia, PA.*

Background: Expression of multiple Notch ligands and receptors has been described in different kidney injury models. During podocyte development, Notch2 is critical for differentiation whereas Notch1 is dispensable. In diabetic glomerulosclerosis, treatment with global Notch inhibitors or podocyte-specific deletion of a Notch transcriptional co-activator, Rbpj, protected glomeruli from diabetic injury; proving that podocyte Notch signaling is critical for regulating glomerulosclerosis. Here we investigated the specific role of Notch1 and 2 receptor isoforms in podocytes under pro-fibrotic stimulus.

Methods: Notch1 or 2 flox/flox and podocin Cre mice were crossed to generate animals with podocyte-specific deletion of Notch1 or 2. We cultured glomeruli and podocytes from these mice and used quantitative RT-PCR, western blots and immunohistochemistry (IHC) to examine Notch signaling.

Results: Previously we showed that podocyte-specific deletion of Notch1, but not Notch2, protects diabetic mice from podocyte loss, albuminuria and glomerular injury. To assess these responses *in vitro*, cultured primary podocytes were treated with transforming growth factor β (TGF-β) a profibrotic cytokine that is highly upregulated in injured glomeruli. In primary wildtype podocytes, TGF-β1 induced the expression of Notch ligands Jagged1 and DLL4 and the activation (cleavage) of Notch1 but not Notch2. TGF-β treatment caused podocyte dedifferentiation evident by a decrease in podocin transcript and protein levels, f-actin stress fiber formation, and upregulation of transcriptional regulator, Snail1. In Notch1 knockout podocytes TGF-β was unable to downregulate podocin and induce Snail1 expression, indicating that Notch1 mediates TGF-β-induced podocyte dedifferentiation. *In vivo,* IHC of kidneys from diabetic mice showed upregulation of Snail1 podocyte expression in wildtype, but not Notch1 knockout, animals. These findings were consistent with the *in vitro* results.

Conclusions: Podocyte Notch1 expression plays an important role in diabetic albuminuria and podocyte dysfunction. Our current experiments indicate that these changes are in part the result of Notch1 induced podocyte dedifferentiation via Snail1.

Funding: NIDDK Support

FR-OR136

Acute Kidney Injury Progresses to Chronic Kidney Disease via Persistent Metabolic Alterations and Oxidative Stress David M. Small,[1] Washington Yamandu Sanchez,[2] Sandrine F. Roy,[3] Christudas Morais,[1] David W. Johnson,[1,4] Glenda C. Gobe.[1] *[1]Centre Kidney Disease Research; [2]Therapeutics Research Centre, Sch Medicine, Univ Queensland; [3]Diamantina Inst; [4]Dept Nephrology, PA Hospital, Brisbane, Australia.*

Background: Acute kidney injury (AKI) often progresses to chronic kidney disease (CKD). Oxidative stress and mitochondrial dysfunction exacerbate AKI but their role in progressive CKD remains unclear. The aim of this project was to determine the metabolic changes associated with oxidative stress during the progression to CKD after AKI.

Methods: Male C57Bl6 mice underwent bilateral ischemia (20min) followed by reperfusion for 21 days (21d). Intravital multiphoton microscopy (MPM) of endogenous nicotinamide adenine dinucleotide (NADH) was performed in kidneys: without ischemia-reperfusion (IR); during ischemia; immediately at reperfusion; and at 21d (n=4). Fluorescence lifetime imaging microscopy (FLIM) measured free/bound NADH (α_1/α_2 ratio), and the average weighted lifetime (τ_m) of NADH in cortex and medulla. Renal perfusion with a mitochondrial dye (TMRM) coupled with MPM assessed mitochondrial health. Molecular and histological assessments were performed in kidneys post MPM and FLIM.

Results: NADH fluorescence intensity (EFI) significantly decreased during ischemia ($p<0.05$ v control). 21d post-IR showed focal tubular atrophy and significantly increased NADH EFI in structurally normal tubules. Tubular epithelial cell uptake of TMRM decreased, the α_1/α_2 ratio increased, and theτ_m of NADH/FAD decreased in cortex ($p<0.05$). Compared with controls, biomolecular analyses showed: nuclear factor-like 2 expression increased at reperfusion ($p<0.001$) but decreased at 21d ($p<0.05$); increased apoptosis, collagen, heme-oxygenase-1, transforming growth factor-β1, proliferating cell nuclear antigen, and 8-hydroxy-2'-deoxyguanosine ($p<0.05$ at 21d); and decreased superoxide dismutase-2 ($p<0.05$ at 21d).

Conclusions: This is the first demonstration of dynamic changes in NADH utilisation by kidney tubular cells promoting oxidative stress in progressive CKD. Mitochondrial dysfunction is persistent in structurally normal tubules of the chronically-damaged kidney following AKI potentially enhancing free radical production.

Funding: Government Support - Non-U.S.

FR-OR137

Defective Fatty Acid Oxidation in Renal Tubular Epithelial Cells Plays Key Role in Kidney Fibrosis Development Hyun Mi Kang,[1] Seon-Ho Ahn,[1] Yi-An Ko,[1] Ae Seo Deok Park,[1] Jianling Tao,[1] Frank S. Chinga,[1] James M. Pullman,[2] Erwin P. Bottinger,[3] Katalin Susztak.[1] *[1]Renal Electrolyte and Hypertension Div, Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA; [2]Dept of Pathology, Montefiore Medical Center, Bronx, NY; [3]Dept of Medicine, Mount Sinai School of Medicine, New York, NY.*

Background: Fibrosis is the histological manifestation of a progressive irreversible process causing chronic and end stage kidney disease. Here we examined the role of fatty acid oxidation (FAO) in patients and mouse models of kidney fibrosis.

Methods: Here we performed gene expression analysis on a large cohort of human kidney tubule samples (n=95) with control and progressive tubulointerstitial fibrosis. In addition, we cultured renal epithelial primary cells from human and mouse to see the effect of FAO *in vitro* and also tested effect of restoring of FAO on tubulointerstitial fibrosis development.

Results: Genome-wide transcriptome studies of normal and fibrotic human kidney tubule samples identified inflammation and metabolism as the top dysregulated pathways. In particular, we found that both human and mouse models with tubulointerstitial fibrosis were characterized by decreased expression of key enzymes and regulators of FAO, loss of mitochondrial density. In vitro experiments indicated that inhibition of fatty acid oxidation in tubule epithelial cells caused ATP depletion, increased cell death, dedifferentiation and intracellular lipid deposition; a phenotype observed in fibrosis. Our results indicate that the TGFB1-induced -and Smad3- mediated repression of Ppargc1a and fatty acid oxidation were critical for the TGFB1-induced profibrotic phenotype. Restoring fatty acid metabolism by genetic or pharmacological methods protected mice from tubulointerstitial fibrosis development.

Conclusions: In summary, we identified that repressed fatty acid oxidation induces a metabolic reprogramming that is critical for the development of fibrosis in the kidney and may present novel therapeutic approaches for the cure of chronic kidney disease.

Funding: Other NIH Support - The work was supported by NIH R01DK087635 and R01DK076077 to K. Susztak, Private Foundation Support

FR-OR138

Sphingosine 1-Phosphate Receptor-1 Modulates Mitochondrial Dynamics in Proximal Tubule Cells Heather M. Perry,[1,3] Amandeep Bajwa,[1,3] Diane L. Rosin,[2,3] Mark D. Okusa.[1,3] *[1]Dept of Medicine, Univ of Virginia; [2]Pharmacology, Univ of Virginia; [3]Center for Immunity, Inflammation and ReGenerative Medicine, Univ of Virginia, Charlottesville, VA.*

Background: Sphingosine 1-phosphate receptor-1 (S1PR1) preserves tubule cells in cisplatin-induced acute kidney injury in mice by augmenting mitochondrial respiration and protecting against mitochondrial oxidative damage. Yet, it is unknown how S1PR1 improves mitochondrial function. The balance of mitochondrial fission and fusion can regulate mitochondrial function, raising the interesting hypothesis that S1PR1 may modulate mitochondrial dynamics.

Methods: Ultrastructural analysis was performed on electron micrographs to determine mitochondrial morphology in TKPTS cells stably overexpressing S1PR1 (TK-P1) or control cells (TK-ctrl). A mitochondrial gene array was performed with total RNA extracted from TK-P1 and TK-ctrl cells. Lastly, mRNA expression of PGC-1α was assessed by quantitative RT-PCR.

Results: Overexpression of S1PR1 resulted in elongated and thinner mitochondria (feret maximum: 0.675±0.027 versus 0.506±0.022, shape factor: 4.400±0.070 versus 4.036±0.036, and compactness: 0.527±0.017 versus 0.609±0.011 in 20-25 cells, 307-346 mitochondria respectively, $p < 0.001$) compared to control cells. In addition, mitochondrial fission (fis1, drp1) and fusion (opa1, mfn1/2) genes were upregulated 4- to 9-fold in TK-P1 cells compared to controls, indicating an overall increase in mitochondrial dynamics. Lastly, consistent with enhanced mitochondrial biogenesis, PGC-1α gene expression was upregulated 20-fold (n = 3) in S1PR1 overexpressing cells relative to controls.

Conclusions: Results provide evidence that S1PR1 may be a novel regulator of mitochondrial dynamics, potentially through promoting biogenesis. Therefore, increasing S1PR1 signaling in proximal tubule cells to boost mitochondrial function may be a new strategy to prevent cisplatin-mediated acute kidney injury.

Funding: NIDDK Support

FR-OR139

Renal Tubular-Specific Lkb1 Deletion Results in Severe Kidney Damage via Altered Metabolic Reprogramming Seung Hyeok Han,[1,2] Laura Malaga-Dieguez,[3] Frank S. Chinga,[1] Katalin Susztak.[1] *[1]Renal Electrolyte and Hypertension Div, Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA; [2]Dept of Internal Medicine, Yonsei Univ College of Medicine, Seoul, Korea; [3]Dept of Pediatrics, Div of Pediatric Nephrology, New York Univ School of Medicine, New York.*

Background: The tumor suppressor Lkb1/STK11/Par-4 is a key regulator of polarity, proliferation and cell metabolism, yet its role in kidney is poorly defined. Here, we investigated the role of Lkb1 in mice with tubule specific deletion of Lkb1.

Methods: To create tubule epithelial Lkb1 specific knock-out (KO) mice, $Lkb1^{flox/flox}$ mice were crossed to transgenic mice expressing Cre recombinase under the cadherin 16 promoter. Male mice were analyzed at 5, 14 and 27 weeks of age. In vitro we used primary cultured renal tubule epithelial cells from wild type and $Lkb1^{flox/flox}$ mice infected with cre(GFP) or empty GFP adenovirus.

Results: Mice with distal tubule specific Lkb1 deletion ($Ksp^{cre}/Lkb1^{flox/flox}$) showed no obvious histological changes at 5 weeks of age, but developed severe kidney fibrosis, including dilated tubules and interstitial matrix accumulation by 27 weeks of age. Lkb1 is an important regulator of cell polarity, however our study showed no obvious polarity defect in renal epithelial cells lacking Lkb1. We also failed to identify a significant difference in mTOR expression between wild type and Lkb1 null tubule cells, even tough Lkb1 has been shown to regulate mTOR signaling in different context. On the other hand expression of enzymes related to fatty acid oxidation was severely reduced resulting in reduced ATP content and increased apoptosis rate. We found that renal tubule epithelial metabolism exclusively depends on fatty acid oxidation. Effects of Lkb1 was mediated by Ampk and Ppara, as the Ampk agonist (A769662) and Ppara agonist fenofibrate restored fatty oxidation defect and ameliorated apoptosis induced by Lkb1 deletion.

Conclusions: In conclusion, here we show that Lkb1 deficiency in renal tubular epithelial cells, via influencing energy metabolism plays an important role in kidney fibrosis development.

Funding: NIDDK Support

FR-OR140

Mechanisms Involved in AMPK-Regulated Tight Junction Assembly Jingshing Wu, Michael J. Caplan. *Cellular and Molecular Physiology, Yale Univ, New Haven, CT.*

Background: Epithelial tight junctions (TJ) serve as the barriers that control paracellular permeability. Formation and maintenance of TJs require calcium-dependent cell adhesion. Removing extracellular calcium leads to dissolution of TJs, while adding calcium back to cells maintained in calcium-free medium ("calcium switch") induces rapid TJ assembly. Previous studies have shown that calcium switch-induced epithelial TJ formation leads to activation of AMP-activated protein kinase (AMPK). Furthermore, activation of AMPK in cells maintained in calcium-free medium is sufficient to cause the TJ protein zonula occludens-1 (ZO-1) to relocalize to sites of junction assembly and to induce its interaction with the F-actin binding protein afadin-l. Our studies were designed to determine whether regulators of TJ assembly including Cdc42, Par3, and atypical protein kinase C (aPKC) contribute to the mechanism involved in AMPK-mediated TJ assembly.

Methods: We assessed whether AMPK regulates the GTP bound state of Cdc42 in Madin-Darby canine kidney (MDCK) cells by using an affinity binding assay and we assessed the subcellular distributions of Cdc42, Par3, and aPKC during AMPK-mediated junction assembly by immunofluorescence microscopy. We monitored the effects of aPKC inhibition on AMPK-mediated junction assembly through immunofluorescence and co-immunoprecipitation studies.

Results: AMPK-mediated junction assembly promoted by AMPK activation by AICAR (2mM) in the absence of extracellular Ca2+ increased the level of GTP-bound Cdc42 and led to Par3/aPKC localization to sites of junction assembly. Furthermore, we found that aPKC inhibition prevented ZO-1 localization to sites of AMPK-induced junction assembly while increasing the level of afadin-l/ZO-1 interaction.

Conclusions: These results demonstrate that AMPK may regulate junction assembly by stimulating nucleotide exchange to increase the size of the Cdc42 pool in the GTP bound form. Accumulation of this active form of Cdc42 may facilitate Par3/aPKC localization to sites of junction assembly, and aPKC activity may in turn regulate the level of afadin-l/ZO-1 interaction.

Funding: NIDDK Support

FR-OR141

Transcriptome-Based Network Analysis Reveals Renal Cell Type-Specific Dysregulation of Hypoxia-Associated Transcripts Maja Lindenmeyer,[1] Natallia Shved,[1] Gregor Warsow,[2] David Hoogewijs,[1] Clemens D. Cohen.[1,3] *[1]Univ of Zurich, Zurich, Switzerland; [2]Univ Medicine Greifswald, Greifswald, Germany; [3]Klinikum Munich, Munich, Germany.*

Background: The best morphologic indicator of disease progression and development of end-stage renal disease is interstitial fibrosis and capillary rarefaction. Accumulating evidence suggests that dysregulation of hypoxia-regulated transcriptional mechanisms is involved in the loss of renal function and the development of chronic kidney disease (CKD) and hypoxia-induced transcription factors (HIFs) have relevance in the dysregulation of gene products in different renal cells.

Methods: Proximal tubular cells and conditionally immortalized podocytes with stable HIF1α and/or HIF2α suppression were generated. Gene expression profiles from cell lines and more than 160 renal biopsies from patients with different CKD stages were obtained using Affymetrix arrays. Weighted Correlation Network Analysis (WGCNA) was applied in order to identify modules of genes that showed highly correlated gene expression across cell groups (WT, HIF1α, HIF2α, HIF1α+2α) and conditions (hypoxia, normoxia). Gene sets from each module underwent GO-enrichment analysis using the topGO library for R, the Pathway System analysis as well as the transcription factor overrepresentation tool from Genomatix.

Results: Microarray analysis of hypoxia-treated renal cells revealed celltype-specific HIF1/HIF2-dependencies as well as dysregulation of several pathways in the cell lines. WGCNA analysis resulted in gene sets (modules) that were highly coregulated within the modules. Further characterization of the modules disclosed common as well as cell group- and condition-specific pathways, GO-Terms and transcription factors for each cell line. Expression of hypoxia-associated genes in genome-wide expression profiles revealed correlation with eGFR in cortical tubulointerstitial and glomerular biopsy specimens. Correlations were both positive and negative and in part compartment-specific.

Conclusions: Our gene expression analysis indicates a condition- and celltype-specific dysregulation of hypoxia-associated transcripts in renal cells.

FR-OR142

Renal Protective Effect of Proximal Tubules-Specific Ghrelin/GHSR System Through the Regulation of Oxidative Stress Levels Keiko Fujimura,[1] Shu Wakino,[1] Kazuhiro Hasegawa,[1] Koichi Hayashi,[1] Motoko Yanagita,[2] Hiroshi Itoh.[1] *[1]Dept of Medicine, Keio Univ, Tokyo, Japan; [2]Dept of Nephrology, Graduate School of Medicine Kyoto Univ, Kyoto, Japan.*

Background: Ghrelin (Ghr) discovered as an endogenous ligand for the Growth hormone secretagogue receptor (GHSR), has been reported to exert organ protective effects. We previously reported renal protective effects of Ghr against angiotensin II (AngII)-induced renal damages. We also demonstrated demonstrated anti-oxidative effects of endogenous Ghr/GHSR system by using GHSR deficient mice ($GHSR^{-/-}$) (PLoS One, 2014). In the present study, we examined the kidney-specific roles of Ghr/GHSR systems in renal function.

Methods: We first analyzed the phenotypes of $GHSR^{-/-}$ mice. We further crossed $GHSR^{-/-}$ mice with the N-Myc downstream-regulated gene 2 (NDRG2) Cre mice that harbor proximal-tubules (PT) specific Cre recombinase gene expression. With Cre recombinase, the expressions of GHSR in $GHSR^{-/-}$ mice were restored by the administration with tamoxifen (Tam) in a PT-specific manner in $GHSR^{-/-}$ mice with NDRG2-Cre gene (NDRG2 Cre^{+}-$GHSR^{-/-}$ mice). Renal senescence and oxidative stress levels were accessed by SA β-Gal and 4-HNE staining, respectively. Renal tissue damages were assayed by the urinary excretion of renal tubular marker,NAG and NGAL as well as by urinary protein excretion. The role of endogenous GHSR was evaluated by the knockdown experiment by using siRNA for GHSR in PT cell line, HK-2 cells.

Results: In $GHSR^{-/-}$ mice, tissue senescence and oxidative stress were enhanced, and renal tubular damages, urinary protein excretion and serum creatinine levels were also increased as compared to those in wild-type litter mice. The elongation of mitochondria was found in $GHSR^{-/-}$ mice in electron microscope. The renal damages in Cre^{+}-$GHSR^{-/-}$ mice with saline infusion were mitigated in Cre^{+}-$GHSR^{-/-}$ mice with Tam administration. By knockdown by siRNA for GHSR in HK-2 cells revealed the increase in mitochondria-derived oxidative stress levels.

Conclusions: Endogenous proximal-tubular Ghr/GHSR system is involved in renal senescence and renal oxidative stress. This system maintains renal function by regulating oxidative stress levels.

FR-OR143

Nox2 as a Modulator of Ischemia-Reperfusion-Induced Renal Fibrosis Aos S. Karim, Shannon Reese, Nancy A. Wilson, Arjang Djamali. *Medicine, Univ of Wisconsin SMPH, Madison, WI.*

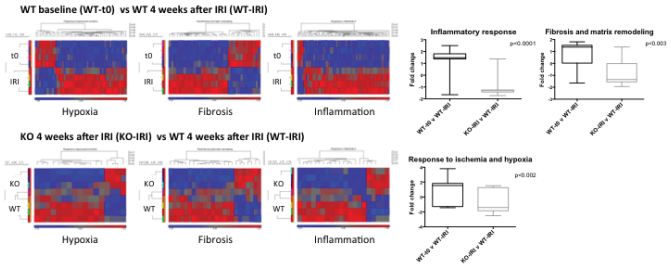
Background: Ischemia-reperfusion injury (IRI) is a significant complication of organ transplantation. Long ischemic time is associated with delayed graft function, which is mostly mediated through reactive oxygen species (ROS). We hypothesized that NADPH oxidase, specifically Nox2, plays an important role in ROS production and kidney injury in IRI.

Methods: Wild-type (WT) and Nox2 knockout (KO) mice were subjected to a right nephrectomy followed by clamping of the left renal artery for 30 min. The animals were followed for 4 weeks, when serum was collected for functional studies, and tissues were harvested for protein and genomic analyses.

Results: Kidney fibrosis (KF) was significantly reduced in tissues of KO compared to WT mice 4 weeks after IRI, as seen by the decline in picrosirius staining (collagen; $p<0.02$) and densitometry of α-smooth muscle actin immunoblots ($p<0.03$). Similarly, oxidative stress (OS; 4-hydroxynonenal and dihydroethidium) was significantly decreased ($p<0.03$ and $p< 0.02$ respectively) in KO compared to WT animals. Although BUN and creatinine levels were significantly increased in both KO and WT mice 4 weeks after IRI ($p<0.001$) compared to baseline, no significant differences were observed between the two groups, suggesting that alterations in OS and KF did not translate into functional changes.

	WT BUN	KO BUN	WT Creatinine	KO Creatinine
Baseline	26.2±0.9 mg/dL	20.4±0.5 mg/dL	0.11±0.01 mg/dL	0.11±0.01 mg/dL
4 weeks after IRI	38.7±1.3 mg/dL	39.7±1.7 mg/dL	0.19±0.01 mg/dL	0.19±0.01 mg/dL

Comparing WT to KO animals 4 weeks after IRI at the genomic level, we found that genes in pathways associated with KF, hypoxia and inflammation were significantly reduced in KO compared to WT animals.



Conclusions: Our studies suggest that Nox2 is a key contributor of oxidative stress and kidney fibrosis in this preclinical model of IRI.

Funding: NIDDK Support, Private Foundation Support

FR-OR144

Endothelial Dysfunction and Injury Control Podocyte Defects in Diabetic Nephropathy Susceptible Mice

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Background: The molecular signaling mechanisms between glomerular cell types during initiation/progression of glomerulosclerosis remain poorly understood. We demonstrated that podocyte dysfunction and depletion characteristic of various models of podocyte-initiated injury were mediated by podocyte-endothelial crosstalk and required endothelial mitochondrial oxidative stress (mtStress) and endothelial dysfunction. Oxidative stress and mitochondrial dysfunction are central mediators in the pathogenesis of diabetic complications including nephropathy (DN). We hypothesize that DN-susceptibility is characterized by glomerular endothelial mtStress-dependent endothelial dysfunction which is required for podocyte injury/loss.

Methods: Type I diabetes was induced by multiple, low-dose streptozotocin (STZ) injections in DN-susceptible DBA/2J (D2) and DN-resistant C57/Bl6 (B6). Osmotic minipumps delivering mitoTEMPO (1mg/kg/day) or vehicle were implanted s.c. prior to onset of hyperglycemia. Kidneys were harvested at 1, 3, 6, 12 weeks following hyperglycemia. Glomerular endothelial cells (mGEC) and podocytes were used in vitro.

Results: One week after hyperglycemia uncoupled oxygen consumption was significantly reduced in STZ-D2 glomeruli, but not in STZ-B6 compared to controls. STZ-D2 mice had increased mtDNA lesions, oxidative stress markers (3NT) and oxidative DNA damage (8-oxoG in mtDNA), exclusively localized to endothelial cells after 3 weeks of comparable hyperglycemia. The endothelial dysfunction and injury in turn was required for podocyte apoptosis as selective mitochondrial-targeted ROS scavenging (mitoTEMPO) prevented podocyte loss, ameliorated albuminuria, and reduced glomerulosclerosis lesions in STZ-D2 mice. In vitro treatment of mGEC cultures with mitoTEMPO prevented mtStress, fragmentation and decreased NOS activity induced by incubation with diluted STZ-D2 serum in media.

Conclusions: Our results demonstrate that mtStress and dysfunction in glomerular endothelial cells precede and mediate in part albuminuria, podocyte defects and depletion, and glomerulosclerosis in diabetic DN susceptible D2 mice. Endothelial dysfunction is not detectable in diabetic DN-resistant B6 mice.

Funding: NIDDK Support

FR-OR145

The Polycomb Repressor Complex and Smad3 Constitute a Switch Enhancing Complex that Interprets TGF-β Signaling to Control Cell Fate in Diabetic Microvascular Complications

Letizia De Chiara, Hayley Beaton, Catherine Godson, John Crean. *UCD School of Biomolecular and Biomedical Science, Univ College Dublin, Dublin, Ireland.*

Background: Developmental cell fate decisions are controlled by the interplay of transcription factors and epigenetic modifiers which together determine cellular identity. During the initiation and progression of diabetic complications cells within affected tissues undergo a process of reprogramming, evoking gene expression profiles reminiscent of ontogenesis.

Results: We have identified a context dependent switch enhancing complex comprising Smad 3 and PRC2 that regulates cell fate during embryonic differentiation and is reactivated during fibrotic processes. Our data suggests that the H3K27 methylating polycomb repressor complex 2 regulates the silencing of TGF-β activated genes during cell differentiation and commitment. Gene expression profiles of renal cells undergoing TGF-β mediated differentiation has revealed a number of transcription factors that co-segregate with Smad3 including the Yamanaka reprogramming factors Oct4, Sox2 and Nanog, while differential expression of these factors in response to TGF-β was clearly demonstrated by western analysis of chromatin associated proteins. Analysis of H3K27 trimethylation revealed that during the process of differentiation the histone mark is turned on, suggesting PRC2 mediated remodeling of the chromatin and switching of gene expression from a permissive or "ON" state to non-permissive or "OFF". Intriguingly proteomic analysis of the EZH2 interactome revealed a number of direct associations with a transcription factor network including phosphorylated Smad 3, TEAD4, Oct 4 and Sox2, suggesting that cooperative recruitment of PRC2 regulates gene repression during TGF-β mediated differentiation. Furthermore, treatment of cells with the specific EZH2 inhibitor 3 deazaneplanocin, inhibited TGF-β mediated epitheial dedifferentiation and blocked the expression of genes associated with fibrosis.

Conclusions: This data demonstrates for the first time a functional association between Smad signaling and the polycomb repressor complex with potential for therapeutic manipulation during the progression of renal disease.

Funding: Government Support - Non-U.S.

SA-OR001

Laboratory and Biological Variation in Serum Creatinine Measurement Results in High False Positive Rate of Acute Kidney Injury

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Background: Inclusion of small serum creatinine changes in the Kidney Disease: Improving Global Outcomes (KDIGO) acute kidney injury (AKI) definition may increase diagnostic false positive (FP) rates.

Methods: We created a data set of simulated non-AKI patients with true creatinine values that were constant over time. We simulated random variation in measured creatinine values using known coefficients of variation for the modified Jaffe rate reaction and a range of expected biological variation. AKI was defined when changes in measured creatinine values met KDIGO criteria. We also generated alternative criteria for AKI diagnosis and applied them to the simulated data set and a pre-existing cohort of 734 adult patients with at least stage 1 AKI (by KDIGO definition) to evaluate if these criteria capture a larger proportion of patients experiencing progression of AKI, initiation of renal replacement therapy, and inpatient mortality.

Results: After four simulated creatinine measurements, subjects with true creatinine values above 1.5 mg/dL had a FP AKI rate of 57.7% compared to 8.8% among those with lower creatinine ($p<0.001$).

AKI Definition	FP Rate at 4 Tests (%)			FP Rate at 7 Tests (%)		
	Total	Cr < 1.5	Cr ≥ 1.5	Total	Cr < 1.5	Cr ≥ 1.5
KDIGO Criteria	17.2	8.8	57.7	32.7	22.2	85.2

In contrast, our alternative approaches to AKI diagnosis had FP rates ranging from 2% to 36.2% in the higher creatinine group and from 0.7% to 4.5% in the lower creatinine group. Compared to KDIGO, our alternative criteria also captured a higher proportion of the AKI cohort that progressed to at least AKIN stage 2, required dialysis, or died ($p<0.05$ for all alternative criteria).

Conclusions: Use of small serum creatinine changes to diagnose AKI is limited by high FP rates due to inherent variability within the analyte. FP rates increase markedly at higher baseline creatinine values, potentially limiting the performance of the KDIGO AKI diagnostic criteria in the CKD population. Alternative approaches to diagnosing AKI may improve prediction of clinically relevant outcomes in an acutely ill population.

Funding: NIDDK Support

SA-OR002

Patterns of Recovery from Acute Kidney Injury (AKI) and Risk of Kidney Disease Progression in the Irish Population

Austin G. Stack,[1,2] Els H. Gillis,[1,2] Mohamed Elsayed,[1,2] Hoang Thanh Nguyen,[2] Ailish Hannigan,[2] Patrick T. Murray,[3] Howard Johnson,[4] Liam F. Casserly,[1] John P. Ferguson.[2] *[1]Nephrology, Univ Hospital Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland; [3]School of Medicine and Medical Sciences, Univ College Dublin, Ireland; [4]Health Intelligence Directorate, Health Services Executive, Dr Steevens Hospital, Dublin, Ireland.*

Background: There are limited data on the patterns of recovery from Acute Kidney Injury (AKI) and its clinical consequences in the Irish Health System. The aim of this study was to describe the patterns of recovery from AKI and risk of kidney disease progression.

Methods: We determined incidence rates and recovery rates from AKI in 207, 336 adults, age > 18 years, in the Irish Health System from 2005-2011. The diagnosis of AKI was based on standardised creatinine measurements using KDIGO classification. Recovery was defined as complete (return of function to within 1.10 times baseline creatinine); partial (1.10 and 1.50 times baseline), or failure to recover (creatinine >1.50 times baseline or dialysis) within 28-days of initial creatinine rise. We modelled time to 50% decline in eGFR among survivors of AKI who did not require immediate dialysis. Cox regression was used to explore associations of recovery status with risk of CKD progression in multivariable models with adjustment for baseline health and location of medical supervision.

Results: There were 25,744 episodes of AKI.Overall, 40.6 % (n=10, 442) of patients had

complete, 5.5 % (n=1, 409) partial, and 12.4 % (n=3, 192) non-recovery of kidney function. Median eGFR declined significantly from baseline to 12 months post AKI event by at least 3ml/min, *P<0.001]*. Compared to patients who had complete recovery (referent, Hazard Ratio =1.00), those with partial or failure to recover experienced significantly higher risks of CKD progression with hazard ratios of 1.62 (95% CI 1.37-1.89) and 6.97 (5.94-8.16) respectively. In total, 282 patients (1.1%) developed immediate ESKD and contributed to between 12 to 45 % of the incident dialysis population.

Conclusions: This is the first study to describe patterns of recovery from AKI in the Irish health system and its deleterious impact on CKD progression.

Funding: Government Support - Non-U.S.

SA-OR003

Development and Validation of a Risk Score for Predicting Acute Kidney Injury in Intensive Care Unit Patients

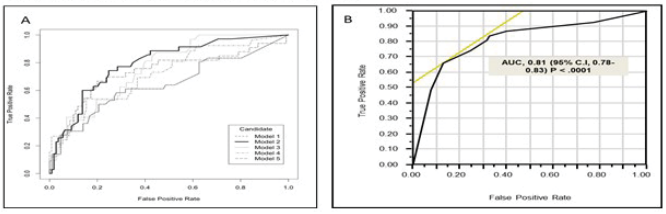
Rakesh Malhotra,[1,5] Etienne Macedo,[2] Josee Bouchard,[3] Kianoush Banaei-Kashani,[4] Ravindra L. Mehta.[5] *[1]VUMC; [2]U Sao Paulo; [3]U Montreal; [4]Mayo Clinic; [5]UCSD.*

Background: Acute kidney injury (AKI) is associated with high morbidity and mortality rates in critically ill patients. Early identification of risk factors for AKI provides an opportunity to develop strategies for prevention, early diagnosis and treatment.

Methods: We undertook multicenter prospective cohort study to develop and validate a risk score to predict AKI. Baseline risk factors were ascertained at ICU admission and additional acute factors within the first 48 hrs of ICU stay. A risk score was developed using multivariable logistic regression based on baseline and acute risk factors in the training cohort (573 patients) and the score was further evaluated in a test cohort (144 patients). Validation was performed in an independent cohort of 1280 patients. Discrimination of the scoring model was examined by the Area Under the Receiver Operating Characteristic Curve (ROC) and model calibration was evaluated by Hosmer-Lemeshow test.

Results: In the multivariate model, chronic kidney disease (CKD), chronic liver disease, congestive heart failure (CHF), hypertension (HTN), atherosclerotic coronary vascular disease (ASCVD), pH≤7.30, nephrotoxin exposure, sepsis, mechanical ventilation and anemia were identified as independent predictors of AKI and the C-statistic for the model in the test cohort was 0.79 (0.70-0.89). Validation revealed predictive accuracy of 0.81 (0.78-0.83). The risk score model demonstrated good calibration in both cohorts.

Figure 1: Area under curve of risk model for prediction of acute kidney injury in a) test cohort (n=144 patients) and b) Mayo Clinic Validation cohort (N=1280)



Conclusions: A risk scoring system based on chronic comorbidities and acute events can identify ICU patients at high risk to develop AKI and adverse outcomes within 48 hours of ICU admission. This risk assessment tool could help clinicians to stratify patients for surveillance, prevention and early therapeutic intervention.

SA-OR004

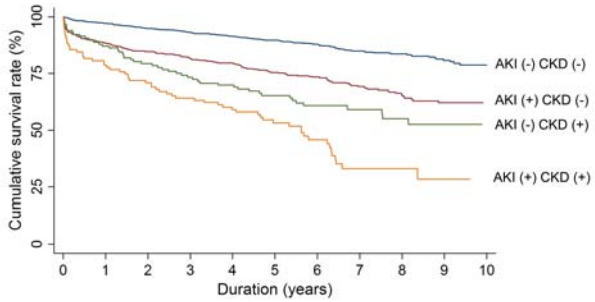
Synergistic Effects of Acute Kidney Injury and Chronic Kidney Disease on the Mortality After Coronary Artery Bypass Graft

Seung Seok Han,[1] Seon Ha Baek,[1,2] Dong Ki Kim,[1] Sejoong Kim,[1,2] Ho Jun Chin,[1,2] Dong-Wan Chae,[1,2] Ki Young Na.[1,2] *[1]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Internal Medicine, Seoul National Univ Bundang Hospital, Gyeonggi-do, Korea.*

Background: Both acute kidney injury (AKI) and chronic kidney disease (CKD) are known to predict mortality in the patients undergoing coronary artery bypass grafting (CABG). However, the synergistic effect between AKI and CKD on the long-term mortality remains unresolved.

Methods: Data on 1904 patients who underwent CABG in two major hospitals from 2004 to 2010 were collected. The hazard ratios (HRs) for all-cause mortality were calculated using Cox model after adjustment of multiple covariates. For evaluating the synergistic effect on 1-year mortality between AKI and CKD, the relative excess risk due to interaction (RERI) was used; positive results for RERI and a value greater than 1 for the synergy index means a positive interaction or more than additivity between variables. The discrimination of predicting mortality was assessed by the area under the curve (AUC) of calculating the receiver operating characteristic curve.

Results: Among the patients, 11.5% had underlying CKD and 33.4% encountered AKI after CABG. During the median follow-up period of 5 years (max. 10 years), patients with AKI or CKD had a high risk of mortality than their counterparts: HR in AKI versus non-AKI, 1.85 (1.513–2.270); HR in CKD versus non-CKD, 2.26 (1.761–2.891).



Number at risk

AKI (-) CKD (-)	1150	1116	1091	1071	974	809	620	437	283	153	40
AKI (+) CKD (-)	533	471	451	435	397	317	252	190	123	72	26
AKI (-) CKD (+)	116	101	92	85	81	65	47	32	23	14	5
AKI (+) CKD (+)	103	81	73	66	57	40	28	14	8	5	2

There was a relative excess risk of mortality when both AKI and CKD were considered together: RERI, 0.72 (-4.786 – 6.225); synergy index, 1.10 (1.962–2.242). When AUCs for predicting mortality were calculated based on the timeframe, the AUC of AKI decreased over time, but the AUC of CKD was relatively steady irrespective of the timeframe.

Conclusions: The present study first demonstrated the synergistic effects of AKI and CKD on the mortality in patients undergoing CABG.

SA-OR005

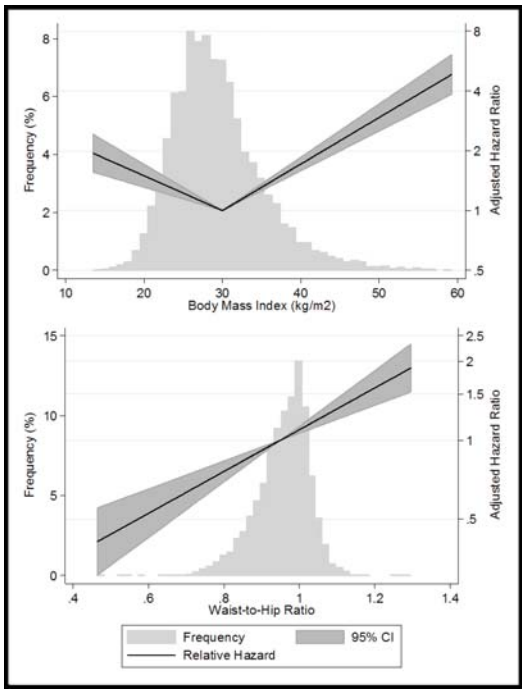
Obesity, Weight Distribution, and the Risk of Acute Kidney Injury: The Atherosclerosis Risk in Communities Study

Keiko I. Greenberg, Josef Coresh, M. Grams. *Dept of Medicine, The Johns Hopkins Univ School of Medicine, Baltimore, MD.*

Background: Elevated body mass index (BMI) at time of hospital admission has been associated with acute kidney injury (AKI) in surgical and critically ill patients. The association of BMI and measures of central obesity with AKI in the population-based setting is unknown.

Methods: Participants from the Atherosclerosis Risk in Communities (ARIC) study visit 4 (1996-8) with eGFR >15ml/min/1.73 m^2 and without a previous history of AKI were included (N=11,063). All participants were followed for subsequent hospitalizations; AKI was defined by billing code. Cox proportional hazards models were adjusted for age, sex, race, hypertension, diabetes, coronary artery disease, eGFR, albuminuria, BMI (linear spline, knot at 30 kg/m^2), waist-to-hip ratio (WHR), and time-varying number of hospitalizations, censoring at 12/31/2010, death, or ESRD.

Results: At baseline, mean age was 63.3 years, mean BMI was 28.8 kg/m^2, and mean waist-to-hip ratio was 0.95. Over 12 years of follow-up, 825 participants developed AKI. Higher BMI and higher WHR were independently associated with AKI. For BMI > 30, a 1 kg/m^2 increase was associated with a 6% increase in risk of AKI (95% CI: 1.05-1.06, p < 0.001); for BMI < 30, a 1kg/m^2 increase was associated with a 4% decrease in AKI risk (95% CI: 0.95-0.97, p < 0.001).



A 0.05 increase in WHR was associated with a 10% increase in risk of AKI (95% CI: 1.06-1.13; p < 0.001). The association of higher WHR and AKI was stronger in those with higher BMI (p for interaction < 0.001).

Conclusions: In the ARIC cohort, BMI over 30 and higher waist-to-hip ratio were independently associated with increased risk of hospitalized AKI. Prevention strategies for AKI may benefit the obese population.

Funding: NIDDK Support, Other NIH Support - The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C)

SA-OR006

Associations of eGFR and Albuminuria with Acute Kidney Injury in Individuals with and without Diabetes and Hypertension: A Collaborative Meta-Analysis

Matthew T. James, M. Grams, Mark Woodward, Carolyn Raina Elley, Jamie Alton Green, David C. Wheeler, Ron T. Gansevoort, Andrew S. Levey, David G. Warnock, Mark J. Sarnak. *CKD Prognosis Consortium.*

Background: Measures of chronic kidney disease, including reduced estimated glomerular filtration rate (eGFR) and increased albuminuria, are risk factors for subsequent acute kidney injury (AKI). Diabetes mellitus and hypertension often coexist with CKD and are risk factors for AKI; whether the associations between eGFR and urine albumin creatinine ratio (ACR) with AKI are consistent in the presence and absence of these conditions has not been established.

Methods: We performed a meta-analysis of 13 cohorts, 8 general population (1,284,751 participants) and 5 chronic kidney disease (79,496 participants) cohorts, within the Chronic Kidney Disease Prognosis Consortium, and identified AKI based on validated administrative codes. We used Cox proportional hazards models to estimate hazard ratios (HR) of AKI associated with eGFR and ACR in individuals with and without diabetes and hypertension. Random effects meta-analysis was used to pool results across cohorts.

Results: Low eGFR and high ACR were associated with higher risks of AKI in individuals with or without diabetes and with or without hypertension in both general population and chronic kidney disease cohorts. The risk gradient for AKI with lower eGFR was greater in those without diabetes than with diabetes (adjusted HR for eGFR 45 compared to 80 mL/min/1.73m^2 4.01 [3.12-5.16] versus 2.85 [2.05-3.96]), but similar with higher ACR in those without versus with diabetes (adjusted HR for ACR 300 compared to 5 mg/g 2.24 [1.89-2.80] versus 2.25 [1.71-2.96]). The risk gradients for AKI with both lower eGFR and higher ACR were greater for those without versus with hypertension (adjusted HR at eGFR 45 compared to 80 mL/min/1.73m^2 5.43 [4.79-6.16] versus 2.78 [2.06-3.76] and for ACR 300 mg/g compared to 5 mg/g 3.19 [2.24-4.56] versus 2.01 [1.69-2.38]).

Conclusions: Lower eGFR and higher albuminuria provide prognostic information about the risk of AKI in people with or without either diabetes or hypertension.

Funding: NIDDK Support

SA-OR007

The Association of Age, Sex, Race, and Kidney Measures with Acute Kidney Injury: CKD Prognosis Consortium Analysis of 1,364,568 Participants in 13 Cohorts

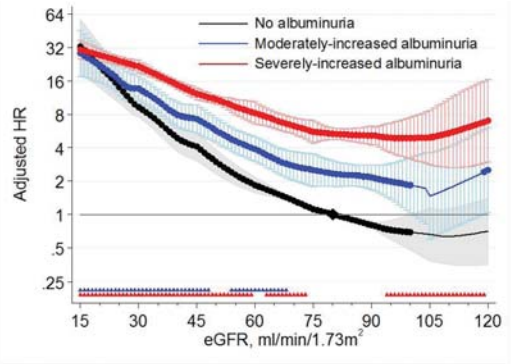
M. Grams, Yingying Sang, Shoshana Ballew, Ron T. Gansevoort, Csaba P. Kovesdy, David M. Naimark, Cecilia Montgomery Øien, David Smith, Josef Coresh, Mark J. Sarnak, Benedicte Stengel, Marcello Tonelli. *CKD Prognosis Consortium.*

Background: Acute kidney injury (AKI) is a global public health problem. The risk of AKI associated with eGFR and albuminuria (albumin to creatinine ratio, ACR) across categories of age, sex, and race has not been quantified.

Methods: Using data on 1,364,568 participants (1,285,049 in general population cohorts; 79,519 in chronic kidney disease cohorts) from 13 cohorts in 8 countries, we tested the association between eGFR, ACR and hospitalization with AKI (identified by diagnostic codes) using a two-stage meta-analysis. We also assessed how the associations of age, sex, and race with AKI vary by eGFR and ACR.

Results: Among general population cohorts, 1% of participants were hospitalized with AKI over 4 years. Lower eGFR and higher ACR were strongly associated with AKI in meta-analysis. Compared with eGFR 80 ml/min/1.73 m^2, the adjusted hazard ratio of AKI at eGFR 45 ml/min/1.73 m^2 was 3.35 (95% CI: 2.75 to 4.07). Compared with ACR 5 mg/g, the adjusted hazard ratio of AKI at ACR 300 mg/g was 2.73 (95% CI: 2.18 to 3.43). Older age was associated with higher risk of AKI than younger age in the higher range of eGFR or lower range of ACR, but this effect was attenuated in lower eGFR or higher ACR. Male sex was associated with higher risk of AKI at all levels of eGFR and ACR, with a slight attenuation in lower eGFR but not in higher ACR. In the 2 cohorts contributing to analysis by race, black race was associated with higher AKI risk than white race at higher levels of eGFR and most levels of ACR.

Conclusions: Reduced eGFR and increased ACR are consistent, strong risk factors for AKI whereas the strength of the associations between AKI, age, and sex is weaker in later stages of CKD.



Bold lines indicate statistical significance compared to the reference (black diamond) at eGFR 80 ml/min/1.73 m^2. Stars along the x-axis represent significant pointwise interactions. HRs are adjusted for sex, race, body mass index, systolic blood pressure, total cholesterol, history of cardiovascular disease, diabetes, and smoking status.

Funding: NIDDK Support

SA-OR008

Volatile Anesthetics in Preventing Acute Kidney Injury following Cardiac Surgery

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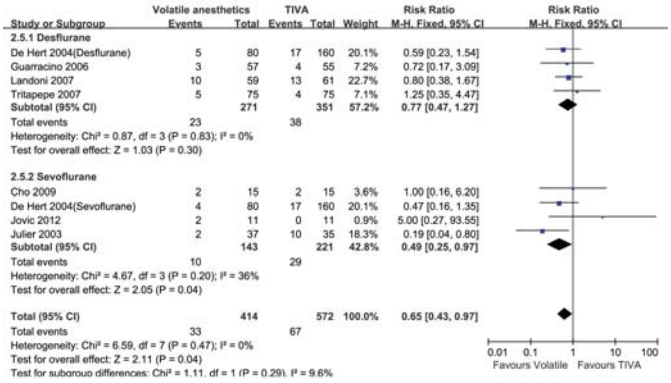
Background: Acute kidney injury (AKI) is a common clinical complication of cardiac surgery. Volatile anesthetics have been shown to protect against AKI injury in animal experiments. However, in clinical setting, the effect of volatile anesthetics was unclear. We conducted a systematic review and meta-analysis of randomized controlled trials (RCTs) to explore whether volatile anesthetics could provide renal protection to patients undergoing cardiac surgery.

Methods: RCTs were identified in the PubMed, OVID, EMBASE, the Cochrane Library, the Current Controlled Trials Register, reviews, and reference lists of relevant articles. 10 trials with a total of 1600 participants were eligible. Data were analyzed using both fixed and random effects model.

Results: Compared with controls, volatile anesthetics significantly reduced AKI incidence [relative risk (RR): 0.65, 95% CI: 0.43 to 0.97; p=0.04)].



Although there was no significant difference between the two groups in absolute postoperative serum creatinine level and mortality, patients receiving volatile anesthetics had significantly (or borderline) lower increase in serum creatinine level from baseline on the first [weighted mean difference (WMD): -0.04 mg/dl, 95% CI: -0.07 to -0.01 mg/dl; p=0.002] and second day postoperatively (WMD: -0.07 mg/dl, 95% CI: -0.14 to -0.00 mg/dl; p=0.05), and reduced incidence of prolonged ICU stay (RR: 0.46, 95% CI: 0.34 to 0.64; p<0.001) and hospitalization (RR: 0.47, 95% CI: 0.27 to 0.83; p=0.009).

Conclusions: Current evidence showed that volatile anesthetics might provide renal protection in patients undergoing cardiac surgery, and supports further RCTs with larger sample size and high-methodological quality.

SA-OR009

Increased Risk of Elevated Blood Pressure (BP) after Acute Kidney Injury (AKI)

Chi-Yuan Hsu,[1] Raymond K. Hsu,[1] Jingrong Yang,[2] Juan Daniel Ordonez,[2] Sijie Zheng,[2] Alan S. Go.[2] *[1]Univ of California San Francisco; [2]Kaiser Permanente Northern California.*

Background: AKI leading to residual renal parenchymal injury may be a hitherto unrecognized cause of elevated BP, a major cardiovascular risk factor.

Methods: We studied adult members of Kaiser Permanente Northern California, a large integrated health care delivery system, who were hospitalized in 2008-2011. All participants had serum creatinine (Cr) and BP documented before admission and were not known to have hypertension or BP >140/90mmHg. AKI was defined using changes in inpatient serum Cr. Study outcome was BP >140/90mmHg measured during an ambulatory, non-emergency department visit within 2 years after discharge.

Results: Among 43,611 eligible patients, mean age was 56 yrs, 60% were women, and median eGFR=137 ml/min/1.73m^2. Overall, 2451 experienced AKI (71.0% AKIN stage 1, 15.3% stage 2 and 13.7% stage 3). AKI survivors were more likely to have BP >140/90mmHg during follow-up (46.1% at 730 days versus 41.2%; p<0.001). This was evident within the first 180 days (30.6% versus 23.1%, p<0.001). After adjustment for potential confounders, AKI was independently associated with a 22% (95% CI 12% to 33%) increased odds of developing elevated BP during follow-up. More severe AKI was associated with higher odds of elevated BP (Table).

Adjusted OR of BP >140/90 mmHg post discharge	**180 days (N=40,861)**	**365 days (N=42,845)**	**540 days (N=43,407)**	**730 days (N=43,611)**
Stage 1 vs no AKI	1.23 (1.10-1.37)	1.21 (1.09-1.34)	1.13 (1.02-1.26)	1.09 (0.98-1.21)
Stage 2 vs no AKI	1.66 (1.33-2.08)	1.53 (1.23-1.90)	1.51 (1.22-1.87)	1.45 (1.17-1.79)
Stage 3 vs no AKI	2.18 (1.74-2.74)	2.17 (1.73-2.71)	1.89 (1.51-2.37)	1.82 (1.45-2.29)

Results are similar in sensitivity analyses after excluding those with pre-admission chronic kidney disease or when the outcome was defined as having at least 2 BP readings >140/90mmHg.

Conclusions: AKI during hospitalization is an independent risk factor for subsequent development of elevated BP, which may be a more subtle sign of residual kidney damage than clinically evident elevations in serum Cr. Preventing the development of AKI may have clinical and public health benefits beyond the immediate hospitalization.

Funding: NIDDK Support

SA-OR010

Impact of Acute Kidney Injury on Mortality Diminishes with Greater Severity of Illness Andrew Moore,[2] Jean-Sebastien Rachoin,[1] Lawrence S. Weisberg.[1] *[1]Medicine, Cooper Univ Hospital-Rowan Medical School of Rowan Univ, Camden, NJ; [2]Medicine, Cambridge Health Alliance-Harvard Univ, Cambridge, MA.*

Background: Acute kidney injury (AKI) is independently associated with mortality. It is unclear, however, whether the impact of AKI on mortality is uniform across the range of severity of illness. We hypothesized that the odds ratio (OR) of death with AKI would be consistent across severity of illness.

Methods: We performed a retrospective observational study of all patients admitted to an ICU in the U.S. and Canada with sepsis or septic shock, from 2003 through 2009, and entered in the Project Impact® database. Patients were categorized by severity of illness using the admission APACHE II score. We defined AKI as that requiring renal replacement therapy (RRT). We analyzed mortality by APACHE II strata.

Results: 68,471 patients were initially considered for inclusion, of whom 40,332 had sepsis or septic shock. 6,773 were excluded because of a diagnosis of chronic kidney disease. 6,039 were excluded because of insufficient information leaving 27,510 records for analysis. The median [IQR] APACHE II score was 20 [10]. 2,625 (9.5%) of these septic patients developed AKI /RRT. 8,795 of the 27,510 patients died in hospital. Using logistic regression models, AKI / RRT was associated with increased mortality, with an adjusted OR 2.7. We divided the sample by APACHE II into the following strata: < 15, 15-20, 21-25, >25. As the APACHE II score increased, so did mortality (Z=-50, p<.01). In the APACHE II <15 group the mortality rate was 13.3% while in the APACHE II >25 group the mortality rate was 56.6%. After adjusting for confounding variables OR of mortality for patients with AKI / RRT declined in the higher APACHE II strata: For the APACHE II < 15 group OR 5.4 (95% CI, 4.1-7.1) and for the APACHE II > 25 OR was 1.8 (95% CI, 1.6-2.0).

Conclusions: Thus, AKI appears to be associated with a disproportionate increase in the risk of death for patients with lower severity of illness scores and its independent impact seems to diminish with higher APACHE II scores. Future studies of AKI in which mortality is an outcome of interest should take this finding into account.

SA-OR011

Association of Urine KIM-1 and NAG with CKD Progression: Results from the CRIC Study Chi-Yuan Hsu, Dawei Xie, Xiaoming Zhang, Sushrut S. Waikar, Venkata Sabbisetti, Joseph V. Bonventre, Josef Coresh, Robert G. Nelson, Clarissa Jonas Diamantidis, Claudia M. Lora, Francis Perry Wilson, Edgar R. Miller, Jiang He, Jeffrey R. Schelling, Mahboob Rahman, Akinlolu O. Ojo, Paul L. Kimmel, Harold I. Feldman, Vasan S. Ramachandran, Kathleen D. Liu. *For the NIDDK CRIC Study and CKD Biomarkers Consortium.*

Background: In chronic kidney disease (CKD), biomarkers of renal tubular injury may be elevated due to ongoing injury and therefore associated with CKD progression.

Methods: Baseline urine kidney injury molecule-1 (KIM-1) and N-acetylglucosaminidase (NAG) concentrations were determined among 2450 Chronic Renal Insufficiency Cohort (CRIC) participants. Cox models were used to examine the independent association between urine KIM-1 and NAG indexed to urinary creatinine concentration and CKD progression, defined as halving of estimated GFR or incident ESRD.

Results: Baseline mean eGFR was 43.6 ml/min/1.73m^2; median protein/Cr ratio 0.14 g/g. 581 events occurred during 5.5 median years of followup. After adjustment for baseline eGFR and proteinuria as well as age, gender, race, education, enrolling center, diabetes, cardiovascular disease, systolic BP, body mass index, and ACE inhibitor/ARB use, KIM-1/Cr was an independent risk factor for CKD progression by quintile analysis (table) or continuous analysis (Hazard Ratio [HR]1.16 per log SD increase, p=0.02). NAG/Cr was significantly associated with CKD progression in univariate analysis and in the continuous analysis (HR per log SD increase 1.13, p = 0.049).

Quintiles of KIM-1/Cr (pg/g)	Unadjusted HR (95%CI)	Adjusted HR	Quintiles of NAG/Cr (U/g)	Unadjusted HR	Adjusted HR
≤661.1	Ref	Ref	≤2.07	Ref	Ref
>661.1-1110.3	1.51 (1.04-2.19)	1.0 (0.66-1.5)	>2.07-3.03	2.2 (1.38-3.51)	1.31 (0.78-2.2)
>1110.3-1829.8	2.25 (1.59-3.19)	1.2 (0.81-1.79)	>3.03-4.97	4.5 (2.93-6.92)	1.3 (0.79-2.13)
>1829.8-2981.7	3.79 (2.73-5.26)	1.33 (0.9-1.95)	>4.97-8.37	8.19 (5.41-12.4)	1.34 (0.82-2.21)
>2981.7	7.9 (5.76-10.83)	1.55 (1.03-2.31)	>8.37	15.81 (10.54-23.72)	1.54 (0.92-2.57)

Conclusions: Higher levels of biomarkers of renal tubular injury are associated with CKD progression after adjustment for traditional risk factors, including eGFR and proteinuria.

Funding: NIDDK Support

SA-OR012

Serum β-Trace Protein and β-2 Microglobulin as Predictors End-Stage Renal Disease in Adults with Chronic Kidney Disease Meredith C. Foster, Josef Coresh, Chi-Yuan Hsu, Andrew S. Levey, Robert G. Nelson, John H. Eckfeldt, Vasan S. Ramachandran, Paul L. Kimmel, Jeffrey R. Schelling, Michael S. Simonson, James H. Sondheimer, Amanda Hyre Anderson, Sanjeev Akkina, Harold I. Feldman, John W. Kusek, Akinlolu O. Ojo, Lesley Inker. *CKD Biomarkers Consortium and the CRIC Study.*

Background: Serum β-trace protein (BTP) and β-2 microglobulin (B2M) independently associate with ESRD and mortality in both general and high risk populations. Less is known about associations with renal outcomes in adults with moderate chronic kidney disease (CKD).

Methods: In the Chronic Renal Insufficiency Cohort (n=3479, mean age 58 yrs; 44% women), we used multivariable (MV) Cox proportional hazards models to estimate hazard ratios (HR) for ESRD by marker quintile for eGFRcr, BTP, and B2M (Referent: Q1, reflecting lowest marker level/highest filtration). We also estimated the HRs for a composite score representing a weighted linear combination of continuous1/BTP, 1/B2M, eGFRcr, and eGFRcys. BTP, B2M, and composite MV models were subsequently adjusted for eGFR creatinine-cystatin C (eGFRcr-cys).

Results: Over 6.1 years median follow-up, 725 (21%) individuals developed ESRD. Higher BTP and B2M and a composite score were associated with increased ESRD risk (**Table**). After adjustment for eGFRcr-cys, associations in the highest quintiles persisted: BTP (HR 2.3, 95% CI 1.2-4.4), B2M (HR 3.1, 95% CI 1.5-6.6), and 4-marker composite score (HR 5.5, 95% CI 2.1-14.7). Overall the composite had stronger HRs but overlapping 95% CI with other markers.

Conclusions: BTP and B2M are associated with increased risk of ESRD in adults with CKD, even with adjustment for clinically available estimates of kidney function.

Table: Adjusted° associations with ESRD

	Q2	Q3	Q4	Q5
	HR(95% CI) (Referent:Q1)*			
eGFRcr	3.4 (2.0, 5.8)	5.0 (3.0, 8.3)	8.7 (5.3, 15)	19 (12, 31)
BTP	2.1 (1.1, 4.0)	4.4 (2.4, 8.1)	8.4 (4.7, 15)	18 (10, 33)
B2M	2.9 (1.5, 5.7)	5.3 (2.8, 10)	12 (6.4, 23)	26 (14, 49)
Composite	2.8 (1.3, 6.0)	7.7 (3.7, 16)	13 (6.6, 27)	36 (17, 73)

°Adjusted for age, sex, race, HDL cholesterol, C-reactive protein, smoking status, cardiovascular disease, hypertension, diabetes, and ln(ACR)
*All p<0.05

Funding: NIDDK Support

SA-OR013

Novel and Traditional Factors Associated with Kidney Function Decline Over Time: Findings From the Chronic Renal Insufficiency Cohort (CRIC) Study Amanda Hyre Anderson,[1] Dawei Xie,[1] Jason Roy,[1] Lawrence J. Appel,[2] Laura M. Dember,[1] Jiang He,[2] John W. Kusek,[2] James P. Lash,[2] Sankar D. Navaneethan,[2] Akinlolu O. Ojo,[2] Mahboob Rahman,[2] Julia J. Scialla,[2] James H. Sondheimer,[2] Susan P. Steigerwalt,[2] Francis Perry Wilson,[1] Myles S. Wolf,[2] Harold I. Feldman.[1] *[1]Univ of Pennsylvania; [2]The CRIC Study.*

Background: Traditional risk factors only partially explain declines in glomerular filtration rate (GFR) among those with chronic kidney disease (CKD). We examined a set of novel risk factors potentially explaining declines in GFR.

Methods: Data from participants of the CRIC Study (N=3,517; median follow-up 6 yrs) with GFR estimated using a serum creatinine and cystatin C equation were analyzed

using mixed effects models to identify traditional (demographic, clinical, behavioral, and biochemical) and novel (mineral metabolism, inflammation, cardiac strain, anthropometric, and carbohydrate metabolism (CHO)) factors associated with declines in eGFR. Prespecified stratified models were fit within diabetic and nondiabetic groups.

Results: Mean (SD) annual change in eGFR was -1.9 (4.3) mL/min/1.73m²/year overall. In multivariable-adjusted models, race/ethnicity (p<0.001), baseline eGFR (p=0.008), systolic blood pressure (p<0.001), proteinuria (p<0.001), body mass index (p=0.04), parathyroid hormone (p=0.04), serum phosphate (p=0.02), and NTproBNP (p<0.001) were all associated with eGFR decline. FGF23, high-sensitivity CRP, high-sensitivity troponin T, fat-free mass from bioelectrical impedance analysis, HOMA-IR, and HbA1c were not significantly associated with eGFR decline over time. Stratified analyses by diabetes revealed similar associations except that higher levels of baseline eGFR were associated with faster declines in eGFR among diabetics and slower declines among nondiabetics.

Conclusions: Among a large set of potentially novel risk factors, selected measures of mineral metabolism and cardiac strain, but not markers of inflammation or CHO, helped to explain eGFR decline among a large cohort with CKD after accounting for traditional factors. The relationship between baseline eGFR and decline in eGFR differed across diabetes groups.

Funding: NIDDK Support

SA-OR014

Urine Collagen Fragments Predict CKD Progression: The Cardiovascular Health Study Joachim H. Ix,[1] Mary L. Biggs,[2] Kenneth J. Mukamal,[3] David Siscovick,[4] Ronit Katz,[2] Joseph Delaney,[2] Dena E. Rifkin,[1] Jan M. Hughes-Austin,[1] Pranav S. Garimella,[5] Mark J. Sarnak,[5] Michael Shlipak,[6] Jorge R. Kizer.[7] *[1]UCSD; [2]UW; [3]BI; [4]NYAM; [5]Tufts; [6]UCSF; [7]Einstein.*

Background: Renal fibrosis on biopsy predicts ESRD and is common with aging, but is poorly captured by eGFR and ACR. Pro-collagen III N-propetide (PIIINP) is cleaved from collagen during fibrosis and urine levels correlate with fibrosis severity on biopsy. Whether urine PIIINP is associated with CKD progression is unknown.

Methods: At the 1996-97 CHS examination, we designed a case-control study to evaluate associations with CKD progression over 9 years. We selected 192 CKD progression cases (30% eGFR decline) and 231 randomly selected non-CKD progressing controls. Separately, we designed a case-cohort study to evaluate incident ESRD (958 random sub-cohort participants and 54 incident ESRD cases). PIIINP was measured in spot urine specimens by radio-immuno assay.

Results: Mean age was 78 years, eGFR was 63 ± 18 ml/min/1.73m², and median ACR was 8 (IQR 5-20) mg/g. Higher urine PIIINP associated with older age, male sex, hypertension, diabetes, lower eGFR and higher ACR. In the case-control study, there was a graded increase in odds of CKD progression across PIIINP quartiles, and each doubling associated with 23% higher odds.

Association of Urine PIIINP with CKD Progression over 9 Years and ESRD*

	Urine PIIINP quartiles				Continuous
Quartile (range [ug/L])	1 (≤ 1.39)	2 (1.40-2.58)	3 (2.59-4.22)	4 (> 4.22)	Per Doubling
CKD Progression					
# cases/controls (%)	40/65 (38%)	56/66 (46%)	45/52 (46%)	51/48 (52%)	
Odds Ratio (95% CI)	1.00 (Ref.)	1.43 (0.81, 2.52)	1.52 (0.77, 3.00)	1.77 (0.82, 3.81)	1.23 (1.02, 1.50)
Incident ESRD					
# events/PY at risk	0 / 2228	4 / 2229	13 / 2174	37 / 1975	
Hazard Ratio (95% CI)	1.00 (Ref.)		2.65 (0.67, 10.54)	3.63 (0.90, 14.70)	1.26 (0.85, 1.88)

*Adjusted for age, sex, race, education, clinical site, urine creatinine, eGFR, and urine albumin.

In the case-cohort study, there was an increasing number of ESRD events across quartiles. The association per doubling of PIIINP were similar in strength to CKD progression, albeit not statistically significant.

Conclusions: Urine PIIINP is associated with CKD progression independent of baseline eGFR and ACR in the community-living elderly. A non-significant association was observed for incident ESRD. Urine PIIINP may mark renal fibrosis and identify those at higher risk of CKD progression beyond eGFR and urine ACR.

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-OR015

Association of Serum Erythropoietin with Cardiovascular Events, Kidney Function Decline, and Mortality: The Health ABC Pranav S. Garimella,[1] Ronit Katz,[2] Kushang V. Patel,[2] Stephen Kritchevsky,[3] Joachim H. Ix,[4] Chirag R. Parikh,[5] Linda F. Fried,[6] Michael Shlipak,[7] Mark J. Sarnak.[1] *[1]Tufts Medical Center; [2]Univ of Washington; [3]Wake Forest Univesrity; [4]UC San Diego; [5]Yale Univ; [6]Univ of Pittsburgh; [7]UC San Francisco.*

Background: Exogenous erythropoietin has been associated with adverse outcomes in CKD and heart failure. In contrast, low erythropoietin levels may reflect kidney tubule dysfunction, which in turn is associated with adverse outcomes. We evaluated the association of endogenous erythropoietin levels with outcomes in a population of older adults.

Methods: Erythropoietin levels were measured in 2,488 women and men 70-79 years of age in the Health, Aging and Body Composition (Health ABC) cohort. Cox proportional hazards were used to evaluate adjusted associations of erythropoietin with incident heart failure, coronary artery disease (CAD), stroke, and all-cause mortality, while Poisson regression was used to evaluate the association with kidney disease progression.

Results: Mean (SD) age was 74 (3) years, 51% were women and 39% were blacks. Median (IQR) erythropoietin was 12.3 (9.0-17.2) mIU/mL. Mean follow-up time was 10.7 (SD=2.9) years. Black participants, as well as those with diabetes, hypertension and CAD had higher levels of erythropoietin (p < 0.05). Higher erythropoietin was independently associated with increased risk of heart failure but not with CAD, mortality, stroke or progression of kidney disease after adjusting for confounding factors.

Table: Association of serum erythropoietin with clinical outcomes

Erythropoietin	Risk factor adjusted* HR (95% CI)	Adjusted for kidney function† HR (95% CI)	Adjusted for hemoglobin‡ HR (95% CI)
Incident heart Failure			
Continuous (per doubling)	1.35 (1.19-.53)	1.31 (1.15-1.48)	1.26 (1.10-1.43)
Quartiles			
< 9.04	1.00 (ref)	1.00 (ref)	1.00 (ref)
9.04 – 12.29	0.99 (0.75-.31)	0.98 (0.74-1.30)	0.97 (0.73-1.29)
12.30 – 17.17	1.18 (0.90-.55)	1.11 (0.85-1.46)	1.09 (0.83-1.44)
≥ 17.7	1.55 (1.19-.01)	1.45 (1.12-1.89)	1.36 (1.04-1.78)
Total Mortality			
Continuous (per doubling)	1.13 (1.04, 1.23)	1.09 (1.00, 1.19)	1.06 (0.97, 1.15)
Incident coronary artery disease			
Continuous (per doubling)	1.08 (0.93, 1.26)	1.06 (0.91, 1.24)	1.08 (0.92, 1.26)
Incident stroke			
Continuous (per doubling)	1.04 (0.84, 1.28)	1.04 (0.84, 1.28)	1.03 (0.83, 1.29)
Incident chronic kidney disease			
Continuous (per doubling)	1.15 (1.00, 1.32)	1.08 (0.94, 1.24)	1.01 (0.87, 1.16)

* Adjusted for age, gender, race, site further adjusted for diabetes, hypertension, systolic BP, prevalent CAD, smoking, albumin, C-reactive protein
†further adjusted eGFR-cysC and urine albumin-creatinine ratio
‡further adjusted for hemoglobin

Conclusions: Higher levels of endogenous erythropoietin are associated with increased risk of heart failure independent of CVD risk factors, kidney function and hemoglobin. It remains to be determined whether this is due a causal relationship, versus a reflection of severity of other heart failure related risk factors.

Funding: NIDDK Support

SA-OR016

Fractional Phosphate Excretion Predicts CKD Progression Independently of FGF23 Carmine Zoccali,[1,2] Patrizia Pizzini,[1] Anna Pisano,[1] Daniela Leonardis,[1] Vincenzo Panuccio,[1] Giovanni Tripepi,[1] Francesca Mallamaci.[1] *[1]CNR-IFC/IBIM & Nephrology and Transplantation Unit, Reggio Calabria, Italy; [2]On Behalf of the MAURO Working Group.*

Background: Hyperphosphatemia is a predictor of accelerated renal function loss in CKD patients. This phenomenon is considered as the trade-off of a counter-regulatory response, possibly mediated by FGF23, aimed at maintaining global phosphate balance by raising phosphate excretion per functioning renal unit (fractional phosphate excretion). However, this hypothesis has never been formally tested in appropriate cohort studies.

Methods: We studied 494 incident patients with stages 2-5 CKD and the study end point was a composite renal outcome (i.e. eGFR reduction > 30%, dialysis or transplantation).

Results: Fractional phosphate excretion (median: 41%, interquartile range 27-61%) was above the upper limit of the normal range (>20%) in the majority of CKD patients (83%). In unadjusted analyses, fractional phosphate excretion was related directly to urinary protein (r=0.24, P<0.001), FGF-23 (r=0.25, P<0.001), male gender (r=0.13, P=0.003), smoking (r=0.13, P=0.004) and diastolic blood pressure (r=0.09, P=0.04) and inversely to eGFR (r=-0.44, P<0.001) and diabetes (r= -0.11, P=0.02) but was independent of serum phosphate (r=-0.03, P=0.54). Over a 2.4 (average) follow-up 165 incident renal events were registered. A 5% increase in fractional phosphate excretion predicted a 6% increase (unadjusted) in the hazard ratio of renal events (HR: 1.06, 95%: 1.03-1.09, P<0.001). In analyses adjusted for age, gender, smoking, diabetes, cholesterol, diastolic pressure, urinary protein and serum phosphate, anti-hypertensive treatment the strength of this relationship remained unmodified (HR: 1.06, 95% CI: 1.03-1.08, P<0.001). Remarkably, further adjustment for FGF23 did not modify the risk for renal events by fractional phosphate excretion (HR: 1.05, 95% CI: 1.02-1.07, P<0.001).

Conclusions: Fractional phosphate excretion is a strong, independent predictor of incident renal events. The predictive power of this parameter is largely independent of other risk factors, including serum phosphate and FGF23. Biological pathways other than FGF-23 play a dominant role in phosphate-related renal damage.

SA-OR017

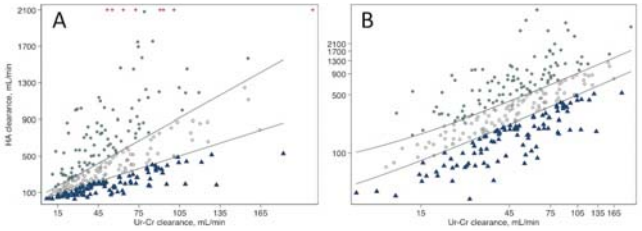
Renal Tubular Secretion in Chronic Kidney Disease Astrid Suchy-Dicey,[1] Thomas J. Laha,[1] Andrew N. Hoofnagle,[1] Cassianne Robinson-Cohen,[1] Ernest Ayers,[1] Tammy L. Sirich,[2] Timothy W. Meyer,[2] Jonathan Himmelfarb,[1] N. David Yanez,[1] Noel Weiss,[1] Bryan R. Kestenbaum.[1] *[1]Univ of Washington, Seattle, WA; [2]Stanford Univ, Stanford, CA.*

Background: The presence and severity of chronic kidney disease are currently assessed by abnormalities in glomerular filtration rate and urinary albumin excretion. Other kidney functions, such as proximal tubular secretion, are not typically quantified. Tubular secretion is capable of clearing metabolites from the blood more efficiently than filtration, suggesting important clinical consequences of secretory dysfunction. Measuring tubular secretion as an independent marker of kidney function may provide insight into kidney disease etiology and prediction of adverse outcomes.

Methods: We developed and validated mass spectrometry assays to measure hippurate and cinnamoylglycine, which are primarily cleared by tubular secretion. We estimated renal secretion function in a prospective cohort study of 299 CKD patients using timed urinary clearance of these molecules. We examined the association between renal secretion and estimated filtration (using average of creatinine and urea clearance), related clinical characteristics, and death.

Results: Tubular secretion correlated with glomerular filtration (R=0.421), but

considerable residual variability remained (Figure: Secretion over filtration, continuous (A) and log scale (B)). Tubular secretion function was significantly better among men, and tended to be lower among non-whites and smokers, although these associations were within the limits of chance. Lower secretion function was associated with increased hazard of death, independent of filtration, age, race, and sex (hazard ratio: 2.6 per halving hippurate clearance; 95% CI: 1.3-5.4).



Conclusions: Prediction of clinical outcomes of patients with CKD may be enhanced by the measurement of proximal tubular secretion.

Funding: NIDDK Support

SA-OR018

WT1 Promotes Fgf Signaling in Nephron Progenitor Cells by Transcriptional Control of Gas1 Martin Kann, [1]Maximilian Otto Lenz,[1] Valerie A. Schumacher,[2] Bernhard Schermer,[1] Chen-Ming Fan,[3] Thomas Benzing,[1] Jordan A. Kreidberg.[2,4] *[1]Nephrolab Cologne & Center for Molecular Medicine, Univ of Cologne, Cologne, Germany; [2]Div of Nephrology, Boston Children's Hospital, Harvard Medical School, Boston, MA; [3]Dept of Embyrology, Carnegie Inst of Washington, Baltimore, MD; [4]Harvard Stem Cell Inst, Cambridge, MA.*

Background: WT1 is a key transcription factor in nephron progenitor cells (NPC) that is required for maintenance of the NPC pool. We had previously identified Gas1 as a direct target gene of WT1, which is also required for NPC maintenance. Here we show that loss of Gas1 causes an intrinsic NPC defect in Fgf signaling and decreased NPC proliferation at an early stage in renal development at which a Gas1-related phenotype is not yet evident.

Methods: NPC proliferation was assayed by BrdU staining. An immortalized murine embryonic kidney cell line was used for assaying Fgf signaling.

Results: Proliferation of Six2 positive NPC at E15.5 was decreased by one third in Gas1 ko as compared to wildtype kidneys as by Six2 and BrdU staining in vivo. At this stage numbers of NPC per ureteric bud did not differ between Gas1 ko and control and nephrogenesis was proceeding normally as evidenced by staining and in situ hybridization for Six2, Wnt4, Jagged1 and WT1. At stage E17.5 a phenotype of a decreased NPC pool together with reduced nephrogenesis was evident in Gas1 ko kidneys. NPC proliferation at E17.5 continued to show the same defect as at E15.5. We hypothesized an intrinsic defect in Fgf signaling in NPC as this pathway is key to NPC proliferation and Gas1 is known to modulate receptor tyrosine kinase signaling. Indeed, comparing Gas1 knockdown to control conditions in a kidney cell line that expresses endogenous WT1 and Gas1, revealed decreased phosphorylation of AKT in Gas1 knockdown cells upon stimulation with Fgf9 and Fgf20. In turn, AKT has been suggested to be a key downstream mediator of Fgf signaling in NPC.

Conclusions: In summary, we describe a novel indirect effect of WT1 on Fgf signaling in NPC: WT1 directly activates transcription of Gas1, which in turn modulates the response to Fgfs in favor of AKT phosphorylation to promote NPC proliferation.

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

SA-OR019

Histone Deacetylases 1 and 2 Repress *Wnt4* During Kidney Development Shaowei Chen, Xiao Yao, Samir S. El-Dahr. *Pediatrics, Tulane Univ School of Medicine, New Orleans, LA.*

Background: *Wnt4*, a member of *Wnt* gene family, is a mesenchymal autocrine signal that is essential for epithelial transformation of metanephric mesenchyme. The expression of Wnt4 is tightly regulated in the cap mesenchyme (CM) cells and their derivatives during kidney development, though the underlying mechanisms remain largely unknown. Histone deacetylases (HDACs) are a superfamily of enzymes, which primarily modulate chromatin structure and thus gene transcription via removal of acetyl moieties from lysine residues of histones. We previously reported that deletion of Hdac1 and Hdac2 from the CM severely impairs nephrogenesis. Here, we examined the role of Hdac1 and Hdac2 in regulation of Wnt4 expression.

Methods: 1. To delete *Hdac1* and *Hdac2* in the CM cells, mice bearing conditional null alleles for *Hdac1* and *Hdac2* were crossed with *Six2-creEGFP* mice. 2. Quantitative real-time RT-PCR (QRT-PCR) and in situ hybridization (ISH) were performed to analyze the mRNA level of *Wnt4*. 3. Chromatin immunoprecipitation (ChIP) was performed to examine the occupancy of Hdac1, Hdac2, and acetylated histones H3 and H4 of *Wnt4* promoter in mK4 cells (immortalized mouse metanephric mesenchyme cells).

Results: 1. QRT-PCR and ISH results revealed that the mRNA level of Wnt4 is elevated in the pretubular aggregates and renal vesicles in $CM^{Hdac1, 2-/-}$ kidneys at E16.5, though no ectopic expression of Wnt4 is observed in the CM. 2. Pharmacological inhibition of HDAC activity (HDACi) or knockdown of Hdac1 and Hdac2 by morpholino oligonucleotides significantly increases the mRNA level of Wnt4 in mK4 cells, indicating a cell-autonomous role for Hdac1 and Hdac2 in regulation of Wnt4 expression. 3. ChIP assays further demonstrated that Hdac1 and Hdac2 occupy the promoter region of Wnt4 in mK4 cells, and HDACi enhances acetylation of H3 and H4 histones associated with Wnt4 promoter.

Conclusions: The *Wnt4* gene is a direct negatively regulated target of Hdac1 and Hdac2. As Hdac1 and Hdac2 themselves lack intrinsic DNA binding activity, these findings suggest that a repressive complex containing Hdac1 and Hdac2 normally represses Wnt4 expression in the pretubular aggregates and renal vesicles during kidney development.

Funding: NIDDK Support

SA-OR020

Nephron Lumen Formation During Kidney Development Denise K. Marciano,[1] Zhufeng Yang,[1] Lei Gao,[1] Paul R. Brakeman,[4] Keith Mostov,[3] Xinchao Pan,[1] Ulrike Schnell,[1] Thomas J. Carroll,[1] Susan E. Zimmerman.[1] *[1]Dept of Medicine, Div of Nephrology, Univ of Texas Southwestern Medical Center, Dallas, TX; [2]Dept of Pediatrics, Univ of California San Francisco, San Francisco, CA; [3]Dept of Anatomy, Univ of California San Francisco, San Francisco, CA.*

Background: A fundamental question in biology is how cells polarize and undergo de novo lumen formation and elongation during tubulogenesis. To date, little is known about lumen formation and elongation in tubules of the kidney nephron. We have previously characterized several steps in nephron tubulogenesis using the mouse kidney as a model system.

Methods: Here we present preliminary studies in which we used time-lapse microscopy with genetic labeling of the nephron lumen to visualize live lumen formation in mouse kidneys. We also use a 3D cell culture model to elucidate the molecular process.

Results: Our live imaging studies revealed dynamic epithelial movements and rearrangements that occur to promote luminal extension of the nephron tubule. In addition, there were small, discrete lumens that formed at the leading edge of the extending lumen, which ultimately fused at the s-shaped body stage. Furthermore, using a 3D cell culture model, we found that multiple, small lumens often coalesced to form a larger lumen in control cells, and that this coalescence was dependent on Afadin, a Nectin adaptor and Rap1 binding protein. Afadin depletion led to a multi-lumen phenotype and loss of Nectin-3 localization at the plasma membrane. Nectin-3 depletion phenocopied the Afadin-depleted cells, giving rise to multiple lumens.

Conclusions: Together these results provide a novel model of nephron lumen extension and suggest that Afadin mediates lumen coalescence in a Nectin-3 dependent manner.

Funding: NIDDK Support, Pharmaceutical Company Support - Satellite Healthcare, Private Foundation Support

SA-OR021

A Novel Mechanism of Glomerular Cyst Formation in Hepatocyte Nuclear Factor 1 Beta (Hnf1b)-Deficiency Armelle Jm Christophorou, Filippo Massa, Arianna Fiorentino, Serge Garbay, Evelyne Fischer, Marco Pontoglio. *Development, Reproduction and Cancer, Institut Cochin, Inserm U1016, CNRS 8104, UP5, Paris, France.*

Background: Glomerular cysts are one of the most characteristic finding in patients carrying MODY5/*HNF1B* mutations. We have previously shown that HNF1beta plays an essential role in the specification and expansion of tubules. In addition, we have shown that the lack of HNF1beta leads to cystic glomeruli connected to collecting ducts with a short and primitive tubule. The mechanisms linked to the formation of glomerular cysts is still unknown.

Methods: With a tissue specific inactivation of Hnf1b in the metanephric mesenchyme (Six2CRE) we studied the mechanism of the formation of glomerular cysts in HNF1beta-deficiency in mouse embryos.

Results: During glomerulogenesis, in S-shaped bodies, the urinary pole (UP) is initially in direct contact with podocyte precursors. Then, UP separates from the podocytes via the intercalation of Bowman's capsule cells. This process leads to the exclusion of the UP from the inner part of the glomerulus. Later on, the UP further slides along the Bowman's capsule to reach the opposite side in respect of the vascular pole (VP). When we inspected the three-dimensional configuration of glomeruli formed in the absence of HNF1beta, we discovered that the UP and VP were structured in an aberrant way. In early mutant glomeruli, the UP is inserted in the glomerular tuft leading to a situation where the tubular urinary outflow of the glomerulus is trapped inside the VP. This configuration implies the possible block of the glomerular outflow, providing the basis for glomerular cyst expansion upon the onset of glomerular filtration. During glomerulogenesis, specific cells in the lower limb of the S-shaped body are destined to remain inside and form the inner glomerular cup (podocyte precursors) whereas others (parietal cells) form the Bowman capsule. Interestingly, HNF1beta is specifically expressed in cells that are destined to be outside of the glomerulus.

Conclusions: Our results indicate that HNF1beta controls the structural architecture of glomeruli and provide the basis for a novel mechanism of glomerular cyst formation.

SA-OR022

The Hippo Pathway Functions in Kidney Branching Morphogenesis Antoine Reginensi,[1] Masato Hoshi,[2] Marco Giovannini,[3] Sanjay Jain,[2] Helen McNeill.[1] *[1]Lunenfeld Tanenbaum Research Inst, Mount Sinai Hospital, Toronto, ON, Canada; [2]Dept of Internal Medicine (Renal Div), Washington Univ School of Medicine, St. Louis, MO; [3]Dept of Head and Neck Surgery, House Research Inst, Los Angeles, CA.*

Background: The Hippo kinase cascade is a highly conserved signaling pathway critical for normal growth and development. Activation of Mst/Lats kinases culminates in the phosphorylation and inactivation of the Hippo targets Yap and Taz. It is still unclear if the Hippo pathway has a critical role in ureteric bud (UB) branching.

Methods: We investigated the role of *Yap, Taz* and *Nf2* in branching morphogenesis using the *Hoxb7Cre* (excision in UB lineage).

Results: We found that *Hoxb7Cre Yap$^{fl/fl}$* embryos harbored striking abnormalities of the urinary tract (92% hydroureters/hydronephrosis, 37% duplicated systems, 8% hypoplastic kidneys). Intrapelvic dye injection revealed that *Yap* deletion results in ureters ending blindly before reaching the bladder. In *Yap* mutants, apoptosis in the cloaca epithelium (E10) is dramatically reduced, preventing fusion of the nephric duct to the cloaca. Importantly, this phenotype is partially rescue by *Ret* haploinsufficency. UB tips are highly dilated in *Yap* and/or *Taz* knock-outs, indicating *Yap* may be involved in Ret signaling. We are currently investigating Yap's role in Ret signaling via regulation of downstream signaling (pERK, MAPK, p38) and target gene activation. We then investigated the function of *Nf2*/Merlin. Merlin has been shown to function as an upstream Hippo pathway regulator in *Drosophila*. *Nf2* knock-outs display severe kidney hypoplasia, due to limited UB branching. Strikingly, the *Nf2* cKO phenotype was rescued by heterozygous deletion of *Yap/Taz*, and phenocopied by Yap overexpression, suggesting that Yap and/or Taz are the major effectors of Nf2 in branching morphogenesis. No changes in *Ret, Etv5* and *Wnt11* were observed in *Nf2* cKO kidneys.

Conclusions: This work demonstrates a novel role for *Yap, Taz* and *Nf2* during branching morphogenesis. Understanding their functions might lead to identification of new players involved in kidney morphogenesis and aid in the development of new therapeutics for patients suffering from kidney diseases.

Funding: Government Support - Non-U.S.

SA-OR023

Patterning of the Renal Stroma Does Not Require Input from the Nephron Progenitors, the Ureteric Bud, or Their Derivatives Amrita Das, Thomas J. Carroll. *Internal Medicine (Nephrology) and Molecular Biology, UT Southwestern Medical Center, Dallas, TX.*

Background: Stromal/epithelial cross-talk is a fundamental event in organ development and maintenance. We have recently shown that in the developing kidney, signals emanating from the stroma play a key role in cell fate specification of the nephron progenitors. However, the regulation of the stromal cells themselves remains unclear.

Methods: All experiments are performed in genetic mouse models. RNA and protein localization using insitu hybridization and immuno histochemistry respectively are used to analyze wildtype and mutant kidneys.

Results: Stromal progenitor cells are a small population of cells within the metanephric mesenchyme, distinguished by their expression of the transcription factor Foxd1 as early as E10.5. Lineage tracing studies have shown that the derivatives of Foxd1-expressing cells populate most of the renal interstitium. These include fibroblasts, glomerular mesangial cells, pericytes and vascular smooth muscle cells. During kidney development, the Foxd1 derived stromal cells acquire a positional identity corresponding to the different nephron segments thereby forming a patterned architecture. Using marker analysis, we are able to segregate the renal interstitium into 6 domains across the cortico-medullary axis. We performed a number of embryological studies to determine if the patterning of the stroma requires signaling from other cell types within the developing kidney, such as the nephron precursor cells, the nephron, the collecting duct epithelia or the vasculature. Surprisingly, although terminal differentiation of the stroma appears to require signals from the medullary collecting ducts, the initial pattering appears to be independent of signals from any of these cell types suggesting that the stroma self-patterns.

Conclusions: Based on these findings and the close association of the distinct stromal domains with different stages of nephron formation, it is tantalizing to speculate that the stroma may affect multiple steps in the differentiation and/or patterning of the nephron.

Funding: NIDDK Support

SA-OR024

Role of Transcription Factor Cp2l1 in Differentiation and Patterning of the Collecting Duct Max Werth,[1] Kai M. Schmidt-Ott,[2] Christian Hinze,[2] Andong Qiu,[1] Jonathan M. Barasch.[1] *[1]Columbia Univ, New York; [2]Max Delbrueck Center for Molecular Medicine, Berlin.*

Background: The collecting duct contains at least three cell types (principal, alpha- and beta-intercalated cells) that are organized in a regular pattern. Little is known about differentiation and patterning of these cells. We have identified a transcription factor, Cp2l1 that regulates both of these processes.

Methods: In vitro: Overexpression of transcription factors in rat metanephric mesenchyme. In vivo: Segment specific (Collecting Duct, Ksp-cre) and cell type specific (Intercalated Cell, V-ATPaseB1-Cre) inactivation of Cp2l1 as well as components of Notch signaling. Genome-wide DNA-binding Assay (ChiP-seq). Gene-Expression Analysis using Affymetrix Gene-Arrays and RNA-seq.

Results: Conditional deletion of Cp2l1 demonstrated the absence of alpha- and beta-intercalated Cells (IC) and their characteristic markers (Foxi1, CA2, H+ATPase, AE1 and Pendrin), leaving a homogenous, single cell type characterized by Aqp2, Krt8, Cdh1 typical of principal cells. Conversely, when Cp2l1 was overexpressed in metanephric mesenchyme, we observed upregulation of IC specific genes like CA2, H+ATPase, BSND. To determine whether Cp2l1 directly regulated IC genes, we used Cp2l1 ChiPseq. A three way comparison of ChIPseq, Cp2l1 overexpression and Cp2l1 knockout revealed that Cp2L1 directly regulated genes most typical of IC. In addition, the three way comparison demonstrated that Notch ligands were also positively regulated by Cp2l1. Notch knockouts did not completely abolish the development of cells expressing IC markers, but rather generated a large number of cells expressing both IC and PC markers. These cells were clustered in patches rather than scattered between principal cells.

Conclusions: Cp2l1 is critical for the initial differentiation of distinct cell types in the collecting duct. It does this by directly activating cell defining proteins such as the H+ ATPase and by regulating components of Notch signaling which in turn determine collecting duct patterning. In contrast, Cp2l1 had only more subtle effects on the IC cell phenotype once the H+ ATPase was expressed and the patterning of the collecting duct was established.

Funding: NIDDK Support

SA-OR025

Cell Fate Patterning along the Kidney Collecting Ducts Is Dependent on Notch/Rbpj Signaling, with Ectopic Activation of Notch1 Capable of Turning on Elf5 and the Principal Cell Program Kameswaran Surendran,[1,2] Justin J. Grassmeyer,[1] Casey Hettinger,[1] Malini Mukherjee,[1] Monica R. Bailey,[3] Satrajit Sinha.[4] *[1]Sanford Children's Health Research Center, Sanford Research, Sioux Falls, SD; [2]Dept of Pediatrics, Sanford School of Medicine, Sioux Falls, SD; [3]Augustana College, Sioux Falls, SD; [4]Dept of Biochemistry, State Univ of New York at Buffalo, Buffalo, NY.*

Background: The mechanisms by which the mammalian kidney collecting ducts are patterned along the cortico-medullary axis with a decreasing ratio of intercalated to principal cells remains unknown. Notch signaling ensures that sufficient number of renal collecting duct progenitors adopt the principal cell fate, without which mice have a reduced number of principal cells, a concomitant increase in intercalated cells and a urine concentrating defect. This study focuses on how Notch signaling regulates the progenitors of collecting ducts to select between principal versus intercalated cell fates.

Methods: We compared gene and protein expression patterns of principal and intercalated markers among others in wild type versus transgenic mice with Notch signaling deficient collecting ducts, or with ectopic Notch1 activation in developing collecting ducts. We also performed transcriptional reporter assays in newly established immature collecting duct cell lines to identify regulators of Elf5 and Avpr2.

Results: Mice with Notch signaling deficient collecting ducts have reduced renal expression of Elf5, which at embryonic day 16.5 is expressed in Foxi1$^-$, Aquaporin2$^-$ cells that surround Foxi1$^+$cells in developing collecting ducts. Consistent with Elf5 being a principal cell specific transcription factor, it is capable of activating an *Avpr2* proximal promoter. Interestingly, ectopic activation of Notch signaling in the developing collecting ducts up regulates Hes1 and Elf5 prior to down regulation of Foxi1. The Notch signaling target Hes1 can transactivate Elf5 proximal promoters.

Conclusions: In summary, Notch signaling regulates the patterning of collecting ducts with intercalated and principal cell types in part by turning on Elf5 to promote and/or to maintain the principal cell identity.

Funding: Other NIH Support - NIGMS

SA-OR026

The Transcription Factor *etv5a* Is Essential for Multiciliated Cell Development During Zebrafish Nephrogenesis Amanda N. Marra, Rebecca A. Wingert. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

Background: The zebrafish pronephros, or embryonic kidney, is comprised of two main types of epithelial cells: transportive and multi-ciliated cells (MCCs). Transportive cells occupy discrete tubule segments and are characterized by expression of solute transporters, while MCCs are dispersed in a "salt-and-pepper" fashion throughout the tubule and are characterized by the presence of motile cilia. The fate choice between epithelial identities is regulated by Notch signaling, in which Notch activity promotes transportive cell formation by restricting MCC fate. Recently, our lab demonstrated that the transcription factor *mecom* restricts MCC identity by promoting Notch, and that retinoic acid (RA) signaling acts upstream to regulate *mecom*. Despite this knowledge, much remains to be learned about MCC formation.

Methods: Previous research has shown that *etv5a* and its ETS family members *etv5b* and *etv4* are required for ciliogenesis in other zebrafish tissues. Here, we mapped *etv5a* expression to renal progenitors that occupy domains where MCCs later emerge. Thus, we hypothesized that *etv5a* is required for ciliogenesis of MCCs in the nephron.

Results: *etv5a* knockdown caused decreased expression of the MCC markers *odf3b, jag2a*, and *centrin*. In addition, *etv5a* knockdown led to alterations in solute transporter domains suggesting that tubule segments expanded and had more transporter cells. In epistasis studies, exogenous RA treatment expanded the *etv5a* domain, indicating that *etv5a* acts downstream of RA. Further, abrogation of Notch with DAPT treatment did not alter *etv5a* expression, suggesting Notch acts downstream of *etv5a*.

Conclusions: Ongoing studies will explore the possible redundancy between ETS factors and continue to examine the epistatic relationships between *etv5a*, RA, Notch and *mecom* to elucidate the mechanism of how these factors interact to direct MCC development during nephrogenesis.

Funding: NIDDK Support

SA-OR027

Injury to the Developing Kidney Leads to Impaired Maturation but Not Inflammation or Fibrosis Scott R. Manson, Julio J. Geminiani, Gino J. Vricella, Qiusha Guo, Paul F. Austin. *Urology, Washington Univ, St. Louis, MO.*

Background: Inflammation and fibrosis are widely accepted as the key processes driving the progression of renal injuries to kidney disease in the mature kidney. However, congenital defects are the leading cause of pediatric kidney disease and the pathogenesis of renal injury in the developing kidney remains poorly understood. In this study, we provide the first direct comparison of injury responses during each of the critical stages of kidney development.

Methods: Disease progression was examined in chronic and reversible murine models of unilateral ureteral obstruction (UUO) at several developmental time points: (1) P1, during nephrogenesis/nephron maturation, (2) P14, during proliferative growth, and (3) P60, in the mature kidney. Renal pathology was assessed by immunostaining for molecular markers of key processes in kidney development and the pathogenesis of renal injury.

Results: UUO at either P1 or P14 in the developing kidney leads to decreased kidney growth, reduced proliferative expansion of nephrons, increased apoptosis in progenitor cells, and impaired nephron differentiation. There is also a notable absence of fibroblast and macrophage recruitment, inflammation, and fibrosis in the developing kidney. This contrasts the mature kidney where there is a marked increase in reparative proliferation, inflammation, and fibrosis. [All results are n=10, p<0.05].

	P1 (Nephrogenesis Phase)	P14 (Growth Phase)	P60 (Mature Phase)
Kidney Weight	- 35.2 %	- 26.3 %	- 11.9 %
Epithelial Proliferation	- 56.2 %	- 61.6 %	+ 538.3 %
Apoptosis	+ 9.5-fold	+ 9.3-fold	+ 6.4-fold
Inflammation	no change	no change	+ 4.3-fold
Renal Fibrosis	no change	no change	+ 6.4-fold

Conclusions: This study reveals that developmental context has a significant impact on the pathogenesis of renal injuries. In contrast to the mature kidney, injury in the developing kidney is characterized by profound developmental deficits and a distinct absence of inflammation and fibrosis. This suggests that treatment strategies for pediatric kidney disease can be optimized by stimulating proliferation and minimizing apoptosis at early stages of development while inhibiting inflammation and fibrosis at later stages.

Funding: NIDDK Support, Private Foundation Support

SA-OR028

Restoring Mitochondrial Superoxide Levels with Bendavia™ Protects against Progression of Diabetic Kidney Disease in db/db Mice Satoshi Miyamoto,[1,2] Manjula Darshi,[1,2] Larkin B. Slater,[1,2] Tai Nguyen,[1] Peter J. Oates,[3] Kumar Sharma.[1,2] *[1]Center for Renal Translational Medicine, Univ of California, San Diego, La Jolla, CA; [2]Veterans Administration San Diego HealthCare System, La Jolla, CA; [3]Stealth Peptides, Inc., Newton, MA.*

Background: We have recently demonstrated that superoxide production and mitochondria biogenesis are reduced in the kidneys of a mouse model of type 1 diabetes (DM), and that mitochondria-related urine metabolites are significantly reduced in human subjects with diabetic kidney disease (DKD) suggesting that reduced mitochondrial function plays a critical role in the progression of DKD. The aim of this study was to determine if type 2 DM is associated with reduced renal superoxide and whether Bendavia™ (B), a mitochondria-targeting peptide, protects against DKD in db/db mice.

Methods: Six weeks-aged male nondiabetic db/m mice and diabetic db/db mice (BKS. Cg-Dock7^{m} +/+ Lepr^{db}/J strain) were randomly assigned to four groups: db/m+vehicle (Control), db/m+B, db/db+vehicle (db/db) and db/db+B, n=15/group. Vehicle or B (3 mg/kg BW) were injected daily s.c. for 4 weeks and then infused subcutaneously via osmotic pumps (3mg/kg per day) over an 8 week period. Glomerular size and mesangial matrix area were assessed by morphometric analysis. Superoxide levels in kidney were measured using i.p. administration of dihydroethidium (DHE), and mounted kidney slices were imaged by confocal microscope.

Results: Urinary albumin/creat ratio, elevated in db/db (738 ± 72, P<0.001) versus Control (74 ± 6) mice, was significantly reduced with B (463 ± 75, p<0.01), whereas there were no significant differences in body weight or HbA1c between both diabetic groups. The increase in glomerular mesangial matrix area in db/db group, was significantly reduced with B (p<0.001). Superoxide levels in glomeruli were significantly reduced by 23.5% in db/db compared with Control (p<0.05) and restored to control levels in db/db+B group.

Conclusions: Our results demonstrate that DKD with type 2 DM is associated with reduced renal superoxide levels and the novel mitochondrial peptide Bendavia™ protected against DKD and restored physiologic levels of superoxide in the db/db model of DKD.

Funding: NIDDK Support, Other NIH Support - DP3DK094352-01, Veterans Affairs Support, Pharmaceutical Company Support - Stealth Peptides, Inc.

SA-OR029

Minichromosome Maintenance Protein 3 Associates with and Regulates Nrf2 in Proximal Tubules Michelle T. Barati, Eric Poulos, Whitney L. Ward, Susan M. Isaacs, Madhavi J. Rane, Jon B. Klein, Michael Merchant. *Univ of Louisville.*

Background: Oxidative stress is a mediator of diabetic nephropathy (DN). Regulation of Nrf2, a primary oxidant-responsive transcription factor, is altered in DN. Our lab identified association of Serine-40 phosphorylated Nrf2 (pS40Nrf2), which occurs upon Nrf2 activation, with minichromosome maintenance proteins (MCM) 2-7 in renal tubule cells, by mass spectrometry (MS). MCM 2-7 proteins form a DNA helicase required for replication and not known to regulate Nrf2 or be regulated in DN. Since MCM3 was highly enriched in pS40-Nrf2 complex in MS findings, this study determined if MCM3 regulates Nrf2 activity or is regulated during DN.

Methods: MCM3/Nrf2 association determined by immunoprecipitation (IP) of pS40Nrf2 and immunobloting (IB) of precipitated proteins for MCM3. MCM3 overexpressed by transfection of a DNA plasmid or knocked down by transfection of siRNA in cultured proximal tubule cells (PTC). Cells were also cultured in media with High glucose (25mM; HG) or normal (5mM) glucose concentration for 24h, alone or after transfections. Kidney sections from 2,4,and 7 month old OVE26 diabetic and FVB control mice were immunostained for MCM3 and PCNA.

Results: IP of pS40Nrf2 from tubule extract and IB for MCM3 confirmed association in the cytosol and nuclei. Overexpression of MCM3 increased pS40Nrf2, Nrf2, and induced a Nrf2 target, NQO1. Knockdown of MCM3 reduced total Nrf2, pS40Nrf2, and NQO1. MCM3 expression increased in tubule nuclei of 4 and 7 month old diabetic mice, while PCNA was unchanged, indicating no proliferation. Culture of PTC with HG concentration increased caspase 3 cleavage, and this was augmented with MCM3 Knockdown. pS40Nrf2/MCM3 association decreased in PTC cultured in HG.

Conclusions: The results identify MCM3 as a novel positive regulator of Nrf2. Induction of MCM3 in tubules during diabetes is not associated with proliferation and a role remains to be defined. Initially, MCM3 may confer protection to tubules by preventing HG-induced apoptotic signaling through activation of Nrf2 and its anti-oxidant targets. Decreased pS40Nrf2/MCM3 association with HG may disrupt this mechanism of Nrf2 activation and increase oxidant injury in diabetes.

Funding: NIDDK Support

SA-OR030

Progression of Tubular Damage in Diabetic Nephropathy (DN) Is Linked to an Altered Ubiquitination of the Cytoskeleton Paola Pontrelli,[1] Francesca Conserva,[1] Massimo Papale,[2] Matteo Accetturo,[1] Margherita Gigante,[1] Grazia Vocino,[2] Anna Maria Di Palma,[1] G. Grandaliano,[1] Salvatore Di Paolo,[3] Loreto Gesualdo.[1] *[1]DETO, Univ of Bari, Italy; [2]Dept of Medical and Surgical Sciences, Univ of Foggia, Italy; [3]Barletta Dimiccoli Hospital, Italy.*

Background: The ubiquitin pathway is a possible key-player in the onset of DN. UBE2V1, an ubiquitin-conjugating E2 enzyme variant, mediates the formation of lysine 63 (lys63)-linked ubiquitin chains, affecting protein localization and cell signaling. Aim of our study was to evaluate the role of ubiquitination in response to hyperglycemia and its possible involvement in the progression of tubular damage in DN.

Methods: Human tubular cells (HK2) were grown in hyperglycemia(HG: 30mM). UBE2V1 expression was evaluated by qPCR and lys-63 ubiquitination by immunoblotting on cells, and immunohistochemistry on kidney biopsies of 6 DN patients and 6 controls. Lys63-ubiquitinated proteins were identified by MALDI-TOF/MS-MS. Lys63-Ubiquitination was confirmed by immunoblotting and confocal microscopy.

Results: UBE2V1 gene expression was increased in HK2 cells in HG (1.51 fold). Also UBE2V1 and Lys63-ubiquitinated proteins were increased under HG *in vitro*, and *in vivo* on DN biopsies (UBE2V1, p=0.004; Lys63: p= 0.008 DN versus control) at tubular level. To identify which proteins underwent Lysin63-ubiquitination under HG, HK2 proteins were immunoprecipitated with an anti-ubiquitin antibody and blotted with an anti-lys63-linked ubiquitin chains antibody. By MALDI-TOF/MS-MS we identified 28 lys63-linked ubiquitinated proteins under HG, involved in cellular assembly and organization (IPA score=50). Beta actin lys63 ubiquitination in HG was confirmed by immunoblotting (p=0.05), and was associated to the depolymerization of actin cytoskeleton (confocal microscopy), and the loss of vimentin (flow cytometry). UBE2V1 silencing in HK2 under HG reported actin ubiquitination in Lys63 to basal condition (immunoblotting).

Conclusions: In conclusion UBE2V1 and Lys63-ubiquitinated proteins increase under HG and persist in DN patients, mainly affecting cytoskeleton organization. Cytoskeletal ubiquitination could represent a potential therapeutic target to reduce tubular damage in the progression of DN.

Funding: Government Support - Non-U.S.

SA-OR031

Proximal Tubular Activated Sirt3 and Proliferated Mitochondrial Ribosomes Compensated for Decreased Sirt1 and Mitochondrial Dysfunction at Very Early stage of Diabetic Nephropathy Kazuhiro Hasegawa, Shu Wakino, Koichi Hayashi, Hiroshi Itoh. *Keio Univ.*

Background: We demonstrated that in diabetic nephropathy (DN), the expression of NAD^{+}-dependent deacetylase, Sirt1 in proximal tubules (PT) decreased before its decrease in podocytes (Pods), which triggered DN-induced albuminuria (Hasegawa K, Nature Medicine 2013). Although previous papers have suggested that PTs are a primary site of changes in DN, it is unknown whether **morphological changes** in PT is present prior to those in Pod.

Methods: We analyzed electron microscopic findings in detail and compared the morphological changes between those in PT and those in Pods in the **early stage of DN,** i.e., in mice 8 weeks after strestozotocin (STZ) treatment or in db/db mice at 8 weeks of age when the glucose levels had already been high.

Results: Among various cellular organelle, we found that **mitochondrial ribosome (Mit-rib) number** was significantly increased in PT according to the decreased Sirt1 expression, although this increase was not observed in Pods. The number of other organelle including mitochondria or peroxisome, did not change. We next explored the relationship between Sirt1 expression and Mit-rib number by using *PT-specific Sirt1 conditional knockout (PT-CKO)* mice and *Pod-Specific Sirt1 CKO (Pod-CKO)*. Of note, the number of Mit-rib increased in PT in *PT-CKO*, but not in PT or Pod in *Pod-CKO*. Increase in Mit-rib remarkably raised the **cytochrome oxidase 18 (COX18)** expression which is a component of respiratory chain subunit and activates mitochondrial oxidative phosphorylation (OXPHOS) to maintain ATP generation. Finally, decreased Sirt1 in PT led to acetylating and activating **Foxa2** (Forkhead protein A2), a putative activator of mitochondrial Sirtuin (**Sirt3**), that is surmised to proliferate Mit-rib.

Conclusions: Recent reports showed that **Sirt3** elongates murine lifespan by increasing **Mit-rib** and activating OXPHOS. Among many cytochrome oxidases, mitochondrial DNA encodes and Mit-rib synthesizes COX18. Our data suggested that activated Foxa2/Sirt3 and proliferated Mit-rib compensated for decreased Sirt1 and mitochondrial dysfunction. These molecular links will provide a novel function for the maintenance of energy by Sirt3 in PT.

SA-OR032

Urinary Semaphorin 3A Correlates with Diabetic Proteinuria and Mediates Diabetic Nephropathy and Associated Inflammation in Mice

Riyaz Mohamed, Punithavathi Vilapakkam Ranganathan, Calpurnia Jayakumar, Ganesan Ramesh. *Vascular Biology Center, Georgia Regents Univ, Augusta, GA.*

Background: Semaphorin3A (sema3A) was recently identified as an early diagnostic biomarker of acute kidney injury. However, its role as a biomarker and/or mediator of chronic kidney disease (CKD) related to diabetic nephropathy is unknown. The aim of this study is to determine the pathogenic role of sema3A in diabetic nephropathy.

Methods: The expression and excretion of sema3A in diabetic animal models and in humans was quantified by immunostaining, RT-PCR and ELISA. To test whether sema3A plays a pathogenic role in the development of diabetic nephropathy, mice with sema3A gene mutation or pharmacological based inhibition of receptor binding or signaling was employed. Diabetes was induced by administering low dose of streptazotocin. Albuminuria was quantified at 12 weeks after induction of diabetes with/without pharmacological inhibitors.

Results: The expression of sema3A was localized to podocytes, distal tubules and collecting ducts epithelium in control animals, and its expression was increased following induction of diabetes. Urinary excretion of sema3A in three different diabetic mouse models showed that excretion was increased as early as 2 weeks after induction of diabetes and increased over time, in conjunction with the development of nephropathy. Consistent with the mouse data, increased sema3A urinary excretion was detected in diabetic patients with albuminuria, particularly in those with macro albuminuria. Genetic ablation of sema3A, or pharmacological inhibition with a novel sema3A inhibitory peptide, protected against diabetes-induced albuminuria, kidney fibrosis, inflammation, oxidative stress and renal dysfunction.

Conclusions: We conclude that sema3A is both a biomarker and a mediator of diabetic kidney disease and could be a promising therapeutic target in diabetic nephropathy.

Funding: NIDDK Support

SA-OR033

Overexpression of Heterogeneous Nuclear Ribonucleoprotein F Attenuates Tubular Apoptosis and Tubulointerstitial Fibrosis in Type 2 Diabetic Mice

Chao-Sheng Lo,[1] Yixuan Shi,[1] Isabelle Chenier,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] Shao-Ling Zhang,[1] John S.D. Chan.[1] *[1]Research Center, CHUM-Tour Viger, Montreal, QC, Canada; [2]Research Center, Maisonneuve-Rosemont Hosp, Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass. Gen. Hosp., Boston, MA.*

Background: We investigated whether overexpression of heterogeneous nuclear ribonucleoprotein F (hnRNP F) inhibits renal angiotensinogen and transforming growth factor-beta 1 (TGF-β1) expression and subsequently attenuate tubular apoptosis and tubulointerstitial fibrosis in type 2 diabetic db/db mice.

Methods: Db/db (BKS strain) transgenic (Tg) mice specifically overexpressing hnRNP F in their renal proximal tubular cells (RPTCs) were studied. Body weight, plasma glucose and albuminuria were monitored bi-weekly in adult male non-db/db littermates, db/db and db/db hnRNP F-Tg mice from 10 to 20 weeks of age. Kidneys were processed for histology and apoptosis studies. Gene expression in renal proximal tubular (RPT) were evaluated by respective real time-qPCR and Western blotting.Urinary Angiotensinogen (Agt), Angiotensin II (Ang II) and Angiotensin 1-7 (Ang1-7) levels were quantified by ELISA. Rat immortalized RPTCs were also studied in vitro.

Results: Db/db mice developed obesity, hyperglycemia, albuminuria and kidney hypertrophy. In contrast, overexpression of hnRNP F in RPTC attenuated the renal hypertrophy and the urinary albumin/creatinine ratio without affecting plasma glucose levels in db/db hnRNP-F Tg mice. Tubular apoptosis, RPT Agt, TGFb1, collagen IV, fibronectin, Bax and caspase-3 expression and urinary Agt and Ang II levels were significantly increased in db/db mice but normalized in db/db hnRNP F-Tg mice. In contrast, RPT Ace2 expression were decreased in db/db mice, whereas urinary Ang I-7 levels were significantly decreased in db/db mice and normalized in db/db hnRNP F-Tg mice. Finally, overexpression of hnRNP F attenuated high glucose-induced apoptosis and pro-apoptotic and fibrotic gene expression in rat RPTCs in vitro.

Conclusions: Overexpression of hnRNP F in renal proximal tubular cells attenuated tubular apoptosis and interstitial fibrosis in type 2 diabetic mice.

Funding: Government Support - Non-U.S.

SA-OR034

Deletion of UNC5B Receptor in Kidney Epithelium Exacerbates Diabetic Nephropathy in Mice

Punithavathi Vilapakkam Ranganathan, Riyaz Mohamed, Calpurnia Jayakumar, Ganesan Ramesh. *Vascular Biology Center, Georgia Regents Univ, Augusta, GA.*

Background: Diabetic nephropathy is a leading cause of end stage renal disease and a major source of morbidity, mortality and health care expenditures. Guidance cue netrin-1 was shown to have protective effects in diabetic nephropathy. However, the role of its receptor UNC5B in diabetic kidney disease is unknown. Moreover, whether netrin-1 is protective against diabetic kidney disease in a genetic model of nephropathy (Ins2Akita mice) and nephropathy prone DBA background mice are also unknown.

Methods: Using Cre-lox technology, UNC5B was specifically deleted in the proximal tubular epithelial cells or single allele whole body deletion. These tissue specific knockout mice, WT mice, and netrin-1 transgenic mice cross bred with DBA background mice were made diabetic by injecting low dose of streptozotocin 50 mg/kg in citrate buffer as multiple doses for every 24h, total of five doses. Blood glucose and urine albumin were measured at 1 week after streptozotocin injection and every four weeks thereafter. 12 week old Akita mice were treated with recombinant human netrin-1 (5μg/animal/every 48h, intraperitoneally) or vehicle (0.1% BSA) for a period of 6 weeks. Then 24h urine was collected for the determination of albumin excretion rate.

Results: WT and UNC5B receptor heterozygous knockout diabetic mice developed significant albuminuria at 8 weeks after induction of diabetes as compared to buffer treated control mice. However, albuminuria was significantly more pronounced in mice with proximal tubule specific deletion of UNC5B. Transgenic overexpression of netrin-1 in proximal tubule in DBA background and administration of recombinant netrin-1 to Ins2Akita mice also significantly reduced diabetes induced albuminuria and suppressed glomerulosclerosis. Moreover, netrin-1 administration also suppressed inflammatory cytokine production in the kidney.

Conclusions: Our data suggest that netrin-1 signaling through UNC5B in proximal tubular epithelium may play a critical role in the protection of kidney against diabetic kidney disease.

Funding: NIDDK Support

SA-OR035

The CCR2 Antagonist CCX140 Improves Renal Function in a Human Transgenic CCR2 Diabetic Mouse Model

Zhenhua Miao, Linda Ertl, Jeffrey P. Mcmahon, Shichang Miao, James J. Campbell, Thomas J. Schall. *ChemoCentryx, Mt View, CA.*

Background: Diabetic nephropathy (DN) is a major complication of uncontrolled diabetes. CCR2 has been implicated in the renal recruitment of blood monocytes in response to hypertension and hyperglycemia. It may also be involved in activation of parenchymal renal cells under pathological conditions. CCX140 is a potent, selective, and orally bioavailable small molecule CCR2 antagonist that is currently being studied in two Phase 2 clinical trials in DN. Here we describe the effects of CCX140 on hyperglycemia and renal function in the models.

Methods: hCCR2KI mice were generated by replacing mouse CCR2 coding region with the human CCR2. Uni-nephrectomized hCCR2KI mice were placed on a high fat/high protein diet for 36 weeks to induce obesity. In an alternative model, hCCR2KI-db/db mice were obtained from breeding of hCCR2KI mouse with $lepR^{wt/db}$ mice. In both models, mice were dosed daily for a total of 6-8 weeks. Assessments included body weight, fasting plasma glucose serum clinical chemistry, and 24 hour urinary output of albumin and creatinine.

Results: CCX140 treatment significantly reduced UAER and ACR in both models. In hCCR2KI-db/db mice: Relative changes in UAER from study start to week 6 were +14% and -50% for vehicle and CCX140, respectively. Relative changes in ACR from study start to week 6 were -3% and -75% for vehicle and CCX140, respectively. In hCCR2KI-DIO mice: Relative changes in UAER from study start to week 8 were +440% and +23% for vehicle and CCX140, respectively. Relative changes in ACR from study start to week 8 were +561% and +72% for vehicle and CCX140, respectively.Treatment-related improvement in fasting blood glucose was also observed (vehicle: 190 mg/dL; CCX140: 150 mg/dL). Also observed at the end of the study were increased podocyte density and reduced interstitial macrophage infiltration in connection with CCX140 treatment.

Conclusions: Rapid and significant improvements in albuminuria and hyperglycemia were seen with CCX140 in 2 mouse models of DN. These data are highly supportive of the clinical evaluation of CCX140 in DN. Results from the two ongoing Phase 2 DN trials are expected in late 2014.

SA-OR036

Inhibition of EGFR Activity Protects against Diabetic Nephropathy in Type II Diabetes By Multiple Mechanisms Ming-Zhi Zhang, Raymond C. Harris. *Medicine, Vanderbilt Univ, Nashville, TN.*

Background: Previous studies by us and others have indicated activation of renal epidermal growth factor receptors (EGFR) in models of diabetic nephropathy (DN) and that inhibition of EGFR activity protects against progressive DN in type I diabetes. In the present studies, we examined whether treatment with erlotinib, an inhibitor of EGFR tyrosine kinase activity, affected the development of DN in a model of accelerated type II diabetes.

Methods: BKSdb/db with eNOS knockout (eNOS$^{-/-}$ db/db) mice received vehicle (water) or erlotinib (80 mg/kg/day) by daily gastric gavage beginning at 8 weeks of age. The animals were sacrificed at 20 weeks of age. A subset of mice was kept until death to achieve a survival curve.

Results: Inhibition of renal EGFR activation by erlotinib was confirmed by decreased phosphorylation of EGFR and ERK1/2. Erlotinib treatment preserved pancreas function with higher blood insulin levels at 20 wks (3.16 ± 0.31 versus 2.08 ± 0.22 ng/ml, $P < 0.05$. N=4), and increased blood levels of adiponectin, an adipocyte-derived hormone that increases insulin sensitivity (20.6 ± 0.7 versus 12.1 ± 1.2 ng/ml, $P < 0.0001$. N= 6). Erlotinib-treated mice had decreased basal blood glucose levels and increased glucose tolerance and insulin sensitivity. ACR (albumin/creatinine ratio) was markedly attenuated by erlotinib treatment (ACR at 20 wks: 588 ± 120 versus1688 ± 141 µg/mg, $P < 0.01$. N = 8). Erlotinib protection against DN was also indicated by less histological glomerular injury as well as decreased renal expression of KIM-1, a kidney injury marker, in association with deceased renal endoplasmic reticulum (ER) stress as indicated by decreased expression levels of C/EBP homologous protein (CHOP, an ER stress marker). Finally, erlotinib treatment led to markedly increased longevity (34.14 ± 1.18 versus 22.08 ± 0.56 weeks, $p<0.0001$. N = 8).

Conclusions: These studies suggest that inhibition of EGFR with erlotinib attenuates the development of DN in type II diabetes through multiple mechanisms, including preservation of pancreas function, increased insulin sensitivity due to increased adiponectin production in adipocytes and inhibition of renal ER stress.

Funding: NIDDK Support, Veterans Affairs Support

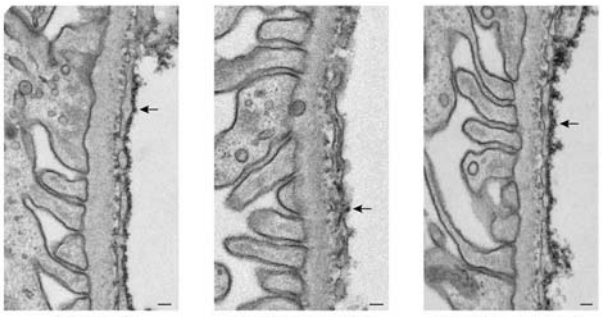
SA-OR037

Reduction in Albuminuria in Diabetic Nephropathy by Atrasentan Is Associated with Restoration of the Glomerular Endothelial Glycocalyx Margien G.S. Boels,[1] Cristina Avramut,[1] Angela Koudijs,[1] Martijn Dane,[1] Dae Hyun Lee,[1] Johan Van der Vlag,[2] Abraham J. Koster,[1] Anton Jan Van Zonneveld,[1] Hermann-Josef Groene,[3] Bernard van den Berg,[1] Ton J. Rabelink.[1] *[1]LUMC, Netherlands; [2]RadboudUMC, Netherlands; [3]DKFZ, Germany.*

Background: Atrasentan, a selective endothelin A receptor (ETAR) blocker, has been shown to reduce albuminuria in T2DM patients. We previously showed that the glomerular endothelial glycocalyx (GEG) is crucial in preventing albuminuria. Therefore, we hypothesized that atrasentan restores the GEG.

Methods: ApoE KO mice were rendered diabetic with STZ (5x60mg/kg) and received a high-cholesterol diet for 12weeks. GEG coverage was quantified using stitches of transmission electron microscopy (TEM) images of cationic ferritin (CF, 5mg/2ml HBSS) perfused glomeruli. CF binds to the negatively charged GEG.

Results: The apoE KO diabetes model recapitulates the well-known features of human DN: mesangial expansion, mesangiolysis, glomerular hypertrophy and aneurism formation, accompanied by increased ACR. ETAR blockade, and consequently endothelial ETBR stimulation was confirmed by NO spin trapping with EPR spectroscopy, which shows almost doubled NO generation**.** 4weeks of atrasentan (7.5mg/kg/day) reduces urinary ACR (~40%, p=0.01), without changes in glomerular gross morphology. Perfusion with CF, however, reveals a clear restoration of GEG: Diabetic apoE KO mice show GEG coverage of only 40.7±3.2%, whereas atrasentan treatment increases this to 89.4±8.6%, p=0.01, comparable to non-diabetic mice, which show GEG coverage of 80.9±5.1%, (>56 capillary loops in 2-3 glomeruli/3 mice).



TEM of cationic ferritin bound to endothelial surface

Conclusions: Similar to observations in T2DM patients, atrasentan reduces albuminuria in diabetic apoE KO mice. This is accompanied by a clear restoration of the glomerular endothelial glycocalyx, confirming a role for the endothelial glycocalyx in prevention of proteinuria in diabetes induced kidney disease.

Funding: Other NIH Support - Dutch Kidney Foundation, Pharmaceutical Company Support - AbbVie

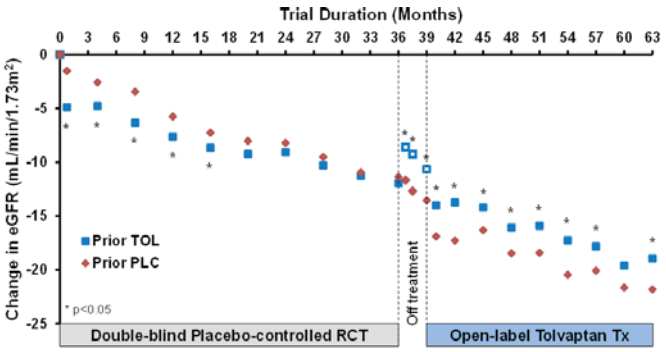
SA-OR038

Tolvptan-Treatment of ADPKD Confers Persistent eGFR Improvement: Results from the TEMPO 4:4 Extension Trial Vicente E. Torres,[1] Arlene B. Chapman,[2] Olivier Devuyst,[3] Ron T. Gansevoort,[4] Eiji Higashihara,[5] Ronald D. Perrone,[6] John Ouyang,[7] Susan E. Shoaf,[7] Jaime Blais,[7] Frank S. Czerwiec.[7] *[1]Mayo Clinic; [2]Emory U; [3]U Zurich; [4]U Med Ctr Groningen; [5]Kyorin U; [6]Tufts; [7]Otsuka.*

Background: TEMPO 4:4 is a two-year open-label extension of the TEMPO 3:4 trial. TEMPO 3:4 showed that the V2-receptor antagonist tolvaptan slowed increase in total kidney volume (TKV) by 49% and decline in kidney function (eGFR) by 26% over 3 years in CKD 1-3 ADPKD patients. This interim analysis (data cut date 7/2013) investigated the persistence of tolvaptan's effect on eGFR.

Methods: Analyses of eGFR response to tolvaptan-treatment during TEMPO 4:4 were prespecified. Subjects outside Japan completing TEMPO 3:4 were eligible to receive 2 years open-label tolvaptan in TEMPO 4:4. Those receiving tolvaptan in TEMPO 3:4 were considered early treatment (ET) and those that received placebo delayed treatment (DT). CKD-EPI eGFR was calculated using central IDMS-traceable serum creatinine measurements taken every 3 months.

Results: Included were data from 871 ADPKD patients. An intra-subject comparison of 314 DT subjects participating in both trials showed significant improvement in eGFR slope after switching from placebo to tolvaptan (-3.59 to -2.85 mL/min/1.73m2/yr treatment effect 21% p=0.048) despite an increased proportion starting in CKD 3 at TEMPO 4:4 baseline compared to their earlier TEMPO 3:4 baseline (37 versus 15%). Analysis of the separation in eGFR between ET and DT subjects post-treatment withdrawal in TEMPO 3:4 demonstrated sustained protection of renal function during two year open label extension ($p<0.05$ for 11/12 time points).



Conclusions: This interim analysis showed deceleration in eGFR decline in placebo treated subjects after initiation of tolvaptan and sustained protection of renal function in tolvaptan treated patients during TEMPO 3:4 and TEMPO 4:4. An updated analysis will be presented.

Funding: Pharmaceutical Company Support - Otsuka PDC

SA-OR039

Refining Genotype-Phenotype Correlation in Autosomal Dominant Polycystic Kidney Disease (ADPKD): The Toronto Genetic Epidemiology Study of PKD (TGESP) Young-Hwan Hwang,[1] John Conklin,[3] Ning He,[2] Kairong Wang,[2] Jamie L. Sundsbak,[4] Christina M. Heyer,[4] Masoom Haider,[3] Peter C. Harris,[4] York P. Pei.[2] *[1]Dept of Medicine, Eulji General Hospital, Eulji Unversity, Seoul, Korea; [2]Divs of Nephrology, Univ Health Network, Toronto, ON, Canada; [3]Dept of Medical Imaging, Univ Health Network, Toronto, ON, Canada; [4]Div of Nephrology, Mayo Clinic, Rochester, MN.*

Background: Renal disease variability in ADPKD is strongly influenced by both a genic and a modifier effect. Non-truncating (NT) *PKD1* mutations have been identified in ~30% of patients but their clinical significance have not been fully defined.

Methods: We recruited a prospective cohort of 220 unrelated patients and 485 of their affected family members through the PKD Clinic at Toronto General Hospital between 2005-2013. All probands had a serum [Cr] <1.4 mg/dL at presentation and underwent comprehensive *PKD1* and *PKD2* mutation screening. In-silico prediction of pathogenicity and segregation analysis was performed for all *PKD1* NT mutations. Height-adjusted total kidney volume (htTKV) was obtained in 162 patients by MRI/CT. Multivariate Cox proportional hazard analysis for renal and patient survival was performed in 646 mutation-positive subjects, adjusting for gender and clustering within the same family.

Results: Overall, 85% of our probands had an identifiable mutation in whom the prevalence of *PKD1* truncating (TR), NT (i.e. non-synonymous missense and atypical splice mutations), in-frame indels (IF indels), and *PKD2* mutations was 32.4%, 23.0%, 3.6%, and 25.7%, respectively. htTKV was smaller in patients with NT compared to TR *PKD1* mutations, but did not differ from those with *PKD2* mutations. Compared to *PKD1* TR mutations, patients with *PKD1* IF indels, *PKD1* NT, and *PKD2* mutations, respectively, had reduced risks of ESRD (hazard ratio (HR) [95%CI]: 0.35 [0.14-0.91], 0.10 [0.05-0.18], 0.03 [0.01-0.05]) and death (HR: 0.31 [0.11–0.87], 0.20 [0.11–0.38], and 0.18 [0.11– 0.31]).

Conclusions: Refining the correlation of genotype with renal disease severity, coupled with targeted exome resequencing, has the potential to advance mutation-based diagnostics to provide useful clinical prognostication in ADPKD.

Funding: Government Support - Non-U.S.

SA-OR040

Enhanced Diagnostics and Gene Discovery in ARPKD-Like and Meckel Syndrome (MKS) Pedigrees Using a Next Generation Sequencing (NGS) Ciliopathy Panel Katharina Hopp, Sarah J. Koon, Christina M. Heyer, Vicente E. Torres, Peter C. Harris. *Mayo Clinic.*

Background: ARPKD and MKS are PKD associated ciliopathies, with one gene, *PKHD1*, implicated in ARPKD and >14 genes in MKS. However, many of our ARPKD-like/MKS pedigrees remain unresolved after Sanger sequencing of commonly affected genes.

Methods: We performed enrichment (Agilent HaloPlex) and NGS (Illumina HiSeq2000) of 258 genes implicated in ciliopathies or cilia biogenesis/function on 18 pedigrees, most of which were outbred, and filtered variants based on quality, population frequency, and substitution significance.

Results: Our analysis solved 50% of pedigrees (6 ARPKD-like, 3 MKS). Of these, 2 ARPKD cases had two mutations in *PKHD1,* one of which was previously missed by Sanger, and 2 MKS fetuses had mutations in *TMEM231* and *TCTN2*, recently identified MKS genes. More interestingly, 4 ARPKD-like probands had pathogenic changes in genes not previously associated with ARPKD. Case (1) presented with PKD at 24wks gestation and inherited *CEP290* p.L552fs homozygous; (2) was diagnosed with ARPKD at 8mo and inherited *TMEM67* p.T244K homozygous (scored as highly likely pathogenic); (3) was diagnosed at 3wks with failure to thrive, ARPKD and hepatic fibrosis, died at 8mo and inherited two *NPHP3* changes (p.W606* and p.D1133fs); and (4) inherited two highly likely pathogenic *IFT172* variants (p.K141E and p.R1134L), a gene recently identified in other ciliopathies. Intriguingly, proband (4) also carried a *PKHD1* mutation (p.Q1665*). We also identified a potentially novel MKS-like gene, *C2CD3*. The fetus inherited a highly likely pathogenic variant (p.V899M) and a rare synonymous change (p.C1114=), which we found to cause exon skipping by fetal tissue analysis. Interestingly, the fetus had typical MKS phenotypes (encephalocele, polydactyly) but without PKD, consistent with the null mouse model.

Conclusions: These results emphasize the suitability of candidate gene NGS for genetic testing of outbred ciliopathy patients and highlight probable additional genetic heterogeneity in unresolved ARPKD-like/MKS patients and/or potential misdiagnoses due to phenotypic overlap with other ciliopathies. Additionally, oligogenic inheritance may play a role in ARPKD-like disease.

Funding: NIDDK Support, Private Foundation Support

SA-OR041

Mutations in *DCDC2* Cause Nephronophthisis with Hepatic Fibrosis in Humans Daniela A. Braun,[1] Markus Schueler,[1] Gayathri Chandrasekar,[2] Heon Yung Gee,[1] Isabel Tapia Paez,[2] Andrea Bieder,[2] Jan Halbritter,[1] Jonathan Porath,[1] Rannar Airik,[1] Detlef Bockenhauer,[3] Tomas Honzik,[4] Richard P. Lifton,[5,6] Juha Kere,[2] Friedhelm Hildebrandt.[1,6] *[1]Div. of Nephrology, Boston Children's Hospital, Harvard Med School, Boston; [2]Dept of Biosciences and Nutrition, Karolinska Inst, Huddinge, Sweden; [3]Univ College London, London, United Kingdom; [4]Dept of Pediatrics, Charles Univ Prague, Prague, Czech Republic; [5]Dept of Genetics, Yale Univ School of Medicine, New Haven; [6]Howard Hughes Medical Inst, Chevy Chase.*

Background: Nephronophthisis-related ciliopathies (NPHP-RC) are a group of autosomal recessive diseases that cause progressive renal failure and various extrarenal manifestations like hepatic fibrosis in the first decades of life. To date, mutations in more than 80 causative genes have been identified that together explain ~70% of all cases.

Methods: To identify novel single gene causes of NHPHP-RC we performed whole exome sequencing combined with homozygosity mapping in 100 individuals. We then screened 1,064 families with NPHP-RC by barcoded multiplex PCR (Fluidigm Access Array™) and next-generation sequencing.

Results: In a consanguineous family with one affected child, we detected a homozygous truncating mutation in the gene DCDC2 (doublecortin domain containing 2). The affected child presented with liver fibrosis at age 11 months and ESRD at age 11 yrs. By screening our NPHP-RC cohort, we identified two compound heterozygous truncating alleles in DCDC2 in a second family with early-onset liver fibrosis. We show by immunofluorescence that DCDC2 localizes to the ciliary axoneme in liver and kidney, as well as to mitotic spindle fibers and the abscission structure in cycling cells. By immunoprecipitation, we show interaction of DCDC2 with Dvl1,2,3 and JIP1 via its N-terminus. Overexpression of DCDC2 inhibits canonical Wnt signaling, but interestingly all three alleles lack this inhibitory effect in vitro. Knockdown of DCDC2 in zebrafish recapitulates a ciliopathy phenotypic with ventral body axis curvature, hydrocephalus, and kidney cysts.

Conclusions: We identified mutations in DCDC2 as a novel cause of NPHP-RC with liver fibrosis in humans and link the pathogenesis to impaired Wnt signaling.

Funding: NIDDK Support

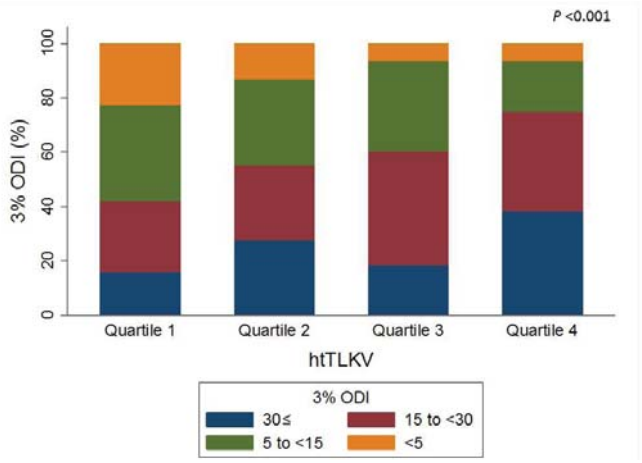
SA-OR042

High Prevalence of Sleep-Disordered Breathing and Its Association with Liver and Kidney Volume in Patients with Autosomal Dominant Polycystic Kidney Disease Keiichi Sumida, Junichi Hoshino, Tatsuya Suwabe, Koki Mise, Kenmei Takaichi, Yoshifumi Ubara. *Nephrology Center, Toranomon Hospital Kajigaya, Kawasaki, Kanagawa, Japan.*

Background: Sleep-disordered breathing (SDB) is prevalent among chronic kidney disease patients, but its prevalence among patients with autosomal dominant polycystic kidney disease (ADPKD) and its association with total liver and kidney volume (TLKV) remain unclear.

Methods: We examined the association between height-adjusted TLKV (htTLKV) and SDB in a cross-sectional study of 304 adult patients with symptomatic ADPKD who were hospitalized at Toranomon Hospital for transcatheter arterial embolization and who underwent pulse oximetry between April 2008 and November 2013. SDB was defined as having a 3% oxygen desaturation index of 15 or more events per hour of sleep. We performed logistic regression with sex-specific quartiles (3,673.9, 5,262.9, and 7,087.3 cm^3/m in men and 3,526.9, 4,680.3, and 5,847.8 cm^3/m in women) of htTLKV as the main predictor, and demographic factors and comorbidities as covariates.

Results: Overall (54.6% women, mean age 56.2 ± 9.4 years, 83.5% on hemodialysis), 177 (58.2%) patients had SDB. Odds ratios (ORs) (95% confidence intervals [95% CI]) for SDB were 1 (reference), 1.73 (0.81–3.67), 2.44 (1.16–5.15), and 4.73 (2.06–10.9) for htTLKV quartiles 1–4, respectively (P for trend <0.001). Older age (OR, 1.84 [95% CI, 1.31–2.58] per increase in 10 years), male sex (OR, 3.85 [95% CI, 1.98–7.50]), receiving hemodialysis (OR, 3.54 [95% CI, 1.49–8.44]), and higher BMI (BMI ≥25 kg/m2) (OR, 4.40 [95% CI, 1.62–11.9]) were also associated with SDB.



Conclusions: SDB is highly prevalent and independently associated with increased htTLKV in patients with symptomatic ADPKD.

SA-OR043

Using Human Pluripotent Stem Cells to Model Kidney Disease Pathophysiology and Therapy Benjamin S. Freedman,[1] Albert Q. Lam,[1] Theodore I. Steinman,[1,2] Peter C. Harris,[3] Jing Zhou,[1] Joseph V. Bonventre.[1] *[1]Brigham and Women's Hospital; [2]Beth Israel Deaconess Medical Center; [3]Mayo Clinic.*

Background: Induced pluripotent stem cells (iPSCs) are adult cells that have been reprogrammed to an embryonic stem cell (ESC)-like state. We tested the ability of iPSCs to model the cellular pathophysiology of cystic kidney diseases, and to differentiate into human kidney cells.

Methods: A cohort of iPSCs was established from 14 patients representing ADPKD, ARPKD, Bardet-Biedl Syndrome, Meckel-Gruber Syndrome, and healthy controls. Pathogenic mutations were identified using next-generation sequencing. Ciliary length and protein expression were quantified in iPSCs and descendant cells. To determine their potential for kidney regeneration, iPSCs were treated with growth factors under serum-free conditions and implanted beneath the murine kidney capsule.

Results: iPSCs underwent extensive self-renewal and differentiation, elaborated primary cilia, and endogenously expressed PKD disease genes. ADPKD iPSCs and descendant cells with PC1 mutations expressed reduced ciliary PC2, which could be rescued by exogenous expression of wild-type PC1. Meckel-Gruber Syndrome iPSCs exhibited abnormally long cilia, with normal PC2 localization. Sequential treatment of ESCs/iPSCs with CHIR99021, FGF2, and retinoic acid induced markers of intermediate mesoderm (PAX2, LHX1), metanephric mesenchyme (SIX2, SALL1), and proximal tubule (KSP, LTL). When implanted in vivo, metanephric cells generated macroscopic growths expressing high levels of aquaporin-1.

Conclusions: We have established a cohort of genetically characterized PKD and ciliopathy iPSCs that recapitulate syndrome-specific ciliary phenotypes, with applicability for therapeutic screens. iPSC-derived kidney cells survive, proliferate, and differentiate in vivo, providing an expandable source of new kidney tissue which is 100 % immunocompatible with the original patient. The ability of iPSCs to provide novel

mechanistic insights into human cellular pathophysiology, together with their potential as regenerative cell therapeutics, makes them a powerful new resource for kidney disease research.

Funding: NIDDK Support, Private Foundation Support

SA-OR044

miR-17~92 microRNA Cluster Promotes Disease Progression in Genetic Models of ADPKD Vishal Patel, Darren Williams, Ronak Lakhia, Sachin S. Hajarnis. *Internal Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent genetic cause of ESRD in the United States. MicroRNAs (miRNAs) are non-coding RNAs that inhibit gene expression primarily by binding to 3'-UTRs of target genes. We have recently shown that the miR-17~92 miRNA cluster inhibits *Pkd1* and *Pkd2*, and promotes kidney cyst growth. However, whether miR-17~92 can modulate the cystic phenotype in ADPKD, where *PKD1* and *PKD2* are already mutated, is not known.

Methods: qRT-PCR analysis was performed to determine miR-17~92 expression in mouse models of ADPKD. To determine whether miR-17~92 played a pathogenic role, miR-17~92 was inactivated from renal tubules of kidney-specific *Pkd1* and *Pkd2* mutant mice. Global gene expression analysis was performed to identify genes that are upregulated in *Pkd1*-miR-17~92 double knockout (KO) mice compared to *Pkd1* single KO mice, and in *Pkd2*-miR-17~92 double KO mice compared to *Pkd2* single KO mice, respectively. Bioinformatic analysis and integration of the available cross-linking and immunoprecipitation (CLIP)-seq datasets identified a common set of genes that contain binding sites and physically interact with miR-17~92 in their 3'-UTRs.

Results: The levels of miR-17~92 are increased in genetic models of ADPKD likely due to c-Myc-mediated transcriptional activation. Deletion of miR-17~92 suppressed cyst growth, decreased kidney injury, improved renal function and nearly doubled the life span of *Pkd1* and *Pkd2* mutant mice. miR-17~92 produced these effects by decreasing the proliferation of cyst epithelial cells. Gene expression analysis, 3'-UTR bioinformatic analysis and integration of CLIP-seq datasets identified a common network of miR-17~92 target genes in *Pkd1* and *Pkd2* mutant kidneys. Functional annotation of the direct targets revealed that miR-17~92 regulates glucose metabolism pathway genes in *Pkd1* and *Pkd2* mutant kidneys.

Conclusions: miR-17~92 promotes cyst growth in mouse models of ADPKD. Our results suggest a new mechanism for disease progression in ADPKD involving miRNA-mediated regulation of metabolic pathway genes.

Funding: NIDDK Support, Private Foundation Support

SA-OR045

Angiotensinogen Inhibition Slows Polycystic Kidney Disease in Mice with a Targeted Mutation in Pkd2 Kameswaran Ravichandran,[1] Abdullah Ozkok,[1] Qian Wang,[1] Adam E. Mullick,[2] Charles L. Edelstein.[1] *[1]Univ of Colorado Denver; [2]Isis Pharmaceuticals.*

Background: Renal cyst enlargement is associated with the activation of both circulating and intrarenal renin-angiotensin-aldosterone (RAAS) system. The aim of the study was to determine the effect of angiotensinogen inhibition in a model of autosomal dominant polycystic kidney disease (ADPKD).

Methods: A Gen 2 angiotensinogen (AGT)antisense oligonucleotide (ASO) that selectively reducesAGT mRNA was injected at 50-100mg/kg/wk i.p. once weekly in PKD2WS25mice (WS25; an orthologous model of human ADPKD) from 4 to 16 weeks of age.

Results:

	+/+ Scr ASO (n=11)	+/+ AGT ASO (n=14)	WS25 Scr ASO (n=10)	WS25 AGT ASO (n=7)
BW (g)	28.2	29.4	27.3	29.4
2KW (g)	0.34	0.35	0.67*	0.47**
2K/TBW (%)	1.2	1.2	2.4*	1.5**
CVD (%)	0.4	0.5	34.1*	22 **
BUN (mg/dL)	32	29	47*	34**
AGT serum (ng/mL)	2489	1233	3643*	1483**
AGT kidney (ng/mL)	1.3	0.08	6.1*	0.7**
TGF-β (pg/mL)	53	40	102*	32 ± 5*
Fibrosis score	0	0	0.4± 0.1*	0.1**
CXCL1 (pg/mg)	0.4	0.3	3.4*	0.6**
IL-12	8	9	37*	9**

*P<0.001 vs. +/+; **p< 0.001 vs. WS25 Scr ASO, NS vs +/+;BW= body weight, CVD= cyst volume density. 2K/TBW (%)=two kidney weight to total body weight ratio.
AGT protein was increased in blood and kidney in PKD and the AGT ASO resulted in a significant decrease in AGT protein.The AGT ASO resulted in a significant decrease in kidney enlargement, cyst volume density and BUN without an effect on body weight. The AGT ASO resulted in a significant decrease in TGF-β and interstitial fibrosis (Sirius red staining) in the kidney. Mice treated with the AGT ASO had a significant decrease in CXCL1, a neutrophil chemokine, and IL-12, a T cell stimulating factor, in the kidney.

Conclusions: AGT inhibition decreased cystogenesis, interstitial fibrosis and pro-inflammatory chemokines/cytokines and represents a possible future therapy for ADPKD.

SA-OR046

Vascular Endothelial Growth Factor C Therapy for Polycystic Kidney Diseases Jennifer L. Huang,[1] Adrian S. Woolf,[2] Maria K. Joannou,[1] Richard N. Sandford,[4] Dorien J.M. Peters,[5] Karen Price,[1] Paul Winyard,[1] David A. Long.[1] *[1]Developmental Biology and Cancer, UCL Inst of Child Health, United Kingdom; [2]Inst of Human Development, Univ of Manchester, United Kingdom; [3]Academic Dept of Medical Genetics, Univ of Cambridge, United Kingdom; [4]Dept of Human Genetics, Leiden Univ, Netherlands.*

Background: Current therapies for polycystic kidney diseases (PKDs) have focussed on ameliorating epithelial aberrations. As an alternative strategy, we hypothesised that the niche surrounding kidney tubules, including the microvasculature, may modulate cystogenesis.

Methods: The renal capillaries and lymphatics were examined in two mouse models of PKD (*Pkd1*^{nl}and *Cys1*^{cpk}). To modulate the vasculature during PKD, mice from both models were administered vascular endothelial growth factor (VEGF)C.

Results: Normal kidney tubules were surrounded by a capillary network expressing CD31 and VEGFR3. PKD led to a significant increase in the area of CD31$^+$/VEGFR3$^+$ vessels, which were disorganised and dilated. PKD did not lead to alterations in Prox1$^+$/LYVE1$^+$ lymphatics. We targeted the aberrant vasculature in PKD using VEGFC, which enhanced the phosphorylation of VEGFR3 in the kidney and led to a normalised renal network of VEGFR3$^+$/CD31$^+$ capillaries. In addition, VEGFC led to hyperplasia of Prox1$^+$/LYVE1$^+$ lymphatics. The changes in the vasculature were associated with a 30-40% reduction in kidney/body weight ratio ($p < 0.001$); lower blood urea nitrogen ($p < 0.01$); lower creatinine concentration ($p < 0.001$); and reduced average cyst size ($p < 0.05$). VEGFC also significantly reduced numbers of M2 alternatively activated macrophages ($p < 0.05$), previously known to be cystogenic.

Conclusions: VEGFC treatment improved PKD by normalising the patterning of VEGFR3$^+$/CD31$^+$ capillaries and causing lymphatic hyperplasia; manoeuvres which would enhance the drainage of excess interstitial fluid. Our results support the contention that the niche surrounding kidney tubules modulates cystogenesis and that VEGFR3$^+$ capillaries and lymphatics are a novel and important target for PKD therapies.

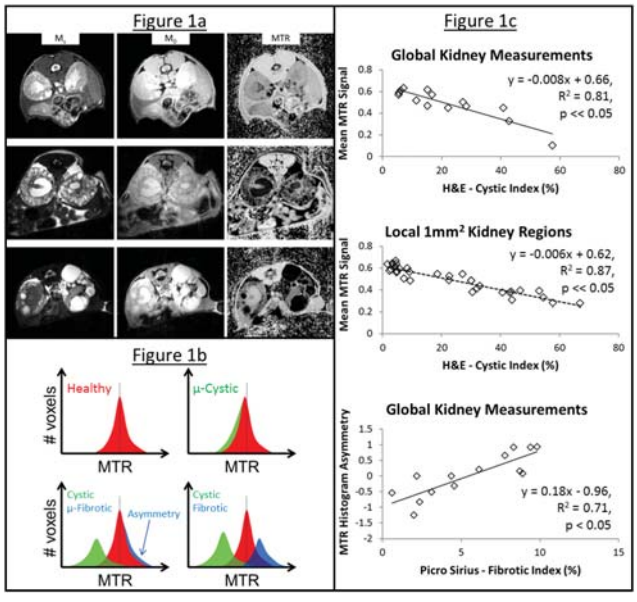
SA-OR047

Magnetization Transfer Imaging for Tissue Remodeling Analysis in a Murine Model of ADPKD Timothy L. Kline, Maria V. Irazabal, Behzad Ebrahimi, Katharina Hopp, Joshua D. Warner, Lilach O. Lerman, Peter C. Harris, Bernard F. King, Vicente E. Torres, Bradley J. Erickson. *Mayo Clinic.*

Background: Magnetization transfer (MT) imaging, a quantitative MR technique, allows the detection of macromolecules based on different magnetic characteristics of free and bound water and has been used to detect lesions and pathophysiological changes in brain, pancreas, and lungs. Early tissue changes in ADPKD may elucidate differences in phenotypically similar patients whose subsequent time-course of the disease may differ. We investigated MT as a source for new tissue remodeling biomarkers.

Methods: Homozygous *Pkd1* R3277C mice at 9 (3F;3M) and 12 months of age (4F;4M) were imaged *in vivo* with a 16.4T MR imaging system, then euthanized. Kidneys were harvested for histological measurement of cystic and fibrotic index. MT ratio (MTR) maps were computed from magnetically saturated (M_s) and unsaturated (M_0) bound proton images by computing $(M_0 - M_s)/M_0$ for each voxel. A Gaussian mixture model (GMM) quantified the presence of tissue remodeling from the MTR intensity distribution.

Results: Figure 1a displays M_s, M_o, and MTR *in vivo* images. Figure 1b conceptualizes how our GMM quantifies various PKD tissue remodeling scenarios from MTR tissue intensity distributions. Compared with normal parenchyma - cysts have decreased MTR, while fibrotic tissues have increased MTR. Figure 1c shows that MTR values correlate with cystic index, and that fibrotic remodeling of tissue is conveyed by MT.



Conclusions: We present the first use of MT imaging for use in PKD assessment. Using a murine model of the disease we found close correlations between parameters extracted from MTR maps and both cystic and fibrotic indices. This information together with traditional total kidney volume assessments will expand the number of useful quantitative measures for monitoring disease progression and predicting outcomes for PKD patients.

Funding: NIDDK Support

SA-OR048

Podocyte Specific Expression of Mutant APOL1 Induces Albuminuria and Global Sclerosis in Mice

Ae Seo Deok Park,[1] Jeffrey B. Kopp,[2] Cheryl Ann Winkler,[3] James M. Pullman,[4] Jeffrey H. Miner,[5] Katalin Susztak.[1] *[1]Perelmen School of Medicine, UPENN; [2]NIDDK; [3]National Cancer Inst; [4]Albert Einstein College of Medicine; [5]Washington Univ in St. Louis.*

Background: Afro-Americans have 3-5 fold-increased risk of developing end stage renal disease. Recently, polymorphism (G1 and G2) in apolipoprotein L1 (APOL1) gene has been identified explaining this increased risk and presenting diverse phenotypes: focal segmental glomerulosclerosis, HIV-associated and hypertensive nephrosclerosis. The pathomechanism of APOL1 remains poorly understood.

Methods: Human kidney samples were collected from nephrectomies and genotyped for G0 (control), G1 or G2 (risk) APOL1 alleles. Gene expression analysis (GEA) was performed on microdissected tubule and glomerular samples using Affymetrix expression arrays. Primary renal epithelial cells were cultured from control and risk kidneys. Generated mice with wild type (G0) and mutant (G1 or G2) APOL1 allele under a tet inducible promoter were crossed with nephrin rtTA and induced on doxycycline diet.

Results: GEA showed that APOL1 transcript level is lower in the risk alleles than controls. APOL1 protein level in cultured renal epithelial cells was also lower in risk samples, but intermediate in G1/G0. Interferon gamma was a strong regulator of APOL1 in vitro. Chromatin immunoprecipitation showed STAT1 binding sites on the APOL1 promoter/enhancer region. IF and immunogold electron microscopy showed that APOL1 in human podocytes follows the endocytic pathway; APOL1 localized to the ER, endocytic vesicles, lysosomes and autophagosomes. Transcriptomic profiling also highlighted differences in synaptic transmission and endocytosis pathways between control and risk alleles samples. Mutant APOL1 mice developed severe albuminuria and glomerulosclerosis at 3-5 weeks after induction, while wild type mice expressing similar levels of G0 APOL1 showed minimal renal abnormalities.

Conclusions: APOL1 expression in risk allele samples was lower than in control allele samples and it could be regulated by interferon. Mutant APOL1 mice developed proteinuria and global sclerosis. APOL1 was localized to the endocytic pathway and transcriptome analysis showed differences in this pathway.

SA-OR049

Identification of Small Molecule Integrin β1 Agonist as a Podocyte Protective Agent Using a Novel Podocyte High Content Screening Assay

Ha Won Lee, Mehmet M. Altintas, Mohd Hafeez Faridi, Hatem A. Elshabrawy, Jochen Reiser, Vineet Gupta. *Dept of Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

Background: Focal segmental glomerulosclerosis (FSGS) is a leading cause of idiopathic nephrotic syndrome with limited therapeutic options. Podocytes are injured early in the disease pathogenesis and represent a crucial cellular target for therapeutic interventions in FSGS and other proteinuric glomerular diseases. However, podocyte-targeted drug discovery has been hampered due to lack of high quality screening assays. Here, we describe a high content screening (HCS) approach to discover podocyte-targetedtherapeutics using differentiated podocyte culturesand reveal that an integrin β1 agonist rescues podocyte injury.

Methods: Using a confocal microcopy based high content imaging system, we developed a high throughput screening assay to measure phenotypic changes in podocytes. Murine podocytes were treated with a podocyte-damaging agent (puromycin aminonucleoside (PAN)). We analyzed >1000 cells per assay condition to computationally and quantitatively determine changes by chemicals in podocyte morphology, cytoskeleton and focal adhesions upon injury.

Results: Our assay consistently produced a Z-prime factor > 0.5, suitable for a high-throughput screening. Subsequently, with a small chemical library of >2200 compounds identified known and novel compounds that protected podocytes from PAN-induced damage (~1% hit rate). Among the hits, we confirmed an integrin β1 agonist as a novel agent that dose-dependently protected podocytes from PAN-induced injury. It restored phenotypic (F-actin cytoskeleton, Akt phosphorylation and focal adhesions) and functional changes in PAN-treated podocytes. We are currently validating the hits using in vivo model systems.

Conclusions: We developed a high-throughput assay for unbiased quantification of changes in podocytes that has wide applicability. Using this assay, we identified novel compounds that prevent podocyte injury. Validation ofan integrin β1 agonistsupports our hypothesis that our podocyte-based HCS assay is a useful technique for the identification of therapeutics for treating proteinuric kidney diseases.

SA-OR050

In Vivo Serial Multiphoton Imaging of the Early Changes in Glomerular Structure and Function in Nephrotic NEP25/Podocin-Confetti Mice

Kengo Kidokoro,[1,2] James L. Burford,[1] Taiji Matsusaka,[3] Naoki Kashihara,[2] Janos Peti-Peterdi.[1] *[1]Physiology & Biophysics, Univ of Southern California, Los Angeles, CA; [2]Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan; [3]Internal Medicine, Tokai Univ, Kanagawa, Japan.*

Background: The development of podocyte injury/dysfunction and albuminuria in nephrotic syndrome (NS) are still incompletely understood due to technical limitations in studying the glomerular filtration barrier (GFB) and podocytes in their intact environment *in vivo*. We aimed to directly visualize the early morphological and functional changes of GFB in vivo in the mouse kidney during the development and progression of NS.

Methods: Podocin-Confetti mice in which single podocytes were genetically labeled and identified by CFP/GFP/YFP/RFP expression were crossed with NEP25 mice, in which selective podocyte injury was induced by a single injection of the immunotoxin anti-Tac(Fv)-PE38 (LMB2). Between 0-7 days after the iv injection of 1.25-5 ng/g LMB2, NEP25/Pod-Confetti mice (4-6 weeks of age) were subjected to multiple surviving surgeries to exteriorize and image the same region and glomeruli of the left kidney by in vivo serial multiphoton microscopy (MPM). Blood vessels were labeled red by Alexa594-albumin. Specific glomeruli were z-scanned to visualize changes in podocyte morphology and dynamics.

Results: After the injection of high dose LMB2 the rapidly progressing development of NS was observed including the detection of detached podocytes and the irregular length, width, shape, and clubbing of podocyte cell processes. Multi-color podocyte clusters on visceral layer and podocyte projections to the parietal Bowman's capsule developed within 7 days. Parallel with these morphological changes, the increased GFB permeability to albumin in denuded areas of the GFB was apparent by the accumulating high level of Alexa594 fluorescence in cells of the proximal tubule and in the tubular lumen.

Conclusions: This MPM imaging study confirmed high podocyte motility, the highly dynamic glomerular remodeling in early NS, and the key role of podocytes in the development of nephrotic proteinuria.

SA-OR051

Microtubule Stabilization By Taxol Promotes Podocyte Process Elongation and Enhances Compensatory Glomerular Adaptation to Podocyte Depletion

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Background: Reminiscent of neuronal repair, upon podocyte depletion, remnant intact podocytes exhibit a considerable adaptive capacity to extend cellular processes and develop collateral branches to cover the denuded glomerular basement membrane. Central to the outgrowth, branching and elongation of cellular processes is remodeling of microtubule cytoskeleton, which is the principal cytoskeletal component in podocyte cell body and major processes. This study examined the role of microtubule remodeling in compensatory glomerular adaptation following podocyte loss.

Methods: The effect of manipulation of microtubule dynamics was examined in adriamycin injured conditionally immortalized mouse podocytes and in murine models with adriamycin nephropathy.

Results: Adriamycin injury induced loss of podocyte phenotypic markers, like synaptopodin and nephrin, elicited cellular shrinkage and progressively reduced cell size as measured by time lapse microscopy, concomitant with disruption of microtubule cytoskeleton integrity. These effects were mimicked by specific microtubule destabilizing agents, including colchicine and vinblastine. In contrast, delayed treatment with taxol, a quintessential microtubule stabilizing agent, abrogated the adriamycin elicited podocyte injury, promoted microtubule assembly and reinstated microtubule scaffolding, associated with a restored shape and size of podocytes and normalized podocyte phenotypes. In mice with adriamycin induced nephropathy, a single low dose taxol treatment was given 5 days after adriamycin injury, when proteinuria and podocyte injury were evident. Taxol therapy attenuated proteinuria and ameliorated progressive glomerular sclerosis despite no correction of podocytopenia. Mechanistically, taxol therapy enhanced microtubule assembly and stabilization in adriamycin injured glomeruli, associated with elongation of podocyte major processes as revealed by electron microscopy.

Conclusions: Stabilization of microtubule cytoskeleton promotes podocyte process elongation, reinforces compensatory glomerular adaptation to podocyte loss and attenuates proteinuria and glomerulosclerosis.

Funding: NIDDK Support

SA-OR052

Protamine Sulfate-Induced Degradation of Synaptopodin and Loss of Stress Fibers Are Mediated by EGF Receptor-Dependent Activation of Src

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Background: Perfusion of rat or mouse kidneys with the polycation protamine sulfate (PS) causes TRPC5-dependent podocyte actin remodeling and foot process effacement. In vitro, exposure of podocytes to PS leads to loss of stress fibers and degradation of synaptopodin in a TRPC5-dependent fashion. PS has previously been show to increase

EGF receptor (EGFR) kinase activity, presumably by exposing a population of cryptic EGFRs. Therefore we hypothesized that PS induces podocyte actin remodeling through EGFR-mediated activation of Src and PI3 kinase signaling.

Methods: The effect of PS on podocytes was analyzed using biochemistry assays, lentiviral approaches and phalloidin staining to visualize the actin cytoskeleton.

Results: We found that PS-induced degradation of synaptopodin and loss of stress fibers were blocked by EGFR inhibitor AG1478, Src inhibitor-1, PI3 kinase inhibitor wortmannin, calcineurin inhibitor CsA, or Cathepsin L inhibitor E64. In a complementary genetic approach, we established that Src-resistant Synpo-Y222A, calcineurin-resistant Synpo-ED or CatL-resistant Synpo-CM1+2 protect from PS-induced loss of stress fibers. Mechanistically, the PS-induced degradation of synaptopodin initiates a signaling cascade including Vav2 activation, enhanced Rac1 signaling, RhoA inactivation and loss of actin stress fibers.

Conclusions: In summary, PS promotes Src signaling through EGFR activation to trigger the degradation of synaptopodin, which results in TRPC5 and Vav2-mediated Rac1 activation. Our results reveal EGFR as the long-sought membrane receptor responsible for PS-induced effects on actin dynamics.

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

SA-OR053

Identification of Podocytopathy-Associated Genes by Translational Profiling of Podocytes Ivica Grgic,[1] Andreas Hofmeister,[1] Hua Sun,[3] Omar H. Maarouf,[4] Vanesa Bijol,[5] Martin R. Pollak,[3] Benjamin D. Humphreys.[4] *[1]Internal Medicine and Nephrology, Philipps-Univ Marburg; [2]Program in Medical and Population Genetics, Broad Inst of MIT and Harvard; [3]Div of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School; [4]Renal Div, Brigham and Women's Hospital; [5]Dept of Pathology and Laboratory Medicine, Brigham and Women's Hospital.*

Background: Research aimed at finding new biomarkers and therapeutic target structures for the management of podocytopathies such as focal segmental glomerulosclerosis (FSGS) requires a detailed analysis of transcriptional changes in podocytes over the course of disease. However, dissociating nucleic acids of specific and particularly underrepresented renal cell populations from the rest of the kidney is challenging.

Methods: Here we have used translating ribosome affinity purification (TRAP) to extract and analyze podocyte-specific mRNA during aging and in two different genetic FSGS models.

Results: We expressed an eGFP-tagged ribosomal fusion protein (eGFP-L10a) in podocytes using the Collagen-1a1 promoter, enabling podocyte-specific mRNA isolation from Col1α1-eGFP-L10a in a one-step process over the course of disease. This TRAP protocol robustly enriched known podocyte-specific mRNAs, validating the approach. We crossed Col1α1-eGFP-L10a mice with the Actn4$^{-/-}$ (severe) and Actn4$^{+/K256E}$ (mild) FSGS models and analyzed podocyte translational profiles at 2, 6 and 44 weeks. Importantly, two identified podocyte genes induced in murine FSGS (Cxcl1 and Dmpk) were also upregulated at the protein level in biopsies from patients with proteinuria and histologically proven podocyte abnormalities. There was no dilution of podocyte-specific transcripts during disease.

Conclusions: These are the first podocyte-specific RNA expression datasets during aging and in two models of FSGS. This approach identified new podocyte proteins that are upregulated in FSGS and may be useful for the discovery of novel biomarkers and therapeutic targets for human glomerular disease.

Funding: NIDDK Support

SA-OR054

Characterization of WT1-Directed Transcription in Podocytes In Vivo by ChIPseq Martin Kann,[1] Sandrine S. Ettou,[2] Maximilian Otto Lenz,[1] Bernhard Schermer,[1] Thomas Benzing,[1] Jordan A. Kreidberg.[1] *[1]Nephrolab Cologne & Center for Molecular Medicine, Univ of Cologne, Cologne, Germany; [2]Div of Nephrology, Boston Children's Hospital, Harvard Medical School, Boston, MA.*

Background: The Wilms' Tumor Suppressor protein WT1 is a key transcription factor (TF) in establishing and maintaining the podocyte pool. While it has long been known that WT1 mutations can cause FSGS, only a few targets of WT1 have been identified in podocytes. Therefore, we addressed WT1 mediated transcription by ChIPseq and mRNAseq in healthy and diseased podocytes.

Methods: ChIPseq for WT1 was carried out on wildtype kidneys. For mRNAseq, GFP was conditionally expressed in podocytes, cells were FAC-sorted from wildtype and adriamycin treated mice, and RNA was extracted.

Results: ChIPseq identified more than 14,000 WT1 peaks within the genome, located within the cis-regulatory regions (CSR) of 5,900 genes. Analysis of histone modifications found in the ENCODE database at WT1 peaks was consistent with the WT1 peaks occurring at transcription start sites (TSS), or within enhancers. While both peak clusters contained WT1 binding motifs, the enhancer cluster was enriched in motifs for TFs with known roles in podocyte speciation such as Fox-class TFs and Lmx1b, suggesting cooperation of WT1 with these TFs to establish podocyte specific gene expression. WT1 binding at TSSs resulted in higher expression levels of bound as compared to unbound genes, indicating a role of WT1 in control of podocyte specific gene expression levels. The highest expression levels were observed for a class of genes that were both, bound by WT1 at their TSS and at enhancers in their CSR. Gene ontology analysis revealed that this class of genes was enriched for hereditary podocytopathy genes, as well as genes within key components of podocytes, such as the actin cytoskeleton, slit diaphragm, and cell adhesions. Induction of podocyte damage by adriamycin resulted in differential expression of WT1 bound genes within cell adhesion pathways, identifying these pathways as novel WT1 targets in podocytes.

Conclusions: In summary, ChIPseq suggests major functions of WT1 in establishing and maintaining podocyte specific gene expression in health and disease.

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

SA-OR055

Tankyrase Inhibition Reduces Elevated Total PARsylation in CD2AP Deficient Podocytes but Aggravates Kidney Injury Sanna H. Lehtonen,[1] Hong Wang,[1] Hani Suleiman,[2] Andrey S. Shaw,[2] Sara Kuusela.[1] *[1]Dept of Pathology, Univ of Helsinki, Finland; [2]Dept of Pathology & Immunology, Washington Univ School of Medicine.*

Background: Mice lacking CD2AP develop nephrotic syndrome with heavy proteinuria and effacement of podocyte foot processes.We found by yeast two-hybrid screening of a glomerular library that CD2AP interacts with tankyrase 2.Tankyrases are poly(ADPribose) polymerases that catalyze PARsylation [poly(ADP-ribosyl)ation], a post-translational modification of proteins involved in diverse cellular processes. Elevated PARsylation may be harmful for cells, as previous studies have shown increased PARsylation in the glomeruli of diabetic db/db mice. The aim of this study was to characterize the significance of tankyrase-CD2AP interaction and the PARsylation activity of tankyrases in podocyte function and injury.

Methods: The interaction of CD2AP and tankyrases was confirmed by co-immunoprecipitation and GST-pull down assays. The activity of tankyrases was blocked with a specific inhibitor, XAV-939. In zebrafish, CD2AP was knocked down with morpholino antisense oligonucleotides. Wild-type and CD2AP knockout mouse kidneys and cultured podocytes were analyzed by immunohistochemistry and colorimetric PARsylation assay. Western blotting and qRT-PCR were used for mRNA and protein expression studies.

Results: Tankyrase 1 and 2 are expressed in normal mouse and rat kidneys *in vivo*, and they form a complex with CD2AP. Tankyrase 1 is downregulated, but the posttranslational modification of both tankyrases is elevated in CD2AP-/- podocytes. Also the total PARsylation activity and the activation of beta-catenin are elevated in the absence of CD2AP, and both can be reduced by treatment with XAV-939. *Lef-1*, a mediator of Wnt/beta-catenin signaling pathway, is upregulated in CD2AP-/- podocytes, and surprisingly, XAV-939 treatment further upregulates *lef-1* and *pai-1*, known to associate with podocyte injury. In zebrafish, administration of XAV-939 to CD2AP-depleted larvae aggravated kidney injury and increased mortality.

Conclusions: The data indicate that maintaining the PARsylation activity of tankyrases in balance by CD2AP is essential for normal kidney function.

Funding: Private Foundation Support

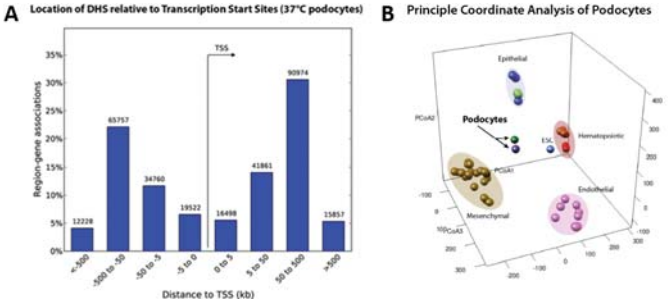
SA-OR056

Regulatory Genomics of the Glomerular Podocyte Shreeram Akilesh. *Pathology, Univ of Washington, Seattle, WA.*

Background: Understanding the genomic regulation of gene expression remains a fundamental problem in biology. As exemplified by the ENCODE project, DNaseI hypersensitivity mapping (DNaseI-seq) identifies regulatory DNA elements (e.g. promoters, enhancers, silencers) that control gene expression and thereby cell identity and their responses to external stimuli. Since these regulatory genomic maps are extremely cell-type specific, we are applying these cutting edge technologies to cultured human podocytes.

Methods: Nuclei were isolated from conditionally immortalized human podocyte cells under undifferentiated/permissive (33°C) and differentiated/non-permissive (37°C) conditions. The nuclei were digested with DNaseI and the released DNA fragments were purified and sequenced to high depth (~25 x 10^6 tags) on the Illumina HiSeq next-generation sequencing platform. The sequenced tags were mapped back to the human genome to generate per-nucleotide cleavage frequency maps across the genome. Data processing was performed using the open-source BEDOPS suite, AWK and R.

Results: DNaseI-seq on cultured podocytes identified 195027 (33°C) and 171397 (37°C) DNaseI hypersensitive sites (DHS) respectively. ~88% of these DHS map to distal regulatory DNA elements >5kb from known gene transcription start sites (TSS) (Fig. 1A). Principle coordinate analysis (PCoA) reveals that podocytes cluster in a previously unseen position between epithelial and mesenchymal cell lines, consistent with their developmental origin (Fig. 1B). Examining enrichment of transcription factor binding motifs within the regulatory DNA landscape of "differentiated" podocytes reveals significant emphasis of POU- and FOX-family transcription factors.



Conclusions: DNaseI-seq is an exceptional tool to study genome regulation. Additional experiments and analyses are in progress which are revealing novel insights into the genomic biology of podocytes and other kidney cells.

Funding: Other NIH Support - NHGRI U54 HG007010-02, Private Foundation Support

SA-OR057

Glomerular Endothelial Cells Form a Functional Syncytium for Calcium Signals Upon Injury – A Multiphoton In Vivo Study Julia Binz, Matthias Hackl, Bernhard Schermer, Thomas Benzing. *Dept II of Internal Medicine and Center for Molecular Medicine Cologne, Univ of Cologne, Cologne, Germany.*

Background: In the renal glomerulus different cell types are located in close proximity and crosstalks, e.g. between podocytes and endothelial cells, have been reported. However the communication between neighboring endothelial cells and their crosstalk with other cell types is incompletely understood. Recently *in vivo* intra-vital imaging of mouse glomeruli has been established, which allows to study cell-cell interactions in the intact kidney.

Methods: 4 weeks old mice expressing the calcium indicator GCaMP3 specifically in endothelial cells (via Tie2:cre) were anaesthetized, an arterial catheter was placed into the right carotid artery and the left kidney was exteriorized for *in vivo* multiphoton microscopy. Blood vessels were labelled by injection of 70 kDa Texas Red dextrane. Acute endothelial injury was induced during by zooming in on a capillary loop on the outside of a glomerulus to cause a localized injury with the laser. The resulting calcium response was recorded with in a time series of the glomerulus including surrounding vessels.

Results: Upon laser induced injury of a capillary loop, a strong calcium response is rapidly propagated across the entire glomerulus extending out to endothelial cells in the afferent and efferent arterioles. At the area surrounding the point of laser damage we observed a sustained elevation of intracellular calcium levels in injured cells. From those injured cells a short calcium wave was periodically propagated towards the vascular stalk and further to the afferent/efferent arteriole.

Conclusions: Glomerular endothelial cells might act as a functional syncytium and propagate a calcium response after injury to every glomerular endothelial cell. The almost instantaneous calcium increases in neighboring cells, which quickly returned to baseline suggest the involvement of gap junctions or the existence of a short-lived local mediator. The periodical waves originating from injured areas suggest that endothelial cells can be repeatedly stimulated.

Funding: Government Support - Non-U.S.

SA-OR058

PiT-1 Signaling through ERK1/2 Promotes Smooth Muscle Cell Osteochondrogenic Phenotype Change and Calcification Nicholas W. Chavkin, Cecilia M. Giachelli. *Bioengineering, Univ of Washington, Seattle, WA.*

Background: Vascular calcification (VC) is prevalent in chronic kidney disease and elevated serum inorganic phosphate (Pi) is a recognized risk factor. Elevated Pi induces vascular smooth muscle cell (VSMC) calcification by a process that requires the Pi transporter, PiT-1. However, Pi-induced osteochondrogenic (OC) phenotype change and calcification occur at concentrations much greater than the Km of Pi transport, suggesting novel Pi transport-independent signaling functions of PiT-1. We tested this possibility using engineered phosphate uptake-deficient PiT-1 mutants.

Methods: Site-Directed mutagenesis was used to create E74K, S132A, and S623A mutations in PiT-1. VSMCs lacking PiT-1 were isolated from PiT-1 deficient mice and stably transduced with wild type (WT) or mutated PiT-1 retroviral constructs. Q-PCR confirmed mRNA expression and immunofluorescence confirmed membrane localization. Pi uptake, ERK1/2 phosphorylation, and calcification were quantitated.

Results: Sodium-dependent Pi uptake kinetic constant, Vmax, was increased in VSMCs transduced with WT PiT-1 (0.91±0.07pmol/ug*min) but not PiT-1-E74K (0.30±0.04pmol/ug*min) compared to vector control (0.41±0.04pmol/ug*min). Elevated Pi induced ERK1/2 phosphorylation in VSMCs overexpressing either WT PiT-1 (1.30±0.17-fold) or Pi uptake deficient PiT-1-E74K (1.30±0.06-fold), compared to vector control (0.83±0.06-fold). OC differentiation as measured by reduced SM22α and increased osteopontin RNA, respectively, was similar in WT PiT-1 VSMCs (0.21±0.06-fold, 14.3±5.1-fold) and PiT-1-E74K VSMCs (0.25±0.02-fold, 21.5±3.8-fold), and greater than vector control (0.98±0.18-fold, 1.9±0.6-fold). WT PiT-1 VSMCs showed higher calcification (1042±81ug Ca/mg protein) than PiT-1^{-}-E74K VSMCs (503±62ug Ca/mg protein), and both were higher than vector control VSMCs (297±30ug Ca/mg protein).

Conclusions: PiT-1 signals ERK1/2 phosphorylation and downstream phenotype change via a pathway that does not require phosphate uptake. On the other hand, Pi uptake through PiT-1 was required for maximal VSMC calcification. These data suggest that both phosphate-uptake dependent and –independent functions of PiT-1 are important for VSMC calcification.

Funding: Other NIH Support - HL062329, HL081785

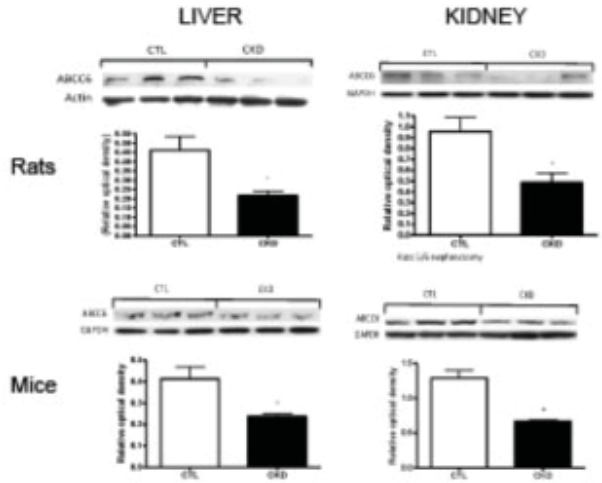
SA-OR059

Chronic Kidney Disease Results in Deficiency of ABCC6, the Novel Inhibitor of Vascular Calcification Shuman Liu, Wei Ling Lau, Nosratola D. Vaziri. *Div of Nephrology, UC Irvine.*

Background: Chronic kidney disease (CKD) is associated with arterial medial calcification which plays a major role in the pathogenesis of cardiovascular disease in this population. Several factors are known to promote soft tissue and accelerated arterial calcification in CKD including systemic inflammation, altered calcium and phosphate homeostasis, hypertension, and deficiency of endogenous calcification inhibitors. The ABCC6 transporter(ATP-binding cassette subfamily C number 6), also known as multidrug resistance-associated protein 6 (MRP6), is highly expressed in the liver and kidney. Mutation of *ABCC6* results in pseudoxanthoma elasticum, an inherited disorder characterized by arterial and soft tissue calcification. Given the prevalence of arterial medial calcification in CKD, the present study was undertaken to test the hypothesis that CKD may lead to acquired ABCC6 deficiency.

Methods: CKD was induced via 5/6 nephrectomy in male Sprague-Dawley rats and by adenine-containing diet to cause chronic interstitial nephropathy in female DBA/2J mice. Sham-operated rats and mice fed regular diet served as controls. Liver and kidney tissues were harvested and processed for ABCC6 protein and mRNA analysis.

Results: ABCC6 protein levels were significantly reduced in the liver and kidney tissues from CKD rats and mice (* $P < 0.05$ compared to CTL). However, ABCC6 mRNA levels were unchanged, pointing to post-transcriptional or post-translational mechanisms for the observed ABCC6 deficiency. Additionally, plasma levels of the calcification inhibitor fetuin-A were significantly decreased in CKD (20.1 + 1.6 mg/L) animals compared to controls (24.8 + 1.1 mg/L, P = 0.03).



Conclusions: CKD results in acquired ABCC6 transporter deficiency. To our knowledge this abnormality has not been previously reported and may contribute to CKD-associated vascular and soft tissue calcification.

SA-OR060

Dietary Protein Restriction Induces Inflammation and Malnutrition, and Exacerbates Vascular Calcification in Uremic Rats with Hyperphosphatemia Shunsuke Yamada,[1] Masanori Tokumoto,[1] Narihito Tatsumoto,[2] Hiroaki Ooboshi,[1] Takanari Kitazono,[2] Kazuhiko Tsuruya.[3] *[1]Div of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan; [2]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [3]Dept of Integrated Therapy for Chronic Kidney Disease, Kyushu Univ, Fukuoka, Japan.*

Background: Dietary protein (Pr) restriction and use of phosphate (Pi)-binder are the two mainstay treatments for hyperphosphatemia in CKD patients. Dietary Pr restriction has the harmful potential to develop malnutrition in CKD patients. However, the differential effects between Pi-binder use and dietary Pr restriction on the inflammation, malnutrition, and vascular calcification have not been fully investigated.

Methods: To compare the effects of dietary Pi and Pr contents on inflammation, malnutrition, and vascular calcification in the uremic model, five groups of rats were fed adenine-containing diets with different combination of Pi and Pr contents for six weeks; high Pi (1.2%), low Pi (0.3%), normal Pr (19%), and low Pr (9.5%). Urine, serum, and tissue samples (aorta, muscle, and liver) were examined at 6 week. Serum biochemistries, serum inflammatory markers, and aortic calcium content were determined.

Results: Rats fed low-Pr diets reduced body weight, muscle mass and serum albumin level, with increased serum inflammatory markers, independent of the dietary Pi loading. High dietary Pi loading induced vascular calcification (increased aortic calcium content), which was enhanced by low-Pr diet. Pi-binder (lanthanum carbonate) reversed all the changes observed in rats fed high Pi and low Pr diets. However, Pr-restriction under low Pi diet did not induce vascular calcification, indicating that malnutrition and inflammation do not cause vascular calcification when high Pi loading is absent.

Conclusions: Dietary Pr restriction induces inflammation and malnutrition independent of Pi loading, and exacerbates vascular calcification under high Pi loading, suggesting that Pi-binder use is more ideal than dietary Pr restriction for the management of patients with CKD.

SA-OR061

Angiopoietin-2 Accelerates Vascular Calcification in Children with Chronic Kidney Disease Undergoing Dialysis Alexandra F. Todd,[1] Karen Price,[1] Maria K. Joannou,[1] Lesley Rees,[2] Rukshana Shroff,[2] David A. Long.[1] *[1]Developmental Biology and Cancer, UCL Inst of Child Health, London, United Kingdom; [2]Renal Unit, Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom.*

Background: Cardiovascular disease (CVD) manifested by calcification is the major cause of mortality in childhood chronic kidney disease (CKD). One of the earliest signs of CVD is disruption to the endothelium which may result from the imbalance of vascular growth factors seen in CKD. Our previous work showed that circulating levels of angiopoietin-2 (Angpt2) - a pro-inflammatory and anti-angiogenic molecule were dramatically increased in children on dialysis. However, whether Angpt2 mediates vascular calcification or is simply a marker of disturbed angiogenesis is yet to be established.

Methods: Muscular arteries were obtained from children undergoing renal transplantation and non-renal patients undergoing intra-abdominal surgery. 1-2 mm wide arterial rings were culture in high calcium and phosphate milieu with/without 25 ng/ml of Angpt2, the highest concentration of measured previously in dialysis serum.

Results: Rings from healthy controls and pre-dialysis patients did not calcify when exposed to pre-calcaemic media with/without Angpt2; in contrast, dialysis rings showed increased calcium content which was significantly enhanced in the presence of Angpt2 ($p<0.04$). Cells explanted from tunica media of dialysis patients also exhibited a significantly increased calcium load following exposure to pre-calcaemic media ($p<0.03$) which was exaggerated with the addition of Angpt2. Angpt2 exposure led to an increase in mRNA levels of the osteogenic marker bone morphogenic protein-2, and decreased levels of matrix-gla protein, a physiological calcification inhibitor. The angiopoietin receptor, Tie-2, was detected by immunohistochemistry and Western blotting on vessel rings and cultured dialysis cells respectively.

Conclusions: Angpt2 accelerated vascular calcification in vessel rings and cultured cells obtained from children with CKD undergoing dialysis, an effect which may be mediated by Tie-2. Modulation of angiopoietins may provide future therapeutic targets to slow vascular calcification in CKD patients.

SA-OR062

Matrix Metalloproteinases Promote Uremic Vascular Calcification Uwe Querfeld,[1,2] Eva Hecht,[2] Christian Freise,[2] Karoline Websky,[2] Berthold Hocher.[3] *[1]Pediatric Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Center for Cardiovascular Research, Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Institut für Ernährungsforschung, Potsdam, Germany.*

Background: Proteolytic matrix metalloproteinases (MMPs) degrade extracellular matrix proteins and have important physiological roles in tissue remodeling, including bone and vascular tissues. Increased MMP activity is associated with several cardiovascular diseases. We studied (1) the presence of MMP-2 and MMP-9 in aortas of uremic rats with calcitriol-induced vascular calcifications and (2) the effect of MMP-2 and MMP-9 in an ex-vivo model of calcium/phosphate induced calcifications in aortas of uremic mice.

Methods: (1) Aortas from sham-op, 5/6-nephrectomized (5/6-Nx) and 5/6-Nx + calcitriol-treated rats were immunhistologically stained for MMP-2 and MMP-9. (2) 5/6-Nx C57BL/6 mice, known to be highly calcification-resistant, were sacrificed after 8 weeks, the aortas were dissected and cultured at 37°C in a calcifying medium containing 2.7 mM calcium und 2.0 mM phosphate, with or without addition of recombinant MMP-2 or -9 or the MMP inhibitor doxycycline. Histological evaluation of the aorta sections for media calcification was performed after 21 days.

Results: An increased presence of MMP-2 was found in the media of uremic (compared to sham-operated) rat aortas, and this was significantly enhanced by calcitriol treatment. Explanted uremic mice aortas but not aortas from normal control mice were susceptible to calcifications induced by a calcifying medium. The addition of MMP-2 or MMP-9 strongly enhanced these calcifications in aortas from uremic and to a lesser extent, even normal mice. In contrast, calcifications were not found in uremic mice aortas treated with doxycycline in spite of calcifying conditions in the medium.

Conclusions: The presence of MMP-2 parallels increases in the formation of media calcifications in a rat model of calcitriol-induced vascular calcifications in uremic rats. Under calcifying conditions, addition of these proteolytic enzymes induces calcification in ex-vivo cultured aortas of mice, whereas their inhibition prevents calcification, indicating that MMP-2 -9 impact the pathophysiology of arterial calcification.

Funding: Private Foundation Support, Clinical Revenue Support

SA-OR063

Chronic Kidney Disease (CKD) Stimulates Activin and Endothelial to Mesenchymal Transition (EnMT), which Causes Vascular Calcification and Is Inhibited by an Activin Ligand Trap Keith A. Hruska,[1] Olga A. Agapova,[1] Yifu Fang,[1] Toshifumi Sugatani,[1] Michael E. Seifert,[2] Victoria Sung.[3] *[1]Pediatrics, Washington Univ School of Medicine, St. Louis, MO; [2]Pediatric Nephrology, Southern Illinois Univ, Springfield, IL; [3]Translational Development, Celgene, San Francisco, CA.*

Background: We have shown that kidney diseases cause vascular calcification by producing systemic Wnt inhibition during kidney repair. We show that the vascular effects of CKD stimulated Wnt inhibition are stimulation of vascular activin as well as induction of Smad dependent EnMT, and that an activin receptor ligand trap, inhibits EnMT and vascular calcification.

Methods: CKD with elevated Wnt inhibitors was induced in the mouse models for lineage tracing and vascular calcification. Activin, Follistatin and Inhibin levels were measured by Elisa, RT-PCR and westerns. Cell lineage tracing was performed in GNZ mice (Stoller et al, Genesis, 2008) bred to endothelial specific Tie2-Cre mice. Mice harboring knock in of GNZ express nuclear GFP and *lacZ* following Cre-mediated recombination.

Results: Activin levels were increased in the vasculature and the circulation without changes in follistatin levels in mouse models of kidney disease and CKD stimulated vascular calcification. CKD induced expression of GFP and lacZ in cells of the adventia and media of GNZ;Tie2-Cre CKD mice compared to GNZ ;Tie2-Cre mice with normal kidney function wherein the GFP and lacZ were limited to the aortic endothelium. Tie2 is an endothelial lineage specific receptor, and this demonstrates that CKD induces aortic EnMT. CKD stimulated aortic EnMT produced decreased vascular smooth muscle function, osteoblastic transition and calcification. Treatment with an activin receptor type 2A (ActR2A) ligand trap, RAP-011, inhibited Smad dependent signaling, blocked aortic osteoblastic transition, increased vascular smooth muscle function and decreased CKD stimulated vascular calcification.

Conclusions: CKD induced vascular activin and EnMT, and RAP-011 decreased activin signaling inhibiting vascular dedifferentiation, osteoblastic transition and vascular calcification.

Funding: NIDDK Support, Pharmaceutical Company Support - Celgene

SA-OR064

Warfarin Promotes Medial Vascular Calcification in Humans Ekamol Tantisattamo, Kum Hyun Han, W. Charles O'Neill. *Renal Div, Emory Univ.*

Background: Matrix gla-protein is a vitamin K-dependent inhibitor of vascular calcification. Genetic deficiency or inhibition with warfarin produces medial vascular calcification in animals but whether this occurs in humans is unclear. As breast arterial calcification(BAC) is exclusively medial, readily identified on screening mammograms(MG), and correlates with peripheral arterial calcification, we compared the prevalence of BAC in patients with and without warfarin therapy.

Methods: A computerized search of medical records from 2011 through 2013 yielded 16,555 patients with screening MG in whom warfarin was listed as a medication in 790. After exclusion of patients with warfarin exposure <1 month, history of ESRD, or serum creatinine ≥2.0, 453 patients were individually matched to subjects without warfarin exposure based on age and diabetes. MG were reviewed visually for BAC.

Results: Mean age was 68±0.6 and 30% had diabetes. The indication for warfarin was atrial fibrillation in 42% and venous thrombosis or embolism in 47%, and mean duration of warfarin was 4.6 yrs (range:0.1 - 39). 219 patients were on warfarin at the time of the MG and the remainder had been off warfarin for a mean of 2.7 yrs (range:0.003 - 11). Serum creatinine was slightly higher (0.91 versus 0.86,$p=0.0015$) and serum calcium was slightly lower (9.29 versus 9.39,$p=0.0005$) in the warfarin patients while serum phosphate did not differ. Prevalence of BAC was 50% greater in warfarin patients (39% versus 26%,$p<0.0001$) but not on MG performed prior to warfarin (27% versus 27%, mean 0.76 yrs), indicating that the increase was due specifically to the warfarin and not to underlying disease or other patient characteristics. The increase correlated with duration (25%, 67%, and 74% at <1, 1 to 5, and >5 yrs) and was greatest (5-fold) in patients under age 60 (12% versus 2.3 %,$p=0.006$). Among warfarin users, age ($p<0.00001$) and warfarin duration ($p=0.003$), but not warfarin indication or duration off warfarin were determinants of BAC in a logistic regression model.

Conclusions: Although limited to women, the results indicate that warfarin is strongly associated with medial arterial calcification independent of other patient characteristics. This effect is cumulative and not reversed when warfarin is discontinued.

SA-OR065

Blood Calcification Propensity and Cardiovascular Events in Hemodialysis Patients in the EVOLVE Trial Andreas Pasch,[1] Matthias Bachtler,[1] Edward Robert Smith,[2] Willi Jahnen-Dechent,[3] Spyridon Arampatzis,[1] Glenn M. Chertow,[4] Patrick S. Parfrey,[5] Yumi Kubo,[6] Jürgen Floege.[3] *[1]Univ Hospital Bern; [2]Royal Melbourne Hospital; [3]RWTH Aachen Univ Hospital; [4]Stanford Univ School of Medicine; [5]Memorial Univ; [6]Amgen Inc.*

Background: Hemodialysis (HD) patients are prone to suffer cardiovascular events. A recently developed novel blood test (T_{50}) determines the individual calcification propensity of blood, where a lower T_{50} reflects a greater tendency towards mineralisation. Whether T_{50} is associated with CV events in HD patients is not known.

Methods: T_{50} was determined in 2,785 baseline serum samples from the Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events (EVOLVE) trial, a study investigating the effect of cinacalcet in HD patients with secondary hyperparathyroidism (sHPT). The associations of T_{50} with the primary composite endpoint (all-cause mortality, myocardial infarction [MI], hospitalization for unstable angina, heart failure, or peripheral vascular event) and its individual components were analyzed.

Results: The mean ± SD calcification propensity T_{50} in the EVOLVE baseline sera was 216 ± 84 min. and was closely related to serum albumin ($p<0.001$) and inversely to serum phosphorus ($p<0.001$). Using multivariate Cox regression models adjusted for patient characteristics, CV morbidities, and baseline labs, lower T_{50} was independently associated with an increased risk of the primary composite endpoint both as continuous (adjusted HR 1.09 [95% CI 1.03, 1.17], $p=0.005$ per 1 SD decrease) and categorical measures (1st tertile, HR 1.25 [1.08, 1.45], $p=0.003$; 2nd tertile, HR 1.09 [0.95, 1.26], $p=0.229$; 3rd tertile, reference). Furthermore, lower T_{50} was associated with an increased risk in MI both as continuous (adjusted HR 1.31 [95% CI 1.12-1.52], $p<0.001$ per 1 SD decrease)

and categorical measures (1st tertile, HR 1.60 [1.14, 2.24], p=0.006; 2nd tertile, HR 1.10 [0.78, 1.54], p=0.591; 3rd tertile, reference). No association was observed between T50 and the other components.

Conclusions: T_{50} was independently associated with the primary composite endpoint and with MI in the EVOLVE trial. Whether an intervention that alters T_{50} will have an impact on CV events remains to be proven.

Funding: Pharmaceutical Company Support - Amgen

SA-OR066

Sodium Thiosulfate and Co-Treatments for Calcific Uremic Arteriolopathy

Sagar U. Nigwekar,[1] Ann Mooney,[2] Jeffrey L. Hymes,[1] Debra Meade,[2] Cindy A. Premo,[2] Kevin Chan,[1,2] Franklin W. Maddux,[2] Ravi I. Thadhani.[1] *[1]MGH; [2]FMCNA.*

Background: Intravenous sodium thiosulfate (STS) and a variety of co-treatments have been proposed to treat calcific uremic arteriolopathy (CUA); however, prospective phase 4 evaluation is lacking.

Methods: Our prospective cohort included 451 chronic hemodialysis patients with CUA treated with STS. Data regarding STS, co-treatments, CUA lesion status, and mortality were derived from surveys sent to treating physicians at STS initiation (baseline) and at 3-month follow-up. Demographic, clinical and laboratory data were abstracted from clinical information systems. Outcomes were CUA lesion status and mortality at follow-up. Logistic regression analyses were conducted to identify whether co-treatments predict a favorable response to STS (defined as lesion stabilization, improvement, or resolution).

Results: Mean age (± standard deviation) was 59 ± 13 years. At baseline, mean serum calcium 8.0 ± 1.7 mg/dL, phosphorous 5.3 ± 1.5 mg/dL, parathyroid hormone 464 ± 446 pg/mL, and albumin 3.3 ± 0.8 g/dL were noted. Positive response to STS was observed in 49%; 12% died and 39% had deteriorated at follow-up. Co-treatments and their association with positive response to STS are tabulated.

Co-treatment	Frequency (%)	Univariate	Multivariable
		Odds ratio (95% Confidence Intervals)	Odds ratio (95% Confidence Intervals)
Cinacalcet	51	14.35 (5.77-35.70)	4.64 (1.45-14.84)
Sevelamer hydrochloride or servelamer carbonate	45	1.10 (0.52-2.33)	1.02 (0.35-2.97)
Calcium acetate	23	2.46 (0.22-27.94)	1.70 (0.07-25.12)
Lanthanum carbonate	10	2.59 (0.61-10.94)	1.25 (0.17-9.33)
Vitamin D therapy discontinuation	42	0.64 (0.30-1.37)	0.97 (0.32-2.94)
Parathyroidectomy	12	1.02 (0.39-2.64)	1.37 (0.27-6.93)
Increased dialysis	27	1.91 (0.84-4.36)	1.53 (0.44-5.27)
Lower calcium bath	33	1.48 (0.68-3.26)	1.31 (0.44-3.94)
Wound care/debridement	68	0.76 (0.26-2.18)	0.44 (0.15-1.31)

In univariate and multivariable (adjusted for demographics, co-morbidities, and laboratory parameters) analyses, cinacalcet co-treatment was associated with a favorable response to STS.

Conclusions: Majority of CUA patients treated with STS and various co-treatments do not improve. Novel therapies and pragmatic clinical trials are needed.

Funding: Pharmaceutical Company Support - Sanofi-Aventis, Private Foundation Support

SA-OR067

Vascular Protective Effect of Spironolactone on Arterial Medial Calcification in Adenine-Induced Chronic Kidney Disease Rats

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Background: Vascular calcification is highly prevalent in patients with chronic kidney disease (CKD) and is closely linked to increased cardiovascular diseases. Studies have shown that spironolactone (SPL), a mineralocorticoid receptor antagonist, has protective effects on cardiovascular systems through suppression of osteoinductive signaling and oxidative stress. However, the impact of SPL on vascular calcification in CKD remains unclear.

Methods: The present *in vivo* study determined the effect of SPL on arterial medial calcification (AMC) in CKD rats fed diets containing 0.3% adenine, 1.2% phosphate, and different concentrations of SPL of 0 mg/kg (CKD-SPL0), 25 mg/kg (CKD-SPL25), 50 mg/kg (CKD-SPL50), or 100 mg/kg (CKD-SPL100) for 8 weeks. We also examined the effect of late treatment with SPL on AMC in CKD rats using adenine-fed CKD rats treated with 100 mg/kg SPL for only the last 2 weeks of the 8-week study period (CKD-SPL100L). Blood pressure levels and urinary chemistries were determined periodically, and tissues and blood samples were collected at week 8.

Results: CKD-SPL0 rats developed hyperphosphatemia and AMC at week 8. Treatment with SPL dose-dependently decreased serum phosphate level and calcium content in the aorta in CKD-SPL50 and CKD-SPL100 rats, in parallel with SPL dose-dependent improvement in kidney function. SPL did not influence the levels of blood pressure and serum potassium. Although the degrees of kidney function and hyperphosphatemia were comparable among CKD-SPL0, CKD-SPL25, and CKD-SPL100L rats, AMC in the latter two groups were attenuated in comparison with CKD-SPL0 rats, suggesting the additional effect of SPL on AMC beyond improvement of kidney function.

Conclusions: SPL can attenuate AMC in CKD rats, by a direct vascular protective effect in addition to an indirect effect through improving kidney function.

SA-OR068

Focal Segmental Glomerulosclerosis Exomes Reveal Candidate Variants Highly Enriched in Cell Movement and Cell Adhesion-Related Genes

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Background: In the past two decades, the findings of focal segmental glomerulosclerosis (FSGS)-causing genes established that genetic components contribute to the development of FSGS. However, the underlying genetic cause is unknown in a majority of families, even when there is strong evidence of inheritance. We analyzed the exomes of families and sporadic cases that have not been explained by known FSGS genes in an extended effort to find the genes responsible for the development of FSGS.

Methods: We performed exome sequencing on 403 probands from 311 familial and sporadic cases of FSGS. We also sequenced 96 unaffected family members. After variant-calling, variants were filtered against common variants that were not known to cause FSGS from the 1,000 Genomes Project, dbSNP, and the Exome Sequencing Project. Novel non-synonymous or splice variants were kept.

Results: Exome analysis revealed that 30.3 percent of all cases contain known FSGS-causing variants, with these variants accounting for 47.9 percent of familial cases and 16.0 percent of sporadic cases. After excluding explained cases, we filtered genes showing more than four novel non-synonymous or splice variants, and grouped genes according to 1) their cellular component, molecular function, and biological process using AmiGO2, a gene ontology service, and 2) their protein-protein interaction using DAPPLE. GO terms of genes from unexplained cases were significantly enriched in cellular component movement and cell adhesion: both had p-values less than 0.0001. DAPPLE results consistently showed two big protein-protein interaction networks, one involving cell movement and the other involving cell adhesion.

Conclusions: Here we show that, in comparison to familial cases, sporadic FSGS cases are less explained by known FSGS-causing genes. These data show that novel non-synonymous or splice variants are highly enriched in cell movement- and cell adhesion-involved genes in the exomes of FSGS patient samples, suggesting the importance of podocyte movement and interaction with neighboring cells and the extracellular matrix.

Funding: NIDDK Support

SA-OR069

KANK Deficiency Leads to Defective Podocyte Function

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Background: KANK proteins (KANK1~4) are characterized by conserved ankyrin-repeat and coiled-coil domains, and a motif (KN motif) at the N-terminus containing motifs for nucear localization and export signals. The *KANK* familly genes are highly expressed in kidney, however, their roles in kidney is not known.

Methods: To search for disease-causing mutations in more than 200 individuals with nephrotic syndrome (NS), we performed homozygosity mapping (HM) and whole exome sequening (WES). The function and localization of KANK proteins were examined in *Drosophila* cardiac nephrocytes, zebrafish, cultured podocytes and rat kidney tissues.

Results: By HM and WES in individuals with NS, we identified recessive mutations in *KANK1* (p.E454K), *KANK2* (p.S181G and p.S684F) and *KANK4* (p.Y801H). Independently, by a functional genetic screen using *Drosophila* cardiac nephrocytes, which are equivalents of mammalian podocytes, we identified *dKank* which is essential for nephrocyte function. RNAi knockdown of *dKank* in nephrocytes led to disrupted slit diaphragm structures, abnormal lacunae structures, as well as a wave-shaped plasma membrane. Intestingly, knockdown of *kank2* in zebrafish recapitulated a human NS phenotype and resulted in edema and proteinuria. Transmission electron microscopy of *kank2* morpants showed podocyte foot process effacement and disorganized slit membranes in glomeruli. In addition, KANK1, KANK2 and KANK4 localize to podocytes in rat glomeruli and KANK1 partially colocalizes with SYNAPTOPODIN. We show by coimmunoprecipitation and GST pulldown that KANK2 interacts with ARHGDIA, of which mutations cause NS and which regulates RHO GTPases in podocytes. In addition, KANK1 regulates RHOA through the interaction with 14-3-3 protein.

Conclusions: These data suggest that *KANK* muations may cause NS and the *KANK* family genes play evolutionarily conserved roles in podocyte cytoskeleton structures and function, likely through regulating RHO GTPase signaling in podocytes.

Funding: Private Foundation Support

SA-OR070

***NUP93* and *NUP205*, which Encode for Nuclear Pore Complex Proteins, Are Mutated in Individuals with Nephrotic Syndrome**

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Background: Steroid resistant nephrotic syndrome (SRNS) is the 2nd most frequent cause of end-stage kidney disease (ESKD) in the first two decades of life. Identification of single-gene causes of SRNS has furthered the understanding of its pathogenesis. However, additional genes and disease mechanisms remain unknown.

Methods: We performed homozygosity mapping and whole exome sequencing (WES) in 100 individuals with SRNS. To identify additional families with mutations in potential SRNS-causing genes, we screened our cohort of ~800 individuals with SRNS by microfluidic multiplex PCR and next generation sequencing (NGS). Immunofluorescence microscopy (IF) was done using a Leica SP5X system.

Results: In 6 individuals with SRNS from 4 consanguineous families, we detected by WES homozygous missense mutations in the genes *NUP93* (3 families) and *NUP205* (1 family), which encode for directly interacting components of the nuclear pore complex. Subsequent high throughput mutation analysis of ~800 individuals with SRNS revealed 3 additional families with compound heterozygous mutations in *NUP93*. The 6 different mutations were either nonsense mutations (n=2) or conserved missense mutations (n=4). The *NUP93* p.Y629C mutation represents a Turkish founder allele, as 2 affected individuals share haplotypes in a 3.1 Mbp segment at the *NUP93* locus. *NUP93* is expressed in podocyte precursors where it localizes to the nuclear envelope and the cytoplasm in IF. All identified patients had an early age of onset of proteinuria (median of 3 years) and most of them progressed rapidly to ESKD. Microscopic hematuria was present in 5 out of 6 patients.

Conclusions: We identify mutations in *NUP93* and *NUP205* as novel causes of SRNS. Further functional studies need to shed light on the involvement of nuclear core complex proteins in the pathogenesis of nephrotic syndrome.

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SA-OR071

Addition of Genome-Wide Linkage Analysis Is Superior to Whole-Exome Sequencing Alone for Identification of Novel Genetic Causes of Hereditary Kidney Diseases

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Background: The advent of Whole-Exome Sequencing (WES) has accelerated gene discovery in familial diseases. Despite considerable advantages, a report of WES for identification of disease-causing mutations estimated a 25% success rate in a population with suspected Mendelian disease [1]. We hypothesized the addition of genome-wide linkage analysis (GWLA) to WES would increase the efficacy of identifying disease-causing variants in patients with familial kidney diseases.

Methods: We performed WES in 35 families with inherited kidney diseases, 14 with combined GWLA. The cohort included 28 families with focal segmental glomerulosclerosis, 5 with childhood-onset steroid-resistant nephrotic syndrome and 2 with vesico-ureteric reflux.

Results: Causal gene variants were identified in 9/35 families, including 3/21 (14%) in the WES alone group and 6/14 (43%) in the GWLA plus WES group. Three of the six mutations identified with GWLA plus WES group were in novel genes whereas in the WES alone group, no novel disease-causing gene was identified. African American (AA) families had more potential disease causing pathogenic variants per exome compared to other races (1 WES per family, p=0.0262; 2 WES per family, p=0.0035) [Table] and no AA families had their causal mutation identified.

Conclusions: Significant limitations remain with the use of WES and large pedigrees are still crucial for efficient identification of single gene causes of hereditary kidney diseases.Sequencing multiple individuals by WES combined with GWLA may be required for gene discovery in Mendelian kidney disease, especially in AA families. (1) Yang Y et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. N Engl J Med. 2013;369(16).

	AA (n=11)	Other (n=14)	p value
1 WES/family (n=13)	186 (n=5)	145 (n=8)	0.0262
2 WES/family (n=12)	90 (n=6)	53 (n=6)	0.0035

Mean (standard deviation) variants after exome filtering per ethnicity

Funding: NIDDK Support

SA-OR072

Identification of the First Gene Mutated in Galloway-Mowat Syndrome, an Association of Early-Onset Nephrotic Syndrome and Post-Natal Microcephaly

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Background: Galloway-Mowat syndrome (GMS) is a rare inherited disorder, with no causal gene reported yet, characterized by nephrotic syndrome associated with neurological impairment including post-natal microcephaly.

Results: We performed whole exome sequencing combined to homozygosity mapping in a family with 2 children harboring a GMS phenotype and identified a non-sense mutation in the *GMS1* gene that encodes a WD40 repeat–containing protein of unknown function**.** Further screening revealed an additional frame-shift mutation in an unrelated patient with GMS from a consanguineous Turkish family. We demonstrated that GMS1 is expressed in the infant brain and in fetal and adult human kidneys. In human immortalized podocytes and fibroblasts, GMS1 staining presented a weak and diffused localization within the cytoplasm during interphase and then it strongly accumulated in the microtubule asters and at the midbody during mitosis. Patient fibroblasts and GMS1-depleted podocytes displayed defects in nuclear morphology, which was associated, in patient fibroblasts, to a decrease of cell survival. Furthermore, we showed that patient fibroblasts and differentiated GMS1-depleted podocytes harbored an atypical morphology resulting from disorganized microtubule network, suggesting microtubule polymerization defects.

Conclusions: Our results point to a critical function of GMS1 in the cell survival and maintenance of cellular architecture, two crucial processes in neurons and podocytes.

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SA-OR073

Integrative Genomics Identifies Novel Associations of *APOL1* Risk Genotype in African-American NEPTUNE Subjects

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Background: To gain new knowledge of *APOL1*-associated nephrotic syndrome, we co-analyzed clinical and genomic datasets from 87 African-Americans in the Nephrotic Syndrome Study Network (NEPTUNE), stratified by *APOL1* risk genotype.

Methods: Adults and children were enrolled at time of first biopsy for proteinuria, histologically classified, and followed longitudinally. We genotyped *APOL1* G1 and G2 risk alleles (RA) and sequenced 18 Mendelian FSGS genes. Analyses used a recessive model to compare those with "high-" versus "low-risk" genotype (2 versus 0/1 RA). We compared characteristics at presentation and multivariate longitudinal models of eGFR, proteinuria, and complete remission (CR). Genome-wide gene expression profiles were obtained from the research biopsy of 26 patients. Transcripts with expression highly correlated with either high- and low-risk *APOL1* mRNA levels were defined and analyzed. Glomerular density and volume and tubulointerstitial (TI) fibrosis were measured with morphometry.

Results: High-risk histologic groups were FSGS(62%), MCD(15%), MN(5%), and "Other"(18%). At baseline, risk groups did not differ in age, immunosuppression, RAS blockade, or hypertension. High-risk individuals had **3.8 higher odds of prematurity**(CI 1.0-13.5 p<0.05). Over time, independent of histologic diagnosis, they had 17ml/min lower eGFR(p<0.01), more proteinuria(p<0.05) and were **2.3x less likely to achieve CR** (CI 1.0-5.3,p<0.05). There was no monogenic NS in high-risk subjects. *APOL1* expression in each risk-genotype group was correlated with expression of distinct transcripts ("low":407 genes, "high":172, shared:7). The top functional concepts of high-risk *APOL1*-correlated genes were **leukocyte activation and migration**. High-risk patients had increased TI fibrosis(23 versus 16%, p<0.05).

Conclusions: Using integrative genomics, we show high-risk genotype subjects have increased prematurity, worse clinical outcomes, and increased interstitial scarring. Intrarenal expression analysis shows the high-risk genotype distinctly associated with inflammatory responses NEPTUNE:U54DK083912.

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SA-OR074

Mutations of the SLIT2-ROBO2 Pathway Genes SLIT2 and SRGAP1 Cause Congenital Anomalies of the Kidney and Urinary Tract Daw-Yang Hwang,[1,10] Stefan Kohl,[1] Xueping Fan,[2] Asaf Vivante,[1] Stefanie Chan,[2] Gabriel C. Dworschak,[1] Heon Yung Gee,[1] Christoph Schell,[4] Tobias B. Huber,[4] Heiko M. Reutter,[3] Neveen Soliman,[5] Radovan Bogdanovic,[6] Elijah O. Kehinde,[7] Richard P. Lifton,[8,11] Velibor Tasic,[9] Weining Lu,[2] Friedhelm Hildebrandt.[1,11] *[1]Boston Children's Hospital, Harvard Medical School; [2]Boston Univ Medical Center; [3]Univ of Bonn, Germany; [4]Univ Hospital Freiburg, Germany; [5]Kasr Al Ainy School of Medicine, Cairo Univ, Egypt; [6]Univ of Belgrade, Serbia; [7]Kuwait Univ, Kuwait; [8]Yale Univ; [9]Univ Children's Hospital, Skopje, Macedonia, The Former Yugoslav Republic of; [10]Kaohsiung Medical Univ Hospital, Taiwan; [11]Howard Hughes Medical Inst.*

Background: Congenital anomalies of the kidney and urinary tract (CAKUT) account for 50% of chronic kidney disease that manifests in the first two decades of life. The wide range of structural malformations results from developmental defects of the kidneys and the urinary tract. Thus far, 31 monogenic causes of isolated CAKUT are known, explaining ~12% of cases.

Methods: To identify additional single-gene causes of CAKUT, we conducted whole exome sequencing in 20 unrelated individuals with CAKUT. In addition we performed high-throughput exon sequencing in a cohort of 749 individuals with CAKUT.

Results: We detected two heterozygous mutations in SRGAP1 in two unrelated families. We examined the candidate gene SLIT2 for mutations in cohort of 749 individuals with CAKUT and identified three unrelated individuals with heterozygous mutations. We showed that SRGAP1 is expressed in early mouse nephrogenic mesenchyme and coexpressed with ROBO2 in SIX2-positive nephron progenitor cells of the cap mesenchyme in developing rat kidney. The newly identified mutations in SRGAP1 lead to an augmented inhibition of RAC1 in HEK293T cells and the SLIT2 mutations compromise the ability of the SLIT2 ligand to inhibit cell migration.

Conclusions: We discovered two novel monogenic causes of isolated CAKUT in humans, emphasizing the importance of SLIT2-ROBO2-SRGAP1 signaling in human kidney development.

Funding: NIDDK Support, Private Foundation Support

SA-OR075

Anti-miR-21 as a Potential Novel Therapy for Both Early and Late Stages of Alport Syndrome Joseph H. Boulanger,[1] Wenping Song,[1] Shweta Pandya,[2] Kelly A. Rogers,[1] Lucy A. Phillips,[1] Deidre Mackenna,[2] Rachel Yabkowitz,[1] Oxana Beskrovnaya,[1] Steven R. Ledbetter,[1] Shiguang Liu.[1] *[1]Genzyme, A Sanofi Company, Framingham, MA; [2]Regulus Therapeutics, San Diego, CA.*

Background: Alport syndrome (AS) is an inherited, progressive glomerulopathy caused by mutations in Col4A3, 4 or 5 and inevitably leads to ESRD. No disease modifying therapy for AS is currently available. An important role of miR-21 in progression of AS and kidney-protective effect of anti-miR-21 oligonucleotide treatment has been shown in Col4a3$^{-/-}$ mice. We set out to further address anti-miR-21 therapeutic effect on late stage disease and on disease progression in combination with ACEi, the current standard of care for AS.

Methods: B6;129-*Col4a3*^{tm1Dec} (F1 hybrid of C57Bl6/J and 129X1/SvJ) and 129-*Col4a3*^{tm1Dec} were used. GFR was measured by inulin clearance. Anti-miR-21 was dosed subcutaneously. Ramipril was given in drinking water. Renal function and lifespan were used as endpoints for efficacy.

Results: B6;129-*Col4a3*^{tm1Dec} mice progressively lose GFR from 20% loss at 8 weeks to 80% loss at 15 weeks of age. Anti-miR-21 had a dose-dependent protective effect on renal function when given once a week at 12.5-50 mg/kg from 5 to 15 weeks of age. Amelioration of renal pathology correlated with renal function improvement. Dose-dependent effect on lifespan and renal function were also observed in 129-*Col4a3*^{tm1Dec}mice. Renal function protection and increased lifespan were observed even when treatment started at 13 weeks of age with 70% GFR loss. A dose of 50 mg/kg/week of anti-miR-21 showed superior renal protective effect relative to 4 mg/kg of Ramipril. Additive effects of combined anti-miR-21 and Ramipril on renal function protection and lifespan extension were observed at late stages.

Conclusions: Anti-miR-21 therapy is more effective compared to Ramipril in slowing kidney disease progression in murine AS. Disease modifying effects of anti-miR-21 are evident when administered in late stage disease where there is substantial loss of renal function. Combination treatment of anti-miR-21 with ACEi may provide additional therapeutic effect. Therefore, anti-miR-21 is proposed as a new and effective therapy for AS.

SA-OR076

Endothelin A Receptor Blockade Prevents Mesangial Filopodial Invasion of Glomerular Capillaries and Delays Alport Glomerular and Interstitial Disease Onset Dominic E. Cosgrove, Brianna Johnson, Daniel T. Meehan, Linda Cheung, Duane C. Delimont. *Genetics, Boys Town National Research Hospital, Omaha, NE.*

Background: The type IV collagen network in Alport GBM is comprised of α1(IV) and α2(IV) chains which contain fewer interchain crosslinks than the networks found in normal GBM. The increase in elasticity of the Alport GBM imparts biomechanical stresses on glomerular cells. This activates Rac1 and CDC42 in mesangial cells, inducing the invasion of the capillary tufts by mesangial filopodia which deposit mesangial proteins in the GBM, activating proinflammatory cell signaling in podocytes. We explored whether endothelin receptor activation on mesangial cells might underlie stretch-mediated CDC42 activation.

Methods: 129 autosomal Alport mice were given either Bosentan (endothelin A and B receptor antagonist) or Sitaxentan (endothelin A receptor antagonist) from 2 to 7 weeks of age. Mice were analyzed longitudinally for proteinuria and BUN, glomerular RNA for gene expression of MMPs and pro-inflammatory cytokines, and tissue by histochemistry and immunohistochemistry for pathologic changes.

Results: Hypertension elevated expression of endothelin-1 in Alport endothelial cells. Endothelin blockade ameliorated mesangial filopodial invasion of glomerular capillaries, delayed disease onset, slowed progression of proteinuria, increased lifespan, and ameliorated GBM dysmorphology. Glomerulosclerosis and interstitial fibrosis were not evident in treated Alport mice when age-matched vehicle-treated Alport mice showed >30% glomerulosclerosis and fibrosis scores between III and IV. Both Bosentan and Sitaxentan were equally effective.

Conclusions: Biomechanical strain-induced activation of endothelin expression in Alport glomerular endothelial cells results in endothelin A receptor-mediated activation of CDC42 in mesangial cells, inducing the invasion of the GBM by mesangial filopodia. This appears to be an important factor contributing to the mechanism of Alport disease initiation, and presents a host of novel therapeutic targets with the potential to delay/inhibit the onset of Alport glomerular and tubulointerstitial pathogenesis.

Funding: NIDDK Support

SA-OR077

Podocyte-Specific *Fat1* Deletion Leads to Focal Segmental Glomerulosclerosis in Mice Alda Tufro, Pardeep Kumar Aggarwal, Sherene Mason, Gilbert W. Moeckel. *Dept of Pediatrics, Yale Univ School of Medicine, New Haven, CT.*

Background: *Fat1*, a cadherin involved in planar cell polarity expressed in podocytes, is an essential slit-diaphragm component. Fat1 localizes to lamelipodia and intercellular junctions, promotes cell migration and regulates actin dynamics through beta catenin and Ena/Vasp interactions.*Fat1* null mice develop congental nephrotic syndrome (CNS), microphtalmia and holoprosencephaly, leading to perinatal lethality.

Methods: To further evaluate *Fat1* function in slit-diaphragm development and maintenance we generated podocyte-specific *Fat1* null mice (*Podocin-Cre;Fat1*$^{flox/flox}$) by Cre recombinase-mediated exon2 deletion. Podocyte *Fat1* gene dosage effect on renal structure and function was examined in neonate and adult mice. Homozygous, heterozygous and single mutant littermates were studied.

Results: Podocyte-specific *Fat1* mutants are viable, born in Mendelian ratios and survive to adulthood. Light microscopy reveals normal renal histology, and no proteinuria is detected in neonates. Adult podocyte-specific *Fat1* mutants develop progressive proteinuria inversely related to *Fat1* gene dosage. Heterozygous mutants have albuminuria ~4-fold > controls at 6 months of age, while homogygous mutants have massive albuminuria ~100-fold >controls at 4 months of age. Creatinine clearance is normal in all adult *Fat1* mutants. LM shows mesangial expansion and proteinaceous casts in podocyte-*Fat1* hets, while homogygous *Fat1* mutants have focal segmental glomerulosclerosis (FSGS). TEM in *Fat1* mutants demonstrates podocyte foot process effacement (FPE) ranging from focal to extensive FPE, microvillus transformation and collapsed F-actin. Control littermates show normal histology and TEM. Podocyte *Fat1* deletion induced nephrin downregulation in a gene dosage dependent manner.

Conclusions: We conclude that *Fat1* is required to maintain slit-diaphragm signaling and structural integrity in adult mice. In contrast with global *Fat1* KO mice, podocyte-specific *Fat1* deletion does not cause CNS or perinatal lethality. Long-term podocyte-specific *Fat1* loss-of-function causes massive proteinuria and FSGS in adult mice, suggesting that *FAT1* might be a candidate gene for human FSGS.

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SA-OR078

Research Priorities in Chronic Kidney Disease: A Partnership among Patients, Caregivers, Clinicians, Researchers, and Policy Makers Allison Tong,[1,2] Sally Crowe,[3] Shingisai Alice Chando,[1,2] Jonathan C. Craig.[1,2] *[1]School of Public Health, Univ of Sydney; [2]Centre for Kidney Research, The Children's Hospital at Westmead; [3]Crowe Associates Ltd.*

Background: Research aims to improve health outcomes for patients, but research priorities are usually determined by clinicians, academics and funders, with little or no involvement by patients or their carers, and in a process which may lack transparency. This study aimed to generate and prioritize research questions in chronic kidney disease (CKD) among all relevant stakeholders at a national level and in an explicit manner.

Methods: A national priority setting partnership workshop was convened in February 2014. The 58 participants from around Australia included patients with CKD (n=23), carers (n=7), nephrologists/surgeons (n=16), nurses (n=8), and allied health professionals and researchers (n=4). In facilitated groups of 8-10, participants generated and voted on intervention questions across four CKD stages/modalities of treatment: early stage (pre-dialysis) CKD, peritoneal dialysis, hemodialysis, and kidney transplantation. Votes were summed to identify the top 20 questions across all CKD stages then ranked.

Results: Eighty-three research questions were generated. The top five research questions were: 1) How effective are lifestyle programs (diet, exercise) for preventing deteriorating kidney function in patients with early CKD? 2) What interventions can improve long-term post-transplant outcomes (drugs, lifestyle)? 3) What strategies will improve family consent for potential deceased donor kidney donation, taking different cultural groups into account? 4) What strategies help patients maintain work while on hemodialysis? 5) How

can we improve and individualize drug therapy to provide better control of side effects? There was broad agreement between patients/caregivers and health professionals in the ranking assigned to most questions.

Conclusions: Priority questions were focused on prevention, lifestyle, quality of life, and long-term impact. These prioritized research questions can inform patient/consumer organizations, researchers, policy makers, and funding agencies in developing a shared CKD research agenda that is relevant to all stakeholders.

SA-OR079

A Sustained Dietary Sodium Reduction Program Reduces Albuminuria: A Large Cluster Randomised Trial Meg J. Jardine,[1] Nicole Yan Li,[1] Toshiharu Ninomiya,[1] Xiangxian Feng,[2] Jianxin Zhang,[3] Jingpu Shi,[4] Yuhong Zhang,[5] Rui Zhang,[6] Vlado Perkovic,[1] Hiddo Jan Lambers Heerspink,[7] Yangfeng Wu,[1] Lijing Yan,[1] Bruce C. Neal.[1] *[1]The George Inst for Global Health; [2]Changzhi Medical College; [3]Hebei Province Center for Disease Prevention and Control; [4]China Medical Univ; [5]Ningxia Medical Univ; [6]Xi'an Jiaotong Univ; [7]Univ of Groningen.*

Background: Albuminuria predicts adverse clinical outcomes. Previous studies suggest dietary sodium restriction reduces albuminuria in the short term. We assessed the impact of a sustained dietary salt reduction intervention on albuminuria.

Methods: We randomised 120 rural villages in China in a 1:1 ratio to an 18 month sodium reduction program, comprising education and access to a reduced sodium, added potassium salt substitute, compared with no intervention. We further randomised intervention villages to price subsidy for salt substitute that neutralised its higher cost, or not. A stratified random sample of adults was selected from each village for outcome evaluation. The primary outcomes were urinary albumin:creatinine ratio (uACR) and albuminuria.

Results: 2,566 survey participants provided 1,903 eligible 24hr urine samples. Sodium was 0.82g/day (0.06 to 1.68g) lower in participants from intervention compared with control villages. The sodium reduction program resulted in lower mean participant uACR in intervention compared with control villages [8.84 (8.04 to 9.81)mg/g, 10.52 (9.72-11.32) mg/g respectively, p=0.008] with a corresponding odds ratio (OR) for albuminuria of 0.67 (0.46-0.99). Secondary randomisation to salt substitute price subsidy produced the lowest rates of albuminuria [respective OR: 0.59 (0.37-0.95), 0.77 (0.44-1.34), 1.00 (ref), p trend 0.02, for intervention villages with price subsidy, intervention villages without subsidy and control villages].

Conclusions: Multiple trials have showed pharmaceutical agents that reduce albuminuria protect against cardiovascular and renal events. Albuminuria reduction through a dietary intervention could potentially be achieved population-wide at low cost. Discovering whether clinical benefits will ensue from dietary sodium reduction should therefore be a global research priority.

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SA-OR080

Promoting Awareness and Screening Indian Youth for CKD Risk Factors: Pilot Program Results Panduranga S. Rao,[1] Ravichandran Rajan,[2] Rachel Perlman,[1] Julie A. Wright Nunes.[1] *[1]Internal Medicine, Univ of Michigan, Ann Arbor, MI; [2]Nephrology, MIOT, Chennai, Tamil Nadu, India.*

Background: Chronic kidney disease (CKD) is a growing epidemic in India. Early detection of risk may ameliorate disease development and improve outcomes. There is little information about early screening to identify at-risk Indian youth.

Methods: Participants were enrolled from colleges throughout Chennai in a cross-sectional pilot screening program from April - May 2013. The program entailed: 1. 30-minute presentation and video on topics general to CKD with specific information on urinalysis (UA) collection techniques. 2. On-site assessment collecting age, sex, height, weight and blood pressure, led by trained study personnel. UA kits were distributed to participants at time of assessment and returned to study personnel within 48 hours. We used logistic regression to examine for associations between proteinuria and participant characteristics.

Results: 2158 students were enrolled from five centers. The mean (SD) age was 19 (1.6) years and 1451 (68%) were men. Measurements and UA kits were complete on 2035 (94%). Mean (SD) BMI was 21.9 (4) kg/m^2 and 388 (19%) had a BMI consistent with being overweight or obese (≥ 25kg/m^2). Mean systolic blood pressure (SBP) was 119 (13) mmHg with 94 (5%) participants > 140. Mean diastolic blood pressure (DBP) was 71 (12) mmHg with 119 (6%) participants > 90. 137 (6%) had glucosuria (UA ≥1+) and 120 (6%) abnormal urine protein levels (UA≥1+). In univariate analysis, sex (OR 1.6 [CI 1.06-2.56] p=0.03 men compared to women) and age (1.13 [1.01, 1.26] p=0.03 older compared to younger) were significantly associated with proteinuria. In analysis adjusted for age, sex, SBP, DBP, glucosuria, and BMI, sex and age remained independently associated with higher odds for proteinuria (1.6 [1.01, 2.56] p=0.04 men compared to women; 1.15 [1.02, 1.29] p=0.02 older compared to younger). Overall, there was active participation by the student community and wide screening of the students.

Conclusions: Early detection of CKD risk factors is important. Our novel pilot program targeting youth offers a unique opportunity to identify and intervene early. More research is needed to determine causal mechanisms.

SA-OR081

Challenges and Opportunities for Effective Chronic Kidney Disease Care Delivery: A Synthesis of Health Systems and Policies from 19 Countries Aminu K. Bello,[1] Adeera Levin,[2] Braden J. Manns,[3] Tilman B. Drueke,[4] Brenda Hemmelgarn,[3] Scott Klarenbach,[1] Giuseppe Remuzzi,[5] Marcello Tonelli.[1] *[1]Univ of Alberta, Canada; [2]Univ of British Columbia, Canada; [3]Univ of Calgary; [4]5Inserm Unit 1088, UFR Médecine/Pharmacie, Univ de Picardie, Amiens, France; [5]Mario Negri Inst, Bergamo, Italy.*

Background: Little is known about the best way to structure health systems to facilitate early chronic kidney disease (CKD) care. We evaluated CKD care programs within the context of the healthcare systems across countries to identify best practices and initiatives in care delivery.

Methods: We collated and synthesized data on existing CKD care policies and structures across 19 developed countries. These included CKD care frameworks within the context of the healthcare system providing a synthesis and comparative analysis of the information across the individual countries. Data were obtained from multiple sources, including renal registries, government reports and published literature, and a detailed survey of key stakeholders from each country(N=1226).

Results: Only three countries have a national specific CKD policy, and governments generally do not consider CKD a priority. For instance, in only three countries did the majority of respondents (>75%) believed that CKD was recognized as a priority by the government. Eleven countries have national CKD guidelines, and none has established schemes to monitor adherence. There were multi-faceted barriers to early CKD care: limited workforce capacity, absence of surveillance systems, lack of a coordinated care strategy, non-integration of CKD with other non-communicable disease (NCD) control initiatives, and low awareness of CKD among stakeholders (policymakers, primary care practitioners, and patients).

Conclusions: There are common challenges faced by diverse health systems on CKD care. Some countries are further ahead than others, but all have considerable work to do. This reflects the need for international cooperation to strengthen health systems and policies for CKD care. This data identifies opportunities for optimal care delivery, and explores potential mechanisms to capitalize on these opportunities.

SA-OR082

Patients That Utilise a Patient Facing Health Record Have Better Health Outcomes Anirudh Rao,[1] David Pitcher,[1] Richard G. Phelps.[2] *[1]UK Renal Registry, United Kingdom; [2]Univ of Edinburgh, United Kingdom.*

Background: Renal Patient View (RPV) has since 2005 been offering patients online access to their test results and information about their condition and treatment. By 2014, 30,000 patients in the UK had registered to use RPV, but how and how often patients utilise RPV varies widely. We examined selected health outcomes for any influence of the level of patient engagement with RPV.

Methods: RPV user database extant was linked with the UK Renal Registry. To assess effect of RPV use on initial RRT, patients who registered to use RPV > 90 days prior to RRT start (early-adopters), where RPV's information provision could have been influential, were compared with patients that registered after RRT start (late adopters). To assess effect of RPV on control of phosphate and URR, patients' phosphate control 6 months before registration was compared with 12 months after registration, stratifying the patients according to their utilisation of RPV inferred from the count of logons per month. Statistical analysis was by CHI sq, T test and logistic models as appropriate.

Results: Early adopters were more likely to start on a home based modality (HBM) compared to late adopters(42% versus 37%,p= .006).In adjusted models,early adopters were three times more likely to begin RRT on a HBM than the overall RRT cohort(OR 2.90,95% CI 2.52-3.34). Early adopters were more likely than late adopters to begin RRT with a living-donor transplant (63.4 versus 54.7 p= 0.03), with more persistent users twice as likely as the overall RRT population(OR 2.08, 95% CI 1.52-2.86) to begin RTT with a living-donor transplant. The greatest reduction of phosphate(0.93 mmol/L) occurred in the subgroup with most frequent login to RPV(>4/month),significantly greater than that observed in the reference group(0.49 mmol/L, p<0.001) or group of RPV registrants that never actually logged on(0.52 mmol/L,p<0.001). In contrast, there was no significant difference in URR between the groups.

Conclusions: Utilisation of RPV is associated with better patient outcomes. The stronger association where RPV utilisation might influence outcome (modality choice and phosphate control) than where influence was less likely (URR) suggests RPV supports patient engagement.

SA-OR083

Evaluating Risk of End-Stage Renal Disease in Public Health Settings Yoshio N. Hall,[1] Marlena Maziarz,[2] Glenn M. Chertow,[3] Jonathan Himmelfarb.[1] *[1]Medicine, Univ of Washington, Seattle, WA; [2]Biostatistics, Univ of Washington, Seattle, WA; [3]Medicine, Stanford Univ, Palo Alto, CA.*

Background: Models to estimate risk of end-stage renal disease (ESRD) have been mostly derived from insured cohorts of predominantly elderly, non-Hispanic white adults. We sought to evaluate the utility of several ESRD risk prediction models for public health application.

Methods: We performed a cohort study of 28,779 adults with moderate-to-advanced chronic kidney disease (CKD) who received ambulatory care in two large urban public health systems in the U.S. during 1996-2009 and who were followed for ESRD through

2011. We developed and assessed the utility of four proportional hazards models for incident ESRD using two recently proposed criteria: "proportion of cases followed" and "proportion needed to follow-up".

Results: Overall, 1,730 persons progressed to ESRD during follow-up (median follow-up=6.6 years). ESRD risk for time frames up to five years was highly concentrated among relatively few individuals. A predictive model (model 2) using five common variables (age, sex, race, eGFR, and dipstick proteinuria) performed as well as more complex models incorporating extensive sociodemographic and clinical data. Using this model, an estimated 80% of individuals who eventually developed ESRD were among the 5% of cohort members at highest estimated risk for ESRD at 1-year. Similarly, 8% and 13% of individuals at highest ESRD risk would have needed follow-up to capture 80% of those who eventually progressed to ESRD at 3- and 5-years, respectively.

Conclusions: In the healthcare safety net, a simple 5-variable model accurately predicts most cases of ESRD within five years. Applying risk prediction at the public health system level may improve CKD surveillance and management by directing resources to a relatively small sub-cohort at highest risk for progressing to ESRD.

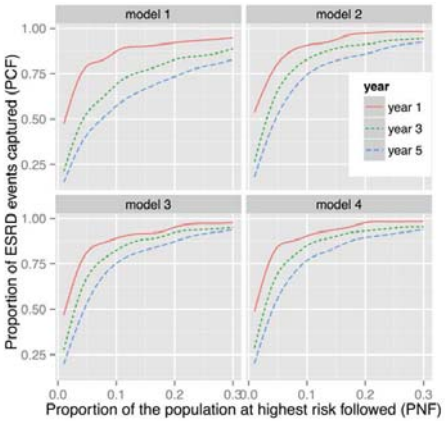

Figure 1. The estimated proportion of ESRD events captured (PCF) among a given proportion of subjects at highest estimated risk of ESRD (PNF) for models 1-4 at 1, 3 and 5-year time frames.

Model 1: adjusted for age, sex, race-ethnicity & eGFR
Model 2: model 1 covariates *plus* dipstick proteinuria & eGFR x proteinuria interaction
Model 3: model 2 covariates *plus* health insurance & comorbidities (diabetes, CVD, HTN, substance abuse and chronic viral disease)
Model 4: model 3 covariates *plus* additional laboratory variables (serum albumin, calcium, hemoglobin, cholesterol)

Funding: NIDDK Support

SA-OR084

Low Awareness and Comprehension of Chronic Kidney Disease Among Japanese Health-Check Subjects

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Background: Chronic kidney disease (CKD) has been highlighted as one of serious risk factors for end stage kidney disease and cardiovascular diseases (CVD). CKD prevalence is high in Japan and it was estimated that approximately one-eighth of Japanese adults were affected with CKD. Although CKD is well understood among Japanese health professionals, CKD awareness among Japanese general population has not been fully elucidated. Thus, CKD awareness and comprehension degree was surveyed among Japanese health-check subjects in this study.

Methods: The study subjects were 7,513 health-check subjects (3,551 females) in a single center in Aichi prefecture, Japan. Questionnaire survey including CKD awareness, self-reported renal function, knowledge questions on diagnosis, risk factors, clinical symptoms and effective life-style modification of CKD was conducted from November 2010 to October 2012 and CKD awareness and comprehension degree was analyzed.

Results: CKD awareness was 15.5% and television was the major source followed by news paper. CKD awareness among subjects who were under treatment of hypertension, diabetes or dyslipidemia was slightly but significantly high at 18.2% ($p<0.001$), but only 20.6% of those knew their renal function. Regarding CKD diagnosis, proteinuria was recognized at 58.9% but eGFR was poorly recognized at 31.5%, and proper understanding for CKD diagnosis was only at 4.6% even among subjects who knew CKD. Regarding clinical symptoms of CKD, edema (69.2%) and hypertension (51.2%) were recognized sufficiently followed by CVD (47.2%), but renal anemia (33.7%) and mineral bone disorder (25.6%) were poorly recognized. CKD risk factors and effective life-style modification for CKD were not well understood.

Conclusions: This study revealed that CKD awareness and comprehension degree remained low in Japan even among health-check subjects. CKD enlightenment campaigns in collaboration with government, the mass media, health professionals and academic societies are essential to improve CKD awareness, and their effect should be adequately monitored at regular intervals.

Funding: Government Support - Non-U.S.

SA-OR085

An Oral Enzyme (ALLN-177) Reduces Dietary Hyperoxaluria

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Background: Hyperoxaluria (HO) is a major cause of calcium oxalate (Ox) nephrolithiasis, resulting primarily from increased absorption of dietary Ox. The only therapy (Rx) available at present is dietary restriction which has suboptimal efficacy. ALLN-177 is a new oral recombinant enzyme with high, substrate-specific, Ox-degrading ability that may be ideal Rx for treating nephrolithiasis from HO. **Objective:** To test the efficacy and safety of ALLN-177 Rx in healthy subjects on a high Ox diet (1g/d).

Methods: Thirty healthy subjects were randomized to crossover between placebo or ALLN-177 Rx with meals (7500 u/meal tid) in a double blind, controlled study in an inpatient research unit. All subjects were placed on a 1g Ox diet alone for 3d and then treated for 7d with ALLN-177 or placebo. Subjects were crossed-over to the opposite Rx after a 7d washout. 24h urine was done daily. The 1^0 endpoint was a comparison of the mean 24h urinary Ox excretion (24hUox) while receiving ALLN-177 versus placebo Rx in the last 4d of each Rx phase. A responder analysis requiring a reduction in mean 24hUox of >5mg in favor of either Rx was done. A mixed model with random effects for crossover designs was used for analysis.

Results: A 1g Ox diet resulted in a sustained increase in 24hUox (mean±SD) similar to levels seen in patients with HO nephrolithiasis (pre-diet=27.1±11.7 to diet=82.2±27.4). ALLN-177 Rx resulted in a 24hUox reduction of 11.5±14.1 versus placebo, $p=0.0002$. There were 18/30 (60%) ALLN-177 responders and none on placebo; responder rates didn't differ by initial Rx period. The mean reduction in responders for 24hUox (23%) ranged from 8.3 to 43 mg. ALLN-177 was well tolerated, and no safety signals were noted.

Conclusions: This is the first well-controlled study of any drug demonstrating an ability to reduce urinary Ox levels of dietary origin. Further clinical studies in patients with recurrent kidney stones and HO are now warranted.

Funding: Pharmaceutical Company Support - Allena Pharmaceuticals

SA-OR086

Oxalobacter Formigenes Colonization Normalizes Oxalate Excretion in a Gastric Bypass Model of Hyperoxaluria

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Background: After Roux-en-Y gastric bypass (RYGB) surgery, calcium oxalate stone risk increases up to 4-fold, driven primarily by post-operative hyperoxaluria. The probiotic *Oxalobacter formigenes* (OF) can reduce urinary oxalate excretion by degrading intraluminal oxalate while promoting enteric oxalate elimination. The objective of the study was to examine the effect of OF colonization on urinary oxalate excretion and intestinal oxalate transport in an established hyperoxaluric RYGB animal model.

Methods: Obese male Sprague Dawley rats underwent sham (n=10) or RYGB (n=18) surgery and were maintained on low oxalate/fat diets. At 10 weeks post-operatively, half of the animals were randomized to OF wild rat strain (OXWR) gavage. Urine and stool were collected weekly to determine oxalate and colonization status, respectively. At 20 weeks post-operatively, [14C]-oxalate fluxes and electrical parameters were measured *in vitro* across isolated distal colon tissue mounted in Ussing chambers.

Results: All rats gavaged with OXWR were confirmed colonized at the time of flux. Urinary oxalate excretion remained low and unchanged in sham animals regardless of colonization status (mean 3.54 ± 0.59 umol/day). RYGB + OXWR had significant reductions in urinary oxalate excretion after gavage (24.94 ± 9.4 versus 6.85 ± 3.0 umol/day, $p<0.001$) while uncolonized RYGB animals remained unchanged (17.2 ± 4.2 versus 14.0 ± 3.3 umol/day, $p=0.62$). Compared to sham, the RYGB procedure increased intestinal oxalate tissue permeability by 36% and transcellular [14C]-oxalate net secretion 2-fold. When compared to non-colonized RYGB, RYGB + OXWR animals had 46% increase in net oxalate efflux with no significant changes in tissue permeability.

Conclusions: In our model, RYGB-related hyperoxaluria can be reduced to that of sham controls by OF colonization. Because intestinal permeability was similar between both RYGB groups, the mechanism for this decrease appears to be induction of distal colonic oxalate secretion by OF. This *in vivo* study suggests OF colonization may benefit RYGB patients with hyperoxaluria and warrants a clinical trial.

Funding: NIDDK Support, Pharmaceutical Company Support - Ethicon Endosurgery, Private Foundation Support

SA-OR087

Urinary Hydroxy-Oxo-Glutarate (HOG) as Diagnostic Factor for Primary Hyperoxaluria Type 3

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Background: Primary Hyperoxaluria (PH) comprises at least 3 recessive diseases characterized by endogenous overproduction of oxalate: PH1 (AGXT); PH2 (GRHPR) and PH3 (HOGA), the latter causes a defect in hydroxyproline metabolism in the mitochondria. We hypothesized that the levels of 4-hydroxy-2-oxoglutarate (HOG) in the urine of PH3-patients would discriminate among the PH diseases.

Methods: The newly created HOG assay was conducted using ion chromatography/mass spectroscopy (IC/MS). We analyzed urine specimen from 62 PH1-, 3 PH2- and of 30 PH3-patients, as well as 54 specimen of patients without a proven PH. Preparation included acidification with HCl and dilution 100x with 0.20M boric acid solution. The IC/MS system was calibrated using five standards of increasing concentrations of HOG (purchased from Santa Cruz Biotechnology). The M/S was calibrated to a span of 0.30 m/z at 161 m/z, negative polarity, dwell time of 0.5s, cone voltage of 25V and a probe temperature of 450ºC. A KOH gradient of 5mM gradually ramping to 100mM over 38 minutes was used in the IC.

Results: HOG concentration (mean 43.49 µmol/l, range 1.06-163.2) and HOG to creatinine ratio (mean 39.86 mmol/mol, range 0.60-260.0) were substantially higher in PH3-patients as compared to that of PH1/2-patients (mean 2.8 µmol/l, range 0.48-5.02

and 1.23 mmol/mol, range 0.07-1.23) or non PH patients (mean 5.18 mmol/l, range 0.08-9.97 and mean 1.45 mmol/mol, range 0.49-4.75). There was a large heterogeneity of HOG excretion in PH3-patients, as well as an overlap of values at the lower range in PH3-, with the higher range in PH1/2- and non PH-patients. However, only PH3-patient had urinary HOG concentration >9.97 µmol/l and >4.75 mmol HOG/mol creatinine. There was no correlation between urinary oxalate and HOG detected.

Conclusions: The current IC/MS assay allows the rapid identification of HOG, which can be used as a diagnostic marker of PH3. We believe that offers a way for diagnosis, and may later be helpful in providing mechanistic insights into the disease itself.

SA-OR088

Characterization of the Urine Proteome in Calcium Oxalate Stone Formers

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Background: Kidney stones form as aggregates of crystals (typically calcium oxalate - CaOx) with organic matrix (mainly proteins). Prior research focused on individual proteins without finding a critical protein. Of >1600 proteins identified by mass spectroscopy (proteomic) analysis in urine, >100 were found in stone matrix. Previously, urine proteins from stone formers were shown to exhibit reduced net negative charge compared to controls, and this study seeks to explain this shift in charge.

Methods: Random urine samples were collected from recurrent, idiopathic CaOx stone formers (SF, n = 25) and from healthy subjects (NU; n = 14), matched for age, sex and race. Urine macromolecules were isolated by ultradiafiltration against 10mM NaCl (10kDa cutoff, Spectrum Industries). Proteomic analysis was obtained at the MCW Innovation Center, with sequence matching to the Uniprot database (uniprot.org) and positive identification based on two unique or overlapping peptides (>85% match). Only samples with adequate signal (>1000 total scan counts and >29 proteins detected) were included.

Results: On average, each sample contained 80 unique proteins from the 409 proteins identified, with 11 proteins common to all samples, and the top 20 proteins accounting for > 70% of the protein mass. Proteins were grouped as anionic or cationic based on isoelectric points calculated from their amino acid content. Cationic proteins (pI > 6.5) were more common in SF urine (SF = 22 ± 6 % SC versus NU = 18 ± 5 %; p=0.04), consistent with a net negative charge reduction in these samples. SF urine showed significant increases in several cationic proteins from the immune response network (www.ingenuity.com), which contains most stone matrix proteins.

Conclusions: Relatively few proteins account for most of the protein signal in these urine samples. Stone formers demonstrated increased abundance of cationic proteins consistent with reduced net negative charge previously reported. This shift in composition would predispose SF urine to protein aggregate formation and loss of crystallization inhibitor properties, which would account for increased risk of stone formation.

Funding: NIDDK Support, Veterans Affairs Support

SA-OR089

Dedifferentiation of Renal Epithelial Cells into Osteogenic Cells and Formation of Randall's Plaque

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Background: Idiopathic calcium oxalate (CaOx) kidney stones develop by deposition of CaOx crystals on Randall's plaques (RP). Mechanisms involved are still unclear. It is our hypotheses that RP formation is similar to vascular calcification in which cells under oxidative stress (OS) acquire osteogenic characteristics. To verify our hypothesis we performed genome wide analysis of differentially expressed genes in the kidneys of hyperoxaluric rats and determine changes consistent with the dedifferentiation of epithelial cells into bone producing cells.

Methods: Male Sprague-Dawley rats received rat chow supplemented with 5% w/w hydroxyl-L-proline. After 28 days, rats were euthanized, total RNA extracted and subjected to genomic microarrays to obtain transcriptome data. Kyoto Encyclopedia of Genes and Genome (KEGG) were used to identify gene clusters. Expression of selected gene products was carried out by RT-PCR and/or immunohistochemistry.

Results: We investigated the expression of genes considered to be involved in epithelial transformation and bone morphogenesis including runt related transcription factor-1 and 2 (RUNX-1 and 2), zinc finger protein Osterix, bone morphogenetic proteins 2 and 7 (BMP2 and 7), bone morphogenetic protein receptor, Type 2 (BMPR2), cytokeratin 8, 10 and 18 (Krt 8, 10, and 18) and vimentin (VIM). Krt 10 and 18 were down regulated while VIM was upregulated and its expression increased. RUNX1 and 2 as well as Osterix were up-regulated. BMP2 and 7 as well as their receptor Bmpr2 were also upregulated.

Conclusions: Down regulation of Krt 8 and 10 and upregulation and increased expression of VIM point to phenotypic changes in epithelial cells. Upregulation of bone morphogenetic proteins and transcriptional regulators of osteogenesis are indicative of cells acquiring osteogenic characteristics. Hyperoxaluria leads to development of OS inducing dysregulated signaling and phenotypic changes. Matrix vesicles are produced and in hypercalciuric conditions, hydroxyapatite deposited on basal side of epithelium lining loops of Henle and/or collecting ducts. Such deposits are likely precursors of Randall's plaque.

Funding: NIDDK Support

SA-OR090

Mechanism for Elevated Urine pH in Patients Who Form Calcium Phosphate Renal Stones

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Background: Calcium phosphate (CaP) stone formers (SF) produce urine of higher pH than calcium oxalate (CaOx) SF or normal people (N). Higher pH raises CaP supersaturation (SS) and may lead to CaP stones, a more destructive kidney disease than CaOx stones. What raises urine pH is not certain.

Methods: In the General Clinical Research Center, we measured urine pH and determinants of renal acidification in 21 N, 12 female (F); 14 CaOx SF, 4 F; 14 with stones >50% CaP, 6 F. We collected 15 urines and 20 blood samples over a 15 hour day; diet was fixed.

Results: For all meal periods combined, urine pH of CaP exceeded N and CaOx (Table). SS CaP was higher in CaP than CaOx or N, but not due to higher urine calcium or phosphate excretion or lower urine volume. Urine NH_4 and citrate excretions, serum potassium and CO_2 did not differ across groups. Titratable acid (TA), net acid excretion (NAE) and fractional excretion (FE) of citrate of CaP did not differ from N. Urine SO_4 of CaP was below N despite a fixed diet, indicating lower endogenous acid production.

Conclusions: CaP SF have normal overall acid excretion, their higher urine pH being insufficient to alter NAE. Urine pH of CaP SF likely represents one end of the normal urine pH distribution rather than a disease affecting renal acidification.

	N	CaOx	CaP
S CO_2	25.2±0.1	25.2±0.1	25.4±0.1
S K	3.98±0.02	3.99±0.02	4.0±0.02
U pH	6.23±0.03	6.12±0.04	6.42±0.04*†
U TA	0.50±0.02	0.61±0.02*	0.45±0.02†
U NH_4	1.12±0.03	1.20±0.04	1.09±0.04
U CO_2	0.82±0.06	0.50±0.07*	0.96±0.06†
U NAE	0.8±0.1	1.3±0.1*	0.6±0.1†
U SO_4	1.68±0.04	1.56±0.04	1.39±0.04*†
U Ca	0.25±0.01	0.40±0.02*	0.43±0.01*
U Phos	1.03±0.03	1.05±0.04	1.02±0.04
U Cit	0.15±0.01	0.15±0.01	0.14±0.01
U Volume	145±7	120±8*	158±8†
FE Cit (%)	9.9±0.4	10.6±0.4	9.0±0.4†
SS CaP	0.76±0.07	1.48±0.08*	1.85±0.08*†
*, differs from N, †, differs from CaOx, p<0.05. Mean ± SEM. Excretions are mMol/hr except SO_4 (mEq/hr), volume (ml/hr).			

Funding: NIDDK Support

SA-OR091

Influence of Hydroxyproline Plasma Concentration on the Metabolism of Oxalate

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Background: The primary hyperoxalurias (PH) are rare but severe inherited diseases characterized by an increased endogenous production of oxalate. In humans the main precursor of oxalate is glyoxylate, whose most prominent source is hydroxyproline, a collagen breakdown. In order to clarify the pathways of endogenous oxalate synthesis, the contribution of hydroxyproline turnover to oxalate and glycolate synthesis and urinary excretion is assessed in a fasted state. Such knowledge would be invaluable for a better understanding of the pathophysiology of PH and idiopathic calcium oxalate stones.

Methods: Patients with PH type 1 (n=4), and normal subjects (n=8) on a 3-days controlled diet were infused with ^{15}N-$^{13}C_5$-hydroxyproline at a constant rate (750 nmol/kg/h) for 6 h. Urine and plasma samples were collected hourly for analysis of total and ^{13}C labelled hydroxyproline and glycine by GC/MS; oxalate and glycolate by IC and IC/MS.

Results: Four PH1 patients with a mean daily excretion of oxalate of 27.3 ± 17.4 mg/1.73 m^2 (14.3 - 52.1), mean glycolate excretion of 77.9 ± 45.3 mg/1.73 m^2 (41.4 - 140.9) and 8 control subjects were analysed (Table I). The mean GFR in PH1 patients was 107 ± 11 ml/mn/1.73m^2. The total urinary oxalate was not increased and no adverse effects observed.

	total Urinary oxalate		Oxalate urine 13C2 enrichment		hydroxyproline plasma 13C2 enrichment
	(mg/h)		(% $^{13}C_2$ oxalate)		(% $^{13}C_5$ hydroxyproline)
	pre-infusion	post-infusion	post-infusion		post-infusion
control	**0.99 ± 0.3**	**0.74 ± 0.19**	**2.42 ± 0.71**	**control**	**18.2 ± 3.12**
(n=4)	(0.7 - 1.37)	(0.58 - 0.95)	(1.79 - 3.17)	(n=8)	(15.2 - 22.0)
PH1	**2.39 ± 1.15**	**2.29 ± 1.21**	**5.84 ± 1.25**	**PH1**	**32.4 ± 6.36**
(n=4)	(1.39 - 3.60)	(1.06 - 3.94)	(4.85 - 7.68)	(n=4)	(26.6 - 41.1)

Conclusions: The increased enrichment in PH1 patients suggests that hydroxyproline makes a significant contribution to oxalate synthesis in PH1 patients. Further study in other types of PH may show an even greater contribution with potential therapeutic implications.

Funding: NIDDK Support

SA-OR092

Distinct Impact of Dabigatran and Warfarin Treatment on Bone Volume and Structure in Rats Maria Fusaro,[1] Luca Dalle Carbonare,[2] Adriana S. Dusso,[3] M. Vittoria Arcidiacono,[3] Sabina Pasho,[4] Giovanni Tripepi,[5] Claudia Torino,[5] Maurizio Gallieni.[4] *[1]CNR Padua, Italy; [2]Univ of Verona, Italy; [3]IRB Lleida, Spain; [4]Dialysis Unit Milan, Italy; [5]CNR Reggio C, Italy.*

Background: Warfarin, a commonly used anticoagulant, inhibits Vitamin K (VK) metabolism and VK-Dependent Protein (VKDP) functions that are essential for bone and vascular health. Dabigatran is a new oral anticoagulant not affecting VK. We compared the impact of warfarin and dabigatran on bone and vascular health in rats with normal renal function.

Methods: Rats received Dabigatran etexilate (0.6 mg/g of chow) or Warfarin (starting from 0.6 mg/kg and adjusted to achieve the desired INR of 2-3 for 6 weeks). Untreated Controls were fed a diet containing 8 mg of VK3 per gram of chow. Femur, tibia and vertebrae were evaluated immunohistochemically and morphometrically. Calcium deposition was examined in aorta and iliac arteries.

Results: Femur and vertebra analysis showed decreased bone volume and increased trabecular separation in rats treated with warfarin compared to control and dabigatran treated rats. vertebra, trabecular number was lower in the warfarin group. In femur, warfarin was associated with higher Activation Frequency (AcF) than in dabigatran or control rats. In vertebra, there was no difference in osteoblast activity and resorption parameters among groups, except for maximum erosion depth, which was higher in warfarin treated rats. Accordingly, warfarin treatment was associated to higher Vertebral Bone Formation Rate/ Bone Surface and AcF than in dabigatran or control rats. There was no arterial calcium deposition in any group.

Femur analysis			
	Warfarin	Dabigatran	Controls
Bone Volume/Tissue Volume (%)	27.3±8.9 (p<0.05 vs dabigatran and controls)	42.4±9.8	43.7±6.5
Trabecular Thickness (μm)	64.1±23.2 (p<0.05 vs dabigatran)	85.4±10.9	66.2±17.4

Conclusions: Warfarin treatment was associated with markedly decreased bone volume, increased trabecular separation and higher turnover than in dabigatran treated and control rats. These differences could translate into a lower incidence of fractures in dabigatran treated patients.

SA-OR093

Raloxifene, but Not Calcitriol, Improves the Structural and Mechanical Properties of Bone in an Animal Model of Chronic Kidney Disease Christopher Newman,[1] Drew M. Brown,[1] Neal X. Chen,[2] Sharon M. Moe,[2,3] Matthew R. Allen.[1] *[1]Anatomy and Cell Biology, Indiana Univ School of Medicine, Indianapolis, IN; [2]Medicine (Div of Nephrology), Indiana Univ School of Medicine, Indianapolis, IN; [3]Roudebush VA Medical Center, Indianapolis, IN.*

Background: Calcitriol (CAL) is used to control elevations in parathyroid hormone (PTH), but its fracture benefits in patients with chronic kidney disease (CKD) are largely unknown. Raloxifene (RAL) has been shown to improve bone density in these patients, but its effects on fracture resistance are likewise unclear. The goal of the current study was to examine the effects of these treatments on bone structural and mechanical properties in an animal model of CKD.

Methods: At 25 weeks of age (30% of normal kidney function), Cy/+ rats with progressive CKD (n=9-11/group) were treated with vehicle (VEH), CAL, or RAL for five weeks. Normal littermates (NL) were used as controls. Serum PTH was assessed at 30 weeks, and tibial bone structure (cortical and trabecular) was assessed using microCT. Bone mechanics were determined by four-point bending of the femur with the main outcomes being strength (bone's resistance to damage) and toughness (bone's tolerance of existing damage).

Results: CKD-VEH had higher PTH than NL (+898%). Both CKD-CAL and CKD-RAL had lower PTH than CKD-VEH (-47% and -43%) but were still higher than NL. CKD-VEH had lower trabecular bone volume compared to NL (-35%). This was normalized by RAL but not CAL. Cortical bone area and thickness were lower in CKD-VEH compared to NL (-16% and -14%). Neither CAL nor RAL eliminated these differences. Strength and toughness in CKD-VEH were both lower than NL (-26% and -25%). While neither treatment improved strength, RAL corrected toughness. Hence, the mechanical benefits of RAL were primarily due to improvements in bone quality.

Conclusions: These data show that while both CAL and RAL lowered PTH, only RAL normalized trabecular bone volume and mechanical properties in CKD animals. Whether these positive skeletal outcomes are primarily due to RAL's anti-bone remodeling effects or its suppression of PTH through a currently unknown mechanism is yet to be determined.

Funding: NIDDK Support

SA-OR094

Identification of a Bone Defect in ADPKD Berenice Y. Gitomer,[1] Renata C. Pereira,[2] Mikaela R. Malaczewski,[1] Myles S. Wolf,[3] Isidro B. Salusky,[2] Jason W. Stoneback,[1] Michel Chonchol.[1] *[1]Univ of Colorado Anschutz Medical Campus; [2]Univ of California, Los Angeles; [3]Northwestern Univ; [4]Univ of California, Los Angeles.*

Background: Autosomal Dominant Polycystic Kidney Diease (ADPKD) is the most common potentially lethal genetic renal disease associated with aberrant expression of polycystin proteins. Polycystin-1 is highly expressed in osteoblasts and osteocytes, which are the main sources of fibroblast growth factor 23 (FGF23). A bone phenotype of reduced bone mineral density, trabecular bone volume and cortical thicknesshas been reported in mice with targeted disruption of pkd1 and normal renal function. To date there has been no systematic investigation of bone phenotype in humans with ADPKD.

Methods: Institutional review board approval was obtained and all subjects gave informed consent. Male ADPKD Caucasian patients with eGFR > 60ml/min/1.73m^2 and normal parathyroid hormone levels underwent standard tetracycline double labeling prior to transiliac crest bone biopsy. Bone samples were processed for histomorphometric analysis of static and dynamic bone parameters.

Results: Osteoid volume, thickness and surface were decreased in ADPKD bone samples compared to bone samples from healthy controls. Bone mineralization, bone formation rate and mineral apposition rate were also decreased in ADPKD bone compared to normal bone.

	PKD1	PKD2	PKD3	PKD4	PKD5	normal male
Ages (years)	35	27	28	27	21	
eGFR (ml/min/1.73m^2)	81	123	119	137.4	160.7	
Osteoid vol/bone vol (%)	0.66	0.61	0.26	0.52	0.48	2.9±2.7
Osteoid thickness (μm)	7.63	9.61	2.75	12.27	5.75	11.7±3.5
Osteoid surface/bone surface (%)	9.91	3.75	4.15	3.99	3.85	16.1±12.6
Mineralizing surface/bone surface (%)	4.43	2.47	0.73	1.55	1.59	18.0±8.0
Mineral apposition rate (μm/day)	0.45	0.42	0.38	0.71	0.48	0.65±0.12
Bone formation rate/bone surface (μm^3/μm^2/year)	7.30	37.41	1.02	40.37	2.74	47.45±25.55

Conclusions: We describe the results of the first detailed histological analysis of bone from human patients with ADPKD. The histomorphometric analyses are consistent with adynamic bone disorder in ADPKD patients despite normal renal function.

Funding: NIDDK Support

SA-OR095

A Renal Olfactory Receptor Aids in Glucose Handling in the Proximal Tubule Blythe D. Shepard,[1] Lydie Cheval,[2] Alain Doucet,[2] Jennifer L. Pluznick.[1] *[1]Dept of Physiology, Johns Hopkins Univ School of Medicine, Baltimore, MD; [2]Centre de Recherche des Cordeliers, Paris, France.*

Background: Olfactory receptors (ORs) are seven transmembrane domain G protein-coupled chemosensors that detect odorants in the nose. We previously reported that OR signaling plays a role in the kidney, and have identified 9 renal ORs including Olfr1393.

Methods: To elucidate the role that Olfr1393 plays in the kidney, we examined nephron localization using reverse-transcribed RNA from microdissected renal segments, and subcellular distribution using stably-expressing MDCK cells. Olfr1393 ligand screening was performed using a luciferase reporter assay with Olfr1393-expressing HEK293T cells. To assay the physiological role of this OR we generated a whole-animal knockout (KO) mouse and measured plasma values by iStat, blood pressure by tail cuff, and GFR by transcutaneous decay of sinistrin in conscious, freely-moving mice.

Results: PCR on microdissected renal segments revealed that Olfr1393 is exclusively expressed in the three proximal tubule segments (S1, S2 and S3, n=3 mice). When stably expressed in polarized MDCK cells, Olfr1393 localizes to the apical PM, yet is excluded from the primary cilia. Olfr1393, like most ORs, is an "orphan receptor" with no known ligand. We comprehensively screened Olfr1393 with over 1400 chemicals and found that Olfr1393 detects preconstrained cyclic molecules containing a carbonyl or alcohol group. At baseline, Olfr1393 KO mice appear to be in homeostatic balance based on plasma electrolytes, BUN, creatinine, blood pressure and GFR. However, despite being euglycemic the KO mice exhibit glycosuria (1.6x increase in glucose/creatinine versus wild-type, WT). When challenged with a glucose bolus (glucose tolerance test, GTT), the KO mice better handled the glucose load as indicated by plasma glucose values (area under curve: WT 22.4 ± 1.9 versus KO 15.7 ± 1.1; P = 0.001).

Conclusions: These data suggest that Olfr1393 is expressed on the apical PM of the proximal tubule where it helps to regulate renal glucose reabsorption. As renal glucose handling is mediated by Sglt1 and Sglt2, we are currently examining the possibility that Olfr1393 interacts with these transporters.

Funding: NIDDK Support, Private Foundation Support

SA-OR096

Nanomolar-Potency, UT-A-Selective Inhibitors of Kidney Tubule Urea Transporters Produce Salt-Sparing Diuresis in Rats Cristina Esteva-Font,[1] Onur Cil,[1] Puay Wah Phuan,[1] Tao Su,[1] Sujin Lee,[1] Marc O. Anderson,[2] Alan S. Verkman.[1] *[1]Depts of Medicine and Physiology, Univ of California San Francisco, San Francisco, CA; [2]Dept of Chemistry and Biochemistry, San Francisco State Univ, San Francisco, CA.*

Background: UT-A inhibitors have the potential to be first in their class salt-sparing diuretics with indications in volume-overload edemas and high vasopressin-associated hyponatremias. UT-A urea transporters are expressed in kidney tubule epithelial cells where they are required for urinary concentration by countercurrent multiplication.

Methods: Using a fluorescence, cell-based functional assay of UT-A1 urea transport developed by our lab (Esteva-Font et al. *Chem. Biol.* 20:1235-1244, 2013) we screened >200,000 synthetic small molecules, drugs, natural products, and urea analogs. Several classes of synthetic small molecule inhibitors were characterized and tested in rats.

Results: Screening of synthetic small molecules produced 9 classes of nanomolar-potency UT-A inhibitors, including indolethiazoles, g-sultambenzosulfonamides and aminocarbonitrilebutenes, some of which had >200-fold UT-A versus UT-B selectivity and metabolic stability *in vivo*, producing sustained therapeutic concentrations in urine. Drug / natural product screening yielded weak UT-A inhibitors (IC50 10-20 μM), including nicotine, sanguinarine and indolcarbonylchromenone; urea analog screening produced low, millimolar-potency inhibitors including dimethylthiourea, benzimidazolylurea and dichlorophenylthiourea. UT-A inhibitor administration to rats increased urine output up to 5-fold and reduced urine osmolality up to 3-fold, both under hydrated conditions with following water deprivation / DDAVP. The diuresis produced by UT-A inhibition was salt-sparing compared to that produced by furosemide.

Conclusions: UT-A1 inhibitors may be useful as diuretics in high-vasopressin, fluid-retaining conditions in which conventional salt transport-blocking diuretics have limited efficacy. Compound prioritization is in progress for efficacy testing in clinically relevant rat models of edema.

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

SA-OR097

Vasopressin-Responsive miRNAs and AQP2-Targeting miRNAs in Kidney Collecting Duct Cells Jae-Eun Kim, Hyun Jun Jung, Tae-Hwan Kwon. *Dept of Biochemistry and Cell Biology, School of Medicine, Kyungpook National Univ, Daegu, Korea.*

Background: Mature microRNA (miRNA) combined with RNA-induced silencing complex acts as an important post-transcriptional regulator. However, miRNAs in the kidney collecting duct cells have not been well understood. We aimed to profile the vasopressin-responsive miRNAs in the kidney inner medullary collecting duct (IMCD) cells, and to identify the aquaporin-2 (AQP2)-targeting miRNAs.

Methods: Microarray chip assay was carried out in the IMCD tubule suspension of rat kidney in the absence or the presence of dDAVP stimulation (10^{-9} M, 2 h). To identify AQP2-targeting miRNAs, *in silico* analysis was performed. *In situ* hybridization was done for examining the expression of AQP2-targeting miRNAs in the kidney. RT-qPCR and immunoblot analysis were done to study the changes of miRNAs and AQP2 expression. Luciferase reporter assay was performed to examine the effects of AQP2-targeting miRNAs on the AQP2 translation.

Results: Microarray chip assay revealed nineteen miRNAs, including both precursor- and mature-miRNAs, as potential candidates that showed significant changes in the expression after dDAVP stimulation ($P<0.05$). Nine mature miRNAs exhibiting more than 1.3-fold changes in the expression on microarray were further examined by RT-qPCR. Four miRNAs (miR-32, miR-137, miR-216a, and miR-216b), which were also identified by microarray assay, targeted 3'UTR of rat AQP2 mRNA. Target seed regions of miR-32 and miR-137 were conserved at the 3'UTR of rat and mouse AQP2 mRNA. *In situ* hybridization demonstrated that miR-32 and miR-137 were expressed in the IMCD cells of rat kidney. Importantly, RT-qPCR and immunoblot analysis demonstrated that dDAVP-induced AQP2 mRNA and protein expression was significantly decreased in mpkCCDc14 cells transfected with miRNA-mimic of miR-32 or miR-137. Moreover, luciferase reporter assay demonstrated a significant decrease of AQP2 translation in mpkCCDc14 cells transfected with miRNA-mimic of miR-32 or miR-137.

Conclusions: We specifically identified miRNAs targeting AQP2 expression in IMCD cells to understand further the molecular mechanisms of the AQP2 regulation for AVP-regulated urine concentration and body water homeostasis.

Funding: Government Support - Non-U.S.

SA-OR098

MiR-132 Regulates Diuresis Through Vasopressin and Prostaglandin-Dependent Alteration of Aquaporin-2 Localization Roel Bijkerk,[1,3] Christiane Trimpert,[2] Ruben de Bruin,[1] Coen van Solingen,[1] Ton J. Rabelink,[1] Benjamin D. Humphreys,[3] Peter M.T. Deen,[2] Anton Jan Van Zonneveld.[1] *[1]Nephrology and Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, Netherlands; [2]Physiology, Radboud Univ Nijmegen Medical Center, Nijmegen, Netherlands; [3]Renal Div, Brigham & Women's Hospital and Harvard Medical School, Boston, MA.*

Background: The collecting duct (CD) principal cells of our kidneys are critical in the maintenance of blood water levels, as binding of vasopressin (AVP) to its V2-receptor and the subsequent translocation of aquaporin-2 (AQP2) water channels to the apical membrane fine-tunes water balance. Cyclooxygenase-2 (Cox2) produces prostaglandins such as PGE2 that counteract renal AVP action by inducing internalization and lysosomal degradation of AQP2. By in silico analysis, we identified miR-132 as a potential post transcriptional regulator of Cox2 expression and subsequently investigated the role of this miRNA in diuresis.

Methods: We used miR-reporter constructs to validate Cox2 repression by miR-132 and generated antagomirs to silence miR-132 function. Synthetic AVP (ddAVP) was administered with osmotic minipumps. Mice were housed in metabolic cages and sacrificed 1 day after i.v. injection of the antagomirs or scrambled controls.

Results: We identified miR-132 to directly target Cox2, and inhibiting miR-132 in vitro increased Cox2 abundance. Silencing of miR-132 in vivo in mice caused an acute diuresis and severe weight loss, characterized by increased plasma osmolality and decreased urine osmolality. Urinary PGE2 levels were elevated and hypothalamic AVP mRNA and blood AVP levels were not increased, despite the increased blood osmolality. This resulted in less translocation of AQP2 to the apical membrane in CD cells. MiR-132 silencing combined with administration of ddAVP partially restored water reabsorption.

Conclusions: Silencing of miR-132 causes acute diuresis. Our data indicate that this is the result of a decrease in AVP synthesis/release and Cox2-mediated increase in renal PGE2 counteracting renal AVP-stimulated water reabsorption through AQP2.

Funding: Government Support - Non-U.S.

SA-OR099

Inhibition of Mitochondrial Complex-1 Restores the Downregulation of Aquaporins in Obstructed Kidney Independently of COX-2/PGE2 Pathway Zhanjun Jia,[2] Ying Sun,[1] Yue Zhang,[1] Guixia Ding,[1] Songming Huang,[1] Aihua Zhang.[1] *[1]Nephrology Dept, Nanjing Children Hospital, Nanjing Medical Univ, Nanjing, China; [2]Nanjing Key Laboratory of Pediatrics, Nanjing, China.*

Background: Downregulation of aquaporins (AQPs) in obstructed kidney has been well demonstrated with elusive mechanism. Recently, mitochondrial abnormality was shown in obstructive kidney disease. However, the role of mitochondrial abnormality in obstructed kidney is still unknown. This study was to investigate the role of mitochondrial abnormality in mediating obstruction-induced AQPs downregulation.

Methods: Following the surgery of unilateral ureteral obstruction, mice were treated with rotenone (an established mitochondrial complex 1 inhibitor) at the dose of 500 ppm in diet.

Results: Following 7 days obstruction, the levels of mitochondrial NADH dehydrogenase 1 (mtNd1), mitochondrial transcription factor (mtFAM), and the copy number of mitochondrial DNA in kidneys were strikingly reduced, suggesting a severe mitochondrial impairment. Meanwhile, AQP1, AQP2, and AQP3 were downregulated by 50-80%as determined by Western blotting and qRT-PCR. Using rotenone to block the activity of impaired mitochondria, downregulation of AQP1 and AQP2 was entirely abolished at both protein and mRNA levels. For AQP3, rotenone partially but significantly restored it by 40%. COX-2/PGE2 pathway has been reported to be associated with the downregulation of AQPs in obstructive kidney disease. Therefore, we examined this pathway in present study. As expected, kidney obstruction markedly elevated expressions of COX-2 and mPGES-1 determined by Western blotting and qRT-PCR), as well as the kidney PGE2 content determined by ELISA. In contrast, the expressions of COX-1, mPGES-2, and cPGES were not affected by ureteral obstruction. Surprisingly, rotenone treatment did not affect COX-2/mPGES-1/PGE2 signaling in present mouse model, which differs from a recent publication showing that rotenone blocked COX-2 upregualtion in obstructed rat kidney (Ostergaard M, et al, AJP-renal, 2014).

Conclusions: Our findings suggest a novel role of mitochondrial dysfunction in mediating the AQPs downregulation in obstructive kidney disease independently of COX-2/PGE2 signaling pathway.

SA-OR100

Cholesterol Depletion of mpkCCD Cells Is Associated with Increased Ubiquitylation of Aquaporin-2 Cecilie Noehr Pedersen, Robert A. Fenton, Hanne Moeller. *Biomedicine, Aarhus Univ, Aarhus, Denmark.*

Background: Arginine vasopressin (AVP) mediates the abundance of the water channel aquaporin-2 (AQP2) in the apical membrane of kidney collecting duct principal cells and thus modulates urinary concentration. AQP2 function is regulated by posttranslational modification (PTM) of its COOH-terminus. AQP2-ubiquitylation and phosphorylation (S269) occurs in the plasma membrane and promotes AQP2 internalization and membrane retention, respectively. We hypothesized that these opposing effects can occur in different domains of the plasma membrane.

Methods: We manipulated the composition of the lipid membrane of a kidney collecting duct cell model, mpkCCD cells, using the cholesterol depleting reagent methyl-β-cyclodextrin (MβCD).

Results: Treatment of mpkCCD cells using MβCD prevented dDAVP-induced phosphorylation of AQP2 at S269. Cholesterol abundance assays demonstrated that MβCD significantly reduced whole cell cholesterol levels after 40 min. MβCD had similar effects on forskolin mediated AQP2 phosphorylation, suggesting that the effects are not indirect via disturbed V2R-signaling. dDAVP washout in the presence of MβCD did not affect dephosphorylation of S269. MβCD increased ubiquitylation of AQP2 both in the presence or absence of dDAVP; which was not a result of an increased abundance of AQP2 in the membrane since MβCD treatment in itself did not increase apical abundance of AQP2. Furthermore, reducing AQP2 endocytosis following dDAVP washout using dynasore and pitstop 2 did not increase the levels of ubiquitylated AQP2.

Conclusions: Our data suggest that cholesterol is critical for modulation of AQP2 PTMs and thereby AQP2 function.

Funding: Private Foundation Support

SA-OR101

NDFIP1: The Missing Adaptor for Ubiquitination and Degradation of the Aquaporin-2 Water Channel by NEDD4 and NEDD4L

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Background: Aquaporin 2 (AQP2) is essential for renal water reabsorption and fine-tuning of its apical membrane expression is critical for reabsorption of the proper volume. This fine-tuning is the result of signals inducing exocytosis and endocytosis of AQP2. Whereas binding of arginine vasopressin (AVP) to its type-2 receptor initiates a cAMP-protein kinase A cascade and AQP2 translocation to the apical membrane, this is counteracted by protein kinase C-activating hormones, resulting in ubiquitination-dependent internalization of AQP2. The involved E3-ubiquitin ligase however is unknown.

Methods: Human embryonic kidney (HEK293) and murine cortical collecting duct (mpkCCD) cells were used to study ubiquitination and degradation of AQP2.

Results: SiRNA knockdown of NEDD4/4L ubiquitin ligases in mpkCCD cells revealed increased AQP2 abundance, but they did not directly interact with AQP2. Yeast Two-Hybrid assay with full-length AQP2 as a bait identified NEDD4 family interacting protein 2 (NDFIP2) to bind AQP2. NDFIP2 and its homologue NDFIP1 are adaptor proteins which bind NEDD4 ubiquitin ligases via their PY-motifs and bring them close to target proteins. In HEK293 cells, NDFIP1/2 bound AQP2 and were essential for NEDD4/NEDD4L-mediated ubiquitination and degradation of AQP2, an effect not observed with PY-lacking NDFIP1/2 proteins. In mpkCCD cells, downregulation of NDFIP1, NEDD4 and NEDD4L, but not NDFIP2, increased AQP2 abundance, but did not affect the extent of AQP2 internalization from the plasma membrane. In mouse kidney, NDFIP1, but not NDFIP2, co-localized and co-immuno-precipitated with AQP2.

Conclusions: Our results reveal for the first time that NDFIP1, NEDD4 and NEDD4L are involved in AQP2 ubiquitination and degradation and position NDFIP1 as a potent and rate-limiting regulator of NEDD4/NEDD4L-mediated ubiquitination and degradation of AQP2. Moreover, as NEDD4/4L also ubiquitinates ENaC in principal cells, the expression and activity of NDFIP1 may be key for principal cells to reabsorb either water or sodium, or both.

Funding: Government Support - Non-U.S.

SA-OR102

Luminal pH Affects Phosphorylation (Serine 256) and Intracellular Trafficking of AQP2 in Inner Medullary Collecting Duct Cells

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Background: Collecting duct cells are continuously exposed to the changes of luminal pH. We aimed to study the effects of altered extracellular pH (pHe) on dDAVP-induced phosphorylation (S256, p-AQP2) and apical targeting of AQP2 in rat kidney inner medullary collecting duct (IMCD) cells.

Methods: Freshly prepared IMCD tubule suspension was exposed to buffer with pH 6.4, 7.4, or 8.4 for 1 h and was treated with dDAVP (10^{-10} M, 3 min). Intracellular pH of IMCD cells were continously monitored when they were exposed to extracellular pH 6.4, 7.4, or 8.4 for 30 min, respectively. To examine whether changes of pH at the luminal side of the cells affect dDAVP-induced AQP2 phosphorylation (S256) and intracellular trafficking, cell surface biotinylation assay and laser-scanning confocal microscopy were performed. Moreover, fluorescence resonance energy transfer (FRET) technique was exploited and time-lapse changes of dDAVP- or forskolin-induced PKA activity and intracellular Ca^{2+} dynamics were monitored.

Results: Increased AQP2 phosphorylation (S256) was seen when tubule suspension was exposed to pH 7.4 and 8.4, compared to pH 6.4. When IMCD cells were exposed to 6.4, 7.4, and 8.4 for 30 min, intracellular pH became 6.1, 7.2, and 8.1, respectively. IMCD cells were exposed to transepithelial pH gradient for 1 h in the transwell chambers (apical pH 6.4, 7.4, or 8.4 versus basolateral pH 7.4). Laser scanning confocal microscopy and cell surface biotinylation assay revealed that exposure to luminal pH 6.4 for 1 h significantly decreased dDAVP (10^{-9} M, 15 min, basolateral)-induced apical targeting of AQP2. Importantly, FRET analysis revealed that dDAVP (10^{-9} M)-induced increase of PKA activity was significantly attenuated when LLC-PK1 cells were exposed to pHe 6.4, compared to pHe 7.4 and 8.4. In contrast, forskolin (10^{-7} M)-induced PKA activation was not affected. Moreover, dDAVP-induced increase of intracellular Ca^{2+} was not affected.

Conclusions: Taken together, acidotic condition is likely to decrease dDAVP-induced phosphorylation and apical targeting of AQP2 in IMCD cells, via an inhibition of V2R-G protein-cAMP-PKA action.

Funding: Government Support - Non-U.S.

SA-OR103

CaSR Signaling Prevents Forskolin-Induced pS256-AQP2 in Isolated Mouse Collecting Duct Tubules and Impairs Osmotic Water Permeability in Renal Cells

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Background: We have recently provided both *in vitro* and *in vivo* evidence that high luminal calcium in the renal collecting duct attenuates short-term vasopressin-induced AQP2 trafficking via activation of the Calcium-Sensing Receptor (CaSR). Here, we evaluated AQP2 phosphorylation in response to specific activation of CaSR with the positive allosteric modulator NPS-R568 in isolated mouse collecting duct tubules and in renal HEK cells stably transfected with CaSR.

Methods: Isolated collecting duct from mouse kidney and HEK cells transfected with wt-hCaSR were exposed FK in the presence of NPS-R568 and the dynamics of pS256-AQP2 and pS261-AQP2 were evaluated. To mimic "tonic" activation of CaSR, parallel experiments were performed in HEK cells transfected with two gain of function variants of CaSR. Osmotic water permeability was analyzed in calcein-loaded cells.

Results: In transfected cells, CaSR activation with NPS-R568 drastically reduced basal pS256-AQP2 levels and increased pS261-AQP2 levels thus having an opposite effect on AQP2 phosphorylation with respect to vasopressin action. Similar, though more pronounced effects were obtained in cells expressing CaSR gain of function variants. When FK stimulation was performed in the presence of NPS-R568, the increase in pS256-AQP2 was totally prevented. In line with these data, the FK-induced increase in the osmotic water permeability was abolished in all CaSR expressing cells reaching values even lower than the basal permeability. The physiological relevance of data obtained in transfected cells was next evaluated in freshly isolated inner medullar collecting duct from mouse kidney. Stimulation with FK in the presence of NPS-R568 completely prevented FK-induced increase in pS256-AQP2. Similar results were obtained in isolated rat kidney inner medulla.

Conclusions: Our in vitro and ex vivo data demonstrate that specific activation of CaSR expressed in the collecting duct prevents FK-dependent increase in pS256-AQP2 and point to a crucial role of CaSR in counteracting short-term vasopressin response.

Funding: Government Support - Non-U.S.

SA-OR104

Glutathionylation of the Aquaporin-2 Water Channel: A Novel Post-Translational Modification Modulated By the Oxidative Stress

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Background: Aquaporin-2 (AQP2) is the vasopressin regulated water channel that controls renal water reabsorption and urine concentration. AQP2 undergoes different regulated posttranslational modifications, including phosphorylation and ubiquitylation, which are fundamental for controlling AQP2 cellular localization, stability and function. The relationship between AQP2 and S-glutathionylation is of potential interest because reactive oxygen species (ROS), produced under renal failure or nephrotoxic drugs, may influence renal function as well as the expression and the activity of different transporters and channels, including aquaporins.

Methods: AQP2 S-Glutathionylation was evaluated by AQP2 immunoprecipitation followed by WB with anti-GSH in mouse kidney and HEK-293 cells stably transfected with AQP2. AQP2 S-Glutathionylation was further analyzed by using BioGEE reagent. ROS content and NADPH oxidase activity were measured with dihydrorhodamine-123 and with lucigenin-enhanced chemiluminescence assay respectively.

Results: Obtained results showed for the first time, that AQP2 is subjected to S-glutathionylation in kidney and in HEK-293 cells stably expressing AQP2. S-Glutathionylation is a redox-dependent posttranslational modification controlling several signal transduction pathways and displaying an acute effect on free cytosolic calcium concentration. Interestingly, we found that, cells expressing wild-type Calcium Sensing Receptor (hCaSR-wt) and its gain of function (hCaSRR990G hCaSRR990G; hCaSR-N124K) had a significant decrease in AQP2 S-glutathionylation secondary to reduced ROS levels and reduced basal intracellular calcium concentration compared to mock cells.

Conclusions: Identifying this novel posttranslational modification provide fundamental insight into cell biological aspects of AQP2 function and is crucial to understand renal diseases characterized by oxidative stress and AQP2-dependent water reabsorption disturbs.

Funding: Government Support - Non-U.S.

SA-OR105

Peritonitis as a Risk Factor for Long-Term Cardiovascular Mortality in Peritoneal Dialysis Patients

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Background: Peritoneal dialysis(PD) related peritonitis is a major complication of the method, related to its association with infectious morbidity and mortality, and to technique failure. The impact of peritonitis on long-term mortality, particularly related to non-

infectious causes, has not been investigated until the present. The aim of this study was to analyze the impact of peritonitis on long-term cardiovascular(CV)mortality in a large cohort.

Methods: The analysis was based on BRAZPDII, a national cohort performed in Brazil from Dec2004 to Jan2011. Incident adult PDpatients with at least 90days on treatment from 122centers were included in the analysis. CVdeath occurring after a minimum of 90 after a peritonitis episode were considered the primary endpoint. Cox regression for time-dependent variables was used for the adjustments. Multivariable analysis included age,gender,educat ion,diabetes,left ventricular hypertrophy,hypertension, previous hemodialysis,pre dialysis care,hemoglobin,creatinine and blood pressure at baseline.

Results: There were 2405episodes of peritonitis in 5707patients(48%males,44%di abetes,73%hypertensive).Patients with 1 episode of peritonitis presented a 25%increase in the hazard ratio of CVmortality compared to those who never experienced peritonitis (HR1.25;CI95%1.04-1.52). Adjusted hazard for CVmortality showed an stepwise effect for patients with additional episodes of infection 2episodes(HR2.00;CI95%1.47-2.72), 3episo des(HR3.41;CI95%2.11-5.24) and 4episodes(HR4.92;CI95%2.58-9.38).

Conclusions: Peritonitis was an independent predictor of CVmortality and the frequency of peritonitis was strongly associated with a linear increase in this risk. This is the first study to demonstrate the impact of peritonitis on late CVmortality of PDpatients, suggesting a link between acute inflammation and CVoutcomes.

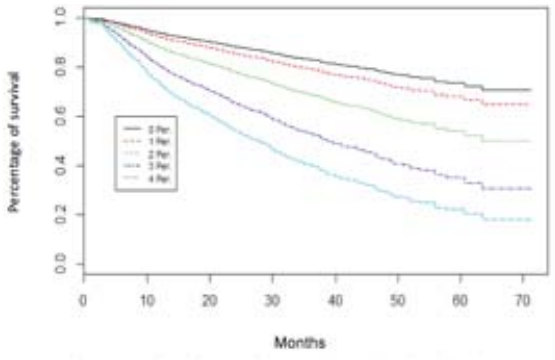


Figure 1 - Unadjusted survival curves por 0, 1, 2, 3 and 4 peritonitis events

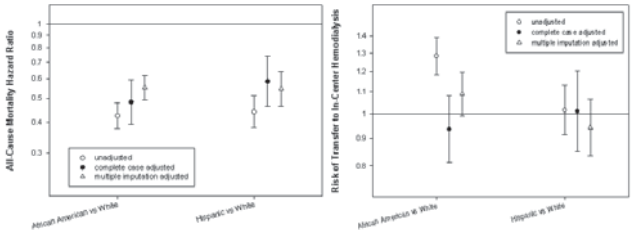
SA-OR106

Association of Race with Risk of Mortality and Transfer to In-Center Hemodialysis in Peritoneal Dialysis Patients Melissa Soohoo,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Sooraj Kuttykrishnan,[2] Miklos Zsolt Molnar,[3] Kamyar Kalantar-Zadeh,[1] Rajnish Mehrotra.[2] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Univ of Washington, Seattle, WA; [3]Univ of Toronto, Toronto, ON.*

Background: Previous studies have reported that African Americans and Hispanics undergoing hemodialysis (HD) have a lower risk for death than Whites. Peritoneal dialysis (PD) is increasingly being used for the treatment of ESRD; however, data on racial differences in survival and time on therapy for patients undergoing PD in the United States have not been well described.

Methods: We examined the probability of treatment with PD among African Americans, Hispanics, and Whites from among the 162,671 patients who started maintenance dialysis in a large dialysis organization from 2007-2011 and the risk for all-cause mortality and transfer to in-center HD for a cohort of 16,896 PD patients (4,187 African-Americans, 2,299 Hispanics, and 10,410 Whites).

Results: African Americans and Hispanics were less likely to use PD (African Americans, OR: 0.63; Hispanics, OR: 0.73) when compared to Whites. Among African Americans, Hispanics, and Whites treated with PD, the mean age (mean + SD) was 51+14, 50+16, and 59+15 years and included 53%, 44% and 40% women; and 63%, 70% and 60% diabetics, respectively. Compared to Whites, African Americans (HR: 0.55, 95% CI: 0.49-0.61) and Hispanics (HR: 0.55, 95% CI: 0.46- 0.64) have a lower risk of mortality. However, there was no significant racial differences in risk for transfer to in-center HD (African Americans, 1.09 (0.99, 1.20); Hispanics, 0.94 (0.84, 1.06)).



Conclusions: Racial-ethnic minorities are less likely to use PD for treatment. However, both African Americans and Hispanics treated with PD have a lower risk for death, compared to Whites. Furthermore, there are no racial differences in risk for transfer to in-center HD. Further studies are needed to examine these racial-ethnic differences.

Funding: NIDDK Support

SA-OR107

Circulating Bacterial-Derived DNA Fragment Level Is a Strong Predictor of Cardiovascular Disease in Peritoneal Dialysis Patients Cheuk-Chun Szeto.[1,2] *[1]Dept of Medicine & Therapeutics, The Chinese Univ of Hong Kong, Shatin, Hong Kong; [2]Dept of Medicine & Therapeutics, Prince of Wales Hospital, Shatin, Hong Kong.*

Background: Circulating bacterial DNA fragment is related to systemic inflammatory state in peritoneal dialysis (PD) patients. We hypothesize that plasma bacterial DNA level predicts cardiovascular events in new PD patients.

Methods: We measured plasma bacterial DNA level in 191 new PD patients, who were then followed for at least a year for the development of cardiovascular event, hospitalization, and patient survival.

Results: The average age was 59.3 ± 11.8 years; plasma bacterial DNA level 34.9 ± 1.5 cycles; average follow up 23.2 ± 9.7 months. At 24 months, the event-free survival was 86.1%, 69.8%, 55.4% and 30.8% for plasma bacterial DNA level quartiles I, II, III and IV, respectively ($p < 0.0001$). After adjusting for confounders, plasma bacterial DNA level, baseline residual renal function and malnutrition-inflammation score were independent predictors of event-free survival. Each fold increase in plasma bacterial DNA level confers a 26.9% (95% confidence interval, 13.0 – 42.5%) excess in risk of developing the composite cardiovascular end point. Plasma bacterial DNA also correlated with the number of hospital admission ($r = -0.379$, $p < 0.0001$) and duration of hospitalization for cardiovascular reasons ($r = -0.386$, $p < 0.0001$). Plasma bacterial DNA level did not correlate with baseline arterial pulse wave velocity (PWV), but with the change in carotid-radial PWV in one year ($r = -0.238$, $p = 0.005$).

Conclusions: Circulating bacterial DNA fragment level is a strong predictor of cardiovascular event, need of hospitalization, as well as the progressive change in arterial stiffness in new PD patients.

Funding: Pharmaceutical Company Support - Baxter Extramural Grant program

SA-OR108

Icodextrin Reduces Insulin Resistance in Incident Non-Diabetic Patients on Peritoneal Dialysis: Results of a Randomized Controlled Trial (STARCH) Thyago Proença de Moraes,[1] Maria Eugenia F. Canziani,[2] Marcia Olandoski,[1] Jose C. Divino-Filho,[4] Roberto Pecoits-Filho.[1] *[1]Pontifícia Univ Católica do Paraná, Brazil; [2]UNIFESP, Brazil; [3]UNESP, Brazil; [4]Karolinska Instt.*

Background: No randomized clinical trial have ever assessed the impact of the substitution of glucose by icodextrin as the osmotic agent in the long dwell on insulin resistance in non-diabetic PD patients.

Methods: This is a phase IV, multicenter, open-label with balanced randomization (1:1) and with two parallel-groups study conducted in Brazil. Inclusion criteria were non-diabetic adult patients on automated PD (APD) for at least 3 months on therapy prior to randomization. Patients assigned to the intervention group were treated with 2 liters of icodextrin 7.5% and the control group with glucose 2.5% during the long dwell and, at night in the cycler, a regular prescription with glucose-based PD solutions. The primary end-point was change in insulin resistance measured by HOMA index at 90 days. Secondary outcomes were changes in fasting glucose, insulin serum levels, glycated hemoglobin and body mass index. The central laboratory was blinded for allocation group.

Results: Sixty patients were randomly assigned to the intervention (n=33) or the control (n=27) group. There was no imbalance in any covariate between groups at baseline. After adjustment for pre-intervention HOMA index levels, the group treated with icodextrin had the lowest post-intervention levels at 90 days in both Intention to Treat analysis 1.49(CI95%1.23-1.74) versus 1.89(CI95%1.62-2.17), F=4.643, p=0.036, partial η^2 =0.078; and in the As Treated analysis 1.47(CI95%1.01-1.84) versus 2.18(CI95%1.81-2.55); F=7.488, p=0.010, partial η^2 =0.195). No significant differences were observed for HbA1c and fasting glucose. Insulin levels were lower in icodextrin group in the ITT analysis (p=0.034) but not in the AT analysis (p=0.060).

Conclusions: The substitution of glucose by icodextrin for the long dwell in APD patients improved insulin resistance measured by HOMA index in non-diabetic patients.

SA-OR109

Longitudinal Effect of Biocompatible Solutions on Peritoneal Solute Transport: Results from the Global Fluid Study Emma H. Elphick,[1] Mark Lambie,[1] Lucy Riley,[1] James A. Chess,[2] Yong-Lim Kim,[3] Marc Dorval,[4] Simon J. Davies.[4] *[1]Keele Univ; [2]Morriston Hospital; [3]Kyungpook National Univ Hospital; [4]Dumont Univ Hospital Centre; [5]Univ Hospital of North Staffordshire.*

Background: Long term peritoneal dialysis (PD) is associated with adverse changes in the peritoneal membrane, such as an increased solute transport demonstrating increased vascularity. Biocompatible solutions (BCS) were developed to mitigate this damage, so we examined their effect on solute transport.

Methods: We analysed the Global Fluid Study, a multinational cohort study. Included adults had 3 or more peritoneal equilibration test measurements more than 2 months from the start of PD and remained on one solution type. Follow up was up to 7.5 years (median of 2.3) in BCS and 12.8 (median 3.2) in standard solutions (SS). A random intercept/slopes linear mixed model assessed the effect of BCS on dialysate to plasma creatinine ratio (D/P Cr) over time for each solution, adjusted for glucose exposure, baseline dialysate IL-6 levels, Icodextrin, residual renal function (RRF) and peritonitis.

Results: Of 366 patients, 71 received BCS (58 Baxter Physioneal(BP), 8 Fresenius Balance(FB) and 5 Gambrosol Trio(GT)). After 2 months of PD the BCS group had a mean

predicted D/P Cr of 0.67 compared to 0.72 for the SS group (p=0.02). The trends over time also differed (p=0.002), with the BCS group D/P Cr increasing initially, peaking at 0.749 by 2.1 years, with no further increase. SS had a continuous increase in D/P Cr. A sensitivity analysis split patients into BP and FB/GT with a difference between groups of borderline significance (p=0.06). Peritonitis correlated with an increase in D/P Cr of 0.019 per episode for SS (p=<0.001), but there was no effect in BCS (p=<0.001). Baseline dialysate IL-6, RRF, glucose exposure and Icodextrin also predicted D/P Cr.

Conclusions: The temporal course of solute transport varies between biocompatible and standard solutions, which may vary between brands. Peritonitis has long term effects on solute transport, but not with biocompatible solutions.

SA-OR110

Maintenance of Volume Status Supported by Bioimpedance Leads to Better Preservation of Cardiac Function Boon Kay Tan,[1] Zanzhe Yu,[3] Simon J. Davies,[1,2] Frauke Wilma Gisela Wenzelburger.[1] *[1]Inst for Science & Technology in Medicine, Keele Univ, Stoke on Trent, North Staffordshire, United Kingdom; [2]Nephrology, Univ Hospital of North Staffordshire, Stoke on Trent, North Staffordshire, United Kingdom; [3]Nephrology, Renji Hospital, Shanghai, China.*

Background: To determine whether optimization of fluid status supported by bioimpedance vector analysis enhances preservation of cardiac function over 12 months.

Methods: Single centre predefined subgroup analysis of non-anuric PD patients from a multicentre prospective randomised controlled trial in which clinicians were blinded to BIA measurements in control subjects. In the active group (Bioimpedance analysis) BIA vector plots were used in combination with clinical assessment (BP, oedema) to manage fluid status with capture of decision making. Full Doppler-2D-echocardiography with colour-coded tissue-Doppler (TDI) was performed at baseline and 12 months by a single blinded operator to determine left ventricular ejection fraction (LVEF). Additionally, early diastolic (E') tissue velocity and early diastolic mitral inflow velocity (E) were measured. E/E' as a surrogate parameter of end-diastolic filling pressures was calculated.

Results: 39 enrolled patients (F/M, 14/25) were non-anuric at baseline and completed the 12 months follow up. The paired t test showed a significant decrease in LVEF in controls over 12 months (56.9±6.1 versus 52.5±6.5, P=0.013), whereas change in the active group was non-significant (54.0±7.4 versus 52.3±5.2, P=0.33). E/E' worsened in control group (8.6±2.3 versus 10.8±2.8 P<0.01) and no change in active group (11.0±3.2 versus 11.5±2.6 P=0.416). On multivariate analysis LVEF and E/E' at 12 months were independently predicted by their baseline values and study randomisation group after controlling for comorbidity status, age, gender and baseline BIA. Urine volume was better maintained in the active group but no between group differences in residual renal clearance or glucose exposure. Decision to reduce target weight was more likely to be made in active group than in control group.

Conclusions: The incorporation of BIA in setting target weight leads to better preservation of cardiac function.

Funding: Pharmaceutical Company Support - Baxter

SA-OR111

Anti-Fibrotic Effect of Valproic Acid in Experimental Peritoneal Fibrosis in Rats Elerson Costalonga, Deise S.J. Pimentel, Filipe M. Silva, Irene L. Noronha. *Renal Div, Univ of São Paulo, São Paulo, Brazil.*

Background: Progressive increase in the thickness of peritoneal membrane (PM) and peritoneal sclerosis are complications of long-term peritoneal dialysis. The aim of this study was to analyze the effect of valproic acid (VPA), a histone deacetylase inhibitor with anti-fibrotic properties, in an experimental model of peritoneal fibrosis induced by chlorhexidine gluconate (CG) in rats.

Methods: Peritoneal fibrosis (PF) was induced by daily intra peritoneal injections of 0.1% CG for 15 consecutive days. Male Wistar rats (250-300 g) were divided into 3 groups (n=6 per group): **CONTROL,** control rats receiving only vehicle; **PF**, peritoneal fibrosis induced in rats; **PF+VPA**, rats with PF treated with VPA (300 mg/Kg/day by gavage). At day 15, the animals were sacrificed. The thickness of PM was measured by Masson thrichrome staining. The number of macrophages (MØ) and the α-smooth muscle actin (α-SMA) expression in the PM was analyzed by immunohistochemistry. mRNA expression of TGF-β, fibronectin, fibroblast specific protein-1 (FSP-1), Smad 3, and cytokines was analyzed by qPCR.

Results: Treatment with VPA significantly reduced the thickness of the peritoneal membrane and α-SMA staining area. Reduction of TNF-α, IL-1β, TGF-β, fibronectin, fibroblast specifif protein-1 (FSP-1), and Smad 3 mRNA levels were also observed.

	CON	PF	PF+VPA
Thickness of peritoneal membrane (mμ)	35±9	120±61*	31±15#
MØ (cells/mm^2)	133±90	826±375*	443±222*
α-SMA (%)	0.04±0.01	4.2±0.4*	0.8±0.1#
TNF-α (qPCR)	1.0±0.4	2.6±0.4*	0.9±0.3#
IL-1β (qPCR)	1.0±0.2	3.8±0.6*	2.1±0.2#
TGF-β (qPCR)	1.0±0.2	2.2±0.1*	1.1±0.2#
FSP-1 (qPCR)	1.0±0.2	3.1±0.2*	1.0±0.2#
Fibronectin (qPCR)	1.0±0.2	2.2±0.2*	1.3±0.5#
Smad 3 (qPCR)	1.0±0.2	2.3±0.5*	1.0±0.2#

*p<0.05 vs. CONTROL, #p<0.05 vs. PF

Conclusions: VPA was effective in reducing the thickness of peritoneal membrane in the experimental model of peritoneal fibrosis induced by CG developed in rats, possible due to its ani-inflamatory and anti-fibrotic effects.

SA-OR112

Inter-State Variability in Home Dialysis Utilization among Minorities in the U.S. Eric L. Wallace,[1] Janice P. Lea,[2] Eric Hammelman,[3] Joshua F. Cohen,[3] James A. Sloand.[4] *[1]Univ of Alabama at Birmingham; [2]Emory Univ; [3]Alavere; [4]Baxter Healthcare Corp.*

Background: The impact of race on dialysis modality use in the U.S. has received limited attention. Studies looking at national data in aggregate may be misleading if large inter-state variability exists. This investigation sought to examine use of home dialysis by U.S. minority populations as a whole and by individual state for Medicare beneficiaries.

Methods: 2012 Medicare 100% Outpatient Standard Analytic File was used to identify all dialysis patients using bill type "72" (outpatient dialysis). PD and HHD patients were identified using revenue and condition codes. The PD or HHD group was defined by having at least one claim during the year that met criteria for the category. Beneficiaries were counted once regardless of the number of different modalities they used that year. Medicare 100% Denominator Standard Analytic File was used to identify race and state of residence. A ratio of the percentage of patients on PD or HHD by race divided by the percentage of total patients on PD or HHD was used to determine the likelihood of being on a modality with respect to average.

Results: Nationally, 9.4% of Medicare dialysis patients received PD and 2.9% received HHD. Use of home dialysis varied across ESRD patients of different races for both PD and HHD as follows: White, 11.4% and 3.5%; Asian, 10.7% and 2.2%; Hispanic, 8.3% and 1.9%; and African American (AA) 6.6% and 2.3%; respectively. AA and Hispanic Medicare beneficiaries with ESRD were approximately 29% and 12%, and 19% and 33% less likely than average to receive PD and HHD, respectively. Significant inter-state variability existed for both total home dialysis use and across racial profile. In 95% of states where AA data was available, use of PD by prevalent AA with ESRD was less than the state's average (Exceptions: Arizona 1.04;Massachusetts 1.05). Similar findings were seen for Hispanics and for HHD use.

Conclusions: AA and Hispanics are underrepresented in the home dialysis population. Inter-state variability exists, but overall, use of PD and HHD by prevalent AA and Hispanics with ESRD was less than state averages. Reasons underlying these disparities deserve further investigation.

Funding: Pharmaceutical Company Support - Baxter Healthcare Corp

SA-OR113

Time from Onset of Symptoms and Administration of Initial Antibiotic Therapy Predicts Peritonitis Outcomes and Hospitalizations Kalindu Muthucumarana,[1] Prue E. Howson,[1] Ramyasuda Swaminathan,[1] Douglas Patrick Crawford,[1] Samantha Chua,[2] Sally A. Burrows,[3] Ashley B. Irish.[1,3] *[1]Dept of Nephrology and Transplantation, Royal Perth Hospital, Perth, WA, Australia; [2]Dept of Nephrology, Sir Charles Gairdner Hospital, Perth, WA, Australia; [3]School of Medicine and Pharmacology, Univ of Western Australia, Perth, WA, Australia.*

Background: Peritoneal Dialysis (PD) peritonitis is a major cause of morbidity and mortality in PD patients. The relationship between treatment delay and outcome is not clearly defined, yet time to treatment is a critical determinant of the outcome of most infectious disorders. This study aims to define this relationship in PD peritonitis.

Methods: Multi-centreprospective study of PD peritonitis from August 2012-January 2014 in Western Australia. Patients were identified at presentation to hospital or community health facility and data pertaining to each PD peritonitis episode was analysed. SC (Symptom-to-Contact time), CT (Contact-to-Treatment time) and ST (Symptom-to-Treatment time) were recorded.

Results: 107 patients (58% Male, 13% Indigenous, 50% Diabetic, mean age 62) had 139 PD peritonitis episodes. 56% were admitted to hospital and the median SC was 5 hours, CT 2.3 hours, and ST 9 hours. 31 catheter removals and 9 deaths occurred during the study period (composite outcome PD failure). Predictors of admission were CT > 4 hours, Female sex and non-Diabetics. SC did not predict PD failure however CT > 4 hours was associated with an increased risk of PD failure by 4% for each hour over 4 hours (HR 1.04 95% CI 1-1.08 P=0.049) which persisted after adjustment for confounders (p=0.054). A ST >12 hours was also predictive of PD failure (HR 2.4 1.08-5.3 p=0.032). No other measured risk factor was found to predict PD failure in this population.

Conclusions: Each hour over 4 hours from initial antibiotic treatment after presentation, or 12 hours after symptom onset, increases the risk of PD failure or death following an episode of PD peritonitis. Delay in contact to treatment time increases hospitalizations. Targeting earlier reporting of symptoms and hospital presentation, and developing an expedited antibiotic strategy may improve outcomes from PD peritonitis.

SA-OR114

Klotho Overexpression Attenuates Peritoneal Fibrosis via Suppression of Wnt/β-Catenin Signaling Pathway in Mice Hiroyuki Kadoya, Minoru Satoh, Seiji Itano, Atsushi Uchida, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Development of peritoneal fibrosis caused by long-term exposure to bioincompatible dialysate is a major limiting factor for continuation of peritoneal dialysis (PD). However, the mechanisms underlying this process have not been fully elucidated. Activation of the wnt/β-catenin signaling pathway has been demonstrated to play an important role in the development of organ fibrosis. Moreover, klotho protein has been indicated to regulate wnt/β-catenin signaling. We investigated whether klotho protein could

reduce peritoneal fibrosis via inhibition of wnt/β-catenin signaling pathway.

Methods: Reporter mice for Wnt/ β-catenin signaling (BAT-LacZ), transgenic mice in which a-klotho is driven by human elongation factor 1 alpha promoter (KLTG), and wild type mice (WT) were used. All the strains are on C57BL/6 background. Experimental group of mice received daily intraperitoneal (i.p) injections of 4.25% glucose with lactate (PD solution) for 4 weeks. Whereas, control mice (Cont) received daily i.p injections of the same volume of saline.

Results: Enhanced Wnt/b-catenin signaling pathway was demonstrated in peritoneal mesothelium by increased β-galactosidase expression in BAT-LacZ mice after 4weeks exposure to PD solution. Concomitant expression of α-SMA, a marker of epithelial mesenchymal transition (EMT), was observed in mesothelium. Experimental group of mice also exhibited peritoneal fibrosis together with increased expressions of TGF-β and Fibronectin. On the other hand, KLTG treated with PD solution showed attenuation of of wnt/β-catenin signaling and amelioration of progression of peritoneal fibrosis.

Conclusions: Overexpression of klotho exerts protective action on peritoneal mesothelium exposed to PD solution through attenuation of wnt/β-catenin signaling.

TH-PO001

Anemia Influences Recovery from Acute Kidney Injury in Hospitalized Patients Melanie Godin,[1] Yu-Ting Christi Kao,[1] Etienne Macedo,[2] Ravindra L. Mehta.[1] *[1]UCSD, San Diego; [2]USP, San Paolo, Brazil.*

Background: Anemia (hemoglobin < 10g/L for cardiac surgery) is increasingly recognized as a modifiable risk factor for the development of AKI and correction of anemia has been associated with improved outcomes. We hypothesized that anemia is a risk factor for recovery from AKI in hospitalized patients.

Methods: 1946 patients hospitalized in the wards at an academic medical center over 24 months were included. Patients <18 years old, ICU, hospital stay <48h or >35 days, with ESRD were excluded. AKI definition was based on AKIN criteria sCr increase > 0.3 mg/dL within 48 hours. Renal recovery was classified as complete (last-ref sCr ≤0.3 g/dL or last/ref sCr ratio ≤1.2), no recovery (death or dialysis dependence at hospital discharge) or partial (meeting neither of the previous criteria). Hemoglobin (Hb) values at hospital admission, discharge (DC) and average (Av) Hb were evaluated as predictors of AKI recovery at hospital discharge.

Results: 348 (18%) patients developed AKI during hospital stay. When compared with non-AKI, the AKI group had lower Hb levels throughout hospitalization (baseline 11.1 versus 11.4; p=0.015, Av Hb 10.2 versus 10,8; p<0.001, discharge 10.1 versus 10.7; p<0.001) for AKI versus Non-AKI respectively. Worsening of anemia was more significant in AKI versus non-AKI patients from admission to DC (decline of 1.05 g/dL versus 0.69g/dL; p=0.001). Of the 348 AKI patients, 69% recovered completely, 23% had a partial recovery and 8% had no recovery. Increasing severity of anemia at baseline was associated with lower likelihood of renal recovery.

Table 1: Logistic regression of baseline Hb quintiles as predictors of renal recovery

	Baseline Hb range (g/dL)	OR of complete or partial recovery (p-value)
Q1	≤9.24	reference
Q2	9.25-10.50	2.74 (0.07)
Q3	10.51-11.60	5.02 (0.02)
Q4	11.61-12.80	4.87 (0.02)
Q5	≥12.81	5.63 (0.01)

Conclusions: AKI patients have lower Hb levels than non-AKI patients. The severity of anemia influences recovery from AKI. Further studies are needed to ascertain the cause of anemia and evaluate its effects on development of CKD post AKI.

Funding: NIDDK Support

TH-PO002

Anemia Is a Risk Factor for Development of Acute Kidney Injury in Hospitalized Patients Melanie Godin,[1] Yu-Ting Christi Kao,[1] Etienne Macedo,[2] Ravindra L. Mehta.[1] *[1]UCSD, San Diego, CA; [2]USP, San Paolo, Brazil.*

Background: Anemia (Hb< 10g/dL) is a recognized risk factor for the development of AKI in cardiac surgery. There is a lack of information on the prevalence of anemia in acute kidney injury (AKI) in other settings. We hypothesized that anemia is common and predicts AKI in hospitalized patients.

Methods: 1946 patients hospitalized at an academic medical center over 24 months who had sequential Hb and creatinine values at admission, during hospitalization and at discharge were included. Patients <18 years old, ICU, hospital stay <48h or >35 days, with ESRD or dialysis were excluded. Comorbidities and CKD status were recorded from discharge ICD-9 codes. AKI definition was based on AKIN criteria sCr increase > 0.3 mg/dL within 48 hours. Hemoglobin (Hb) values at hospital admission, discharge (DC) and average (Av) Hb were evaluated as predictors of AKI during hospital stay.

Results: At admission 26% of pts had Hb levels <10g/dL while 40.3% met this criteria during hospitalization. 348 (18%) patients developed AKI and had significantly lower levels of Hb with more patients with Av Hb <10g/dL than the non-AKI group (51.1% versus 20.7%; p<0.001). Worsening of anemia was more severe in AKI than non-AKI patients (Hb decline of 1.05 versus 0.69 g/dL between admission and DC; p=0.001). Multivariable logistic regression adjusting for co-morbidities (age, gender, BMI, diabetes, CKD and heart failure), identified Hb levels <10g/dL as an independent predictor of AKI (OR 1.82; p<0.001).

Table 1: Multivariable Logistic Regression Model for development of AKI

	OR (p-value)
Av Hb <10g/dL	1.84*
Age	1.01**
Male	1.72*
BMI	1.03**
CKD	2.19*
Diabetes	1.73*
Heart failure	3.01*
*p<0.001 ** p<0.02	

Hb levels did not correlate with the severity of AKI. Although patients with pre-existing CKD had lower levels of Hb at all time points during hospitalization, they did not experience a worsening of anemia.

Conclusions: Anemia is a risk factor for AKI in hospitalized patients. Correction of anemia could potentially modify this risk. Further studies are needed to define the cause of anemia and its relationship to AKI.

Funding: NIDDK Support

TH-PO003

Patterns of Serum Creatinine Predict Renal Recovery After Acute Kidney Injury in Critically Ill Patients Melanie Godin,[1] Etienne Macedo,[2] Yu-Ting Christi Kao,[1] Josee Bouchard,[3] Ravindra L. Mehta.[1] *[1]Neph, UCSD, San Diego, CA; [2]Neph, USP, San Paolo, Brazil; [3]Neph, HSC, Montreal, QC, Canada.*

Background: The severity and duration of AKI are important factors determining outcomes from AKI. Currently, it is difficult to determine if recovery is occurring to target interventions. We hypothesized that the patterns of sCr change would provide a method to predict the magnitude and time to renal recovery.

Methods: We studied 685 ICU patients screened for a prospective study from Jun 06 to Dec 08. AKI was defined by the AKIN Scr criteria (≥ .3mg/dL change within 48 hours). We calculated the ratio of ICU discharge/reference sCr to evaluate renal recovery with a ratio of ≤1.2 reflecting complete recovery. Duration of AKI, sCr Upslopes (US; from ref to time point) and downslopes (DS;peak to time point) at 12, 24 and 48h as well as peak, magnitude of increase or decrease were evaluated to predict renal recovery and AKI duration.

Results: 164 (23.9%) patients met AKIN criteria and 37 were treated with RRT and were not included in the ratio of sCr and DS analysis. Complete recovery was met in 36%. sCr US and DS at 48h, peak, magnitude of increase and decrease were individually correlated with the extent of renal recovery and with AKI duration.

Liner regression predictor	Ratio of Renal Recovery (DC/reference sCr)		AKI duration (h)	
	B (p-value)	r	B (p-value)	r
Peak sCr	.66(<.001)	.39	18.7181 (.002)	.34
Max–ref sCr (magnitude increase)	.01(<.001)	.64	35.7043 (<.001)	.37
US48h	1.15(.001)	.42	82.1456 (.029)	.28
Peak–last ICU sCr (Magnitude decrease)	-.58(<.001)	.28	-6.8564 (.608)	.06
DS48h	-.53(.039)	.42	-25.9102 (.084)	.10
*US=upslope; DS=downslope;				

The ratio of recovery and duration of AKI were moderately correlated (R=.459, p<0.001). RRT dependency at ICU discharge was predicted by sCr peak (OR 2.65 p=.04), magnitude increase (OR 3.13 p<.001) and US at 24 (OR 4.03 p=.002) and 48h (OR 21.39 =.003).

Conclusions: Patterns of sCr change and AKi duration are predictive of renal recovery at ICu discharge. Future prospective studies should evaluate these parameters to predict recovery and identify time points for intervention.

Funding: NIDDK Support

TH-PO004

Risk Factors for Recurrent AKI Edward D. Siew,[1] Sharidan Parr,[1] Khaled Abdel-Kader,[1] Nisha Bansal,[3] Svetlana Eden,[1] James Fly,[2] Adriana Hung,[1] Theodore Speroff,[1,2] T. Alp Ikizler,[1] Michael Edwin Matheny.[1,2] *[1]Vanderbilt Univ Medical Center; [2]GRECC, Tennessee Valley Healthcare System (TVHS); [3]Kidney Research Inst, Univ of Washington, WA.*

Background: Recurrent AKI compounds the risk for progressive kidney disease. We examined risk factors for hospitalized recurrent AKI in U.S. Veterans.

Methods: We identified AKI survivors hospitalized within a regional VA healthcare network from 2002-2010. We excluded patients with ESRD; patients whose hospitalization involved nephrectomy, glomerulonephritis, or urinary tract obstruction; or dialysis dependence at discharge. We defined AKI as a 0.3 mg/dl or 50% increase from baseline to peak serum creatinine (SCr). Baseline SCr was defined as the most recent outpatient value 7-365 days prior to AKI (index and recurrent). Risk factors examined included demographics, comorbidities, and inpatient diagnoses and procedures. Fine and Gray Proportional Hazards regression was used to examine time to first recurrent AKI with death as a competing risk.

Results: Among 17,323 patients, 4,377(25.3%) experienced hospitalized recurrent AKI within a year, and 2,524(15%) died before experiencing recurrent AKI. Median time to recurrent AKI was 56(IQR:16-157) days. Risk factors for recurrent AKI included age, low serum albumin(SAlb), congestive heart failure(CHF), diabetes(DM), peripheral vascular disease(PVD), and advanced liver disease(ALD). Inpatient factors associated with recurrent AKI included cancer, chemotherapy, CHF, and ALD.

Risk Factor	**Adjusted Hazard Ratio aHR (95%CI)**
Age(50-80 yrs)	1.2(1.1-1.4)
SAlb(4-3 g/dl)	1.3(1.2-1.5)
Comorbidities	
CHF	1.3(1.2-1.4)
ALD	1.3(1.1-1.6)
DM	1.2(1.1-1.2)
PVD	1.2(1.1-1.2)
Inpt Dx/Proc.	
CHF	1.3(1.2-1.4)
ALD	1.6(1.3-1.9)
Cancer	1.1(1.0-1.2)
Chemotherapy	2.0(1.5-2.6)
Cardiac surgery	0.7(0.5-0.9)
Abd surgery	0.8(0.6-0.9)
Mech Ventilation	0.9(0.8-1.0)

* aHR >1 indicates a higher risk for recurrent AKI

Conclusions: Elderly patients with CHF, ALD, or receiving chemotherapy are at high risk for recurrent AKI and may benefit from risk reduction strategies. Future studies should examine how care delivery influences this risk.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO005

Identification of Risk Factors of Community-Acquired Acute Kidney Injury in Outpatients Takeshi Matsubara, Tatsuo Tsukamoto, Motoko Yanagita. *Dept of Nephrology, Graduate School of Medicine, Kyoto, Japan.*

Background: Compared with hospital-acquired AKI, little is known about community-acquired AKI (CA-AKI). The definition of AKI is a small increase in Cre from baseline within 2-7 days, and extending this duration could cause an increase of false positives such as the patients with Cre fluctuation or chronic kidney disease (CKD). Therefore, the aim of study is to clarify the prevalence, risk factors, and outcome of CA-AKI in outpatients.

Methods: Firstly, we enrolled the patients whose Cre is increased by 50% or +0.3mg/dl in electric health record from baseline Cre, defined as the most recent Cre during preceding 12 months between Sep 2011 and Mar 2012 in our hospital. Next, the enrolled patients were divided into "False positive" and "CA-AKI" group. False positive group was consisted of two subgroups; one is a group of patients with Cre fluctuation, who did not fulfill the criteria when baseline Cre was redefined as the mean value of Cre obtained one year before the enrolled date, and the other is a group of patients with CKD, whose slope of the reciprocal Cre curves was constant during 6 months before and after the enrolled date. The rest of above patients were all classified into CA-AKI group. Finally, the prevalence rates of comorbidities and the outcome (AKI stage 2 and 3, or death within one year) determined from chart review were compared between CA-AKI and False positive group by multivariate analysis.

Results: Among 85172 outpatients in the analysis, 1298 patients (1.5%) were enrolled, and classified into False positive (457 patients: 0.51%), and CA-AKI (841 patients: 0.99%) group. CA-AKI group had higher prevalence of active malignancy (OR 2.98: CI 1.91-4.76), cardiovascular disease (OR 1.88: 1.36-2.62), age>65 (OR 1.71: 1.23-2.36), NSAIDs (OR 3.74: 2.22-6.63), Calcineurin Inhibitor (OR 3.08: 1.39-7.37), and ACEi/ARB (OR 1.68: 1.23-2.30) compared with False positive group. The proportion of AKI stage 2 and 3, or death within a year was higher in CA-AKI group (OR 6.81 p<0.001).

Conclusions: CA-AKI could occur in approximately 1% of outpatients, and showed poor outcome. Several comorbidities and nephrotoxic agents listed above could be candidates of the risk factors for CA-AKI.

Funding: Government Support - Non-U.S.

TH-PO006

Incidence of and Risk Factors for Acute Kidney Injury After Major Surgery M. Grams,[1] Yingying Sang,[1] Shoshana Ballew,[1] Zoltan Szabo,[4] Kunihiro Matsushita,[1] Kamyar Kalantar-Zadeh,[3] Josef Coresh,[1] Csaba P. Kovesdy.[2] *[1]Johns Hopkins Univ; [2]Memphis VA; [3]UC Irvine; [4]Linkoping Univ.*

Background: Acute kidney injury (AKI) is a frequent complication after major surgery; yet surgery-associated risk of AKI has not been well characterized, particularly for non-cardiovascular surgery. We sought to determine rates, predictors, and consequences of AKI after major surgery in a nationally representative cohort.

Methods: The study population included 3.5 million veterans with eGFR >60 ml/min/1.73 m^2 in 2004-6. The patient's first major surgical hospitalization occurring before 2011 was selected for analysis. Inpatient, post-surgical AKI was classified according to KDIGO criteria, with baseline kidney function defined as mean outpatient creatinine in the year prior to surgery.

Results: There were 167,262 veterans with eligible hospitalizations, most commonly for general (GI/abdominal procedures, 28%), orthopedic (20%), vascular (16%), and cardiac (14%) surgeries. Overall, 12% of hospitalizations were complicated by AKI (Stage 1: 75%, Stage 2: 14%, Stage 3 (No RRT): 7%, AKI-RRT: 4%). Transplant (63%), cardiac (19%), and general surgeries (13%) had the highest rates of AKI. Overall, older age, black race, lower baseline eGFR, and the presence of hypertension, diabetes, congestive heart failure, and liver disease were associated with higher risk of AKI. Diuretic and ACE-inhibition/ARB use were also associated with higher risk of AKI, whereas statin use was associated with lower risk of AKI. Higher AKI stage during surgical hospitalization was associated with longer length of stay and higher risk of end-stage renal disease and mortality.

	No AKI	AKI			
		KDIGO Stage 1	*KDIGO Stage 2*	*KDIGO Stage 3 (no RRT)*	*AKI-RRT*
N, %	147,369 (88.1%)	14,893 (8.9%)	2,837 (1.7%)	1,348 (0.8%)	815 (0.5%)
Length of stay, days	8.8	14	19	22	48
In-hospital mortality, %	1.0	4.8	15	25	31
1-year mortality, %	8.8	16	29	40	51
1-year ESRD, %	0.05	0.47	0.67	1.3	9.7

Conclusions: AKI is common after many types of surgery, in particular transplant, cardiac, and general surgeries, and it is associated with higher risk of adverse outcomes. Evaluation of preventative and treatment strategies for AKI is needed.

Funding: NIDDK Support

TH-PO007

Effect of Pre-Operative Proteinuria on Post-Operative Outcomes after Coronary Artery Bypass Grafting Lekha K. George,[1] Miklos Zsolt Molnar,[1] Santhosh K. Koshy,[2] Jun Ling Lu,[1] Kamyar Kalantar-Zadeh,[3] Csaba P. Kovesdy.[1,4] *[1]Univ of Tennessee; [2]Regional One Medical Center; [3]Univ of California Irvine; [4]Memphis VAMC.*

Background: Proteinuria is associated with reduced coronary flow reserve and increased microvascular resistance. We hypothesized that pre-operative albuminuria carries a poor prognosis after coronary artery bypass surgery (CABG).

Methods: Among 17,812 patients who underwent CABG during 2006-2012 in any VA hospital, we identified 5,968 with available preoperative urine microalbumin-creatinine ratio (UACR) measurements. We examined the association of UACR <30, 30-299 and >=300 mg/g with 30-day mortality and hospitalization length >10 days using logistic regression, and with acute kidney injury (AKI) (defined by K-DIGO criteria) using ordinal logistic regression. Adjustments were made for age, gender, race, baseline BP, pre- and perioperative ACEI and statin use, baseline eGFR and cholesterol, and comorbidities.

Results: Mean±SD baseline age and eGFR were 65±8 years and 77±19 ml/min/1.73m^2, respectively. 99% of patients were men, 10% were black and 88% were diabetic. 102 patients (1.71%) died within 30 days of CABG, and 26.8% patients developed AKI. Median length of hospitalization was 9 days (IQR: 6-14). Higher UACR did not confer a significant increase in 30-day mortality, but was associated with significantly longer hospitalizations and higher incidence of all stages of AKI (Table).

	UACR <30 mg/g (N=3,852)	UACR 30-299 mg/g (N=1,671)	UACR ≥300 mg/g (N=445)
30-Day Mortality (OR, 95%CI)	1.0	1.14 (0.73-1.79)	1.50 (0.75-2.97)
Length of Hospitalization >10 days (OR, 95%CI)	1.0	1.22 (1.07-1.39)*	1.73 (1.38-2.18)*
AKI (estimated incidence, %±SD)			
Stage 1	21.1±6.2	26.0±7.0***	33.0±8.0**
Stage 2	2.5±1.1	3.3±1.4***	5.0±2.3**
Stage 3	0.7±0.3	0.9±0.4***	1.4±0.7**

*p<0.001, **p<0.01, ***p<0.05, compared to UACR <30 mg/g group

Conclusions: Higher UACR is associated with significantly longer post-CABG hospitalization and AKI incidence. The effects of antiproteinuric treatments on post-CABG outcomes will need to be examined in randomized trials.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO008

Risk Factors for Progression of Acute Kidney Injury to Chronic Kidney Disease in Critically Ill Patients Jung Nam An,[1] Dong Ki Kim,[2] Hajeong Lee,[2] Yun Kyu Oh,[1] Yon Su Kim,[2] Chun Soo Lim,[1] Jung Pyo Lee.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Seoul National Univ Hospital, Seoul, Republic of Korea.*

Background: Acute kidney injury (AKI) is an independent risk factor for progression to advanced stage chronic kidney disease (CKD). Survivors after AKI episode have also increased over time. We tried to analyze the effects of AKI episode during hospitalization on the risk for CKD.

Methods: Among 950 adult (age > 20 years old) patients who started continuous renal replacement therapy (CRRT) in intensive care units at Seoul National University Hospital and Boramae medical center from 2010 to 2013, a total of 173 surviving AKI patients at three months after initiation of CRRT were enrolled in this study. Primary outcome is composite of RRT and increased serum creatinine (sCr) level more than 50% over 3 months.

Results: The frequency of primary outcome was 33.5%. In the patients with primary outcome, sCr levels at baseline and after 3 months were 2.07 ± 1.82 mg/dL and 3.43 ± 2.76 mg/dL (p < 0.001), respectively. Compared with the others with recovered renal function, underlying diabetic nephropathy was more frequent (22.4% versus 5.2%, p = 0.001), sCr level at 72 hours after initiation of CRRT was higher (3.10 ± 1.29 mg/dL versus 1.59 ± 0.74 mg/dL, p = 0.031), and far more patients failed in RRT weaning (63.8% versus 2.6%, p < 0.001). In logistic regression analysis, old age, diabetic nephropathy, post-operative AKI, increased sCr level at 24 and 72 hours after initiation of CRRT, and RRT weaning failure increased the risk for prolonged kidney injury. After adjusting other risk factors, total duration of RRT (Odds ratio [OR] 1.25; 95% confidence interval [CI] 1.42; p < 0.001) and decreased heart rate at 72 hours after initiation of CRRT (OR 0.95; 95% CI 0.92-0.99; p = 0.014) were significantly associated with progression to CKD.

Conclusions: AKI episodes requiring CRRT are independent risk factors for CKD progression. The severity of AKI episodes is closely related to clinical outcomes, and strong predictor of advanced CKD.

TH-PO009

A Novel Risk Score of Contrast-Induced Nephropathy After Percutaneous Coronary Intervention Wei Qin, Ling Ji, Lichuan Yang. *Div of Nephrology, West china Hospital of sichuan Univ, Chengdu, Sichuan, China.*

Background: Contrast-induced nephropathy (CIN) is a major cause of acute kidney injure. Although several studies had been done to identify risk factors and score system of CIN, yet their application was limited. We aim to establish a novel risk score system could be easily used in clinical environment.

Methods: 806 PCI patients, randomly divide into analysis cohort (70%) and validation cohort (30%), were enrolled retrospectively. Risk factors of CIN were identified using univariate and multivariate logistic regression in analysis cohort. Risk score system was developed based on regression coefficients of them. Sensitivity and specificity of the new risk score system was validated and compared with previous models.

Results: Incidence of CIN was 12% in total cohort. High CIN incidence (50%) was observed in CKD patients. 62 potential risk related variables were included in univariate and multivariate analysis. Age, BMI, myoglobin, cardiac function, hypoproteinemia, CKD, IABP (intra-aortic ballon pump) and PVD (peripheral vascular disease) were identified as independent risk factors. A novel risk score system was established.

Risk factors	Weighted score in model
Age>75 years	1
BMI>26 kg/m^2	1
Myoglobin (ng/mL)	
<1000	1
1000-2000	2
>2000	3
Hypoproteinemia (<35g/L)	1
Cardiac Function	
Killip I	0
Killip II	1
Killip III	2
Killip IV	3
History of CKD	3
IABP	1
PVD	2
Contrast Volume>200mL	2

The new risk score system was validated in validation cohort. Highest sensitivity and specificity (c-statistic: 0.917, 95%CI 0.877–0.957) was observed when comparing with previous models.

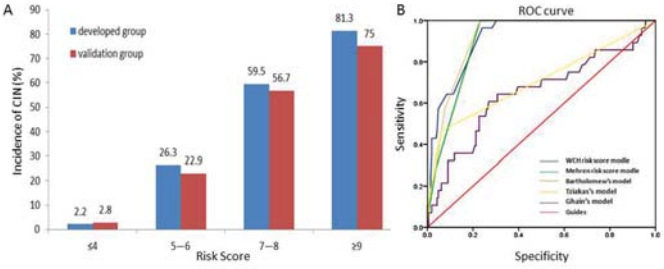


Figure 1: (A) Risk stratification of CIN according to the present risk score system. (B) Comparing of prediction accuracy among different CIN risk score models.

Conclusions: A novel CIN risk score model was developed based on a retrospective study of 805 patients undergoing PCI procedure. This model is the best score model in comparison with previous models. Application of this model might be helpful to predict CIN in patients undergoing PCI.

TH-PO010

Prospective Observational Study of Acute Kidney Injury after Cardiac Surgery: Enrichment Strategy - on Behalf of the NephroNet Investigators James A. Tumlin, Ravindra L. Mehta, Jorge Cerda, Kevin W. Finkel, Claude Mabry Galphin, Jesse M. Goldman, Sudarshan Hebbar, Nelson P. Kopyt, Michelle W. Krause, Andreas Orfanos, Amber S. Podoll, Diane Potvin, Jon R. Von Visger, John Jason White, Lakhmir S. Chawla. *Nephronet Investigators.*

Background: To design and appropriately power clinical trials it is important to have an accurate estimation of the event rate. Typically, the rates of post-surgical AKI have been derived from national registries or epidemiologic studies. To better select the patients at increased risk of developing AKI, we conducted an observational study to determine the AKI rates in an "enriched" population. Well-defined risk factors were selected as pre-specified inclusion/exclusion criteria. The occurrence of cardiac surgery associated-AKI was determined and other clinical outcomes were collected for these patients for 90 days.

Methods: The entry criteria were patients scheduled for on-pump cardiac surgery and two or more of the following risk factors: Class III/IV CKD, IDDM, NIDDM + proteinuria, COPD, EF < 40%, anemia, or contrast exposure within 7days postoperatively (without evidence of AKI). Primary endpoint was proportion of patients with AKI. Secondary endpoints included major adverse kidney events for 90 days including the need for dialysis; or renal dysfunction as compared to baseline eGFR.

Results: 100 patients were enrolled. 97 patients were included in the Per Protocol (PP) analysis. In PP, the mean (SD) age was 65.7+ 10.0 years, 73% were male, and 86% were white. Of the 97 patients, 56 (57.7%) developed AKI within 7 days of the operation (Stage I: 71%, Stage II: 21%, Stage III: 7%). Among the patients with AKI, 34 (61%) had sustained (> 48 hours) or severe AKI (Stage II or III) within the 90 day period. 29 patients out of 34 with sustained AKI had pre-existing CKD.

Conclusions: In this prospective observation study, we demonstrate that our inclusion/exclusion study design can enrich the AKI event rate to > 50%. Pre-existing CKD appears to be a critical risk factor for sustained or severe AKI.

Funding: Pharmaceutical Company Support - Thrasos Therapeutics, Inc.

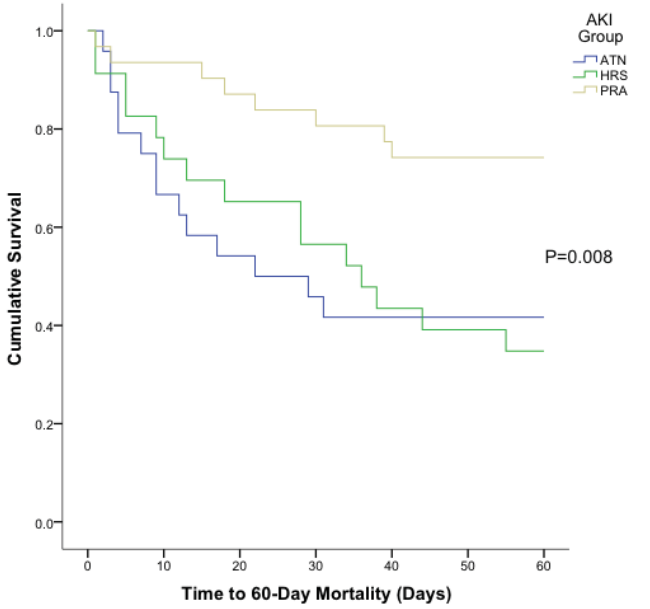
TH-PO011

Acute Kidney Injury and Mortality in Cirrhosis – Is MELD Score Enough? Guillermo Ortiz-Sanjuan,[1] Andrew S. Allegretti,[1] Julia Beth Wenger,[1] Joseph James DeFerio,[1] Sahir Kalim,[1] Hector Tamez,[2] S. Ananth Karumanchi,[2] Ravi I. Thadhani.[1] *[1]Massachusetts General Hospital; [2]Beth Israel Deaconess Medical Center.*

Background: Acute kidney injury (AKI) increases the risk for mortality in cirrhosis. It remains uncertain whether the type of AKI increases mortality risk beyond the Model of End-Stage Liver Disease (MELD) score.

Methods: 75 hospitalized patients with cirrhosis and AKI were prospectively followed. MELD score and markers of liver and kidney function were collected on enrollment. AKI was classified as hepatorenal syndrome (HRS), acute tubular necrosis (ATN), prerenal azotemia (PRA), or other by review of records and independent physician adjudication. HRS was diagnosed using the 2006 Ascites Club Criteria. Kaplan Meier curves and log-rank tests were used to assess differences in 60-day survival by AKI group and multivariable Cox Proportional-Hazards were used to adjust for MELD score.

Results: 19 (25%) patients were diagnosed with ATN, 23 (31%) with HRS, 27 (36%) with PRA, and 6 (8%) with other etiologies. MELD scores were lower for PRA, but similar for ATN and HRS[Median (Q1,Q3): ATN 28 (21,34), HRS 28 (24,36), PRA 20 (17,26); overall p=0.002; ATN versus HRS p=0.48]; Creatinine was lower in PRA, but similar between ATN and HRS [ATN 2.1 (1.8,3.5)mg/dL, HRS 2.7 (2.1,3.0), PRA 1.3 (1.2,2.0); overall p<0.001; ATN versus HRS p=0.25]; total bilirubin (TB) and INR were similar across groups [INR: ATN 3.9 (2.5,10.4)mg/dL, HRS 5.1 (2.1,12.3), PRA 4.1 (1.5,9.1)mg/dL; overall p=0.51] [TB: ATN 1.8 (1.5,2.5), 1.8 (1.6,2.0), PRA 1.7 (1.4,2.1); overall p=0.50]. 60-day mortality was 58% for ATN, 65% for HRS, and 26% for PRA (p=0.008); after adjusting for MELD score, survival differences by AKI group were no longer significant



Conclusions: In our cohort, patients with ATN and HRS were at the highest risk for 60-day mortality. Nonetheless, the type of AKI did not add predictive value above and beyond MELD score.

Funding: Clinical Revenue Support

TH-PO012

Postoperative Serum Chloride Levels and Acute Kidney Injury in Liver Transplant Recipients Ho Geol Ryu, Hannah Lee, Se-Hee Min. *Anesthesiology, Seoul National Univ Hospital, Seoul, Korea.*

Background: Acute kidney injury (AKI) is a common complication after liver transplantation with a reported incidence between 17 and 95%. Risk factors of AKI after liver transplantation include intraoperative blood loss and hypotension, postoperative sepsis, and calcineurin inhibitors. Chloride plays an important role in acid base balance and is regulated by the kidneys. Previous reports have suggested that metabolic acidosis caused by perioperative hyperchloremia may cause intrarenal vasoconstriction leading to decreased GFR and urine output. However, the association between AKI and perioperative chloride level is lacking in liver transplantation patients and tudy was performed to see if there was a relationship.

Methods: Patients who received liver transplantation between July 2010 and December 2012 at Seoul National University Hospital were included in the study. Patients were grouped according to their average serum chloride level for 72 hours after surgery into 3 groups (average chloride level <101, 101-110, >110 mEq/L) and the difference in the risk of AKI was analyzed.AKI was defined according to the RIFLE criteria. Risk factors for developing AKI after liver transplantation were also analyzed.

Results: A total of 363 patients had received liver transplantation during the study period and 86 (23.7%) had developed AKI. There was no difference in the incidence of AKI between the 3 groups that were divided according to the average serum chloride level (p=0.85). Preoperative serum chloride levels were also not associated with the development of postoperative AKI. Multivariate regression analysis identified high MELD scores (1.037 [1.006, 10.68], p=0.0174), deceased donor (2.746 [1.515, 4.987], p=0.0009), and preoperative renal replacement therapy (21.918 [4.459, 107.738], p=0.0001) as independent risk factors of AKI.

Conclusions: Perioperative serum chloride levels were not associated with the development of AKI after liver transplantation. Risk factors associated with postoperative AKI after liver transplantation include high MELD scores, deceased donor, and preoperative renal replacement.

TH-PO013

High and Low Level of Serum Uric Acid Is a Novel Risk Factors for Acute Kidney Injury: A Retrospective Database Analysis By Using the Integrated Medical Information System at Kochi Medical School Hospital Taro Horino,[1] Kazunori Otomo,[2] Takeo Miki,[2] Hiromi Kataoka,[3] Yutaka Hatakeyama,[3] Tatsuki Matsumoto,[1] Kazu Hamada,[1] Yoshiko Shimamura,[1] Koji Ogata,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Yoshio Terada,[1] Yoshiyasu Okuhara.[3] *[1]Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi Univ, Nankoku, Japan; [2]Center for Innovative and Translational Medicine, Kochi Medical School, Kochi Univ, Nankoku, Japan; [3]Center of Medical information Science, Kochi Medical School, Kochi Univ, Nankoku, Japan.*

Background: Serum uric acid (SUA) levels before cardiovascular surgery had been reported to be independent risk factors for postoperative acute kidney injury (AKI). These studies however had some limitations. Here, we investigated the association between SUA levels and AKI by performing a retrospective database analysis using 30 years of data.

Methods: A total of 78,626 hospitalized patients aged > 18 years were enrolled. AKI was diagnosed according to AKIN criteria. Multivariate logistic regression analyses were performed to investigate the independent association between SUA levels and AKI. Kidney function and comorbidities, medication, procedures performed prior to AKI onset were taken into account as possible confounding risk factors.

Results: Adjusted odds ratios were higher in both high and low SUA strata in all sexes and age classifications. In high SUA strata, odds ratios were higher in women aged ≤50 years than in men of both age groups and women aged >50 years, and this observation was particularly remarkable in the strata corresponding to SUA levels > 7 mg/dL (OR 10.0; 95% CI, 6.43-15.58; P < 0.001). In low SUA strata, odds ratios were higher in men aged ≤50 years than in women of both age groups and men aged >50 years, and this observation was remarkable in the strata corresponding to SUA levels < 2 mg/dL (OR 9.89; 95% CI, 5.46-17.91; P < 0.001).

Conclusions: We suggest that SUA could be an independent risk factor for AKI development in hospitalized patients. Additionally, we found that patients aged ≤50 years have a greater predilection to develop AKI than those aged >50 years and the correlation between SUA levels and AKI risk are different in gender dependent manners.

TH-PO014

Risk of Acute Kidney Injury According to Each Valve Involved During Cardiac Surgery Jacqueline Villase?or, Armando Vazquez-Rangel. *Nephrology, Inst Nacional de Cardiologia Ignacio Chavez, Mexico, Mexico, DF, Mexico.*

Background: Valve surgery is known to confer a higher risk for acute kidney injury (AKI) or renal support therapy (RST), but there is limited information dissecting the relation among the involved valves and their renal prognosis in cardiac surgery. **Aim**: To compare renal prognosis according to each valve involved or their combination during cardiac surgery.

Methods: Retrospective cohort study including patients who underwent coronary artery bypass grafting surgery (CABG) as control group or open heart valve procedures from March 1st 2009 to February 28th 2011. We excluded patients with pre-existing chronic kidney disease stage 5 or preoperative AKI. AKI was defined by serum creatinine according to KDIGO criteria.

Results: A total of 1155 patients were analyzed. AKI was present in 375 patients (32.5%) from which 72 (6.2%) required RST. There were 299 (25.9%) patients for CABG, 41 (3.5%) only tricuspid, 158 (13.7%) only mitral, 353 (30.6%) only aortic valve, 200 (17.3%) involved 2 valves, 41 (3.5%) for 3 valves, 51 (4.4%) for CABG+1 valve, and 12 (1%) for CABG+2 or 3 valves. The percentages of AKI and RST respectively were: 29.1 and 5.7% for CABG, 24.4 and 9.8% for tricuspid, 31.0 and 4.4% for mitral, 31.7 and 4.0% for aortic, 34.5 and 8.5% for 2 valves, 39.0 and 12.2% for 3 valves, 49.0 and 13.7% for CABG+1 valve, and 58.3 and 8.3% for CABG+2 or 3 valves. In multivariable analysis adjusting for pre-, trans-, and post-operative variables, only aortic valve (OR 1.81 [1.05-3.13], p=0.033) and CABG+valve (OR 2.27 [1.18-4.38], p=0.014) showed an increased risk for AKI; while for RST only tricuspid valve showed a trend (OR 3.76 [0.93-15.15], p=0.063).

Conclusions: Renal prognosis differs according to each valve involved during cardiac surgery. While aortic valve shows a higher incidence of AKI with lower requirement for RST, tricuspid valve shows less AKI with higher requirement for RST. When more than 1 valve is involved there is a higher risk for both outcomes, but they could be at least partially due to some other pre-, trans- or post-operative factors, while the single valve observations tended to be independent from them.

TH-PO015

Combining Risk Factors Improves Acute Kidney Injury Prediction in Intensive Care Unit Rakesh Malhotra,[1,5] Etienne Macedo,[2] Josee Bouchard,[3] Kianoush Banaei-Kashani,[4] Ravindra L. Mehta.[5] *[1]VUMC; [2]U Sao Paulo; [3]U Montreal; [4]Mayo Clinic; [5]UCSD.*

Background: Several individual risk factors have been reported to be associated with Acute Kidney Injury (AKI). A previous study by Chawla et al has proposed combining individual risk factors to improve predictive value for AKI. We evaluated the performance of a modified AKI risk sampling tool in a multicenter prospective cohort study of critically ill patients.

Methods: Our study included two independent cohorts: 717 patients admitted to a surgical intensive care unit (SICU) and medical intensive care unit (MICU) at UCSD from June 2006 to December 2008 and 1280 patients admitted to SICU and MICU at Mayo Clinic, Rochester from Jan 2010 to December 2010. Risk factors were classified as chronic major comorbidities (e.g., advanced age, diabetes mellitus, chronic kidney disease, or cardiovascular disease), chronic minor (e.g., hypertension, morbid obesity, cancer, chronic lung disease) and acute factors (e.g., hypotension, sepsis, high-risk surgery, mechanical ventilation or nephrotoxin exposure). Within 48 hours of ICU admission we stratified patients as no risk, baseline risk, acute risk, and enhanced risk based on their risk factor profile. The incidence of AKI, need for renal replacement therapy (RRT) and mortality were assessed among risk groups. AKI was defined by an absolute increase of 0.3mg/dL from reference sCR within 48 hours.

Results: The incidence of AKI in UCSD cohort was 22.9% (n=164) and 5.6% of patients required dialysis (n=40). In MCR cohort, 40% (n=511) developed AKI and 1.5% (8) required dialysis. Patients classified as enhanced risk in both UCSD and Mayo cohorts had a higher incidence of AKI (31.1% versus 9.1%; p < 0.001 and 56% versus 5%; p < 0.001), higher mortality (10.1% versus 0.9%; p < 0.001 and 4% versus 1%; p < 0.001) and need for RRT (8.2% versus .0%; p< 0.001 and 1.5% versus .0%) in comparison to baseline risk group.

Conclusions: The use of simple risk classification system based on chronic comorbidities and acute events can identify patients at high risk to develop AKI and adverse outcomes. Future studies should incorporate these factors to stratify patients for prevention and early therapeutic intervention.

TH-PO016

Validating a Scoring Tool to Predict Acute Kidney Injury (AKI) following Cardiac Surgery Brian L. Wong,[1] Jennifer St.Onge,[2] Bhanu Prasad Tikkisetty.[3] *[1]College of Medicine, Univ of Saskatchewan, Regina, SK, Canada; [2]Research and Performance Support, Regina Qu Apelle Health Region, Regina, SK, Canada; [3]Nephrology, Regina Qu Appelle Health Region, Regina, SK, Canada.*

Background: Acute kidney injury (AKI) after cardiac surgery is associated with increased mortality. Pre-operative risk scores may simplify identification of AKI patients to improve patient management. Current validated scoring tools are used to predict AKI requiring dialysis (AKI-D); less is known about whether these tools can predict less severe forms of AKI. The purpose of this study was to evaluate the Cleveland Clinic scoring tool in predicting both AKI-D and less severe AKI in patients in the Regina Qu'Appelle Health Region (RQHR).

Methods: Data on risk factors for AKI were collected from a retrospective chart review of 2343 cardiac surgery patients between 2007 and 2011 in the RQHR. The primary outcome was AKI as defined by KDIGO staging: Stage 1 (increase in serum creatinine 1.5-1.9 times baseline within 7 days), Stage 2 (increase in creatinine 2.0-2.9 times baseline) and Stage 3 (increase in creatinine 3.0 times baseline or more OR initiation of dialysis during hospital stay). We assessed the performance of a modified version of the Cleveland Clinic tool using all available risk factor data in predicting KDIGO Stage for AKI using receiver operating curve analyses (ROC).

Results: The incidence of AKI was 6.1% in Stage 1, 2.6% Stage 2, and 5.8% in Stage 3. Using ROC analyses, the area under the curve (AUC) for the Cleveland score was 0.61 (95% CI: .56 to .65; p<.001) for Stage 1, 0.61 (95% CI: .54 to .68; p<.01) for Stage 2 and 0.77 (95% CI: .74 to .82; p<.001) for Stage 3. Greater level of risk on the Cleveland tool was more associated Stage 3 AKI.

Conclusions: We found the Cleveland Clinic tool to be valid in identifying patients with different stages of AKI in the RQHR, particularly those that require dialysis, which could help improve patient management following cardiac surgery. Future prospective studies would further validate the use of this tool for predicting earlier stages of AKI in addition to AKI-D.

TH-PO017

Culture Positive Infection Is a Risk Factor for Acute Kidney Injury in Intensive Care Unit Patients Jamie S. Hirsch,[1,2] Andrew H. Chiang,[1] Sumit Mohan,[2] Jonathan M. Barasch,[2] Noemie Elhadad.[1] *[1]Biomedical Informatics, Columbia Univ, New York, NY; [2]Nephrology, Columbia Univ, New York, NY.*

Background: Sepsis and acute kidney injury (AKI) are frequent complications in critically ill patients admitted to the intensive care unit. While hemodynamic instability associated with septicemia can cause AKI, the impact of infections alone on the development

of AKI is unclear. We hypothesized that a culture positive infection increases the risk of developing AKI in the subsequent 48 hours even in the absence of hemodynamic instability.

Methods: Data was obtained from the Multiparameter Intelligent Monitoring in Intensive Care II database, which presents anonymized ICU patient records for 32,535 patients. All adult patients with length of stay >48 hours were included, and AKI was defined by AKI Network stage I criteria (rise in serum creatinine of ≥0.3 mg/dL). Patients with AKI on admission or preexisting end-stage renal disease were excluded. Chi-square and logistic regression analyses were conducted for AKI risk 48 hours subsequent to a culture positive infection at any site, with adjustment made for hypotension and requirement of vasopressor medications.

Results: We identified 957 unique patients with a total 1494 instances of positive cultures and 222 episodes of AKI. Among patients with positive cultures, those who developed AKI were not significantly older (65.4±21.9 versus 63.3±19.3 years, p=0.2), but were less likely to be female (48.0% versus 51.8%, p<0.001) or White (66.2% versus 71.0%, p<0.001), and had a higher number of comorbidities (2.9±1.8 versus 2.6±1.7, p=0.008). Patients with culture positive infection appear to have an increased risk of developing AKI within 48 hours (OR 1.24, p=0.005). The risk of AKI was not attenuated by adjustment for the need for vasopressor support (OR 1.28, p=0.001) or for the cardiovascular component of the SOFA score (OR 1.25, p=0.003).

Conclusions: Culture positive infection, even in the absence of hemodynamic instability, appears to be temporally associated with the development of AKI within the subsequent 48 hours. Further study is required to understand the mechanism underlying this relationship.

Funding: Other NIH Support - NLM T15 LM007079-22

TH-PO018

Uncontrolled Hyperglycemia Is Associated with Greater Risk of Acute Kidney Injury in Critically Ill Patients

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Background: Hyperglycemia is frequently associated with adverse outcomes, particularly among critically ill patients in the intensive care unit (ICU). Its influence on the subsequent development of acute kidney injury (AKI) remains unclear. We hypothesized that uncontrolled hyperglycemia during the first 24 hours of ICU admission increases the risk of developing AKI.

Methods: Data was obtained from the Multiparameter Intelligent Monitoring in Intensive Care II database, which presents anonymized ICU patient records for 32,535 patients. All adult patients with length of stay >48 hours were included, and AKI was defined by AKI Network stage I criteria (rise in serum creatinine of ≥0.3 mg/dL). Patients with AKI on admission or preexisting end-stage renal disease were excluded. Chi-square and multivariate logistic regression analyses were used to estimate the risk of AKI associated with persistent hyperglycemia (mean 24 hour glucose >160 mg/dL) on admission.

Results: We identified 5239 unique patients with 5910 ICU admissions. There were 1301 admissions with mean glucose >160 mg/dL during the first 24 hours. Patients presenting with hyperglycemia were of similar gender (female 47.1% versus 46.2%, p=0.81), but were older (66.1±18.2 versus63.7±21.7 years, p<0.001), had a higher number of comorbidities (2.5±1.7 versus 2.3±1.7, p<0.001), and higher admission simplified acute physiology score (SAPS; 14.6±5.3 versus 13.3±5.1, p<0.001) and SOFA score (6.0±3.9 versus 5.3±3.7, p <0.001). Patients with persistent hyperglycemia at the time of ICU admission were at increased risk of AKI (OR 1.41, p<0.001). This risk persisted even after adjustment for gender, age, history of peripheral vascular disease, diabetes mellitus, and chronic kidney disease, admission SOFA score, need for vasopressor support, and presence of culture-positive infection (OR 1.23, p=0.003).

Conclusions: Persistent hyperglycemia at ICU admission appears to be associated with the development of AKI. Controlled trials are required to examine if improved glucose control would ameliorate the risk of AKI.

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TH-PO019

Acute Kidney Injury Risk Prediction in Critically Ill Patients Using Fluctuating Covariates

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Background: AKI is a common complication in the ICU, and dramatically increases patient morbidity and mortality. We aimed to improve AKI risk prediction in the general critical care population by utilizing multiple covariates that vary over time.

Methods: Data was obtained from the Multiparameter Intelligent Monitoring in Intensive Care II database, which contains records for 32,535 ICU patients. Adult patients with length of stay >48 hours and without AKI or end-stage renal disease at admission were included. AKI was defined by AKI Network stage I criteria (rise in serum creatinine of ≥0.3 mg/dL). AKI risk over a subsequent 48 hour period was estimated sequentially for patients during their ICU stay. Demographics, laboratory data, medications, infection status, and illness severity were included as covariates in a multivariate logistic regression model. Forward selection using Bayesian information criterion was performed; evaluation was conducted with area under the receiver operating characteristic curve (AUC).

Results: We identified 4058 unique patients, 4464 ICU admissions, and 2787 episodes of AKI. Patients who developed AKI were of similar gender (male 56.1% versus 54.4%, p=0.27), but were older (66.6±19.7 versus61.7±21.5 years, p<0.001), had a higher number of comorbidities (2.5±1.8 versus 2.0±1.5, p<0.001), and higher admission SOFA (6.2±3.9 versus 4.9±3.4, p <0.001). 15 variables predicted the development of AKI within 48 hours, with AUC 0.73, comparing favorably against using SOFA score alone (AUC 0.65). The final feature set included age; laboratory data (hemoglobin variance, white blood cell deviance, sodium minimum, creatinine mean and maximum, blood urea nitrogen minimum, and mean anion gap, partial activated thromboplastin time, and lactate); 24 hour volume balance; medications (furosemide and vancomycin); and overall and neurologic subpart of SOFA.

Conclusions: A model incorporating covariates that vary over time provides acceptable performance in predicting temporally proximate AKI risk in ICU patients. A 48-hour fixed-window risk assessment may be valuable in the ICU, where patient status is dynamic.

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TH-PO020

Cardiac Surgery-Associated Acute Kidney Injury in Neonates and Infants: Comparison of Creatinine and Urine Output Criteria

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Background: Cardiac surgery-associated acute kidney injury (AKI) in infants and neonates is common and is associated with poor outcomes. Most studies to date do not report AKI incidence with urine output (U/O) criteria. We compared AKI incidence and AKI-outcome associations using Acute Kidney Injury Network (AKIN) delta SCr (ΔSCr) and U/O to examine the effect of including U/O.

Methods: In a single centre prospective cohort study, infants and neonates requiring cardiac surgery from Mar 2013-Jan 2014 were eligible. We collected post-operative hourly U/O and ΔSCr up to 3d. Kappa statistic was used to evaluate agreement between U/O and ΔSCr staging. We defined 3 groups: No AKI, ΔSCr alone, U/O stage higher than ΔSCr stage (U/O>ΔSCr). We compared intensive care length of stay (LOS) and ventilation days (Kruskall-Wallis test) between groups.

Results: 96 infants and 34 neonates were enrolled. Mean age at surgery was 149d (infants) and 7d (neonates); 97% had post-op diuretics; dialysis incidence was 1.5%; mortality was 3.8%. AKI incidence was 39% (infants), 26% (neonates) with ΔSCr criteria alone and increased to 48% for infants and neonates when including U/O criteria. Agreement between ΔSCr and U/O stage was poor in infants (kappa 0.234) and neonates (kappa 0.121). The median (range) ventilation days in neonates with no AKI was 1.5(0-12) *versus* ΔSCr alone 7.7(6-10) *versus* U/O>ΔSCr 7.6(0-31) (p=0.003). Median LOS for infants with no AKI was 2.6(1-30) *versus* ΔSCr alone 7.7(1-78) *versus* U/O>ΔSCr 7.5(1-13) (p=0.03).

Conclusions: The incidence of cardiac surgery-associated AKI in infants and neonates rises significantly by including AKIN UO criteria. The outcomes for those diagnosed with the added U/O criteria and ΔSCr criteria are both poor compared to those without AKI, suggesting that U/O criteria may have clinical implications for AKI in this young population.

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TH-PO021

Community-Acquired Acute Kidney Injury: Incidence and Etiologic Factors

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Background: Acute kidney injury (AKI) affects 5-10% of hospitalized patients and is often a complication of medical or surgical interventions. However, limited information exists on the epidemiology and causes of community-acquired AKI. The aim of this study was to examine the incidence, etiology and outcome in patients presenting with AKI to an emergency department (ED) of an urban university hospital.

Methods: In this retrospective study we used the electronic patient information system at Landspitali – The National University Hospital of Iceland, the only major hospital in the Reykjavik area, to identify all patients aged ≥18 years, who upon arrival to the ED in the year 2010 had an elevated serum creatinine (SCr) level. We used the KDIGO criteria to determine the presence of AKI by reviewing all SCr measurements of these patients available at the University Hospital. Clinical information on patients with AKI was extracted from their medical records.

Results: A total of 74,822 patients ≥18 years came to the ED, of whom 2878 had an elevated SCr. Of those, 1110 patients (1.5%) had AKI. Their median age was 78 years (range, 21-102), 55.1% were men and 29.3% had a baseline eGFR <60 ml/min/1.73 m^2. To date we have examined clinical data for 605 patients (56.2% men), of whom 360 (59.5%) had stage 1 AKI, 170 (28.1%) stage 2 and 75 (12.4%) stage 3. The AKI was of prerenal nature in 90.7% of patients, with various and often multiple underlying contributing factors, including sepsis (22.1%), volume depletion (19.8%) and exacerbation of chronic heart, lung or liver disease (16.8%). In 61.2% of these patients medications were considered a contributing factor, most frequently renin-angiotensin system blockers (52.2%) and NSAIDs (8.9 %).

Conclusions: These preliminary results show that 1.5% of patients presenting to the ED of a metropolitan hospital have AKI which is similar to previous studies. Most patients with community-acquired AKI are elderly and prerenal conditions account for the overwhelming majority of cases. Commonly used medications appear to be a dominant contributing factor.

Funding: Government Support - Non-U.S.

TH-PO022

Elevated Serum Uric Acid Can Increase the Risk of Acute Kidney Injury After Cardiac Surgery in Elderly Patients Jiaqi Xu, Yuanhan Chen, Xinling Liang, Penghua Hu, Lu Cai, Wei Dong, Zhilian Li, Wei Shi. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: To investigate the effect of pre-operative serum uric acid on acute kidney injury after cardiac surgery in elderly patients.

Methods: Clinical data was collected from elderly patients(age ≥60 year)undergoing cardiac surgery with cardiopulmonary bypass in Guangdong General Hospital between January 2005 and May 2011. The baseline serum creatinine was defined as the latest before surgery, and the primary outcome was AKI according to RIFLE criteria (Risk, Injury, Failure, Loss of renal function and End-stage renal disease). Multivariate analysis by logistic regression was used to obtain the independent risk factors for AKI.

Results: Among 936 elderly patients, 576 cases (61.5%) developed AKI. Mean uric acid concentration is higher in AKI group than Non-AKI group(436.55±119.13 VS 397.98±107.21 umol/l, P<0.001). The sex-specific cutoff points for serum uric acid tertiles were: tertile 1: uric acid ≤384.65umol/l in men and ≤354umol/l in women; tertile 2: 384.66-476.99umol/l in men and 354.01-437.96umol/l in women; tertile 3:≥477umol/l in men and ≥437.97u mol/l in women. People were divided into three groups according to the cutoff points above. The incidence of AKI is highest in tertile 3(tertile 1:56.1%; tertile 2:56.3%; tertile 3:72.2%; P<0.001). The data were adjusted for age, gender, co-morbidities(hypertension, DM, cerebrovascular disease, COPD), previous cardiac surgery, eGFR<60ml/min/1.73m^2, NYHA≥Grade 3, urine protein dipstick results of ≥±,combination of CABG and valvular surgery, CPB time, aortic cross-clamping time, exposuring to ACEI/ARB and lipid-lowering drugs pre-operative, early postoperative variables (use of ACEI/ARB, diuretics and digoxin, central venous pressure during the first 24 h after surgery but before AKI). Compared with the lowest tertile, the highest had an OR for incident AKI of 1.897(95%CI 1.270-2.833) after fully adjustment.

Conclusions: Pre-operative elevated uric acid can increase the risk of AKI after cardiac surgery in elderly patients. Attention should be paid for uric acid in order to early prevention of cardiac surgery-associated AKI.

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TH-PO023

Proteinuria: An Independent Risk Factor for Development of Cardiorenal Syndrome Type I and In-Hospital Mortality Lu Cai, Zhilian Li, Xinling Liang, Ruizhao Li, Lixia Xu, Wei Shi. *Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, Guangdong, China.*

Background: Proteinuria is an established risk factor for acute kidney injury (AKI), but the role of proteinuria for cardiorenal syndrome type(CRS) I is still unclear.The aim of the study is to analyse the association between proteinuria and CRS type I and in-hospital mortality.

Methods: We studied all consecutive patients hospitalized with AHF from July 2005 to July 2012 in Guangdong General Hospital and the First Affiliated Hospital of Sun Yat-sen University.Demographic and clinical data were collected retrospectively.Urinary protein in the first 48 hours after admission were recorde and AKI was defined by KDIGO criteria. Proteinuria was defined as mild (trace to 1+) or heavy (2+ to 4+) according to the results of the dipstick test. Urinary protein in the first 24 hours after admission were recorded. Baseline serum creatinine was defined as the first serum creatinine on admission. Logistic regression analysis was used to determine whether proteinuria is an independent risk factor for the occurrence of CRS type I and in-hospital mortality or not.In-hospital survival of patients with CRS type I along with proteinuria was assesed by Kaplan-Meier curves.

Results: 1058 patients were enrolled.The incidence of CRS type I with proteinuria was significantly higher than non-proteinuria group (67.9% versus 34.3%, P <0.001). Logistic regression analysis showed that proteinuria was an independent risk factor for the development of CRS type I and in-hospital mortality (OR 3.335, 95% CI 2.516 ~ 4.420; OR 1.785, 95% CI 1.164 ~ 2.737).Mild and heavy proteinuria exhibited a stepwise increased ratio for both development of CRS type I (Mild proteinuria: OR 2.801, 95%CI 2.076 ~3.778;Heavy proteinuria: OR 5.607, 95%CI 3.583 ~8.775) and in-hospital mortality (Mild proteinuria: OR1.735, 95%CI1.107~2.718;Heavy proteinuria: OR2.021, 95%CI1.169~-3.496).K-M curve indicated that in-hospital survival of CRS type I patients with proteinuria were lower than CRS type I patients without proteinuria (Long rank p <0.001).

Conclusions: Proteinuria is an independent risk factor for the development of CRS type I and in-hospital mortality.

Funding: Government Support - Non-U.S.

TH-PO024

The Incidence and Risk Factors of Acute Kidney Injury in the Very Elderly Patients with Mechanical Ventilation Qingli Cheng, Qinglin Li, Yang Liu, Guang Yang. *Dept of Geriatric Nephrology, Chinese PLA General Hospital, Beijing, China.*

Background: To investigate the incidence, pathogenetic and risk factors of acute kidney injury (AKI) in the very elderly patients with mechanical ventilation therapy.

Methods: A total of 260 elderly patients with mechanical ventilation therapy in the geriatric department of our hospital from June 2008 to December 2012 were enrolled in this study. The clinical data of all the patients were analyzed to explore the incidence and risk factors of AKI in the very elderly patients after mechanical ventilation therapy.

Results: The average age of the patients was (88.9±5) years. 125 cases (48.1%) suffered from AKI which emerged at a mean time of (1.6±0.7) days after mechanical ventilation. The incidence of the history with chronic kidney disease (66.4% versus 51.1%, *P*=0.012) and the history with diabetes (51.2% versus 38.5%, *P*=0.040) in the patients with AKI (AKI group) were higher than that in the patients without AKI (Non-AKI group). Compared with the Non-AKI group, lower level of PaO_2 (59.7±12.8 versus 63.6±14.1, *P*=0.031), lower level of oxygenation index (PaO_2/FiO_2, 122.5±42 versus 145.7±46, *P*=0.000), higher level of hemoglobin (105±23 versus 100±17, *P*=0.046) and hyperglycemic (10.2±4.1 versus 8.8±3.7, *P*=0.004) were found in AKI group. The incidence of AKI increased in the patients with mechanical ventilation if they were used higher level of positive end-expiratory pressure (PEEP≥4cmH_2O). Logistic regression analysis showed that the history of chronic kidney disease (OR=1.964), hyperglycemia (OR=1.076), the lower level of PaO_2/FiO_2 (OR=2.142) and using higher level of PEEP (≥4cmH_2O, OR=0.990) were the risk factors of the prognosis of AKI in those patients with mechanical ventilation therapy (*P*<0.05).

Conclusions: The incidence of AKI in the very elderly patients with mechanical ventilation was 48.1% in this study. The mean time of suffered from AKI in those patients was about 1~3 days after the therapy of mechanical ventilation. The history of chronic kidney disease, hyperglycemia, low level of PaO_2/FiO_2 and using higher level of PEEP (≥4cmH_2O) were the risk factors of AKI in the very elderly patients with mechanical ventilation therapy.

Funding: Government Support - Non-U.S.

TH-PO025

Acute Kidney Injury After Heart Transplant in Children; Risk Factors and Outcomes Christine L. Macdonald,[2] Gwen Alton,[2] Simon Urschel,[1] Ari Joffe,[1] Gwen Rempel,[2] Colleen Norris,[2] Catherine Morgan.[2] *[1]Pediatrics, Univ of Alberta, Edmonton, AB, Canada; [2]Nursing, Univ of Alberta, Edmonton, AB, Canada.*

Background: Heart transplant is life-saving for children with end-stage congenital heart disease or acquired heart failure. Critical illness after transplant can include acute kidney injury (AKI). There is little data on the epidemiology of, risk factors for, or impact on outcomes of AKI after pediatric heart transplant.

Methods: Using secondary analysis of data from an ongoing prospective cohort study, we evaluated 72 children (0- 5 yrs) who had a heart transplant between 2001 and 2012. We evaluated: 1) postoperative AKI rate (defined by pRIFLE); 2) pre-, intra-, and early postoperative AKI risk factors (days on waitlist, inotrope use and ventilation pre-transplant, ECMO / ventricular assist device at transplant, preoperative estimated glomerular filtration rate (eGFR), ABO incompatibility, donor ischemic time, peak intraoperative lactate, tacrolimus level early postoperatively) using stepwise logistic regression; 3) effect of AKI on short-term outcomes (duration of ventilation and length of PICU stay).

Results: AKI occurred in 73% of children. Independent predictors of AKI were pre-transplant ventilation (OR 8.6, p=0.007) and higher eGFR (p=0.032). Preoperative inotrope reduced the risk of AKI (OR 0.13, p=0.016). Sixteen percent of children had a tacrolimus level >15 on day 3 post-transplant and these children had more AKI than children without (OR 7.8, p=0.086). Although not statistically significant, automated model selection retained tacrolimus level >15 as a predictor (using multiple different modeling strategies). AKI resulted in longer ventilation days and ICU stay (p=0.038 and p=0.003, respectively).

Conclusions: AKI was common after heart transplant and was associated with important outcomes. As in other pediatric cardiac surgery populations, lower preoperative GFR was protective against postoperative AKI; the role of modified immune suppressive strategies in this context needs to be further evaluated. Although not statistically significant, elevated early postoperative tacrolimus is likely biologically important in the prediction of AKI risk and needs further evaluation in a larger cohort.

TH-PO026

Postoperative Ileus Is an Important Risk Factor of Acute Kidney Injury in Patients Undergoing Colorectal Surgery Jun Yong Lee, Hyojeong Chang, Sun Chul Kim, Myung-Gyu Kim, Won-Yong Cho. *Korea Univ, Republic of Korea.*

Background: Recently, emerging evidence suggest a presence of gut-kidney crosstalk. Despite major advance in surgical techniques from open to robot assisted surgery, acute kidney injury (AKI) is still major postoperative complication. The purpose of this study is to compare the incidence of postoperative AKI according to different surgical techniques and also the risk factors, outcome of AKI in patients undergoing colorectal surgery.

Methods: This is a single center, retrospective study. A total of 199 patients who received colectomy due to colorectal cancer from 2010 to 2012 were enrolled and their clinical data were reviewed.

Results: The mean age was 66±12 years and male was 66.3%. Preoperative blood urea nitrogen and creatinine were 14.92±5.2 and 0.96±0.26 mg/dL. Stage I, II, III and IV cancer were found in 31.7%, 25.6%, 25.6% and 11.6% respectively. Open surgery was performed in 12% and laparoscopic assisted surgery or robot assisted surgery were performed in 55.7% or 32% of patients. AKI developed in 12 patients (6.0%), and 3 (25%) of them received acute hemodialysis. Postoperative ileus developed in 58 patients (29%). Incidence of AKI was not different according to surgical techniques and the presence of diabetes, hypertension, chronic kidney disease (CKD), intraoperative shock, postoperative ileus, postoperative infection were associated with the development of AKI. Interestingly, postoperative ileus was found to be the only independent risk factor of AKI in multivariate analysis (odds ratio : 14.73, p=0.004). In addition, AKI patients showed significantly longer hospital stay and higher mortality than non AKI patients.

Conclusions: Despite advances in surgical techniques, paralytic ileus is a common manifestation after colorectal surgery and it showed strong association with the development of AKI. These results suggest that enhanced bacterial translocation or increased

intraabdominal pressure possibly resulting from postoperative ileus might be partially responsible for the development of AKI following colectomy. More careful attention should be paid on the development of posoperative ileus or AKI in patients who undergo colectomy regardless of surgical techniques.

TH-PO027

Risk of Acute Kidney Injury in the General Population Arnar Jan Jonsson,[1] Bjarni Gunnarsson,[2] Hrefna Gudmundsdottir,[1,3] Margret B. Andresdottir,[3] Thor Aspelund,[1,2] Vilmundur Gudnason,[1,2] Runolfur Palsson,[1,3] Olafur S. Indridason.[3] *[1]Faculty of Medicine, Univ of Iceland, Reykjavik; [2]Icelandic Heart Association, Kopavogur; [3]Div of Nephrology, Landspitali - The National Univ Hospital of Iceland, Reykjavik, Iceland.*

Background: Acute kidney injury (AKI) is common in hospitalized patients and is associated with adverse outcomes. However, limited data exist on the epidemiology of AKI in the general population. The aim of this study was to determine the risk of AKI and associated risk factors in the general population.

Methods: We studied subjects from the Reykjavik Study, a population-based cohort study on cardiovascular disease in Iceland. The participants were recruited from 1967 to 1991 and baseline data included a measurement of serum creatinine (SCr) and assessment of cardiovascular disease (CVD) risk factors. For the 16,504 participants, we obtained all available SCr measurements at medical laboratories in the Reykjavik area from study entry until March 2012. AKI was defined according to the KDIGO criteria. Logistic regression was used to examine the relationship between AKI and baseline variables.

Results: We identified at least one follow-up SCr for 14,513 subjects, of whom 47.1% were men, 28.7% had hypertension, 2.9% had diabetes and 5.9% had eGFR <60 mL/min/1.73 m^2 at baseline. A total of 3,065 subjects (21.1%, 95%CI 20.4-21.8%), 51.1% men, developed AKI during a follow-up of 29.6 (range, 0.4-45.5) years. The frequency of a first AKI episode for different age groups is shown in the Table. Increasing age (OR 1.012, 95%CI 1.007-1.018), sex (OR 0.80, 95%CI 0.73-0.86 for women) and eGFR <60 mL/min/1.73 m^2 at baseline (OR 1.24, 95%CI 1.05-1.46), were associated with AKI. Classical CVD risk factors were not associated with future AKI.

Age group (years)	Percent men (95% CI)	Percent women (95% CI)
60-70	4.7 (4.1-5.3)	3.3 (2.8-3.8)
70-80	14.8 (13.8-15.8)	9.9 (9.2-10.7)
>80	18.5 (17.1-19.8)	15.8 (14.7-16.9)

Conclusions: Estimated lifetime risk of AKI in the general population appears quite high. Our study did not identify modifiable risk factors for AKI. Nevertheless, since many AKI episodes are known to be preventable, raised awareness among physicians may improve patient outcomes.

Funding: Government Support - Non-U.S.

TH-PO028

Urine Output Thresholds for Cardiac Surgery-Associated Acute Kidney Injury Joana Balderas Juarez, Armando Vazquez-Rangel. *Nephrology, Inst Nacional de Cardiologia Ignacio Chavez, Mexico, DF, Mexico.*

Background: In cardiac surgery there is a high incidence of short term transient oliguria, with a questionable impact on hard outcomes. Current urine output (UO) criteria for acute kidney injury (AKI) by KDIGO have not been deeply tested in terms of different thresholds and times in this population.

Methods: A retrospective cohort study ind adult patients after cardiac surgery in a third-level academic center in México City from March 1st 2010 to June 30th 2012. UO vas evaluated every 4hrs for 72hr. Progression into CKD was defined as having beyond 3 months both, an estimated glomerular filtration rate (eGFR) by CKD-EPI <60ml/min/1.73m2 and a reduction from baseline eGFR of a least 15ml/min/1.73m2.

Results: A total of 794 patients were included. RST was required in 55 (6.9%) patients, and 68 (8.6%) died during hospitalization. With a median follow-up time of 365 days, 78 (9.8%) died and 52 (6.5%) progressed into CKD. UO cut-off values obtained by ROC curves for RST at 4,8,12,16,20 and 24hr were 0.25, 0.35, 0.45, 0.5, 0.6 and 0.65ml/kg/hr respectively, with AUCs between 0.82 and 0.86 at all times. After adjusting for preoperative risk for RST (Thakar score), postoperative SOFA score and fluid balance, all cut-offs remained significant. Meeting any of these UO cut-offs showed an OR of 7.89 (3.75-16.59) (p=0.001) for RST, OR 3.99 (2.14-7.48) (p=0.001) for severe AKI by serum creatinine, OR 1.79 (1.05-3.04) (p=0.032) for in-hospital death, and HR 2.28 (1.59-3.27) (p=0.001) for follow-up death or progression into CKD.

Conclusions: While and UO cut-off of 0.5ml/kg/hr in 12hr matched the KDIGO criteria, a lower cut-off (around 0.3ml/kg/hr) should be implemented in shorter periods of time (4-8hr) and higher cut-offs (0.6ml/kg/hr) are significant for longer periods (20-24hr), with a proven impact on hard in-hospital and out-patient follow-up outcomes. This was no dependent on preoperative risk categories in contrast to renal angina criteria.

TH-PO029

A Rare Case of Acute Kidney Injury Secondary to Isoflurane Induced Malignant Hyperthermia Rohan V. Mehta,[1] Rahul Mehta,[2] John Doran.[3] *[1]Nephrology, Emory Univ School of Medicine, Atlanta, GA; [2]Internal Medicine, Univ of Virginia, Charlottesville, VA; [3]Nephrology, Emory Univ School of Medicine, Atlanta, GA.*

Introduction: Malignant hyperthermia (MH) is a rare muscular disorder provoked by exposure to halogenated anesthetics and succinylcholine. Signs of MH include increase in CO2 production and O2 consumption, acidosis, refractory hyperkalemia, hyperthermia, tachycardia, muscle rigidity, and rhabdomyolysis.

Case Description: A 58-year old male with a pancreatic mass was taken to the operating room (OR) for a Whipple's procedure. Vital signs included BP 142/73 mm Hg, HR 75 per minute, O2 saturation 100% on room air, and oral temperature of 36.5 F. In the OR, anesthesia was induced with propofol and rocuronium, and maintained with isoflurane. 6.5 hours into the procedure, sinus tachycardia ensued; BP declined to 80/40 mm Hg and minute ventilation doubled. High fever and extreme rigidity developed. ABG showed acidemia and hyperkalemia (pH 7.12, K 5.6 mmol/L). A presumed diagnosis of MH was made. All anesthetic gasses were discontinued and dantrolene was given with transient clinical improvement. However in the next 24 hours, anemia and hypotension required blood transfusions, and he developed non-oliguric acute kidney injury with persistant hyperkalemia, rhabdomyolysis and worsening metabolic acidosis for which CVVHD was initiated.

Discussion: MH is an abnormally high metabolic state of skeletal muscle in which a sudden increase in intracellular calcium caused by a defect in the RYR1 gene for the ryanodine receptor causes uncontrolled myocyte contractions. Halothane-caffeine contraction test on muscle biopsy specimen is the confirmatory test for MH. Dantrolene treats MH effectively by blocking calcium release and stopping uncontrolled muscle contractions. Discontinuation of the triggering agents, changing the breathing circuits and switching to an agent-free O2 source is recommended. In our case, CVVHD effectively treated rhabdomyolysis and hyperkalemia and helped avoid cardiac arrhythmias. In summary, we report a case of MH precipitated by isoflurane in a patient undergoing Whipple's procedure, which was successfully managed with dantrolene and CVVHD.

TH-PO030

Dialysis-Requiring Acute Kidney Injury from Pyonephrosis in Pregnancy: A Case Report Rizza Ann B. Lio, Brian Michael I. Cabral. *Section of Nephrology, Dept of Medicine, Univ of the Philippines-Philippine General Hospital, Manila, Philippines.*

Introduction: Acute Kidney Injury (AKI) during pregnancy presents a unique challenge. The medical team has to contend with the structural and physiologic changes in pregnancy and must also balance the needs of both mother and fetus. Pyonephrosis which is a life-threatening complication of ureteric obstruction is uncommon in pregnant patients because ureteral dilatation is one of the anatomic alterations of pregnancy.

Case Description: A 34-year-old G3P2 patient in her 25th week of pregnancy presented with a 3-day history of high-grade fever, right flank pain, and anuria. On admission, evaluation revealed a febrile patient with stable vital signs and a right costovertebral angle tenderness. Patient remained anuric despite foley catheter insertion. Initial laboratory findings showed leukocytosis, high anion gap metabolic acidosis, a creatinine level of 11mg/dL, and potassium of 5.7 mmol/L. Culture studies were obtained and the patient was started on Ceftriaxone as empiric therapy. An abdominal ultrasound was done which showed acute pyonephrosis and bilateral ureteropelvocaliectasia. Emergency hemodialysis was initiated to address the uremia. Subsequent dialysis sessions were done to maintain BUN <40mg/dL and to target bicarbonate of 18-22 mmol/L. On the third hospital day, a right percutaneous nephrostomy was done and 50mL of purulent discharge was drained. Culture studies revealed growth of Klebsiella sensitive to Ceftriaxone which was given for 14 days. The nephrostomy tube was removed after two weeks and patient was discharged well with a creatinine 1.2 mg/dL and BUN 8mg/dL. On subsequent out-patient consults, there was note of recovery of normal renal function. Patient then gave birth via spontaneous vaginal delivery on the 38th week of pregnancy.

Discussion: Severe cases of AKI requiring dialysis occurs in less than 20,000 pregnancies. When pyonephrosis complicates pregnancy, a multidisciplinary approach to management is necessary to balance the risks of the disease and the interventions to both mother and fetus. Timely diagnosis and urgent but safe interventions are needed because a uremic milieu is devastating for both.

TH-PO031

Supplement Associated Acute Renal Injury in Weight Athletes: A Report of 4 Cases Alaa A. Ali,[1] Safa E. Almukhtar,[2] Michael D. Hughson.[1] *[1]Shorsh General Hospital, Sulaimaniyah, Iraq; [2]Hawler Univ College of Medicine, Erbil, Iraq.*

Introduction: Athletes participating in weight lifting and body building routinely use nutritional supplements to develop and maintain a large muscle mass. This is consumed as protein powders combined with vitamins and calcium, creatine powder, and capsular forms of testosterone supplements, the later alleged to raise natural testosterone levels. Some nephrologists have expressed concern about excess protein induced glomerular hyperfiltration placing athletes at risk of developing focal segmental glomerulosclerosis (FSGS). Nevertheless, FSGS is not a demonstrated complication of weight training, and renal injury in athletes is mainly acute tubular injury complicating rhabdomyolysis during intense hot weather training.

Case Description: Case Description: In an academic Iraqi nephrology center, 4 body builders (18, 18, 23, and 24 years old) were referred who presented with renal insufficiency. All used commercial testosterone supplements, protein powders, vitamins, and creatine for periods ranging from 3-6 months. All athletic activity was indoors; none participated in endurance events. The presenting complaints were weakness and lethargy. Temperatures and blood pressures were normal. Serum creatinine levels ranged from 2.6 to 3.8 mg/dl with MDRD eGFR ranging from 22-34 mL/min/1.73m^2. Urine analyses with Labstix glucose, protein, and hemoglobin were unremarkable. Renal biopsies revealed foci of regenerating tubular epithelium, desquamated necrotic cells, hyaline casts, and intratubular calcifications. The biopsies contained 12 to 16 glomeruli in which no FSGS or pigmented casts suggesting rhabdomyolysis was found. Following the diagnoses of acute tubular injury, patients were re-evalated at 6 months and one year. None of the patients continued using supplements and serum creatinine and eGFR were in the normal range.

Discussion: Acute tubular injury was found to be the cause of renal dysfunction in weight athletes using nutritional supplements. Weight athletes should be warned about potential adverse affects, and if supplements are used, health services examinations with serum creatinine testing may be warranted even with mild symptoms.

TH-PO032

Hemodialysis for Acute Gabapentin Toxicity Abraham Cohen-Bucay, Herbert T. Cohen. *Renal Section, Boston Medical Center and Boston Univ School of Medicine, Boston, MA.*

Introduction: Gabapentin is a small molecule (171 Dalton) that is minimally protein bound, not metabolized and exclusively renally cleared. Its half-life with normal glomerular filtration rate is 5-7 hrs, but this increases up to 130 hrs in anuric patients. It is a dialyzable molecule, and its half-life on dialysis is 3.8 hrs. Serum gabapentin levels are higher in patients with chronic kidney disease (CKD), and symptoms of gabapentin toxicity are more common in those CKD patients who are elderly, have multiple comorbidities or have a pre-existing central nervous system disorder. Typically, symptoms of gabapentin toxicity appear once serum levels exceed 15 mcg/ml and include reduced consciousness, ataxia, myoclonus and asterixis. Here we present a case of acute gabapentin toxicity treated with hemodialysis.

Case Description: A 91 year-old woman with stage 4 CKD (creatinine 2.0 mg/dl) and peripheral neuropathy was admitted with chest pain and shortness of breath. Although her creatinine remained stable, on her second hospital day, she developed a change in mental status consisting of somnolence and myoclonus. Her outpatient record had indicated she was on gabapentin 800 mg three times daily, which was started on admission, although she had not been taking this recently. The altered mental status was attributed to gabapentin, as work up was otherwise negative. Because the patient had advanced CKD, and gabapentin is exclusively renally cleared, we did one session of hemodialysis for 6 hrs using a large dialyzer and high blood and dialysate flows to maximize clearance. With dialysis, her mental status improved substantially and returned to baseline in 1 day. Her serum gabapentin level decreased from 27.4 mcg/ml before dialysis to 6.9 mcg/ml 3 hrs after dialysis.

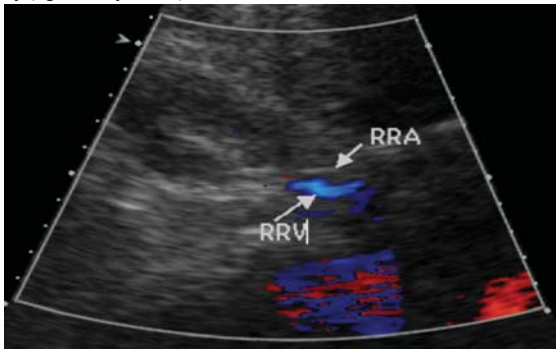
Discussion: Hemodialysis is an effective treatment for acute gabapentin toxicity. Although hemodialysis is an invasive therapy, we believe it is a safe option for patients with advanced CKD who would have no other means to excrete gabapentin in a timely manner. To our knowledge, this is the first report of the treatment of gabapentin toxicity with hemodialysis in a patient not otherwise on dialysis.

TH-PO033

Reversible Acute Kidney Injury due to Bilateral Renal Artery Thrombosis in a Patient with a Thrombophilia Mario A. Mendoza, Eddie M. Rodriguez, Emmanuel O. Gonzalez, Cristy Gianna Martinez, Carlos S. Rosado-Rodriguez, Hector R. Cordova. *Medical Service, VA Caribbean Healthcare System, San Juan, PR.*

Introduction: Hereditary thrombophilias are rare prothrombotic states, usually associated with venous thrombotic complications. We report a patient with prothrombin gene mutation who developed renal artery thrombosis after an emergency surgical procedure.

Case Description: A 46 y/o man with history of hypertension was admitted due to jaundice, choluria, acholia, and right upper quadrant pain. Imaging studies confirmed the presence of biliary tract obstruction due to choledocolithiasis. It was not possible to remove the gallstones endoscopically. Therefore, the patient underwent open cholecystectomy. The procedure was complicated by acute blood loss. The patient developed acute kidney injury post-operatively. Renal anatomy and blood flow were assessed with Doppler ultrasound due to persistent oilgo-anuria. There was evidence of absent renal artery (RA) blood flow bilaterally (right kidney shown).



Renal replacement therapy was started. Anticoagulation therapy was initially contraindicated due to the presence of a large liver subscapular hematoma. Coagulation studies revealed: INR: 1.07, PT: 14.3, PTT: 32.4, Protein S: 68%, Protein C: 104%, prothrombin gene mutation (+). The patient recovered renal function (s creat: 1.2 mg/dl) after 3 weeks of renal replacement therapy.

Discussion: Hereditary thrombophilias may predispose to venous and/or arterial thrombosis. Most reports of renal artery thrombosis are described in patients with unsuspected thrombophilias who undergo kidney transplant surgery. The renal Doppler ultrasound findings lead us to the discovery of the hereditary thrombophilia. We identified the presence of an heterozygous prothrombin gene mutation. The initiation of chronic anticoagulation therapy coincided with the slow recovery of renal function in our patient.

Funding: Veterans Affairs Support

TH-PO034

A Rare Case of Acute Kidney Injury Caused by Mantle Cell Lymphoma Kana N. Miyata, Nazia A. Siddiqi, Lawrence P. Kiss, Nikolas B. Harbord, James F. Winchester. *Mount Sinai Beth Israel, New York.*

Introduction: Renal involvement in non-Hodgkin lymphoma, especially mantle cell lymphoma(MCL) is rare.

Case Description: A 77 year old Filipino man with hypertension, hypothyroidism, and bladder cancer developed acute kidney injury(AKI) (baseline creatinine of 2.2 mg/dL rose to 4.5 mg/dL). Two months prior he was noted to have a lung nodule on chest X ray and a PET scan showed diffuse lymphadenopathy in the neck, chest, abdomen, and pelvis. On admission, he complained of fatigue and decreased appetite. Vital signs were stable. Physical exam revealed submandibular and right axillary lymph nodes. Urinalysis showed protein 1+, large blood, eosinophils, dysmorphic RBCs, and urine protein/creatinine ratio of 2.9 g/gCr. He had low C3 49 mg/dL and C4 16 mg/dL, positive ANA of 160, negative MPO-ANCA, and positive PR-3 ANCA. Ultrasound revealed increased echogenicity with right kidney 12.4 cm and left 11.4 cm. Hospital course was complicated by pulmonary edema associated with non-ST-elevation myocardial infarction. Kidney function continued to decline requiring dialysis on hospital day 16. Biopsy revealed patchy dense monotonous lymphocytic aggregates. The lymphocytes were B cells, positive for CyclinD1, consistent with atypical CD5-negative MCL as confirmed by the detection of translocation t(11;14) by FISH. There were crescents in 3 out of 26 glomeruli, consistent with pauci-immune glomerulonephritis. Chemotherapy was started.

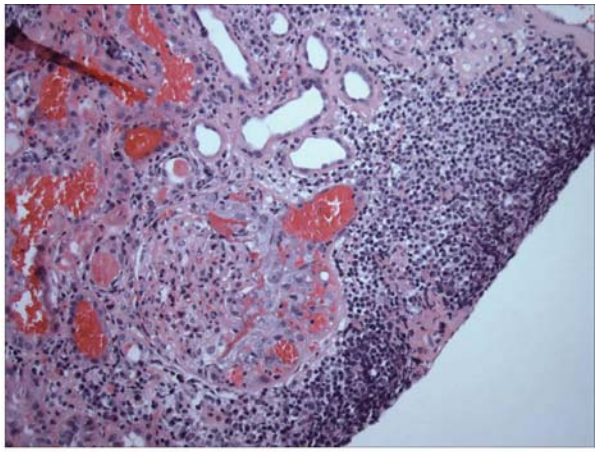


Fig.1 Crescent formation and atypical lymphoid cells infiltration.

Discussion: We present the first case of a patient with coexistence of renal MCL infiltration and pauci-immune glomerulonephritis. Renal involvement in non-Hodgkin lymphoma has been reported, including AKI, glomerulonephritis, and infiltration of renal parenchyma by lymphoma cells. Renal manifestations of MCL are especially rare and there are only 14 cases published to date. Four of them showed renal MCL infiltration and this will be the 5th case.

TH-PO035

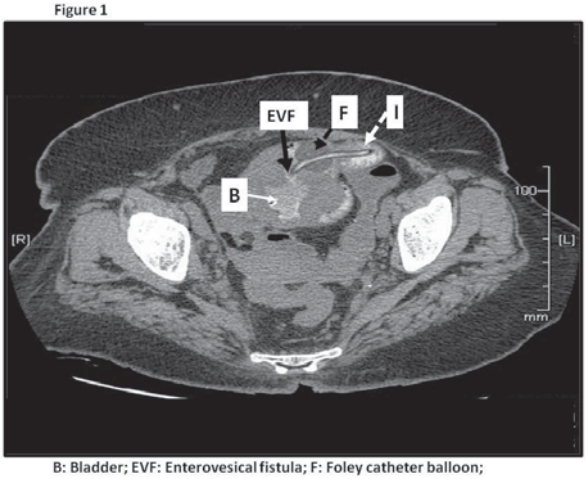
Severe Azotemia and Hyperchloremic Metabolic Acidosis in a Patient with Reverse Flow Enterovesical Fistula Ike Ezumba,[1] Elvira Gosmanova,[1] Leigh Darryl Quarles,[1] Barry M. Wall.[1,2] *[1]Nephrology, UTHSC, Memphis, TN; [2]Nephrology, VAMC, Memphis, TN.*

Introduction: Enterovesical fistula (EVF) usually has flow from intestine to the bladder and commonly manifests with recurrent urinary tract infections, fecaluria and pneumaturia. We report a rare case of EVF with reverse flow from the bladder to intestine, leading to severe azotemia and hyperchloremic metabolic acidosis.

Case Description: 55-year old female underwent transurethral resection of bladder cancer and soon developed watery diarrhea and fecal incontinence. On exam she had low BP and dry mucous membranes. Lab. findings: Na 133meq/L, K 3.6meq/L, Cl 110meq/L, HCO_3 12meq/L, BUN 100mg/dL, and Creatinine 0.7mg/dL. Urine studies could not be obtained as patient was not passing urine even after placing a Foley catheter. CT scan of the abdomen and pelvis showed a markedly contracted and nodular bladder with a large bladder

wall defect and the Foley catheter extending through the bladder wall into a small bowel loop [Figure 1]. A diagnosis of EVF with reverse flow from the bladder to intestine was made. Sodium bicarbonate infusion was started to correct volume depletion and metabolic acidosis. The patient underwent partial cystectomy with creation of an ileal conduit. These measures led to rapid resolution of azotemia and metabolic acidosis within 48 hrs.

Figure 1



B: Bladder; EVF: Enterovesical fistula; F: Foley catheter balloon; I: Intestine

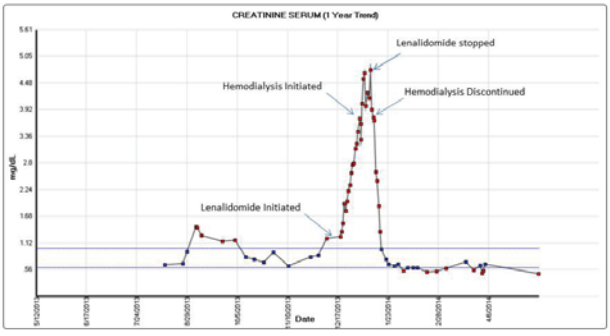
Discussion: EVF rarely can have reverse flow leading to urine excretion via GI tract in patients with diminished bladder capacity. The diverted urine stimulates HCO_3 secretion in exchange for urine Cl via Cl/HCO_3 anion exchanger located both in the ileum and the colon leading to hyperchloremic metabolic acidosis. Urinary NH_4Cl is reabsorbed in the colon and metabolized in the liver to to NH_3 and H^+. The NH_3 is metabolized to urea causing azotemia out of proportion to the kidney function. Surgical closure of EVF is required to correct these metabolic abnormalities.

TH-PO036

First Reported Case of Acute Tubular Necrosis Induced by Lenalidomide in a Patient with Follicular Lymphoma Tho M. Luong,[1] Maen Abdelrahim,[2] Ala Abudayyeh.[2] *[1]Nephrology, The Univ of Texas Medical School at Houston, TX; [2]Nephrology, The Univ of Texas MD Anderson Cancer Center, TX.*

Introduction: Lenalidomide is an immunomodulatory drug used to treat relapsed and refractory multiple myeloma, light-chain amyloidosis and certain lymphomas. Because lenalidomide is renally secreted, dose reduction is required to avoid toxic effects (pancytopenia, infections, pneumonitis, and thromboembolism). We discuss the first published case of lenalidomide-induced acute kidney injury from acute tubular necrosis.

Case Description: A 54 year old female with a history of follicular lymphoma treated with R-CHOP was evaluated for hematuria, nephrotic range proteinuria, edema, and renal failure. Renal biopsy showed membranoproliferative glomerulonephritis with C3 and IgG lambda deposition and reoccurrence of her lymphoma. Lenalidomide was initiated for further treatment and a week after initiation; the patient was hospitalized for worsening renal failure, pulmonary edema, and respiratory failure requiring intubation and hemodialysis. Urinalysis showed dense, diffuse granular casts indicative of acute tubular necrosis. After discontinuing lenalodomide the patient's renal function improved and no longer required hemodialysis



Discussion: Few associations of acute kidney injury with lenalidomide toxicity have been reported, and most have been attributed to allergic interstitial nephritis. An ongoing study at a single center is documenting the incidence of acute kidney injury after initiation of lenalidomide, but the etiology of the injury has not yet been established in this study. Lenalidomide is still recommended to treat relapsed and refractory multiple myeloma. However, our case represents a possible new complication of treatment with lenalidomide. Providers should be aware of this potential complication as the use of lenalidomide continues to rise.

TH-PO037

Two Cases of Household Product Poisoning with Hyperosmolarity Payam Shakouri, George N. Coritsidis, Ishita Rajnish, Karendip Braich, Jasjit Singh, Salwa Rhazouani, Aaron S. Stern. *Nephrology, Elmhurst Hospital Center, Elmhurst, NY.*

Introduction: In our investigation we were trying to find new etiologies for increased osmolar gap.

Case Description: Case A:43 y/o M with DM1 presented to the ICU after being found unresponsive. He presented with tachypnea, tachycardia and hypotension. He was found to be in DKA and AKI. He had a profound serum osmolar gap of 74 mOsm/kg. He was treated with IV insulin, fluids with Bicarbonate and fomepizole.Assays for toxic ingestions were later found to be negative.On hospital day 8 he admitted to spraying a common household pesticide sublingually 6-7 times daily for 7 days for its euphoric effect. Case B: 57 y/o F with multi-substance abuse and frequent overdoses presented with respiratory and renal failure after overdose of an unknown substance. She had a profound metabolic acidosis and an increased osmolar gap. Drug screen was negative for toxic ingestion.Patient underwent resuscitation, and dialysis. Upon recovery she admitted to drinking a common household cleaner (Pine Sol).

	Sodium (mEq/L)	Potassium (mEq/L)	Bicarbonate (mEq/L)	Creatnine (mmol/L)	Measured Serum Osmolality (mOsm/kg of H20)	Calculated Serum Osmolality (mOsm/kg of H20)	Osmolar Gap (mOsm/kg of H20)
Case A	112	8.8	3	3.8	372	298	74
Case B	133	3.1	6	2.6	313	288	25

Discussion: The pesticide is Case A contains imiprothrin and cypermethrin as active components. Other ingredients include: Isobutene, propane, isopropanol. There have been no reports of metabolic or serum osmolality disturbances concerning imiprothrin or cypermethrin.Increased osmolar gap is likely from other compounds present in higher concentrations and not imiprothrin or cypermethrin. We believe this is the first reported case of pesticide induced hyper osmolarity. Patient in Case B presented with AKI and anion gap metabolic acidosis.Given the patient's history of polysubstance ingestion, it was suspected that she may have also ingested ethylene glycol. On further questioning she admitted to ingesting Pine Sol. Pine Sol has two formulations, one containing Isopropanol, the other contains glycolic acid. Glycolic acid, the toxic metabolite of ethylene glycol, could account for all of the metabolic disturbances observed in this patient.

TH-PO038

Sitagliptin Associated Acute Interstitial Nephritis Julie Lesage,[1] Sebastien Savard,[1] Fabrice Mac-Way.[1] *[1]Div of Nephrology, CHU Hôtel-Dieu de Québec, Québec, QC, Canada; [2]Dept of Pathology, CHU Hôtel-Dieu de Québec, Québec, QC, Canada.*

Introduction: Inhibitors of dipeptidyl peptidase (DDP-4 inhibitors) are a new class of oral hypoglycaemic agents with increasing usage in the treatment of type II diabetes. In this paper, we describe two cases of biopsy-proven acute interstitial nephritis (AIN) attributed to treatment with sitagliptin, the first DDP-4 inhibitor to have been approved by FDA.

Case Description: Case 1 was an asymptomatic 63-year-old man that showed an elevation in creatinine, from 75 µmol/L to 225 µmol/L, twenty-two months after the introduction of a combination of sitagliptin and metformin. Renal biopsy revealed interstitial inflammation mainly composed of eosinophils. The renal function gradually improved after cessation of sitagliptin. Case 2 was a 60-year-old male with an asymptomatic increase in serum creatinine level from 103 µmol/L to 425 µmol/L over three months, which matched the introduction of sitagliptin-metformin. We could find an urinary concentration of eosinophils of 0,7% but no serologic abnormalities. Renal biopsy revealed severe tubulointerstitial inflammation composed of eosinophils, lymphocytes, plasmocytes and neutrophils, with severe fibroedema. The creatinine significantly improved with cessation of sitagliptin and an oral prednisone treatment.

Discussion: To our knowledge, this is the first report of sitagliptin-induced AIN. In both cases, the temporal association between sitagliptin initiation, renal failure and improvement of renal function after drug discontinuation supports the diagnosis of sitagliptin-induced AIN as documents by kidney biopsy. In the context of increasing popularity of this class of drugs, clinicians should be aware of this potentially adverse reaction with sitagliptan.

TH-PO039

A Case of Acute Kidney Injury from Crystal Nephropathy Secondary to Pomalidomide Use Sam Leung,[1] Phylicia Baird,[2] Olawumi O. Babalola,[2] Rimda Wanchoo,[1] Kenar D. Jhaveri.[1] *[1]Kidney Diseases and Hypertension, Hofstra North Shore LIJ School of Medicine, Manhasset, NY; [2]Internal Medicine, Hofstra North Shore LIJ School of Medicine, Manhasset, NY.*

Introduction: Pomalidomide is an analog of thalidomide indicated for treatment of refractory Myeloma. It has a reported incidence of <5% for renal failure. We report the first case of crystal nephropathy with pomalidomide use.

Case Description: A 76 year-old female with a history of refractory IgG Kappa myeloma presented with fevers, productive cough and weakness. Prior to admission, she had taken four days of a planned 21 day course of pomalidomide. Upon admission, she was noted to have a fever of 102.2F and a WBC of 2.6 K/ul with a nadir ANC of 0.3 K/ul. Respiratory

viral panel was positive for respiratory syncytial virus. In the setting of neutropenic fever, she was started on vancomycin, piperacillin/tazobactam and levofloxacin for multifocal pneumonia, seen on CT of the chest without contrast. Additionally, pamolidomide was held. On hospital day 2, her creatinine was noted to increase to 1.65 mg/dl from 1.10mg/dl on admission. Her creatinine continued to trend upwards despite adequate hydration and avoidance of nephrotoxins. Urinalysis was significant for pH of 5, and protein of 25 mg/dl with no granular casts. Microscopic analysis of urine sediment collected on second day of admission was significant for long, spindle shaped crystals. A diagnosis of pomalidomide induced crystal associated tubular damage was made. Levofloxacin was discontinued on day 2 and the other antibiotics were continued. Vancomycin levels ranged between 10-27 µg/ml. Over the following 3 weeks, her serum creatinine peaked at 3.99mg/dl, but returned to a baseline of 0.9mg/dl after three months. Due to thrombocytopenia, a kidney biopsy could not be performed.

Discussion: Given the time course of chemotherapy, urine microscopy findings and lack of use of other medications that are known to cause crystal nephropathy, pomalidomide is the most likely cause of her crystal nephropathy. This rare case of acute kidney injury due to crystal nephropathy after treatment with pomalidamide illustrates an unreported and potentially serious side effect of pomalidamide.

TH-PO040

Kinetic Estimated Glomerular Filtration Rate: Another Approach for Management of Acute Kidney Injury Ekamol Tantisattamo, Harold A. Franch, James L. Bailey. *Renal Div, Emory Univ.*

Introduction: Acute kidney injury (AKI) is common in hospitalized patients. In non-oliguric AKI, we often rely on the trend and stability of serum creatinine (SCr) to determine the onset of renal recovery. This limits an assessment of renal function when SCr is changing rapidly. Kinetic estimated glomerular filtration rate (KeGFR) is an existing simplified formula which is not widely recognized but helpful to determine renal function in AKI. We report a case of man with non-oliguric AKI requiring continuous veno-venous hemodialysis (CVVHD) and demonstrate the utility of KeGFR for management of AKI.

Case Description: Case description: A 64 year-old man with history of stage 3 CKD was admitted with urosepsis. Initial labs revealed SCr of 11.15 mg/dL from the baseline of 0.9 mg/dL. Serum K was 6.7 and HCO_3 was 5 mmol/l. ABG showed pH of 6.8, $PaCO_2$ of 19, and PaO_2 of 106 mmHg. Urine microscopy revealed muddy brown casts. He was non-oliguric. CVVHD was initiated. Forty-four hours later, acidosis was improved and CVVHD was discontinued. Around 22 hours after discontinued CVVHD, SCr was 1.6 mg/dL and had trended up to 3.06 mg/dL within 24 hours (hospital day4) when KeGFR was almost 0 ml/min (see formula in the figure1). From hospital day4-8, SCr still had trended up; however, KeGFR showed improving renal function. Therefore, dialysis catheter was removed on hospital day7. He was discharged with SCr of 1.64 mg/dL.

Discussion: SCr is not a sensitive marker and eGFR from MDRD routinely reported with SCr cannot be applied in AKI. To calculate eGFR from KeGFR formula, only SCr which is routinely monitored during AKI, is required. By using KeGFR, more accurate renal function can be confidently determined at bedside in the setting of acute change in SCr. Our case demonstrates utility of KeGFR for management in AKI especially to determine the onset of renal recovery and time to discontinue dialysis.

$$KeGFR = \frac{SSP_{Cr} \times CrCl}{MeanP_{Cr}} \times \left(1 - \frac{24 \times \Delta P_{Cr}}{\Delta Time(h) \times Max\Delta P_{Cr}/Day}\right)$$

KeGFR = Kinetic estimated glomerular filtration rate (ml/min)
SSP_{Cr} = Steady-state plasma creatinine (mg/dl)
CrCl = Corresponding creatinine clearance (mg/dl)
$MeanP_{Cr}$ = Mean maximal change in plasma creatinine /day (mg/dl)
ΔP_{Cr} = Change in plasma creatinine between 2 consecutive values (mg/dl)
ΔTime = Interval between 2 consecutive creatinines (mg/dl)
$MaxΔP_{Cr}/Day$ = Maximal change in plasma creatinine / day (use 1.5 mg/dl/day)

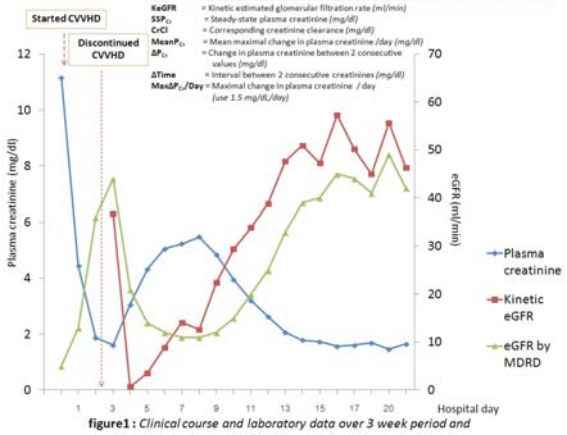


figure1 : *Clinical course and laboratory data over 3 week period and Kinetic glomerular filtration rate formula.*

TH-PO041

Fatal Rhabdomyolysis due to Massive Number of Bees' Stings Ghayyath Sultan, Gaurav Agarwal, Mohammed Ahmad, Christopher A. Dyer. *Dept of Nephrology, Univ of Texas, San Antonio, TX.*

Introduction: Rhabdomyolysis due to massive number of bee's stings with fatality has been reported in infants, elderly and debilitated patient but fatal outcomes in a previously healthy young adults has not yet been reported.

Case Description: A 30 year old athletic man with no prior history of hypersensitivity to insect stings was stung by approximately 300 bees after he accidently encountered a bee's nest. He was treated with subcutaneous epinephrine and intravenous diphenhydramine en route to the emergency department. On arrival, he was tachycardic and hypertensive and subsequently developed a progressive decline in respiratory status and required intubation. Multiple bees' stings were noted over the arms, neck, chest, face, ears and abdomen. He developed oliguric acute kidney injury accompanied by hyperkalemia, hyperuricemia, hyperphosphatemia and hypocalcemia. Laboratory findings revealed, serum creatinine 1.98[thinsp]mg/dl, AST of 1851 IU/L on admission and peaked at > 20,000 IU/L next day, uric acid of 24.9 mg/dl, phosphorus of 13.8 g/dl, myoglobin of > 1000 ng/ml and creatine phosphokinase (CPK) of 40,000[thinsp]IU/l reaching a peak of 361,200 IU/L second day of admission. He was treated with aggressive fluid resuscitation, intravenous sodium bicarbonate and emergent hemodialysis. He was converted to continuous venovenous hemofiltration (CVVH) due to persistent hyperkalemia. On the second hospital day, the patient became hypotensive and required pressor support with epinephrine. He subsequently developed ventricular fibrillation and cardiopulmonary resuscitation was attempted for over 60 minutes without success.

Discussion: Although rhabdomyolysis may be caused by multiple etiologies; it is rarely reported after bees' stings. The mechanism of myotoxicity is attributed to the direct toxicity of the venom to myocytes and fatality is a potential outcome even in otherwise young and healthy patients.

TH-PO042

High Flux Dialysis for Acute Nephrotoxicity following High Dose Methotrexate Ghayyath Sultan, Gaurav Agarwal, Nyan W. Phyo, Hanna E. Abboud. *Dept of Nephrology, Univ of Texas, San Antonio, TX.*

Introduction: High dose methotrexate (HDMTX) can be safely given to most patients with supportive care measures. Few patients, however, develop nephrotoxicity. Early recognition and treatment is essential to prevent the accumulation of toxic methotrexate (MTX) levels that can lead to significant morbidity and mortality.

Case Description: A 71 year old man with primary CNS lymphoma was started on high dose methotrexate (4g/m2). Prior to starting HDMTX he was treated with rescue leucovorin therapy and IV sodium bicarbonate to achieve urine Ph of greater than 7 for 2 consecutive days. Baseline serum creatinine on admission was 0.8 mg/dl. 24 hours after receiving HDMTX his creatinine increased to 2.3 mg/dl and peaked at 4.37 mg/dl. He continued to have adequate urine output. MTX levels were 253 µmol/L post infusion and 129 µmol/L 17 hours after the treatment. MTX levels remained in the toxic range along with worsening kidney function. High flux hemodialysis (HD) was initiated. MTX levels decreased from 32.57 µmol/L pre HD to 6.59 µmol/L after two four hours each of HD treatments. MTX levels continued to decrease gradually with ongoing treatment of sodium bicarbonate and leucovorin and reached a value of less than 1 µmol/L two weeks later. Serum creatinine also improved gradually reaching new base line of 1.2 mg/dl three weeks later. In addition to renal toxicity; patient developed thrombocytopenia with platelets count of 64000/mcl (normal 150000 - 450000/mcl) and liver toxicity with ALT of 140 IU/L (normal 10 – 40 IU/L) and AST of 62 IU/L (normal 10 – 40 IU/L). Both thrombocytopenia and liver toxicity resolved with complete clearance of methotrexate from the circulation.

Discussion: Methotrexate is a direct renal toxin that can lead to renal failure with decrease in MTX clearance and systemic toxicity. Additional treatment is required when standard measures fail to prevent MTX- induced nephrotoxicity. High flux hemodialysis is very effective in reducing MTX levels. Pharmacotherapy with glucarpidase has been used successfully to decrease MTX levels. However, the drug is prohibitively expensive and may not be available in many centers.

TH-PO043

Novel Endovascular Treatment of Supra-Renal Caval Thrombosis and Acute Kidney Injury Akshta Pai, Mohamad Alkhouli, Grayson Wheatley, Eric T. Choi, Riyaz Bashir, D. B. Johnstone. *Temple Univ Hospital, Philadelphia, PA.*

Introduction: Hospital-acquired acute kidney injury is most often seen as a result of decreased renal perfusion from any cause, including nephrotoxic medications, contrast media, surgery and sepsis. Catastrophic vascular obstruction is a relatively rare and controversial cause of acute kidney injury.

Case Description: We present a case of a morbidly obese 55-year-old male hospitalized with a large abdominal wall hematoma who suddenly became oligo-anuric for unclear reasons. Our diagnosis was renal vein thrombosis and supra-renal vena caval thrombosis. Treatment with systemic heparin unfortunately resulted in expansion of the large abdominal wall hematoma, and his obesity precluded surgical intervention. As an alternative, endovascular extraction of the thrombus using the AngioVac veno-venous filtration system was utilized, and afterwards and the patient regained normal renal function.

Discussion: To our knowledge, this is the first report of utilizing the AngioVac system in the treatment of renal vein and supra-renal vena caval thrombosis without concomitant thrombolytics.

TH-PO044

Ethylene Glycol Toxicity Masquerading as Lactic Acidosis Sami Alasfar, Mohamed G. Atta. *Johns Hopkins Univ, Baltimore, MD.*

Introduction: Ethylene glycol toxicity is manifested by gastrointestinal and nervous system involvement with metabolic acidosis. Its metabolites are structurally similar to lactate and can cause false elevation of lactate. We present a case of ethylene glycol toxicity demonstrating the substantial potential for misdiagnosis.

Case Description: A 53-year-old male with diabetes presented with confusion, nausea, and vomiting. His only home medication was Metformin with unknown dose.

On examination, pulse was 121 beats/min and blood pressure was 188/91 mmHg. He was agitated and had Kussmaul respiration. Laboratory tests on admission are shown below:

Test	Value
pH	6.3
CO2	44 mmHg
Bicarbonate	5 mEq/L
Osmolality	351 mOsm/kg
Osmolar gap	22
Sodium	142 mEq/L
Potassium	6.2 mEq/L
Cholride	97 mEq/L
Anion gap	40
Creatinine	1.6 mg/dL
Glucose	486 mg/dL
Lactate	>30 mmol/L
Ethanol and Salicylate	Undetectable
Urine drug screen	Negative
Urinalysis	Negative for ketones or crystals

He was intubated for airway protection and emergently started on sodium bicarbonate drip and continuous renal replacement therapy. After 24 hours, lactate level started to decrease and normalized by 36 hours. Three days later, admission Metformin level came back normal and admission ethylene glycol was elevated at 167 mg/L. Subsequent ethylene glycol levels normalized. Later, a friend reported that the patient purchased an online product with the intent to commit suicide. Patient developed multiple complications including strokes and septic shock. His renal function did not recover and remained on dialysis.

Discussion: Elevated lactate level suggests tissue hypoxia or, as in this case, possible metformin toxicity. However, because of misinterpretation of glycolate as lactate by analyzers that utilizes L-lactate oxidase method, ethylene glycol toxicity may be overlooked. Although lactate can be elevated in patients with ethylene glycol toxicity, such elevation is usually minimal. This case shows the potential to misdiagnose ethylene glycol toxicity as lactatic acidosis when analyzers utilizing L-lactate oxidase method are used. Thus, elevated lactate level may be the first sign of ethylene glycol toxicity in this setting and it should be considered in patients presenting with metabolic acidosis and elevated lactate level.

TH-PO045

Vancomycin Associated Interstitial Nephritis, Acute Tubular Necrosis and Leucocytoclastic Vasculitis Sherif Y. Isshak, Jiries S. Dahu, John C. Edwards. *Nephrology, Saint Louis Univ, St. Louis, MO.*

Introduction: Vancomycin (VANC) can cause leukocytoclastic vasculitis (LV), occasionally accompanied by acute kidney injury (AKI).While VANC is known to cause both acute tubular necrosis (ATN)and acute interstitial nephritis (AIN), the pathology of AKI in context of LV has not been reported.

Case Description: A 46 year old man with normal kidney function suffered a gunshot wound to the head. He had cranioplasty complicated by MRSA infection and was treated with VANC, target serum level 15-20 mg /dl. VANC level did not exceed 25 mg/dl throughout the course. On the eighth day of VANC therapy, He developed altered mental status. On day 11, serum creatinine (Scr) rose from baseline 0.8 to 1.5 mg/dl and he developed erythematous papulovesicular and petechial rash. There was no documented hypotension, urinary retention, intravenous contrast or nephrotoxic drugs exposure. Skin biopsy showed leucocytoclastic vasculitis; direct immunofluorescence stain was negative for C3, C1q, IgM, IgA, and IgG. On day 12, VANC was changed to daptomycin and rifampin. Rash improved. Five days later, VANC was restarted because of its superior CNS penetration. The rash recurred and Scr worsened to 2.9 mg/dl. Urine sediment was notable for WBC and RBC casts. Renal biopsy showed diffusely increased mesangium, interstitial mononuclear infiltrate with prominent eosinophils and tubules with focal cytoplasmic vacuolization, attenuated brush border and numerous tubular epithelial mitotic figures. Immunofluorescence was negative. VANC was changed to linezolid. The rash resolved and Scr improved to 1.6 where it stabilized.

Discussion: This patient developed manifestations of VANC toxicity, including LV and AKI that on kidney biopsy showed no evidence of vasculitis, but rather components of both AIN and ATN, both of which have been associated with VANC in the absence of vasculitis.The injury occurred without excessive VANC blood level. VANC toxicity has been associated with serum levels above 15mg/dl, daily dose 4 g or more, and concomitant drugs, particularly pipercillin and gentamicin. With stopping VANC, Scr usually improves to baseline, but may progress to CKD or ESRD.

TH-PO046

Thrombotic Thrombocytopenic Purpura with Acute Kidney Injury in a Patient with Systemic Sclerosis Bernice Kim, Dong Jun Oh, Sung Joon Shin, Kyung Soo Kim. *Div of Nephrology, Dept of Medicine, Dongguk Univ Ilsan Hospital, Go-yang, Republic of Korea.*

Introduction: Thrombotic thrombocytopenic purpura (TTP) is a rare complication of systemic sclerosis (SSc). Distinguishing TTP from scleroderma renal crisis (SRC) is difficult, because both diseases can present with thrombocytopenia, microangiopathic hemolytic anemia (MAHA), and renal impairment, but the pathogenesis and treatments are totally different. In this report, we present a case of TTP in a patient with SSc treated with plasmapheresis.

Case Description: A previous healthy 81-year-old woman was admitted because of stuporous mentality. On admission, she was afebrile with blood pressure of 141/87 mmHg. Physical examination revealed skin thickening in both hands and Raynaud's phenomenon, strongly suggestive of systemic sclerosis. Initial laboratory tests were as follows: platelet count $76x10^3$ /μL, hemoglobin 7.9 g/dL, corrected reticulocyte count 4.0%, lactate dehydrogenase (LDH) 866 IU/L, and creatinine 6.39 mg/dL. Peripheral blood smear revealed schistocytosis, indicating MAHA. Anti-Scl 70 antibody was positive, and diagnosis of systemic sclerosis was confirmed by a rheumatologist. Activity of ADAMTS13 was mildly decreased to 39%. Presumptive diagnosis of TTP was made and emergent treatment with plasmapheresis with systemic corticosteroid was initiated. After 12 sessions of plasmapheresis, she had clinical therapeutic response as evidenced by improvement of mentality to nearly alert state, and normalization of LDH and MAHA. However, thrombocytopenia persisted, but further treatments for refractory TTP were not administered, considering her age. Renal function did not improve, and she remained dialysis dependent.

Discussion: Although distinguishing TTP from SRC is difficult, urgent decision has to be made based on clinical findings, as in the presented case. Plasmapheresis should be considered when TTP cannot be ruled out, since TTP is a potentially fatal disease whereas early treatment can be lifesaving. When there is no clinical response to plasmapheresis, SRC should be reconsidered and appropriate therapy with angiotensin converting enzyme inhibitor should be taken into account.

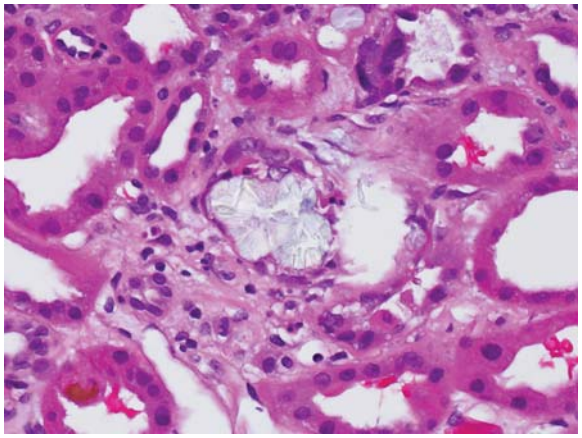
TH-PO047

Acute Kidney Injury due to Oxalosis Secondary to Pancreatic Insufficiency Irfan K. Moinuddin,[1] Machaiah M. Madhrira,[1] Amy Nicole Sussman,[2] Erika R. Bracamonte.[3] *[1]Div of Nephrology, Univ of Arizona Medical Center, Tucson, AZ; [2]Dept of Nephrology, Univ of Arizona at Tucson, Tucson, AZ; [3]Dept of Pathology, Univ of Arizona at Tucson, Tucson, AZ.*

Introduction: AKI due to pancreatic carcinoma can be the result of hypoxemia, release of pancreatic amylase from the injured pancreas with impairment of renal microcirculation, decrease in renal perfusion pressure due to abdominal compartment syndrome or hypovolemia. We describe a case of AKI due to oxalosis in a patient in whom pancreatic cancer caused severe pancreatic insufficiency.

Case Description: We report a case of a 66 year old female with past medical history of ovarian cancer, uterine cancer, colon cancer and newly diagnosed pancreatic adenocarcinoma was admitted for the evaluation of nausea, vomiting and diarrhea. She reported diarrhea of several months duration. Her admission serum creatinine was 10.4mg/dl, one month prior being 1.0mg/dl. Urinalysis showed protein 300, WBC >150, RBC 43 and no bacteria and urine protein/creatinine of 1.8gm/gm.Renal ultrasound showed no hydronephrosis. She was volume resuscitated adequately for three days with no significant improvement in her renal functions and hence a renal biopsy was pursued.

Histopathology demonstrated a tubulointerstitial process with calcium oxalate crystals in the tubules consistent with oxalosis. Patient was diagnosed with AKI due to secondary oxalosis. On enzymatic evaluation she was found to have marked pancreatic insufficiency and fat malabsorption.



Discussion: Fat malabsorption leads to saponification of calcium and absorption of free oxalate. Oxalate deposition in the kidneys can lead to nephrocalcinosis, sterile pyuria, hematuria and mild proteinuria. In a patient with pancreatic adenocarcinoma who presents with AKI associated with diarrhea, oxalosis due to fat malabsorption must be considered.

TH-PO048

Lactic Acidosis Leading to Shock: A Rare Cause and a Suggestion for Change in Guidelines Dennis Moledina, Barry R. Gorlitsky. *Nephrology, Yale, New Haven, CT.*

Introduction: Metformin is a first-line agent for treatment of type 2 DM. It has been linked to lactic acidosis in at-risk patients and current guidelines suggest avoiding Metformin if serum creatinine (mg/dl) is >1.4 in women and >1.5 in men. We describe a case of lactic acidosis with metformin use in a patient who did not meet the criteria for avoiding Metformin based on creatinine cut-off but did have a decreased eGFR. We make the case for utilizing eGFR rather than serum creatinine as a cut-off value for avoiding Metformin.

Case Description: 72-year-old woman with a baseline creatinine of 1.3 mg/dl (eGFR=41 ml/min) presents as a stroke code with severe hypotension. Her daughter provides

the history of a week of malaise and vomiting prior to presentation. Her laboratory work demonstrated a pH of 6.80, serum bicarbonate <5 mEq/L, lactic acid level of 26.0 mmol/L, and creatinine of 7.2 mg/dl. There was recovery in hemodynamics with initiation of CRRT and no source of sepsis could be identified. Due to a high level of suspicion a Metformin level was submitted from a sample drawn on admission, however discussion with patient's daughter and pharmacy regarding home medications did not reveal Metformin use. We did obtain a history of Furosemide, Lisinopril and Ibuprofen explaining the AKI. The Metformin level was found to be elevated at 33 mcg/ml (therapeutic range is 1-2) and on extubation the patient admitted to using Metformin 500 mg twice daily, which was filled from an unlisted pharmacy. She was able to achieve recovery of creatinine to her baseline.

Discussion: The FDA received 47 confirmed reports of Metformin-associated lactic acidosis in 1996–the year after its introduction in the U.S. CKD is a major risk factor for this due to renal route of elimination. While our patient clearly did not meet the defined criteria for avoiding Metformin, she did have CKD IIIb based on her eGFR. This case highlights the need for a change in the current FDA guideline to an eGFR based approach which is the case in many countries. Thus, while Metformin is a safe and effective therapy for type 2 DM, its use carries a risk of lactic acidosis in our CKD population and prescribers must consider a dose reduction or discontinuation in the at-risk patient.

TH-PO049

An Unusual Cause of Acute Kidney Injury due to Oxalate Nephropathy in Systemic Scleroderma Heather M. Mascio,[1] Michael F. Flessner,[2] Thomas P. Baker,[3] Maryann T. Ally,[4] Christie Alyce Joya,[4] Christina M. Yuan,[1] Kevin C. Abbott,[1] Robert Nee.[1] *[1]Nephrology Service, Walter Reed National Military Medical Center, Bethesda, MD; [2]NIDDK, National Insts of Health, Bethesda, MD; [3]Joint Pathology Center, Defense Health Agency, Silver Spring, MD; [4]Dept of Medicine, Walter Reed National Military Medical Center, Bethesda, MD.*

Introduction: Oxalate nephropathy is an uncommon cause of acute kidney injury. Far rarer is its association with scleroderma, with only one other published case report in the literature.

Case Description: We present a case of a 75 year-old African-American female with a history of systemic scleroderma with chronic pseudo-obstruction and small bowel bacterial overgrowth (SIBO) treated with rifaximin admitted for anorexia. The patient was normotensive. Her initial laboratory evaluation showed creatinine 4.01 mg/dL (0.65 mg/dL one week prior). Urinalysis was unremarkable. She had no serum osmolar gap. Urine microscopy revealed calcium oxalate monohydrate crystals. An extensive serologic workup was negative. Renal ultrasound was unremarkable. A renal biopsy was performed, demonstrating a diffuse tubulo-interstitial process with numerous calcium oxalate crystals consistent with oxalate nephropathy. The patient was subsequently initiated on hemodialysis.

Discussion: In this case no obvious cause for her nephropathy was found. We hypothesize that her treatment with rifaximin for SIBO may have caused decolonization of oxalate degrading bacteria in her GI tract. Also, her chronic pseudo-obstruction with delayed intestinal transit may have increased intestinal oxalate absorption. In the previous published case report of scleroderma, oxalate nephropathy was attributed to malabsorption from chronic pancreatitis and the AKI resolved without requiring renal replacement therapy. Oxalate nephropathy should be considered in the differential diagnosis for unexplained acute kidney injury in scleroderma, particularly with normotension, and subsequent evaluation should be focused on bowel function to include alterations in the gut flora due to antibiotic administration.

TH-PO050

Acute Kidney Injury and Primary Hyperoxaluria Type 1: A Case Discussion and Review of the Literature Louis R. Spiegel, Shailaja Chidella, Sam Leung, Nicole M. Ali. *Hofstra North Shore - LIJ School of Medicine.*

Introduction: Primary hyperoxaluria is characterized by an overproduction of oxalate and an increased demand on the kidneys. Oxalate is renally excreted and if the GFR decreases to less than 30-45mL/min there is a tendency towards systemic oxalate deposition in a variety of organ systems. In the setting of decreased GFR the optimal method of oxalate removal is unknown. We present a patient with primary hyperoxaluria type 1, the most common form of the three primary hyperoxalurias, who develops acute kidney injury (AKI) and subsequently requires renal replacement therapy.

Case Description: A 61 year old female with Primary Hyperoxaluria Type 1 (PH Type I), hypertension, status post deceased donor renal transplant (DDRT) in 1999, chronic kidney disease (CKD) 4, presented with vomiting, decreased oral intake, diarrhea, decreased urine output and was found to have AKI with creatinine of 12mg/dL (baseline-2.6mg/dl),severe acidosis, anemia and thrombocytopenia. She was volume resuscitated and was initiated on aggressive hemodialysis (HD) for oxalate clearance. She underwent renal biopsy which revealed severe oxalate nephropathy and tubular injury. Bone marrow biopsy showed hypocellular bone marow, renal osteodystrophy and oxalate deposition. She continued to receive HD 6 days a week for 5 hours per session. Patient has subsequently received a combined liver-kidney transplant.

Discussion: Traditional methods of obtaining clearance, such as three times per week hemodialysis and daily peritoneal dialysis are unable to clear oxalate faster than it is generated in primary hyperoxaluria. Many different approaches have been attempted with varying success. Comparisons between 4 hours of daily HD to three times per week HD to hemodiafiltration have been performed, as well as combinations of HD and peritoneal dialysis with nocturnal hemodialysis.We present a unique case of a patient with PHT Type 1 responsive to pyridoxine who received a DDRT 13 years prior. Due to hemodynamic factors she developed AKI that resulted in impaired oxalate excretion and systemic deposition. We report our experience using 6 days per week, 5 hours per session hemodialysis.

TH-PO051

Novel Features of Methylmalonic Acidemia Associated Nephropathy Jatinder K. Hothi,[1] Luan D. Truong,[2] Biruh Workeneh.[1] *[1]Div of Nephrology, Baylor College of Medicine, Houston, TX; [2]Pathology, Houston Methodist Hospital, Houston, TX.*

Introduction: Patients with methylmalonic academia have in-born errors of propionate metabolism, but given the low incidence of the disease the renal lesions associated with the disorder have not been fully characterized.

Case Description: We report a case of a 20-year-old man who presented with a slightly elevated serum creatinine and proteinuria. A biopsy of his kidney revealed mild tubulointerstitial nephritis, which has been previously described, but also 2 novel features related to the glomerulus. The glomeruli showed focal mesangial sclerosis, which has not been described at early stage. Secondly, the pathology demonstrated glomerulomegaly (enlarged glomerular volume). The glomeruli on average were 260 microns in diameter, and this in addition to the focal mesangial sclerosis indicates a previously unreported pattern of injury. In addition to starting an ARB, the patient was given a course of corticosteroids empirically to determine if it would be beneficial, but a 3-month course did not result in improved serum creatinine or proteinuria.

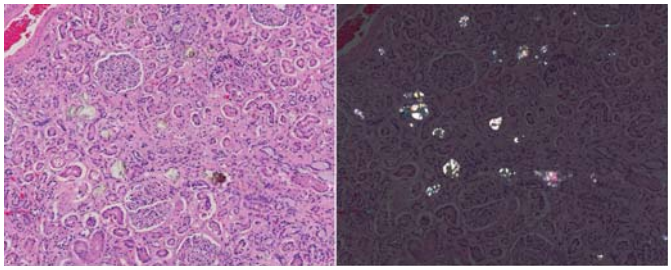
Discussion: If additional cases or review of pathologic lesions confirm the glomerulomegaly then this feature should be added to the description of pathologic injury pattern associated with MMA. Currently, there is no specific treatment for nephropathy associated with MMA and further study is necessary to determine whether specific therapy can be designed to prevent this complication in patients.

TH-PO052

Rapidly Progressive Renal Failure due to Oxalate Nephropathy in a Patient with Pancreatic Neuroendocrine Tumor Anna Malkina, Jean L. Olson, Ursula Lang, Raymond K. Hsu. *UCSF.*

Introduction: 50-year-old man with history of pancreatic neuroendocrine tumor presented with cachexia and non-oliguric acute kidney injury (AKI) with sub-nephrotic range proteinuria. Clinical history and non-invasive tests did not reveal the etiology or prognosis of AKI.

Case Description: Patient's history was notable for anorexia, 35 lbs unintentional weight loss in prior 10 months, absence of nutritional supplements use, and 14 year history of insulin-dependent diabetes mellitus. Neuroendocrine tumor was diagnosed 10 months ago with head of pancreas mass and metastases to 40% of liver parenchyma. Creatinine declined from 0.9 to 4.61 mg/dL in prior 4 months. Urine spot protein to creatinine ratio was 1.34 mg/mg. Renal ultrasound was without hydronephrosis, masses, or calculi. Urine sediment showed renal tubular epithelial cells. Patient was initiated on renal replacement therapy for treatment of severe metabolic acidosis and uremia. He sustained multi-organ failure with hypotension, hypercarbic respiratory failure, and pancytopenia; and was transitioned to comfort care. Autopsy revealed oxalate nephropathy with widespread tubular injury in the background of diabetic nephropathy.



Discussion: Oxalate deposition may lead to rapidly progressive renal failure in setting of enteric overabsorption in absence of excessive dietary oxalate intake. In this case pancreatic insufficiency due to tumor burden resulted in fat malabsorption. Accumulation of intestinal dihydroxy bile acids and fatty acids 1) increased intestinal mucosal permeability to oxalate, 2) increased amount of soluble oxalate that would otherwise complex with luminal calcium and be excreted in stool, and 3) led to inhibition of intestinal oxalate-degrading bacteria. Renal failure was due to secondary hyperoxaluria.

TH-PO053

Ibuprofen-Induced Coma and Renal Replacement Therapy Weeraporn Srisung,[1] Pavis Laengvejkal,[1] Kunut Kijsirichareanchai,[2] Sorot Phisitkul.[1] *[1]Texas Tech Univ HSC; [2]Univ of Nebraska.*

Introduction: Ibuprofen is a nonsteroidal anti-inflammatory drug that has been widely used for several years due to its relatively safe side-effect profile. Ibuprofen overdose is very common and intoxication usually occurs with ingestion exceeding 400 mg/kg. We report a case of an uncommon presentation of Ibuprofen-induced encephalopathy with severe neurological deficits mimicking brain death, which resolved with dialysis.

Case Description: A 21-year-old female presents with unresponsiveness 1 day after ingesting an unknown amount of Ibuprofen. She was intubated prior to arrival. She was hypotensive, tachycardic and afebrile. Respiration was fully supported by the ventilator. Her Glasgow Coma Scale was 2T. Pupils were 6 mm and unreactive to light. Gag, corneal and oculocephalic reflexes were absent with no muscle tone nor posturing. The remainder of the physical examination was unremarkable. Laboratory studies were notable for serum

pH 6.71, pCO2 34.4 mmHg, sodium 149 mEq/L, potassium 5 mEq/L, chloride 114 mEq/L, carbon dioxide 5 mEq/L, BUN 37 mg/dl, Creatinine 1.9 mg/dl, lactate 11 mmol/L, and no significant osmolal gap. Fractional excretion of sodium was 18.49%. She was polyuric. Renal replacement therapy was started. She became more responsive after four hours of treatment. A few hours later, she was able to follow simple commands. Her pupillary response returned and all other neurological deficits resolved. Her creatinine normalized prior to discharge.

Discussion: Ibuprofen-induced neurological deficits typically manifest as altered mentation, nystagmus, blurred vision, diplopia, and tinnitus. However, fixed dilated pupils and absent brainstem reflexes in this clinical setting are atypical. We suspect this patient's encephalopathy was secondary to Ibuprofen overdose and concomitant hypoperfusion with resultant acidosis. Dialysis does not have a clinically important effect on plasma clearance of Ibuprofen, so the patient's neurologic recovery is likely due to correction of acidosis. This case demonstrates that even in those with severely impaired neurologic status, renal replacement therapy can dramatically improve the clinical outcome in patients with ibuprofen-induced encephalopathy.

TH-PO054

Synthetic Cannabinoids and Acute Kidney Injury - A Case Series

Weeraporn Srisung, Sharma S. Prabhakar. *Internal Medicine, Texas Tech Univ HSC, Lubbock, TX.*

Introduction: Synthetic cannabinoids(SC) have been increasingly used for recreation, however, their side effects have not been well recognized. We report here 3 cases of SC-induced acute kidney injury(AKI).

Case Description: Case 1 A 31-year-old Hispanic male SC abuser presented after a physical assault. Significant soft tissue injury and fractures were excluded, but he was found to have high serum creatinine (SCr). Physical examination (PE) was unremarkable. Basal metabolic panel (BMP) showed BUN/Cr of 26/2.6. Urinalysis was negative. Fractional excretion of sodium (FENa) is 1.99%. Creatinine kinase (CK) was normal. Renal ultrasound (US) showed normal size kidneys without hydronephrosis. Soon SCr rose to 3.0 mg/dL despite intravenous fluid (IVF), however, it decreased to 1.4 mg/dL with good urine output before discharge on 6th day. **Case 2**. A 32-year-old male, drug abuser presented after amphetamine and SC overdose. PE was unremarkable. SCr was 1.1 mg/dl but rose to peak at 5.7 mg/dl despite IVF. Urine output dropped to 10-20 ml/hr. CK was 3,987 IU/L. FENa was 2.13%. Renal US showed no hydronephrosis. With dialysis and supportive care, he improved. SCr upon follow-up was 0.9 mg/dl. **Case 3.** A 31-year-old male was admitted for sensorial and behavioral problems. PE was unremarkable. Later on, he admitted to abusing SC daily. BMP showed BUN/SCr 25/3.5. FENa is 2.13%. CK was 1,137 IU/L. Urine studies and renal US were unremarkable. His SCr improved on the next day with IVF.

Discussion: SC are shown to have temporal association with AKI but the pathogenic mechanisms remain unclear. Renal biopsy showed acute tubular necrosis or acute interstitial nephritis although biopsies were done in only some of the published cases. XLR-11 or [1-(5-fluoropentyl)indol-3-yl]-(2,2,3,3-tetramethylcyclopropyl methadone) were found in products of SC used by subjects as well as clinical specimens in one case series. This raises hypothesis that XLR-11 or its metabolites might be the culprit causing AKI in this group. Potential binding of XLR-11 to renal cannabinoid receptor 1 could offer a possible pathogenic link. We conclude that AKI is a common complication of SC abuse and warrants increased awareness among medical and general communities.

TH-PO055

Correction of Acidemia and Cardiac Hemodynamics by Modified Continuous Veno-Venous Hemofilteration for Severe Lactic Acidosis

Gaurav Alreja, Joseph Palmisano, Andre A. Kaplan. *Internal Medicine, Univ of Connecticut, Farmington, CT.*

Introduction: Correction of severe lactic acidosis often results in extracellular volume expansion, hypocalcemia, hypernatremia, acceleration of lactate generation and worsening of acidemia. Previous studies failed to show improvement in hemodynamics or outcome with correction of lactic acidosis. We present a case with severe lactic acidosis that corrected with a modified replacement solution during continuous veno-venous hemofiltration (CVVH) resulting in marked improvement in cardiac hemodynamics.

Case Description: 61 year old female presented with elevated lactate level (10 mmol/L), liver damage (AST 13000 IU/L, ALT 5041 IU/L) and metabolic acidemia: HCO3 7meq/L, pH 7.094. CT scan showed cervical cancer, retroperitoneal lymphadenopathy and hepatic metastases. Despite aggressive bicarbonate administration (75 mEq/hr) and maximal minute ventilation, acidemia persisted and massive volume overload ensued. Swan Ganz catheterization showed hyperdynamic circulation with distributive shock (cardiac output 18.2 L/min, cardiac index 7.8L/min/m2, SVR 237 dyn/cm5, SvO2 82%). CVVH with predilution was initiated with a modified solution (150 mEq NaHCO3 in 1000 cc D5W) at a rate of 2000 cc/hr (delivering 300 mEq/hr NaHCO3). Although, serum lactate level increased from 10 to 30 mmol/L, both HCO3 level and pH improved to 23 meq/L and 7.42 respectively in a span of 7 hours. The patient received 14 gm of calcium gluconate during this time period through a separate infusion site. The patient's hemodynamic parameters improved significantly (Cardiac output 7L/min, SVR-800 dyn/cm5, and SvO2 63%) allowing discontinuation of vasopressors.

Time (Hours)	-11	0 (CVVH Started)	2	4	7
HCO3 (mEq/L)	7	10	14	19	23
Lactate (mmol/L)	10.7	23	27	29	30
pH	7.09	7.19	NA	NA	7.47

Discussion: Although prior studies of CVVH showed only 3% removal of generated lactic acid and difficulty in controlling acidemia (due to increased lactate generation with rising bicarbonate), the modified replacement solution utilized in this case may be valuable for correcting acidemia and improving cardiac hemodynamics. At the same time, ongoing CVVH was able to control hypervolemia, hypernatremia and hypocalcemia.

TH-PO056

Severe Tubulointerstitial Nephritis from Acute Epstein-Barr Virus Infection

Craig A. Mackaness. *Dept of Medicine, Nephrology Div, Lehigh Valley Health Network, Allentown, PA.*

Introduction: Infectious Mononucleosis from Epstein Barr Virus (EBV) is a rare etiology of severe acute renal failure.

Case Description: Our patient is a 21 year old Caucasian woman with no significant past medical history who presents to our center with complaints of a two week prodrome of nausea, vomiting, cough, sore throat, and malaise. Examination revealed a temperature of of 40 degrees C, bilateral pustular and exudative pharyngeal erythema. Tender and prominent anterior cervical lymphadenopathy with palpable hepatosplenomegaly were present. On presentation, serum creatinine was elevated at 2.24 which over the ensuing four days worsened to 7.1 mg/dL. Urinalysis demonstrated 300-600 protein with 3-5 RBC/hpf, 6-10 WBC/hpf and Granular casts of >10/lpf. A urine protein/creatinine ratio was elevated at 2.3. The patient's white blood cell count was elevated at 14,000 with 39% atypical lymphocytes. A Monospot was positive and EBV IgM was elevated at 1.68. An ASO titer was increased at 947 with a subseqently negative rapid strep and culture. Her renal function progressively worsened with oliguria. Serologic workup was otherwise negative and she underwent native kidney biopsy. The biopsy revealed patchy foci of active lymphocytic tubulitis, necrotic tubules with neutrophils, and many tubules with granular cell casts. These findings established the diagnosis of acute tubulointerstitial nephritis. EBV in situ hybridization stain (EBER) demonstrated a single small lymphocyte positive stain. She was placed on dexamethasone for her symptomatic tonsilar enlargement and concern for airway compromise. Her renal function dramatically improved over the next three days and she was discharged home without requring renal replacement therapy.

Discussion: Subclinical renal dysfunction from acute EBV is common occurring in as many as 16% of patients, (Infect Dis Clin Pract 2008; 16:127-128) but severe oliguric renal failure from acute EBV is rare. This patient had direct evidence of EBV virus in the renal parenchyma, suggesting pathogenesis. Clinicians treating patients with suspected or confirmed acute EBV should include monitoring of renal function for this rare, but serious complication.

TH-PO057

Case Report of Synthetic Cannabinoids Use Combined with Quetiapine Presenting as Rhabdomyolysis and Acute Kidney Injury

Aiyu Zhao, Maybel M. Tan, Moses A. Lee, Moro O. Salifu, Mary C. Mallappallil. *State Univ of New York at Downstate, Brooklyn, NY.*

Introduction: The use of synthetic cannabinoids (SCBs), commonly known as "Spice" or "K2", is associated with many severe adverse effects that are not observed with marijuana use. We are reporting a unique case of a patient who developed rhabdomyolysis and acute kidney injury (AKI) required dialysis after use of K2 combined with quetiapine.

Case Description: A 39 year old man with history of cannabis abuse and schizophrenia presented with generalized bodyache. He was using K2 daily for years more heavily for one week. He attempted suicide by ingesting 10 tablets of quetiapine the day before presentation. Physical exam was significant for moderate lower extremities edema and diffuse muscle tenderness without rigidity. Serum creatinine was 1md/dl one year ago which was 6.09 mg/dl on presentation and increased to 9.39 mg/dl. Peak serum Creatine Kinase (CK) level was 148,643 IU/L. Urinalysis showed muddy brown casts. Toxicity screen including urine cannabinoid was negative, which is expected with SCBs use. Fractional excretion of sodium was more than 1% compared to typical rhabdomyolysis-induced AKI when it is frequently less than 1%. All other workup for AKI was unrevealing. After trial of intravenous fluid, hemodialysis (HD) was initiated for anuria and persistent hyperkalemia. He required 10 sessions of HD after which he recovered renal function over 3 weeks. Serum creatinine was 2.25mg/dl and CK was 299 IU/L on discharge.

Discussion: Causes for the different adverse effects profile between SCBs and marijuana are not defined yet. Cases reported in literature with K2 use and AKI have been associated with reversible AKI characterized by acute tubular necrosis and interstitial nephritis. Recent studies has showed the involvement of cytochromes P450s (CYPs) in biotransformation of SCBs. The use of quetiapine which is a substrate of the CYP3A4 and is excreted 73% as urine metabolites may worsen the side effect profiles of both quetiapine and K2. SCBs use should be included in the differential diagnosis of AKI and CK level should be monitored. Further research is needed to identify the mechanism of SCBs nephrotoxicity.

TH-PO058

Renal Sarcoidosis with Vasculocentric Granulomatous Interstitial Nephritis Pattern

Swee-Ling Levea,[1] Thomas A. Golper,[1] Agnes B. Fogo.[2] *[1]Nephrology, Vanderbilt Univ Medical Center, Nashville, TN; [2]Pathology, Microbiology and Immunology, Vanderbilt Univ Medical Center, Nashville, TN.*

Introduction: Sarcoidosis is an inflammatory disorder characterized by non-caseating epitheloid cell granulomas that can involve multiple organ systems. Involvement of the kidneys is rare, most commonly showing granulomatous interstitial nephritis.

Case Description: A 69 years old white man with history of nephrolithiasis, prior pulmonary histoplasmosis, and chronic interstitial cystitis was referred by Infectious Disease for evaluation of elevated creatinine, 2.0mg/dL. He had a two month history of fever, night sweats and 20 lbs unintentional weight loss. Ground glass opacities on CT chest prompted a bronchoscopy with bronchoalveolar lavage and lung biopsy, which was negative for malignancy, granulomas or infections. Broad infectious workup was unremarkable including a tagged WBC scan. Renal biopsy was performed, which revealed a granulomatous interstitial nephritis with well-formed granulomas with epitheliod giant cells encroaching and invading vascular walls, but without necrosis. He was started on prednisone 1mg/kg/day for four months with minimal improvement in his renal function.

Discussion: Acute kidney injury with associated constitutional symptoms has a broad differential diagnosis. Renal sarcoidosis is rare and can manifest clinically as renal insufficiency, hypercalcemia, proteinuria or nephrocalcinosis. The renal lesions associated with sarcoidosis include non-caseating granulomatous interstitial nephritis, nephrolithiasis and rarely glomerular disease, including IgA nephropathy and proliferative or crescentic glomerulonephritis. The most common renal lesion is non-caseating granulomatous interstitial nephritis, and renal sarcoidosis is then a diagnosis of exclusion of other causes of granulomas. Vasculocentric granulomatous interstitial nephritis is a rare and unusual histologic variant of sarcoidosis, which carries a worse prognosis. In our patient, the biopsy finding of vasculocentric granulomatous interstitial nephritis in the absence of drug-induced, autoimmune or infectious cause is supportive of the diagnosis of renal sarcoidosis.

TH-PO059

Eight Cases of Acute Kidney Injury in Children Administered Tosufloxacin Tosilate and Non-Steroidal Anti-Inflammatory Drugs Hideki Matsumura,[1] Akira Ashida,[1] Akihiko Shirasu,[1] Hyogo Nkakura,[1] Motoshi Hattori,[2] Hiroshi Tamai.[1] *[1]Pediatrics, Osaka Medical College, Takatsuki, Osaka, Japan; [2]Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

Introduction: Tosufloxacin tosilate (TFLX), a fluoroquinolone antimicrobial, and non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used individually on a safe basis, but few reports have documented their association with AKI. Here, we describe eight cases of AKI that occurred after administration of TFLX and NSAIDs in children.

Case Description: The age of eight patients was ranging from 8 to 13 years. All patients visited their family physician because of fever, and were administrated drugs including TFLX and NSAIDs. Most patients suffered vomiting after drug administration. Patients visited our hospital 1 to 4 days after starting the drugs, and AKI then became apparent. Two patients received overdoses of TFLX, one received a low dose and the remaining five received appropriate doses. Their maximum levels of plasma creatinine were 0.70 – 2.90 mg/mL. Urine volumes were not decreased in all patients, and four patients had occult hematuria with isomorphic erythrocytes. Markers of AKI were not elevated. Renal functions of all patients became normalized spontaneously within five days after discontinuation of the drugs.

Discussion: As AKI resolved spontaneously and quickly, tubulointerstitial nephritis and acute tubular necrosis were unlikely. Because we did not perform biopsy, it was difficult to make an accurate diagnosis of AKI, but hematuria with isomorphic erythrocytes in some patients suggested crystal nephropathy. Our hypothesis for the mechanism of AKI is that administration of NSAIDs under conditions with insufficient oral intake results in decreased renal and urine flow. Formation of TFLX crystals is facilitated by slow urine flow, resulting in crystal nephropathy. The crystals are washed out and the AKI resolves quickly after discontinuation of TFLX and NSAIDs. Some reports have documented crystal nephropathy after long-term use of fluoroquinolones. As our patient who took NSAIDs and TFLX only once also developed AKI, we speculate that AKI due to these combined medications was not related to the dose or duration of therapy.

TH-PO060

Relapsing Interstitial Nephritis Secondary to DRESS Syndrome Abhijin Das, Elijah Stiefel, Lauren C. Hughey, Huma Fatima, Eric L. Wallace. *UAB, Birmingham, AL.*

Introduction: Allergic interstitial nephritis (AIN) is a well known cause of kidney injury and presents with eosinophiluria, rash, and can be accompanied by fever. Differentiating simple AIN from DRESS, drug reaction with eosinophilia and systemic symtpoms, is difficult but important as more aggressive management of DRESS is necessary as it carries significant morbidity and mortality.

Case Description: A 36 year old female recently treated with terbinafine for onychomycosis presented with fever, to 105^0 F and emesis prompting her to go to her local emergency room(ER). Baseline laboratory evaluation revealed a creatinine of 9.8 mg/dl. Urinalysis dipstick showed 500mg of proteinuria and trace blood and renal ultrasound showed 14cm kidneys bilaterally. Hemodialysis was initiated. Kidney biopsy revealed acute granulomatous interstitial nephritis. The drug was discontinued, and creatinine improved to 2.7mg/dl. She was discharged on prednisone. 3 days later she presented again to the ER with fever to103.8 F. Work up for infectious causes and autoimmune disorders were negative. During this hospitalization, kideny failure recurred requiring reinstitution of dialysis. Furthermore, she developed pancytopenia and erythroderma. Skin biopsy showed necrotic cells at all levels of the epidermis. The dermis contained a sparse perivascular infiltrate with occasional eosoniphils. Taken together with her relapsing course and systemic symptoms a diagnosis of DRESS was made. Due to lack of response with steroids and the desire to avoid cyclosporine,intravenous immunoglobulin and mycophenolate were administered. The patient had improvement in her rash and blood counts. Kidney function recovered to a last creatinine of 1.7mg/dl. HHV-6 viral PCR which has been associated with DRESS was negative 2 months after the episode. 5 months after discharge, she remains off dialysis and without further relapse.

Discussion: DRESS syndrome should be considered in patients with a drug history and AIN when it is associated with systemic manifestations and particularly when those manifestations have a relapsing and remitting course. Correct diagnosis is crucial as DRESS can carry significant mortality and aggressive treatment is needed to prevent subsequent relapses.

TH-PO061

Acute Kidney Injury (AKI) Caused by Synthetic Cannabinoids (SC) in 3 Teens: A Growing but Under-Recognized and Preventable Public Health Problem Saurabh Dasgupta,[1,2] Pavan Kumar Gona,[1] David A. Myers,[2] Kai Lau.[1] *[1]Medicine, Univ of Oklahoma, OKC, OK; [2]Pediatrics, Univ of Oklahoma, OKC, OK.*

Introduction: Since 2009, "herbal incenses" are increasingly popular as many contain SC escaping urine drug screen (UDS). Impaired cognition, acute coronary syndrome and seizures have been caused by these agents, besides AKI in 22 cases in U.S. as of 2013. We recently cared for 3 teens with AKI due to SC. We here share our experience on this issue.

Case Description: The clinical course, lab, therapy and follow up data were reviewed to infer insights.

Case 1, a boy aged 15, and case 2, a girl aged 15, were admitted for nausea and vomiting. Initial serum creatinine (Scr) was 7.1 and 3.0 mg%. It peaked respectively at 11.5 and 6.3 mg% 5 d later. Both admitted to using "Flame 2." The boy was hemodialysed (HD) 4 x in 9 d. Scr fell to 4.3 by d 11 and spontaneously to 2.4 mg% by d 13 and to 1 mg% 4 wk later. The girl was HD x 2. Scr fell to 2.3 on d 5, then spontaneously to 0.9 on d 9 and to 0.6 mg% 4 wk later. Case 3, a boy aged 17 admitted for altered mental status and seizures, had initial Scr of 4.6 and peak of 8.4 mg% on d 5. Fluids were given. Hyperuricemia (17.9 mg %) was treated. He admitted to using K2. Without HD, Scr fell to 1.7 mg% on d 8 and 0.9 mg% on d 15. None had oliguria or eosinophiliuria. All 3 biopsies showed acute tubular necrosis (ATN). As all known causes of AKI were excluded and the clinical course resembled published cases, SC-induced AKI was diagnosed.

Discussion: We confirm and extend published experience on the entity of SC-induced AKI. The specific nephrotoxin and exact mechanism are obscure. ATN is supported by pathology and typical clinical course, with Scr peaking ~5 d post exposure, then initial and eventually full recovery in 2-4 wk. Long-term renal impact is unknown. Legalizing marijuana expands access but also increases the incidence of SC and AKI. Our data illustrate the need for raised awareness of "herbal incenses" by all physicians, for uncovering relevant history and for recognizing various presentations. SC eludes current UDS and diagnosis needs a high index of suspicion. We urge counselors and student health staff to be familiar with SC-induced AKI and play active roles in education and prevention.

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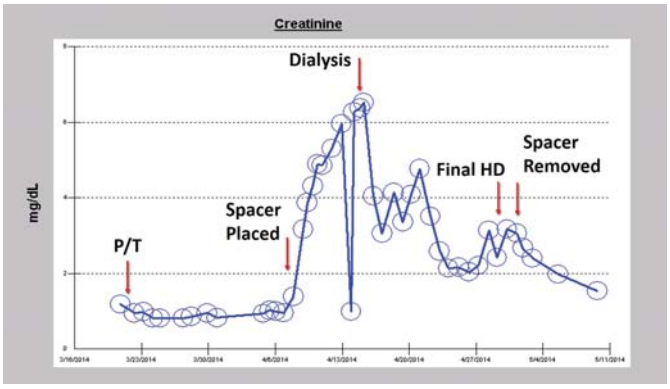
TH-PO062

Multifactorial Acute Kidney Injury and Polymethylmethacrylate Joint Spacers; Who to Blame? Sarah A. Johnson,[1] Thomas E. Rogers,[2] Frederic F. Rahbari-Oskoui.[1] *[1]Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA; [2]Dept of Pathology, Emory Univ School of Medicine, Atlanta, GA.*

Introduction: Antibiotic impregnated polymethylmethacrylate (PMMA) spacers are used for treatment of recurrent prosthetic joint infections assuring local delivery. We report a case of a patient with bilateral septic arthritis with acute tubulointerstitial nephritis (TIN) secondary to piperacillin/tazobactam (P/T), then complicated by aminoglycoside (AG) induced acute kidney injury (AKI) because of the PMMA spacer.

Case Description: Case Description: 58 yo male with melanoma on steroids post parietal mass resection presented with hip pain found to have septic arthritis with a normal serum creatinine (SC). P/T was started but caused a diffuse macular rash on day 5. P/T was changed to ceftriaxone and then levofloxacin (LFX). Recurrent sepsis on day 16 necessitated re-debridement of bilateral hips with insertion of a PMMA spacer containing 3.6 gm of tobramycin (TM) and 3 gm of vancomycin on day 18. Day 19, SC rose to 3.1 mg/dl with a peripheral eosinophilia of 940 cells/mcl. Prednisone at 40 mg daily was started. Serum TM level was 2.5 mcg/ml on Day 20 and peaked at 4.5 mcg/ml with a peak SC of 6.5 mg/ml. Daily hemodialysis was initiated for TM clearance but failed to maintain serum TM levels below 1 ug/ml. A renal biopsy showed TIN, mild tubular necrosis and tubular vacuolization consistent with TM injury. PMMA spacer was removed after which the patient's SC and TM levels rapidly improved (1.5 mg/ml and < 0.5 ug/ml respectively).



Discussion: AG-impregnated PMMA spacers may cause persistent AKI and should be removed if the serum AG levels are in the toxic range and they are not necessary for vital prognosis. Rare case reports describe renal injury secondary to PMMA spacers. The renal safety profile and kinetics of these spacers is undetermined and requires further investigation.

TH-PO063

Safety of Warfarin in Chronic Kidney Disease Patients with Solitary Kidney in the Era of Warfarin-Related Nephropathy

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Introduction: Independent of systemic hemorrhage, warfarin coagulopathy is an emerging phenomenon in the pathogenesis of AKI especially in CKD patients. Warfarin-Related Nephropathy (WRN) was first described by Brodsky et al in 2009, as an acute kidney injury secondary to glomerular hemorrhage and renal tubular obstruction by RBC casts. We report a case of WRN in a high risk geriatric patient with stable CKD and solitary kidney.

Case Description: An 80 years old male with medical history of controlled HTN, chronic atrial fibrillation, stage 3 CKD (baseline Cr 1.8-2.2 mg/dl) and left nephrectomy due to low grade papillary urothelial carcinoma, who presented to the hospital with dyspnea, peripheral edema and decreased urine volume for a week. He has been on warfarin for many years with no history of coagulopathy. Laboratory and radiological data was pertinent for elevated Cr (15.2mg/dl), BUN (124mg/dl), INR (5.1), normal CBC, bland urine sediment and unremarkable renal US. He was diagnosed with oligouric AKI of unclear etiology after exclusion of pre-renal and post renal causes. Despite IV fluids hydration, holding ACE inhibitor and normalization of coagulopathy, his renal function did not improve. As he developed uremic symptoms, hemodialysis was initiated after which his encephalopathy, volume and acid-base status became satisfactory. A renal biopsy was then performed which revealed glomerular hemorrhage and RBC casts obstructing numerous tubules suggestive of WRN. Unfortunately, renal function did not recover and patient progressed to ESRD.

Discussion: Our case illustrates the high risk associated with warfarin therapy in CKD population even with mild degree of coagulopathy and regardless of anticoagulation therapy duration. This risk is even higher in a setting of solitary kidney due to diagnostic dilemma and fear of biopsy complications. This case may be an example of when anticoagulation monitoring should be very intense and the decision to remain on warfarin is thoughtfully reviewed.

TH-PO064

A Unique Case of Pemetrexed-Associated Renal Injury

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Introduction: Pemetrexed is a novel antifolate chemotherapy increasingly used in treatment of metastatic non-small cell lung cancer. Acute renal injury is infrequently reported as a side effect of this agent. Vootukuru et al. discussed the association of AKI with nephrogenic diabetes Insipidus (DI) and distal renal tubular acidosis (RTA) as manifestations of drug-related renal toxicity.

Case Description: Herein, we present a 69 year-old gentleman with metastatic non-small cell lung cancer who developed acute kidney injury in association with nephrogenic DI and distal RTA following sequential treatments with Pemetrexed. Patient presented to the hospital with worsening fatigue and shortness of breath, along with 1 month of polyuria and polydipsia. Patient had been treated with pemetrexid over the last seven months and received his 7th cycle two weeks prior to presentation. Patient had also received four cycles of Carboplatin and Taxol over one year prior to initiating pemetrexid with no changes in GFR (Cr 0.8mg/dl). Patient was euvolemic on exam with stable Vitals, producing 4 liters of urine/day with intact thirst sensation. Serum creatinine was elevated (3.6mg/dl) Na 142mg/dl, Glucose 84mg/dl, HCO3 15mmol per liter, Albumin 3.5 g/dl. Venous blood gas revealed Ph 7.30, CO2 33, AG 8. This was consistent with nonanion gap metabolic acidosis. Urine analysis showed Urine PH of 6.0. Specific gravity 1.005, with urine AG 13. Urinary gap in the setting of Non-AGMA was suggestive of distal RTA. Diagnosis of RTA should be considered with caution in the setting of AKI. However, metabolic panel and VBG consistent with RTA were evident three months prior to the elevation in creatinine. Urine microscopy showed several muddy granular casts consistent with ATN. Nephrogenic DI was also diagnosed based on Urine specific gravity, polyuria and failure of improvement in SG with water deprivation. Desmopressin test was not done but brain MRI did not show any evidence to account for the DI.

Discussion: Pemetrexed-associated renal toxicity can manifest as a unique pattern of distal tubular dysfunction. Distal RTA can precede and potentially portend a decline in renal function.

TH-PO065

Filgastrim Nephrotoxicity due to Capillary Leak Syndrome

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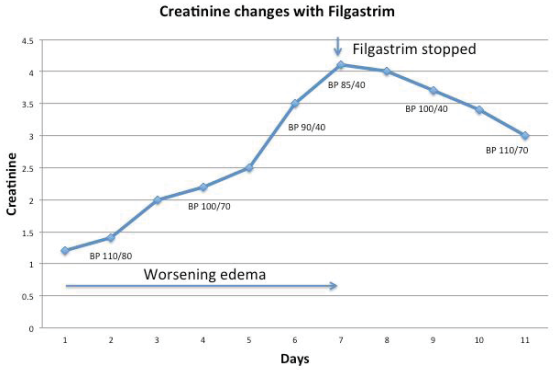
Introduction: Filgastrim (Neupogen) is frequently used in Hematology/Oncology. It is indicated to decrease the incidence of infection and neutropenic fever in patients with these conditions. This medication has many side effects, which include hypertension, ostealgia, nausea, leukocytosis, and capillary leak syndrome. Capillary leak syndrome presents in less than 1% of the patients and is a rare disorder that is due to endothelial dysfunction with subsequent leakage of fluid into the interstitial space and decreased organ perfusion.

Case Description: A 60-year-old man with autologous stem cell transplant due to primary Amyloidosis was admitted to the hospital due to neutropenia, he was started on Filgastrim. Two days after Filgastrim was started, the patient's renal function deteriorated. Nephrology was consulted for Acute Kidney Injury (AKI); baseline serum creatinine was 1.2 mg/dL and increased to 4.1 mg/dL



On physical exam, his systolic blood pressures were between 80-110 with notable lower extremity edema. Laboratory data showed hypoalbuminemia and renal ultrasound was normal. Creatinine peaked at 4.1 mg/dL and with the clinical findings suggestive of capillary leak syndrome, a decision was made with the Hematology service to stop the Filgastrim. The renal function improved and creatinine decrease to 1.4 mg/dL.

Discussion: Acute Kidney Injury (AKI) in patients with hemato-oncological conditions is frequent; it could be related to pre-renal, intrinsic, and/or post-renal conditions. Filgastrim rarely affects the kidney and when it happens, it is due to a described mesangioproliferative glomerulonephritis. This is the first case in the literature of AKI due to Filgastrim-induced capillary leak syndrome. This is a diagnosis of exclusion and Nephrologist and Oncologists need to be aware of this potential side effect and complication of this medication.

TH-PO066

Xanthine Nephropathy, a Rare Manifestation of Tumor Lysis Syndrome

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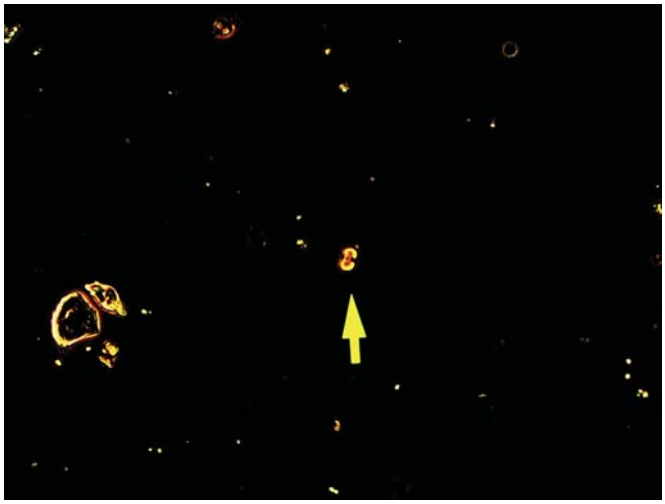
Introduction: Tumor lysis syndrome is an important cause of renal failure in hematological malignancies. Tumor cells lyse spontaneously or due to therapy. Renal failure results from deposition of uric acid, calcium phosphate and xanthine which are released from cells. Allopurinol used to prevent tumor lysis syndrome paradoxically causes renal failure in some patients due to xanthine crystallization. We present a case of xanthine nephropathy in a patient with T cell lymphoma.

Case Description: A 78 year old gentleman with history of chronic lymphocytic leukemia, chronic kidney disease and hypertension was admitted with pneumonia and pancytopenia. Bone marrow biopsy revealed a new diagnosis of T cell lymphoma. His baseline creatinine was 1.4 mg/dl which was elevated to 2.0 mg/dl. Chemotherapy with high dose steroid and nitrogen mustard was initiated after treatment of pneumonia. His acute renal failure progressed despite optimization of volume and discontinuation of nephrotoxic agents. Urine analysis showed ph 5.2, hyaline cases and amorphous xanthine crystals.



Serum uric acid and phosphorus levels were elevated consistent with tumor lysis. Renal biopsy was deferred due to thrombocytopenia. Urinary xanthine and hypoxanthine levels were measured due to suspicion for xanthine nephropathy and found to be very elevated. Hydration and discontinuation of allopurinol resulted in improvement of renal function.

Discussion: Xanthine nephropathy should be suspected in patients who develop acute renal failure despite tumor lysis prophylaxis. Accumulation of xanthine is due to the inhibition of xanthine oxidase by allopurinol. This paradoxically causes acute renal failure due to xanthine toxicity. Measurement of xanthine levels in the urine aids in the diagnosis. Allopurinol should be discontinued to prevent further renal injury.

TH-PO067

Mannitol-Induced Acute Renal Failure (ARF): Prompt and Full Reversal by Solute-Free Water Supports the Pathogenic Role of Hyperosmolality in Osmotic Nephrosis Joe Ghata,[1] Pavan Kumar Gona,[1] Kai Lau.[1,2] *[1]Nephrology, Univ of Oklahoma, OKC, OK; [2]Medicine, VAMC, OKC, OK.*

Introduction: Osmotic nephrosis refers to ARF following sucrose, dextrans, dye or mannitol. Morphologically there is proximal tubule cell vacuolization, swelling and collapsed lumens. The pathophysiology is unknown with theories ranging from volume depletion, tubuloglomerular feedback activated by increased distal delivery, afferent arteriolar vasoconstriction, to proximal cell swelling occluding lumen. To date, treatment is stopping mannitol and hemodialysis.

Case Description: The most proximate factor is marked hyperosmolality from excessive mannitol. We tested the therapeutic role of diluting mannitol and serum (S) Osm by solute-free fluids in a 64-year-old man with mannitol-induced ARF. He had a recent resection of recurrent glioblastoma multiforme but developed weakness and dysarthria.

For cerebral edema, he received 7 doses of 100 g of mannitol in 1 h q 6 h (total of 700 mg in 44 h). S creatinine (cre) rose from 1 to 2.5 mg% in 2 d. S Na fell from 137 to 124 mM in 2 d, measured S osmolality (Osm) rose from 293 to 333 mosm/kg whereas calculated S Osm fell from 282 to 272, yielding an osmolar gap of 62. S cre climbed to 5.9 by d 4. There were no signs of sepsis, hypotension, volume depletion or nephrotoxic drug. In response to 2 L of D5W in 36 h, S Osm fell from peak of 340 to 299 in 2 d and to 291 in 3 d as S cre fell promptly from 5.9 to 2.2 in 2 d and to the baseline of 1.1 by d 3. eGRF was inversely related to S Osm ($r=-0.92$, $p<0.001$) and to S osmolal gap ($r=-0.93$, $p<0.001$), indicating the higher the mannitol level, the worse the renal function. eGFR was negatively related to cumulative fluid balance, arguing against volume depletion. 29 cases of mannitol-induced ARF with full recovery are available for full review. 22 were successfully treated by dialysis, 7 by supportive measures (recovery in 8 d) and none by dilute fluids.

Discussion: Our patient confirms the nephrotoxic risk of mannitol infused at high dose and fast rate. The data show the utility of osmolal gap in estimating mannitol level to guide dosing. We show the efficacy of solute-free solution in reducing levels and supports the pathogenic role of hyperosmolality.

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

TH-PO068

Granulomatous Interstitial Nephritis with Rare Circulating Lymphocytes Positive for Epstein-Barr Virus Encoded Small Ribonucleic Acid (EBER-1) in a Patient with Crohn's Disease Vanessa Moreno,[1] Laura R. Kidd,[1] Constance C. Foreman,[2] J. Bryan Carmody,[3] William F. Glass.[1] *[1]Pathology and Laboratory Medicine, The Univ of Texas - Health Science Center, Houston, TX; [2]Eastern Virginia Medical School, Norfolk, VA; [3]Nephrology, The Children's Hospital of the King's Daughters, Norfolk, VA.*

Introduction: Granulomatous interstitial nephritis (GIN) is an uncommon disorder found in <1% of all renal biopsies. Several cases of GIN-associated with Crohn's disease (CD) have been reported in adults, with only 3 pediatric cases described in the literature. The majority of these pediatric patients did not receive 5-ASA therapy and have occurred during the exacerbation of CD.

Case Description: We describe a case of GIN-associated CD in a 16 year old African American male who received treatment with 5-ASA for one month, followed by two months of prednisone. His baseline creatinine (Cr) was 0.8 prior to administration of 5-ASA and 1.1 mg/dl afterward. Two months after stopping 5-ASA, he developed gastrointestinal CD exacerbation, retinal vein thrombosis, and acute renal failure with elevated BUN (45 mg/dl) and Cr (2 mg/dl). Urinalysis showed trace proteinuria, 25-50 WBC/hpf and 3-5 RBC/hpf. Urine culture was negative. Protein/creatinine ratio was 0.7 mg/mg. Serology was positive for ANA and Epstein-Barr virus (EBV), which was consistent with past infection (VCA IgG+, VCA IgM-, EA-, EBNA+) and reactivation (EBV viral load by PCR of 2406 copies/ml). The kidney biopsy revealed chronic GIN, consistent with extraintestinal CD. In addition, EBER-1 in situ hybridization was positive in rare circulating lymphocytes within peritubular capillaries whereas the granulomas, infiltrating lymphocytes and plasma cells were negative, suggestive of EBV reactivation and not an EBV+ lymphoproliferative disorder.

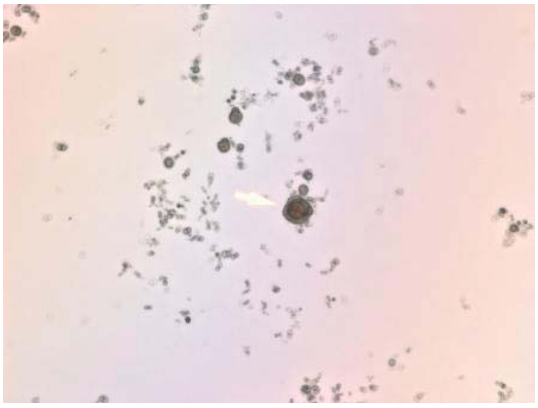
Discussion: These observations suggest the possibility that reactivation of EBV plays a role in the pathogenesis and exacerbation of this patient's CD, including involvement of extraintestinal tissues. We described the fourth case of GIN-associated CD in the pediatric population and the first known case of this entity associated with EBV reactivation and retinal vein thrombosis in a child.

TH-PO069

Xanthinuria: An Unusual Cause of Pink Diaper in an Infant Rasheda Z. Amin,[1] Loai Akram Eid,[2] Vidar O. Edvardsson,[3] Asha Moudgil.[1] *[1]Pediatric Nephrology, Children's National Medical Center, Washington, DC; [2]Pediatrics, Latifa Hospital, Dubai, United Arab Emirates; [3]Pediatric Nephrology, Landspital Univ Hospital, Reykjavik, Iceland.*

Introduction: Hereditary xanthinuria, an autosomal recessive disorder of purine metabolism, commonly presents as urolithiasis and rarely with renal failure. We report the case of an infant with pink diaper stains and failure to thrive who was found to have hereditary xanthinuria.

Case Description: A nine month old, full term, white, male infant presented with intermittent pink diaper staining and failure to thrive for five months. Past medical and family history, review of systems and physical exam were unremarkable. Urine microscopy of a first morning void showed crystals closely resembling 2,8 Dihydroxyadenine, seen in adenine phosphoribosyltransferase deficiency.



Workup showed increased urinary excretion of xanthine and hypoxanthine, low serum and urine levels of uric acid, highly suggestive of hereditary xanthinuria. Genetic testing of the proband revealed a known heterozygous mutation, T910M, in exon 25 of the XDH gene (c.2729C>T) and a second, previously unidentified, heterozygous variant, R830C, in exon 23 of the XDH gene (c.2488C>T), confirming the diagnosis. The proband's mother was heterozygous for the T910M mutation and his father was heterozygous for the R830C variant. The infant is being successfully managed with a low purine diet and high fluid intake with resolution of crystalluria and improved growth. Subsequent urine exams have not shown crystals, but pink diaper staining recurs in episodes of acute illness. His urine xanthine- and hypoxanthine-to-creatinine ratios remain elevated.

Discussion: Our case highlights the importance of manual urine microscopy for detection of crystalluria on a first morning void. It underscores hypouricemia as an important diagnostic aid. We report a new, likely pathogenic variant, R830C, in the XDH gene.

TH-PO070

Disseminated Histoplasmosis in Immunocompetent Female Presenting as Pseudoobstructive Uropathy Buthayna Dinary, Keyvan Ravakhah. *St.Vincent Medical Center, Cleveland, OH.*

Introduction: Progressive Disseminated Histoplasmosis (PDH) is a rare condition in immunocompetent host. This endemic mycosis can affect multiple systems including skin, lungs, GI, kidney, adrenal glands, blood and central nervous system. PDH characterized by variable Clinical presentation that ranges from asymptomatic infection to life-threatening illness. Here we describe a case of sudden onset of acute renal failure (ARF), hydronephrosis, in association with diffuse retroperitoneal lymphadenopathy in the context of disseminated histoplasmosis.

Case Description: A 74 year-old previously healthy female resident of Ohio presented with intermittent dyspepsia, 30- lbs weight loss and decreased appetite for 1 month duration. Upper endoscopy revealed a duodenal ulcer and biopsy was positive for H. pylori. She was treated with omeprazole, amoxicillin and clarithromycin. Her condition improved partially, but 6 weeks later she was readmitted to the hospital with dull abdominal pain, fever and decreased urine output. On physical exam she was febrile and had bilateral flank tenderness and left leg pitting edema. Her WBC was 12.6 k/ul with a neutrophil of 86%. Laboratory studies showed potassium (6.4mmol/l), creatinine (3.1 mg/dl), BUN (70 mg/dl), Hg (10.9 gm/dl) and proteinuria. US showed bilateral hydronephrosis. CT abdomen revealed retroperitoneal LAP. Duplex US of renal veins revealed left RVT. Lymph node biopsy demonstrated diffuse collections of large histiocytes with oval narrow-based budding yeast. She was found to be negative for HIV. Fungal serologies and Blood cultures were negative. She was hydrated, anticoagulated and treated with IV amphotericin for 2 weeks. Patient creatinine improved to 2.5 mg/dl. She was discharged on Lovenox and itraconazole for 6 months.

Discussion: Disseminated Histoplasmosis should be suspected in any patient presenting with unexplained ARF and enlarged kidneys, especially in the setting of widespread lymphadenopathy. Prompt diagnosis of PDH improves chances of preserving renal function. Liposomal amphotericin B are typically used to treat PDH, followed by Itraconazole. The outlook is usually good, but, if not appropriately diagnosed or treated, the disease can cause severe complications, such as ESRD.

TH-PO071

An Interesting Case of Iced Tea Nephropathy Fahd Syed,[1] Alejandra Mena-Gutierrez,[1] Neriman Gokden,[1] Sudhir V. Shah,[1,2] Umbar Ghaffar.[1,2] *[1]Div of Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Renal Section, Medicine Service, Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Introduction: Oxalate nephropathy caused by a combination of tubular toxicity, nephrocalcinosis, and obstruction from stones has been reported in the setting of primary and acquired hyperoxaluria. We present the case of a patient with kidney biopsy findings of oxalate nephropathy in which dietary history revealed excessive intake of iced tea as a potential etiology.

Case Description: A 56-year-old male presented to the hospital in 5/14 with weakness, fatigue, body aches and pains and his serum creatinine was 4.5 mg/dL. Review of his medical record indicated a creatinine level of 1.2 mg/dL in 10/13 and 2.5 mg/dL in 2/14. He had no proteinuria or hematuria. His urine sediment was remarkable for the presence of numerous calcium oxalate crystals. He did not have a past history of kidney stones or any family history of renal disease. He denied using ethylene glycol. Due to the rapidly progressive nature of renal failure and normal kidney size on ultrasound, a renal biopsy was performed which demonstrated many oxalate crystals, interstitial inflammation with eosinophils and interstitial edema consistent with a diagnosis of oxalate nephropathy. A 24-hour urinary collection revealed oxalate of 99 mg (7-44). No evidence of malabsorption or any dietary history previously associated with oxalate nephropathy was found. On further review of the patient's history, the patient admitted to drinking 16 glasses of iced tea daily. Advanced renal failure necessitated initiation of dialysis.

Discussion: Black tea has high oxalate content (1150 mg /100g) which is higher than several other foods like rhubarb, star fruit and fruit juice diets which have been reported to cause oxalate nephropathy. We are not aware of any reports of oxalate nephropathy from excessive tea ingestion. A finding of large amount calcium oxalate crystals in urine sediment in a setting of renal failure of unknown etiology led to kidney biopsy and to a detailed dietary history resulting in this interesting diagnosis of iced tea nephropathy.

TH-PO072

Primary Sjogren Syndrome with Renal Fanconi Syndrome Jing Wang, Xiaoxiao Shi, Yubing Wen, Xue-Mei Li, Limeng Chen. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Introduction: Proximal renal tubular acidosis (RTA) presenting as Fanconi Syndrome is rarely seen in Primary Sjogren Syndrome (pSS). We reported 4 cases of pSS with Fanconi Syndrome.

Case Description: Renal biopsy were performed in 117 pSS patients diagnosed by the 2002 American-European consensus classification criteria, in Peking Union Medical College Hospital. Clinical records were reviewed.

Remarkable tubulointerstitial lesion were observed in 55 cases (47.0%), 87.5% of them developed distal RTA. Only 4 cases were characterized by Fanconi Syndrome.

	Patient 1	Patient 2	Patient 3	Patient 4
Age/Gender	41/F	31/F	41/F	35/F
Clinical Characteristic				
Blood Pressure (mmHg)	105/65	100/60	105/70	110/70
Polyuria	-	+	-	-
Osteopathy	-	+	+	-
Fatigue	+	+	+	+
Anoxia	+	+	+	+
Proximal RTA	+	+	+	+
Aminoaciduria	+	-	+	-
Glycosuria	+	-	+	+
Blood Evaluation				
HGB (g/L)	98↓	77↓	125	81↓
Scr (μmol/L)	151↑	88	176↑	309↑
eGFR (ml/min/1.73 ㎡)	37	75	30	16
Na (mmol/L)	NA	139.3	143.0	140.6
K (mmol/L)	3.40↓	2.10↓	3.30↓	2.70↓
Ca (mmol/L)	2.28	2.10↓	2.42	2.37
P (mmol/L)	0.75↓	0.54↓	0.80↓	0.65↓
UA (μmol/)	NA	302	91↓	132↓
Urine Analysis				
Urine pH	6.0	8.5↑	7.0	6.5
24h-Urine Protein (g/24h)	0.92	0.92	0.32	1.90
Immune Profile				
ESR(mm/h)	68↑	NA	72	108
hsCRP(mg/L)	2.5	NA	2.6	NA
ANA	1:80	1:160	1:64	1:80
SSA/SSB	-	1:64/1:4	-	-
C3/C4	C3↓	NA	Normal	Normal
IgG (g/L)	20.9↑	21.4↑	17.6↑	17.9↑
IgM (g/L)	4.32	1.78	11.2↑	1.63
IgA (g/L)	1.41	2.77	4.38	4.14
Confirmation of pSS Diagnosis				
Ocular Symptoms	+	+	+	+
Oral Symptoms	+	+	+	+
Ocular Signs	+	+	+	+
Salivary Flow Test	+	NA	+	-
Labial Biopsy	NA	NA	+	NA

Renal biopsy revealed typical tubulointerstitial nephritis, with tubule epithelial cell degeneration, mild tubule atrophy, interstitial inflammation and fibrosis. After treating with steroid (1mg/kg/d),improvement of symptom, electrolyte disorder and Scr level(8%-65% decline)were observed.

Discussion: We reported 4 cases of pSS with Fanconi Syndrome,with coexisting acute or chronic tubulointerstitial lesion and renal function injury. Patients generally showed good response to steroid.

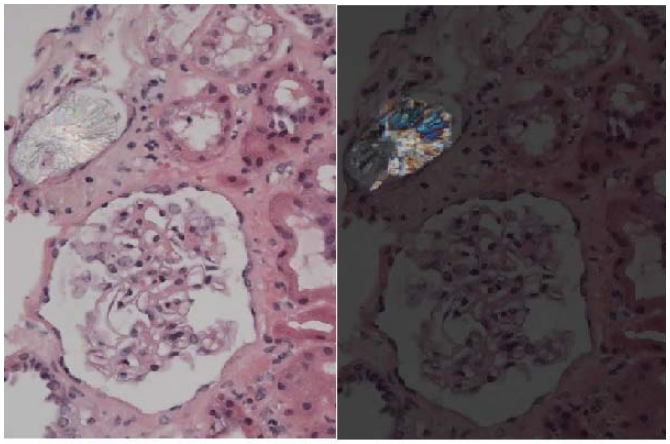
Funding: Government Support - Non-U.S.

TH-PO073

Chronic Nephropathy from Dietary Hyperoxaluria: Sustained Improvement of Renal Function after Dietary Intervention Nhan Trong Luu,[1,2] B. Horowitz,[1,2] Antonios Tzamaloukas.[1,2] *[1]Nephrology, Univ of New Mexico, Albuquerque, NM; [2]Nephrology, Dept of Veteran Affairs, Albuquerque, NM.*

Introduction: Calcium oxalate may cause renal parenchymal damage by urolithiasis, nephrocalcinosis, acute kidney injury (AKI) and chronic kidney disease (CKD). Few patients with hyperoxaluric CKD have been reported. We present a case of CKD which resulted from dietary oxalate, with renal function improvement after reduction in dietary oxalate intake.

Case Description: A 56-year old man with CKD which had been stable for two years following a single episode of calcium oxalate urolithiasis developed progressive elevation of his serum creatinine with eGFR from 60 to 15 mL/min per 1.73 m2. Basic workups including urinalysis, urine microscopy, and gallium scintigraphy disclosed chronic interstitial nephritis. Culture was sterile. Despite discontinuing proton pump inhibitor and initiation of a short course of oral corticosteroids, pyuria persisted and renal function declined. Renal biopsy is shown below:



Histology showed deposition of oxalate crystals in the tubules and interstitium, pronounced tubular changes, interstitial nephritis and interstitial fibrosis.

Urinary oxalate excretion was more than 5x the upper limit of normal secondary to high dietary oxalate intake. Investigations for primary or enteric hyperoxaluria were negative. Institution of a diet low in oxalate resulted in rapid normalization of urinary oxalate excretion and improvement of renal function from eGFR of 15 to 59 mL/min/1.73 m2 after 3.5 years dietary oxalate intake change.

Discussion: CKD from dietary hyperoxaluria may be reversed by simply avoiding oxalate-rich food items even when it is diagnosed at an advanced stage. Its presentation, including the urine findings, can be indistinguishable from other types of interstitial nephritis. The diagnosis of dietary hyperoxaluria requires careful dietary history. Recognition of dietary hyperoxaluria as the cause of CKD is critical for salvage of renal function.

TH-PO074

IgG4-Related Tubulointerstitial Nephritis Associated with Only Lymphadenopathy, and without Elevated Serum IgG4 Level or Renal Imaging Abnormalities Xi Qiao, Lihua Wang. *Nephrology, Second Hospital of Shanxi Medical Univ, Taiyuan, Shanxi, China.*

Introduction: IgG4-related kidney disease (IgG4-RKD) usually presents as plasma cell–rich tubulointerstitial nephritis (TIN) associated with renal mass lesions. Serology usually demonstrates high levels of serum total IgG and IgG4. Most patients have accompanying IgG4-related extrarenal lesions. However, IgG4-TIN with only lymphadenopathy has not been well recognized. Here, we present a rare case of IgG4-TIN associated with swelling of many lymph nodes as the only extrarenal lesions, and without elevated serum total IgG or IgG4 levels or renal imaging abnormalities.

Case Description: A 61-year-old man was admitted to our hospital for renal dysfunction and swelling of many lymph nodes. He didn't have other organ involvement except for the lymphadenopathy. His renal imaging was normal radiographically. Histological findings of the kidney indicated IgG4-positive plasma cell infiltration with storiform fibrosis, which was consistent with IgG4-related TIN. Repeated lymph nodes biopsy revealed IgG4-related lymphadenopathy. However, the patient did not have elevated serum total IgG or IgG4 levels. Oral prednisolone therapy, initially 30 mg/day and gradually tapered over 10 months, improved his renal function and the swelling of his affected lymph nodes. These findings supported our final diagnosis of IgG4-TIN.

Discussion: We describe a case of IgG4-TIN associated with only lymphadenopathy and without elevated serum IgG or IgG4 levels imaging abnormalities. This case highlights

the need for renal biopsy in cases with unknown renal insufficiency. It is necessary to accumulate cases of IgG4-TIN with detailed clinical and laboratory manifestations. Early diagnosis and treatment is desirable in IgG4-TIN.

TH-PO075

Life-Threatening Metabolic Disorders Associated to Drug-Induced Hepatic and Renal Mitochondrial Dysfunction Alberto Manuel Martin Cordova, Mario A. Mendoza, Carlos Antonio Cortes Sanchez, Emmanuel O. Gonzalez, Rodolfo Estremera-Marcial, Eddie M. Rodriguez, Hector R. Cordova. *Medical Service, VA Caribbean Healthcare System, San Juan, PR.*

Introduction: Renal tubular toxicity is reported infrequently (1.5%) in patients on Tenofovir therapy. Risks factors include advanced age, low body weight, chronic kidney disease, and the use of other nephrotoxic drugs. Tenofovir can cause a rise in lactic acid levels, particularly, in patients with subjacent liver disease. We describe a case of a patient with HIV infection treated with Tenofovir who developed Fanconi syndrome and lactic acidosis simultaneously.

Case Description: A 66 y/o man with history of HIV and Hepatitis C virus infection, in treatment with Tenofovir, complained of weakness, muscle cramps, and polyuria for one week. There were no focal motor deficits. Laboratories results revealed serum creatinine of 2.7 mg/dl, mixed hyperchloremic and high anion gap metabolic acidosis (tCO2: 15 meq/L), hypokalemia (1.8 meq/L), hypophosphatemia (1.0 mg/dl), elevated lactic acid level (3.6 mmol/L), and glycosuria without hyperglycemia. Intravenous fluids and electrolyte replacement lead to improvement in symptoms and the metabolic disorder, only after discontinuation of Tenofovir. Liver enzymes were normal. However, there was evidence of liver steatosis in a previous ultrasound study. Renal function (serum creatinine: 1.5 mg/dl), serum electrolytes and lactic acid levels (1.2 mmol/L) improved.

Discussion: Accumulation of Tenofovir in the mitochondria of the proximal renal tubular cells is associated with the development of Fanconi syndrome. It can also disrupt mitochondrial function in the hepatocytes causing steatosis and a reduction in the hepatic lactate clearance. Discontinuation of Tenofovir was crucial in our case because electrolyte replacement alone failed to correct the metabolic derangements. There are few reports of Fanconi syndrome and lactic acidosis in patients receiving Tenofovir, almost all fatal. In those cases, another drug was the culprit of the lactic acidosis. Patients on Tenofovir therapy should be closely followed for the development of the Fanconi syndrome and/or lactic acidosis, particularly in the presence of liver disease.

Funding: Veterans Affairs Support

TH-PO076

Tumor Lysis Syndrome from a Malignant Peripheral Nerve Sheath Tumor Gerald Denny, Sharidan Parr, Anna Marie Burgner. *Dept of Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

Introduction: Tumor lysis syndrome (TLS) represents a clinical entity that can lead to acute kidney injury (AKI) and prove life threatening if not treated appropriately. We report the case of a malignant peripheral nerve sheath tumor that resulted in TLS and AKI requiring renal replacement therapy.

Case Description: A 36-year-old Caucasian male presented for definitive treatment of a known malignant peripheral nerve sheath tumor of the right chest diagnosed previously following blunt force trauma to the chest. He was found at admission to have acute renal failure and hyperkalemia. He had no other previous medical problems. Plasma lab values: creatinine, 1.64 mg/dL (previously 0.92 mg/dL), potassium 6.6 mEq/L, bicarbonate 13 mmol/L, uric acid 19.2 mg/dL, phosphorus 6.7 mg/dL, LDH 3346 U/L, lactate 8 mEq/L, and creatinine kinase 85 U/L. Urine lab values: urine protein to creatinine ratio 0.17, FENa 0.1%, and eosinophils negative. Abdominal ultrasound revealed no evidence of hydronephrosis. His labs were consistent with spontaneous tumor lysis syndrome. He was transferred to the intensive care unit and hemodialysis was emergently performed. He was subsequently placed on continuous renal replacement therapy secondary to concern for ongoing tumor lysis. He further decompensated over the next 24 hours with hypoxemic respiratory failure preventing surgical intervention. The decision was made by the patient to forego intubation and resuscitation and he subsequently died due to cardiac arrest secondary to hypoxemic respiratory failure.

Discussion: Malignant peripheral nerve sheath tumors are a subset of sarcomas and to our knowledge, this is the first reported case of spontaneous TLS in the setting of not only malignant peripheral nerve sheath tumors, but also sarcomas. The rapid enlargement of this tumor likely led to outgrowth of its blood supply resulting in TLS. Recognition of characteristics such as rapid growth are important when considering the possibility of complications from novel malignancy. While acute leukemias and non-Hodgkin's lymphoma are most likely to spontaneously result in TLS, rapidly growing sarcomas must also be followed closely.

TH-PO077

Bilateral Renal Infiltration as Initial Clinical Presentation of Non-Hodgkin's Lymphoma Suellen Klein, Maria C. Piraciaba, Carlos A.T. Sampaio, Simone C. Lo, Roberto Zatz, Lectícia Jorge, Cristiane B. Dias, Leonardo Abreu Testagrossa. *Univ of Sao Paulo, Brazil.*

Introduction: Diffuse renal lymphomatous infiltration is a rare and severe condition mostly associated with aggressive non-Hodgkin's lymphomas (NHL). We report a case of a large B-cell lymphoma in which the initial clinical manifestation was bilateral renal infiltration accompanied by severe anemia and uremic syndrome.

Case Description: A 49-year-old white man was hospitalized with severe nausea, vomiting and emaciation. He had been well until 6 months earlier, when fatigue, anorexia and progressive weight loss developed. He had been diagnosed with anemia and treated with iron sulfate, but symptoms had worsened steadily. At admission, BUN was 210 mg/dL creatinine 11 mg/dL, K+ 7.0 mmol/L and HCO_3 15 mmol/L. Hemoglobin was 7.5 g/dL, and serum iron was 23 µg/dl. Urinalysis showed: protein >1g/L, WBC 15/HPF, RBC >100/HPF. Ultrasound showed markedly enlarged kidneys (19.8 and 17.3 cm), with no hydronephrosis. A percutaneous renal biopsy revealed diffuse neoplastic infiltration. Immunofluorescence detected mild mesangial C3 granular deposition. Immunohistochemistry was positive for CD 20, CD 68, Ki-67 and BCL-6, confirming the diagnosis of large B-cell NHL. A myelogram showed no abnormalities. The patient is currently on hemodialysis, and chemotherapy has been started.

Discussion: Renal involvement in NHL is common, especially among men in their late forties, but antemortem diagnosis is made in only 1% of cases, because clinical manifestations are usually nonspecific. Pathogenic mechanisms may include recruitment of lymphoid cells into renal parenchyma through preexisting inflammation; and cell migration through lymph vessels. Renal dysfunction is common at diagnosis. Clinical and radiological findings often mimic those of renal carcinoma. Diagnosis requires renal histological examination. Chemotherapy usually includes cyclophosphamide, doxorrubicin, vincristine, and prednisolone, with unsatisfactory results, which may be improved by the recent introduction of rituximab.

Funding: Government Support - Non-U.S.

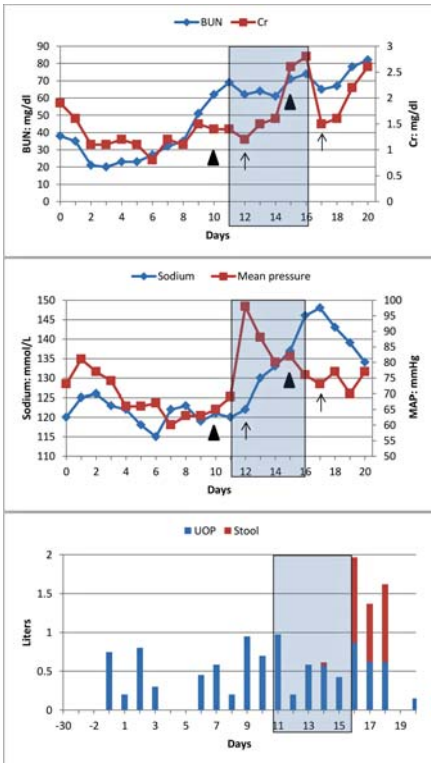
TH-PO078

Case Report: Ileus and Severe Hepatic Encephalopathy in a Liver Cirrhosis Patient with Hepatorenal Syndrome after Starting Octreotide Mohammad G. Abu-Farsakh. *Nephrology, Baylor College of Medicine, Houston, TX.*

Introduction: Hepatorenal Syndrome (HRS) is generally associated with poor prognosis. Octreotide has been used in a combination with Midodrine and Albumin to treat Hepatorenal Syndrome. As far as we know, there was no previous reports linking Octreotide to the development of ileus, precipitating severe hepatic encephalopathy.

Case Description: Here, we report a case of a 41 year old man with alcoholic liver cirrhosis who presented with hyponatremia and refractory ascites requiring recurrent large volume paracentesis. The patient's hyponatremia did not improve with fluid restriction, and only partially and transiently corrected with Normal Saline administration. The patient was started on Octreotide, Midodrine, and Albumin for Hepatorenal syndrome but unexpectedly developed ileus which precipitated severe hepatic encephalopathy although his serum sodium corrected appropriately to normal level during therapy period. Both ileus and encephalopathy resolved after stopping Octreotide, suggesting a possible causal relationship to the event.

Discussion: In the setting of new onset ileus and hepatic encephalopathy after starting Octreotide therapy, consider stopping Octreotide as it may be the precipitating factor, especially if no other alternative explanation could be found. The attached figure below shows changes in serum BUN, Cr, Sodium, MAP, UOP and stool output in our patient before, during, and after therapy. Black arrow heads represent the onset and end of Octreotide treatment, the other two arrows represent the onset and end of encephalopathy. The shaded area represents the duration of ileus.

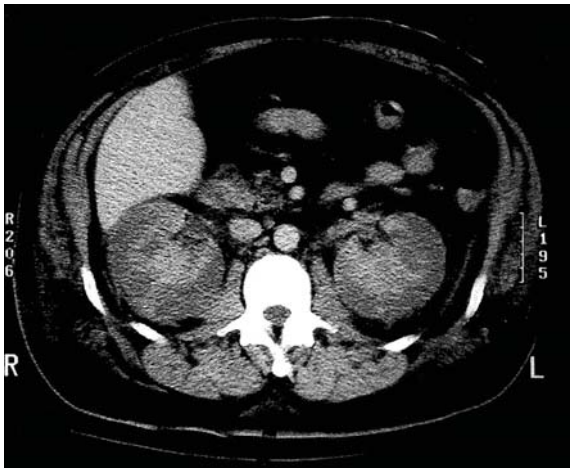


TH-PO079

Bilateral Renal Cortical Necrosis in Sepsis Mark I.C. Hong, Seyed-Ali Sadjadi. *Nephrology, Jerry L Pettis VA Medical Center, Loma Linda, CA.*

Introduction: Acute bilateral renal cortical necrosis (BRCN) is a rare cause of renal failure due to ischemia from thrombosis or vasospasm. Consequently, necrosis of the renal cortex, including Bertin columns, ensue with sparing of the medulla and a thin layer of subcapsular cortex. Etiology includes obstetric causes that account for 50 – 70% of cases and non-obstetric cases account for 20 – 30%. Herein we report a case of BRCN associated with E. Coli sepsis.

Case Description: 44 year old Caucasian male with history of diabetes and hypertension presented with upper respiratory tract infection, costovertebral angle tenderness, and anuria for 24 hours. He was admitted for urosepsis and acute kidney injury with serum blood urea nitrogen (BUN) 41 mg/dL and creatinine (Cr) 6.6 mg/dL. Contrast enhanced CT scan demonstrated bilateral renal medullary enhancement, non-enhancement of cortex and lack of contrast medium excretion in the collecting system.



Blood and urine culture were positive for E. Coli. The patient was diagnosed with BRCN due to E. Coli sepsis and started on dialysis shortly after admission.

Discussion: Acute renal cortical necrosis is a rare cause of renal failure. Though kidney biopsy is the gold standard, it may not be possible to do in critically ill patients. Contrast enhanced CT scanning is a sensitive and specific alternative modality for early diagnosis of BRCN. Recovery of kidney function depends on the extent of the renal cortical necrosis and most patients eventually become dialysis dependent with overall an mortality of 36%. Our patient remained dialysis dependent.

TH-PO080

Neurological Complications in Nephropathic Cystinosis: A Rare Case Masaaki Yamada, Aneesha A. Shetty, James F. Simon. *Cleveland Clinic, OH.*

Introduction: Nephropathic cystinosis (NC) is a rare genetic disease characterized by lysosomal cystine accumulation in cells. Early manifestations include Fanconi's syndrome, nephrolithiasis and renal failure associated with involvement of other organs if left untreated. Cysteamine therapy and renal transplantation have significantly increased the life expectancy of these patients making later manifestations of the disease important to identify. Cystinosis encephalopathy is one such late complication.

Case Description: The patient was a 23 year-old male with NC treated with cysteamine since diagnosis at age 6 months who presented with intermittent confusion and hallucinations. His renal function stayed near normal (serum creatinine 1.0 - 1.3 mg/dL) until date. Neurological examination showed no obvious cranial nerve, focal motor or sensory deficit. Initial tests including routine labs and culture, toxicology, CSF and EEG were unremarkable except presence of cerebral atrophy on CT. Brain MRI revealed chronic microvascular ischemic changes and small old infarcts. Continuous EEG showed subclinical epileptiform activity. Cystinosis encephalopathy was considered the most likely diagnosis. Cysteamine therapy was continued and subclinical seizures were treated with phenytoin and lamotrigine. His neurological symptoms improved gradually and he was discharged to a long-term care facility but subsequently developed expressive aphasia. Repeat brain MRI revealed subacute stroke involving the left frontal lobe which was managed conservatively. As of last contact, he had recovered significant neurologic functions with minimal residual deficits.

Discussion: Cystinosis encephalopathy should be considered if an adult NC patient develops unexplained neurological symptoms. Manifestations include short-term memory loss, intermittent confusion, motor and cerebellar dysfunction, cerebral ischemia and seizures. Cerebral atrophy and mineralization are typical radiographic findings. However, these are not specific and an extensive investigation into other possible causes is warranted. Cystine depleting therapy appears to slow the progression of neurological complications but the overall prognosis is poor.

TH-PO081

The Unusual Course of Severe Rhabdomyolysis in H1N1 Patient Mansumeet Singh, Ravkiran Kaur Khurana, Siddiq Anwar, Tingting Li. *Nephrology, Washington Univ School of Medicine, St. Louis, MO.*

Introduction: Rhabdomyolysis is a common and potentially lethal clinical entity that results from acute muscle necrosis with leakage of muscle constituents into circulation. A variety of causes can lead to rhabdomyolysis, including trauma, overexertion, toxins, electrolyte disorders, and infection. H1N1 influenza A infection is an under recognized cause of rhabdomyolysis. We present a case of H1N1-induced severe rhabdomyolysis leading to acute kidney injury in an adult male who recovered uneventfully.

Case Description: A 32 year old Gambian male initially presented to the outside hospital emergency room with complaints of cough, sore throat and brown streaked sputum. Rapid influenza antigen test was negative, and he was discharged on Levaquin. His symptoms persisted along with the new development of severe muscle aches which prompted a visit to our hospital. Initial labs were: creatine kinase (CK) 806,000 units/L, serum creatinine 3.96 mg/dL, Potassium (K)-4.1 mEq/L, AST 2568 units/L, and ALT 857 units/L. H1N1 influenza A was positive by RT-PCR and was thought to be the etiology of the rhabdomyolysis. He was initiated on oseltamivir and was aggressively volume repleted. Surprisingly, he remained non-oliguric (urine output consistently > 1.5 L/day) with no evidence of hyperkalemia, hyperphosphatemia, or metabolic acidosis. His creatinine peaked at 9.5 mg/dL and trended down thereafter. He did not require renal replacement therapy. His CK levels decreased slowly as did his transaminases. He was discharged to home in good condition after two weeks.

Discussion: H1N1 influenza infection should be recognized as an important cause of severe rhabdomyolysis with acute kidney injury as an expected consequence. However, the severity of rhadomyolysis does not necessarily predict severity of renal injury or need for dialysis.

TH-PO082

Diagnostic Accuracy of Neutrophil Gelatinase-Associated Lipocalin, as Marker of Acute Kidney Injury, Decreases in Chronic Kidney Disease Patients with Impaired Glomerular Filtration Rate Carlo Donadio. *Clinical and Experimental Medicine, Univ of Pisa, Pisa, Italy.*

Background: Cardio-renal syndromes are characterized by the impairment of cardiac and renal functions. Plasma and urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL), and plasma B-type natriuretic peptide (BNP) are markers of acute kidney injury (AKI) and heart failure (HF), respectively. The aim of this study was to assess the effect of the reduction of GFR on plasma BNP and on plasma and urinary NGAL concentration**s** in stable chronic kidney disease (CKD) patients at different functional stages.

Methods: GFR (^{99m}Tc-DTPA), plasma BNP, and plasma and urinary concentrations of NGAL were measured in 310 clinically stable CKD patients, at functional stages from 1 to 5. Serum and urinary low-molecular-weight proteins cystatin C and β2-microglobulin, and urinary tubular enzymes were measured for comparison. Plasma BNP, NGAL, cystatin C and β2-microglobulin were measured also in 31 maintenance hemodialysis patients.

Results: Plasma NGAL increased with the reduction of GFR in CKD patients from stage 2.

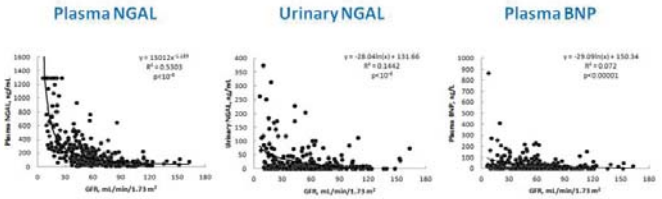


Fig 1. Correlations with GFR of plasma and urinary NGAL and plasma BNP.

In the different CKD stages modest differences were found for BNP values. Urinary NGAL increased slightly but significantly in patients at CKD stages 4 and 5, similarly to urinary cystatin C and β2-microglobulin. In maintenance hemodialysis patients, plasma NGAL and BNP were markedly increased, and high-flux hemodialysis significantly decreased their plasma concentrations.

Conclusions: Plasma NGAL increases markedly with the reduction in GFR, generating a very high number of false positive diagnoses of AKI in stable CKD patients. The grade of GFR impairment and the cause of kidney disease have a lower effect on urinary NGAL and on plasma BNP. In any case, specific reference values of NGAL and BNP should be used in chronic kidney disease patients, according to their functional stage.

Funding: Government Support - Non-U.S.

TH-PO083

Association of Peak Changes in Cystatin C and Creatinine after Cardiac Surgery with 3-Year Mortality in Adults Meyeon Park,[1] Michael Shlipak,[1,2] Heather Thiessen Philbrook,[2] Amit X. Garg,[2] Jay L. Koyner,[2] Steven G. Coca,[2] Chirag R. Parikh.[2] *[1]UCSF; [2]TRIBE-AKI Investigators.*

Background: Serum creatinine (Cr) and cystatin C (CysC) have different temporal profiles in the post-operative setting. Consideration of changes in both markers may be required to capture associations of in-hospital changes in kidney function with prognosis following hospital discharge.

Methods: This is an analysis of the TRIBE-AKI (Translational Research Investigating Biomarker Endpoints for Acute Kidney Injury) multicenter prospective cohort study. We examined in-hospital peak changes of Cr and CysC for 3 days after cardiac surgery among 1199 hospital survivors, and evaluated associations with 3-year mortality, adjusting for pre-operative eGFR, elective surgery, race, gender, age, cardiopulmonary bypass time, diabetes, hypertension, heart failure, myocardial infarction, surgery type, pre-operative urinary albumin to creatinine ratio, and site.

Results: During the first 3 days of hospitalization, nearly twice as many patients had a peak rise in Cr >20% (37%) as had a >20% peak rise in CysC (20%). Patients who had elevations of >20% in both Cr and CysC were at higher adjusted risk for 3 year mortality (Table) (HR 2.4, 95% CI 1.7-3.4). However, patients who had a 20% rise in only one marker had much smaller elevations in risk.

Association of In-Hospital Changes in Cr and CysC with Mortality after Discharge		
Change in Filtration Marker	N	Adjusted HR (95% CI)
Cr<20%, CysC<20%	630	1.0 (referent)
Cr>20%, CysC<20%	273	1.32 (0.98, 1.78)
Cr<20%, CysC>20%	76	1.50 (1.05-2.12)
Cr>20%, CysC>20%	152	2.37 (1.68, 3.35)

Conclusions: In adults undergoing cardiac surgery, elevations in Cr post-operatively are twice as common as elevations in CysC. Elevations in both markers are associated with much greater increases in mortality risk at 3 years, compared with elevations of only one filtration marker.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO084

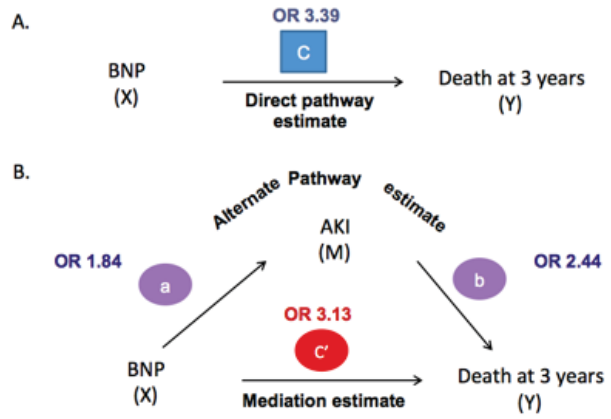
Acute Kidney Injury Mediates the Association Between Brain Natriuretic Peptide and Long-Term Mortality After Cardiac Surgery Uptal D. Patel, Heather Thiessen Philbrook, Jay L. Koyner, Steven G. Coca, Michael Shlipak, Amit X. Garg, Chirag R. Parikh. *Translational Research Investigating Biomarker Endpoint (TRIBE) in AKI Investigators.*

Background: Both brain natriuretic peptide (BNP) and AKI are associated with increased mortality after cardiac surgery. Pre-operative BNP levels are also associated with AKI, but whether post-operative AKI mediates the association between pre-operative BNP with long-term mortality is unclear.

Methods: We utilized the TRIBE AKI adult cohort (n=1121) to examine the direct association between pre-operative BNP and death at 3 years after cardiac surgery. We then examined the indirect association through post-operative AKI, defined as AKIN Stage 1 or higher. We estimated the impact of mediation by AKI with the Sobel test, which required BNP to be dichotomized (1st versus 2nd/3rd tertiles) for logistic regression models.

Results: After 3 years, there were 126 deaths. The median BNP values were significantly higher among those who died (181 mg/dL [IQR 73, 357] versus 70 [32, 165]; p<0.001) with an odds ratio (OR) for death among the highest BNP tertile of 3.4 (95% CI 2.3, 5.1; C in Fig). BNP was associated with AKI (OR 1.8; a in Fig), and AKI was associated with death (OR 2.4; b in Fig). After examining these indirect associations, the direct association between BNP and death was lower (aOR 3.1; C' in Fig). The proportion of the association mediated by at least mild AKI was found to be 30% (p=0.01).

Figure. Direct (A) and alternate pathways (B) between BNP and death.



Conclusions: We found that AKI mediates a substantial proportion of the association between BNP and mortality. These findings provide further insight into the potential pathways by which BNP is associated with mortality after cardiac surgery, and suggest that reducing the incidence of AKI may reduce the mortality risk associated with elevated BNP.

Funding: NIDDK Support

TH-PO085

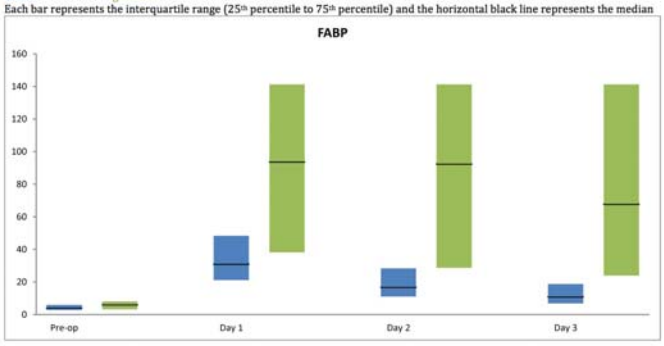
Peri-Operative Heart-Type Fatty Acid Binding Protein Predicts Acute Kidney Injury after Cardiac Surgery Jennifer A. Schaub,[1] Steven G. Coca,[1] Michael Shlipak,[2] John Eikelboom,[3] Amit X. Garg,[4] Peter Kavsak,[3] Eric Mcarthur,[4] Heather Thiessen Philbrook,[4] Richard P. Whitlock,[3] Chirag R. Parikh.[1] *[1]Yale Univ School of Medicine, New Haven, CT; [2]UCSF School of Medicine, San Francisco, CA; [3]McMaster Univ, Hamilton, ON, Canada; [4]Western Univ, London, ON, Canada.*

Background: Acute Kidney Injury (AKI) after cardiac surgery is a common and heterogenous disorder for which there is no meaningful treatment. We conducted a prospective study to evaluate if blood levels of heart FABP (hFABP) predicted incidence of AKI and helped distinguish AKI due to true tubular damage versus cardiac dysfunction.

Methods: The Translational Research Investigating Biomarker Endpoints in Acute Kidney Injury (TRIBE-AKI) cohort is a prospective cohort of 1219 patients who underwent cardiac surgery at six academic institutions across North America who were at high risk for post-operative AKI. The primary outcome was severe post-operative AKI defined as doubling of serum creatinine or requiring dialysis.

Results: On average, patients who experienced severe AKI had higher levels of hFABP both pre and post-operatively than patients who did not experience AKI.

Figure 1 – Biomarker Box Plots by Severe AKI Status
Blue = No Severe AKI green = Severe AKI Cases
Each bar represents the interquartile range (25th percentile to 75th percentile) and the horizontal black line represents the median



In analyses that adjusted for known AKI risk factors, hFABP levels within the first 6 hours post-operatively remained an independent predictor for incidence of AKI (adjusted OR 5.4 [2.9, 10.1]). Notably, this relationship persisted even after adjustment for change in serum creatinine and interleukin-18 levels (IL-18) (OR 2.9 [1.4, 5.8]).

Conclusions: Elevated hFABP levels within the first six hours post-operatively are an independent predictor for developing severe AKI. hFABP levels seem to identify a subset of patients with severe AKI whom did not sustain severe tubular injury.

Funding: Other NIH Support - RO1HL085757

TH-PO086

Increased Plasma Pro-Thrombin Fragment 1.2 (PF 1.2) Is an Independent Risk Factor for Acute Kidney Injury (AKI) in Anaemic Patients After Cardiac Surgery Simona Simone, Giuseppe Scrascia, Crescenzia Rotunno, Eustacchio Montemurno, Loreto Gesualdo, Domenico Paparella, Giovanni B. Pertosa. *DETO, Univ of Bari, Italy.*

Background: Acute Kidney Injury (AKI) following cardiac surgery is a common complication associated to short and long-term mortality. Aim of the study was to evaluate the association between coagulation system, inflammation/oxidative stress and AKI after cardiopulmonary bypass (CPB) in patients with pre-operative higher EuroSCORE points (>6) and anaemic status (Hb <12 g/dL in women, <13 g/dL in men).

Methods: Forty-one anaemic patients were prospectively enrolled. Twenty-six patients (Group A) showed a <25% postoperative Glomerular Filtration Rate (eGFR) reduction, whereas 15 patients evidenced a eGFR reduction >25% (Group B). Plasmatic Pro-thrombin Fragment 1.2 (PF 1.2, coagulation activation marker), 8-oxoDG (oxidative stress marker to DNA), IL-6 (pro-inflammatory marker), IL-10 (anti-inflammatory marker), urinary Il-18 and NGAL were evaluated.

Results: An increase in inflammatory markers (IL-6, IL10) was observed in both groups immediately after the operation compared to baseline, but no differences were found between groups. Twenty-four hours (T24) after the operation PF1.2 (999±704.1 versus 506.6±548 pmol/L; p=0.018) and 8-oxo-DG (98.8±66 versus 65.2±26 ng/mL; p=0.02) plasma levels significantly increased in group B as compared to group A. PF1.2 was independently associated with eGFR reduction in multivariate analysis. T24 PF1.2 values had an Area under the Receiving Operating Characteristic (ROC) of 0.744 for eGFR reduction. Urinary IL-18 had similar values in both groups 2 hours after the operation, but NGAL increase (2 hours versus baseline) was significantly higher in Group B patients (0.3 versus 4.4 ng/mL; p: 0.03). Five patients of Group B required renal replacement therapy (average 38.4 hours after the operation).

Conclusions: An elevated T24-postoperative plasma PF1.2 level is an independent risk factor for AKI and dialysis in anaemic patients with higher EuroSCORE points. Coagulation activation along with an increased oxidative stress may have a role in renal microcirculation impairment (ischemia-reperfusion damage) in these setting of patients.

TH-PO087

Increased Urinary Liver-Type Fatty Acid-Binding Protein (L-FABP) Linked with Incident Acute Kidney Injury and Poor Survival After Allogeneic Hematopoietic Stem Cell Transplantation Naoki Shingai,[1] Minoru Ando,[2,3] Ken Tsuchiya,[3] Kosaku Nitta.[3] *[1]Dept of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Dept of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [3]Dept of Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

Background: Hematopoietic stem cell transplantation (HCT) involves a great risk of acute kidney injury (AKI). Liver-type fatty acid-binding protein (L-FABP) is generated in renal proximal tubules and its urinary concentration is a sensitive biomarker to detect tubular injury mainly due to ischemic stress.

Methods: A one-year prospective study was conducted in 85 allogeneic HCT recipients, who had neither renal dysfunction (eGFR less than 60 ml/min/1.73 m^2) nor prior HCT at entry, to investigate the impact of baseline urinary L-FABP (uL-FABP) level on incidence of AKI and mortality after HCT. AKI was defined as one that emerges within the first 100 days after stem-cell infusion, according to the serum Cr criteria of the AKIN. The median patient age and observation period were 49 years and 710 days, respectively. The recipients were stratified into a high and a low uL-FABP group according to the reference value for healthy subjects (8.4 mg/gCr). The associations of high uL-FABP with either AKI incidence or mortality were analyzed using the Gray's method and multivariate Fine-Gray proportional hazards regression, adjusted for age, gender, disease risk, preparative regimen, and HCT-comorbidity indices.

Results: The cohort included 17 with high uL-FABP and 68 with low uL-FABP, between whom there was no significant difference in baseline eGFR. The high uL-FABP group showed significantly greater cumulative incidence of AKI (88.2% versus 59.4%, P <0.001), non-relapse mortality (NRM) at 1 year (58.8% versus 13.5%, P<0.001), and all-cause mortality (ACM) at 1 year (76.5% versus 25.5%, P<0.001), compared to the low group. The risks of high uL-FABP were statistically significant, compared to the low uL-FABP: HRs [95% CI] were 4.24 [1.7-10.6] for AKI incidence, 8.35 [3.09-22.6] for NRM, and 4.66 [2.09-10.4] for ACM, respectively.

Conclusions: Increased uL-FABP at baseline may be associated with high risk of incidence of AKI and poor survival after HCT.

TH-PO088

Endogenous Ouabain Levels Predict Survival Rate in Septic Acute Kidney Injury Marco Simonini, Simona Pozzoli, Cristina Sorlini, Andrea Duca, Chiara Lanzani, Stefano Franchini, Moreno Tresoldi, Paolo Manunta. *San Raffaele Scientific Inst, Italy.*

Background: Acute kidney injury (AKI) is a common complication of sepsis, which is associated with higher risks of adverse outcomes. Despite identification of several cellular mechanisms being thought to underlie the development of septic AKI, the pathophysiology of AKI is still poorly understood. Recently it was demonstrated that elevated levels of endogenous ouabain (EO), an adrenal stress hormone with hemodynamic and renal effects, were able to predict post-operative AKI. The aim of this work was to verify if EO dosage could be useful in the identification of subject with increased risk of AKI in severe septic state.

Methods: A group of more than 200 patients presented at Emergency Room (ER) of our hospital with severe sepsis or septic shock were studied. The incidence of AKI (stage II or III by AKIN) was 63%. Total survival rate was 41.9%. In a subgroup of subjects (40) EO was measured at the hospital admission and for the next 48h.

Results: No difference was observed in EO level in patient with or without AKI at ER admission. However patient with persistent high levels of EO (>300 pmol/L) didn't resolve AKI (prevalence at 48h from admission 60% versus 17.6%; p=0.024). Moreover patients with a more severe clinical presentation (septic shock) presented a higher EO level (411.95±202.20 versus 317.14±76.93 pmol/L). Lastly higher EO plasmatic level were associated with a lower survival rate already in the early hours of observation (p=0.013 at 72h) and with higher total in-hospital mortality of 79.6% versus 27.8% (RR 8.7 (CI95% 1.67-45.21); p<0.001). These results were statistical significant after adjustment for covariates, including clinical biomarker of sepsis severity.

Conclusions: Our data suggest that plasma EO may have a role in persistence of renal damage in evidence of severe hemodynamic alterations as presence of septic shock. Moreover EO level is able to identifies patients with more severe presentation and with an increased risk of early mortality. We believe that these preliminary findings are of great clinical impact, indeed an inhibitor of EO (rostafuroxin) has been recently developed and may help minimize AKI and improve outcomes.

TH-PO089

Storage Time and Urinary Biomarker Levels in ASSESS-AKI Kathleen D. Liu, Edward D. Siew, William Brian Reeves, Jonathan Himmelfarb, Alan S. Go, Chi-Yuan Hsu, T. Alp Ikizler, James S. Kaufman, Paul L. Kimmel, Vernon M. Chinchilli, Chirag R. Parikh. *For the NIDDK ASSESS-AKI Consortium.*

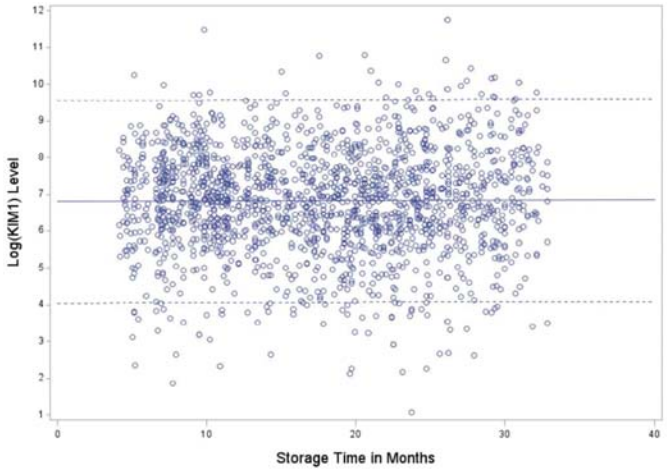
Background: Renal tubular injury biomarkers have been studied in the context of acute kidney injury (AKI). Many studies rely on banked specimens stored for months-years before biomarker measurement, but the impact of storage time on biomarker levels is not well understood.

Methods: Urine samples were obtained during initial hospitalization ("baseline") and 3 months later from 866 ASSESS-AKI participants. Samples were collected, processed and stored at -80°C per protocol until kidney injury molecule-1 (KIM-1), neutrophil-gelatinase associated iipocalin (NGAL), interleukin-18 (IL-18) and liver fatty acid binding protein (LFABP) measurements were made by ELISA at variable times after initial collection. Mixed effects models were used to determine the impact of storage time on biomarker levels. P < 0.05 was considered statistically significant.

Results: Mean age was 64 years; 39.1% were female; 34.2% had a baseline eGFR < 60 mL/min/1.73m^2. Storage time before biomarker measurement ranged from 4-34 months. There was no relationship between storage time and NGAL, IL-18 or LFABP levels after accounting for age, gender, diabetes, ICU stay, clinical center, visit, baseline and peak inpatient serum creatinine levels.

Biomarker	P value for storage time effect
NGAL	0.53
KIM-1	0.051
IL-18	0.41
LFABP	0.12

Although the p value for the overall effect of storage time on KIM-1 levels bordered on statistical significance, there was no clear relationship between time and KIM-1 levels, as shown in the Figure.



Conclusions: We found no significant association between storage time (up to 34 months) on urine NGAL, IL-18, LFABP and KIM-1 levels among urine samples collected, processed and stored under protocolized conditions. Further studies are needed to determine the impact of longer storage times on biomarker levels.

Funding: NIDDK Support

TH-PO090

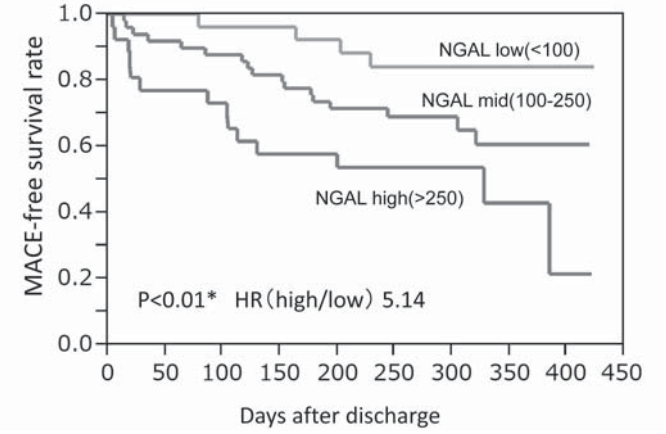
Plasma Neutrophil Gelatinase-Associated Lipocalin Predicts Major Adverse Cardiovascular Events After CCU Discharge Kent Doi,[1] Masamichi Ito,[2] Masao Takahashi,[2] Naoki Yahagi,[1] Masaomi Nangaku,[3] Eisei Noiri.[3] *[1]Emergency and Critical Care Medicine, The Univ of Tokyo; [2]Cardiovascular Medicine, The Univ of Tokyo; [3]Nephrology and Endocrinology, The Univ of Tokyo.*

Background: Acute exacerbation of renal dysfunction has a great impact on the outcomes of cardiovascular patients in critical conditions. Emerging acute kidney injury (AKI) biomarkers including neutrophil gelatinase-associated lipocalin (NGAL) have a high potential for detecting renal tissue injury better than serum creatinine. This study was aimed to evaluate whether plasma NGAL can predict the mortality and major adverse cardiovascular events after discharge from cardiac care unit (CCU).

Methods: The patients who admitted in the CCU of Tokyo University Hospital were prospectively enrolled. Blood and urinary markers including blood NGAL, brain natriuretic peptide, creatinine, and cystatin C, and urinary albumin, N-acetyl-β-D-glucosaminidase, and L-type fatty acid-binding protein were measured at their discharge from CCU. The primary outcome was major adverse cardiac event (MACE) within 6 months.

Results: Of 101 enrolled patients, 34 patients experienced MACE (34%). Among the clinical parameters and biomarkers, only plasma NGAL was significantly higher in patients with MACE than those without MACE (311.4 versus 186.3 ng/mL, p<0.01). Multivariate logistic analysis revealed plasma NGAL and complications of diabetes and heart failure were independent predicting factors for MACE. The patients with the highest NGAL at

discharge (>75th percentile) showed a significantly higher risk of MACE than those with the lowest NGAL (<25th percentile) (log-rank test, Hazard ratio 5.15 [95% confidential interval 1.84–18.20], p<0.01).



Conclusions: Plasma NGAL at CCU discharge has a significant prognostic indicator of 6 months outcomes in critically ill cardiac patients treated at CCU.

Funding: Government Support - Non-U.S.

TH-PO091

Comparative Analysis of Diagnostic and Predictive Performance of Novel Renal Biomarkers in Plasma and Urine of Acute Kidney Injury Patients Gunnar Schley, Kai-Uwe Eckardt, Carsten Willam. *Nephrology and Hypertension, Univ of Erlangen-Nuremberg, Erlangen, Germany.*

Background: Renal biomarkers represent an attractive new diagnostic tool which not only implies early diagnosis of AKI, but provides also information about patients at risk for AKI and prediction of adverse clinical outcome parameters like the need for renal replacement therapy, length of stay in ICU and mortality. This study directly compared the diagnostic and prognostic performance of biomarkers in urine and plasma.

Methods: This prospective cohort study included 110 unselected adults undergoing cardiac surgery. Plasma and/or urine concentrations of creatinine, cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), liver fatty acid-binding protein (L-FABP), kidney injury molecule 1 (KIM1), and albumin and furthermore a multiplex panel of 15 biomarkers in plasma and urine were measured during the perioperative period. The primary outcome was AKI defined by AKIN criteria.

Results: Biomarkers in plasma showed markedly better predictive and diagnostic performance than their urinary counterparts. Discriminative power of urinary biomarkers improved when concentrations were related to urinary creatinine but still did not achieve AUC values of markers measured in plasma samples. Before surgery plasma IP10 (interferon-γ-induced protein 10, CXCL 10), cystatin C and MIG (monokine induced by interferon-γ, CXCL9) best predicted postoperative AKI (AUC 0.73-0.70). Best diagnostic performance 4h after surgery had NGAL (AUC 0.83), cystatin C (0.76), and MIG (0.74) in plasma. Combination with clinical scores (EuroSCORE and Cleveland Clinic Foundation Score) or combinations of several biomarkers did not significantly improve predictive or diagnostic power of either plasma or urine markers.

Conclusions: In our cohort plasma biomarkers had higher discriminative power to predict and to diagnose AKI than urine biomarkers. Plasma IP10 and NGAL performed best in predicting and diagnosing AKI respectively. Their performance could not be improved by combining with clinical scores or additional biomarkers.

TH-PO092

Higher Urinary Sediment Score Is Related to Increased Risk of AKI, and Mortality Rolando Claure-Del Granado,[1] Vania Cecilia Prudencio Ribera,[1] Susana Ledezma,[2] Ravindra L. Mehta.[3] *[1]IIBISMED, Univ Mayor de San Simon, School of Medicine, Bolivia; [2]Hospital Obrero #2, CNS, Bolivia; [3]Univ of California San Diego.*

Background: AKI diagnosis is based on changes of creatinine and diuresis, which could delay identification of AKI at earlier stages. Findings in urinary sediment have been proposed as an earlier AKI biomarker to detect kidney damage before a drop in kidney function occurs. We hypothesized that the use of urinary sediment score (Perazella et al Clin J Am Soc Nephrol 3:1615-19; 2008) in critically-ill patients could predict the development of AKI, and be related to in-hospital mortality.

Methods: We included 50 consecutive patients admitted to our Institution Medical ICU. We measured serum creatinine and collected urine every 24 hours for 7 consecutive days following ICU admission. Urine sediment was evaluated daily and assigned a score from 1 to 3 using Perazella et al criteria. We analyzed the predictive value of the urinary sediment score for the subsequent development of AKI as well to predict survival.

Results: We identified 26 (52%) patients with AKI (KDIGO guidelines sCr criteria), and 24 (48%) patients who did not develop AKI. Urinary sediment at 24 and 48 hours showed a mean score of 1.88±0.68 points in non-AKI patients versus 2.88±0.32 points in AKI patients (p=0.001); and 2.04±0.75 points in non-AKI patients versus 2.96±0.19 points in AKI patients (p=0.001); respectively. Patients with an urinary sediment score ≥ 3 at 24 and 48 hours were at higher risk of developing AKI within 7 days of follow-up (RR 5.87 95% CI 2.34 – 14.7;p < 0.001 and RR 4.31 95% CI 2.22 – 8.38;p < 0.001 respectively). The sediment score was able to predict the development of AKI (AUC 0.876 [95% CI 0.772-0.980], p < 0.0001; and AUC 0.840 (95% CI 0.720-0.959), p < 0.0001 at 24 and 48 hours; respectively). A urinary sediment score ≥2 was related to increased 28 day in-hospital mortality as compare to a score ≤1.

Conclusions: A urinary sediment score of ≥2 predicts the development of AKI before a rise in sCr occurs. This low cost tool would permit the early identification of AKI to initiate preventive and treatment strategies minimizing extension of kidney injury.

TH-PO093

Urinary α-Glutathione S-Transferase and π-Glutathione S-Transferase in Acute Kidney Injury following Cardiovascular Surgery Kai-Hsiang Shu,[1] Taomin Huang,[2] Vincent Wu.[1] *[1]Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ Hospital Yun-Lin Branch, Dou-Liu, Taiwan.*

Background: Urinary biomarkers have been proposed to augment the diagnosis of acute kidney injury (AKI), with AKI after cardiovascular surgeries being the prototype of evaluating scenario. Glutathione S-transferse (GST), scavenging free radicals, with α-GST localized in the proximal tubule and π-GST in the distal tubule, are thus evaluated prospectively as biomarkers.

Methods: Urine samples were collected in 142 adults undergoing cardiac surgeries for analyses of urinary α-GST and π-GST using enzyme-linked immunosorbent assay (ELISA), with and without normalization to urinary creatinine concentration. AKI events were defined as AKI network (AKIN) criteria. The composite outcome of renal replacement therapy and all cause mortality were also recorded.

Results: Twenty-one (14.8%) subjects had AKIN stage 1; 4 (2.8%) patients had AKIN stage 2; 8 (5.6%) patients progressed to AKIN stage 3. Hospital mortality rate was 7.2% (9 events). Urinary π-GST concentration at 3 hours after cardiovascular surgery was able to predict all AKI (AUC = 0.61, p = 0.055), severe AKI (stage 2 or 3, AUC = 0.81, p < 0.001), stage 3 AKI (AUC = 0.78, p = 0.008), and mortality (AUC = 0.82, p = 0.001). In contrast, urinary α-GST concentration at 24 hours after cardiovascular surgery was better at predicting severe AKI (stage 2 or 3, AUC = 0.68, p = 0.04), stage 3 AKI (AUC = 0.72, p = 0.037), and mortality (AUC = 0.77, p = 0.008). Normalization of urinary π-GST and α-GST concentration to urinary creatinine concentration did not further improve the diagnostic performance.

Conclusions: Urinary π-GST predicts adverse renal outcome early and hospital mortality, while α-GST performs better at 24 hours following cardiovascular surgery. Both are more accurate at predicting advanced AKI. The differential time frame of the two biomarkers heralds further elucidation of the pathogenesis. This prospective observational study demonstrates that urinary GST can be a highly versatile marker for early detection of AKI and outcome in AKI patients.

TH-PO094

Incorporation of Urinary Biomarkers with Renal Angina Index Refines Prediction of Severe AKI in a Heterogeneous Patient Population Shina Menon,[1] Theresa A. Mottes,[2] Lin Fei,[3] Stuart Goldstein,[2] Rajit K. Basu.[2] *[1]Pediatric Nephrology, Indraprastha Apollo Hospitals, New Delhi, India; [2]Center for Acute Care Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [3]Biostatistics and Bioepidemiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Accurate prediction of acute kidney injury (AKI) in heterogeneous patient populations is challenging. We previously demonstrated optimized prediction of AKI via incorporation of novel biomarkers into the renal angina index (RAI) risk stratification model in children with *sepsis.*

Methods: We conducted a prospective observational study of RAI prediction of AKI in all patients admitted to a single tertiary pediatric intensive care unit. Renal angina fulfillment was determined by the RAI on the day of PICU admission (Day 0). Urinary neutrophil gelatinase associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), and liver-fatty acid binding protein (L-FABP) were measured on Day 1. The primary outcome was KDIGO Stage II AKI (100% SCr rise from baseline) 3 days after admission (Day 3-AKI).

Results: 137 patients enrolled from September 2012 to December 2013 were included. Renal angina was present in 50 pts on Day 0 (36.4%). Day 3-AKI occurred in 33 pts (24%) with a three-fold higher incidence in patients with renal angina. RAI had high negative predictive value (NPV) for Day 3-AKI. Incorporation of NGAL and l-FABP with RAI increased predictive discrimination, measured by area under the curve (AUC) for Day 3 – AKI (0.69 to 0.77 with inclusion of either biomarker). The specificity of prediction was augmented (71% to 90% with either biomarker), increasing positive likelihood ratio (LR) (2.8 to 4.3 and 4.4, respectively) and resulting in significant net reclassification improvement (NRI) (0.39 and 0.67, respectively) and integrated discrimination improvement (IDI).

Conclusions: Incorporation of biomarkers with RAI yields refined AKI prediction, increasing specificity by reducing the rate of false positivity. We suggest renal angina risk stratification, strengthened by biomarker incorporation, identifies children at very low risk of AKI 72 hours after PICU admission with near certainty.

TH-PO095

Pattern of Urinary NGAL Elevation and Acute Kidney Injury Severity in Critically Ill Patients Fernando de Almeida Soares, Lilian Carmo, Camila Lima, Vivian Lumi Onusic, Liliany P. Repizo, Emmanuel A. Burdmann, Etienne Macedo. *Nephrology, Univ of Sao Paulo - USP, Sao Paulo, SP, Brazil.*

Background: Biomarkers are a promising tool for early Acute Kidney Injury (AKI) recognition that could lead to improvement in patients' outcomes. In clinical intensive care units (ICU), the time of insult leading to kidney injury is frequently unknown and biomarkers cutoff points are cumbersome. The purpose of this study is to evaluate if the pattern of elevation of urinary neutrophil gelatinase-associated lipocalin (NGALu) could be useful to predict AKI severity in critical ill patients.

Methods: We performed a prospective study between Jan12 and July13. We enrolled patients with high risk for AKI who were admitted in the ICU. Urinary NGAL were measured every 12h during the first 2days of ICU stay. We used the terms NGAL(+)/(-) according to study-specific NGAL cutoff for optimal AKI prediction, and the terms sCr(+)/(-) according to KDIGO criteria. Maximum delta uNGAL was defined by the greatest 12h difference between the sequential intervals. We categorized patients according to uNGAL and sCr classification.

Results: Of the 272 screened patients, 46 met the inclusion criteria. Thirty (62.5%) patients met KDIGO criteria for AKI. Four patients had AKI before ICU admission and so were excluded. In patients reaching a max KDIGO stage2/3, the delta uNGAL was 58,0 (-4,5-546,6) versus 10,8 (-3,3-50) in patients reaching max KDIGO1 (p= 0,04). There was a progressive increase in delta uNGAL with increasing biomarker positivity as shown in Table 1.

Biomarker Posivity and Maximum Delta Urinary NGAL levels

NGAL + / sCr +	152.8 (6.2- 1097)
NGAL + / sCr -	32.5 (-8.0-57.3)
NGAL - / sCr +	13.3 (1.2-31.5)
NGAL - / sCr -	6.8 (-6.0-35)

Seven (16,7%) patients needed RRT: six in the group NGAL+/sCr+ and one in the group NGAL -/sCr +. The mortality rate was higher in the NGAL+/sCr + group (23,8%), and lower in the others groups: NGAL-/sCr+ 4,7%; NGAL+/sCr– 2,3%; NGAL-/sCr– 2,3%.

Conclusions: Delta NGAL can be a predictor of AKI severity and the need for RRT. The association of groups of positive biomarkers and delta values in addition to the absolute values could improve their performance to predict AKI severity and the need for dialysis.

Funding: Government Support - Non-U.S.

TH-PO096

Identifying and Evaluating Combinations of Markers to Predict AKI Allison Meisner,[1] Kathleen F. Kerr,[1] Heather Thiessen Philbrook,[2] Steven G. Coca,[3] Chirag R. Parikh.[3] *[1]Dept of Biostatistics, Univ of Washington, Seattle, WA; [2]Div of Nephrology, Dept of Medicine, Western Univ, London, ON, Canada; [3]Section of Nephrology, Yale School of Medicine, New Haven, CT.*

Background: Urinary biomarkers of kidney injury provide modest prediction of acute kidney injury after cardiac surgery when considered individually. We developed and applied a rigorous framework to search for biomarker combinations to improve performance of AKI risk prediction models after cardiac surgery. We carefully addressed several important statistical issues, including the effect of center and the impact of optimism, when developing these combinations.

Methods: The framework involves splitting the data into training and test datasets, using logistic regression to fit models with combinations of markers and clinical covariates, and evaluating these models using center-adjusted AUC that is corrected for optimistic resubstitution bias. We applied this framework to the TRIBE-AKI study, which prospectively followed 1219 adults after cardiac surgery.

Results: Based on the training dataset, we identified 3 promising models from over 1000 models with 3 variables. The models included cardiopulmonary bypass time and change in serum creatinine plus a post-operative biomarker (urine IL-18, urine KIM-1 or urine albumin) and showed promising performance in the training dataset (Table below). The optimism-corrected AUCs for each model were higher in the training dataset than in the test dataset (Table) but still suggested good performance in the test dataset. The test dataset was limited by the small number of events.

Conclusions: Linear combination methods suggest limited improvement in accuracy for predicting AKI events. External validation of these models is required in other cardiac surgery datasets.

Center-Adjusted AUC

	Training Dataset* n=830, # severe AKI cases = 39	Test Dataset n=389, # severe AKI cases = 21
CPB Time, ΔSCr and Urine IL-18	0.858	0.751
CPB Time, ΔSCr and Urine KIM-1	0.854	0.743
CPB Time, ΔSCr and Urine Albumin	0.851	0.731

* AUC corrected for optimistic resubstitution bias

Funding: Other NIH Support - NHLBI RO1HL085757

TH-PO097

Cardiac Biomarkers in the Prediction of Postoperative Acute Kidney Injury in Children Undergoing Cardiac Surgery Emily Marie Bucholz,[1] Chirag R. Parikh.[1,2] *[1]Yale Univ School of Medicine, New Haven, CT; [2]Translational Research Investigating Biomarkers and Endpoints for Acute Kidney Injury (TRIBE-AKI) Consortium, Yale Univ, McMaster Univ.*

Background: Preliminary evidence suggests that renal biomarkers can help identify pediatric patients at risk for developing AKI after cardiac surgery; however, little is know about the prognostic utility of cardiac biomarkers.

Methods: Data from TRIBE-AKI, a prospective study of children <18 years of age undergoing cardiac surgery, were used to examine the association of cardiac biomarkers (NT pro-BNP, CK-MB, and troponins I and T (cTnI and cTnT)) with the development of postoperative AKI. Cardiac biomarkers were collected prior to and 0-6 hours after surgery. Severe AKI was defined as a doubling of creatinine or dialysis within 7 days of surgery. Logistic regression and receiver-operating characteristic curves were used to evaluate the association between cardiac biomarkers and postoperative AKI.

Results: Of the 106 patients, 23(22%) developed severe AKI after cardiac surgery. Patients who developed severe AKI had higher median levels of pre- and postoperative NT pro-BNP, CK-MB, cTnI, and cTnT compared to patients without AKI ($p<0.01$). Preoperatively, higher levels of CK-MB were associated with increased odds of developing severe AKI (OR 3.26, 95%CI:0.94-11.31). When combined with other clinical variables, preoperative CK-MB provided good discrimination of AKI status (area under the curve (AUC) 0.83, 95%CI:0.58-0.84). Among postoperative biomarkers, cTnI and cTnT were associated with increased odds of developing severe AKI (cTnI: OR 1.44, 95%CI 0.81-2.57; cTnT: OR 1.62, 95%CI:0.83-3.18) and yielded high AUCs when combined with the clinical model (cTnI: AUC 0.87, 95%CI:0.79-0.96; cTnT: AUC 0.86, 95%CI:0.78-0.95).

Conclusions: Preoperative CK-MB and postoperative cTnI and cTnT are associated with increased risk of postoperative AKI and provide good discrimination between patients who develop severe AKI and those who do not. These cardiac biomarkers may be useful for risk stratification of pediatric patients undergoing cardiac surgery; however, larger studies are needed to confirm these results.

Funding: NIDDK Support

TH-PO098

Plasma Interleukin-6 and Interleukin-10 Predict AKI and Death in Adults after Cardiac Surgery William R. Zhang, Amit X. Garg, Steven G. Coca, John Eikelboom, Peter Kavsak, Eric Mcarthur, Colleen Shortt, Heather Thiessen Philbrook, Michael Shlipak, Richard P. Whitlock, Chirag R. Parikh. *TRIBE-AKI Consortium.*

Background: Inflammation plays an integral role in the pathophysiology of acute kidney injury (AKI). We investigated the utility of plasma cytokines interleukin (IL)-6 and IL-10, two known components of inflammation in acute injury, as biomarkers of AKI and mortality in adults at high risk for AKI undergoing cardiac surgery.

Methods: Patients in the TRIBE-AKI cohort study were enrolled at six academic centers. AKI was defined as a doubling in serum creatinine concentration from baseline or receipt of acute dialysis during hospitalization. Pre- and postoperative (0-6 hours after surgery) measurements of IL-6, IL-10, and IL-6:IL-10 ratio were categorized into tertiles and evaluated for associations with outcomes of in-hospital AKI or post-discharge all-cause mortality at a median of 3 years after surgery.

Results: Elevated postoperative IL-6 was associated with increased risk of AKI (OR 6.4, 95% CI 2.2-18.7), though the association was attenuated following adjustment (OR 3.0, 95% CI 0.94-9.5). IL-6 was not associated with risk of mortality. While IL-10 was not associated with risk of AKI, elevated postoperative IL-10 was independently associated with lower risk of mortality (HR 0.72, 95% CI 0.56-0.93). There was significant interaction by neutrophil gelatinase-associated lipocalin (NGAL), an established AKI biomarker, on the association of IL-10 and mortality, such that the independent protective effect conferred by IL-10 was only observed in patients with plasma or urine NGAL levels above the median (p=0.01). Elevated IL-6:IL-10 ratio was independently associated with risk of AKI preoperatively (OR 3.2, 95% CI 1.1-9.6) and with risk of mortality pre- and postoperatively (HR 1.4, 95% CI 1.1-2.0; HR 1.4, 95% CI 1.2-1.6, respectively).

Conclusions: After cardiac surgery, elevated plasma IL-6 approached independent association with increased AKI, and elevated plasma IL-10 was independently protective against long-term mortality, suggesting that these cytokines may serve as biomarkers for perioperative outcomes.

Funding: Other NIH Support - NHLBI

TH-PO099

NTpro-BNP Levels Are Associated with Severe Acute Kidney Injury and 1-Year Mortality after Cardiac Surgery Emilie P. Belley-Cote,[1] Chirag R. Parikh,[3] Colleen Shortt,[1] Steven G. Coca,[3] John Eikelboom,[1,2] Amit X. Garg,[4] Peter Kavsak,[1] Eric Mcarthur,[5] Heather Thiessen Philbrook,[4] Richard P. Whitlock.[1,2] *[1]McMaster Univ, ON, Canada; [2]Population Health Research Inst, ON, Canada; [3]Yale Univ, CT; [4]Western Univ, ON, Canada; [5]Inst for Clinical Evaluative Sciences, ON, Canada.*

Background: Acute kidney injury (AKI) is a frequent complication after cardiac surgery and is associated with higher post-operative morbidity and mortality. Perioperative cardiac biomarkers levels may predict AKI.

Methods: In a prospective multicenter cohort of adults undergoing cardiac surgery, we measured the following biomarkers in pre and post-operative banked plasma samples: high-sensitivity cardiac troponin T (hs-cTnT), troponin I (TnI), CK-MB and NTpro-BNP. Our objectives were to evaluate whether these biomarkers are associated with severe AKI after surgery, defined as a doubling in serum creatinine or AKI requiring hemodialysis, and 1-year mortality.

Results: In the patients who were discharged alive, severe AKI occurred in 37/960 (3.9%) and 43/960 (4.5%) died within 1 year of follow-up. AKI occurred a median of 3 days (IQR 2-4) after surgery. NTpro-BNP was the only pre-operative biomarker that was independently associated with severe AKI (adjusted OR=1.4, 95% CI (1.0, 1.9)). Day 1 post-operative biomarkers (measured within 6 hours post-operatively) were all associated with severe AKI.

Day 1 Biomarkers and Association with Severe AKI

Biomarker	Adjusted OR (95% CI)
hs-cTnT (ng/L)	2.1 (1.3, 3.3)
TnI (ug/L)	1.6 (1.1, 2.3)
CK-MB (ug/L)	2.2 (1.3, 3.7)
NTpro-BNP (pg/mL)	1.5 (1.1, 2.0)

Pre-operative NTpro-BNP was also associated with 1-year mortality (adjusted OR=1.7, 95% CI (1.2-2.2)). Day 1 NTpro-BNP was associated with 1 year mortality independently of change in creatinine from pre-operative level (adjusted OR=1.6, 95% CI (1.2-2.2)).

Conclusions: Early increases in post-operative cardiac biomarkers are associated with severe AKI after cardiac surgery. Of the studied biomarkers, NTpro-BNP is the only pre-operative biomarker independently associated of severe AKI and 1-year mortality. Future research should focus on whether interventions that lower NTpro-BNP can impact upon post-operative outcomes.

Funding: Other NIH Support - NIH and NHLBI

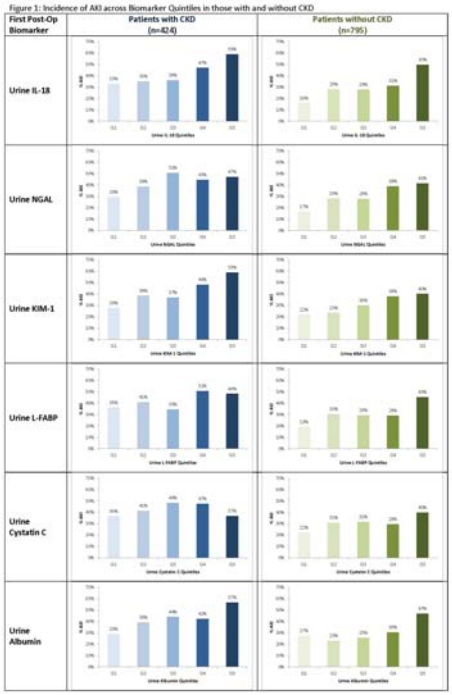
TH-PO100

Chronic Kidney Disease Does Not Impact the Utility of Urinary Acute Kidney Injury Biomarkers Jay L. Koyner, Heather Thiessen Philbrook, Steven G. Coca, Uptal D. Patel, Michael Shlipak, Amit X. Garg, Chirag R. Parikh. *Translational Research Investigating Biomarker Endpoint (TRIBE)-AKI Investigators.*

Background: The impact of underlying chronic kidney disease (CKD) on the performance of AKI biomarkers is unclear.

Methods: We stratified the TRIBE AKI adult cohort (n=1219) into those with (n=424) and without (n=795) CKD (eGFR <60ml/min), and examined the association of the first post-operative urinary concentration of 6 biomarkers (neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), and liver fatty acid binding protein (L-FABP), cystatin C and albumin) with the development of post-operative AKIN Stage 1 AKI following cardiac surgery. We categorized those with and without CKD into quintiles for each of the 6 biomarkers and determined the adjusted relative risk (aRR) of developing AKI. We also assessed the interaction with CKD using continuous log-transformed values of the biomarkers.

Results: A total of 180(42%) patients with CKD developed AKI compared to 246(31%) in the non-CKD group; p<0.001. Figure 1 displays the incidence of AKI across quintiles for each biomarker in those with and without CKD.



There was a significant difference in the incidence of AKI across quintiles for NGAL (p=0.01), L-FABP (p=0.04) and Albumin (p=0.04). For these biomarkers the aRR's were higher in those without CKD versus those with CKD. We only observed a significant interaction by CKD on the relationship between the biomarker and AKI for IL-18 using the continuous log-transformed values (non-CKD aRR [95%CI] 1.17[1.11-1.23] versus CKD 1.06[0.99-1.13]; interaction p=0.007).

Conclusions: Although interactions by CKD on the association between biomarkers and AKI may be present, they are not uniformly significant and the magnitude of the effect modifications were small. Adjusting cutoffs of urinary biomarkers for AKI in those with pre-existing CKD is not warranted.

Funding: NIDDK Support, Other NIH Support - NHLBI - R01HL-085757

TH-PO101

Adjudication of Etiology of Acute Kidney Injury: Experience from the TRIBE-AKI Multi-Center Study Jay L. Koyner,[1] Amit X. Garg,[1] Heather Thiessen Philbrook,[1] Steven G. Coca,[1] Lloyd G. Cantley,[2] Aldo J. Peixoto,[2] Chirag R. Parikh.[1] *[1]TRIBE-AKI Investigators; [2]Section of Nephrology, Yale Univ.*

Background: Minimal data exists on the adjudication process in the setting of Acute Kidney Injury (AKI) to confirm our ability to judge different etiologies (e.g. Acute Tubular Necrosis (ATN), Pre-renal Azotemia (PRA)) in research setting.

Methods: We enrolled 475 consecutive adults undergoing cardiac surgery at 4 sites for this sub-study of the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE-AKI) study. Three expert nephrologists performed independent chart review, utilizing clinical variables and retrospective case report forms with pre, intra, and post-operative data, and then adjudicated all cases of AKI (n=67). AKI was defined as a >50% increase in serum creatinine from baseline (RIFLE "Risk"). We examined the patterns of AKI diagnoses made by the adjudication panel as well as association of these diagnoses with pre and postoperative AKI biomarkers.

Results: Based on the agreement of 2 out of 3 reviewers, ATN was the adjudicated diagnosis in 41 cases (61%) while PRA in 13 (19%) and the rest were indeterminate.

	Final Adjudication Result			Total
Etiology by Panelist Adjudication	ATN	PRA	IND	n (%)
3 ATN	11			11 (16)
3 PRA		2		2 (3)
2 ATN; 1 PRA	16			16 (24)
2 ATN; 1 IND	11			11 (16)
2 PRA; 1 ATN		4		4 (6)
2 PRA; 1 IND		6		6 (9)
2 IND; 1 ATN			2	2 (3)
2 IND; 1 PRA			5	5 (8)
1 ATN; 1 PRA; 1 IND	3	1	6	10 (15)
Total	41	13	13	67 (100)
ATN= Acute Tubular Necrosis; PRA = Pre-Renal Azotemia; IND= Indeterminate				

Neither serum creatinine nor any biomarker of AKI (urine or serum) was associated with an adjudicated diagnosis of ATN within the first 24 post-operative hours. Patients adjudicated as ATN had a longer duration of AKI and worse clinical outcomes. There was poor agreement across the panel of reviewers with adjudicated diagnoses being independent of each other (Fleiss' Kappa=0.046).

Conclusions: The etiology of AKI after cardiac surgery is multi-factorial and pure AKI etiologies, such as ATN and PRA may not exist in this setting. Biomarkers did not appear to correlate with the adjudicated etiology of AKI; however, the lack of agreement among the adjudicators impacted these results.

Funding: NIDDK Support, Other NIH Support - NHLBI, Private Foundation Support

TH-PO102

Association of Plasma Inflammatory Biomarkers with Acute Kidney Injury after Pediatric Cardiac Surgery Jason Henry Greenberg,[1] William R. Zhang,[1] Heather Thiessen Philbrook,[2] Michael Zappitelli,[3] Prasad Devarajan,[4] Richard P. Whitlock,[5] John Eikelboom,[5] Peter Kavsak,[5] Amit X. Garg,[2] Colleen Shortt,[5] Chirag R. Parikh.[1] *[1]Yale Univ; [2]Western Univ, Canada; [3]McGill Univ, Canada; [4]Cincinnati Children's; [5]McMaster Univ, Canada.*

Background: Children undergoing cardiac surgery may exhibit a pronounced inflammatory response to cardiopulmonary bypass(CPB). Inflammation is recognized as an important pathophysiologic process leading to acute kidney injury(AKI). The aim of this study was to evaluate the association of two inflammatory cytokines interleukin(IL)-6 and IL-10 with AKI and other adverse outcomes in children after CPB surgery.

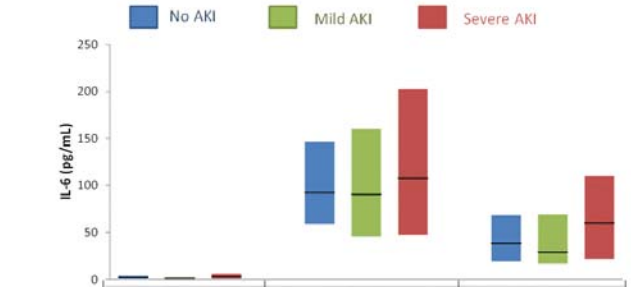
Methods: This is a sub-study of the TRIBE-AKI cohort, including 106 children from 1 month to 18 years old, undergoing CPB. Plasma IL-6 and IL-10 were measured preoperatively and postoperatively on Days 1(within 6 hours after surgery) and 3.

Results: Mild AKI, defined by a ≥50% rise of baseline serum creatinine, and severe AKI, defined by dialysis or a doubling of baseline serum creatinine, was diagnosed in 31(29%) and 24(23%) patients respectively. Preop IL-6 was significantly higher in patients with severe AKI versus without severe AKI(median 2.59(0.6-4.88) versus 1.66(0.6-3.08) p=0.03). After adjustment for age, gender, race, and study site, the highest preop IL-6 tertile was associated with an eight-fold increased risk for severe AKI compared with the lowest tertile(adjusted OR 7.9(CI: 1.7-37.3)). IL-6 and IL-10 increased significantly after

surgery, peaking on Day 1 postoperatively(Figure 1). First postoperative IL-6 and IL-10 did not significantly differ between patients with versus without severe AKI. Elevated IL-6 on day 3 was associated with longer hospital stay(p=0.0001).

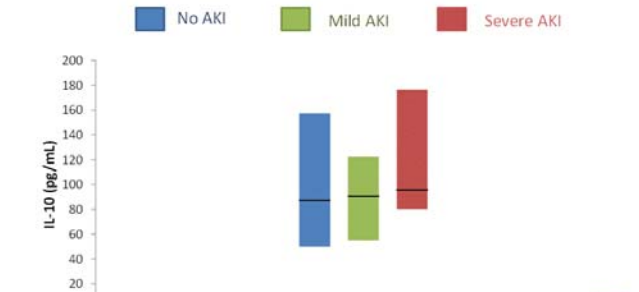
Figure 1. Inflammatory Biomarker Distribution by AKI Status

A. IL-6



	Pre-op			Day 1			Day 3		
25th pct	0.6	0.6	0.6	58.73	45.57	47.33	19.08	16.7	21.61
Median	1.66	0.6	2.59	91.73	89.81	107.27	38.08	28.5	59.39
75th pct	3.08	1.44	4.88	145.36	158.85	201.15	67.15	67.9	108.7

B. IL-10



	Pre-op			Day 1			Day 3		
25th pct	0.9	0.9	0.9	49.67	54.65	79.805	0.9	0.9	0.9
Median	0.9	0.9	0.9	86.53	90.29	95	0.9	0.9	2.64
75th pct	1.95	0.9	0.9	156.2	121.6	175.2	0.9	2.39	6.33

Conclusions: Pre-operative IL-6 is associated with development of severe AKI and may be prognostic of resource utilization.

Funding: Other NIH Support - NHLBI

TH-PO103

Intraabdominal Hypertension Did Not Predict Short Term Renal Recovery or in Hospital Mortality in Critically Ill Patients with AKI

Hyojeong Chang, Myung-Gyu Kim, Sang-Kyung Jo, Won-Yong Cho. *Korea Univ Anam Hospital, Seoul.*

Background: Although emerging evidence suggest that intraabdominal hypertension (IAH) is a useful predictor of the development of acute kidney injury (AKI), whether the presence of IAH could also predict renal recovery or mortality remains unclear. The purpose of this study was to determine the prevalence of IAH and also whether the presence of IAH could predict short term renal recovery or in-hospital mortality in critically patients with AKI. Prognostic value of urinary biomarkers including neutrophil gelatinase-associated lipocalin (NGAL) and liver-type fatty acid-binding protein (L-FABP) in predicting renal recovery or in-hospital mortality was also determined.

Methods: This is a prospective observational study enrolling 52 patients who admitted to intensive care unit with AKI from February 2012 and January 2014. Urine NGAL and L-FABP at the time of ICU admission were examined, and intra-abdominal pressure(IAP) was measured for initial three days and their average value was calculated. The IAH was defined as a mean IAP>12mmHg.

Results: Mean age was 69.2±14.5 years and male was 48.0%. At the time of admission, mean simplified acute physiology score (SAPS) II score was 43.5±15.2 and IAH was found in 84.6% of patients. AKI with failure grade according to RIFLE criteria developed in 65.4% and 57.7% of patients needed renal replacement therapy. In hospital mortality was 21.2% and renal recovery defined as serum creatinine level below 0.45mg/dL or within above 20% of baseline value during hospitalization was achieved in 42% of patients. Although both high urine NGAL (OR, 1.018) and L-FABP (OR, 1.032) were found to be independent predictors of renal recovery, presence of IAH did not predict renal recovery. For predicting in-hospital mortality, high SAPS II score (OR, 1.094) and high urine NGAL (OR, 1.032) were independent predictors in multivariate analysis; however IHA and urine L-FABP were not.

Conclusions: IAH is prevalent in critically ill patients with established AKI. However, it did not predict short term prognosis including renal recovery or in-hospital mortality. Urine NGAL was found to be a useful predictor of both renal recovery and in-hospital mortality.

TH-PO104

The Role of Circulating Tumor Necrosis Factor Receptor 1 and 2 in Contrast-Induced Nephropathy

Jung Nam An,[1] Dong Ki Kim,[2] Yun Kyu Oh,[1] Yon Su Kim,[2] Chun Soo Lim,[1] Jung Pyo Lee.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Seoul National Univ Hospital, Seoul, Republic of Korea.*

Background: Contrast-induced nephropathy (CIN) is the important cause of hospital acquired acute kidney injury. Accurate understanding of the pathogenesis and the prevention and early intervention of CIN are crucial. Thus, the aim of this study was to evaluate the clinical role of circulating tumor necrosis factor receptors (cTNFRs) as predictors for CIN.

Methods: During the period of May 2013 to February 2014, a total of 262 patients who underwent coronary angiography and/or percutaneous coronary intervention in Seoul National University Boramae Medical Center, were enrolled. CIN was defined as either an increase in serum creatinine (sCr) ≥ 0.25 mg/dL or ≥ 25% within 48 hours after procedure.

Results: Male gender was 64.1%, mean age was 64.6 ± 10.9 years, and the patients with diabetes and chronic kidney disease were 27.5% and 17.6%, respectively. Overall patients had fluid therapy before and after contrast use, and 36.3% underwent percutaneous coronary intervention. CIN developed in 4.2% of patients and the levels of cTNFRs were as follows (CIN group versus non-CIN group): cTNFR1, 3223.8 ± 2899.4 pg/mL versus 1330.8 ± 841.5 pg/mL ($P = 0.026$); cTNFR2, 6535.8 ± 5353.1 pg/mL versus 3226.4 ± 2926.3 pg/mL ($P = 0.090$). The risk factors for CIN were younger age, underlying diseases as stroke and chronic kidney disease, the use of N-acetylcysteine, and higher levels of ln(cTNFRs). Increased serum levels of ln(cTNFR1) (odds ratio 16.97; 95% confidence interval 4.24-67.95; $P < 0.001$) and ln(cTNFR2) (odds ratio 4.16; 95% confidence interval 1.55-11.18; $P = 0.005$) were significantly associated with the development of CIN after adjusting other risk factors including baseline renal function and diabetes.

Conclusions: Markedly elevated circulating TNFR levels are independent predictors of decreased renal function after contrast-related procedure. Further studies, establishing the significance of cTNFRs as prognostic markers of CIN, will be needed.

TH-PO105

Urine Vitamin D Binding Protein and Complement C 3: Novel Prognostic Biomarkers in Acute Kidney Injury

Nithin Karakala,[1] Joseph Alge,[1] Michael G. Janech,[1] James A. Tumlin,[4] Lakhmir S. Chawla,[2] Andrew Shaw,[3] John M. Arthur.[1] *[1]Medicine, Nephrology, Medical Univ of South Carolina; [2]Medicine, Nephrology, Washington DC VA Medical Center; [3]Anesthesiology, Vanderbilt Univ Medical Center; [4]Medicine, Nephrology, UTCOM.*

Background: Improved biomarkers of acute kidney injury (AKI) that can predict the prognosis of the patient early in the injury are needed. We previously identified novel AKI biomarker proteins by mass spectrometry. The aim of this study was to validate the use of two novel markers, urine vitamin D binding protein (VDBP) and complement C3, as potential prognostic biomarkers to predict outcomes in patients with AKI.

Methods: We used a cohort of 153 patients who underwent cardiovascular surgery and developed AKI stage 1 within 72 hours after the procedure. Urinary VDBP and complement C3 were measured by ELISA, urine creatinine was measured by Jaffe's method. The primary outcome was a composite of death, renal replacement therapy (RRT) and AKIN stage 3. Secondary outcomes were death, RRT, and RRT or death.

Results: There were no significant differences between the outcome groups with respect to demographics, underlying medical conditions, type of surgery or pre-op creatinine. Urine VDBP (AUC 0.67) and complement C3 (AUC 0.74) concentrations were highly discriminative for the composite primary outcome. The ability to predict primary outcome further improved when the urine biomarkers were normalized to urine creatinine (uCr), VDBP/uCr (AUC: 0.73) and complement C 3 (AUC: 0.77). The AUC values of VDBP/uCr for predicting the secondary outcomes were: death (0.71), RRT (0.68), RRT or death (0.73) The AUC values for complement C3/uCr were: death (0.77), RRT (0.70) and RRT or death (0.75).

Urine Biomarker	Primary Outcome Group (n=17) Median (IQR)	No Outcome Group (136) Median(IQR)	p Value	AUC
VDBP (ng/ml)	0.44 (0.11,1.130)	0.17 (0.06,0.34)	0.02	0.67
Comp C 3 (ng/ml)	100 (37.5, 192.5)	19.9 (5,68.8)	0.001	0.74
VDBP/uCr (ng/mg)	0.43 (0.18, 2.27)	0.14 (0.07, 0.41)	0.001	0.73
Comp C 3/uCr (ng/mg)	152.7 (48, 497)	19.9 (5.4, 77.9)	<0.001	0.77

Conclusions: Urine VDBP and complement C3 are novel biomarkers useful in predicting outcomes of death and renal replacement therapy in patients with early AKI.

Funding: NIDDK Support

TH-PO106

Urinary Biomarkers of Acute Kidney Injury in Children after Cardiac Surgery Michael A. Ferguson,[1] Venkata Sabbisetti,[2] Satish Rajagopal,[3] Joshua Blinder,[3] Joseph V. Bonventre,[2] Sushrut S. Waikar.[2] *[1]Div of Nephrology, Boston Children's Hospital, Boston, MA; [2]Renal Div, Brigham and Women's Hospital, Boston, MA; [3]Dept of Cardiology, Boston Children's Hospital, Boston, MA.*

Background: Acute kidney injury (AKI) is common after pediatric cardiac surgery and associated with increased morbidity and mortality. Current metrics used to assess for AKI have suboptimal sensitivity and specificity. Urinary biomarkers may identify those with or at risk for AKI earlier than conventional markers.

Methods: We conducted a prospective study of children undergoing cardiac surgery to evaluate the diagnostic performance of urinary biomarkers of AKI, including kidney injury molecule 1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), *N*-acetyl-β-glucosaminidase (NAG), microalbumin (MAB), and total protein (TP). Urine samples were obtained at multiple time points. AKI was stratified using the pediatric modified RIFLE criteria (pRIFLE). Diagnostic performance was assessed using the area under the receiver operating characteristic curve (AUC-ROC).

Results: 16 (7%) subjects developed significant AKI (decrease in estimated GFR ≥ 50%). Those who developed AKI were younger, had longer surgical times, longer bypass times, and longer cross clamp times. Biomarker performance characteristics are detailed in Table 1. 2-hours post-operatively, no biomarker achieved an AUC-ROC > 0.70. The highest performing biomarker 8 hours post-operatively was NGAL (AUC-ROC of 0.79). The highest performing biomarker on day 1 was KIM-1 (AUC-ROC of 0.72).

Biomarker	AUC-ROC	Cutoff	Sensitivity	Specificity
2-Hour				
KIM-1 (ng/mg Cr)	0.65	0.93	88	50
MAB (mcg/g Cr)	0.65	34.3	71	63
NAG (mU/mg Cr)	0.70	64.9	77	68
NGAL (ng/mg Cr)	0.70	772	65	75
Protein (mg/mg Cr)	0.68	0.62	82	62
8-Hour				
KIM-1 (ng/mg Cr)	0.65	1.39	78	46
MAB (mcg/g Cr)	0.67	22.4	67	72
NAG (mU/mg Cr)	0.65	7.84	72	58
NGAL (ng/mg Cr)	0.79	35.7	83	79
Protein (mg/mg Cr)	0.75	0.20	72	73
Day-1				
KIM-1 (ng/mg Cr)	0.72	5.72	73	70
MAB (mcg/g Cr)	0.65	32.5	53	77
NAG (mU/mg Cr)	0.60	5.32	67	62
NGAL (ng/mg Cr)	0.67	24.3	73	57
Protein (mg/mg Cr)	0.62	0.09	87	45

Conclusions: Urinary biomarkers of kidney injury had only moderate AUC-ROCS when compared against creatinine-based definitions of AKI.

Funding: Pharmaceutical Company Support - Genentech, Inc., Private Foundation Support

TH-PO107

Midkine Levels in Acute Kidney Injury After Cardiac Bypass - A Pilot Study Victoria K. Campbell,[1,2] Chris Anstey,[1] Sharron T. Hall,[2,4] Shay Mcguinness,[3] Rachael L. Parke.[3] *[1]Dept of Intensive Care, Sunshine Coast Hospital and Health Service, Nambour, Queensland, Australia; [2]Cellmid LTD, Sydney, New South Wales, Australia; [3]Dept of Intensive Care, Auckland City Hospital, Auckland, New Zealand; [4]Pathology North, Hunter New England, Newcastle, New South Wales, Australia.*

Background: There is great interest in biomarkers for early detection of acute kidney injury (AKI), and to identify those who will develop chronic kidney disease (CKD). Midkine (MK) is a pro-inflammatory and pro-fibrotic cytokine, implicated in both. The aim of this study was to quantify pre and post-operative MK levels in cardiac-bypass patients, and to identify any association with degree of cardiac-surgery associated acute kidney injury (CSA-AKI).

Methods: Stored samples of plasma and urine from a trial cohort of cardiac-surgery patients (Sodium Bicarbonate to reduce Cardiac Surgery Associate Acute Kidney Injury: A Phase ll Multi-centre, Double-blind, Randomized Controlled Trial.2013. Mcguiness et al. Crit Care Med 41(7) 1599), from before, immediately after, and the morning after surgery were obtained. Samples were assayed for MK levels using a commercially available validated MK-ELISA kit (Cellmid Ltd, Sydney, Australia). Plasma and urine had been stored at -70ºC. CSA-AKI was defined as an increase in plasma creatinine concentration greater than 25% from baseline to peak within 5 days of bypass.

Results: Sixty patients were selected. Plasma MK levels significantly increased in most patients immediately post-operative and fell on day 1 ($p<0.001$). Urine MK significantly increased from baseline on day 1 ($p=0.008$). Patients were evenly divided into 3 AKI groups: a <25% increase in creatinine (non-AKI controls), an increase 25-50%, and an increase of >50%. The plasma MK levels immediately post surgery and on day 1 correlated with those most severely affected (a >50% increase, $p=0.04$), but not over all degrees of AKI($p=0.14$). There was no correlation with urine MK levels and AKI.

Conclusions: The results of this study do not suggest a role of plasma and/or urine MK levels for predicting all degrees of CSA-AKI, but there is an association with the most severe CSA-AKI, and with cardiac bypass surgery itself, which warrant further exploration.

Funding: Pharmaceutical Company Support - Cellmid LTD

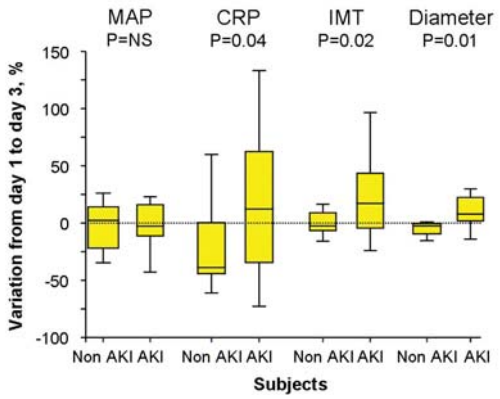
TH-PO108

Echotracking System and Bioelectrical Impedence Analysis Parameters Link to Acute Kidney Injury in Critical Ill Patients Paolo Lentini,[1] Luca Zanoli,[2] Massimo de Cal,[1] Stefania Rastelli,[2] Anna Basso,[1] Andrea Contestabile,[1] Antonio Granata,[3] Roberto Dell'Aquila.[1] *[1]Nephrology, S. Bassiano Hospital, Bassano Del Grappa, Italy; [2]Internal Medicine, Univ of Catania, Catania, Italy; [3]Nephrology, S: Giovanni di Dio Hospital, Italy.*

Background: Acute Kidney Injury (AKI) in the Intensive Care Unit (ICU) is a risk factor for mortality. Echotracking is the reference system for the non-invasive measure of arterial parameters, including intima-medial thickness (IMT), an independent predictor for cardiovascular events, and arterial diameter. Arterial wall thickness has been widely considered a structural vascular characteristic that changes slowly over years. However, acute changes in vascular smooth muscle tone and several cytokines may theoretically alter arterial wall thickness. Little is known about these changes in AKI. Bioelectrical impedance analysis(BIA) has been used to manage volume in chronic hemodialysis patients (pts) for decades: however, the use of BIA in ICU has not been extensively studied. We aimed to assess if pts that are developing AKI have parallel alterations of arterial structure and fluid overload.

Methods: Case-control study. 17 pts with AKI and 17 without AKI (age76±13yrs, 44% males) matched for baseline C-reactive protein (CRP) were selected and followed for three consecutive days. Arterial parameters were measured with an Echotracking system device using a 13MHz probe. BIA was performed with a multifrequency device to evaluate the hydration status.

Results: Reasons for ICU admission, age, SAPS II, SOFA and APACHE II were similar between cases and controls. During follow-up CRP, IMT and carotid diameter increased significantly in AKI, suggesting that inflammation may alter acutely arterial structure in AKI. At baseline AKI pts have a significant hyperhydration.



Conclusions: Inflammation, IMT, diameter and BIA are associated with AKI in ICU pts.

TH-PO109

Urinary L-Type Fatty Acid-Binding Protein Can Predict Long-Term Renal Outcome in the ICU Patients Rei Isshiki,[1] Kent Doi,[1,2] Maki Sumida,[1] Yoshifumi Hamasaki,[1] Naoki Yahagi,[2] Masaomi Nangaku,[1] Takeshi Sugaya,[3] Eisei Noiri.[1] *[1]Nephrology and Endcrinology, Univ of Tokyo, Tokyo, Japan; [2]Emergency and Critical Care Medicine, Univ of Tokyo, Tokyo, Japan; [3]CMIC Co., Ltd., Tokyo, Japan.*

Background: Although AKI was previously considered to be reversible, recent studies have suggested that AKI will increase the risk of CKD development/progression. However, it remains unclear whether any biomarker can predict progressive CKD in AKI survivors. This study evaluated the performance of urinary biomarkers for long-term renal outcome prediction in ICU population.

Methods: We prospectively enrolled 495 adult patients who had been admitted to the ICU of the University of Tokyo Hospital. We measured three urinary biomarkers at ICU admission; L-type fatty acid-binding protein (L-FABP), neutrophil gelatinase-associated lipocalin (NGAL), and N-acetyl-β-D-glucosaminidase (NAG). Serum creatinine(sCr) values within 7 days after ICU admission were used for AKI diagnosis. Patients were followed up for three years after ICU discharge and long-term renal outcomes of halving of eGFR or incident end-stage renal disease were evaluated.

Results: Of 495 patients, 169 patients (34.1 %) could be followed up for 3 years after ICU discharge. Among them, 30 patients (17.8 %) showed progression of renal dysfunction. Compared with the non-progressors, the progressors had a significantly higher rate of AKI during ICU stay (90.0% versus 40.3%; $p<0.0001$). L-FABP and NGAL level were significantly higher in the progressors (L-FABP 110.45 versus 21.4 μg/gCre; $p=0.0014$ NGAL 215.5 versus 45.0 μg/gCre; $p=0.0017$), but NAG level was not significantly different ($p=0.62$). A step-wise multiple logistic regression analysis revealed baseline eGFR

before ICU admission, sCr increase during ICU stay, and L-FABP at ICU admission were significantly associated with progression of renal dysfunction after ICU discharge (Hazard ratio 1.53; 95% CI 1.02-2.32; p=0.038). ROC analysis demonstrated L-FABP could predict progression of renal dysfunction after ICU discharge with statistical significance (AUC-ROC 0.69 [95%CI 0.57-0.78]).

Conclusions: Measurement of urinary L-FABP at ICU admission will be useful for predicting long-term renal function after ICU discharge.

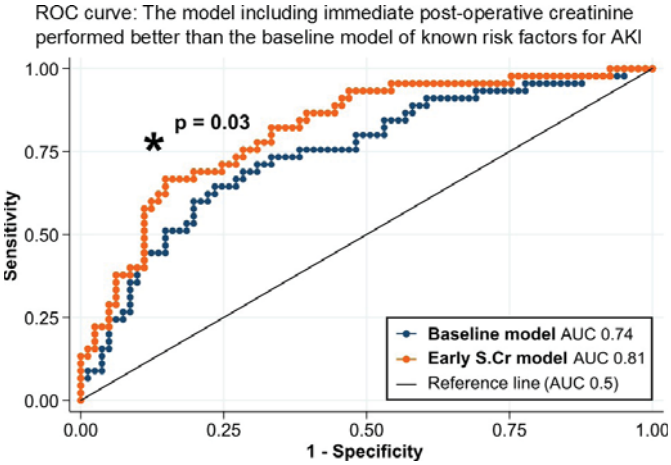
TH-PO110

Serum Creatinine Predicts Acute Kidney Injury Immediately following Cardiac Surgery Shaun Michael Hutchinson,[1] Kevan Polkinghorne,[1,2] Stephen R. Holdsworth,[1,2] Shaun A. Summers.[1,2] *[1]School of Clinical Sciences, Monash Univ, Melbourne, Victoria, Australia; [2]Dept of Nephrology, Monash Medical Centre, Melbourne, Victoria, Australia.*

Background: AKI commonly complicates cardiac surgery, affecting up to 42% of patients post-operatively. It causes significant morbidity, mortality and increased length of stay. New predictive markers are not widely available for clinical use. We aimed to investigate whether serum creatinine may predict later AKI in the early post-operative period following cardiac surgery.

Methods: We prospectively studied 127 patients who underwent cardiac surgery at Monash Medical Centre between February and July 2013. Baseline and post-operative clinical data including creatinine values were obtained. The predictor was serum creatinine taken within 15 minutes of arrival to intensive care following surgery. The primary outcome was development of AKI within 7 days of surgery.

Results:



46 patients (36.2%) developed AKI in the study. In univariate analysis, patients who developed AKI were more likely to be older (OR 1.06 per year increase, p=0.004), have higher BMI (OR 1.14 per 1kg/m^2 increase, p=0.002), poorer pre-operative renal function (OR 1.03 per 1µmol/L increase, p=0.002) and have undergone mixed procedures (coronary artery bypass plus another procedure) (OR 4.21, p=0.007). Immediate post-operative creatinine was independently associated with AKI (OR 1.2 per 5µmol/L increase, p<0.001). In multivariate logistic regression analysis, the predictive model including early creatinine was a better discriminator than the baseline model of known risk factors (AUC 0.81 versus 0.72, p=0.03).

Conclusions: Early serum creatinine is a cheap, readily available test which may help to predict AKI in patients having cardiac surgery, in combination with other known clinical risk factors. This finding could allow more accurate early risk stratification, enhancing decision-making and patient outcomes.

TH-PO111

Paraquat Induced Proteinuria Amplifies Renal Biomarker Excretion and Increases Biomarker Diagnostic Thresholds Fahim Mohamed, Nicholas Buckley, John W. Pickering, Klintean Wunnapuk, Philip Peake, Zoltan H. Endre. *Nephrology, PoW Hospital, Sydney, Australia.*

Background: Paraquat (PQ)-induced nephrotoxicity is a major problem in rural Asia. AKI biomarkers (BM) may provide early diagnosis for initiation of treatment since dialysis availability is limited. Our initial observations of significant proteinuria in this population prompted both pre-clinical and clinical studies of the effect of proteinuria on novel biomarker cut-offs for AKI diagnosis and prediction of outcome.

Methods: Male Wistar rats (6 rats per dose group) were gavaged with paraquat dichloride (dose; 15, 30, 60 and 90 mg/kg). Controls were gavaged with water. Urine and blood samples were collected at regular intervals. In an additional clinical study, all consenting patients who presented to 5 adult medical units following acute PQ self-poisoning were similarly monitored until discharge. BM concentrations were quantified using ELISA and Bio-Plex assays and correlated (Spearman rank-order correlation) with urinary albumin.

Results: A dose-dependent increase in urinary albumin excretion was observed in PQ treated rats. Increased albumin excretion was associated with increased excretion of urinary CysC (r=0.9, p<0.0001), NGAL (r=0.7, p<0.0001), β2-microglobulin (β2M) (r=0.7, p<0.001) and KIM-1 (r=0.6, p<0.01). A moderate correlation (r=0.3) was also observed between urinary osteopontin (OstP) and clusterin (Clu). Following PQ ingestion, 34 of 50 patients had albuminuria [albumin creatinine ratio (ACR) ≥30 mg/g], which increased with AKI severity. In patients with albuminuria, BM concentration was higher (p<0.001) for urinary NGAL, CysC, Clu, β2M, KIM-1 and OstP; a good correlation with ACR was observed for all BMs (r>0.6, p<0.01). BM cut-offs for prediction of death were higher in the albuminuric group.

Conclusions: Proteinuria increases excretion of most low-molecular weight protein BMs following paraquat poisoning irrespective of origin. Diagnostic cut-offs for AKI BMs and for outcome prediction must be modified in the presence of albuminuria.

Funding: Government Support - Non-U.S.

TH-PO112

Urinary Cystatin C, Clusterin and NGAL Are Early Predictors of Acute Kidney Injury (AKI) after Paraquat Poisoning Fahim Mohamed, Nicholas Buckley, John W. Pickering, Philip Peake, Zoltan H. Endre. *Nephrology, PoW Hospital, Sydney, Australia.*

Background: AKI is common following self-poisoning with the herbicide paraquat. We have observed that serum creatinine increases independently of GFR after paraquat poisoning. This study prospectively compared the diagnostic performance of serum creatinine with the panel of novel biomarkers proposed by the Predictive Safety Testing Consortium for the early diagnosis of nephrotoxic AKI (ToxAKI).

Methods: Consenting patients were recruited following paraquat ingestion. Serial blood and urine samples were collected at a regular intervals until discharge or death. The AKIN criteria were used for AKI diagnosis and staging.

Results: Of patients who provided more than two samples (n=50), 38 patients (76%) developed AKI [stage I (n=12), II (n=7) and III (n=19)] and of these most died (n=12) within 48 hours of ingestion. Urinary cystatin C (uCysC), clusterin (uClu) and NGAL levels increased many folds (p<0.01) between 16-24 hours post ingestion compared to patients who did not develop AKI and to healthy controls. Each biomarker showed good diagnostic performance [AUC-ROC for uCysC 0.79 (95% CI 0.62-0.96), uNGAL 0.79 (0.65-0.92), uClu 0.68 (0.48-0.88)], for diagnosis of AKI at 16-24 hours post ingestion. Diagnostic performance improved when biomarker levels were normalised to urinary creatinine concentration. Increases amongst these biomarkers were positively correlated with serum creatinine (r=0.6 for uCysC, 0.5 for uNGAL and uClu) and negatively correlated with creatinine clearance (r=-0.7 for uCysC, -0.3 for uNGAL, -0.6 for uClu). This study also determined the biomarker cut-off values (in ng/ml) with 70% sensitivity and 70% specificity for detecting AKI (uCysC >46, uNGAL > 25, uClu >280).

Conclusions: Many-fold increases in uCysC, uNGAL and uClu within 24 hours of poisoning confirmed paraquat induces tubular injury and can be used to detect AKI early. This could guide early intervention for reno-protection. Point-of-care biomarker analysis may accelerate early intervention in these settings.

Funding: Government Support - Non-U.S.

TH-PO113

Impact Analysis of Prognostic Factors in Patients with Paraquat Poisoning and Discussion of the Treatment Options From One Single Center in China Jianghua Chen, Ying Xu, Jingyun Le. *The kidney Disease Center, the First Affiliated Hospital, Zhejiang Univ, Hangzhou, Zhejiang, China.*

Background: To analyze the prognostic factors and discuss treatment options in order to decrease the mortality rate in paraquat poisoning patients.

Methods: 148 cases of paraquat poisoning patients from June 2010 to March 2014 in our hospital were enrolled. The general situation (gender, age), clinical data (dose of paraquat poisoning, interval from poisoning to treatment), laboratory parameters (blood oxygen, serum creatinine level, glomerular filtration rate) and treatment regimen (gastric lavage, total dose of cyclophosphamide, total dose of methylprednisolone, frequency of blood perfusion) were retrospectively analyzed.

Results: Of the 148 cases of paraquat poisoning patients, 85 cases survived and 63 cases died, the survival rate was 57.4%. All cases were divided into the survival group and the death group. There were no statistical differences in gender and age between two groups. The paraquat ingestion dose was lower in the survival group [(14.77 ± 18.9) mmHg] than the death group [(41.00 ± 36.1) mmHg] (p <0.05). The serum creatinine level and glomerular filtration rate in admission had statistically significant differences. [(85.05 ± 75.9) µmol/L and (116.80 ± 47.6) ml/min in survival group, (172.72 ± 182.8) µmol/L and (49.37 ± 52.3) ml/min in death group (p <0.05)]. The total dose of methylprednisolone therapy in the survival group (1.85 ± 1.37) g was significantly higher than the death group (1.35 ± 1.14) g (p<0.05). The gastric lavage or not, total dose of cyclophosphamide treatment and frequency of blood perfusion had no significant differences between two groups. (p> 0.05).

Conclusions: This study found that paraquat ingestion dose, renal function in admission and total dose of methylprednisolone were important factors affecting the prognosis of paraquat poisoning patients, high-dose of methylprednisolone therapy can significantly improve survival, the impact on prognosis of cyclophosphamide and hemoperfusion therapy are still pending further investigation.

TH-PO114

Delta Neutrophil Index Is an Independent Predictor of Mortality in Patients with Septic Acute Kidney Injury Requiring Continuous Renal Replacement Therapy

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Background: Delta neutrophil index (DNI), defined as the fraction of circulating immature granulocytes, is known to increase in infectious and/or septic conditions. In this study, we investigated whether DNI had a predictive value for mortality in patients with septic acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT).

Methods: A total of 285 patients with septic AKI, who were treated with CRRT at Yonsei University Health System between August 2009 and September 2012, were included. The patients were dichotomized into 'high' and 'low' DNI groups based on the cut-off value from receiver operating characteristics curve of DNI at the time of CRRT initiation. Log-rank test and Cox proportional hazards analysis were conducted to ascertain the prognostic value of DNI for 28-day all-cause mortality.

Results: The mean age was 61.0 ± 14.7 years and 180 patients (63.2%) were male. The high DNI group (DNI > 5.6%) was composed of 149 patients (52.3%). During the study period, 192 patients (67.1%) died. 28-day mortality rates were significantly higher in the high DNI group compared to the low DNI group (79.9% versus 53.3%, P <0.01) (log-rank test, P <0.01). In multivariate Cox proportional hazard analysis, DNI at the time of CRRT initiation was found to be an independent predictor of 28-day all-cause mortality after adjustment for age, gender, mean arterial blood pressure, hemoglobin, platelet, serum albumin levels, PT, aPTT, and APACHE II and SOFA scores (per 1% increase, hazard ratio = 1.010, 95% confidence interval = 1.001-1.019, P = 0.035).

Conclusions: DNI at the time of CRRT initiation may be a useful predictor of mortality in septic AKI patients requiring CRRT.

TH-PO115

Can Neutrophil-Lymphocyte Ratio Be Independent Risk Factor for Predicting Acute Kidney Injury in Patients with Severe Sepsis?

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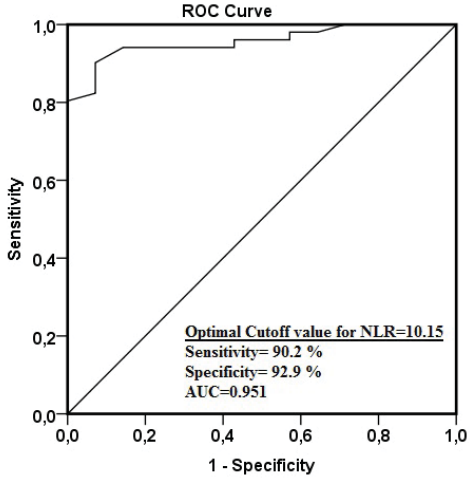
Background: Neutrophil-Lymphocyte Ratio (NLR) is an easily calculated, sensitive, and accurate marker for prognosis and diagnosing sepsis, cardiovascular disease and cancer. As sepsis and septic shock are main causes of acute kidney injury (AKI) intensive care unit (ICU), we investigated whether NLR is an early predictor of AKI in patients with severe sepsis. We compared NLR's predictive power with that of other inflammation-related variables.

Methods: Between December 2011 and November 2013, we enrolled 118 consecutive cases with severe sepsis admitted to ICU in this retrospective study. Levels of C-reactive protein (CRP), NLR, and white blood cell count (WBC) were recorded on admission and patients' renal function was monitored for 7 consecutive days.

Results: The rate of AKI occurrence 7 days after enrollment was 57.6%. NLR levels were higher in the AKI group (Group 1) than in the non-AKI group (Group 2) on the day of ICU admission (p<0.001). AKI development was independently associated with NLR, APACHE II and duration of invasive mechanical ventilation in multivariate logistic regression analysis. The area under the receiver-operating characteristic (ROC) curve of NLR for predicting AKI was 0.951, which was superior to WBC and CRP (p<0.05). The cut-off value of 10.15 for NLR had the highest validity for predicting AKI in patients with severe sepsis. The sensitivity, specificity, negative-predictive value (NPV), positive-predictive value (PPV), for this cut-off value were 90.2%, 92.9%, 90.4%, 92.7%, respectively.



Conclusions: NLR is superior to CRP, and WBC for predicting the development of AKI in patients with severe sepsis.

TH-PO116

Diagnostic Accuracy of Urinary Neutrophil Gelatinase Associated Lipocalin (uNGAL) for the Early Detection of Contrast Induced Nephropathy – The ANTI-CIN Study

Werner Ribitsch, Gernot Schilcher, Franz Quehenberger, Stefan Pilz, Rupert H. Portugaller, Martie Truschnig-Wilders, Robert Zweiker, Marianne Brodmann, Alexander R. Rosenkranz, Joerg H. Horina. *Medical Univ of Graz, Graz, Austria.*

Background: In the ANTI-CIN Study (clinicaltrials.gov NCT01292317) we tested the diagnostic accuracy of uNGAL as an early biomarker of contrast induced nephropathy (CIN) and investigated whether patients with an early rise of NGAL benefit from an intensified volume expansion with regard to CIN incidence.

Methods: We conducted a prospective randomized trial on patients with an eGFR < 70ml/min undergoing intraarterial angiography. All subjects received 12ml/kgBW NaCl 0.9% pre-interventionally. NGAL(ARCHITECT® NGAL Test, Abbott Laboratories,U.S.) was measured the day before and 4-6hrs after angiography. In the event of a significant rise of uNGAL(uNGAL > 150ng/ml if baseline was < 75ng/ml, otherwise a doubling of uNGAL) patients were randomized into two groups. Group A received 22ml/kgBW NaCl 0.9%, Group B did not receive any i.v. fluids. Both groups had an unrestricted oral fluid intake. CIN was defined according to a ≥ 25% increase of creatinine from baseline to either 24hrs or 48hrs after angiography.

Results: We studied 617 (287 female) patients with a mean eGFR of 48.7±12.7ml/min. Ten patients(1.62%) exhibited a significant rise of uNGAL from 84ng/ml(47-226ng/ml) at day -1 to 423 ng/ml (189-1121ng/ml) 4-6hrs after angiography.The incidence of CIN was 9.4%(58 patiens) in the entire cohort and 10%(1 patient) among the subgroup with a rise of uNGAL resulting in a specificity of 0,98 and a sensitivity of 0,017 of uNGAL for the diagnosis of CIN.

	No CIN (n=559)	CIN (n=58)	p
Age (yrs)	74 (37-91)	76 (52-89)	n.s.
Creatinine d_{-1} (mg/dl)	1.38±0.42	1.19±0.27	<0.001
Creatinine d_1 (mg/dl)	1.28±0.39	1.47±0.39	<0.001
Creatinine d_2 (mg(dl)	1.32±0.40	1.58±0.53	<0.001
uNGAL d_{-1} (ng/ml)	19 (0.5-2035)	18 (2-396)	n.s.
uNGAL d_0 (ng/ml)	11 (0.5-1298)	13 (0.5-480)	n.s.

Conclusions: A significant rise of uNGAL shortly after angiography does not facilitate an early recognition of CIN in patients with impaired kidney function.

Funding: Pharmaceutical Company Support - Abbott Laboratories,US

TH-PO117

Membrane Attack Complex (MAC) Staining in Acute Kidney Injury

Eva Rodriguez,[1] Javier Gimeno,[2] Maria Jose Soler,[1] Judit Rigol,[1] Marta Riera,[1] Julio Pascual.[1] *[1]Nephrology, Parc de Salut Mar-IMIM, Barcelona, Spain; [2]Pathology, Parc de Salut Mar-IMIM, Barcelona, Spain.*

Background: There is growing evidence that the complement pathway is involved in the pathophysiology of several kidney diseases, like haemolytic-uremic syndrome, experimental models of membranous nephropaty and murine models of ischemia-reperfusion. The final component of the complement system is the membrane attack complex (MAC or C5b-C9). The role of MAC is to settle on the tubular membrane surface causing a direct cel·lular lytic effect. The aim of this study is to test if the complement system is activated in acute kidney injury (AKI), through deposition of MAC in proximal tubules (PT) and medullary collecting tubules (MCT).

Methods: Immunohistochemical staining for MAC (1:50, anti-Human terminal complement complex, Hycult Biotech©) was performed in paraffin embedded renal tissue obtained from autopsies. We evaluated the percentage of the stained tubules (0-49%, 50-100%), the perimeter of the stained tubules (0-49%, 50-100%) and the intensity of the staining (0-49%, 50-100%) in either the proximal cortical tubules (PT) and the medullary collecting tubules (MCT) per high power field (HPF),comparing between samples of patients who died presenting normal renal function and those with AKI, considering the plasmatic creatinine criteria at least 3 days before the death.

Results: 32 samples were evaluated, 21 of them (65%) had AKI and 11 of them (35%) had normal renal function. The percentage of MAC-stained PT, the perimeter stained and the intensity of their staining were significantly higher in AKI-patients when compared with those without AKI (65.6% versus 34.4%, respectively, p<0,001). In MCT we found similar results, the percentage of MAC-stained MCT, the perimeter stained and the intensity of their staining were significant higher in AKI-patients when compared with those without AKI (65.6% versus 34.4%, p< 0,001).

Conclusions: Our data showed that complement system is activated in AKI and could play a role in the physiopathology of the acute tubular injury.

TH-PO118

Alternative Complement Components as Predictors of Acute Kidney Injury Risk Michael Merchant,[1] Michael E. Brier,[1] Emily F. Anggelis,[1] Jon B. Klein,[1,2] Kenneth R. McLeish.[1,2] *[1]Medicine, Univ of Louisville, Louisville, KY; [2]Robley Rex VAMC, Louisville, KY.*

Background: Acute kidney injury (AKI) contributes to the morbidity and mortality of post-surgical and critically ill patients. The ability to intervene is limited by the availability of biomarkers that identify patients likely to develop AKI. The current study used patients undergoing cardiac surgery to test the hypothesis that the urine proteome contains surrogate biomarkers that identify patients at risk for post-surgery AKI.

Methods: Urine was obtained prior to cardiac surgery from patients at increased risk of developing AKI based on clinical criteria, and patients were followed for 48 hr post-surgery. AKI was defined as a 0.5 mg/dl increase in serum creatinine above baseline. Urine proteins were isolated from 16 patient samples (n=7 without AKI (AKI-neg) and n=9 with AKI (AKI-pos), trypsinized and analyzed by 2D-LCMS. Spectral count data were compared as an unlabeled quantitative approach using t-test examining the direction and magnitude difference in expression. Analysis of regulated proteins was performed using Ingenuity™ Pathway Analysis.

Results: Of 599 proteins identified, 15 were more abundant in the AKI-neg group and 24 were more abundant in AKI-pos group. These proteins could be sub-grouped into markers of (1) tubular injury (haptoglobin, complement components C3, C9, Factor B), (2) previously described biomarkers of established AKI (angiotensinogen, alpha1-microglobulin) and (3) novel markers (histidine rich glycoprotein, afamin, corticosteroid-binding globulin). Ingenuity™ Pathway Analysis of protein groupings identified members of the alternative complement pathway as strongly regulated in patients that later developed AKI.

Conclusions: Alternative complement pathway components are highly expressed in the urine of patients that developed AKI following cardiac surgery. The data suggest that the urine contains proteins that identify patients at risk of developing AKI.

Funding: NIDDK Support, Veterans Affairs Support, Private Foundation Support, Clinical Revenue Support

TH-PO119

Prognostic Value of Presepsin in Predicting Adverse Renal Outcomes and Death in Cardiosurgical Patients Maria Jimena Mucino-Bermejo, Grazia Maria Virzì, Alessandra Brocca, Davide Giavarina, Tommaso Hinna Danesi, Claudio Ronco. *IRRIV-S Bortolo Hosp.*

Background: Accurate evaluation of surgical risk is crucial to clinical decision-making in cardiosurgical (CCH) patients (pts). In a large number of clinical scenarios, biomarkers are reliable tools in stratifying morbidity and mortality risk. As a biomarker presepsin is used in acute care settings, in order to distinguish between sepsis due to bacterial infections and Systemic Inflammatory Response due to non-bacterial cause and has been reported to be useful for stratifying mortality risk among septic pts in the ICU and may also be helpful to predict infective-related morbidity and mortality in different clinical settings, including cardiosurgery. The main aim of this study is to assess the usefulness of presepsin in predicting adverse outcomes among CCH pts.

Methods: Observational single center study that includes 122 adult CCH pts (83 male, 66.4±11.2 yrs). Presepsin was dosed by the PATHFAST Immunoanalyzer system. Procalcitonin quantitative analysis was performed using BRAHMS PCT sensitive KRYPTOR.

Results: We observed that presespin is a better predictor of inhospital, 30-day and global mortality than procalcitonin. ($p<0.05$) Patients with worse renal outcome (defined as AKI, change on CKD stage or need for CRRT) have significantly higher presepsin levels. ($p<0.005$).

Conclusions: Presepsin levels correlate with the risk of death better than procalcitonin. Higher presepsin levels are associated with adverse renal outcomes. Presepsin may be a reliable biomarker for stratifying renal and mortality risk among CCH pts.

TH-PO120

Serum Cystatin C Is a Predictor of Long-Term Outcomes of Critically Ill Patines with Acute Kidney Injury Karina Soto,[1,4] Liliana Maria Goncalves Cunha,[1] Iola Pinto,[2] Alberto Ortiz,[3] Ana Luisa Papoila.[4] *[1]Hospital Fernando Fonseca; [2]ISEL; [3]Fundacion Jimez Diaz, Spain; [4]Univ Nova de Lisboa, Portugal.*

Background: Outcomes following Acute Kidney Injury (AKI) in intensive care unit (ICU) and prognostic markers are poorly known. We explored longterm kidney and patient outcomes and the prognostic value of serum Cystatin C (SCysC) following AKI.

Methods: Prospective study of 128 consecutive ICU adults. Serum creatinine (SCr) and SCysC were measured daily up to discharge. Kidney function and mortality followed up to 4y. Statistical analysis: Friedman's; Cochran's Q; Kruskal-Wallis; Chi-Square and Fisher's tests. Odds ratios by logistic regression model.

Results: 20% (26) of patients died in ICU (85% had AKI) and 2.3% at hospital discharge. Overall mortality was 44.5%, higher in AKI than in no-AKI ($p<0.001$). At 2y (92), 31.5% were AKI, 47% normal function (NF); 19% Transitory Azotemia (TAz) and 3.3 % stable CKD. 36 % of AKI patients needed RRT and 23% became RRT-dependent. During follow-up 7,6% new patients needed RRT for ESRD (86% AKI); and CKD => stage III developed in 65.5% of AKI patients; 23.5% in TAz and 11.6 % in NF groups $p<0.001$. CKD proportion increased during follow-up $p<0.001$. CKD rates were 60% at discharge; 65% at 2y; and 83% at 4y in AKI patients compared with no-AKI patients 4.0%;17.5%; 20.6%; respectively ($p<0.001$). AKI patients lost more kidney function than non-AKI patients $p<0.001$. KDIGO CKD stage =>1 was determinant of CKD progression (OR 7.3 (2.8-19.3) p=<0.001). Odds increase with severity. Daily SCysC were associated with CKD progression; OR 7.3 (2.9-17.8) p=<0.001. Variables which determined CKD were: Age (OR 1.06); CVD (OR 4.55); Shock (OR 4.9), MOF (OR 4.76), prior CKD and comorbidities (OR 17), all p<0,05. Multivariable logistic regression: prior CKD; daily SCysC and SCr desviation (baseline - discharge SCr) were predictors of CKD (OR 4.4; 3.4; and 3.5; p<0,005; HLS=0.598; ROC 0.88; $p<0.001$). Odds for mortality was 6.3 for AKI and 2.9 for SCysC $p<0.001$.

Conclusions: Patients with one episode of AKI have significantly lower long-term survival rates and higher incidence of CKD than critically ill patients with no AKI. SCysC was a determinant for CKD and death.

TH-PO121

Troponin I Serum Levels Predict the Need of Dialysis in Incident Sepsis Patients in the Intensive Care Unit Miguel Luis Graciano,[1] Daniel Almeida Thiengo,[1] Jocemir R. Lugon.[1] *[1]Clinical Medicine, Univ Federal Fluminense, Niteroi, RJ, Brazil; [2]Clinical Medicine, Univ Federal Fluminense, Niteroi, RJ, Brazil; [3]Clinical Medicine, Univ Federal Fluminense, Niteroi, RJ, Brazil.*

Background: Sepsis, an extremely prevalent condition in the intensive care unit, is usually associated with organ dysfunction, which can commonly affect the heart and the kidney. A crosstalk between these organs has already been reported in a number of circumstances.

Methods: This prospective study aimed to determine whether the cardiac dysfunction in sepsis patients predicts the occurrence of acute renal failure. Cardiac dysfunction was assessed by transthoracic echocardiography and serum troponin I levels and renal impairment by AKIN criteria and the need of dialysis. Twenty-nine patients with incident sepsis without previous cardiac or renal dysfunction were enrolled.

Results: Patients averaged 75.3±17.3 years old and 55% were male. Median APACHE II severity score at ICU admission was 16 (9.7 - 24.2) and mortality rate in 30 days was 45%. On the fifth day, 59% had ventricular dysfunction. Troponin serum levels on day 1 in the affected patients were 1.02±0.6 ng/mL compared with 0.23±0.18 ng/mL in patients without heart dysfunction (P=0.01). Eighteen out of 29 patients (62%) underwent renal replacement therapy (RRT) and the percent of patients with ventricular dysfunction who required dialysis was higher (94% versus 16%, P=0.0001). Values of troponin at day 1 were used to develop a ROC curve to determine their ability to predict the need of dialysis. The area under the curve was 0.89 and the cutoff value was 0.4 ng/mL.

Conclusions: In conclusion, we found that an elevation in serum troponin levels, while guarding a relationship with ventricular dysfunction, can be a precious tool to predict the need for dialysis in sepsis patients.

Funding: Government Support - Non-U.S.

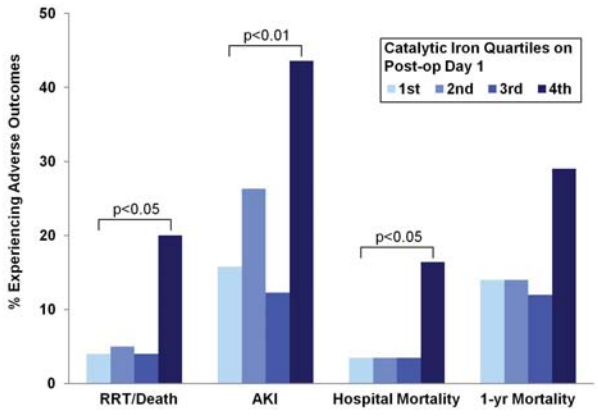
TH-PO122

Plasma Catalytic Iron, Acute Kidney Injury, and Death following Cardiac Surgery David E. Leaf, Sushrut S. Waikar. *Div of Renal Medicine, Brigham and Women's Hospital.*

Background: Catalytic iron, the chemical form of iron capable of participating in redox cycling, has been shown to be a key mediator of AKI in multiple animal models. In humans, the role of elevated plasma catalytic iron levels in AKI has not been studied.

Methods: We performed a prospective cohort study of 250 patients who underwent cardiac surgery. Plasma catalytic iron, free hemoglobin, and other iron markers were measured at four time points (preoperatively, end of cardiopulmonary bypass, and on postoperative days 1 and 3). The primary outcome was AKI requiring renal replacement therapy or in-hospital mortality (RRT/death). Secondary outcomes included AKI, hospital mortality, postoperative myocardial injury, and postoperative vasopressor requirement.

Results: Plasma catalytic iron levels (but not other iron markers) rose significantly at the end of cardiopulmonary bypass [median (IQR) 1.1 (0.7-1.6) compared to 0.4 (0.3-0.4) µmol/L pre-operatively, $p<0.001$] and were directly associated with bypass time ($p<0.001$) and number of pRBC transfusions ($p<0.01$). In multivariate analyses adjusting for age and pre-operative renal function, patients in the highest compared to lowest quartile of catalytic iron on postoperative day 1 had a 6.71 (1.37-32.87) greater odds of RRT/death, and also had greater odds of AKI, hospital mortality, postoperative myocardial injury, and post-operative vasopressor requirement.



Conclusions: These data are consistent with and expand on the findings from animal models demonstrating a pathologic role of catalytic iron in mediating adverse renal outcomes. Interventions aimed at reducing plasma catalytic iron levels in humans as a strategy for preventing AKI are warranted.

Funding: NIDDK Support

TH-PO123

Serum Kidney Injury Molecule-1 Levels in Acute Kidney Injury

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Background: Urine Kidney Injury Molecule-1 (KIM-1) is a well-known marker in clinical acute kidney injury (AKI). However, there are limited data on serum KIM-1 levels in AKI. We hypothesized that serum KIM-1 levels can also predict AKI.

Methods: We conducted a prospective, multicenter observational study to evaluate the role of urine and serum KIM-1 levels as early biomarkers for AKI in critically ill patients. AKI was defined according to the AKIN serum creatinine criterion. Samples were collected every 12 hours after intensive care unit (ICU) admission for a ≥48 hours and up to 10 days.

Results: From the 80 patients enrolled, 18 developed AKI after a median of 1.0 (IQR 0 – 2.3) days after ICU admission. Mean serum KIM-1 levels were higher in AKI than in non-AKI patients over the entire ICU stay (2.47±0.42 versus 2.25±0.31,p<0.001) and similar results were obtained for urine KIM-1 (3.27±0.60 versus 2.82±0.56,p<0.001). The area under the curve (AUC) to predict AKI over 7 days using serum and urine log10 KIM-1 levels at ICU admission were 0.68 (95% CI, 0.50-0.87) and 0.75 (95%CI, 0.57-0.93), respectively. Urine KIM-1 levels at ICU admission could predict AKI (OR 4.9 95%CI, 1.2-20.1); however serum KIM-1 levels at ICU admission did not predict AKI. Neither serum nor urine KIM-1 levels at ICU admission could predict mortality and/or renal recovery.

Conclusions: Serum KIM-1 levels may help to predict AKI. Further studies are required to evaluate its role compare to urine KIM-1 in AKI.

Funding: Private Foundation Support, Clinical Revenue Support

TH-PO124

Early Prediction of Acute Kidney Injury Severity after Liver Transplantation by Neutrophil Gelatinase-Associated Lipocalin

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Background: In post-liver transplantation, AKI is a frequent complication associated with an increased morbid-mortality rate. We hypothesized that the pattern of NGAL elevation could provide information on the risk of progression of AKI, the need for RRT and mortality.

Methods: We analyzed 32 patients > 18 years undergoing liver transplantation from June 2013- 2014. Urine and blood samples were collected preoperatively before induction of anesthesia, after portal reperfusion, 6, 18, 24 and 48 hours after surgery. AKI diagnosis was based on the sCr KDIGO criterion.

Results: Of 32 patients, 22(69%) developed AKI during the first 7 days after transplantation and 10(31%) needed RRT during the hospital stay. AKI and no–AKI patients had similar urine biochemistry and microscopy during the sample collection period. Plasma NGAL was significantly higher in AKI patients, starting at 6h after portal reperfusion.

Eighteen hours after surgery the AUC for AKI prediction was 0,85. Urinary NGAL was higher in AKI patients starting 18h after surgery, with an AUC of 0,82 to predict AKI at 24h. Six hours after surgery non-survivors had significantly higher PNGAL.

Conclusions: NGAL is a promising biomarker for predicting AKI, the need for RRT and mortality after liver transplantation. Future studies should evaluate whether the clinical use of these biomarkers could improve patient outcomes.

TH-PO125

Utility of Fractional Excretion of Sodium and Urea in Discerning Etiology of Acute Kidney Injury in End-Stage Liver Disease

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Background: Acute kidney injury (AKI) is a common complication in advanced cirrhotics, seen in up to 20% of hospitalized patients. Determining the cause of renal dysfunction, whether due to pre-renal, acute tubular necrosis (ATN), or the hepatorenal syndrome (HRS) is important due to therapeutic and prognostic implications. Traditionally, fractional excretion of sodium (FeNa) <1% has been said to imply tubular integrity and thus favor a pre-renal state or HRS versus ATN. Fractional Excretion of Urea (FeUrea) <35% is used in patients on diuretics to indicate the same. However, there lacks evidence on their use in patients with ESLD. We thus aimed to see these indices were truly of value in predicting the type of AKI in ESLD patients.

Methods: We conducted a 2 year, retrospective study on ESLD patients presenting with a rise in creatinine of 0.3 mg/dL or 1.5 times baseline. AKI was defined as pre-renal, ATN (>72 hours kidney injury despite volume resuscitation with hypovolemic or septic shock preceding the AKI, +/- granular casts), or HRS, as diagnosed per guidelines of the International Ascites Club. We excluded patients with acute liver failure, MPGN or CKD. Urine indices had to be collected within 8 hours of presentation.

Results: We found 130 cases of ESLD patients with AKI, of whom 55 met inclusion criteria. 21 had ATN, the rest were equally divided between prerenal and HRS. 83% (46/55) of all AKI had FeNa<1 % and FeUrea <35%.

	Pre Renal	ATN	HRS
Mean FeNa	0.50	0.48	0.36
With Diuretics	0.49	0.45	041
W/O Diuretics	0.60	0.51	0.31
Mean FeUrea	29.4	18	22.7
With Diuretics	28.1	17.5	24.7
W/O Diuretics	18.7	19.0	20.9

FeNa and FeUrea were almost universally <1% and <35% in ESLD with AKI, even in 90% of ATN cases.

Conclusions: Our study indicates in patients with advanced ESLD, the diagnostic utility of FeNa and FeUrea is probably unreliable. A FeNa and FeUrea of <1% and <35% respectively appears to be the norm in ESLD despite tubular injury, likely secondary to chronic renal hypoperfusion.

TH-PO126

Urinary Exosomal mRNA Analysis for Non-Invasive Diagnosis of Acute Kidney Injury

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Background: Acute kidney injury (AKI) is most commonly recognized through changes in serum creatinine (sCr) or urine output. Novel biomarkers such as NGAL or KIM-1 reflect underlying kidney damage in specific sites of nephron however are limited in defining underlying mechanisms of injury. Urinary exosomes and microvesicles (EMV) offer a promising biomarker source to define the underlying pathways contributing to damage as they are released throughout the nephrons by encapsulating the functional cytoplasmic molecules. To discover new AKI biomarkers, we analyzed EMV mRNA in urine of hospitalized patients in different settings.

Methods: Urine samples were obtained from healthy subjects N=6), ICU (N=6), cirrhosis (N=9), or cardiac surgery (N=9) groups. 12 patients developed AKI (based on AKIN sCr criteria; ≥0.3mg/dl in 48 hrs). After initial centrifugation, urine supernatants including ENV were applied to ENV collection tubes, followed by lysis, mRNA isolation, cDNA synthesis using oligo(dT)-immobilized microplate and quantification by real-time PCR. The mRNA data were analyzed further based on AKI diagnosis.

Results: *Among 36 mRNA, Calmodulin 1 (CALM1), Aquaporin 2 (AQP2) and Osteopontin (SPP1) were dysregulated in AKI patients.* Logistic regression analysis was conducted to select the best combinations of genes (up to 4 out of the 36) to diagnose AKI. By ROC analysis, the top formulae include the *above genes frequently and allow diagnosis of AKI among patients with more than 90% specificity and sensitivity.*

Conclusions: Urinary EMV mRNA showed differential expression in patients with AKI regardless of the setting, compared to those without AKI. These initial findings support further development of EMV biomarkers as a non-invasive tool to profile AKI and establish mechanisms of kidney injury.

Funding: Other NIH Support - O'Brien Center for AKI Research

TH-PO127

AKI-Associated Mitochondrial Alterations Revealed by Urine Exosomes in Patients Undergoing Cardiac Surgery

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Background: Acute Kidney Injury (AKI) occurs in up to 40% of patients undergoing cardiac surgery (CS) and is currently recognized through changes in serum creatinine (sCr) or urine output (UO). Novel biomarkers of kidney damage e.g. NGAL and KIM-1 provide earlier diagnosis but are limited in identifying underlying injury mechanisms. Urinary exosomes are bioactive nanoparticles of 10-400 nm diameter released by renal tubule cells that encapsulate cellular content that can be studied to identify underlying cellular processes. We hypothesized that urinary exosomal proteins would be qualitatively and quantitatively different in patients developing AKI post CS in comparison to those without AKI.

Methods: Urine exosomes were isolated from12 subjects undergoing CS at different time points (pre surgery and at 2, 6, 12, 24 and 48 hrs after CS with normal kidney function (N=6); and cardiac surgery subjects with documented kidney dysfunction (N=6). 1-dimensional gel electrophoresis and liquid chromatography / tandem mass spec was used to identify proteins and normalized spectral abundance for semi-quantification of proteins. AKI was diagnosed in 6 subjects (AKIN sCr criteria ≥0.3 mg/dl change in 48 hrs).

Results: A total of 1176 urine exosome proteins were identified of which 401 proteins were unique to AKI patients, and 233 proteins were unique to patients that did not develop AKI. Mitochondrial proteins were more prominently expressed in AKI patients in comparison to those without AKI. Furthermore, 16 proteins had more than 10 peptide representations each, and potentially qualified as robust biomarkers tracking AKI in CS. The western blotting validation of the proteomic data is underway.

Conclusions: Urinary exosome protein analysis may be useful to identify unique molecular signatures for CS AKI. Future studies processing serial samples from the AKI and non-AKI subjects should assess the temporal sequence of mitochondrial events after CS and their role in the pathogenesis of CS AKI.

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TH-PO128

Urinary Exosome Biomarkers of AKI in Patients with Liver Disease Satish P. Ramachandrarao, Michelle Pearlman, Linda Awdishu, Ravindra L. Mehta, Heather M. Patton. *Medicine, UCSD, San Diego, CA.*

Background: Acute kidney injury (AKI) occurs in 19% of patients with cirrhosis and ascites. Serum creatinine elevation is a poor measure of kidney failure or function in this population. Exosomes are membrane-bound bioactive nanoparticles of 10-400 nm diameter and released by all cell types including renal tubules, and can provide crucial cellular information on kidney health. We hypothesized that urinary exosome protein analysis distinguishes patients with AKI from those without AKI in Liver disease setting.

Methods: Urine exosomes from patients with 1) compensated cirrhosis, 2) decompensated cirrhosis without AKI 3) cirrhosis with AKI, and healthy controls (N=6 each) and their clinical data were collected. 100 micro gm protein equivalent per subject of urinary exosome proteins were resolved by 1d SDS-PAGE, Coomassie stained, in-gel trypsinized, and the trypsinized fragments were subjected to Liquid Chromatography followed by Tandem Mass Spectrometry. The proteins with at least 2 peptides were qualified for subsequent analysis.

Results: 12/16 patients had cirrhosis. Overall, 1573 proteins were identified and the number of unique proteins varied according to patient group. Normal controls had 75 (46%) proteins in common with group 1, patients with decompensated cirrhosis had 99 (63%) proteins in common with group 1, while patients with AKI had only 85 (31%) proteins in common with decompensated cirrhotics with normal kidney function, and only 52 (24%) in common with patients with compensated cirrhosis.

Variable	Group 1	Group 2	Group 3	Control Group
Age (years)	60.1 ± 9.8	57.8 ± 5.7	48.6 ± 7.4	28.7 ± 13
Gender	1 (16.7)	4 (66.7)	5 (83.3)	6 (100)
Child Pugh Turcotte Class A/B/C	6/0/0	0/5/1	0/2/4	N/A
MELD	7.8 ± 2.1	12 ±3.2	24 ± 9.1	N/A

Conclusions: Urinary exosome protein analysis in patients with various stages of cirrhosis with or without AKI identifies pathways unique to AKI in Cirrhosis setting. The quantification analysis of these unique proteins and their relationship to clinical outcomes will help clarify the potential role of this noninvasive test in assessing AKI in cirrhosis patients.

Funding: Other NIH Support - O'Brien Center for AKI Research

TH-PO129

Acute Kidney Injury following Administration of Gadolinium-Based Contrast Agents: Early Detection by Biomarkers Habib Mawad, Louis-Philippe Laurin, Jean-François Naud, François A. Leblond, Vincent Pichette, Martine Leblanc. *Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.*

Background: Gadolinium-based contrast agents have been traditionally used as non-nephrotoxic alternatives to iodinated contrast agents. Their well-established role in nephrogenic systemic fibrosis has limited their use in patients with moderate to severe kidney dysfunction. Recent literature has raised concern regarding their potential nephrotoxicity since cases of acute kidney injury have been reported in patients with normal renal function. The aim of our study was to determine if these agents could induce nephrotoxicity detected by early biomarkers of kidney injury.

Methods: We studied four biomarkers of acute kidney injury: interleukin-18 (IL-18), N-acetyl-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL) and Cystatin C in 28 volunteers with normal renal function after intravenous administration of a gadolinium-based contrast agent. Urinary and serum levels of biomarkers were analyzed at 0, 3 h and 24 h following injection. All urinary biomarkers were normalized for creatinine concentration.

Results: Baseline serum creatinine was 57.8 ± 34.5 µmol/L and remained stable throughout the first 24 h. IL-18 urinary concentrations increased significantly at 3 h (10.66 versus 7.29 ng/mg creatinine; $p < 0.05$). Similarly, urinary NAG levels were significantly higher at 3 h (3.93 versus 2.19 IU/mg creatinine; $p < 0.001$). Serum Cystatin C levels decreased at 3 h from 933.72 to 897.82 ng/mL ($p = 0.0506$). For all three of these markers, the difference was no longer significant at 24 h. No difference was observed at 3 h or 24 h for urinary and serum NGAL levels.

Conclusions: Gadolinium-based contrast agents may cause transient sub-clinical kidney injury shortly after their administration as suggested by significant variations of specific urinary and serum biomarkers in patients with normal kidney function. These results provide new insight about their potential nephrotoxicity.

TH-PO130

Peptidylarginine Deiminase-4 Exacerbates Kidney Ischemia and Reperfusion Injury May M. Rabadi, Mihwa Kim, Ahrom Ham, Kevin M. Brown, H. Thomas Lee. *Anesthesiology, Columbia Univ, New York, NY.*

Background: Peptidylarginine deiminase-4 (PAD4)-mediated post-translational conversion of arginine to citrulline is implicated in several inflammatory autoimmune diseases including rheumatoid arthritis and multiple sclerosis. Here, we tested the hypothesis that PAD4 exacerbates acute kidney injury (AKI) by promoting the inflammatory response after renal ischemia reperfusion (IR).

Methods: After IACUC approval, mice were pretreated with vehicle or with a PAD4 inhibitor (2-chloroamidine) before 30 min renal ischemia. Some mice were treated with recombinant PAD4 (rPAD4) before 20 min renal ischemia. In addition, cultured mouse kidney proximal tubule cells were treated with rPAD4 to test whether rPAD4 directly induces renal tubular inflammation. Finally, we performed PAD4 immunohistochemistry in archived kidney specimens from patients subjected to nephrectomy for tumor and from patients subjected to living-related kidney transplantation (Columbia University IRB determined this as non-human subject research).

Results: Renal IR increased PAD4 expression and activity with cytosolic translocation in mouse kidneys. In human kidneys, PAD4 is localized in the nucleus in non-ischemic kidneys whereas kidneys subjected to IR showed cytosolic PAD4 translocation. After 30 min renal IR, vehicle-treated mice developed severe AKI at 24 hr (Cr=2.9±0.2mg/dL, N=5). In contrast, mice pretreated with a PAD4 inhibitor had significantly reduced renal injury (Cr=1.9±0.2mg/dL, N=6, $P<0.05$) with decreased kidney neutrophil chemotactic cytokine (MIP-2, KC) expression and neutrophil infiltration. Furthermore, mice pretreated with rPAD4 and subjected to mild (20 min) renal IR developed exacerbated ischemic AKI (Cr=2.4±0.3mg/dL, N=6) compared to vehicle-treated mice (Cr=0.8±0.2mg/dL, N=6, $P<0.01$) with increased kidney MIP and KC induction and neutrophil infiltration. Finally, cultured mouse kidney proximal tubules treated with rPAD4 had significantly increased MIP-2 and KC mRNA expression.

Conclusions: Our studies suggest that PAD4 plays a critical role in renal IR injury by promoting neutrophil-mediated inflammation. Inhibition of renal tubular PAD4 activity may reduce the morbidity and mortality due to ischemic AKI.

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TH-PO131

Increased Kidney Injury after Ischemia-Reperfusion in G6PD Deficient Mice Zhihong Yang, Robert C. Stanton. *Vascular Cell Biology, Joslin Diabetes Center, Boston, MA.*

Background: Ischemia/reperfusion (I/R) is a common cause of acute renal failure (ARF). Diabetic patients are at greatly increased risk for I/R induced ARF. Reactive oxygen species (ROS), which are excessively produced during reperfusion, are important mediators of cell damage due to I/R. Glucose-6-phosphate dehydrogenase (G6PD) is an essential antioxidant as it is the main source of the essential reductant, NADPH. Previous work from our lab has shown that G6PD deficient mice have increased renal oxidative stress. And that diabetes leads to decreased G6PD. We hypothesized that G6PD is central to the protection against damage from I/R.

Methods: Wild type and G6PD deficient mice (about 20% of wild type function) were studied. Mice were divided into 4 groups (n=4-7 per group): wild type sham (wt+sham), wild type I/R (wt+I/R), hemi sham (hemi+sham), hemi I/R (hemi+I/R). Renal arteries and veins were clamped for 30 min. After clamp removal, kidneys were observed for a further 5 minutes to ensure blood reflow. Mice were kept at 37ºC during the procedure and allowed to recover. After 24h, mice were sacrificed.

Results: G6PD activity, mRNA and protein expression were significantly decreased in kidneys from hemi groups. The histologic kidney damage level was determined in a blinded manner. Tubules were analyzed: 0, no damage; 1, mild: rounding of epithelial cells and dilated tubular lumen; 2, moderate: flattened epithelial cells, loss of nuclear staining, dilated lumens, and congestion of lumens; 3, severe: destroyed tubules with flat epithelial cells lacking nuclear staining and congestion of lumen.10-15 fields per slide were used for counting. Hemi+I/R group had the highest damage score, 4 folds increase compared to wt+I/R group. Serum creatinine was increased 3 fold in hemi+I/R group compared to wt+I/R group. We measured neutrophil gelatinase-associated lipocalin(NGAL), a biomarker of kidney injury. Real time PCR and western blot of kidney tissues showed significant increase in NGAL in the hemi+I/R group. NGAL was also significant increased in the plasma of hemi+I/R group.

Conclusions: In conclusion, G6PD deficient mice are more sensitive to kidney I/R injury. G6PD is essential for protecting the kidney from renal injury during I/R.

TH-PO132

Kidney Injury Molecule-1 (Kim-1) Protects From Renal Ischemia-Reperfusion Injury Ola Ziyad Ismail,[1] Xizhong Zhang,[1] Junjun Wei,[1] Giulia Michela Martone,[1] Sahra Nathoo,[1] Aaron R. Haig,[1] Rita Suri,[2] Lakshman Gunaratnam.[1] *[1]Schulich School of Medicine and Dentistry, Western Univ, London, ON, Canada; [2]Univ of Montreal, Montreal, QC, Canada.*

Background: Phagocytic clearance of apoptotic cells (AC) prevents inflammation as uncleared AC undergo secondary necrosis, releasing danger associated molecular pattern proteins (DAMPs). Kidney Injury Molecule-1 (Kim-1) is a receptor for phosphatidlyserine, an "eat-me" signal on the AC surface. After acute kidney injury (AKI), Kim-1 is upregulated on surviving renal proximal tubular epithelial cells (PTECs), allowing them to become

semi-professional phagocytes for AC. We hypothesized that absence of Kim-1 expression precludes phagocytosis by PTECs, resulting in increased release of DAMPs from uncleared AC and more severe tissue damage after AKI.

Methods: We subjected previously generated Kim-1 knockout (KO) and wild-type (WT) mice to bilateral renal ischemia and measured serum creatinine and tissue injury. We exposed PTECs from WT and KO mice to fluorescently labelled AC. We assessed phagocytosis by flow cytometry and HMGB1 (a DAMP that mediates renal damage in AKI) release by Western blot.

Results: After 30 min of ischemia and 24h of reperfusion, renal function was significantly worse in KO compared to WT mice (median creat 202 versus 68 μmol/L, respectively, p=0.001, n=9/gp). After 48h of reperfusion, 7/11 KO but 0/5 WT mice had died (p=0.017). When we reduced ischemia time to 25 min, WT mice maintained near-normal renal function (median creat 13 μmol/L, n=11) while KO mice developed severe renal dysfunction (median creat 111 μmol/L, n=12) (p=0.001) after 48 h of reperfusion. KO mice exhibited worse histological renal damage compared to WT mice (median IRI score = 4 versus 2, p=0.038). The number of AC was higher in the KO compared with WT kidneys. When we exposed primary PTECs to AC, PTECs not expressing KIM-1 exhibited significantly reduced efferocytosis over 24h, compared to PTECs expressing KIM-1 (p=0.0005, n=3 x 6 time points). As expected, we detected significantly more HMGB1 in the extracellular media of PTECs from KO versus WT mice.

Conclusions: Our results suggest that Kim-1 is essential to limit renal damage after ischemic AKI in mice by enabling PTECs to clear AC.

Funding: Government Support - Non-U.S.

TH-PO133

Arginase2 Is Up-Regulated in Renal Tubules of AKI, and Inhibition of Arginase2 Ameliorates the Prognosis of AKI by Regulated NO In Vivo

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Background: Arginase (AGase) is an enzyme that breaks down L-arginine and ornithine to urea, and was identified as NO metabolism-related factor. Because the biological role for AGase2 in AKI is poorly understood, we studied the expression/modulation mechanism and role of AGase2 axis in AKI pathogenesis, and the aging effects.

Methods: To clarify the role of AGase2 in AKI, we used a mouse ischemia/reperfusion (I/R) AKI model and cultured renal tubular cells (NRK-52E cells). S-(2-Boronoethyl)-L-cysteine (BEC), a potent arginase inhibitor was administered using a mini-osmotic pimp, then bilateral renal arteries were occluded for 28min and reperfused. Moreover, to assess aging effects, we studied AGase2 expression in aged mice (42- and 87-week-old).

Results: Western blot analysis showed that AGase2 expression was increased at 12-48 h after I/R. Immunohistological examination revealed that AGase2 expression was increased in proximal tubules. In vitro AGase2 expression was also increased in NRK-52E cells under hypoxia. Overexpression of HIF-1α increased AGase2 expression. When increased AGase2 under hypoxia was suppressed by AGase2 siRNA, phosphorylation of eNOS (Ser-177) was increased. Overexpression of AGase2 resulted in suppressed phosphorylation of eNOS. Administrarion of BEC significantly increased serum NOx levels, and ameliorates prognosis of AKI. BUN levels was reduced from 89.9 (contol) to 43.2 mg/dl (BEC), Cr levels was reduced from 0.74 (contol) to 0.17 mg/dl (BEC) at 24h. Finally, AGase2 expression was significantly increased in 42- and 87-week-old aged mice, and showed further increase after AKI, in addition to the reduction of phosphorylation of eNOS.

Conclusions: In summary, AGase2 is up-regulated in renal tubular cells under AKI and hypoxic conditions. Inhibition of AGnase2 ameliorates the prognosis of AKI. AGase2 might play a pivotal role on AKI pathogenesis via NO modulation and eNOS activity. Furthermore AGase2 might be critical for aging-related AKI pathogenesis.

TH-PO134

Activation of the Calcium-Sensing Receptor Before Renal Ischemia/Reperfusion Exacerbates Kidney Injury in Mouse

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Background: The calcium-sensing receptor (CaSR) belongs to the family C of G-protein coupled receptors. In cardiomyocytes, hepatocytes and neurons, CaSR activation at the time of ischemia/reperfusion (I/R) promotes cell death. Its role in renal I/R is unknown.

Methods: We used a 12-week-old C57BL/6J mouse model of renal 30-min ischemia followed by 48-hour reperfusion. Prior to I/R, mice were administered daily with the CaSR agonist, R-568 (250μg, i.p.), or with vehicle (4% DMSO) for 48 hours.

Results: Blood analyses (serum blood urea nitrogen (BUN) levels), examination of kidney histology (using Jablonski's score) and comparative metabolomics by nuclear magnetic resonance on urine and kidney samples showed that R-568 treatment was not associated with significant nephrotoxicity in comparison to vehicle (n=2x8 mice). Still, serum Ca^{2+} level was significantly decreased in R-568-treated animals (2.37 ± 0.27 versus 2.66 ± 0.21 mmol/L, p<0.05). Following kidney I/R (n=2x8 mice), blood analyses indicated that serum BUN levels increased higher in R-568-treated animals than in controls (4.54 ± 0.82 versus 0.78 ± 0.21 g/L, p<0.05). Jablonski's score was higher in R-568-treated kidneys than in controls (3±1 versus 1±1, p<0.05). Immunodetection and quantification (www.cytomine.be) of PCNA (proliferating cell nuclear antigen) and ApopTag expression revealed that R-568-treated kidneys were characterized by a significantly higher rate of cell proliferation and apoptosis in comparison to controls (p<0.05).

Conclusions: The activation of CaSR before renal I/R increases the structural and functional damage in mouse. Modulating CaSR activity might serve as a novel pharmacological approach to prevent I/R-associated kidney injury.

Funding: Government Support - Non-U.S.

TH-PO135

Metabolomics Study of Mouse Kidney and Urine After Renal Ischemia/Reperfusion Injury

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Background: Ischemia/reperfusion (I/R) is one of the most common causes of acute kidney injury (AKI). Several cellular and tissular pathways have been implicated in renal I/R, including metabolism, ion transport, polarization, apoptosis, oxidative stress, and inflammation. Still, the pathophysiology of I/R-related AKI remains unclear, which confines the management of patients with AKI to supportive maneuvers. Metabolomics approach helps to identify the metabolites involved in physiological and pathological changes of integrated living systems, as well as their dynamic fluctuations. In kidney diseases, metabolomics demonstrated enormous potential in the research on drug-induced nephrotoxicity, diabetic nephropathy, as well as AKI.

Methods: We performed a ^{1}H Nuclear Magnetic Resonance (NMR) metabolomics analysis on urine and kidney samples from a 12-week-old C57BL/6J mouse model of renal 30-min ischemia followed by 48-hour reperfusion in order to further investigate the metabolic changes in I/R-induced AKI. The urine spectra were normalized to creatinine. Sham-operated mice were used as controls.

Results: Classical metabolomics post-treatment and non-supervised statistical analysis (i.e. Principal Component Analysis, PCA) significantly distinguished urine samples of mice with I/R-induced AKI from sham-operated animals. Additional investigations led to the identification of relevant changes in various metabolites including taurine, creatine, lactate, alanine, citrate and succinate. A similar discrimination was found after testing and processing kidney samples. Renal metabolite profile, including lactate, lipids, amino acids and taurine, was significantly affected by I/R injury.

Conclusions: In mouse, kidney I/R induces significant changes of metabolite profiles in both urine and renal parenchyma. Such investigations may help better understand the pathophysiology of I/R-related AKI, thereby leading to the identification of novel therapeutic targets.

Funding: Government Support - Non-U.S.

TH-PO136

P2X7 Receptor Inhibition Protects against Acute Kidney Injury in Mice

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Background: Activation of the purinergic P2X7receptor (P2X7R) has been associated with development of experimental nephritis and diabetic and hypertensive nephropathy. However, its role in acute kidney injury (AKI) remains unknown.

Methods: In this study, we examined the effects of P2X7R inhibition in a murine model of ischemia/reperfusion (IR)-induced AKI using A438079, a selective inhibitor of p2x7R.

Results: At 24 hours after IR, mice developed renal dysfunction and renal tubular damage, which was accompanied by elevated expression of P2X7R. Administration of A438079 at the early time (immediately or 6 hours) in the onset of reperfusion protected against renal dysfunction and attenuated kidney damage whereas delayed administration of A438079 at 24 hours after onset of reflow had no protective effects. The defending actions of A438079 were associated with inhibition of renal tubule injury and apoptosis and suppression of renal expression of chemokines (monocyte chemotactic protein-1, RATES). Moreover, IR injury led to an increase in phosphorylation (activation) of extracellular signal-regulated kinases 1/2 in the kidney and early treatment with A438079 diminished this response.

Conclusions: Collectively, these results indicate that early P2X7R inhibition is effective against renal tubule injury and proinflammatory response after IR and suggest that targeting P2X7R may be a promising therapeutic strategy for clinical treatment of AKI.

Funding: NIDDK Support

TH-PO137

Changes in Metabolic Profiles during Acute Kidney Injury and Recovery following Ischemia/Reperfusion

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Background: Changes of metabolism have been implicated in renal ischemia/reperfusion injury (IRI). However, a global analysis of the metabolic changes in renal IRI is lacking and the association of the changes with ischemic kidney injury and subsequent recovery are unclear.

Methods: In this study, mice were subjected to 25 minutes of bilateral renal IRI followed by 2 hours to 7 days of reperfusion. Kidney injury and subsequent recovery was

verified by serum creatinine and blood urea nitrogen measurements. The metabolome of plasma, kidney cortex, and medulla were profiled by the newly developed global metabolomics analysis.

Results: Renal IRI induced overall changes of the metabolome in plasma and kidney tissues. The changes started in renal cortex, followed by medulla and plasma. In addition, we identified specific metabolites that may contribute to early renal injury response, perturbed energy metabolism, impacted osmotic regulation and the induction of inflammation. Some metabolites, such as 3-indoxyl sulfate, were induced at the earliest time point of renal IRI, suggesting the potential of being used as diagnostic biomarkers. There was a notable switch of energy source from glucose to lipids, implicating the importance of appropriate nutrition supply during treatment. In addition, we detected the depressed polyols for osmotic regulation which may contribute to the loss of kidney function. Several pathways involved in inflammation regulation were also induced. Finally, there was a late induction of prostaglandins, suggesting their possible involvement in kidney recovery.

Conclusions: In conclusion, this study demonstrates significant changes of metabolome kidney tissues and plasma in renal IRI. The changes in specific metabolites are associated with and may contribute to early injury, shift of energy source, inflammation, and late phase kidney recovery.

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TH-PO138

DNA Methylation in Ischemic Acute Kidney Injury Chunyuan Guo,[1,2] Xiao Xiao,[1,2] Qingqing Wei,[1,2] Zheng Dong.[1,2] *[1]Cellular Biology and Anatomy, Georgia Regents Univ, Augusta, GA; [2]Research Dept, Charlie Norwood VA Medical Center, Augusta, GA.*

Background: DNA methylation is an important mechanism of epigenetic regulation, which is heritable during cell division, but does not involve the change of DNA sequence. DNA methylation plays an essential role in the regulation of gene transcription in physiological and disease conditions. However, very limited is known about DNA methylation in renal diseases, especially in acute kidney injury (AKI).

Methods: We conducted a genome-wide DNA methylation analysis in kidney cortical tissues aftrer renal ischemia-reperfusion AKI in mice using reduced representation bisulfite sequencing (RRBS) technology. The genes with differentially DNA methylated regions (DMRs) within their promoter regions were further analyzed for mRNA and protein to determine if DNA methylation changes correlates with gene transcription.

Results: Totally 1.5 and 1.4 millions of CpG sites were analyzed in the sham and ischemic injured kidney samples respectively. Comparing the methylation profiles of the sham and ischemic injured kidney, there were 235 DMRs in 425 genes. 24 genes were identified containing DMRs at 5' end regulatory regions. Several genes of interest with DMRs in the promoter region showed significantly differential expression in after ischemia-reperfusion, such as Bcl6 and Hoxc10. Hypermethylation of their promoter regions suppressed gene expression. Western blot analysis further showed a decrease in Bcl6 and Hoxc10 protein in ischemic AKI.

Conclusions: The results reveal significant changes in DNA methylation during ischemic AKI, which may contribute to gene regulation in the pathogenesis.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO139

Lipidomics Analysis of Serum from Rats with Acute Kidney Injury Reveals Increased Levels of Renally Generated N–acylphosphatidylethanolamines, which Are Precursors of Anandamide, an Inducer of Oxidative and Mitochondrial Stress Jon D. Ahlstrom,[1] Zhuma Hu,[1] Ping Zhang,[1] Landon Shay Wilson,[2] Christof Westenfelder,[1,3] Stephen Barnes,[2] Janusz Kabarowski.[2] *[1]Medicine, U of Utah and VAMC, Salt Lake City, UT; [2]Pharmacology and Toxicology, Univ of Alabama, Birmingham, AL; [3]Physiology, U of Utah, SLC, UT.*

Background: The uremic state that is induced by Acute Kidney Injury (AKI) adversely affects multiple organ systems by mechanisms that are still poorly characterized.

Methods: To further investigate the effects of exposing cells x 48 hrs (Normal Rat Kidney/tubular cells and therapeutically used Mesenchymal Stem Cells) to 10% SHAM or AKI sera obtained 24 hrs post IRI-AKI (50 min bilateral pedicle clamp) or bilateral nephrectomy (NPHX). To identify the potential mediator(s) of the oxidative and mitochondrial stress found in AKI serum, sera were subjected to comprehensive lipidomics analysis by direct infusion electrospray-mass spectrometry using SWATH Acquisition on an AB Sciex 5600 tripleTOF instrument at the UAB-UCSD O'Brien Core Center for Acute Kidney Injury Research.

Results: Incubation of both cell types to 10% IRI-AKI serum caused, compared to incubation with NPHX or SHAM rat sera, up regulation of anti-oxidant genes (catalase, HO-1), increased reactive oxygen species and mitochondrial complex I activities, and reduced mitochondrial reserve capacity. The most significantly abundant lipids in the IRI-AKI samples were N–acylphosphatidylethanolamines (NAPEs), which are precursors to the hormone anandamide, an endogeneous cannabinoid known to inhibit oxidative phosphorylation. Accordingly, these NAPEs may in part mediate systematic cellular and mitochondrial stress following AKI.

Conclusions: At comparable levels of azotemia (AKI versus NPHX), AKI serum causes distinct oxidative and mitochondrial stress in cultured cells, and it shows higher levels of NAPEs, and derivatives of these are known to impair mitochondrial function.NAPEs may represent an as yet inadequately understood "toxin" generated by the injured kidney that may adversely affect renal tissue, distant organs, and administered MSCs.

Funding: Other NIH Support - O'Brien Core Center for Acute Kidney Injury Research, Veterans Affairs Support, Private Foundation Support

TH-PO140

Role of Chemokine Receptor 5 in Renal Ischemia-Reperfusion Injury Kyung Don Yoo,[1] Seung Seok Han,[1] Ji In Park,[1] Jung Nam An,[2] Ran-Hui Cha,[3] Hajeong Lee,[1] Dong Ki Kim,[1] Yon Su Kim,[1,4] Seung Hee Yang.[4] *[1]Internal Medicine, Seoul National Univ Hospital, Korea; [2]Internal Medicine, Seoul National Univ Boramae Hospital, Korea; [3]Internal Medicine, National Medical Center, Korea; [4]Kidney Research Inst, Seoul National Univ Hospital, Korea.*

Background: Chemokine receptor 5 (CCR5) is known for a key member of inflammatory chemokines in ischemic acute kidney injury. Signal transducer and activator of transcription 3 (STAT3) was recently revealed to play roles in CCR5 expression in human monocytes. CCR5 promoter regions contain STAT3 binding sites. In kidney disease, its role is unclarified. Therefore, we studied the interaction of STAT3 and CCR5 signals on renal ischemia-reperfusion injury (IRI).

Methods: B6 wild type and CCR5 deficient mice were performed in bilateral renal artery pedicles clamping for 30 min followed by reperfusion. After 6 hours of reperfusion, intrarenal lymphocytes were analyzed by flow cytometry.

Results: IRI produced more severe tubular damage in B6 wild type mice than in CCR5 deficient mice (BUN, 191.0 ± 2.7 versus 176.3 ± 2.3 mg/dL; creatinine, 2.37 ± 0.04 versus 1.75 ± 1.16 mg/dL, $p < 0.05$). Although inflammatory cytokines/chemokines, such as monocyte chemotactic protein-1, IFNγ,TNFα, CCL3, CCL4, and CCL5 were increased by IRI in wild type mice compared to sham mice, they were significantly attenuated in CCR5 deficient mice.Especially, CCR5 deficient mice reduced a greater number of inflammatory leukocytes and STAT3 in the kidney after IRI than wild type mice.These findings were supported by in vitro study with human tubular epithelial cells (TECs). The level of CCR5 and pSTAT3 were elevated in the hypoxia-conditioned TECs; however, those were decreased in CADPE (caffeic acid 3,4-dihydroxy-phenethyl ester, JAK2/STAT3 inhibitor) treated cells. The protective effect of the STAT3 inhibitor was achieved by suppression of proinflammatory cytokines and up-regulation of regulatory cytokines. The absence of CCR5 resulted in the protection of kidney damage via less apoptosis and tubulointerstitial inflammation.

Conclusions: These results elucidate that CCR5 regulates ischemic reperfusion injury via STAT3–dependent activation of immune system.

TH-PO141

Role of TRPV1 Channels in Ischemia/Reperfusion-Induced Acute Kidney Injury Lan Chen,[1] Lajos Marko, Mario Kassmann,[2] Kaiyin Wu, Ye Zhu, Maik Gollasch. *[1]Nephrology, Xiamen Zhongshan Hospital, Xiamen Univ, Fujian Province, China, Xiamen; [2]Nephrology, Experimental and Clinical Research Center (ECRC), a joint cooperation between the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany.*

Background: Transient receptor potential vanilloid 1 (TRPV1) receptor-positive sensory nerves are widely distributed throughout the kidney, suggesting that TRPV1-mediated action may participate in the regulation of renal function under pathophysiological conditions. Stimulation of TRPV1 channels protects against ischemia/reperfusion (I/R)-induced acute kidney injury (AKI). However, it is unknown whether inhibition of these channels is detrimental in AKI or not. We tested the role of TRPV1 channels in I/R-induced AKI by modulating these channels with capsaicin (TRPV1 agonist), capsazepine (TRPV1 antagonist) and using *Trpv1-/-* mice.

Methods: Anesthetized C57BL/6 mice were subjected to 25 min of renal ischemia and 24 hrs of reperfusion. Mice were pretreated with capsaicin (0.3 mg/kg body weight) or capsazepine (50 mg/kg body weight).

Results: Capsaicin ameliorated the outcome of AKI, as measured by serum creatinine levels (154.35 ± 6.58 µmol/L, versus I/R control 190.84 ± 6.13 µmol/L, $p < 0.05$), tubular damage (tubular necrosis score, 2.0 ± 0.27 versus I/R control, 3.0 ± 0.0, $P < 0.05$), neutrophil gelatinase-associated lipocalin (NGAL) abundance(5.44 ± 1.13% versus I/R control, 12.78 ± 2.40%, $p < 0.05$) and Ly-6B.2 positive polymorphonuclear inflammatory cells in injured kidneys (17.95 ± 4.54 per field view versus I/R control, 41.62 ± 6.23). Neither capsazepine nor deficiency of TRPV1 did deteriorate renal function or histology after AKI.

Conclusions: Activation of TRPV1 channels ameliorates I/R-induced AKI, but inhibition of these channels does not affect the outcome of AKI. Our results may have clinical implications for long-term safety of renal denervation to treat resistant hypertension in man, with respect to the function of primary sensory nerves in the response of the kidney to ischemic stimuli.

Funding: Private Foundation Support

TH-PO142

Activation of Inflammatory Mediators following Renal Ischemia-Reperfusion Injury Leads to Rapid Suppression of Mitochondrial Biogenic Signaling Ryan Whitaker, Rick G. Schnellmann. *Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC.*

Background: Mitochondrial dysfunction is an important mediator of acute kidney injury (AKI) development and progression. Our group demonstrated persistent disruption of mitochondrial homeostasis and inhibition of mitochondrial biogenesis (MB) in multiple models of AKI. While the mechanism(s) of negative regulation of MB following AKI are poorly understood, there is increasing evidence for a link between inflammation and metabolism. These studies sought to examine the role of inflammatory mediators in the early suppression of MB in I/R-induced AKI.

Methods: Cell culture experiments were performed in mouse immortalized TKPTS cells. *In vivo* studies used male C57BL/6 mice subjected to bilateral clamping of the renal pedicles for 18 min to induce AKI. Mice were euthanized at 1 or 3h post-reperfusion. Blood was collected for assessment of BUN and kidneys were collected for various biochemical analyses.

Results: MB was disrupted beginning 3h post-I/R as evidenced by decreased renal cortical mRNA expression of the mitochondrial genes PGC-1α, Tfam, ATPβ, NDUFS1, NDUFB8, COX1 and ND1. Additionally, mtDNA copy number was also decreased by 50% at 3h. No changes were observed 1h after I/R. Increased renal TNF-α mRNA levels and nuclear translocation of NF kappa ß protein were observed beginning 1h after reperfusion. MicroRNA-494, a TNF-α controlled negative regulator of MB, was upregulated 2-3-fold 1h after reperfusion. To evaluate the causal role of TNF-a on the observed mitochondrial genes, TKPTS cells were treated with recombinant TNF-α (10 ng/mL) for 3h. mRNA expression of PGC-1α, NDUFS1 and COX1 were down regulated 75%, 55% and 40%, respectively.

Conclusions: These results demonstrate a potential link between renal TNF-a-mediated inflammation following AKI and the persistent suppression of MB transcriptional signaling and suggest a possible role of mir-494. Pharmacological targeting of inflammatory pathways may serve as viable therapeutic modality to promote recovery of mitochondrial and renal function following AKI.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO143

Urinary ATP Synthase Subunit Beta Is a Novel Biomarker of Mitochondrial Dysfunction in Acute Kidney Injury

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Background: Diverse acute insults from surgery, ischemia/reperfusion (I/R), trauma, and drugs lead to mitochondrial dysfunction and acute kidney injury (AKI). Consequently, there is a great need for non-invasive biomarkers of renal mitochondrial dysfunction. We hypothesized that urinary mitochondrial ATP synthase subunit beta (ATPSβ) protein is a sensitive and specific marker of mitochondrial dysfunction in AKI.

Methods: Myoglobinuric AKI was induced by glycerol injection into rats, and mice were subjected to I/R-induced AKI.

Results: Animals in both models had persistent mitochondrial disruption and elevated serum creatinine, indicative of renal dysfunction, from 24 h through 144 h. Preliminary LC-MS/MS studies of 24 h urine samples revealed increased urinary ATPSβ protein in both AKI models. To investigate further, mice were subjected to sham or different degrees (5, 10 and 15 min ischemia times) of I/R-induced AKI. Serum creatinine, BUN and NGAL were only elevated in the 15 min I/R group at 24 h with overt histological damage. Immunoblot analysis of urinary ATPSβ revealed two bands (~52 and 25 kDa) that were confirmed to be mouse ATPSβ by LC-MS/MS. These bands increased at 24 h in the 10-and 15-min I/R groups, respectively. A time course using the 15-min I/R group revealed elevated urinary ATPSβ at 72 h that returned to control values at 144 h after reperfusion, a time when renal function was returning. LC-MS/MS analysis of urine from patients with AKI identified ATPSβ. Immunoblot analyses of patient urine samples collected 1.5 days after cardiac surgery revealed increased urinary mitochondrial ATPSβ levels in AKI patients when compared to control patients without AKI.

Conclusions: These translational studies provide evidence that ATPSβ may be a new and early urinary biomarker of renal mitochondrial dysfunction and enable evaluation of potential therapies for AKI.

Funding: Other NIH Support - NIEHS STTR

TH-PO144

Sildenafil Interferes with the Extension of the Ischemic Acute Kidney Injury

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Background: Ischemia is a critical event to extending renal injury. Besides, reperfusion initiates cellular injury by increasing generation of reactive oxygen species and induction of protection mechanisms such as heme oxygenase-1 (HO-1) enzyme. Sildenafil citrate (SIL) catalyzes the breakdown of cGMP nvolved in smooth muscle relaxation. This study evaluated the effect of SIL on the extension of renal damage provoked by different periods of ischemia.

Methods: Male Wistar rats weighing ±280 g were divided: **SHAM**; **Ischemia 30** (30 min renal pedicles clamping); **Ischemia 30+SIL** (0.25 mg/kg 60 min before renal ischemia); **Ischemia 45** and **Ischemia 45+SIL**. Renal function, hemodynamics, renal blood flow-RBF, oxidative injury, quantitative PCR of HO-1 and kidney histological analysis were evaluated.

Results: SIL pretreatment in Ischemia 30 and 45 ameliorated renal function by improving RBF, decreasing the oxidative metabolites with expression of HO-1 and reducing tubuleinterstitial injury.

Groups	Inulin clearance (ml/min)	RBF (ml/min)	Urinary Peroxides (nmmol/g cr)	Thiols (nmol/g protein)	Urinary TBARS (nmol/g cr)	Urinary NO (µmol/g cr)
SHAM	0.65±0.04	11.8±0.7	2±0.1	170±16	57±7	30±2
Ischemia 30	0.34±0.02α	4.2±0.3α	6±0.2α	117±10α	114±16α	76±7α
Ischemia 30+SIL	0.51±0.04αβ	7.8±1.0β	2±0.3β	111±11α	47±2β	35±3β
Ischemia 45	0.19±0.02αβ	2.3±0.1α	10±1.8αβ	129±17α	117±33α	76±14α
Ischemia 45+SIL	0.31±0.03αγ	5.8±0.4αγ	4±0.6γ	68±12αγ	45±7βγ	47±5γ

$^{\alpha}p<0.05$ vs SHAM, $^{\beta}p<0.05$ vs Ischemia 30, $^{\gamma}p<0.05$ vs ischemia 45.

Conclusions: The study concludes that the functional and histological damage induced by ischemia/reperfusion period determine the AKI extension and SIL confirms protective effect, involving mechanisms of HO and NO system.

TH-PO145

Adaptation of Rat Descending Vasa Recta to Ischemic Renal Injury

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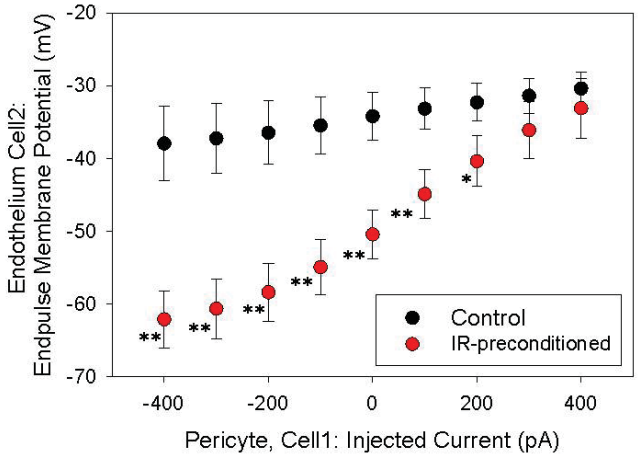
Background: Prior renal ischemia-reperfusion (IR) can protect the kidney against future insults. We tested whether cross clamp of the renal artery modifies descending vasa recta (DVR) that supply the renal medulla.

Methods: Two days after 30 minute IR, DVR were harvested from right and left, control and IR treated kidneys, respectively. NO generation, vasoactivity, pericyte and endothelial membrane potentials and mural conduction of injected currents were measured in isolated vessels.

Results: DAF fluorescence, indicating NO generation, increased over 20 minutes in IR treated DVR by 23±3% and in controls by 5±4% (P<0.01, n=7). The difference was eliminated by iNOS inhibition (1400W, 1 uM). The percent reduction of luminal diameter by AngII (10 nM) was reduced in IR pretreated DVR, 28±6% versus 54±8% (P<0.01, n=5). Membrane potentials of IR pretreated DVR were hyperpolarized; pericytes (-49±2 versus -58±4 mV, P<0.05) and endothelia (-44±3 versus -61± 4 mV, P<0.01). In dual patch clamp studies, injection of hyperpolarizing current (-400 pA) into a pericyte led to greater hyperpolarization of adjacent pericytes (-55±4 versus -77±3 mV, P<0.01 n=7) or endothelia (-38±5 versus -62±4 mV, P<0.01, n=7).



Conclusions: We conclude that IR preconditioning enhances DVR NO generation, reduces vasoconstriction, hyperpolarizes both endothelia and pericytes. It also enhances conduction of hyperpolarization, between pericytes and across myoendothelial junctions. These adaptations uniformly favor vasodilation, preservation of medullary blood flow and tissue oxygenation.

Funding: NIDDK Support

TH-PO146

C/EBP Homologous Protein (CHOP)-Gene Deficiency Attenuates Renal Ischemia/Reperfusion Injury in Mice

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Background: Endoplasmic reticulum (ER) has been implicated in the pathology of renal ischemia/reperfusion (I/R). C/EBP homologous protein (CHOP) is an important mediator of ER stress-induced cell and organ injury. Here, we investigated the role of CHOP in I/R-induced kidney injury using CHOP-knockout ($CHOP^{-/-}$) and their wild-type ($CHOP^{+/+}$) mice.

Methods: Mice were subjected to either 30 minutes of bilateral renal ischemia or sham-operation in CHOP-knockout ($CHOP^{-/-}$) and their wild-type ($CHOP^{+/+}$) mice.

Renal functional and histological changes were evaluated by concentrations of plasma creatinine (PCr) and blood urea nitrogen (BUN) and the periodic acid Schiff (PAS) staining, respectively. TUNEL assay was performed to determine apoptotic cells.

Results: Twenty-four hours after ischemia, the increases of PCr and BUN concentrations were less in $CHOP^{-/-}$ than in $CHOP^{+/+}$ mice. Disruption and congestion in tubules appeared in the outer medulla in PAS-stained kidney sections of both $CHOP^{-/-}$ and $CHOP^{+/+}$ mice 24 hours after ischemia. The damage scores of kidneys after ischemia were lower in $CHOP^{-/-}$ mice than $CHOP^{+/+}$ mice. Apoptosis was evaluated with Terminal deoxynucleotidyl transfer-mediated dUTP nick end-labeling (TUNEL) assay. The number of TUNEL-positive cells were less in $CHOP^{-/-}$ mice than in $CHOP^{+/+}$ mice. The activation of caspase3 and pro-apoptotic Bax was less in $CHOP^{-/-}$ mice than in $CHOP^{+/+}$ mice. In contrast, the activation of anti-apoptotic Bcl-2 and Bcl-x_L was more in $CHOP^{-/-}$ mice than in $CHOP^{+/+}$ mice validated TUNEL results.

Conclusions: These results indicate that CHOP-deficiency attenuates susceptibility to kidney injury by inhibition of necrosis and apoptosis in tubular epithelial cells, suggesting CHOP is a potential therapeutic target protein in I/R injury.

TH-PO147

MicroRNA-34a Is Up-Regulated in Acute Kidney Injury and by Aging and Regulates PNUTS (PPP1R10) Expression and Apoptosis of Renal Tubular Cells In Vitro and In Vivo Kazu Hamada, Tatsuki Matsumoto, Yoshiko Shimamura, Koji Ogata, Kosuke Inoue, Yoshinori Taniguchi, Taro Horino, Yoshio Terada. *Endocrinology, Metabolism and Nephrology, Kochi Univ, Nankoku, Japan.*

Background: MircoRNAs(miR) have emerged as critical regulators of physiological as well as pathological conditions. PNUTS (also known as PPP1R10; protein phosphatase 1 regulatory subunits 10) has recently reported to be regulated in cardiac aging. Both ischemia and aging are risk factors of acute kidney injury (AKI). Because the biological role for miR-34a and PNUTS in AKI is poorly understood, we studied the regulation and role of miR-34a and PNUTS in AKI and aging.

Methods: We used an in vivo mice cisplatin (CDDP)-induced AKI model and cultured renal tubular cells (NRK-52E cells). To elucidate the regulation of miR-34a and PNUTS mRNA, we used RT-PCR. The protein expression of PNUTS was evaluated by western blot analysis and immunohistological examination. Moreover, to assess aging effects, we studied miRNA-34a and PNUTS in aged mice (50- and 90-week-old).

Results: Immunohistological examination revealed the reduced expression of PNUTS in the proximal tubule cells in CDDP-induced AKI. PNUTS mRNA and protein expression were down-regulated in 12–48 h and 24–48 h after CDDP injection. miR-34a was inversely up-regulated at 6–48 h. PNUTS mRNA and protein expression showed a dose-dependent reduction by H_2O_2 in NRK-52E cells. Transfection of miR-34a mimic inhibited, and miR-34a inhibitor stimulated PNUTS expression. Overexpression of PNUTS reduced H_2O_2-induced caspase3 activity and apoptotic (TUNEL positive) cell number. Finally, PNUTS expression was significantly reduced in aged mice kidney, and inversely miR-34a increased by aging.

Conclusions: We showed that PNUTS expression was reduced and miR-34a was increased during oxidative stress and CDDP-induced AKI. Expression of PNUTS was negatively regulated by miR-34a, and controlled apoptosis. These results indicate that regulation of miR-34a and PNUTS may play a key role in the pathophysiology of AKI. Furthermore miR-34a and PNUTS might be critical for age-related renal damage.

TH-PO148

Preconditioning Mice with Activators of 5'-Adenosine Monophosphate-Activated Protein Kinase Ameliorates Ischemic Acute Kidney Injury Wilfred Lieberthal,[1] Jerrold S. Levine.[2] *[1]Dept of Medicine, Stony Brook Medical Center, Stony Brook, NY; [2]Dept of Medicine, Univ of Illinois at Chicago, Chicago, IL.*

Background: We have previously shown that preconditioning cultured proximal tubular cells (PTCs) with pharmacologic activators of 5'-Adenosine Monophosphate-activated Protein Kinase (AMPK), reduces the severity of PTC injury induced by ATP depletion. We now examine the effects of preconditioning mice with the AMPK activators AICAR or A-769662 on the severity of acute kidney injury (AKI) induced by renal ischemia-reperfusion injury (IRI).

Methods: Mice were preconditioned with AICAR or A-769662 or their vehicles. The following day the mice were subjected to right nephrectomy, after which IRI was induced by clamping the pedicle of the left kidney for 30 minutes. Serum creatinine was measured before and 24 hours after IRI.

Results: In mice preconditioning with the vehicles for AICAR or A-769662, IRI increase the serum creatinine from 0.66±0.08 to 1.97±0.009 and from 0.87±0.07 to 2.08±0.06 mg/dl respectively (both $p<0.001$). In mice preconditioned with AICAR or A-769662, IRI increased the creatinine from 0.62±0.03 to 1.23±0.13 mg/dl and from 0.72±0.05 to 1.2±0.09 mg/dl respectively (both $p<0.001$). Creatinine values after IRI were lower after preconditioning mice with AICAR and A-769662 than after preconditioning with their vehicles (both $p<0.01$). However, creatinine values after IRI are comparable in mice preconditioned with AICAR and with A-769662.

Conclusions: The severity of ischemic AKI is substantially ameliorated by AICAR and A-769662. In addition, the beneficial effect of preconditioning mice with AICAR or A-769662 was comparable.

Funding: Veterans Affairs Support

TH-PO149

Meclofenamate Elicits a Nephropreventing Effect in a Rat Model of Ischemic Acute Kidney Injury (AKI) by Suppressing Indoxyl Sulfate (IS) Production and Restoring Renal Organic Anion Transporters Yui Nomura,[1] Chika Saigo,[1] Yuko Yamamoto,[1] Hirofumi Jono,[1,2] Hideyuki Saito.[1,2] *[1]Dept of Clinical Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto Univ, Kumamoto, Japan; [2]Dept of Pharmacy, Kumamoto Univ Hospital, Kumamoto, Japan.*

Background: Indoxyl sulfate (IS) is excreted in the urine under normal kidney function, but is retained in the circulation and tissues during renal dysfunction in AKI, inducing oxidative stress in the kidney and cardiovascular system. IS is enzymatically produced in the liver from indole by cytochrome P450-mediated hydroxylation to indoxyl, followed by sulfotransferase-mediated sulfate conjugation.

Methods: Meclofenamate (10 mg/kg) was adminstered intravenously to rats treated with renal ischemia/reperfusion, and examined serum and renal levels of IS, renal function and PGE2 level, and expression of organic anion transporters rOat1 and rOat3.

Results: After testing about 200 compounds, including phytochemical polyphenols, we identified meclofenamate as a potent inhibitor of IS production in both rat and human liver S9 fractions. Ischemia/reperfusion (I/R) of rat kidney caused a marked elevation in the serum IS concentration 48 hr after surgery. However, administration of meclofenamate significantly suppressed this increase in the serum IS level. Moreover, IS concentrations in both kidney and liver were dramatically elevated by renal I/R treatment, but this increase was blocked by meclofenamate. Serum creatinine and BUN were markedly elevated in rats after renal I/R treatment, but were significantly attenuated by administration of meclofenamate. Renal expression of both rOAT1 and rOAT3 was downregulated by I/R treatment. However, the expression of both transporters recovered after administration of meclofenamate, which was associated with the inhibition of I/R-evoked elevation of PGE2. These results suggested that meclofenamate inhibits hepatic production of IS, thereby suppressing serum and renal accumulation of IS. Meclofenamate also prevents the I/R-PGE2-dependent downregulation of rOAT1 and rOAT3.

Conclusions: In conclusion, meclofenamate was found to elicit a nephropreventive effect in ischemic AKI.

Funding: Government Support - Non-U.S.

TH-PO150

Mouse Kidney Resident Double Negative Alpha/Beta T Cells Have Distinct Properties From Other Lymphocytes and Can Reduce Inflammation During AKI Maria Noel Martina Lingua, Samatha Bandapalle, Sanjeev Noel, Ankit Saxena, Abdel Hamad, Hamid Rabb. *Medicine, Johns Hopkins Univ, Baltimore, MD.*

Background: TCRαβ+CD4-CD8- double negative (DN) T cells with immunoregulatory function are a significant component of T cells residing in the kidney. However, the role of DN T cells in the regulation of renal homeostasis in the steady state and during AKI is unknown. We have further explored the functional role of DNT cells in mouse kidney under normal condition and following AKI.

Methods: CD45+ lymphocytes were purified from kidney mononuclear cells isolated from normal and ischemic AKI mouse kidneys and were analyzed for activation marker and co-stimulatory molecules by flow cytometry. Microarray analysis of flow sorted DNT cells was performed to decipher unique genomic signatures distinct from CD4 and CD8 cells. Adoptive transfer experiment was performed to define the immune function of DNT cells during AKI. We also measured intracellular cytokines in the kidney-infiltrating lymphocyte.

Results: The frequency of DNT cells in the kidney was higher than other T cell subsets in normal (30% of the T cells) and 3 hours post-ischemia (>60%). DNT had a baseline activated phenotype indicated by high expression of CD40L, CD28 co-stimulatory molecules and activation marker CD69. DNT cells proliferate at an unexpectedly high rate in normal kidney (30% BrdU incorporation) with further increase in proliferation after IRI (80% BrdU incorporation). Adoptive transfer of DN T cells 3h pre-ischemia resulted in significant reduction in inflammatory response (reduced γ-IFN and granzyme) in recipient mice as compared to the control group. Microarray analysis revealed a significant up-regulation of IL-27 gene in kidney DNT cells in comparison to CD8/CD4 cells isolated from kidney.

Conclusions: Our data demonstrate that mouse kidney resident DNT cells are innate-like cells and very early responder to IRI. They have a unique molecular response compared to CD4 and CD8 cells. Understanding the role of DNT in the kidney could have important implications for new therapies for AKI as well as other immune-mediated kidney diseases including glomerulonephritis, allograft rejection and interstitial nephritis.

Funding: NIDDK Support

TH-PO151

***In Vitro* Suppression Assays Do Not Predict *In Vivo* Functional Activity of Regulatory T Cells in Kidney Ischemia Reperfusion Injury** Gilbert R. Kinsey, Katarzyna Jobin, Joanna Ratajczak, Liping Huang, Brian K. Stevens. *Div of Nephrology and Center for Immunity, Inflammation and ReGenerative Medicine, Univ of Virginia.*

Background: Regulatory T cells (Tregs) play an important protective role in mouse models of acute kidney injury. Understanding the mechanisms used by Tregs to protect the kidney and the target cells that are influenced by Tregs would facilitate the development of therapeutic strategies based on Treg activity. Previous studies with programmed death

1 (PD-1) blocking antibodies suggest a critical role for PD-1 on Tregs in kidney ischemia reperfusion injury (IRI). $CD4^+$ T cells and natural killer T (NKT) cells promote renal injury during IRI. This study was to determine whether *in vitro* assays of Treg suppressive function are representative of Treg actions during kidney IRI.

Methods: Wild type (WT) and PD-1 KO Tregs were adoptively transferred to naïve WT mice 18 hr prior to bilateral renal IRI. Plasma creatinine and tubular necrosis were measured at 24 hr of reperfusion. *In vitro*, WT and PD-1 KO Tregs were tested for their ability to suppress $CD4^+$ T cell proliferation over 72 hrs (measured as CFSE dilution by flow cytometry) and α-galactosylceramide (α-galcer) induced-NKT cell interferon gamma (IFN-γ) production over 24 hrs (measured by ELISA).

Results: The *in vivo* experiments demonstrated that Tregs absolutely require expression of PD-1 to suppress kidney IRI. The *in vitro* experiments revealed that both WT and PD-1 KO Tregs effectively and equally suppressed the proliferation of $CD4^+$ T cells. Interestingly, whereas WT Tregs modestly inhibited α-galcer induced NKT cell IFN-γ production over 24 hr (65 ± 18% of NKT cells activated in the absence of Tregs (i.e. control)), the PD-1 KO Tregs were significantly better at inhibiting NKT cell IFN-γ production (20 ± 12% of control).

Conclusions: The lack of PD-1 expression by Tregs negates their protective function in an *in vivo* model of kidney IRI, but has no effect ($CD4^+$ T cell proliferation) or enhances (NKT cell IFN-γ production) Treg suppressive ability *in vitro*. These results suggest these *in vitro* models are not useful for determining the mechanism of action for Tregs in kidney IRI.

Funding: NIDDK Support

TH-PO152

Mice Lacking CD4 T Cells Have Worse Lung Injury After Ischemic Acute Kidney Injury (AKI) due to Reduced IL-10 Production

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Background: We have previously demonstrated that the spleen is an important source of the anti-inflammatory cytokine IL-10 to ameliorate AKI induced lung injury. The goal of this study was 1) to identify the splenic source of IL-10 and 2) to characterize the mechanism whereby IL-10 expression is up-regulated in AKI.

Methods: Splenic analysis of different cell populations in mice undergoing AKI revealed that IL-10 expression colocalized with molecular markers of macrophages (CD11b), T (CD4) and B-cells (B220) with little expression in neutrophils. Among these, CD4+ T cells demonstrated the highest expression of IL-10 suggesting a key role of this cell type in ameliorating lung injury post-AKI. AKI or sham operation was performed in CD4 deficient mice and lung injury was assessed 4 hours post AKI.

Results: Of interest, despite no significant changes in the degree of renal injury induced, lung injury, determined by measuring lung CXCL1 and MPO (a marker of neutrophil infiltration), was exacerbated in CD4 deficient mice compared to wild type animals. We have previously shown that circulating levels of the pro-inflammatory cytokine IL-6 are elevated in mice after AKI. In some settings, IL-6 has anti-inflammatory effects. We therefore tested the hypothesis that in splenocytes, including CD4+ T cells, IL-6 induces IL-10 expression. Addition of IL-6 to splenocytes and CD4+ T-cells increased IL-10 production in a dose-dependent manner. IL-10 production is mediated by IL-6-induced activation of STAT-3 as demonstrated by STAT-3 phosphorylation in a time-dependent manner and by the observation that IL-10 expression is reduced by the specific STAT-3 inhibitor stattic (20 μM). Consistent with this, in vivo, splenic IL-10 expression is significantly lower in IL-6 deficient mice with AKI compared to wild type.

Conclusions: In conclusion, our data indicates that in AKI, CD4+ T-cells are stimulated by IL-6 to produce IL-10 and this mechanism is important to ameliorate AKI-induced lung injury.

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TH-PO153

Divergent Control of Dendritic Cell Maturation by S1P Receptors Is Critical in Modulating Acute Kidney Injury in Mice

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Background: Regulation of dendritic cell (DC) maturation maybe a key therapeutic approach for the treatment of AKI. S1P, a sphingolipid is a natural ligand for family of 5 GPCRs(S1P1-5). Activation of the S1P1 reduces kidney ischemia-reperfusion injury (IRI), whereas activation of S1P3 may lead to loss of epithelial barrier and increase in IRI. $S1P3^{-/-}$ mice are protected for kidney IRI. DC maturation is necessary to initiate kidney IRI; depletion of DCs or adoptive transfer of $S1P3^{-/-}$ DCs protects kidneys from IRI.

Methods: We used WT, CD11cCre and $CD11cCreS1Pr1^{fl/fl}$ (DC deficiency of S1P1) mice and VPC01091 (S1P1 agonist and S1P3 antagonist) in kidney IRI. Injury was assessed by plasma creatinine (PCr; mg/dl), FACS, and RT-PCR.

Results: $CD11cCreS1Pr1^{fl/fl}$ mice result in more severe injury compared to CD11cCre (PCr: 0.9±0.18 versus 0.4±0.09; $p<0.01$). DCs $S1P1^{-/-}$ mice have higher number of mature kidney DCs ($CD11c^+MHCII^+$) and more neutrophil infiltration after IRI. These results suggest that in contract to the protective effect of deficiency of S1P3 in DCs, deficiency of S1P1 worsens AKI. VPC01091 (1mg/kg) treatment of WT mice led to: 1) less kidney injury after IRI compared to vehicle (1.2±0.2 versus 0.23±0.01; $p<0.001$), 2) reduced peripheral blood lymphocyte counts 3)significantly less total number of mature kidney DCs and neutrophils, and 4) less pro-inflammatory kidney cytokines (TNF-α, CXCL1 and IL-6). VPC01091 treatment led to a reduction of injury in CD11cCre mice (0.4±0.09 versus 0.2±0.01; $p<0.05$) and DC $S1P1^{-/-}$ (0.9±0.18 versus 0.44±0.09; $p<0.05$) mice.

Conclusions: S1P1 and S1P3 expression in DCs regulate maturation and have divergent effects in kidney IRI. Whereas it is known that deficiency of S1P3 inhibits DC maturation and reduces kidney IRI, deficiency of S1P1 enhances DC maturation exacerbates injury and inflammation. VPC01091 takes advantage of the protective effect of S1P1 activation and S1P3 inhibition reduces kidney IRI. Furthermore, the effect of VPC01091 is effective in mice deficient of S1P1 in DCs suggests that pharmacological activation of S1P1 extends beyond DC S1P1. VPC01091 may be useful in clinical aspects for the prevention of AKI or treatment of established AKI.

TH-PO154

Dissociation of Renal Filtration Function Failure from Leukocyte Infiltration in Proximal Tubule CD73-Deficient Mice Undergoing Ischemia-Reperfusion

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Background: Renal ischemia-reperfusion injury (IRI) is characterized by inflammatory cell infiltration, damages to the endothelium and tubules, and compromised renal filtration functions. CD73 is thought to protect mice from IRI by generating adenosine which inhibits leukocyte effector functions through the adenosine receptors. However, it is unclear whether a hierarchy of IRI protection efficiencies occurs with CD73 expression by various renal cell type.

Methods: Sub-threshold bilateral renal pedicle clamping (Sub-IRI) was performed in mice with global CD73 deletion or cell type-specific targeted CD73 deletion in proximal tubular cells and CD11c+ myeloid cells, as well as in bone marrow chimeras of WT and CD73 deficient mice. Creatinine measurements, confocal microscopy, and flow cytometry were used to assess kidney functions and inflammation 24 h after ischemia-reperfusion.

Results: CD73 was expressed at high levels by proximal tubule epithelial cells and mesangial cells, intermediate levels by interstitial cortical type I fibroblasts, and low levels by CD11c+ myeloid cells. Adoptive transfer experiments showed that increased IRI was found only in hosts with CD73 deletion in non-hematopoietic cells. Cell type-specific targeted CD73 expression further defined the contribution of CD73 expression on different renal cell types. Enhanced IRI was found only in PepCK-Cre-CD73fl/fl mice but not in CD11c-Cre-CD73fl/fl mice, indicating that CD73 on proximal tubular cells but not on cortical fibroblasts or myeloid cells is key to IRI protection. Interestingly more than 5-fold increases in PMN and CD11b+F4/80Low macrophages but no detectable increase in creatinine over sham-operated mice was found in wild-type and CD11c-Cre-CD73fl/fl mice undergoing sub-IRI.

Conclusions: The studies showed that CD73 expression on proximal epithelial cells and not on cortical fibroblasts and myeloid cells was critical for renal IRI protection. Furthermore, a biphasic IRI disease course with leukocyte infiltration followed by renal cell damage may occur with CD73 interfering only with the latter phase.

Funding: NIDDK Support

TH-PO155

Double Knockout of PARP-1 and Cyclophilin D Reduces Ischemic Renal Injury in Mice

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Background: The key features of Ischemic renal injury (IRI) are vascular damage, inflammation and proximal tubular cell apoptosis and necrosis, particularly in the outer medulla. Poly (ADP-ribose) polymerase 1(PARP-1) and cyclophilin D play important roles in inducing necrotic cell death after various types of cell stress. Our laboratory has previously established key roles for both PARP-1 and cyclophilin D in necrosis after IRI. However, whether these molecules induce necrosis independently of each other or if their signaling pathways integrate to induce necrosis is unclear. In this study, we hypothesized that genetic deletion of both PARP-1 and cyclophilin D additively or synergistically protects mice from ischemic renal injury.

Methods: Wild type, PARP-1 knockout, cyclophilin D knockout and PARP-1/cyclophilin D double knockout mice underwent either sham surgery or clamping of the renal pedicle for 30 min followed by reperfusion for 1 and 5 days. Renal function was assessed by creatinine and blood urea nitrogen (BUN) assays, histological damage and necrosis by Periodic acid-Schiff staining, inflammation by neutrophil staining. Apoptosis was determined by TUNEL staining.

Results: Plasma levels of creatinine and BUN were significantly decreased 24 h after reperfusion in double knockout mice compared with wild type and single knockout mice for cyclophilin D and PARP-1. Double knockout mice also had less renal histological damage, necrotic proximal tubules, infiltration of neutrophils, and TUNEL positive cells.

Conclusions: Genetic deletion of both PARP-1 and cyclophilin D protects mice kidneys from functional and histological deterioration after IRI. Inhibition of both PARP-1 and cyclophilin D may provide more protective effect in IRI.

Funding: NIDDK Support

TH-PO156

Nrf2 and HIF1α Cooperatively Regulates Heme Oxygenase 1 Expression in Renal Epithelial Cells during Hypoxia and Reoxygenation

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Background: Nrf2 and HIF1α transcription factors play protective roles in ischemia reperfusion (IR)-induced acute kidney injury (AKI) *in vitro* and *in vivo* by upregulating cytoprotective enzymes that mitigate cellular stress, including heme oxygenase 1 (Hmox1).

We hypothesized that Nrf2-HIF1α crosstalk is critical for Hmox1 induction during IR-induced AKI. Indeed, we now report existence of such crosstalk in the regulation of Hmox1 expression in renal epithelial cells (RECs) following hypoxia and reoxygenation.

Methods: We have utilized human renal proximal tubular epithelial cells, HK-2, and primary cultured mouse RECs from *Nrf2*$^{-/-}$ and *Nrf2*$^{+/+}$ mice.Cells were exposed to acute (2 h) and chronic (12 h) hypoxia followed by reoxygenation (up to 6 h) with or without HIF1α inhibitor, digoxin. Cells were treated with HIF1α activator, $CoCl_2$ HIF1α, Nrf2 and Hmox1 expression levels were analyzed by qRT-PCR and immunoblot analysis.

Results: Both acute and chronic hypoxia, as expected, induced HIF1α activation, which was attenuated during reoxygenation in HK2 cells. Hmox1 expression remained significantly ($p<0.05$) elevated following acute (2-fold) and chronic (10-fold) hypoxia and during reoxygenation of chronic hypoxia (2.5-fold). Intriguingly, chronic hypoxia markedly decreased Nrf2 levels (90%), but reoxygenation restored these levels to room air controls. Digoxin inhibited hypoxia-induced HIF1α activation and Hmox1 expression, and decreased Nrf2 levels. In contrast, $CoCl_2$ increased Nrf2 expression (3-fold) but digoxin blocked this induction. We also found reduced HIF1α activation and Hmox1 expression in *Nrf2*$^{-/-}$ RECs exposed to hypoxia and reoxygenation. Consistent with this result, $CoCl_2$ markedly stimulated Hmox1 expression in *Nrf2*$^{+/+}$ (5-fold) but not in Nrf2$^{-/-}$ RECs.

Conclusions: Our results show for the first time that HIF1α and Nrf2 cooperatively modulate Hmox1 induction during hypoxia and reoxygenation in RECs, and deregulation of this HIF1α-Nrf2 crosstalk may contribute to enhanced susceptibility to RECs injury during IR-induced AKI.

Funding: NIDDK Support

TH-PO157

Heterozygous Knockout of SerpinC1 Exacerbates Renal Ischemia/Reperfusion Injury in Rats Feng Wang,[1] Guangyuan Zhang,[2] Kristie Usa,[3] Aron M. Geurts,[4] Niansong Wang,[5] Mingyu Liang.[6] *[1]Nephrology and Rheumatology, Shanghai Jiao Tong Univ Affiliated Sixth People's Hospital, Shanghai, China; [2]Physiology, Medical College of Wisconsin, Milwaukee, WI; [3]Physiology, Medical College of Wisconsin, Milwaukee, WI; [4]Physiology, Medical College of Wisconsin, Milwaukee, WI; [5]Nephrology and Rheumatology, Shanghai Jiao Tong Univ Affiliated Sixth People's Hospital, Shanghai, China; [6]Physiology, Medical College of Wisconsin, Milwaukee, WI.*

Background: SerpinC1-encoded antithrombin III (ATIII) is the major anti-coagulation molecule in vivo and has anti-inflammatory effects. SerpinC1 homozygous knockout is embryonically lethal. We hypothesized that partial loss of SerpinC1 would exacerbate renal ischemia/reperfusion injury.

Methods: SerpinC1 heterozygous knockout rats were established in a congenic model SS.BN-(D13Rat151-D13Rat197)/Mcwi using zinc-finger nucleases targeting exon 1, resulting in a 29 base pair deletion removing the endogenous translation initiation codon, and is therefore predicted to result in a complete null allele for SerpinC1. The left renal ischemia was induced by nontraumatic vascular clamps over the renal artery for 30 minutes after right nephroctomy and 24 hours' reperfusion followed in wild type (WT, n=6) and heterozygous knockout (Het, n=6) littermate groups. Sham operation was performed in additional groups of rats.

Results: Renal tubular injury scores were increased from 0.50±0.10 to 3.58±0.71 in the Het group and to 2.15±0.30 in the WT group after renal ischemia/reperfusion ($P<0.05$ for Het versus WT). Serum creatinine was increased from 0.21±0.04 mg/dl to 1.09±0.40 mg/dl in the Het group and from 0.20±0.02 to 0.67±0.20 in the WT group ($P<0.05$ for Het versus WT). Concomitantly, renal levels of malondialdehyde and apoptosis were 1.6±0.2 fold and 1.4±0.1 fold, respectively, greater in the Het group compared to the WT group ($P<0.05$). Significant thrombosis was not observed in the kidneys of any of the groups.

Conclusions: In summary, heterozygous knockout of SerpinC1 exacerbates renal ischemia/reperfusion injury in rats possibly via increased oxidative stress and apoptosis.

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TH-PO158

ADAMTS13-Von Willebrand Factor (vWF) Axis Is Involved in the Pathophysiology of Renal Ischemia Reperfusion Injury Myung-Gyu Kim, Sun Chul Kim, Hyojeong Chang, Sang-Kyung Jo, Won-Yong Cho. *Dept of Internal Medicine, Korea Univ Anam Hospital, Seoul, Republic of Korea.*

Background: Recent epidemiologic studies showed that impaired activity of ADAMTS13, a protease cleaving Von Willebrand factor (vWF) multimer, could predict renal complications in diabetic patients. The ADAMTS13- vWF axis has also been suggested to play a critical role in the pathophysiology of ischemia-reperfusion injury (IRI) in different organs.The purpose of this study was to investigate whether ADAMTS13-ultralarge vWF (UlvWF) multimer axis was involved in the pathophysiology of IRI induced AKI.

Methods: We performed renal IRI in ADAMTS13 knockout (KO) mice or wild type (WT) mice. Functional, histological kidney damage, inflammation were compared and the effect of recombinant human ADAMTS13 (rhADAMTS13) in ADAMTS13 KO mice was also assessed.

Results: Following IRI, the ADAMTS13 level in blood was significantly reduced and vWF was detected in both medulla and cortex of injured kidney by immunohistochemistry. Western blot analysis also showed increased expression of ULvWF-muiltimer in ischemic kidneys, and the expression was significantly higher in ADAMTS13 KO mice than WT mice. The higher level of vWF-multimer in ADAMTS13 KO mice was correlated with more functional deterioration and severe tubular injury, suggesting an important role of VWF in renal IRI. In addition, the number of Gr-1 (+) neutrophils was significantly higher in the kidney of ADAMTS13 KO mice compared to that of WT mice, whereas F4/80 macrophages were not. In ADAMTS13 KO mice, administration of rhADAMTS13 after IRI partially restored renal injury, suggesting that ADAMTS13 may be an important player that regulates IR injury associated with vWF.

Conclusions: Our data shows that ADAMTS13-vWF axis is involved in the pathophysiology in renal IRI. This is first report showing the involvement of UlvWF-multimer in renal IRI indicating that ADAMTS13 could have therapeutic value to limit renal ischemia/reperfusion injury.

TH-PO159

Dichotomy of STAT3 Phosphorylation in Ischemic Acute Kidney Injury Istvan Arany,[1] Dustin Reed,[1] Kiran B. Chandrashekar,[2] Luis A. Juncos.[2,3] *[1]Pediatrics, Univ of Mississippi Medical Center; [2]Medicine, Univ of Mississippi Medical Center; [3]Physiology and Biophysics, Univ of Mississippi Medical Center.*

Background: Studies suggest that the transcription factor STAT3 (signal transducer and activator of transcription-3) can promote either injury or protection depending on its phosphorylation state in extrarenal organs. In the present study we tested the hypothesis that tyrosine (Y705) phosphorylation is adaptive while serine (S727) phosphorylation of STAT3 is maladaptive during renal IR-AKI.

Methods: Male wild type (w.t.), STAT3 ß k.o. (the regulatory STAT3 ß isoform is deleted) or homozygous SA/SA mice (S727A mutant, which is impaired in S727 phosphorylation) were subjected to 18 minutes of warm ischemia followed by 24 hours of reperfusion (IR). A group of w.t. mice were subjected to chronic nicotine (Ch-NIC) exposure prior to IR. Renal function (creatinine) and injury (KIM1) as well as STAT3 phosphorylation and HO-1 expression were determined. In vitro, STAT3 tyrosine/serine phosphorylation was manipulated by either mutation (Y705F or S727A, respectively) or pretreatment with LIF (↑pTyrSTAT3), ET-1 or NIC (↑pSerSTAT3) in renal proximal tubule cells (LLC-PK1) that were subjected to oxidative stress (400 μM H_2O_2). Cell injury (LDH release) as well as activity of the HO-1 promoter or a STAT3-binding element reporter (SBE; that is a part of the HO-1 promoter) was determined.

Results: IR-AKI increased pTyrSTAT3 and HO-1 but ameliorated renal dysfunction/injury in SA/SA mice while decreased pTyrSTAT3 and HO-1 and increased pSerSTAT3 and renal dysfunction/injury in STAT3 ß k.o. mice or w.t. mice exposed to Ch-NIC. In vitro, LIF (↑pTyrSTAT3) increased induction of HO-1 and SBE and attenuated oxidant injury while Y705F mutation (↓pTyrSTAT3) resulted in opposite effects. In contrast, ET-1 or Ch-NIC (↑pSerSTAT3) decreased HO-1/SBE induction and augmented injury while S727A mutation (↓pSerSTAT3) showed opposite effects.

Conclusions: Our studies suggest that pTyrSTAT3 is adaptive while pSerSTAT3 is maladaptive during IR-AKI that involves differential modulation of HO-1 transcription via the SBE. Hence, manipulation of this pathway might offer therapeutic means to ameliorate acute kidney injury.

Funding: NIDDK Support

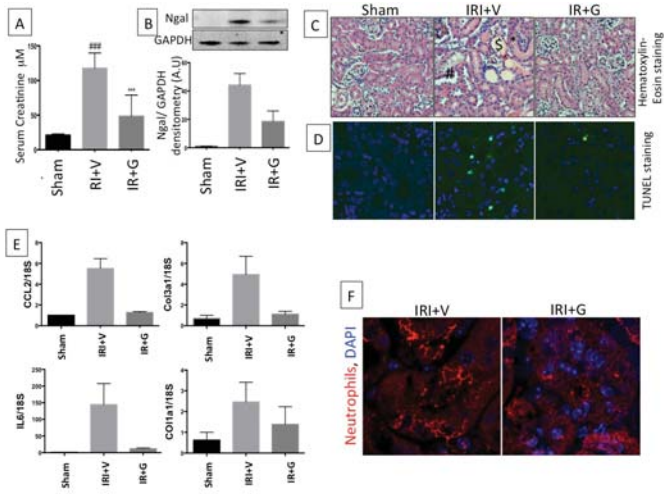
TH-PO160

The Role of G Protein-Coupled Receptor Gβγ Signaling in Acute Kidey Injury Fadia A. Kamal, Joshua Travers, Prasad Devarajan, Burns C. Blaxall. *Cincinnati Children's Hospital Medical Center, OH.*

Background: Acute kidney injury (AKI) is a significant condition of increasing morbidity. The pathogenesis of AKI is complex and variable with renal tubular death and tubulointerstitial inflammation being two determinants of tissue damage and renal failure. G protein–coupled receptors (GPCRs) are widely expressed in the kidney and their role in AKI is understudied. Prolonged agonist stimulation of the GPCR induces pathological upregulation of its Gβγ-GRK2 signaling causing receptor phosphorylaion and internalization and activation of pathologic downstream signaling pathways.

Methods: We explored the protective effect of the small molecule Gβγ inhibitor *gallein* in a bilateral ischemia reperfusion injury (IRI) mouse model. Mice were pretreated with either vehicle (V) or galleon (G) three days pre-IRI. Twenty-four hours after IRI mice were sacrificed, urine and blood were collected before and after IRI and kidneys were harvested for histology and protein and mRNA analysis.

Results: Functionally, serum creatinine (A) and tissue Ngal (B) levels were elevated post-IRI. Histologically, tubular expansion, tubular damage and cast formation (C) and tubular apoptosis (D) revealed tissue damage post-IRI. Further, kidney inflammatory and profibrotic gene expression (CCL2, IL-6, Collagen-I and Collagen-III) (E) and neutrophil infiltration (F) were elevated post-IRI. Gallein pretreatment attenuated all these pathological changes and protected renal function post-IRI. Interestingly, renal GRK2 protein expression was upregulated post-IRI and was attenuated in gallein treated mice.



Conclusions: In conclusion, preserved renal function and structure and attenuated GRK2 upregulation twenty-four hours post-IRI by the small molecule Gβγ inhibitor gallein, indicate a direct role for Gβγ-GRK2 signaling in renal IRI and a potential protective role for gallein in AKI.

TH-PO161

Mechanisms of the Renal Protection By Cyclosporine After an Ischemia Reperfusion Early at the Reperfusion: Role of HSP70 Sandrine Lemoine,[1,2] Bruno Pillot,[1] Lionel Augeul,[1] Michel Ovize,[1,3] Laurent Juillard.[1,2] *[1]Edouard Herriot Hospital-Nephrology, HCL, Lyon, France; [2]Carmen 1060-IHU OPERA, Univ of Lyon 1, Univ of Lyon, Lyon, France; [3]Louis Pradel Hospital-Cardiology, HCL, Lyon, France.*

Background: The aim of this study is to show that the preconditioning with CsA provides a protection against renal ischemia reperfusion (IR) by two pathways allowing together the delay of mPTP opening: the HSP70 pathways and by the direct inhibition of cyclophiline D (cypD).

Methods: We performed a right unilateral nephrectomy with a 30-minutes contralateral clamping of the left renal artery in C57BL6 male mice. First, we measured in 3 groups (a sham group, an ischemic group and a pre-conditionning group with 10 mg/kg of CsA (preCsA) injected 10 min before ischemia) the calcium overload (CRC) in order to test mPTP, the oxydative phosphorylation to test respiratory chain, the renal expression of HSP70 and GSK 3-b by western blotting at 20 min of reperfusion. Secondly, we performed the same ischemia in CyclophilineD deficient mice to evaluate the role of CypD in mPTP. Finally, we used the inhibitor of HSP70 (Quercetin, 100mg/kg, 2h before ischemia) in mice preconditioned with CsA to evaluate the possible role of HSP70 in mPTP.

Results: At 20 min of reperfusion, we showed a delay of mPTP opening in the preCsA group compared to the ischemic group. However, we found no protection in the oxidative phosphorylation that is dramatically decreased at 20 min of reperfusion in all groups, as previously reported. Renal HSP70 and GSK3 phosphorylated protein (P-GSK3b) levels were higher in the preCsA group than in the ischemic group, suggesting an enhancement of these 2 proteins by CsA. The pretreatment with Quercetin decreased HSP70 and P-GSK3b levels compared to preCsA group and eliminated the preconditionning protection with an early opening of mPTP.

Conclusions: Our study suggested that preCsA promotes two pathways of protection, the HSP70 pathways, mediated by P-GSK3B and by inhibition of cyclophilline D. These 2 pathways allow delaying the mPTP opening and protects against IR, suggesting that mPTP is the key event in the renal lesion of IR.

Funding: Pharmaceutical Company Support - Amgen

TH-PO162

Small Heat Shock Protein Beta-1 (HSPB1) Is Up-Regulated and Regulates the Autophagy and Apoptosis of Renal Tubular Cells in Acute Kidney Injury In Vitro and In Vivo Tatsuki Matsumoto, Madoka Urushido, Haruna Ide, Kazu Hamada, Yoshiko Shimamura, Koji Ogata, Kosuke Inoue, Yoshinori Taniguchi, Taro Horino, Shinpei Fujimoto, Yoshio Terada. *Kochi Medical School, Kochi Univ, Japan.*

Background: Heat shock protein Beta-1 (HSPB1)(HSP27) is a small heat shock protein that is involved in many cellular processes, and it reportedly protects cells against oxidative stress. Autophagy is a mechanism that protects cells from many types of stress and is thought to play an important role in preventing stress in acute kidney injury (AKI). However, little is known about the role of HSPB1 in autophagy and apoptosis in AKI pathogenesis.

Methods: We used an in vivo rat ischemia/reperfusion AKI model and cultured renal tubular cells as an in vitro model. To elucidate the regulation of HSPB1, we evaluated the promoter activity and expression of HSPB1 in NRK-52E cells in the presence of H_2O_2. To examine the regulation of autophagy by HSPB1, we established NRK cells that were stably transfected with a fusion protein between green fluorescent protein and LC3 as a marker of autophagy.

Results: Immunohistological examination showed the expression of HSPB1 in the proximal tubule cells after AKI. RT-PCR and western blot analysis showed that HSPB1 mRNA and protein expression were upregulated 6–72 h and 12–72 h after ischemia/reperfusion. HSPB1 promoter activity, mRNA, and protein expression showed a dose-dependent induction by H_2O_2. Overexpression of HSPB1-induced autophagy in NRK cells under normoxic conditions was confirmed by confocal microscopy, which revealed the presence of LC3-positive granules. Furthermore, autophagy induced by H_2O_2 (400 µM) was inhibited by the transfection of small interfering RNAs for HSPB1. Overexpression of HSPB1 reduced Bax activation and H_2O_2-induced-apoptosis as measured by caspase 3 activity and the terminal deoxynucleotidyl transferase dUTP nick end labeling assay.

Conclusions: We showed that HSPB1 expression increased during oxidative stress in AKI. Incremental HSPB1 expression caused autophagy and inhibited apoptosis in renal tubular cells. These results indicate that up-regulated HSPB1 plays a role in the pathophysiology of AKI.

TH-PO163

Phosphoinositide 3 Kinase γ Plays a Critical Role in Acute Kidney Injury Xiaogao Jin, Yanlin Wang. *Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Acute kidney injury (AKI) is a common clinical condition that is associated with high morbidity and mortality. Ischemia-reperfusion injury (IRI) is a common cause of AKI. There is no effective therapy for this serious clinical condition except renal replacement. Therefore, a better understanding of the pathogenic mechanisms underlying IRI is essential for ultimately developing effective therapy. Inflammation plays a critical role in the pathogenesis of IRI. However, the signaling mechanisms underlying inflammatory cell infiltration are not fully understood.

Methods: We examined the role of phosphoinositide 3 kinase γ (PI3Kγ) in inflammatory cell infiltration into the kidney in mice subjected to 30 minutes of ischemia followed by 24 hours of reperfusion.

Results: Compared with wild-type mice, PI3Kγ knockout mice protected the kidney from IRI. Tubular cell apoptosis induced by ischemia-reperfusion injury was decreased in the kidney of PI3Kγ knockout mice. Furthermore, PI3Kγ deficiency inhibited the infiltration of neutrophils, macrophages, and T cells into the kidney, which was associated with a decrease in the expression of the proinflammatory molecules in the kidney. Moreover, wild-type mice treated with AS605240, a selective PI3Kγ inhibitor displayed less tubular damage and apoptosis, accumulated fewer inflammatory cells and expressed less proinflammatory molecules in the kidney following IRI.

Conclusions: Our results demonstrate that PI3Kγ plays a critical role in the pathogenesis of acute renal ischemia-reperfusion injury, suggesting that inhibition of PI3Kγ may be a potential therapeutic strategy in acute kidney injury.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO164

Angiotensin 1-7 (Ang1-7) Protects against Renal Ischemia Reperfusion (I/R)-Induced Acute Kidney Injury (AKI) and Its Deleterious Cardiac Effect; Possible Role of Vascular Endothelia Growth Factor (VEGF) Arnaldo F. Lopez-Ruiz,[1] Andrea P. Soljancic,[1] Kiran B. Chandrashekar,[1] Ruisheng Liu,[2] Luis A. Juncos.[1] *[1]Medicine/Nephrology, Univ of Mississippi Medical Center, Jackson, MS; [2]Physiology, Univ of Mississippi Medical Center, Jackson, MS.*

Background: Ang1-7 is a contra-regulatory peptide of the renin-angiotensin system with cytoprotective effects. AKI has been shown to induce distant deleterious effect in other organs including the heart. AKI-induced cardiac dysfunction is associated with impaired VEGF expression. We tested whether exogenous Ang1-7 protects against I/R-induced AKI and its cardiac deleterious effects. We also tested if Ang1-7 upregulates VEGF expression.

Methods: AKI was induced by clamping both renal pedicles for 40 min. SD rats were divided into 4 groups, namely; sham, AKI, AKI+Ang1-7, AKI+Ang1-7+A779 (Ang1-7 antagonist). Drugs were given 1 day before I/R and continued for for 2 extra days. During follow up, plasma creatinine, sFlt-1 (anti-angiogenic factor) and urine NGAL (tubular injury marker) were measured. 48h after I/R, kidneys (K) and heart (H) were harvested to evaluate TNFα (pro-inflammatory signal), BNP (cardiac distress) and VEGF (cytoprotective factor).

Results:

	Creat (mg/dl)	NGAL (UI/ml)	K-TNFα (pg/ml)	H-TNFα (pg/ml)	sFlt-1 (pg/ml)	K-VEGF (pg/ml)	H-VEGF (pg/ml)	H-BNP (pg/ml)
Sham	0.6±0.1	155±70	9.5±2	3.2±1	45±6	382±64	340±45	32±8
AKI	2.3±0.1*	1380±92*	155±21*	88±6*	755±35*	95±12*	88±7*	245±15
AKI+ Ang1-7	1.6±0.1#	635±115#	72±8#	38±4#	390±58#	228±25#	185±15#	115±18
AKI+ Ang1-7 +A779	2.1±0.2	1270±125	141±15	76±9	725±42	110±10	98±6	228±26

* $p<0.05$ vs Sham, # $p<0.05$ vs AKI

Conclusions: AKI leads to systemic increase of anti-angiogenic factor and impaired renal VEGF expression.AKI contributed to cardiac dysfunction by inducing pro-inflammatory signaling which also resulted in impaired cardiac VEGF expression. Ang1-7 attenuated I/R-induced AKI and ameliorated its cardiac deleterious effect perhaps by upregulating VEGF expression and reducing sFlt-1 release.

Funding: Other NIH Support - NIH DK073401

TH-PO165

Potential Role of Asymmetric Dimethylarginine in AKI Yosuke Nakayama, Nana Obara, Seiji Ueda, Miyuki Yokoro, Seiya Okuda. *Div of Nephrology, Dept of Medicine, Kurume Univ, Kurume, Fukuoka, Japan.*

Background: Injury to the renal vasculature plays important roles in the pathogenesis of acute kidney injury (AKI). However, roles of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthease, in AKI remain unclear. So, we investigated the kinetics and the roles of ADMA in ischemia/ reperfusion (IR)-injured mice and patients undergoing elective coronary angiography (CAG).

Methods: We first examined the kinetics of ADMA, and DDAH-1, a key enzyme for ADMA degradation, levels in the kidney of IR-injured mice. Further, we examined the effects of continuous infusion of ADMA on renal IR injury, and studied whether the IR injury could be attenuated in DDAH-1 transgenic (Tg) mice. Furthermore, we collected blood and urine samples of 52 patients before and after elective CAG at our institution.

Results: After the IR injury, DDAH-1 levels were decreased and renal and plasma ADMA levelswere increased in association with renal injury. Infusion of subpressor dose of ADMA exacerbated renal dysfunction,capillary loss and tubular necrosis in the kidney of IR-injured wild mice, while theseIR-induced damages were attenuated in DDAH-1 Tg mice. In contrast-induced nephropathy (CIN) study, no case of obvious AKI assessed by changes increatinine level was identified. However, levels of ADMA, high sensitivity C-reactive protein (hs-CRP), N-acetyl-β-D-glucosaminidase (NAG) and L-type fatty acid binding protein (L-FABP) were significantly increased by administration of contrast medium. Further, elevated hs-CRP levels but not ADMA, NAG and L-FABP levels were significantly associated with semiannual increased creatinine levels in these patients.

Conclusions: Our present studysuggests that IR injury may reduce DDAH-1 expression andresultantly cause ADMA accumulation, which could lead to capillary loss and tubularnecrosis in the kidney, thereby being involved in renal injury.Although, further study is needed to clarify the long term impacts of ADMA elevation in CIN patients on future of organ damage, ADMA could be a novel predictive maker for AKI.

Funding: Private Foundation Support

TH-PO166

Renoprotective Effect of Testosterone Propionate in Ischemia-Reperfusion (I/R) Induced Acute Kidney Injury (AKI) Chetan N. Patil,[1,2] Rodrigo Maranon,[1,2] Carolina Dalmasso,[1,2] Huimin Zhang,[1,2] Luis A. Juncos,[3] Jane F. Reckelhoff.[1,2] *[1]Women's Health Research Center, Univ of Mississippi Medical Center, Jackson, MS; [2]Dept of Physiology and Biophysics, Univ of Mississippi Medical Center, Jackson, MS; [3]Dept of Medicine, Div of Nephrology, Univ of Mississippi Medical Center, Jackson, MS.*

Background: Acute Kidney injury (AKI) is a leading cause of morbidity and mortality among patients, and men are more prone to AKI compared to women. Our lab has recently shown that serum testosterone levels are significantly reduced by 3 hrs post ischemia and the levels remain reduced at 24 hrs. We also found that infusion of testosterone 3 hrs post reperfusion attenuated renal injury following ischemia/reperfusion (I/R). In the present study we evaluated the dose response effect of testosterone propionate in I/R induced AKI.

Methods: Male SD rats (8-14 wks; n=3-5/grp) were subjected to sham surgery or I/R induced AKI with bilateral clamping of renal vessels for 30 min. Three hrs after reperfusion, rats were administered with vehicle (0.75%EtOH) or fixed dose of testosterone propionate (i.v. over 10 min). Rats were placed in metabolism cages for 24hrs for nitrate/nitrite excretion (UNOxV), and euthanized and blood taken for creatinine (PCr).

Results: I/R increased PCr and decreased UNOxV, compared to shams (0.58±0.05 versus 3.99±0.36 mg/dL, p<0.0001; UNOxV: 8.54±1.7 versus 1.7±0.78 µmol/day/kg BW, p<0.001). Dose response study showed that administration of low dose (10ugm/kg, PCr 4.05±0.41mg/dL) as well has very high dose of testosterone (50ugm/kg, PCr 3.89±0.23 mg/dL; 100ugm/kg, PCr 4.56±0.21 mg/dL) failed to show any improvement in renal function in I/R induced AKI rat model. But 20µgm/kg testosterone propionate attenuated the increase in PCr (2.03±0.23 mg/dL, p< 0.01) and tended to decrease UNOxV (3.79±0.91 µmol/day/kg, p=0.0596).

Conclusions: These results suggest that modest doses testosterone propionate can improve renal function in males with I/R induced AKI. Hence testosterone propionate could be a potential therapeutic agent for the treatment of AKI in men. Supported by NIH R01HL66072, P01HL05971 and AHA 14POST18640015.

Funding: Other NIH Support - NIH R01HL66072, P01HL05971, Private Foundation Support

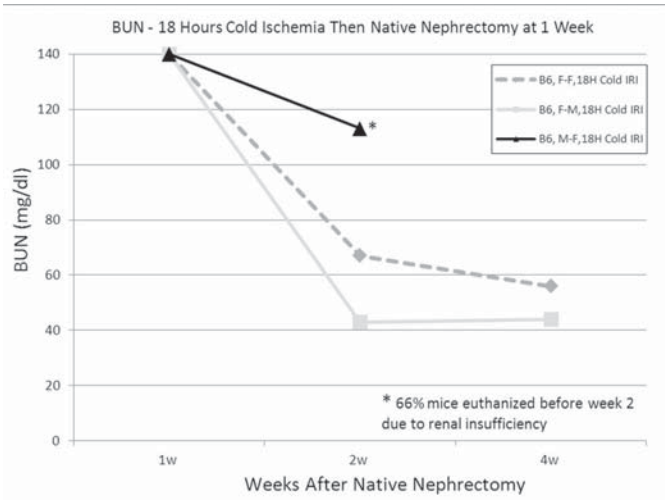
TH-PO167

Gender Differences in Renal Ischemia Tolerance Are Mutable and Based on Reperfusion Environment Matthew H. Levine,[1] Zhonglin Wang,[1] Shayan Cheraghlou,[1] Liqing Wang,[2] Tricia Bhatti,[2] Wayne W. Hancock.[2] *[1]Surgery, Univ of Pennsylvania, Philadelphia, PA; [2]Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: It is known that female mice have greater renal ischemia reperfusion injury (IRI) than males but the mutability of these differences and whether the environment at ischemia or reperfusion is unknown. We here utilize several models of transplantation to assess these factors.

Methods: A murine renal transplant model followed by native nephrectomy and subsequent 25 mins of warm IRI on the transplanted kidney was used in c57/b6 mice with readouts including daily BUN/creatinine for 4d after injury and automated Sirius Red fibrosis score at 30d after IRI. A cold ischemia model involving 18h of cold storage in UW solution followed by transplantation was used to separate donor and recipient gender effects.

Results: At baseline, B6 males tolerated approximately half the ischemic time of females to yield equivalent IRI. Female recipients of either gender kidney had superior tolerance to subsequent IRI than male recipients of either gender kidney, indicating that the IRI tolerancecan be altered by the local environment. Utilizing a cold ischemia transplant model, female donor kidneys yielded superior IRI tolerance regardless of recipient gender, compared to male donor kidneys (Fig 1).



This indicates that the gender environment at the time of ischemia is more important than at the time of reperfusion in determining IRI tolerance.

Conclusions: Syngeneic mouse gender can alter IRI tolerance significantly, indicating a role for hormonal manipulation. IRI tolerance of male kidneys can be made equal to female after transplantation to a female environment, proving mutability of the process. The cold ischemia data indicate that treatment at the time of ischemia (ie in the donor) is likely to yield maximal benefit.

Funding: NIDDK Support

TH-PO168

Recombinant Alkaline Phosphatase Ameliorates Creatinine Clearance and Glomerular Filtration Rate in Rat Ischemia-Reperfusion-Induced Acute Kidney Injury: An Intravital Microscopy Study into the Mode-of-Action Andrea van Elsas,[1] Ruben M. Sandoval,[2] Silvia B. Campos-Bilderback,[2] Bruce A. Molitoris.[2] *[1]AM-Pharma, Bunnik, Netherlands; [2]Indiana Univ School of Medicine, Indianapolis, IN.*

Background: In two clinical studies bovine purified intestinal Alkaline Phosphatase (AP) displayed intriguing activity in sepsis-associated acute kidney injury (AKI) (Heemskerk et al., 2009. CCM 37: 417; Pickkers et al., 2012. CC 23:R14). A human recombinant AP was designed by replacing the crown domain from intestinal AP with that derived from placental AP to obtain a stable highly active chimeric enzyme called 'recAP' (Kiffer-Moreira et al., 2014. PLOS One 9:e89374).

Methods: To gain further understanding of its mode-of-action, recAP was used to treat ischemia-reperfusion (I/R)-induced AKI in rats, RecAP was dosed immediately following nephrectomy and 30' clamping of the blood supply to the remaining kidney. Kidney function was determined after 24 h by plasma creatinine (PCr) and by ratiometric GFR quantification by two-photon microscopy using rhodamine-dextran (~150 kDa) and FITC-dextran (~5 kDa). Time-lapse video recording was used to determine RBC and WBC flow and vascular endothelial adhesion in the injured kidney.

Results: A significant rise in PCr developed from ~0.4 mg/dL in control nephrectomized SD rats to 3.2 ± 0.59 mg/dL after I/R (n=6). Single dose i.v. administration of recAP (50-2000 U/kg) significantly reduced PCr (1.7 ± 0.65 mg/dl at 500 U/kg) (p<0.01). In Munich-Wistar Frömter rats, PCr was 0.38 ± 0.04 mg/dL at t=0 rising to 2.28 ± 0.64 mg/dL 24 h post-injury (control). A single dose of 1000 U/kg recAP limited PCr to 0.82 ± 0.15 mg/dL. At 24 h post injury, control AKI rats demonstrated a ratiometric GFR of 0.09 ± 0.09 ml/min, compared to 0.75± 0.29 ml/min (p<0.005) in recAP treated rats. RecAP did not appear to alter RBC and WBC flow or adherence to vascular endothelium.

Conclusions: In conclusion, recAP demonstrated significant efficacy in the treatment of rat I/R-induced AKI maintaining GFR and plasma creatinine close to normal levels. The mode-of-action of recAP in this model of AKI is under further investigation.

Funding: Pharmaceutical Company Support - AM Pharma

TH-PO169

Human Recombinant Alkaline Phosphatase Protects against Lipopolysaccharide-Induced Renal Inflammation Esther Peters,[1,2] Norbert Gretz,[3] Peter Pickkers,[1] Rosalinde Masereeuw.[2] *[1]Intensive Care Medicine, Radboudumc, Nijmegen, Netherlands; [2]Pharmacology and Toxicology, Radboudumc, Nijmegen, Netherlands; [3]Medical Research Centre, Medical Faculty Mannheim of Univ of Heidelberg, Mannheim, Germany.*

Background: Two phase-II trials showed improved renal function in critically ill patients with sepsis-induced AKI after treatment with the enzyme alkaline phosphatase (AP). Here we investigated the protective mechanism in vitro and in vivo, possibly related to dephosphorylation of lipopolysaccharide (LPS).

Methods: Human proximal tubular epithelial cells (ciPTEC) were incubated with LPS (10µg/ml). Human recombinant AP (10U/ml) was added 2h prior to LPS and cytokine production was studied after 24h by ELISA (n=5 per group). Male SD rats were treated with placebo (n=6), LPS (n=6) or LPS+AP (n=5). At t=0, LPS (IV, 0.3mg/kg) was administered to induce AKI, followed by an AP bolus (IV, 1000U/kg) at t=2h. GFR was assessed by transcutaneous measurement of FITC-sinistrin in freely moving awake rats. Urine was collected during 16h. At t=24h animals were sacrificed followed by blood and organ sampling.

Results: In ciPTEC, AP significantly reduced LPS induced cytokine response of TNF-α (40.4±7.9%), IL-6 (47.5±3.1%) and IL-8 (39.6±2.4%). Inactive AP had no effect on LPS-induced cytokine response, confirming the relevance of dephosphorylation. *In vivo,* LPS significantly prolonged FITC-sinistrin half-life (placebo: 20.7±1.6min, LPS: 54.6±8.9min) and decreased creatinine clearance (placebo: 3.1±0.3ml/min, LPS:2.3±0.2ml/min), for which AP treated animals were protected (half-life: 37.0±5.7min; creatinine clearance: 2.7±0.2ml/min). Also, AP prevented LPS-induced increase in fractional urea and KIM-1 excretion and renal KIM-1 expression.

Conclusions: Our data show a renal protective effect of AP in LPS-induced inflammation. Cell studies showed that the dephosphorylating property of AP is responsible for this effect. The ability of AP to reduce renal inflammation may account for the observed attenuated acute kidney injury in sepsis patients.

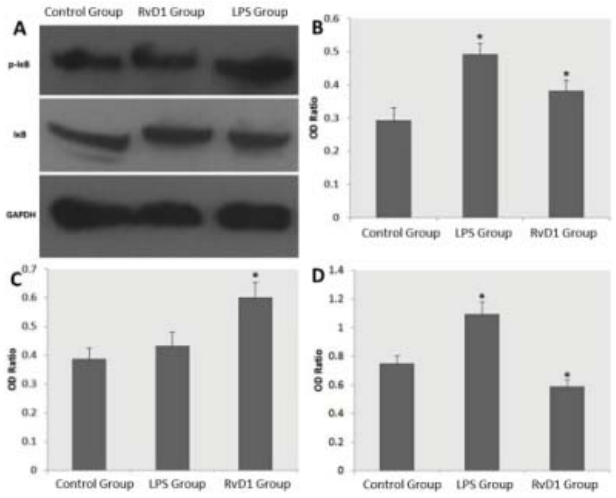
TH-PO170

Resolvin D1 Protects Lipopolysaccharide-Related Acute Kidney Injury by Downregulating NF-κB Signaling Pathway Yuliang Zhao,[1] Ling Zhang,[2] Ping Fu.[3] *[1]Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China; [2]Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China; [3]Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan Province, China.*

Background: Resolvin D1 (RvD1), a newly found bioactive compound derived from DHA, has been described to promote inflammation resolution in various inflammatory disease models. The current study aims to explore the protection of RvD1 on lipopolysaccharide (LPS)-related AKI and its possible mechanism, so as to provide potential insights into therapeutic targets of septic AKI.

Methods: Male BALB/c mice and human proximal tubule epithelial (HK-2) cells were randomly divided into control group (saline), LPS group (LPS) and RvD1 group (RvD1+LPS). The drugs were intraperitoneally injected or added into medium. The animals' general condition were recorded. The blood, kidneys and HK-2 cells were harvested at different time points.

Results: We recorded the distribution of lipoxin A4 receptor (ALX, RvD1 receptors) on tubular epithelial. Kaplan-Meier analysis indicated that RvD1 improved 48h animal survival compared with LPS group, while RvD1 also ameliorated kidney pathological injury under HE staining and TEM scan. The blood creatinine and TNF-α level of LPS group were significantly increased, however RvD1 prohibited the trend. Quantitative PCR indicated after LPS stimulation, the mRNA expression of TLR4, MyD88 and TNF-α in both mice kidneys and HK-2 cells were all up-regulated, while RvD1 substantially inhibited the up-regulation of these genes. Western blot showed the p-IκB/IκB ratio in LPS group was significantly higher than the control group, while being inhibited in RvD1 group.



Conclusions: In LPS-related AKI, RvD1 could decrease TNF-α level, ameliorate kidney pathological injury, protect kidney function and improve animal survival by down-regulating NF-κB signaling pathway.

TH-PO171

Klotho Deficiency Aggravates Kidney Injury and Central Autonomic Modulation in Sepsis Lectícia Jorge,[1] Fernanda O. Coelho,[1] Fernando Santos,[2] Makoto Kuroo,[3] Lucia Andrade.[1] *[1]Nephrology, Univ of Sao Paulo, SP, Brazil; [2]Heart Inst, Univ of Sao Paulo, SP, Brazil; [3]Jichi Medical Univ, Tochigi, Japan.*

Background: Sepsis may be associated with a loss in cardiovascular variability and a heart sympathetic modulation impairment, suggesting that central autonomic regulatory dysfunction contributes to circulatory failure. Last year, we have demonstrated that Klotho(Kl) deficiency aggravates sepsis-related multiple organ dysfunction. The aim of this study was to evaluate the relationship among cardiovascular autonomic modulation, renal function and Kl deficiency, during sepsis.

Methods: 8-12 week old male Kl+/- and Kl+/+ mice (n=32) underwent CLP or sham operation. The mean arterial pressure (MAP) and heart rate (HR) were recorded 24 h post-surgery. Overall variability for high- and low-frequency components (HF, and LF, ms^2/Hz or mm Hg^2/Hz) from heart rate (HR) and systolic (SBP) blood pressures spectra were obtained from 60-min recordings. LF(HR)/HF(HR) and the square root of LF(HR)/LF(SBP) (alpha) were used as indices of sympathovagal interaction and baroreflex control of the heart, respectively. We also measured renal function (urea, mg/dl) at 24h after surgery.

Results: At 24h post-CLP, MAP decreased in both CLP groups compared with Sham groups (Kl+/Kl- CLP: 125±17 versus Kl+/Kl- Sham: 141±10 versus Kl+/Kl+ CLP: 116±23 versus Kl+/Kl+ Sham: 135±18, $p<0.05$). However, variability was significantly decreased in Kl+/Kl- CLP than in Kl+/Kl+ CLP (27±13 versus 77±39), as was LF power (1.8±1.5 versus 9±10), LF(HR)/HF(HR) (0.26±0.29 versus 0.57±0.5), LF(HR)/LF(SBP) (alpha) (0.6±0.38 versus 1.52±0.84). Urea was increased in Kl+/Kl- CLP group compared with Kl+/Kl+ CLP group (77±14 versus 54±4, $p<0.05$).After 24h, Kl+/Kl- CLP remained with an important impairment of autonomic control of the heart and baroreflex sensitivity, while, in Kl+/Kl+ CLP, those were recovered.

Conclusions: These results suggest that, in Kl deficiency, the decrease in central autonomic regulation contributes to circulatory failure and acute kidney injury.

Funding: Government Support - Non-U.S.

TH-PO172

Nrf2 Activation Protects against Sepsis-Induced Acute Kidney Injury (AKI) Andrea P. Soljancic,[1] Arnaldo F. Lopez-Ruiz,[1] Kiran B. Chandrashekar,[1] Richard L. Ogletree,[2] Ruisheng Liu,[3] Luis A. Juncos.[1] *[1]Medicine/Nephrology, Univ of Mississippi Medical Center, Jackson, MS; [2]Pharmacology, Univ of Mississippi Medical Center, Jackson, MS; [3]Physiology, Univ of Mississippi Medical Center, Jackson, MS.*

Background: Sepsis is a systemic response to an infection that often leads to AKI. Sepsis-induced AKI is mediated by increased oxidative stress, inflammation and renal vasoconstriction. Activation of the Nrf2 pathway is an endogenous cytoprotective mechanism induced by sepsis. Nrf2 pathway upregulates antioxidant, anti-inflammatory and cytoprotective enzymes such as heme oxygenase-1 (HO-1). Bardoxolone (Bx) is a synthetic activator of Nrf2 pathway that has shown to protects against renal diseases. We tested if enhanced activation of the Nrf2 pathway using Bx protects against sepsis-induced AKI by inducing HO-1.

Methods: Sepsis was induced in SD rats by cecal-ligation-puncture (CLP). Four groups of rats were used; 1) sepsis control (CLP), 2) CLP + SnPP (HO-1 inhibitor), 3) CLP + Bx, 4) CLP + Bx + SnPP. At 24 hs plasma creatinine was measured and rats were then sacrificed. Kidneys were harvested to evaluate the expression of HO-1 and TNFα by Elisa. Urine was collected to evaluate prox. tubular injury (KIM-1).

Results:

	PL. Creatinine (mg/dl)	TNFα (pg/ml)	KIM-1 (pg/ml)	HO-1 (ng/ml)
CLP	1.97±0.1	219±7.7	390.2±15.8	11.07±0.5
CLP+SnPP	2.4±0.03*	322.5±7.4*	1079.8±47.4*	4.3±0.3*
CLP+Bx	1.3±0.1*	84.1±4.2*	224.2±9.8*	17.9±0.6*
CLP+Bx+ SnPP	1.5±0.1*	110.5±5.2*	272.6±12.8*	10.9±0.6#

Mean±SEM: *p<0.05 vs CLP; # p<0.05 vs CLP+Bx

Conclusions: CLP contributes to tubular injury (KIM-1) and activation of pro-inflammatory signals (TNFα) that are exacerbated during HO-1 inhibition. Bx further upregulates intrarenal HO-1, reduces tubular injury and improve renal function. HO-1 inhibition does not attenuate the protective effect of Bx. In conclusion, exogenous activation of Nrf2 pathway upregulates renal HO-1 and confers protection against sepsis-induced AKI; however this protective effect was not blocked by SnPP suggesting that the protective effect may not be mediated by HO-1.

Funding: Other NIH Support - NIH DK073401

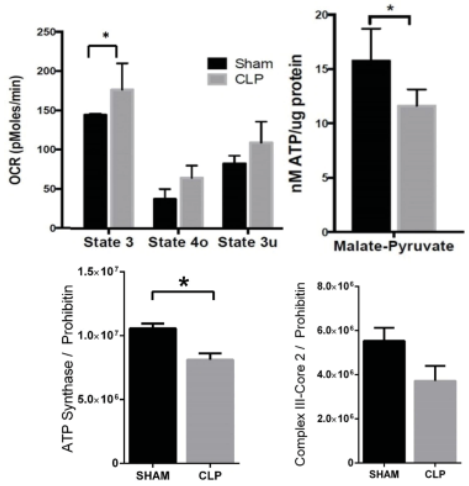
TH-PO173

Role of AMPK in Mitochondrial Dysfunction in Sepsis-Induced AKI Noureddin D. Nourbakhsh, Joanna Thomas, Prabhleen Singh. *Medicine-Nephrology, UC San Diego and VASDHS.*

Background: Sepsis-associated AKI is frequently observed and has poor prognosis. The lack of complete understanding of its pathogenesis is a significant barrier to progress and fresh insights into its pathogenesis are critically needed.

Methods: We examined renal function, mitochondrial oxygen consumption, ATP generation and other functional and molecular analyses to examine mitochondrial dysfunction in the pathogenesis of sepsis using a model of cecal ligation and puncture (CLP) at 24 hours post-injury. We also evaluated therapies targeting mitochondrial dysfunction. Data presented as mean±sem.

Results: GFR was significantly reduced in CLP mice 345±19 versus 155±35 uL/min; p=0.004. HIF expression was significantly increased in the CLP kidneys demonstrating hypoxia. Mitochondria from CLP animals exhibited elevated rates of both coupled and uncoupled respiration, but ATP generation measured in the same samples was significantly lower, along with decreased expression of complex III and complex IV.



CLP mitochondria also demonstrated increased fission (DRP-1 expression), decreased biogenesis (PGC-1a expression) and reduced AMPK expression. AMPK activation with AICAR significantly improved GFR post CLP compared to untreated mice (267±59 versus 155±35 uL/min, p=0.02).

Conclusions: Renal mitochondria in the setting of sepsis demonstrate dissociation between oxygen consumption and ATP generation with increased fission and reduced biogenesis. Lower AMPK levels may be the primary event leading to mitochondrial dysfunction. Specific downstream pathways including biogenesis, fission-fusion balance and autophagy to explain mitochondrial dysfunction and response to AMPK activation are being examined.

Funding: NIDDK Support

TH-PO174

Mitochondrial DNA Contributes to Sepsis Induced Acute Kidney Injury via Toll-Like Receptor 9 Naoko Tsuji,[1] Hideo Yasuda,[1] Takayuki Tsuji,[1] Naro Ohashi,[1] Akihiko Kato,[2] Yoshihide Fujigaki.[3] *[1]1st Dept of Medicine, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Blood Purification Unit, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [3]Dept of Medicine, Teikyo Univ, Tokyo, Japan.*

Background: Inhibition of Toll-like Receptor (TLR) 9, whose ligand has been recently revealed to be not only bacterial DNA but also endogenous mitochondrial DNA (mtDNA), prevents septic acute kidney injury (AKI). We investigated roles of mtDNA for septic AKI.

Methods: Polymicrobial sepsis was induced in C57BL/6 male mice and TLR9 knockout (KO) mice by cecal ligation and puncture (CLP). The blood and kidney tissue were collected for quantitative analysis of mtDNA by PCR methods and renal injury at 2, 6 and 24 h after CLP. The intravenous mitochondrial debris (MTD) including mtDNAwas given to TLR9 KO and wild type mice. The renal expressions of neutrophil gelatinase-associated lipocalin (NGAL) by immunohistochemistry and western blot and serum creatinine (sCr) levels were assessed as renal injury and function markers respectively.

Results: The renal expressions of NGAL increased in proximal tubules at 2h and sCr levels increased at 24 h after CLP. (24h: sCr 0.41±0.05 versus 0.08±0.01 mg/dl, P<0.01). Plasma mtDNA levels increased at 2 and 6 h after CLP (2h: 4.5±1.8 versus 0.4±0.1 $\times 10^5$ copy/μl, p<0.05, 6h: 4.6±1.8 versus 0.5±0.1 $\times 10^5$ copy/μl, p<0.05, 24h: 2.0±0.9 versus 0.8±0.2 $\times 10^5$ copy/μl, p=0.20). TLR9 KO reduced renal NGAL expressions at 2h and sCr levels at 24 after CLP (sCr: 0.38±0.05 versus 0.17±0.02 mg/dl, p<0.01). MTD injection increased renal expressions of NGAL at 2 h, which was reduced by TLR9 KO.

Conclusions: Endogenous mtDNA entered into circulation of the blood at early phase after CLP and might activate TLR9 cascade, which contributed to development of AKI.

TH-PO175

TLR4-Dependent MEK/ERK Activation Suppresses Mitochondrial Biogenesis (MB) in a Mouse Model of Sepsis-Induced Acute Kidney Injury (AKI) Joshua Andrew Smith, L. Jay Stallons, Justin B. Collier, Rick G. Schnellmann. *Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC.*

Background: Sepsis is the most common contributing factor to the development of AKI. Recent evidence indicates that disruption of mitochondrial homeostasis and biogenesis is an important cause of renal pathology in sepsis. We investigated the molecular mechanisms responsible for suppression of MB following endotoxin-induced AKI in mice.

Methods: AKI was induced by injecting male wild-type and TLR4-deficient mice with lipopolysaccharide (LPS; 10 mg/kg). A separate group of wild-type mice were treated with the MEK1/2 inhibitor Trametinib (1 mg/kg) 1 h prior to LPS. Serum and kidneys were harvested at 1, 3 and 18 h post-LPS. Renal function was determined by BUN. Renal mitochondrial homeostasis and biogenesis were assessed by RT-qPCR and immunoblot analysis of PGC-1α, its downstream targets, and nuclear- and mitochondrial-encoded components of the electron transport chain (ETC). Mitochondrial DNA content was measured by qPCR.

Results: Renal dysfunction was observed at 3 and 18 h post-LPS as demonstrated by increased BUN. PGC-1α transcript levels were decreased as early as 3 h after LPS exposure and remained suppressed at 18 h. mRNA expression of downstream targets of PGC-1α (NRF-1, TFAM, NDUFS1) as well as ETC components (NDUFB8, ATPSβ, and COXI) and mitochondrial DNA content was decreased at 18 h. TLR4-deficient mice were protected from LPS-induced renal dysfunction and suppression of MB. Immunoblot analysis demonstrated activation of ERK1/2 at 1 and 3 h post-LPS. Renal injury and loss of PGC-1α mRNA expression following LPS administration were attenuated by inhibition of MEK/ERK signaling.

Conclusions: Mitochondrial homeostasis is disrupted in a mouse model of sepsis-induced AKI. Our data also reveal an essential role for TLR4/MEK/ERK signaling in suppression of renal MB in this model. We propose that the TLR4/MEK/ERK signaling pathway is a novel therapeutic target to reverse mitochondrial dysfunction following AKI.

Funding: Other NIH Support - 5R01GM084147-05, Veterans Affairs Support

TH-PO176

Influence of Lipopolysaccharides (LPS) on Neurogenic and Paracrine Function of Neurons with Renal Afferents Kristina Rodionova,[2] Stefan Karl,[2] Tilmann Ditting,[2] Wolfgang Freisinger,[2] Peter Linz,[2] Peter Reeh,[3] Kerstin U. Amann,[1] Roland Veelken.[2] *[1]Pathology, Universitätsklinikum Erlangen, Erlangen, Germany; [2]Nephrology, Universitätsklinikum Erlangen, Erlangen, Germany; [3]Physiology, Universitätsklinikum Erlangen, Erlangen, Germany.*

Background: Renal afferent nerves exert sympathomodulatory and paracrine effects (e.g. release of vasodilator CGRP) on kidney and cardiovascular system. Recently we demonstrated that inflammatory mediator CXCLR1 decreased the ability of cultured renal afferent neurons to generate sustained action potential (AP) firing upon electrical stimulation. Hence we tested the hypothesis that mediator of septic shock – LPS – likewise decreases number of renal neurons exhibiting sustained AP firing upon current injection.

Methods: Dorsal root ganglion neurons (Th11-L2) of rats were incubated with LPS (E.coli O127/B8, 20mg/l medium) 12 hours before patch clamp recording. Current clamp mode was used to characterize neurons as "tonic", i.e. sustained AP firing or "phasic", i.e. <5 APs in response to current injection. In voltage clamp mode, inward currents were assessed during stimulation of TRPV1 receptors with protons. In organ bath of kidney slices – incubated with or without LPS - we stimulated TRPV1 receptors with Capsaicin and measured CGRP content in organ bath supernatant (ELISA).

Results: Renal afferent neurons exhibited in 42% tonic firing pattern. Number of neurons with tonic response pattern was not altered by exposure to LPS. However, LPS exposure significantly increased inward currents due to TRPV1 receptor stimulation with protons (LPS: -3.62±0.41 pA/pF, control: -1.7±0.48 pA/pA, *p<0.05). CGRP release due to TRPV1 stimulation was also significantly increased. (LPS-incubation: 58±20 pg/ml, control: 14±2, *p<0.05).

Conclusions: LPS exposure did not decrease number of tonic afferent renal neurons as previously described for CXCL1, but increased inward currents upon TRPV1 receptor stimulation with protons. Furthermore, LPS sensitizes TRPV1 receptors to induce increased release of CGRP from afferent axons in kidney. Hence, renal afferent nerves in septic shock are rather likely to influence renal perfusion by CGRP release than sympathetic nerve activity by altered afferent nerve traffic.

TH-PO177

Reduction of Tubular Flow Rate as a Mechanism of Oliguria in Endotoxemia Revealed by Intravital Imaging Daisuke Nakano,[1] Kent Doi,[2] Takashige Kuwabara,[3] Kiyoshi Mori,[3,4] Masashi Mukoyama,[3] Akira Nishiyama.[1] *[1]Dept of Pharmacology, Kagawa Univ, Kagawa, Japan; [2]Dept of Emergency and Critical Care Medicine, Univ Hospital, The Univ of Tokyo, Tokyo, Japan; [3]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [4]Medical Innovation Center, Kyoto Univ Graduate School of Medicine, Kyoto, Japan.*

Background: Urine output is widely used as a criterion for acute kidney injury (AKI) diagnosis. Although a number of investigations have identified potential mechanisms of septic AKI, regulation of urine flow post-glomerular filtration has not been evaluated. The aim of this study was to evaluate changes of urine flow in septic AKI by intravital imaging to enable real-time monitoring of intratubular urine flow rate.

Methods: Intravital imaging was performed by using 2-photon laser microscopy. Urine flow rate was estimated by the timing of the appearance of injected dyes in distal nephron segments.

Results: The tubular flow rate measured by freely filtered dye (FITC-inulin or Lucifer yellow) time-dependently declined after lipopolysaccharide (LPS) injection; it was already slower at 2 h in LPS-injected mice compared with saline-injected mice (saline: 38±3 s, LPS 2 h: 67±8 s, LPS 6 h: 125±17 s, n=5-8, p<0.05), while the blood pressure and GFR were normal. Importantly, fluorophore-conjugated LPS accumulated in the proximal tubules, selectively retaining the tubular fluid and inducing luminal obstruction owing to cell swelling. Delipidation of LPS or deletion of Toll-Like receptor 4 in mice abolished these effects (49±4 and 42±3 s, at 6 h, respectively). In contrast, neutralizing TNF-α showed little effect on LPS-induced tubular flow retention (112±20 s, at 6 h). Rapid intravenous fluid resuscitation started within 6 h could improve the tubular flow rate only when it was accompanied by the dilation of obstructed proximal tubules exhibiting LPS-accumulation.

Conclusions: The present findings suggest that LPS-induced signaling via TLR4 in the proximal tubules plays an important role in the reduction of urine flow in the early phase of endotoxemia, and that the efficacy of fluid resuscitation may depend on the response in LPS-accumulated tubules.

Funding: Government Support - Non-U.S.

TH-PO178

Removal of LPS Binding Protein (LBP) Prevents Endothelial Dysfunction and Renal Fibrosis in Endotoxiemia-Induced Acute Kidney Injury Giuseppe Castellano,[1] Alessandra Stasi,[1] Angelica Intini,[1] Margherita Gigante,[1] Anna Maria Di Palma,[1] C. Divella,[1] Giuseppe Stefano Netti,[2] Clelia Prattichizzo,[2] Paola Pontrelli,[1] Enrico Fiaccadori,[3] Nicola Brienza,[1] G. Grandaliano,[2] Giovanni B. Pertosa,[1] Loreto Gesualdo.[1] *[1]Dept of Emergency and Organ Transplantation, Univ of Bari; [2]Dept of Medical and Surgical Sciences, Univ of Foggia; [3]Dept of Clinical and Experimental Medicine, Univ of Parma.*

Background: The pathophysiology of LPS-induced AKI is characterized by an intense activation of renal resident cells by endotoxin and derived pro-inflammatory products. The occurrence of renal fibrosis has been poorly investigated. Aim of the present study is to evaluate the association between endothelial dysfunction and acute fibrosis and the possible effects of coupled plasma filtration adsorption (CPFA) in this setting.

Methods: After 3h from LPS infusion, 8 pigs were treated with CPFA for 6h; 8 control pigs receive no treatment. Renal biopsies were performed before (T0) and 9h (T9) after LPS infusion. LBP levels were quantified in sera by ELISA. Endothelial cells (EC) were cultured in presence ofswine sera for 12h and were analyzed by FACS and RT-PCR.

Results: In a swine model of LPS-induced AKI, we found that CPFA significantly prevented fibrosis in septic pigs (Masson's Thricrome, p=0.04) by preserving EC phenotype in peritubular capillaries and renal arteries (α-SMA^{pos}EC 5.58±0.52 versus T9LPS:18.10±1.58, p=0.0002). The removal of LBP from endotoxemic sera (0.21±0.03ug/ml versus LPST9:9.6±0.69, p=0.0001) was critical to abrogate the effects of LPS on EC dysfunction in vitro, by blocking LPS-induced collagen I production (normalized mRNA expression, LPS CPFA sera:1.03±0.28 versus LPS sera: 4.17±0.72, p=0.03) and preserving EC phenotype (%CD31^{pos}EC, LPS CPFA sera:94.96±3.4 versus LPS sera:49±9.05, p=0.02). The addition of exogenous LBP in LPS CPFA sera induced EC dysfunction (CD31:62.83±6.09; collagen I:3.29±0.61) like the untreated endotoxemic animals sera.

Conclusions: Selective removal of the LPS adaptor proteins LBP might represent a therapeutic option to prevent EC dysfunction and tissue fibrosis in LPS-induced AKI.

TH-PO179

Endotoxemia-Induced Decrease in GFR Is Mediated in Part by COX-1 Derived Prostanoids in Mice Klaus Höcherl. *Dept of Pharmacology and Toxicology, Friedrich-Alexander Univ Erlangen-Nürnberg (FAU), Erlangen, Germany.*

Background: Thromboxane (Tx) A_2 has been suggested to be involved in the development of sepsis-induced acute kidney injury (AKI). Therefore, we investigated the impact of cyclooxygenase (COX)-1 and COX-2 activity on lipopolysaccharide (LPS)-induced renal TxA_2 formation, and on endotoxemia-induced AKI in mice.

Methods: Male C57BL/6 mice, 8-10 wk old, were used. Endotoxemia was induced by the injection of lipopolysaccharide (LPS; 3 mg/kg; i.p.). TxA_2 levels were measured by assay kits. Glomerular filtration rate (GFR) was determined by FITC-sinistrin clearance in conscious mice. Pimonidazole (60 mg/kg) was used to investigate renal hypoxia. Renal vascular permeability was measured by using Evans blue dye.

Results: Injection of LPS (3 mg/kg; i.p.) decreased glomerular filtration rate (GFR) and the amount of thrombocytes to about 50% of basal values after four hours. Plasma and renocortical tissue levels of TxB_2 were increased about 10- and 1.7-fold in response to LPS, respectively. The COX-1 inhibitor SC-560 attenuated the LPS-induced fall in GFR and in platelet count to about 75% of basal levels. Further, SC-560 abolished the increase in plasma and renocortical tissue levels of TxB_2 in response to LPS. The COX-2 inhibitor SC-236 further enhanced the LPS-induced decrease in GFR to about 40% of basal values. SC-236 did not alter thrombocyte levels nor the LPS-induced increase in plasma and renocortical tissue levels of TxB_2. Pretreatment with Clopidogrel inhibited the LPS-induced drop in thrombocyte count, but did not attenuate the LPS-induced decrease in GFR and the increase in plasma TxB_2 levels. In addition, SC-560 reversed LPS-induced vascular leakage and renal tubular hypoxia.

Conclusions: This study demonstrates that inhibition of COX-1 improves endotoxemia-induced AKI. Our data further suggest that vascular- rather than platelet-derived thromboxane could be responsible for the fall in GFR in response to LPS.

Funding: Government Support - Non-U.S.

TH-PO180

Hydrogen Sulfide Accelerates Recovery of Kidney after Ischemia/Reperfusion Injury Sang Jun Han,[1] Jee In Kim,[2] Kwon Moo Park.[1] *[1]Dept Anatomy and BK21 Plus, Kyungpook National Univ School of Medicine, Daegu, Republic of Korea; [2]Dept Molecular Medicine and MRC, Keimyung Univ, Daegu, Republic of Korea.*

Background: Hydrogen sulfide (H_2S), a novel biological gas, plays a protective role in ischemia/reperfusion (I/R)-induced acute kidney injury. However, the role of H_2S in the repair process after kidney injury remains to be defined. Here, we investigated the role of H_2S in recovery of kidney function and tubular epithelial cells following ischemia/reperfusion (I/R) injury.

Methods: Mice were subjected to 30 min of bilateral renal ischemia. Some mice were administered with NaHS, an exogenous H_2S donor, and propargylglycine (PAG), an inhibitor of cystathionine gamma lyase (CSE) which is a H_2S producing enzyme, beginning at 2 day after ischemia until sacrifice daily.

Results: I/R resulted in severe tubular cell damage and functional loss in the kidney as evaluated by PAS staining and plasma creatinine (PCr) concentration. Eight days after ischemia, the kidney was functionally recovered with a partial restoration of damaged tubules. I/R reduced expression level and activity of CSE and cystathionine beta synthase (CBS). The reduced activity and expression levels were gradually recovered over time. Administration of NaHS accelerated return to normal level of PCr, whereas administration of PAG delayed that. Furthermore PAG treatment increased mouse mortality after I/R. Administration of NaHS accelerated proliferation of tubular epithelial cells as evaluated by 5-Bromo-2′-Deoxyuridine (BrdU) incorporation whereas PAG delayed that.

Conclusions: Our findings demonstrate that H_2S accelerated recovery of kidney tubule cells following I/R by a increase in proliferation of tubular cells, suggesting that H_2S could be a therapeutic target for recovery after acute kidney injury.

TH-PO181

Indole Derivatives Are Renoprotective in Acute Kidney Injury Takehiro Suzuki,[1] Takaharu Ichimura,[2] Hisato Shima,[2] Yoichi Takeuchi,[1] Eikan Mishima,[1] Yasutoshi Akiyama,[2] Chitose Suzuki,[2] Sadayoshi Ito,[2] Takaaki Abe,[2] Joseph V. Bonventre.[1] *[1]Brigham and Women's Hospital, Boston, MA; [2]Tohoku Univ, Sendai, Miyagi, Japan.*

Background: In patients with chronic kidney disease, the uremic toxins may promote renal damage and anemia. We analyzed the effects of uremic solutes on erythropoietin (Epo) production in Hep3B cells and identified indole compounds that enhanced Epo-production. These indoles also had protective effects on HK2 cells against cytotoxic agents. The aim of this study was to clarify the effects of the indoles in kidney injury and to identify new drugs to prevent renal disease progression.

Methods: Forty-one indole compounds were synthesized and screened by monitoring Epo-production in Hep3B cells. Cytoprotective effects were examined by cell viability in HK2 cells treated with cisplatin or aristolochic acid. The most potent compound (#5) was administrated, at 50mg/kg body weight by gavage, to mice 3 hr before they were exposed to kidney ischemia (26 min) reperfusion injury (IRI) or cisplatin (20 mg/kg body weight by intraperitoneal injection). Plasma creatinine (Cr), blood urea nitrogen (BUN), renal pathology and Epo mRNA expression in kidney were assessed.

Results: Indole compound #5 (Indole #5) was most potent in Epo-production and cytoprotection. In IRI, Cr at 48 hr after ischemia was significantly reduced in the Indole #5-treated group (0.88 + 0.38 mg/dl versus 1.60 + 0.66, control.). Indole #5 treatment resulted in reduced tubular cell injury on histological examination, reduced expression of the acute kidney injury biomarker, KIM-1, and increased numbers of Ki67 positive cells. BUN, at 96 hr after cisplatin injection, was significantly reduced in Indole #5 treated groups (70.9 + 10.1 versus 101 + 13.0 mg/dl). Tubular cell injury and KIM-1 expression were reduced in the Indole #5-treated group. Epo expression in the kidney was not significantly different between Indole #5-treated and control groups.

Conclusions: Indole #5 has renoprotective effects in both IRI and cisplatin nephrotoxicity. Indole derivatives may be effective kidney protective therapeutic agents.

Funding: Government Support - Non-U.S.

TH-PO182

Thioredoxin-Interacting Protein (TXNIP) Is Up-Regulated and Regulates the Mitochondrial Function, NOX4, and Apoptosis of Renal Tubular Cells in Acute Kidney Injury In Vitro and In Vivo Yoshio Terada, Haruna Ide, Tatsuki Matsumoto, Kazu Hamada, Yoshiko Shimamura, Koji Ogata, Kosuke Inoue, Yoshinori Taniguchi, Taro Horino, Shinpei Fujimoto. *Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Nankoku, Kochi, Japan.*

Background: Thioredoxin-interacting protein (TXNIP) has been found to regulate the cellular reduction-oxidation (redox) state by binding to and inhibiting thioredoxin in a redox-dependent fashion. However, little is known about the role of TXNIP in acute kidney injury (AKI) pathogenesis.

Methods: We used an in vivo rat ischemia/reperfusion AKI model and cultured renal tubular cells as an in vitro model. To elucidate the regulation of TXNIP, we evaluated the promoter activity and expression of TXNIP in NRK-52E cells in the presence of H_2O_2. We also evaluated mitochondrial enzymes, morphology, and apoptotic change in overexpression and siRNA of TXNIP.

Results: Immunohistological examination showed the expression of TXNIP in the proximal tubule cells after AKI. RT-PCR and western blot analysis showed that TXNIP mRNA and protein expression were up-regulated 6–48 h and 12–48 h after ischemia/reperfusion. TXNIP promoter activity, mRNA, and protein expression showed a dose-dependent induction by H_2O_2. Overexpression of TXNIP stimulated infammasome (ASC, IL-1β) and caspase3 activity, and caused apoptosis (TUNEL positive) in NRK-52E cells. Furthermore, inflammasome activation and apoptosis induced by H_2O_2 (400 μM) was inhibited by siRNAs for TXNIP. Protein and mRNA levels of ATP5a, complex(COX) IV, UCP2, PGC-1α in NRK-52E cells were significantly decreased by H_2O_2, and siRNA for TXNIP ameliorates reduction of these enzymes. NOX(NADPH oxidase)4 and oxidative stress marker (CellROX) was up-regulated by overexpression of TXNIP and down-regulated by siRNA of TXNIP.

Conclusions: We showed that TXNIP expression increased during oxidative stress in AKI. Incremental TXNIP expression induced NOX4 expression, oxidative stress, mitochondrial damage, and caused apoptosis in renal tubular cells. These results indicate that up-regulated TXNIP plays a key role in the pathophysiology of AKI.

Funding: Government Support - Non-U.S.

TH-PO183

Protective Effect of Allopurinol on Myoglobinuric Acute Kidney Injury Pedro H.F. Gois, Daniele Canale, Weverton M. Luchi, Rildo A. Volpini, Daniela Ferreira, Antonio C. Seguro, Maria H.M. Shimizu. *Univ of São Paulo.*

Background: Myoglobinuric acute kidney injury (AKI) is the most severe complication of rhabdomyolysis.**Objective:**To evaluate the efficacy of allopurinol (Allo) in protecting against glycerol-induced AKI.

Methods: Male wistar rats (258±5g) were injected intramuscularly with 5ml/Kg BW of either 50% glycerol(Gly) or 0.9% saline(SF). Five groups were studied:SF(n=5), SF+Allo(n=6), Gly(n=7), Gly+Allo(n=7), Gly+ivAllo(n=7). SF+Allo and Gly+Allo rats received Allo(300mg/L) in the drinking water 7 days prior to and for 24h after Gly/SF injection. Gly+ivAllo rats received intravenous Allo(50mg/Kg) 30 minutes after Gly/SF injection and Allo(300mg/L) in the drinking water thereafter.Analyzed data(24h after infusion):inulin clearance(GFR),blood pressure(BP),renal blood flow(RBF),tubular injury score(TIS),serum uric acid(UA),renal tissue immunoblotting for heme oxygenase(HO-1),manganese superoxide dismutase(MnSOD),cleaved caspase-3 and p21.Data are mean±SEM.

Results: Gly rats showed markedly reduced GFR associated with increased oxidative stress and apoptosis. Histopathological findings confirmed the renal dysfunction in the Gly group and myoglobin casts were observed in both Gly and Gly+Allo groups. Prophylactic Allo attenuated all of these alterations. BP and RBF did not differ among groups.

	SF	SF+Allo	Gly	Gly+Allo
GFR (ml/min/100gBW)	1.12±0.03	1.02±0.05	0.49±0.04^{ace}	0.78±0.08^{af}
UA (mg/dL)	1.7±0.1	0.3±0.1^{a}	1.4±0.3^{ce}	0.4±0.1^{a}
TIS	0.06±0.01	0.22±0.05	1.48±0.13^{ad}	0.81±0.17^{ae}
HO-1 (%)	100±6	77±8	647±40^{ace}	487±32^{ae}
MnSOD (%)	100±2	113±27	311±55^{ace}	126±25
Cleaved Caspase-3 (%)	100±8	103±6	158±18^{bce}	67±5
p21(%)	101±10	85±12	44±8^{adf}	76±9

^{a}p<0.001,^{b}p<0.01 **vs. SF**; ^{c}p<0.001,^{d}p<0.01 **vs. Gly+Allo**; ^{e}p<0.001,^{f}p<0.01 **vs. SF+Allo**

Therapeutic Allo also improved GFR(0.78±0.06ml/min/100gBW), TIS(0.89±0.17) and UA(0.3±0.1mg/dL).

Conclusions: Our data shows that Allo protects, both prophylactic and therapeutic, against renal dysfunction in a model of Gly-induced AKI by reducing oxidative stress and inhibiting apoptosis.This may represent a new therapeutic approach for myoglobinuric AKI.

Funding: Government Support - Non-U.S.

TH-PO184

Pharmacological Modulation of Wnt Signaling in a 3D Cell Culture Model Geurt Stokman, Sandrine Florquin. *Pathology, Academic Medical Center, Amsterdam, Netherlands.*

Background: Tubular morphology is tightly regulated by growth factor and morphogen signaling. Direct pharmacologic modulation of these pathways in animal models is difficult with respect to controlling adverse or off-target effects. To study the role of the morphogen Wnt in tubule formation and repair following injury we developed a 3D culture assay using proximal tubular epithelial cells.

Methods: HK-2 cells were cultured in a Matrigel-collagen matrix. Tubule formation was stimulated with Transforming Growth Factor-β1 and Hepatocyte Growth Factor resulting in formation of a branched network. Tubule morphology was analyzed by immunostaining and electron microscopy. Pharmacological activation of Wnt signaling was induced by exposure to GSK3 inhibitors or conditioned medium of L-cells expressing Wnt ligands. Inhibition of Wnt signaling was achieved by a tankyrase inhibitor. A fluorescent reporter cell assay confirmed that all Wnt signaling activators and inhibitors affected Wnt signaling in HK-2 cells. Digital image analysis was used to quantify morphological characteristics.

Results: Activation and inhibition of Wnt signaling during the full 5 day culture period significantly reduced tubule outgrowth in the culture assay and correlated to a decrease in cell proliferation. Activation of Wnt signaling in established tubules resulted in more pronounced intratubular cilia compared to controls. Inhibition of Wnt signaling led to cilia formation outside of the tubular structures and centrosome misorientation. Wnt signaling activation did not affect tubular damage by diethyl maleate-induced oxidative stress compared to controls, whereas treatment with the antioxidant N-acetyl cysteine prevented tubular damage.

Conclusions: Our results suggest that pharmacological modulation of Wnt signaling may have adverse effects on developing or regenerating tubules after injury. This model may be used to pre-screen the effect of pharmacological intervention in animal models for renal disease. In addition this model can predict the outcome on morphological alterations of tubules in the absence of biasing factors, providing a rationale for development of novel therapeutics.

Funding: Private Foundation Support

TH-PO185

Effect of Erythropoietin on Bone Marrow-Derived Mesenchymal Stem Cells Proliferation In Vitro under Acute Kidney Injury Micro-Environment and Its Mechanism Nanmei Liu. *Dept of Nephrology, Jimin Hospital of Shanghai, Shanghai, China.*

Background: Bonemarrow-derived mesenchymal stem cells (BMSC) transplantation is helpful for the repair of acute kidney injury (AKI) but with limited efficiency. Apoptosis or necrosis of the BMSC under the transplanted microenvironment is the main reason. This study investigated the effect of erythropoietin (EPO) on the proliferation of BMSC under the AKI microenvironment and the possible mechanism.

Methods: C57BL/6 mice's BMSC had been successfully isolated by the Percoll method. The AKI mouse model was prepared. Then we sacrificed the AKI mice and cut the renal cortex to make the AKI kidney homogenate supernatant. P3-BMSC was divided into the following groups:Group A: low glucose DMEM medium with the 10% FBS;Group B: low glucose DMEM medium with the 10% FBS plus the AKI kidney homogenate supernatant;Group C: low glucose DMEM medium with 10% FBS plus the AKI kidney homogenate supernatant and EPO(1, 5, 10, 50IU/ml). Each group was incubated for 1d, 3d, 5d, 7d. CCK-8 and TUNEL were used to detect proliferation and apoptosis of BMSC. Erythropoietin receptor (EPOR) and the level of the proliferation/apoptosis-related signal pathway protein were examined by the Western blot.

Results: The AKI microenvironment decreased BMSC's proliferation while the apoptotic proportion of BMSC was significantly higher than that of Group A. EPO intervention could enhanced the proliferation of BMSC, and at the same time, the apoptotic proportion of BMSC decreased significantly. Western blot showed that the EPO intervention decreased the Caspase-3 level in BMSC under the AKI microenvironment, while the protein expression of Bcl-2 in BMSC decreased significantly. The protein expressions of phosphor-Janus kinase2 (pJAK2) and phosphor-signal transducer and activator of transcription (pSTAT-5) were significantly increased by EPO intervention at the concentration of 10 IU/ml for 5d.

Conclusions: Erythropoietin can promote the proliferation of BMSC under the AKI microenvironment in vitro. EPO/EPOR and the activation of the proliferation/apoptosis-related signal pathway protein are the possible mechanisms.

Funding: Government Support - Non-U.S.

TH-PO186

Effective Treatment of Acute Kidney Injury with Mesenchymal Stem Cells Depends Critically on Their Early Administration That Is Triggered by an Early Diagnosis with SDF-1, an Injury- and Therapy-Specific Biomarker Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Jon D. Ahlstrom,[1] Florian Toegel,[2] Christof Westenfelder.[1,3] *[1]Medicine, U of Utah and VAMC, Salt Lake City, UT; [2]Medicine, Brigham and Women's Hospital, Boston, MA; [3]Physiology, U of Utah, SLC, UT.*

Background: A rise in serum creatinine (SCr) after a potential AKI in patients generally occurs only within 36 or more hours. Since it is not injury-specific, it is not suited to diagnose an actual renal insult that must be promptly treated. It is well established that

injury-specific biomarkers, such as NGAL, Kim-1, IL-18, etc., permit the diagnosis of AKI within a few hours post injury and thereby facilitate the timely institution of an effective therapy such as administration of Mesenchymal Stem Cells (MSCs). We reported previously that kidney tissue and urinary levels of the chemokine SDF-1 rise within 2-3 hrs post IRI AKI in rodents and in the urine of patients, and that this transient increase mediates the recruitment of administered, SDF-1 receptor-expressing MSCs.

Methods: To further examine the temporal relationship between induction, diagnosis and effective treatment of experimental AKI with allo-MSCs, groups (n=8) of SD rats with AKI (45 min bilateral renal pedicle clamp) were either infused with vehicle or with 2x10E6 allogeneic MSCs (Fischer344). Urinary SDF-1 and creatinine levels were monitored over 48 hrs post AKI.

Results: Urinary SDF-1/Cr ratios peaked at 2-5 hrs post insult and declined to baseline by ~ 24 hrs. The administration of MSCs post reflow and up to 6-8 hrs later was most effective in protecting and repairing renal function, while the comparative renoprotective activity declined by 24 hrs and became minimal by 36 hrs post AKI.

Conclusions: These data confirm that the early, biomarker-guided diagnosis of AKI identifies the optimal time for MSC-based, highly effective therapy of IRI AKI. Administration of MSCs at a time when injury-specific biomarker levels decline while SCr levels begin to rise appears futile because the compromised microvascular perfusion may limit the delivery of therapeutic MSCs into the kidney or may even be harmful if infused MSCs further impair renal blood flow.

Funding: Veterans Affairs Support, Private Foundation Support

TH-PO187

Inhibition of the TGF-β Receptor 1 with SB-505124 Fails to Block the Mobilization of Mesenchymal Stem and Endothelial Progenitor Stem Cells following Remote Ischemic Pre-Conditioning Jon D. Ahlstrom,[1] Zhuma Hu,[1] Ping Zhang,[1] Christof Westenfelder.[1,2] *[1]Medicine, U of Utah and VAMC, Salt Lake City, UT; [2]Physiology, U of Utah, SLC, UT.*

Background: Acute kidney injury (AKI) is a common and serious condition for which there is currently no effective and approved treatment. Our laboratory has previously shown in animal models and in a Phase I Clinical Trial that Mesenchymal Stem Cells (MSCs) are a safe and effective therapy for AKI. Remote Ischemic Pre-Conditioning (remote IPC) has shown promise as a therapy for AKI. Remote IPC has also been shown to mobilize MSCs and endothelial progenitor cells (EPCs) into the circulation. However, the importance of MSCs and EPCs to the therapeutic effects of remote IPC for AKI is not known. To test the hypothesis that increased numbers of MSCs or EPCs from remote IPC therapy are required for the treatment of AKI with remote IPC, we evaluated the use of SB-505124 (a TGF-β R1-in inhibitor which has previously been shown to block the mobilization of MSCs) as a tool to prevent the mobilization of MSCs and EPCs in SD rats following remote IPC.

Methods: Groups (n=6) of adult, male Sprague Dawley rats were randomized into either remote IPC, remote IPC + SB-505124 (3 mg/kg I.P.), or SHAM rats. Remote IPC was performed in both hind limbs with 3 cycles of 4 min ischemia/4 min reperfusion. At 24 hrs post remote IPC, whole blood was evaluated by flow cytometry for the relative abundance of MSCs (CD45lo/CD271+), EPCs (CD45lo/CD34+), and hematopoietic stem cells (HSCs, CD45hi/CD34+).

Results: At 24 hrs following remote IPC, the numbers of circulating MSCs and EPCs were significantly increased. However, treatment with SB-505124 did not significantly block the mobilization of MSCs or EPCs following remote IPC.

Conclusions: Remote IPC significantly increased the numbers of circulating MSCs and EPCs. However, a more suitable method for blocking stem cell mobilization than SB-505124 is needed in order to test the importance of MSCs and EPCs for the treatment of AKI with remote IPC.

Funding: Veterans Affairs Support, Private Foundation Support

TH-PO188

Effects of Human Adipose Tissue-Derived Mesenchymal Stem Cells with Expression Level Variants of HO-1 on the Cisplatin-Induced Acute Kidney Injury in Rats Hyun-Jung Kim,[1] Eun Ju Lee,[1] Hyun Seop Cho,[1] Dong Jun Park,[1] Se-Ho Chang,[1] Jin H. Kim,[2] Myeong H. Jung.[2] *[1]Internal Medicine; [2]Biomedical Research Inst Gyeongsang National Univ Hospital, Jinju, Republic of Korea.*

Background: Mesenchymal stem cells (MSCs)-based therapies are currently being investigated for the treatment of acute kidney injury (AKI), although the mechanisms involved remain controversial. Kidneys with injury were exposed to various environments such as oxidative stress, inflammation, and hypoxia. Heme oxygenase-1 (HO-1) that is induced in response to various stresses plays roles for anti-apoptotic, anti-inflammatory, and proangiogenic properties in AKI. This study designed to examine whether HO-1 plays a role in the beneficial effects of MSCs in AKI.

Methods: We isolated MSCs from human adipose tissue, found MSCs with different HO-1 expression level dependent on passages or individuals, and divided MSCs carrying the expression level variants of HO-1. Rats were divided into four groups: control, injected with cisplatin (10 mg/kg), and cisplatin followed by infusion of Ad-MSCs ($5X10^5$ cells) carrying the higher or lower HO-1 expression.

Results: Animal survival and renal function were decreased and histological damage was increased in cisplatin-treated rats at day 3. Infusion of Ad-MSCs ameliorated renal dysfunction, tissue injury, and apoptotic cell death caused by cisplatin, leading to increased survival. MSCs carrying the higher HO-1 expression are much more resistant to injury than those with the less expression of HO-1. The viability of cultured renal proximal tubular cells exposed to cisplatin was also improved by coculture with Ad-MSCs or with conditioned medium. The viability was more effective in MSCs or conditioned medium with higher HO-1 expression than less HO-1 expression. MSCs itself with higher HO-1 expression are more resistant to oxidative stimuli (and inflammatory environments) than those with the less expression of HO-1.

Conclusions: We demonstrated that Ad-MSCs with expression level variants of HO-1, naturally employed, can protect against cisplatin-induced AKI. Our results show that human Ad-MSCs exert a paracrine protective effect on cisplatin nephrotoxicity.

TH-PO189

Human Umbilical Cord Mesenchymal Stem Cells Attenuate Kidney Injury and Endothelial Dysfunction in Sepsis Jose Manuel Condor Capcha, Camila Eleuterio Rodrigues, Daniele Canale, Maria H.M. Shimizu, Roberto De Souza Moreira, Lucia Andrade. *Nephrology, Univ of Sao Paulo, Brazil.*

Background: The pathophysiology of sepsis involves complex cytokine and inflammatory mediator networks, a mechanism to which nuclear factor kappa B (NF-κB) activation is central. Downregulation of endothelial nitric oxide synthase (eNOS) contributes to sepsis-induced endothelial dysfunction. Human umbilical cord mesenchymal stem cells (hUCB-MSCs) are known to express genes and secreted factors involved in angiogenesis, including vascular endothelial growth factor (VEGF) and e-NOS. We used a cecal ligation and puncture (CLP) model to analyze the role of hUCB-MSCs in sepsis-related organ dysfunction.

Methods: We used flow cytometry to evaluate hUCB-MSC phenotypes. We divided Wistar rats into groups: sham (sham-operated); CLP; and CLP+MSC (10^6 hUCB-MSCs, i.p., 6 h after CLP). At 24 h post-CLP, we evaluated renal function (inulin clearance, Cl_{in}); biochemical variables in serum and urine; and Klotho, eNOS, VEGF, Bax and NF-κB (immunoblotting) in kidney tissue (protein expression expressed as % of sham). Data are mean±SEM.

Results: hUCB-MSCs were negative for CD3, CD34, CD45 and HLA-DR, whereas they were positive for CD73, CD90 and CD105.

	Sham	CLP	CLP+MSC
Cl_{in}, ml/mim/100 g BW	0.84±0.02	0.26±0.031[a,b]	0.99±0.12
Klotho, %	100±3.1	43.3±5.5[a]	65.2±3.4[a,c]
eNOS, %	102.5±2.5	49.0±2.1[a,b]	97.4±2.8
VEGF, %	98.4±3.5	141.6±2.1	208.7±24.45[a,c]
NF-κB, %	98.0±4.1	164.8±3.9[a,b]	94.7±3.5
Bax, %	95.0±5.0	118.3±4.4[d]	95.2±2.3[c]

ap<0.001 vs. sham; bp<0.001 vs. CLP+MSC; cp<0.01 vs. CLP; dp< 0.05 vs. sham

Fractional excretion of sodium (FENa) was lower in CLP+MSC rats than in CLP rats (0.67±0.12 versus 2.0±0.64%, p<0.05), as was FEK (9.24±1.5 versus 18.5±4.3%), FEH_2O (1.52±0.25 versus 4.76±1.3%, p<0.002), aspartate aminotransferase (142.5±11.4 versus 217.0±25 IU, p<0.02) and alanine aminotransferase (39.6±4.2 versus 56.3±3.3 IU, p<0.02).

Conclusions: hUCB-MSCs protected renal and liver function, as well as upregulating VEGF and eNOS, and might therefore play a protective role in sepsis. (Supported by FAPESP).

Funding: Government Support - Non-U.S.

TH-PO190

Human Umbilical Cord Mesenchymal Stem Cells in Acute Kidney Injury-Induced Renal Senescence Camila Eleuterio Rodrigues, Jose Manuel Condor Capcha, Ana C. de Bragança, Maria H.M. Shimizu, Barbara A. Santana-Lemos, Danilo Candido Almeida, Niels O.S. Camara, Lucia Andrade. *Univ of Sao Paulo, Brazil.*

Background: Ischemia/reperfusion-induced acute kidney injury (IR-AKI) induces kidney senescence, leading to cell-cycle arrest, telomere shortening and transient Klotho (Kl) deficiency. Although stem cells ameliorate renal injury, the effects might depend on their age. Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) might better protect against IR-AKI.

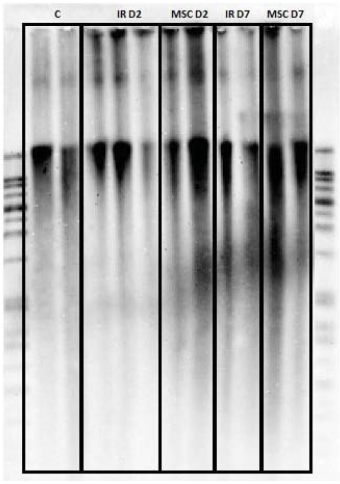
Methods: We used flow cytometry to evaluate hUC-MSC phenotype. Male rats were induced to IR-AKI by 45-min clamping of both renal arteries; some rats received 10^6 hUC-MSC i.p. 6 h later. Rats were euthanized on post-IR days 2 (D2) and 7 (D7). We studied four groups: IR-D2 (n=9); IR+MSC-D2 (n=5); IR-D7 (n=2); and IR+MSC-D7 (n=2). On D2, we measured creatinine clearance, fractional excretion of sodium (FENa) and FEK. In kidney tissue, we used Western blotting (for p21, Kl, TGFβ and ßgalactosidase) and Southern blotting (for telomere length). Data are mean±SEM.

Results: hUC-MSCs were negative for CD3, CD34, CD45 and HLA-DR, whereas they were positive for CD73, CD90 and CD105. Renal function was better in IR+MSC-D2 rats than in IR-D2 rats (0.22±0.06 versus 0.1±0.02 ml/min/100 g BW, p<0.05). FENa was lower in IR+MSC-D2 rats than in IR-D2 rats (0.73±0.25 versus 2.85±1.12%, p<0.05), as was FEK (12.41±3.96 versus 30.4±5.92%, p<0.02).

	IR-D2	IR+MSC-D2	IR-D7	IR+MSC-D7
p21, %	189.0±4.3	117.5±11.8[a]	99.5±1.5	99.5±0.5
Kl, %	42±4.0	73±8.8[a]	22.5±2.5	67.5±2.5[b]
TGFß, %	168±7.8	100±13.5[a]	99.5±1.5	98.5±0.5
ßgalactosidase, %	97.7±1.4	97.3±1.4	162±1.4	96±4.0

[a]p<0.05 vs. IR-D2
[b]p<0.05 vs. IR-D7

Telomeres length is shown.



Conclusions: hUC-MSCs improve renal function by preventing senescence. Supported by FAPESP.

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TH-PO191

Exosomes (EXOs) Derived from Mesenchymal Stem Cells (MSCs) Minimized the LPS Acute Kidney Injury by Renal Pluripotent Cells Activation (rPCs) Luciana Aparecida Reis,[1] Gerson D. Keppeke,[2] Manuel De J. Simoes,[3] Nestor Schor.[1] *[1]Nephrology Div, UNIFESP/EPM, Sao Paulo, Brazil; [2]Rheumatology Div, UNIFESP/EPM, Sao Paulo, Brazil; [3]Morphology Dept, UNIFESP/EPM, Sao Paulo, Brazil.*

Background: Since acute kidney injury (AKI) has high morbidity and mortality, it is of relevance to search for alternative therapeutics. We investigated the effects of MSCs, your conditioned medium (CM) or EXOs in a LPS-induced nephrotoxicity model and the effects rPCs in this model.

Methods: Rats received *i.v.*: LPS (10 mg/B.W.) or PBS (CTL) with MSCs ($1x10^6$), CM (500 µl) or its EXOs (100 µg/ml) from MSCs incubated or not for 12 hours with cytochalasin B (CB; 1 µM) or actinomycin D (AD; 2.6 µM) and given in 1 or 3 doses and sacrificed after 72 hours. Blood and urine samples were collected for creatinine (sCr), urea (sU) and FENa. Kidneys were analyzed for HE, KI67, caspase 3, BrDU markers of RPCs as Wnt1, PAX2 and CD24. Also markers of EXOs as CD63, presence of Y chromosome, IL6, TNF-α, INF-γ and IL10 were also evaluated.

Results: As expected, it was observed increases in sCr, sU, FENa, caspase 3 marking, proinflammatory cytokines and reduction of KI67 with lesions in proximal tubules induced by LPS. However, these parameters were ameliorated with MSCs, CM or EXOs treatments. EXOs increased BrDU, Wnt1, PAX2, CD24 and CD63 expressions indicating activation of RPCs. CB and AD inhibited the protective effect of EXOs. It was impressive the effect of 3 times administration of MSCs or CM or EXOs decreasing the mortality in LPS group.

Conclusions: Therefore, results support that the MSCs and its CM and EXOs protected from AKI induced by LPS. It is reasonable to suggest that the mediation of by EXOs is, at least in part, by stimulating rPCs evaluated by PAX2, Wnt1 and CD24 positive staining. Also, suggest at least in part, participation of rPCs in this cascade of events and those EXOs alone could be employed in order to ameliorate LPS nephrotoxicity.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO192

Granulocyte Macrophage-Colony Stimulating Factor Promotes Macrophage Alternative Activation After Renal Ischemia/Reperfusion Injury Sarah C. Huen,[1] Larry Huynh,[1] Arnaud Marlier,[1] Yashang Lee,[1] Gilbert W. Moeckel,[2] Lloyd G. Cantley.[1] *[1]Internal Medicine, Section of Nephrology, Yale Univ, New Haven, CT; [2]Pathology, Yale Univ, New Haven, CT.*

Background: Following kidney ischemia/reperfusion (I/R) injury, monocytes home to the kidney and differentiate into activated macrophages. While proinflammatory macrophages contribute to the initial kidney damage, an alternatively activated phenotype is required for normal renal repair. The microenvironment of the kidney during the repair phase mediates the transition of macrophage activation from a proinflammatory to a reparative phenotype. How signals from renal tubular cells influence these macrophage phenotypes is unknown.

Methods: Mouse proximal tubular (MPT) cells were cultured in serum free media for 48 hours to generate MPT conditioned media (CM). Bone marrow-derived macrophages (BMM) isolated from C57BL/6 mice were treated with MPT CM. Male C57BL/6 mice, 8-10 weeks old, were subjected to bilateral renal I/R for 24 minutes and given intraperitoneal neutralizing GM-CSF or isotype IgG antibody daily from day 1.5-5 after I/R. Kidney macrophages were isolated at various time points for gene expression analysis.

Results: *In vivo* kidney macrophages isolated during the tubular repair phase after I/R and *in vitro* BMM treated with MPT CM exhibit an alternative activation gene profile (induction of *Arg1*, *Cd206*, *Msr1*, but not *Dectin-1*, *Fizz1, Ym1*) that differs from macrophages induced by IL-4 (induction of *Arg1*, *Cd206*, *Dectin-1, Fizz1*, *Ym1*, but not *Msr1*). MPT CM activates STAT3 and STAT5, but not STAT6, in BMM, leading to induction of alternative activation. Using BMM from *LysM-Cre;Stat3$^{fl/fl}$* mice and pharmacologic inhibition of STAT5, we found that tubular cell mediated macrophage alternative activation is regulated by STAT5 activation. Blockade of GM-CSF, a known STAT5 activator, both *in vitro* and *in vivo* after renal I/R injury attenuated kidney macrophage alternative activation and suppressed tubular proliferation *in vivo*.

Conclusions: This data suggests that tubular cells can instruct macrophage activation by secreting GM-CSF, leading to a unique macrophage reparative phenotype and tubular proliferation after sterile ischemic injury.

Funding: NIDDK Support, Private Foundation Support

TH-PO193

Accelerated Recovery from Acute Kidney Injury through 5-Hydroxytryptamine 1F Receptor Agonism Sara M. Garrett,[1] Ryan Whitaker,[1] Rick G. Schnellmann.[1,2] *[1]Center for Cell Death, Injury, and ReGeneration, Dept of Drug Discovery and Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC; [2]Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC.*

Background: Mitochondrial dysfunction is a feature of diverse acute insults and chronic conditions (e.g., acute kidney injury (AKI), chronic kidney disease), with the greatest impact affecting tissues primarily reliant on aerobic respiration (e.g., kidney). Because pharmaceutical promotion of mitochondrial biogenesis (MB) has been shown to alleviate mitochondrial dysfunction in several model systems, we explored the role of the renal 5-hydroxytryptamine 1F receptor in MB and recovery from ischemia/reperfusion (I/R)-induced AKI.

Methods: Rabbit renal proximal tubules were isolated, cultured, treated with the selective 5-HT_{1F} receptor agonist LY344864, and tested for MB (FCCP-uncoupled oxygen consumption rate (OCR), mitochondrial protein level). Renal cortices from mice treated with LY344864 for various times and doses were analyzed for changes in MB gene expression. Mice subjected to I/R were treated daily with vehicle or LY344864, beginning 24 hr after I/R, and assessed for changes in blood urea nitrogen (BUN), kidney injury molecule-1 (KIM-1), and mitochondrial DNA (mtDNA) copy number.

Results: LY344864 (1 – 100 nM) increased FCCP-OCR and the mitochondrial proteins ATP synthase β, cytochrome c oxidase 1 (Cox1), and NADH dehydrogenase (ubiquinone) 1b subcomplex subunit 8 (NDUFB8) *in vitro*; this MB response was blocked with small interfering RNA knockdown of the 5-HT_{1F} receptor. LY344864 increased peroxisome proliferator–activated receptor coactivator 1α, Cox1, and NDUFB8 transcript levels and mtDNA copy number in renal cortex of naïve mice and accelerated recovery of renal function following I/R-AKI, as evidenced by decreased BUN and KIM-1 and increased mtDNA copy number.

Conclusions: Promotion of MB and accelerated recovery from I/R-AKI through agonism of the 5-HT_{1F} receptor represents a new approach to treat mitochondrial dysfunction in the setting of renal injury.

Funding: Other NIH Support - UL1-RR029882,5T32-DK083262-03, Veterans Affairs Support

TH-PO194

Nephron-Specific Kidney Injury Molecule-1 Expression Induces Tubular Damage and Kidney Failure in Transgenic Zebrafish Wenqing Yin, Dirk M. Hentschel, Joseph V. Bonventre. *Renal Div, Brigham and Women's Hospital, Boston, MA.*

Background: Mammalian Kidney Injury Molecule-1 (KIM-1) is upregulated after kidney injury in proximal tubular cells, and also in animal models and patients with chronic kidney disease. In our murine model, sustained KIM-1 expression in renal epithelial cells causes kidney fibrosis and progression of CKD, suggesting KIM-1 could be a potential therapeutic target in chronic kidney disease. Here, we characterized zebrafish KIM-1 (zKIM-1) family members. By using the zebrafish model, we further verified the pathogenic role of KIM-1 in kidney injury and validated therapeutic screening approaches for chronic kidney disease.

Methods: We cloned zKIM-1 and compared biochemical and functional aspects with that of human KIM-1 (hKIM-1), using PCR, western blotting and immunostaining. We created two KIM-1 transgenic zebrafish models, one utilized a constitutively active promoter, the other tamoxifen-inducible. We also used the mTOR inhibitor (rapamycin) to ameliorate the KIM-1-induced phenotypes in murine and zebrafish models.

Results: As with hKIM-1, zKIM-1 was not expressed in healthy nephrons and was markedly upregulated after gentamicin-induced injury. zKIM-1 also showed a conserved phagocytic activity. Overexpression of zKIM-1 in the pronephros under control of the cdh17 promoter caused pericardial edema, reduced GFR and a higher mortality. Kidney tubular damage was present in nephrons overexpressing zKIM-1. Cre-mediated overexpression of zKIM-1 at 96 hours post fertilization caused a similar kidney maladaptive phenotype marked by tubular damage, edema and higher mortality. In KIM-1 transgenic mice and zebrafish models, rapamycin significantly protected and decreased zebrafish pericardia edema and mortality.

Conclusions: zKIM-1 has high sequence and functional similarities to hKIM-1 and is a biomarker for zebrafish kidney injury. Similar to the murine model, our zKIM-1 transgenic zebrafish also had tubular damage and renal failure, indicating that this genetic model can be used as a tool to identify potential therapeutics for KIM-1-potentiated CKD. mTOR may play an important role in maladaptive KIM-1 signaling.

TH-PO195

Magnesium Protects against Cisplatin-Induced Kidney Injury in Tumor-Bearing Mice, without Compromising Cisplatin's Anti-Tumor Efficacy
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Background: Cisplatin (CIS) is a potent chemotherapeutic drug for treating ovarian and other cancers. CIS-induced acute kidney injury (AKI) occurs in ~30% of patients with accompanying hypomagnesemia. Using an ovarian tumor xenograft model, we examined the role of magnesium (Mg) in CIS-AKI and cisplatin's anti-tumor efficacy.

Methods: Athymic nude mice (female, n=7-8/group, 7wks old) were started on normal (100%Mg) or 10%Mg (MgD) diets on day1. One group of MgD mice was switched to normal diet on day15 + $MgCl_2$ in water (7days) + $MgSO_4$ (s.c., 4days) (MgS). A2780 human ovarian cancer cells were injected (s.c., flank) on day2. Saline or CIS (10mg/kg, i.p.) were injected on days 12, 19 and 21; mice were euthanized on day22. Tumor growth was recorded, and blood urea nitrogen (BUN), renal apoptosis (TUNEL), and renal cell-death markers (by PCR array) were measured to assess renal damage.

Results: CIS-treated MgD mice had elevated BUN (P< 0.05) and renal apoptosis (P<0.05) compared to CIS alone. MgD mice treated with CIS had higher renal pro-apoptotic gene (*Apaf1, Atp6v1, Bax, Bcl2l11, Casp1/3/7, Fas, Gadd45, Tnf, Tnfrs10b,* and *Trp53*), and necrosis-related gene expression (*Ccdc103, Commd4, Dennd4a, Grb2, Mag, Mapk8, Parp2, Pvr* and *Txn14b*)compared to CIS-treated mice. Renal autophagy-related gene expression (*Akt, Atg3/5, Bax, Becn1, Casp3, Ctsb, Ctss, Map1lc3a, Mapk8, Rps6kb1, Sqstm1* and *Ulk*) was also upregulated in CIS-treated MgD mice versus CIS alone mice. MgS reversed the adverse renal effects of MgD and downregulated many of the upregulated genes (P<0.05). Tumor growth over time was inhibited by CIS and further inhibited by MgS on days 21-22. MgD did not significantly affect tumor growth regardless of CIS treatment.

Conclusions: MgS protects against CIS-induced AKI, as evidenced by improved kidney function, reduced activation of apoptosis and cell death-related pathways in the kidneys. While improving kidney function, MgS also improved CIS-induced tumor killing. These findings warrant future large scale studies assessing Mg status and aggressive Mg replacement therapy in CIS-treated patients.

Funding: Private Foundation Support

TH-PO196

CD4 T Cell Knockout Worsens Lung Cancer and Has No Effect on Cisplatin-Induced AKI in Mice
Kameswaran Ravichandran,[1] Hyun-Jung Kim,[1] Abdullah Ozkok,[1] Qian Wang,[1] Quocan Nguyen,[1] Alkesh Jani,[1] Raphael A. Nemenoff,[1] Howard Y. Li,[1] Danica Ljubanovic,[2] Charles L. Edelstein.[1] *[1]Univ of Colorado Denver; [2]Univ Of Zagreb.*

Background: We have developed a model of 4 week, low dose cisplatin (Cis)-induced AKI in mice with cancer that closely resembles the Cis dosing regimen used in humans with non small cell lung cancer. In the 4 week model of AKI, there is an increase in CD4 T cells that precedes the AKI and tubular injury suggesting that CD4 T cells may play a causative role. The aim of the study was to determine the effect of CD4 T cell depletion on AKI and the growth of lung cancer.

Methods: Wild type (WT) C57BL/6 or CD4 T cell -/- mice were injected with lung cancer cells in to the flank at day 1 subcutaneously. Ten days later, Cis (10 mg/kg/week) was given for 4 weeks.

Results:

	WT Vehicle	WT Cisplatin	CD4-/- Vehicle	CD4-/- Cisplatin
BUN (mg/dL)	20.6	128*	19	87**
SCr (mg/dL)	0.3	0.7*	0.3	0.6**
Tumor weight (g)	0.7	0.06*	1.4***	0.4**
Tumor volume week 1 (mm^3)	68	62	102	89
Tumor volume week 4 (mm^3)	682	67*	1152***	238**
Perf (n=3)	4+	4+	1+***	2+**
GrB (n=3)	5+	5+	2+***	3+**
CC3 (n=3)	1+	2+*	4+***	2+**

N=5-6 per group.* P< 0.01 vs. WT Vehicle.* P< 0.01 vs. CD4-/- Vehicle.* P< 0.05 vs. CD4-/- week 1. *P< 0.01 vs. WT vehicle.

The decrease in BUN and SCr in CD4-/- mice versus WT mice with AKI was not statistically significant. Tumor weight was double in CD4 -/- mice. Cis prevented the increase in tumor volume in WT mice but not in CD4-/- mice. Tumors in CD4-/- mice started enlarging at 1 week and were significantly larger at 4 weeks. To determine the mechanism of increased tumor growth, perforin (perf), granzymeB (grB) and cleaved caspase-3 (CC3) that decrease tumor growth by increasing apoptosis were measured by immunoblot. Perf, GrB were decreased in CD4-/- versus WT tumors. CC3 was increased by Cis in WT but not CD4-/- tumors. Despite larger tumors, CC3 was increased in CD4-/- versus WT tumors.

Conclusions: CD4-/- mice with AKI are not protected against AKI. Increased tumor growth in CD4-/- tumors is associated with decreased perf and grB. Cis increases CC3 in WT but not CD4-/- tumors (may explain less effect of Cis in -/- tumors). CC3 is increased in larger CD4-/- tumors perhaps as a compensatory response.

TH-PO197

NFKB Inhibition Protects against Cisplatin-Induced Acute Kidney Injury
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Background: NFKB is a cell signaling pathway important in inflammation and cell survival. Inflammation, acute tubular necrosis (ATN) and apoptosis in the kidney are features of cisplatin-induced AKI. The role of NFKB in cisplatin-induced AKI was determined.

Methods: C57BL/6 miceinjected were with cisplatin (Cis) (25 mg/kg) get AKI on day 3. The NFKB (p65) inhibitor JSH-23 (20 mg/kg)IP was injected on day 1 and 2 after cisplatin.

Results:

	Vehicle (Veh)	Veh +JSH-23	Cisplatin	Cis+JSH-23
Creatinine (mg/dL)	0.29	0.25	1.04*	0.43 ***
BUN (mg/dL)	33	26	164 *	93 ***
ATN score	0	0	3	1.3
Apoptotic tubular cells per 10 HPF	1.5	0	8.5	5
Serum NGAL (ng/mL)	0.18	0	19.87 **	2.38 ***
IL-6 (pg/mg)	3.42	3.49	27.12 *	7.90 ***
IL-8 (pg/mg)	0.94	1.04	19.81 *	11.16 ***
TNF-alpha (pg/mg)	0.79	0.56	0.99 **	0.57 ***
MPO activity (unit/min/µg protein)	0.042	0.034	0.068 *	0.036 ***

*P<0.01 vs Veh, **P<0.05 vs. Veh, ***P<0.05 vs Cis.

Kidney function (BUN, SCr), tubular injury (ATN,serum NGAL), pro-inflammatory cytokines (IL-6, CXCL1, TNF alpha) and MPO activity (a marker of neutrophils and macrophages) were significantlyincreased in AKI and decreased by JSH. On immunoblotof kidney tissuesNFKB (p65), MEKK-1, ERK, JNK, cleaved caspase-3 were significantly increased in AKI and significantly decreased by JSH-23 (n=4 immunoblots/group. Statistical analysis of densitometry data was performed.

Conclusions: NFKB inhibition protects against Cis-induced AKI, decreases ATN but not apoptosis, decreases MAPK pathway proteins and pro-inflammatory cytokines and MPO.

TH-PO198

Paracrine Fibroblast to Epithelial Signaling Mediates Retinoic Acid-Dependent Repair after Acute Kidney Injury
Mark P. De Caestecker, Nataliya Skrypnyk, Leslie S. Gewin, Takuto Chiba. *Depts of Medicine, Cell and Developmental Biology, Vanderbilt Univ Medical Center, Nashville, TN.*

Background: Retinoic acid (RA) is a secreted ligand that activates RA signaling in neighboring cells to induce a variety of different responses. We have shown that RA signaling is activated in proximal tubular epithelial cells (PTECs) after acute kidney injury (AKI), and that inhibition of RA signaling in PTECs inhibits post-injury repair. During kidney development RA is synthesized locally from retinol by retinaldehyde dehyrogenase (Raldh) expressed in stromal mesenchyme and activates RA signaling in ureteric bud epithelium to regulate growth and branching. However the source and regulation of RA synthesis post AKI is unknown.

Methods: We used ischemia reperfusion AKI (IR-AKI) in mice to assess the regulation and localization of RA synthetic machinery post AKI. Renal cortical fibroblasts (RCFs) were isolated and treated with hypoxia and re-oxygenation, H2O2 or Antimycin A to activate oxidative stress and/or TEMOL to reduce oxidative stress. Expression of RA synthetic machinery in vivo and in vitro was assessed by QRT-PCR, Western blot and immunostaining, and RA synthesis assessed by co-culture with F9-RARE-LacZ reporter cells.

Results: Raldh3 and Raldh2 are strongly induced in SMA positive peri-tubular fibroblasts and in occasional F4/80 positive interstitial macrophages early (Raldh3: 6-12 hours) and late (Raldh2: 1-7 days) after IR-AKI. Raldh3>Raldh2 expression by cultured RCFs is increased by H2O2, hypoxia/re-oxygenation and Antimycin A, and inhibited with TEMPOL. RCFs exposed to oxidative stress secrete active RA.

Conclusions: These data suggest that RA synthesis by peri-tubular fibroblasts is induced by oxidative stress and promotes RA signaling in neighboring PTECs. Since RA signaling promotes tissue repair after AKI, these data suggest a novel paracrine-signaling pathway whereby peri-tubular fibroblasts activated by oxidative stress after AKI enhance RA-dependent regenerative repair of PTECs.

Funding: NIDDK Support

TH-PO199

Elucidating the Mechanisms of Therapeutic Augmentation of Kidney Repair After Acute Kidney Injury
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Background: Acute kidney injury (AKI) is a serious disorder for which there is no targeted clinical treatment. A promising approach to improve treatment options lies in developing novel post-AKI therapies that enhance innate renal regenerative processes.

Our lab has used zebrafish to identify small molecules that enhance renal tubular epithelial cell (RTEC) regeneration after AKI. Using this approach we identified methyl-4-phenylthiobutanoate (m4PTB), an HDAC inhibitor that promotes renal progenitor cell expansion in zebrafish embryos and accelerates recovery after AKI in zebrafish and mice. The ability of m4PTB to enhance RTEC proliferation post-AKI depends on intact retinoic acid (RA) signaling, suggesting that the RA pathway is critical to promote innate renal regeneration.

Methods: We utilize a nephrotoxic model of gentamicin-induced AKI in zebrafish larvae that demonstrates the same hallmarks of renal injury and regeneration post-AKI as the mammalian kidney. *Tg(12XRARE:GFP)* transgenic larvae are used to assess RA signaling, and heat shock is used to induce expression of a dominant negative RA receptor (RAR) in *Tg(hsp70:dnRARa)* larvae. Pharmacological RAR inhibitors are also used to block RA signaling.

Results: Expression of RA pathway components, including *aldh1a2* and *rarab*, is increased in the zebrafish kidney post-AKI, and RA target expression is further increased by m4PTB treatment by qRT-PCR in a mouse model of ischemia reperfusion AKI. Live imaging of *Tg(12XRARE:GFP)* zebrafish larvae after injury shows activation of RARE:GFP in RTECs that lose cadherin17 expression, suggesting that RA signaling is associated with RTEC dedifferentiation post-AKI. Finally, we show that blocking RA signaling either by heat shock in *Tg(hsp70:dnRARa)* larvae or by chemical RAR inhibition exacerbates injury and abrogates m4PTB-stimulated RTEC proliferation post-AKI.

Conclusions: This work implicates RA pathway activation as a critical response in renal regeneration post-AKI and suggests that m4PTB action is mediated by activating RA-dependent regenerative responses. Funding support: F30 DK101143.

Funding: NIDDK Support

TH-PO200

Antagonism or Genetic Deficiency of PDGF-C Does Not Affect Renal Recovery in Two Models of Acute Kidney Injury Taizo Nakagawa,[1] Pia Paffenholz,[2] Ina V. Martin,[2] Peter Boor,[2] Stephanie Zok,[2] Sonja Djudjaj,[2] Jürgen Floege,[2] Tammo Ostendorf.[2] *[1]Toyamaken-Saiseikai Toyama Hospital, Toyama, Japan; [2]Diviision of Nephrology and Immunology, RWTH Aachen, Aachen, Germany.*

Background: Acute kidney injury (AKI) accelerates progression of chronic kidney disease. We recently identified platelet-derived growth factor C (PDGF-C) as a central pro-fibrotic factor in the kidney. Studies in AKI with non-specific PDGF/PDGF-receptor-antagonists reported an impaired tubular recovery. We therefore investigated this potential safety concern and assessed whether treating renal fibrosis with anti-PDGF-C strategies compromises renal recovery after AKI.

Methods: We compared the renal outcome following renal ischemia/reperfusion (I/R)- and cisplatin (Cis)-induced damage in wildtype- versus PDGF-C-deficient mice, and in anti-PDGF-C-antibody versus IgG-treated mice. We quantified tubular injury, apoptosis, cell proliferation, chemokine expression, leukocyte infiltration, expression of AKI markers, and expression of PDGF ligands/receptors in the cortical and outer medullary region.

Results: All mice showed severe tubular injury after induction of AKI by Cis or I/R, including overexpression of NGAL and KIM-1 and infiltration of leukocytes (day 5). mRNA expression of PDGF-A, -B and both PDGF receptors markedly increased following Cis or I/R (day5). Compared to control mice (PDGF-C WT littermates or WT mice treated with IgG) no significant differences were detected for most investigated parameters in all settings in the acute and early phase of AKI (d1 and d5 following I/R- or d3 and d5 in Cis-induced damage). In I/R (d5) infiltrating monocytes/macrophages and T cells were significantly reduced in PDGF-C$^{-/-}$-mice but not by anti-PDGF-C treatment. In the Cis model, infiltrating monocytes/macrophages (d5, 15 mg Cis, PDGF-C$^{-/-}$) and T cells (d3, 25 mg Cis, anti-PDGF-C) were rather increased.

Conclusions: In two AKI models, PDGF-C deficiency or anti-PDGF-C treatment resulted in no major difference compared to controls, suggesting, that antagonism of PDGF-C in AKI is safe at least in the short term. However, given variable effects of PDGF-C antagonism on infiltrating leukocytes, long term outcome studies in these models are ongoing.

Funding: Government Support - Non-U.S.

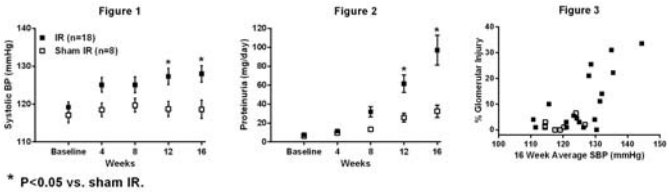
TH-PO201

Role of Blood Pressure and Hemodynamic Factors in the Progression of CKD following AKI in Rats Aaron J. Polichnowski,[1,2] Karen A. Griffin,[1,2] Maria M. Picken,[2] Jianrui Long,[3] Geoffrey A. Williamson,[3] Rongpei Lan,[4] Manjeri A. Venkatachalam,[4] Anil K. Bidani.[1,2] *[1]Hines VA Hospital, Hines, IL; [2]Loyola Univ Medical Center, Maywood, IL; [3]Illinois Inst of Technology, Chicago, IL; [4]Univ of Texas Health Science Center, San Antonio, TX.*

Background: The mechanisms by which AKI accelerates the progression of CKD remain controversial. We have recently suggested that hemodynamic mechanisms may contribute to the accelerated rate of *de novo* glomerular injury following AKI (Polichnowski et al., *JASN* 2014); however, the quantitative relationships between radiotelemetrically measured blood pressure (BP) and renal injury have not been rigorously examined over an extended period following ischemia-reperfusion (IR) injury in rats.

Methods: Male SD rats underwent right uninephrectomy and were chronically instrumented with a BP radiotelemeter. Two weeks later, rats were subjected to 40 min IR (n=18) or sham IR (n=8). BP was assessed every 10 min, 24 hr/day and 24-hr urine collections were conducted every 4 weeks for 16 weeks following IR or sham IR. Kidneys were then perfused-fixed for blinded assessment of glomerulosclerosis (GS).

Results: Modest, but significant, increases in systolic BP (Fig. 1) and robust increases in proteinuria (Fig. 2) were observed by 12 weeks following IR versus sham IR. The % GS was significantly higher in rats with IR (11±3%) versus sham IR (2±1%). Moreover, the most robust levels of GS were observed in those rats whose average 16-week systolic BP exceeded ~128 mmHg (Fig. 3).



* P<0.05 vs. sham IR.

Conclusions: The modest hypertension that develops following IR in rats with pre-existing reduced renal mass may nevertheless lead to substantial increases in proteinuria and GS. These data suggest that further reductions of functional renal mass following IR in rats with pre-existing CKD may increase the renal BP transmission and susceptibility to additional BP-induced glomerular injury.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO202

Id1 and 3 Expression Protects against Capillary Rarefaction and Fibrosis following Acute Kidney Injury Matthew D. Plotkin. *Dept of Nephrology, Central Arkansas Veterans Healthcare System, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

Background: Inhibitor of differentiation (Id) proteins are a family of dominant negative inhibitors of HLH transcription factors that inhibit progenitor cell differentiation and promote angiogenesis. Id1 and 3 are expressed in endothelial cells in the adult mouse kidney and are increased following injury. The aim of this study was to determine if Id1 and 3 prevent capillary rarefaction and fibrosis following acute tubular injury.

Methods: The response to folic acid (FA) induced tubular injury was examined in wild type (WT) and Id1 homozygous/Id3 heterozygous knockout (KO) mice along with the effect of oxidative stress in endothelial cells and fibroblasts isolated from these mice.

Results: Id1 and 3 levels were increased 1 day following FA injection with maximum 10-fold increased levels at day 5, returning to baseline by day 14. Elevated expression occurred in dilated collecting ducts and surrounding endothelial cells and fibroblasts. KO mice had an 8-fold increase in mortality at 2 days compared with WT mice, associated with increased collecting duct injury and nearly complete loss of medullary peritubular capillaries. At 2 weeks, KO mice had a 4-fold increase in medullary capillary rarefaction with a marked increase in fibrosis compared with WT mice. *In vitro*, cells isolated from WT mice had an immediate 5 fold increase in Id1 and 3 levels in response to H_2O_2 and a dose and time dependent increase at later time points. Compared with WT cells, KO cells displayed increased anti-oxidant and DNA repair gene expression and a 75% reduced level of apoptosis. No differences in ROS levels were detected. Surviving cells expressed αSMA, suggesting transition to a myofibroblast phenotype. Primary cultures of fibroblasts from adult WT and KO mice showed a similar response to oxidative injury and reduced αSMA expression with increased Id expression.

Conclusions: Id1 and 3 levels are increased in epithelial and mesenchymal cells following FA induced kidney injury. Id1 and 3 KO results in increased capillary rarefaction and fibrosis, a difference that may be due to endothelial-mesenchymal transition and increased fibroblast survival associated with decreased apoptosis.

Funding: Veterans Affairs Support

TH-PO203

H-Ras Gene Deletion in SVJ-129 Mice, Protects against Cisplatin-Induced Acute Kidney Injury Paloma Martín-Sánchez,[1] Laura Calleros,[1] Mercedes Griera,[1] Jose Luis Cano-Peñalver,[1] Manuel Rodriguez-Puyol,[1] Diego Rodriguez-Puyol.[2] *[1]Dept of Physiology, Univ de Alcalá, Alcalá de Henares, Madrid, Spain; [2]Research Unit, Hospital Univ Principe de Asturias, Alcalá de Henares, Madrid, Spain.*

Background: Arterial hypertension is one of the main causes of kidney dysfunction. Renal damage is often associated with certain drugs, as Cisplatin, a pharmacological compound used in chemotherapy that cause nephrotoxicity, altering glomerular filtration surface area. The importance of small GTP-binding protein Ras in arterial pressure regulation has been poorly studied. It has been demonstrated that H-Ras isoform genetic activation induce hypertension, and our group demonstrated that H-Ras gene deletion produces hypotension in mice. Therefore, in this work we consider the following OBJECTIVE: Check the protective effect of H-Ras deletion against Cisplatin-induced AKI.

Methods: Cisplatin was administered to SVJ-129 H-Ras lacking (H-Ras -/-) and WT mice (one single intraperitoneal 10 mg/kg dose). 72 hours after plasma and urine were collected (for creatinine determination) and kidneys were extracted to measure PKG expression. Tubular damage was analyzed in H&E stained sections. To verify that Cisplatin has a contractile effect, glomerular contraction experiments were done ex vivo.

Results: We demonstrated a significant reduction of plasma creatinine in H-Ras -/- mice, treated with Cisplatin, compared to WT group. When analyzing kidney tissue sections stained with H&E, a more important damage was observed in control samples treated with Cisplatin than in H-Ras -/- mice, showing a higher proportion of morphological alterations than in control kidneys.The increased PKG expression in H-Ras -/-, was demonstrated in

renal cortex of both animals groups (Cisplatin treated or not), suggesting the involvement of the PKG up-regulation in H-Ras deleted mice renal damage protection. Ex vivo glomerular contraction was higher in WT mice treated with Cisplatin than in H-Ras -/- mice.

Conclusions: The present results demonstrated that H-Ras gene deletion reduced acute renal damage induced by Cisplatin in mice, possibly trough a renal cortex PKG expression up-regulation mechanism that produces glomerular vasorelaxtion.

Funding: Government Support - Non-U.S.

TH-PO204

Oat1/3 Restoration Protects against Renal Damage after Ischemic AKI

Reinhard Schneider,[1] Marcus Meusel,[1] Boris Betz,[1] Christopher Held,[1] Kerstin Möller-Ehrlich,[2] Maike Julia Buettner,[3] Christoph Wanner,[1] Christoph Sauvant.[4] *[1]Div.Nephrology, Univ Hospital, Wuerzburg; [2]ZEMM, Julius-Maximilians Univ, Wuerzburg; [3]Nephropathologic Inst Univ, Erlangen; [4]Clinic for Anaesthesia, Halle/Saale, Germany.*

Background: Expression of proximal tubular organic anion transporters Oat1 and Oat3 is reduced by prostaglandin E2 (PGE2) after renal ischemia and reperfusion (I/R) injury. We hypothesized that impaired expression of Oat1/3 is decisively involved in the deterioration of renal function after I/R injury. Therefore, we administered probenecid, which blocks proximal tubular indomethacin uptake, to abolish the indomethacin mediated restoration of Oat1/3 regulation and its effect on renal functional and morphological outcome.

Methods: Ischemic AKI was induced in rats by bilateral clamping of renal arteries for 45 min with 24h follow up. Low-dose indomethacin (1mg/kg) was given i.p. at the end of ischemia. Probenecid (50mg/kg) was administered i.p. 20 min later.

Results: Indomethacin restored the expression of Oat1/3, PAH net secretion and PGE2 clearance. Additionally, indomethacin improved kidney function as measured by GFR and morphology, whereas in opposite it reduced renal cortical apoptosis and nitric oxide production. Notably, indomethacin did not affect inflammation parameters in the kidneys (e.g. MCP-1, ED1+-cells). On the other hand, probenecid blocked the indomethacin induced restoration of Oat1/3 and moreover abrogated all beneficial effects.Notably, indomethacin did not affect parameters of inflammation in the kidneys (e.g. MCP-1, ED1+-cells). On the other hand, probenecid blocked the indomethacin induced restoration of Oat1/3 and moreover abrogated all beneficial effects.

Conclusions: Our study indicates that the beneficial effect of low-dose indomethacin in iAKI is not due to its anti-inflammatory potency, but in contrast to its restoration of Oat1/3 expression and function. Inhibition of proximal tubular indomethacin uptake abrogates the beneficial effect of indomethacin by resetting the PGE2 mediated Oat1/3 impairment, thus re-establishing renal damage. This provides evidence for a mechanistic effect of Oat1/3 in a new model of the induction of renal damage following ischemic AKI.

Funding: Government Support - Non-U.S.

TH-PO205

HDAC2 Deletion Leads to Substantial Increase in Tolerance of Ischemia Reperfusion Injury

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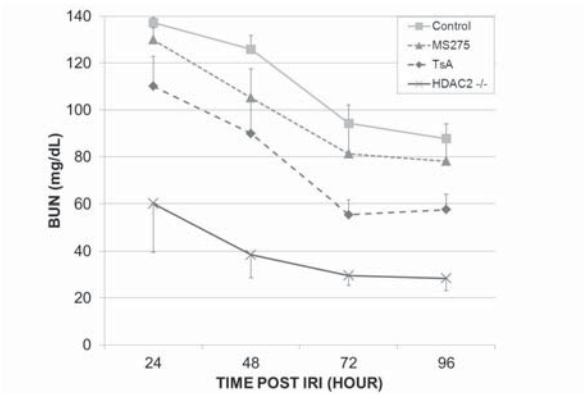
Background: We have identified that pan-histone deacetylase (HDAC) and class I-HDAC specific drug inhibition leads to increased tolerance of ischemia reperfusion injury (IRI), preserving early renal function and limiting long term fibrosis. We have investigated the role of individual deletion of three of the class I HDACs (HDAC 1, 2, and 3) on IRI.

Methods: A murine model producing 28 minutes of unilateral warm renal IRI with contralateral nephrectomy was performed in mice with inducible ERT2-Cre mediated deletion on floxed HDAC 1 and 2. Pan-HDAC3 deletion led to murine death so a kidney specific deletion was produced by transplanting kidneys from floxed HDAC3:ERT2-Cre kidneys into wildtype recipients and performing renal IRI once the transplant had stabilized with equivalent wildtype controls. Renal function was assessed by BUN or creatinine measurement daily for 4 days and fibrosis scoring was by automated Sirius Red quantification at 30d after IRI.

Results: Global HDAC2 deletion led to substantial improvement in IRI tolerance by BUN and creatinine assay - greater than with pan HDAC (TSA) or class I HDAC (MS275) drug inhibitiors (Fig1). HDAC2 deletion was protective of renal fibrosis development.



HDAC1 deletion had no significant impact on renal IRI tolerance. Global HDAC3 deletion led to lethality at a mean of 17 days. HDAC3 deletion confined to the kidney was well tolerated but did not alter tolerance of renal IRI.

Conclusions: HDAC2 plays a key role in IRI tolerance with substantial improvement in renal function and fibrosis formation in HDAC2 deficient animals. The other tested class I HDAC knockouts did not alter renal IRI tolerance. Further investigation into the role of HDAC2 in IRI tolerance are ongoing.

Funding: NIDDK Support

TH-PO206

Non-Muscle Myosin Heavy Chain Type II A Is Required for TRIM72-Mediated Proximal Tubular Cell Protection in AKI

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Background: Trim -72 is a TRIM family member protein essential for membrane repair. It was originally clone in striated muscle and was recently identified in proximal tubular epithelial cells after injury. We have previously demonstrated using myofibroblast cell line C2C12, it immune-colocalized with non-muscle myosin heavy chain type IIA (myh9), at early stages in myotubule differentiation. Genetic studies conducted on May-Hegglin anomaly patients, myh9 mutation is the molecular basis results victim patients highly susceptible to steroid resistant glomerulonepohropathy. In genetically ablated rodents, kidney phenotype such as glomerulopathy were observed similar to May-Hegglin patients. However, we and other groups observed that the NMIIA also abundantly expressed in tubular epithelial cells besides podocytes. We previously observed TRIM -72 are capable to protect tubular epithelial cells in acute kidney injury. And TRIM -72 null mice (mg 53-/-) are highly susceptible To IRI-AKI. It is not clear if the interaction between myh9 and TRIM -72 contributes to TRIM-72-medated PTEC protection.

Methods: We use in vitro system human proximal tubular epithelial cells (HKC8) cotransfected with TRIM -72 and various myh9 constructs (a full length, truncated or mutants: pcDNA-Myh9-wt, pcDNA-myh9-ΔTail, and pcDNA-myh9-ΔIQ2 respectively) to assess intermolecular interaction by immune coprecipitation and observe membrane repair capability.

Results: We observed the immune-coprecipitation between NMIIA and TRIM -72 was complete abolished upon tail-domain truncation (pcDNA-myh9-ΔTail) and partially altered if their light chain regulatory motif were mutated. The TRIM -72 molecules travel along the cellular tracks composed of NMIIA to seal injured membrane. The TRIM72 apparently lost its membrane repair capability with truncated (pcDNA-myh9-ΔTail) or mutant NMIIA (pcDNA-myh9-ΔIQ2).

Conclusions: NMIIA in PTEC cells may contribute to TRIM -72 mediated tubular protection in AKI. NMIIA in PTEC cells, distinct to podocyte in May-Heggin anomaly, is required for TRIM -72-mediated membrane repair at PTEC in AKI.

TH-PO207

Progranulin Protects against Renal Ischemia/Reperfusion Injury in Mice

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Background: Progranulin (PGRN), an autocrine growth factor, has multiple physiological functions and is widely involved in the pathogenesis of many types of diseases. However, the role of PGRN on the regulation of renal function keeps unclear. The pivotal anti-inflammatory role of PGRN in rheumatoid arthritis and other inflammatory disease models encourages us to further elucidate the function of PGRN in ischemic acute kidney injury (AKI).

Methods: Acute kidney injury was induced by renal ischemia/reperfusion (I/R) injury in PGRN knockout ($PGRN^{-/-}$) mice and age-matched C57BL/6 wild type was (WT) mice. Western blot analysis, real time RT-PCR, immunohistochemistry, TUNEL assay, and renal function and morphological examinations were used in this study.

Results: In this study, we found that PGRN was significantly reduced in the kidney from a mouse model of renal I/R injury. Furthermore, we observed that PGRN deficiency significantly aggravated renal injury as demonstrated by higher serum creatinine, more severe morphological injury, increased tubular epithelial cell apoptosis and tubulointerstitial neutrophil and macrophage infiltration in $PGRN^{-/-}$ I/R mice versus control mice. In vitro, we further found that PGRN decreased hypoxia-induced inflammatory response and apoptosis in proximal tubule epithelial cells, which was associated with NOD2-mediated immune response. To our surprise, both wild type mice pretreated with and delayed administration of recombinant PGRN (rPGRN) could effectively protect against renal I/R injury or promotes recovery from AKI.

Conclusions: Our results provide new evidence for a better understanding of the biological activities of PGRN in the kidney and suggest that PGRN may be an innovative therapeutic strategy for treating patients with ischemia and reperfusion–associated disease.

Funding: Government Support - Non-U.S.

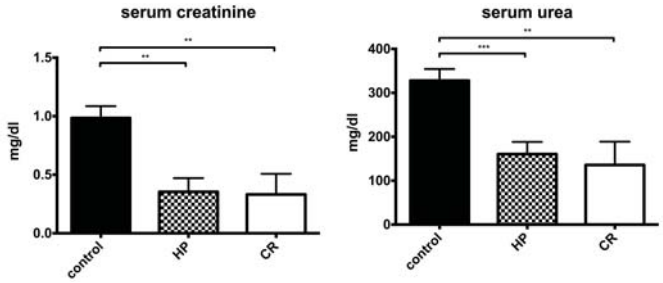
TH-PO208

Transcriptional Regulation of Renal Organ Protection Marc Johnsen,[1] Torsten Kubacki,[1] Bernhard Schermer,[1,2,3] Thomas Benzing,[1,2,3] Volker Rolf Burst,[1] Roman-Ulrich Mueller.[1,2,3] *[1]Dept 2 of Internal Medicine and Center for Molecular Medicine Cologne, Univ of Cologne, Cologne, Germany; [2]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, Univ of Cologne, Cologne, Germany; [3]Systems Biology of Ageing Cologne, Univ of Cologne, Cologne, Germany.*

Background: Preconditioning strategies have been used successfully to attenuate AKI but the underlying mechanisms remain largely unknown. Both, caloric restriction and exposure to hypoxia, are organ protective when applied acutely and expand lifespan when applied chronically in all species examined. We hypothesized that there are evolutionary conserved pathways which we tried to identify on the mRNA-level.

Methods: After subjecting male BL6 mice either to a 40% caloric reduction (CR) or to intermittent hypoxia treatment (HP) we performed unilateral nephrectomy and ischemic AKI in the contralateral kidney induced by clamping of the left vascular pedicle. Transcriptional analysis was performed using RNA sequencing on the unharmed kidney, which was harvested at first.

Results: There was a marked protective effect of preconditioning on serum parameters (creatinine, urea) and survival that was most prominent in the CR group.



Analysis of the RNA sequencing results revealed an overlap of a set of genes in both preconditioning groups which were confirmed by qPCR. By performing qPCR on preconditioned hearts and livers, we further reduced the number of candidate genes.

Conclusions: We were able to narrow down the group of putative candidate genes involved in a postulated, conserved, organ independent mechanism of preconditioning warranting further investigation.

Funding: Government Support - Non-U.S.

TH-PO209

microRNA Let-7C Delivery Using Engineered Mesenchymal Stem Cells to Combat Kidney Fibrosis Bo Wang, Andrea F. Wise, Brooke M. Huuskes, Sharon D. Ricardo. *Dept of Anatomy and Developmental Biology, Monash Univ, Melbourne, Victoria, Australia.*

Background: MicroRNA is highly effective at reducing renal fibrosis and reversing progression of disease. However, the advancement of miR therapies is hampered by difficulties indelivering miR in a robust and sustainable manner. The ability of MSC to transfer large molecules, and even organelles, suggests their potential usefulness as delivery vehicles for therapeutic miRtreatmentas an innovative approach.

Methods: C57BL6/J mice after unilateral ischemia/reperfusion (IR) injurywere injected with $1x10^6$ enhanced green fluorescent protein$^+$/firefly luciferase$^+$MSCs and imaged from 0-7 days using whole body bioluminescence imaging for cell tracing. miR-let7c modified MSCs were co-cultured with NRK52E, a kidney proximal tubular cell line, using a Transwell system, and the expression of fibrotic genes assessed using qPCR.

Results: Following IR, MSCs homed to the injured kidney where they remained for up to 3 days. miR-let7c was successfully engineered and expressed in MSCs. The modified miR-let7c-MSCs produced miR-let7c into the exogenous environment through exosome delivery. MSC-delivered miR-let7c was endocytosed into NRK52E cells and strongly inhibited the up-regulation of smooth muscle actin, collagen and *TGF-b* type I receptor, a specific target of miR-let7c.

Conclusions: MSCs home to the injured kidney in mice with IR injury. *In vitro* studies show that miR-let7 produced from modified MSC can be endocytosed into kidney epithelial cells leading to the inhibition of fibrotic genes induced by *TGF-b1*. This data will pave the way for the application of miR, or even siRNA, as an innovativeRNAi therapeutic strategy for renal disease therapy, but may also offer promise for other degenerative chronic disorders.

Funding: Government Support - Non-U.S.

TH-PO210

Hypoxia Inducible Factor 1 Alpha (Hif-1a) and Oxidative Stress as Triggers of Chronic Kidney Disease (CKD) Induced by an Acute Kidney Injury (AKI) Episode Roxana Rodríguez Romo,[1,2] Arturo Gómez,[1,2] Kenia Benitez,[1,2] Sara Huerta,[3] Gerardo Gamba,[1,2] Rosalba Perez-Villalva,[1,2] Norma Bobadilla.[1,2] *[1]Molecular Physiology Unit, Inst de Investigaciones Biomédicas, UNAM; [2]Nephrology, Inst Nacional de Ciencias Médicas y Nutrición; [3]Hospital Infantil Federico Gómez.*

Background: There is a lack of knowledge about the temporal influence of different potential mechanisms that trigger the progression to CKD induced by an AKI episode. This study was designed to evaluate the role of Hif-1a and oxidative stress in participating in chronic renal injury induced by ischemia/reperfusión.

Methods: Twenty three Wistar male rats underwent to renal bilateral ischemia for 45 min were studied and sacrificed after 1, 2, 3 or 4 months after ischemia. Nine sham-operated rats were studied 1, 2 and 4 months after. At the end of each experimental period, mean arterial pressure (MAP), renal blood flow (RBF), creatinine clearance (CCr), urinary protein (UProt) and urinary peroxide excretion (UH_2O_2) were measured, as well as total and nuclear Hif-1a expression assessed by tissue microarray and immunohistochemistry.

Results: Rats underwent to ischemia remained normotensive and exhibited a progressive increment in UProt that was significant since the 3rd month. In contrast oxidative stress was apparent since the 1st month. Although renal dysfunction was not observed after 4 months of ischemia, there was a significant increment in the glomerular area, which was accompanied by a significant increase in the nuclear expression of Hif-1a.

Conclusions: Our results suggest that oxidative stress plays an early role in provoking renal injury, whereas the activation of Hif-1a occurs lately, however both mechanismsare undoubtedly involved in promoting long-term functional and structural alterations in this model of renal injury induced by an AKI episode. Moreover, this study pointed out the importance of studying the effect of anti-oxidants just when ischemic insult has happened or the use of Hif-1a inhibitors to determine their impact in reducing the CKD progression.

Funding: Government Support - Non-U.S.

TH-PO211

The PPAR-α Agonist Fenofibrate Ameliorates Aging-Related Progressive Renal Injury Eun Nim Kim, Ji Hee Lim, Min Young Kim, Byung Ha Chung, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim, Yoonsik Chang, Bumsoon Choi. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

Background: Aging is a multifactorial process characterized by a progressive decline in physiological function. Peroxisome proliferator-activated receptor-α (PPAR-α) is key regulators in various age-associated physiological processes related to energy metabolism and oxidative stress. We therefore examined the activation of PPAR-α by PPARa agonists fenofibrate would improve changes of aging and oxidative stress in the kidney.

Methods: Male 19-month-old C57/BL6 mice were used in this study. Fenofibrate (0.1%) was provided to old mice for 6month. We measured histological change, oxidative stress, and aging-related protein expression in the kidneys.

Results: Fenofibrate-treated old-mice displayed decreased albuminuria (59.4 ± 31 ng/24hr versus 22.7 ± 8.5 ng/24hr; $p < 0.05$ versus VH). Creatinine clearance increased with Fenofibrate-treated old-mice (0.07 ± 0.04 ml/min versus 0.16 ± 0.05 ml/min; $p < 0.05$ versus VH). Serum creatinine was decreased in Fenofibrate-treated old-mice, although this was not statistically significant (0.75 ± 0.3 mg/dL versus 0.44 ± 0.2 mg/dL). There were decreases in mesangial volume (56.1 ± 2.06% versus 42.87 ± 1.25%; $p < 0.001$ versus VH) and tubulointerstitial fibrosis (16.1 ± 4.32% versus 4.41 ± 3.61%; $p < 0.001$ versus VH) in Fenofibrate-treated old-mice. In our study, expression of PPARa (1 ± 0.2 fold versus 1.31 ± 0.11 fold; $p < 0.05$ versus VH) was increased in Fenofibrate-treated old-mice. SIRT1 expression (1 ± 0.2 fold versus 1.97 ± 0.23 fold; $p < 0.05$ versus VH) was increased in Fenofibrate-treated old-mice compared with control-old mice. Also, expression of PGC-1a (1 ± 0.07 fold versus 1.85 ± 0.13 fold; $p < 0.05$ versus VH) was increased in Fenofibrate-treated old-mice.

Conclusions: These results suggest that PPAR-α agonists may benefit aging-related renal injury by SIRT1 activation. Pharmacologically targeting PPAR-α and SIRT1 signaling molecules may reduce the pathologic changes of aging in the kidney.

TH-PO212

AMPK Activation and Angiotensin II Receptor Blockade Have Synergic Action in Reducing Angiotensin II-Induced Extracellular Matrix Accumulation in Human Mesangial Cells Jose B. Lopes de Faria, Kamila Silva, Jacqueline M. Lopes de Faria, Alexandros Papadimitriou. *Renal Pathophysiology Laboratory, Investigation on Diabetes Complications, Univ of Campinas (UNICAMP), Campinas, Sao Paulo, Brazil.*

Background: A rise in angiotensin II (ANG II) and 5' adenosine monophosphate-activated protein kinase (AMPK) reduction are both associated with progression to kidney fibrosis. However, the mechanism of interaction between ANG II and AMPK in extracellular matrix (ECM) accumulation is not known. The aims of the current study were to assess 1) the contribution and mechanism of AMPK leading to ECM accumulation in human mesangial cells (HMCs) exposed to ANG II and 2) the role of AMPK activation under a renin angiotensin system (RAS) blockade.

Methods: HMCs were exposed for 24 h under ANG II (1 μM) with or without an ANG II type I receptor blocker (losartan, LS, 10 μM); silencing NOX4 (siRNA 200 nM); AMPK

activators (AICAR, metformin (MTF), 1 mM); LS with AICAR or metformin; LS or AICAR with NOX4 siRNA; or an AMPK blocker (Compound C, CC, 10 μM). Western blot was used to evaluate the expression of NOX4, phosphorylated LKB1 (p-LKB1), phosphorylated activated AMPK (p-AMPK), phosphorylated SMAD3 (p-SMAD3), and collagen IV (C-IV).

Results: In HMCs, ANG II reduced p-AMPK and increased NOX4, p-SMAD3, and C-IV levels, all of which were reversed by LS or AICAR ($P<0.0014$). LS increased p-AMPK via a rise in p-LKB1 ($p<0.0023$). NOX4 siRNA, LS, and AICAR equally reduced an ANG II-induced rise in p-SMAD3 and C-IV levels ($p<0.0018$). Reduction in NOX4, p-SMAD3, and C-IV by LS or AICAR was reversed with CC ($P<0.0012$). AICAR or MTF with LS further increased p-AMPK and further decreased NOX4, p-SMAD3, and C-IV levels under ANG II compared to LS ($p<0.0001$).

Conclusions: HMCs under ANG II, AICAR, or MTF with LS confer an additional reduction in ECM accumulation compared to LS via a synergistic AMPK activation and hence inhibition in NOX4/TGF-β1 signaling.

Funding: Government Support - Non-U.S.

TH-PO213

Renoprotective Effects of Metformin in Response to Unilateral Ureteral Obstruction in Mice Michael Christensen, Jorgen Frokiaer, Rikke Norregaard. *Inst of Clinical Medicine, Aarhus Univ, Aarhus, Denmark.*

Background: Metformin is the first choice treatment for type-2 diabetes where it among others has glucose stabilizing effects. Besides the effects in diabetes metformin has been used as an anti-inflammatory agent preventing inflammation in several disease models, including kidney injury models. In this study, we examined the effect of metformin on the progression of tubular injury, inflammation and oxidative stress in response to unilateral ureteral obstruction (UUO).

Methods: C57bl/6 mice were treated with metformin (500mg/kg/day) 7 days prior to obstruction, as well as 3 days post obstruction. Treated and sham-operated mice were sacrificed and plasma as well as kidneys was harvested for both RNA and protein analyses as well as histological and immunohistochemical analysis.

Results: Hematoxylin and eosin staining showed lesser tubular dilation in the metformin treated UUO group (UUO-MET) compared to UUO. Kidney injury molecule-1 a specific marker for proximal tubule damage was markedly increased in the obstructed kidney of UUO mice, and this increase was partly normalized in the UUO-MET. UUO increased inflammatory markers TNFalpha and interleukin-6 which was attenuated in response to metformin administration. The M1 macrophage marker integrin alpha X was expressed to a lesser extent in the metformin treated UUO mice, indicating lesser macrophage infiltration. Besides a down regulation of inflammatory markers there was an increase in the abundance of the antioxidants protein heme oxygenase-1 and superoxide dismutase-1 in the UUO-MET mice compared to untreated UUO mice. Mice receiving metformin had elevated plasma levels of lactate both in SHAM and UUO groups, indicating that metformin was successfully administrated to the mice. Plasma creatinine and urea were unchanged after metformin administration in response to UUO.

Conclusions: In conclusion, this study indicates that metformin attenuates the progression of tubular injury, inflammation and oxidative stress in mice exposed to a 3 days UUO.

TH-PO214

PGC-1a Attenuates Reactive Oxygen Species Induced Kidney Injury By Activating Nrf2-Mediated Mitochondrial Antioxidants Hoon In Choi,[1] Jung Sun Park,[1] Eun Hui Bae,[2] Seong Kwon Ma,[2] Jong Un Lee,[1] Soo Wan Kim.[2] *[1]Physiology, Chonnam National Univ Medical School, Gwangju, Korea; [2]Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea.*

Background: The peroxisome proliferator activated receptor (PPAR) gamma co-activator 1α (PGC-1α) has been known as a transcriptional master regulator of mitochondria biogenesis and antioxidant response. Ischemia/reperfusion (I/R) triggers acute kidney injury (AKI) via aggravating oxidative stress and inflammation. Therefore we postulated PGC-1α may reduce oxidative stress and inflammation in renal tubule cells.

Methods: I/R induced AKI was developed by clamping both renal pedicles for 30 min. Mice were sacrificed 1 day later after operation. We developed stable PGC-1α over-expressing cell line in human renal proximal tubule epithelial cells (HK-2 cells). Cell viability was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Apoptosis was assessed by flow cytometry analysis after the cells were stained by fluorescein isothiocyanate-conjugated annexin V and propidium iodine. The fluorescent probe 2',7'-dichlorofluorescein diacetate and MitoTracker were used to measure intracellular and mitochondrial levels of reactive oxygen species (ROS), respectively. The expression of antioxidant related gene was measured by real time PCR and Western blot analysis. The protein expression of pro-apoptotic and pro-inflammatory proteins was determined by immunoblotting.

Results: Plasma creatinine level was increased in I/R-induced AKI compared with controls. The mRNA and protein expressions of PGC-1α were decreased in I/R-induced AKI. H_2O_2 treatment resulted in decreased expression of PGC-1α and cell viability, while increased ROS levels in HK-2 cells. These changes were counteracted by PGC-1α over expression. Over-expression of PGC-1α reduced pro-apoptotic proteins expression (Bax, P-p53, cleaved caspase3) and pro-inflammatory proteins expression (COX2 and iNOS). PGC-1a over-expression induced increased expression of NRF2 as well as induced NRF2 target genes such as Prx3, Prx5, and MnSOD.

Conclusions: PGC-1a protects ROS-mediated kidney injury by up-regulating NRF-2 dependent antioxidant enzymes.

Funding: Government Support - Non-U.S.

TH-PO215

Nrf2 Mediates the Oxidative Stress-Dependent Hypertension Associated with Renal-Selective Depletion of DJ-1 Santiago Cuevas, Yu Yang, Prasad Konkalmatt, Laureano D. Asico, Jun B. Feranil, John Edward Jones, Ines Armando, Pedro A. Jose. *Div of Nephrology, Univ of Maryland School of Medicine, Baltimore, MD.*

Background: Renal dopamine D2 receptor (D2R) dysfunction is associated with increased oxidative stress and high blood pressure (BP). We have reported that the renal expression of DJ-1, an antioxidant, is regulated by D2R. Nrf2 is involved in the upregulation of several antioxidant genes. We hypothesized that Nrf2 plays a role in the antioxidant effect of DJ-1 in the kidney.

Methods: D2R and DJ-1 renal expression have been silenced in mice by renal subcapsular infusion of specific siRNA.

Results: DJ-1 and Nrf2 co-immunoprecipitate in the mouse kidney. Silencing D2R expression in mouse renal proximal tubule cells (MPTCs) decreases D2R expression (78±11%,n=4), Nrf2 promoter activity (53±2%, n=5) and increases ROS production (56±8%;n=6). Selective renal silencing of D2R in mice decreases renal expression of D2R (48±2% n=3), DJ-1 (45±2%,n=7), Nrf2 (22%±2,n=7) and GST (63±6%,n=3) and increases systolic BP (119±7%,n=3); NQO1 expression was not affected. Silencing DJ-1 expression in MPTCs decreases the expression of DJ-1 (75±15%,n=4), Nrf2 (63±11%, n=3), and its targets NQO1 (75±15%,n=4), and GST (61±8%,n=4), Nrf2 promoter activity (58±1%,n=5) and increases ROS production (182±10%,n=6). Selective renal silencing of DJ-1 in mice decreases renal expression of DJ-1 (30±6%,n=3), Nrf2 (57±6%, n=3), NQO1 (46±11%,n=3), and GST (28±7%, n=3) and increases systolic BP (115.2±3.7%, n=3). DJ-1-/- mice have decreased expression of Nrf2 (46.8±6.8%,n=5), NQO1 (19.4±2%,n=5) and GST (51±12%, n=3) and increased systolic BP (130±3%,n=5) and renal expression of nitro-tyrosine (177±31%,n=5). Immunoprecipitation experiments showed increased Nrf2 ubiquitination in DJ-1 knockout mice. Systolic BP and renal MDA expression returned to normal after treatment with both either tempol, a SOD-mimetic agent or bardoxolone, a Nrf2 inducer in DJ1-/- mice but had no effect in their wild-type littermates.

Conclusions: Our results suggest that the antioxidant effect of renal DJ-1 is, in part, mediated via decreased Nrf2 degradation. Altered DJ-1 expression results in oxidative stress-mediated hypertension.

Funding: Other NIH Support - 5P01HL068686-11; Renal vascular oxidative stress in hypertension

TH-PO216

Contribution of NADPH Oxidase 4 Signaling to the Smooth Muscle Fibroproliferative Phenotype in Arteriovenous Fistulae David Jourd'heuil,[1] Roman G. Ginnan,[1] Xiaochun Long,[1] Paul B. Kreienberg,[2] David J. Conti,[2] Harold A. Singer,[1] Arif Asif.[3] *[1]Center for Cardiovascular Sciences, Albany Medical College, Albany, NY; [2]Dept of Surgery, Albany Medical College, Albany, NY; [3]Div of Nephrology and Hypertension, Albany Medical College, Albany, NY.*

Background: Although arteriovenous fistulae (AVFs) are the preferred form of dialysis access, they suffer significant problems with high incidence of both early and late failures. NADPH oxidase (NOX) and reactive oxygen species contribute to the smooth muscle fibroproliferative phenotype during pathological vascular remodeling. However, the contribution of NOXs to the smooth muscle neoplastic response in an AVF and activation of downstream pathways are poorly characterized. We investigated the expression of NOXs during the neointimal hyperplasia associated with an AVF and analyzed their role in regulating two potential downstream targets in smooth muscle, namely the multifunctional Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII) and the cytoprotective hemoprotein cytoglobin (CYGB).

Methods: Histomorphometric, immunostaining, mRNA, and protein analysis were performed on tissue samples obtained from the venous segments of AVFs collected from patients undergoing surgical access creation or at revision. Cultured smooth muscle cells from primary placement veins were derived and the significance of NOX expression on CYGB and CaMKII expression and activation was determined.

Results: The NOX isoform NOX4 was upregulated in the revision compared to the primary placement veins. Coincidental was a differential expression of CaMKII isoforms with upregulation CaMKIIΔ, an isoform previously linked to neointimal hyperplasia. Similarly, CYGB was upregulated with significant association with both medial smooth muscle and neointimal cells. NOX4 and CYGB were upregulated through imuno-stimulation of cultured venous smooth muscle cells and CaMKII was activated by autophosphorylation and oxidation.

Conclusions: Our results identified NOX4, CaMKIIΔ, and CYGB as novel targets of inflammatory and hyperplastic conditions associated with AVF. Our results also suggest a functional link between NOX4-dependent ROS production and CaMKII and CYGB activation.

Funding: Pharmaceutical Company Support - Dialysis Clinic, Inc. Paul Teschan Research Fund

TH-PO217

Effect of NADPH Oxidase Inhibition on the Development of Interstitial Fibrosis in Unilateral Ureter Obstruction Rats Xizi Zheng, Liqiang Meng, Yu Wang, Jiawei Tang, Lei Qu. *Inst of Nephrology, Peking Univ.*

Background: Unilateral ureteral obstruction (UUO) is a well-established model of renal interstitial fibrosis. The involvement of intra-renal oxidative stress in UUO pathogenesis has been reported. However, the source of reactive oxygen species (ROS) production remains unclear. The present study was designed to clarify the contribution of NOXs family to the ROS production and subsequent interstitial fibrosis in UUO rats.

Methods: Male Wistar rats were subjected to UUO or sham operation. Either vehicle or apocynin (100 mg/kg per day) were given by daily oral gavage for 1, 3 and 7 days after surgery, respectively. Level of 8-isoprostaglandinF2α (8-iso-PGF2α) and activity of total superoxide dismutase (T-SOD) and catalase in renal homogenates were measured by ELISA. Expressions of NOX2, NOX4, α-SMA, COL-I and pERK1/2 were measured by Western Blot. Immunohistochemistry was performed to determine the expression of α-SMA and ERK1/2 in renal tissues.

Results: Compared to the sham, UUO rats showed increased oxidative stress, as detected by increased renal tissue 8-isoPGF2α level. Meanwhile, there was an increase in NOX2 and NOX4 expression on day1, day3 and day7 in UUO rats (all $P<0.01$). There was a gradual but sustained reduction of catalase activity in UUO rats by 49%, 70% and 81% on day 1, day3 and day7, respectively(all $P<0.05$),while SOD activity increased on day 1 and day 3 ($P<0.05$) with a subsequent decrease to sham level on day 7. Furthermore, UUO rats developed interstitial fibrosis on day7 with increased COL-I and a-SMA expression, accompanied with activation of ERK1/2. Apocynin treatment significantly decreased 8-isoPGF2α level (-31.6%) and the expressions of NOX2 (-28.7%) and NOX4 (-31%) in UUO rats on day7.Significantly decreased expression of COL-I (-26.4%) and a-SMA (-80%) and activation of ERK1/2(-45%) were also observed, which were detected by IHC staining. Neither T-SOD nor catalase activity in UUO rats was affected by apocynin treatment.

Conclusions: The NOXs family contributes mainly to the production of ROS, subsequent myofibroblast activation and interstitial fibrosis after UUO. Inhibition of the NOXs family may be a choice for preventing interstitial fibrosis.

Funding: Government Support - Non-U.S.

TH-PO218

Smad2/3 and Nox4-Mediated Mitochondrial Dysfunction Is Critical in PAN-Induced Podocyte Damage and Albuminuria Development Jianming Ye,[1] Lixia Yu,[1] Yanbo Liu,[2] Qingfeng Fan.[3] *[1]First People's Hospital of Kunshan; [2]Jilin Univ First Hospital; [3]Univ of Pennsylvania.*

Background: Podocyte damage plays an important function in the initiation and progression of proteinuric glomerular diseases, whereas the underlying mechanisms are still elusive. This study investigated the potential role and proximal signaling of mitochondrial dysfunction in a PAN-induced podocyte injury model both *in vitro* and *in vivo*.

Results: In PAN-treated podocytes, cellular apoptosis and FITC-labeled albumin uptake are increased significantly in a time- and dose-dependent manner. Podocytes treated with 50 μg/ml of PAN shows a time-dependent mitochondrial DNA (mtDNA) damage, displaying as obvious decrease of mtDNA level revealed by Q-PCR, and significant reduction of mitochondrial proteins (CytC, CoxI and SDHA) revealed by immunoblotting. Dysfunction of mitochondria are accompanied with obvious decrease of oxygen consumption rate (OCR assay) and mitochondrial membrane potential (JC-1 assay), as well as significant increase of reactive oxygen species (DCF assay) and MnSOD and catalase protein level in PAN-treated podocytes. Furthermore, PAN induces obvious activation and remarkable translocation of Smad2/3 from cytoplasm to nuclei, whereas expression of Nox4 is significantly increased in mitochondria, and the translocation of CytC from mitochondria to cytoplasm is also detected in PAN-treated podocytes. Knockdown of Smad2/3 or Nox4 obviously decreases PAN-induced podocyte damage. Smad2/3 knockdown decreases mitochondrial Nox4 level, alleviates mitochondria DNA damage and improves mitochondria functions, also inhibites upregulation of cytoplasmic CytC and cleaved caspase 3 level in PAN-treated podocytes. In PAN rats, Q-PCR shows that mtDNA level decreases evidently in isolated glomerular mitochondria at day 10 following PAN injections, while expression of Nox4 and p-Smad2/3 are upregulated. Treatment with prednisone decreases albuminuria, and prevents upregulation of Nox4 and p-Smad2/3.

Conclusions: These findings provide evidences that Smad2/3 and Nox4-mediated mtDNA damage and mitochondrial dysfunction plays a crucial role in PAN-induced podocyte damage and proteinuria development.

TH-PO219

Sphingomyelinase Induces Podocyte Ferroptosis through Oxidative Stress in HIV Milieu Kamesh R. Ayasolla, Maria Sultana-Syed, Shallu Sharma, Rivka Lederman, Ashwani Malhotra, Mohammad Husain, Pravin C. Singhal. *Medicine, North Shore LIJ Medical School, Great Neck, NY.*

Background: Ferroptosis is a programmed cell death but it is distinct from apoptosis. Mechanistically it is initiated by cellular non-chelated iron and driven by altered lipid environment (reduced glutathione and lipid alterations). Since HIV-induced loss of podocytes has been incriminated in the development as well as progression of HIV-associated Nephropathy (HIVAN), we asked whether lipid alteration mediated ferroptosis is contributing to it. In the present study, we tested the role of sphingomyelinase (SMase) in induction of podocyte ferroptosis in HIV milieu, both *in vivo* and *in vitro*.

Methods: SMase activities of renal tissues of 4 wk old control (FVB/N, n=4) and HIVAN mice (Tg26, n=4) as well as empty vector (EV)- or HIV-transduced human podocytes (HPs) were measured. EV/HPs and HIV/HPs were assayed for reactive oxygen species (ROS) generation by DCFDA labeling and for ferroptosis by staining with H3342 and propidium iodide (porous cells with condensed nuclei). To establish a causal relationship between SMase activity and down stream outcomes, EV/HPs and HIV/HPs were evaluated for redox activity (superoxide dismutase[SOD], catalase, glutathione peroxidase and lipid peroxidase), NF-κB activity (gel shift assay and luciferase activity) and morphologic assay for ferroptosis in the presence or absence of GW4869 (SMase inhibitor)/TEMPOL (SOD mimetic agent).

Results: Renal tissues of Tg26 mice displayed 2-fold increase in SMase activity when compared to control mice. Similarly, HIV/HPs showed enhanced ($P<0.01$) in SMase activity when compared to EV/HPs. HIV/HPs enhanced ($P<0.01$) ROS generation and activation of NF-κB but mitigated redox activity when compared to EVs. GW4869 not only down graded HIV-induced ROS generation and NF-κB activity but also enhanced redox response in HIV milieu. In addition, GW4869 treatment markedly decreased number of ferroptosed podocytes in HIV milieu. TEMPOL also attenuated ROS generation, NF-κB activity and percentage of ferroptosed podocytes in HIV milieu.

Conclusions: HIV enhances SMase activity in kidney cells. HIV-induced activation of podocyte SMase activity contributes to ferroptosis.

Funding: NIDDK Support

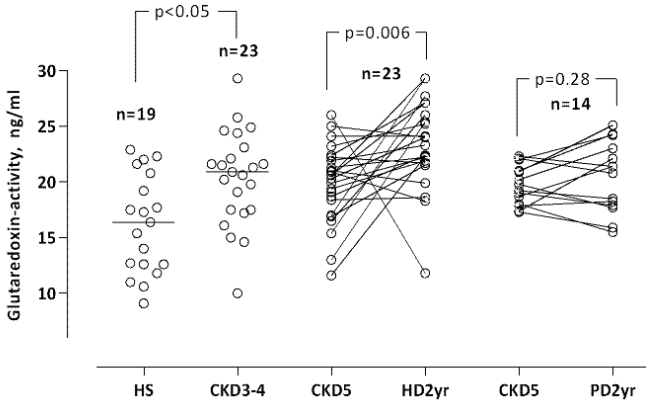
TH-PO220

Plasma Glutaredoxin as a Marker of Oxidative Stress in Chronic Kidney Disease and Dialysis Anna Levin,[1] Annette Bruchfeld,[1] Peter F. Barany,[1] Olof Heimburger,[1] Björn Anderstam,[1] Peter Stenvinkel,[1] Johanna Ungerstedt.[2] *[1]Dept of Clinical Science, Intervention and technique, Karolinska Inst, Stockholm, Sweden; [2]Dept of Medicine, Huddinge, Karolinska Inst, Stockholm, Sweden.*

Background: Oxidative stress is known to play a major role in progression and complications of CKD. Glutaredoxin1 (Grx1) is an intracellular antioxidant, recently shown to be catalytically active also in serum. Grx1 activity levels correlate to disease severity in type 2 diabetes, and high Grx1 activity is associated with abrogated reperfusion after arterial ischemia. In the present study we investigated Grx1 activity in CKD 3-4, at dialysis start and after 2 years of dialysis.

Methods: Grx1 activity was analyzed with a novel fluorescence assay kit (Imco Corp, Stockholm, Sweden). Healthy subjects (HS, n=19), CKD 3-4 (n=23), CKD 5 starting dialysis and after two years of dialysis Hemodialysis (HD, n=23), Peritoneal Dialysis (PD, n=14) were assessed. All samples were analyzed in triplicate.

Results: Grx1 activity was significantly higher in CKD3-4 compared to healthy subjects ($p<0.05$, fig. 1), indicating an oxidized extracellular environment in CKD patients. After two years of dialysis, HD pts had a further increase in Grx1 activity ($p=0.006$, fig. 1), however a subgroup with persistently low Grx1 was identified. PD patients did not increase Grx1 at 2 years suggesting a beneficial effect of PD on extracellular oxidative state. Grx1 activity did not correlate with age (tested in both healthy subjects and CKD 3-4) norwith GFR in CKD 3-5.



Conclusions: Grx1 activity is a marker of oxidized extracellular environment. In the present study we demonstrate higher Grx1 activity in CKD than in healthy subjects. The effect on Grx1 activity seems to differ between dialysis modalities suggesting a more favorable effect in PD. Grx1 may be a useful marker of oxidative state and possibly also risk of ischemic events in CKD.

Funding: Government Support - Non-U.S.

TH-PO221

Constitutive Shedding of Angiotensin-Converting Enzyme 2 from Proximal Tubular Cells Is Mediated by Protein Kinase C Isoforms Delta (Δ) and Epsilon (ε) Fengxia Xiao, Joe A. Zimpelmann, Kevin D. Burns. *Nephrology, Medicine, Kidney Research Centre, OHRI, Univ of Ottawa, Ottawa, ON, Canada.*

Background: Angiotensin-converting enzyme 2 (ACE2) degrades angiotensin (Ang) II to Ang-(1-7), and thereby protects against renal injury. Soluble ACE2 fragments are shed from the proximal tubule, and are found at higher urinary levels in humans with diabetes or

chronic kidney disease. Although high glucose-induced shedding of ACE2 from proximal tubular cells is mediated by a pathway involving a disintegrin and metalloproteinase-17 (ADAM-17), the mechanism for constitutive shedding of ACE2 is unknown.

Methods: Primary cultures of mouse proximal tubular cells were grown to near confluence, and ACE2 shedding into the media was assessed by enzyme activity assay, using an ACE2 fluorogenic substrate in the presence or absence of the ACE2 inhibitor MLN-4760. Cells were incubated with pharmacologic inhibitors, or transfected with siRNA directed at protein kinase C (PKC)-Δ or -ε.

Results: Incubation of proximal tubular cells with increasing concentrations of D-glucose stimulated ACE2 shedding, which peaked at 16 mM ($p<0.006$ versus 4 or 7.8 mM, n=5). Increasing levels of L-glucose (osmotic control) had no effect on shedding. In cells maintained in 7.8 mM D-glucose, constitutive shedding of ACE2 was significantly inhibited by the general PKC antagonist, sotrastaurin (10^{-5} M, 43.8% inhibition, $p<0.001$, n=6), but not by an inhibitor of ADAM-17 (TAPI-1). Incubation of cells with the PKC-α and -β1-specific inhibitor Go6976, the PKC β1 and β2-specific inhibitor ruboxistaurin, inhibitors of matrix metalloproteinases-2,-8, and -9, or an inhibitor of ADAM-10 (GI250423X) had no effect on basal ACE2 shedding (n=4-5). By contrast, transfection of cells with siRNA directed against PKC isoforms Δ or ε reduced ACE2 shedding by 23.2% and 24.1%, respectively, compared to transfection with scrambled siRNA.

Conclusions: These results indicate that constitutive shedding of ACE2 from proximal tubular cells is mediated by PKC isoforms Δ and ε. Targeting these PKC isoforms might maintain membrane-bound ACE2 in proximal tubule in disease states and diminish Ang II-stimulated adverse signaling.

Funding: Government Support - Non-U.S.

TH-PO222

FGF23 Enhances the Release of Nitric Oxide via Secretion of Soluble α-Klotho in Human Vascular Endothelial Cells In Vitro

Maren Leifheit-Nestler, Beatrice Richter, Jacqueline Haller, Wolfgang H. Ziegler, Dieter Haffner. *Dept of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany.*

Background: Endothelial dysfunction is the first detectable manifestation of cardiovascular disease in CKD patients and is associated with increased degradation of secreted nitric oxide (NO) in the presence of reactive oxygen species (ROS). Elevated levels of the phosphaturic hormone fibroblast growth factor 23 (FGF23) correlate with cardiovascular mortality in CKD patients. The role of FGF23 in the pathophysiology of endothelial dysfunction is unclear. The aim of our study was to investigate the impact of FGF23 and its co-factor a-klotho on NO production, and ROS formation in vascular endothelial cells.

Methods: Human coronary artery endothelial cells (HCAEC) were stimulated with FGF23 in a time and dose-dependent manner and evaluated for proliferation, activation of downstream pathways, soluble α-klotho, the α-klotho shedding transmembrane metalloprotease ADAM17, and endothelial NO synthase (eNOS) by western blotting and immunofluorescence, respectively. FGF23-dependent NO production was assessed by the modified Griess assay, and formation of ROS was detected by fluorescence microscopy and flow cytometry.

Results: FGF23 induces proliferation of HCAEC. In HCAEC, FGF23 activates FGFR1, and increases NO production, as well as eNOS phosphorylation and dephosphorylation of S1177 and T495, respectively in a dose-dependent fashion. FGF23 stimulates the release of soluble α-klotho and enhances glycosylated ADAM17 protein expression. ADAM17 and α-klotho are co-localized in HCAEC. The NO stimulating effect of FGF23 is blunted by co-incubation of HCAEC with a klotho neutralizing antibody, and FGF23-dependent activation of eNOS is decreased after klotho inhibition. FGF23 has no impact on ROS production under physiological conditions, *in vitro*. Klotho neutralization slightly enhances ROS production. In an angiotensin induced stress model, FGF23 prevents HCAEC from oxidative stress, demonstrated by reduced ROS.

Conclusions: FGF23 stimulates NO release via secretion of soluble α-klotho, and protects HCAEC from angiotensin-induced ROS production.

TH-PO223

$1,25(OH)_2D_3$ Promotes High Glucose Induced Macrophage Activation from M1 to M2 via VDR-PPARγ Signalling Pathway in RAW264.7 Cells

Min Zhou, Yinfeng Guo, Zhixia Song, Xiaoliang Zhang. *Nephrology, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Macrophage, especially its activation state closely relates to the progression of diabetic nephropathy. Classically activated macrophages (M1) are proinflammatory effectors, while alternatively activated macrophages (M2) exhibit anti-inflammatory properties.PPARγ, a nuclear receptor, is essential for macrophage polarization, and $1,25(OH)_2D_3$ has renoprotective roles that extend beyond the regulation of mineral metabolism. This study tries to investigate the effect of $1,25(OH)_2D_3$ on glucose induced macrophage activation and whether this is associated with VDR-PPARγ pathway.

Methods: RAW264.7 cells were used to perform cell culture, activity of intracellular iNOS was measured.VDR siRNA and PPARγ antagonist pre-treatment with macrophages were done before using 10^{-8}M $1,25(OH)_2D_3$ to intervene high glucose treated macrophages. TNF-α, IL-12 and IL-10 in supernatant, M1 marker iNOS, M2 markers including ARG-1, MRC and nuclear receptors, VDR and PPARγ were examined.

Results: The iNOS activity was increased in a glucose-dose and time dependent manner. Particularly, 25mM glucose at 24h gave the maximum response. After being treated with 25mM glucose for 24h, not only inflammatory cytokines of TNF-α, IL-12 were increased, but PCR and Western blot analysis showed iNOS was also upregulated ($P<0.05$). However, M2 markers, i.e. IL-10, ARG-1 and MRC were decreased ($P<0.05$). When in the presence of $1,25(OH)_2D_3$, the trends were reversed: the markers of M1, including TNFα, IL-12 and iNOS were reduced by 17, 39 and 77%, while the markers of M2, IL-10, ARG-1 and MRC were increased by 192, 180 and 39% respectively. In addition, VDR and PPARγ were also increased ($P<0.05$). However, the above effects of $1,25(OH)_2D_3$were abolished when inhibited the expression of VDR and PPARγ by VDRsiRNA and PPARγ antagonist. Besides, PPARγ was also decreased upon treatment with VDRsiRNA ($P<0.05$).

Conclusions: $1,25(OH)_2D_3$ can promote high glucose induced classically activated macrophages (M1) convert to alternatively activated macrophages (M2) and this is achieved through VDR-PPARγ pathway.

Funding: Government Support - Non-U.S.

TH-PO224

Vitamin D Receptor Dynamics Modulates Tubular Cell Phenotype during Low and High Expression of p53 via mdm2

Shabirul Haque, Nirupama Chandel, Kamesh R. Ayasolla, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Mdm2 induces transcriptional repression and proteosomal degradation of p53, a tumor suppressor gene. P53 plays a bimodal role in cellular injury-enhanced expression is known to stimulate apoptosis via phosphorylation of p66ShcA and attenuated expression is associated with the activation of proliferation via mTOR pathway. We hypothesized that vitamin D receptor (VDR) dynamics will not only modulate tubular cell p53 expression through alteration of mdm2 but would also alter tubular cell phenotype.

Methods: To achieve high and low expressoin of p53 in tubular cells, primary human proximal tubular epithelial cells (HPTCs) were either transfected with p53 plasmid (p53/HPTC) or p53-siRNA (si-p53/HPTC) followed by Western blot analysis for p53 expression. Control (C)/HPTCs, p53/HPTCs, and si-p53/HPTCs were evaluated for tubular cell phenotype by morphologic assays for proliferation and apoptosis. To evaluate the involved molecular mechanisms, protein blots of C/HPTCs, p53/HPTCs and si-p53/HPTCs were probed for phospho-p66ShcA, phospho-mTOR, mdm2 and reprobed for actin. To evaluate relationships amongst VDR, mDm2, and p53, protein blots of VDR agonist treated-p53/HPTCs and si-p53/HPTCs were evaluated for mdm2 and p53 expressions. C/HPTCs and VDR knockout HPTCs was probed for mdm2 expression.

Results: p53/HPTCs displayed enhanced phosphorylation of p66ShcA, associated downstream signaling and induction of tubular cell apoptosis when compared to C/HPTCs; whereas, si-p53/HPTCs displayed enhanced phosphorylation of mTOR, associated downstream signaling and increased number of PCNA +ve cells when compared to C/HPTCs. p53 expression displayed inverse relationship with tubular cell expression of mdm2 both in high and low expressing p53 cells. Treatment of p53/HPTCs and si-p53/HPTCs with a VDR agonist not only reversed the expression of p53 and mdm2 but also restored tubular cell phenotype. Since VDR knockout HPTCs displayed attenuated expression of mdm2, it appears that VDR determines the expression of mdm2.

Conclusions: VDR dynamics determines tubular cell phenotype during high and low p53 states through modulation of mdm2.

Funding: NIDDK Support

TH-PO225

Impaired Cell-Cell Singalling via microRNA-126 Represents a Novel Pathomechanism in Asymmetric Dimethylarginin Induced Endothelial Dysfunction

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Background: Asymmetric Dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), is elevated in patients with chronic kidney disease and is known to contribute to endothelial dysfunction. We investigated the role of microRNA-126 signalling through exosomes, which is essential for endothelial development and biology, in ADMA induced endothelial dysfunction.

Methods: We measured ADMA-Plasma levels in 39 patients with coronary artery disease (CAD) by ELISA assay and correlated them with circulating miR-126 levels assessed by qRT-PCR. We infused ADMA into healthy rats (250μMol/kg/day) and healthy human volunteers (0.1mg/kg/min, 40minutes) and quantified plasma levels of different microRNAs. Stimulating HUVECs in vitro with ADMA, we quantified extra-and intracellular levels of miR-126. Furthermore we used another eNOS-Inhibitor L-NAME. Immunefluorescence staining and confocal microscopy was performed to detect multivesicular bodies (MVB) and exosomes after ADMA/L-NAME treatment. We performed exosome isolation and characterization.

Results: Plasma levels of ADMA in patients with CAD correlated inversely with levels of circulating levels of miR-126 ($r=-0.52$; $p<0.001$). ADMA infusion reduced circulating levels of miR-126 in rats and significantly also in healthy human volunteers. Time dependent alteration of extra- (decrease) and intracellular (increase) amounts of miR-126 was detectable in vitro. Stimulation with L-NAME showed a significant reduction of extracellular levels of miR-126. Confocal microscopy of CD-63 positive MVBs and exosomes revealed an obvious change in MVB structure due to fusion and/or swelling of the vesicles. This was detectable after ADMA and L-NAME stimulation. HUVEC derived exosomes exhibited typical characteristics.

Conclusions: MiR-126 signalling via exosomos, which is known to convey alarm signals to injured endothelium and thereby contribute to repair mechanisms and cellular survival, is impaired by ADMA. This represents a novel mechanism how ADMA could contributes to endothelial function.

TH-PO226

Podocyte Microparticles: Mechanisms of Formation and Downstream Effects on Proximal Tubular Epithelial Cells Dylan Burger, Shareef Akbari, Maddison Turner. *Kidney Research Centre, Ottawa Hospital Research Inst, Ottawa, ON, Canada.*

Background: Microparticles (MPs, also known as microvesicles) are 0.1-1.0 μm phospholipid vesicles shed from membranes of stressed/injured cells. We recently reported that podocytes produce MPs (pMPs) in response to high glucose and that pMPs may be found in the urine of diabetic mice in advance of albuminuria. In the present study we assessed the role of Rho kinase (ROCK) and ATP binding cassette A1 (ABCA1) in pMP formation and determined whether pMPs interact with proximal tubular cells and influence function.

Methods: Conditionally immortalized human podocytes (HPODs) were exposed to 25 mM glucose in the presence and absence of fasudil (ROCK inhibitor, 10 μM) or PSC833 (ABCA1 inhibitor, 5μM). Mannitol served as an osmotic control. pMPs were enumerated by flow cytometry (Annexin V labeling). pMPs were isolated from media and cultured human proximal tubular epithelial cells were exposed to pMPs (10 μg/ml). MP-cell interaction was examined by fluorescence microscopy and effects on intracellular signaling and fibronectin expression were examined by Western blot. Reactive oxygen species (ROS) production was assessed by lucigenin chemiluminescence.

Results: High glucose treatment increased pMP formation at 24 hours (~2.5-fold, $P<0.05$, n=3). Treatment with fasudil or PSC833 blocked high glucose-induced pMP formation ($P<0.001$, $P<0.05$ respectively). In proximal tubular cells, fluorescence microscopy revealed cell surface binding of pMPs, suggesting a paracrine effect. Treatment with pMPs increased p38 phosphorylation (~3 fold) after 30 minutes ($P<0.05$, n=4). By contrast, JNK and ERK phosphorylation levels were unchanged over 24 hours. Expression of fibronectin was increased following 72 hour treatment with pMPs ($p<0.05$, n=6). Proximal tubular ROS production was increased by pMP treatment at 16 and 24 hours ($P<0.05$, n=4).

Conclusions: Our results suggest that pMP formation involves activation of ROCK and ABCA1. Once formed, pMPs interact with proximal tubular epithelial cells and induce intracellular signaling, increased synthesis of matrix proteins, and ROS production. Such effects may play a role in the development of tubular injury in glomerular disease.

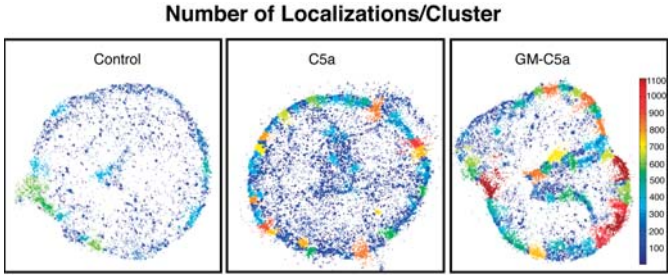
TH-PO227

Neutrophil Activation Organizes Leukotriene Synthetic Enzymes Into Supramolecular Nanoclusters Angela Bair Schmider,[1] Hunter Elliott,[2] Roy J. Soberman.[1] *[1]Nephrology, Massachusetts General Hospital/Harvard Medical School, Boston, MA; [2]Cell Biology, Harvard Medical School, Boston, MA.*

Background: The recruitment and activation of neutrophils (PMN) is a prominent component of tissue injury in inflammatory kidney diseases. The chemotactic lipid, leukotriene (LT)B_4 plays a non-redundant role in the initial recruitment of neutrophils from the vasculature, and controls the participation of these cells in the key pathological process of swarming. We previously identified a critical structure, the LT biosynthetic complex. The core members of this structure are the enzyme 5-lipoxygenase (5-LO) and its nuclear membrane scaffold protein, 5-lipoxygenase-activating protein (FLAP). The latter presents arachidonic acid (AA) to 5-LO.

Methods: We paired single molecule superresolution microscopy (*S*tochastic *O*ptical *R*econstruction *M*icroscopy; STORM) with cluster analysis to show the reorganization of 5-LO and FLAP into supramolecular nanoclusters on the nuclear envelope of mouse PMN in response to cell "priming" by GM-CSF and activation by C5a. These processes are known to stimulate and augment LTB_4 synthesis. STORM data is generated as a "map" of intensity signals and presented as a pseudo image based on defined parameters. It does not query the relationship between individual molecules in terms of groups. To test the hypothesis that LT synthetic complexes were organized into larger groups, we developed cluster analysis algorithms to analyze primary STORM data.

Results: The activation of PMN by C5a results in reorganization of FLAP and 5-LO into supramolecular nanoclusters. Priming with GM-CSF augmented this process.



Conclusions: We have identified novel supramolecular complexes of LT synthetic enzymes that are likely to play a major regulatory role in PMN activation and have developed and employed a novel approach for analysis of single molecule fluorescence data.

Funding: NIDDK Support

TH-PO228

Selective Proximal Tubule Injury Causes Interstitial Fibrosis, Distal Tubule Impairment, and Proteinuria Koji Takaori,[1] Jin Nakamura,[1] Tadashi Yamamoto,[2] Motoko Yanagita.[1] *[1]Nephrology, Kyoto Univ, Kyoto, Japan; [2]Structural Pathology, Niigata Univ, Niigata, Japan.*

Background: Recently we clarified that renal fibroblasts including erythropoietin (Epo) producing cells transdifferentiate into myofibroblast and predominantly contribute to fibrosis, with concomitant loss of Epo production in the diseased kidney. It remains unclear, however, what triggers the transdifferentiation of fibroblasts to myofibroblasts and how proximal tubule injury affects other segment of the nephron.

Methods: For in vitro analysis, we utilized co-culture of renal fibroblasts and tubular epithelial cells. For in vivo analysis, we utilized *N-myc downstream-regulated gene-1 (Ndrg1)CreERT2* inducible simian diphtheria toxin receptor (DTR) transgenic mice (*Ndrg1CreERT2:iDTR* mice) in which Cre-mediated excision of a STOP cassette is achieved after the administration of tamoxifen, and renders *proximal tubule*s sensitive to diphtheria toxin (DT). Furthermore, we utilized *Uterine sensitization-associated gene-1 (U.S.A.G-1) LacZ* mice in which LacZ is expressed in distal tubules for distal tubule cell sorting.

Results: First, we confirmed the expression of DTR in almost all proximal tubules in the kidney of *Ndrg1-CreERT2:iDTR* mice. A single DT injection causes proximal tubule injury, interstitial fibrosis, and distal tubule injury. While electron microscopy examinations reveal the normal glomerular structure, massive proteinuria was observed after the injection of DT. Repeated DT injections caused severer interstitial fibrosis, while most glomeruli remained intact. We also confirmed the induction of collagen expression in fibroblasts when co-cultured with damaged tubular epithelial cells.

Conclusions: Our data provide the new evidence that selective proximal tubule injury induces the transdifferentiation of fibroblast as well as distal tubule injury. Our data also demonstrates that dysfunction of proximal tubules leads to albuminuria. These results indicate the importance of protecting tubule epithelial cells to suppress kidney disease progression. Further understanding of the crosstalk between proximal tubule and other cellular components in the kidney will give us new insight into the mechanism of kidney disease progression.

TH-PO229

The Small Heterodimer Partner SHP Attenuates Renal Inflammation Through Inhibition of ROS Production in Ischemia Reperfusion Injury Jung Sun Park,[1] Hoon In Choi,[1] Eun Hui Bae,[2] Seong Kwon Ma,[2] Jong Un Lee,[1] Soo Wan Kim.[2] *[1]Physiology, Chonnam National Univ Medical School, Gwangju, Chonnam, Korea; [2]Kidney, Chonnam National Univ Hospital, Gwangju, Chonnam, Korea.*

Background: The orphan nuclear receptor, small heterodimer partner (SHP; NR0B2), appears to play a negative regulatory role in innate immune responses and contribute to various inflammatory signaling and transcriptional regulation. We investigated whether SHP attenuates renal inflammation in ischemia reperfusion (I/R) and H2O2-induced kidney injury in human proximal tubular (HK2) cells.

Methods: Mouse I/R injury was induced by clamping both renal pedicle for 30 min, and sacrificed 24 hr later. In vitro study, human proximal tubular (HK2) cells were incubated with H2O2 300 μM for 6 hr. Cell viability was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.The fluorescent probe 2',7'-dichlorofluorescein diacetate was used to measure intracellular levels of reactive oxygen species (ROS). The protein expression of NF-κB, mitogen-activated protein kinase (MAPK), iNOS, COX-2, TNFa, and IL-1β was determined by semiquantitative immunoblotting.

Results: I/R kidney injury and H2O2 treatment in HK2 cells decreased cell viability, increased production of ROS and induced expression of inflammatory proteins such as iNOS, COX-2, TNFa, and IL-1β. I/R kidney injury and H2O2 treatment in HK2 cells induced the activation of MAPK signal pathway and NF- κB signal pathway, while reduced the activation of Nrf-1 and Nrf-2. Transfection of SHP increased cell viability and prevented the IR injury and H2O2-induced ROS production as well as reduced expression of inflammatory proteins. The pretreatment of MAPK inhibitors (PD980590, SB203580) and NF-κB inhibitor (Bay11-7082) counteracted activation of the Nrf-1 and Nrf-2 signal pathway and induced SHP promoter activity. Accordingly, ROS scavenger (N-acetyl cysteine, NAC) prevented the IR injury and H2O2-induced ROS production and increased of the Nrf-1 and Nrf-2 activation and induced SHP promoter activity.

Conclusions: These findings suggest that SHP attenuates I/R and H2O2-induced kidney injury by counteracting inflammatory response through inhibition of NF-κB and MAPK signal pathway.

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TH-PO230

Cytosolic Phospholipase A2 Derived Eicosanoids Regulate the Epithelial Phenotype of Human Proximal Tubular Epithelial Cells John Ross Montford, Seth B. Furgeson, Raphael A. Nemenoff. *Renal Disease and Hypertension, Univ of Colorado Anschutz Medical Campus, Aurora, CO.*

Background: Renal fibrosis represents the final common pathway in the development of progressive chronic kidney disease (CKD). Cytosolic phospholipase A2 (cPLA2) is the rate limiting enzyme in the production of eicosanoids that regulate a host of physiologic processes and have been implicated in renal fibrosis. The goal of this study was to assess the role of cPLA2 in regulating the epithelial phenotype of proximal tubule cells.

Methods: Human proximal tubular epithelial (HK-2) cells were silenced for cPLA2 using lentiviral shRNA constructs. Expression of the epithelial marker E-cadherin, and

mesenchymal markers N-cadherin, vimentin, and αSMA were measured by Western blotting for protein and by qRT-PCR for mRNA. Epithelial to mesenchymal transformation (EMT) was induced via administration of TGF-β (10ng/ml). In addition, prostaglandin E2 (PGE2) at various concentrations was administered to the non-silenced and cPLA2 silenced shRNA clones. For measurement of promoter activity cells were transfected with E-cadherin luciferase constructs and transfection efficiency normalized to b-galctosidase.

Results: cPLA2 silenced cells demonstrated altered morphology and higher expression of E-cadherin protein and mRNA with lower levels of mesenchymal markers at baseline. This effect was associated with increased E-cadherin promoter activity, suggesting transcriptional regulation. Exposure of cells to TGF-β induced EMT in control cells, characterized by suppression of E-cadherin and induction of mesenchymal markers. These effects were blunted in cells silenced for cPLA2. PGE2, an eicosanoid produced by proximal tubular epithelial cells, reversed the effects of $cPLA_2$ silencing and promoted a more mesenchymal phenotype.

Conclusions: cPLA2 derived eicosanoids regulate the epithelial phenotype of human proximal tubular epithelial cells. This effect is mediated, at least in part, by the inhibition of PGE2 expression. These data indicate that targeting eicosanoid pathways will have effects on EMT in proximal tubule cells, and provides an additional mechanism for the effects of these agents in renal fibrosis.

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TH-PO231

Ste20-Like Kinase, SLK, Activates the Hsp70 Pathway Andrey V. Cybulsky, Julie Guillemette, Joan Papillon. *Medicine, McGill Univ, Montreal, QC, Canada.*

Background: Expression and activation of SLK increases during renal ischemia-reperfusion injury. When highly expressed, SLK signals via c-Jun N-terminal kinase and p38 to induce apoptosis, and it exacerbates apoptosis induced by ischemia-reperfusion injury. Overexpression of SLK in glomerular epithelial cells (GEC)/podocytes in vivo induces injury and proteinuria. In response to various stresses, cells enhance expression of chaperones or heat shock proteins (e.g. Hsp70), which are involved in the folding and maturation of newly synthesized proteins, and can refold denatured or misfolded proteins. We address the interaction of SLK with Hsp70.

Methods: cDNAs and shRNAs were expressed in GEC and COS-1 cells by transient transfection. To induce in vitro ischemia-reperfusion injury, cells were incubated with 2-deoxyglucose+antimycin A, followed by re-exposure to glucose (anoxia/recovery). Heat shock involved incubation at 42°C followed by recovery at 37°C.

Results: Increased expression of SLK (following transfection) induced Hsp70. Exposure of cells to anoxia/recovery also induced Hsp70, and this effect was amplified by SLK overexpression. Hsp70 is regulated at the transcriptional level by heat shock factor (HSF)-1. SLK stimulated HSF1-luciferase reporter activity. Moreover, HSF1-luciferase reporter activity was induced by anoxia/recovery and heat shock, and in both instances was further amplified by SLK overexpression. To address the requirement for SLK kinase activity, we employed a fusion protein consisting of the SLK catalytic domain (amino acids 1-373) and a modified FK506 binding protein, Fv (Fv-SLK 1-373). Addition of AP20187 (a FK506 analog) stimulated dimerization and kinase activity of Fv-SLK 1-373, leading to an increase in HSF1-luciferase reporter activity. Transfection of constitutively active HSF1 enhanced Hsp70 expression and inhibited SLK-induced apoptosis. Conversely, the proapoptotic action of SLK was augmented by HSF1 shRNA, or the Hsp70 inhibitor, pifithrin-μ.

Conclusions: Increased expression/activity of SLK activates the HSF1-Hsp70 pathway. Hsp70 attenuates the primary proapoptotic effect of SLK. Modulation of chaperone expression may potentially be harnessed as cytoprotective therapy in renal ischemia-reperfusion injury.

Funding: Government Support - Non-U.S.

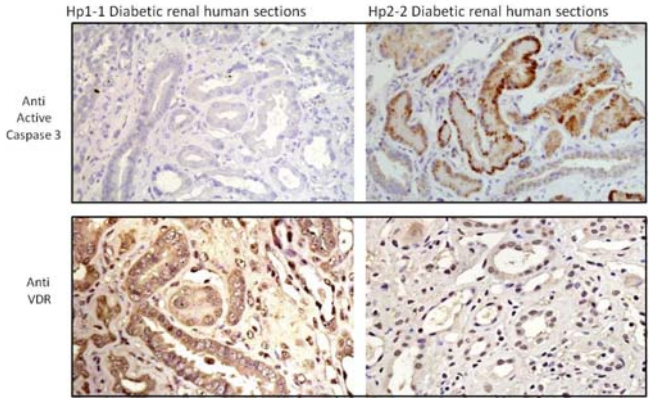
TH-PO232

Haptoglobin 2-2 Genotype Is Associated with Decreased Levels of Active Vitamin D, Accelerated Renal Apoptosis and Injury in Diabetic Nephropathy Mice and Patients Farid M. Nakhoul,[1,2] Nadia Thawho,[2] Farber Evgeny,[1] Rabea Asleh,[3] Andy Levy,[3] Inbal Dahan.[2] *[1]Poriya Medical Center; [2]Diabetic Nephropathy Lab, Poriya Medical Center, Lower Galilee; [3]Vascular Biology lab Technion, Haifa.*

Background: Diabetic mice with different Haptoglobin (Hp) genotype (1-1, 2-2) have a different susceptibility to developed Diabetic Nephropathy (DN). Hp 2-2 diabetic mice have impaired Hb clearance and increased iron depositions in the lysosomes of kidney proximal tubules (PCT) compared with Hp 1-1diabetic mice. Patients and mice with chronic kidney disease have low levels of active vitamin D and decrease expression of the anti-oxidant- klotho. We are exploring the influence of the Hp genotype and klotho expression on active vit. D deficiency in DN mice and patients.

Methods: All patients screened for Hp genotyping. Slides from kidney biopsies of DN patients and mice with different Hp genotype (1-1, 2-2) were subjected to immunohistochemistry staining of iron, active caspase 3, vitamin D receptor (VDR) and 1-alpha hydroxylase by using specific antibodies. Blood samples subjected to laboratory evaluation and ELISA assays.

Results: 1. There were increased iron deposits in the renal lysosomal PCT of Hp 2-2 DN patients, similarly to what we have demonstrated in mice with Hp 2-2. **2.** In the PCT of Hp 2-2 mice and patients there was increase expression of active caspase-3 that was accompanied with decrease levels of klotho and decrease expression of renal VDR.



Conclusions: 1. Hp 2-2 genotype in mice and patients is associated with increased iron deposits in renal PCT, decrease levels of klotho and VDR and high levels of PCT apoptosis. **2.** The decreased levels of the anti oxidant klotho and the increase levels of PCT apoptosis, predispose to vitamin D deficieny and DN progression. **3**. Active vitamin D deficiency correlates with sever renal damage in DN patients with Hp 2-2 genotype.

TH-PO233

Pro-Fibrotic Role of Tumor Suppressor ATM Downstream of TGF-beta1 in Orchestrating Maladaptive Renal Repair Jessica Overstreet,[1] Diana Cardona-Grau,[2] Samik H. Patel,[1] Rohan Samarakoon,[1] Roel Goldschmeding,[3] Paul J. Higgins.[1] *[1]Center for Cell Biology and Cancer Research, Albany Medical College, Albany, NY; [2]Div of Urology, Albany Medical Center, Albany, NY; [3]Dept of Pathology, Univ Medical Center Utrecht, Netherlands.*

Background: Ataxia telangiectasia mutated (ATM) has been implicated in the oxidative stress response and acute ischemic renal injury. However, the mechanistic involvement of ATM in the TGF-β1 pathway related to renal fibrosis is largely unknown.

Methods: The unilateral ureteral obstruction (UUO) mouse model and genetic and pharmacological approaches were used to investigate ATM function in TGF-β1-induced pro-fibrotic responses in renal epithelial cells (HK-2) and fibroblasts (NRK-49Fs).

Results: Increased activation of ATM ($pATM^{Ser1981}$) in the tubulointerstitial region of the UUO kidney of mice correlated with SMAD3 and p53 phosphorylation and pro-fibrotic gene expression. ATM is rapidly phosphorylated at Ser^{1981} by TGF-β1 stimulation. Stable silencing and pharmacological inhibition of ATM ablated TGF-β1-induced p53 activation and PAI-1, fibronectin and p21 expression while completely relieving TGF-β1-stimulated proliferative arrest in HK-2 cells. Interestingly, stable gene depletion of the NADPH oxidase (NOX) subunits, $p22^{phox}$ and $p47^{phox}$ blocked TGF-β1-induced ATM activation and target gene induction via p53-SMAD3 dependent mechanisms. Furthermore, increased fibroblast proliferation promoted by addition of conditioned media from TGF-β1-stimulated mock transduced/control HK-2 cells were not detected by the stimulation of conditioned media derived from ATM-depleted cells treated with TGF-β1.

Conclusions: TGF-β1 promotes ATM activation via redox/NOX-dependent mechanisms leading to p53-dependent pro-fibrotic gene expression and growth inhibition in HK-2 cells. TGF-β1/ATM-initiated tubular dysfunction promotes fibroblast growth suggestive of crucial role of ATM in epithelial-mesenchymal cross-talk in fibrosis.

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TH-PO234

Targeting of ROCK1 Protects Mice From Hyperlipidemia-Induced Glomerlosclerosis By Improving Podocyte Lipophagy Yangyang Zuo, Wenjian Wang, Lei Fu, Jianteng Xie, Jianxun Wu, Jing Li, Yuxiong Lai, Wei Shi. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, Guangdong, China.*

Background: Foam cells and lipid deposits contributes to glomerulosclerosis. Intrestingly, the trapping of lipid-laden podocytes is a critical but reversible step in glomerulosclerosis. Recently, lipophagy, an alternative pathway of lipid degradation through the lysosome is observed in several cell lines. However, whether and how lipophagy is involved in the progression of lipid-induced nephropathy remains unclear.

Methods: To verify Rho kinase(ROCK) pathway plays a citical role in lipophagy, we inhibited and induced the expression of ROCK in podocytes,which were treated with oxidative low density lipoprotein in vitro. And a further study was carried out on ROCK deficient mice model.

Results: Overexpression of ROCK increase of lipid retention in oxidative low density lipoprotein treated podocytes, which consequently activates, and results in a marked increases of reactive oxygen species production. Interestingly, this process is reversed by ROCK inhibition evidenced by increased lipophagy which associated to low phosphorylated Akt and actin-related protein 2 levels. Furthermore, targeted deletion of ROCK1 in high fat diet mice exhibits a significantly ameliorated albuminuria and mesangial matrix accumulation compared to control. ROCK deficient mice with high fat diet demonstrated a marked decrease of lipid retention and improved lipophagy in the kidney.

Conclusions: Our data suggests a previously unknown contribution of ROCK to lipid-induced nephropathy. Constitutive inhibition of ROCK may be a major protective strategy to ameliorate lipid-overload in podocyte and lipid-related loss of renal function.

Funding: Government Support - Non-U.S.

TH-PO235

Inflammasomal Upregulation in a Mouse Model of Hepatorenal Syndrome Type 1 Hiba Sheikh, Sam Righi, Essa Abuhelaiqa, Siddhartha S. Ghosh, Daniel E. Carl. *Dept of Internal Medicine-Div of Nephrology, Virginia Commonwealth Univ Health System, Richmond, VA.*

Background: Hepatorenal Syndrome Type 1 (HRS-1) manifests when an acute insult leads to renal vasoconstriction and secondary impairment of glomerular filtration in the setting of liver disease. The aim of this study is to pioneer a mouse model for HRS-1 that would aid in the identification of inflammatory markers, specifically at the level of the inflammasome, which influence the pathophysiology of HRS-1 and serve as potential therapeutic targets.

Methods: C57bl mice received 1mL/kg of CCl4 biweekly for 12 weeks induce liver cirrhosis confirmed by histopathology and echocardiography. A pre-standardized dose of Lipopolysaccharide (LPS) was given intraperitoneally to control and CCl4-treated mice to induce acute kidney injury by simulating the inflammatory stressor caused by acute infection. 4 mouse populations were studied: (1)control mice (2)control mice injected with LPS (3) CCL4 treated mice (4) CCL4 treated mice with LPS (N=6 per group). Renal function was monitored by measuring urine output, urinary sodium, and serum creatinine measured at 4,6, and 12 weeks post injection. The mouse kidneys were then harvested and analyzed by western blot for the presence of inflammasomal components: xanthine oxidase, caspase 1, and ASC.

Results: The same dose of LPS in control animals had no effect on renal function. The CCl4+LPS treated mice had a significant decrease in urine volume and urinary Na ($p<0.05$) and marked increase in creatinine ($p<0.05$) compared to the other 3 mouse groups. Both xanthine oxidase and caspase 1 were significantly elevated ($p<0.05$) in the CCL4+LPS treated group compared to the CCL4 mice. ASC was significantly elevated ($P<0.05$) in CCL4+LPS mice compared to the other 3 mouse populations.

Conclusions: In the CCL4-induced cirrhotic mouse model, we found an increase in the expression of xanthine oxidase and Caspase 1 in the presence of a stressor such as LPS. This translates to an increase in downstream pro-inflammatory markers such as reactive oxygen species and IL1β. ASC was elevated in the CCL4+LPS mouse population only suggesting the activation of specific markers exclusively in HRS.

TH-PO236

Activation of the Nlrp3 Inflammasome by Mitochondrial Reactive Oxygen Species: A Novel Mechanism of Albumin-Induced Tubulointerstitial Inflammation Dan Liu, Min Xu, Linli Lv, Bi-Cheng Liu, Kun Ling Ma. *Inst of Nephrology, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Albuminuria is not only an important marker of chronic kidney disease, but also a crucial contributor to tubulointerstitial inflammation (TIF). Here we determine whether activation of the Nlrp3 inflammasome is involved in albuminuria induced-TIF and the underlying mechanisms of inflammasome activation by mitochondrial reactive oxygen species (mROS).

Methods: We established an albumin-overload induced rat nephropathy model. The adult male Wistar rats that were uninephrectomized or sham operated under anesthesia 5 days before starting bovine serum albumin (BSA) injection. In vitro, tubular epithelial cell line (HK-2) was cultured with or without Nlrp3 gene siRNA transfection and then stimulated with BSA for different time durations (6h, 12h, 24h) and concentrations (5, 10, 20 mg/ml). Cell lysates and supernatants were collected and determined by Western blotting and ELISA. IgA nephropathy biopsy samples were used for Nlrp3 and IL-18 detection by immunohistochemistry.

Results: The experimental rats were characterized by albuminuria, renal infiltration of inflammatory cells, tubular dilation and atrophy with albumin-overload. Renal expressions of the Nlrp3 inflammasome, IL-1β and IL-18 were significantly increased in this animal model. Invitro, albumin time- and dose-dependentlyincreased expression of the Nlrp3 inflammasome, IL-1β, and IL18. Moreover, silencing of the Nlrp3 gene or use of caspase-1 inhibitor, Z-VAD-fmk, significantly attenuated albumin induced IL-1β and IL-18 expression in HK2 cells. In addition, mROS generation was elevated by albumin stimulation, whereas the ROS scavenger, N-acetyl-L-cysteine (NAC) inhibited Nlrp3 expression and release of IL-1β andIL-18. In kidney biopsy specimens from patients with IgA nephropathy, Nlrp3 expression localized to the proximal tubular epithelial cells, which closely correlated with the extent of proteinuria and TIF.

Conclusions: This study demonstrates that albuminuria might serve as an endogenous danger associated molecular pattern (DAMP) that stimulates TIF via mROS-mediated activation of the cytoplasmic Nlrp3 inflammasome.

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TH-PO237

Indoxyl Sulfate Promotes Modulation of CD14+/CD16+ Inflammatory Monocytes and Increases ROS Generation Andrea Novais Moreno-Amaral,[1] Natalia Borges Bonan,[1] Gabriela Ferreira Dias,[1] Lia S. Nakao,[2] Fellype C. Barreto,[1] Roberto Pecoits-Filho.[1] *[1]School of Medicine, PPGCS, PUCPR, Brazil; [2]Basic Pathology Dept, UFPR, Brazil.*

Background: Circulating monocytes can be modulated into an intermediate CD14+/CD16+ inflammatory phenotype. Here we evaluate the monocyte modulation induced by the uremic toxin indoxyl sulfate (IS). In addition, we aimed to analyze the capacity of these cells to generate ROS.

Methods: Monocytes from 6 healthy controls (HC) were isolated from whole blood. Cells were incubated for 24h with N-acetyl cysteine (5mM NAC) before 24h incubation with RPMI 2% FBS, IS (0,6, 53 and 236 mg/L) or LPS+IFN-γ (25ng/ml+150U/ml). Cells were stained with anti-CD14-PE and anti-CD16-FITC or with DCFH-DA and analyzed by flow cytometry.

Results: IS induced monocytes modulation in a dose-dependent manner. The highest IS concentration (236 mg/L) promoted 46±10% of CD14+/CD16+-positive monocytes compared to 7±4.7% in control cells (RPMI 2% FBS). IS also showed high ability to induce ROS generation by inflammatory monocytes in dose-dependent manner (18±9.5% with 0.6mg/L; 37±17.5% with 53mg/L; and 81±16.9% with 236 mg/L, compared with control cells 5±1.1%). This was similar when compared to cells treated with LPS+IFN-γ (91±8.8%). Both IS effects on HC monocytes were inhibited in presence of NAC. Monocyte modulation was significantly decreased by NAC (IS 236 mg/L promoted 4±1.2% of CD14+/CD16+ positive cells) and ROS generation was decreased to 12±3.1%, 27±15.3% and 35±16.4% in an IS dose response assay.

Conclusions: Our results show that IS was able to modulate monocytes to assume an inflammatory phenotype capable of ROS generation, and that theses response was attenuated by NAC.

TH-PO238

SIRT2 Ameliorates Lipopolysaccharide-Induced Inflammation in Macrophages Won Kim, Yujin Jung, Dal Kim, Kyung Pyo Kang, Sik Lee, Sung Kwang Park, Tung Nguyen-Thanh, Aesin Lee. *Dept of Internal Medicine, Chonbuk National Univ Medical School, Jeonju, Republic of Korea.*

Background: SIRT2 is a NAD(+)-dependent deacetylases and associated with numerous processes such as infection, carcinogenesis, DNA damage and cell cycle regulation. However, the role of SIRT2 in inflammatory process in macrophage remains unclear.

Methods: In the present study, we have evaluated the regulatory effects of SIRT2 in lipopolysaccaride (LPS)-stimulated macrophages isolated from SIRT2 knockout (KO) and wild type (WT) mice or Raw264.7 macrophage cells. As inflammatory parameters, expression of inducible nitric oxide synthase (iNOS), the productions of nitric oxide, reactive oxygen species (ROS) and M1-macrophage-related factors were evaluated. We also examined the effects of SIRT2 on activation of nuclear factor-kappaB signaling.

Results: SIRT2 deficiency inhibits LPS-induced iNOS mRNA and protein expression in bone marrow derived macrophages. SIRT2-siRNA transfection also suppressed LPS-induced iNOS expression in Raw264.7 macrophage cells. Bone marrow derived macrophages isolated from SIRT2 KO mice produced lower nitric oxide and expressed lower levels of M1-macrophage related markers including iNOS and CD86 in response to LPS than WT mice. Decrease of SIRT2 reduced the LPS-induced reactive oxygen species production. The phosphorylation of p65 was significantly decreased in SIRT2-deficient macrophages after LPS stimulation.

Conclusions: Our data suggested that deficiency of SIRT2 ameliorates iNOS, NO expression and reactive oxygen species production with suppressing LPS-induced phosphorylation of p65 in macrophages.

Funding: Government Support - Non-U.S.

TH-PO239

Recombinant Pentraxin-2 Directly Protects Human Proximal Tubular Epithelial Cells Tubular From Injury During Cell Stress Naoki Nakagawa,[1] Ivan G. Gomez,[1,2] Allie M. Roach,[1,2] Bryce Gordon Johnson,[1,2] Mark L. Lupher,[3] Jeremy Stuart Duffield.[1,2] *[1]Renal Div, Univ of Washington, Seattle, WA; [2]Biogen Idec, Cambridge, MA; [3]Promedior Inc., Boston, MA.*

Background: Pentraxin-2 is a naturally produced circulating plasma protein involved in innate immunity. We have previously shown that recombinant human Pentraxin-2 (rhPTX-2) therapy attenuates the progression of Alport nephropathy in Col4a3 deficient mice and rhPTX-2 is distributed not only to macrophages but also to proximal tubular epithelial cells (PTECs) in Col4a3 deficient mice. We hypothesized that rhPTX-2 would directly prevent tubular injury in human PTECs.

Methods: Human PTECs were from discarded human kidneys digested using a collagenase based method and purified by magnetic immunoaffinity separation and characterized. To induce cell-stress, PTECs were treated with TGFβ (5ng/ml) or 10% human plasma with or without rhPTX-2 (25mg/ml) for 16h or 24h and evaluated for immunostaining for mesenchymal marker (Vimentin) and epithelial marker (E-cadherin), mitochondrial Reactive oxygen species (ROS) production and qPCR for epithelial-mesenchymal-transition (EMT) markers.

Results: Immunostaining for Vimentin was increased and for epithelial marker E-cadherin was decreased in response to cell stress but those changes were dramatically attenuated by pretreatment with rhPTX-2. The mRNA expression levels of vimentin,

a-smooth muscle actin and Twist1 were increased by cell stress but significantly suppressed by pretreatment with rhPTX-2 compared to the vehicle. In response to cell stress, PTECs rapidly generated mitochondrial ROS, and lost mitochondrial function but PTECs pretreated with rhPTX-2 produced far less mitochondrial ROS accompanied by decreased caspase-3 activity. rhPTX-2 has a specific binding activity for proximal tubule cells and is internalized via a clathrin mediated pathway, suggesting an epithelial receptor.

Conclusions: rhPTX-2 protects epithelium against plasma or TGFβ mediated EMT changes, mitochondrial dysfunction and cell death. rhPTX-2 is a potential new therapy for human chronic kidney diseases.

TH-PO240

Connexin43 as a Determinant of Renal Cell Susceptibility to Nephrotoxic Drug-Induced Cell Injury Kun Gao,[1,2] Yuan Chi,[1] Hui Zhang,[1] Wei Sun,[2] Jian Yao.[1] *[1]Depts of Molecular Signaling, Univ of Yamanashi, Chuo, Yamanashi, Japan; [2]Dept of Nephrology, Affiliated Hospital of Nanjing Univ of Chinese Medicine, Nanjing, Jiangsu, China.*

Background: Drug-induced renal injury represents a frequent clinical entity. It is desirable to identify the factors that influence renal cell susceptibility to nephrotoxicity. Given that gap junctions (GJs) play a determinant role in tumor cell sensitivity to chemotherapy, we tested whether GJ affects renal cell response to nephrotoxic drugs.

Methods: Rat tubular epithelial cells and murine podocytes were exposed to nephrotoxic agents. The generation of ROS was monitored and cell injury was evaluated. The expression and function of GJ protein connexin43 (Cx43) were measured through dye transfer, IF staining and Western blot analysis. The role of Cx43 was determined through interference of its expression and function with chemical inhibitors or siRNA.

Results: 1)Exposure of renal tubular epithelial cells and podocytes to aminoglycoside or puromycin caused morphological change, cellular viability loss and caspase 3 activation, which was preceded by an elevation in ROS level and P38 activation. Treatment of cells with antioxidants (GSH and NAC) significantly prevented cell injury, suggesting a causative role of oxidative stress in cell injury. 2) The cell injury was associated with enhanced Cx43 phosphorylation, which was also preventable by antioxidants. 3) The altered Cx43 contributed to aminoglycoside- and puromycin-induced cell injury. Treatment of cells with Cx43 siRNA or GJ inhibitors enhanced cell resistance to the cytotoxicity of drugs. 4) Suppression of Cx43 expression and/or function caused a dramatic reduction in TXNIP level and an increase in AKT activation. Further analysis revealed that downregulation of TXNIP with siRNA enhanced AKT activation and augmented cell resistance to the cytotoxicity of aminoglycoside and puromycin.

Conclusions: Collectively, our results indicate that Cx43 is a determinant of cell susceptibility to nephrotoxic drugs. This effect of Cx43 is at least partially due to its regulation on TXNIP expression and AKT activation. Cx43 could be a promising therapeutic target for prevention and treatment of drug-induced renal cell injury.

TH-PO241

Examination about Various Receptors of Sphingosine 1-Phosphate Receptor Shunji Shiohira, Miki Nishida, Kosaku Nitta, Ken Tsuchiya. *Kidney Center, Tokyo women's Medical Univ, Shinjuku, Tokyo, Japan.*

Background: In our past report, S1P induced fibrosis. In addition, there are various reports S1P has another effects besides fibrosis. The diversity of responses mediated by Sphingosine 1-phosphate receptors (S1PRs), including immunity, cell migration, angiogenesis, heart development, oxidative stress and fibrosis, depends on the pattern of receptor expression and the associated downstream effectors. To get more insight into roles for S1P and receptor subtype effects, we performed using agonists, antagonists and siRNAs knockdown of receptor subtypes. And also, in order to investigate participation with S1P in oxidative stress of acute renal injury, in vivo, we adapted ischemia-reperfusion injury model (IRI) of mice.

Methods: Normal rat kidney interstitial fibroblast (NRK-49F) cells were stimulated with exogenous S1P. And also NRK-49F cells were stimulated with S1P after the addition of S1PR agonist and antagonist, or siRNAs targeted to S1PRs were evaluated. And also, in vivo, IRI stimulated by agonists and antagonists of S1PRs were evaluated. Moreover, the SOD activity of serum was also evaluated.

Results: S1P stimulated various markers of NRK-49F cells in a dose-dependent manner. Increase in a-SMA, collagen type 1 (COL1), collagen type 4 (COL4), tissue inhibitor of matrix metalloproteinase-1 (TIMP1) and plasminogen activator inhibitor-1 (PAI1) expressions and decrease in E-cadherin expression were observed by addition of S1P. siRNA transfection to NRK-49F cells of S1PR3 attenuated the expression of markers. And, agonist of S1PR1 only attenuated the expression of COL1 and PAI1. In the presence of agonist of S1PR1, the serum BUN/Creatinine (Cre) and the activity of SOD in IRI mice were attenuated in day 1. These results suggest S1PR1 participates in the oxidant stress related to acute phase of IRI. In the presence of antagonist of S1PR3, the serum BUN/Cre in IRI mice were improved. Especially, it is remarkable in day 2. And antagonist of S1PR3 did not affect the activity of SOD. These results suggest S1PR3 affect subacute phase of organ injury rather than acute phase.

Conclusions: S1PR1 is participating in oxidant stress and S1PR3 is participating in fibrosis.

TH-PO242

High Glucose Milieu (HGM) Induces Kidney Cell Pyroptosis and Inflammasome Formation through Activation of Caspase-1 Shabirul Haque,[1] Maria Sultana-Syed,[1] Shallu Sharma,[1] Rivka Lederman,[1] Himanshu Vashistha,[2] Ashwani Malhotra,[1] Leonard G. Meggs,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Medicine, Ochsner Clinic, New Orleans, LA.*

Background: The inflammasome is a multiprotein oligomer which promotes the maturation of inflammatory cytokines such as interleukin (IL)-1 ß. The inflammasome is responsible for activation of inflammatory processes and has been shown to induce cell pyroptosis, a process of programmed cell death distinct from apoptosis. Hyperglycemia is one of the important mediators of the development and the the progression of renal lesions in diabetic nephropathy. In *in vitro* studies, HGM has been known to cause both podocyte and tubular cell injury. However, the role of inflammasomes in kidney cell injury in HGM in general and pyroptosis of kidney cells in particular, has not been investigated. We hypothesized that HGM would enhance both podocyte and tubular cell injury through induction of kidney inflammasome formation and pyroptosis.

Methods: Human podocytes (HPs) or human renal proximal tubular cells (HRPTCs) were incubated in media containing either buffer or variable concentrations of glucose (10, 25, and 35 mM) for 24 and 48 hours and assayed for pyroptosis. To determine the involved mechanisms, HPs/ HRPTCs treated with HGM (35 mM) for 48 h and then followed by RNA and protein extraction. cDNA as well as protein blots were probed for NLRP3, IL-1ß and caspase-1. To establish a causal relationship between caspase-1 activation and pyroptosis, HPs/HRPTCs were treated with HGM in the presence or absence of caspase-1 inhibitor and evaluated for occurrence of pyroptosis.

Results: HGM enhanced pyroptosis both in HPs and HRPTCs in a dose and time dependent manner. HGM enhanced transcription of NLPR3 and pro-caspase-1. HGM also enhanced protein expression of IL-1 beta and caspase-1. Since caspase-1 inhibitor not only inhibited HP/HRPTC IL-1ß expression but also reduced number of kidney cell displaying pyroptosis, it appears that this effect of HGM is mediated through caspase-1 activation.

Conclusions: High glucose enhances kidney cell pyroptosis and inflmmasome formation through the activation of caspase-1.

Funding: NIDDK Support

TH-PO243

Bilirubin Activates Transcription of HIF-1α in Human Proximal Tubular Cells Cultured in the Physiologic Oxygen Content Ho Jun Chin, Youn-Su Park, Seon Ha Baek, Seong Woo Lee. *Internal Medicine, Seoul National Univ Bundang Hospital, Seong nam, Kyeongkido, Republic of Korea.*

Background: Hypoxia-inducible factor (HIF) is a master gene involved in the regulation of hypoxia. Hypoxic and non-hypoxic stimuli, such as oxidative stress, regulate the expression of HIF. We investigated the effect of bilirubin on HIF-1 expression under physiological oxygen concentration (approximately 5%) in the proximal tubular cells and the possible mechanism of HIF-1 regulation by bilirubin in human proximal tubular cells *in vitro*.

Methods: The human kidney (HK2) cells were cultured for 1–5 h in 5% oxygen with or without bilirubin. HIF-1α protein expression increased by bilirubin treatment at 0.01–0.2 mg/dL concentration.

Results: The messenger RNA (mRNA) expression of HIF-1α that was suppressed by actinomycin D was increased by 1.69 ± 0.05 folds in the cells cultured with 0.1 mg/dL bilirubin, compared to the control cells. The inhibitors of PI3K/mTOR, PI3K/AKT, and ERK 1/2 pathways did not attenuate increased HIF-1α expression by bilirubin. HIF-1α expression decreased by 10 μM exogenous hydrogen peroxide (H_2O_2); scavenger of ROS with or without bilirubin in the HK2 cells increased HIF-1α concentration more than that in the cells without bilirubin. Exogenous H_2O_2 decreased the phosphorylation of P70S6 kinase, which was completely reversed by bilirubin treatment. Knockdown of *NOX4* gene by small interfering RNA (siRNA) increased HIF-1α mRNA expression.

Conclusions: Bilirubin enhanced HIF-1α mRNA transcription as well as the upregulation of HIF-1α protein translation through the attenuation of ROS and subunits of NADPH oxidase in human proximal epithelial cells.

Funding: Private Foundation Support

TH-PO244

All-Trans-Retinoic Acid Prevents Oxidative Stress-Induced Loss of Renal Tight Junction Proteins in Type-1 Diabetic Model Eduardo Molina-Jijon, Rafael Rodriguez-Munoz, Maria del Carmen Namorado, Pablo Bautista-Garcia, Alejandro Perez-Lopez, Jose L. Reyes. *Physiology, Biophysics and Neurosciences, Center for Research and Advanced Studies. National Polytechnic Inst, Mexico City, DF, Mexico.*

Background: Renal complications in diabetes are severe and may lead to renal insufficiency. We previously reported that decreased expression of renal tight junction (TJ) proteins: occludin and claudin-2 and -5 in diabetic nephropathy (DN) were associated to increased oxidative stress in glomeruli and proximal tubules. Now we investigated whether all trans retinoic acid (atRA), a compound that plays a relevant role in kidney maintenance and that posses antioxidant properties, prevents loss of TJ proteins in streptozotocin (STZ)-treated rats.

Methods: atRA was administered daily by gavage (1 mg/kg) from days 3-21 after STZ administration. Renal expression of kidney injury molecule (KIM)-1, NADPH oxidase

subunits ($p47^{phox}$ and $gp91^{phox}$), endothelial nitric oxidase (eNOS), occludin, claudin-2 and -5 were analyzed by Western blot. Localization of occludin, claudin-2 and -5 were analyzed by confocal microscopy. Claudin-2 nitration and phosphorylation were analyzed by immunoprecipitation assays.

Results: atRA treatment attenuated loss of body weight and proteinuria but it did not prevented hyperglucemia. Other alterations, such as: increased KIM-1, reactive oxygen species (ROS) production, $p47^{phox}$ and $gp91^{phox}$ expressions and eNOS uncoupling were also attenuated by atRA. Decreased expressions of occludin, claudin-2 and -5 induced by diabetes were ameliorated by atRA. We also found that diabetes induces tyrosine nitration, and phosphorylation in serine residues of claudin-2 and atRA prevented these changes.

Conclusions: In conclusion, the findings described above indicated that atRA exerted nephroprotective effects by attenuating oxidative stress and in consequence loss of renal tight junction proteins was also prevented at early stages of DN. (supported by grant CONACyT 179870 to JLR).

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TH-PO245

IGF-1 Receptor and E-Cadherin Form a Complex to Maintain Tubular Epithelial Cells Phenotype via β-Catenin Signaling Pathway Lei Jiang, Junwei Yang. *Center for Kidney Disease, Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

Background: In epithelial cells, intercellular adhesion is primarily mediated by E-cadherin. Previous studies have demonstrated a physical interaction between insulin-like growth factor-1 receptor (IGF-1R) and E-cadherin/catenin complex suggesting a crosstalk between the signaling pathways mediated by IGF-1R and E-cadherin. In this work, we investigated whether a crosstalk between IGF-1R, and E-cadherin was involved in the change of tubular epithelial cells phenotype.

Methods: Immunoprecipitation experiments were performed to determine the interaction of IGF-1R and E-cadherin in NRK-52E cells. Next we used IGF-1R siRNA and IGF-1R inhibitor PPP to decrease the IGF-1R expression, and detected the E-cadherin expression. We further intraperitoneal injected the CD-1 mice with PPP and observed the tubular epithelial cells phenotype by western blot and immunofluorescence assay.

Results: 1. IGF-1R and E-cadherin interact at cell–cell contact sites. 2. Inhibition of IGF-1R expression by IGF-1R siRNA transfection or PPP could reduce E-cadherin expression and change the epithelial phenotype. 3. PPP induced the change of epithelial phenotype is independent on the intracellular smad, AKT, p42/44 MAPK and p38MAPK signaling pathway, and is regulated by β-catenin/snail pathway. 4. PPP injection could decrease IGF-1R expression and change the tubular epithelial cells phenotype in CD-1 mice.

Conclusions: These results suggest that regulation of the E-cadherin/IGF-1R scaffolding is essential for maintain the phenotype of tubular epithelial cells. Its alteration could be of major importance to tubular epithelial cells injury.

TH-PO246

Connective Tissue Growth Factors Domain I and Domain IV Show Similar Capacity to Regulate Pericyte/Fibroblast Transition Shuyu Ren,[1] Bryce Gordon Johnson,[3] Scott MacDonnell,[2] Jeremy Stuart Duffield.[3] *[1]Divsion of Nephrology, Univ of Washington, Seattle, WA; [2]Boerhinger Ingelheim, New York, NY; [3]Biogen Idec, Cambridge, MA.*

Background: Connective tissue growth factor (CTGF or CCN2), is a secreted matricellular protein of the CCN family. CTGF has four domains (I to IV) with each sharing homology to different proteins (eg: IGFBP, VWC, TSP1) and lead to the interaction of CTGF with many other ligands and receptors to modulate cell function. CTGF has been shown to promote fibrosis in the kidney but its mechanism of action has been unclear. Pericytes are mesenchymal derived cells attached to peritubular capillaries which identified as a major progenitor of scar-forming myofibroblasts. However, the cellular and molecular mechanisms modulating fibrogenesis are still largely unknown. We recently showed that the canonical WNT extracellular regulator Dickkopf related protein-1 (DKK-1), potently inhibits kidney fibrosis and blocks CTGF mediated activation of pericytes and myofirboblasts (Ren et al PNAS 2013). We determined to understand how CTGF and DKK1 signal in this cell lineage to regulate fibrogenic responses.

Methods: Pericytes were purified from transgenic mice and human kidneys. Cells were assessed in migration, morphology and Western blot assays. CTGF domains I, I + II, were synthesized and purified by Boerhinger Ingelheim. Domain IV was purchased from peprotech.

Results: CTGF domain IV activates WNT/bcatenin signaling in pericytes, which is inhibited by recombinant DKK-1. CTGF Dom-IV rapidly phosphorylates the co-receptor of WNT/bcatenin signaling - LRP6. DKK-1 blocks CTGF domain IV mediated fibrotic responses in culture including fibrotic gene activation, pericyte morphology changes and migration in JNK MAP kinase dependent, WNT partially dependent pathway. CTGF Dom-I also activates pericyte migration which is also inhibited by DKK-1, JNK inhibition or Wnt ligand secretion. CTGF Dom-I or -IV induced migration and activation of human kidney pericytes.

Conclusions: Our studies suggest CTGF Dom-I may also play an important role in the regulation of human renal fibrogenesis and that DKK1 is sufficient to inhibit all the effects of CTGF on this lineage.

TH-PO247

RNA Extraction from Urinary Extracellular Vesicles isolated by Hydrostatic Dialysis Harry B. Holthofer, Dorota Ewa Tataruch, Luca Musante. *Centre for BioAnalytical Sciences, Dublin City Univ, Dublin, Ireland.*

Background: Urinary Extracellular vesicles (UEVs) carry proteins and nucleic acids characteristic for their cell of origin in the kidney (nephron) and urogenital tract. The presence of small RNAs in UEVs has gained interest as potential disease biomarkers. Although urine can be obtained in large amounts, vesicle enrichment on a large cohort of patients is still a challenging task. Differential centrifugation is considered as a "golden" standard technique in spite of its inherent problems and requirement of expensive instrumentation.

Methods: Here we report the performance of novel hydrostatic dialysis (HD) based method of urinary vesicles enrichment to yield high quality and quantity RNA from the UEVs. First, we compared RNA profile coming from above 1000kDa (HDa) fraction versus pellets 17,000g and 200,000g coming from differential centrifugation. Second, we evaluated three different RNA extraction methods with starting material from HDa fraction. These included the TRIzol, miRNeasy Micro/Mini Kit (Qiagen®) and Urine Exosome RNA Isolation Kit (Norgen Biotek®). RNA quality and yield was verified by Nanodrop Spectrophotometer ND-1000 and Agilent Bioanalyzer.

Results: RNA extracted from pellets after differential centrifugation presents closely similar results to our HDa fraction in terms of spectrophotometer data. However, qualitative analysis using Agilent Bioanalyzer showed major differences in RNA profile between these two isolation methods. Analysis of HDa derived RNA shows fully comparable profile with all three extraction techniques.

Conclusions: Use of hydrostatic dialysis method for RNA isolation is preferred for: 1) standardization of physical-chemical conditions of samples and to obtain stable RNA profiles independent of the extraction procedure; 2) extraction of RNA from UEVs coming from widely variable volume of sample instead of aliquots and to obtain high yield of nucleic acids. This high quality material can be used for further analysis like qPCR or Next Generation Sequencing. Hydrostatic dialysis appears as a superior way to process desired volumes of urine. In conjunction with optimized RNA extraction protocol this favors procurement of high quality and quantity of RNA.

Funding: Government Support - Non-U.S.

TH-PO248

Internal Proteomic Profile of Human Urinary Extracellular Vesicles Harry B. Holthofer,[1] Xinyu Liu,[1,2] Clizia Chinello,[3] Luca Musante,[1] Wenxiao Zeng,[1] Dorota Ewa Tataruch,[1] Giulio Calzaferri,[1] Fulvio Magni.[3] *[1]Centre for BioAnalytical Sciences, Dublin City Univ, Dublin, Ireland; [2]Inst of Nephrology and Urology, The Third Affiliated Hospital of Southern Medical Univ, Guangzhou, China; [3]Dept of Health Sciences, Univ of Milano-Bicocca, Monza, Italy.*

Background: Urinary extracellular vesicles (UEVs) comprise subgroups such as exosomes, microvesicles and apoptotic bodies. These membrane confined structures carry characteristic intracellular proteinsand RNA, reflecting the physio- pathological processes of their parent cell. Previous studies have identified over 3300 UEV proteins despite the presence ofTamm-Horsfall Protein (THP) which complicatesdetection of low abundant proteins. Here we established a protocol to eliminate THP to focus on the intraluminal content of UEVs.

Methods: Urine was collected from healthy volunteers and UEVs isolated by hydrostatic dialysis. UEV fraction was subjected to ***reduction*** and ***alkylation*** followed by ***trypsin***digestion. Thereafter sample was ultrafiltered by 30kDa molecular weight cut off device and theretained fraction ***solubilized*** by sodium deoxycholate (DOC) followed by yet another ***reduction***, ***alkylation*** and ***trypsin***digestion for UEV internal contents. Western-blot (WB) analysis for the exosome marker Tumor Susceptibility Gene 101 (TSG101) and podocin was carried out to estimate vesicle integrity whereas proteome mapping was performed by mass spectrometry (LC-MS).

Results: WB results showed that TSG101 was not affected by reduction-alkylation-trypsin treatment whereas signal disappeared after DOC and trypsin. Podocin was not affected by reduction-alkylation but it vanished after trypsin digestion. Notably, THP was fully digested with this treatment. LC-MS analysis identified 881 proteins 60% of which were previously found in human UEVswhile the remaining 40% were new.Gene ontology classification revealed that the unique proteins were cytoplasmic(37%), organelle specific (24%), macromolecular complexes (12%)and membrane proteins (10%).

Conclusions: Our UEV isolation and proteomic approach allows the characterization of vesicle contents, expanding the list of intraluminal UEV proteins without loss due to THP interference.

Funding: Government Support - Non-U.S.

TH-PO249

The Effect of Autologous Adipose-Derived Mesenchymal Stem Cell Transplantation on Vascular Calcification in Rats with Adenine-induced Kidney Disease Shinya Yokote,[1] Shuichiro Yamanaka,[1,2] Yuichi Jimmy Katsuoka,[2] Susumu Tajiri,[1,2] Ichiro Ohkido,[1] Makoto Ogura,[1] Takashi Yokoo.[1] *[1]Div of Nephrology and Hypertention, Dept of Internal Medicine, Jikei Univ School of Medicine, Tokyo, Japan; [2]Div of ReGenerative Medicine, Jikei Univ School of Medicine, Tokyo, Japan.*

Background: Previous studies have investigated using mesenchymal stem cells (MSC) to treat damaged kidneys. However, the effect of adipose-derived MSC (ASC) on vascular calcification in chronic kidney disease (CKD) is still poorly understood. The purpose of this work was to investigate the potential of ASC as an alternative treatment for the treatment of CKD and vascular calcification by using an adenine-induced CKD model.

Methods: CKD was induced in 13-week-old male Sprague-Dawleyrats (n=16) by feeding a diet containing 0.75% adenine for 4 weeks. Time course changes in serum inorganic phosphorus (Pi), calcium (Ca) and creatinine were measured. Rats were randomized into two groups: control (phosphate buffered saline, n=9); and MSC (intravenous transplantation of 5×10^5 autologous ASC on days 28, 35, 42, 49 and 56 following adenine-feeding, n=7).

Results: ASC transplantation significantly reduced serum Pi compared with control ($P<0.05$). Histopathology of the kidney showed greater dilation of tubular lumens and more interstitial fibrosis in the control group than the MSC group. Calcium and Pi content of the aorta in the MSC group were lower than in the control group ($P<0.05$). Von Kossa staining of the thoracic aorta media also revealed that ASC transplantaiton suppressed vascular calcification compared with the control group. There was a positive correlation between the Ca and Pi content of the thoracic aorta and the serum Pi level at the end of study, suggesting that the anti-calcification effect of ASC transplantation is due to suppression of the elevation of serum Pi levels via a suppression of kidney injury.

Conclusions: This study found that autogenic ASC transplantation improved kidney function and suppressed the progression of vascular calcification, suggesting that autogenic transplantation of ASC is a novel approach for preventing the progression of CKD and vascular calcification.

Funding: Government Support - Non-U.S.

TH-PO250

Novel Generation and Characterisation of Human Renal Progenitor/Stem Cell Lines Chiara Mari, Karen Price, Satyamaanasa Polubothu, Amnah Alharbi, Paul Winyard. *Developmental Biology and Cancer, UCL, Inst of Child Health, London, United Kingdom.*

Background: Therapy based on stem/progenitor cells ameliorates many experimental kidney diseases. CD24 and CD133 are often used as markers to identify appropriate cells, generally based on studies from adult tissues. We rationalised that fetal kidneys should contain more stem cells, with greater potential for multi-differentiation and long term efficacy. It is not certain, however, whether CD24 and CD133 are the best markers for fetal-derived stem cells. Therefore, we generated multiple early fetal human cell lines and have begun to assess expression of renal progenitor genes and differentiation potential.

Methods: Human fetal kidneys were supplied by the MRC/Wellcome-funded Human Developmental Biology Resource with full ethical permission.CD24 and CD133 expression was evaluated by immunofluorescence. Human fetal cell lines were isolated by FACS based on CD24 and CD133 expression. Real-time PCR was used to define their molecular phenotype.

Results: CD24 and CD133 were detected in developing epithelial structures from 7 weeks gestation, particularly in cells that have undergone mesenchymal-epithelial transition. We used explant culture to generate mixed cell populations from which CD24+CD133+ (double positive cells, DP) and CD24-CD133- (double negative cells, DN) were isolated. 6 DP and 6 DN lines from 8 to 13 weeks gestation were generated. DP and DN lines were clearly different morphologically: DP cells were predominantly epithelial while DN were more mesenchymal. Culture for up to 7 passages demonstrated consistent DP or DN differences. Real-time PCR showed higher expression of characteristic cap mesenchymal markers such as *SIX2*, *EYA1* and *NCAM1* in DN compared to DP lines.

Conclusions: We have generated novel human fetal kidney lines with and without CD24/CD133 co-expression. Immunostaining and PCR raises the possibility that DP cells are at a later epithelial stage of differentiation whereas the DN population may include earlier pre-epithelial progenitors. Our next steps are to assess multi-lineage differentiation, clonogenic potential and contribution to nephrogenesis *in vitro*, before comparing DN and DP efficacy in kidney diseases.

TH-PO251

Cancer Stem/Progenitor Cells Isolated from Clear Cell Renal Cell Carcinoma Express Genes Conferring Resistance to the Cisplatin Treatment Fabio Sallustio,[1,2,3] Vanessa Galleggiante,[1] Grazia Serino,[1] Monica Rutigliano,[1] Claudia Curci,[2] G. Lucarelli,[1] P. Ditonno,[1] M. Battaglia,[1] Francesco Paolo Schena.[1,2] *[1]DETO, Univ of Bari, Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]Disteba, Univ of Salento, Lecce, Italy.*

Background: Recent studies have shown the presence of a stem-like cell population in several human cancers that is crucial for the tumor development. These "cancer stem cells" can maintain the ability to self-renew and sustain the tumor via the expression of tumor-progenitor genes. Because cancer stem cells are believed to be responsible for tumor initiation as well as resistance to chemo- and radiotherapy, their persistence may account for relapsing disease.

Methods: With the aim to study and characterize Renal Cancer Stem Cells (RCSCs) derived from clear cell renal cell carcinoma, we isolated CD133+/24+ from healthy and tumoral renal tissue of 40 patients. Cells were characterized for their mesenchymal phenotype and stemness proteomic profile. Microarray analysis was performed on 24 healthy Adult Renal Stem/Progenitor Cells (ARPCs) and 24 RCSCs. Results were validated using qRT-PCR analysis. Putative markers were evaluated by FACS.

Results: We showed that the CD133+/CD24+ tumoral cells did not express mesenchymal stem cell markers and that they were more undifferentiated than ARPCs. RCSCs were clonogenic and able to differentiate in adipocytes, epithelial and osteogenic cells. Microarray analysis identified 52 genes differently modulated between RCSCs and ARPCs. 23 genes were expressed exclusively in RCSCs but not in ARPCs and CD133- tumoral cells. Among the most significant pathways and biological processes differently modulated we identified the Cancer biological processes and in particular the proliferation of vascular endothelial cells. The gene expression profile identified NRCAM and CTR2 as putative markers for renal cancer stem cells. Moreover, we identified 2 genes (ERCC1 and CTR2) that are involved in the mechanism conferring resistance to the cisplatin treatment in patients.

Conclusions: The identification of these cancer cells in ccRCC and related markers could have a role in supporting the prognosis of patients with RCC and the improving of therapeutic strategies.

Funding: Government Support - Non-U.S.

TH-PO252

***In Situ* Cell Lineage Tracing of the Clonal Architecture of Normal Human Adult Kidney Tubules Suggests Regular Cell Turnover** Jonathan E. Zuckerman,[1] Charles Lassman,[1] Ira Kurtz.[2] *[1]Pathology and Laboratory Medicine, UCLA; [2]Nephrology Div, UCLA.*

Background: Because no clear tubule progenitor cell niche has been identified in the adult human kidney, the mechanism of tubular cell regeneration in the normal kidney and after acute injury is uncertain. We sought to use spontaneous mitochondrial DNA (mtDNA) mutations as lineage markers of progenitor cells for progenitor cell niche identification in the adult human kidney. Spontaneous mtDNA mutations in humans are common. Because of uneven partitioning of mtDNA copies (100's per cell) during cell division, a copy of mutated mtDNA can become the sole form of mtDNA within a cell that is long lived and mitotically active (e.g., a progenitor cell). This type of event, a homoplasmic transformation, can be detected in human tissues and is sufficiently rare as to mark individual cells. We hypothesized that we could identify clonal expansions of progenitor cells that have undergone this type of transformation to identify their niche within adult human kidney tubules.

Methods: Frozen sections from normal human kidney specimens were stained for both cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) activity. Cell patches with deficient COX activity but normal SDH activity (indicates homoplasmic mtDNA mutations) were identified via light microscopy. Serial sections were imaged to determine 3D spatial relationships of COX deficient cell patches.

Results: Strong COX and SDH activities were observed throughout the human kidney tubules, but not in glomeruli or interstitial structures. Dual staining for COX and SDH activity allowed for easy identification of COX deficient cells. These patches were focal and evenly distributed throughout the kidney tubules in both cortex and medulla. Patches of COX deficiency spanned only short segments of tubules; often with multiple independent patches within a single nephron.

Conclusions: The presence of small clonal patches of cells distributed discontinuously throughout the nephron suggests new cells arise from locations throughout the nephron and that cell replacement occurs normally, challenging the widely held view that there is limited proliferation of normal human tubular cells in the absence of acute tubular injury.

TH-PO253

c-Kit$^+$Cells Are Kidney-Specific Stem Cells Maintained Throughout Adult Life Erika B. Rangel,[1] Samirah A. Gomes,[2] Garrett Goss,[3] Konstantinos Chatzistergos,[3] Sabine Klein,[4] Bradley J. Goldstein,[3] Dieter Saur,[4] Joshua M. Hare,[3] Erika B. Rangel.[1] *[1]Inst Israelita de Ensino e Pesquisa, Albert Einstein Hospital, Sao Paulo, Brazil; [2]Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil; [3]ISCI, Univ of Miami, Miami, FL; [4]Dept of Internal Medicine, Medizinische Klinik und Poliklinik der Technischen, Munich, Germany.*

Background: Identification of stem cell populations in mammalian tissues is important for therapeutic applications and for understanding biological processes. We recently reported that c-kit$^+$ cells isolated from developing rat kidneys exhibit stem cell properties. We hypothesize that c-kit$^+$ cells represent a tissue-specific stem cell population that contribute to kidney development and are maintained throughout adult life.

Methods: For lineage tracing, we crossed the inducible c-kit Cre reporter mice with the IRG and R26R lacZ mice. By varying the timing of tamoxifen treatment, c-kit$^+$ cells and their descendants were specifically labeled with EGFP or lacZ and their spatiotemporal distribution was followed.

Results: C-kit expression was more abundant in early post-natal (P) period (7.91 in P0.5-3.5; 10.6 in P7-14 versus 3.13 in embryonic [E]17.5-18.5, $P<0.0001$), but was maintained throughout adult life, although at lower levels (5.7 in P30 and 2.2 in P90-180). When tamoxifen was administered during E7.5-9.5, a few EGFP/LacZ$^+$ cells were observed in tubular segments from the cortex to the medulla, and at E10.5-12.5, when ureteric bud invades the metanephric mesenchyma, ribbons of c-kit-EGFP/LacZ$^+$ cells expanded to form

tubular structures and were detected in structures resembling the S-shaped bodies. In the post-natal period, the number of c-kit-EGFP/LacZ^{+} cells increased in the cortex, medulla, and papilla. No overlap between c-kit-EGFP^{+} cells and differentiation markers (AQP1, AQP2, NKCC2) was observed, suggesting that these cells correspond to undifferentiated cells. In adult mice, c-kit-LacZ^{+} cells were found in distinct tubular segments.

Conclusions: C-kit marks a stem cell population dedicated to generating different kidney tubular segments from the cortex to the medulla during kidney development and adult life, which indicates important biological and therapeutic implications.

TH-PO254

Sox9 Positive Cells Contribute Renal Epithelial Cell Regeneration and Kidney Fibrosis Development Hyun Mi Kang, Katalin Susztak. *Renal Electrolyte and Hypertension Div, Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA.*

Background: Sox9 is one of the important transcription factors controlling cell proliferation, fate and differentiation in the development of many tissues and organs. Here we examined the role of Sox9 in renal epithelial cells.

Methods: To test the role of Sox9 in renal epithelial cells, we generated mice with tubule epithelial specific Sox9 deletion, by intercrossing the PAX8 rtTA-TetO cre and Sox9^{floxed} animals. In addition, we also isolated Sox9 positive cells from the mouse kidney to examine the proliferation and differentiation capacities *in vitro.*

Results: To define the transcription factor related to progenitors in the kidney, we isolated tubule epithelial cells which showed proliferation and differentiation capacity in vitro. We performed genome wide gene expression analysis using microarrays and found cells that show high proliferation and differentiation capacity had high Sox9, Lgr4, Foxd1 and Pax8 expression. In control mouse and human kidneys, Sox9 is rarely detected in the tubules but in injury models its expression is highly increased most tubules, especially dilated proximal tubules. To test whether Sox9 contributes to kidney regeneration we generated mice with tubule specific deletion of Sox9. These animals developed more severe injury after folic acid injection than wild type animals. Fibronectin, collagen1a and 4a were significantly increased and proliferation markers, cycline A, B, D and E were remarkably decreased in Sox9 deleted kidney. Similar results were observed in different time courses, 4 weeks and 16 weeks after folic acid injection. In particular, we isolated Sox9 positive cells from mouse kidney and they showed the ability to self-renew and expressed progenitor cell markers. They also showed multi lineage differentiation potential especially renal tubule cells under the specific condition *in vitro.*

Conclusions: In summary, we demonstrated that Sox9 plays an important role in proliferating and regenerating the renal tubule epithelial cells. Deletion of Sox9 leads to decreasing proliferation and inducing fibrosis development. Sox9 positive cells may represent progenitor cells in the regenerating kidney.

Funding: Private Foundation Support

TH-PO255

Isolation of Putative Podocyte Precursors and Comparison at Different Stages of Differentiation Jianxiang Xu, Patricia N. Kralik, Shirong N. Zheng, Lu Cai, Paul N. Epstein. *Pediatrics, Univ of Louisville, Louisville, KY.*

Background: Continued loss of podocytes will ultimately impair renal function. Since differentiated podocytes are unable to replicate, replacements must come from non-podocyte, precursors. However the identity of podocyte precursors in adults is uncertain.

Methods: Mice were generated with two fluorescent markers: One a red podocyte lineage marker and the other a green marker of podocyte transcription. Differential fluorescence was used to identify and purify potential podocyte precursors.

Results: FACS analysis of glomerular cells showed almost all podocytes expressed both red and green markers, but consistently 2% expressed only the green marker. Confocal microscopy demonstrated green only cells on the glomerular tuft, near the vascular pole. Structurally green only cells varied from simple ovoid cells, to complex cells with fine foot processes indistinguishable from normal podocytes. Immunostaining for podocyte proteins WT1, synaptopodin and nephrin was heterogeneous, varying from negative to strongly positive. Green only cells could be separated from normal red/green podocytes by FACS and cultured or extracted for RNA. In culture activation of the red lineage system was rare. Gene array of freshly purified cells showed differential ($P<0.05$) expression of 1392 genes in green versus red/green podocytes. The 5 most common GO processes in up-regulated RNAs of green cells were: extracellular matrix organization, axon guidance, negative regulation of cell proliferation, angiogenesis and kidney development. Surprisingly, podocyte specific RNA expression was equivalent in normal and green only podocytes.

Conclusions: Putative podocyte precursors were identified and purified using expression of a green podocyte transcription marker and absence of a red podocyte lineage marker. Green only cells were considered podocyte precursors based on robust expression of all podocyte-specific RNAs and morphology and immunostaining results showing differentiation from simple cells to nearly differentiated podocytes.

Funding: NIDDK Support, Private Foundation Support

TH-PO256

Podocytes Derived from Undifferentiated Cells of Amniotic Fluid and Urine Fanny Oliveira Arcolino,[1] Lambertus P.W.J. Van den Heuvel,[1] Silvia Zia,[1] Jaan Toelen,[1,2] Joris Vriens,[1] Patricia Murray,[3] Elena N. Levtchenko.[1,2] *[1]Dept of Development and ReGeneration, KU Leuven, Leuven, Belgium; [2]Dept of Pediatrics, UZ Leuven, Leuven, Belgium; [3]Dept of Translational Medicine, Univ of Liverpool, Liverpool, United Kingdom.*

Background: Loss of podocytes is the main cause of many glomerular diseases. Subpopulations of cells found in amniotic fluid (AF) and urine have been shown to express progenitor cell features and may have the potential to differentiate into several cell lineages. We aimed to obtain podocytes derived from kidney stem/progenitor cells (KSPC) isolated from (AF), principally composed of fetal urine, and freshly voided urine from neonates and adult donors.

Methods: Fetal progenitor cells (fPC) were isolated from AF and urine progenitor cells were isolated from neonates (nPC) and healthy adult donors (aPC). Clonal cell lines were characterized as KSPC and KSPC-derived podocytes by gene expression analyses using quantitative PCR and protein expression by flow cytometry and immunofluorescence. Podocytes differentiation was induced by incubation of KSPCs in culture medium supplemented with retinoic acid and vitamin D.

Results: KSPCs expressed mesenchymal stem cell markers, but not hematopoietic markers. While fPCs and nPCs were positive for the fetal renal markers SIX2 and PAX2, aPCs expressed the adult renal epithelial markers CD133 and CD24. KSPC-derived podocytes presented mesenchymal-to-epithelial transition and many of the cells became bi- or multi-nucleated with arborized cytoplasm comparable to conditionally immortalized podocytes. Cells presented up-regulation of podocyte-specific genes such as Lmx1b, podocalyxin, synaptopodin, CD2AP and nephrin, they presented typical podocyte cytoskeleton and focal adhesion molecules distribution.

Conclusions: Stem/progenitor cells can be isolated from amniotic fluid, neonatal and adult urine and are committed to the renal linage. These cells presenting renal phenotype can be differentiated into podocytes and may provide a novel and non-invasive source of cells for regenerative medicine aiming kidney repair.

Funding: Government Support - Non-U.S.

TH-PO257

Lineage Tracing Reveals Generation of New Podocytes By Renal Progenitors of Bowman's Capsule After Injury Laura Lasagni, Elisa Ronconi, Maria Lucia Angelotti, Anna Julie Peired, Duccio Lombardi, Elena Lazzeri, Paola Romagnani. *Excellence Center Denothe, Univ of Florence, Italy.*

Background: Renal progenitor cells (RPC) are well characterized in humans, but the available data in the mouse are still limited because of the lack of appropriate models to study them. This is mostly related to the fact that the markers used to identify RPC in human cannot be applied in mouse. Based on our and others previous observation that Pax2 selectively costains CD133 in human nephron, we established a PAX2.rtTA;tetO.cre;mT/mG conditional transgenic mouse in which, following doxycyclin (dox) treatment, Pax2 promoter drives the expression of GFP specifically in RPC in Bowman's capsule and in scattered cells of tubules. With this model, we aimed to clarify the contribution of RPC to podocyte regeneration after injury and whether it could be modulated by drugs.

Methods: Following 10 days dox treatment at 5th week of age and a washout period of one week, podocyte loss has been induced in PAX2.rtTA;tetO.Cre;mT/mG mice by adriamycin administration, and animals divided on the basis of the course of proteinuria in regressing versus progressing. At day 30, the presence of GFP+ cells migrated within the tuft, expressing podocyte proteins and showing morphological characteristics of fully differentiated podocytes was evaluated by confocal and electron microscopy.

Results: In mice showing regression of proteinuria, GFP+ cells expressing podocyte markers and showing foot processes indistinguishable from resident podocytes could be detected in the glomeruli, while in mice with progressive glomerular damage, regeneration could not be detected and GFP+ cells were observed as components of glomerular lesions. To test whether podocyte regeneration could be enhanced by drug treatment, following adriamycin administration, mice were treated with a GSK3s-inhibitor or vehicle. After 15 days, the number of regenerated GFP+podocytes was significantly higher in GSK3s treated mice than in control.

Conclusions: These data support the hypothesis that podocyte regeneration is carried out by RPC after injury, is associated with regression of damage, and can be pharmacologically enhanced with important perspectives for treatment of CKD.

TH-PO258

Expression and Secretion of VEGF and SDF-1, Cytokines Essential for Mesenchymal Stem Cells' Renoprotective Effects, Vary Significantly under Different Culture Conditions Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Christof Westenfelder.[1,2] *[1]Medicine, U of Utah and VAMC, Salt Lake City, UT; [2]Physiology, U of Utah, SLC, UT.*

Background: Mesenchymal Stem Cells (MSCs) are increasingly evaluated for therapeutic applications such as AKI and CKD. Their off-the-shelf use relies on culture expansion and banking. Recent publications caution that MSC-based therapies may yield inconsistent therapeutic results due to variability in culture protocols. Using siRNA gene knock-down technology, we showed that the cytokines VEGF-A and SDF-1, as elaborated by MSCs, are critical to their renoprotective actions in rats with AKI. We investigated

whether routine cell culturing changes VEGF-A and SDF-1 expression in 7 human MSC cell lines at passages 2-6. Four lines were clinical grade and had been used at Passage (P) 2 in a successful Phase I AKI Trial.

Methods: Cells were plated at 1,000 cells/cm^2, cultured in α-MEM + 5% human platelet lysate, grown to (a) 70% or (b) full confluence, passaged up to P6, and SDF-1 and VEGF-A expression/secretion was assessed throughout and compared to P2 levels.

Results: Gene expression levels were unchanged at all passages tested. Secretion of SDF-1 was unchanged for P3 cells grown to 70% confluence, but fell by ~50% at P3 when cells were grown to confluence. Starting at P4, secretion levels became inconsistent. By contrast, VEGF-A secretion rose significantly with each passage, regardless of cell confluence.

Conclusions: MSC-expressed SDF-1 is an autocrine survival factor and does mediate survival effects in renal cells. Since a 50% reduction in SDF-1 expression diminishes MSCs' renoprotective effects in experimental AKI, a similar reduction seen here at P3 could similarly reduce their therapeutic efficacy in clinical AKI. MSC-expressed VEGF is angioprotective and pro-angiogenic in experimental AKI. It remains to be examined whether the increased VEGF expression seen with higher passaging numbers offsets the loss in the kidney protective actions of MSCs that are under-expressing SDF-1, and potentially other cytokines. Together, our data underscore the fact that it is essential that culture conditions must be stringently defined when MSCs are used therapeutically.

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TH-PO259

Transgenic Expression of Human Alkaline Phosphatase by Allogeneic Rat Mesenchymal Stem Cells Renders Them Ineffective in the Treatment of 5/6th Nephrectomy-Induced Chronic Kidney Disease

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Background: We showed that allogeneic rat and human Mesenchymal Stem Cells (MSCs) protect renal function post IRI AKI and appear to be renoprotective in on-pump cardiac surgery patients at risk for post-op AKI. Such paracrine effects in allogeneic settings are obtained without the elicitation of an antibody response, confirming MSCs' immune privileged properties. Here, we examined the possibility of antibody (Ab) production to allo- or xenogeneic genes and secondary loss of therapeutic efficacy as a result of multiple doses of Fischer344 allo-MSCs (F344-MSCs) or human Alkaline Phosphatase transgenic Fischer344 allo-MSCs (hPAP MSCs) given to Sprague Dawley (SD) rats with 5/6th Nephrectomy (NX)-induced CKD.

Methods: Four groups (n= 6) of SD rats underwent 5/6 NX and were treated with two, bi-weekly i.v. infusions (1x10^6 cells/kg bw) at 3 and 4 weeks post NX of (a) F344-MSCs; (b) hPAP-MSCs; (c) vehicle in Controls; (d) Sham surgery. Serum Creatinine (SCr), systolic blood pressure (BP) and urinary albumin (U Alb) excretion were assessed weekly, and at 12 weeks renal histopathology was examined, and serum samples were FACS analyzed for potential antibody production to allo- or xenogeneic genes.

Results: Rats treated with F344-MSCs showed at endpoint significantly lower SCr levels, U Alb excretion and BPs versus Controls, as well as lower scores for interstitial fibrosis and glomerulosclerosis. Serum from these animals contained no IgG to F344-MSCs, while rats treated with hPAP-MSCs showed no differences in any measured endpoints versus Controls, and their sera contained IgG to hPAP-MSCs.

Conclusions: Repeated administration of allo-MSCs in CKD appears to arrest the progressive loss of renal function and rise in albuminuria, supporting the possibility that these cells may be of use clinically in treating CKD. However, use of MSCs containing xenogeneic epitopes eliminates their protective effects, likely due to the destruction of these cells by the elicitation of antibodies to such epitopes.

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TH-PO260

Engineering Transplantable 3D Renal Tissue From a Suspension of Human Amniotic Fluid Stem Cells and Mouse Metanephric Cells

Valentina Benedetti,[1] Christodoulos Xinaris,[1] Susanna Tomasoni,[1] Paola Rizzo,[1] Rubina Novelli,[1] Daniela Corna,[1] Takashi Yokoo,[2] Marina Morigi,[1] Ariela Benigni,[1] Giuseppe Remuzzi.[1,3] *[1]IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; [2]The Jikei Univ School of Medicine, Tokyo, Japan; [3]Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy.*

Background: Stem cell-based engineering of kidneys may offer prospects for alternatives to dialysis and transplantation. Amniotic fluid stem cells (AFSCs) have been successfully used for therapeutic applications in kidney injury models. However, the capacity of these cells to contribute to the creation of efficient kidney tissue is still unknown.

Methods: Here, we tested whether human AFSCs can be patterned by the spatial information of self-organizing metanephric cells to form a 3D tissue applicable to transplantation. Mixed suspensions of AFSCs and E11.5 mouse kidney cells were centrifuged, and pellets were cultured *in vitro* up to 5 days.

Results: Immunofluorescence analysis revealed minimal integration of labeled-AFSCs into condensing metanephric mesenchyme (MM), with the majority of AFSCs distributed between Pax-2-positive structures without acquiring renal markers. To enhance their integration in self-forming tissue, AFSCs were genetically modified to express Glial cell-derived neurotrophic factor (GDNF), a factor secreted by MM. GDNF-expressing AFSCs incorporated into Pax-2-positive structures and formed 3D chimeric tubules that were surrounded by laminin-positive membranes. Chimeric organoids were then implanted under the kidney capsule of host rats and allowed to grow *in vivo* for 7 days. Engrafted tissue became vascularized, and developed well-formed tubules and glomeruli that contained red cells. Several AFSCs were localized into developing glomeruli and expressed the podocyte marker α-actinin-4. AFSC were also found in vessels and expressed the endothelial marker CD31. Finally, only few AFSCs were found in tubules.

Conclusions: Hence, AFSCs can be effectively directed by self-organizing menatephric cells to form chimeric nephrons, which can further develop *in vivo*. This ability can assist the development of tissue-engineered kidney alternatives and facilitate more focused investigations on AFSCs biology.

Funding: Private Foundation Support

TH-PO261

Amniotic Fluid Stem Cells: Mediators of Crosstalk Between Glomerular Endothelial Cells and Podocytes

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Background: Emerging evidence strongly support the notion of podocyte depletion as a major mechanism driving glomerulosclerosis. We have previously shown that stem cells derived from amniotic fluid (AFSC) significantly delay chronic kidney disease progression in Alport Syndrome mice via preservation of podocyte numbers among other mechanisms. However, exactly how stem cells mediate such protection has not been fully elucidated. Endothelial cell integrity and healthy cross-talk with the podocyte is necessary for maintenance of glomerular filtration barrier. Since injection of AFSC protect glomerular structure but do not differentiate into podocytes in vivo, we hypothesize that AFSC preserve the filtration barrier and detachment of podocytes by acting directly on endothelial cells through VEGF signaling mechanisms.

Methods: Primary glomerular endothelial cells were subjected to angiotensin II exposure for 24 hours as control and treated with AFSC in experimental groups. In vivo, Alport mice were injected with AFSC. Whole kidneys, glomeruli and glomerular cells (endothelial cells, podocytes and AFSC) derived from injected mice were harvested and analyzed.

Results: Angiotensin II stimulation resulted in endothelial cell injury after 24 hours in culture characterized by cell quiescence and necrosis with downregulation of VEGF and upregulation of necrotic markers such as Jph3, S100a7a and sycp2. In contrast, cells co-cultured with AFSC had normal morphology and proliferation with increased VEGF and decreased necrosis gene expression. The data indicate AFSC induce increased production of VEGF providing endothelial cells ample amount of the key survival signal. Injection of AFSC in Alport mice regulated VEGF expression within damaged glomeruli near normal level which in turn protects glomerular filtration barrier from subsequent deterioration.

Conclusions: Taken together our data suggest that AFSC contribute to podocyte preservation by regulating endothelial cell survival via active cytokine regulation controlling VEGF expression level both in vitro and in vivo.

Funding: Private Foundation Support

TH-PO262

Adipose Tissue-Derived Mesenchymal Stem Cells in Long-Term Dialysis Patients Display Downregulation of PCAF Expression and Poor Angiogenesis Activation

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Background: We previously demonstrated that mesenchymal stem cells (MSCs) differentiate into functional kidney cells capable of urine and erythropoietin production, indicating that they may be used for kidney regeneration. However, the viability of MSCs from dialysis patients may be affected under uremic conditions.

Methods: In this study, we isolated MSCs from the adipose tissues of end-stage kidney disease (ESKD) patients undergoing long-term dialysis (KD-MSCs; mean: 72.3 months) and from healthy controls (HC-MSCs) to compare their viability. KD-MSCs and HC-MSCs were assessed for their proliferation potential, senescence, and differentiation capacities into adipocytes, osteoblasts, and chondrocytes. Gene expression of stem cell-specific transcription factors was analyzed by PCR array and confirmed by western blot analysis at the protein level.

Results: No significant differences of proliferation potential, senescence, or differentiation capacity were observed between KD-MSCs and HC-MSCs. However, gene and protein expression of p300/CBP-associated factor (PCAF) was significantly suppressed in KD-MSCs. Because PCAF is a histone acetyltransferase that mediates regulation of hypoxia-inducible factor-1α (HIF-1α), we examined the hypoxic response in MSCs. HC-MSCs but not KD-MSCs showed upregulation of PCAF protein expression under hypoxia. Similarly, HIF-1α and vascular endothelial growth factor (VEGF) expression did not increase under hypoxia in KD-MSCs but did so in HC-MSCs. Additionally, a directed *in vivo* angiogenesis assay revealed a decrease in angiogenesis activation of KD-MSCs.

Conclusions: In conclusion, long-term uremia leads to persistent and systematic downregulation of PCAF gene and protein expression and poor angiogenesis activation of MSCs from patients with ESKD. Furthermore, PCAF, HIF-1α, and VEGF expression were not upregulated by hypoxic stimulation of KD-MSCs. These results suggest that the hypoxic response may be blunted in MSCs from ESKD patients.

Funding: Private Foundation Support

TH-PO263

***In Vitro* Culture of Human Renal Progenitors** Stefano Da Sacco, Astgik Petrosyan, Sargis Sedrakyan, Kevin V. Lemley, Roger E. De Filippo, Laura Perin. *Children's Hospital Los Angeles.*

Background: How different renal cells respond to injury may determine their survival under disease conditions. A better understanding of the developmental pathways active in these cells may shed light on their response to injury and provide insights into novel therapies. Currently, no culture system exists, without the use of genetic manipulation, that can stably propagate human nephron progenitors and differentiate them into mature renal cells. We previously showed the presence of a renal progenitor population within human amniotic fluid (hAKPC-P) that can be induced to differentiate into functional podocytes. Most importantly, hAKPC-P contain a sub-population of cells that express specific developmental renal genes, like Six2 and Cited1, suggesting their identity as nephrogenic precursors with self-renewal and nephron specification capabilities.

Methods: A live subpopulation of Six2+Cited1+ cells was isolated by FACS sorting by specifically tagging RNA with fluorescent probes. The isolated population was characterized for gene and protein expression by RT-PCR, microarrays, staining, western blot and secretome profiling. Effect of growth factors like BMP7 and Wnt9b was evaluated. Ability of Six2+Cited1+ cells to integrate into developing renal structures was also assessed by disaggregation/aggregation assays.

Results: Six2+Cited1+ cells represent 0.3-0.5% of the amniotic population as assessed by FACS analysis for both protein and RNA. Cells exhibit a fast growth rate and can be easily expanded. This subpopulation expressed nephrogenic markers including HOX genes, Eya1, and Notch. Moreover, cells respond to BMP7 and Wnt9b by losing Six2 and Cited1 expression and losing self-renewal capability. Additionally, the ability of Six2+Cited1+ cells to differentiate in vitro was assessed by culture with human fetal kidney in a disaggregation/aggregation assay.

Conclusions: Second trimester human amniotic fluid harbors a small population of renal progenitors that can be easily isolated without the use of genetic manipulation and expanded in vitro maintaining self-renewal and renal differentiation. This research could lead to important discoveries concerning renal developmental pathways in humans.

Funding: Private Foundation Support

TH-PO264

Amniotic Fluid Kidney Progenitors as a Tool to Study Podocyte-Endothelial Cell Interaction In Vitro Stefano Da Sacco, Astgik Petrosyan, Sargis Sedrakyan, Kevin V. Lemley, Roger E. De Filippo, Laura Perin. *Children's Hospital Los Angeles.*

Background: The glomerular filtration barrier (GFB) is formed by three major components: the podocyte, a complex network of membranous proteins known as the glomerular basement membrane (GBM) and fenestrated endothelial cells. While podocytes are the only cells in the glomerulus to produce and assembly collagen IV, endothelial cells play a key role in the formation of a functional GFB. We have previously shown that progenitor cells from human amniotic fluid (hAKPC) differentiates into mature podocytes producing collagen IV alpha 3-4-5 chains in vitro. However, an in vitro system that can mimic the GFB is still lacking. In this study, we evaluate the ability of hAKPC to interact with glomerular endothelial cells in a 3D in vitro system to possibly resemble a GBM like-assembly.

Methods: hAKPC were differentiated for 20 days. Immunofluorescence, qPCR, SEM and TEM were performed to confirm formation of slit diaphragm on differentiated podocytes. To investigate the formation of GBM-like structures in vitro, hAKPC were co-cultured with glomerular endothelial cells in a 3D system and analyzed by PCR, Western Blotting, light and electron microscopy. In addition, response to active molecules and toxins was tested.

Results: When 3D co-cultured with endothelial cells, hAKPC are capable of forming spheroids, express podocyte specific markers including nephrin and form peculiar morphological structures when interacting with endothelial cells as confirmed by TEM and histology. These structures present deposition of collagen IV and laminin with the same spatial deposition of in vivo glomerular GBM. When stimulated with angiotensin II, spheroids showed marked contraction with modification of the structures and loss of slit diaphragm proteins.

Conclusions: hAKPC can be differentiated into mature podocytes in vitro and exhibit the ability to interact with endothelial cells to form a GBM-like structure. These preliminary results suggest that hAKPC might prove useful to understand the molecular pathways leading to formation of GBM, the most important structure in kidney ultrafiltration, as well as serve as a platform for testing specific podocyte and endothelial related drugs.

Funding: Private Foundation Support

TH-PO265

Extrarenal Progenitor Cells Do Not Contribute to Endothelial Repair in the Mouse Kidney Jan Sradnick,[1] Song Rong,[2] Anika Luedemann,[1] Vladimir T. Todorov,[1] Faikah Gueler,[2] Christian Hugo,[1] Bernd Hohenstein.[1] *[1]Div of Nephrology, Dept of Internal Medicine III, Univ Hospital Carl Gustav Carus, Dresden, Germany; [2]Div of Nephrology and Hypertension, Dept of Internal Medicine, Hannover Medical School, Germany.*

Background: The role of endothelial progenitor cells for endothelial cell (EC) regeneration unclear and we previously showed that mainly endothelial colony forming cells (ECFC) can be found in kidneys after selective EC- or ischemia/reperfusion (IR) injury. Using bone marrow (BM) transplantation (BMT), we excluded the BM origin of these cells. To clarify the role of extrarenal versus BM derived cells for EC repair, we now investigated the regenerative processes of EC-IR injury following kidney transplantation (KTx) in transgenic mice with combined BMT.

Methods: Ubiquitously tdTomato (tdt) expressing reporter mice served as recipients (R), C57/Bl6 mice were donors (D). A group of R also underwent BMT from D prior to renal injury, allowing us to differentiate the extrarenal niche. Reverse KTx served as control (D:tdt+;R:C57/Bl6). Ischemia time (25 min) was chosen to detect significant numbers of putative progenitor cells (pPC) in kidneys. On days 2 and 5, kidneys, blood and spleens were analyzed extensively using multicolor FACS-analysis and histology. pPC (CD34+,Flk-1+,CD31+,CD105+,CD146+,CD45-,CD133-,CD115-,CD14-), hematopoietic stem cells (c-kit+,Sca-1+,lin-), macrophages, dendritic-, b- and t-cells were detected by FACS and tdt co-staining by histology.

Results: On days 2 and 5, pPC were increased (+300%;$p<0.01$). Many inflammatory cells were recruited to injured kidneys, 94% of them were tdt+ (extrarenal). Following KTx, tdt+ pPC were rarely found in blood (2‰) and spleens (8‰). In contrast, no extrarenal tdt+ pPC were identified in KTx kidneys by FACS or histology at any time point. pPc makers were exclusively found on renal, tdt- cells. Only few tdt+ inflammatory cells, but no tdt- pPC, were found in KTx/BMT-chimera mice.

Conclusions: In contrast to inflammatory cells, no extrarenal pPC are recruited to the kidney during endothelial repair. Under pathophysiological conditions endothelial repair occurs exclusively via local mechanisms that need to be specified in more detail.

TH-PO266

Attenuation of Renal Fibrosis through the Combined Therapy of Mesenchymal Stem Cells and the Hormone Serelaxin Brooke M. Huuskes,[1] Andrea F. Wise,[1] Chrishan S. Samuel,[2] Sharon D. Ricardo.[1] *[1]Dept of Anatomy and Developmental Biology, Monash Univ, Clayton, Victoria, Australia; [2]Dept of Pharmacology, Monash Univ, Clayton, Victoria, Australia.*

Background: MSCs protect and repair the kidney in experimental models of acute kidney disease, however the development of fibrosis may hamper MSC-dependent repair. Here we tested the novel combination of MSCs with the antifibrotic, serelaxin to repair and protect the kidney post unilateral ureteric obstruction (UUO), when fibrosis is established.

Methods: Male C57Bl6 mice (n=4-6/group) underwent UUO surgery and received vehicle (PBS); luciferin/enhanced green fluorescent protein (eGFP)-tagged MSCs ($1x10^6$); serelaxin (0.5mg/kg/day); or the combination of MSCs and serelaxin; and were culled after 7 days. Sham-operated mice were used as a control. Bioluminescence imaging was used for live MSC cell tracing and the development of fibrosis was assessed by hydroxyproline assay, quantitative morphometry, gelatin zymography and immunostaining.

Results: *In vivo* tracing studies showed MSCs migrated to the UUO-injured kidney within 1 hour post transplantation where they remained for 36 hours, compared to sham-treated animals, confirmed by PCR for the eGFP gene. Combination therapy conferred significant protection from UUO-induced fibrosis as indicated by hydroxyproline assay ($p<0.001$ versus vehicle, $p<0.05$ versus MSC or serelaxin alone). This was accompanied by preserved structural architecture, decreased tubular epithelial injury ($p<0.01$ versus vehicle), macrophage infiltration ($p<0.01$ versus vehicle) and α-smooth muscle actin expression ($p<0.01$ versus vehicle). Gelatin zymography revealed that MMP-2 levels were significantly increased with combination therapy ($p<0.01$ versus vehicle, $p<0.05$ versus MSC or serelaxin alone). Serelaxin significantly increased MSC proliferation *in vitro* (~14%; $p<0.05$). Additionally, TNF-α stimulated MSCs showed an increased migration capacity in the presence of serelaxin (10-100ng/mL; $p<0.001$ versus negative control).

Conclusions: This study is the first to demonstrate that when used in combination, MSCs and the antifibrotic hormone serelaxin, ameliorate pathological fibrosis and improve MSC-mediated repair, in part, via through the up-regulation of MMP-2.

Funding: Private Foundation Support

TH-PO267

Nephron Regeneration After Acute Kidney Injury in the Zebrafish Kristen K. McCampbell, Kristin Springer, Rebecca A. Wingert. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

Background: The zebrafish kidney is an excellent system for renal regeneration and disease studies, as it is composed of functional units known as nephrons that contain highly conserved proximal and distal tubule segments similar to other vertebrates including mammals. After zebrafish nephrons incur damage, there is robust epithelial regeneration within existing nephrons and new nephrons are produced from renal progenitors. The mechanisms responsible for these kidney regeneration phenomena remain poorly understood and a major limitation in the field has been the paucity of methods to label adult nephron cell populations.

Methods: Here, we present novel combinations of labeling methods that can be used in whole mount preparations or tissue cryosections to gage renal composition and assess nephron functionality.

Results: We demonstrate the existence of pan-proximal and pan-distal markers, as well as methods to distinguish the individual proximal tubule domains (proximal convoluted and straight tubule). We validated these markers by comparisons to the expression domains of solute transporter genes that uniquely identify each nephron segment. Next, we characterized the cellular changes resulting from acute gentamicin injury to establish the timing of renal cell death after injury, the proliferative compartments within the kidney, and the gene expression changes associated with nephron regeneration.

Conclusions: Taken together, these data have provided a greater understanding of cellular changes associated with regenerative events. This information is applicable to the future study of adult zebrafish kidney injury paradigms.

Funding: NIDDK Support

TH-PO268

Rejuvenation of Kidney Aging By Exposure to a Young Systemic Environment Xiang-Mei Chen, Yichun Ning, Xuefeng Sun, Qi Huang, Diangeng Li, Quan Hong, Guangyan Cai. *Dept of Nephrology, Chinese PLA General Hospital, Chinese PLA Inst of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center of Kidney Diseases, Beijing, China.*

Background: Kidney is a typical target organ of age-associated tissue damage, and the increased incidence of CKD and AKI in the elderly is a health problem worldwide. Our previous study found that the internal environment has an important impact on the kidney aging process. Changes of the state of the environment can accelerate or reverse the kidney aging process.

Methods: To clarify the biological mechanisms that drive kidney aging process, we tested the influence of circulating factors using heterochronic parabiosis, a surgical technique in which joining of animals of different ages leads to a shared circulation. All mice were sacrificed after parabiosis for five weeks. Blood samples were collected from angular vein of each mouse for the measurement of biochemical efficacy markers. The renal tissues from each group of mice were removed and perfused with ice-cold, isotonic phosphate-buffered saline (PBS; pH 7.4). Aging index p16, apoptotic index cleaved caspase-3, autophagy indicators LC3, p62, polyubiquitin were measured by western blot. Microarray analysis was used for filter out cytokines that had a significant difference among all groups. Cluster analysis of cytokines was to identify key factors that may affect renal aging.

Results: The survival rate of mice in heterochronic parabiosis (HP) was significantly lower than their controls. After 5 weeks of exposure to the circulation of young mice, there was no significant change in the old mice of HP on kidney morphology and function. Aging-related indicators p16 and SA-β-gal, apoptosis and autophagic activity in old mice parabiosed to young mice were all in improvement trend compared with unjoined old mice or old mice parabiosed to old mice. Using high-throughput screening methods in the blood circulatory system, we identified 6 kinds of cytokines that may be closely associated with aging; namely IGFBP-2, Eotaxin-2, Fractalkine, CD27, LIX and SCF.

Conclusions: These results suggest that kidney aging can be modulated by systemic factors that change with age.

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TH-PO269

Cross Talk between p66 and Wnt Gene Networks Regulates Stress -Induced Senescence in Mesenchymal Stem Cells and Aging Phenotypes in Diabetic Kidneys Pradeep Vaitla,[1] Ryan C. Mascarenhas,[1] Allyson E. Bradley,[1] Ashwani Malhotra,[2] Pravin C. Singhal,[2] Himanshu Vashistha,[1] Leonard G. Meggs.[1] *[1]Nephrology, Ochsner Clinic Foundation, New Orleans, LA; [2]Medicine, Hofstra, North Shore LIJ Medical School, Great Neck, NY.*

Background: Stem cell therapy has emerged as a promising tool for the treatment of a variety of diseases. Gene based strategies that attenuate or prevent entry to senescent programs provide an innovative approach to extend the life cycle of stem cells and their regenerative potential. The p66 longevity gene plays a key role in the activation of gene programs that regulate cell entry to apoptotic and senescent phenotype(s), which are prevalent in diabetes. Here we show kidney mesenchymal stem cells (MSCs) genetically deficient in p66 exhibit enhanced canonical Wnt signaling, escape stress-induced senescent programs and participate in maintenance and repair diabetic kidneys.

Methods: We isolated lineage negative, stem cell antigen-1 positive cells from kidneys of adult wild type and p66 KO mice, that exhibit immunophenotype and differentiation characteristics of MSCs. To test whether p66 KO-MSCs delay or prevent expression of senescent phenotype(s) in diabetic kidneys, we crossed Akita diabetic ($Ins2^{+/C96Y}$) mouse with p66 KO to generate p66 KO-Akita.

Results: Microarray of cultured p66 KO-MSCs at high glucose detected differential expression of Wnt network genes that antagonize stem cell senescence. Increased accumulation of β-Catenin a key signaling molecule of the canonical Wnt pathway, was detected in p66 KO-MSCs. Expression of senescent associated proteins ($p16^{INK4a}$; p21; p53) was upregulated in WT-MSCs at high glucose and growth curves were markedly attenuated. The number of kidney nuclei staining positive for the senescent protein $p16^{INK4a}$ was substantially increased in in kidneys of Akita, as were the senescent phenotype(s) of interstitial fibrosis and tubular atrophy. By contrast, these changes were barely detectable in kidneys of p66 KO Akita.

Conclusions: These findings reveal the key role played by Wnt regulatory genes in maintaining a viable and functional population of p66 KO-MSCs under simulated HG and in diabetic kidneys.

Funding: Private Foundation Support

TH-PO270

Endothelial Progenitor Cell Extract (EPC-E) Ameliorates Interstitial Fibrosis in Mice with Unilateral Ureteral Obstruction (UUO) Kei Matsumoto, Sandhya Xavier, Reina Ikeda, Michael S. Goligorsky. *New York Medical College, NY.*

Background: Accumulation of myofibroblasts leads to excessive matrix deposition, fibrotic distortion of tissue architecture and the loss of organ function. Given that a significant proportion of myofibroblasts is generated by the process of endothelial-mesenchymal transition, we inquired whether the reversal of this process is achievable. Hence, we attempted to reprogram myofibroblasts using in vitro and in vivo cell-free murine EPC-E.

Methods: Renal fibroblasts were isolated from αSMA-GFP mice and treated with TGF-b1 alone or TGF-b1 and EPC-E for 4 days. UUO surgery was performed in αSMA-GFP mice on C57BL/6J background and either EPC-E (10μl) or buffer (10μl) injected into the lower pole of UUO kidneys. Histologic, immunofluorescence staining for αSMA, CD31, and F4/80, and real-time RT-PCR for αSMA, collagen I, TIMP2 were performed.

Results: Fibroblasts derived from αSMA-GFP kidneys showed massive expression of GFP 24 h after TGF-β1 treatment. When cells were co-treated with EPC-E, their conversion to GFP-positive myofibroblasts was significantly halted. EPC-E inhibition of TGF-β1-induced phenotypic changes did not require prior cell permeabilization, raising the possibility of a similar effect in vivo setting. Indeed, when UUO, but not contralateral, kidneys were injected with Texas red dextran, 70kD, it was readily detectable in the renal parenchyma. Injection of the EPC extract was associated with the nearly 60% reduction of renal fibrosis. The number of myofibroblasts was reduced by 35% and CD31 positive area was increased by 35% compared to vehicle-treated mice. Furthermore, EPC-E-treated mice showed significantly reduced interstitial F4/80 macrophage infiltration, as well as reduced αSMA, collagen I, TIMP2 mRNA expression.

Conclusions: These studies establish 1) the possibility to prevent in vitro TGF-β1-induced fibroblast activation and acquisition of the myofibroblastic phenotype using EPC-E treatment of non-permeabilized cells; studies show that 2) EPC-E treatment ameliorates the progression of renal fibrosis in the UUO model through the suppression of myofibroblast differentiation, macrophage infiltration and preservation of vascular density.

Funding: NIDDK Support

TH-PO271

Plasticity of Mesenchymal Stem Cells (MSCs) during Repair of Injured Mesangium Jiamin Teng, Elba Turbat-Herrera, Guillermo A. Herrera. *Pathology, LSU Health, Shreveport, LA.*

Background: It has been shown that MSCs play a crucial role in repairing the damaged mesangium. Using an ex-vivo kidney platform, it has been observed that in a model of mesangial injury by glomerulopathic light chains (GLCs), rat MSCs perfused through the renal artery migrate to the sites of mesangial injury where either amyloid deposition or increased extracellular matrix was present. MSCs engage in the disposal of altered mesangial constituents, as they clean the damaged areas and create a new mesangium.

Methods: Two platforms were used: 1- Ex-vivo kidney perfusion model and 2- 6D live cell imaging platform. In the first platform, mesangial damage was created by delivering GLCs for 2 days via renal artery followed by perfusion with rat MSCs; the process was monitored for up to 96 hours by sequential sampling of the perfused kidneys using light and TEM. In the second model, rat MCs grown on a matrix were made quiescent for 2 days and incubated with GLCs for 4 days and after mesangial damage was confirmed, RMSCs marked with PKH2 and Lysotracker / CD68 to monitor for phagocytic capabilities were introduced. The entire process was monitored for up to 5 weeks using sequential photography. Immunofluorescence/histochemical stains for MSCs (CD 44, 56 and 117) were also performed.

Results: After the MCs were treated with GLCs and damage occurred, MSCs migrated to the sites of injury. MSCs which show an undifferentiated appearance acquired a macrophage phenotype and proceeded to phagocytose the damaged mesangium, including apoptotic mesangial cells. In the early phases of the process, MSCs acquired prominent Lysotracker and CD68 cytoplasmic staining. After the cleaning process was completed, MSCs progressively acquired more cytoplasm, developed cytoplasmic processes with myofibrils and attachment plaques (TEM) and concomitantly expressed smooth muscle actin and smoothelin.

Conclusions: MSCs manifest marked plasticity as they engage in the process of mesangial remodeling. First, they acquire a macrophage phenotype endowing them to phagocytose apoptotic MCs, amyloid fibrils, and other debris. As they proceed to rebuild the damaged mesangium, they develop morphologic and immunohistochemical features typical of mature MCs.

TH-PO272

Wnt6 Regulates Epithelial Cell Fate Specification during Kidney Development and in Adult Renal Cells Hayley Beaton, Andrew Gaffney, Catherine Godson, Debra F. Higgins, John Crean. *Conway Inst of Biomolecular and Biomedical Research, Univ College Dublin, Dublin, Ireland.*

Background: Diabetic nephropathy (DN) is the leading cause of chronic kidney disease. Recent studies from our group and others identified a role for Wnt signalling in the pathogenesis of DN. Gene expression analysis of Wnt family members on human renal biopsies from DN patients identified differential expression of the novel ligand, Wnt6, correlating with severity of the disease.

Methods: Mouse embryonic whole mounts at Theiler stage 17.5 were analysed by in *situ* hybridisation. Wnt6 expression was determined in the unilateral ureteral obstruction (UUO) mouse model of tubulinterstitial fibrosis. Wnt6 was overexpressed in renal epithelial cell lines (HKC8s and MDCKs) and bone marrow derived mesenchymal stem cells. Signalling networks were assessed by Western blot.

Results: Wnt6 and FzD7 expression are prominent in the pronephros of the developing mouse kidney. TCF/Lef reporter activity is also prominent in the developing mouse pronephros. In the UUO mouse model, Wnt6 was down-regulated suggesting that loss of Wnt6 may have pathogenic significance. *In vitro* Wnt6 expression leads to *de novo* tubulogenesis in MDCK cells grown in 3D culture and increased expression of epithelial cell specific markers. Overexpression of Wnt6 in mesenchymal stem cells led to commitment to adipogenic/epithelial phenotypes. Wnt6 rescued epithelial to mesenchymal transition in

response to TGFβ. Wnt6 overexpression in renal epithelial cells inhibited TGFβ-mediated p65-NFκB nuclear translocation highlighting possible crosstalk pathways between Wnt and NFkB signalling.

Conclusions: We propose that Wnt6 is involved in determining cell fate specification and as such its increased expression activates transcriptional programmes that determine phenotypic transition. If these mechanisms can be established, they may be of pharmacological and clinical relevance in terms of developing new therapeutic strategies to slow or reverse the progression of DN.

TH-PO273

The Effects of High Glucose on Kidney Stem Cells and Its Injury Mechanism
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Background: To explore the effects of high glucose on the biological characteristics and paracrine action of kidney stem cells (KSC).

Methods: The KSC were isolated from the renal papilla in SD rats. The phenotye characteristics of KSC were identified. The ability of adipogenic and osteogenic differentiation of KSC was evaluated. The differences of gene expression between KSC and rat renal tubular epithelial cell (RTEC) were compared using qRT-PCR. The changes of the cell morphology, proliferation, phenotypic markers, chemokine expression and anti-hypoxia ability of SKC were investigated after cultured with high glucose (30mmol/L) media. The effects of KSC conditional media (pre-treated with high glucose) on the hypoxia/reoxygenation (H/R) RTEC were investigated.

Results: The positive staining of CD29, CD90, CD73 and CD45 in the KSC were 99%, 95.8%, 99.9% and 3.4% respectively. The positive stainings of CD133 and Nestin were 33.2% and 70.2%, their double staining rate was 31.4%. The differentiation to adipogenic and osteogenic were induced successfully in the KSC. Compared with RTEC, the expression of Nanog, Oct4/pou5f1, Sox2/sry-box-2 andα-SMA,Vimentin were higher, and the expression of E-Cadherin, CK18 were lower in the KSC.The KSC grew slowly in the high glucose media. However, the synthesis of Fibronectin, Collagean I and Collagean IV were increased in the KSC which cultured with high glucose media. Meanwhile, the gene expression of a-SMA, Vimentin, TGF-β, CTGF and PAI-1 were higher. After cultured in hypoxia environment for 24 hrs, the cell proliferation index, the gene expression of SDF-1 and CXCR4 of KSC were lower in the KSC which pre-treated with high glucose.The cell apoptosis rate, the level of LDH and MDA were lower, and the level of SOD were higher in the H/R RTEC after co-cultured with KSC conditional media. However, the renovated ability of the KSC was damaged by high glucose.

Conclusions: The KSC were isolated successfully from the rat renal papilla. The ability of proliferation, differentiation, anti-hypoxia and renovated of the KSC may injure in the high glucose media. These effects may via the changes of cell apoptosis and the oxidative stress in the KSC.

Funding: Government Support - Non-U.S.

TH-PO274

Erythropoietin Ameliorates Renal Damage in 5/6 Nephrectomized Rats via Endothelial Progemtor Cells
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Background: To investigate the effects of erythropoietin (EPO) on renal protection in rats with chronic renal failure (CRF) and its mechanism.

Methods: The CRF model was established by a two stage 5/6 nephrectomy procedure in rats. Experimental rats were randomly divided into four groups: sham operation group (control group), CRF group, CRF rats treated with 30 U/kg EPO (low-dosage group) and with 50 U/kg EPO (high-dosage group). CRF rats received EPO by hypodermic injection for 6 weeks and then were sacrificed. Serum creatinine (Scr), blood urea nitrogen (BUN), urine protein, haematoglobin (Hb) and blood pressure were measured. The CD34 and CD31 expressions in glomerulus were detected by immunohistochemistry method. The mRNA of ET-1, eNOS and VEGF were detected by RT-PCR. Peripheral blood endothelial progenitor cells (EPCs) were isolated by density gradient centrifugation from peripheral blood mononuclear cells. The ability of cell proliferation, adhesion and vasculogenesis in vitro was further observed.

Results: The expressions of CD34 and CD31 protein in glomerulus, and the expressions of eNOS and VEGF mRNA in renal tissue were higher in EPO treatment group than those in CRF model group (all $P<0.05$). The expression of ET-1 mRNA in renal tissue was lower in EPO treatment group than that in CRF model group. In addition, the Scr, BUN, urine protein and blood pressure in EPO treatment group were significantly lower than those in CRF model group (all $P<0.05$). Haematoglobin in EPO treatment group was higher than that in CRF model group ($P<0.05$). Reanl pathological injury was improved by EPO treatment in dose-dependent manner. Compared to sham operation group, the ability of cell proliferation, adhesion and vasculogenesis vitro in CRF rats was remarkably decreased ($P<0.05$, respectively). Such ability was promoted significantly in dose-dependent manner by EPO treatment ($P<0.05$, respectively).

Conclusions: EPO can ameliorate renal pathological injury and renal function in rats with chronic renal failure, maybe through improving the number and ability of endothelial progenitor cells and then promoting the renovation of glomerular capillary endothelium.

Funding: Government Support - Non-U.S.

TH-PO275

The Secretome of Hydrogel Co-Embedded EPC and MSC Instructs Macrophage Polarization
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Background: We previously reported delivery of endothelial progenitor cells (EPC) embedded in HA-hydrogels protects renal function during acute kidney injury (AKI) and promotes angiogenesis. We attempted to further ameliorate renal dysfunction by co-embedding EPC with renal mesenchymal stem cells (MSC), while examining their paracrine mechanisms that affect proinflammatory macrophages.

Methods: A live/dead assay determined if EPC-MSC co-embedding in HA-hydrogels improved viability during LPS treatment, while embedded delivery of cells to LPS-induced AKI mice was assessed for effects on mean arterial pressure (MAP), renal blood flow (RBF), serum creatinine, proteinuria and angiogenesis (femoral ligation). The cyto-/chemokine release from embedded stem cells was examined including effects on modulating the polarization and release of proinflammatory molecules from macrophages.

Results: EPC-MSC co-culturing improved stem cell viability during LPS exposure, an effect augmented by MSC hypoxic preconditioning. Delivery of co-embedded EPC with hypoxic preconditioned MSC to AKI mice demonstrated additive improvement (as compared to EPC delivery alone) in medullary RBF and proteinuria, with no differences observed for serum creatinine, MAP or angiogenesis. Exposure of proinflammatory M1 macrophages to EPC-MSC conditioned culture medium changed their polarization to anti-inflammatory M2 macrophages, while EPC-MSC delivery to endotoxemic mice elevated levels of circulating M2 macrophages. Incubation of co-embedded EPC-MSC with macrophages altered their release of cyto-/chemokines including enhanced release of anti-inflammatory IL-10 from macrophages.

Conclusions: Co-embedding EPC-MSC 1) improved their resistance to stress, 2) impelled macrophage polarization from M1 to M2, 3) altered macrophage release of cyto-/chemokines, and 4) improved renal and vascular function when MSC were preconditioned by hypoxia.

Funding: NIDDK Support, Private Foundation Support

TH-PO276

Creating Filtration Structure From a Suspension of Embryonic Kidney Cells
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Background: Worldwide, the lack of donor kidneys available for transplantation has driven a strong interest in developing methods for engineering new kidney tissue. Recently, we have shown that organoids constructed *in vitro* from embryonic kidney cells can integrate into living recipients, and perform kidney functions.

Methods: Here, we examined whether engineered organoids could provide the adequate environment to obtain *i)* mature 3D ultrastructural organization of filtration slits, and *ii)* selective transglomerular filtration and tubular uptake of fluorescent probes. Organoids made from suspensions of E11.5 mouse kidney cells were cultured *in vitro* for 5 days, then implanted under the renal capsule of a rat host, and allowed to grow for 2 weeks.

Results: Electron microscopy (EM) analysis of implanted organoids showed glomeruli in various developmental stages, with glomerular capillary walls covered by podocytes. The foot processes were regularly spaced and bridged by slit diaphragms, displaying differentiated ultastructure. A closer analysis of slit diaphragms ultrastructure by scanning EM in-lens detector revealed the presence of heterogeneous pores within the junction, similar to those of adult kidney. To test the physiological permselectivity of the filtration barrier, fluorescent dextrans of various molecular weights (MW) (10, 70 or 155 kDa) were systemically injected into the host rat. Analysis of implanted organoids showed that all dextrans were localized in α-actinin 4-positive glomeruli, while only those of low MW (10 and 70 kDa) were found in proximal tubuli where they colocalized apically with megalin-receptor, indicating transglomerular passage and tubular uptake of selectively ultrafiltered probes.

Conclusions: Thus, the proposed technology allows indeed to create nephrons with essential functional properties starting from cell suspensions. The present approach may open future perspectives for cell-based therapeutics and offers a methodological basis for developmental or disease-modeling studies.

Funding: Private Foundation Support

TH-PO277

Renal Tissue Decellularization and Regeneration: Therapeutic/Curative intervention Paradigm for Polycystic Kidney Disease
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Background: Tissue regeneration through use of stem cell is a novel way of meeting the challenges both at the level of cure as well as understanding the molecular mechanisms involved in tissue regeneration process. The Chemical base decellularization followed by recellularization with stem cells is opening newer vistas for understanding both the renal tissue functioning as well as pathology, with special focus onPKD.

Methods: Two different experimental approaches were used. One of the approaches exploited chemical based decellularization of an adult kidney followed by recellularization with stem cells.The characterization for the decellularization/ recellularization was analysed employing histologic, Scanning Electron Microscopic analysis while the stem cell characterization was followed through flowcytometry.

Results: The de-cellularization produced renal scaffold devoid of any cells, confirmed by light microscopy followed by SEM. 3D porous structures along with Nanofibrous structures of ECM were found to be well maintained. The Masson's trichrome staining demonstrated well maintained collagen protein and decellularization did not show any adverse effect on ECM. The recellularization using mesenchymal stem cells isolated from bone marrow demonstrated their relocation to emptied scaffolds as seen by nuclear staining. Mass spectrometry allowed to determine the novel proteins important to development of the recelluarized structures. The similar approach was used to generate decellularized structures of *in vitro* renal cyst model system (metanephric system) and its recullularization using stem cells to find out the potentially common chemico-molecular components important for tissue regeneration both under normal and polycystic kidney disease condition.

Conclusions: The present work thus would delineate the importance of different chemico-molecular entities in understanding the disease patho-physiology, treatment and organ regeneration, specifically the one associated with polycystic kidney disease.

TH-PO278

Strategies for Proximal Tubule Differentiation of Human Induced Pluripotent Stem Cells

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Background: The ability to generate human induced pluripotent stem cells (hiPSCs) from any individual has huge potential for several branches of clinical research, from disease elucidation to drug development. However, the development and optimization of differentiation protocols for target cells is not trivial. Successful differentiation protocols have been developed for several target cell types including neurons, cardiomyocytes and hepatocytes. However, there is currently no successful differentiation strategy to direct iPS cells into proximal tubular cells.

Methods: To this end we tested different combinations of growth factors, including bone morphogenetic proteins (BMPs), activin A, retinoic acid, and fibroblast growth factors (FGFs) in an attempt to differentiate hiPSC into proximal tubular like cells. hiPSC were grown on matrigel under feeder-free conditions in mTeSR1 medium. To induce differentiation, factors for maintenance of pluripotency were withdrawn and cells were cultured in DMEM/F12 supplemented with above mentioned growth factors, in different combinations for different times.

Results: In all cases, after 24 h cells had exhibited a radical change in gross morphology from small compact stem cell morphology to larger cobblestone shaped colonies. At day 4 in culture the pluripotent marker Oct4 was undetectable indicating a loss of pluripotency. The majority of cells could be maintained for up to 28 days in culture. Two of the combinations tested looked promising as cells expressed proximal tubule markers including aquaporin 1, claudin 2 and the renal epithelial marker cadherin 16. In addition solute and water transport could be observed by dome formation.

Conclusions: These results represent the first report of successful proximal tubule orientated iPSC differentiation. However, more characterization work is needed and further optimization will be required to improve proximal tubule differentiation status and purity.

TH-PO279

Epigenetic Induction of Kidney Lineages in Human Kidney Derived iPS Cells

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Background: OSR1 is one of the earliest intermediate mesoderm markers, and OSR1+ cells induced from human iPS cells (201B7: established from human fibroblast) were reported to be able to differentiate into multiple cell types of intermediate mesoderm-derived organs in vitro and in vivo (Nature Commun. 4, 1367, 2013). However, OSR1+ cells were reported to fail to induce metanephric progenitors (Cell Stem Cell 14, 53, 2014). Recently epigenetic memory such as DNA methylation signature of iPS cells derived from parental cells were reported to determine the differentiation fate of the iPS cells (Nature 2012), and this discrepancy could be explained by epigenetic memory in fibroblast derived iPS cells. In this study, we tried to confirm the kidney specific induction protocol (Nature Commun, 2013) by using two different kinds of human iPS cells established from fibroblast (F-iPS) and kidney epithelial cells (K-iPS), and also tried to establish efficient induction of kidney lineages by epigenetic induction.

Methods: According to the kidney specific induction protocol, we treated F-iPS and K-iPS with Activin A, GSK-3β inhibitor, BMP-7 and combination of HDAC inhibitor (TSA). We examined expression of markers of kidney development (WT-1, Pax-1, Sall-1) and differentiation (AQP-1, Nephrin) by Western blot, RT-PCR, and real time-PCR.

Results: Our results confirmed induction of these markers both in F-iPS and K-iPS. Interestingly, we found more efficient induction of these markers in K-iPS treated with kidney specific induction protocol in a time-dependent manner as compared with F-iPS cells. Moreover combination of kidney specific induction protocol and TSA significantly augmented induction of these markers.

Conclusions: These results demonstrate that epigenetic induction with kidney specific induction protocol can augment epigenetic memory-determined differentiation of human iPS cells towards kidney lineages, and additional *in vivo* date using K-iPS and epigenetic induction will be discussed.

TH-PO280

Transposon Mediated Long-Term Kidney-Specific Transgene Expression after Gene Transfer In Vivo

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Background: Dialysis has a ten-year survival rate of 10% and there is currently a shortage of donor organs for transplantation. Therefore, alternative breakthrough methods to treat patients with kidney disease are needed.

Methods: We have developed a procedure to efficiently and specifically deliver plasmid DNA to the kidneys of mice that is based on the principles of hydrodynamic tail vein injection: a high volume of injection and a rapid delivery of the DNA solution. We found that a fast injection of the renal pelvis with a DNA solution of sufficient volume was effective for delivery of plasmid DNA to the kidney.

Results: Mice given renal pelvis hydrodynamic injections had high levels of luciferase transgene expression that was localized specifically to the injected kidney and no other organs by IVIS imaging. The volume and amount of DNA injected were not as important as the speed of the injection. When we injected a plasmid containing the CMV promoter to drive the luciferase transgene, the majority of the gene expression was lost within the first month after the injection. For long-term expression, we co-injected a plasmid to produce a hyperactive piggyBac transposase in order to permanently integrate the luciferase transgene carried by the transposon plasmid into the genome. We tested several promoters (CMV, EF1alpha, gamma-GT, and podocin). EF1alpha produced the highest levels of long-term gene expression. Steady-state levels of long-term expression were seen from 35 days to 89 days following injection in mice injected with the transposon and transposase plasmids, significantly higher than the group of mice that did not receive transposase. Staining suggested EF1alpha-luciferase expression is mainly localized to the renal tubules, whereas the CMV promoter expressed mainly in the fibroblast population.

Conclusions: We have developed a method of kidney-specific gene expression that may be employed to introduce exogenous transgenes to study kidney function in mice with the potential to be translated to the clinic for renal gene therapy.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO281

Population-Based Screening for Monogenic FSGS: Feasibility and Challenges

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Background: Sequencing Mendelian focal segmental glomerulosclerosis (FSGS) genes can identify causal mutations in familial cases. But it is more challenging to diagnose monogenic FSGS in sporadic cases. Using sequence data of 9 FSGS genes from population-based cases and controls, we sought to create a filter that can distinguish causal mutations from harmless rare or novel variants.

Methods: We sequenced *NPHS1, NPHS2, PLCE1, LAMB2, INF2, WT1, LMX1B, TRPC6, and ACTN4* in 94 Nephrotic Syndrome Study Network (NEPTUNE) subjects with FSGS and 60 ancestry-matched controls from the 1000Genomes Project Phase3 (1KG). We verified a high concordance of called variants for the 60 control subjects sequenced both by our lab and 1KG. We thus used the genotypes of all 1KG subjects as controls (n=2535). Our **gene-level filter** required variants to be *nonsense* and at <0.1% minor allele frequency (MAF) in Exome Sequencing Project (ESP). Or if *missense*; absent in ESP (or in the Human Gene Mutation Database and ESP MAF <0.1%) and predicted "damaging" in 2 of 3 of PolyPhen2, SIFT, and MutationTaster. We removed novel ESP variants that were common in any continental population. Our **isoform-level filter** classified *INF2 and LAMB2* variants as causal if they met the above requirements and were in isoforms under much stronger negative selection. We then calculated the prevalence and odds ratio of putative monogenic FSGS in NEPTUNE versus 1KG.

Results: With a prevalence of 5.3% versus 1.5% in 1KG, FSGS cases had 3.7 higher odds of putative monogenic FSGS (CI 1.4-9.6; p<0.01). Removing the isoform filter or relaxing the missense MAF filter to 0.1% did not change monogenic case prevalence. But prevalence increased in controls by 65% and 15%, respectively.

Conclusions: Our method used very stringent filters and isoform level data to reduce false positives. And we see significantly higher odds of causal mutations in cases versus controls, with 5.3% putative monogenic FSGS in NEPTUNE. Yet a 1.5% prevalence in controls is much higher than expected. Incomplete penetrance may contribute, but we aim to further reduce false positives by refining our method. Thus, when sequencing sporadic patients for FSGS, classifying them as monogenic should be done cautiously.

Funding: NIDDK Support

TH-PO282

Exome Sequencing of > 30 Genes Associated with Steroid Resistant Nephrotic Syndrome

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Background: Steroid Resistant Nephrotic Syndrome (SRNS) is a glomerular disorder characterised by proteinuria, hypoalbuminemia, oedema and lack of response to immunosuppression. In unselected Paediatric SRNS, the incidence of disease causing gene mutations is around 20%. So far over 30 genes have been associated with this disease.

We followed up our previous findings that the increasing reliability of Next Generation Sequencing (NGS) provides the potential for revolutionising genetic investigation of this and similar patient groups.

Methods: We used whole exome sequencing to screen 196 paediatric SRNS patients for genes known to be associated with hereditary SRNS as well as to look for novel variants in other potential disease causing genes. Patients were collected via a national UK Renal Registry with comprehensive detail of phenotype. Significant variants detected by NGS were confirmed by Sanger sequencing.

Results: 21% of the sequenced patients had either a previously described mutation or a variant likely to be disease causing. Analysis revealed known as well as novel disease associated variations in the four commonest Nephrotic genes: *NPHS1, NPHS2, LAMB2* and *WT1* and also in other, more rare genes such as *LMX1B, MYO1E, ADCK4* and the recently identified *CRB2*. One of the tested patients was found to have 4 mutations in 2 genes. Phenotypically unexpected mutations were a hemizygous missense mutation in *COL4A5* in a patient without hearing loss and a *TRPC6 mutation* in a patient who presented at age of 8 years. Increased burden of certain common polymorphisms were noted in the known SRNS genes in cases versus controls.

Conclusions: Our results demonstrate the obvious clinical need for sequencing multiple genes in SRNS where genetic heterogeneity is a defining feature. We identified mutations and potentially pathogenic variants in genes that would not routinely be screened under current testing practice. Our detailed phenotypic information also enabled us to identify potential modifier variants from this cohort.

TH-PO283

CKD-Y: Next Generation Sequencing in Search of Primary Renal Disease Diagnoses in Young CKD/ESRD Patients Albertien M. van Eerde,[1] Edith Peters,[1] Kirsten Y. Renkema,[1] Bert van der Zwaag,[1] Marc Lilien,[2] Martin H. De Borst,[3] Rachel H. Giles,[4] Gerjan Navis,[3] Nine V. Knoers.[1] *[1]Dept of Medical Genetics, UMC Utrecht, Utrecht, Netherlands; [2]Paediatric Nephrology, Wilhelmina Children's Hospital, UMC Utrecht, Utrecht, Netherlands; [3]Dept of Nephrology, UMC Groningen, Groningen, Netherlands; [4]Dept of Nephrology, UMC Utrecht, Utrecht, Netherlands.*

Background: In the Netherlands, 25% of patients with early onset (<30 years) renal replacement therapy (RRT) have not been diagnosed with a primary renal disease (PRD) according to the ERA-EDTA registry classification. An additional 20% are registered with a CAKUT phenotype (congenital anomalies of the kidney and urinary tract) and approximately 10% have other congenital or hereditary disorders registered. These numbers are similar to other national registry data of similar renal disease patients. It is likely that in these young patients, part of the unknown PRD diagnoses represent CAKUT and other congenital/inherited diseases. If the PRD diagnosis is unknown, this hampers the classification and genetic counseling. Project CKD-Y aims to improve genetic diagnostics in and diagnostic classification of Young CKD/ESRD patients.

Methods: We have designed the "RENome" using SureSelect, Agilent technology to include 408 genes involved in hereditary renal disease and/or urinary tract development. The regions are captured and subsequently sequenced (SOLiD™ 5500XL platform; Applied Biosystems, Inc). The ~200 samples of all early onset (<30 years) RRT patients from the REGaTTA cohort are being sequenced. Variants detected by RENome sequencing will be validated by Sanger sequencing and for selected variants the pathogenicity will be functionally studied.

Results: RENome Next Generation Sequencing results will be presented, and applicability in diagnostic infrastructure and genetic counselling will be discussed.

Conclusions: RENome sequencing will for the first time show in how many young RRT patients rare variants in known renal disease genes are likely to have contributed to their renal disease. This will improve the possibilities for genetic counseling and diagnostic classification. *AMvE is supported by the Dutch Kidney Foundation (as are NK, RG, MdB) and Fonds NutsOhr*a.

Funding: Private Foundation Support

TH-PO284

Rationalizing the Genetic Diagnosis of SRNS/FSGS Using Next Generation Sequencing Kits Olivia Boyer,[1,2,3] Beata S. Lipska-Zietkiewicz,[4,6] Olivier Gribouval,[1,2] Patrick Nitschke,[2] Christine Bole-Feysot,[1] Annelies Rotthier,[7] Jurgen Del Favero,[7] Franz S. Schaefer,[5,6] Corinne Antignac.[1,2,3,8] *[1]Inserm U1163, Imagine Inst, Paris, France; [2]Paris Descartes Univ; [3]Pediatric Nephrology, Necker Hospital, APHP; [4]Biology and Genetics, Medical Univ of Gdansk, Gdansk, Poland; [5]Pediatric Nephrology, Center for Pediatrics and Adolescent Medicine, Heidelberg, Germany; [6]Podonet Consortium; [7]Multiplicom NV, Niel, Belgium; [8]Medical Genetics, Necker Hospital, APHP.*

Background: Steroid-resistant nephrotic syndrome (SRNS) and idiopathic FSGS are genetically heterogeneous with > 25 causative genes identified to date. The stepwise genetic analysis through classical Sanger sequencing has become rather long and expensive. Conversely, exome sequencing analyses millions of genes in parallel but accumulates large amounts of data and may lead to ethically challenging incidental findings.

Methods: Through the EurenOmics consortium, our group designed a custom multiplex PCR kit (Multiplicom) that analyses 31 known or plausible SRNS/FSGS genes. This kit was validated on 23 mutated patients (97% sensitivity) then used to perform genetic testing in 71 patients without any identified mutation by classical screening, and 56 new patients (127 families). The median age at diagnosis was 4 yrs (0-48), and 39% had reached ESKD at a median age of 12 yrs (0-67). 54% had a positive family history and 21% extra-renal features.

Results: Mutations were identified in 15% of families previously tested negative with conventional screening and 20% of newly diagnosed patients. This translated into a mutation rate of 13% in autosomal dominant families, 50% in autosomal recessive or consanguineous families and 14% in sporadic cases. The mutated genes were: *NPHS1* (3 families), *NPHS2* (4), *PLCE1* (2), *WT1* (1), *INF2* (2), *LMX1B* (2), *MYO1E* (1) and *COQ2* (1), *COQ6* (1), *ADCK4* (2), *LAMB2* (1) and *SMARCAL1* (2). Interestingly, we identified a homozygous *MYO1E* missense mutation in 2 siblings with late onset autosomal recessive FSGS and ESKD around 35 years of age.

Conclusions: Targeted Next Generation Sequencing kits are powerful and cost-effective techniques that will allow the rationalization of genetic diagnosis of SRNS/FSGS.

Funding: Government Support - Non-U.S.

TH-PO285

Sequence Kernel Association Test to Discover Associated Regions in a Large National Pediatric Steroid Resistant Nephrotic Syndrome Cohort Denis Andrew Baird,[1] Agnieszka Bierzynska,[2] Ania B. Koziell,[3] Gavin Iain Welsh,[2] Ian N. Day,[1] Moin Saleem.[2] *[1]School of Social and Community Medicine, Univ of Bristol, Bristol, United Kingdom; [2]Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom; [3]Dept of Immunobiology, King's College London, London, United Kingdom.*

Background: Steroid Resistant Nephrotic Syndrome (SRNS) is a genetically and phenotypically heterogeneous, with a wide mutation spectrum, ranging from rare highly penetrant mutations to common risk contributing variants. In this sense, SRNS behaves more like a complex trait. Genome Wide Association Studies (GWAS) techniques have associated common variants underpinning many complex traits. However, for most diseases a large proportion of heritability still remains to be explained, directing GWAS research efforts towards detecting the contribution of rare variants. Collapsing methods, based on grouping rare variants across a region to increase power, have been developed to facilitate this. SKAT, a variance component collapsing method, was run on a cohort of 64 childhood SRNS cases, collected from the UK RADAR Registry, in order to determine specific regions of the exome associated with SRNS.

Methods: SKAT combined common and rare [1] was run on NPHS1. Multiple controls obtained from UK10K were used. The analysis is currently being repeated on a larger sample of 97 additional patients to confirm the NPHS1 association, and will be extended to incorporate all genes across the genome. In addition, collapsing over protein domains will be carried to reduce noise and localise signals within genes, and separate SKAT iterations will be performed for all, rare and common point mutations. 1. Sequence Kernel Association Tests for the Combined Effect of Rare and Common Variants. *Am. J. Hum. Genet.* **92,** 841–853 (2013).

Results: NPHS1 showed a strong association with SRNS overall.

Conclusions: SKAT is a commonly applied tool to elucidate rare variants in common, complex disease. However, often rare variant signals are not detected by collapsing methods. The association with NPHS1 indicates that SKAT could be a powerful tool for detecting regions which harbor functional mutations in rare, polygenetic diseases such as SRNS.

TH-PO286

Collagen Mutations Identified by Targeted Next Generation Sequencing Are the Most Frequent Mutations Underlying Adult Focal Segmental Glomerulosclerosis Christine Gast,[1,2] Reuben J. Pengelly,[2] Gopalakrishnan Venkat-Raman,[1,2] Sarah Ennis.[2] *[1]Wessex Renal and Transplant Service, Queen Alexandra Hospital, Portsmouth, Portsmouth, Hampshire, United Kingdom; [2]Human Genetics and Genomic Medicine, Univ of Southampton, Southampton, Hampshire, United Kingdom.*

Background: Multiple genes underlying focal segmental glomerulosclerosis (FSGS) and/or steroid resistant nephrotic syndrome (SRNS) have been identified, with the recent inclusion of collagen mutations. We aimed to investigate the distribution of gene mutations in adult patients with FSGS/SRNS by targeted next generation sequencing.

Methods: 91 adults from 86 families with familial or sporadic FSGS/SRNS were recruited as part of a study on familial kidney diseases. All individuals were ascertained through a single renal unit in the South of England. All but 2 were of Caucasian ethnicity, 34 had a family history of renal disease. DNA was extracted from whole blood or saliva using standard protocols. An Illumina Truseq Custom Amplicon Targeted Next Generation sequencing (NGS) panel was designed covering 39 genes for FSGS/SRNS including *COL4A3-5*. NGS sequencing was performed on the MiSeq system. Data were analysed using our standard Mendelian disease pipeline.

Results: Confirmed pathogenic mutations were identified in 14 patients, 11 with positive family history. Pathogenic *COL4A5* mutations were found in 4 patients from 3 families, all with a diagnosis of familial FSGS. Two siblings were compound heterozygous for different pathogenic *COL4A3* mutations. A single pathogenic *COL4A3* and *COL4A4* mutation were found in two individuals with sporadic FSGS, one had a family history of haematuria. Before NGS, Alports had only been suspected in two of 91 probands. Other pathogenic mutations included *LAMA5* (2), *INF2* (1), *CD2AP* (1), *NFX5* (1) and *NPHS2* (1). In other probands, novel mutations predicted to be pathogenic were found in *COL4A3*, *COL4A4*, *COL4A5*, *COQ2*, *INF2*, *TRPC6*, *WT1* and *SYNPO*.

Conclusions: We have shown that collagen mutations frequently underlie FSGS/SRNS and should be considered, particularly with a positive family history. Targeted NGS allows identification of these gene mutations.

Funding: Private Foundation Support

TH-PO287

Whole Exome Sequencing Identifies Mutation of the *Exportin-5* as a Novel Single-Gene Cause of Nephrotic Syndrome Shazia Ashraf,[1] Carolin Sadowski,[1] Stefan Kohl,[1] Svjetlana Lovric,[1] Werner Pabst,[1] Heon Yung Gee,[1] Friedhelm Hildebrandt.[1,2] *[1]Div of Nephrology, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Howard Hughes Medical Inst, Chevy Chase, MD.*

Background: Identification of single-gene causes of nephrotic syndrome (NS) has furthered the understanding of its pathogenesis. However, many genes and disease mechanisms remain unknown. To identify new genes, that if mutated cause NS, we combined homozygosity mapping (HM) and whole human exome sequencing (WES) in consanguineous families with NS.

Methods: An individual of consanguineous parents from Turkey with NS was cyclosporinA responsive and histologically showed minimal change NS. HM yielded >15 segments of homozygosity by descent with a cumulative homozygous segments of ~300 Mb. We performed WES in this individual to identify the underlying single-gene disease-causing mutation.

Results: By WES in this consanguineous family, we identified a homozygous missense mutation (p.Val552Ile) in the *Exportin-5* (*XPO5*) gene in an amino acid residue conserved since *Ciona intestinalis*. The mutation segregated with the affected status in this family and was absent from >6,500 European controls in the Exome Variant Server. XPO5 is a scaffold protein that belongs to a large family of karyopherins and thus mediates the transport of proteins between the nuclear and cytoplasmic compartments. XPO5 is known to bind the nucleoporins NUP214 and NUP153 in a Ran-GTP-independent manner and also to RAN in its GTP-bound conformation. Immunoflorescence microscopy showed that XPO5 is highly expressed in rat glomerular podocytes. XPO5 also co-localizes with SYNPO (synaptopodin) at the slit membrane in mature rat glomeruli.

Conclusions: We, thus, identified mutation of XPO5 as a potential novel single-gene cause of NS. Further genetic and functional studies will shed light on the involvement of nuclear pore proteins in the pathogenesis of NS and will provide a further step in understanding the disease mechanism.

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TH-PO288

Exome Capture Identifies Mutation of NUP107 as a Novel Cause of Nephrotic Syndrome Svjetlana Lovric, Shazia Ashraf, Carolin Sadowski, Heon Yung Gee, Daniela A. Braun, Werner Pabst, Friedhelm Hildebrandt. *Nephrology, Boston Children's Hospital, Boston, MA.*

Background: Nephrotic syndrome is characterized by proteinuria, hypoalbuminemia, edema and hypertriglyceridemia. 20% of childhood NS is steroid resistant (SRNS). Whereas over 30 single-gene causes of SRNS are known, a large proportion of SRNS remains genetically unsolved.

Methods: To identify novel single-gene causes of SRNS we applied homozygosity mapping with whole exome sequencing (WES) to 100 families with SRNS. We than screened a worldwide cohort of ~800 families with NS with a barcoded array based multiplex PCR (48x48 Fluidigm Access Array™) and next generation re-sequencing.

Results: In a consanguineous family with 3 affected individuals we identified a homozygous missense mutation (p.Met101Ile) in the gene NUP107 (nucleoporin 107 kDa) by WES. NUP107 is a scaffold protein of the nuclear pore complex, which is responsible for the transportation of small molecules between the nucleus and the cytoplasm, e.g. mRNA export by small Ran GTPases. The identified amino acid substitution is located in an N-terminal phosphorylation site and in addition may result in loss of function by skipping exon 4. The mutation in NUP107 leads to a very distinct phenotype of nephrotic syndrome, with focal segmental glomerulosclerosis and microcephaly.

Conclusions: We here identified a mutation in NUP107 as a novel disease-causing gene of SRNS, revealing the nuclear core complex as a new pathomechanism of SRNS.

Funding: Other NIH Support - DK086542 and DK076683

TH-PO289

Functional Analysis of New Steroid-Resistant Nephrotic Syndrome Candidate Genes Sara Gonçalves,[1,2,3] Christelle Arrondel,[1,2] Martin Helmstädter,[4] Olivia Boyer,[1,2,5] Olivier Gribouval,[1,2] Christine Bole-Feysot,[1,2] Patrick Nitschke,[1,2] Marie-Claire Gubler,[1,2] Tobias B. Huber,[4,6] Matias Simons,[1,4,7] Geraldine Mollet,[1,2] Corinne Antignac.[1,2,8] *[1]Inserm U1163, Paris, France; [2]Paris Descartes – Sorbonne Paris Cité Univ, Imagine Inst, Paris, France; [3]Nephrology, Santa Maria Univ Hospital, Lisbon, Portugal; [4]Renal Div, Univ Hospital Freiburg, Freiburg, Germany; [5]Pediatrics Nephrology, Necker Hospital, Paris, France; [6]BIOSS, Albert-Ludwigs Univ, Freiburg, Germany; [7]ZBSA, Univ of Freiburg, Freiburg, Germany; [8]Genetics, Necker Hospital, Paris, France.*

Background: Inherited forms of steroid-resistant nephrotic syndrome (SRNS) are genetically heterogeneous and the disease-causing mutations remain unknown in approximately half of familial cases and the majority of sporadic cases. We sought to identify new genes implicated in SRNS.

Results: Exome sequencing was performed on 2 affected members of a consanguineous family, presenting SRNS, cardiomyopathy and neurologic impairment. We identified two homozygous missense variants in two candidate genes, ADD3 and KAT2B, both expressed in podocytes. Segregation analysis in non-affected family members did not exclude any mutation. The first gene encodes adducin γ, an important regulator of the actin cytoskeleton, and the second gene encodes the lysine acetyl transferase2B (KAT2B), which is involved in acetylation of histones. Using xCELLigence technology we found that adducin γ knock-down (KD) in a human podocyte cell line led to decreased adhesion and migration, whereas KAT2B KD led to decreased adhesion only. Additionally, validation experiments using the GAL4-UAS expression system were performed in Drosophila nephrocytes: KD of the ADD3 orthologue in garland nephrocytes disrupted the actin cytoskeleton, decreased the expression of the NEPH1 orthologue, kirre, and led to decreased endocytosis in adult nephrocytes.

Conclusions: Our results point to a major effect of ADD3 in podocyte biology. Transgenic fly lines carrying the human mutations will help to define the precise role of each mutation.

Funding: Government Support - Non-U.S.

TH-PO290

Exome Sequencing Identified Novel Mutations of *FKRP* in Familial Atypical Hemolytic Uremic Syndrome Hirofumi Watanabe,[1] Shin Goto,[1] Hajime Yamazaki,[2] Tadashi Yamamoto,[3] Ichiei Narita.[1] *[1]Div of Clinical Nephrology and Rheumatology, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Dept of Internal Medicine, Nagaoka Red Cross Hospital, Nagaoka, Japan; [3]Dept of Structural Pathology, Inst of Nephrology, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan.*

Background: Atypical hemolytic uremic syndrome (aHUS) is a rare renal thrombotic microangiopathy. Previously, disease-causing mutations of aHUS have been identified in the complement-related genes. However, with the advancement of sequencing technology, novel causative genes not related to complement regulation were recently reported. To dissect causative genes for aHUS thoroughly, we performed comprehensive genetic analysis in a family with aHUS harboring no mutations in complement-related gene.

Methods: We studied a Japanese multiplex family composed of three patients diagnosed with aHUS in infancy, clustered in a dominant transmission mode. Unequal crossing over at the *CFH* and *CFHR* loci was screened with multiplex ligation-dependent probe amplification (MLPA). Moreover, we conducted whole exome sequencing of three affected and three unaffected individuals using Ion Proton Sequencer. Variants identified by exome sequencing were filtered on the basis of variant annotation, functional expectation, and allele frequency. Genome-wide linkage analysis was also carried out using exome sequencing data.

Results: In this family, copy number abnormalities in *CFH/CFHR* co-segregating with the disease were not detected by MLPA analysis. Exome sequencing detected a total of 130 heterozygous and non-synonymous variants shared only by the affected individuals. Filtering against several variant databases and excluding variants harbored by additional unaffected members of the family, we identified five variants located in the region with maximum LOD scores. The mutation of two consecutive amino acids in *FKRP* (p.R167S and p.C168R) was predicted to have substantial effect on the protein structure or function.

Conclusions: We identified novel mutations of *FKRP* in a family with aHUS, and functional analyses of these mutations are in progress to reveal their roles in the development of aHUS.

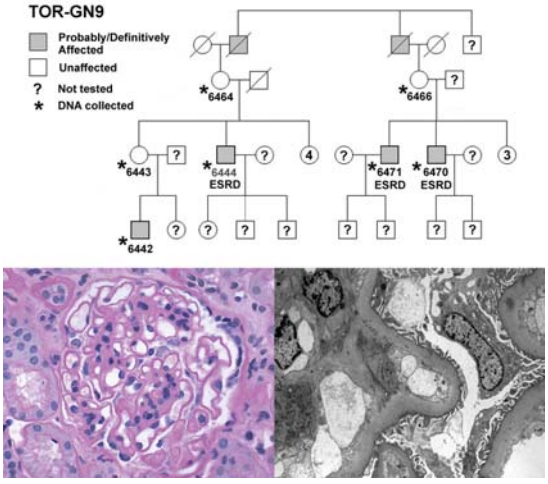
TH-PO291

A Familial Syndrome Associated with an Unusual Glomerular Pathology and Putative X-Linked Recessive Inheritance Moumita Barua,[1] Ginette Starkell,[2] Rohan John,[3] Daniel C. Cattran,[1] York P. Pei.[1] *[1]Div of Nephrology, Univ Health Network, Toronto, ON, Canada; [2]Dept of Pathology, Brampton Civic Hospital, Mississauga, ON, Canada; [3]Dept of Pathology, Univ Health Network, Toronto, ON, Canada.*

Background: The identification of Mendelian genes underlying rare familial disorders have yielded important insights in glomerular pathobiology.

Methods: We clinically ascertained the proband (6442) and other key or affected members of TOR-GN9. Whole exome sequencing (WES) of two affected members (6442 and 6471) is in progress.

Results: The pedigree of TOR-GN9 is shown in the upper panel of Figure.



The clinical course of our proband (6442) is characterized by recurrent tonsillitis and nephrotic range proteinuria up to 10 g/day. A renal biopsy revealed diffuse glomerular basement membrane thickening and mesangial expansion with focal global and segmental glomerulosclerosis by light microscopy (LM) and intramembranous immune complex deposition by electron microscopy (EM) (lower panel). Linear staining of IgG, IgA, kappa and lambda as well as IgM staining in the mesangium and capillary wall by immunofluorescence (IF) microscopy was also noted. He was treated with a course of steroids with partial remission of his proteinuria (~2 g/day) at the last follow-up. Review of the renal biopsy report from 6444 described similar findings on LM but no EM/IF was available. He was given a diagnosis of membranous nephropathy.

Conclusions: The absence of disease in three putative obligate female carriers (i.e. 6464, 6466, and 6443) in this family suggest an X-linked recessive inheritance. WES is in process and has the potential to identify the disease-causing genetic defect of this unusual syndrome.

Funding: Government Support - Non-U.S.

TH-PO292

Autosomal Dominant Alport Syndrome: Molecular Analysis of the COL4A3/COL4A4 Genes and Clinical Outcome

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Background: Alport syndrome encompasses a group of inherited, heterogeneous disorders involving chronic kidney disease progressing to end-stage renal disease (ESRD), sensorineural hearing loss and ocular abnormalities. Autosomal dominant Alport syndrome (ADAS) caused by heterozygous mutations in either *COL4A3* or C*OL4A4* gene is a rare mode of inheritance. Until now, only about 30 families with ADAS have been reported and the phenotype/genotype correlation is not well known.

Methods: A retrospective analysis of 24 genetically diagnosed patients with ADAS in 11 unrelated families was conducted. The analysis of *COL4A3/COL4A4* genes was performed by PCR and direct sequencing of genomic DNA for all exons and exon-intron boundaries.

Results: In our study, the mean age at first detection of proteinuria was 15.8 years. Five patients(20.8%) reached ESRD at the mean age of 60.6 years. No patient had hearing loss or ocular lesion. Molecular analysis revealed six novel mutations including four missenses, one splicing-site mutation and one in-frame mutation. Four mutations were reported as causative mutations for autosomal recessive Alport syndrome in the previous studies. Interestingly, heterozygous carrier parents of these mutations in those reports were asymptomatic or only presented microhematuria.

Conclusions: The present study showed the renal phenotypes in ADAS patients were much milder than X-linked or autosomal recessive Alport syndrome patients. Hearing loss and ocular abnormalities were quite rare in our cohort. Thus, it is difficult to make a diagnosis in the early phase of the disease. The correlation between mutation and phenotype was unclear. Especially, we detected four mutations previously reported as pathogenic mutations for ARAS in five ADAS families. These results indicate the existence of modifier genes to affect the phenotype of kidney disease in ADAS. Additional research is needed to confirm the predictive indicator for phenotypic variation of ADAS.

TH-PO293

Drug Repurposing for the Treatment of Experimental Alport Syndrome

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Background: Alport syndrome (AS) is a hereditary disorder due to mutations in any of the genes encoding collagen-IV-a3a4a5 chains in the glomerular basement membrane, leading to ESRD. There are few treatment options for AS. Accordingly, we sought to identify existing drugs for the repurposed treatment of AS by studying gene expression in the kidneys of Col4a3-/-mice.

Methods: We performed gene profiling of renal cortices from male 129/Svj Col4a3-/-mice (KO) and wild type (WT) mice at 4 and 7 weeks of age (N=8/group). Gene expression was analyzed with Affymetrix_Mouse_Gene2.0_ST array. We used Significance Analysis of Microarrays (SAM) and 2-way ANOVA to identify differentially expressed genes. These genes were utilized for drug repurposing by Connectivity Map (CMAP) and Drug Pair Seeker (DPS) to find drugs that reverse AS gene expression changes.

Results: We used SAM to identify the "disease signature", composed of 769 significantly up-regulated and 113 significantly down-regulated genes in 7-week-old KO versus WT mice (FDR<1%). The top drug combination that reversed this disease signature in DPS included vorinostat and genistein. We next identified the "progression signature", composed of 426 differentially up-regulated and 501 down-regulated genes in 7-week-old compared to 4-week-old KO mice, when adjusted for age. The top drug that reversed the AS progression signature in CMAP was vorinostat (p=0.00032). Data reanalysis with 2-way ANOVA, accounting for the interaction between age and genotype, revealed that vorinostat and genistein were among the top drug combinations that reversed the AS progression signature. Both vorinostat and genistein inhibit tyrosine kinases.

Conclusions: Gene expression profiling and *in silico* analysis in a murine model of AS identified a novel drug combination that may reverse gene expression profile associated with renal disease progression. *In vivo* studies of this drug combination will determine if it impacts disease progression.

TH-PO294

Fourteen Different Monogenic Genes Account for 15% of Nephrolithiasis/ Nephrocalcinosis in a Mixed Pediatric and Adult Cohort

Jan Halbritter,[1] Michelle Baum,[1] Ann Marie Hynes,[2] Sarah Rice,[2] David T. Thwaites,[3] Zoran Gucev,[4] Jonathan Porath,[1] Ari Wassner,[5] Caleb Nelson,[6] Velibor Tasic,[4] John Andrew Sayer,[2] Friedhelm Hildebrandt.[1] *[1]Dept of Internal Medicine, Div of Nephrology, Boston Children's Hospital, Boston, MA; [2]Inst of Genetic Medicine, Univ of Newcastle, Newcastle, United Kingdom; [3]Inst of Cell & Molecular Biosciences, Univ of Newcastle, Newcastle, United Kingdom; [4]Medical Faculty Skopje, Univ Children's Hospital Skopje, Skopje, Macedonia, The Former Yugoslav Republic of; [5]Dept of Internal Medicine, Div of Endocrinology, Boston Children's Hospital, Boston, MA; [6]Dept of Urology, Boston Children's Hospital, Boston, MA.*

Background: Nephrolithiasis is a prevalent condition with a high morbidity. Although dozens of monogenic causes have been identified, the fraction of single-gene disease has never been studied.

Methods: To determine the percentage of cases that can be molecularly explained by mutations in one of 30 known monogenic causes of nephrolithiasis, we conducted a high-throughput mutation analysis in a cohort of 272 genetically unresolved individuals (106 children, 166 adults) from 268 families with one of the following diagnoses: nephrolithiasis (n=256), or isolated nephrocalcinosis (n=16).

Results: We detected 52 likely causative mutations in 14 of 30 genes analyzed, leading to a molecular diagnosis in 15.3% (41/268) of all cases. Twenty out of 52 detected mutations were novel (38.5%). The cystinuria gene *SLC7A9* (n=19) was most frequently mutated. The percentage of monogenic cases was surprisingly high, both in the adult (11.4%) and the pediatric cohorts (21.7%). Recessive causes were more frequent among children, while dominant disease occurred more abundantly in adults.

Conclusions: Our data represents one of the most in-depth studies of monogenic causes of kidney stone disease. We outline how knowledge of the molecular cause of nephrolithiasis and nephrocalcinosis has prognostic implications and will facilitate personalized treatment.

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TH-PO295

PAX2, a Key Molecule for Embryonic Kidney Development, Participates Progression of Kidney Dysfunction and Interstitial Fibrosis After Birth

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Background: The PAX2 gene, which encodes a developmental transcription factors, plays critical roles in development of the urogenital tract, eyes, ears, and central nervous system. Renal coloboma syndrome is a very rare condition that affects kidney and eye development. It was reported that around half cases of renal coloboma syndrome had PAX2 gene mutations. Here, we provide the evidence of PAX2 gene in kidney dysfunction after birth.

Methods: Twenty-six cases were clinically diagnosed as renal coloboma syndrome, and four cases had coloboma without any kidney abnormality (disease control). DNA of all cases was analyzed for PAX2 gene mutations by direct sequencing.

Results: Eleven out of 26 renal coloboma syndrome cases had PAX2 gene mutations. Four cases had novel mutations. Six cases (54.5% 6/11) were end stage kidney disease (ESKD) in PAX2 gene mutation group, and only two cases (13.3% 2/15) were ESKD in non-PAX2 gene mutation group. There was no ESAD case in coloboma only group. Proteinuria of renal coloboma syndrome with PAX2 mutations was tend to higher than that of renal coloboma syndrome without PAX2 mutations or coloboma only group (mena±SEM; 1.61±0.45, 0.64±0.32 g/gCr). Kidney biopsy of 6 cases with PAX2 gene mutation revealed that three were focal segmental glomerulonephritis and one was glomerulomegaly. Other two cases were non-IgA mesangial proliferative glomerulonephritis, and tubular interstitial nephritis, respectively.

Conclusions: Thus, our human data of renal coloboma syndrome indicate that PAX2 is not only a key molecule in embryonic kidney development, but also a key molecule in progression of kidney disease after birth.

Funding: Government Support - Non-U.S.

TH-PO296

Renal Biopsies after 6-11 Years of Enzyme Replacement Therapy in 9 Young Classic Fabry Disease Patients

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Background: Data on renal morphologic effects of long-term ERT is limited. Previous studies have shown correlation between dose of Enzyme Replacement Therapy (ERT) and GL3-clearing from baseline biopsy to 5 years (y) biopsy (Tøndel et al., JASN, 2013). The purpose of this study was to evaluate the effect of ERT in patients treated up to 11 years.

Methods: 9 classic Fabry patients (8 male/1 female) with median age of 17 y (range 7-30 y) at start of ERT had their last kidney biopsies performed after a median duration of ERT of 8 y (range 6-11 y). 6 patients had a baseline biopsy before start of ERT, and 3 patients had their first renal biopsy 2-3 y after initiation of ERT. The patients were divided into a high dose group (cumulative dose > 75 mg/kg, 5 patients) and a low dose group (cumulative dose < 75 mg/kg, 4 patients). Various dosing regimen were given. The podocyte-GL3 was scored in toluidine blue sections using the scoring system of the International Scoring Group of Fabry Nephropathy (Fogo et al., NDT, 2010). All patients had full podocyte score at baseline.

Results: Glomerular endothelial and mesangial cells were cleared in all patients. There was a significantly higher clearance of the podocyte GL3 score in the high dose group than in the low dose group; median change -1.9 (range -0.50 to -3.33) and -0.20 (range 0 to -0.42), respectively, p-value =0.016. A small increase in podocyte scores was observed at 8 years in all patients (no=3) who were switched from agalsidase beta 1.0 mg/kg/eow to agalsidase alpha 0.2 mg/kg/eow after 5 y of ERT; + 0.57, + 0.81 and + 0.92.

Conclusions: Reduction of podocyte GL3 deposits may be achieved with long-term ERT across all dosing regimen, and the clearing is dose-dependent. Switch from high dose to low dose regimen after 5 y ERT may result in reaccumulation of GL3 in the podocytes. Serial kidney biopsies after long-term ERT are valuable in evaluating treatment response.

Funding: Government Support - Non-U.S.

TH-PO297

Enzyme Replacement Therapy (ERT) in Fabry Disease (FD) Reduces Podocyte (PC) Globotriaosylceramide (GL3) Content within a Year (yr)

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Background: CKD is a common FD complication. Recent studies support a role for PC injury in FD nephropathy. Previous studies using flawed methodologies failed to show PC GL3 clearance after 1yr of ERT. We aimed to re-evaluate PC response to ERT using unbiased electron microscopic morphometry.

Methods: Paired biopsies (Bx) at baseline (BL) (ERT naïve) and after ~1yr of ERT (1 mg/kg Fabrazyme every 2 weeks) from 6 males with FD (age 31[18-46], median [range]) were studied. Fractional volume of GL3 inclusions per PC [Vv(Inc/PC)], mesangial [Vv(Inc/Mes)] and endothelial cells [Vv(Inc/Endo)] were estimated using point counting. Average PC volume [V(PC)] and volume of GL3 inclusions per PC [V(Inc/PC)] were estimated using the point sampled intercept method.

Results: V(Inc/PC) decreased in all cases from BL (3126±2091 μm^3) to 1yr (797±291 μm^3) (p=0.03). This reduction paralleled reduction in PC size from BL to 1yr (p=0.03). Vv(Inc/PC) was not statistically different between BL and 1yr. There was a strong relationship between V(Inc/P) or V(PC) at BL and % V(Inc/PC) reduction at 1yr (r=0.90, p=0.01), indicating that Bx with larger PC with more abundant GL3 at BL showed the most prominent GL3 loss at 1yr. While 0[0-10]% of PC at BL contained no GL3 inclusions, this increased to 15[4-25]% at 1yr. This increase did not correlate with change in Vv(Inc/PC), arguing that this phenomenon was likely not due to random sectioning. Vv(Inc/Endo) and Vv(Inc/Mes) approached to 0 at 1yr ERT.

Conclusions: These data, for the first time, document reduced GL3 in PC after 1yr ERT in male FD patients. The unchanged Vv(Inc/PC) explains why scoring methods failed to detect this in previous studies as our finding depended on reductions in PC size, a parameter very difficult to detect by subjective methods. Although further studies are needed to determine if this response is maintained with longer ERT duration, these results suggest that shorter studies may be adequate to detect important early treatment effects in FD. Increased PC with no GL3 following ERT is also novel and may suggest PC regeneration.

Funding: Private Foundation Support

TH-PO298

Mosaicism of Podocyte Involvement Is Related to Podocyte Injury in Females with Fabry Disease

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Background: Fabry disease (FD). an X-linked deficiency of α-galactosidase A,leads to intracellular globotriaosylceramide (GL-3) accumulation. Although less common than in males, CKD occurs in ~15% of females. Recent studies highlight the importance of podocyte (PC) injury in the development and progression of FD nephropathy. We hypothesized that the greater the % of PC with active wild-type GLA gene (due to X-inactivation of the mutant copy) the less is the overall PC injury.

Methods: Kidney biopsies from 11 treatment-naive females with FD, ages 14 [8-39] (median [range]) years were studied by electron microscopy and compared with 4 treatment-naive males. PC profiles with GL-3 inclusions, consistent with Fabry PC phenotype (FPC) and those without GL-3 inclusions, consistent with non-Fabry PC phenotype (NFPC) were counted in glomeruli. Volume fraction of GL-3 inclusions per PC [Vv(Inc/PC)], mesangial cells [Vv(Inc/Mes)], endothelial cells [Vv(Inc/Endo)] and foot process width (FPW) were estimated using unbiased stereology.

Results: In females, 50 [13-100]% of PC were NFPC. Vv(Inc/PC) in FPC was not different between females and males, consistent with little or no cross-correction between FPC and NFPC. However, Vv(Inc/Mes) and Vv(Inc/Endo) were ~20 times less in females compared to males. % NFPC per glomerulus (%NFPC/glom) correlated with age in females (r=0.69, p=0.04), suggesting a survival disadvantage for FPC over time. Age-adjusted %NFPC/glom was inversely related to FPW (r=-0.80, p=0.02), an indicator of PC injury. Vv(Inc/PC) in FPC in females correlated directly with FPW.

Conclusions: These findings support important relationships between PC mosaicism and PC injury in female FD patients. FPC may have discernable survival disadvantages in females. More rapid cell replication rates may provide more marked survival advantages to non-Fabry endothelial and mesangial cells in FD females. Kidney biopsy, by providing information about PC mosaicism, may help to stratify females with FD for kidney disease risk and to guide treatment decisions.

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TH-PO299

Lyso-Gb3 Activates Fibrogenic Signaling in Human Podocytes

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Background: Fabry disease leads to progressive proteinuric chronic kidney disease leading to glomerular sclerosis and interstitial fibrosis. However, the link between the metabolic abnormality and kidney fibrosis is poorly characterized. TGFbeta-1 and Notch1 are known mediators of kidney fibrosis, while chronic inflammation frequently leads to fibrosis. Globotriaosylsphingosine (lyso-Gb3) was recently identified as a bioactive molecule accumulating in Fabry disease. We hypothesized that lyso-Gb3 could modulate fibrogenic signaling in glomerular podocytes.

Methods: Notch1, Jagged and Hes1 expression was determined by immunohistochemistry in human renal biopsies from Fabry patients. The effects of Lyso-Gb3 in cultured human podocytes was assessed by qRT-PCR and Western blot. The regulation of Notch1 patchway was studied in cultured human podocytes exposed to Lyso-Gb3 and Notch1 function was explored by siRNA knock-down of Notch1. Using Electrophoretic mobility shift assay we studied NFkappaB activation.

Results: In human podocytes lyso-Gb3, at concentrations found in serum of Fabry patients: a) increased production of type IV collagen and fibronectin, b) increased TGFbeta-1 expression, c) promoted activation of the transcription factor NFkappaB, d) increased expression of Notch1 and its target Hes. Studies with inhibitors or antagonists of TGFbeta-1 (receptor antagonists, neutralizing antibodies), Notch1 (gamma-secretase inhibitor, Notch1 siRNA) and NFkappaB (parthenolide) illustrated the functional consequences of the activation of these pathways in response to lyso-Gb3 and unraveled the relationships between them.

Conclusions: Lyso-Gb3, a molecule accumulated in Fabry disease, promotes fibrogenic signaling even in podocytes expressing alpha-galactosidase. These data identify novel potential therapeutic targets to prevent progression of fibrosis in Fabry disease.

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TH-PO300

Severity of Fabry Nephropathy as a Marker of Cardiovascular Outcome: 10-Year Data from an Australian Cohort on Enzyme Replacement Therapy

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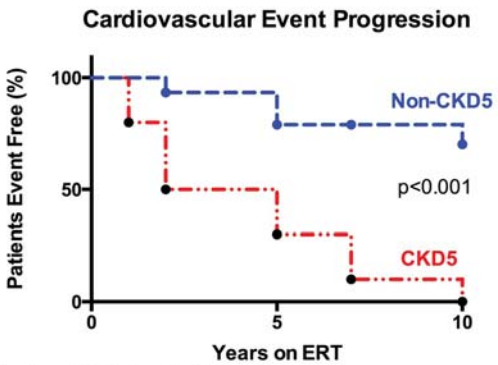
Background: Fabry Disease (FD) is an X-linked lysosomal storage disease caused by deficiency in the enzyme α–galactosidase A. Renal involvement results from progressive glomerular podocyte injury causing proteinuria, leading to focal segmental glomerulosclerosis and ultimately end-stage kidney disease (CKD5). Renal disease is a risk factor for cardiovascular (CVS) disease.

Methods: We performed a retrospective analysis of long-term outcomes of male FD (n=25) patients, from a single Australian centre, after 10-years on enzyme replacement therapy (ERT). Clinical outcomes were separated into patients with CKD5 (n=10) and those without (non-CKD5, n=15). Fabry nephropathy was monitored by proteinuria and nuclear ^{Cr}-51 EDTA glomerular filtration rate (GFR). Fabry cardiomyopathy was assessed by echocardiography and frequency of CVS events (death, arrhythmia, pacing device insertion, systolic dysfunction).

Results: GFR, in the non-CKD5 cohort, was unchanged from baseline to 10 years (86.9 ± 16.6 versus 82.2 ± 25.4 ml/min/1.73m^2, p=0.57). Proteinuria was stable to 10 years (baseline 0.37 ± 0.33 g/day versus 0.46 ± 0.42 g/day, p=0.42). CVS events were higher in patients with CKD5 over 10 years follow-up (100% versus 33%, p<0.001).



Number of Patients at Risk

Time (yr)	0	1	2	5	7	10
Non-CKD5	15	15	15	14	12	12
CKD5	10	10	10	9	7	6

Left ventricular mass index in CKD5 patients increased by 89.4 ± 78.3 g/m² versus 18.6 ± 29.1 g/m²p=0.014, in non-CKD5, driven by changes in interventricular wall thickness (7.7 ± 5.5mm versus 1.5 ± 2.0mm, p=0.004).

Conclusions: Severity of Fabry Nephropathy at commencement of ERT is a marker of CVS involvement and outcome. CKD5 is associated with worse baseline CVS parameters and significant progression despite ERT. Mild renal disease at commencement of ERT is associated with stable long-term renal function and reduced cardiac progression.

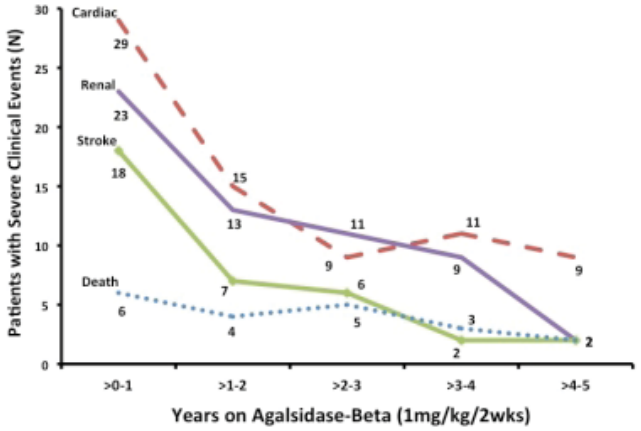
TH-PO301

Occurrence of Severe Clinical Events by Time on Enzyme Replacement Therapy with Agalsidase Beta among Patients with Fabry Disease David G. Warnock,[1] Sonia S. Maruti,[2] Gustavo Horacio Cabrera,[3] Joel Charrow,[4] Christoph Wanner,[5] Alberto Ortiz.[6] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Genzyme a Sanofi Company, Cambridge, MA; [3]Grupo Medico Del Viso, Buenos Aires, Argentina; [4]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; [5]Univ of Würzburg, Würzburg, Germany; [6]Fundacion Jiménez Díaz, Madrid, Spain.*

Background: Fabry disease is an X-linked disorder that has been treated with enzyme replacement therapy (ERT) for nearly 15 years, but there is uncertainty about its effects on severe clinical events. We hypothesized that there is a "lag time" to benefit or reduction of severe clinical events after the first year of ERT, like the time needed for clearance of vascular endothelial globotriaosylceramide deposits by agalsidase beta.

Methods: Patients in the Fabry Registry (sponsored by Genzyme, a Sanofi company) were followed from the time they started ERT to the time they had a severe clinical event, or they stopped receiving agalsidase beta, or June 25, 2009 (start of the shortage of agalsidase beta). Severe clinical events included death, cardiac intervention, arrhythmia, heart failure, ESRD, or stroke. Patients were excluded if they had late-onset genetic variants or had ESRD before starting ERT.

Results: There were 186 severe clinical events among 1,298 patients treated with ERT (average dose 1 mg/kg/2 weeks) for up to 5 years. The number of events decreased after the first year of ERT. The incidence rate was 67 (95% CI:52-84) events per 1,000 patient-years during year 1, and 39 (95% CI:22-64) events per 1,000 patient-years after year 1 with 5 years follow-up.



Conclusions: Longer time on agalsidase beta appears to be associated with lower rates for severe clinical events, consistent with a lag time to benefit after starting agalsidase beta at 1 mg/kg/2 weeks.

Funding: Pharmaceutical Company Support - Genzyme

TH-PO302

Identification of Urate Transporter 1 Gene Mutations in Spanish Patients with Idiopathic Renal Hypouricemia Felix Claverie-Martin,[1] Elizabeth Cordoba-Lanus,[1] Hilaria Gonzalez-Acosta,[1] Ricardo Enriquez,[2] Cesar Loris,[3] Cristina Aparicio Lopez.[4] *[1]Hospital Nuestra Señora de Candelaria, Santa Cruz de Tenerife; [2]Hospital General de Elche, Elche; [3]Hospital Infantil Miguel Servet, Zaragoza; [4]Hospital de Getafe, Madrid, Spain.*

Background: Renal hypouricemia is an inherited and heterogeneous disorder characterized by impaired uric acid (UA) reabsorption in the proximal tubule. Patients present low serum levels of UA associated with excessive urinary wasting of UA, and some have severe complications like exercise-induced acute renal failure or nephrolithiasis. Mutations in the *SLC22A12* gene, encoding the renal tubular uric acid transporter 1 (URAT1), are the major cause of renal hypouricemia. Most mutations have been identified in Japanese patients, and only a few have been detected in Europeans. Here, we report clinical and molecular data of five Spanish patients diagnosed with idiopathic renal hypouricemia.

Methods: Blood and urinary samples were collected for measurement of UA and creatinine levels, and for genetic analysis. Patients were evaluated for renal stones or other renal diseases. Genomic DNA was isolated using a commercial kit. *SLC22A12* exons were amplified by PCR and the products were sequenced. Mutation prediction software was used to score the pathogenicity of genetic variants.

Results: The patients had persistently low serum UA levels ranging from 0.8 to 1.3 mg/ml and elevated fractional excretion of UA (27%-60%). One of the patients had nephrolithiasis and was also diagnosed with polycystic kidney disease. The other patients were asymptomatic. Sequence analysis revealed mutations in each patient. A new missense mutation, p.A476D, affecting transmembrane domain 11 of URAT1 was identified. Analysis with informatics tools predicted this change to be pathogenic. Two previously reported mutations, missense mutation p.T467M and deletion p.L415_G417del, associated with renal hypouricemia in families from the Czech Republic were also detected.

Conclusions: This is the first report of genetically diagnosed cases of renal hypouricemia in Spain. Although this disorder has been described mainly in Asian patients it should also be contemplated in other regions.

Funding: Government Support - Non-U.S.

TH-PO303

Comparison of Allopurinol and Febuxostat in Adenine Phosphoribosyltransferase Deficiency: Effect on Urinary 2,8-Dihydroxyadenine Excretion Vidar O. Edvardsson,[1,2] Hrafnhildur L. Runolfsdottir,[2] Steinunn Oddsdottir,[1] Inger Maria Agustsdottir,[1] Finnur Freyr Eiriksson,[3] Margret Thorsteinsdottir,[2,3] Runolfur Palsson.[1,2] *[1]Landspitali - The National Univ Hospital of Iceland, Reykjavik, Iceland; [2]Univ of Iceland, Reykjavik, Iceland; [3]ArcticMass, Reykjavik, Iceland.*

Background: The xanthine dehydrogenase (XDH) inhibitor allopurinol is known to prevent nephrolithiasis and chronic kidney disease in patients with adenine phosphoribosyltransferase (APRT) deficiency by decreasing the synthesis of 2,8-dihydroxyadenine (DHA). The aim of this exploratory pilot study was to compare the efficacy of allopurinol and the non-purine XDH inhibitor febuxostat in reducing urinary DHA excretion.

Methods: Patients with eGFR >60 mL/min/1.73 m² who are listed in the APRT Deficiency Registry of the Rare Kidney Stone Consortium and currently receiving allopurinol therapy, were enrolled in a 42-day pilot study. After 7-day washout period, the subjects were prescribed 400 mg of allopurinol in a single daily dose for 14 days. After a second 7-day washout period, all subjects were prescribed 80 mg febuxostat in a single daily dose for another 14 days. Twenty-four hour urinary DHA excretion was evaluated at the end of the first washout period and at the end of allopurinol and febuxostat treatment periods (days 7, 21 and 42). Urinary DHA was measured using UPLC-MS/MS and expressed as DHA-to-creatinine ratio (ng/mmol).

Results: To date, 6 of 10 patients have completed their participation in the study. The median (range) urinary DHA-to-creatinine ratio was 3207 (1620-5374) ng/mmol off therapy, 2105 (813-4014) ng/mmol on allopurinol and 422 (141-446) ng/mmol on febuxostat treatment. Statistical significance was not calculated due to the small number of subjects.

Conclusions: A marked decrease in the DHA-to-creatinine ratio was observed with both allopurinol and febuxostat therapy. In the prescribed doses, febuxostat appears to be more efficacious than allopurinol in reducing DHA excretion in patients with APRT deficiency. These results need to be confirmed in a larger patient sample.

Funding: NIDDK Support, Other NIH Support - Office of Rare Diseases

TH-PO304

Dysfunctional Autophagy-Lysosome Pathway Triggers Apical Dedifferentiation in Nephropathic Cystinosis Alessandro Luciani, Jenny A. Kuerth, Olivier Devuyst. *Physiology UZH, Zurich, Switzerland.*

Background: Nephropathic cystinosis, a lysosomal storage disease caused by mutations in the *CTNS* gene encoding the lysosomal cystine transporter cystinosin, is characterized by generalized proximal tubule (PT) dysfunction that progresses, if untreated, to end-stage renal disease. The mechanisms linking lysosomal accumulation of cystine to PT cell dysfunction and the transition to renal failure remain largely unknown.

Methods: Using a combination of techniques, including electron microscopy and three dimensional tomography reconstruction, as well as functional lysosomal degradative assays, we analyzed endocytic uptake, lysosome function, and differentiation and proliferation markers in primary cultures of PT cells derived from micro-dissected kidneys of age- and gender-matched *Ctns*$^{-/-}$ and *Ctns*$^{+/+}$ mice.

Results: Studies in *Ctns*$^{-/-}$ PT cells demonstrated that the loss of cystinosin leads to perinuclear positioning of enlarged and dysfunctional lysosomes, with impaired clearance of autophagosomes containing ubiquitinated proteins and damaged mitochondria, compared to *Ctns*$^{+/+}$ cells. The defective mitochondrial clearance of *Ctns*$^{-/-}$ cells was associated with excessive production of reactive oxygen species (ROS) and increased tyrosine phosphorylation of ZO-1, loss of tight junction integrity, and abnormal nuclear translocation of ZONAB, a transcription factor that promotes cell proliferation but directly represses differentiation markers such as the megalin/cubilin receptors. Similar changes were observed in *Ctns*$^{-/-}$ kidneys *in vivo*. Scavenging of ROS with either mitochondria-targeted antioxidant mito-TEMPO or antioxidant synthetic SOD mimetic EUK-134 prevented the disruption of ZO-1 and restored the expression of megalin/cubilin in *Ctns*$^{-/-}$ PT cells.

Conclusions: These data reveal that the absence of cystinosin in *Ctns*$^{-/-}$ PT cells impairs the autophagy/lysosome pathway, which in turn induces oxidative stress, loss of tight junction integrity, abnormal ZONAB signaling and dedifferentiation and dysfunction of the cells. This chain of events can be rescued by anti-oxidants, opening new therapeutic perspectives to prevent the progression of nephropathic cystinosis.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO305

Long-Term Outcome of Renal Transplantation in Adult Cystinosis Patients
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Background: Cystinosis is a rare lysosomal disorder leading to end stage renal disease (ESRD) in more than 90% of patients before 20 years of age. We report outcome of renal transplantation in 30 adult cystinosis patients.

Methods: data of 30 adult patients were retrospectively analysed in 5 French university centers. A control cohort of 93 patients was constituted, matching with a 3/1 ratio to age, graft date, living/deceased donor and center to cystinosis patients.

Results: 31 transplantations in 30 patients were performed between1980 and 2013. Median age at transplantation was 20.4 years (7-36.5). At transplantation (D0), all patients had corneal cystine deposits, 3 diabetes and 7 hypothyroidism. All patients except one were treated by oral and ocular cysteamine at D0. Median leucocyte cystin level was 1.4 nmol ½ cystin/mg protein (0.4-4.7) at D0, and 1.9 (0.5-10) during follow up. Cystinosis complications occurred during follow up: diabetes mellitus (n=4), hypothyroidism (n=1), liver involvement (n=1), neurologic involvement (n=2). At 10 years, patient survival was respectively 97% and 99% in cystinosis and control group, and graft survival was not different between the two groups (86.5 and 72% respectively). After a median follow up of 144 months (6-340), six cystinosis patients (19%) reached ESRD (graft survival 53%). Number of graft rejections or infections was not different between cystinosis and control. Diabetes after transplantation occurred as frequently in cystinosis as in control patients (13% and 5% respectively, not significant), without difference for calcineurin inhibitors treatment.

Conclusions: Renal transplantation appears to be safe and efficient in adult cystinosis patient, with as good outcomes as aan age-matched population of kidney recipients. Diabetes risk after transplantation is not increased in patients with cystinosis.

TH-PO306

The Swan-Neck Lesion: Proximal Tubular Adaptation to Oxidative Stress in Nephropathic Cystinosis
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Background: Cystinosis is a congenital metabolic disorder resulting from a mutation in cystinosin (*CTNS*), causing progressive proximal tubular cell flattening and the "swan-neck lesion" (SNL). Therapy with cysteamine can delay, but does not prevent, eventual renal failure.

Methods: To determine the role of oxidative stress in cystinosis, histologic sections of kidneys from C57BL/6 *Ctns*$^{-/-}$ mice were examined from 1 week to 9 months of age. This mutant strain develops Fanconi syndrome by 2 months (mo), and decreased GFR by 12 mo. Additional mice were treated with mitoquinone (MitoQ), an antioxidant targeted to mitochondria, or vehicle (dTTP) from 3-6 mo. Oxidative stress was revealed by staining with 4-hydroxynonenal (4HNE), and superoxide (formed by functioning mitochondria) was localized by nitroblue tetrazolium. Onset of SNL and proximal tubular volume fraction were determined by *Lotus tetragonolobus*, and tubular remodeling was revealed by transgelin immunostaining.

Results: 4HNE staining localized to the junction of the SNL and columnar tubular cells in 1-3 mo *Ctns*$^{-/-}$ mice, as well as to collapsing proximal tubules and interstitial cells at 9 m. Superoxide persisted around columnar cells distal to SNL, but was no longer generated by cells of the SNL, which expressed transgelin and lost *Lotus*-staining as well as most mitochondria. Treatment with MitoQ increased the fraction of Lotus-stained glomeruli in 6 mo mice from 28±1% to 38±4% ($p<0.05$), but did not significantly enhance proximal tubular volume fraction (54±1% versus 57±1%, $p=0.22$).

Conclusions: We conclude that oxidative stress is present at the "leading edge" of the early SNL, and in atrophied tubules and interstitial cells in advanced disease. Cells of the SNL adapt to oxidative stress by flattening with loss of apical cystine binding and mitochondrial loss, temporarily preventing ongoing cystine uptake and oxidative injury. Antioxidant treatment delays initiation of the SNL, and may provide therapeutic benefit in cystinotic children.

Funding: Private Foundation Support

TH-PO307

Intermediate Phenotypes of Dent-2 Disease and Lowe Syndrome in Patients with OCRL Mutations
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Background: Mutations in the *OCRL* gene cause Lowe syndrome and Dent disease-2, which are generally discriminated by presence or absence of congenital cataract and mental retardation. Here, we report five patients with intermediate phenotypes of Dent-2 disease and Lowe syndrome, who carry novel mutations in the *OCRL* gene.

Methods: Direct DNA sequencing was performed in five patients from three unrelated families who were clinically diagnosed with Lowe syndrome or Dent disease. Clinical manifestations were also reviewed.

Results: Prominent low molecular weight proteinuria and cataract were noted in all patients. Patients 1-1 and 1-2 are brothers with mild developmental delay (IQ >70). Patient 1-3, their uncle, can fully care for himself. No treatment has been required for cataract in patients 1-2 and 1-3. Mental retardation in patient 2 was also mild (IQ 83). Of note, mental development in patient 3 was apparently normal. No patients needed alkali therapy for metabolic acidosis. Analyses in the *OCRL* gene revealed that families 1 and 2 carried the same missense mutation I271T in exon 9, and patient 3 carried a missense mutation V636E in exon 18.

Conclusions: We reported five patients with novel missense mutations in the *OCRL* gene who presented with cataract and mild or no mental retardation. To our knowledge, patient 3 is the first case with a mutation in the *OCRL* gene who presented with cataract requiring surgery but without mental retardation, which is an intermediate phenotype of Dent-2 disease and Lowe syndrome. These findings confirm phenotypic heterogeneity in patients with *OCRL* mutations.

Funding: Government Support - Non-U.S.

TH-PO308

Clinical and Genetic Spectrum of Type 3 Bartter Syndrome
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Background: Type 3 Bartter syndrome (BS) is a rare salt losing tubulopathy caused by mutations in the *CLCNKB* gene, coding for the basolateral chloride channel ClCkb. Patients with type 3 BS may have variable phenotype: antenatal/neonatal (A/N) BS, classical (C) BS or Gitelman syndrome (GS). The aim of this study was to analyze the phenotype/genotype correlation in a large cohort.

Methods: We retrospectively analyzed results of 106 patients belonging to 102 families with Type 3 BS addressed to our department during last 13 years (29 A/NBS, 50 CBS and 27 GS). The *CLCNKB* gene was analyzed by sequencing and MLPA.

Results: Clinically, 27% patients presented as A/NBS 47% as CBS and 26% as GS. Median [IQR] ages at diagnosis were 0.4[0.7-11.4] months, 1 [0.41-11.25] and 26 [16.5-42.5] years for A/NBS CBS and GS respectively. Polyhydramnios and prematurity were present in 93 and 36% cases of A/NBS. Main symptoms at diagnosis for CBS were failure to thrive and polyuria; for GS asthenia, cramps and fortuitous discovery of hypokalemia. Plasma Na and Cl were significant lower in A/NBS (p 0.0008 and 0.007) or CBS (p 0.001) compared with GS; GS had significantly lower Ca and Mg as compared to A/NBS (p 0.006, 0.004) or CBS (p 0.03, 0.02). Renin was significantly higher in A/NBS compared with CBS (p 0.004) or GS (p 0.002). Genetics: 56 different mutations were detected (5 splicing, 6 large del, 7 nonsense, 8 frameshift and 30 missense). 51 patients were homozygous (18 A/NBS, 20 CBS and 13 GS), 42 were compound heterozygous (10 A/NBS, 26 CBS and 6 GS) and only one heterozygous mutation was detected in 12 patients (1 A/NBS, 4 CBS and 7 GS). Percentages of mutated alleles by phenotype and mutation type were:

	Large deletions	Splicing	Frameshift/Nonsense	Missense
A/NBS	56	12	7	24
CBS	38	5	24	32
GS	21	0	17	62

Conclusions: 2/4 cases of type 3 BS correspond to CBS and ¼ either to A/NBS or GS. Large deletions and severe mutations are more frequently associated with A/NBS and CBS and missense mutations with GS. Biochemical data are similar in A/NBS and CB, in contrast a mild biochemical phenotype was observed in GS patients.

Funding: Government Support - Non-U.S.

TH-PO309

From Bench to Bed: T60M-the Most Frequent Mutation in Chinese Patients with Gitelman Syndrome
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Background: To investigate the function of the most frequent mutation of Na-Cl cotransporter (NCC) in Chinese Gitelman Syndrome (GS) patients in vivo and in vitro.

Methods: Peripheral blood DNA samples of 63 patients with a clinical suspicion of GS were tested by direct sequencing of slc12a3 gene. Then we summarized the slc12a3 gene mutations of Chinese GS patients reported previously to figure out the most frequent mutations. In vitro, wild type human NCC cDNA-pGEM T vector and 6 missense mutations were generated by site-directed mutagenesis. $^{22}Na^{+}$ uptake experiment was carried out in xenopus laevis oocyte expression system. In vivo, hydrochlorothiazide test was carried out in 13 GS patients and 20 healthy controls.

Results: 47 patients were diagnosed GS genetically, 43 different mutations were identified, including 14 novel mutations, T60M was carried by 6 patients. Totally, 107 Chinese families were diagnosed GS genetically, 74 mutations and 196 mutated alleles were detected in these families. T60M, D486N, R913Q and c.2877_2878delAG were frequent mutants (≥5%), and T60M is the most frequent one (16.3%). Compared with wild-type NCC, mutants present different extent sodium uptake dysfunction, and T60M only remain

20% activity. Compared with health volunteers, Fractional excretion maximal/ baseline (FEmax/b) of potassium, sodium and chloride were observed significantly lower in 2 T60M homozygous mutation patients and other 11 GS patients.

Conclusions: T60M is the most frequent SLC12A3 gene mutation in Chinese GS patients. In vitro, $^{22}Na^{+}$ uptake experiment in xenopus laevis oocyte expression system indicate the dysfunction of it; in vivo, HCT test confirmed the impairment in GS patients.

Funding: Government Support - Non-U.S.

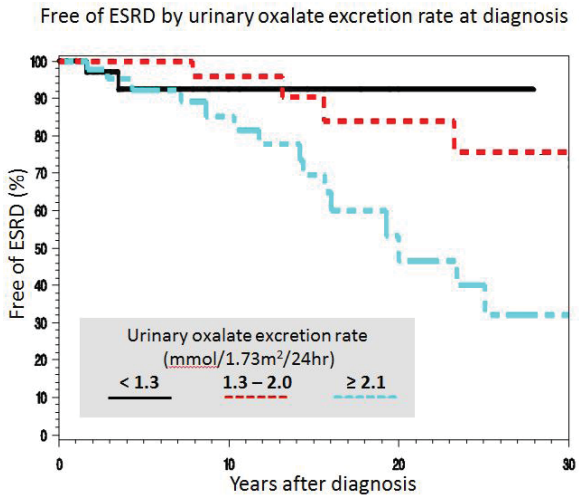
TH-PO310

Analysis of Urinary Risk Factors for ESRD among Patients with Primary Hyperoxaluria Fang Zhao, Eric J. Bergstralh, Ramila A. Mehta, Barbara M. Seide, Andrea G. Cogal, John C. Lieske, Dawn S. Milliner. *Mayo Clinic, Rochester, MN.*

Background: Clinical manifestations of primary hyperoxaluria (PH) are heterogenous, including time from diagnosis to end stage renal disease (ESRD). Factors that influence the rate of renal function decline are poorly understood. Thus urinary parameters at PH diagnosis were examined as potential predictors of kidney outcome.

Methods: Clinical data, including urinary chemistries, were analyzed among patients in the Rare Kidney Stone Consortium (RKSC) PH Registry without ESRD at diagnosis. The modified Schwarz (children) and MDRD (adults) equations were used to calculate eGFR. Data are expressed as median (25^{th}, 75^{th}). Renal survival was estimated using the Kaplan-Meier method and factors associated with renal survival determined by a Cox's proportional hazard model.

Results: Among 298 patients without ESRD at diagnosis, 192 (64.4%) had PH1, 35 (11.7%) had PH2, 38 (12.8%) had PH3 and 33 (11.1%) were of unclassified type. Median age at diagnosis was 7.9 (3.8, 18.2) years with a median eGFR of 73 (56.4, 97.5) ml/min/1.73m^2. Baseline urinary oxalate was 1.6 (1.1, 2.4) mmol/1.73m^2/24hr, while citrate was 398.0 (196.9, 697.5), calcium was 72.9 (43.4, 134.2), and phosphorus was 823.9 (551.5, 1044.1)mg/1.73m^2/24hr. ESRD developed in 59 (20%) patients during follow-up at a median of 13.2 (4.5, 20.3) years. Urinary oxalate excretion stratified by tertile at diagnosis significantly associated with renal survival [HR 2.3, 95%CI (1.1, 4.9), P=0.02], while excretion rates of citrate, calcium and phosphorous did not (P=0.16, 0.19 and 0.68, respectively). ESRD-free rate at 20 years was 92% for those with a urinary oxalate excretion rate <1.3, 84% for those 1.3 - 2, and 47% for those ≥ 2.1mmol/1.73m^2/24hr.



Conclusions: A higher urinary oxalate excretion rate at diagnosis predicts earlier progression to ESRD in PH.

Funding: NIDDK Support, Private Foundation Support

TH-PO311

Clinical and Biochemical Features in Primary Hyperoxaluria Type 3 Prince Singh, Linda Hasadsri, Ramila A. Mehta, Andrea G. Cogal, Katharina Hopp, Peter C. Harris, John C. Lieske, Dawn S. Milliner. *Mayo Clinic, Rochester, MN.*

Background: Primary hyperoxaluria (PH) types 1 and 2 result in repeated stone episodes and kidney failure due to hepatic overproduction of oxalate. Recently identified PH3 is caused by mutations of *HOGA1*. Due to its rarity, the clinical course of affected individuals is uncertain.

Methods: The Rare Kidney Stone Consortium PH registry (n=420) was queried. PH3 diagnosis had been established in 39 hyperoxaluric individuals via bidirectional sequencing. Urine 4-hydroxy-2-oxoglutarate (HOG) excretion was measured in 16 patients (pts), 6 first-degree relatives genetically confirmed as carriers, and 252 controls. eGFR was calculated from modified Schwarz (children) and MDRD (adults) equations.

Results: Among 39 PH3 pts, median age at symptom onset was 2.9 years (0.9, 6.0) mean 25^{th}, 75^{th} %ile. Initial findings included urolithiasis (85%), hematuria (28%) and nephrocalcinosis (5%). Median eGFR was 95 ml/min/1.73m^2 (68,117).

Table 1. UHOG and Uox in PH3 patients, carriers, and control subjects

	PH3, n=16	Carriers, n=6	Controls, n=252
U_{ox}, µg/mg creat (mean, range)	188.6 (44.9-541.2)	57.6 (20.6-137.9)	45.9 (0-481)
UHOG, µg/mg creat (mean, range)	116.3 (25.8-582.1)	21.3 (11.2-53.9)	0.61 (0-3.8)
UHOG PH3 vs carriers p=0.01, PH3 vs control p<0.0001. UHOG carriers vs controls p=0.59			

Pts <2 years old had higher HOG excretion (298.5±180.3 µg/mg Cr, p<0.0001)) which declined with age (73.3±21.8 for pts >10 years old, p<0.0001). No gender association was observed for HOG excretion in pts even after age-adjustment. Patient follow-up was 6.9±9.2 years. Most (36/39) maintained stable eGFR (99.7 ml/min/1.73m^2/24h). In 3 pts eGFR was lower at last followup: 60 (age 19 yrs), 34 (age 71 yrs), and one progressed to ESRD (age 8 yrs).

Conclusions: Urolithiasis and hematuria, often starting in infancy, were common presenting features in this PH3 cohort. Renal function remained excellent in 36/39 during followup. HOG levels were higher in PH3 pts compared to carriers and carriers versus controls. Thus urine HOG may prove useful in diagnosis. PH3 appears to have a less severe phenotype than PH1, although a larger clinical experience is needed to confirm this observation.

Funding: NIDDK Support, Other NIH Support - NCaTS, Private Foundation Support

TH-PO312

Inactivation of Dstyk in Mice Causes Congenital Obstructive Uropathy Katarina Vukojevic, Miguel Verbitsky, Esther Lopez Rivera, Vivette D. D'Agati, Jonathan M. Barasch, Cathy Mendelsohn, Ali G. Gharavi, Simone Sanna-Cherchi. *Columbia Univ.*

Background: Congenital anomalies of the kidney and urinary tract account for most cases of pediatric end-stage kidney disease. We identified mutations in *DSTYK* (encoding a dual serine/threonine and tyrosine protein kinase) in 2.3% of patients with dominantly inherited urinary tract malformations. To gain insight into the mechanism linking mutations in *DSTYK* to human urinary tract malformations, we characterized a *Dstyk* knock-out mouse model.

Methods: Inactivation of *Dstyk* was performed in C57BL/6 mice by introducing an insertional gene-trapping vector designed to abrogate translation after exon 1. Mice and tissues were analysed at different time-points during development (E13.5, E15.5, E18.5) and postnatally (P1, P3, P7, 3 months).Morphological analysis was conducted on paraffin sections stained with hematoxylin and eosin. Because gene-trap models have been associated with incomplete gene inactivation, we conducted qPCR on cDNA from brain or kidney tissue to assess for residual expression.

Results: A total of 162 mice (34 embryos and 128 postnatal) were obtained by intercross between *Dstyk*$^{+/-}$ mice. Both embryos and mice were generated at expected proportions indicating absence of embryonic lethality. While all *Dstyk*$^{+/+}$ and *Dstyk*$^{+/-}$ mice survived, we observed high perinatal lethality in *Dstyk*$^{-/-}$ (8/26, 30.7%).Analysis of null embryos at different time-points showed no abnormalities in nephrogenesis at E13.5 and E15.5, while high penetrance of isolated bilateral hydronephrosis was observed at E18.5 (5/5, 100%) and P1-3 (12/17, 71%). No other overt extra-urinary phenotypes were observed in Dstyk$^{-/-}$ mice. The incomplete penetrance of the lethality and obstructive uropathy phenotypes can be explained at least in part by residual expression of Dstyk in Dstyk$^{-/-}$ mice. All mice surviving after P3 and 3/17 null mice between P1 and P3 without ureteric phenotype showed significant Dstyk cDNA espression (>15%).

Conclusions: Our results indicate that global inactivation of *Dstyk* in mice results in high-penetrance of isolated bilateral obstructive uropathy and perinatal lethality, identifying a novel player in ureteric development and function.

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TH-PO313

Uretero-Pelvic Junction Obstruction and *TBX18* Mutations Asaf Vivante,[1] Stefan Kohl,[1] Daw-Yang Hwang,[1] Julian Jakob Schulz,[1] Shirlee Shril,[1] Amita Sharma,[2] Andreas Kispert,[4] Harald Jüppner,[2,3] Friedhelm Hildebrandt.[1,5] *[1]Div of Nephrology, Dept of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Pediatric Nephrology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA; [3]Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA; [4]Institut für Molekularbiologie, Medizinische Hochschule Hannover, Hannover, Germany; [5]Howard Hughes Medical Inst, Chevy Chase, MD.*

Background: Congenital anomalies of the kidney and urinary tract (CAKUT) are the leading causes of chronic kidney disease in children (50%). Uretero-pelvic junction obstruction (UPJO) comprises about 10% of CAKUT and its pathogenesis is poorly understood. We had previously shown that a causative gene can be identified in about 10% of CAKUT cases.

Methods: To identify a causative gene for UPJO we investigated a four-generation Hispanic-White family with isolated dominant UPJO by whole exome sequencing followed by targeted sequencing in seven affected members. We also performed exon sequencing in 100 unrelated UPJO patients.

Results: We detected a protein truncating heterozygous mutation in *TBX18,* which encodes a transcription factor known to be involved in ureteral smooth muscle development.

This p.Gly337Valfs*19 mutation was present in all affected family members and absent from unaffecteds. An additional *TBX18* missense mutation, affecting a highly conserved residue at the T-box domain, was detected in 1 of 100 unrelated patients.

Conclusions: We identified *TBX18* mutations as the first genetic cause of UPJO suggesting that UPJO in humans may result from lack of ureteral smooth muscle differentiation.

Funding: NIDDK Support

TH-PO314

Porphyria-Associated Chronic Kidney Disease Nicolas Pallet, Iadh Mami, Eric Thervet, Alexandre Karras. *Hopital Europeen Georges Pompidou.*

Background: Acute Intermittent Porphyria (AIP) is a genetic disorder of the synthesis of heme caused by a deficiency in hydroxymethylbilane synthase (HMBS), leading to the overproduction porphyrin precursors in the blood and urine. Rare reports have associated AIP with chronic kidney disease. The aim of this study is to describe the clinical and biological characteristics, the renal pathology, and the cellular mechanisms of kidney disease associated with AIP.

Methods: All individuals (415) with HMBS deficiency who were followed-up in the French Porphyria Center were enrolled in 2003 in a population-based study. A follow-up study was performed in 2013 assessing patients for clinical, biological and histological parameters. In vitro and in vivo models were used to determine the mechanisms of the nephrotoxicity of porphyrin precursors.

Results: Chronic kidney disease occurred in up to 59% of the symptomatic AIP patients, with a decline in the glomerular filtration rate of approximately 1 ml/min/1.73 m^2 annually. Proteinuria was absent in the majority of the cases. The renal pathology was a chronic tubulointerstitial nephropathy, which was associated with fibrous intimal hyperplasia and focal cortical atrophy that is reminiscent of antiphospholipid syndrome-associated arteriopathy. Our experimental data provide evidence that porphyrin precursors promote apoptosis and epithelial phenotypic changes in the proximal tubular cells and might cause an active fibrogenic process.

Conclusions: Chronic kidney disease is a highly prevalent complication of AIP. Taking into account that HMBS mutations are frequent in the general population, the diagnosis of chronic kidney disease associated with AIP should be considered in cases of chronic tubulointerstitial nephropathy and/or focal cortical atrophy with severe proliferative arteriosclerosis.

TH-PO315

Targeted Sequencing of 208 Candidate Genes in 460 Patients with Congenital Anomalies of the Kidney and the Urinary Tract Improves Genetic Testing Nayia Nicolaou,[1] Isaac J. Nijman,[1] Albertien M. van Eerde,[1] Ernie M.H.F. Bongers,[2] Kirsten Y. Renkema,[1] Nine V. Knoers.[1] *[1]Medical Genetics, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]Human Genetics, Radboud Univ Medical Center, Nijmegen, Netherlands.*

Background: Congenital anomalies of the kidney and urinary tract (CAKUT) comprise a spectrum of renal structural malformations that form the most common cause of end-stage renal failure in children. Familial cases implicate genetic factors, although genetic testing for human CAKUT is currently insufficient due to inadequate knowledge of the possible genes involved. Therefore, we aim to identify rare mutations in candidate genes and elucidate their involvement in disease aetiology to improve DNA diagnostics for CAKUT.

Methods: The coding regions of 208 candidate genes were sequenced in 460 CAKUT patients using a targeted next-generation sequencing approach. The genes selected were known to either play a role in human CAKUT or be involved in disrupted nephrogenesis in animal models. Average depth of sequencing coverage was 130X. Filtering and prioritization of variants, based on population frequency and in silico predictions on the effects of the mutations on protein function, resulted in the selection of 200 potentially pathogenic variants that were confirmed by Sanger sequencing.

Results: Validated variants were found in 94/208 genes in 236/460 patients, underlining the heterogeneity of CAKUT. 27/460 (6%) probands were diagnosed with a known disease-causing mutation. Of note, the identification of variants in DCHS2, FAT1 and FAT4 in multiple patients, implicates the planar cell polarity pathway in CAKUT. Furthermore, de novo variants included a well-known causal truncating variant in PAX2 and deleterious variants in the LGR4 and GNB3 genes.

Conclusions: To conclude, we identified novel mutations in a large candidate gene panel in a cohort of 460 CAKUT patients. Our method allows sequencing of 192 patients in a single sequencing run in a high-throughput and cost-effective manner. We detected clinically relevant variants, which facilitates genetic counselling for these patients, demonstrating the clinical implications of our gene panel.

TH-PO316

Microvascular Disease and BP Control in Males and Females with X-linked Alport Syndrome James Durham Smith,[1] Deb J. Colville,[1] Ecosse L. Lamoureux,[3] Tien Yin Wong,[2] Andrew Catran,[1] Judith A. Savige.[1] *[1]Medicine (Northern Health and Melbourne Health), The Univ of Melbourne, Melbourne, VIC, Australia; [2]Singapore Eye Research Inst, Univ of Singapore, Singapore, Singapore; [3]Dept of Ophthalmology, The Univ of Melbourne, Melbourne, VIC, Australia.*

Background: Poorly-controlled hypertension and secondary microvascular disease contribute to declining renal function. The aim of this study was to determine the prevalence and severity of microvascular disease in Alport syndrome from retinal images.

Methods: Individuals were diagnosed with X-linked Alport syndrome on the basis of a lamellated glomerular membrane, and gene studies. Retinal images from 17 males pre- and 11 post-transplantation and 28 females, all pre-transplantation, were examined for microvascular retinopathy (Wong and Mitchell classification), and for arteriole and venular calibre using a semiautomated system (University of Wisconsin).

Results: The males had an average age pre-transplant of 30 ± 19 years, and mean eGFR of 68 ± 28 ml/min/1.73 m^2. Fifteen (88%) had a mild or moderate retinopathy,11 (65%) had focal arteriole narrowing and 6 (35%) had haemorrhages. Their mean arteriole calibre was 142.8 ± 19.9 µm and mean venular calibre was 207.5 ± 29.7 µm. (Normal age-matched values were 148.4 ± 4.7 and 228.6 ± 11.6 µm respectively.) The post-transplantation males were older with a mean age of 38.2 ± 14.9 with eGFR of 42 ± 26 ml/min/1.73 m^2. Eight (72%) had a mild or moderate retinopathy, 5 (45%) had focal arteriole narrowing and 3 (27%) had haemorrhages. Arteriole and venular calibre were not different post transplantation (141.9 ± 17.6 and 208.3 ± 15.9 µm respectively). The females had an average age of 40.3 ± 16 year, and eGFR of 79 ± 16 ml/min/1.73 m^2. Twenty-one (75%) had a mild or moderate retinopathy, 17 had focal arteriole narrowing (61%) and 6 (21%) had haemorrhages. Their arteriole and venular calibre were 151.5 ± 19.8 and 217.7 ± 31.2 µm. (Normals were 163.9 ± 8.1 and 239.4 ± 15.8 µm respectively.)

Conclusions: Systemic microvascular disease is common in males and females with X-linked Alport syndrome and is not reversed by renal transplantation. Blood pressure control is not optimal in these individuals.

TH-PO317

Missense Mutations in the COL4A5 Gene, and the Effect of a Chemical Chaperone on ER Size and the Transport of the Collagen a5 Chain Extracellularly Judith A. Savige. *Medicine (Northern and Melbourne Health), The Univ of Melbourne, Melbourne, VIC, Australia.*

Background: Forty % of mutations in X-linked Alport syndrome are caused by missense variants in the COL4A5gene. This study examined the effects of COL4A5 missense mutations on the cell, and the consequences of treatment with a chemical chaperone, PBA.

Methods: Cell lines were produced from skin fibroblasts from two male and four female subjects with X-linked Alport syndrome caused by missense mutations, and from 4 non-hematuric normals. Growth curves were examined over five days. Levels of mRNA corresponding to ER stress (BiP, CHOP and ATF6), autophagy (ATG5, BECN1 and ATG7) and the pro- and antiapoptotic pathways (caspase 3, BAD and Bcl_2) were quantitated using RT-PCR (Applied Biosystems 7500). Levels of intra- and extracellular collagen IV alpha 5 chains were measured using an inhibition ELISA, before and after 10 mM PBA treatment. ER size was also estimated from electron micrographs, and mRNA levels for ER stress, autophagy and apoptosis compared before and after PBA treatment.

Results: Cell growth rates were reduced in the affected male but not female cell lines. Affected male and female cell lines had larger ER than unaffected individuals ($p<0.05$, $p<0.05$), and increased mRNA corresponding to ER stress, autophagy and apoptosis. Treatment with PBA reduced the size of the ER, reduced the amount of intracellular collagen a5(IV) chain, increased the amount of extracellular collagen IV a5 chain, but also increased apoptosis and autophagy activity.

Conclusions: Missense *COL4A5* mutations result in the ER accumulation of the mutant collagen IV a5 chain, and increased ER stress. They have an adverse effect on cell growth. Treatment with the chemical chaperone, PBA, increases the transport of the mutant collagen IV a5 chain extracellularly but also has adverse cellular effects.

Funding: Private Foundation Support

TH-PO318

Drosophila, a Highly Efficient and Relevant System for Validating Genetic Variants Causing Kidney Diseases Zhe Han,[1] Fujian Zhang.[2] *[1]Children's National Medical Center, Washington, DC; [2]301 Hospital, Beijing, China.*

Background: Large numbers of genetic variants are being identified from exome sequencing of kidney disease patients worldwide, but how to validate the causal factors remains a central question to be addressed. Mammalian models are cost-prohibitive to be used in large-scale. A low-cost, high-efficient and relevant *in vivo* model to test the effects of genetic variants on renal function is highly desirable.

Methods: We found that the fruit fly Drosophila has a specialized cell type called nephrocyte, which combines the function of mammalian glomerular podocytes and renal proximal tubules. We generated a transgenic fly line carrying a renal functional readout, and performed a genetic screen to discover hundreds of renal genes conserved from flies to humans. Moreover, we developed a novel genetic approach to test human genetic variants in the background of nephrocyte-specific removal of the corresponding fly gene products.

Results: We showed that the genes essential for filtration and protein reabsorption are highly conserved from Drosophila to humans. Using specific mutations identified from kidney patient exome sequencing, we generated Drosophila models in which the fly genes are replaced by the corresponding human genes (with or without variants) specifically in the nephrocytes. In two specific examples with the ADCK4 (COQ8) and ARHGDIA (RhoGDI) genes, we showed that nephrocyte function defects caused by knocking down of the corresponding fly genes can be rescued by targeted expression of these human wild-type genes in the nephrocytes but not by their disease-causing variants. Furthermore, Coenzyme Q10 treatment can rescue the renal function of COQ8 knockdown in nephrocytes, providing an example for feasible and relevant drug treatment testing in fly models of kidney diseases.

Conclusions: We have established an inexpensive, quick and relevant *in vivo* system for validating genetic variants identified from exome sequencing of kidney disease patients. This new model system also has the potential of testing potential drugs.

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TH-PO319

A High Frequency of Rare COL4A3/COL4A4 Variants in a Familial FSGS Cohort Andrew F. Malone,[1] Paul J. Phelan,[1] Gentzon Hall,[1] Alison Homstad,[1] Guanghong Wu,[1] Thomas Lindsey,[1] Matthew A. Sparks,[1] Stephen R. Smith,[1] Nicholas J. Webb,[5] Philip A. Kalra,[7] Andrey S. Shaw,[2] Peter J. Conlon,[3] J. Charles Jennette,[4] David Howell,[6] Michelle P. Winn,[1] Rasheed A. Gbadegesin.[1] *[1]Depts of Pediatrics and Medicine, Duke Univ Medical Center, NC; [2]Dept of Pathology and Immunology, Washington Univ, MO; [3]Beaumont Kidney Centre, Beaumont Hospital, Ireland; [4]Dept of Pathology and Laboratory Medicine, Univ of North Carolina at Chapel Hill, NC; [5]Royal Manchester Childrens Hospital, United Kingdom; [6]Dept of Pahology, Duke Univ Medical Center; [7]Hope Hospital, Salford, United Kingdom.*

Background: FSGS has many causes including inherited genetic defects. Proteinuria is the predominant clinical finding at presentation. Mutations in COL4A3 and COL4A4 are known to cause Alport syndrome and result in pathognomonic glomerular basement membrane findings on electron microscopy. There is phenotypic overlap of FSGS and Alport nephritis, yet the approach to treatment is very different for these distinct clinical entities.

Methods: To determine the prevalence of novel COL4A3 and COL4A4 variants in a cohort of families enrolled in a familial FSGS study, we performed next generation sequencing or direct sequencing on 70 families with a diagnosis of familial FSGS.

Results: We excluded mutations in known FSGS genes in all the families. Seven families (10%) from the cohort have rare or novel variants in COL4A3 or COL4A4. In all seven families, nephrotic range proteinuria and hematuria was present. Light microscopy showed classical FSGS histology. Glomerular basement membrane histology was variable but with no features typical of Alport nephritis. There was no recurrence of disease after kidney transplantation. In this study, families with COL4A3 and COL4A4 variants that segregate with disease represent 10% of our cohort of familial FSGS.

Conclusions: We propose that COL4A3 and COL4A4 variants be considered in the interpretation of next-generation sequencing data from such patients. This study illustrates the power of molecular genetic diagnostics in the clarification of renal phenotypes.

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TH-PO320

Intercalated Cells Are Critical to Kidney Innate Immunity Andrew L. Schwaderer,[2] Xi Chen,[2] Vijay Saxena,[2] Evan Barr-Beare,[2] David S. Hains.[1] *[1]Le Bonheur Children's Hospital, Memphis, TN; [2]The Research Inst at Nationwide Children's Hospital, Columbus, OH.*

Background: Intercalated cells regulate acid-base homeostasis, but recent findings indicate a role in the innate defense of the kidney. Mice deficient in Carbonic anhydrase 2 (*Car2*$^{-/-}$) are deficient in normal intercalated cells. The objective of this study is to evaluate the biological consequences of intercalated cell disruption in a murine UTI model.

Methods: *Car2*$^{-/-}$mice were compared to wild-type littermate controls. Mice were transurethrally challenged with uropathogenic *Escherichia coli.* Bladder and kidney bacterial burden was measured at 24 and 48 hours. The 24-hour experiment was repeated on mice with and without base supplementation. The RT^2 profiler antibacterial response array (Qiagen) was used to determine innate immune differences between genotypes at baseline and in response to infection.

Results: Mean kidney 24 hour post inoculation CFUs were higher in *Car2*$^{-/-}$compared to *Car2*$^{+/+}$mice at 301,357 ± 749,330 and 19,987 ± 53,965 respectively, p = 0.04. Kidney clearance of bacteria was achieved in 47% of *Car2*$^{+/+}$versus 16% of *Car2*$^{-/-}$ kidneys at 24 hours and 50% of Car2$^{+/+}$ versus 6% of *Car2*$^{-/-}$kidneys at 48 hours, p values of 0.014 and 0.012 respectively. Oral alkali supplementation to standardize the urine pH and serum bicarbonate levels did not rescue Car2$^{-/-}$ from increased kidney infection susceptibility. *Car2*$^{-/-}$mice have significantly increased neutrophil-gelatinase-associated lipocalin (Ngal) mRNA and protein and expression at baseline and a marked decrease ability to upregulate key bacterial response gene expression during kidney infection.

Conclusions: Our findings provide in vivo evidence that supports a role for carbonic anhydrase 2 and intercalated cells in promoting renal bacterial clearance. Decreased and/or deficient intercalated cells result in increased antimicrobial peptide production by other cells, which is not sufficient to prevent increased bacterial burden. Ngal has anti-inflammatory properties, thus its increased expression likely contributes to the decreased ability of *Car2*$^{-/-}$ kidneys to upregulate bacterial response genes following experimental UTI.

Funding: NIDDK Support

TH-PO321

RNase6 Is an Up-Regulated Antimicrobial Peptide with Pyelonephritis Robert Easterling, Tad Eichler, Brian Becknell, John David Spencer. *The Research Inst at Nationwide Children's, Columbus, OH.*

Background: Pyelonephritis (pyelo) is marked by infiltrating leukocytes that secrete bactericidal molecules like antimicrobial peptides (AMP). Ribonuclease 6 (RNase6) is a potent AMP expressed by myeloid cells and rapidly kills uropathogenic *E.coli* (UPEC). The factors that regulate RNase6 expression are not characterized. We hypothesize that RNase6 production by monocyte/macrophages is regulated by UPEC and inflammatory cytokines associated with pyelo.

Methods: Primary peripheral blood mononuclear cells (PBMC) were isolated from healthy humans. CD14+ monocytes were selected and challenged with UPEC. THP-1 monocytic cells were differentiated with phorbol myristate acetate (PMA) or IFNγ+lipopolysaccharide (LPS). Fully differentiated THP-1 cells were stimulated with UPEC and inflammatory cytokines. RNA, protein lysates, and media were collected. qRT-PCR, ELISA, and immunoblot measured RNase6 expression. UPEC growth in stimulated and unstimulated media aliquots was assessed.

Results: Unstimulated CD14+ monocytes did not express RNase6 protein by ELISA or immunoblot *ex vivo*. *RNASE6* expression increased 3.5fold after 30 minute UPEC stimulation in CD14+ monocytes. RNase6 protein was detected within 30 minutes of UPEC exposure and secreted RNase6 concentrations reached 521.85±31.71ng/mL two hours after UPEC treatment (p=0.041). To evaluate the mechanisms of UPEC induced RNase6 secretion, we utilized THP-1 monocytic cells. Undifferentiated THP-1 cells express low levels of RNase6 mRNA and protein. When THP-1 cells were activated with PMA or IFNγ+LPS, *RNASE6* expression increased 25fold (p<0.001). RNase6 protein secretion increased from 8.16±2.1ng/mL to 221.27±48.78ng/mL (p=0.021). Stimulation of activated THP-1 cells with UPEC or inflammatory cytokines (IFN-γ, IL-1β, TNF-α) did not affect *RNASE6* expression. When UPEC was added to media samples with increased RNase6 concentrations, UPEC growth was suppressed (p=0.007).

Conclusions: RNase6 is a myeloid derived AMP that is induced by UPEC in primary human monocytes and up-regulated during macrophage differentiation. Elucidation of the factors that regulate RNase6 production may lend novel insight into the pathogenesis and prevention of pyelo.

Funding: NIDDK Support

TH-PO322

Molecular Characterization of Serum IgA1 *O*-Glycosylation from Patients with IgA Nephropathy Kazuo Takahashi,[1] Stacy D. Hall,[2] Zina Moldoveanu,[2] Knud Poulsen,[3] Mogens Kilian,[3] Yoshiyuki Hiki,[3] Yukio Yuzawa,[1] Bruce A. Julian,[2] Jan Novak,[2] Matthew B. Renfrow.[2] *[1]Fujita Health Univ School of Medicine, Toyoake, Japan; [2]Univ of Alabama at Birmingham, Birmingham, AL; [3]Fujita Health Univ School of Health Sciences, Toyoake, Japan.*

Background: IgA1 with galactose (Gal)-deficient hinge-region *O*-glycans (Gd-IgA1) plays a key role in IgA nephropathy (IgAN). Serum level of Gd-IgA1 is elevated in most IgAN patients. To characterize IgA1 involved with IgAN, *O*-glycan microheterogeneity and attachment sites should be analyzed, as each hinge region has nine potential sites for *O*-glycosylation.

Methods: We developed a high-resolution mass spectrometry (MS) protocol and individually analyzed IgA1 from sera of 2 IgAN patients (IgAN-IgA1) and 2 healthy controls (HC-IgA1). Serum IgA1 was purified by affinity chromatography using anti-human IgA antibody. Neuraminidase-treated IgA1 proteins were digested with bacterial IgA-specific proteases and trypsin; hinge-region glycopeptides were analyzed by on-line liquid chromatography (LC) LTQ Orbitrap Velos Pro. Relative abundance of each glycopeptide was determined using a label-free quantitation method. Glycosylation sites in the hinge-region were determined by activated ion-electron capture dissociation (AI-ECD) Fourier transform ion-cyclotron resonance (FT-ICR) tandem MS.

Results: The combined LC-MS profiling using IgA-specific proteases and AI-ECD tandem MS data provided a comprehensive analysis of all sites of *O*-glycans and overall *O*-glycan microheterogeneity distribution of IgA1 hinge-region *O*-glycoforms. The total amount of *O*-glycans in IgAN-IgA1 was higher than that in HC-IgA1. Moreover, the amount of Gal-deficient *O*-glycans in IgAN-IgA1 was greater than that in IgA1 from HC-IgA1. AI-ECD FT-ICR tandem MS revealed that specific sites with Gal-deficient *O*-glycans or nonglycosylated sites.

Conclusions: Identification of *O*-glycosylation patterns of serum IgA1 provides useful information relevant to pathogenesis of IgAN. Glycoforms predominating in IgAN-IgA1 may be candidates for biomarkers specific for IgAN.

Funding: NIDDK Support, Other NIH Support - NIGMS

TH-PO323

TGF-β1 Release from Human Proximal Tubular Epithelial Cells Is Stimulated by Galactose-Deficient Polymeric IgA1 Chee Kay Cheung,[1,2] Saarah Bashir,[1] Karen Molyneux,[1] Nigel J. Brunskill,[1,2] Jonathan Barratt.[1,2] *[1]Infection, Immunity and Inflammation, Univ of Leicester, Leicester, Leicestershire, United Kingdom; [2]John Walls Renal Unit, Univ Hospitals of Leicester, Leicester, Leicestershire, United Kingdom.*

Background: Progression in IgA nephropathy (IgAN) is dependent upon tubulointerstitial injury and not on severity of mesangial IgA1 deposition. Our previous data indicated that IgA1 is able to stimulate pro-fibrotic cytokine release from human proximal tubular epithelial cells (PTEC) to a far greater extent than albumin, IgG or IgM. We aimed to ascertain the structural properties of IgA1 required to elicit this response.

Methods: Total IgA1 was isolated from serum from 5 healthy subjects and 5 patients with IgAN by ammonium sulphate precipitation followed by Jacalin-agarose affinity chromatography. IgA1 was further separated into monomeric and high molecular weight/ polymeric IgA1 (mIgA1 and pIgA1) by size exclusion chromatography. An identical process was carried out in a patient with IgA deficiency as a control. Human HK2 PTEC were incubated with 100μg/mL mIgA1 and pIgA1 for 48h. TGF-β1 and IL-6 were measured in the supernatants by ELISA and mRNA in the cell lysates by qPCR. The galactosylation profile of individual IgA1 preparations was tested by their ability to bind to the GalNac specific lectin *Helix Aspersa (HA).*

Results: Both pIgA1 and mIgA1 significantly upregulated TGF-β1 release from PTEC, with pIgA1 having the strongest effect. pIgA1 also produced a significant upregulation in TGF-β1 mRNA expression. No significant increase in IL-6 release was observed. No significant increase in TGF-β1 or IL-6 release was observed after incubation with the IgA deficient control. pIgA1 showed lower HA-lectin binding versus mIgA1, and the level of IgA1 galactosylation inversely correlated with TGF-β1 release. No significant difference in cytokine release was seen between IgA1 prepared from patients with IgAN versus healthy subjects.

Conclusions: PTEC TGF-β1 production is significantly upregulated by IgA1, with a stronger effect observed with galactose-deficient polymeric IgA1. Leakage of this form of IgA into the urinary space through the damaged glomerulus may drive tubulointerstitial fibrosis in IgAN.

Funding: Private Foundation Support

TH-PO324

Abnormal Signaling in IgA1-Producing Cells from Patients with IgA Nephropathy Alters Glycosylation of Autoantigen, Galactose-Deficient IgA1 Colin Reily,[1] Koshi Yamada,[1,5] Zhi Qiang Huang,[1] Milan Raska,[1,4] Joshua Charles Anderson,[1] Hitoshi Suzuki,[1,5] Bruce A. Julian,[1] Christopher D. Willey,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Palacky Univ, Olomouc, Czech Republic; [3]Faculty of Medicine, Juntendo Univ, Tokyo, Japan.*

Background: IgA nephropathy (IgAN) is an autoimmune disease characterized by elevated production of autoantigen, IgA1 with galactose-deficient *O*-glycans (Gd-IgA1). Gd-IgA1, recognized by autoantibodies, forms immune complexes, some of which deposit in the kidneys and induce glomerular injury. Very limited information is available about signaling pathways involved in Gd-IgA1 production by IgA1-secreting cells from patients with IgAN.

Methods: IgA1-secreting cells from IgAN patients and healthy controls were stimulated with IL-6 or mock-stimulated and IgA1 production and glycosylation were assessed. Kinome profiling using PamStation 12 PTK (tyrosine kinome PamChip) and Western blotting were used for assessment of signaling using cell lysates from IgA1-secreting cells with or without IL-6 stimulation. RealTime RT-PCR was used to assess expression of specific genes.

Results: IL-6 enhanced production of Gd-IgA1 in IgA1-secreting cells from IgAN patients but not healthy controls. Kinome profiling and Western blotting revealed that IL-6 signaling was mediated by STAT3 and that IgA1-secreting cells from IgAN patients compared to the cells from healthy controls had enhanced and longer lasting phosphorylation of STAT3. siRNA knock-down confirmed the key role of STAT3 in IL-6-induced enhancement of Gd-IgA1 production. Moreover, differences in several other signaling pathways in IgA1-secreting cells from IgAN patients *versus* healthy controls were identified.

Conclusions: Studies of cytokine signaling revealed aberrancies in IgA1-secreting cells from IgAN patients centered around STAT3 signaling. The exaggerated and prolonged STAT3 activation was linked to increased Gd-IgA1 production. We postulate that abnormal signaling mechanisms in IgA1-producing cells may offer potential targets for future disease-specific therapy of IgAN.

Funding: NIDDK Support, Other NIH Support - RO-1, Private Foundation Support

TH-PO325

Pathogenic Role of Aberrantly-Glycosylated IgA1 and Corresponding Autoantibodies in Patients with Henoch-Schoenlein Purpura Nephritis Hitoshi Suzuki,[1,2] Zina Moldoveanu,[2] Bruce A. Julian,[2] Yusuke Suzuki,[2] Yasuhiko Tomino,[1] Robert J. Wyatt,[3] Jan Novak.[2] *[1]Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Univ of Alabama at Birmingham, Birmingham, AL; [3]Univ of Tennessee Health Sciences Center, Memphis, TN.*

Background: Patients with Henoch-Schoenlein purpura with nephritis (HSPN) and IgA nephropathy (IgAN), but not with Henoch-Schoenlein purpura without nephritis (HSP) have elevated serum levels of galactose-deficient IgA1 (Gd-IgA1). Gd-IgA1 bound by Gd-IgA1-specific autoantibodies forms pathogenic immune complexes. Mucosal infections are often associated with clinical presentation and exacerbation of HSPN and IgAN. In IgAN, some Gd-IgA1 and autoantibodies originate from tonsillar Ig-secreting cells and some cytokines enhanceproduction of Gd-IgA1. Here, we assessed common pathogenic features of HSPN and IgAN.

Methods: Serum and Ig-secreting cells from patients with HSPN and HSP and healthy controls were used for analysis of IgA1 glycosylation and Gd-IgA1-specific IgG. Serum Gd-IgA1 and Gd-IgA1-specific IgG were measured before and after tonsillectomy in adult HSPN patients.

Results: IgA1 secreted by cells from HSPN patients had Gal-deficient *O*-glycans, whereas IgA1 from HSP patients and healthy controls was normally galactosylated. Levels of Gd-IgA1-specific IgG in sera and culture supernatants of Ig-secreting cells were higher in HSPN than in HSP patients or healthy controls ($P<0.01$). We subcloned IgA1-producing cells from 5 HSPN patients and determined that the secreted IgA1 had Gal-deficient *O*-glycans. This finding was consistent with decreased expression of ß1,3-galactosyltransferase observed in cells from HSPN patients compared to cells from HSP patients. Serum levels of Gd-IgA1 and Gd-IgA1-specific IgG were elevated in HSPN patients with active disease, as manifested by hematuria/proteinuria ($P<0.01$). Tonsillectomy in HSPN patients that reduced proteinuria and hematuria was accompanied by reduction of serum levels of Gd-IgA1 and Gd-IgA1-specific IgG ($P<0.001$).

Conclusions: Gd-IgA1 and Gd-IgA1-specific autoantibodies are elevated in patients with HSPN, supporting the hypothesis that HSPN and IgAN have common pathogenetic components.

TH-PO326

Expression of microRNAs in Kidney Biopsy, Plasma and Urine From Patients with IgA Nephropathy Zhangsuo Liu,[1] Lu Wen,[2] Junjun Zhang,[3] Guo Jia.[4] *[1]The First Affiliated Hospital of Zhengzhou Univ; [2]The First Affiliated Hospital of Zhengzhou Univ; [3]The First Affiliated Hospital of Zhengzhou Univ; [4]The First Affiliated Hospital of Zhengzhou Univ.*

Background: MicroRNAs(miRNA) are demonstrated to play important roles in the pathophysiology of IgA nephropathy (IgAN). In the present study, the expression of the four miRNA species (miR-148b, miR-194, miR-200a and miR-382) in patients with IgAN were evaluated,investigating whether these miRNAs can be used as non-invasive biomarkers in IgAN.

Methods: The expression of miRNAs in plasma and urine of 34 patients with biopsy-proven IgAN and kidney specimens of 7 patients was quantified by Real-time PCR technology. The results were compared to 13 healthy volunteers. The correlation between the miRNA levels and clinical parameters and Hass classification were analysed.

Results: We found that the levels of intra-renal miR-148b, miR-194 and miR-200a were down-regulated in patients with IgAN, while miR-382 was up-regulated ($P<0.05$); the IgAN group had significantly lower plasma miR-148b, miR-194 and miR-382, but higher miR-200a levels than controls($P<0.05$); patients with IgAN had lower urinary miR-194 and miR-200a, but higher miR-148b and miR-382 levels than controls ($P<0.05$). Plasma expression of miR-148b and miR-194 inversely correlated with Hass classification($P<0.017$). Urinary expression of miR-148b was significantly higher in Hass grade IV-V than those in grade III($P<0.017$) while miR-194 expression was significantly higher in Hass grade I-II and grade III than those in grade IV-V($P<0.017$). Urinary level of miR-148b negatively significantly correlated with complement 4(C4) ($P<0.05$), while miR-382 levels positively correlated with urine red blood cell counts ($P<0.05$).

Conclusions: MiRNAs may be involved in the pathogenesis of IgAN. Plasma and urinary expression of miRNAs has the potential of further development as non-invasive biomarkers of IgAN.

Funding: Government Support - Non-U.S.

TH-PO327

Salivary Microbiota Associated with Immunoglobulin A Nephropathy (IgAN) Maria De Angelis,[1] Maria Piccolo,[1] Eustacchio Montemurno,[2] Gabriella Lauriero,[2] Carmela Cosola,[2] Marco Gobbetti,[1] Loreto Gesualdo.[2] *[1]DiSSPA, Univ of Bari, Italy; [2]DETO - Nephr Unit, Univ of Bari, Italy.*

Background: Immunoglobulin A nephropathy (IgAN) is one of the most common forms of primary glomerular disease. Along structural IgA abnormalities, hyperproduction of poorly galactosylated IgA1 is thought to play a role in the pathogenesis of primary IgAN. The downstream effector mechanisms triggered by mesangial IgA1 deposition and its etiology are poorly understood. Recently, a probable role of the enteric microbiota in educating the immune system and disease development was shown.

Methods: Aim of our study was to compare the composition of the salivary microbiota between IgAN pts and Healthy Controls (HC). Based on clinical data, IgAN pts were grouped into "non-progressors" (NP) and "progressors' (P). Each group was composed by 14 volunteers. The total salivary microbiota was characterized through an integrated approach of culture-dependent and -independent methods. Bacterial tag-encoded FLX-titanium amplicon pyrosequencing (bTEFAP) of 16S rRNA genewas carried out for genomic.

Results: The abundance of some OTUs belonging to Firmicutes phylum significantly ($P<0.05$) differed between NP, P and/or HC. *Gemella haemolysins*, *Granulicatella adiacens*, *Veillonella parvula* were positively associated ($P<0.05$) with HC. Within the phylum Bacteroidetes, *Prevotella* species were the highest in HC. The only exception was for *P. aurantiaca*. Compared to HC, the percentage of abundance of some OTUs belonging to Pasteurellaceae family (e.g., *Haemophylus parainfluenzae*) increased in P and even more in NP. Fusobacteriaceae with the genera Fusobacterium were the lowest in P while no statistically significant ($P>0.05$) differences were found between HC and NP samples.

Conclusions: Our results indicate significant differences in microbiota distribution among NP, P and HC, with interesting potential outputs for the development of specific diagnostic tests, and/or for treatment and prevention.

TH-PO328

GWAS Follow-Up Studies Identify Abnormal Signaling in B Cells from Patients with IgA Nephropathy, an Autoimmune Disease with Aberrantly *O*-Glycosylated Autoantigen

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Background: IgA nephropathy (IgAN) is an autoimmune glomerulonephritis wherein immune complexes consist of IgA1 with galactose-deficient *O*-glycans (Gd-IgA1) and anti-Gd-IgA1 autoantibodies. Our GWAS identified a locus on chromosome 22q12.2 that contains genes encoding leukemia inhibitory factor (LIF) and oncostatin M (OSM), IL-6-related cytokines using gp130 for signal transduction and implicated in mucosal immunity and inflammation. Recently, we observed that abnormal IL-6/gp130/STAT3 signaling enhances production of Gd-IgA1 in IgAN. In this study, we characterized signaling mechanisms involved in Gd-IgA1 production induced by LIF, using immortalized IgA1-secreting cells derived from the circulation and tonsils of IgAN patients and controls.

Methods: IgA1-secreting cells derived from the circulation and tonsils of IgAN patients and controls were stimulated with LIF. Levels of secreted IgA1 and Gd-IgA1 were determined by ELISA. LIF/gp130 signaling pathways were analyzed by Western blotting, and the role of signaling pathways induced by LIF in Gd-IgA1 production was confirmed by using siRNA knock-down.

Results: LIF increased production of Gd-IgA1 in cells from the circulation and tonsils of IgAN patients but not in those from controls. STAT1 phosphorylation (Y705) induced by LIF was enhanced in cells from the circulation and tonsils of IgAN patients compared to those from controls. siRNA knock-down confirmed the central role of LIF/gp130/STAT1 signaling pathway in the enhanced production of Gd-IgA1 ($p<0.05$).

Conclusions: IgA1-secreting cells from IgAN patients responded abnormally to stimulation by LIF, a cytokine encoded in an IgAN-associated locus identified by GWAS. This abnormal signaling enhanced production of Gd-IgA1, the key autoantigen in IgAN.

TH-PO329

The Level of IgA Antibodies to Endothelial Cells Correlate with Histological Evidence of Disease Activities in Patients with Lupus Nephritis

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Background: Although anti-endothelial cell antibodies (AECA) have been frequently detected in patients with systemic lupus erythematosus (SLE), their pathological role remains unclear. Because antigens expressed on the endothelial cell (EC) surface are pivotal for autoimmune reactions, methods that detect antibodies only to EC surface molecules are required. Therefore, we developed a solubilized cell surface protein capture enzyme-linked immunosorbent assay (CSP-ELISA) that is able to detect antibodies against membrane proteins.

Methods: Sera from 51 patients with biopsy-proven lupus nephritis (LN), 25 with SLE without renal involvement (non-LN SLE), 10 disease controls (DC) and 80 healthy controls (HC) were tested for IgG- and IgA-AECA to human umbilical vein EC (HUVEC) or human glomerular EC (HGEC) by CSP-ELISA.

Results: Titers of IgG- and IgA- AECA were significantly higher in LN and non-LN SLE patients than in the combined DC and HC ($P < 0.001$) groups. The significant correlation of titer of AECA to both HGEC and HUVEC indicated AECA in LN patients recognize membrane proteins expressed on HGEC and HUVEC. The level of IgG-AECA did not correlate with active lesions defined by ISN/RPS classification, but the level of IgA-AECA did correlate with histological evidence of active lesions in LN patients ($P < 0.001$). The level of IgA-AECA correlate with the presence of endocapillary hypercellularity and fibrinoid necrosis ($p < 0.001$). The sensitivity as diagnostic test of IgA-AECA for histological evidence of active lesions in LN patients was 0.92, with specificity 0.72.

Conclusions: The significant correlation of IgA-AECA to endocapillary hypercellularity indicate IgA-AECA is observed to be associated with endothelial damages in LN.

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TH-PO330

Novel Nephritogenic Goodpasture Autoantibodies Target Accessible Quaternary Epitopes of α345(IV) Collagen in Patient Kidneys

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Background: Goodpasture (GP) disease is a severe autoimmune glomerulonephritis mediated by IgG autoAbs that bind to the GBM. Serum GP autoAbs mainly target two epitopes on α3NC1 monomers which are relatively inaccessible in cross-linked α345NC1 hexamers, the native form of the GP autoantigen in the GBM. Though anti-α3NC1 autoAbs are found in patient kidneys, whether they alone are sufficient to cause nephritis is not clear. In a classic study, GP autoAbs eluted from patient kidneys induced severe nephritis and death in monkeys, while serum autoAbs caused mild transient nephritis, despite linear fixation to the GBM. We tested the hypothesis that some GP autoAbs that bind in the kidney and cause nephritis are different from circulating autoAbs.

Methods: We analyzed the specificity of kidney-eluted autoAbs from three GP patients who developed fatal glomerulonephritis. Transgenic mice expressing human α3(IV) collagen were used for in vitro binding studies and passive immunization experiments.

Results: In one GP patient, kidney-eluted autoAbs bound to native α345NC1 hexamers, with minimal binding to α3-α5NC1 monomers, though anti-α3NC1 autoAbs were found in serum. In other two patients, kidney-eluted autoAbs bound both α3NC1 monomers and α345NC1 hexamers. Further analyses of eluted autoAbs identified two subsets with distinct properties. One bound to α3NC1 monomers but not to cross-linked α345NC1 hexamers nor to the native GBM, similar to classical serum GP autoAbs. Another bound to cross-linked α345NC1 hexamers and the native GBM, but not to α3NC1 monomers.

Conclusions: A novel type of anti-GBM autoAbs, recognizing quaternary epitopes present in α345NC1 hexamers but not NC1 monomers, was identified in the kidneys of GP patients. The hallmark feature of these autoAbs was the accessibility of their epitopes in the native GBM, in contrast to classical GP anti-a3NC1 autoAbs. GP autoAbs to quaternary a345NC1 epitopes can mediate nephritis in the absence of anti-a3NC1 autoAbs, though the severity of nephritis is likely exacerbated when both types of autoAbs are present in the GBM.

Funding: NIDDK Support, Private Foundation Support

TH-PO331

Moesin Is a Target of Autoantibodies in Proliferative Lupus Nephritis

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Background: The mechanisms by which nephritogenic antibodies are generated in SLE and the basis for their deposition in the glomerulus are unclear. The present study tests the hypothesis that autoantibodies to native glomerular antigens contribute to the development of lupus nephritis (LN).

Methods: Human podocyte membrane extracts and human glomerular extracts were separated by SDS-PAGE and immunoblotted with sera from patients with proliferative LN (Class III and IV), membranous LN (Class V), SLE without nephritis, and normal individuals. Proteins in the MW regions corresponding to bands reactive with the LN samples were characterized by LC-MS/MS.

Results: Patients with LN demonstrated a reactive band at approximately 50 kDa in the podocyte membrane extracts and between 43-46 kDa in the glomerular extracts. LC-MS/MS of those bands identified 296 proteins from podocytes and 351 proteins from glomeruli. Bioinformatic analysis of those proteins determined that 68 podocyte proteins and 94 glomerular proteins were plasma membrane-associated. Of those plasma membrane-associated proteins, 14 were common to both the podocytes and glomeruli, including proteins previously implicated in autoimmune diseases: annexin A2, heat shock protein 60, alpha enolase, and moesin. Sera from patients with proliferative LN contained significantly increased anti-moesin IgG by ELISA, compared to the other groups ($p < 0.01$). IHC of kidney biopsies from patients with proliferative LN showed increased moesin expression along glomerular capillaries, compared to normal controls.

Conclusions: We conclude that moesin may be a target for glomerular autoantibody deposition in proliferative LN. Quantitative serum anti-moesin levels may be a useful biomarker for proliferative LN.

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TH-PO332

The CKD Biomarkers Consortium Differentiating Treatment Responders from Non-Responders Using Biomarkers Identified from the Molecular Analysis of Lupus Nephritis Kidney Biopsies Samir Parikh,[1] Huijuan Song,[1] Paul L. Kimmel,[3] John W. Kusek,[3] Jianying Zhang,[1] Lianbo Yu,[1] Ana Malvar,[2] Brad H. Rovin.[1] *[1]Nephrology and Biostatistics, Ohio State Univ Medical Center, Columbus, OH; [2]Nephrology and Pathology, Hospital Fernandez, Buenos Aires, Argentina; [3]NIDDK, NIDDK, Bethesda, MD.*

Background: The molecular events occurring within the kidney during treatment of lupus nephritis (LN) are unknown. This work evaluated whether complete treatment responders (CR) and non-responders (NR) could be distinguished by differences in renal gene expression at diagnostic (Bx1) and repeat kidney biopsy (Bx2).

Methods: The expression of 511 immune-response genes was determined in kidney biopsies from 19 patients with proliferative LN and 4 normal controls using Nanostring technology. Biopsies were done at LN flare, and after completion of induction therapy. The median interval between biopsies was 8 (6-28) months. Using clinical criteria, 5 patients achieved CR, 10 achieved partial remission (PR) and 4 had NR. After normalization, a linear mixed model and paired t-test was used to compare transcript expression at baseline and between biopsies respectively. A 2-fold difference between transcript levels and p-value < 0.01 was required for differential expression. RT-PCR was used to validate selected transcripts.

Results: At Bx1, principal components analysis demonstrated complete segregation of CR from NR and normal controls from all LN. Interferon alpha, T and B cell signaling, and interleukin 10 pathways were activated in NR while apoptotic and T-cell regulatory pathways were activated in CR. A three gene signature differentiated CR from NR over the course of induction therapy. Neural cell adhesion molecule 1 (NCAM-1) and interleukin 28 (IL-28) increased 3-fold in CR compared to NR between Bx1 and Bx2 ($P<0.007$), whereas interleukin 17 (IL-17) decreased 65% in CR compared to NR ($P<0.004$).

Conclusions: These data suggest that the molecular profile of a responder is different than a non-responder at flare. The molecular analysis of the kidney biopsy at LN flare may be useful in predicting treatment response. For non-responders, response rates may improve through therapies that decrease IL-17 or increase NCAM-1 and/or IL-28.

Funding: NIDDK Support, Pharmaceutical Company Support - Questcor Pharmaceuticals

TH-PO333

Circulating Apoptotic Cell-Derived Microparticles Drive the Activation of Blood Dendritic Cell Subsets in Systemic Lupus Erythematosus Patients Jurgen Dieker,[1] Jurjen Tel,[2] Elmar Pieterse,[1] Astrid Thielen,[1] Marinka Bakker-van Bebber,[1] Jo H.M. Berden,[1] Jolanda De Vries,[2] Luuk Hilbrands,[1] Johan Van der Vlag.[1] *[1]Dept of Nephrology, Radboud UMC, Nijmegen, Netherlands; [2]Dept of Tumor Immunology, Radboud UMC, Nijmegen, Netherlands.*

Background: Persistent circulation of chromatin-containing apoptotic material and/or neutrophil extra cellular traps (NETs) has been proposed as an important driving force of the anti-chromatin autoimmune response in patients with systemic lupus erythematosus (SLE).

Methods: We analyzed microparticles isolated from plasma of SLE patients or healthy subjects, and assessed their effect on human blood-derived dendritic cells (DCs) and neutrophils.

Results: In SLE patients, we identified microparticles highly positive for annexin V and apoptosis-modified chromatin, not present in healthy subjects. These were mostly $CD31^+$/$CD45^-$ (endothelial), partially $CD45^+$/$CD66b^+$ (granulocyte), and negative for B and T cell markers. Microparticles isolated from plasma of SLE patients increased the expression of co-stimulatory surface molecules and production of pro-inflammatory cytokines in blood-derived plasmacytoid DC (pDCs) and myeloid DCs (mDCs). SLE microparticles also enhanced NETosis in blood-derived neutrophils. Microparticles from healthy subjects exhibited no significant effects on mDCs, pDCs and NETosis.

Conclusions: Concluding, circulating microparticles in SLE patients include a population of apoptotic cell-derived microparticles, not present in healthy subjects, which has pro-inflammatory effects on pDCs and mDCs, and enhance NETosis. These results underline the important role of apoptotic microparticles in driving the autoimmune response in SLE patients.

Funding: Private Foundation Support

TH-PO334

Treg17 Cells Are Programmed by Stat3 to Mediate Lupus Nephritis Malte A. Kluger, Simon Melderis, Matthias C. Meyer, Michael Luig, Boeren Goerke, Rolf A. Stahl, Ulf Panzer, Oliver M. Steinmetz. *III. Med. Klinik, Univ Hospital Eppendorf, Hamburg, Germany.*

Background: Th17 cells are central mediators of glomerulonephritis. The mechanisms underlying their counter regulation are largely unknown. We recently showed that Th17 specific regulatory T cells (Treg17) which depend on activation of Stat3 are mediators of acute crescentic GN. It is unknown to date whether they also play a role in chronically developing forms of nephritis. We therefore studied the function of Treg17 in the pristane model of lupus nephritis (LN).

Methods: Specific deletion of Stat3 was achieved using Foxp3Cre and CD4Cre x Stat3fl/fl mice. LN was induced by i.p. injection of pristane and peritoneal immunity was assessed at 1 week. Renal histology and function as well as systemic immunity were studied at 9 months.

Results: Foxp3Cre x Stat3fl/fl mice lacking Treg17 cells showed selectively enhanced peritoneal Th17 inflammation one week after induction of LN. In line, after 9 months, systemic immune responses were skewed towards Th17. Importantly, Foxp3Cre x Stat3fl/fl mice showed much greater mortality, as well as aggravated nephritis in terms of histology and inflammatory cell infiltration. Aggravation of disease was independent of humoral immune responses as shown by unchanged systemic autoantibody production as well as renal antibody and complement deposition. However, while analyses of systemic Tregs revealed unchanged numbers and percentages, renal Treg infiltration was strikingly reduced. FACS analyzes of renal Tregs from nephritic Foxp3Cre x Stat3fl/fl mice revealed near abscence of the trafficking receptor CCR6 which was abundantly expressed on wildtype Tregs. Importantly, the observed aggravation of disease was completely reversible in the absence of Th17 responses as shown at 9 months after LN induction in CD4Cre x Stat3fl/fl mice which lack not only Treg17 but also Th17 cells.

Conclusions: Our data indicate a protective role of Stat3 induced Treg17 cells in chronic lupus nephritis. Treg17 cells specifically target nephritogenic Th17 responses by using the chemokine receptor CCR6.

Funding: Government Support - Non-U.S.

TH-PO335

Deficiency for TNF Receptor 2 and TNF Receptor 1 and 2 Accelerates Autoimmunity and Lupus Nephritis in Lupus-Prone MRL-Fas/lpr Mice John M. Hoppe, Anela Taubitz, Nuru Eltrich, Volker Vielhauer. *Nephrologisches Zentrum, Medizinische Klinik und Poliklinik IV, Ludwig-Maximilians-Univ, Munich, Germany.*

Background: Tumor necrosis factor (TNF) contributes to organ damage in systemic lupus erythematosus (SLE). However, experimental data from SLE-prone mice and clinical experience in humans demonstrate that TNF is both a potent proinflammatory mediator, and an immune regulator limiting autoimmunity. This has prevented the successful implementation of TNF blocking therapies in SLE. The pleiotropic functions of TNF may be differentially mediated by the two TNF receptors TNFR1 and TNFR2. We therefore investigated the role of these receptors in lupus-prone MRL-*Fas*^{lpr} mice, in which inflammatory organ disease correlates with induced levels of TNF.

Methods: We generated MRL-*FAS*^{lpr} mice genetically deficient in TNFR1, TNFR2, or both receptors and phenotypically characterized these mice until 24 weeks of age.

Results: TNFR1-deficiency in MRL-*FAS*^{lpr} mice did not alter systemic autoimmunity or renal injury compared to wild-type littermates, despite a significantly increased formation of intrarenal extranodular lymphoid tissue with increased expression of cytokines and chemokines. In contrast, TNFR2- and particularly TNFR1,2 double-deficient MRL-*FAS*^{lpr} mice were characterized by accelerated autoimmunity, as revealed by increased mortality, more severe lymphoproliferation, higher anti-Sm autoantbody titers, increased renal accumulation of autoimmune CD4-CD8- T lymphocytes, and a reduced apoptotic rate in intrarenal lymphoid tissue. Proteinuria was more severe in TNFR1,2 double-deficient mice. Renal histology revealed exacerbated glomerular injury in TNFR2- and TNFR1,2-deficient MRL-*FAS*^{lpr} mice, and increased glomerular and tubulointerstitial infiltration of T cells in TNFR1,2-deficient mice. Renal expression of the proinflammatory cytokines IL-6 and IL-17A was increased in TNFR2- and TNFR1,2-deficient mice.

Conclusions: Our data demonstrate that selective deficiency of TNFR1 or 2 is not protective in lupus of MRL-*FAS*^{lpr} mice, but that TNFR2 and TNFR1 and 2 deficiency accelerates autoimmunity and organ damage.

Funding: Government Support - Non-U.S.

TH-PO336

Anti-Perlecan Antibodies in Murine Lupus Nephritis Luc Pomerleau, Melanie Dieude, Katia Hamelin, Julie Turgeon, Marie-Josee Hebert, Guillaume Bollee. *Centre de Recherche du Centre Hospitalier de l'Univ de Montreal, Montreal, QC, Canada.*

Background: LG3 is a proteolytic fragment of perlecan released by apoptotic endothelial cells. Autoantibodies reactive to LG3 were identified in renal transplant recipients with acute vascular rejection. In a mouse model, passive transfer of anti-LG3 antibodies enhanced vascular rejection. Lupus nephritis is closely associated with endothelial injury and immune-mediated injury to the glomerular capillaries. Here, we sought to explore the association between anti-LG3 antibodies and the progression of murine lupus nephritis.

Methods: Serum anti-LG3 titers and urinary excretion of LG3 were monitored from early to advanced stages of glomerular injury (21, 27 and 32 weeks) in NZB/W F1 mice, a common model of lupus nephritis. C57Bl6 mice were used as controls. Urinary excretion of LG3 was measured by Western blot and normalized to urine creatinine. Anti-LG3 titers were measured by ELISA. Urine albumin to creatinine ratios was also measured at 21, 27 and 32 weeks. At 32 weeks, mice were sacrified and renal histology was analyzed.

Results: At 21 weeks of age, NZB/W F1 mice showed no increase in albuminuria, LG3 was not detected in urine and anti-LG3 titers were similar in NZB/W F1 and controls. At 27 weeks, albuminuria was significantly higher in NZB/W F1 than controls. At 32 weeks, NZB/W F1 mice showed increased blood urea nitrogen, high-grade albuminuria and glomerular crescents. Serum anti-LG3 titers gradually increased in parallel with progression of glomerular injury and were significantly increased in NZB/W F1 mice compared to controls at 27 and 32 weeks ($n=5$-8 per group, $p<0.05$). At 32 weeks, LG3 was detected in the urine of NZB/W F1 mice but not in controls ($n=5$ per group, $p<0.05$).

Conclusions: We observed a progressive increase in serum anti-LG3 titers that parallels development of murine lupus nephritis. This suggests that LG3 released secondary to immune-mediated vascular injury induces the production of anti-LG3 antibodies, which

may aggravate glomerular microvascular injury, therebyleading to a vicious circle. Further studies are warranted to characterize the significance of urinary LG3 excretion and anti-LG3 antibodies in lupus nephritis.

TH-PO337

Different Glycosylation Patterns Influence the Physiological and Pathological Functions of Myeloperoxidase Jia Wang, Zhao Cui, Jian-Nan Li, Ming Hui Zhao. *Renal Div, Dept of Medicine, Renal Div, Dept of Medicine, Peking Univ First Hospital, Beijing, China.*

Background: Myeloperoxidase (MPO) has been proven to be pathogenic in anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis. It also acts as bactericidal agent by catalyzing the production of hypochlorous acid, and hence reactive oxygen species, from hydrogen to peroxide and chloride ions. MPO has five N-linked glycosylation sites on its two heavy chains, which are occupied by either complex or high mannose structures. The effect of glycosylation pattern to the functions of MPO is barely known.

Methods: We used different glycosidases to remove different glycans on MPO molecule separately, including the innermost GlcNAc, alpha 2,3, alpha 2,6 and alpha 2,8-linked sialic acid, branched terminal fucose, β-D-galactopyranoside, β-linked mannose, α1-2 and α1-3 linked D-mannopyranosyl, unbranched α1-6 linked D-mannopyranosyl, and chitobiose core.

Results: Compared to MPO treated in the absence of glycosidases, there was a significant decrease in chlorination activity of MPO treated with glycosidases, in which the removal of D-galactopyranoside (0.35±0.02 versus 0.50±0.04, P<0.001) and alpha-linked sialic acid (0.35±0.02 versus 0.50±0.04, P<0.001) presented the most remarkable decline. Deglycosylation could reduce the binding between MPO and its physiological inhibitor-ceruloplasmin, with the most significance on the removal of innermost GlcNAc at the asparagine residues of MPO (0.37±0.04 versus 1.06±0.11, P<0.001). The binding between MPO and ceruloplasmin was hardly reversed by MPO-ANCA after deglycosylation to MPO, especially the removal of innermost GlcNAc at the asparagine residues (0.38±0.03 versus 0.12±0.04, P=0.002) and alpha-linked sialic acid (0.28±0.03 versus 0.12±0.04, P=0.009).

Conclusions: Different glycosylation patterns might influence the physiological and pathological functions of MPO, which deserved consideration in the investigations of ANCA-associated vasculitis pathogenesis.

Funding: Government Support - Non-U.S.

TH-PO338

Longitudinal Measurement of PR3 and MPO mRNA Levels in a Large Cohort of Patients with ANCA Disease Reveals That Increased Expression Per Neutrophil Correlates with Disease Activity Jia Jin Yang,[1] Caroline J. Poulton,[1] Candace Henderson,[1] Elizabeth J. Brant,[1] Britta E. Jones,[1] Elizabeth Alderman McInnis,[1] J. Charles Jennette,[2,1] William Franklin Pendergraft,[1] Ronald J. Falk,[1,2] Dominic J. Ciavatta.[3,1] *[1]Medicine, UNC-CH; [2]Pathology, UNC-CH; [3]Genetics, UNC-CH, Chapel Hill, NC.*

Background: We have demonstrated up-regulation of autoantigen genes in mature neutrophils and monocytes from patients with ANCA disease (J Am Soc Nephrol 2004, 15:2103-14). Here, we investigated levels of autoantigen gene expression in ANCA-patients during the disease course.

Methods: A total of 1270 samples were collected from 318 ANCA-patients during various stages of disease activity from 1999 to 2014. Levels of PR3 and MPO gene transcripts were determined by quantitative PCR, compared to calibrator samples and expressed as number of positive cells in 10^4 total cells.

Results: PR3 (n=1270, 158±515, p<0.0001) and MPO (387±937, p<0.0001) mRNA levels were significantly up-regulated in leukocytes from ANCA patients compared to healthy donors (n=135, PR3:16±36; MPO:56±56). Levels of PR3 and MPO gene transcription were closely correlated with disease activity during the disease course. PR3 (n=511, 246±648, p<0.0001) and MPO (556±1151, p<0.0001) mRNA levels were statistically elevated during active disease and reduced during remission (PR3: 96±394; MPO: 258±702). The mRNA test had a higher specificity than ANCA titer because ANCA titer was not decreased in some patients when remission was achieved. PR3 and MPO gene expression was affected by treatment (e.g. temporarily decreased with pulse corticosteroids; increased with rituximab). Higher absolute neutrophil counts, seen in 62% of active patients, did not explain increased PR3 and MPO gene expression, as expression per neutrophil was significantly higher in patients with increased expression (n=210, PR3:86±250, p<0.0001; MPO:163±306, p<0.0001) than that in patients with normal expression (n=236, PR3:3±3; MPO:15±8).

Conclusions: Active ANCA patients have significantly increased PR3 and MPO gene transcription per neutrophil that correlates with activity status during the course of disease and suggests that this aberrant expression is involved in the pathogenesis of ANCA disease.

Funding: NIDDK Support

TH-PO339

Serum Antibodies against Linear Peptides of Myeloperoxidase Were Identified in over Half Patients with Anti-Glomerular Basement Membrane Disease and Were Associated with Disease Severity Jian-Nan Li,[1] Zhao Cui,[1] Ming Hui Zhao.[1,2] *[1]Renal Div, Dept of Medicine, Peking Univ First Hospital; [2]Peking-Tsinghua Center for Life Sciences.*

Background: Some patients with anti-glomerular basement membrane (GBM) disease had coexisting anti-neutrophil cytoplasmic antibodies (ANCA), especially anti-myeloperoxidase (MPO) antibodies. Our previous study indicated that most sera from double positive patients recognized c-terminus of MPO heavy chain (H1, $MPO_{279-409}$). This study investigated the frequency of antibodies against overlapping linear peptides derived from H1 and intact MPO, in sera from patients with anti-GBM disease.

Methods: Sera from 76 patients with anti-GBM disease were collected on diagnosis. Thirteen overlapping linear peptides (H1-1~H1-13) covering $MPO_{279-409}$ were synthesized. IgG antibodies were screened from the 76 sera by ELISA using intact human MPO and the overlapping pepetides as solid phase ligands, respectively. Their clinical significance was further investigated.

Results: Of the 76 patients with anti-GBM disease, sera from 17 (22.4%) patients recognized intact human MPO, while, sera from 48 (64%) patients recognized linear peptides of $MPO_{279-409}$. No cross-reaction was detected among antibodies against α3(IV) NC1, linear peptides and intact MPO. Of the thirteen linear peptides, H1-4 (40.8%) and H1-12 (42.1%) had the highest recognition frequencies. Patients with antibodies against linear peptides presented with older age than those without the antibodies (58.4±15.1 versus 35.9±15.9 years; P=0.008). In comparison with the patients without antibody against H1-11~12, patients with antibodies against H1-11~12 showed significantly higher serum creatinine at the time of diagnosis [645.0 (378.5-1037.0) versus 464.5 (200.8-645.3) μmol/L, P=0.02], had significantly higher probability for progressing to ESRD (HR 2.22; 95% CI 1.08-4.58; P=0.031), and recognized significantly more types of linear peptides [0 (0-1) versus 4 (2.0-8.5), P<0.001].

Conclusions: Antibodies against linear peptides of MPO can be detected in majority of patients with anti-GBM disease and some were associated with disease severity, implying a common pathogenic mechanism of anti-GBM antibody and MPO-ANCA.

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TH-PO340

The High Binding Affinity of HLA-DRB1*0405 and MPO Epitope aa 447–459 Determines the Poor Prognosis of ANCA Disease Yali Cao. *Nephrology, China-Japan Friendship Hospital, Beijing.*

Background: The HLA system plays a central role in the distinction between self antigens and non-self antigens. It has been demonstrated that DRB1*0405 is an independent risk factor for the poor response to treatment and the deterioration of renal function in Chinese patients with ANCA disease. MPO-ANCA reactive with MPO epitope aa 447–459 (RKIVGAMVQIITY) are exclusively associated with active disease.We asked if DRB1*0405 could be involved in antigen presentation and predict the prognosis.

Methods: Capture ELISAs were used to detect the bind affinity of MPO447–459 and DRB1*0405. Flow cytometry and immunofluorescence staining were used to identify the bind of MPO447–459 and DRB1*0405 on the surface of neutrophil.

Results: Capture ELISAs demonstrated MPO447–459 bound DRB1*0405 with high affinity. Neutrophils of healthy individuals are reported to contain cytoplasmic reservoirs of MHC II (DR) antigen. MHC II surface expression is detected on neutrophils of patients with active ANCA disease but not on neutrophils of patients with inactive disease.We asked if surface-expressed DRB1*0405 would bind MPO447–459 peptides. Surface expression of DRB1*0405 was detected in patients with activie disease who were genotyped as DRB1*0405,*0405. Surface-expressed DRB1*0405 protein bound MPO447–459, consistent with in vitro binding studies. With the remmission of ANCA disease, DRB1*0405 was undetectable and the surface binding was decreased. In contrast, neutrophils from a patient expressing DRB1*01,*14 and one expressing DRB1*03,*15 molecules were found not to bind MPO447–459. Specificity of MPO447–459 binding was verified by a competition study. Biotinylated MPO447–459 binding was decreased with increasing concentrations of nonbiotinylated peptide (54 to 12%). Flow cytometry data were validated by immunofluorescence staining.

Conclusions: These data indicate that DRB1*0405 molecules expressed on the surface of neutrophils are capable of binding certain pathogenic antigenic-MPO peptide that results in presentation to and activation of T cells. Taken together, the study suggest HLA-DRB1*0405 alleles might contribute to the pathogenesis of MPO-ANCA disease and determine the poor disease prognosis.

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TH-PO341

Proteinase-3 T Cell Epitope Mapping in Systemic Vasculitis Vincent P. O'Reilly,[1] Simone A. Scollard,[2] Alice M. Coughlan,[1] Conleth F. Feighery,[2] Mark Alan Little.[1] *[1]Trinity Health Kidney Centre, Dublin, Ireland; [2]Dept of Immunology, Trinity College Dublin, Dublin, Ireland.*

Background: Granulomatosis with polyangiitis (GPA) is a severe autoimmune vasculitis that affects multiple organ systems and is characterised by antibodies against proteinase-3 (PR3). T cells are required for antibody class switching and secretion by plasma cells, suggesting a role for autoreactive T cells specific for PR3. Autoreactive T cells are present in healthy individuals where they are tightly supressed by regulatory T cells. Although much is known about the PR3 B cell epitope, specific T cell responses have not been defined.

Methods: We performed *in silico* analysis in an attempt to predict which PR3 peptide sequence best fitted the HLA-DP binding groove. Then, control and patient PBMCs or T-reg depleted CD4+ sorted T cells were stimulated with whole PR3 protein or 8 pools of linear 20-mer PR3 peptides. Proliferation was measured by tritiated thymidine incorporation or CellTrace loss. Cytokine responses (IL-6 secretion) were assessed with ELISA.

Results: In silico analysis predicted binding of amino acids 222-242 to HLA-DP. 44% and 0% of GPA patients and controls respectively showed reactivity to whole heat inactivated PR3 (p<0.05); only patients that were ANCA positive at the time of the assay showed proliferation. Monocytes pulsed with pool 7 peptides (AA 193-236) stimulated most CD4+ T cell proliferation (1.7X increase over vehicle). Pool 7 also stimulated secretion of IL-6

from the T cell monocyte co-cultures (2.6X increase over vehicle). An even stronger IL-6 signal (6.5-fold increase over vehicle) was observed in GPA patient co-cultures stimulated with pool 7, which included the predicted peptide sequence adjacent to the catalytic triad.

Conclusions: PBMCs from GPA patients proliferate in response to PR3 stimulation. T-reg depleted CD4+ cells from healthy controls respond to linear peptides of PR3, with a peptide binding sequence adjacent to the catalytic triad being the most likely candidate for key T cell epitope.

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TH-PO342

Monocyte Subsets and Their Role in ANCA Vasculitis Eóin O'Brien, Mark Harrington, Mark Alan Little, Fionnuala B. Hickey. *Clinical Medicine, Trinity College Dublin, Dublin, Ireland.*

Background: Anti-neutrophil cytoplasm autoantibody (ANCA)-associated vasculitis (AAV) is an autoimmune condition affecting the microvasculature. It is the leading cause of rapid, progressive glomerulonephritis which leads to loss of kidney function. The autoantibodies are primarily directed against myeloperoxidase (MPO) or proteinase-3 (PR3), proteins expressed mainly in neutrophils and monocytes. Much of the research to date has been directed on the role of neutrophils with little research into the role monocytes play in disease. Similar to other immune cells, monocytes can be divided into subsets based on their inflammatory phenotype. These are categorised as Classical, Intermediate and Non-Classical, with non-classical being the most pro-inflammatory. The purpose of this study was to investigate the role of specific monocyte subsets in AAV.

Methods: Blood was obtained from AAV patients (active disease and remission), healthy donors and control individuals with other forms of kidney disease. Flow cytometry was used to evaluate the proportions of each subtype of monocyte (based on cell-surface expression of CD14 and CD16) and the amount of MPO or PR3 expressed on their surface. In order to investigate the effect of ANCA on monocytes, sorted monocytes were treated with patient ANCA, or with monoclonal antibodies (mAb) directed against MPO or PR3 and IL-1β production was measured by ELISA and Western blot. IL-1β in serum from patients and control individuals was measured by ELISA.

Results: This study indicates that in patients with active AAV, MPO and PR3 antigens are expressed on the cell surface of a higher proportion of monocytes compared to patients in remission. In addition we demonstrate that in these patients, MPO and PR3 are preferentially expressed on the more pro-inflammatory cells, (intermediate and non-classical). We also report that incubation of monocytes from control individuals with patient ANCA or mAb against MPO results in increased IL-1β production indicating inflammasome activation by ANCA. IL-1β was also detected in the serum of patients.

Conclusions: In summary, these data implicate a key role for monocytes as targets of autoantibodies in the pathogenesis of ANCA vasculitis.

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TH-PO343

Soluble CD163 Level as a Biomaker of Active Disease in Systemic Vasculitis Limy Wong,[1] Claire Kennedy,[1] Vincent P. O'Reilly,[1] Louise Ann Elliott,[2] Alice M. Coughlan,[1] Paul O'Hara,[1] Fionnuala B. Hickey,[1] Emma Connolly,[1] Conleth F. Feighery,[1] Anthony M. Dorman,[4] Sarah Margaret Moran,[3] Michael Clarkson,[3] George S. Mellotte,[1] Mark Alan Little.[1] *[1]Trinity Health Kidney Centre; [2]Immunology, Trinity College Dublin; [3]Cork Univ Hospital; [4]Pathology, Beaumont Hospital.*

Background: A sensitive and specific biomarker to distinguish small vessel vasculitis (SVV) from mimics and infection, and to identify those at risk of relapse is lacking. We hypothesised that urinary and serum sCD163, which is upregulated in inflammation, mirror disease activity in SVV.

Methods: Using the Rare Kidney Diseases (RKD) Biobank, 323 urine and 420 serum samples from patients with SVV, disease controls (DC) and healthy controls (HC) were assayed for sCD163 by ELISA. Clinical data were derived from the linked RKD registry. Immunohistochemistry and rtPCR were performed on renal tissue from patients with SVV and from rats with MPO-ANCA vasculitis.

Results: Urinary sCD163 levels were significantly higher in those with active SVV (3.7±0.5ng/mL) than DC (1.4±0.4ng/mL), HC (0.6±0.02ng/mL) and SVV in remission (0.9±0.1ng/mL). The level was highest in active anti-GBM disease (17.6±9.4ng/mL). In SVV, only those with active renal disease displayed elevated levels (6.7±1.6); those with active extra-renal disease were indistinguishable from all control groups (0.7±0.1). Serum sCD163 levels were not significantly different in patients with SVV (332± IQR 240 to 528ng/mL) and DC (298,IQR 236 to 475ng/mL) or HC (287, IQR 226 to 463ng/mL); the highest serum sCD163 levels were observed in those with active anti-MPO disease (482, IQR 335 to 745ng/mL) and untreated SVV (518,IQR 324 to 1039ng/mL). There was no correlation between serum and urinary sCD163. CD163 was not detectable in rodent MPO-ANCA vasculitis kidney lesions, but was heavily expressed in the vascular adventitia of renal vessels. In human renal tissue, the degree of glomerular CD163 expression was tightly correlated with urine but not serum levels.

Conclusions: Urinary sCD163 associates very tightly with active renal vasculitis and provides a non-invasive measure of active vasculitic GN.

TH-PO344

Epigenetic Control of CD177 – A Receptor Presenting the ANCA Antigen Proteinase 3 Claudia Eulenberg, Sylvia Bähring, Friedrich C. Luft, Ralph Kettritz. *Experimental and Clinical Research Center, Charité Medical Faculty and the Max-Delbrück Center for Molecular Medicine, Berlin, Germany.*

Background: Proteinase 3 is the major ANCA antigen in granulomatosis with polyangitis (GPA). PR3-ANCA binding to membrane-expressed PR3 (mPR3) is a key event for neutrophil activation and subsequent necrotizing vasculitis. The neutrophil-specific CD177 receptor enables $mPR3^{high}$ expression on a neutrophil subset ($CD177^{pos}/mPR3^{high}$). ANCA patients show a higher percentage of $CD177^{pos}/mPR3^{high}$ neutrophils compared to healthies and the higher the percentage the worse the clinical disease course. We reported previously that CD177 protein and mRNA expression is restricted to $CD177^{pos}/mPR3^{high}$ neutrophils with a random monoallelic mRNA expression pattern. We hypothesized that epigenetic mechanisms control CD177 gene expression.

Results: Using the Infinium HumanMethylation450 BeadChip (Illumina) and cell sorting we found three CpGs in the potential CD177 promotor that were methylated in $CD177^{neg}$, but not in $CD177^{pos}$ cells ($p<0.001$; n=6). We next performed chromatin immunoprecipitation (ChIP) analysis to assess chromatin conformation in the two CD177 subsets. We identified a 75bp region upstream of the ATG that showed H3K4me3 mark (euchromatin) enrichment in $CD177^{pos}$ neutrophils ($p<0.05$; n=4). This putative promotor region contains a TATA box and binding sites for several transcription factors (TF). Finally, we established a $CD34^{+}$ hematopoietic stem cells (HSC) model system that is more suitable than terminally differentiated neutrophils for further studies, such as reporter assays. By haplotype analysis, undifferentiated HSC showed biallelic CD177 mRNA expression with one allele being silenced during neutrophilic differentiation. The resulting monoallelic expression reflected the status observed in neonatal neutrophils from the same cord blood donor.

Conclusions: Together, our data strongly implicate epigenetic mechanisms that are responsible for the generation of distinct CD177/mPR3 neutrophil subsets. Further studies need to identify the transcription factor(s) that control this process.

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TH-PO345

Drug Bioactivation by Myeloperoxidase and Covalent Binding to Target Proteins: Implications for Drug-Induced Anti-Neutrophil Cytoplasmic Antibody-Associated Vasculitis Olivier Lardinois,[1] Leesa Deterding,[2] Jacob Hess,[1] Candace Henderson,[1] Ronald J. Falk,[1] William Franklin Pendergraft.[1] *[1]UNC Kidney Center, Dept of Medicine, Univ of North Carolina, Chapel Hill, NC; [2]Mass Spectrometry Group, National Inst of Environmental Health Sciences, Research Triangle Park, NC.*

Background: Drug-induced vasculitis is a serious side effect of certain medications and there is a strong association between drug-induced vasculitis and the formation of anti-neutrophil cytoplasmic autoantibodies (ANCA). The most often implicated drugs are hydralazine, propylthiouracil, and levamisole-adulterated cocaine. The precise mechanism by which these drugs cause vasculitis is still unknown. ANCA in drug-induced vasculitis are most frequently directed against the neutrophil granule enzyme MPO, which generates hypochlorous acid (HOCl). Most drugs that cause vasculitis are low molecular weight substances that can potentially be metabolized by MPO to reactive free radical compounds. We hypothesized that these electrophilic compounds react with MPO or with other target proteins to form hapten adducts that stimulate autoantibody formation. To our knowledge, the presence and specific location of any such drug-adducts on proteins has never been determined.

Methods: Drug-protein adducts were detected and quantified using immunochemical and liquid chromatography-mass spectrometry techniques.

Results: Our analysis revealed that all causal drugs investigated, except for hydralazine, form drug-protein adducts after in vitro exposure to HOCl and are bioactivated to reactive metabolites by MPO. We also detected intense adduct trapping in proteins exposed first to acrolein, an aldehyde product of endogenous lipid peroxidation, and then to hydralazine. Hydralazone derivatives formed during these reactions are sufficiently stable to unequivocally identify sites of adduction on proteins by liquid chromatography-tandem mass spectrometry.

Conclusions: All offending drugs investigated here have a high propensity to form adducts with proteins. Some derivatives formed during these reactions show strong structural similarities to that of other chemicals found in the environment, including pesticides.

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TH-PO346

Reduced $IL\text{-}10^{+}$ Regulatory B Cells in Patients with Active ANCA-Assoiated Vasculitis (AAV) Permit Increased Circulating Autoantibodies Lydia Aybar, JulieAnne G. McGregor, Susan L. Hogan, Yichun Hu, Carmen E. Mendoza, Elizabeth J. Brant, Caroline J. Poulton, Candace Henderson, Ronald J. Falk, Donna O. Bunch. *Nephrology and Hypertension, UNC, Chapel Hill, NC.*

Background: AAV Pathogenesis is B cell dependent, yet how B cell subsets modulate immunopathogenesis is unknown. Although their phenotype remains controversial, regulatory B cells (Bregs) play a role in immunological tolerance via IL-10.

Methods: We investigated putative $CD24^{hi}CD38^{hi}$ and $CD24^{hi}CD27^{+}$ Bregs, in addition to their $CD5^{+}$ subsets, in 69 patients with AAV. IL-10 production was determined following culture of peripheral blood mononuclear cells with CD40 ligand and CpG DNA.

Results: The $CD5^+$ subset of$CD24^{hi}CD38^{hi}$ B cells, but not $CD24^{hi}CD38^{hi}$ or $CD24^{hi}CD27^+$ B cells, was decreased in patients with active disease relative to patients in remission ($p\leq0.001$) and healthy individuals ($p\leq0.0001$). IL-10^+ B cells were reduced during active disease compared to patients in remission ($p=0.005$) and healthy controls ($p=0.001$). $CD5^+$ B cells produce more IL-10 than $CD5^{neg}$ cells (18% versus 11%). In paired analysis of 9 patients, as IL-10^+, $CD5^+CD24^{hi}CD38^{hi}$ and $CD24^{hi}CD38^{hi}$ B cells increased in disease remission, ANCA titers decreased.

Conclusions: $CD5^+$ $CD24^{hi}CD38^{hi}$ and IL-10-producing B cells similarly decrease in active disease and rebound during remission; $CD5^+$ B cells were enriched in IL-10 production. These data suggest that CD5 may identify IL-10-producing Bregs that malfunction during active disease, resulting in increased ANCA production.

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TH-PO347

Changing Mast Cell Phenotype with Cromogycate Provides a Novel Therapeutic Strategy to Treat Autoimmune Myeloperoxidase Anti-Neutrophil Cytoplasmic Antibody Associated Glomerulonephritis

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Background: The aim of the study was to test the hypothesis that cromoglycate can inhibit proinflammatory mast cell (MC) degranulation and enhance modulatory IL-10 synthesis resulting in therapeutic treatment of anti-MPO autoimmunity and glomerulonephritis (GN).

Methods: We compared MC frequency and degranulation status in 48 renal biopsies from patients with MPO-ANCA GN and 10 thin membrane disease (controls). Experimental anti-MPO GN was induced by immunization with MPO and GN triggered by a subnephritogenic dose of anti-GBM globulin. Cromoglycate (or saline control) was administered daily commencing either 2 days before or 10 days after (established disease) immunization.

Results: MCs were prominent in the interstitium of MPO-ANCA GN kidney biopsies (5±0.9 versus 1±0.2 cells/high power field [c/hpf] $p<0.05$) and were mainly degranulated (57±8 versus 0±0% $p<0.0001$) compared with controls. In experimental anti-MPO GN, cromoglycate treatment reduced MC degranulation (5±1 versus 15±4c/hpf $p=0.05$) at the immunization site and significantly reduced DC activation (CD40 and MHC-II expression) in draining lymph nodes. This was associated with significant reduction in anti-MPO autoimmunity (DTH, MPO stimulated T cell proliferation, IFNγ production; 3.6±1.3 versus 13.5±3.7ng/ml $p<0.05$) but increased IL-10 (198±16 versus 124±10pg/ml $p<0.05$). Glomerular injury was significantly reduced (albuminuria and segmental glomerular necrosis; 34±4 versus 62±6% of glomeruli affected $p<0.01$). Cromoglycate treatment of established disease also similarly attenuated GN (segmental glomerular necrosis; 32±4 versus 55±5% $p=0.01$). Cromoglycate effects were MC specific as administration to MC deficient mice developing GN had no effect on autoimmunity or GN. In vitro MPO stimulated proliferation of CD4+ Teffector cells (Teff) from MPO immunized mice was enhanced in the presence of MCs. Addition of cromoglycate to CD4+ Teff and MCs enhanced MC IL-10 production and inhibited Teff proliferation.

Conclusions: Cromoglycate specifically alters MC phenotype from injurious to immunomodulatory in anti-MPO GN and may offer new therapeutic opportunities.

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TH-PO348

Treatment of Experimental Autoimmune Vasculitis with a SYK Inhibitor

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Background: Spleen tyrosine kinase (SYK) is phosphorylated during ANCA-induced neutrophil activation, and thus it represents a potential therapeutic target in AAV. We have shown previously that SYK inhibition with fostamatinib, a selective tyrosine kinase inhibitor, is an effective treatment in experimental models of anti-GBM disease, and that SYK is expressed and activated in human ANCA-GN. We have now studied the effects of SYK inhibition with fostamatinib in a rat model of anti-MPO vasculitis.

Methods: Disease was induced by immunising Wistar Kyoto rats (n=8/group) with the ANCA target antigen, myeloperoxidase (MPO). Disease onset was confirmed by development of haematuria and proteinuria 4 weeks after immunisation, Animals were then treated with vehicle, fostamatinib 20 mg/kg, or fostamatinib 30 mg/kg by twice daily oral gavage from week 4 to 6 and then assessed for disease severity at the end of week 6.

Results: Are shown in the Table below. Reported as median±IQR per group. Comparison by Kruskall Wallis test.

	Vehicle	Fostamatinib 20mg/kg	Fostamatinib 30mg/kg	p value
Proteinuria (mg/day)	2.88 (1.8-6.3)	0.13 (0.0-0.8)	0.23 (0-0.6)	0.0004
Haematuria (au)	3 (2-3)	0 (0-0.4)	0 (0-0.1)	0.0018
Abnormal Glomeruli (%)	12 (3-29)	5 (2-9)	0 (0-6)	0.0262
Macrophages/Glomerulus	1.77 (1.2-2.6)	0.25 (0.2-0.4)	0.2 (0.0-0.4)	0.0004
Serum Creatinine (μmol/l)	54 (52-58)	48 (47-52)	46 (43-47)	0.047
Lung Haemorrhage Score (au)	3 (2-3.8)	1 (0.3-1.8)	0 (0.1-0.4)	0.0001
Lung Haemosiderin Score (au)	3.0 (0.9-4.1)	0.5 (0.2-0.7)	0.2 (0.1-0.4)	0.0087
MPO-ANCA titre (au)	115 (95-160)	95 (75-120)	100 (70-120)	ns

Conclusions: In a preclinical model, SYK inhibition with fostamatinib is an effective treatment for crescentic glomerulonephritis and lung haemorrhage, the life-threatening manifestations of AAV, even after onset of disease. This therapeutic effect was seen at a low dose range of fostamatinib, estimated to be consistent with doses achieved in clinical studies using fostamatinib in non-renal disease. These observations support the potential investigation of targeting SYK in clinical studies of AAV.

TH-PO349

Human PTEC Modulate Autologous B Cell Function

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Background: Research on infiltrating inflammatory cells in the kidney interstitium have, until fairly recently, not examined B cells other than in the specific environment of transplantation. In these reports, B cells, like dendritic cells, are localised almost exclusively within the kidney tubulointerstitium where they would be ideally placed to interact with proximal tubular epithelial cells (PTEC). We have previously reported that activated PTEC are able to down-modulate autologous lymphocyte and dendritic cell function.

Methods: B cells were cultured with autologous PTEC and readouts included proliferation, Ab/cytokine secretion and Ag expression.

Results: PTEC decreased B cell proliferative responses to the mitogen PWM and the TLR agonist R848. Interestingly, autologous PTEC also significantly decreased the number of B cells secreting both IgG and IgM Ab in response to these stimuli and overall levels of Ab production were also decreased. Transwell studies demonstrated that this modulation was primarily contact-dependant and blocking studies with anti-PD-L1 led to partial restoration in Ab production. Further blocking studies against soluble HLA-G and IDO, two other immuno-inhibitory molecules also up-regulated in our activated PTEC, demonstrated minor restoration of Ab responses. B cells cultured in the presence of PTEC displayed increased levels of CD38 but decreased levels of CD27, a marker of B cell blasts and memory cells. Cytokine analysis demonstrated increased levels of B cell-derived immuno-regulatory IL-10 in 75% of donors stimulated with R848 in the presence of PTEC.

Conclusions: We report that PTEC are able to modulate autologous B cell phenotype and function. This modulation is complex as our results demonstrate a contact-dependant component operating though PD-L1 but with other soluble and intra-cellular factors such as sHLA-G and IDO also playing a role. We hypothesize that such mechanisms may have evolved to maintain peripheral immune-homeostasis, especially within the inflammatory milieu that exists within many kidney diseases.

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TH-PO350

Coronin 1a Deficiency Protects Mice against Anti-Glomerular Basement Membrane Induced Humoral Immune Response

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Background: The family of Coronin's consists of cytoskeletal, actin binding proteins involved in regulation of actin-based motility-related processes. Furthermore, leukocyte-specific protein Coronin 1a (Cor1a) is essential for macrophage, T and B cell signaling. Previously, using microarray analyses we observed an upregulation of Cor1a during the time course of experimental glomerular injury. To date, nothing is known about the role of Cor1a in cytoskeleton-dependent processes such as cell migration, trafficking and cytokinesis during renal disease.

Methods: To investigate the role of Cor1a in glomerular injury, crescentic nephrotoxic glomerulonephritis was induced in Cor1a deficient (-/-) mice and wild-type (wt) littermates. Disease progression was analyzed by collecting serum and urine samples throughout the experiment weekly. To examine morphological changes a renal survival biopsy was taken on day 10 before mice were sacrificed for histological and flow cytometric analyses on day 21 after disease onset.

Results: In response to anti-glomerular basement membrane (GBM) glomerulonephritis we detected significantly reduced titers of IgG antibodies generated against the induction antibody in Cor1a-/- compared to wt mice. Since, Cor1a is essential for T cell homing and activation Cor1a-/- mice had strongly reduced numbers of renal CD4+ and CD8+ T cells during GBM-nephritis on day 21. Intriguingly, flow cytometric analyses of renal granulocytes and more precisely the neutrophils revealed a significant increase in Cor1a deficient mice compared with wt littermates whereas renal macrophages were not affected. However, histologic evaluation showed that GBM-nephritis in Cor1a-/- mice causes only minor changes on renal damage compared to wt mice and that these mice were not protected from crescentic glomerular injury.

Conclusions: Cor1a deficient mice fail to induce a humoral immune response during GBM-nephritis which is accompanied by reduced renal T cell numbers but an enhanced recruitment of neutrophils during crescentic nephrotoxic glomerulonephritis.

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TH-PO351

IL-17F Drives Renal Tissue Injury in Crescentic GN
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Background: The T_H17 immune response is associated with the pathogenesis of autoimmune diseases, implicating the T_H17 "master cytokine" IL-17A as the critical mediator of autoimmune diseases such as human and experimental crescentic glomerulonephritis (GN). However, the relative importance of additional T_H17 effector cytokines such as IL-17F in renal tissue injury is unknown.

Methods: To study the impact of IL-17F on renal inflammation, we induced experimental crescentic glomerulonephritis (NTN) in wild-type, IL-17F, IL-17A, and IL-17 A/F gene-deficient mice.

Results: In line to recent data by us and others IL17A-deficiency leads to an attenuated course of disease after induction of glomerulonephritis when compared to wild-type animals. Interestingly, after induction of NTN, IL17A/F double knockout mice showed an even greater reduction of renal tissue injury and a conservation of renal function compared to IL17A gene-deficient mice indicating a unique role of IL-17F. Next we were able to identify significant production of IL17F by $CD4^+$ T cells and γΔ T cells in the kidneys of nephritic mice. To specifically analyze the role of IL17F we analyzed the course of NTN in IL17F gene-deficient mice. Intriguingly, these animals showed a highly significant reduction of renal tissue injury (glomerular crescent formation 24% versus 34% and tubulointerstitial damage score 20% versus 28%; $p<0.05$ and $p<0.001$, respectively) and preserved renal function (BUN 35mg/dL versus 82 mg/dL; $p<0.001$) when compared to wild-type mice.

Conclusions: We therefore conclude that both IL-17A and IL-17F, each of them prominently expressed by renal $CD4^+$ T_H17 cells and gamma delta T cells, significantly contribute to the development of renal tissue injury in experimental GN. This finding might be of great importance for the development of anti-IL-17-cytokine therapies in crescentic GN.

TH-PO352

Leptin Deficiency Inhibits IL-23/Th-17 Axis and Monocyte Chemoattractant Protein-1 Production in a Murine Model of Nephrotoxic Serum Nephritis
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Background: Recent studies have demonstrated that leptin promotes Th17 cell responses and enhances the production of proinflammatory cytokines. To clarify the effect of leptin in the regulation of IL-23/Th-17 axis and the activation of macrophages, we used a murine model of nephrotoxic serum nephritis (NSN).

Methods: Nephrotoxic serum was administered in C57BL/6J mice (WT) and food restricted C57BL/6J-ob/ob (FR-ob/ob) mice after preimmunization with sheep IgG in Complete Freund's adjuvant. Kidneys and blood were collected at sacrifice 7 days after nephrotoxic serum injection. Cytokine or chemokine expressions and Th17 cell proliferation in the kidneys were analyzed by quantitative real-time PCR. Murine macrophage RAW264.7 cell line or murine peritoneal exudate macrophages (PEMs) were used to investigate the direct effect of leptin by real-time PCR and ELISA.

Results: Following the induction of NSN, blood urea nitrogen was remarkably elevated in WT mice ($n=8$; $p<0.001$). Glomerulosclerosis were significantly inhibited in FR-ob/ob mice, and 75% of WT mice died until day 14, while all FR-ob/ob mice survived ($n=8$). Serum levels of murine IgG1 and IgG2b against sheep IgG were significantly reduced in FR-ob/ob mice, followed by decreased deposition of murine anti-sheep IgG in glomeruli. Expression levels of mRNA for Th17 related cytokines such as IL-17A, IL-22, IL-23p19, IL-6, TGF-β1, which were related with anti-sheep IgG antibody production, and that for F4/80 and monocyte chemoattractant protein (MCP)-1 were significantly reduced in FR-ob/ob mice kidneys, whereas those for Th1 and Th2 related cytokine were not different. Leptin directly upregulated IL-23p19 mRNA expression in RAW264.7 cells, and also induced MCP-1 secretion and mRNA expression in PEMs from db/db mice, which lack long form of the leptin receptor OB-Rb followed by phosphorylation of STAT3.

Conclusions: Leptin deficiency suppresses IL-23/Th-17 axis and secondary antibody production. Furthermore, leptin induced MCP-1 expression in macrophages in OB-Rb/STAT3-independent signaling pathways.

TH-PO353

Regulatory Macrophages Derived From Peritoneal Dialysate Reduce Renal Injury in Adriamycin Nephropathy
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Background: Patients undergoing peritoneal dialysis (PD) discard huge numbers of peritoneal macrophages in dialysate daily. Macrophages (MΦ) have shown great promise in cell therapy of different types of experimental kidney disease and human kidney transplantation. This study aimed to examine the potential of using peritoneal macrophages (PMs) from peritoneal dialysate to treat renal disease.

Methods: Peritoneal macrophages derived from PD patients were examined. Peritoneal dialysate was collected also from mice undergoing PD dailyfor 7 days via a silicon catheter in the peritoneal cavity. Murine adriamycin nephropathy (AN) was induced by 10 mg/kg adriamycin in BALB/c mice. Adoptive transfer of modulated macrophages derived from PD mice into mice with AN was used to assess their *in vivo* functions.

Results: PMs from PD patients and mice accounted for more than 40% of total peritoneal leukocytes. PMs from PD patients and mice expressed low levels of CCR2, but high levels of CD86 and MHC-II. Ninety % of PMs from PD patients maintained their viability after storage in liquid nitrogen for at least one year. PMs from PD patients and mice displayed normal macrophage function and could be modulated into an M1 or M2 phenotype. *In vivo*, adoptive transfer of M2 PMs from PD mice protected against renal injury in mice with AN.

Conclusions: M2 macrophages derived from PD mice are able to reduce renal injury in AN, suggesting that peritoneal macrophages from PD patients may have the potential for clinical therapeutic application.

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TH-PO354

Blocking Myeloid Growth Factors to Protect against Ischemia/Reperfusion Injury
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Background: Inflammation is a hallmark of ischemia/reperfusion injury (IRI), involving inflammatory cell infiltration. A subsequent remodeling phase is in part mediated by M2 macrophages. GM-CSF and CSF-1 are two growth factors that mediate myeloid cell function. This study aimed to protect mice from IRI by blocking GM-CSF or CSF-1R.

Methods: Mice received 40min unilateral IRI (25min bilateral IRI for renal function) and were administered αGM-CSF or αCSF-1R antibodies under two regimes: short-term (day -2, 0, 2 post-IR) and prolonged (day -2, 0, 2, 4, 7, 10 post-IR). Infiltrating myeloid cells were analyzed by flow cytometry, serum cytokines were assessed with a multiplex array and picrosirius red staining was used to measure collagen. Serum creatinine and urea were assessed at day 14.

Results: Short-term CSF-1R blocking, but not GM-CSF, delayed the infiltration of $Ly6C^+$ monocytes and reduced $F4/80^+$ macrophage numbers. With prolonged CSF-1R blocking, macrophages, including a $CD206^+$ M2 subset, were reduced at day 7. This also increased circulating CSF-1 levels and the M2 associated cytokines IL-4, IL-10, CCL2 and CXCL2. Levels of the pro-inflammatory cytokines IL-1β, IL-12 and IL-17 were also elevated. Neither antibody treatment altered collagen accumulation at days 7 or 14 post-IR compared to the IR control group. Increased creatinine and urea concentrations, reflecting reduced renal function, were observed at day 14 compared to the sham-IR control. Prolonged GM-CSF blockade did not improve renal function. Urea and creatinine concentrations appeared lower with CSF-1R blockade compared to the IR control group but assessment at different time-points is required to identify a true protective effect.

Conclusions: $Ly6C^+$ monocyte infiltration and $CD206^+$ M2 macrophage numbers can be altered with αCSF-1R antibody administration. These effects were not observed with GM-CSF blockade.

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TH-PO355

Mitochondrial Dysfunction Confers Albumin-Induced NLRP3 Inflammasome Activation and Renal Tubular Injury
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Background: Proteinuria is involved in the development of tubular lesions and in the progressive loss of renal function in chronic kidney diseases (CKDs) via uncertain mechanisms. The present study aims to define the roles of mitochondria in proteinuria-induced renal tubular injury and their underlying mechanisms.

Methods: Eight-week-old 129/Sv male mice were subjected to an intraperitoneal injection with low-endotoxin albumin for 11 days to produce albumin-overload model. Mitochondial function including ROS production, mitochondrial membrane poteintial (MMP), and ATP content was detected.

Results: Employing the albumin-overload mouse model, we observed severe tubular structure damage and striking tubular cell apoptosis. Furthermore, the tubular epithelial cells displayed a loss of E-cadherin expression and gained expression of α-SMA and vimentin, indicating a cellular phenotypic alteration. Strikingly, these albumin overload-induced abnormalities were robustly blocked by a mitochondrial superoxide dismutase-2 mimic, MnTBAP. In agreement with these results, we observed a marked change in mitochondrial morphology accompanied by mitochondrial Cytochrome c release and mtDNA copy number reduction. These alterations were largely reversed by MnTBAP, suggesting a key role for mitochondria-derived oxidative stress in mediating the albumin effect on mitochondrial dysfunction and subsequent tubular injury. Moreover, the NLRP3/caspase-1/cytokine cascade was activated in the kidney by albumin-overload and was entirely abolished by MnTBAP. In albumin-treated mouse proximal tubular cells, albumin directly induced, mitochondrial dysfunction, NLRP3/caspase-1/cytokine cascade activation, cell apoptosis, and cellular phenotypic transition. Similar to our in vivo results, treatment with either MnTBAP or cyclosporin A, a mitochondrial permeability transition pore inhibitor, remarkably attenuated these abnormalities in cells.

Conclusions: These novel findings demonstrate a potential role for mitochondrial dysfunction/NLRP3 inflammasome axis in the pathogenesis of proteinuria-induced renal tubular injury.

TH-PO356

VEGF Derived From Dendritic Cells Affects Their Maturation and Is Protective in Unilateral Ureteral Obstruction Joanna Kalucka,[1] Susanne Olbrich,[1] Daniel Engel,[2] Christian Kurts,[2] Kai-Uwe Eckardt,[1] Alexander Weidemann.[1] *[1]Medical Clinic Nephrology & Hypertension 4, Univ of Erlangen-Nuremberg, Erlangen, Germany; [2]Inst of Experimental Immunology, Univ Clinic of Bonn, Bonn, Germany.*

Background: Progression of chronic kidney injury is correlated with an increase of inflammation. Dendritic cells (DCs) constitute one of the major leukocyte populations in fibrotic kidney.Vascular Endothelial Growth Factor (VEGF) has been shown to be expressed and secreted by DCs in inflamed tissues; however its pathophysiologic role in DCs during renal inflammation and fibrosis is unknown. Thus, we investigate the role of VEGF in DCs during chronic kidney injury.

Methods: We generated a conditional knock-out mouse strain using the Cre-loxP technology (CD11c:cre/VEGFf/f). Conditional knock-out (cKO) VEGF mice and their wild-type (WT) littermates were subjected to unilateral ureteral obstruction (UUO) for 7 days and kidneys were analyzed for biochemical, histological, RNA and FACS parameters.

Results: VEGF cKO mice are born at normal Mendelian ratios without any obvious pathological changes, renal dysfunction, or changes in immune cell composition within the kidneys. However, 7 days after UUO, VEGF cKO mice exhibit significantly worse renal function than WT animals, suggesting that DC-derived VEGF affects immune responses which in turn affect the function of the contralateral, unobstructed kidney. Irrespective of the genotype, UUO kidneys display significantly less VEGF mRNA than control kidneys indicating that DCs do not contribute to overall VEGF expression in the kidneys. FACS analysis confirmed that DCs in the UUO kidney become functionally activated. Moreover, we noticed a significant reduction of $CD11c^{hi}$ MHCII+ DCs in VEGF cKO UUO kidneys, suggesting that VEGF plays a role in DC maturation.

Conclusions: Our results suggest that VEGF in DCs is necessary for their maturation and has a protective function in a model of chronic kidney injury. Further analysis is needed to investigate the downstream effectors of VEGF-dependent DC function, also on the healthy kidney. To our knowledge these intriguing data are the first to establish a significant function of VEGF in DCs in an in vivo model of kidney fibrosis.

TH-PO357

Natural Killer Cells Regulate Renal Fibrosis after Acute Kidney Injury Isaah Vincent,[1] Sun-Sang J. Sung,[1] Liping Huang,[1] Diane L. Rosin,[2] Mark D. Okusa.[1] *[1]Div of Nephrology, Univ of Virginia, Charlottesville, VA; [2]Dept of Pharmacology, Univ of Virginia, Charlottesville, VA.*

Background: Acute kidney injury (AKI) may lead to progressive renal disease and in some cases ESRD. Fibrosis is a key feature in the pathogenesis of progressive kidney disease yet the mechanisms that regulate fibrosis is unknown. Certain immune cells contribute to fibrosis while others attenuate fibrosis. Natural killer (NK) and Natural Killer T (NKT) cells have been linked to regulation of fibrosis in several different organ systems thus these studies were done to determine the role of these cells in renal fibrosis following AKI.

Methods: Kidney injury was induced by using either 24 minutes of unilateral renal ischemia followed by reperfusion (IRI) or an ip injection of 250 mg/kg folic acid (FA) in sodium bicarbonate. To determine the role of NK and NK T cells during fibrosis in the period after the initial inflammatory injury in AKI, we treated C57BL/6 mice with 200 µg PK136 monoclonal antibody against NK1.1, which depletes both NK and NK T cells, on days 5, 10, and 15 after inducing injury. Nineteen days after injury, mice were euthanized and kidneys collected and processed for histology (Masson's Trichrome), mRNA, and flow cytometry.

Results: In both IRI and FA models, compared to IgG-treated mice, PK136 treatment increased the fibrotic area within the kidney (by Masson's Trichrome), mRNA message of ECM component collagen 1, and the presence of myofibroblasts (indicated by a-SMA immunoreactivity). This may be due to changes in inflammatory environment, as PK136 depleted mice have decreased IFN-γ expression and increased IL-10 expression compared to IgG-treated mice. To determine whether NK or NK T cells play a role in fibrosis, AKI was induced in $Ja18^{-/-}$ and $CD1d^{-/-}$ mice, which lack NK T cells. Compared to WT mice, $Ja18^{-/-}$ and $CD1d^{-/-}$ mice had no increase in fibrotic area or collagen message, indicating that the enhanced fibrosis after PK136 depletion is due to the absence of NK cells.

Conclusions: NK cells, not NK T cells, aid in the suppression of fibrosis after AKI. Further investigation will focus on determining the mechanism by which NK cells regulate fibrosis within the kidney.

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TH-PO358

Pericytes as Critical Innate Immune Sentinels following Ischemic Acute Kidney Injury Ivan G. Gomez,[1,2] Jeremy Stuart Duffield.[1,2] *[1]Depts of Medicine & Pathology, Univ of Washington, Seattle, WA; [2]Biogen Idec, Cambridge, MA.*

Background: Following kidney ischemia reperfusion injury (IRI), the innate immune system is activated, initiating inflammatory process and pericyte/myofibroblast activation. Previous studies have demonstrated that Toll-like receptor (TLR2 and TLR4) and Myd88 signaling is important in this process, but myeloid lineage TLRs and Myd88 are dispensable. However, the role of mesenchymal cells (pericytes [PCs]) in the innate immune response has not been explored in detail, but their function and known roles implicate them as sensors of injury and guardians of leukocyte recruitment to the interstitium.

Methods: Murine model of Unilateral-IRI was used for sterile kidney injury. Crude Danger Associate Molecular Patterns (DAMPs) were purified and characterized from IRI kidneys 24h after injury. PCs isolated from normal kidney of wild-type, *Tlr2-4-/-* and *Myd88-/-*, *Nalp3-/-* and *Caspase 1-/-* mice were cultured with stimulation of TLR ligands, DAMPs and LPS. For in vivo experiment, *Coll1a1-GFP (Coll-GFP)* and *FoxD1-Cre* crossed with *Myd88fl/fl* transgenic mice were used.

Results: Stimulation of PCs by TLR ligands resulted in secretion of a broad array of pro-inflammatory cytokines and chemokines including IL-6 and MCP-1, responses attenuated by Myd88 deficiency. DAMPs activated TLR-2/-4, Myd88 signaling pathways inkidney PCs, but not primary tubular epithelial cells or vascular endothelial cells. DAMP-mediated PC activation resulted in secretion of IL-6, MCP-1 but also IL-1b and IL-18, indicative of activation of inflammasome signaling. These responses were dependent on TLR-2/-4, Myd88 pathway, and the inflammasome receptor NLRP3. In vivo, Myd88 depletion in Foxd1 lineage mesenchymal cells, resulted in reduced inflammation and fibrosis, implicating that transition of pericytes to myofibroblasts requires active MyD88 signaling.

Conclusions: Our findings suggest that kidney PCs act as sentinel cells that actively modulate signaling through activation of innate receptors and contribute to inflammatory responses in sterile IRI.

TH-PO359

Bone Marrow Precursor Cells Produce Soluble Urokinase Receptor That Causes Focal Segmental Glomerulosclerosis Eunsil Hahm, Changli Wei, Andrew Zloza, Jevgenijs Lusciks, Jing Li, Isabel Fernandez, Jochen Reiser. *Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

Background: Soluble urokinase receptor (suPAR) has been considered a circulating factor that causes focal segmental glomerulosclerosis (FSGS), yet the origin of pathological suPAR in FSGS remains unclear. Given there are variations in the biochemical forms by which suPAR can occur in FSGS, we analyzed suPAR expression in stem-like cell niches.

Methods: Adoptive transfer, humanization techniques, ELISA, and flow cytometry were performed.

Results: We observed that urokinase receptor (uPAR) expression is increased in bone marrow (BM) cells, but not circulating blood cells in the LPS proteinuric mouse model. Interestingly, LPS treatment increased uPAR expression in BM $Gr\text{-}1^{+}$ cells with a massive expansion of $Gr\text{-}1^{low}$ populations. Despite depletion of circulating $Gr\text{-}1^{+}$ cells, proteinuria was potentiated in LPS-challenged mice. In addition, granulocyte colony-stimulating factor (G-CSF) further elevated albumin-creatinine ratio (ACR) and plasma suPAR levels, compared to LPS alone. These data suggest that increased demand for BM cell production facilitates proteinuria. Stem-like $c\text{-}kit^{-}/Gr\text{-}1^{low}/sca\text{-}1^{+}$ BM cells were required for suPAR production and proteinuia induction. Ablation of BM cells by gamma irradiation significantly ameliorated proteinuria and reduced suPAR production in both balb/c and B6 mice in response to LPS, while transfer of BM cells restored proteinuria with an enhanced plasma suPAR levels upon LPS administration. However, uPAR null mice failed to restore LPS-induced proteinuria in irradiated WT mice. Moreover, NOD-*scid IL2rg*^{null} (NSG) immunocompromised mice given BM cells from LPS-challenged WT, but not uPAR null mice, exhibited increased ACR with augmented circulating and urinary suPAR levels. This indicated BM-derived uPAR is required for development of proteinuria. Humanized mice that engrafted with peripheral blood mononuclear cells (PBMC) of patients with FSGS developed proteinuria and elevated mouse suPAR in both plasma and urine.

Conclusions: These results suggest that stem-like $c\text{-}kit^{-}/Gr\text{-}1^{low}/sca\text{-}1^{+}$ BM cells are responsible for pathological suPAR causing FSGS.

Funding: NIDDK Support

TH-PO360

Role of Apol1 in HIV-1 Internalization in Human Podocytes Pravin C. Singhal,[1] Manuela Valsecchi,[2] Massimo Aureli,[2] Xiqian Lan,[1] Karl Leon Skorecki,[3] Domenico Mavilio,[4] Joanna Mikulak.[4] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Dept of Medical Biotechnologies and Translational Medicine, Univ of Milan, Milan, Italy; [3]Medicine, Rambam Health Care Campus, Haifa, Israel; [4]Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Milan, Italy.*

Background: Genetic variants of Apolipoprotein-1 (Apol1) are associated with FSGS and HIV-associated nephropathy (HIVAN). Apol1 is a serum trypanolytic factor linked to high density lipid particles. Intracellular Apol1 expressed by several cell types, including podocytes, appeared to be a lipid-binding protein relevant for the maintenance of cellular homeostasis through regulation of lysosomal activity as well as inhibition of HIV-1 replication. However, the molecular mechanisms involved in Apol1-mediated preservation of physiological conditions as well as in the onset of FSGS and HIVAN are still unresolved. We explore the role of Apol1 in HIV entry and accumulation in human podocytes in relation to membrane traffic and sphingolipid metabolism.

Methods: Expression of Apol1 in human podocytes was assessed by Western blot, qPCR and immunoflourescence analysis. Human podocytes were transfected with either wild type Apol1 (WT) or Apol1 G1 genetic variant and analyzed for glycohydrolases (α-mannosidase,ß-galactosidase,ß-hexosaminidase,ß-glucocerebrosidase, glucocerebrosidase-1 and glucocerebrosidase-2) activity or for HIV-1 accumulation of the specific strong-stop HIV-1 DNA by qPCR analysis. Trans-infection of HIV-1 to T cells was detected by qPCR.

Results: WT Apol1 in human podocytes co-localizes with Rab5 expressing early endosomes, and regulates lysosomal activity through inhibition of enzymatic activity of several lysosomal and membrane-associated glycohydrolytic enzymes. Expression of Apol1 G1 genetic variant resulted in the further decline in the activity of glycohydrolases and correlated with increased HIV-1 entry and accumulation compared to WT. Moreover, both WT and G1 were able to increase HIV infectivity after recovery by $CD4^+$ T cells from HIV-1 harboring podocytes.

Conclusions: Apol1 regulates the activity of glycohydrolases that is relevant for HIV-1 entry and accumulation in human podocytes.

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TH-PO361

Renin Enhances HIV Replication through Protease Activity and Activation of Pro (P)-Renin Receptor (RR) Nirupama Chandel, Xiqian Lan, Kamesh R. Ayasolla, Mohammad Husain, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Blockade or inhibition of Ang II production are currently used to slow down the progression of HIV-associated nephropathy. HIV is known to subvert cellular machinery to enhance its replication. Recently, HIV has been reported to enhance T cell renin expression. We hypothesized that HIV induces a high renin state to promote its own replication in T cells (TCs). Since Ang II blockade enhances renin production, use of AT1 receptor blockers may enhance HIV replication and thus renin inhibition may be a better alternative to block production of Ang II in patients with HIV infection. However, if a patient with HIV infection is being treated effectively with antiviral therapy, the effect of AT1R blockade-induced HIV replication would not be of any consequence.

Methods: Freshly isolated human T cells (TCs) were pulsed with primary strain of HIV ($HIV\text{-}1_{92HT599,}$ HTC)for 2 h followed by treatment with different concentrations of renin. To establish a causal relationship, HTCs were treated with either buffer or renin with/without aliskiren (a renin inhibitor). To determine the role of (P)-RR and renin, control TCs and TCs silenced for (P)-RR/renin were pulsed with HIV. After 24 hours, HIV replication was assayed by mRNA expression for HIV-1LTR and protein expression by p24 ELISA. Additionally, renin-induced down stream molecular mechanisms were evaluated. Proteolytic activity of renin and HIV protease (Hpr) on angiotensinogen (Agt, renin substrate) and Gag polyproteins (Hpr substrate) was evaluated.

Results: Renin enhanced HIV replication in TCs in a dose dependent manner. (P)-RR deficient TCs as well as those lacking renin displayed attenuation of both NF-κB activity and HIV replication. TCs treated with Hpr and renin displayed binding of (P)-RR to promyelocytic zinc finger protein (PLZF). Renin, HIV, and Hpr activated PI3K pathway. Both renin and Hpr cleaved Agt to Ang I and also cleaved Gag polyproteins to p24. Furthermore, aliskiren inhibited renin- and Hpr-induced cleavage of Agt and Gag polyproteins.

Conclusions: Renin contributes to HIV replication in TCs via (P)-RR and through cleavage of Gag polyproteins.

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TH-PO362

HIV-1-Induced Trimethylation of Histone H3K49[me]^3 in Kidney Cells Is Associated with Enhanced Snail Expression but Down Regulation of E-cadherin and Vitamin D Receptor (VDR) Nirupama Chandel, Rivka Lederman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: HIV infection of kidney cells plays a key role in the development of HIV-associated nephropathy (HIVAN). We previously reported that kindey cell phenotype in HIVAN is a consequence of epthelial mesenchymal transition (EMT). We hypothesized that HIV infection may be modulating kidney cell phenotype though the induction of epigenetic factors. Since epigenetic factors-induced alterations are reversible, identification of their role in the development of HIVAN may be used as a therapeutic strategy. In the present study, we evaluated the effect of HIV on trimethylation of Histone H3K49[me]^3 in kidney cells both *in vivo* and *in vitro*.

Methods: Renal tissues were harvested from four week old control (FVB/N) and HIV-transgenic (Tg26) mice (n=3 for each group). *In vitro* studies, human renal proximal tubular cells (HPTCs) were transduced with either empty vector (EV) or NL4-3 without *gag* and *pol* (HIV). Protein blots of renal tissues, EV/HPTCs and HIV/HPTCs were probed for trimethylation (H3K49 [me]^3). To explore a causal relationship between Snail expression and down regulation of E-cadherin and VDR, 293T cells were transfected with Snail plasmid. Control 293T and Snail/293T cells were probed for Snail. The same blots were stripped and reprobed for E-cadherin, VDR, and actin (n=3).

Results: Renal tissues from Tg26 mice (n=3) displayed enhanced triemethylation (H3K49 [me]^3) as well as enhanced expression of Snail, but down regulation of E-cadherin and VDR. Similarly, HIV/HPTCs (n=3) displayed enhanced trimethylation (H3K49 [me]^3) and Snaill expression but down regulation of E-cadherin and VDR. Since enhanced expression of Snail in 293T cells, led to down regulation of E-cadherin and VDR, HIV-induced down regulation of tubular cell E-cadherin and VDRappears to be mediated through enhanced expression of Snail.

Conclusions: HIV-1 down regulates tubular cell E-cadherin and VDR expression through upregulation of Snail via trimethylation of Histone H3K49[me]^3. Since loss of E-cadherin is a marker of loss of epthelial phenotype, HIV likely enhances kidney cell EMT via epigenetic factors.

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TH-PO363

Complement Activation in the Kidney during Preeclampsia Jamie S. Chua,[1] Maria Elisabeth Penning,[1] Cees van Kooten,[2] Aletta Buurma,[1] Jan A. Bruijn,[1] Ingeborg M. Bajema,[1] Eliyahu V. Khankin,[4] Kitty Bloemenkamp,[3] S. Ananth Karumanchi,[4] Hans J. Baelde.[1] *[1]Pathology, LUMC, Leiden, South Holland, Netherlands; [2]Nephrology, LUMC, South Holland, Netherlands; [3]Obstetrics, LUMC, Leiden, Netherlands; [4]Howard Hughes Medical Inst and Dept of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.*

Background: A growing body of evidence suggests that complement dysregulation plays a role in the pathogenesis of preeclampsia. The kidney is one of the major organs affected in preeclampsia. Because the kidney is highly susceptible to complement deposits, we hypothesized that preeclampsia is associated with renal complement activation.

Methods: We performed a nationwide search for renal autopsy material in the Netherlands using a computerized database (PALGA). Renal tissue was obtained from 11 women with preeclampsia, 25 pregnant controls, and 14 non-pregnant controls with hypertension. The samples were immunostained for C4d, C1q, MBL, properdin, C3d, C5b-9, IgA, IgG and IgM. Our findings in human samples were validated using a soluble fms-like tyrosine kinase 1 (sFlt-1) mouse model of preeclampsia.

Results: Preeclampsia was significantly associated with renal C4d —a stable marker of complement activation— and the classical pathway marker C1q. In addition, the prevalence of IgM was significantly higher in the kidneys of the preeclamptic women. No other complement markers studied differed between the groups. In the preeclampsia mouse model, the kidneys in the sFlt-1-injected mice had significantly more C4 deposits than the control mice.

Conclusions: The strong association between preeclampsia and renal C4d, C1q, and IgM levels suggests that the classical complement pathway plays a role in the pathogenesis of renal injury in preeclampsia. Moreover, our finding that sFlt-1-injected mice develop excess C4 deposits indicates that angiogenic dysregulation may play an important role in complement activation within the kidney. We suggest that inhibiting complement activation may be beneficial for preventing the renal manifestations of preeclampsia.

TH-PO364

Podocytes Produce Complement Proteins which Contribute to Local Glomerular Complement Activity Lindsay S. Keir,[1,2] Anne Katrin Dettmar,[3] Gavin Iain Welsh,[1] Christoph Licht,[4] Anna Richards,[5] Jun Oh,[3] Moin Saleem.[1] *[1]Univ of Bristol, United Kingdom; [2]Scripps Research Inst, CA; [3]Univ Medical Center, Hamburg, Germany; [4]The Hospital for Sick Children, Toronto, Canada; [5]Univ of Edinburgh, United Kingdom.*

Background: Disordered complement activation is implicated in the pathogenesis of many glomerular diseases. The reason the glomerulus is vulnerable to complement dysregulation is unknown. Local production of complement components has been reported but remains controversial. The contribution of podocytes to this is unclear but podocyte damage occurs in complement related glomerulopathies. We hypothesised that podocytes produce complement proteins that are involved in local complement activation and regulation. This may explain the susceptibility of the glomerulus to defective complement control.

Methods: Complement protein expression of human conditionally immortalized podocytes was determined before and after interferon gamma (IFNg), interleukin 6 (IL-6) or vascular endothelial growth factor (VEGF) treatment. C3 convertase and complement challenge assays detected activity of secreted proteins. Murine kidneys (C57BL/6) were analysed for complement protein expression using immunohistochemistry and in situ hybridization. Complement activation was studied in primary rat podocytes treated with anti Fx1a antibodies and exposed to normal, C2 and C3 deficient sera.

Results: Human podocytes express mRNA and protein for both activatory and regulatory complement proteins. Some proteins were secreted, notably C3 and CFH which were functionally active. IFNg upregulated production of C3 and CFH. VEGF increased CFH and CD46 while IL-6 reduced them. Murine glomeruli expressed C3 and CFH mRNA and CFH, crry and CD59a proteins. Podocyte derived C2 and C3 contributed to complement activation in a model of membranous nephropathy.

Conclusions: Podocytes synthesize functional complement proteins which contribute to local glomerular complement activation and regulation. This may explain why podocytes are targeted in complement mediated diseases. Defining the role of podocyte derived complement proteins may advance our understanding of glomerular diseases and identify new treatment strategies.

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TH-PO365

Effect of Interleukin-2 in Murine Podocytes Diego H. Aviles,[1] Jeannine Ory Ascani,[2] Arnold H. Zea,[1] William E. Smoyer,[3] Beatriz E. Finkel-Jimenez,[4] David Tate,[1] Anna Magdalena Wilk,[1] Krzysztof Reiss,[1] Tyrus Stewart.[1] *[1]LSU Health Sciences Center, New Orleans, LA; [2]Children's Hospital Research Inst, New Orleans, LA; [3]Nationwide Children's Hospital, Columbus, OH; [4]American Univ of the Caribbean School of Medicine, Coral Gables, FL.*

Background: The podocyte is a highly differentiated cell that serves as a key component of the glomerular filtration barrier. Injury to these cells can result in proteinuria. Evidence suggests that cytokines may play a role in the development of proteinuria. Previous studies have shown that patients with nephrotic syndrome have increased levels of IL-2. The purpose of this study was to investigate the effects of IL-2 on kidney podocytes.

Methods: A conditionally immortalized mouse podocyte cell line was used for all experiments. Expression of IL-2 receptor subunits was measured by real-time PCR, flow cytometry, and western blot. The activity of the Jak/Stat pathway was measured by real-time PCR and western blot. Markers of autophagy and apoptosis were measured by western blot. Mitochondrial depolarization and apoptosis were measured by flow cytometry.

Results: Murine podocytes express IL-2 receptor subunits, as shown by flow cytometry, real-time PCR, and western blot. Stimulation of murine podocytes with IL-2 resulted in significant increases in mRNA levels of STAT5a and STAT5b, in addition to significant increases in cytosolic STAT5a and JAK3 protein expression. The autophagy marker LC3II had significantly decreased protein expression following stimulation. The pro-apoptotic protein Bax and Caspase-8 inhibitor $cFlip_L$ also had significantly increased protein expression after stimulation. Mitochondrial depolarization and apoptosis were both significantly increased following stimulation.

Conclusions: The results of this study showed that murine podocytes express a functional IL-2 receptor and that IL-2 induces podocyte injury. This effect is mediated by JAK/STAT signaling. Furthermore, IL-2 causes a decrease in autophagy, which may contribute to the observed apoptosis. This study offers insight to cytokine induced podocyte injury.

TH-PO366

Live Imaging of Immune Complex Formation In Vivo Lai Guan Ng. *Singapore Immunology Network (A*STAR), Singapore.*

Background: Compelling evidence shows that immune complexes (IC) play a pathogenic role in various immune-related diseases. The hallmarks of IC-induced acute inflammatory responses are characterized by massive neutrophil infiltration, edema and tissue injury. While it is well established that neutrophils have a central role in IC-mediated inflammation, the dynamics of neutrophil-IC interactions are still poorly defined.

Methods: Here, using a combination of in vitro, in vivo genomic and functional assays together with multiphoton intravital microscopy in a mouse ear skin model of the reverse Arthus reaction, we investigated the mechanisms underlying neutrophil responses in IC-mediated inflammation.

Results: We found that, following the initial IC deposition, neutrophils started to accumulate in close proximity to the perivascular areas with deposited IC. This was followed by the interaction between neutrophils and IC, which eventually led to the subsequent extravasation of these cells and vascular leakage. Interestingly, we observed that neutrophil-IC interactions appeared to amplify the rate of IC deposition and neutrophils were able to internalize IC while transmigrating from the blood into the tissue. In addition, we discovered that neutrophils coordinate their accumulation and recruitment in response to IC-activation via a CXCR2 ligand-dependent quorum-sensing system.

Conclusions: We thus elucidate previously unknown mechanisms for IC-mediated inflammation, which may have significant implications for designing clinical therapies.

Funding: Government Support - Non-U.S.

TH-PO367

The Influence of Renal Compartments on Dendritic Cell Signature Under Homeostatic Conditions and During Allograft Rejection Federica Chessa, Shijun Wang, Daniel Mathow, Zoran Popovic, Hermann-Josef Groene. *Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany.*

Background: Renal dendritic cells (rDCs), a component of the renal mononuclear phagocytic system (rMoPh), play major roles in renal homeostasis and disease. A predominant distribution of rDCs in homeostatic renal cortex has been described. The functional relevance of the compartment-specific distribution and reactivity of rDCs remains unknown. Allograft biopsy studies have shown that the histopathological features of kidney allograft rejection mainly develop in renal cortex.

Methods: The aim of this study is 1) to comprehensively describe the renal leukocyte population of cortex and medulla with a focus on rDCs and 2) to investigate the gene expression profile of cortical and medullary rDCs, under homeostatic conditions and during transplant rejection.

Results: Our results confirm that the leukocyte density in renal cortex (2800 cells/mg of tissue) is significantly higher than in medulla (1680 cells/mg of tissue). Expression profile of rDCs in homeostasis shows upregulation of CD36 in cortex in comparison to medulla, indicating compartmental regulation of antigen uptake capacity of rDCs independent from inflammation. Upon allogeneic stimuli, a compartment-specific signature, including genes involved in antigen uptake, processing and presentation, occurs preferentially in cortical DCs of donor and host origin. Flow cytometry analysis of host leukocytes during rejection reveals a remarkable tendency of host alloreactive DCs to infiltrate cortex, where they reach a cell density of 7000 cells/mg of tissue, 7-times higher than in medulla. In addition, we observe a progressive loss of donor-derived rDCs, which is complete at 7 days post-transplantation, likely due to either cell death or migration towards host lymphoid tissues.

Conclusions: We conclude that, inspite of discrete differences of cortical and medullary rDCs under homeostatic conditions, upon allogeneic stimuli both resident donor rDCs and infiltrating host DCs in cortex acquire an activated profile with regard to alloantigen uptake, processing and presentation, thereby contributing to compartment-specific post-transplant kidney damage.

TH-PO368

Human Antibody-Drug Conjugate Target Glomeruli to Resolve Nephritis Nino Kvirkvelia,[1] Maggie McMenamin,[1] Besarion Lasareishvili,[2] Vanessa Iris Gutierrez,[1] Michael P. Madaio.[1] *[1]Medicine, Georgia Regents Univ, Augusta, GA; [2]Agricultural Univ of Georgia, Tbilisi, Georgia.*

Background: Current therapies to limit kidney disease progression, lack specificity, have suboptimal efficacy and often have systemic toxicity. To approach this problem, we postulated that human monoclonal (m) anti-α3 (IV) antibodies (Ab) that localize in glomeruli could serve as vehicles for targeted drug delivery for glomerular diseases. Given enhanced glomerular expression of α3 (IV), with very limited epitope exposure in other organs, it provides an ideal target for delivery of glomerular disease modifying agents. As a potential disease-modifying agent, we took advantage of recent observations that PGE_2 enhanced renal cellular recovery and regeneration after established immunologic injury during the course of nephrotoxic serum nephritis (NTN).

Methods: To enhance efficacy, limit systemic effects and specifically target the kidney, PGE_2 was chemically coupled to a human m anti-α3 (IV) Ab using the zero length crosslinker 1-Ethyl-3-[3-dimethylamino-propyl]carbodiimide HCL (EDC) and the conjugates (10-15μg/g of body weight) were injected in NTN mice.

Results: Chemical composition of conjugates was confirmed by western blotting, and anti-α3 (IV) activity was assessed by two site ELISA. Glomerular localization of the anti-α3 (IV) Ab–PGE_2 conjugates was visualized in NTN mice by IF and live animal in vivo imaging. Thereafter, the capacity of the conjugates to modify disease was determined during established NTN. Glomerulonephritis resolved, and BUN levels were reduced in the conjugate treated mice (66.6 ± 2.12mg/dL), as compared to mice treated with only NTS (99.3±13.7mg/dL, $p<0.005$). Podocyte injury, assessed by alterations in synaptopodin staining, was also limited in anti-α3 (IV) Ab–PGE_2 conjugate treated mice.

Conclusions: The results provide a novel means of targeting glomeruli with human reagents by providing efficient drug delivery to the site of injury. The approach has potential application for treatment of human glomerular diseases, independent of etiology.

Funding: NIDDK Support

TH-PO369

A Transgenic Model of Randall-Type Heavy Chain Deposition Disease Amélie Bonaud,[1] Sébastien Bender,[1,3] Michel Cogné,[1,3] Frank Bridoux,[2] Christophe Sirac,[1] Guy Touchard.[2] *[1]CRIBL, CNRS, Limoges, France; [2]Nephrology, Hospital, Poitiers, France; [3]Immunology, Hospital, Limoges, France.*

Background: Randall-type heavy chain deposition disease (HCDD) is a rare disorder characterized by glomerular and peritubular amorphous deposits of a truncated monoclonal immunoglobulin (Ig) heavy chain (HC) bearing a deletion of the first constant domain (CH1).However, the natural history and pathophysiological mechanisms of HCDD remain poorly described. If deletion of CH1 seems to be required for secretion of an isolated HC, mutations in the variable region probably account for tissue deposition.

Methods: To better understand the mechanisms of HC deposition in kidney, we have created a transgenic mouse model of HCDD. Using gene-targeted insertion into the Ig kappa locus, we have introduced a truncated human γ1 HC extracted from a patient with HCDD and added a CH1 domain flanked by two loxP sites. This KI design allowed the expression in plasma cells of a complete human γ1 HC that can be deleted of the CH1 domain upon CRE-mediated recombination.

Results: The complete HC is well produced in mice in association with murine light chains (LC) (6g/L). By contrast, the germline deletion of the CH1 domain induces a dramatic decrease of the human HC in sera (0.3g/L). Despite this low level of truncated HC, histological studies reveal significant peritubular and glomerular basement membrane depositions that are absent in mice expressing the complete human HC. These results demonstrate that the glomerular damages in HCDD rely on the production of an isolated truncated HC which, in absence of a LC partner, display a high propensity to aggregate even at very low concentration. Treatment of mice with proteasome inhibitors results in a strong decrease of renal deposits.

Conclusions: Further studies carried out on this model, including the conditional CRE-mediated deletion of the CH1 domain and a precise follow-up of renal function during the evolution of the pathology, will certainly shed lights on the physiopathological mechanisms of HCDD and provide new therapeutic perspectives.

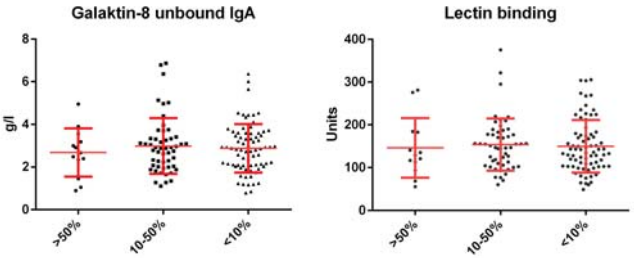
TH-PO370

Several Different Glycosylation Variants of IgA Are Elevated in Plasma of Patients with IgA Nephropathy But No One Seems to Correlate with Severity or Outcome Marten Segelmark,[4] Sigrid Lundberg,[1] Michael C. Carlsson,[2] Hakon Leffler,[2] Peter Pahlsson.[3] *[1]Clinical Science Intervention and Technology, Karolinska Inst, Stockholm, Sweden; [2]Laboratory Sciences, Lund Univ, Lund, Sweden; [3]Clinical and Experimental Medicine, Linköping Univ, Linköping, Sweden; [4]Medical and Health Sciences, Linköping Univ, Linköping, Sweden.*

Background: There are several glycosylation sites in the hinge region of IgA1 and plasma cells secrete a mixture of IgA1 O-glycoforms. The predictive value of plasma levels of such glycoforms remains unclear.

Methods: We analyzed IgA1 O-glycoforms in a cohort of 141 patients with IgAN and correlated the results with urinary albumin excretion at baseline and decline in GFR during follow-up (median 92.6 months; IQR 63.8-125.0). IgA1 O-glycoforms were measured by binding to human galactin-8 (Gal8) and a Helix aspersa (HA) lectin binding assay. Gal8 binds 2-3 sialyated galactosides while HA binds terminal GalNac.

Results: The two assays enabled us to separate four glycosylation variants (HA-binding, non-HA-binding, Gal8-binding, Gal8-non-binding). All variants were elevated but none correlated with proteinuria at inclusion or GFR slope during follow-up.



The figure show the plasma levels of two variants in patients with rapid progression, slow progression and in stable patients. When the relative binding to HA was compared with the relative binding to Gal8 we found no significant correlation (r=-0.06, p=0.49). A total of 80 follow-up samples from 25 patients were analyzed with the HA assay (range 0.5-16 years). Patients with stable GFR had a median change in the HA assay of 0.017 units per year compared to 0.015 units/year for patients with rapid progression (NS).

Conclusions: The glycosylation pattern of IgA1 is heterogenous but does not seem to be linked to severity and outcome. Glycosylation patterns are stable over time in both progessors and non-progressors.

Funding: Private Foundation Support

TH-PO371

Reactivity of Tn-Specific Antibodies with Galactose-Deficient IgA1 from Patients with IgA Nephropathy Qi Bian,[1,2] Zina Moldoveanu,[1] Colin Reily,[1] Stacy D. Hall,[1] Audra A. Hargett,[1] Matthew B. Renfrow,[1] Bruce A. Julian,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Div of Nephrology, Changhai Hospital, Shanghai, China.*

Background: Most patients with IgA nephropathy (IgAN) have elevated levels of IgA1 with galactose-deficient hinge-region *O*-glycans (Gd-IgA1); Gd-IgA1 plays a key role in the disease pathogenesis. Gd-IgA1 *O*-glycans with terminal *N*-acetylgalactosamine (GalNAc) are also termed Tn antigen. Detection of Tn antigen can be accomplished either with GalNAc-specific lectin from *Helix aspersa* (HAA) or with antibodies. In this study, we assessed the reactivity of a panel of monoclonal Tn-specific antibodies with serum IgA1 and with IgA1 produced by cells from IgAN patients and healthy controls.

Methods: Mouse monoclonal Tn-specific antibodies 4G5, 3E8, 5F6 and 6E5 were obtained from Drs. Schjoldager, Mandel, and Clausen (Center for Glycomics, University of Copenhagen, Copenhagen, Denmark). Antibody reactivity with IgA1secreted by cells from IgAN patients and healthy controls was assessed by immunofluorescence microscopy. Standard Gd-IgA1 proteins were analyzed by high-resolution mass spectrometry and relative abundance of hinge-region glycopeptides was determined using a label-free quantitation method. IgA1 was purified from sera by affinity chromatography and polymeric and monomeric molecular forms were isolated by size-exclusion chromatography. Binding of HAA and Tn antibodies to IgA1 preparations was determined by ELISA.

Results: Of the four tested Tn antibodies, 6E5 bound to standard Gd-IgA1 proteins in ELISA. The binding was enhanced by enzymatically removing sialic acid. Using purified serum IgA1, 6E5 bound preferentially to polymeric forms. There was a direct correlation between binding of HAA lectin and 6E5 antibody ($R^2 = 0.772$). Moreover, 6E5 antibody bound to IgA1 produced in cells from IgAN patients but not from healthy controls.

Conclusions: A Tn-specific antibody 6E5 bound a glycoform(s) of standard Gd-IgA1 proteins as well as serum polymeric IgA1. Moreover, this antibody reacted with IgA1-secreting cells from IgAN patients but not healthy controls. Thus, glycoform-specific antibodies may provide a new tool for future testing of Gd-IgA1 in IgAN.

Funding: Other NIH Support - NIGMS, Private Foundation Support

TH-PO372

Serum Galactose-Deficient IgA1 Detected by Specific Monoclonal Antibody KM55 Is Increased in IgA Nephropathy Patients Yusuke Suzuki,[1] Hitoshi Suzuki,[1] Junichi Yasutake,[1,2] Yasuhiko Tomino.[1] *[1]Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Nephrology Research Labs, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan.*

Background: Galactose-deficient IgA1 (Gd-IgA1) has been revealed to be an important factor for the onset and progression of IgA nephropathy. When measuring serum Gd-IgA1, lectin-based assay is known to be the only available method, but has some critical problems in stability and convenience. Thus we aimed to obtain Gd-IgA1 specific monoclonal antibody and establish more robust and reliable measurement method.

Methods: Rats were immunized with human Gd-IgA1 hinge region peptide to obtain Gd-IgA1 specific monoclonal antibody KM55. Enzyme linked immunosorbent assay with KM55 (KM55 ELISA) for specifically detecting serum Gd-IgA1 was consequently constructed. Serum Gd-IgA1 concentrations in human subjects were measured using KM55 ELISA assay.

Results: The measurement of serum Gd-IgA1 by KM55 ELISA revealed increased serum Gd-IgA1 concentration in IgA nephropathy compared to that in other renal diseases and non-renal diseases. In addition, the results obtained from KM55 ELISA and lectin-based assay positively correlated within the same serum samples.

Conclusions: KM55 ELISA, which provides a simple and robust measurement system, is an alternative method for determination of serum Gd-IgA1 levels in patients and is helpful for better understanding of IgA nephropathy.

Funding: Pharmaceutical Company Support - Kyowa Hakko Kirin Co., Ltd.

TH-PO373

Specificity of Monoclonal Antibodies against a Synthetic Glycopeptide, an Analogue to the Hypo-Galactosylated IgA1 Hinge Region in IgA Nephropathy Yoshiyuki Hiki,[1] Hideo Hori,[1] Koichiro Yamamoto,[2] Yoshihiro Yamamoto,[2] Nobuya Kitaguchi,[1] Yukio Yuzawa,[2] Kazuo Takahashi.[2] *[1]Dept of Health Sciences, Fujita Health Univ, Toyoake, Aichi, Japan; [2]Dept of Medicine, Fujita Health Univ, Toyoake, Aichi, Japan.*

Background: Increased levels of hypo-galactosylated IgA1 (HypoGal-IgA1) in IgA nephropathy (IgAN) have been detected using a *Helix aspersa* agglutinin (HAA) lectin ELISA. In this study, we developed monoclonal antibodies to evaluate the HypoGal-IgA1 in IgAN, aiming to gain a more consistent and reproducible assay.

Methods: As an analogue to the HypoGal-IgA1 hinge region, a 19 mer synthetic peptide with five N-acetylgalactosamine (GalNAc) residues at positions 4, 7, 9, 11 and 15 (sHGP) [VPST(GalNAc)PPT(GalNAc)PS(GalNAc)PS(GalNAc)TPPT(GalNAc) PSPS-NH2] was synthesized. Two monoclonal antibodies against sHGP (35A12(IgG3 k) and 44H8 (IgG1 k)) that reacted with human IgA and sHGP but not with synthesized hinge peptide without GalNAc were developed.Their reactivities to serum IgA from IgAN patients (n=49), patients with other forms of kidney diseases (OKD, n =48), and healthy controls (HC, n = 41) were evaluated using ELISA assays. Each of monoclonal antibodies was coated on plates and HRP labelled anti-human IgA was used as a detector. All sera were treated with neuraminidase beforehand to remove neuraminic acid on GalNAc residues in IgA1 hinge region.

Results: The binding levels of the two antibodies against serum IgA were significantly higher (all, $p < 0.0001$, Steel-Dwass non-parametric test) in IgAN patients compared to HC and OKD patients. There were no significant differences between HC and OKD groups in both antibodies.There was close correlation of IgA binding levels between 35A12 and 44H8 ($R^2 = 0.734$). The areas under the curve (AUC) calculated from the receiver operating characteristic (ROC) curves of the IgAN patients against HC and OKD of 35A12 were 0.861 and 0.741, respectively. Those of 44H8 were 0.854 (versus HC) and 0.760 (versus OKD).

Conclusions: These results indicated that the monoclonal antibodies recognized similar epitopes in HypoGal-IgA1, which is found predominantly in IgAN patients. The developed antibodies are proposed to be a clinically useful tool for IgAN screening.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO374

Urine Sediment Specific miRNAs as Biomarkers for IgA Nephropathy Derived Mainly from Urinary Erythrocyte Guangyan Cai,[1,2,3] ZhiYu Duan,[1,2,3] Ru Bu,[1,2,3] Yang Lu,[1,2,3] Kai Hou,[1,2,3] Xiang-Mei Chen.[1,2,3] *[1]Dept of Nephrology, Chinese PLA General Hospital, Beijing, China; [2]State Key Laboratory of Kidney Diseases, Beijing, China; [3]National Clinical Research Center of Kidney Diseases, Beijing, China.*

Background: IgA nephropathy (IgAN) is a kind of slowly progressive disease and biomarkers are required to reflect disease severity and progression. miRNAs in urine sediment seems to be promising candidate biomarkers.

Methods: Urine sediments from IgAN patients, primary glomerulonephritis (non-IgAN) patients and healthy controls were collected. Total miRNAs were extracted and analyzed by miRNA microarray. Urine erythrocytes in urine sediment were separated by CD235a magnetic bead and microvesicles were collected by ultracentrifugation to detect differential miRNAs sources.

Results: 214 differential miRNAs in urine sediment were screened out by miRNA microarray. Compared with normal control and disease control groups, miR-25, miR-144 and miR-486 levels were increased in IgAN. miR-486 showed a positive correlation with serum creatinine level. miR-144 and miR-486 levels were highest in Lee's grade III. miR-144 had a positive correlation with segmental sclerosis. miR-25, miR-144 and miR-486

levels were significantly higher in patients with glomerular hematuria. miRNAs levels of urinary sediment were greatly increased in the erythrocyte group (positive sorting group) compared with erythrocyte-free group (negative sorting group) by magnetic bead separation. The baseline levels of miR-25, miR-144 and miR-486 were significantly higher in erythrocytes than in tubular epithelial cells and polymorphonuclear leukocytes (major cell components in urine sediment). However, the levels of miR-144 and miR-486 in red blood cells had no difference between IgAN and control groups. The levels of miR-144 and miR-486 in microvesicles extracted from urine supernatant were higher in IgAN than control group.

Conclusions: Urine sediment miRNAs can be used as promising non-invasive biomarkers of IgAN. miR-25, miR-144 and miR-486 in urine sediment mainly comes from urine erythrocytes. Urine erythrocytes may play a biological role through releasing microvesicles.

Funding: Government Support - Non-U.S.

TH-PO375

Morphological and Qualitative Alterations of Glomerular Basement Membrane in IgA Nephropathy Yukinari Masuda, Kiyotaka Nagahama, Akira Shimizu. *Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

Background: The glomerulus contains well-developed capillaries, which are at risk of injury due to high hydrostatic pressure, hyperfiltration, hypertension and inflammation. However, the pathological alterations of the injured glomerular basement membrane (GBM), are still uncertain in cases of glomerulonephritis. In the present study, we examined the morphological and qualitative alterations of the injured GBM in IgA nephropathy, in addition to the routine observation by transmission electron microscopy (TEM).

Methods: We examined the alterations of the GBM in 50 renal biopsy cases with IgA nephropathy using double immunostaining for the α2(IV) and α5(IV) chains of type IV collagen, and examining the ultrastructural alterations by TEM, and a new technique, low-vacuum scanning electron microscopy (LV-SEM), which can evaluate three dimensional surface of GBM. GBM alterations in IgA nephropathy were compared with cases with normal control.

Results: In normal control cases, an intact morphology of GBM was recognized with linear strong expression of α5(IV), but almost no expression of α2(IV), and smooth and regular appearance of their surface. By contrast, the GBM of IgA nephropathy cases showed various morphological and qualitative alterations. In the TEM findings, thinning, gaps, rupture, thickening with a lamellar and reticular structure and double contours were detected in the GBM. Double immunostaining for α5(IV) and α2(IV) showed thickening of the GBM with reduced α5(IV) and increased α2(IV), or mosaic images of α5(IV) and α2(IV), and multiple holes, fractures, spiny projections and rupture of α5(IV) in the GBM. In addition, LV-SEM showed an etched image and multiple holes in a widening and wavy GBM. These findings might be associated with the development of a brittle GBM in IgA nephropathy.

Conclusions: GBM alterations were frequently noted in IgA nephropathy, and were easily evaluated by double immunostaining for α2(IV) and α5(IV) of type IV collagen and LV-SEM. The evaluation of GBM alterations may enhance our understanding of the pathophysiology of the progression of glomerular diseases.

TH-PO376

Expression of Prorenin Receptor in Renal Biopsies from Patients with IgA Nephropathy Nagisa Miyazaki, Ichijiro Murata, Genzou Takemura, Jun Matsumoto-Miyazaki, Gakuro Yoshida, Kaori Niimi, Ayuko Nishiwaki, Shinya Minatoguchi. *Second Dept of Internal Medicine, Gifu Univ Graduate School of Medicine, Gifu, Japan.*

Background: Prorenin receptor (PRR) is critically involved in the tissue renin-angiotensin system, and plays a key role in the development of organ damage in various renal disease. In Asia, immunoglobulin A (IgA) nephropathy is the most common form of primary glomerulonephropathy, but information is entirely lacking on the possible involvement of PRR in IgA nephropathy. In the present study, we assessed PRR levels in renal biopsy specimens from patients with IgA nephropathy and evaluated its relevance to the clinical and pathological features of the disease. We also examined the relevance of PRR expression to autophagy, as PRR is known to be an integral component of vacuolar-type H^+-ATPase which is essential for activation of the autophagic process.

Methods: We retrospectively reviewed the medical records of 48 patients with biopsy-proven IgA nephropathy diagnosed between February 2010 and January 2013. Paraffin sections stained with hematoxylin-eosin, PAS, Masson's trichrome, PAM and Sirius red were examined using a light microscope. Immunohistochemistry and immunofluorescence with a primary antibody against PRR and LC3, and immunoelectron microscopy for PRR were also performed. We assessed PRR levels in those specimens and evaluated its relevance to the clinical and pathological features of the disease.

Results: PRR was immunohistochemically localized on proximal tubular cells. The PRR positive area (%PRR area) correlated with daily urinary protein, which is known to reflect disease severity (r=0.286, p=0.049). PRR levels were weaker in tubular cells bordering areas of severe interstitial fibrosis. We also noted a positive correlation between autophagy activation and PRR levels in the same proximal tubular cells under a confocal microscope.

Conclusions: Our findings suggest renal expression of PRR in IgA nephropathy may be a compensatory response slowing disease progression by preventing tubular cell death and subsequent fibrosis through activation of cytoprotective autophagic machinery.

TH-PO377

Association of Serum C3 and Mesangial C3 Deposition with IgA Nephropathy Xiaoyan Zhang, Jingyuan Xie, Weiming Wang, Xiaoxia Pan, Zhaohui Wang, Jing Xu, Pingyan Shen, Nan Chen. *Dept of Nephrology, Ruijin Hospital, Shanghai Jiaotong Univ, School of Medicine, Shanghai, China.*

Background: Recent studies indicated that complement activation is involved in the pathogenesis of IgA nephropathy(IgAN).The aim of this study is to investigate association between complement activation and severity and progression of IgAN.

Methods: 528 biopsy-proven IgAN patients were retrospectively recruited. Clinical,histological and progression data were recorded.Serum levels and renal depositions of factors related to alternative complement pathway were measured.Low C3 level was defined as serum C3 less than 85mg/dl.

Results: Severe mesangial C3 deposition was detected in 245 (46.4%) patients and low C3 level was found in 93 (21.7%) patients.When renal biopsy was taken,patients with more severe C3 deposition in kidney had higher serum levels of Cr,uric acid and IgA,lower levels of eGFR and BMI,more severe endocapillary hypercellularity,tubular atrophy and interstitial fibrosis.Patients with lower serum C3 level had lower white blood cell count (WBC),hemoglobin,triglyceride, cholesterol,eGFR and higher serum Cr.Negative correlation was found between mesangial C3 deposition and serum C3.Patients with lower serum C3 had a higher risk to develop to ESRD. Multivariate Cox regression analysis showed low C3 level was an independent risk factor for ESRD(HR=1.03,95%CI 1.04-1.01,p<0.001)after adjusted by sex,age and clinical indicators.By ELISA, we found IgAN patients with lower serum C3 had higher serum levels of C3a,SC5b-9 and lower levels of C4,CFH,CFI,CFB.Interestingly, patients with lower serum CFH had higher serum levels of C3a and SC5b-9 and stronger C3 deposition in mesangial area.Serum CFH was negatively correlated with complement activation products such as serum C5b-9(r=0.-254,P<0.05) and C3a(r=-0.239,P=0.05) in addition to positively correlated with CFB(r=0.375,P<0.01).

Conclusions: Complement activation is associated with clinical and histological severities and renal progression in IgAN patients.Alternative pathway activation with CFH as a key regulator is demonstrated in IgAN patients with low serum C3 level.

TH-PO378

Validation of the Japanese Histologic Classification 2013 of Immunoglobulin A Nephropathy for Prediction of Long-Term Prognosis in a Japanese Single-Center Cohort Ryuta Sato,[1] Hideki Wakui,[1] Kensuke Joh,[2] Atsushi Komatsuda.[1] *[1]Hematology, Nephrology, and Rheumatology, Akita Univ Graduate School of Medicine, Akita, Japan; [2]Pathology, Tohoku Univ Graduate School of Medicine, Sendai, Japan.*

Background: The Japanese histologic classification (JHC) 2013 of immunoglobulin A nephropathy (IgAN) for prediction of long-term prognosis was newly proposed by Kawamura T et al (J Nephrol 2013). The glomerular lesion percentage score (GLPS) [number of glomeruli with cellular crescents, fibrocellular crescents, global sclerosis, segmental sclerosis, or fibrous crescents / number of total obtained glomeruli × 100 (%)] was assessed in the patients with IgAN and categorized into histologic grades (HGs) of HG1 (<25%), HG2 (25-49%), HG3 (50-74%), and HG4 (≥75%) in the JHC 2013. The aim of our study was to validate the correlation between the JHC 2013 system based on GLPS (HGs) and long-term prognosis in a Japanese single-center retrospective cohort.

Methods: 198 adult patients with IgAN were collected from 1980 to 2001 in Akita University Hospital. Renal biopsy specimens and clinical findings such as blood pressure, urinary protein, estimated glomerular filtration rate (eGFR), and steroid therapy were obtained. GLPS was assessed and categorized into HG1, HG2, and HG3/4 (≥50%) because only 9 patients had a HG4 (≥75%). Disease progression (50% eGFR decline or introduction of dialysis) was defined as a first endpoint. The risk of disease progression among three HGs and The association of GLPS (per 10%) with disease progression within 10 years after biopsy were examined by logistic regression analysis adjusted by clinical findings.

Results: Median follow up period was 12 years. Median age was 42 y/o (18-73). Disease progression occurred in 12.8% (12/94) of HG1 patients, 32.3% (21/65) of HG2 patients, and 46.2% (18/39) of HG3/4 patients. The risk of disease progression was significantly higher in the HG2 and HG3/4 groups than in the HG1 group (OR 3.3, P=0.004, and OR 5.9, P=0.0001 versus 1). A higher GLPS was significantly associated with disease progression (OR 1.3, P=0.004 in GLPS per 10%).

Conclusions: The JHC 2013 system was well correlated with long-term prognosis in our cohort of Japanese adult patients with IgAN.

TH-PO379

Oxford and Electron Microscopy Based Classifications in IgA Nephropathy Byoung Geun Han, Youngsub Kim, Jae Seok Kim, Jae Won Yang, Seung-Ok Choi. *Div of Nephrology, Dept of Internal Medicine, Yonsei Univ Wonju College of Medicine, Wonju, Republic of Korea.*

Background: The oxford classification that was recently introduced is believed to improve the prediction of prognosis in IgA nephropathy. This study aims to investigate the relationship of the oxford classification and clinical prognostic factors (age, sex, proteinuria, creatinine) and whether this classification could predict renal survival. In addition, we developed a new classification that is based on electron microscopic findings and investigated the usefulness of it.

Methods: The subjects included 259 patients with IgA nephropathy which was proven by renal biopsy. We reclassified tissue samples by oxford classification. We applied the new

classification based on the electron microscopic findings (foot process fusion, glomerular basement membrane thickness and electron dense deposit site). We investigated the relationships of two classifications and clinical prognostic factors.

Results: The results showed that M variable of oxford classification was related to serum creatinine (M0 / M1, 0.9±0.5 / 1.1±0.6 mg/dL, p=0.007), S variable to urinary protein creatinine ratio (S0 / S1, 1.1±2.1 / 1.9±1.8 mg/g, p=0.012), T variable to age (T0 / T1 / T2, 31±14 / 44±16 / 29±10 years, p<0.001), sex (T0 / T1 / T2, 110 / 20 / 2 males, p=0.015), and serum creatinine (T0 / T1 / T2, 0.6±0.3 / 1.5±0.8 / 1.9±1.4 mg/dL, p<0.001). In electron microscopy, glomerular basement membrane thickness only was related with sex (p=0.023), and others showed no significant relationship. In addition, both oxford and electron microscopy based classifications could not predict renal survival.

Conclusions: This study indicates that oxford classification can be used to predict the prognosis of IgA nephropathy. However, we believe that this classification needs a representative variable which integrates the individual variables (M,S,E,T) to predict of prognosis more easily. Electron microscopy based classification did not show any significant relationship with prognostic factors. Therefore, we believe that the light microscopy based classifications including oxford classification are superior than the electron microscopy based classification in IgA nephropathy.

TH-PO380

Does Expression of B7-1 mRNA Correlate with Proteinuria in Patients with IgA Nephropathy? Jennie Lönnbro-Widgren,[1] Peidi Liu,[2] Kerstin Ebefors,[2] Jenny C. Nystrom,[2] Borje Haraldsson.[1] *[1]Medicine, Univ of Gothenburg, Gothenburg, Sweden; [2]Neuroscience and Physiology, Univ of Gothenburg, Gothenburg, Sweden.*

Background: The glomerular filtration barrier is a highly specialized structure that allows high filtration of water but a total restriction of large molecules. Co-stimulatory protein B7-1 is normally not expressed on podocytes but when it is expressed it reduces the capacity of the podocyte to attach to surrounding matrix through β1-integrin. This leads to morphological changes of the podocytes and development of proteinuria. Expression of B7-1 has now been reported in patients with proteinuric kidney diseases but has been found difficult to stain for morphological analysis. Therefore, we investigated if expression of B7-1 mRNA is up regulated in patients with IgA Nephropathy (IgAN) and if this correlates with the degree of proteinuria.

Methods: We collected 24 IgAN patient biopsies and 47 controls from kidney donors and extracted the mRNA from the glomeruli. Microarrays were performed using Affymetrix GeneChip human genome U133 2.0+. Gene expression raw data was normalized using the Robust Multi-array Average (RMA) method. Fold-changes and p-values were processed using moderated T-Test statistics for both up and down regulated genes. The p-values were adjusted using FDR (False Discovery Rate). The analysis was performed in R. The expression of B7-1 was adjusted based on the average expression value of each sample and later compared with clinical data including U-albumin/creatinine, eGFR, s-creatinine, and disease progression rate.

Results: B7-1 mRNA was significantly up regulated compared to the control group, p<0.001. Plotting the value for B7-1 against the clinical data did not show any correlation between B7-1 mRNA expression and U-albumin/creatinine, s-creatinine, eGFR and disease progression rate.

Conclusions: Even if B7-1 mRNA expression was significantly up regulated in patients with IgAN the expression did not correlate with the degree of proteinuria. However, only three patients in this pilot study had proteinuria > 3.5 gram/24 hours. B7-1 mRNA expression in patients with heavy proteinuria needs to be further investigated, as well as the role of B7-1 in the pathogenesis of proteinuria.

TH-PO381

Primary IgA Nephropathy in Elderly Patients Wisit Cheungpasitporn,[1] Samih H. Nasr,[2] Charat Thongprayoon,[1] Michael A. Mao,[1] Qi Qian.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Anatomic Pathology, Mayo Clinic, Rochester, MN.*

Background: The objective of this study is to investigate and compare the clinicopathological features, treatment and outcomes of IgA nephropathy (IgAN) between elderly and younger adults, on which there has been no previously published data from the United States.

Methods: All native kidney biopsies with a pathological diagnosis of IgAN (n=1084) from Mayo Clinic Rochester between January 1, 1994 and December 31, 2013 were screened; relevant clinical data reviewed. Patients with secondary IgAN and Henoch-Schönlein purpura were excluded from analysis. All biopsies were classified based on the Oxford classification.

Results: A total of 45 elderly patients (age ≥65 years) with primary IgAN were identified and enrolled. 162 younger adults (age: 18-64 years) with primary IgAN were randomly selected from the same specimen collection for comparison. Elderly patients were in poorer general health as reflected by a higher Charlson Comorbidity Index (2.0 versus 0.9, p=0.007), higher pulse pressures (67 versus 47 mmHg, p<0.001), greater number of BP medications (2.3 versus 1.4, p<0.001) and more severe anemia (HGB: 10.6 versus 12.9 g/dL, p<0.001) at time of kidney biopsy. Pathologically, kidney specimens from elderly showed a higher degree of tubular atrophy and interstitial fibrosis, p=0.04, and vascular sclerosis, p<0.001, compared to the younger cohort. Treatments for IgAN (including ACE inhibitor, ARB and immunosuppressants) were similar between the two groups. Elderly IgAN patients had significantly worse renal and overall survival with more end-stage renal failure at 6 months (HR 5.1, 95% CI: 1.02-28.1, p=0.04) and lower percentage of 5-year survival (67.5 versus 91.8%, log-rank p<0.001). After adjusting for age and comorbidity differences, the harmful effects of IgAN on elderly patients' renal and overall survival remain significant, p<0.05.

Conclusions: Compare to younger adult IgAN patients, elderly IgAN patients show (1) a higher degree of baseline comorbidity, (2) higher degree of cortical scarring and vascular disease on biopsy, and (3) faster progression to ESRD and mortality (independent of age and comorbidity) despite receiving similar treatments for IgAN.

TH-PO382

IgA Nephropathy Clinical-Morphological Study Revealed Gender-Related Differences Mai Ots-Rosenberg,[1] Zivile Riispere.[2] *[1]Internal Medicine, Univ of Tartu, Tartu, Estonia; [2]Dept of Pathological Anatomy and Forensic Medicine, Univ of Tartu, Tartu, Estonia.*

Background: The Oxford classification of IgA nephropathy (IgAN) based on the MEST score (mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity, and tubular atrophy/interstitial fibrosis). The study aimed to investigate IgAN clinical-morphological correlations using MEST score and to find gender-related peculiarities in IgAN progression.

Methods: All native kidney biopsies (n=578) performed during 2001–2010 at the Tartu University Hospital were retrospectively reviewed. IgAN formed the main part (35.5%) of primary glomerulopathies (Riispere et al. Scand J Urol Nephrol, 2012). A total of 88 cases of IgAN during the 11 years were registered. To compose the patient cohort, we followed the recommendations of the International Consensus of IgAN study – the Oxford's classification of IgAN – and, thus, 73 IgAN cases were selected for the study. Baseline clinical data were collected within 3 months of the biopsy and at the end of a follow-up.

Results: Pathology findings for each biopsy were registered: 73% had diffuse mesangial hypercellularity (M1) and 32% had endocapillary hypercellularity (E1); segmental glomerulosclerosis/adhesion was found in 52% of the cases; tubular atrophy/interstitial fibrosis (T1) was present in few cases (10%); arteriosclerosis (A1 and A2) was registered in 35% of cases. The frequency of the findings was similar in males and females, except arteriosclerosis which was more frequent in male patients (P=0.004). M1, E1, S1, T1 and A2 scores were associated with the levels of MAP, eGFR and proteinuria. Patients were followed for an average of 4.1 years.

Conclusions: The main findings of the study revealed, similarly to the Oxford study, our cohort's pattern of MEST confirmed having a predictive value independent of clinical data as their higher score was linked to a worse outcome; second, contrary to the results of the whole cohort, we found gender-related differences in the mesangial hypercellularity category as one predictive value of disease progression, which was correlated to eGFR and measured as a mean rate of renal function decline.

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TH-PO383

T Cell Regulation in Children with Primary IgA Nephropathy and Henoch Schoenlein Purpura Licia Peruzzi,[1] Roberta Camilla,[1] Elisa Loiacono,[1] Alessandro Amore,[1] Luca Vergano,[1] Carla Guidi,[1] Giulio Mengozzi,[2] Rosanna Coppo,[1] Paola Puccinelli.[3] *[1]Nephrology Dialysis Transplantation, Citta della Salute e della Scienza. Regina Margherita Children's Hospital, Turin, Italy; [2]Laboratory Medicine, Citta della Salute e della Scienza, Turin, Italy.*

Background: Indoleamine 2,3-dioxygenase (IDO) catabolizes tryptophan to kynurenine (Kyn), a metabolite with immunoregulatory activity. IDO-expressing dendritic cells possess potent T cell regulatory properties. Primary IgA Nephropathy (pIgAN) and Henoch Schoenlein Purpura (HSP) are immune-mediated glomerular diseases with similarities but also differences in presentation and outcome.

Methods: We aimed at investigating IDO metabolites and regulatory T cells (Treg) in 24 children with pIgAN and 23 with HSP (3-14 years) with or without renal involvement; 25 healthy controls (HC) were investigated as well. IDO activity was assessed in sera as change in tryptophan (Trp) and its catabolic product kynurenine (Kyn), which were simultaneously determinated using an isocratic RP HPLC method with UV detection. A Kyn/Trp ratio was also calculated. In mononuclear cells (PBMC) real time PCR (Taqman) was adopted to measure mRNA levels of FoxP3mRNA and TGF-β1mRNA. Results were normalized to Abl gene and expressed as fold increase.

Results: Children with pIgAN and with HSP had significantly increased levels of the Trp metabolite Kyn (pIgAN 2.61 ± 0.72; HSP 2.31 ± 0.54 versus 2.02 ± 0.32 μmol/l; for both diseases P= 0.048 versus HC), while Trp levels were similar to controls. The ratio Kyn/Trp, expression of IDO activity, was significantly increased in pIgAN only (5.74±2.27, p=0.0004). FoxP3 mRNA and TGFβ1 mRNA transcriptional levels were significantly depressed (FoxP3 mRNA: pIgAN 0.94 ± 0.19; HSP 0.85 ± 0.07; HC 1.26 ± 0.09, for both diseases p=0.042 versus HC) (TGFβ1mRNA pIgAN pIgAN 0.85 ± 0.09; HSP 0.91 ± 0.07; HC 1.44 ±0.09, for both diseases p<0.0001).There was a significant inverse correlation between Kyn levels and TGFβ1mRNA values (P= 0.023).

Conclusions: Children with pIgAN and HSP present with a similar reduced expression of regulatory T cells transcription factors, while those with pIgAN have also a selective higher activity of IDO enzyme which regulates T cells reprogramming.

Funding: Government Support - Non-U.S.

TH-PO384

Diagnositic and Predictive Value in Progressive IgA Nephropathy by Measurement of Measurement of MCP-1 and NGAL Jianghua Chen, Pingping Ren. *Kidney Disease Center, The first Affiliated Hospital, College of Medicine, Zhejiang Univ, Hangzhou, Zhejiang Province, China.*

Background: To investigate the relationship among IgAN and the level of MCP-1 and NGAL in urine, explore the diagnostic and predictive value in progressive IgAN and the renal survival.

Methods: We examined serum samples from IgAN patients between Januray 2004 and December 2010. 173 patients were enrolled, divided into two subgroups: subgroup 1 (eGFR≥60ml/min) and subgroup 2 (eGFR≥90ml/min). MCP-1 and NGAL was measured in serum samples using a commercial human MCP-1 enzyme-linked immunosorbent assay(ELISA) kit (R&D Systems). A conventional receiver operating characteristic (ROC) curve was applied to determine the sensitivities and specificities for MCP-1, NGAL measurements for mild, moderate, severe IgAN, and for patients with progressive and nonprogressive IgAN, compared to the ROC curve measured by proteinuria, eGFR, sCr. Association was analyzed among urine MCP-1 level, urine NGAL and clinical and histopathological parameters.

Results: The level of urine MCP-1 and NGAL in IgAN were higher than that in healthy controls (P<0.05). It was significant difference among the urine MCP-1 of mild, moderate and severe IgAN in each subgroup (p<0.05). The area under ROC curve of MCP-1 was the largest, and it was significant differcence compared to that of UP, eGFR and sCr in subgroup 1 (eGFR≥60ml/min) and subgroup 2 (eGFR≥90ml/min) (p<0.01). ROC curve was constructed to determine the discriminatory power of MCP-1 and NGAL levels for diagnosis of progressive and nonprogressive IgAN. The area under ROC curve of MCP-1 was the largest, and it was significant differcence compared to that of UP, eGFR and sCr in subgroup 2 (eGFR≥90ml/min) (p<0.01).Urine MCP-1 and NGAL level had association with clinical and histopathological parameters. The level of serum RAGE, urine MCP-1 and NGAL may predict the renal survival.

Conclusions: The monitoring of MCP-1 in urine may be a new and noninvasive approach for early detecting severe IgAN and progressive IgAN, and help predict the renal survival.

Funding: Government Support - Non-U.S.

TH-PO385

Renalase Is Associated with Renal Disease Activity in Human Lupus Nephritis Chaojun Qi, Ling Wang, Shan Mou, Minfang Zhang, Qin Wang, Zhaohui Ni. *Dept of Nephrology, Renji Hospital, School of Medicine, Shanghai Jiao Tong Univ, China.*

Background: Lupus nephritis (LN) is one of the most serious complications of systemic lupus erythematosus (SLE) and accounting for significant morbidity and mortality. Renalase is a novel secretory flavo-protein which have the ability to protect kidney from ischemic and toxic injury independent of its ability to metabolize catecholamine. The current study aimed to explore the association of renalase and LN clinically and propose the preliminary mechanism of renalase in LN.

Methods: 70 LN patients who fulfilled the ACR revised classification criteria after renal biopsy and 35 healthy controls (HC) were included. The blood and renal biopsy samples were collected on the site of recruiting. 20 active LN patients who received prednisone and immunosuppressive therapy were selected and blood samples before and 6 months after therapy were collected. Serum renalase levels were detected by ELISA. Renalase and macrophage in the kidney was measured by immunohistochemistry.

Results: Serum renalase was elevated in LN patients compared with HC (72.95±35.36versus39.80±14.63 μg/ml, *P*<0.001) and it was significantly higher in active LN patients than inactive LN patients (92.19±34.07versus 53.70±24.75μg/ml, *P*<0.001). Serum renalase was positively correlated with 24hours proteinuria (r=0.435, *P*<0.001), ds-DNA(r=0.351, *P*=0.003), ESR(r=0.402, *P*=0.001), active index (AI, r=0.277, *P*<0.001) and negatively correlated with serum C3(r=-0.371, *P*=0.002) and eGFR(r=-0.694, *P*<0.001). Moreover, most of 20 active LN patients got remission and their serum renalase levels went back to lower level after immunosuppressive therapy (80.77±28.46 versus 61.79±24.90 μg/ml, P<0.05).We also found that patients with LN class V had the lowest serum renalase level in all classes. Immunohistochemistry showed that renalase had strong signal and co-localized with macrophages inside the glomeruli but not in tubules of proliferative LN.

Conclusions: Serum renalase levels were associated with disease activity and severity of LN. Serum renalase can indicate different pathological types of LN. Renal macrophages infiltration with high renalase expression may be one of the important mechanisms in the process of LN.

Funding: Government Support - Non-U.S.

TH-PO386

Evaluation of Clinical Outcomes and Renal Vascular Lesions in Proliferative Lupus Nephritis Beatriz Seves Holanda, Mariana Pin Andrade, Jonatas Dantas, Cristiane B. Dias, Luis Yu, Lectícia Jorge, Viktoria Woronik. *Div of Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil.*

Background: Few studies have analyzed the impact of renal vascular lesions(RVLs) on the outcome of proliferative lupus nephritis(pLN). The aim of this study was to evaluate the influence of RVL in the renal outcome of pLN.

Methods: A retrospective analysis was carried out on 196 SLE patients submitted to a kidney biopsy between 2000-13.Clinical and laboratory data were collected at baseline and at the end of follow up. Treatment was decided by the clinical staff based on conventional literature protocols. VRLs were defined as thrombotic microangiopathy (TMA) or arterial sclerosis (AS).

Results: Clinical features are showed in table 1.

	TMA (n=9)	AS (n=80)	No RLVs (n=106)
Age(y)	27±6	33±12*	29±10
Proteinuria(g/day)	3±3.48	4.2±3.5	4.8±5*
Anti-cardiolipin +	3(38%)	21(34%)	27(29%)
eGFR Initial(ml/min)	20.5±12*	44±28	48±33
Follow up(y)	3.5±3.7	4.6±3.7	7.2±9.9*
eGFR end(ml/min)	41.2±40.3	60.5±40.4	63.8±42
ESRD	3(38%)	16(20%)	22(21%)
eGFR<30	5(56%)	25(31%)	29(27,4%)
eGFR<60	6(66.7%)	40(50%)	43(41%)

Results are shown as median±SD or n(%). *p<0,05

TMA, AS, no VRLs groups were similar regarding complement level, ANA, anti-DNA antibody and hemoglobin. TMA was significantly associated with higher histological activity index (TMA 7.1±4.2 versus AS+No VRLs 5.4±2.4,p<0.05). Chronicity index was higher in all renal vascular lesions subgroups versus controls (TMA+AS 3.8±2.3 versus No VRLs 3±2.2, p<0.05). Finally, multiple logistic regression analysis show that TMA or AS weren't significantly associated with a worse renal outcome. Chronicity index and eGFR at baseline were the only predictors of outcome.

Conclusions: Renal vascular lesions are common in SLE patients with nephritis. The RVLs were associated with higher chronicity index and diminished renal function at baseline. These two variables were the independent determinants of poor renal function in the logistic multivariate regression. The associations between renal vascular lesions with renal outcomes were not significant, likely because of insufficient statistical power.

TH-PO387

Mannan-Binding Lectin and C1q and Their Impact on the Immunological Activity of Lupus Nephritis Zofia I. Niemir,[1] Karol Wozniczka,[1] Anna Swierzko.[2] *[1]Dept of Nephrology, Univ of Medical Sciences, Poznan, Poland; [2]Polish Academy of Sciences, Lodz, Poland.*

Background: Mannan-binding lectin (MBL) and C1q are important molecules in the immunity. Serum MBL levels correlate with the presence of low (0/0 and XA/0), intermediate (XA/XA and YA/0) or high producing (YA/YA) MBL2 genotypes. A more severe SLE course in patients deficient in MBL and C1q is implied. We compared the immunological activity of lupus nephritis (LN) in patients with different MBL2 genotypes in relation to C1q serum levels.

Methods: The study involved 57 patients with LN and 65 healthy controls (C). MBL2 genotyping on blood DNA was performed by the PCR-RFLP analysis. Serum MBL, C1q and antibodies to C1q (anti-C1q Ab), double-stranded DNA (anti-dsDNA Ab) and nucleosome (anti-Ns Ab) were determined by the enzyme-linked immunosorbent assays. The activity of SLE was measured using the SLE Disease Activity Index.

Results: In the LN group, the YA/YA, YA /XA, YA /0 or XA/XA, and 0/0 or 0/XA genotypes were carried by 38.6%, 29.8%, 21.1%, and 10.5% of patients. The matching values in the C group were 33.8%, 33.8%, 23.2%, and 9.2%. In both groups, the respective genotypes had a significant effect on serum levels of MBL. Of interest, in LN patients with the MBL-insufficient genotypes median serum C1q was significantly lower compared to those carrying the high and intermediate alleles (p=0.02). In the C group, an opposite trend was observed and subjects presenting MBL-insufficient genotypes had a significantly higher median level of C1q than the patients with corresponding genotypes did (p=0.02). In the whole group of LN patients, a negative correlation between serum levels of C1q and anti-C1q Ab was found (r=-0.38; p=0.0038). Moreover, in patients with active phase of LN (aLN) and MBL-insufficient genotypes, significant negative correlations between serum levels of MBL and anti-dsDNA Ab (r=-0.59; p=0.0251) and MBL and anti-Ns Ab (r=-0.65; p=0.0116) were noted. The highest levels of anti-dsDNA Ab, anti-Ns Ab, and anti-C1q Ab were observed in aLN patients presenting 0/0 or 0/XA genotypes.

Conclusions: Our results show that in LN MBL deficiency associates with that of C1q and they both contribute to the immunological activity of the disease.

Funding: Government Support - Non-U.S.

TH-PO388

Macrophage Differentiation During Pathogenesis of Lupus Nephritis Gregor Leonahrd Olmes, Christoph Daniel, Maike Julia Buettner, Kerstin U. Amann. *Nephropathology, Erlangen, Germany.*

Background: The role of macrophages in pathogenesis of Lupus nephritis, in particular their differentiation to a certain subtype (e.g. M1 or M2) modulating the inflammatory reaction, is unknown. Here we investigated if differentiation in M1 or M2-macrophages is dependent on the stage of Lupus nephritis and correlates with clinical parameters.

Methods: Using immunohistochemistry we analyzed renal biopsies from 73 patients with lupus nephritis (ISN/RPS classes II-V) for infiltration with M1-macrophages (iNOS/CD68 pos.), M2a (CD206/CD68 pos.), M2c (CD163/CD68) as well as FoxP3-positive regulatory T-cells. In addition, retrospective clinical parameters, i.e. blood pressure, proteinuria and serum urea were correlated with the macrophage infiltration using Pearson-test.

Results: The mean number of CD68+ macrophages was related to the diagnosed ISN/RPS class, showing the highest macrophage infiltration in biopsies from diffuse proliferative class IV and the lowest number in ISN/RPS class V. In all ISN/RPS classes we detected more CD163+/CD68+ than CD206+/CD68+ cells. Surprisingly we predominantly detected M2-macrophages independent of ISN/RPS classification in renal biopsies, while M1-macrophages played only a minor role. The majority of M2a- and M2c-macrophages were localized in the tubulointerstitium. Number of renal FoxP3+ cells correlated with CD163+ cells (r=0,785; p<0.001), whereas serum creatinine correlated positively with the number of CD68 positive (r=0.511; p<0.001) and CD206+/CD68+ macrophages in the tubulointerstitium (r=0.359; p=0.005). In addition, the number of CD163+/CD68+ cells was related to serum urea (r=0.420; p=0.01). Interestingly, the number of renal CD163+/CD68+ macrophages correlated negatively with blood pressure (r=-0.386; p=0.042). However, hypertension was positively associated with the number of CD206+/CD68+ macrophages in the glomeruli and the tubulointerstitium (r=0.338; p=0.015 and r=0.304; p=0.03).

Conclusions: M2-type macrophages are key players in lupus nephritis and macrophage subpopulations might be involved in the development of disease progressing hypertension.

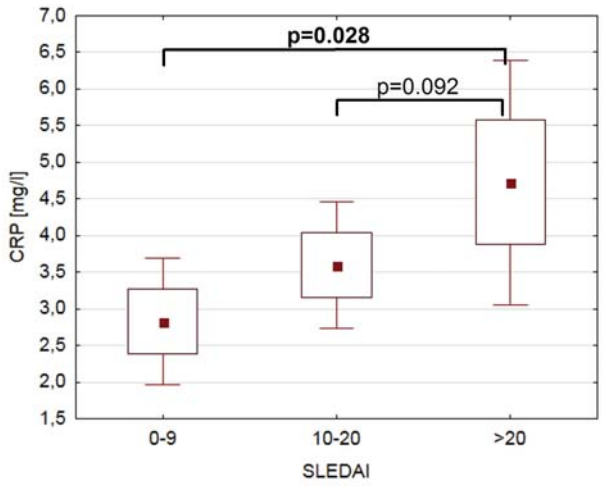
TH-PO389

C-Reactive Protein - Low or High in Systemic Lupus Erythematosus? Katarzyna Jakuszko, Magdalena Krajewska, Katarzyna Koscielska-Kasprzak, Marta Myszka, Dorota Bartoszek, Hanna Bartosik, Marian Klinger. *Dept of Nephrology and Transplantation Medicine, Wroclaw Medical Univ, Wroclaw, Poland.*

Background: Interferon alfa (IFN-α) has a role in the pathogenesis of systemic lupus erythematosus (SLE) and an inhibitory effect on the C-reactive protein (CRP) synthesis. The aim of this study was to evaluate the relationship between serum CRP and the manifestation and activity of SLE.

Methods: The study included 121 patients with lupus nephritis (LN) and 55 with non-renal SLE. A subset of 21 patients with infection (12), pleural effusion (5), pericarditis (2) and chronic synovitis (2) were excluded to eliminate the effects on the CRP levels. The levels of IFN-α, interleukin-6 (Il-6) and CRP were measured using commercially available tests. Disease activity was estimated by SLE Disease Activity Index (SLEDAI).

Results: The CRP levels were lower in patients with measurable (>1pg/ml) IFN-α levels (3.1±4.5 versus 4.6±5.4mg/l, p=0.04), however SLEDAI was significantly higher in this group (16.3±3.6 versus 12.7±7.2, p=0.0003). Moreover CRP levels were lower in patients with LN (3.3±4.7 versus 5.2±6.2mg/l, p=0.03) and hematologic disorder (3.3±4.8 versus 4.2±5.4mg/l, p=0.007) and higher in patients with arthritis (3.7±5.0 versus 2.4±4.1mg/l, p=0.007) and malar rash (3.7±5.1 versus 2.5±4.4mg/l, p=0.03). After excluding patients with measurable IFN-α levels there were significant differences in CRP levels between group with low and high SLE activity (SLEDAI 0-9: 2.8±4.2mg/l; >20: 4.7±5.3mg/l, p=0.028).



Significant correlations between CRP and SLEDAI (r=0.18, p=0.002) and Il-6 (r=0.41, p<0.001) were found.

Conclusions: The range of CRP in SLE exhibits the differentiation related to disease manifestations. The study showed the influence of IFN-α on CRP levels, a potential role and usefulness of CRP evaluation as an indicator of lupus inflammation activity in patients without nephritis and measurable IFN-α levels.

TH-PO390

Efficacy of Treatment for Class III/IV Lupus Nephritis Studied in a Historic Cohort Emilie Rijnink,[1] Ron Wolterbeek,[3] Jan A. Bruijn,[1] Marlies Reinders,[2] Ingeborg M. Bajema.[1] *[1]Pathology, Leiden Univ Medical Center, Leiden, Netherlands; [2]Nephrology, Leiden Univ Medical Center, Leiden, Netherlands; [3]Medical Statistics, Leiden Univ Medical Center, Leiden, Netherlands.*

Background: According to the current guidelines, patients with class III or IV lupus nephritis (LN) require aggressive immunosuppressive therapy including prednisolone and intravenous cyclophosphamide (ivCYC) or mycophenolate mofetil (MMF), which can cause severe side effects. However, studies on the risk of progressive renal failure in mild class III LN are limited. We aimed to answer the question whether these aggressive regimens are justified in patients with class III LN.

Methods: We identified patients with class III and IV LN biopsied at the Leiden University Medical Center between 1969 and 2009. Biopsies were rescored according to the ISN/RPS 2004 classification. Renal function was studied retrospectively during 5-years follow-up and was compared amongst 4 types of induction therapy: corticosteroids (CS) alone; CS combined with azathioprine (AZA); MMF or ivCYC.

Results: In total 22 patients were identified with LN class III and 61 patients with LN class IV. At baseline, serum creatinine, GFR, proteinuria, age and time since diagnosis of systemic lupus erythematosus did not differ significantly between treatment groups for both class III and IV LN. The occurrence of renal impairment (GFR <60mL/min), ESRD (GFR <15mL/min), and doubling of serum creatinine relative to baseline did not differ significantly between treatment groups during 5-year follow-up for class III LN (resp. p=0.66, p=0.57, and p=0.36) and for class IV LN (resp. p=0.85, p=0.92, and p=0.96).

Conclusions: This retrospective, single-center study shows no difference in renal outcome between class III and class IV lupus nephritis irrespective of the type of treatment. Our data may indicate that currently, class III and class IV lupus nephritis are over treated.

Funding: Private Foundation Support

TH-PO391

Histopathological Features of Lupus Nephritis in Patients with Positive ANCA Serology Tabitha Turner-Stokes, Massimiliano Morreale, Tom Cairns, H. Terence Cook, Charles D. Pusey, Ruth M. Tarzi, Liz Lightstone. *Imperial College Renal & Transplant Centre, United Kingdom.*

Background: The ISN/RPS classification divided class IV lupus nephritis (LN) into segmental (IV-S) and global (IV-G) subclasses. IV-S lesions tend to have more necrosis and fewer deposits, more characteristic of ANCA associated lesions found in vasculitis. ANCA are found in up to 10% of patients with SLE. The aim of this study was to determine whether Class IV-S LN was more common in ANCA-positive compared with ANCA-negative SLE patients and determine whether this was associated with other histopathological differences.

Methods: Patients with LN were identified from our renal biopsy database (1997-2013). 32/305(11%) patients who had ANCA serology at time of biopsy were MPO or PR3 antibody positive (ANCA+ve) and compared to a control group (n=83) selected randomly and confirmed to have negative serology (ANCA-ve). Biopsy reports were reviewed to determine: ISN class of LN, and presence of necrosis, crescents, endocapillary proliferation and subendothelial deposits.

Results: The ANCA were anti-MPO in 26(84%), anti-PR3 in 2(6%) and dual positive in 3(10%). Comparing the ANCA+ve with ANCA-ve group there was no significant difference in mean age or duration of LN. Histology was different however: ANCA+ve group was significantly more likely to have Class IV-S (37% versus 16%, p=0.02) and less likely to have Class V alone (10% versus 28%, p=0.04). Comparing Class III and IV LN between the 2 groups, endocapillary proliferation was more common in the ANCA+ve group (92% versus 63%, p=0.016). Despite these differences, the extent of endocapillary deposits on EM did not differ between the 2 groups (p=0.50). There was a trend towards more necrosis (36% versus 20%, p=0.11) and crescents (64% versus 42%, p=0.06) in the ANCA+ve group.

Conclusions: Our data suggest that class IV-S LN is more common in SLE patients who are ANCA+ve compared to those who are ANCA-ve, with more endocapillary proliferation and crescent formation. Interestingly there was no difference in the extent of endocapillary deposits in the 2 groups. Further study is required to determine the immunological mechanisms by which ANCA cause these histopathological differences in patients with SLE.

TH-PO392

Autoantibodies to Erythropoietin Receptor Are Related to Disease Activity in Systemic Lupus Erythematosus Junya Yamahana, Akinori Hara, Yasunori Iwata, Norihiko Sakai, Miho Shimizu, Kengo Furuichi, Takashi Wada. *Div of Nephrology, Kanazawa Univ Hospital, Kanazawa, Ishikawa, Japan.*

Background: In addition to hematopoiesis, erythropoietin (EPO) has protective effects in some injuries, such as ischemia and progressive inflammation. We have reported that anti-EPO receptor (EPOR) antibodies were related to EPO resistant anemia in some diseases. However, little is known about the involvement of anti-EPOR antibodies in progressive tissue injury. Thus, we evaluated anti-EPOR antibodies in systemic lupus erythematosus (SLE) related organ injury in this study.

Methods: Thirty-five SLE cases (32 females and 3 males, 37.5 ± 3.0 years old) were evaluated in this study. Anti-EPOR antibodies were detected by ELISA and the titer was considered as positive when the OD450 ratio of patient serum/ control serum was >1.5. Correlations between anti-EPOR antibodies, SLE disease activity index (SLEDAI), British isles lupus assessment group index (BILAG index) were evaluated. Furthermore, the relevance between clinico-pathological findings and the titer of anti-EPOR antibodies was also analyzed.

Results: Ten out of 35 patients were anti-EPOR positive. Serum levels of anti-EPOR antibodies were correlated with SLEDAI and BILAG index scores (r=0.601, 0.668, respectively, p<0.01). In the anti-EPOR antibodies positive group, the number of injured organs based on BILAG criteria was higher than those of antibodies negative group (4.6 ± 0.4 versus 2.3 ± 0.3, p<0.01). In renal pathology, activity index (r=0.99, p<0.01), cellular proliferation (r=0.82, p=0.04), leukocyte proliferation (r=0.95, p<0.01), cellular crescent (r=0.97, p<0.01) were respectively correlated with serum levels of anti-EPOR antibodies in ISN/RPS 2003 Class IV cases. In the patients with higher level of serum anti-EPOR antibodies, renal function tended to decline rapidly.

Conclusions: Serum levels of anti-EPOR antibodies were related to organ injury in SLE patients.

TH-PO393

The Prognostic Significance of IgG4 Deposition in Membranous Nephropathy and Its Impact on the Therapeutic Regimen Hala M. KFoury, Sufia Husain, Abdulkareem Alsuwaida, Mohammed A. Al-Ghonaim, Saad S. Alobaili, Jamal S. Al Wakeel. *Medicine, King Saud Univ, Riyadh, Saudi Arabia.*

Background: Antiphospholipase A2 receptor antibody(PLA2R1) is identified in most cases of primary/idiopathic membranous nephropathy (iMN) and the auto-antibody to conformational epitope of PLA2R1 is an IgG4 in the majority of cases. On the other hand, renal involvement by IgG4 associated immunological diseases usually presents either as tubulointerstitial nephritis or as membranous nephropathy. The aim of this study is to assess the prognostic value of IgG4 reactivity in a cohort of biopsy proven cases of iMN and to outline its potential in guiding therapy.

Methods: A retrospective study of biopsy proven iMN cases from January 1997 to August 2013 was undertaken. A total of 52 patients were identified and an extensive analysis of the clinical and histological parameters was performed. The primary endpoint was a worse renal outcome which was defined as doubling of serum creatinine of the baseline value.

Results: The study included 52 patients (37 males and 15 females). The mean age of the participants was 38.3 years. The mean baseline creatinine was 96.8 ± 60.2 umol/l among those with positive staining for IGg4 and 118.5 ±172.6umol/l among those with negative staining for IgG4 (P=0.5). The probabilty of doubling serum creatinine was similar among those with or without IgG4 deposition. Follow up of the patients revealed that 23.4% of those with positive IgG4 and 14% of those with negative IgG4 had deteriorationof the renal function. The prevelance and severity of tubulo-interstitial inflammation was the same in the two groups and did not have any statistical significance (P=0.9).

Conclusions: We found that there was no relationship between IgG4 positivity and the severity of clinical and/or histological parameters in patients with iMN. In addition, the IgG4 reactivity had no impact on longterm outcome of the patients, however, further studies are needed to outline the potential use of targeted therapy against IgG4 auto-antibody in a ceratin category of patients with membranous nephropathy.

Funding: Government Support - Non-U.S.

TH-PO394

Expression of PLA2R in Renal Tissues of Children and Adults with Idiopathic Membranuous Nephropathy Xiaoqing Yang, Yanjie Huang, Xia Liu. *Pediatrics, The First Affiliated Hospital of Henan Univ of Traditional Chinese Medicine, Zhengzhou, Henan, China.*

Background: To investigate the location and diagnostic value of Antiphospholipase A2 receptor (PLA2R) in the renal tissue of children and adult with idiopathic membranuous nephropathy (IMN).

Methods: 99 cases of IMN, including 16 children and 83 adults, 17 cases of secondary membranous and 15 cases of non-membranous glomerulopathy were collected between 2012 and 2013. The intrarenal PLA2R and IgG expression, colocalization of PLA2R and IgG, PLA2R and COL4α5, were examined by immunofluorescence (IF) staining, then the positive rates of PLA2R expression were compared between adult and children. The intrarenal IgG4 expression were measured by IF staining in 55 cases. The relationship of PLA2R expression with the atypical pathological features of IMN were also analysed.

Results: The strong positive staining of PLA2R were observed in outer of glomerular capillary basement membrane, its positive rate in adults and in children was respectively 89.16% and 87.50%. PLA2R and IgG colocalized along the peripheral capillary walls. The double positive rate of PLA2R and IgG4 is 90%. PLA2R expression were all negative in renal tissues of secondary membranous glomerulopathy and non-membranous glomerulopathy.

Conclusions: The renal expression of PLA2R combined with IgG4 in glomeruli can be used as a reliable marker to differeratiate IMN from secondary membranous glomerulopathy and non-membranous glomerulopathy. PLA2R is not only a target antigen in adult IMN, but also in children in this study.

Funding: Government Support - Non-U.S.

TH-PO395

Reappraisal of a PLA2R1 Immunofluorescence Study on Idiopathic Membranous Nephropathy: Influence of the Immunostaining Method and Correlation with the IgG4-Dominant/Codominant Immunophenotype Shigeo Hara,[1] Shunsuke Goto,[2] Nozomu Kamiura,[3] Akihiro Yoshimoto,[3] Motoko Yanagita,[4] Shinichi Nishi,[2] Tomoo Itoh.[1] *[1]Div of Diagnostic Pathology, Kobe Univ Graduate School of Medicine, Kobe, Japan; [2]Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan; [3]Div of Nephrology, Kobe City Medical Center General Hospital, Kobe, Japan; [4]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan.*

Background: The M-type phospholipase A2 receptor (PLA2R1) was recently identified as a specific target antigen in idiopathic membranous nephropathy (iMN). However, the influence of different sample preparation techniques in immunostaining on the results is unclear. Published evidence identified IgG4 as the dominant subclass of PLA2R1 antibody; however, it remains unclear whether the IgG subclass profiles of the glomerular immune complex of PLA2R1 positive and negative iMN are similar.

Methods: The subjects were 44 cases of biopsy-proven iMN. The PLA2R1 positivity rates of paraffin-embedded or frozen sections were compared. The comprehensive IgG subclass profile of the glomerular immune deposit was compared between PLA2R1-positve and PLA2R1-negative iMN cases.

Results: The PLA2R1 positivity rate for the paraffin-embedded sections was 58.1%, while that for the frozen sections was 54.3%, although background non-specific staining was observed for the frozen sections. In 3 cases, discrepancy was observed between the results for the paraffin-embedded and frozen sections. With regard to IgG subclass immunoprofiling, 82.3% PLA2R1-positive iMN cases demonstrated an IgG4-dominant/codominant phenotype, whereas 35.7% PLA2R1-negative iMN cases demonstrated this phenotype ($p = 0.009$).

Conclusions: PLA2R1 immunofluorescence results are not affected by the different techniques used for sample preparation, although paraffin-embedded sections merit the histological detection of PLA2R1 because of non-specific background staining in frozen sections. Higher frequency of the IgG4-dominant/codominant phenotype in PLA2R1-positive iMN cases may suggest immunologically heterogeneous subgroups in iMN.

TH-PO396

IgG Subclasses and Phospholipase A2 Receptor in Glomerular Deposits of Membranous Nephropathy: A Retrospective Analysis Hong-Rui Dong, Yan-Yan Wang, Guo-Qin Wang, Li-Jun Sun, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

Background: To investigate the differences in the IgG subclasses distribution and phospholipase A2 receptor (PLA2R) expression in glomeruli between patients with idiopathic membranous nephropathy (IMN), lupus-related MN (LN-MN) and hepatitis B-associated MN (HBV-MN).

Methods: The IgG subclasses and PLA2R in glomeruli were examined by immunofluorescence and/or immunohistochemistry staining.

Results: 77 patients with IMN, 15 with LN-MN and 8 with HBV-MN were enrolled in this study. In IMN patients, the deposition frequencies of IgG1, IgG2, IgG3 and IgG4 were 19.5%, 37.7%, 46.8% and 97.4%, respectively. The frequency of IgG4 was the highest ($P<0.01$) and its immunofluorescence intensity was also the most strong among the four IgG subclasses. The deposition frequencies of IgG1, IgG2, IgG3 and IgG4 in were 73.3%, 93.3%, 66.7% and 6.7%, respectively. The frequency of IgG4 in LN-MN was the lowest in the four IgG subclasses ($P<0.01$). The deposition frequencies of IgG1, IgG2, IgG3 and IgG4 in HBV-MN were 12.5%, 62.5%, 75.0% and 12.5%, respectively. Enhanced expression of PLA2R in glomeruli was found in 96.1% patients of IMN, but not in patients of LN-MN or HBV-MN. In 93.5% patients of IMN, IgG4 deposition and high expression of PLA2R occurred simultaneously in glomeruli.

Conclusions: In IMN patients, IgG4 is the predominant deposit in glomeruli, which is often accompanied with enhanced PLA2R expression. However, In LN-MN and HBV-MN patients, the other IgG subclasses except IgG4 were predominant deposits in glomeruli, and no enhanced PLA2R expression is found. Therefore, this examination may help to make differential diagnosis between IMN and the above two kinds of secondary MN.

Funding: Government Support - Non-U.S.

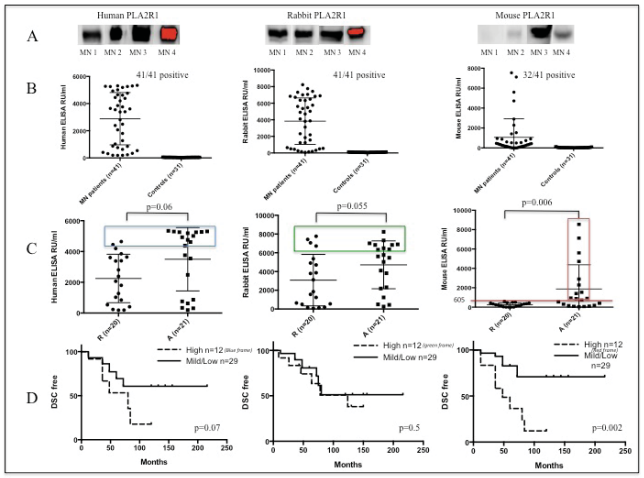
TH-PO397

A Specific Anti-PLA2R1 Mouse ELISA Can Predict Long-Term Outcome in Patients with Membranous Nephropathy Barbara Seitz-Polski,[1,2] Nicola M. Tomas,[2,3] Rolf A. Stahl,[3] Vincent L.M. Esnault,[1] Gerard J. Lambeau.[2] *[1]Nephrology, Pasteur Univ Hospital, Nice, France; [2]Inst of Molecular and Cellular Pharmacology, CNRS and Univ of Nice, Valbonne, France; [3]Nephrology, Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

Background: Membranous nephropathy (MN) is an autoimmune disease associated with autoantibodies against the phospholipase A2 receptor (PLA2R1) in 70% of cases. About 30% of MN patients progress to end-stage kidney disease (ESKD) while 30% undergo spontaneous remission. The aim of this study was to identify new prognostic factors for patients with anti-PLA2R1.

Methods: We analyzed the cross-reactivity of sera from 41 MN patients positive against human PLA2R1 on rabbit and mouse PLA2R1 orthologs by Western blotting. We designed three ELISAs using recombinant human, rabbit and mouse PLA2R1.

Results: All sera recognized equally human and rabbit PLA2R1 in Western blot analyses and ELISA, but only 32 sera (78%) were reactive against mouse PLA2R1, suggesting the presence of distinct epitopes that are differentially conserved among species. During the follow-up of 36 months, 21 patients had a persistent nephrotic syndrome and 20 patients went into remission. We analyzed the association between anti-PLA2R1 titers in the different ELISAs and the clinical outcome. The mean anti-PLA2R1 activity was significantly different between active and remission groups in the mouse ELISA in univariate and multivariate analyses (p=0.006, p=0.02 respectively) but not in human and rabbit ELISAs. A ROC curve analysis of the mouse ELISA titers defines a threshold of 605 RU/ml above which 100% of patients had poor prognosis and lower survival without doubling of serum creatinine (p=0.002). A similar threshold could not be defined for rabbit and human ELISAs.



Conclusions: Our results suggest that measuring anti-PLA2R1 titers using a mouse PLA2R1 ELISA can identify MN patients at risk for ESKD.

Funding: Government Support - Non-U.S.

TH-PO398

Glomerular Endothelial Cell Injury and Focal Segmental Glomerulosclerosis Lesion in Idiopathic Membranous Nephropathy Megumi Fukui,[1] Akiko Mii,[1] Akira Shimizu,[2] Shuichi Tsuruoka.[1] *[1]Nephrology, Nippon Medical School, Tokyo, Japan; [2]Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

Background: Focal segmental glomerulosclerosis (FSGS) lesions have often been discussed as a negative predictor in idopathic membranous nephropathy (MGN). The mechanism of the development of FSGS lesion in MGN is still uncertain.

Methods: From 250 cases of MGN, 26 cases contained FSGS lesion. We compared the clinicopathological characteristics between MGN cases with FSGS lesion (MGN+FSGS) and age, sex, and stage matched MGN cases without FSGS (MGN-FSGS).

Results: The glomerular filtration rate (eGFR) was significantly lower in MGN+FSGS cases compared to MGN-FSGS, although nephrotic syndrome, hematuria, and systolic blood pressure levels were not significantly different between the two groups. Pathologically, glomeruli in MGN+FSGS cases showed narrowing and loss of glomerular capillaries with separating from GBM or disappearance of CD34+ endothelial cells, and accumulation of extracellular matrix (ECM) in capillary walls, indicating the development of glomerular capillary injury. These findings of endothelial injury were seen even in MGN-FSGS cases, but they were more prominent in MGN+FSGS than MGN-FSGS by computer assessed morphometric analysis. In MGN+FSGS cases, 44 out of 534 glomeruli (8.2%) contained FSGS lesions (n=31, NOS lesion; n=13, perihilar lesion). Significant thickness of GBM with ECM accumulation was evident in MGN+FSGS cases. Podocyte injury with effacement of foot processes was also noted, although the expression of VEGF on podocytes was not different between the two groups, which suggests that the significant thickness of capillary walls may influence the function of VEGF from podocyte resulting in the glomerular capillary injury that contribute to the development of FSGS lesion in MGN.

Conclusions: Glomerular capillary injury was seen in all MGN cases. Furthermore, the prominent injuries of glomerular capillaries may be associated with the deterioration of eGFR and the formation of FSGS lesions in MGN.

TH-PO399

Glomerular Endothelial Cell Injury in Focal Segmental Glomerulosclerosis Megumi Fukui,[1] Akiko Mii,[1] Yukinari Masuda,[2] Akira Shimizu,[2] Shuichi Tsuruoka.[1] *[1]Nephrology, Nippon Medical School, Tokyo, Japan; [2]Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

Background: Focal segmental glomerulosclerosis (FSGS) is considered to be podocyte disease manifesting foot process effacement and nephrotic proteinuria but the mechanism of the development of segmental sclerosis in FSGS is still uncertain.

Methods: We selected idiopathic FSGS cases (n=34, male; n=22, female; 12) from a series of biopsies in our department from 1997 to 2013 and examined the clinicopathological characterizations.

Results: The average age was 45±22 (2-83), about 73.5% developed nephrotic syndrome, and mean eGFR was 51 ± 26.6 ml/min/1.73m^2. According to the Columbia classification of FSGS, frequencies were 55.9% cellular, 14.7% perihilar, 11.8% tip or not otherwise specified (NOS), and 5.9% collapsing. All FSGS cases showed endothelial cell injuries characterized by dissociation from GBM and disappearance of CD34+ endothelial cells and narrowing of the capillary lumen. Especially, tip variant showed mildest endothelial cell injury localized to tip lesion, but collapsing variant presented most severe injury in whole glomeruli with tuft collapse, corresponding to the difference in prognosis. Furthermore, by computer assessed morphometric analysis, FSGS cases had significantly smaller capillaries even in non-sclerotic glomeruli than MCD cases, despite the similar pathogenesis in terms of podocyte disease. Electron microscopy findings showed an increase of endothelial cells, loss of fenestra, and widening of the subendothelial space suggesting the endothelial cell damages. Especially in cellular variant, more severe endothelial cell injuries were seen with interposition of macrophage into the widened subendothelial space and differentiation to foam cells. On the other hand, in NOS and perihilar variant, foot processes of podocytes and fenestra of endothelial cells were relatively preserved. It may be suggested that cellular variant reflects acute phase, and NOS or perihilar variant presents chronic phase of FSGS.

Conclusions: All FSGS cases showed endothelial cell injury. Not only podocyte injury but also endothelial cell injury may contribute to develop the sclerotic lesion in FSGS.

TH-PO400

Venous Thromboembolism in ANCA Associated Vasculitis Eoin D. O Sullivan, Sarah Margaret Moran, Michael Clarkson. *Dept of Renal Medicine, Cork Univ Hospital, Cork, Ireland.*

Background: ANCA associated vasculitis (AAV) is associated with increased risk of venous thrombo-embolisim (VTE) 1. The mechanisms underlying this are not fully elucidated but increased endogenous thrombin potential as well as higher circulating factor VIII are potential contributing factors[2]. Our aim was to measure the incidence of VTE in renal AAV, and to observe whether any clinical features or therapies were associated with higher VTE rates.

Methods: Using the Cork Vasculitis Registry, we retrospectively identified 101 patients with renal AAV from 2005 to 2014. VTE events included radiologically confirmed pulmonary emboli and deep vein thrombosis. The incidence of VTE was calculated (VTE/100 person-years) and related to periods of disease activity.

Results: 100 patients were ANCA positive. The mean age at diagnosis was 65 years, and mean follow up was 50 months. VTE occured in 11 patients (11%), an incidence rate of 2.6/100 patient years. The presence of cresentic histology on renal biopsy was associated with development of VTE (p 0.026).Nearly twice as many VTE were found in patients with MPO positive disease. Odds ratio of VTE in MPO disease 2.5 (CI 0.68-9.1) We found no association between VTE rates and age, Birmingham vasculitis activity score, treatment modalities, ANCA titres or glomerular filtration rate.

Conclusions: 11% of patients with renal AAV were diagnosed with VTE. 90 % during disease activity. This has important implications for anticoagulation. Anticoagulation has been shown to be effective in ANCA positive patients despite the risk of pulmonary haemorrhage[3]. We found lower rates of VTE were observed in PR3 positive disease; previous research has demonstrated conflicting rates in this regard[4,1].

TH-PO401

Decreased Degradation of Neutrophil Extracellular Traps Is Associated with Low MPO-ANCA Affinity in MPO-ANCA Associated Vasculitis Patients with Deep Vein Thrombosis Muneharu Yamada, Takashi Oda, Taito Oshima, Yui Tsukano, Shuuhei Komatsu, Go Hirose, Tadasu Kojima, Yasuyo Sudo, Tomohiro Tomiyasu, Noriko Yoshikawa, Masaharu Yoshida. *Dept of Nephrology, Tokyo Medical Univ Hachioji Medical Center, Hachioji, Tokyo, Japan.*

Background: We have recently reported that the ability of neutrophil extracellular traps (NETs) induction correlated with the MPA disease activity as well as with the ANCA affinity to MPO (*JASN 2014*).NETs contribute to deep vein thrombosis (DVT). We therefore investigated the ability of sera from patients with MPO-ANCA associated vasculitis (MPO-AAV) to degrade NETs, and evaluated the correlation between ANCA affinity and NETs degradation in MPO-AAV patients with/without DVT.

Methods: First we measured the level of ANCA affinity to MPO in sera collected from 30 patients with MPO-AVV and 5 healthy volunteers by using the competitive inhibitory ELISA method (*Clin Exp Rheumal 2009*). Then NETs degradation activity in those sera was evaluated. Phorbol myristate acetate-induced NETs from neutrophils isolated from healthy volunteers were incubated with 10% patient sera for 6 hours. NETs degradation ability was evaluated by quantitating the DNA content in the supernatant by the use of fluorescence spectrometry assay with Picogreen.

Results: MPO-AAV patients were divided into two groups based on their ANCA affinity (high and low). Sera from MPO-AAV patients had significantly lower degradation ability than that of healthy controls (36.01±2.29 ng/ml versus 57.38±11.25 ng/ml, p<0.01). High affinity group showed higher degradation potential of NETs compared to low affinity group. DVT was associated more frequently in low affinity group than those in high affinity group. The ability to degrade NETs in patients of low affinity group with DVT was lower than those patients without DVT. Thus, low ANCA affinity group showed lower NETs degradation and more frequent DVT association.

Conclusions: These results suggest that sustained NETs existence due to decreased degradation ability in low ANCA affinity MPO-AAV patients may induce sustained inflammation and DVT.

TH-PO402

MPGN and C3 Glomerulopathies in Children: Does the Presentation and Outcome Vary Based on Histological Findings? Shefali Vyas,[1] Isabel Roberti,[1] Brittany M. Singh,[3] Anup Singh.[2] *[1]Children'S Kidney Center, SBMC, Livingston, NJ; [2]Pediatric Nephrology, SPUH, New Brunswick, NJ; [3]Virginia Tech, VI.*

Background: MPGN has a variable clinical presentation and recently the separation of C3 glomerulopathies (C3GN) has been identified.

Methods: We reviewed charts of children with idiopathic MPGN classified in the traditional categories (Type I, II or DDD and III) and reclassified them as C3GN

(non-immune mediated MPGN) or IM MPGN (immune mediated MPGN) comparing clinical presentation, therapy and outcome data. All patients received steroids and ACEi ± ARBs. Additional therapies, such as CIN, MMF or cyclophosphamide were used if no improvement was seen.

Results: A total of 22 children were studied with a follow-up time of 4 months to 15 years (median=4 yrs). Clinical data at presentation and outcome is presented in Table 1. C3 nephritic factor was done in 5 (2 negative/1 equivocal/1 + titer). Three patients with DDD had genetic testing and were all negative for Factor H and I mutations.

Characteristics	C3GN (N=8)	Immune Mediated MPGN (N=14)
Age at Presentation	5-16 yrs (median 11 yrs)	4-17 years (median 10 yrs)
Male/Female	4/4	5/9
*At presentation: aUA/NS/GH	2/5/3	1/13/5
AKI at presentation	3 (37%)	2 (14%)
Low C3/ C4	8/1	11/6
Hypertension	4 (50%)	10(71%)
** CIN/MMF/CYP	1/1/1 (37%) ***	9/3/1 (71%)
At last F/up: Remission/ Partial remission/ CKD	6/2/0	9/4/1

*aUA = asymptomatic with abnormal UA;NS= nephrotic syndrome, GH= gross hematuria
**CIN=calcineurin inhibitors, MMF= mycophenolate mofetil, CYP=cyclophosphamide
***P=0.01.

There was no difference in age, gender or ethnicity, clinical presentation and outcome among both groups. HTN was noted in 14 children (64%) - 12 stage II and 2 stage I HTN. All were normotensive on last follow-up visit. However, the group with IM MPGN required significantly more therapy including the use of 3 immunosuppressants in 3 patients. A total of 68% were in complete remission, 27% in partial at last follow up- similar among both groups. 80% of the children with AKI achieved remission with only 1 progressing to ESRD (IM MPGN).

Conclusions: C3GN and IM MPGN have excellent long term outcome if treated aggressively with steroids and immunosuppressants.

TH-PO403

Use of C4d Biomarker as a Diagnostic Tool to Classify Membranoproliferative Glomerulonephritis Nirupama Gupta, Eduardo H. Garin. *Pediatric Nephrology, Univ of Florida, Gainesville, FL.*

Background: Membranoproliferative glomerulonephritis (MPGN) in 2013 was reclassified into MPGN type I and C3 glomerulopathy (C3G) based on its pathogenesis involving the complement cascade, either the classical or alternative pathway respectively. This study aimed to evaluate whether C4d biomarker, a cleavage protein of C4, could be used as a diagnostic tool to differentiate between classical or alternative pathway activation in MPGN.

Methods: We conducted a retrospective study including adult and pediatric patients diagnosed with MPGN and minimal change disease (MCD) from January 2000 to December 2012 by using the Informatics for Integrating Biology and the Bedside software**.** Pertinent data from chart review were recorded. The formalin-fixed paraffin-embedded tissues were stained for C4d deposition using rabbit anti-human C4d polyclonal antibody via an immunoperoxidase method. Statistics were done using Mann-Whitney and chi-squared tests.

Results: Eighteen MPGN and 10 MCD patients were included in the study. The control MCD biopsies had none or minimal C4d staining. Using the 2013 MPGN classification, the MPGN group was sub-classified as: MPGN type I (8), C3G (7), and unclassified MPGN (3). Based on C4d immunohistochemical staining, 14 patients had C4d glomerular staining and four patients did not. Seven of these patients had C1q (+) (classical pathway activation) and seven had C4d (+) and C1q (-) (lectin pathway activation). Of the four C4d negative, three had evidence of alternative pathway activation and one was unknown (C4d (-), C1q (-), and C3 (-)). Of the eight MPGN 1 patients categorized by the 2013 classification, two were reclassified as C3G; of the seven C3G patients, three were reclassified as MPGN 1; and of the three unknown patients, two were reclassified as MPGN 1 after C4d staining. The lectin pathway was involved in four of the MPGN 1 and three of the C3G patients.

Conclusions: C4d stain can help differentiate between MPGN type I and C3G. This study also reports for the first time that the lectin pathway also seems to play a prominent role in the pathogenesis of MPGN in some patients.

Funding: Clinical Revenue Support

TH-PO404

Histopathologic Glomerular Damage Score Predicts Outcome in ANCA-Associated Necrotizing Glomerulonephritis Silke R. Brix,[1] Martin Busch,[2] Fedai Özcan,[3] Ulf Panzer,[1] Rolf A. Stahl,[1] Thorsten Wiech.[4] *[1]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Klinik für Innere Medizin III, Universitätsklinikum Jena, Jena, Germany; [3]Klinik für Nephrologie und Notfallmedizin, Klinikum Dortmund, Dortmund, Germany; [4]Institut für Pathologie, Sektion Nephropathologie, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.*

Background: To improve its prognostic value, a detailed analysis of kidney biopsies of 50 patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated necrotizing glomerulonephritis was performed.

Methods: 612 Glomeruli were categorized for different stages of damage, i.e. fibrinoid necrosis, cellular, fibrocellular, and fibrous crescents, and sclerosis. Crescents and glomerular sclerosis were differentiated in segmental (<50%) and global damage (>50%) assuming that glomeruli with less than 50% destruction were able to sustain residual function. A glomerular damage score was determined by counting segmental damage as 0.5 and global damage as 1.

Results: The glomerular damage score correlated with renal function during the two year follow up ($p<0.0001$, $r=0.73$). Renal outcome differed depending on the score when grouped into mild (0-25%), moderate (25-50%) and severe glomerular damage (>50%), respectively ($p<0.01$). Patients who reached end stage renal disease had a higher initial score than patients who preserved renal function ($p<0.001$). This score showed a stronger correlation to renal function than the percentage of unaffected glomeruli ($p<0.01$, $r=0.45$). Using the ANCA classification proposed in 2010, we found nine focal, 19 crescentic, 20 mixed and two sclerotic cases. These groups did not significantly differ in their renal function at two year follow up.

Conclusions: The glomerular damage score predicts renal outcome in ANCA-associated necrotizing glomerulonephritis.

Funding: Government Support - Non-U.S.

TH-PO405

Collapsing Glomerulopathy Is the Dominant Variant of Focal Segmental Glomerulosclerosis in Native Kidney Biopsies with Thrombotic Microangiopathy David Buob,[1] Mélanie Decambron,[2] Viviane Gnemmi,[3] Maxime Hoffmann,[4] Raymond Azar,[5] Christian Noel,[2] Copin Marie-Christine,[3] Francois Glowacki.[2] *[1]Pathology, Tenon Hospital, Paris, France; [2]Nephrology, CHRU de Lille, Lille, France; [3]Pathology, CHRU de Lille, Lille, France; [4]Nephrology, Hôpital Privé de La Louvière, Lille, France; [5]Nephrology, Centre Hospitalier de Dunkerque, Dunkerque, France.*

Background: Acute vaso-occlusive disease, such as thrombotic microangiopathy (TMA), is an under-recognized cause of focal segmental glomerulosclerosis (FSGS), particularly the collapsing variant. The frequency and significance of TMA-associated FSGS have not been specifically studied so far in native kidney.

Methods: Clinicopathological features were retrospectively studied in 53 patients with histologically proven TMA on native kidney biopsies. TMA cases superimposed on immune glomerulonephritis and diabetic nephropathy were excluded.

Results: Histological TMA was related to hypertensive nephropathy (n=30, 56%), drugs (n=10, 19%), complement abnormalities (n=9, 17%), antiphospholipid syndrome (n=2, 4%), and post-partum (n=2, 4%). 47% (n=25) presented with arteriolar TMA, 11% (n=6) with glomerular TMA, and 42% (n=22) with mixed TMA. Acute TMA changes were significantly associated with acute renal failure. Cases with diffuse TMA were significantly associated with severe anemia and higher arterial pressure. Among the 53 patients with histological TMA, 33 (63%) had concurrent FSGS lesions: collapsing FSGS was the dominant variant (n=17, 52% of the FSGS cases), followed by NOS (n=10, 30%), cellular (n=4, 12%), perihilar (n=1, 3%), and tip (n=1, 3%). Cases with FSGS had significantly less laboratory evidence of TMA. There was no significant association between FSGS classification and proteinuria or renal failure. Although non-significant, we noticed a trend toward an association between FSGS lesions and renal survival.

Conclusions: FSGS lesions, and in particular collapsing changes, are frequent in native kidney biopsies with TMA. Although no association was found between TMA-associated FSGS and proteinuria or evolution towards chronic renal failure, our results suggest that endothelial injury related to TMA might play an important role in the pathophysiology of FSGS.

TH-PO406

Characteristics of Renal Biopsies in Children of Low Birth Weight Kentaro Koike,[1] Nobuo Tsuboi,[1] Yohei Ikezumi,[2] Go Kanzaki,[1] Makoto Ogura,[1] Akihiko Saitoh,[2] Takashi Yokoo.[1] *[1]Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan; [2]Dept of Pediatrics, Niigata Uuniversity Medical and Dental Hospital, Niigata, Japan.*

Background: Recent autopsy studies have demonstrated much larger variability in the total number of nephrons in normal populations than previously suspected. In addition, human nephron number has been shown to be closely related to the intrauterine environment and the birth weight. We previously reported a relationship between low birth weight (LBW) and the development of focal segmental glomerulosclerosis (FSGS) accompanied by podocytopenia (Ikezumi Y et al. Am J Nephrol, 2013). We also demonstrated that a low glomerular density (GD; glomerular number per renal cortical area) in renal biopsy specimens can be a marker of future progression to renal failure in adult patients with primary glomerular disease, suggesting the significance of GD as a surrogate for the total number of nephrons.

Methods: The renal biopsy findings of LBW children were compared to those of age-matched normal birth weight (NBW) controls who had been histopathologically diagnosed with FSGS or minimal change nephritic syndrome (MCNS). The GD and glomerular volume (GV) were estimated based on measurements taken using a computed imaging analyzer.

Results: A total of 31 patients, consisting of 8 patients with LBW-FSGS, 10 patients with NBW-FSGS, and 13 patients with NBW-MCNS, were analyzed. In general, GD was significantly correlated with birth weight ($r=0.477$, $p<0.05$). In addition, GD showed significant inverse correlations to the GV($r=-0.61$, $P<0.05$). There were no significant differences in age, body mass index (BMI), or urine protein excretion at renal biopsy between the groups. Compared to the other groups, the LBW-FSGS patients had a significantly lower GD and larger GV

	LBW-FSGS	NBW-FSGS	NBW-MCNS
Birth weight (g)	944±537	3133±323†	3166±393†
Glomerular volume ($\times 10^6 \mu m^3$)	4.2±1.5	1.8±0.5†	1.5±0.4†
Glomerular density ($/mm^2$)	1.4±0.6	3.3±1.2†	3.6±1.1†

(†;p<0.05 versus LBW-FSGS).

Conclusions: LBW children show renal biopsy findings that are characteristic of a decreased number of nephrons.

TH-PO407

IgD Heavy Chain Deposition Disease: Detection by Laser Microdissection and Mass Spectrometry

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Background: Monoclonal immunoglobulin (Ig) deposition disease (MIDD) is a rare complication of monoclonal gammopathy. It is characterized by deposition of monoclonal Ig light, heavy or both heavy and light chains along the glomerular and tubular basement membranes. Most commonly, MIDD is associated with light chain deposition, in particular λ light chains. Less commonly MIDD is associated with heavy chain deposition, with γ chain being the most common heavy chain. IgD heavy chain deposition disease has not been described.

Methods: We describe a 77-year man who presented with proteinuria and renal failure. Kidney biopsy showed a nodular sclerosing glomerulonephritis with extensive focal global glomerulosclerosis and tubular atrophy and interstitial fibrosis. IF was negative for Ig deposits, while EM showed powdery deposits in the glomeruli and along tubular basement membranes. This prompted us to perform proteomic analysis to determine the composition of deposits.

Results: Laser microdissection and mass spectrometry (LMD/MS) of the glomeruli showed large spectra number for IgD.

Probability Legend: over 95% | 80% to 94% | 50% to 79% | 20% to 49% | 0% to 19%

#	Visible?	Starred?	Bio View: Identified Proteins (12)	Accession Number	Molecular Weight	Protein Grouping Ambiguity	Pre Sample blank	Sample 1	Sample 2
1	☑	☆	Ig delta chain C region	IGHD_HUM...	42 kDa			62	54
2	☑	☆	Apolipoprotein E	APOE_HUM...	36 kDa			39	41
3	☑	☆	Complement component C9	CO9_HUMAN	63 kDa			17	22
4	☑	☆	Complement C3	CO3_HUMAN	187 kDa			12	19
5	☑	☆	Complement C5	CO5_HUMAN	188 kDa			15	17
6	☑	☆	Complement component C6	CO6_HUMAN	105 kDa			14	12
7	☑	☆	Complement component C7	CO7_HUMAN	94 kDa			14	11
8	☑	☆	Apolipoprotein A-IV	APOA4_HU...	45 kDa			10	12
9	☑	☆	Complement component C8 alpha...	C08A_HUM...	65 kDa			6	9
10	☑	☆	Complement factor H-related pro...	FHR1_HUM...	38 kDa				7
11	☑	☆	Ig kappa chain C region	IGKC_HUMAN	12 kDa			5	
12	☑	☆	Ig lambda-2 chain C regions	LAC2_HUM...	11 kDa			4	

The findings were validated by immunohistochemistry for IgD that showed intense glomerular and tubular staining for IgD. Together with the kidney biopsy, the findings are consistent with IgD deposition disease. Bone marrow biopsy showed 5% plasma cells. The patient was treated with bortezomib and dexamethasone which resulted in improvement of hematological parameters but with no improvement of renal function.

Conclusions: We describe a unique case of IgD deposition disease. From the diagnostic standpoint, IgD deposition is difficult to diagnose since routine IF does not detect IgD. The diagnosis was based on proteomic analysis by LMD/MS and underscores the value of LMD/MS in further evaluating renal biopsies where routine assessment fails to reach an accurate diagnosis.

TH-PO408

Immune Complex Disease Associated with Chronic Bevacizumab Treatment of Vestibular Schwannomas in Neurofibromatosis 2 Patients

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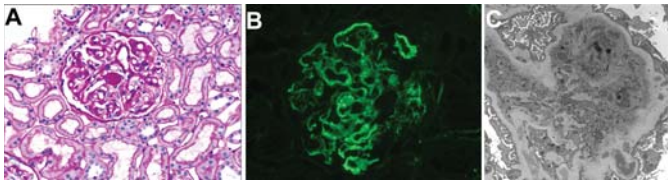
Background: Bevacizumab, a humanized monoclonal antibody to VEGF is included in treatments of many epithelial malignancies and is also used to treat hearing loss and tumor growth of vestibular schwannomas in patients with neurofibromatosis 2, NF2. Three patients developed persistent proteinuria (>2 gms).

Methods: Patients were treated every two weeks with 5 mg/kg. Patients were treated for 3, 5, or 6 years. One patient had a low C3 and C4 without cryoglobulins, rheumatoid factor and with a normal SPEP. The other two patients had normal SPEP but complements, cryoglobulins, and rheumatoid factors were not evaluated. Creatinines were normal.

Results: Kidney biopsies showed prominent intracapillary hyaline (resembling pseudothrombi), mild mesangial hypercellularity, and diffuse glomerular basement membrane (GBM) duplication (Figure 1A). By immunofluorescence, granular GBM deposits stained for IgM (2+), IgG (none), C3 (1+), IgA (trace – 1+), C1q (none), kappa and lambda (trace-1+) (Figure 1B). Ultrastructurally, prominent duplication, variable subendothelial deposits, subendothelial hyaline like deposits were present (Figure 1C). Diagnoses of mild chronic thrombotic microangiopathy and immune complex disease were made.



Conclusions: Intracapillary hyaline like plus immune deposit disease is a histologic variant of TMA associated with long term bevacizumab. The intracapillary hyaline like deposits stained poorly for immunoglobulins so that they are unlikely to be true immunoglobulin containing pseudothrombi. Long term bevacizumab treatment of NF2 patients may create risk of an immune complex disease superimposed on mild TMA.

TH-PO409

Rituximab Therapy in Patients with Primary and Secondary Glomerulonephritis: Relationship between Clinical Response and CD19+ Trend

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Background: Rituximab is an effective therapy in primary and secondary glomerulonephritis (GN). Usually it is given as a single dose (375 mg/m2) or two repeated doses of 1 g (rheumatologic regimen). Little is known for how long CD19+ depletion persists following a single administration and whether CD19+ trend may be predictive of relapses.

Methods: Retrospective analysis of 21 patients (10 M/ 11 F; mean age 58.94 ± 16.47 years), treated with Rituximab in single or multiple doses for ANCA vasculitis (3), membranous GN (8), cryoglobulinemia (6), Lupus Nephritis (3), FSGS (1) between Jun '09 and Feb '14 (median follow up 13 months, range 1 - 47.03 months).

Results: Patients received from 1 to 4 Rituximab infusions according to clinical needs and CD19+ trend over time. 4 patients received a rheumatologic dose regimen. The mean dose for each Rituximab administration was 747 ± 185 mg with a mean cumulative dose of 1305 ± 934 mg. Complete CD19+ depletion (< 5 cell/mm^3) was obtained in all the patients; CD19+ returned ≥ 5 cell/mm^3 in 16 patients after a mean of 8.72 ± 5.2 months (min 3.6, max 20.4 months). The trend of CD19+ was related to clinical manifestations in 4 out of 16 patients (5 patients were excluded for inconclusive data). Treatment response was obtained in 17 patients (4 partial remission, 13 full response).

Conclusions: Rituximab is an effective therapy in adult patients with primary and secondary GN. According to our limited experience, CD19+ depletion cannot always predict patient response to Rituximab and/or disease relapses, even if increased CD19+ levels may anticipate disease relapses in some cases.

TH-PO410

Atypical Immunofluorescence Pattern in Adult Patients with X-Linked Alport Syndrome Using Anti Type IV Collagen α1–α6 Antibody

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Background: X-linked Alport syndrome (X-AS) is caused by mutations in COL4A5, which prevents the proper production of type IV collagen (COLIV) of the basement membranes (BM). Immunofluorescence (IF) using anti COLIV α1-α6 antibodies (α1-α6) can detect these antigens to diagnose X-AS, where staining patterns for α5 are principally completely negative in male and mosaic in female on the glomerular BM (GBM), whereas the BM of Bowman capsule (BBM) is positive. Electron microscopy (EM) confirms the diagnosis. However, we encounter the cases with atypical pattern of α5 in adult patients (pts) and prompt us to compare the patterns of α5 with other subtypes of α.

Methods: We analyzed 9 pts of X-AS who were diagnosed by clinical findings, IF, and EM. The localization of IF was expressed by GBM pattern (G) and BBM pattern (B). Staining pattern included complete negative as (-), mosaic as (±), and positive as (+).

Results: α1-α4 were normal in 9 pts. α5–α6 showed normal on G and B in 4 pts (3F,1M), who were diagnosed only by EM showing basket-weave appearance. In 2 pts(1F,1M), α5 showed completely negative on BGM and mosaic on GBM, whereas α6 showed negative on BGM. The remaining 3 female pts showed typical mosaic pattern on G and B by α5 stain.

Conclusions: Some adult pts with X-AS appeared to be normal in IF and could not be diagnosed only by IF. In the other pts, phenotypic expression of α5 was atypical together with complete loss of α6 stain suggesting involvement of genotypic abnormality of α6.

		α4	α4	α5	α5	α6	α6
case No	Age/Sex	B	G	B	G	B	G
1	45F	–	+	±	±	±	–
2	43F	±	+	±	±	±	–
3	49F	–	+	+	+	+	–
4	50F	–	+	+	+	+	–
5	38F	±	+	±	±	±	–
6	38F	–	+	–	±	–	–
7	51M	–	+	–	±	–	–
8	34M	±	+	+	+	+	–
9	42F	–	+	+	+	+	–

TH-PO411

Clinicopathological Comparison Between Nodular and Diffuse Lesion in Diabetic Glomerulopathy Kensuke Joh,[1] Katsuya Ishiyama.[2] *[1]Dept of Pathology, Tohoku Univ Graduate School of Medicine, Sendai-City, Miyagi-ken, Japan; [2]Kidney Center, JCHO Sendai Hospital, Sendai-City, Miyagi-ken, Japan.*

Background: Histological subtype of diabetic glomerulopathy (DG) consists of nodular type and diffuse type. The purpose of this study is to elucidate the clinicopathological difference between these 2 subtypes in Type II diabetics undergoing renal biopsy.

Methods: Among 1077 patients (pts), 217 pts were diagnosed as diabetes mellitus (DM) and were treated more than one year. 75 pts were combined with other glomerulonephritis and were excluded. The remaining 142 pts were divided into 3 groups histologically; these were group with minor lesion in 58 pts (A), group with diffuse lesion in 55 pts (B), and group with nodular lesion in 29 pts (C). Correlation between these subgroups and clinicopathological findings was evaluated statistically.

Results: The clinical profiles were as follows: mean age; 64.2±11.4 yrs old, male in 77%, mean known duration of DM until biopsy (13.2±9.6 yrs), retinopathy in 44%, mean arterial pressure (MAP); 99±13 mmHg, HbA1c; 6.4±1.2 mg/dl, eGFR; 47±21 ml/min/1.73 m^2, amount of proteinuria 0.84 (0.2-2.4) g/d, hematuria (≥5 /hpf) in 26%. There was no difference among A, B, and C in age, eGFR, and MAP. Clinically, C showed shorter known duration of DM (A: 10.7±9.0 yrs, B: 16.7±9.9 yrs, C: 11.3±8.5 yrs, $p<0.05$), lower HbA1c (A: 6.3±0.8 %, B: 6.8±1.4%, C: 6.1±1.2%, $p<0.05$), higher amount of proteinuria (A: 0.2 (0.1, 0.6) g/d, B: 1.3 (0.5, 2.9) g/d, C: 2.7 (1.5, 4.5) g/d, $p<0.01$), and higher frequency of retinopathy (A: 14.6%, B: 51.9%, C: 75.9%, $p<0.05$) in comparison with those of B. In pathology, C showed higher frequency of mesangiolysis (MES) than that of B (A: 0%, B: 16.4%, C: 75.9%, $p<0.001$). There was no difference in extent of interstitial fibrosis and global sclerosis between B and C. In logistic regression, retinopathy (4.8 (1.8-13.0) for C, $p<0.001$ and 5.3 (2.0-14.6) for MES, $P<0.001$) and HbA1c (0.5 (0.3-0.9) for C and MES, $p<0.05$) were selected as independent predictors for C and MES among clinical parameters.

Conclusions: Nodular lesion (C) was not a subsequent lesion of diffuse lesion (B) and was produced together with MES, which was not mainly involved in the formation of B.

TH-PO412

The Significance of Tubuloreticular Inclusions (TRIs) in Allograft Kidney Biopsies Carla L. Ellis,[1] Alton Brad Farris,[1] Gaurav Gupta,[2] Lorraine C. Racusen,[3] Lois J. Arend.[3] *[1]Pathology and Laboratory Medicine, Emory Univ Hospital and School of Medicine, Atlanta, GA; [2]Nephrology, Medical College of Virginia, Richmond, VA; [3]Pathology and Laboratory Medicine, The Johns Hopkins Hospital and School of Medicine, Baltimore, MD.*

Background: Tubuloreticular inclusions (TRIs) are found in renal endothelial cells ultrastructurally. Although they can be seen in native biopsies (lupus nephritis, HIV, interferon therapy) their significance in allograft biopsies has not been fully characterized.

Methods: Renal allograft biopsies with TRIs present (from two medical centers) were selected (n=53) between the period of Mar '08 – May' 14. A control group in which TRIs were not described was identified (n=48). A descriptive analysis of patient and biopsy characteristics for the TRI group was compared with the control group for prevalence of viral infections and other clincopathologic characteristics.

Results: A sizeable proportion (11/28; 40%) of biopsies was obtained from 'high-risk' transplant recipients (ABO incompatible and/or positive-crossmatch) at one institution. 21/53 (40%) biopsies had some form of allograft rejection (cellular and/or antibody mediated rejection). Focal segmental glomerulosclerosis was the predominant cause of end-stage kidney disease (when known, n=41) in the TRI group (8/41; 20%). Only 3 patients in the TRI group had 'classic' pre-transplant associations with TRIs (two with HIV and one with lupus). In comparison with the control group (1/48; 2%), the TRI group had a significantly higher prevalence of hepatitis C infection (10/53; 19%; p=0.007), BK viremia/nephropathy (7/53; 13%) and EBV or CMV viremia (3/26; 12%).

Conclusions: Preliminary findings indicate that the presence of TRIs in renal allograft biopsies is not explained adequately by traditionally reported associations (HIV, lupus or interferon therapy). An associated viral infection (particularly hepatitis C) should be sought in renal allograft biopsies with demonstrable TRIs. Further analyses are needed to find other putative etiologies for the presence of TRIs, including the observed intriguing preponderance in 'high-risk' renal allografts.

TH-PO413

Urinary Sediment Podcalyxin (u-sed-PCX) Indicates Estimated Urinary Podocyte Number (eUPN) in Diabetic Nephropathy Masanori Hara,[1] Yohei Ikezumi.[2] *[1]Dept of Pediatrics, Yoshida Hospital, Tsubame, Niigata, Japan; [2]Dept of Pediatrics, Niigata Univ Hospital, Niigata, Japan.*

Background: Our previous studies by immunofluorescence (IF) revealed that urinary podocyte(pod) number in diabetic nephropathy is low as 0.1-1.0 cells/ml, which does not well explain for the cause for podocytopenia in diabetic nephropathy. In this study we would like to propose the term "estimated urinary podo number (eUPN)" as a better indicator for the detached podo into urine in diabetic nephropathy.

Methods: Urine samples from patients type 2 diabetes (n=71) and healthy controls (n=69) were used for IF study and urinary podocalyxin (PCX) quantification by ELISA. Urine samples were centrifuged at 12000g (to obtain whole cells, fragmented cells and plasma membranes) and the precipitate was air-dried on the slide glass, and stained by IF using the monoclonal antibody to PCX. The amount of PCX in the precipitate (u-sed-PCX) was quantified by ELISA as described previously (diabetologia, 55:2913, 2012).

Results: IF study revealed that the precipitates contained PCX positive cells (whole cells) and other PCX positive structures such as cell-fragment like and also fine granular structures in diabetic patients and the amount of non-whole cell structures is much higher than the whole cells, indicating that u-sed-PCX is originates mostly from these structures rather than whole cells. The level of u-sed-PCX in diabetic patients was significantly correlated with the amount of PCX positive structures including whole podo ($p<0.05$). The amount of u-sed-PCX was elevated in micro- and macroalbumunuric patients ($p<0.05$, respectively). The amount of PCX belonging to one podo was calculated by u-sed-PCX and the lost podo number during the course of diabetic nephropathy (Diabetes 51;3083-3089, 2002), showing 145.6 pg each. Level of u-sed-PCX (u-podo by IF) in micro- and macroalbuminuric patients was 3.7±1.0 (0.2±0.1) and 8.8±3.2 (0.4±0.2) ng/mg creatinine (cells/mg creatinine), which are equivalent to 41.7 and 60.3 cells/mg creatinine as eUPN, suggesting that eUPN is 150-200 times more than u-podo by IF.

Conclusions: U-sed-PCX might indicate eUPN and be a better indicator for detached podo in diabetic nephropathy.

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TH-PO414

Development of Abnormal Urinalysis in Mixed Connective Tissue Disease Predicts Development of Other Connective Tissue Disease Takeshi Zoshima, Ichiro Mizushima, Kazunori Yamada, Mitsuhiro Kawano. *Div of Rheumatology, Kanazawa Univ Hospital, Kanazawa, Japan.*

Background: Renal involvement occurs in 10-50% of mixed connective tissue disease (MCTD) patients (pts), most commonly membranous nephropathy (MN). It is usually mild, and corticosteroid therapy is effective. MCTD pts sometimes develop other connective tissue diseases (CTDs) such as systemic lupus erythematosus (SLE), and show severe nephropathy. Few reports have evaluated the significance of new development of abnormal urinalysis (AU) in MCTD.

Methods: We enrolled 41 Japanese MCTD pts [34 women, 7 men; mean age 53.9 years (yrs); observation period 11.7 yrs (1-34 years)] at 5 hospitals from 2000 to 2013. All pts met the Kasukawa criteria, and were observed for >1 yr after diagnosis. We defined AU as persistent proteinuria (≥1+) and/or hematuria (≥1+) by dipstick.We analyzed retrospectively the relationships between AU and clinical findings.

Results: AU developed newly in 16 pts (39%).There were no significant differences between normal urinalysis (NU) and AU group in the prevalence of anti-dsDNA antibodies (Abs) or anti-Sm Abs at diagnosis, or hypertension, diabetes mellitus, hyperlipidemia, obesity, or hyperuricemia before AU. Renal biopsy was performed in 12 AU pts, and revealed 5 cases of lupus nephritis (LN) (3 class IV, 1 IV+V, 1 V), 3 MN, and one each of necrotizing crescentic glomerulonephritis (GN), tubulointerstitial nephritis, and mesangial proliferative GN. Eight AU pts (50%) developed other CTDs [5 SLE, and 3 rheumatoid arthritis (RA)], as did 4 NU pts (16%) (2 SLE, and 2 RA) ($p=0.03$). Eight AU pts developing other CTDs were younger at diagnosis ($p<0.01$) than the other 8 AU pts. Five of these 8 pts developed SLE simultaneously with AU, while the remaining 3 did RA >1 yr later. Two of 8 AU pts not developing other CTDs manifested ANCA associated vasculitis (AAV) simultaneously with AU.

Conclusions: New AU in the course of MCTD predicts the appearance of other CTDs, especially LN or AAV. It is important to follow urinalysis frequently in MCTD, and consider the simultaneous development of other CTDs when AU is newly noted, particularly in pts diagnosed with MCTD when young.

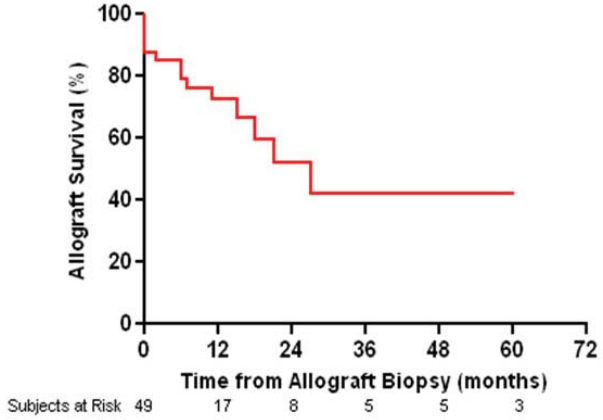
TH-PO415

Diabetic Nephropathy in Kidney Transplant Biopsies Is an Important Contributor to Allograft Failure Steven Salvatore, Sean R. Campbell, Mohamad M. Alkadi, Thangamani Muthukumar, Surya V. Seshan. *Weill Cornell Medical College.*

Background: Diabetic nephropathy (DN) is the most common cause of ESRD in the U.S. and therefore many transplant recipients have or develop diabetes. Our aim is to characterize DN in allograft biopsies and to study its role in long-term graft dysfunction.

Methods: From 2008-2014, 49 patients with kidney transplant biopsy proven DN were studied. Clinicopathologic parameters including biopsy results and outcome data were analyzed.

Results: The 49 patients ranged from 30-81 yrs (mean 57) with a male:female ratio of 32:17. The original cause of ESRD was DN (27 pts), hypertension (2), ADPKD (5), glomerulonephritis (4), FSGS (2), post-nephrectomies (1), or unknown (8). Prior to transplantation, 34 patients had known diabetes, 15 did not. Transplant biopsies were performed for elevated creatinine in 69% of patients and proteinuria 27%. The time to biopsy was mean 5.8 yrs, range 5 d to 15 yrs with average creatinine of 2.8 mg/dL (range 0.84-12.7) and proteinuria mean 2.7 (0-8.6) g/24 hrs. The type of DN was recurrent 61%, de novo 10%, donor related 4%, and unclear 24%. The DN was class I 8%, IIa 69%, IIb 8%, III 6%, and IV 8%. 21 patients (43%) had concomitant rejection: cellular 5, antibody mediated 16. Other superimposed glomerular and tubulointerstitial diseases were found in 27 patients. 30% of the patients lost their grafts in a mean 6.3 years following transplantation (range: 1 d to 15 yrs). An allograft survival curve is shown below.



Conclusions: In diabetic patients, DN is an important contributor to allograft dysfunction which may recur in transplants more rapidly than in native kidneys, or may be present in donor kidneys. Once clinically detected, biopsy proven DN in transplant leads to graft loss in approximately 50% of cases within 2 years. Earlier detection for at risk patients may be amenable for appropriate management and help prevent progression.

TH-PO416

Birmingham Vasculitis Activity Score Does Not Consistently Discriminate Active Disease from Remission in Patients with Anti-Neutrophil Cytoplasmic Autoantibody Vasculitis Elizabeth J. Brant, JulieAnne G. McGregor, William Franklin Pendergraft, Caroline J. Poulton, Susan L. Hogan, Ronald J. Falk. *UNC Kidney Center, Univ of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: The Birmingham Vasculitis Activity Score (BVAS) has been used since 1994 to characterize degree of disease activity in patients with anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis (AAV). The objective of this study was to evaluate whether BVAS, determined at single time points, accurately defines active disease versus remission in AAV.

Methods: A retrospective review of prospectively collected clinical data was performed to distinguish persistent or recurrent AAV from chronic and/or non-AAV symptoms in patients who had been given a BVAS during prior clinic visits or hospitalizations. Patients with a BVAS greater than 0 were designated 'active.' To be classified in remission, patients had to have a BVAS of 0 and no clinical evidence of active disease. Patients who had been assigned a BVAS of 0 in the setting of non-specific or mild symptoms that were believed due to AAV but that did not meet strict BVAS criteria were designated as 'active' despite the BVAS of 0, without reference to severity.

Results: A total of 630 visits from 170 patients were included in the analysis and assigned to 1 of 3 groups: 1) BVAS >0 with active disease, 2) BVAS of 0 in remission, and 3) BVAS of 0 with active disease. All 257 visits given a BVAS > 0 were determined to have active disease. Three hundred twenty-seven of 373 visits (327/373, 87.7%) with a BVAS of 0 were classified as being in remission. However, 46 (46/373, 12.3%) visits with a BVAS of 0 were determined to have active disease.

Conclusions: The BVAS is used at discrete points in time and has strict criteria, so does not consistently discriminate active disease from remission in patients with AAV, particularly in the lower range of disease activity. This may have significant implications with regard to management decisions, as well as development and analysis of research studies of patients with AAV. The BVAS should not be the sole method of classifying disease activity in patients with AAV.

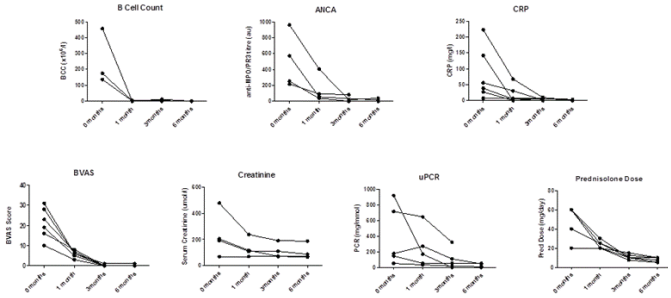
TH-PO417

Use of a Fully Humanized Anti-CD20 Monoclonal Antibody for B Cell Depletion Therapy in ANCA-Associated Vasculitis Stephen Paul McAdoo, Rachna Bedi, Megan Griffith, Ruth M. Tarzi, Charles D. Pusey, Tom Cairns. *Imperial College London and Imperial College Healthcare NHS Trust, London, United Kingdom.*

Background: Rituximab (RTX), a chimeric anti-CD20 mAb that results in B cell depletion, is an effective treatment for remission-induction in AAV. Ofatumumab is a fully humanized anti-CD20 mAb that is licensed for use in haematological malignancies, although its use in AAV has not been reported.

Methods: This is an uncontrolled open-label cohort study using ofatumumab, in addition to low-dose cyclophosphamide and corticosteroids, for remission induction in new or relapsing disease in patients with AAV.

Results: To date, 8 patients with AAV have been treated, and 6 month follow-up data is available for 7 cases: 4 male, 3 female; 3 GPA, 2 MPA, 2 CSS; 3 new, 4 relapsing. Circulating B cell depletion was achieved in all patients by 1 month and sustained at 6 months. As shown below, this was associated with reduced BVAS scores, inflammatory responses and circulating ANCA. In those with renal involvement (n=6), there was improved renal function in 5 cases. One patient with MPA, who was dialysis-dependent at presentation with renal biopsy showing 50% tubular atrophy, failed to recover renal function (excluded from graph below). One patient had a minor infusion reaction to ofatumumab.



Conclusions: This is the first report of ofatumumab use in AAV. These preliminary data suggests that treatment with ofatumumab results in B cell depletion with similar kinetics to that observed in historical patients from our centre treated with a comparable RTX-based induction regimen. This was also associated with similar clinical and serological responses. Ofatumumab may be an alternative B cell depleting agent for patients with AAV who are intolerant or unresponsive to rituximab. In addition, ofatumumab may be a most cost-effective B cell depletion therapy (cost per course in th UK ≤2548 versus ≤3492 for RTX).

TH-PO418

Rituximab for Remission Induction in Elderly Patients with ANCA Associated Vasculitis Duvuru Geetha, Homa Timlin, Rebecca Manno. *Johns Hopkins.*

Background: Advancing age is a risk factor for treatment related side effects and mortality in AAV patients treated with cyclophosphamide (CYC) and glucocorticoids (GC) for remission induction. The efficacy and safety of rituximab (RTX) in elderly AAV patients has not been well described.

Methods: We performed a single center retrospective review of 31 consecutive AAV patients aged 60 or more at the time of RTX use for remission induction. All patients received RTX with GC for remission induction. 4 patients received concomitant CYC for a mean duration of 52 days. We evaluated clinical and laboratory variables at diagnosis, rates of complete remission defined as Birmingham Vasculitis Activity Score/Wegener's Granulomatosis [BVAS/WG] = 0 and patient survival, renal survival, infections requiring hospitalization and vasculitis relapse 24 months following RTX use.

Results: Of the 31 patients, 77% were Caucasian, 68% female, mean age was 71 ± 6 years, 58% were MPO ANCA positive and 42% had relapsing disease. The mean BVAS/WG score at entry was 4.4 ±1.5, 71% had glomerulonephritis (GN) and 10% had alveolar hemorrhage. The mean baseline e- GFR was 40 ±28 ml/min/1.73 m^2. Thirty patients achieved remission with a mean time to remission of 57 ± 27 days. The single patient with refractory vasculitis responded to CYC. The mean prednisone dose at 6 months was 5.6 ± 4 mg. Remission maintenance therapy was started within 12 months of RTX induction in 6 patients (4 with RTX, 1with azathioprine and 1 with mycophenolate mofetil). One patient suffered a limited relapse 10 months post RTX use. Among the 22 patients with GN at baseline, one developed ESRD. Patient survival was 100%. There were no episodes of infusion reaction or leukopenia. There were 3 episodes of bacterial pneumonia, one episode of candida pneumonia and 1 episode of disseminated cutaneous zoster.

Conclusions: This study demonstrates that rituximab is effective for remission induction in elderly patients with AAV. Furthermore, we observed a high incidence of infectious complications. Our experience was limited by its retrospective design, and further studies are needed to evaluate the efficacy and safety of RTX in elderly AAV patients.

TH-PO419

Rituximab Treatment of ANCA-Associated Vasculitis with Severe Renal Disease Duvuru Geetha, Zdenka Hruskova, Marten Segelmark, Jonathan J. Hogan, Jessica Anne Dale, Matthew David Morgan, Lorraine Harper, Vladimir Tesar, David R.W. Jayne. *Johns Hopkins.*

Background: Rituximab (RTX) is approved for remission induction in ANCA associated vasculitis (AAV). However, data on use of RTX in patients with severe renal disease is lacking.

Methods: We conducted a retrospective multi-center study to evaluate the efficacy and safety of RTX with glucocorticoids (GC) (Group A) versus RTX + GC + concomitant cyclophosphamide (CYC) (Group B) in patients presenting with e GFR less than 20 ml/min/1.73 m^2. We evaluated outcomes of remission at 6 months (6M), renal recovery after acute dialysis at diagnosis, e-GFR rise at 6M, patient and renal survival and adverse events. Remission was defined as stabilization of serum creatinine and resolution of hematuria and absence of signs of extra-renal vasculitis.

Results: Of the 28 patients, 9 patients were in Group A and 19 were in Group B. There were no differences in age (63 versus 58, p = 0.6), baseline e GFR (12 ± 5 versus14± 4, p = 0.4), use of plasmapheresis (17% versus 60%, p = 0.2), RTX dosing regimen (375 mg/m^2 x 4 or 1000 mg x2)(44% versus 63%, p = 0.4) or median follow up days between the groups (790 versus 1118, p = 0.4). No significant differences in outcomes were observed.

	Group A (n=9)	Group B (n=19)	p-value*
Remission (%) (n=25)	100	94	1.00
Median 6 month Prednisone dose (mg) (range) (n=24)	5(0 to 10)	8(0 to 10)	0.12
Median GFR rise at 6 months (range) (n=24)	4(-1 –to 34)	4(-10 to 64)	0.28
Renal recovery %(n=10)	50 (n=2)	50 (n=3)	1.00
Infections (%) (n=21)	25	29	0.61
Leukopenia (%)	13	6	0.55
ESRD (n=28)	3	7	0.86
Death in the first 6 months (n=28)	1	2	0.96

p-value by t-test, Fisher's exact or Wilcoxon rank-sum test

Conclusions: This analysis of AAV patients with severe renal disease treated with RTX and GC demonstrates that the outcomes are equivalent to treatment with RTX + CYC + GC.

TH-PO420

A Single Center Experience of Rituximab for Remission Induction in ANCA Associated Vasculitis

Duvuru Geetha, Homa Timlin, Rebecca Manno. *Johns Hopkins.*

Background: Rituximab (RTX) is approved for remission induction in ANCA associated vasculitis (AAV). This study was undertaken to describe clinical response to RTX and evaluate safety profile.

Methods: We performed a single center retrospective review of 48 consecutive AAV patients who received RTX for remission induction(2005 to 2013). All patients received RTX with glucocorticoids. Nine patients with glomerulonephritis (GN) received concomitant CYC. We evaluated clinical and laboratory variables at diagnosis, rates of complete remission defined as Birmingham Vasculitis Activity Score/Wegener's Granulomatosis [BVAS/WG] = 0 and patient survival, renal survival, infections requiring hospitalization and vasculitis relapse 24 months following RTX use.

Results: Of the 48 patients, 81% were Caucasian, 65% male, mean age at the time of RTX use was 56 ± 22 years, 50% were PR3 ANCA positive and 46% had relapsing disease. The mean BVAS/WG score at entry was 4.4 ±1.6, 69% had GN with 8% requiring dialysis at entry and 21% had alveolar hemorrhage. The mean baseline e- GFR was 49 ±34 ml/min/1.73 m^2. Forty seven patients achieved remission with a mean time to remission of 70 ± 32 days. One patient with refractory vasculitis responded to CYC. The mean prednisone dose at 6 months was 5.0 ± 4 mg. No relapses were observed in 19 patients who received remission maintenance therapy following RTX induction whereas 6 of the 29 patients who did not receive remission maintenance therapy experienced a total of 6 relapses with median days to relapse of 319. All relapsing patients were PR3 ANCA positive. Among the 33 patients with GN at baseline, 2 developed ESRD. Patient survival was 100% at 2 years. One patient experienced an infusion reaction. There were no episodes of leukopenia. There were 3 episodes of bacterial pneumonia, one episode of candida pneumonia and 1 episode of disseminated cutaneous zoster.

Conclusions: This study demonstrates that rituximab is effective for remission induction in patients with AAV. Vasculitis relapse was common in patients who did not receive maintenance therapy. We propose that maintenance immunosuppression be considered after RTX induction therapy.

TH-PO421

Survival Outcomes of Elderly Patients Diagnosed with ANCA Associated Vasculitis

Steven Whatmough, Niamh Sweeney, Ajay Prabhakar Dhaygude. *Dept of Renal Medicine, Royal Preston Hospital, Preston, Lancashire, United Kingdom.*

Background: ANCA associated vasculitis (AAV) is a multisystem disorder with peak incidence at 60 years. Data regarding outcomes of patients older than 75 years is lacking. Mortality rates of untreated generalised AAV approaches 90% at 1 year. In this study we compared outcomes of patients with AAV presenting before and after 75 years of age.

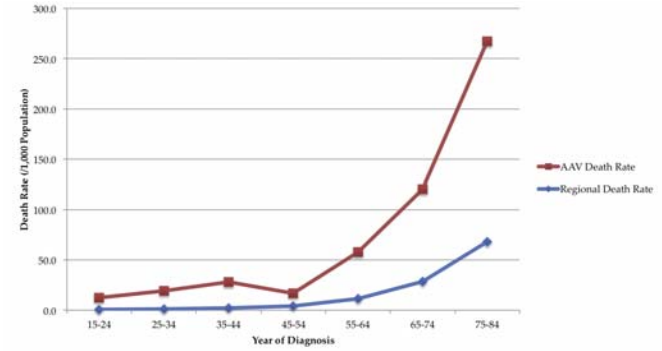
Methods: All patients with biopsy proven pauci immune glomerulonephritis (PIGN) or received cyclophosphamide (CYP) for AAV, between the years 1988 to 2010, were included in this study. Patient demographics, renal survival, patient survival and cumulative CYP dose were compared.

Results: In total 284 patients were identified. The median age at diagnosis was 58 years (range 16 to 87 years). The regional incidence was 12.5 cases per million population.

Table 1. A summary of the patient/renal survival rates and the average CYP dose.

	<75 Years (n=240)	>75 Years (n=44)	P. Value
Male:Female	137:103	25:19	0.1308
Presenting Creatinine (µmol/L)	514.90	548.62	0.6361
Mean Cumulative CYP dose (mg)	4329	2241	0.0126
Patient Survival			
Median (months)	107	45	0.0047
3 Year	72%	54%	0.0266
5 Year	63%	38%	0.0034
Renal Survival			
Median (months)	33	4	0.0738
3 Year	49%	36%	0.1002
5 Year	43%	29%	0.0770
Standardised Mortality Ratio	1106%	393%	

Figure 1. A graph comparing regional mortality rates of general population and AAV patients, by age.



Conclusions: 1. Patients who present with AAV at a younger age have improved patient survival rates.

2. There was a trend towards improved renal survival in younger patients at five years.

3. Older patients received lower CYP dose.

4. Older patients treated with CYP have improved survival compared to untreated counterparts.

Limitations - We only included biopsy proven PIGN and CYP treated patients.

TH-PO422

Improving Outcomes of ANCA Associated Vasculitis - A 22 Year Follow Up

Steven Whatmough, Niamh Sweeney, Ajay Prabhakar Dhaygude. *Dept of Renal Medicine, Royal Preston Hospital, Lancashire, United Kingdom.*

Background: ANCA associated vasculitis (AAV) is a multisystem disorder associated with end stage renal disease and mortality. Early diagnosis and advances in treatment in recent years have improved patient outcomes. A retrospective study of the outcomes of patients treated with AAV, was performed.

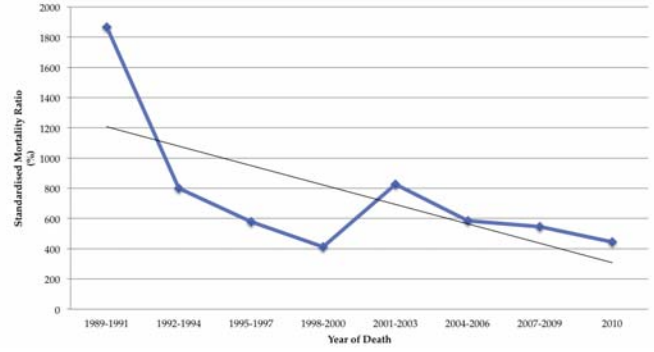
Methods: Data regarding demographics, renal and patient survival were collected for all patients diagnosed with AAV between the years 1988 and 2010. These were divided into two groups - those presenting between years 1988-1999 and 2000-2010.

Results: A total of 314 patients with AAV were identified, of which 30 were lost to follow up. The regional annual incidence was found to be 12.5 cases/million population. A summary of results is presented in Table-1.

Table 1. A table summarising the patient and renal survival rates for each era.

	1988-1999 (n=107)	2000-2010 (n=177)	P. Value
Presenting Age	57	59	0.4905
Presenting Creatinine (µmol/L)	764	427	<0.0001
Patient Survival			
Median (months)	67	125	0.0087
6 Month	79%	88%	0.0214
10 Year	35%	51%	0.0072
Median Survival + Presenting Creatinine <500µmol/L (months)	77	125	0.0536
Renal Survival			
Median (months)	9	43	0.0119
6 Month	35%	51%	0.0463
10 Year	51%	61%	0.0101
Median Survival + Presenting Creatinine <500µmol/L (months)	38.5	121	0.0404

Figure 1. A graph illustrating the standardized mortality ratio.



Conclusions: The results indicate the later cohort had;

1. Significantly lower presenting creatinine, suggesting an early diagnosis.
2. Improved short-term renal and patient survival, probably a result of protocolised treatment, early diagnosis and an improved management of the complications during the first year.
3. Improved long-term renal and patient survival, likely due to improved management of cardio-vascular morbidity.

TH-PO423

Efficacy of Oral C5aR Inhibitor CCX168 on Non-Renal Disease Activity in Patients with Antineutrophil Cytoplasmic Antibody-Associated Vasculitis

David R.W. Jayne,[1] Annette Bruchfeld,[2] Matthias Schaier,[3] Michel Y. Jadoul,[4] Marten Segelmark,[5] Daina Selga,[6] Istvan Szombati,[7] Michael Venning,[8] Christian Hugo,[9] Antonia Potarca,[10] Thomas J. Schall,[10] Pirow Bekker.[10] *[1]Univ of Cambridge, United Kingdom; [2]Karolinska Inst, Sweden; [3]Univ Hosp Heidelberg, Germany; [4]Cliniques Saint-Luc, Belgium; [5]Linkoping Univ, Sweden; [6]Lund Univ, Lund, Sweden; [7]Budaclinic, Hungary; [8]Manchester Univ, United Kingdom; [9]Dresden Univ, Germany; [10]ChemoCentryx.*

Background: A clinical trial in ANCA-associated vasculitis showed renal disease efficacy of oral 30 mg CCX168 twice daily based on eGFR, urinary ACR and MCP-1.

Methods: The purpose here was to evaluate the non-renal disease activity of CCX168 based on BVAS. 25 patients completed this blinded clinical trial; 9 received placebo+cyclophosphamide (CYC)+full dose prednisone (60 mg/day), 8 received CCX168+CYC+low dose prednisone (20 mg/day), and 8 received CCX168+CYC+no prednisone.

Results: Baseline BVAS and Week 12 results are shown in the table.

		CCX168+ CYC+ Low-Dose Steroids (N=8)	CCX168+ CYC+ No Steroids (N=8)	SOC: CYC+ High-Dose Steroids (N=9)
Baseline BVAS Score, median (range)	Total Renal Non-renal	11 (5-30) 5.5 (1-10) 3.5 (0-20)	11 (5-28) 5.5 (4-10) 6 (1-18)	9 (3-15) 5 (0-12) 4 (0-7)
BVAS Total	Response* Remission**	6 of 7 (86%) 2 of 7 (29%)	7 of 8 (88%) 2 of 8 (25%)	4 of 9 (44%) 3 of 9 (33%)
BVAS Renal	Response Remission	5 of 7 (71%) 2 of 7 (29%)	4 of 8 (50%) 2 of 8 (25%)	3 of 8 (38%) 2 of 8 (25%)
BVAS Non-Renal	Response Remission	5 of 6 (83%) 4 of 6 (67%)	7 of 8 (88%) 4 of 8 (50%)	4 of 7 (57%) 3 of 7 (43%)
BVAS Total BVAS Renal BVAS Non-Renal	Mean (SE) %Change	-71 (9)% -64 (10)% -81 (33)%	-65 (11)% -50 (15)% -83 (29)%	-26 (25)% -16 (26)% -15 (6)%
eGFR, mL/ min/1.73 m²	Mean (SE) Change	6.8 (2.1)	0.6 (3.6)	2.2 (3.3)
Urinary ACR	%Change#	-63%	-59%	-9%
Urinary MCP-1:creat	%Change#	-72%	-52%	-37%

* Remission: BVAS 0 plus prednisone dose ≤10 mg/day; ** BVAS response: BVAS decrease ≥50% plus no worsening in any body system. # Based on Week 12/Baseline ratio of geometric means.

Conclusions: Results show that CCX168 has promise in treating not only renal but also non-renal disease activity in patients with ANCA-associated vasculitis.

Funding: Pharmaceutical Company Support - ChemoCentryx, Inc.

TH-PO424

Mycophenolate Mofetil for the Treatment of Membranous Lupus Nephritis

Jason Cobb, Jose E. Navarrete, Nnaemezie Emmanuel Odioemene. *Renal Div, Emory Univ, Atlanta, GA.*

Background: Treatment options for membranous (class V) lupus nephritis (LN) includes prednisone, cyclophosphamide (CYC), and calcineurin inhibitors (CNI). Most of the research examining the treatment of pure membranous LN occurred before mycophenolate mofetil (MMF) was readily available. Post-hoc analyses from studies for the treatment of proliferative LN have compared MMF to CYC for pure membranous LN treatment. We are reporting the clinical characteristics and response rates of our pure membranous LN patients treated with MMF in comparison to other treatment options in our single center predominantly black patient population.

Methods: Retrospective chart review of LN patients from 2005-2013 in our single center academic medical practice. We excluded membranous LN with proliferative changes. We analyzed data comparing LN class V patients treated with MMF to other therapy options.

Results: From 2005-2013: 36 patients with membranous LN without proliferative changes, black race (n=35), and 35:1 female to male ratio. For all patients (mean values): initial serum creatinine (sCr) 1.1 mg/dL ± 0.9, final sCr 1.6 mg/dL ± 1.5, initial urine protein 3.3 g/24 h ± 3.2, final urine protein 1.5 g/24 h ± 2.2, initial serum albumin 2.7 g/dL ± 0.8, and final serum albumin 3.3 g/dL ± 0.9. MMF treated (n=15) and other therapies group (n=21). Comparison of MMF treated to other therapies initial sCr 0.78 mg/dL versus 1.3 mg/dL, p=.08. Comparison of MMF treated to other therapies final sCr 1 mg/dL versus 1.88 mg/dL, p =.10. MMF treated versus other therapies initial serum albumin 2.45 g/dL and 2.42 g/dL respectively, p=.94. MMF treated versus other therapies final serum albumin 3.32 g/dL and 2.97 g/dL, p=.34. Initial urine protein levels in MMF treated 3.52 g/24 h and 3 g/24 h in other therapies patients, p=0.62. Final urine protein levels in MMF treated 1.35 g/24 h and 1.25 g/24 h in others, p =.90.

Conclusions: In our Class V LN patient population we did not observe differences in final sCr, final serum albumin, and final urine protein levels in our MMF treated in comparison to other therapies (predominantly CYC, CNI, and prednisone). More data is needed but MMF seems to be a viable treatment option for pure membranous LN.

TH-PO425

Comparison of Lupus Nephritis Evolution According to the Gender in Mexican Patients

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Background: Systemic lupus erythematosus (SLE) is less frequent, has a worse prognosis and more serious kidney involvement in males. Nevertheless, evolution of lupus nephritis (LN) according to gender is still controversial. We compare the evolution of LN according to gender.

Methods: Retrospective cohort. Medical records of SLE patients with LN diagnosis (biopsy proven) between January 2005 and December 2012 were reviewed; clinical, kidney function (GFR estimated by MDRD simplified formula) and sociodemographical information were obtained during the follow–up. Histopathological findings of the kidney biopsy, LN class and patient status (live/deceased) were also recorded.

Results: 144 patients included, mean follow–up 41±14 months, 83% females, mean age 32±11 years. At follow–up, males had a tendency to higher increase in serum creatinine (Figure 1). Predictors of eGFR decline were: Education level (RR -7.21; IC95% [-12.4 to -2.0], *p* 0.007*)*, Baseline urea level (RR -0.45, IC95% [-0.76 to -0.14], *p* 0.004*)*, Baseline serum creatinine (RR -13.27, IC95% [-20.0 to -6.6], *p*<0.0001*)* and Age (RR -0.38, IC95% [-0.8 to 0.6], *p* 0.09).

Figure 1. Evolution of serum creatinine according to gender in LN patients

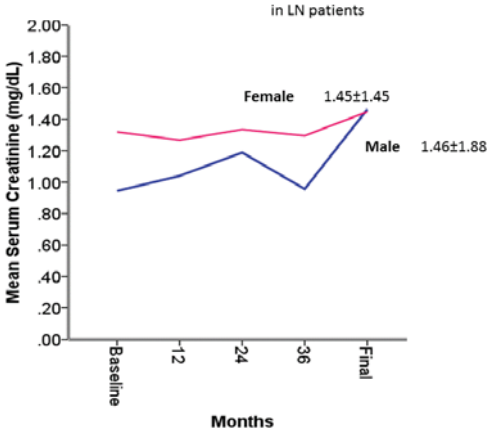

Table 1.Comparison of variables

Variable	Male, n = 25/17%		Female, n= 119/83%	
	Baseline	Final	Baseline	Final
Hypertension, N/%	8/32		42/35	
LN Classification, N/%				
Class II	3/12		20/17	
Class III	8/32		53/45	
Class IV	10/40		29/24	
Class V	4/16		17/14	
Proteinuria (mg/24h)	1500 (626-2400)	652 (124-1375)*	1800 (549-3500)†	360 (210-1200)*

†p‹0.05 vs the same evaluation in male *p‹0.05 vs baseline of the same group.

Conclusions: Males with LN had a tendency to higher increase in serum creatinine compared with females, which might be related to the greater proportion of LN Class IV in males (40% VS females 24%).

TH-PO426

Efficacy and Safety of Sirukumab in Patients with Active Lupus Nephritis: Results From a Phase 2,Multicenter, Randomized, Double-Blind, Placebo-Controlled, Proof-of-Concept Study Brad H. Rovin,[1] Ronald Van Vollenhoven,[3] Cynthia Aranow,[4] Carrie Wagner,[2] Robert Gordon,[2] Benjamin Hsu,[2] Bei Zhou.[2] *[1]Nephrology, Ohio State Univ, Columbus, OH; [2]Janssen Research and Development; [3]Karolinska Inst, Sweden; [4]Feinstein Inst.*

Background: To examine the efficacy and safety of sirukumab, an anti-interleukin-6 monoclonal antibody, in patients (pts) with active class III or IV lupus nephritis (LN).

Methods: Inclusion criteria were class III or IV LN on biopsy within 14 mo of randomization, persistent proteinuria (≥0.5 g/d) despite immunosuppression (MMF or AZA ± corticosteroids), and stable RAAS blockade. Pts were randomized to IV sirukumab 10 mg/kg (n=21) or placebo (Pbo,n=4) q4wks through wk24. The primary endpoint was the percent reduction in proteinuria from baseline. Major secondary endpoints included the proportion of pts with: 1) ≥50% reduction in proteinuria; 2) a meaningful reduction in proteinuria (P/C ratio <0.5 or if nephrotic P/C ratio <3 and ≥50% reduction in P/C ratio; 3) no worsening of glomerular filtration rate (GFR); 4) percent change from baseline in Patient's and Physician's Global Assessments of Disease Activity.

Results: Primary and secondary results are shown in Table 1. There was no consistent reduction in proteinuria in the sirukumab group. Among the 12 sirukumab-treated pts positive for anti-dsDNA at baseline, there was a mean 62.3% reduction in anti-dsDNA at wk 24. Sirukumab was discontinued in 6 patients, 5 because of an AE. Half of the sirukumab group had at least one serious AE, mostly infectious. No deaths occurred and no serious AE occurred in Pbo.

Primary and Secondary Efficacy Endpoints at Week 24	Pbo	Sirukumab
Intent-to-treat patients	4	20
Median % Change in proteinuria from baseline (95% CI)	43.4	0 (-61.8,39.6)
% patients with ≥50% decrese in proteinuria	0	20%
% patients with meaningful reduction in proteinuria	0	15%
% patients with no decline in GFR	75%	55.6%

Conclusions: Sirukumab treatment in patients with active, refractory LN did not result in a percent improvement in proteinuria, but 15-20% of treated pts did show an overall reduction. A high frequency of SAEs was observed in this immunosuppressed population.

Funding: Pharmaceutical Company Support - Janssen Research and Development

TH-PO427

Low Dose IV Cyclophosphamide (IVC) Induction Is Efficacious in Blacks with Lupus Nephritis (LN) Simon K. Winn, Andrea Cove-Smith, Catherine Susanna Vinen. *Renal Unit, Kings College Hospital, London, United Kingdom.*

Background: Based on post-hoc analysis of ALMS[1], it is suggested that MMF may be superior to IVC as induction therapy for LN in black patients[2]. Further subanalysis reveals that MMF was superior to IVC only in Hispanics, not in blacks. Moreover, ALMS compared MMF treatment with a high-dose modified NIH IVC regimen (monthly pulses of 0.5–1.0 g/m2). We report our use of the low-dose Eurolupus strategy (6 fortnightly pulses of 0.5g) to treat black LN patients in an urban centre.

Methods: Retrospective data were obtained for all African, Afro-Caribbean or black patients with biopsy-proven LN treated with IVC from our database. Treatment failure at 6mos was defined as per Eurolupus (serum creatinine (sCr) not <1.3mg/dL if baseline (B/L) 1.3-2.6mg/dL, sCr not <50% if B/L >2.6mg/dL, or no resolution of nephrotic syndrome).

Results: 20 patients given IVC for class III(2), IV(14) or V(4) LN were identified. Average age at biopsy was 30.6yrs (range 17-45); B/L sCr 1.95+/-1.2mg/dL. Other characteristics are tabulated. Average dose of IVC was 2.6g. 15 patients (75%) achieved treatment response at 6mos. Overall, sCr fell by 51% and sAlb increased by 34%. Infective complications were seen in 4 patients.

	Baseline +/- SD (Range)	Remission 6mos +/- SD (Range)	No Remission 6mos +/- SD (Range)
sCr mg/dL	1.95 +/-1.2(0.65-5.5)	0.95+/-0.3(0.7-1.5)	4.13+/-3.6(1.8-10.3)
sAlb g/dL	2.68 +/-0.8(1.2-4.2)	3.58+/-6.8(2.4-4.4)	3.40+/-0.46(2.6-3.7)
C3	0.61 +/-0.32 (0.22-1.46)	0.98+/-0.31 (0.47-1.45)	1.2+/-0.18(0.81-1.13)
dsDNA	144.2 +/-109(4.3-291)	84.23+/-98.8 (6-289)	22.0+/-8.5(16-28)
uPCR mg/mmol	424.1 +/-371(33-1230)	191+/-128(17-409)	415.3+/-191.5 (158-587)
Prednisolone dose mg	34+/-12(20-60)	12+/-7(3.75-20)	17+/-12(7.5-30)

Conclusions: Treatment response rate is comparable to studies in largely white cohorts such as the Eurolupus trial, in which 71% of low-dose IVC group achieved renal remission. Furthermore, disease severity in our cohort appears higher than in Eurolupus and ALMS trials (B/L sCr 1.1 and 1.14mg/dL, respectively). These data support the efficacy of low-dose IVC for induction therapy of class III, IV and V LN in a UK black population. 1 Rheumatology (Oxford). Jan 2010; 49(1):128–140. 2 ACR guidelines 2012.

TH-PO428

A Long-Term Follow-Up Study of Multi-Target Therapy Using a Combination of Tacrolimus and Mycophenolate Mofetil in Patients with Active Lupus Nephritis Hidekazu Ikeuchi, Keiju Hiromura, Ken Kayakabe, Kazuhiko Uchiyama, Hiroko Hamatani, Noriyuki Sakurai, Toru Sakairi, Yoriaki Kaneko, Akito Maeshima, Yoshihisa Nojima. *Dept of Medicine and Clinical Science, Gunma Univ Graduate School of Medicine, Maebashi, Gunma, Japan.*

Background: We previously reported that multi-target therapy using tacrolimus (TAC) and mycophenolate mofetil (MMF) was effective in inducing early remission and in yielding a high remission rate in patients with active class III, IV, V lupus nephritis (LN) (Mod Rheumatol, 2013). Here, we conducted a follow-up study to evaluate the long-term effect and safety of multi-target therapy.

Methods: All 16 patients in the previous study (2 men and 14 women, 34.3±8.3 years old, 9 new-onset and 7 flared) were followed-up for a median of 36 months (range, 27-53) after the initiation of multi-target therapy. Urinary protein/Cr ratio was 4.6±2.8 g/gCr and serum Cr was 0.73±0.29 mg/dl at the initiation of multi-target therapy. Eight patients had mixed membranous and proliferative LN.

Results: All patients achieved a complete remission (CR) at a median of 3.6 months (range, 0.3-14.3). CR rates at 6 and 12 months were 81% and 94%, respectively. After achieving CR, MMF was switched to azathioprine (AZA) in 13 patients and to mizoribine in 2 patients. MMF was stopped in 1 patient because of cytomegalovirus (CMV)-associated gastric ulcer and TAC+AZA was stopped in 1 patient due to abdominal cellulitis. Fourteen patients (88%) remained well without a renal flare. The mean dose of prednisolone was 4.1±2.5 mg/day at the final observation in these patients. Two patients (12%) had a renal flare. Both the patients had experienced renal flares more than 2 times before multi-target therapy. Infection was the most frequent adverse event. CMV viremia was detected in 6 patients (37.5%), which develpoed during the first 6 months. Two out of 6 patients was diagnosed with CMV infection (gastric ulcer in 1 patient and pancytopenia in 1 patient). Varicella zoster was observed in 3 patients (18.8%).

Conclusions: Although some patients experienced a renal flare or severe infection, most patients had a favorable clinical course during 2- to 4-year follow-up after the initiation of multi-target therapy.

TH-PO429

Efficacy of Rituximab in Adult Frequently Relapsing Minimal Change Disease (MCD) Catherine King, Stuart W. Smith, Peter Hewins. *Nephrology, Univ Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.*

Background: Corticosteroids are the basis of treatment for nephrotic syndrome due to minimal change disease (MCD) but 25% of patients have frequent relapses (FRNS) and 30% become steroid dependent. Prolonged use of steroids and conventional additional immunosuppressants causes significant toxicity. Rituximab (RTX) is now included in KDIGO guidelines for childhood MCD. Evidence for RTX usage in adult MCD is limited. This report describes a single-centre experience of RTX use for adult MCD.

Methods: Outcomes of all adult MCD patients treated with RTX for FRNS 2008-2012 were analyzed retrospectively using patient records. Treatment comprised 2x1g infusions.

Results: 7 MCD patients (5 male) received RTX. All patients had FRNS and 4 were steroid dependent. All had previously taken ≥2 immunosuppressants in addition to steroids. 6 patients had experienced ≥1 major treatment side effect: diabetes (n=1), hypertension (n=3), infection requiring hospitalisation (n=2), osteoporosis (n=1), weight gain (n=4) and biopsy-proven calcineurin inhibitor induced renal damage (n=3). At time of RTX treatment, 4 patients were relapsing. All entered remission post RTX. Median length of follow up was 13 months (range 4-61). After RTX, rate of relapse was reduced (Wilcoxon-signed-rank, p≤0.05). Number of additional immunosuppressants, steroid dependency and anti-hypertensive agents usage also reduced.

	Pre Rituximab	Post Rituximab
Median relapses/year	4	1
Median no. Immunosuppressants (range)	2 (1-4)	0 (0-2)
No. Patients on continued steroids	4	1
No. Patients on antihypertensive therapy	4	2

Four of 7 patients relapsed post RTX after a median of 7 months (range 1-10). All 4 relapsing patients were successfully re-treated with RTX and none developed RTX resistant nephrosis. Median number of courses of RTX per patient was 1 (range 1-4). No RTX related adverse events were observed.

Conclusions: Rituximab was effective and safe in adults with FRNS due to MCD. Median rate of relapse was significantly reduced following RTX. All but one patient remained off all other immunosuppressive medications at follow up. These data support the case for further assessment of the efficacy and safety of Rituximab in adult MCD.

TH-PO430

Long-Term Prognosis of Patients in Adults with Steroid-Dependent Minimal Change Nephrotic Syndrome Treated with a Biannual, Single-Dose Rituximab Regimen Takashi Takei, Yuko Iwabuchi, Kosaku Nitta. *Deaprtment of Medicine, Kidney Center, Tokyo Women's Medical Univ, Shinjuku-ku, Tokyo, Japan.*

Background: In recent years, several clinical trials have shown the efficacy of rituximab in patients with steroid-dependent minimal-change nephrotic syndrome (MCNS). We previously reported the efficacy and safety of a single dose of rituximab administered twice at an interval of 6 months for patients with steroid-dependent MCNS in adults (Nephrol Dial Transplant 28: 1225, 2013). The objective of this study was to evaluate prognosis after four single-dose rituximab infusions during 24-months.

Methods: We conducted a prospective cohort study with a historical control to evaluate the effect of single-dose infusions of rituximab at 375 mg/m^2 BSA per dose at intervals of 6 months during 24-months followed by continuous rituximab treatment as a maintenance therapy. Seventeen patients were continuously treated with rituximab infusion (RTX group). On the other hand, seven patients discontinued RTX administration after four single-dose infusions of rituximab (non-RTX group).

Results: In RTX group, three of seventeen patients stopped rituximab treatment after fifth infusion and two of seventeen patients stopped after sixth infusion. Complete remission was maintained in all the seventeen patients for a 12-month period after four single-dose rituximab infusion. In non-RTX group, two of seven patients developed relapse after four rituximab infusion and rituximab retreatment started.

Conclusions: : In our trial, rituximab therapy was associated with maintenance of complete remission. Ten patients with B-cell repletion who stopped rituximab therapy also were able to maintain complete remission. The rituximab treatment is rational and should be considered as an important treatment alternative in patients with steroid-dependent MCNS.

TH-PO431

Efficacy of Combined Very Low-Dose Prednisolone and Cyclosporine as an Initial Treatment for Minimal Change Nephrotic Syndrome (MCNS) Yoshihiko Inoue, Eri Kawashima, Yoshikuni Nagayama, Kiyoko Inui, Hironori Tayama, Ashio Yoshimura. *Div of Nephrology, Showa Univ Fujigaoka Hospital, Yokohama, Kanagawa, Japan.*

Background: The mainstay of MCNS therapy is glucocorticoid (prednisolone, PSL) achieving complete remission, however frequent relapses may occur and subsequent necessity of increasing amount of PSL dosage is a critical problem. We evaluated combination of very low-dose of PSL to 50% of conventional therapy with cyclosporine (CsA) as an initial treatment for MCNS in adults.

Methods: Twenty patients who had all biopsy-proven MCNS were studied prospectively for three years. They were randomly assigned to two groups, Group A (PSL 0.8mg/kg/day, n=10) and Group B (PSL 0.4mg/kg/day + CsA 1.5-2mg/kg/day, n=10). eGFR, serum albumin levels (sA), amount of proteinuria (UP), Initial PSL dosage, total PSL dosage, duration for complete remission (CR), average length of hospital stay (HS), side effects by PSL treatment and the number of relapse (NR) were compared between two groups. The daily CsA dosage was sustained by using concentration monitoring with 2-h post-dosing level (C2) (600-1000ng/ml).

Results: There were no differences in eGFR, sA or UP between both groups before treatment. Initial PSL dosage was 49.0±7.0mg/day (mean±SD) in Group A and 24.5±4.4 in Group B ($p<0.01$). Total PSL dosage for three years in Group A (7580.5±719.1mg) was also more than in Group B (4624.0±669.4)($p<0.01$). However, there were no differences in duration for CR between Group A (16.2±6.3days) and Group B (14.7±3.0). Average length of HS was significantly longer in Group A (50.9±16.2days) than in Group B (19.3±3.6) ($p<0.01$). NR was 3 patients in Group A and 2 in Group B. Side effects by PSL treatment was observed in two patients in Group A (steroid induced DM and hypercholesterolemia) but none in Group B. C2 level of CsA was 736.6±167.5ng/ml.

Conclusions: The combination of very low-dose prednisolone with CsA as an initial treatment was effective as well as the conventional therapy with PSL alone. It also didn't increase relapse rate of nephrotic syndrome. Therefore this treatment may provide a new way to decrease PSL dosage, shortening of hospital stay and reduction of side effects by PSL for adult MCNS patients.

TH-PO432

Low-Dose Cyclosporine in Treatment of Nephrotic Syndrome: Effectiveness and Renal Safety Xiao-Juan Yu, Lin Ruan, Xiao-Jing Liu, Gang Liu, Li Yang. *Renal Div, Peking Univ First Hospital.*

Background: To observe the effectiveness and renal safety of low-dose cyclosporine (CsA) in treating patients with membranous nephropathy (MN) and focal segmental glomerulosclerosis (FSGS).

Methods: Seventy-nine patients with primary nephrotic syndrome and normal renal function (baseline Scr<133umol/L), pathologically identified as MN in 72 and FSGS in 7, were enrolled in this prospective cohort study. Prednisone of 0.2-0.5mg/kg/d and CsA of 1.5-2.5mg/kg/d were prescribed on top of RAS inhibition. A decrease in eGFR of >30% or increase in Scr of >30% within the first 3 months of CsA commencement was defined as CsA associated acute renal injury (CsA-ARI). A decrease in eGFR of >30% and a development of eGFR<60ml/min/1.73m2 after 3 months was defined as CsA associated chronic renal injury (CsA-CRI). The median follow-up time was 18 (3-54) months.

Results: Thirty-one patients (39.2%) achieved complete remission at 11 (2-44) months and 25 patients (31.6%) got partial remission at 10 (3-49) months. Fourteen patients (20.3%) developed CsA-ARI during the first 3 months (2.5±0.8 months) and 22 patients (27.8%) developed CsA-CRI at 11.2±6.5 months. The remission rates of patients having ARI, CRI, or no renal injury were 42.9%, 50.0% and 85.1% respectively (P=0.001). By the end of follow up, the decrease of eGFR from baseline was 42.29±16.74, 28.20±18.05 and 15.15±21.93 (P<0.001)ml/min/1.73m2. Of all the factors that were analyzed, urinary protein levels at the time of CsA-ARI development was identified as independent risk factors for CsA-ARI (RR=1.147,95%CI 1.050-1.254,P=0.002); whereas baseline eGFR (RR=0.978,95%CI 0.961-0.996,P=0.015) and a history of CsA-ARI (RR=6.156,95%CI 2.022-18.742,P= 0.001) were identified as independent risk factors for the development of CsA-CRI. Neither the dosage nor the overtime plasma concentration of cyclosporine was found to be associated with the occurrence of acute/chronic renal injury in our patients.

Conclusions: Low dose CsA was effective in treating nephrotic patients of MN and FSGS, with a total remission rate of 70.8%. Whereas CsA associated renal injury was still common at low dosage and needs more attention by nephrologists.

TH-PO433

Prospective Study of Tacrolimus in Adult Onset Steroid Dependent Cyclophosphamide Unresponsive MCD Raja Ramachandran, Harbir Singh Kohli, Ritambhra Nada, Vivekanand Jha, Krishan L. Gupta. *PGIMER, Chandigarh, India.*

Background: KDIGO guidelines recommend cyclophosphamide (CYC) as first line agent in the management of adult onset steroid dependent (SD) minimal change disease (MCD) and limits use of calcineurin inhibitors to patients relapsing after CYC. However, the above recommendation is not based on strong evidence. The present study was undertaken to see the efficacy and safety of Tacrolimus (TAC) in adult patients with SD MCD unresponsive to CYC.

Methods: Adult onset nephrotic syndrome with SD (≥ 2 relapse during taper or within 2 weeks of stopping steroids) CYC unresponsive (relapse occurring within 6 months of a 3-month course of CYC) MCD were enrolled in the study. TAC was started to achieve a target trough level of 5–10 ng/mL and oral prednisolone was tapered by 0.1 mg/kg/week to stop and TAC was continued for 48 weeks from then onwards. Outcome viz complete remission (CR) (reduction of proteinuria to <0.5 g/d and sr albumin ≥3.5gm/dl), partial remission (PR) (reduction of proteinuria to 0.5–3.5 g/d and sr albumin ≥3.5gm/dl) were assessed at the end of 48 weeks. Relapses defined as increased proteinuria after complete or partial remissions were recorded. Adverse events were recorded.

Results: A total of 11 patients completed the study. Ten (91%) patients achieved CR and 01 (9%) patient was unable to maintain remission without steroids. Two patients had relapse of nephrotic syndrome after achieving remission with TAC and responded to short course of oral prednisolone. Reversible nephrotoxicity was seen in 2 (18%), TAC related diarrhea in 3 (27%), infections in 2 (18%), DM in 2 (18%), tremors in 2 (18%) and gum hypertrophy in 1 (9%). There was significant reduction in eGFR at the end of 48 weeks (p=0.04). Seven (64%) patients had a follow-up of >96 weeks, of which 4 (57%) patients relapsed on stopping TAC and were restarted on TAC. At the end of 96 weeks all 4 patients underwent kidney biopsy and 3 (75%) patients had evidence of chronic TAC toxicity.

Conclusions: TAC is an effective steroid-sparing agent in the management of SD CYC unresponsive adults with MCD. However, strict renal function and blood sugar monitoring is required due to its nephrotoxicity and diabetogenic potential.

TH-PO434

Rituximab-Induced B Cell Depletion for Treatment of Refractory Minimal Change Glomerulopathy Landon C. Brown,[1,2] Meghan E. Free,[2] Emily H. Chang,[2] Louis-Philippe Laurin,[3] Ronald J. Falk,[2] Patrick H. Nachman,[2] William Franklin Pendergraft.[2] *[1]Univ of North Carolina (UNC) School of Medicine, Chapel Hill, NC; [2]UNC Kidney Center, Chapel Hill, NC; [3]Dept of Medicine, Univ de Montréal, Montréal, QC, Canada.*

Background: Minimal change glomerulopathy is responsible for 10-25% of nephrotic syndrome cases in adults. The majority of these patients are treated successfully with corticosteroids; however, a subset of patients requires additional immunosuppression. Recent case series suggested that rituximab-induced B cell depletion may be beneficial for inducing remission in patients with minimal change glomerulopathy.

Methods: We performed a retrospective analysis of clinical data from patients at our institution with biopsy-proven minimal change glomerulopathy on initial biopsy who were corticosteroid dependent or resistant and were subsequently treated with rituximab.

Results: Ten adult patients (mean age 49.6 years (S.D. 20.4), 20% female) received rituximab for corticosteroid dependent (n=5) or resistant (n=5) disease. Eight patients received 2 doses of 1 gram IV rituximab spaced two weeks apart, and two patients received 375 mg/m^2 IV weekly for 3 and 4 weeks. Median duration of follow-up after B cell depletion was 17.9 months. Mean urine protein to creatinine ratio was 7.5 (S.D. 4.2) just prior to rituximab therapy and decreased to 0.9 (S.D. 1.7) at date of last test. Mean serum albumin increased from 2.6 g/dL (S.D. 0.8 g/dL) to 4.0 g/dL (S.D. 0.7 g/dL). Seven of the 10 patients achieved sustained remission off other forms of immunosuppression. Median time to remission was 3 months. One patient achieved remission, subsequently relapsed after 23 months, and was successfully re-treated with additional rituximab. This same patient then relapsed 13 months later and was again successfully re-treated. Two patients did not achieve remission; one of whom had not attained peripheral B cell depletion.

Conclusions: These findings suggest that rituximab may have a therapeutic role in adults with treatment refractory minimal change glomerulopathy. A clinical trial is warranted to determine the true efficacy of this approach.

Funding: NIDDK Support

TH-PO435

Renal Histological Lesions due to CNI Exposure Are Exceptional in Pediatric Patients with Steroid Dependent Idiopathic Nephrotic Syndrome

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Background: Childhood onset idiopathic nephrotic syndrome (INS) is steroid sensitive in 90% of patients but more than 25% run a steroid dependent course and require immunosuppressive drugs. Calcineurin inhibitors (CNI) are efficient in steroid dependent INS. However, CNI can induce two different types of renal toxicity: renal vasoconstriction and arteriolar hyalinosis (AH), the latter is considered irreversible. No biological parameter can distinguish these types of toxicity.

Methods: We investigated prospectively histological lesions in protocol renal biopsies for all INS patients under 18 years who have received CNI for at least 12 months and analysed correlations with other clinical and biological key parameters.

Results: We included 17 patients (12 boys), 5 on cyclosporine (CyA) and 12 on tacrolimus (TAC). All had normal serum creatinine and normal blood pressure. At the time of renal biopsy, serum albumin was normal and was proteinuria negative in all patients. Median (range) age was 7.2 (3.5 – 15.2) years, CNI treatment duration was 2.4 (1.2 – 5.2) yrs, and CyA trough level was 90 (75 – 130) ng/ml and TAC trough level was 5 (3.7 – 8) ng/ml. We found histological signs of CNI renal toxicity (arteriolar hyalinosis) only in one patient who was on a high dose of tacrolimus (0.31 mg/kg per day) with trough levels of 6 to 7 ng/ml for 4.5 years. None of the other patients showed renal lesions attributable to CNI exposure, despite long term exposure in three other patients of 4.2, 4.6, and 5.2 years respectively.

Conclusions: The vast majority of pediatric patients with steroid dependent INS on CNI did not reveal any histological signs of CNI toxicity even after prolonged and relatively high exposure. However, one patient had arteriolar hyalinosis and fibrosis despite tacrolimus trough levels and AUC in the therapeutic range. No biological and clinical parameter seems to predict histological lesions for pediatric INS patients on CNI. Therefore, protocol renal biopsies may be of interest for patients who require long term CNI treatment for severe steroid dependent INS.

TH-PO436

Response to Cyclosporine A in Primary Idiopathic and Genetic Steroid Resistant Nephrotic Syndrome: Discontinuation Is Possible

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Background: Despite recent advances the prognosis of SRNS is still guarded as uncertain, because the presence of FSGS carries a high risk for the development of chronic renal failure. Duration of treatment in responders and treatment of patients with genetic causes is a matter of debate. With the availability of cyclosporine A (CSA) an improvement has been noticed but long-term follow-up data are scarce.

Methods: We report on 36 patients with SRNS diagnosed since 1991 at a median age of 3 (range 0 .08- 15.0 years) years who were treated with CSA after initial non-response to steroids. Genotyping for at least 3 common mutations (NPHS1 and 2, WT1) was performed in 33/36 (92) patients.

Results: Median follow-up was 15 (range 1.3-29.3 years). 23 (64%) had histological changes that were consistent with FSGS, 13 children had minimal change NS on histology. 19 patients (53%), especially those with MCNS at initial biopsy (12/13 versus 7/23, p<0.002) entered complete remission (CR) with CSA monotherapy. Ten patients entered patients partial remission (PR; 28%, all FSGS). Seven patients (6 FSGS, 1 MCNS) did not respond to treatment and ultimately 8 went into end-stage renal disease; 4 of these patients had mutations in the NPHS2 gene. Two patients with NPHS2 mutations achieved PR, one patient with compound heterozygote NPHS1 and one with heterozygous (dominant) ACTN4 mutation went into CR. In 14 of 19 responders to calcineurin inhibitors discontinuation was attempted after a treatment time of 4.5±3.9years. No further relapses occurred in 10/14 (73%) patients with a median follow-up of 9.7 (0.7-21.6) years.

Conclusions: In summary and conclusion CSA monotherapy is effective in SRNS, even in individual patients with genetic causes. In patients with complete remission a dose reduction and even discontinuation of CSA can be achieved in a significant proportion of patients. In the majority of patients renal function remained stable over prolonged periods indicating, that the prognosis of SRNS may be viewed more optimistic when a response to CSA treatment can be achieved.

TH-PO437

Treatment Response to H.P. Acthar® Gel in Patients with Idiopathic Focal Segmental Glomerulosclerosis and Idiopathic Membranous Nephropathy: A Retrospective Case Series

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Background: This retrospective case series examined treatment with H.P. Acthar® Gel (repository corticotropin injection, Questcor Pharmaceuticals, Inc., Hayward, CA), an FDA-approved treatment for remission of proteinuria associated with nephrotic syndrome, in patients with idiopathic focal segmental glomerulosclerosis (FSGS) or idiopathic membranous nephropathy (iMN).

Methods: Eligible cases (N=26) were patients with FSGS or iMN treated with H.P. Acthar Gel, had biopsy-verified FSGS or iMN, and had assessment of 24-hour proteinuria level or spot urine protein:creatinine ratio prior to and following ≥6 months' H.P. Acthar Gel therapy. Percent proteinuria reduction, remission response, estimated glomerular filtration rate (eGFR, mL/min/1.73m^2), and serum albumin (g/dL) were examined.

Results: Of 16 patients with FSGS and 10 patients with iMN, 88% (14/16) of FSGS patients and 90% (9/10) of iMN patients had failed prior immunosuppressive or cytotoxic therapy. After H.P. Acthar therapy, proteinuria reduction from baseline ranged from 32% to 81% in patients with FSGS and from 52% to 94% in patients with iMN, excluding patients with no response (n=1 FSGS [3% reduction]; n=1 iMN [13% reduction]) or early termination (n=2 FSGS; n=1 iMN). Complete remission (n=2 iMN), partial remission (n=9 FSGS; n=4 iMN), or clinical response (n=4 FSGS; n=2 iMN) occurred in 81% of patients. eGFR was stable or increased in 4/13 FSGS patients and 7/10 iMN patients, and serum albumin was stable or increased in 9/10 FSGS patients and 7/10 iMN patients.

Conclusions: The findings from this multisite retrospective case series indicate that H.P. Acthar Gel may help meet an important treatment need in patients with FSGS or iMN. The trend shown in percent proteinuria reduction following ≥6 months H.P. Acthar Gel treatment in patients with FSGS or iMN suggests patients may benefit from treatment extended beyond 6 months.

Funding: Pharmaceutical Company Support - Questcor

TH-PO438

Long Term Effects of Rituximab (RTX) in Adult Patients with Idiopathic Membranous Nephropathy (MN) and Focal Segmental Glomerular Sclerosis (FSGS)

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Background: MN and FSGS are the major causes of adult nephrotic syndrome (NS). RTX appeared effective in reducing proteinuria in MN (but long-term follow-up data are lacking), while the efficacy in adult FSGS is debated and seems to be related to RTX dose.

Methods: Seventeen MN patients (mean age 67 (29-86) years, 6 women, 11 men) and 6 FSGS patients (mean age 65.6 (45-81) years, 3 women, 3 men) with major risk factors precluding corticosteroids or conventional immunosuppression were treated with RTX (4 weekly doses of RTX 375 mg/m2 for MN patients, and 8 weekly doses for FSGS).

Results: In MN patients proteinuria decreased from 5.6 (3-8) g/24h to 2.4 (0.06-13) g/24h after 6 months (p<0.05) and 1.3 (0.06-8) after 12 months (p< 0.01). Four patients received a 2nd course of RTX (one at 6 months because of persistent nephrotic syndrome, and three at 12, 18, or 30 months for relapse. Three out of these 4 became proteinuria-free (< 0.5 g/24 h) in 6 months. The unresponsive patient was lost from the follow-up. Among the 12 patients reaching 24 months of observation two had 1 g/24 h and 10 <0.5 g/24 h of proteinuria at the last observation. Creatinine remained stable: 1 (0.7-1.6) mg/dl at 12 months and 1.1 (0.7-1.7, # 12 patients) at 24 months versus 1 (0.5-2.4) at baseline. In FSGS patients proteinuria did not change: 4.9 (1.7-7.9) g/24 h mean baseline value; 4.9 (1.9-6.7) at six, 4.7 (1.7-9.3) at twelve, and 4.7 (1.3-9.9, # 4 patients) at 24 months. Only 1 out of 6 patients had a substantial response (basal proteinuria 7 g/24 h, 4.9 g/24 h at 12, and 1.3 g/24 h at 24 months) despite a prolonged B cell depletion (lasting more than 2 years). Renal function decreased: mean creatinine 2.2 (1.2-3.7) mg/dl at basal, 3.2 (1.6-6.4) at 12 months. No serious adverse effects were observed.

Conclusions: The 4 weekly infusion protocol of RTX is a reasonable option MN patients with co-morbidities limiting the use of corticosteroids and/or conventional immunosuppression, providing a good safety profile. An intensified regimen with 8 weekly infusions of RTX is instead ineffective in most adult FSGS patients.

TH-PO439

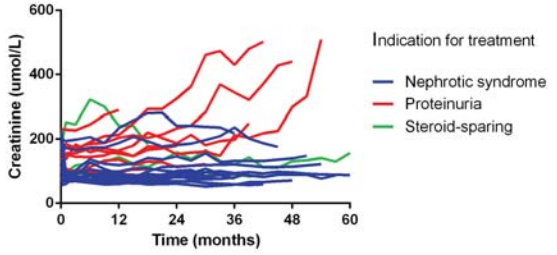
Long-Term Outcome of Tacrolimus Mono-Therapy for Primary Focal Segmental Glomerulosclerosis Thomas Michael Connor, Jack W. Galliford, Ruth M. Tarzi, Liz Lightstone, H. Terence Cook, Charles D. Pusey, Tom Cairns, Megan Griffith. *Hammersmith Hospital, London, United Kingdom.*

Background: Focal segmental glomerulosclerosis (FSGS) is a significant cause of end-stage renal failure. Patients with nephrotic syndrome have a worse prognosis. Tacrolimus (tac) is an effective treatment for the nephrotic syndrome in FSGS, but long-term therapy is often necessary, and there are few data on long-term toxicity. This study reports the long-term outcome of patients treated with tac monotherapy.

Methods: Retrospective review of all patients with primary FSGS treated with tac for >12 months. All patients received maximum tolerated ACE inhibitors +/- ARBs. Complete remission (CR) was defined as normal serum albumin with UPCR <50.

Results: 1. Demographics: 27 patients with primary FSGS were treated with tac (17 for nephrotic syndrome, 7 nephrotic-range proteinuria, 3 steroid sparing). Mean age 48 (19-80). 18 were male. Ethnicity: 10 Caucasian, 6 Afro-Caribbean, 11 Asian. 11/27 patients had received prior immunotherapy. Mean albumin was 22 (9-40) g/L and creatinine 128 (57 – 232) umol/L at presentation. **2. Remission:** 16/17 nephrotic patients achieved CR at a mean 145 days (12-651). 17/27 patients had a stable creatinine over treatment; patients with a lower starting GFR had an increased risk of deterioration;.

Serum creatinine in patients with FSGS during tacrolimus treatment



3. Relapse: 5/17 patients had a relapse of nephrotic syndrome while on tac at 24 (6-39) months, 2/5 following poor adherence. Tac was withdrawn in 3/17 patients; 2/3 relapsed after ceasing therapy. **4. Side effects:** Tac was stopped in 4/27 patients; 3 for reduced GFR, and 1 with metastatic cancer. No admissions for infection.

Conclusions: Most patients on long-term tac for primary FSGS exhibit a stable GFR. Tac offers a good alternative to steroids in these patients. Relapse after long-term therapy is still a problem in some patients and prolonged treatment is often required.

Funding: Government Support - Non-U.S.

TH-PO440

16 Cases of Steroid-Dependent /Resistant Minimal Change Disease Treated with Continuous B-Cell Depletion (Rituximab) John Niles, Andrew P. Murphy, Karen A. Laliberte. *Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Minimal change disease accounts for 10 to 25% of nephrotic syndrome in adults. Of these,over 50% relapse and approximately 25% become steroid dependent or steroid resistant. For those who require additional immunosuppression, Rituximab induced B cell depletion is showing promise for inducing and maintaining remision in patients with minimal change disease.

Methods: We performed a retrospective analysis of clinical data from patients at our clinic with minimal change disease that were corticosteroid dependent or resistant, treated with rituximab and continuous B-cell depletion.

Results: 16 adult patients,12 male and 4 female: mean age 47.5 (IQR 40-56) received rituximab for corticosteroid dependent n=9 or resistant n=7 disease. All 16 received 2 doses of 1gram of Rituximab IV, ~2 weeks apart. Induction also included cytoxan and prednisone. B cell depletion was maintained with 1gm of Rituximab every 4 - 6 months. Median duration of follow up after B cell depletion was 22 months. Mean pre treatment peak urine protein to creatinine ratio was 10.8 and decreased to .62 at date of last test. Mean pre treatment serum albumin increased from 2.0 to 4.2. 12 patients achieved sustained remission, 7 of the 12 were off all other immunosuppression. 1 of 12 patients achieved remission but had early B cell return and was changed to 1gm every 3 months. 1 of the 16 patients died due to a stroke after 24 months of remission. 4 patients achieved remission but follow up is less than 1 year. 1 of these 4 required additional cytoxan and pred. No infections requiring hospitalization occured.

Conclusions: These finding suggest that continuous B-cell depletion with Rituximab has a promising role in the treatment of adults with minimal change disease. Furthermore, induction with overlap of other immunosuppresive agents followed by maintained b cell depletion with Rituximab led to remission in all patients.

TH-PO441

No Additional Effect of Oral Immunosuppressive Agents, Mizoribine, with Steroid Pulse Therapy in Patients with IgA Nephropathy: A Prospective Randomized Controlled Trial Kosuke Masutani,[1] Akihiro Tsuchimoto,[1] Tomomi Yamada,[2] Koji Mitsuiki,[3] Ritsuko Katafuchi,[4] Hideki N. Hirakata,[3] Kazuhiko Tsuruya,[1] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Kyushu Univ Graduate School of Medical Science, Fukuoka, Japan; [2]Dept of Clinical Epidemiology and Biostatistics, Osaka Univ Graduate School of Medicine, Osaka, Japan; [3]Kidney Center, Fukuoka Red-Cross Hospital, Fukuoka, Japan; [4]Dept of Internal Medicine, National Fukuoka-Higashi Medical Center, Koga, Fukuoka, Japan.*

Background: The significance of immunosuppressive agents as an adjunct treatment with corticosteroids for IgA nephropathy (IgA-N) has not been well-demonstrated. This study evaluated the effect of two different treatment regimens, steroid pulse therapy and combined with mizoribine (MZR), imidazole nucleotide which inhibits purine synthesis and T-helper functions.

Methods: Forty IgA-N patients with moderate to severe glomerular injuries were randomly given either pulse methylprednisolone followed by 2-year course of oral prednisolone (P group, n=20) or in combination with MZR (150 mg/day for 2 years, M group, n=20). The primary endpoint was the reduction of proteinuria by 50% or more of the baseline value. The secondary endpoints were increase in serum creatinine (Cr) by 50% or more, or decrease in estimated glomerular filtration rate by 50% or less of the baseline levels.

Results: During the observation period, the urinary protein excretion declined from 1.42 ± 1.25 to 0.33 ± 0.37 g/gCr in P group ($p<0.01$) and from 1.22 ± 0.90 to 0.43 ± 0.42 g/gCr in M group ($p<0.01$), and the lack of statistical difference was found between 2 groups ($p=0.11$). All patients in P group and 19 patients (95.0%) in M group reached the primary endpoint ($p=0.70$). No patient reached the secondary endpoint through the observation period.

Conclusions: Both therapeutic regimens remarkably reduced the levels of proteinuria. We did not find the beneficial effect of MZR on IgA-N in combination with steroids, and terminated the trial. Steroid pulse therapy with 2-year course of oral steroid was effective even in the moderate to severe IgA-N.

Funding: Private Foundation Support

TH-PO442

The NEFIGAN Trial: A Randomized, Placebo-Controlled Study to Evaluate the Efficacy and Safety of NEFECON® in IgA Nephropathy Patients at Risk of Developing ESRD: Preliminary Data from the Run-In Phase Bengt C. Fellstrom,[1] Rosanna Coppo,[2] John Feehally,[3] Jürgen Floege,[4] Alan G. Jardine,[5] Francesco Locatelli,[6] Bart D. Maes,[7] Alex Mercer,[8] Manuel Praga,[9] Vladimir Tesar.[10] *[1]Uppsala Univ Hospital; [2]Univ Hospital Regina Margherita; [3]Univ of Leicester; [4]RWTH Univ of Aachen; [5]Univ of Glasgow; [6]Ospedale A.Manzoni; [7]Heilig Hartziekenhuis; [8]Pharmalink AB; [9]Hospital 12 de Octubre; [10]Charles Univ.*

Background: NEFECON is a new oral modified-release capsule formulation of the corticosteroid, budesonide, for the treatment of IgAN patients at risk of developing ESRD despite optimized RAS blockade. The safety and efficacy of NEFECON is currently under evaluation in a prospective, randomized, placebo-controlled study (NEFIGAN Trial). Patients entering the study undertake a 6-month Run-in Phase during which standard-of-care ACEI/ARB therapy is optimized according to KDIGO guidelines. Run-in Phase data are presented.

Methods: 149 primary IgA nephropathy (IgAN) patients at risk of ESRD completed the 6 month Run-in Phase in which ACEI and/or ARB dose was optimized to a maximum recommended or tolerated dose. UPCR, eGFR and systolic / diastolic blood pressure (SBP/DBP) were compared at entry and completion of the Run-in Phase. Run-in Phase inclusion criteria: Biopsy verified IgAN ≥18 years, UPCR ≥0.5 g/g OR urine protein ≥0.75 g/day, eGFR ≥45 mL/min/1.73m^2.

Results: Over the course of the Run-in Phase, mean changes in UPCR, eGFR and SBP/DBP were -1.6%, -1.1% and -3.7/-1.2 mmHg, respectively. For patients entering the Run-in Phase already on an optimized ACEI/ARB dose (n=92), mean changes for UPCR, eGFR and SBP/DBP were +1.7%, -1.8% and -3.4/-0.3 mmHg, while for those requiring ACEI/ARB dose optimization (n=57), mean changes were -7.0%, 0% and -4.2/-2.7 mmHg, respectively.

Conclusions: The majority of patients entering the NEFIGAN Trial had optimized RAS blockade according to KDIGO guidelines at enrolment. Overall, no substantial changes in UPCR, eGFR or SBP/DBP were noted over the duration of the 6 month Run-in Phase. These data illustrate the need for new treatments for IgAN as patients remain at risk of progression to ESRD despite optimized RAS inhibition.

Funding: Pharmaceutical Company Support - Pharmalink AB

TH-PO443

Prominent IgM Deposition in Glomerulus Is Associated with Initial Higher Level of Proteinuria and Favorable Outcome After Combined Treatment of Tonsillectomy with Steroid Pulse Therapy in Patients with IgA Nephropathy

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Background: IgA nephropathy (IgAN) is characterized by mesangial deposition of IgA and C3,often with co-deposits of IgM. Its significance has not fully clarified, therefore we studied the relationship between IgM deposition, clinical features and renal outcome in patients with IgAN with combined treatment of tonsillectomy with steroid pulse therapy (Tx-SP).

Methods: We retrospectively reviewed 74 IgAN patients treated with Tx-SP form December 2006 to March 2014. The patients were divided in two groups: moderate (2+) to severe (3+) measngial IgM deposition (IgM-positive patients, group 1) and negative to faint it (IgM-negative patients, group 2A). Using propensity scores to minimize confounding factors, we selected 22 matched IgM-negative patients (group 2B) from among the 63 unmatched IgM-negative ones and compared them with the 11 IgM-positive ones. The amount of proteinuria, the number of RBC in urine sediment, serum creatinine and histological severity by renal biopsy were also compared between two groups.

Results: Group 1 showed increase in proteinuria than group 2A (urinary protein grade 1.55±0.52 versus1.00±0.97, p = 0.022) and group 2B(1.32±1.09. p =0.057) before Tx-SP. After Tx-SP, proteinuria in group 1 decreased significantly to the same level as the IgM-negative patients (the slope of urinary protein qualitative -1.27±0.47 versus -0.71±0.92 in group 2A, p = 0.006 or -0.95±1.00 in group 2B, p = 0.005). There were no significant differences between the two groups in level of microhematuria, serum creatinine and histological severity.

Conclusions: Prominent IgM deposition in glomerulus is associated with severe proteinuria in patients with IgAN. However, Tx-SP induces sufficient reduction of proteinuria in them as well as IgM-negative IgAN.

TH-PO444

Clinical Characteristics of IgA Nephropathy Associated with Low Complement 4 Levels

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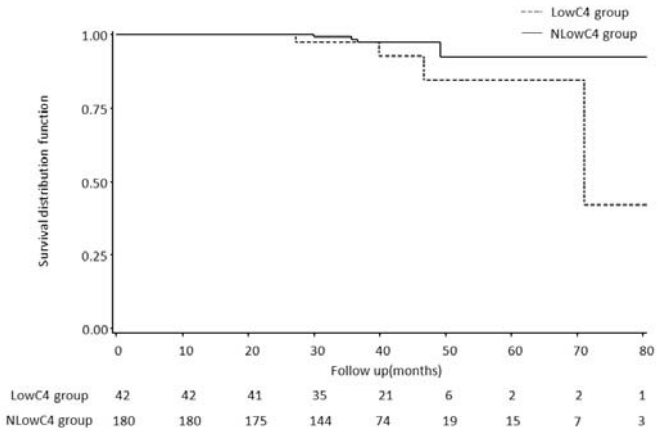
Background: C4 mediated lectin complement pathway plays an essential role in IgAN. C4 deficiency is the most commonly inherited immune disorder in human. The present study investigated the characteristics of the IgAN patients with low serum C4 levels.

Methods: We performed a cross-sectional and cohort study. Renal clinical as well as histopathologic parameters according to Oxford IgAN classification were assessed. A Kaplan-Meier survival analysis was performed concerning the primary outcome defined as the serum creatinine increased 1.5 fold from baseline. The prognostic significances of clinical and histopathologic parameters were determined using Cox proportional hazards models.

Results: Data from 512 biopsy proven IgAN cases were available for analysis with a median follow-up of 38.4 months. 99 cases (19.34%) presented with low C4 levels (LowC4 group) and the other 413 cases did not (NlowC4 group). At the time of renal biopsy, renal injury were significantly attenuated in the LowC4 group compared with the NlowC4 group which was supported by a subgroup analysis concerning C3 levels. Renal C4 depositon was significantly decreased while IgM deposition was notably increased in the LowC4 group. A spearman correlation analysis shown that low C4 levels were associated with the improved renal injury at biopsy. However, the risk of developing primary outcome was significantly greater in those with low C4 levels. Specifically, during the follow-up period, the risk of developing primary outcome was nearly ten fold higher in those with low C4, compared to those without low C4.



Conclusions: There is a high prevalence of low C4 levels in IgAN patients. These patients with low C4 levels presented attenuated renal injury at the time of renal biopsy, whereas were associated with a poor prognosis.

Funding: Government Support - Non-U.S.

TH-PO445

Urinary Complement Factor H: A Biomarker Predicts Progression of IgA Nephropathy

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Background: Activation of complement system participates in the pathogenesis of IgA nephropathy (IgAN). Complement factor H (CFH) is a crucial factor in inhibiting alternative pathway. The study was to investigate whether urinary CFH associated with renal outcome in IgAN.

Methods: 351 IgAN patients, followed for 51.8±26.6 months, participated in the study. Renal outcome was defined as composite endpoints, including death, ESRD, eGFR decline≥ 50% or doubling of plasma creatinine. Urinary CFH level was measured by ELISA and calculated as urinary CFH over creatinine ratio (uCFH/uCr).

Results: In the whole cohort, uCFH/uCr was independently associated with disease progression either as continuous [log(uCFH/uCr)] or categorical traits (dichotomous and quartile variables) after adjustment (eGFR, proteinuria, mean arterial blood pressure, histological grading, immunosuppressive therapy) in the Cox proportional hazard model. Kaplan-Meier analysis showed that higher uCFH/uCr at baseline predicted worse renal outcome during follow-up (Log rank, P<0.001). ROC curve showed that log(uCFH/uCr) had a predictive value for renal outcome (AUC=0.761), and the AUC increased to 0.818 after being incorporated into eGFR and proteinuria. At early stage of IgAN with eGFR≥60 ml/min/1.73m^2, log(uCFH/uCr) had a better predictive value (AUC = 0.739) for renal outcome compared with eGFR (AUC = 0.604) or proteinuria (AUC = 0.637).

Conclusions: Urinary CFH associated with renal function decline and higher urinary CFH may be an early risk factor for progression of IgA nephropathy.

TH-PO446

Time-Averaged Albumin Predicts the Long-Term Prognosis of IgA Nephropathy Patients Who Achieved Remission

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Background: Primary IgA nephropathy (IgAN) is the most common idiopathic glomerulonephritis worldwide. Although most patients are able to achieve remission after therapy with the currently available treatments, a large number of patients will still progress to end-stage renal disease. This study aimed to evaluate the risk factors for progression in patients who achieved remission.

Methods: Patients from a prospective database with IgAN and who were followed up for at least 36 months were included in this study. All the subjects had achieved a complete remission or partial remission following 6 months' therapy. The relationship between the clinical parameters and composite renal outcomes were assessed.

Results: The study comprised 878 IgAN patients recruited between January 2005 and December 2010. Overall, 632 patients were enrolled in this study. The data from the 369 patients who achieved remission were analyzed; the mean follow-up time was 49 months. The median serum creatinine (SCr) was 91.3 μmol/L, and the time-averaged creatinine (TA-SCr) was 91.8 μmol/L. The mean serum albumin (ALB) was 39.4 g/L, and the time-averaged serum albumin (TA-ALB) was 42.1 g/L. Multivariate Cox regression analyses revealed that the TA-ALB and TA-SCr levels were associated with the composite renal outcome.

Characteristics	Univariate analysis				Multi variate analysis	
	HR	95% CI	P value	HR	95% CI	P value
ALB g/L at month 6	0.86	0.76-0.96	0.01			NS
TA-ALB g/L	0.80	0.70-0.93	0.002	0.86	0.75-0.98	0.03
Baseline SCr μmol/L	1.02	1.01-1.04	0.003			NS
TA-SCr μmol/L	1.02	1.01-1.03	<0.001	1.02	1.01-1.03	<0.001
Baseline eGFR (ml/min/1.73 m2)	0.97	0.94-0.99	0.01			NS
TA-eGFR (ml/min/1.73 m^2)	0.96	0.93-0.98	0.001			NS
TA-UPE (g/d)	1.00	1.000-1.002	0.01			NS

The patients with a TA-SCr value > 120 μmol/L and a TA-ALB level < 38 g/L were less likely to recover from renal progression.

Conclusions: The strong predictive relationship of low TA-ALB and high SCr levels with progression observed in this study suggests that TA-ALB may serve as a marker of the long-term renal prognosis of IgAN patients who achieved remission.

Funding: Government Support - Non-U.S.

TH-PO447

Effect of Mycophenolate Mofetil on Expression of Serum microRNA-141 in Immunoglobulin A Nephropathy

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Background: IgAN is also an important cause of end-stage renal disease.Previous studies showed that a number of miRNAs species,such as miR-141 and miR-200b, miR-205 and miR-192, were regulated in renal cells that are undergoing epithelial-to-mesenchymal transition (EMT). In IgAN, activated tubular epithelial cell may develop EMT and change into activated fibroblast, the main effector of renal fibrosis.In this study,we aim to explore the serum level of miR-141 and the effect of MMF on it in IgAN patients.

Methods: 47 patients treated with MMF as MMF treatment group, 33 patients without any treatment as NIgAN group which matched with MMF treatment group, 15 healthy volunteers as health control group. To explore the serum level of miR-141 through real-time PCR, and compare serum level of miR-141 between the three groups.

Results: As compared with health control group, the serum level of miR-141 in NIgAN group is increased (*P<0.001*). The serum level of miR-141 in MMF treatment group is significantly lower than NIgAN group (*P<0.001*).There is no statistically difference of serum level of miR-141 between MMF treatment group with health controls. Serum level of miR-141 inversely correlates with glomerular filtration rate(GFR)(*r=-0.398, P=0.024*) in IgAN patient,and there is no significant correlation between serum level of miR-141 with urinary protein excretion, blood pressure, and other clinical factors. As comparing Lee grade ≤ 3 with> Lee grade 3 patients, the serum level of miR-141 is higher in the latter, but the difference is not statistically significant.

Conclusions: The serum level of miR-141was significantly elevated in IgAN patients,and its expression is closely related to the progress and severity of renal function. MMF could make the serum level of miR-141 in IgAN significantly reduced. Serum level of miR-141 may be considered as therapeutic target, reflecting the effect of MMF treat with IgAN.

TH-PO448

Clinical Significance and Pathological Characteristics of CD147 in Patients with IgA Nephropathy

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Background: Recent studies demonstrated that tubulointerstitial injury can predict renal outcome in patients with IgA nephropathy (IgAN).CD147 is a key regulator of renal tubulointerstitial fibrosis in cellular and animal models. However, it is not clear whether the expression of CD147 correlates with tubulointerstitial injury in IgAN patients.

Methods: We analyzed the degree of CD147 expression and localization in renal biopsies from 86 patients with IgAN and correlated their immunostaining scores with clinical and histological parameters. In particular, we also retrospectively analyzed whether the degree of CD147 expression in the renal interstitium correlated with renal survival.

Results: Elevated CD147 expression was found in the basolateral membrane of renal tubules in IgAN patients; however, in normal kidney samples, positive staining for CD147 was not found in the tubular epithelial cells (p=0.000). CD147 protein expression in the tubulointerstitium showed a negative correlation with estimated glomerular filtration rate (eGFR;r= -0.600, p= 0.000) and a positive correlation with serum creatinine (Scr;r=0.322, p=0.002) and tubulointerstitial lesions (r=0.525, p=0.000). Kaplan-Meier survival curves showed that elevated CD147 expression, which was measured by immunohistochemistry, was associated with decreased renal survival. Multivariate analyses further demonstrated that a high CD147 immunostaining score was an independent predictor of renal outcome in patients with IgAN (HR= 8.731, p=0.041).

Conclusions: CD147 expression is associated with tubulointerstitial injury and predicts renal prognosis in IgA nephropathy. CD147 could be a new marker for tubulointerstitial fibrosis.

TH-PO449

Could Tonsillectomy plus Methylprednisolone Pulse Therapy Improve Renal Outcome in Patients with IgA Nephropathy? Results from 15-Year Follow-Up Study

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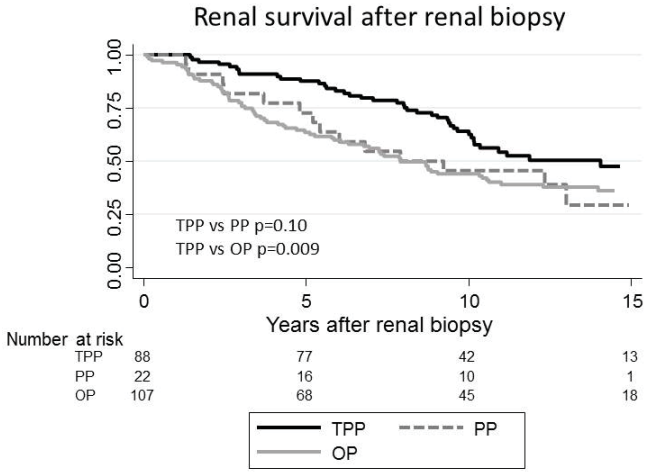
Background: RAS inhibitors and steroids are considered as first-line therapies for patients with IgA nephropathy. However, it is still unknown whether tonsillectomy plus prednisone pulse therapy (TPP) could decrease risk of ESRD beyond these treatments in patients with IgA nephropathy (IgAN).

Methods: Data on 217 IgAN patients diagnosed by renal biopsy between 1995 to 2005 in our hospital were followed until ESRD or death (mean follow-up, 9.1±0.3 years). Their renal survival was compared after dividing by their initial treatments; TPP, prednisone pulse (PP), or oral prednisone (OP). Factors affecting survival were analyzed using the Cox's hazard model after adjusting the IgAN prognostic score (Goto M et al., NDT 2009) at baseline and other treatment factors (use of RAS inhibitors and antiplatelet/anticoagulants).

Results: 5- and 10-year renal survival were, respectively, 0.88 (95% confidence interval [CI], 0.78-0.93) and 0.64 (0.53–0.73) with TPP (n=88); 0.73 (0.49–0.87) and 0.45 (0.24–0.64) with PP (n=22); and 0.64 (0.54–0.72) and 0.44 (0.34–0.53) with OP (n=107).



Survival curves were significantly different between TPP and OP (p<0.01). After adjusting baseline and treatment factors, hazard ratios for ESRD, with OP as reference, were 0.68 (0.44–1.05, p=0.08) for TPP, 0.77 (0.40–1.47, p=0.43) for PP.

Conclusions: Tonsillectomy plus methylprednisolone pulse therapy may decrease risk of ESRD in patients with IgAN comparing to oral or pulse prednisone therapy.

TH-PO450

The Long Term Efficacy and Safety of Immunosuppression on the Progression of IgA Nephropathy: A Meta-Analysis of Randomized Controlled Trials with More Than 5-Year Follow Up

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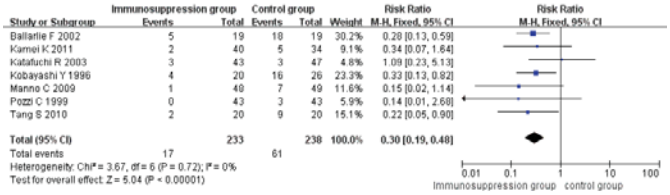
Background: Immunoglobulin A nephropathy (IgAN) is a common glomerulonephritis usually treated with immunosuppression. However, the long-term efficacy and safety of immunosuppression for IgAN is still controversial.

Methods: Articles were identified from PubMed, ISI Web of Science, EMBASE, Google Scholar, Scopus, Science direct and Cochrane library. Randomized controlled trials (RCTs) at least 5 years follow-up were selected to investigate the efficacy and safety of immunosuppression for IgAN. The primary outcome was the progression to end stage renal disease (ESRD). Another outcome was deterioration in renal function defined as doubled serum creatinine or 50% reduction of estimated glomerular filtration rate (eGFR) without going into ESRD.

Results: The eligible 7 RCTs with 471 patients were enrolled in this study. Generally, immunosuppression could lower the risk for the progression to ESRD (RR, 0.30, 95% CI 0.19-0.48) and deterioration in renal function (RR, 0.19, 95% CI 0.07-0.54).



As far as the pooled RRs of progression to ESRD, 4 studies with less than 7-year follow-up, 3 followed for more than 7 years, 4 adopted corticosteroids monotherapy, 2 used corticosteroids plus other immunosuppression, 4 fromAsia, and 3 from Europe were 0.32 (95% CI, 0.18-0.58), 0.28 (95% CI, 0.13-0.59), 0.34 (95% CI, 0.17-0.67), 0.29 (95% CI, 0.15-0.58), 0.37 (95% CI, 0.20-0.68), and 0.23 (95% CI, 0.11-0.47), respectively. Immunosuppression was associated with an increased risk for adverse events (RR=2.13, 95% CI 1.17-3.86).

Conclusions: Immunosuppression for IgAN might reduce long-term risk of progression to ESRD and deterioration in renal function but increase the risk of adverse events to some extent.

Funding: Government Support - Non-U.S.

TH-PO451

Pathological Prognosticators and Therapeutic Impact of Tonsillectomy in IgA Nephropathy

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Background: The Oxford classification of IgA nephropathy consists of four markers, MEST, as the prognosticators. We retrospectively examined the relevance of extracapillary proliferation and arterial sclerotic changes which might correlate prognosis of chronic kidney disease, and efficacy of tonsillectomy in IgA nephropathy.

Methods: Of 727 Japanese patients who were diagnosed with IgAN based on kidney biopsy in our institution, 314 patients with 12 months or more follow-up period and with 8 or more glomeruli per biopsy were included in this study. Patients with complication of systemic disease that would be expected to cause IgA deposition in glomeruli, such as anaphylactoid purpura, or those with complication of diabetes mellitus or with severe kidney injury (initial eGFR <30 ml/min/1.73m^2) were excluded. Presence of extracapillary proliferation (Ex), arteriole hyalinosis (A), and intimal thickening of small artery (SA) were scored 0 in the absence and 1 in the presence of each lesion.

Results: Each intraclass correlation coefficient demonstrated moderate correlation shown as; M:0.409, E:0.489, S:0.616, T:0.533, Ex:0.577, A:0.575, SA:0.59. Then, we investigated the correlation between these pathological scores and renal outcome. The end point was determined as the 50% reduction in initial eGFR or ESRD defined as eGFR <15ml/min/1.73m^2. The median duration of follow-up was 137 months (range 12-491). Steroid therapy was conducted in 126 patients and tonsillectomy was undergone in 54 patients. In the univariate analysis, kidney survival was significantly lower in patients with S1, Ex1 and A1 than in those without, and hazard ration for S, Ex and A was 2.16, 2.01, and 2.07, respectively. In the multivariate model, eGFR at biopsy was the sole risk factor. Tonsillectomy did not improve renal outcome, regardless of combination of steroid theapy.

Conclusions: Segmental sclerosis, extracapillary proliferation and arteriole hyalinosis could be useful as prognosticators, although renal function at baseline would be more important predictor of renal outcome. Tonsillectomy did not have any significant effect in renal outcome.

Funding: Government Support - Non-U.S.

TH-PO452

Efficacy and Safety of Rituximab plus Cyclosporine in Idiopathic Membranous Nephropathy; Results of an Ongoing Prospective Trial

Meryl A. Waldman, Michelle Braun, Howard A. Austin. *NIDDK, National Inst of Health, Bethesda, MD.*

Background: Cyclosporine (Csa) has efficacy in reducing proteinuria in idiopathic membranous nephropathy (IMN) but partial remissions (PR), rather than complete (CR) are more common and relapse upon drug withdrawal is problematic. Extending treatment may increase remissions and reduce relapses but potential for nephrotoxicity exists. Rituximab (RTX) monotherapy has shown promise in IMN but PRs are more common and effect on proteinuria tends to be delayed. We are conducting a prospective phase 2 trial in 30 pts with IMN to investigate whether "induction" with RTX + Csa for 6 mos followed by "maintenance" RTX may achieve greater reduction in proteinuria than either agent alone, increase number of remissions (especially CR) and reduce relapse rates. Here we report interim data.

Methods: Patients with IMN, persistent high grade proteinuria despite conservative rx for min 6 mos and eGFR ≥40 ml/min/1.73 m^2 receive RTX (1gm D 1 and 15) + Csa x6 mos, then tapered. A 2nd course of RTX is given after min of 6 mos and evidence of B cell recovery.

Results: To date, 13 pts have enrolled. Mean baseline proteinuria 10.7g/d. All patients have shown response with reduction of proteinuria. Of 8 pts with 24 mos follow up, there were 4 (50%) complete remissions (protein reduction ≤0.3g/d) and 3 (38%) partial remissions (protein reduction≥50% and ≤3.5g/d); 2 pts relapsed (R:≥50% increase in proteinuria) after achieving PR and again achieved PR after RTX monotherapy. Regimen was well tolerated.

Pt#	Baseline proteinuria g/24 hr	3m	9m	12m	18-20m	24m	most recent f/u
1	11.2	10.9	14	5.7	1.8	0.8	1.3
2	10.5	0.1	0.3	0.1	0.1	0.1	0.1
3	14.1	11.3	2.1	1.4	0.8	0.4	0.1
4	13.9	1.1	0.3	0.2	0.2	0.2	0.2
5	8.0	1.9	0.7	0.5	0.6	3.4(R)	0.4
6	9.8	7.2	2.1	7.4 (R)	2.8	0.6	0.7
7	9.6	1.3	0.2	0.2	0.1	0.1	0.1
8	12.6	3.5	0.3	0.2	0.2	0.1	
9	15.9	2.9	1.4	1.3			
10	8.3	1.0	0.2	0.3			
11	5.9	1.4	0.5				
12	12.8	1.1					
13	7.1						

Conclusions: "Induction" with RTX + Csa followed by "maintenance" RTX may be a treatment approach for IMN to achieve a greater number of remissions (complete) and may obviate the need for long term immunosuppression. It appears to be well tolerated. Enrollment continues and longer term follow up is needed.

Funding: NIDDK Support

TH-PO453

Synthetic ACTH in Patients with Idiopathic Membranous Nephropathy: Long Term Follow-Up

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Background: Although alkylating agents improve outcome in idiopathic membranous nephropathy (iMN), these agents often have serious side effects. Synthetic ACTH may be advantageous in patients with iMN with reported remission rates up to 85 % and few side effects.

Methods: We have previously shown preliminary data in 20 patients with high risk iMN treated with ACTH (NCT00694863/kidney week 2011: SA-PO2851). Here we report long term renal outcome. Patients with iMN, a nephrotic syndrome, eGFR> 60 ml/min/m² and high risk for progression were treated with i.m. injections of synthetic ACTH during 9 months (m). Maximal dose was 1 mg twice a week. For comparison, we selected historical controls, treated with cyclophosphamide (CP) (1.5 mg/kg/day for 12 m) and steroids, matched for serum creatinine, proteinuria, age, sex and previous immunosuppressive treatment.

Results: We compared 20 patients treated with ACTH and 20 historical controls treated with CP; the patient characteristics are shown in Table 1. Cumulative remission (PCR <3.5 g/10 mmol) after first therapy was seen in 13 (70 %) patients treated with ACTH versus 19 (95 %) patients in the CP group (p=0.04). Relapse rates were respectively 31% and 26 %. Overall ten (50 %) patients in the ACTH group versus two (10%) in the CP group needed additional immunosuppressive agents because of initial resistance, relapse or progressive disease (p=0.01). At the end of follow-up 15 (75 %) patients in the ACTH group and 17 (85 %) CP group patients were in remission (p=0.70).

	ACTH group (n=20)	CP/Prednisone group (n=20)
Sex (male/female)	15/5	16/4
Age (years)	54 (±14.5)	50 (±13.2)
Serum creatinine (µmol/l)	104 (74-156)	97 (68-165)
Protein creatinine ratio (g/10 mmol)	8.7 (2.4-13.2)	9.4 (4.6-18.6)
Follow-up (months)	46 (±13)	64 (±35)

Conclusions: Treatment with synthetic ACTH is less effective than CP in inducing a remission in high risk patients with iMN. At the end of follow-up an equal rate of remissions was achieved in both groups at the cost of additional treatment in 50 % of patients in the ACTH group. Still 40 % of patients have long term remission with ACTH monotherapy.

TH-PO454

Anti-PLA2R Antibody Titers During ACTH Treatment in Patients with Idiopathic Membranous Nephropathy

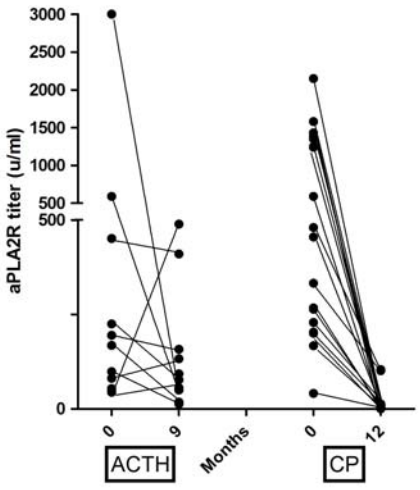
Anne-Els van de Logt,[1] Julia M. Hofstra,[1] Jack F. Wetzels,[1] Paul E. Brenchley.[2] *[1]Nephrology, Radboud Univ Medical Center, Nijmegen, Netherlands; [2]Nephrology and Transplantation, Manchester Inst, Manchester, United Kingdom.*

Background: Synthetic ACTH may be advantageous in patients with idiopathic membranous nephropathy (iMN) with reported remission rates up to 85 %. In a separate study, we showed that cumulative remission rate (protein creatinine ratio <3.5 g/10 mmol) after first therapy was lower (70 %) in patients treated with ACTH versus 95 % in patients treated with cyclophosphamide (CP). Here we compare antibody response during treatment with either ACTH or CP.

Methods: We selected patients treated with synthetic ACTH (9 months) or with CP (12 months) with positive anti-PLA2R antibodies (aPLA2R) at baseline and a follow-up serum sample available at end of treatment. In all serum samples anti-PLA2R antibodies were measured with an ELISA (Kanigicherla, KI 2013).

Results: The CP treated patients had more severe disease (Table). The course of aPLA2R titers in individual patients is showed in a Figure. At the end of therapy, proteinuria was reduced in both groups (ACTH 1.8 versus CP 1.1 ;p=0.436); remissions were achieved in 6/10 versus 15/18 patients (p=0.207); aPLA2R was negative in 2/10 versus 16/18 patients (p=0.001). Anti-PLA2R antibodies were still present in 4/6 ACTH-treated patients in remission, versus 0/15 CP-treated patients in remission.

	ACTH group (n=10)	CP/Prednisone group (n=18)
Sex (male/female)	8/2	15/3
Age (years)	54 (±12.4)	55 (±9.4)
Serum creatinine (µmol/l)	95 (79-129)	136 (105-278)
Protein creatinine ratio (g/10 mmol)	8.8 (±2.8)	15.5 (±8.8)
Follow-up duration (months)	55 (33-61)	123 (26-192)



Conclusions: aPLA2R antibodies disappear less often or slower with ACTH therapy. This might explain the lower efficacy of ACTH compared to CP. However, our data point to non-immunological effects of ACTH in some patients.

TH-PO455

Prognostic Value of Remission of Proteinuria for the Development of ESRD in the Patients with Idiopathic Membranous Nephropathy Hideo Tsushima, Ken-Ichi Samejima, Masaru Matsui, Yasuhiro Akai, Yoshihiko Saito. *First Dept of Internal Medicine, Nara Medical Univ, Kashihara City, Nara, Japan.*

Background: Prognostic factors for the development of end-stage renal disease (ESRD) in the patients with idiopathic membranous nephropathy with nephrotic syndrome (niMN) have not fully understood.

Methods: We retrospectively investigate the clinical and histological renal prognostic markers in the patients with niMN who were clinically and histologically evaluated from 1981 to 2011.

Results: Seventy-two patients (54 males and 18 females) who had the diagnosis of niMN were enrolled. Mean age at the time of renal biopsy was 59.1±14.1 years old. Diurnal urinary protein was 6.7±3.9 g and serum albumin 2.6±1.5 g/dl at renal biopsy. When clinical parameters were evaluated by univariate analysis, the development of ESRD was closely associated with the remission of proteinuria (< 1.0g/d) but not with the degree of proteinuria, serum albumin, blood pressure, and estimated glomerular filtration rate. Interestingly, the rate of glomerularsclerosis and interstitial fibrosis at initial biopsy did not contribute to the renal prognosis.

Conclusions: Our findings demonstrated that remission of proteinuria is a pivotal prognostic index for the preservation of renal function. The severity of glomerulosclerosis and interstitial fibrosis at initial renal biopsy did not correlated with future development of ESRD.

TH-PO456

Long-Term Outcomes of Idiopathic Membranous Nephropathy: Two-Centre UK Experience Nadia Sarween,[1] Stuart W. Smith,[1] Daniela Farrugia,[2] Mohammed Awais Hameed,[2] Sarah J. Logan,[1] Catherine King,[1] Paul Carmichael, Peter Hewins.[1] *[1]Renal Medicine, Queen Elizabeth Hospital, Birmingham, West Midlands, United Kingdom; [2]Renal Medicine, New Cross Hospital, Wolverhampton, West Midlands, United Kingdom.*

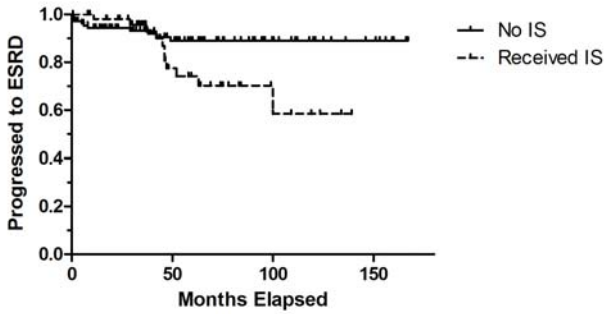
Background: Idiopathic membranous nephropathy (IMN) can respond to immunosuppression (IS). Serious side effects and a high spontaneous remission rate create debate over optimal management and differing practices. We retrospectively reviewed treatment and outcomes of IMN patients at 2 United Kingdom teaching hospitals serving an interconnected urban area.

Methods: Patients with biopsy proven, new-diagnosis membranous nephropathy without a secondary cause were identified from hospital records (2000-2012).

Results: Data were available for 168 patients (centre 1 n=106, centre 2 n=62). Patients that received IS had a higher baseline ACR (p=0.016) and were more likely to have reached ESRD (p=0.03).

Median age (range) years	55 (18-83)
Sex	62% male
Ethnicity	68% Caucasian, 17% South Asian, 6% Black
Median creatinine (µmol/L)*	94 (45-1526)
Median serum albumin (g/L)*	27 (10-50)
Median urine ACR (mg/mmol)*	565 (26-2091)
Median follow up	59.2 months
Received IS	31%
Average time to IS	5.7 months (0-65)
Median urine ACR (mg/mmol) no IS vs IS	487 (no IS), 640 (IS)
Spontaneous remission	73%
Median time to spontaneous remission	19.7 months (0.8-160)
Remission in IS patients	80.8%
Median time to remission in IS patients	14.8 months (1.1-124)
ESRD: no IS vs IS	9.5% (no IS), 21% (IS)
Mortality: no IS vs IS	16.4% (no IS), 13% (IS)

* At presentation. Remission=complete or partial. IS was calcineurin inhibitor (n=36), modified Ponticelli (n=29), steroids only (n=5) and other (n=7)



Conclusions: Outcomes were favourable for most IMN patients including nephrotic patients not treated with IS. Belated spontaneous remissions occur and deferred IS can be considered. A subset of patients develop ESRD and an optimal IS strategy is yet to be devised.

TH-PO457

Hemolytic Uremic Syndrome Induced by Shiga Toxin-Producing Escherichia Coli O104:H4 (STEC) in Adults: A Follow-Up for 12 Months with a Focus on the Outcome of Kidney Function Inge Derad,[1] Birgit Obermann,[2] Martin Nitschke,[1] Juergen Steinhoff.[1] *[1]Dept of Nephrology, Univ of Lübeck, Lübeck, Germany; [2]Dept of Microbiology and Hygiene, Univ of Lübeck, Lübeck, Germany.*

Background: During the Northern German endemic in 2011, hemolytic uremic syndrome (HUS) was induced in 855 patients by Shiga toxin-producing, enteroaggregative Escherichia coli bacteria (STEC). 60 patients were treated according to a standardized protocoll. The present single center, observational study describes the 1-year course of the disease with an emphasis on kidney function. Outcome data are associated with treatment and patient characteristics at onset of HUS.

Methods: According to the rescue guidelines of the German Society of Nephrology patients with severe HUS were treated with eculizumab, on top of the regular treatment with best supportive therapy and a limited number (5) of plasmapheresis. In order to protect patients from an increased risk of meningococcal disease inherent in the therapy with eculizumab, the antibiotic azithromycin was concomitantly administered for two weeks. This decision - to start or omit an additional therapy with eculizumab separated the patients into two groups (EcAz, Non-EcAz), and marked the day zero of the present study. After discharge, treatment was continued in our outpatient clinics. Standardized visits assessed the patients' well-being, bodily functions, kidney function, neurological symptoms, hematologic signs and blood pressure.

Results: 56 patients were regularly seen during the follow-up. All patients had survived without end-stage renal disease. After one year kidney function was affected with increased creatinine (4.4%, maximun:129µmol/l), proteinuria (26.7%), increased cystatin C (46.7%), reduced estimated glomerular filtration rate (<90 ml/min) (46.7%), and newly developed hypertension (25%).

Conclusions: Although STEC-HUS was a severe, life-threatening acute illness, after one year patients showed a good recovery. Kidney function in the severely ill patients treated with eculizumab plus azithromycin improved to the same level as the less severly ill controls. Only HUS patients with severe illness developed chronic hypertension.

Funding: Government Support - Non-U.S.

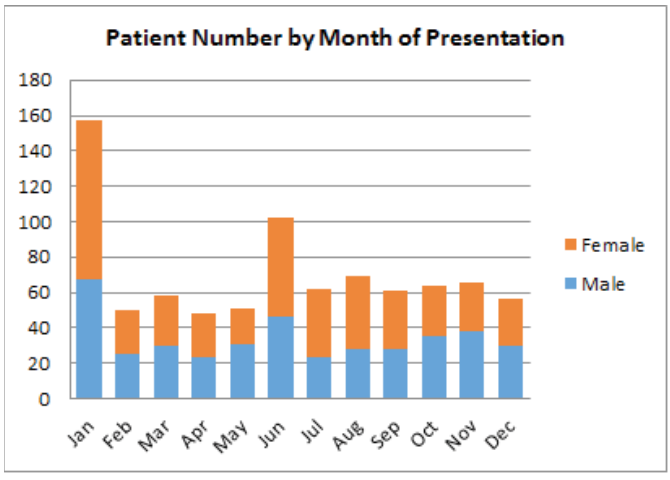
TH-PO458

Effect of Seasonal and Temperature Variations on Incidence of Systemic Vasculitis Hsu-Han Wang,[1] Neil Basu,[2,5] Mark Alan Little,[3,5] Michael G. Robson,[4,5] Alan D. Salama.[1,5] *[1]Centre for Nephrology, Univ College London; [2]Musculoskeletal Collaboration, Univ of Aberdeen; [3]Clinical Medicine, Trinity College Dublin; [4]MRC Centre for Transplantation, King's College London; [5]UKIVAS Registry, United Kingdom.*

Background: Seasonal variation has been reported in primary systemic vasculitis, but the cause of this variation remains unknown. The influence of temperature, which affects neutrophil degranulation, has not been investigated.

Methods: Using the UKIVAS national vasculitis registry, residential post code and temperature data provided by UK Climate Projections (UKCP09) which is to a 5x5 km resolution, we analysed the impact of environmental temperature on vasculitis presentation. A ranking of the temperature by month covering the study duration was made to standardize seasonal temperature differences.

Results: 916 patients were included, 844 with accurate presentation dates; 727 (79.3%) had ANCA-associated vasculitis (AAV). There was an increased incidence in January and June months.



We selected two sites (Aberdeen, A, and London, L, N=284) for detailed analysis of the AAV subset. Gender and ANCA distributions were similar in A and L. The mean monthly temperature at diagnosis, one and two month(s) prior were similar for MPO- and PR3-ANCA. However, when we compared mean temperature with historic records we found that the temperature 2 months prior to diagnosis was significantly higher historically for PR3-ANCA compared to MPO-ANCA patients (ranked 62% versus 51% p=0.017), suggesting a higher temperature preceding diagnosis compared to historical means for that month.

Conclusions: There are seasonal variations in presentation of AAV, with peaks in January and June. The mean environmental temperature is historically higher 2 months before diagnosis in PR3-ANCA patients. Further investigations including other environmental factors are necessary to explain the seasonal variation of vasculitis in UK.

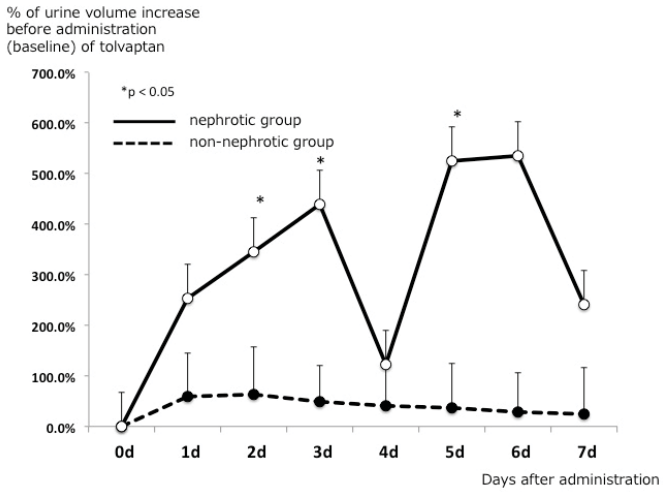
TH-PO459

Effectiveness of Tolvaptan in Chronic Kidney Disease Patients with Systemic Edema Kazuhito Fukuoka, Shinya Kaname, Yoshihiro Arimura. *1st Dept of Internal Medicine, Div of Nephrology and Collagen Disease, Kyorin Univ School of Medicine, Mitaka, Tokyo, Japan.*

Background: Tolvaptan is a V2R antagonist which has been used for heart failure in Japan. Although most of those patients complicate various degrees of kidney dysfunction, its effectiveness in CKD is unclear.

Methods: This study was a single center, retrospective study that compared the efficacy of tolvaptan in heart failure patients complicated with CKD due to diabetic mellitus, chronic glomerulonephritis and nephrosclerosis. The subjects admitted in our hospital for tolvaptan treatment from 2012 to 2013 were recruited.

Results: A total of 23 patients were analyzed. An average age was 70.3 years and the estimated GFR was 26.7±23.7ml/min/1.73m^2. None of the patients discontinued tolvaptan because of adverse events such as worsening of renal function or hypernatremia. The urine volumes increased at day 2 and reached their peak within 3 days, although the extent of the increase varied by patient. We divided the patients into two groups; the responder group (3%< body weight reduction and/or 10%< urine volume increase) and non-responder group (neither criteria fulfilled). The significant differences in urine protein excretion was observed between the two groups, but there were no differences in other clinical parameters at the baseline such as eGFR, urine and serum osmotic pressure and sodium concentration. Of note was that in 8 cases of nephrotic syndrome the urine volume were more prominently increased after administration of tolvaptan as compared to the non-nephrotic cases.



Interestingly, these nephrotic cases showed higher serum vasopressin levels before applying tolvaptan(8.9±4.67 versus0.43±0.75).

Conclusions: Tolvaptan is effective as a diuretic for some CKD patients, particularly for those with gross proteinuria with increased vasopressin levels due to intra-vascular dehydration.

TH-PO460

Galectin-3 Inhibition with GCS-100 Improves eGFR in Patients with Chronic Kidney Disease Pablo E. Pergola,[1] Geoffrey A. Block,[2] Bhupinder Singh,[3] Robert S. Cohen,[4] William T. Durham,[5] James A. Tumlin,[6] George Tidmarsh,[7] James Rolke.[7] *[1]Renal Associates PA, San Antonio, TX; [2]Denver Nephrology, Denver, CO; [3]Apex Research of Riverside, Riverside, CA; [4]Southwest Kidney Inst, Tempe, AZ; [5]Mountain Kidney and Hypertension Associates, Ashville, NC; [6]SouthEast Renal Research Inst, Chattanooga, TN; [7]La Jolla Pharmaceutical Company, San Diego, CA.*

Background: Galectin-3 has been implicated in interstitial and glomerular fibrosis resulting in progressive loss of kidney function.

Methods: GCS-100, a polysaccharide inhibitor of galectin-3, was evaluated in CKD stages 3b/4 in a multicenter, randomized, placebo-controlled Phase 2 study. 121 consenting adults received placebo or GCS-100 at doses of 1.5 or 30 mg/m^2 IV weekly for 8 weeks followed by a 4 week observation period. The primary endpoint was the change in eGFR from baseline to end of treatment versus placebo. Both T-test and ANCOVA were used for analyses.

Results: Demographics: 117 patients (65 Stage 4) completed treatment (mean age 65; 43 female). Baseline eGFR was 29.2 ml/min/1.73m2 ± 9.00 (SD). Diabetes and HTN were the most common etiologies of CKD. Efficacy: In the 1.5 mg/m^2 group, a change in eGFR of +1.26 ±0.77 versus placebo (-0.58±0.46) was observed (p=0.045). When restricted to diabetic etiology, for 1.5 mg/m^2 the change in eGFR was 2.33 ± 1.13 versus placebo (-0.53±0.56; p=0.028). Galectin-3 and K+ were significantly reduced (p=0.07 each). Uric acid and BUN were significantly reduced versus baseline (p=0.07, 0.08 respectively). No significant changes in eGFR, galectin-3, K+, uric acid or BUN were observed among those receiving 30 mg/m^2. Safety: Four SAEs occurred, 2 in the placebo, 2 in the 30 mg/m^2 group and none at 1.5 mg/m^2. None of the SAEs were drug-related.

Conclusions: Short term therapy with the galectin-3 inhibitor, GCS-100 at 1.5 mg/m^2, resulted in small but significant improvement in eGFR. If future, long term studies confirm these findings, GCS-100 could be used as a disease-modifying agent to slow and potentially reverse the renal fibrosis common in CKD.

Funding: Pharmaceutical Company Support - La Jolla Pharmaceutical Company, San Diego, CA

TH-PO461

Cathepsin L Is Essential for the Development of Proteinuria in Diabetic Mice Marjolein Garsen,[1] Angelique Rops,[1] Toin Van Kuppevelt,[2] Ton J. Rabelink,[3] Jo H.M. Berden,[1] Johan Van der Vlag.[1] *[1]Nephrology, Radboud Univ Medical Center, Nijmegen, Netherlands; [2]Matrix Biochemistry, Radboud Univ Medical Center, Nijmegen, Netherlands; [3]Nephrology, Leiden Univ Medical Center, Leiden, Netherlands.*

Background: Heparan sulfate (HS) is a negatively charged polysaccharide that is abundantly expressed in the glomerular filtration barrier. Glomerular HS expression is reduced in proteinuric patients, which is associated with an increased expression of the HS-degrading enzyme heparanase (HPSE). HPSE is essential for the development of proteinuria in diabetic nephropathy (DN). Cathepsin L (CTSL) is a lysosomal cysteine protease that cleaves pro-HPSE, thereby making it biologic active. CTSL also degrades the actin-associated protein synaptopodin in podocytes. Both mechanisms may contribute to the development of proteinuria.

Methods: To precise the exact role of CTSL in the development of proteinuria, type 1 diabetes mellitus was induced in wildtype (wt) and CTSL knockout (ko) mice by streptozotocin. Mice were sacrificed after 4, 8 and 16 weeks.

Results: Diabetic wt mice developed proteinuria, starting at 4 weeks, whereas diabetic CTSL ko mice failed to develop proteinuria. 16 weeks after induction of diabetes, CTSL expression was increased in wt mice. Simultaneously, synaptopodin expression was reduced in diabetic wt mice (p=0.1), but not in diabetic CTSL ko mice. Heparanase expression and activity were increased 4 weeks after the induction of diabetes in wt mice, and remained high until 16 weeks. Glomerular HS expression was reduced in diabetic wt mice, but preserved in diabetic CTSL ko mice at all time-points. Finally, macrophage infiltration, a characteristic of DN, was increased in diabetic wt mice, but normal in diabetic CTSL ko mice.

Conclusions: Our data suggest that CTSL is causally involved in the pathogenesis of proteinuria in DN. Most likely CTSL-deficiency causes loss of HPSE activity, which prevents the loss of glomerular HS expression and the development of albuminuria.

TH-PO462

The Role of Krüppel-Like Factor 2 in Endothelial Injury of Diabetic Nephropathy Fang Zhong,[1,2] Peter Y. Chuang,[1] Hongyu Chen,[2] Yongjun Wang,[2] Sandeep K. Mallipattu,[3] John C. He.[1,4] *[1]Dept of Medicine/Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Dept of Nephrology, Hang Zhou Hospital of Traditional Chinese Medical, Zhejiang Chinese Medical Univ, Hang Zhou, Zhejiang, China; [3]Div of Nephrology, Dept of Medicine, Stony Brook Univ School of Medicine, Stony Brook, NY; [4]Renal Section, James J Peters VAMC, New York, NY.*

Background: Elucidating mechanisms that mediate the early stage of diabetic nephrology (DN) may help us identify novel preventive and therapeutic measures for patients with DN. Krüppel-like Factor 2 (KLF2), a shear-stress inducible transcription factor, has endo-protective effects. The role of KLF2 in DN has not been studied.

Methods: Both STZ rat and mouse models were used. The endothelial cell specific Klf2 heterozygous knockout mice (KO) were used to determine the role of KLF2 in DN. HUVEC was used for in vitro studies. The expression of angiogenesis markers and podocyte-specific genes were examined in glomeruli of the mice. The KLF2 expression was examined on human biopsies.

Results: In STZ-rats, *Klf2* expression was reduced in comparison to non-diabetic rats. However, normalization of hyperglycemia by insulin treatment increased *Klf2* expression to a level higher than that of non-diabetic rats, suggesting that Klf2 might be regulated positively by hyperfiltration but negatively by hyperglycemia. In vitro, high glucose suppressed KLF2 expression in HUVEC. KLF2 expression was also reduced in human diabetic kidneys. We found that KO-STZ developed more kidney/glomerular hypertrophy and proteinuria than WT-STZ. Glomerular expression of Vegf, Flk, and angiopoietin 2 increased but expression of Flit, Tie2, and angiopoietin 1 decreased in KO-STZ compared to WT-STZ. Glomerular expression of ZO-1, glycocalyx, and eNOS was also decreased in KO-STZ. Podocyte injury was also more prominent in KO-STZ.

Conclusions: Our data suggest KLF2 is likely regulated by both shear stress and high glucose, and has protective effects against diabetes-induced glomerular endothelial cell injury at the early stage of DN. The data also highlight a potential crosstalk from the glomerular endothelial cell to podocyte.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO463

High Spatial and Spectral Resolution Mass Spectrometry Imaging Reveals Reduced AMP and Elevated ATP Levels in Glomeruli of Diabetic Mice

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Background: AMPK is a master cellular energy sensor that is activated in states of caloric depletion (high AMP/low ATP). Although renal AMPK activity is reduced in the kidney from humans with diabetic kidney disease (DKD) and in mouse models of DKD, the basis for the reduced AMPK activity is unclear. As it is difficult to quantitatively and spatially assess ATP and AMP in tissues ex vivo, we applied MALDI mass spectrometry imaging (MALDI-MSI) to localize AMP and ATP in intact kidney compartments.

Methods: Kidney tissues were obtained from 23 weeks-aged male diabetic Akita (C57BL/6J-Ins2Akita) mice and age-matched C57BL/6J control mice (n=3/group). Snap frozen kidneys were embedded in CMC and sectioned, and then thaw mounted onto an ITO glass slide. MALDI-MSI was performed in negative ion mode using a SolariX MALDI-FTICR 7.0T Mass Spectrometer and data were processed using Quantinetix™.

Results: ATP and AMP were widely distributed in the normal kidney glomerulus and tubules under standard spatial resolution. Quantitation and visualization of these analytes specifically in the glomerular compartment of control and diabetic kidneys were achieved by analysis under high-special resolution (30 μm). Importantly, relative abundance of AMP and ATP were significantly reduced by 62% and increased by 58%, respectively, in the glomeruli of diabetic mice versus control mice ($p< 0.01$).

Conclusions: Using high resolution MSI we demonstrate for the first time that the AMP/ATP ratio is markedly reduced in intact diabetic glomeruli in mice with type 1 diabetes. MALDI-MSI can provide novel spatial metabolite insights and will be a useful tool for investigating the basis for biochemical processes in kidney disease.

Funding: NIDDK Support, Other NIH Support - DP3DK094352-01, Veterans Affairs Support

TH-PO464

Pathological Angiogenesis in Diabetes Is Regulated by Slit2 and its Robo Receptors

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Background: Diabetes is the leading cause of kidney failure in the United States. A hallmark of this disease is glomerular endothelial injury, where pathological angiogenesis contributes to hyperfiltration, a common feature linked to renal injury progression. No therapies safely target this process. Slit2 has emerged as a regulator of endothelial function, eliciting either pro- or anti-angiogenic effects through its Robo1 and Robo4 receptors respectively. **Objectives:** To examine whether Slit2-Robo signaling regulates high glucose (HG)-induced glomerular endothelial responses *in vitro* and *in vivo.*

Methods: Glomerular endothelial cell (GEC) expression of Slit2, Robo1, and Robo4 was examined under normal glucose (NG) or HG conditions *in vitro*, and *in vivo* in two models of diabetic nephropathy (db/db mice, streptozotocin (STZ)-induced diabetic Wistar rats). GEC responsiveness to VEGF in tube formation and migration assays was examined under both NG and HG conditions. The effects of Robo4 deficiency (Robo4 knockout mice) and Slit2 administration (2 μg thrice weekly i.p. injections) on diabetes-induced glomerular angiogenesis and hyperfiltration were also analyzed.

Results: While healthy GEC expressed high levels of Robo4 and low levels of Robo1, Robo4 levels fell while Robo1 levels remained unchanged under HG conditions both *in vitro* and *in vivo*, leading to a reduction in the Robo4:Robo1 ratio. Slit2 protein levels were also reduced in rat glomeruli 3 wks after STZ-diabetes induction. These changes in expression of Slit2-Robo signaling components were accompanied by STZ-diabetes-induced glomerular capillary growth and hyperfiltration, both of which were attenuated by exogenous Slit2 administration. Four weeks post-STZ-induced diabetes, Robo4 KO mice kidneys demonstrated increased glomerular endothelial density compared with wild type controls.

Conclusions: Our data suggest that diabetic glomerular angiogenesis and hyperfiltration are promoted by a reduction in anti-angiogenic Slit2-Robo4 signaling, a change which can be overcome with Slit2 therapy. Our results illustrate the potential for Slit2 as a novel treatment for early diabetic nephropathy.

TH-PO465

Inhibition of ASK1/p38 Signaling Suppresses Diabetic Nephropathy in eNOS Deficient Mice

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Background: Inflammation and oxidative stress induce injury via p38 and JNK signaling and play major roles in the development of diabetic nephropathy. Apoptosis signal-regulating kinase 1 (ASK1), an upstream mediator of p38 and JNK signaling, is activated by inflammatory cytokines and oxidative stress, suggesting that ASK1 may be a therapeutic target for diabetic nephropathy.This study examined whether blockade of ASK1 can prevent the induction and progression of diabetic nephropathy in hypertensive mice.

Methods: eNOS-/- mice were made diabetic by injections of streptozotocin (5x50mg/kg/day). Groups of diabetic eNOS-/- mice received ASK1 inhibitor (0.1% GS-444217 in food) given as either an early intervention (weeks 0-6 of diabetes) or late intervention after renal injury was established (weeks 6-13 of diabetes). Control diabetic and non-diabetic eNOS-/- mice received normal chow. Glomerulosclerosis was assessed on PAS-stained kidneys using a 0-4+ score.

Results: Early intervention with GS-444217 inhibited development of diabetic glomerulosclerosis (diabetic 2.2±0.3 versus treated diabetic 1.2±0.1; $p<0.001$), despite having no effect on albuminuria, ongoing hypertension or fasting blood glucose levels. Late intervention with GS-444217 reduced glomerulosclerosis (non-diabetic 0.6±0.1; diabetic 2.4±0.3 versus treated diabetic 1.9±0.2, $p<0.01$) and improved renal function relative to diabetic controls (serum cystatin-C μg/ml: non-diabetic 320±42; diabetic week 6,765±102; diabetic week 13,701±111 versus treated diabetic week 13, 526±93; $p<0.01$). In addition, kidney gene expression of markers of fibrosis (TGF-β1, PAI-1, collagen-1) and tubular injury (KIM-1) were reduced in treated mice ($p<0.05$). Furthermore, both early and late intervention resulted in a marked suppression of p38 activation in diabetic kidneys.

Conclusions: Intervention with an ASK1 inhibitor can halt the progression of diabetic nephropathy in eNOS-/- mice, which can be attributed to reduced activation of the ASK1/p38 signalling cascade in the kidney.

Funding: Pharmaceutical Company Support - Gilead Sciences, Government Support - Non-U.S.

TH-PO466

Inhibition of Factor Xa with Rivaroxaban Ameliorates Proteinuria and Reduces Inflammatory Cytokines with Diminished Loss of Endothelial Glycocalyx in Diabetic Nephropathy

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Background: Activated factor Xa(FXa) binds to protease-activated receptors PAR-1 and PAR-2 and may exert cellular function in the endothelium. We and others have shown that the endothelium (EC) and its glycocalyx surface layer (ESL) are important for the initiation of proteinuria and contribute to the early inflammatory changes in diabetic nephropathy. We therefore tested the hypothesis that (1) treatment with a FXa inhibitor prevents or ameliorates proteinuria and inflammatory changes in diabetic mice and (2) that this effect is mediated via endothelial mechanisms.

Methods: Streptozotocin -treated mice (STZ), db/db mice and controls received either sham or rivaroxaban RIVA (300 mg or 150 mg) before (prevention) or during hyperglycemia (intervention). After 4 and 8 weeks of hyperglycemia, the animals were analyzed. Urinary albumin was measured by ELISA. Immunohistochemistry was performed on cryostat or on paraffin sections. Gene and protein expression was analyzed by real-time qPCR and western blot analysis.

Results: Treatment with RIVA reduced the hyperglycemia – induced increase in proteinuria in both STZ and db/db mice. The hyperglycemia-induced reduction in heparansulfate expression and endothelial glycocalyx density was prevented by RIVA. The hyperglycemia-induced increase of Il-6, TNF-alpha and ICAM-1 was also reduced by RIVA treatment. Macrophage infiltration in the diabetic kidney was reduced by RIVA. Lastly, the increased pERK in diabetic glomeruli was diminished by RIVA.

Conclusions: In hyperglycemic mice thetreatment with the factorXa inhibitor rivaroxaban reduces albuminuria and has a significant anti-inflammatory effect. This effect seems to be mediated by a stabilization of the endothelial glycocalyx by rivaroxaban.

Funding: Pharmaceutical Company Support - Bayer

TH-PO467

Mitochondrial Protection Preserves Kidney Function in Swine Metabolic Syndrome

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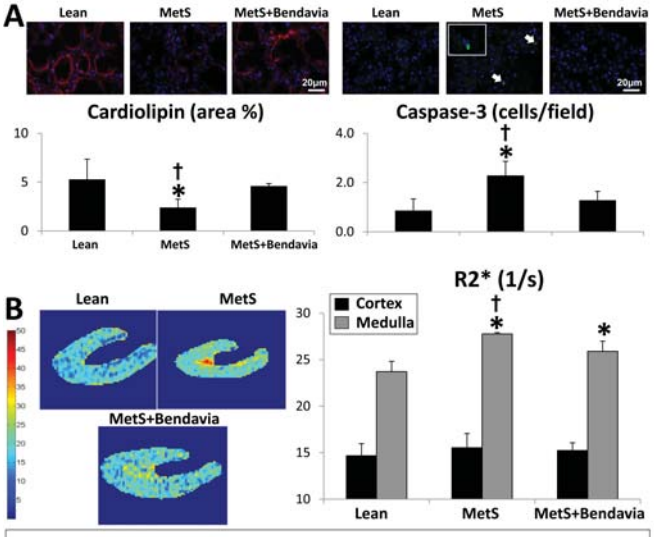
Background: The metabolic syndrome (MetS) induces kidney hyperfiltration, which may accelerate progression to chronic renal failure. Mitochondrial dysfunction might be associated with MetS, thus strategies aimed to preserve mitochondria may attenuate MetS-induced renal structural and functional injury. Bendavia™, a peptide targeting the mitochondria, is reno-protective in experimental ischemic nephropathy. We hypothesized that chronic treatment with Bendavia would attenuate hyperfiltration-elicited renal tissue injury and dysfunction in swine MetS.

Methods: Pigs were studied after 16 weeks of diet-induced MetS (n=6), MetS treated for the last 4 weeks with Bendavia (0.1mg/kg SC q.d, n=4), and Lean controls (n=6). Single-kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were measured with multidetector CT, and oxygenation with BOLD-MRI. Mitochondrial cardiolipin content (nonyl acridine orange) and apoptosis (caspase-3) were assessed ex-vivo.

Results: Blood pressure was elevated in all MetS pigs, and their kidneys were larger compared to Lean. Regional RBF was relatively preserved, yet GFR was elevated in MetS, and medulla oxygenation fell. Bendavia normalized GFR in Bendavia-treated pigs (Table), restored cardiolipin content, decreased apoptosis, and improved medullary oxygenation (Figure).

Conclusions: Chronic treatment with Bendavia preserved mitochondrial cardiolipin, decreased apoptosis, attenuated glomerular hyperfiltration, and improved medullary oxygenation in MetS. These observations implicate mitochondrial damage in renal injury in experimental MetS, and suggest a potential role for Bendavia in preserving kidney function in MetS.

	Lean	MetS	MetS+Bendavia
Weight (kg)	70.0±4.5	93.2±1.0*	92.5±3.3*
Mean arterial pressure (mmHg)	101.3±3.9	124.2±4.0*	114.3±4.8*
Renal Volume (ml)	135.8±9.0	220.9±10.3*	228.5±14.1*
RBF (ml/min/cc of tissue)	3.8±0.3	3.9±0.3	4.0±0.6
GFR (ml/min/cc of tissue)	0.70±0.02	0.81±0.04*†	0.65±0.05



A: Fluorescent staining for cardiolipin (left, red) and caspase-3 (right, green) in renal tissue sections (DAPI=blue) showing restored cardiolipin expression and decreased apoptosis in Bendavia-treated pigs.
B: Blood oxygen level-dependent magnetic resonance imaging (BOLD-MRI) showing hypoxic regions (red), and quantification of the BOLD hypoxia index R2* in the cortex and medulla. *p<0.05 vs. Lean; †p<0.05 vs. MetS+Bendavia.

Funding: Pharmaceutical Company Support - Stealth Peptides

TH-PO468

C3a Receptor Antagonist Ameliorates Fibrosis in Type 2 Diabetic Nephropathy By Suppressing the Activation of TGF-β/smad3 Pathway

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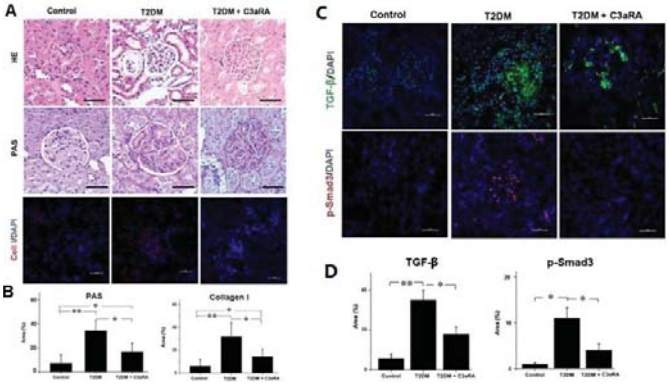
Background: Diabetic nephropathy (DN) is a serious complication for patients with diabetes mellitus (DM). Emerging evidences suggested that complement C3a was involved in the progression of DN. The aim of this study was to investigate the effect of C3a Receptor Agonist (C3aRA) on DN and its potential mechanism in type 2 diabetes mellitus (T2DM) rats.

Methods: SD rat was induced T2DM by high fat diet (HFD) plus repeated low dose of streptozocin (STZ) injection. T2DM rats were treated with vehicle or C3aRA for 8 weeks. Biochemical analysis, HE and PAS stains were performed to evaluate the renal function and pathological changes. Human renal glomerular endothelial cells (HRGECs) were cultured and treated with normal glucose (NG), high glucose (HG), HG + C3a, HG + C3a + C3aRA and HG + C3a + SIS3 (Smad3 Inhibitor), respectively. Real-time PCR, immunofluorescent staining and western blot was performed to detect the mRNA and protein levels.

Results: T2DM rats showed worsened renal morphology and impaired renal function compared to control rats, including elevated levels of serum creatinine (CREA), blood urea nitrogen (BUN) and urine albumin excretion (UACR), as well as increased levels of C3a, C3aR, Collagen I, TGF-β and p-Smad3 in the kidney of T2DM rats and C3a-treated HRGECs. In contrast, C3aRA treatment improved renal function and morphology, reduced CREA, UACR and the intensity of PAS and collagen I staining in the kidney of T2DM rats, and decreased C3a, TGF-β, p-Smad3 and collagen I expressions in HRGECs and T2DM rats.



Conclusions: C3a mediated pro-fibrotic responses and therefore aggravated renal injury in T2DM rats. C3aRA ameliorated T2DN through inhibiting TGF-β/Smad3 signaling and ECM deposition. Complement C3a receptor was a potential therapeutic target for DN.

TH-PO469

PBI-4050 Protects against Diabetic Nephropathy in Type II Diabetes

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Background: Extensive kidney fibrosis occurs in several types of chronic kidney diseases such as severe diabetic nephropathy (DN). PBI-4050, a novel first-in-class orally active low molecular weight compound, has been shown to exhibit anti-fibrotic and anti-inflammatory properties in different *in vivo* models, including CKD models. Phase II clinical trials are underway to test its efficacy in patients with CKD and DN. In the present studies, we examined whether PBI-4050 affected the progression of DN in a mouse model of accelerated type II diabetes.

Methods: eNOS$^{-/-}$*db/db* mice received vehicle (water) or PBI-4050 (200 mg/kg/day) by gavage either from 8 to 20 weeks of age (early treatment) or from 16-24 weeks of age (late treatment). A subset of mice with late treatment was kept until death to achieve a survival curve.

Results: Early PBI-4050 treatment ameliorated the fasting hyperglycemia and abnormal glucose tolerance tests seen in vehicle-treated mice. In addition, PBI-4050 led to higher blood insulin levels (20 wks: 5.29 ± 1.10 versus 2.23 ± 0.42 ng/ml, $P<0.05$. n=4). Early PBI-4050 treatment preserved kidney function, indicated by higher glomerular filtration rate (GFR at 20 wks: 0.223 ± 0.024 (n=10) versus 0.132 ± 0.031 ml/min/mouse (n=5), $P<0.05$) and lower ACR (20 wks: 1537 ± 201 versus 2808 ± 541 μg/mg, $P<0.05$. n=9). PBI-4050 had no effect on blood pressure. Late PBI-4050 treatment had similar effects on glucose control and prevention of further increases in ACR. Late PBI-4050 treatment also led to increased longevity, with 7/8 PBI-4050-treated mice alive at 25 weeks of age versus only 2/8 in vehicle-treated mice. Both early and late PBI-4050 treatment protected against progression of DN, as indicated by reduced histological glomerular injury and decreased fibrosis and collagen I and collagen IV expression. PBI-4050 inhibited kidney macrophage infiltration, oxidative stress and TGF-β-mediated fibrotic signaling pathways but increased autophagy.

Conclusions: These studies suggest that PBI-4050 attenuates development of DN in type II diabetes through improvement of glycemic control and inhibition of renal TGF-β-mediated fibrotic pathways in association with decreases in macrophage infiltration and oxidative stress and increases in autophagy.

Funding: NIDDK Support

TH-PO470

PPARalpha Inhibits the Canonic Wnt Pathway Through Regulating NOX4/ROS in Diabetic Nephropathy

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Background: Peroxisome proliferator-activated receptor alpha (PPARα) has displayed reno-protective effects in diabetic animal models. This study was to determine if the beneficial effects of PPARα on renal fibrosis are through suppressing the canonical Wnt pathway and to investigate the mechanism by which PPARα regulates the canonic Wnt pathway.

Methods: Streptozotocin (STZ)-induced diabetic PPARα$^{-/-}$ and age-matched wild-type (Wt) mice were used. Primary renal tubular cells were cultured from Wt and PPARα$^{-/-}$ mice. Reactive oxygen species (ROS) was measured using CM-H_2DCFDA. The extracellular domain deletion mutant of LRP6 (LRP6ΔN) and a point mutant of β-catenin (S37A) were used, both of which can activate the canonic Wnt pathway in the absence of Wnt ligand.

Results: Compared with diabetic Wt mice, diabetic PPARα$^{-/-}$ mice showed more severe proteinuria and prominent activation of the canonic Wnt pathway.The over-expression of PPARα using adenovirus (Ad-PPARα) significantly inhibited Wnt3a-induced increases of phosphorylated LRP6 levels and transcriptional activity of β-catenin. Ad-PPARα has no inhibitory effect on LRP6ΔN- or S37A-induced increases of transcriptional activity of β-catenin. LRP6 stability was decreased by the over-expression of PPARα and increased in PPARα$^{-/-}$ cells compared with Wt cells. We have reported that oxidative stress could activate the canonic Wnt pathway by stabilizing LRP6. ROS scavenger NAC dramatically inhibited Wnt3a-induced increases of phosphorylated LRP6 levels and transcriptional activity of β-catenin. Ad-PPARα significantly decreased 4-hydroxynonenal (4-HNE)-

induced ROS production. 4-HNE-induced ROS production was greater in PPARα$^{-/-}$ cells compared with Wt cells. Over-expression of NOX4, an important enzyme in ROS generation, significantly increased ROS levels and Wnt3a-induced transcriptional activity of β-catenin. Diabetic PPARα$^{-/-}$ mice showed more prominent NOX4 over-expression compared to diabetic Wt mice.

Conclusions: The inhibitory effect of PPARα on the canonical Wnt pathway is mediated, at least in part, through the destabilization of LRP6 and its antioxidant effect.

Funding: Other NIH Support - OCAST HR13-076, NIH EY018659, EY019309, EY012231 and GM104934

TH-PO471

Transcriptomic Analysis of Short Duration Endoplasmic Reticulum Stress on Renal Tubular Cells Michelle T. Barati,[1] Yan Zhang,[2] Eric C. Rouchka,[3] Michael Merchant.[1] *[1]Medicine, Univ of Louisville, Louisville, KY; [2]Neuroscience Training, Univ of Louisville, Louisville; [3]Computer Engineering and Computer Science, Univ of Louisville, Louisville.*

Background: Heat, oxidant, or endoplasmic reticulum (ER) stresses are known to induce assembly of ribonucleoprotein structures called stress granules (SG). SG's role is to salvage important proteins and RNA. We have shown diabetic nephropathy (DN) can affect renal SG formation and possibly though the Receptor for Activated C Kinase 1 (RACK1). We hypothesized that RACK1 expression may modify ER stress driven SG-scaffolding of RNA; some of which are relevant to DN.

Methods: Human proximal tubule cells (HK2) were transfected with GFP, GFP-RACK1, or GFP-dnRACK1 for 24hr, then treated with 0.5uM thapsigargin (Tg) or vehicle (DMSO) for 30min. Cells were lysed and 8-fractions isolated using density gradient ultracentrifugation (UC); then analyzed by (A) Agilent 2100 Bioanalyzer to characterize RNA size and quality, (B) immunoblot for SG marker proteins (TIA-1, G3BP), or GFP and. SG-enriched fractions were used for RNAseq analysis with a 50 bp single-end run on an Illumina GAIIx system at Cofactor Genomics (St. Louis, MO). Bioinformatics analysis was conducted by Kentucky Biomedical Research Infrastructure Network using Tophat and Metacore.

Results: Fractions 1-4 contained low MW RNA (no rRNA) and 2-3 contained SG markers (TIA1 or G3BP) and RACK1/dnRACK1 proteins. RNAseq analysis of fractions 1-3 show that Tg induced >2-fold changes (p-value <0.05) in 1,300-1,500 genes (lincRNA, antisense RNA, miRNA, and coding RNA transcripts) in each transfection condition. RACK1 transfection + Tg caused a 80+ fold increase in the coding RNA for CERCAM (ER luminal protein). dnRACK1 transfection +Tg caused a 300+ fold decrease in the coding RNA for KRAP (actin interacting protein regulating IP3R-mediated Ca++ release). The strongest difference for Tg+GFP transfections was increased coding RNA for histone H2 and H3 cluster variants.

Conclusions: ER stress and RACK1 overexpression can alter the HK2 transcriptome. Further work is needed to determine if these RNA are sequestered into SG. Metacore analysis suggests transcriptome changes may modify AMPK and PKA signaling pathways.

Funding: NIDDK Support, Other NIH Support - NIH-NIGMS P20GM103436

TH-PO472

Chronic HIV Infection Aggravates Diabetic Kidney Disease through Suppression of Sirt1 and Increase of NF-κB and Stat3-Mediated Inflammatory Response Yifei Zhong,[1] Ruijie Liu,[2] John C. He.[2] *[1]Longhua Hospital, Shanghai Univ of Traditional Chinese Medicine, China; [2]Mount Sinai Medical School.*

Background: Our recent studies suggest that chronic HIV infection aggravates diabetic kidney disease in human and in mice. Recently, we found that Sirt1 is suppressed in diabetic patients and knockout of Sirt1 in podocytes promotes NF-κB and Stat3 acetylation leading to the activation of inflammatory genes. Thus, we liked to determine here whether chronic HIV infection aggravates diabetic kidney disease through suppression of Sirt1 expression and enhancing NF-κB and Stat3 acetylation and activation.

Methods: Sirt1 expression, NF-κB and Stat3 phosphorylation and acetylation, expression of NF-κB and Satt3 target genes were determined in podocytes infected with HIV with or without high glucose and in kidneys from HIV-1 transgenic mice with or without diabetes.

Results: HIV infection of podocytes suppressed Sirt1 expression and induced acetylation and phosphorylation of both NF-κB and Stat3. It also induced expression of NF-κB and Stat3 target genes. High glucose together with HIV infection induced further suppression of Sirt1 and activation of NF-κB and Stat3 target gene expression in podocytes. In Tg26 mice, Sirt1 expression was also suppressed and NF-κB and Stat3 acetylation was enhanced. Expression of NF-κB and Stat3-mediated pro-inflammatory genes was also increased. In HIV-infected diabetic kidney, there was an additive effect on the expression of NF-κB and Stat3-mediated pro-inflammatory genes in HIV-infected diabetic mice. And then we developed a new Sirt1 agonist which suppressed significantly Sirt1 activity and NF-κB and Stat3 acetylation. In vivo, we found that this Sirt1 agonist improves kidney injury in diabetic Tg26 mice.

Conclusions: These findings suggest that chronic HIV infection could aggravate diabetic kidney disease through suppression of Sirt1 and increase of NF-κB and Stat3 acetylation leading to activation of NF-κB and Stat3–mediated pro-inflammatory gene expression in diabetic kidney. Therefore, restoration of Sirt1 expression and inhibition of acetylation of NF-κB and Stat3 could be potential therapy for HIV-infected patients with diabetes.

TH-PO473

Mice Transgenic for Apolipoprotein C1 Develop Proteinuria and Nodular Glomerulosclerosis Pascal Bus,[1] Rosalie Bor,[1] Jimmy F.P. Berbée,[2] Jan A. Bruijn,[1] Louis M. Havekes,[2] Emile De Heer,[1] Hans J. Baelde.[1] *[1]Dept of Pathology, Leiden Univ Medical Center, Leiden, Zuid-Holland, Netherlands; [2]Dept of Endocrinology and Metabolic Diseases, Leiden Univ Medical Center, Leiden, Zuid-Holland, Netherlands.*

Background: An association between an *APOC1* polymorphism and the development of diabetic nephropathy has been identified and confirmed in several meta-analyses.In this study we investigate the role of apolipoprotein (apo)C1 in the development of glomerular damage and the role of macrophages in this process.

Methods: Mice transgenic for human apoC1 (apoC1-tg mice) and control wild-type littermates were generated and sacrificed at various ages. Proteinuria was measured using dipstick analysis. Kidneys were sectioned and stained for apoC1, FA-11 (macrophages), WT1 (podocytes), collagen types I, IV and VI and fibronectin. The number of macrophages and podocytes in addition to the glomerular damage and glomerular hypertrophy, were scored. Correlations were assessed using the Pearson's correlation test.

Results: ApoC1-tg mice developed proteinuria (+ to ++) whereas all control mice were negative. Mesangial matrix expansion started at 13 months of age and progressed into nodular glomerulosclerosis at 15 - 16 months of age. Lesions consisted of collagen types I, IV and VI and fibronectin. The amount of podocytes per glomerulus was found to be decreasing with age. An increase of glomerular macrophages was observed at 15 weeks of age and continued to increase in time. These macrophages expressed high amounts of apoC1. There was a strong linear correlation between the number of podocytes, the number of macrophages, glomerular hypertrophy, glomerular damage and age ($P < 0.001$).

Conclusions: Our data demonstrated that apoC1-tg mice develop proteinuria and nodular glomerulosclerosis with increased accumulation of collagen types I, IV and VI and fibronectin in the extracellular matrix, similar to lesions found in patients with diabetic nephropathy. As the increase of apoCI-expressing macrophages in the glomerulus precede glomerular damage, these macrophages may play a crucial role in the development of glomerular damage in apoC1-tg mice.

TH-PO474

A Soluble Guanylate Cyclase Activator Protects from Diabetic Nephropathy in the ZSF1Rrat Paul Harrison, Hongxing Chen, Kathleen A. Lincoln, Hong Wang, Nicholas F. Brown, Holly Clifford, Hu Sheng Qian, Glenn A. Reinhart, Diane Wong, Christopher Sarko, John Ginn, Jehrod Brenneman, Todd Bosanac, Ryan M. Fryer, Jeremy G. Richman, Carine Boustany, Steven S. Pullen. *Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.*

Background: Therapies which restore cyclic GMP levels within the kidney are hypothesized to slow disease progression. We evaluated the effect of BI 703704, a soluble guanylate cyclase (sGC) activator, on the progression of diabetic nephropathy.

Methods: BI 703704 was administered at 0.3, 1, 3 and 10 mg/kg/day to male ZSF1 obese rats for 15 weeks, during which mean arterial pressure (MAP), heart rate (HR), and urinary protein excretion (UPE) were determined. At term, histological assessments of glomerular and renal interstitial lesions were performed in addition to markers of myofibroblast activation (a-SMA) and tubular damage (KIM-1). Renal cyclic GMP (cGMP) levels were also quantified as a measure of target engagement.

Results: BI 703704 resulted in sGC activation as evidenced by dose-dependent increases in renal cGMP levels (Veh: 18 ± 2, BI 703704 at 0.3, 1, 3 and 10 mg/kg: 17 ± 2; 24 ± 3; 24 ± 2 and 29 ± 3 pmol/kg, respectively). sGC activation resulted in dose-dependent decreases in UPE (Veh: 463 ± 58, BI 703704 at 0.3, 1, 3 and 10 mg/kg, 328 ± 55; 339 ± 24; 258 ± 41 and 105 ± 26 mg/day, respectively). Importantly, these effects were accompanied by a significant reduction in the incidence of glomerulosclerosis (from 16% to 71% dose-dependently) and interstitial lesions (from 14% to 67% dose-dependently). Decreased expression of tubulo-interstitial a-SMA (66-68%) and KIM-1 (from 38% to 72%) was also observed following sGC activation. Decreased MAP (13.5 mmHg) and increased HR (26 bpm) were only observed at the highest dose of BI 703704.

Conclusions: These results support the efficacy of a sGC activator in preventing the progression of diabetic nephropathy. Importantly beneficial effects were observed at doses that did not significantly alter MAP and HR.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim Pharmaceuticals, Inc.

TH-PO475

Nicotinamide n-Methyltransferase, an NAD Metabolic Enzyme, Protects Proximal Tubular Cells From Lipotoxicity Yuki Tanaka,[1] Shinji Kume,[1] Hisazumi Araki,[1] Masami Kanasaki,[1] Shin-Ichi Araki,[1] Daisuke Koya,[2] Masakazu Haneda,[3] Takashi Uzu,[1] Hiroshi Maegawa.[1] *[1]Medicine, Siga Univ of Medical Science, Otsu, Japan; [2]Diabetology and Endocrinology, Kanazawa Medical Univ, Kahoku, Japan; [3]Metabolism and Biosystemic Science, Asahikawa Medical Univ, Asahikawa, Japan.*

Background: Free fatty acid-bound albumin (FFA-albumin) filtered from glomeruli is reabsorbed in the proximal tubule cells (PTCs). Lipotoxicity induced by FFA-albumin may be one of the major causes of renal failure, especially in the diabetic condition. Nicotinamide adenine dinucleotide (NAD) metabolism has been focused as a therapeutic target for modern diseases. We examined the role of NAD metabolism in the renal lipotoxicity.

Methods: mRNA expression levels of 19 candidate enzymes were analyzed in the kidneys of a FFA-albumin-overload mouse model and cultured PTCs stimulated by FFA-albumin. The functional role of an identified enzyme was also examined.

Results: Of 19 enzymes, mRNA expression level of nicotinamide n-methyltransferase (NNMT) significantly increased in both the kidneys and cultured PTCs stimulated with FFA-albumin. NNMT-overexpressing cultured PTC was established using a retrovirus-mediated gene transfer system. Overexpression of NNMT inhibited FFA-albumin-induced apoptosis; determined by cleavage of caspase 3 and annexin-V-positive cells in FACS analysis. 1-methylnicotinamide (1-MNA) is the methylated product by NNMT. Intracellular 1-MNA concentration decreased in the kidneys and PTCs stimulated FFA-albumin, which was reversed by overexpression of NNMT. Supplementation of 1-MNA significantly inhibited FFA-albumin-induced apoptosis in cultured PTCs, and ameliorated apoptosis, inflammation determined by the expression levels of F4/80 and MCP-1, and fibrosis determined by the expression levels of fibronectin and PAI-1 in the kidneys of FFA-albumin-overload mouse model.

Conclusions: We identified NNMT as a novel enzyme that can reduce proximal tubular cell damage from lipotoxicity. NNMT and its metabolic product, 1-MNA, may become a new target to protect renal damage from lipotoxicity in diabetes.

TH-PO476

Sulforaphane Protection from Diabetic Nephropathy Is Possibly Mediated by Increasing Nuclear Factor (Erythroid-Derived 2)-Like 2 Transcriptional Regulation of Metallothionein via Inhibition of Histone Deacetylase2 Activity Hao Wu,[1,2] Zhiguo Zhang,[2,3] Lili Kong,[1,2] Yangwei Wang,[1,2] Paul N. Epstein,[2,4] Lining Miao,[1] Lu Cai.[2,4] *[1]Nephrology, 2nd Hospital of Jilin Univ, Changchun, Jilin Province, China; [2]Pediatrics, Univ of Louisville, Louisville, KY; [3]Cardiology, 1st Hospital of Jilin Univ, Changchun, Jilin Province, China; [4]Pharmacology and Toxicology, Univ of Louisville, Louisville, KY.*

Background: Sulforaphane (SFN) prevents diabetic nephropathy (DN) via upregulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) expression and function. However, how Nrf2 protects the kidney from diabetes remains unclear. Gene array analysis revealed that SFN drastically induced metallothionein (MT) which had been repeatedly reported to prevent DN; Reportedly SFN was able to inhibit histone deacetylases (HDACs) and HDAC inhibitor could up-regulate MT expression; Therefore, we hypothesize that SFN protects kidney from diabetes by inhibiting HDACs, which increases the acetylation level at and the occupancy of Nrf2 at the promoter of MT gene, to transcriptionally up-regulate MT expression.

Methods: Mouse model of type 2 diabetes was induced by high-fat diet followed with a single injection of STZ and were then treated with and without SFN for 4 months.

Results: Diabetic mice exhibited albuminuria, renal fibrosis, inflammation, and oxidative damage along with increased HADC activity, effects significantly alleviated by SFN treatment along with Nrf2 and MT upregulation. Mechanistically SFN significantly reduced the binding amount of HDAC2 at MT1 promoter, increased histone acetylation and Nrf2 levels at MT1 promoter in diabetic kidney, resulting in MT transcriptional up-regulation. Immunohistochemical staining of diabetic kidneys showed the same location of Nrf2 and MT expression predominantly in renal tubules with diabetes-induced oxidative damage, inflammation and fibrosis.

Conclusions: These results suggest that SFN-mediated up-regulation of Nrf2 followed by epigenetically transcriptional up-regulation of MT expression may play a critical role in its renal protection from diabetes.

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TH-PO477

Peroxiredoxin 2 Deficiency Exacerbates Diabetic Nephropathy via an Induction of Inflammation, Apoptosis and Phenotype Transition of Renal Tubules Eun Sun Ryu, Hyun-Soo Shin, Hack Sun Choi, Kyu Bok Choi, Duk-Hee Kang. *Div of Nephrology, Ewha Womans Univ School of Medicine, Seoul, Korea.*

Background: Enhanced ROS generation is known to play a key role in the pathogenesis of DN. Peroxiredoxins (PRDXs) are the most recently identified family of antioxidant enzymes that catalyze the reduction reaction of peroxides, such as H_2O_2. Our preliminary study revealed an abundant expression of PRDX2 in renal tubular cells, which was up-regulated rapidly with an exposure to HG. We therefore investigated whether PRDX2 deficiency aggravated the progression of DN. Based on a growing body of evidence indicating the importance of renal tubular cells as a pro-fibrotic/inflammatory mediator in DN, we examined the role of PRDX2 on phenotype transition, inflammation and apoptosis of renal tubular cells.

Methods: Diabetes was induced by the injection of STZ in PRDX2 KO and WT mice. Renal functional parameters such as BUN, creatinine and albuminuria were measured with an assessment of renal pathology, inflammatory cell infiltration, apoptosis of tubular cells. In-vitro study was also conducted in NRK52E with an evaluation of phenotype transition, inflammatory markers and apoptosis under HG condition. Effect of PRDX2 gene silencing or overexpression was also investigated.

Results: PRDX2 deficiency in diabetic mice resulted in higher BUN, albuminuria and more accelerated interstitial fibrosis compared to WT mice despite a comparable hyperglycemia. E-cadherin in renal cortex of diabetic PRDX2 KO mice was decreased with an increase in α-SMA and apoptosis of renal tubular cells. NAC administration ameliorated functional and morphologic changes in diabetic PRDX2 KO mice. Interestingly, PRDX2 gene silencing decreased E-cadherin with an increased fibronectin and MCP-1 in NRK52E cells, which was further evident with HG. HG-induced alterations in E-cadherin, fibronectin and MCP-1 were alleviated with PRDX2 over-expression in NRK52E cells. HG-induced apoptosis was also ameliorated by PRDX2 over-expression.

Conclusions: PRDX2 is a key anti-oxidant enzyme which plays an important role in protecting the kidney against HG-induced inflammation, apoptosis and phenotype transition of renal tubular cells in diabetic nephropathy.

TH-PO478

BMP7 Reduces Tubular Inflammation and Oxidative Stress in Diabetic Nephropathy Ruixi Li, Wai Han Yiu, Hao-Jia Wu, Dickson W.L. Wong, Loretta Y.Y. Chan, Miao Lin, Joseph C.K. Leung, Kar Neng Lai, Sydney C.W. Tang. *Div of Nephrology, Dept of Medicine, The Univ of Hong Kong, Queen Mary Hospital, Hong Kong, China.*

Background: Bone morphogenetic protein-7 (BMP7) has been reported to confer renoprotective effects in acute and chronic kidney disease models, but its potential role in type 2 diabetic nephropathy remains unknown.

Methods: Primary human proximal tubular epithelial cells (PTECs) were growth-arrested and exposed to glycated human serum albumin (AGEs) with or without BMP7. Nine-week-old db/db mice and their db/m littermates underwent uninephrectomy (Unx) or sham operation, and received BMP7 (300 μg/kg body weight) or vehicle intraperitoneally every other day for 8 weeks before sacrifice.

Results: In cultured human PTECs, exposure to AGEs induced overexpression of ICAM1, MCP1, IL-8 and IL-6, involving activation of p44/42 and p38 MAPK signaling. BMP7 dose-dependently attenuated AGE-induced upregulation of ICAM1, MCP1, IL-8 and IL-6 at both mRNA and protein levels. Moreover, BMP7 suppressed AGE-induced p38 and p44/42 MAPK phosphorylation and reactive oxygen species production in PTECs. Compared with vehicle control, Unx db/db mice treated with BMP7 for 8 weeks had significantly lower urinary albumin-to-creatinine ratio (3,549±816.2 μg/mg versus 8,612±2,037 μg/mg, p=0.036), BUN (33.26±1.09 mg/dL versus 37.49±0.89 mg/dL, p=0.006), and renal cortical expression of ICAM1 and MCP1 at both gene and protein levels. In addition, BMP7-treated animals had significantly less severe tubular damage, interstitial inflammatory cell infiltration, renal cortical p38 and p44/42 phosphorylation, and lipid peroxidation.

Conclusions: BMP7 attenuates tubular pro-inflammatory responses in diabetic kidney disease by suppressing oxidative stress and multiple proinflammatory signaling pathways including p38 and p44/42 MAPK. Its potential application as a therapeutic molecule in diabetic nephropathy warrants further investigation. *This study is supported by a General Research Fund of the Research Grants Council (Grant number: HKU7770/09M) of Hong Kong, and the National Basic Research Program of China 973 program no. 2012CB517600 (no. 2012CB517606).*

Funding: Government Support - Non-U.S.

TH-PO479

Pioglitazone Suppresses miR-124 Leading to Alleviates Dysfunction of Glomerular Endothelial Cells Induced By High Glucose Hui Peng,[1,2] Yuanqing Li,[1,2] Yan-Ru Chen,[1] Jun Zhang,[1] Meirong Zhong,[1] Tan-Qi Lou,[1] Zhaoyong Hu.[2] *[1]Nephrology Div, Dept of Medicine, The third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Nephrology, Dept of Medicne, Baylor College of Medicine, Houston, TX.*

Background: We previously reported that ROCK1 activation in glomerular endothelial cells (GEnCs) is one of the key mechanisms leading to diabetic nephropathy (DN). We also found that miR-124 was decreased in urinary sediment of DN patients, indicating the involvement of miR-124 in DN. According to bioinformatic analysis, ROCK1 contains in its mRNA 3'UTR the combining sites of miR-124. These findings led us to investigate the role of miR-124 in regulation of ROCK1 and the development of DN.

Methods: We assessed miR-124 expression in glomeruli of diabetic db/db mice and in patients with DN. We also examined how high glucose (HG) inhibits the expression of miR-124 in GEnCs, as consequence, leading to ROCK1 up-regulation and GEnCs dysfunctions including down-regulation of tight junction proteins and increased permeability in monolayer of GEnCs. Furthermore, we investigated how pioglitazone (PIO) promotes miR-124 expression and hence, suppressing ROCK1 activation and the development of DN.

Results: Using hybridization in situ, we found miR-124 expression decreased in glomeruli of DN patients, which was confirmed by SmartFlare miR-124 in the glomeruli of db/db and db/m mice, especially in endothelial cells. Meanwhile, HG decreased miR-124 in cultured GEnCs, accompanied with overexpression of ROCK1 protein but not mRNA. Redistribution of tight junction, and hyperpermeability of monolayer cells occurred under HG culture, all of which, however, were alleviated when miR-124 was overexpressed in GEnCs. Furthermore, PIO administration rescued HG-induced down-regulation of miR-124 as well as the dysfunctions of GEnCs.

Conclusions: our results show miR-124 decreased in the glomerular endothelia of DN, which is downregulated by HG. The downregulation of miR-124 results in ROCK1 up-regulation, and causes GEnC dysfunctions. The down-regulation of miR-124 can be rescued by PPAR-γ agonist Pioglitazone, providing a novel mechanism of preventing glomerular endothelial injury under DN.

Funding: Government Support - Non-U.S.

TH-PO480

Diabetic Nephropathy (DN) or Diabetic Nephritis (DNs): Inflammasome, Existed in Both Kidney of DN Patients and db/db Mice, Was Rereduced by NF-κB Inhibitor and Therefore Ameliorated the DN

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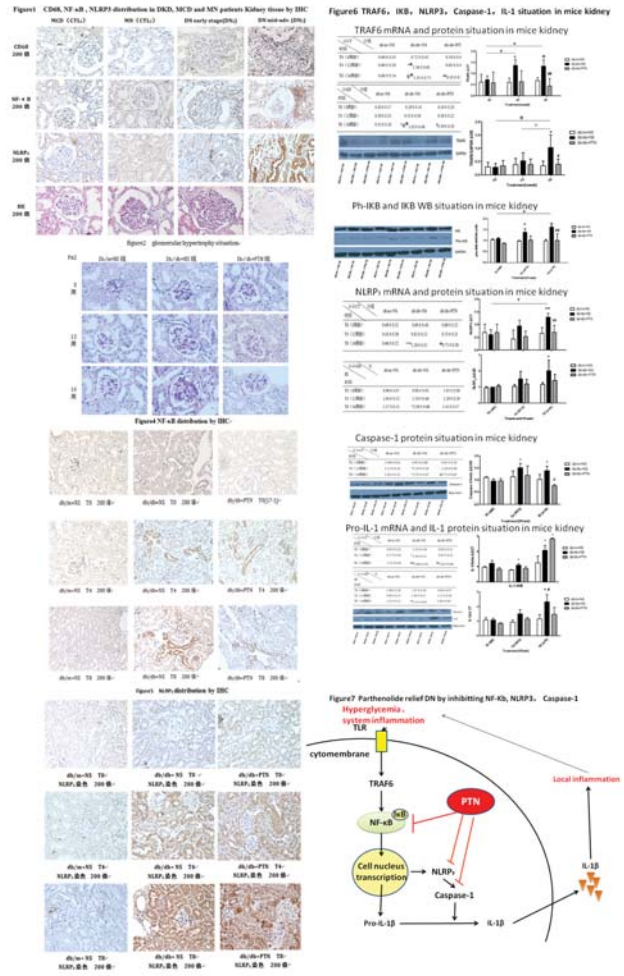
Background: To confirm whether or not the NF-κB inhibitor parthenolide, could relief the DN through inflammasome and NF-κB.

Methods: Retrospective analysis 37 DN patients by kidney biopsy, in which 7 of them (DN1) showing only glomerular hypertrophy and the others (DN2) showed as middle-advanced stage. 10 MCD and 15 MN patients were taken as control. CD68, NF-κB and $NLRP_3$ inflammasome pathway were detected through IHC. Meanwhile, 42 eight weeks of age db/db mice and 15 db/m mice were used and divided into 3 groups: 1) db/m+saline (NS), 2) db/db+NS and 3) db/db+parthenolide (PTN). TRAF6—IκB—NF-κB and $NLRP_3$—Caspase-1—IL-1 were detected by IHC, WB, Rt-PCR and IHC at time point 0 week (T0), T4 and T8 respectively.

Results: Most of DN patients have a elevation of hsCRP (6.6±12.1mg/L) compared with MCD and MN patients. CD68 and NF-κB showed the positive result in DN1 group. While the $NLRP_3$ was found only in advanced stage DN2 group. In db/db mice, the level of urinary albumin, blood glucose, serum MCP-1, IL-6 and glomerular hypertrophy is significantly higher than db/m mice ($p < 0.05$). And the expression of TRAF6, ph-I-κB, NF-κB, $NLRP_3$, Caspase-1 and IL-1 (WB T0 1.07±0.20, T4 1.49±0.51, T8 2.33±0.83) increased in db/db mice. All these parameters got decreased after PTN (IL-1 WB T0 0.84±0.07, T4 1.15±0.18, T8 1.46±0.81) (in T8 $P<0.05$). PTN may ameliorate the kidney inflammations in db/db mice.



Conclusions: There was inflammation in both DN patients and db/db mice kidney. NF-κB pathway and $NLRP_3$ pathway were involved in. Parthenolide, the NF-κB inhibitor, could relief both the clinical and pathological characters of DN by these pathways.

TH-PO481

High-Fat Diet-Induced Renal Disease Is Aggravated in Clusterin Deficient Mice

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Background: We examined the role of clusterin in high fat-induced renal disease in high fat-fed clusterin knockout (KO) and wild-type mice.

Methods: The Seven-week-old male C57BL/6 wild-type and the clusterin KO mice were fed a normal chow diet or a high-fat diet for 12 weeks. Kidney tissues were stained with periodic acid Schiff (PAS) for mesangial expansion and trichrome for collagen fibrosis. Gene expression was measured using real time polymerase chain reaction and protein levels were measured using Western blotting.

Results: Body weight and fat mass were increased in both wild-type and clusterin KO mice after being fed high-fat diet, but there was no difference between wild-type and clusterin KO mice. The plasma levels of lipid were increased by high-fat diet, but there was no difference between the two groups. However, triglyceride contents of the kidney were markedly elevated in the high fat-fed clusterin KO mice when compared with high fat-fed wild-type mice. High-fat diet increased mesangial area and tubulointerstitial fibrosis in both groups. Deficiency of clusterin in the high-fat group considerably increased mesangial matrix accumulation and tubulointerstitial fibrosis compared to the high fat-fed wild-type mice. Under a high-fat diet, clusterin KO mice exhibited a significant rise in urinary albumin-to-creatinine ratio, when compared with high fat-fed wild-type mice. The protein levels of clusterin in wild type mice were increased by high-fat diet. High-fat diet increased the gene expression of scavenger receptor A1 and lectin-like oxidized low-density lipoprotein receptor-1 in clusterin KO mice, but decreased peroxisome proliferator-activated receptor α and acyl-Coenzyme A oxidase compared with high fat-fed wild type mice. Proteins level of sterol regulatory element-binding protein and megalin were also significantly higher in high fat-fed clusterin KO mice than in high fat-fed wild-type mice.

Conclusions: These results suggest that clusterin may have protective effects on renal disease caused by high-fat diet.

TH-PO482

Decreased Autophagy Exacerbates the Development of Renal Hypertrophy and Diabetic Nephropathy

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Background: Renal hypertrophy is an early characteristic of diabetic nephropathy (DN) that may contribute to the pathogenesis in DN. Autophagy is a cellular process of degradation of cytoplasmic contents, including dysfunctional organelles and proteins. Recent studies have suggested the dysregulation of autophagy in DN. However, role and regulation of autophagy in DN, especially in DN-related renal hypertrophy, is largely unknown.

Methods: In this study, autophagy was examined in kidneys of diabetic Akita mice and STZ-induced B6 mice, and in high glucose treated renal proximal tubular cells (RPTC). The role of autophagy was further examined by using proximal tubule-specific Atg7-deficient (PT-Atg7-KO) mice.

Results: Autophagy was shown to be suppressed in diabetic kidney tissues and in high glucose-treated tubular cells, which was accompanied by decreased expression of autophagy genes, such as LC3 I and LC3 II. In cultured renal tubular cells, autophagy inhibition by chloroquine induced hypertrophy. Both Akita and STZ-induced diabetic mice showed tubular hypertrophy, which was further exacerbated by the deletion of Atg7 from proximal tubules. Moreover, DN-associated renal tubular injury and apoptosis were significantly increased in PT-Atg7-KO mice.

Conclusions: The results indicate that autophagy is suppressed in DN. The suppression of autophagy contributes to tubular hypertrophy and subsequent kidney injury.

TH-PO483

The Role of NEPRILYSIN in Diabetic Nephropathy

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Background: Hyperglycemia is a manifestation of diabetes that leads to diabetic nephropathy, which progresses to end-stage renal disease that requires dialysis or kidney transplant. There is a critical need for new therapeutic approaches to preserve kidney function.

Results: Our recent data demonstrate that NEPRILYSIN is downregulated in kidneys in several models of diabetes, including mice and medaka fed a high fat diet (HFD) and rats treated with streptozotocin. Results were confirmed by proteomics, western blot, and subcellular immunohistochemical analysis. NEPRILYSIN is a membrane metallo-endopeptidase expressed in the glomerulus and apical membrane of proximal and distal tubules. It regulates peptides modulating lipid metabolism and glucose maintenance. Downregulation of renal NEPRILYSIN reduced levels of ANGIOTENSIN-(1-7). Subsequent treatment with TELMISARTAN, an ANGIOTENSIN II receptor blocker, restored angiotensin-(1-7) levels and modulated nephropathy and hyperglycemia induced by *NEPRILYSIN* knockdown in medaka. We also confirmed that NEPRILYSIN protein localization in human kidney sections is lower in kidneys from patients with diabetic

nephropathy than in kidneys from healthy patients. Moreover, we discovered that *NEPRILYSIN* knockdown in both mice and medaka adults resulted in diabetic nephropathy, and produced decreased kidney function in response to hyperglycemia.

Conclusions: These data support our hypothesis that NEPRILYSIN in the kidney is indispensable for preventing diabetic nephropathy and modulating hyperglycemia by maintaining a balance between ANGIOTENSIN-(1-7) and ANGIOTENSIN II. Our combination of an innovative discovery-based approach followed by genetic validation in adult animal models will provide mechanistic insight into the roles of NEPRILYSIN, and unveil the potential of new therapeutic approaches.

TH-PO484

Chronic Nicotine Exposure Augments High Fat Diet-Induced Renal Oxidative Stress: Potential Relevance to Augmented Renal Lipotoxicity in Obese Smokers Istvan Arany, Samuel Hall, Dustin Reed, Mehul P. Dixit. *Pediatrics, Univ of Mississippi Medical Center.*

Background: Obesity and smoking are independent risk factors in development and progression of chronic kidney disease that involve free fatty acid (FFA)- and nicotine (NIC)-dependent induction of renal oxidative stress and consequent injury. However, the renal risk in obese smokers is elusive. In this work we tested the hypothesis that NIC exposure exacerbates high fat diet-associated renal oxidative stress in mice and in a corresponding in vitro model of proximal tubule cells.

Methods: 8-9-week-old male C57BL/6J mice had ad libitum access to either normal diet (ND: 13% kcal from fat) or high fat diet (HFD: 39.7% kcal from fat) and drinking water that contained either 2% saccharine (vehicle) or 200 mg/ml nicotine-bitartrate in 2% saccharine (NIC) for 12 weeks when markers of renal function (creatinine), injury (KIM1) and oxidative stress (HNE content, MnSOD expression) were determined. In vitro, renal proximal tubule cells (NRK52E) were treated with 100 μM oleic acid (OA) in the presence or absence of 200 μM NIC: ROS production, LDH release as well as promoter activity of MnSOD were determined.

Results: HFD increased body weight by 15±2.7 g versus 3.5±1.13. NIC did not affect this increase significantly but further augmented the already high circulating free fatty acid level in obese animals by 35%. Kidneys of HFD mice showed sign of oxidative stress (↑HNE) as well as injury (↑KIM) that were exacerbated by NIC (↑30 and 40%, respectively). HFD increased renal expression of MnSOD (5-fold), which was significantly attenuated (↓40%) in the presence of NIC. On the other hand, renal function (serum creatinine) remained normal. In vitro, NIC augmented OA-dependent mitochondrial ROS production and consequent mitochondrial depolarization/injury (↑50%) but suppressed OA-dependent induction of the MnSOD promoter (↓50%).

Conclusions: NIC augments renal oxidative stress and consequent injury by increasing FFA-dependent production of mitochondrial ROS and by inhibition of MnSOD transcription. Our results call attention to potential adverse effects of smoking/NIC use (NIC patches, E-cigarettes) on obesity-associated renal lipotoxicity.

TH-PO485

Transgenic Mice Over-Expressing Human Gremlin Develop Higher Renal Damage in Experimental Diabetes Alejandra Droguett,[1] Vanessa Marchant,[1] Yenniffer Sanchez,[1] Graciela Valderrama,[1] Maria Eugenia Burgos,[1] Bredford Kerr,[2] Daniel Carpio,[1] Marta Ruiz-Ortega,[3] Jesus Egido,[3] Sergio A. Mezzano.[1] *[1]Nephrology, Univ Austral Valdivia, Valdivia, Los Rios, Chile; [2]Centro Estudios Cientificos, Valdivia, Los Rios, Chile; [3]Nephrology, Fundación Jiménez Díaz, Univ Autónoma, CIBERDEM, Madrid, Spain.*

Background: Gremlin is a glycoprotein that acts as a mediator of Transforming Growth Factor beta (TGF-β) profibrotic events. There is in vitro evidence that a high glucose level induces Gremlin expression in mesangial cells and it has been shown that allelic Gremlin depletion attenuates experimental diabetic kidney disease. Additionally, we have described that Gremlin is over expressed in biopsies from patients with diabetic nephropathy (DN), co-localized with TGF-β, suggesting a role for Gremlin in this nephropathy.

Methods: To study the *in vivo* role of Gremlin in DN, we developed a streptozotocin (STZ) diabetic model in transgenic mice expressing human Gremlin in proximal tubular epithelial cells. In this experimental model, the mice developed blood glucose between 300 and 500 mg/dl. The urinary albumin /creatinine ratio (ACR), determined at week 20 was significantly increased in the diabetic animals, however, no significant differences between transgenic (TG/STZ) and wild type (WT/STZ) mice were observed.To assess the level of kidney damage, renal tissue was analyzed by light microscopy (PAS and Masson staining), electron microscopy (EM) and qPCR.[/italic]

Results: At glomerular level, TG/STZ mice had significant thickening of the basement membrane, increased mesangial matrix and podocytopenia (WT-1 staining) versus WT/STZ mice. At tubulointerstitial level, TG/STZ animals showed increased cell infiltration (CD3, F4/80 staining) and mild interstitial fibrosis. Additionally, the molecular analysis demonstrated a significant decrease expression of podocin and increased expression of inflammatory and fibrosis markers (MCP-1, TGF-b 1, Col1a1, and α-SMA).

Conclusions: Together, these results show that transgenic mice overexpressing Gremlin develop higher diabetic renal damage and suggests that Gremlin plays a role in the development of glomerular and tubulointerstitial injury. FONDECYT 1120480.

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TH-PO486

Insulin Prevents Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)-Stimulation of Renal Angiotensinogen Gene Expression, Hypertension and Kidney Injury in Diabetic Mice Shaaban Abdo,[1] Anindya Ghosh,[1] Isabelle Chenier,[1] Janos G. Filep,[2] Julie R. Ingelfinger,[3] Shao-Ling Zhang,[1] John S.D. Chan.[1] *[1]Research Center, CRCHUM, Univ of Montreal, Montreal, QC, Canada; [2]Res. Ctr, Maisonneuve-Rosemont Hosp., Montreal, QC, Canada; [3]Pediatr Nephrol Unit, Mass Gen Hosp., Boston, MA.*

Background: We previously reported that Nrf 2 stimulates renal angiotensinogen (Agt) gene expression in Akita mice. We tested whether insulin inhibits Nrf2 stimulation of renal Agt gene expression and thus prevent hypertension and kidney injury in Akita mice.

Methods: Male Akita mice were studied from 10-16 weeks of age with or without insulin implants from week 12. Age- and sex-matched non-Akita served as wild type (WT) controls. We performed insulin clamp studies in WT mice using constant intravenous insulin infusion (10 mU/min/kg) plus variable rate of glucose infusion to clamp glycemia at ~ 8 mM for 3 hours (euglycemic-hyperinsulinemic clamp). WT mice infused with saline served as controls. Kidneys were examined by light microscopy and immunohistochemistry were performed for Agt and Nrf2. Gene and protein expression in renal proximal tubules (RPTs) was evaluated by qPCR and immunoblotting, respectively.Immortalized rat renal proximal tubular cells (IRPTCs), stably transfected with rat Agt or Nrf2 gene promoter, were also studied in vitro.

Results: Treatment of Akita mice with insulin normalized blood glucose levels, hypertension and renal oxidative stress, inhibited renal Nrf2 and Agt geneexpression, attenuated renal hypertrophy, glomerular hyperfiltration and tubulointerstitial fibrosis. In WT mice subjected to euglycemic-hyperinsulinemic clamp, renal Agt and Nrf2 gene expression were down-regulated as compared to saline-infused mice. In vitro, insulin inhibited Nrf2 and Agt gene promoter activity induced by high glucose media via the p44/42 MAPK signaling pathway. Transfection with small interfering RNA of Nrf2 enhanced insulin inhibition of Agt gene promoter activity in IRPTCs.

Conclusions: Our data indicate that insulin prevents hypertension and attenuates kidney injury, at least in part, through suppressing Nrf2 stimulation of renal Agt gene transcription in Akita mice, independent of its glucose lowering action.

Funding: Government Support - Non-U.S.

TH-PO487

The Dysregulation of LDL Receptor Pathway Induced by Inflammation Contributes to Podocyte Injury in Diabetic Nephropathy Kun Ling Ma, Yang Zhang, Jing Liu, Wu Yu, Zebo Hu, Bi-Cheng Liu. *Inst of Nephrology, Southeast Univ, Nanjing City, Jiangsu Province, China.*

Background: Diabetic nephropathy (DN) is accepted as a chronic inflammatory disease and often accompanied with lipid disorder. Inflammation and lipid disorder play crucial roles in synergistically accelerating the progression of DN. This study aimed to investigate the potential mechanisms of inflammation with lipid disorder in podocyte injuries of DN via *in vivo* and *in vitro* studies.

Methods: Male non-diabetic db/m mice and diabetic db/db mice were randomly divided into four groups: db/m, db/m+casein, db/db, and db/db+casein for 8 weeks. Casein was subcutaneously injected to induce chronic inflammation. In in vitro study, podocytes were treated with cholesterol loading or IL-1β without or with high concentration of glucose. The levels of inflammatory cytokines in plasma and kidneys were checked by enzyme-linked immunosorbent assay and Western blotting. The renal pathology was checked by pathological staining and electron microscopy. Immunofluorescent staining and Western blotting were used to check the protein expressions of podocyte specific molecules, inflammatory cytokines, and low density lipoprotein receptor (LDLr) related molecules in kidneys.

Results: The kidney morphological changes, podocyte injuries, and epithelial mesenchymal transition (EMT) were more significant in casein-injected db/db mice compared to db/db mice and the control. Moreover, inflammation increased lipid droplet accumulation in kidneys of db/db mice, which was resulted from the increased protein expressions of LDLr, sterol regulatory element–binding protein (SREBP) cleavage activating protein (SCAP), and SREBP-2 in kidneys of db/db mice. In vitro studies further demonstrated that the inflammation increased lipid accumulation in podocytes and induced podocyte EMT, which were correlated with inflammation-mediated increased expressions of LDLr, SCAP, SREBP-2, and enhanced translocation of SCAP/SREBP-2 complex from endoplasmic reticulum to Golgi in podocytes.

Conclusions: Inflammation induced lipid accumulation and EMT of podocytes through the dysregulation of LDLr pathway, which contributed to podocyte injuries and accelerated the progression of DN.

TH-PO488

Comparison of GLP-1 Analog versus DPP4 Inhibitor in the Acute Hemodynamic and Renal Effects Xiaoyan Zhou,[1] Chin-Hu Huang,[1] Julie Lao,[1] Alessandro Pocai,[1] Michael J. Forrest,[2] Olga Price,[2] Gail M. Forrest,[2] Sophie Roy,[1] David E. Kelley,[1] Kathleen A. Sullivan.[1] *[1]Dept of Cardiometabolic Diseases, Merck & Co., Kenilworth, NJ; [2]Dept of In Vivo Pharmacology, Merck & Co., Kenilworth, NJ.*

Background: Glucagon-like peptide 1 (GLP-1) analogs and dipeptidyl peptidase-4 (DPP4) inhibitors belong to a new class of anti-hyperglycemic agents known as incretin-based therapies that have shown beneficial effects on the cardiovascular system. However,

there is limited information on their renal effects. We compared the acute effects of a GLP-1 analog, liraglutide, versus a DPP4 inhibitor, MK-0626, on renal function and hemodynamic parameters in rats. Furthermore, we evaluated the GLP-1 dependent effects of MK-0626 by infusing GLP-1 to obtain varying sustained plasma levels of GLP-1 in an acute experimental paradigm.

Methods: Three ascending doses of Liraglutide (3, 9, and 27 nmol/kg) or MK-0626 (1 mg/kg) either alone or with GLP-1 (2.4, 4.8, or 9.6 pmol/kg/min) was administered to the rats. Blood pressure (BP) and heart rate (HR) were recorded from an indwelling catheter. Glomerular filtration rate (GFR) and renal blood flow (RBF) were assessed by Inulin and para aminohippurate clearance, respectively. Renal excretory function was assessed by urine collection from the bladder.

Results: Liraglutide or MK-0626 plus GLP-1 evoked significant diuretic (up to 7 fold) and natriuretic (up to 50 fold) responses and increased GFR (1.3~1.5 fold). The natriuretic and diuretic response evoked by Liraglutide was dose-dependent; MK-0626 plus GLP-1 caused natriuresis and diuresis that correlated with active plasma GLP-1 levels. MK-0626 either alone or with GLP-1 increased RBF (1.2~1.5 fold). The high dose of Liraglutide or MK-0626 plus a high dose of GLP-1 also increased HR (~7.5%), whereas BP was not affected.

Conclusions: Our results demonstrated that Liraglutide and MK-0626 may have beneficial effects on renal sodium and water handling; additionally, the DPP4 inhibitor favorably affected renal hemodynamics by increasing RBF. However, high doses of Liraglutide or supraphysiological levels of GLP-1 may adversely affect cardiovascular system by increasing HR.

Funding: Pharmaceutical Company Support - Merck & Co.

TH-PO489

Dapagliflozin Ameliorates Diabetic Nephropathy by Oxidative Stress in Akita Mice Takashi Hatanaka, Daisuke Ogawa, Naoto Terami, Hiromi Tachibana, Naoko Nishii, Jun Wada, Hirofumi Makino. *Dept of Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Recently, sodium glucose cotransporter 2 (SGLT2) inhibitors has been prescribed as a novel therapeutic approach for treating type 2 diabetes. SGLT2 inhibitors have been reported to be also effective for type 1 diabetes.

Methods: We examined the effects of Dapagliflozin (Dapa)on early stage of diabetic nephropathy using a mouse model of type 1 diabetes. We also investigated the protective effects of Dapa on cultured renal cells usingmurine proximal tubular epithelial (mProx24) cells. Four groups of male Akita and C57BL6/J mice were used in this study. Eight-week-old Akita mice were treated with 1.0 mg / kg Dapa or insulin for 12 weeks. Diabetic Akita control group and non-diabetic C57BL/6J control group were not treated. We measured blood glucose and adjusted insulin dose to maintain blood glucose levels as same as Dapa treated group.

Results: Compared with non-treated Akita mice, Dapa and insulin improved blood glucose, hemoglobin A1c (Dapa: 6.68±0.46%; insulin: 6.53±0.33%; non-treated: 9.18±0.63%; $p<0.05$), and urinary albumin excretion (Dapa: 22.6±6.5 μg / mgCr; insulin: 26.2±9.1 μg / mgCr; non-treated: 91.3±11.0 μg / mgCr; $p<0.05$). Urine volume and water intake were significantly increased in Dapa treated group compared with those in insulin treated group, but, there were no differences in body weight, creatinine clearance, and blood pressure between the two groups. Interstitial fibrosis and mesangial matrix accumulation were evaluated by histological analysis.Dapa and insulin inhibited interstitial fibrosis and mesangial matrix accumulation of the kidney. Oxidative stress was evaluated by dihydroethidium and NADPH oxidase 4 staining. Compared to insulin, Dapa improved oxidative stress. Moreover, Dapa suppressed oxidative stress in a dose-dependent manner in the flow cytometry with mProx24 cell. Oxidative stress was also decreased by knockdown of gene, when SGLT2 was inhibited by siRNA.

Conclusions: In conclusion, Dapa may ameliorates diabetic nephropathy by improving hyperglycemia along with inhibiting oxidative stress in Akita mice.

TH-PO490

Dapagliflozin AmelioratesGlucose Homeostasis and Diabetic Nephropathy in *db/db* Mice Naoto Terami, Daisuke Ogawa, Takashi Hatanaka, Hiromi Tachibana, Atsuko Nakatsuka, Naoko Nishii, Jun Wada, Hirofumi Makino. *Dept of Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Inhibition of sodium glucose cotransporter 2 (SGLT2) has been reported as a novel therapeutic approach for treating diabetes. However, the effect of SGLT2 inhibitors on the kidney is unknown. In addition, whether SGLT2 inhibitors have an anti-inflammatory or antioxidative stress effect is still unclear. To resolve these issues, we evaluated the effects of the SGLT2 inhibitor, dapagliflozin, using a mouse model of type 2 diabetes and cultured proximal tubular epithelial (mProx24) cells.

Methods: Eight-week-old male *db/db* mice were treated with 0.1 or 1.0 mg/kg of dapagliflozinfor 12 weeks. Body weight, blood glucose, hemoglobin A1c, urinary albumin excretion, creatinine clearanceand blood pressure were measured. Mesangial matrix accumulation andinterstitial fibrosis in the kidney and pancreatic β-cell mass were evaluated by histological analysis. Furthermore, gene expression of inflammatory mediators, such as monocyte chemoattractant protein-1, transforming growth factor-β and osteopontin, was evaluated by quantitative reverse transcriptase-polymerase chain reaction. In addition, oxidative stress was evaluated by dihydroethidium and NADPH oxidase 4 staining.

Results: Administration of 0.1 or 1.0 mg/kg of dapagliflozin ameliorated hyperglycemia, β-cell damage and albuminuriain *db/db* mice. Serum creatinine and blood pressure were not affected by administration of dapagliflozin, but glomerular mesangial expansion and interstitial fibrosis were suppressed in a dose-dependent manner. Dapagliflozin treatment markedly decreased macrophage infiltration and the gene expression of inflammation and oxidative stress in the kidney of *db/db* mice. Moreover, dapagliflozin suppressed the high-glucose-induced gene expression of inflammatory cytokines and oxidative stress in cultured mProx24 cells.

Conclusions: These results demonstrate that dapagliflozin ameliorates diabetic nephropathy by improving hyperglycemia along with inhibiting inflammation and oxidative stress in *db/db* mice.

TH-PO491

Oral Treatment with PBI-4547, a Novel First-in-Class Anti-Diabetic and Anti-Fibrotic Compound, Improves Blood Glucose Level and Kidney Function in the Diabetic db/db Mouse Model Lyne Gagnon, Kathy Hince, Liette Gervais, François Sarra-Bournet, Mikaël Tremblay, Marie-Pier Cloutier, Shaun Abbott, Jean-Simon Duceppe, Boulos Zacharie, Pierre Laurin, Brigitte Grouix. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: Worldwide, 171 million people have diabetes, and a number of complications are associated with poorly controlled hyperglycemia. Diabetic nephropathy is one of the most common complications of diabetes. The aim of this study was to investigate the effect of PBI-4547 on blood glucose, insulin level, and kidney function in uninephrectomized (NX) diabetic (db/db) mice.

Methods: Total nephrectomy of the right kidney was performed on day 0 and animals were treated with vehicle or PBI-4547 (10 and 50 mg/kg, oral once a day) from day 1 through 103. Kidney function (GFR) and kidney mesangium lesions were examined.

Results: Blood glucose was significantly increased in NX-db/db mice (45 ng/ml) compared to NX-C57BL/6 mice (less than 10 ng/ml), but reduced, in a dose dependent manner, to the normal level in PBI-4547-treated NX-db/db mice (4-7 ng/ml). Insulin secretion level was reduced overtime in the NX-db/db mice, this insulin secretion loss was reversed with the treatment with PBI-4547. Furthermore. glucose/insulin ratio was significantly reduced to normal level with PBI-4547 treatment. Blood triglyceride level was also reduced to normal level in PBI-4547-treated NX-db/db mice. Kidney function assessed by GFR (inulin clearance) was significantly reduced in NX-db/db mice and significantly increased with PBI-4547 treatment. Db/db mice had larger glomeruli with increased mesangial matrix as shown by periodic acid-Schiff staining.Mesangium lesions scores were significantly reduced in NX-db/db mice treated with PBI-4547.

Conclusions: Taken together, these results suggest that PBI-4547 offers the potential as a novel therapy for regulation of glucose metabolism and reduction of diabetic nephropathy.

Funding: Pharmaceutical Company Support - ProMetic Life Sciences Inc.

TH-PO492

Acceleration of Diabetic Nephropathy Progression in Mice via Renin AAV Delivery Shannon Marie Harlan, Matthew D. Breyer, Josef G. Heuer. *Biotherapeutic Discovery Research, Eli Lilly and Company, Indianapolis, IN.*

Background: Progress in the development of novel therapies for diabetic nephropathy (DN) has been hampered by the lack of a mouse model that completely mimics human DN. Available mouse models of DN typically lack hypertension, exhibit early changes of DN and fail to progress to end stage renal disease (ESRD). We hypothesize that inducing hypertension on diabetic models will drive progression to ESRD.

Methods: An adenoviral associated virus (AAV) that expresses mouse renin 1d (ReninAAV) was administered via retro-orbital injection on type 1 (Akita) and type 2 (db/db) diabetic models. Arterial pressure was measured by tail-cuff plethysmography. Mice were followed for 3 months post injection and kidney disease progression was monitored by weekly urine ACR (albumin creatinine ratio) and measurement of serum creatinine and kidney pathology at necropsy.

Results: In all studies, the effects of ReninAAV on arterial pressure and ACR were seen within a week and persisted for the 3 months of study (end point values for all parameters shown versus LacZAAV control). Akita/B6 mice injected with ReninAAV developed elevated arterial pressure (+33mmHg versus control), but not elevated ACR or serum creatinine, consistent with B6 mice being disease resistant. In contrast, Akita/DBA mice injected with ReninAAV had a 7.5x elevation in ACR and increased kidney pathology versus control. We further tested the ReninAAV on db/db or uninephrectomy (uNx) db/db mice. This resulted in significantly elevated ACR (11x db/db, 56x uNx db/db versus db/db controls) that translated into increased histopathology for tubular protein, interstitial inflammation and fibrosis, and glomerulopathy. ReninAAV on the db/db background appeared to be the best model, and uNx db/db mice had the most severe disease progression with increased ACR values (56,000μg/mg versus 3,467μg/mg), increased serum creatinine (0.16±0.01 versus 0.13±0.01, $p<0.04$) and interstitial fibrosis scores (2.4±0.2 versus 1.0±0.0) as compared to control uNx db/db.

Conclusions: These data support the conclusion that renin driven hypertension contributes to the progression of diabetic nephropathy in mice and is influenced by genetic background.

Funding: Pharmaceutical Company Support - Eli Lilly and Company

TH-PO493

IFN-gamma Plays an Important Role in the Progression of Diabetic Nephropathy Akiko Sato, Saori Nishio, Tatsuya Atsumi, Daigo Nakazawa. *Dept of Medicine II, Hokkaido Univ School of Medicine, Sapporo, Hokkaido, Japan.*

Background: Type 2 diabetes is the most frequent type of diabetes, and the increasing prevalence of diabetic nephropathy worldwide is a major societal issue because of the enormous expense associated with kidney replacement therapy. It has been reported that inflammatory cytokines, mainly IL-6, and IL-18 and TNF-α, are involved in the development and progression of diabetic nephropathy. IL-18 is a potent pro-inflammatory cytokine capable of inducing IFN-gamma production by TH1 cells. We hypothesized that IFN-gamma is one of the key cytokine in the progression of diabetic nephropathy. We examined the effects of IFN-gamma in experimental diabetic nephropathy.

Methods: We created IFN-gamma$^{-/-}$·db/db mice by crossing IFN-gamma$^{-/-}$ mice with db/m mice. IFN-gamma$^{-/-}$·db/db mice as the experimental group were compared with IFN-gamma$^{-/-}$·db/db mice as the control group. Body weight, blood glucose and urinary albumin/creatinin ratio (UACR) were determined from 8 weeks of age up to 24weeks of age every 4 weeks. Mice were sacrificed at 24weeks of age and samples of kidney tissue were taken for pathological analysis. The kidney tissues were stained with Periodic acid-Schiff (PAS) stain. We analyzed the proportion of mesangial area per a glomerulus. We performed Elastica-Masson staining to assess degree of interstitial fibrosis.

Results: Blood glucose levels were in excess of 300mg/dl in all of mice. At 24 weeks of age, there were no significant differences in body weights. There were significant differences in UACR ($p<0.01$), 1357.0±406.2 versus 875.8±552.3 mg/g·Cre at 24weeks of age. The proportion of mesangial area were 0.208 ± 0.02 in IFN-gamma$^{+/+}$·db/db mice, 0.164± 0.03 in IFN-gamma$^{-/-}$·db/db mice ($p<0.01$). Interstitial fibrosis was noted in IFN-gamma$^{+/+}$·db/db mice (2.78 ± 0.75%) and IFN-gamma$^{-/-}$·db/db mice (1.85 ± 0.68%). It was significantly decreased in IFN-gamma$^{-/-}$·db/db mice ($p<0.05$). The renal pathological changes in IFN-gamma$^{-/-}$·db/db mice were significantly attenuated compared with IFN-gamma$^{-/-}$·db/db mice.

Conclusions: IFN-gamma$^{-/-}$·db/db mice had less severe diabetic nephropathy. IFN-gamma may play the important role in the pathogenesis and progressions of diabetic nephropathy.

TH-PO494

Knockout (KO) of TRPC6 Attenuates Kidney Disease in Diabetic Akita Mice Liming Wang, Robert F. Spurney. *Medicine, Duke Univ and Durham VA Medical Centers, Durham, NC.*

Background: Diabetic nephropathy (DN) is the most common cause of end-stage kidney disease in developed countries (ESKD). As a result, much effort is directed at identifying new treatment strategies. Accumulating evidence suggests that the calcium-activated phosphatase calcineurin (CN) plays a key role in DN. An important source of enhanced intracellular calcium levels is the transient receptor potential channel C6 (TRPC6). Indeed, gain-of-function mutations in TRPC6 that induce sustained elevations in intracellular calcium cause familial forms of focal segmental glomerulosclerosis (FSGS). Moreover, the AT1 receptor for angiotensin II actives TRPC6 and blockade of AT1 receptors is an established treatment for DN.

Methods: We investigated the effect of TRPC6 KO on diabetic kidney disease in a mouse model of type 1 diabetes mellitus (male FVB/NJ Akita mice).

Results: Wild type (WT) and KO Akita mice had similar levels of hyperglycemia at 12 wks (482 ± 33 [WT Akita] versus 429 ± 23 [KO Akita] mg/dl; P = NS) and 16 wks of age (470 ± 17 [WT Akita] versus 444 ± 19 [KO Akita] mg/dl; P = NS). Systolic blood pressure was also similar at 12 wks of age (129 ± 8 [WT] versus 135 ± 3 [Akita] mm Hg; P=NS). Albuminuria was decreased in KO animals at 12 wks of age compared to WT Akita mice (233 ± 34 [WT Akita] versus 104 ± 16 [KO Akita] ug/24H; P=0.0012). A similar trend was observed at the 16 wk time point but the difference was not statistically significant (276 ± 45 [WT Akita] versus 205 ± 27 [KO Akita] ug/24H; P=0.16). To determine if the diabetic milieu activates CN, we measured mRNA levels of the CN target genes TRPC6 and regulator of CN 1 (RCAN1), using mRNA prepared from enriched glomerular preparations. Diabetes up-regulated both TRPC6 (1.51 ± 0.23 relative expression; P = 0.0342) and RCAN1 (32.3 ± 6.97 relative expression; P = 0.0041) compared to non-diabetic WT mice. KO of TRPC6 blocked the increase in RCAN1 (1.1 ± 0.39 relative expression; P = 0.0002).

Conclusions: These data suggest that TRPC6 plays a key role in the pathogenesis of diabetic kidney disease. Inhibition of TRPC6 is a promising target for the treatment of DN.

Funding: NIDDK Support, Veterans Affairs Support

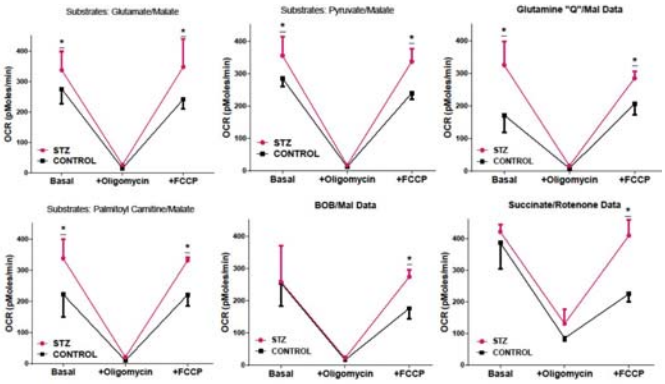
TH-PO495

Elevated Renal Mitochondrial and Proximal Tubule Oxygen Consumption in Early Diabetic Nephropathy Joanna Thomas, Scott C. Thomson, Prabhleen Singh. *Medicine-Nephrology, UC San Diego and VASDHS.*

Background: Diabetic nephropathy is the leading cause of ESRD. Early hypoxia despite an increase in renal blood flow and oxygen delivery has been described in early diabetic nephropathy. We investigated alterations in renal metabolism that may contribute to early hypoxia by examining renal mitochondrial function and tubular oxygen consumption in type 1 diabetes.

Methods: Rats were made diabetic with streptozotocin (STZ) and hyperglycemia was treated with daily insulin. Renal mitochondria and proximal tubules were harvested at 15 days following STZ. The utilization of different energy substrates by mitochondrial oxidative phosphorylation was measured via Seahorse Extracellular Flux Analyzer. Other functional and molecular analyses to evaluate oxidative metabolism and gluconeogenis were also performed. Data represents mean ± SEM.

Results: Basal and protonophore (FCCP) stimulated mitochondrial oxygen consumption were significantly increased for all substrate combinations aside from beta-hydroxybutyrate/malate and succinate/rotenone in diabetic rats (*$p<0.05$).



Ex-vivo proximal tubule oxygen consumption was also significantly increased in diabetic rats (378 ± 40 versus 264 ± 33 pmol O_2/min; $p \leq 0.04$). Renal expression of electron transport chain proteins (OxPhos Complex V-ATP Synthase, IV-1, and I-ND6) and gluconeogenesis proteins (PCK1 and FBP2) were significantly increased in diabetic rat kidneys supporting our renal oxygen consumption results ($p \leq 0.05$).

Conclusions: In early diabetes, there are significant alterations in renal metabolism including increased mitochondrial oxidative phosphorylation and renal gluconeogenesis which leads to increased tubular oxygen consumption. This likely contributes to the early hypoxia which has subsequent detrimental effects on renal function and morphology. Further investigations into therapeutic targets to reverse the metabolic phenotype are being conducted.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO496

Differential Regulation of Smad Signaling in Experimental Models of Diabetic Nephropathy Lise Thomsen,[1,2] Amaya Garcia de Vinuesa,[3] Troels Krarup Hansen,[2] Jenny Norlin, Emile De Heer,[5] Alexander Rosendahl,[1] Peter Dijke.[3] *[1]Diabetes Complications Biology, Novo Nordisk A/S, Måløv, Denmark; [2]Clinical Medicine – Dept of Endocrinology and Internal Medicine, Aarhus Univ Hospital, Aarhus, Denmark; [3]Molecular Cell Biology, Leiden Univ Medical Center, Leiden, Netherlands; [4]Translational Pharmacology, Novo Nordisk A/S, Malov, Denmark; [5]Pathology, Leiden Univ Medical Center, Leiden, Netherlands.*

Background: Diabetic Nephropathy (DN) is the leading cause of end-stage-renal-disease. In human DN, the development of glomerulosclerosis and tubulointerstitial fibrosis has been suggested to be driven by dysregulation of SMAD signalling by members of the TGF-β superfamily, which includes TGF-bs, activins and bone morphogenetic proteins (BMPs). Experimental models of DN do however not develop progressive renal disease. To identify potential novel therapeutic treatment modalities and improve translation to man, the TGF-β superfamily was evaluated in pre-clinical models.

Methods: Kidneys from db/db mice and streptozotocin induced SV129 mice were stained for H&E, PAS, Sirius red, pSmad1/5/8 and pSmad2, two downstream nuclear effectors of BMP and TGF-b/activin respectively. A transcriptional signature of 44 genes including TGF-β and BMP end-genes, BMP modulators, TGF-b-superfamily ligands and EMT markers was determined.

Results: Limited structural interstitial changes were present in the examined mouse models, except for dilation of distal tubules in the STZ mice. In these mice enhanced nuclear pSmad1/5/8 expression in glomeruli was observed, but in the whole kidney no concomitant regulation was noted on BMP target ID-1 gene level. A highly significant increase of nuclear pSmad2 expression was present in distal tubules in the STZ mice accompanied with an increased transcription level of TGF-β2 and activinA, as well as the TGF-β/activin target-gene PAI-1. By contrast db/db mice showed down-regulated pSmad2 expression in PTECs.

Conclusions: In early experimental DN where only minimal tissue remodelling occurs, nuclear pSmad2 was induced in distal tubules which correlated to dilated lumen of distal tubuli, upregulation of PAI-1, and increased transcription of TGF-β2 and activinA. Our results suggest that this pathway may provide novel therapeutic target opportunities in the treatment of DN.

Funding: Government Support - Non-U.S.

TH-PO497

Translational Validation and Molecular Characterization of Progressive End Stage Renal Disease (ESRD) in Diabetic eNOS-/-;LepRdb/db Mice Zhonghua Qi, Martin S. Cramer, Dianna L. Jaqua, Yuan Su, Yan Ding, Bhaskarjyoti Sarmah, Shannon Marie Harlan, Josef G. Heuer, Matthew D. Breyer. *BioTDR LRL, Eli Lilly and Company, Indianapolis, IN.*

Background: Most studies of animal models of diabetic nephropathy (DN) focus on renal pathology and albuminuria (ACR) as experimental endpoints, however these are not accepted endpoints for clinical registration trials for DN which requires evidence for slowing renal function loss, measured by serum creatinine (S_{cre}). Preclinical discovery of

therapeutics that confer clinical benefits have been hampered by the absence of studies demonstrating similar endpoints can be used in mice with DN. The diabetic *eNOS$^{-/-}$;LepR$^{db/db}$* mouse exhibits robust albuminuria, renal histopathologic lesions of DN, and reduced inulin clearance. The purpose of the present studies was to determine the feasibility of detecting progressive loss of GFR reflected by increased S_{cre}, to be utilized as experimental endpointinstudies of *eNOS$^{-/-}$;LepR$^{db/db}$ mice.*

Methods: ACR and Enzymatic S_{cre} (mg/dl) were measured every 4 weeks for up to 16 weeks, in *eNOS$^{-/-}$;LepR$^{db/db}$* mice up to 28 weeks of age. Kidneys were analyzed for pathology and genome-wide mRNA expression (M430_2 chip).

Results: S_{cre} was highly correlated with FITC inulin clearance (r^2=0.70). In 259 *eNOS$^{-/-}$;LepR$^{db/db}$* mice a significant increase in S_{cre} from 0.12 to 0.18 was seen ($p<0.004$). A progressive increase in S_{cre} was observed in 77/259 mice (baseline 0.14±0.01 to final 0.35±0.03, $p<0.0001$). In the remaining 70% of mice S_{cre} did not increase (0.120±0.002 to 0.11±0.002). There was no difference in urinary ACR between these two groups. Baseline S_{cre} was significantly greater in progressors ($p<0.001$). Microarray mRNA expression showed that of 679 significantly changed genes, 575 overlapped with those reported to change in human DN (e.g.Smad3, NfKB, and EGFR/RAS/ATK/mTOR pathways) versus 104 genes discordant with human DN changes.

Conclusions: These findings underscore the similarity between renal failure progression in human DN and DN in *eNOS$^{-/-}$;LepR$^{db/db}$* mice. They further support the feasibility of testing therapies that slow the rate of creatinine increase in this model.

Funding: Pharmaceutical Company Support - Eli Lilly and company

TH-PO498

Amelioration of Podocyte Injury by an Oral Adsorbent AST-120 in Metabolic Syndrome/Diabetes Rats Rieko Aoki, Fujio Sekine, Kaori Kikuchi, Shigeaki Miyazaki, Yusuke Yamashita, Yoshiharu Itoh. *Pharmaceuticals Div, Kureha Corporation, Tokyo, Japan.*

Background: Metabolic syndrome is known to be an important risk factor involved in the development of diabetic nephropathy which is a major complication of diabetes and the leading cause of end-stage renal disease. An oral adsorbent AST-120 has been used clinically as a medicine for patients with chronic kidney disease (CKD) to slow down the progression of CKD. However, there is little evidence to support therapeutic efficacy of AST-120 for early stage overt diabetic nephropathy. In this study, we aimed to assess the effect of AST-120 on SHR/NDmcr-cp rats, model rats of metabolic syndrome/ type 2 diabetes.

Methods: Male SHR/NDmcr-cp (SHR/ND) rats, aged 7 weeks, were divided into two groups. One group was administered 0% and the other group was administered 8% of AST-120 for 12 weeks in their diets. WKY rats were used as a normal control. At every 4 weeks, serum and 24-hour urine samples were collected for biomedical studies. We examined the podocyte foot process width (FPW) using Transmission Electron Microscopy as foot process effacement, and urinary podocalyxin by ELISA as podocyte injury. We also investigated the dose-response effect of AST-120 administered at 0%, 1%, 2%, 4% and 8% for SHR/ND rats.

Results: 8% AST-120-administered SHR/ND rats showed significantly lower levels of urinary protein excretion, urinary albumin excretion, urinary podocalyxin excretion and FPW compared to the unadministered SHR/ND rats. The FPW was significantly correlated with the levels of urinary protein excretion (r = 0.9498) and urinary albumin excretion (r = 0.9532). AST-120 reduced the urinary protein excretion and urinary albumin excretion in a dose-dependent manner.

Conclusions: The amelioration of podocyte injury by AST-120 may contribute to the reduction of proteinuria and albuminuria. These results indicate that the administration of AST-120 at an early stage of diabetic nephropathy would be beneficial to protect from the disease progression.

TH-PO499

Involvement of Autophagy-Triggered Apoptosis in the Advanced Glycation End Products-Induced Renal Mesangial Cell Injury Chih-Kang Chiang,[1,2,3] Tien-Fong Lu,[1] Tzu-Ming Jao,[2] Jenq-Wen Huang,[2,3] Kuan-Yu Hung,[2] Shing-Hwa Liu.[1] *[1]Graduate Inst of Toxicology, National Taiwan Univ, College of Medicine, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan; [3]Dept of Integrated Diagnostics & Therapeutics, National Taiwan Univ Hospital, Taipei, Taiwan.*

Background: There are 25-40 % of diabetic patients, who develop diabetic nephropathy (DN) within 20-25 years after the onset of diabetes. DN could lead to disability and high mortality rate in diabetic patients. Also, DN is the most common cause of end-stage renal disease (ESRD). It has been reported that the amount of advanced glycation end products (AGEs) in tissue of diabetic patients with ESRD is twice as much as diabetic patients without ESRD. Still, the influence of AGEs on renal glomerular mesangial cells remains unclear. Here, we investigated the effects of AGEs on the activation of autophagy and ER stress, which might influence the growth and function of mesangial cells.

Methods: Mouse mesangial cells (MMCs) were obtained from Food Industry Research and Development Institute. MMCs were treated with 10, 20, 40, 80 and 160 μg/ml AGEs to evaluate the cell viability by MTT assay and flow cytometry. The protein expressions were measured by Western blot assay. Furthermore, MMCs were treated with 3-Methyladenine (3MA), siATG5 and 4-phenylbutyric acid (4PBA) to study the influence of autophagy and ER stress.

Results: AGEs significantly decreased cell viability in a dose-dependent manner. And the result of flow cytometry indicated that the decreasing of cell viability might due to apoptosis. Further, AGEs induced the protein expressions of LC3-II, p-eIF2α, CHOP and caspase-3 in MMCs in a dose-dependent manner. Also, pre-treatment of 3MA reversed the decreasing of cell viability and treatment with 4PBA reversed the number of apoptotic cells. Finally, treatment with siATG5 reversed the protein expressions of LC3-II, p-eIF2α, CHOP and caspase-3. Therefore, knockdown of autophagy might reverse AGEs-induced ER stress and apoptosis.

Conclusions: These results indicated that autophagy-triggered and/or ER stress-triggered apoptosis is involved in the AGEs-induced MMCs injury.

Funding: Government Support - Non-U.S.

TH-PO500

Role of wnt/β-Catenin Signaling in Alsostertone Mediated Podocyte Injury in Experimental Obesity Related Glomerulopathy Jia-Jia Zhu, Jing Dong, Bo-Li Liu, Yang Min, Hong-Liang Rui, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

Background: To investigated role of aldosterone (ALD) in obesity-related glomerulopathy(ORG) and podocyte lesion and if wnt/β-catenin signaling was involved in such process.

Methods: 1. 24 male C57BL/6J mice were randomly divided into 3 groups (n=8) for 12w: control group (low fat diet), model group (high fat diet) and intervention group (HFD for 12w and spirolactone for last 4w). All mice were sacrificed at 12w, the serum, urine and renal tissue were collected and measured. 2. Cultured mouse podocytes (MPC) were divided into different groups with varieties of stimuli, The expression ofnephrin, podocin, podoplanin and podocalyxin of MPC were determined by real-time PCR and Western blots.

Results: 1. The model groups with HFD developed obesity and renal injury, including albuminuria, glomerular hypercellularity and higher tubule injury scores. Furthermore, the mRNA and protein expression of nephrin, podocin, podoplanin and podocalyxin in renal tissue were significantly down-regulated ($P<0.05$). Intervention with ALD receptor antagonist spirolactone could ameliorate HFD -induced obesity and renal injury.

2. Stimulation of ALD up-regulate expression of Wnt1, Wnt2b, Wnt6 in cultured podocyte and downo-regulated phosphorylatd beta-catenin. Intervention of EPL attenuated such effects. These changes also could be attenuated by Dickkopf-1(DKK1) ($P<0.05$). The mRNA and protein expression of nephrin, podocin, podoplanin and podocalyxin were significantly down-regulated ($P<0.05$) in ALD group; and these changes were attenuated by EPL and DKK1.

3. Expression of Wnt1, Wnt2b and Wnt6 were significantly increased in ORG model mice. Spironolactone therapy attenuated such effects.

Conclusions: ALD receptor antagonists can ameliorate HFD-induced obesity and renal injury in mice. ALD-induced podocyte injury is partially through activating Wnt/β-catenin signaling.

Funding: Government Support - Non-U.S.

TH-PO501

Coronary and Renal Vascular Function in Spontaneously Diabetic Leptin Deficient Mice Helena Westergren,[1,2] Julia Gronros,[3] Suvi E. Heinonen,[3] Tasso Miliotis,[3] Karin Jennbacken,[3] Alan Sabirsh,[3] Anette E. Ericsson,[3] Ann-Cathrine Jönsson-Rylander,[3] Sara Svedlund,[2] Li-Ming Gan.[1,2,3] *[1]Dept of Molecular and Clinical Medicine, Inst of Medicine, Univ of Gothenburg, Gothenburg, Sweden; [2]Dept of Clinical Physiology, Sahlgrenska Univ Hospital, Gothenburg, Sweden; [3]AstraZeneca R&D, AstraZeneca R&D, Molndal, Sweden.*

Background: Type 2 diabetes is associated with increased microvascular disease in man. Microvascular dysfunction affects both cardiac and renal function and is recognized as a main driver of cardiovascular risks. For further mechanistic understanding, there is need for validation of a relevant diabetic mouse model with coronary- and renal microvascular dysfunction.

Methods: A longitudinal study was performed in male diabetic C57Bl/6J-*lep^{ob}* mice (ob/ob) lacking atherosclerotic plaque formation, and lean controls. Cardiac echocardiography and coronary flow velocity reserve (CFVR) was measured at 10, 16 and 21 week of age by ultrasound Doppler technique. Renal blood flow velocity was measured prior to termination at 21 weeks of age and renal vascular resistance was assessed by resistivity- and pulsatility indexes. Cardiac and renal capillary density was evaluated histologically, and the nitric oxide-pathway investigated through plasma analysis of L-arginine and asymmetric dimethylarginine (ADMA).

Results: HbA_{1c} was increased in the ob/ob mice at all time points ($p<0.001$). CFVR was reduced in ob/ob mice at 16 (ob/ob: 2.0±0.4; lean: 2.6±0.5, $p<0.05$) and 21 weeks (ob/ob: 2.2±0.5; lean: 2.7±0.5, $p<0.05$) of age. Renal resistivity index (ob/ob: 0.81±0.04; lean: 0.69±0.06, $p<0.001$) as well as pulsatility index (ob/ob: 1.50±0.13; lean: 1.18±0.19, $p<0.001$) were higher in ob/ob mice. Plasma L-arginine was lowered in ob/ob mice (ob/ob: 1.2±2.2; lean: 5.5±2.2, $p<0.001$), while ADMA was unaltered. Furthermore, a significant decrease in renal capillary density was observed in the ob/ob mice ($p<0.05$).

Conclusions: Leptin-deficient ob/ob mice display cardio- and renal microvascular dysfunction in parallel with metabolic disturbances. This model appears suitable for translational mechanistic and interventional studies to further understand diabetic microvascular complications in type 2 diabetes.

Funding: Pharmaceutical Company Support - AstraZeneca R&D, Government Support - Non-U.S.

TH-PO502

Septin 7 and Nonmuscle Myosin IIA Compete for Binding to the SNARE Complex to Regulate Glucose Uptake into Podocytes Sanna H. Lehtonen,[1] Vincent Dumont,[1] Markku Lehto,[2,3] Christopher Fogarty,[2,3] Per-Henrik Groop,[2,3] Tuula A. Nyman,[1] Jukka Pekka Tienari,[3] Anita A. Wasik.[1] *[1]Univ of Helsinki, Finland; [2]Folkhälsan Research Center, Finland; [3]Helsinki Univ Central Hospital, Finland.*

Background: Podocytes are insulin responsive and can develop insulin resistance. However, the molecular mechanisms leading to the development of insulin resistance in podocytes are poorly characterized.

Methods: Protein-protein interactions were studied by co-immunoprecipitation and Duolink proximity ligation assay. Protein knockdowns were achieved by siRNAs. Glucose uptake was measured by 2-deoxy-D-glucose uptake assay, and immunohistochemistry was utilized for protein expression and localization analyses.

Results: We found previously that the small GTPase septin 7 is a negative regulator of glucose transporter trafficking in podocytes. Here we show that nonmuscle myosin IIA (NMHC-IIA) forms a complex with septin 7 and nephrin, and that depletion of NMHC-IIA by siRNA decreases insulin-stimulated glucose uptake into podocytes. Thus septin 7 and NMHC-IIA play opposite roles in glucose transport. Both septin 7 and NMHC-IIA interact with SNAP23, a plasma membrane component of the SNARE complex participating in the GLUT4 storage vesicle exocytosis. Overexpression and siRNA studies further indicate that septin 7 and NMHC-IIA compete for binding to SNAP23. Importantly, we found that depletion of septin 7 increases the complex formation between phosphorylated myosin regulatory light chain (pp-RLC) and SNAP23. Previous studies indicate that phosphorylation of RLC activates NMHC-II and thereby facilitates GLUT4 translocation and glucose uptake. We found that pp-RLC is upregulated in cultured human podocytes exposed to sera obtained from patients with type 1 diabetes with macroalbuminuria, and in the glomeruli of Zucker obese rats. Furthermore, immunohistochemical analysis revealed increased expression of pp-RLC in the podocytes of human patients with type 2 diabetes.

Conclusions: The data indicate that NMHC-IIA facilitates glucose transporter trafficking in podocytes. Activation of NMHC-IIA in podocytes in diabetes suggests that NMHC-IIA may play a role in the development of diabetic nephropathy.

Funding: Private Foundation Support

TH-PO503

MicroRNAs as Potential Targets in Diabetic Nephropathy Malte Kölling,[1,2] Joon-Keun Park,[2] Hermann G. Haller,[2] Thomas Thum,[1] Johan M. Lorenzen.[1,2] *[1]Inst of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Hannover, Germany; [2]Dept of Medicine, Div of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.*

Background: MicroRNAs (miRs) are important mediators of diabetic nephropathy (DN). In addition, PKC-β loss is associated with amelioration of long-term complications of DN, including mesangial expansion and tubulointerstitial fibrosis. We aimed to identify PKC-β related microRNAs in the induction of DN and the potential of their therapeutic modulation to prevent diabetic nephropathy.

Methods: DN was induced by streptozotocin in PKC-β knock out and wildtype mice. Microarray analysis revealed several deregulated miRs. Mesangial cells (MC) and renal fibroblasts were subjected to high glucose (HG) and TGF-β treatment. PKC-β was activated by PMA. Ruboxistaurin inhibited PKC-β. The synthetic retinoid 11302 inhibited AP-1 and was analyzed by Luciferase Assay. Real time PCR and Western Blotting were performed. Electrophoretic mobility shift assay revealed transcriptional activation. Proliferation was examined by WST-1 Assay. Elevated miR-21 was silenced in vivo in streptozotocin-induced diabetic mice by locked-nucleic acid (LNA) treatment targeting miR-21.

Results: Several miRs, including miR-21, were upregulated in diabetic mice. In mesangial cells and fibroblasts, HG, TGF-β and PMA increased miR-21 and fibrotic gene expressions (all $p<0.05$). Ruboxistaurin normalized miR-21 levels ($p=0.001$) and CTGF ($p=0.05$) in MC, confirming PKC-β as key factor. AP-1 was activated by TGF-β. Inhibition of AP-1 rescued miR-21, CTGF- and Col1a2 upregulation (all $p<0.0001$) after TGF-β stimulation in MC, indicating AP-1 as a key transcriptional activator. TGF-β induced phosphorylation of AKT, ERK and GSK-3β. Overexpression of miR-21 resulted in both upregulated inflammatory genes, including Il-6 and MCP-1, and increased proliferation. MiR-21 was succesfully silenced in vivo by LNA-21 treatment.

Conclusions: This study elucidates a pathway including PKC-β and miRs. Interfering with this pathway results in less glomerular and interstitial injury. Thus, miRs are novel targets to ameliorate diabetic nephropathy.

Funding: Private Foundation Support

TH-PO504

Identification of Novel microRNAs Regulating mTORC1-Dependent Proximal Tubular Cell Apoptosis in Diabetes Shogo Kuwagata,[1] Shinji Kume,[1] Hisazumi Araki,[1] Masami Kanasaki,[1] Shin-Ichi Araki,[1] Daisuke Koya,[2] Masakazu Haneda,[3] Takashi Uzu,[1] Hiroshi Maegawa.[1] *[1]Medicine, Shiga Univ of Medical Sciecne; [2]Medicine, Kanazawa Medical Univ; [3]Medicine, Asahikawa Medical Univ.*

Background: Proximal tubular cell (PTC) damage is associated with progressive renal dysfunction in diabetic nephropathy. Hypoxia from vascular damage is a cause of PTC damage. Cells have mechanisms to cope with hypoxia: inhibition of mTORC1 signal and activation of Hif1α transcriptional activity. Both mechanisms are also altered by diabetic stimuli such as hyperglycemia and free fatty acids (FFA). However, the effects of diabetic stimuli on hypoxic response in cells remains unclear.

Methods: We examined the effect of high glucose and FFAs on stress responses to hypoxia and apoptosis in cultured PTC, and identified novel microRNAs (miRNAs) regulating PTC apoptosis by diabetic stimuli.

Results: Hypoxia adaptively decreased mTORC1 signal and activated Hif1α transcriptional activity to protect PTC from apoptosis. Co-stimulation with high glucose and palmitate, a FFA, abolished hypoxia-induced inhibition of mTORC1 without affecting Hif1α activation, and induced apoptosis; determined by cleavage of caspase 3 and PARP, and TUNEL staining. Rapamycin, an mTORC1 inhibitor, and siRNA against Raptor, a critical component of mTORC1, inhibited co-stimulation-induced exacerbation of apoptosis from hypoxia. MicroRNAs regulate apoptosis. We thus conducted a miRNA microarray to identify novel miRNA regulating PTC apoptosis. Microarray analysis revealed five candidate miRNAs related to co-stimulation-induced exacerbation of apoptosis from hypoxia. Of these, miR-450a-5p and miR-3100-3p were upregulated and miR128-3p, miR148b-3p and miR-185-5p were downregulated. Transfection of inhibitor miRNAs against miR-450a-5p and miR-3100-3p did not influence apoptosis, but mimic miRNAs of miR128-3p, miR148b-3p and miR-185-5p significantly inhibited co-stimulation-induced exacerbation of apoptosis from hypoxia.

Conclusions: Diabetic stimuli impaired mTORC1-dependent cell adaptive mechanism against hypoxia, leading to apoptosis in PTCs. We identified novel miRNAs that can inhibit this process and may improve PTC damage in diabetes.

TH-PO505

Proximal Tubular Sodium Transporter Studies with Azilsartan Identified the New Pharmacological Cues Leading to Salt-Sensitivity Improvements Masaki Hatanaka, Satoko Yamamoto, Jun-Ya Kaimori, Hiromi Rakugi, Yoshitaka Isaka. *Dept of Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Azilsartan, the brand-new potent angiotensin receptor brocker (ARB), is reported to reduces blood pressure more strongly than candesartan and, interestingly, improve sodium sensitivity. However, the mechanism of those effects remain unclear. To explore the mechanistic insight of azilsartan effects, we examined the change in renal tubular sodium handling in mice with this drug.

Methods: 5/6 nephrectomized C57Bl6 mice were given high sodium diet and administrated orally azilsartan, candesartan, and vehicle. After 2 weeks, we examined blood pressure, sodium excretion, and renal sodium transporter protein expressions.

Results: Blood pressure decreased significantly in azilsartan group (93.9 ± 5.0mmHg) compared with candesartan (111.7 ± 2.7mmHg : $p<0.05$) and vehicle (129.7 ± 1.3mmHg:$p<0.0001$) groups. From the pressure-diuresis curve analyses, salt-sensitivity was improved in azilsartan group, but not in candesartan nor vehicle group. From immunohistochemistry and western blotting, the protein of sodium-proton exchanger 3 (NHE3), the major sodium transporter in the proximal tubule, decreased in azilsartan group, but not in candesartan nor vehicle group. However, the transcript level of NHE3 didn't decrease in azilsartan group. The other sodium transporters were not dramatically changed. The same results were obtained from opossum kidney (OK) proximal tubule cells experiments under angiotensin II loading. The farther study revealed that the azilsartan-induced decrease in NHE3 protein was partially regulated by proteasome but not by lysosome, suggesting the sodium sensitivity improvement by azilsartan was possibly through proteasomal NHE3 regulation.

Conclusions: Azilsartan, not candesartan, possibly improves salt-sensitivity by inhibiting NHE3 by increasing NHE3 proteasomal degradation. This new ARB mechanism could lead to new pharmacological cues about salt-sensitivity.

Funding: Government Support - Non-U.S.

TH-PO506

Abstract Withdrawn

TH-PO507

$GABA_B$ Receptor May Regulate the Translocation of AQP2 in the Renal Collecting Duct Kozue Takano,[1] Junichi Yatabe,[1,2,3] Midori Sasaki Yatabe,[1,3] Hironobu Sanada,[3] Tsuyoshi Watanabe,[3] Junko Kimura.[1] *[1]Dept of Pharmacol, Fukushima Med Univ, Fukushima, Japan; [2]Dept of CKD Initiatives, Fukushima Med Univ, Fukushima, Japan; [3]Dept of Nephrol, Hypertens, Diabetol, Endocrinol and Metab, Fukushima Med Univ, Fukushima, Japan.*

Background: Gamma-aminobutyric acid (GABA) induces diuresis/natriuresis and lowers blood pressure. However, its mechanism is unknown. We have shown $GABA_B$ receptor R1 and R2 subtype expressions in rat kidney. Water reabsorption in the collecting duct increases with vasopressin V2 receptor signaling coupled with Gs, which stimulates cAMP production and increases aquaporin 2 (AQP2) on the apical membrane. On the other hand, $GABA_B$ receptor is coupled with Gi/o. In this study, we examined whether $GABA_B$ receptor signal inhibits AQP2 expression and/or translocation through the inhibition of cAMP production.

Methods: To identify the localization of $GABA_B$ receptor protein expression, renal cortices of Wistar-Kyoto rats (WKY) were used for immunofluorescent staining. Expression levels of $GABA_B$ receptor R1 and R2 subtype messenger RNAs in Madin-Darby Canine

Kidney (MDCK) cells were examined by RT-PCR. Cyclic AMP assay was also performed using MDCK cells treated with arginine vasopressin (AVP)/ forskolin and GABA/ baclofen.

Results: $GABA_B$ receptor R1 subtype protein stained mainly in the arterioles and glomeruli in WKY. R2 subtype protein showed strong signal in the collecting duct. Costaining of $GABA_B$ receptor R2 subtype and AQP2 was observed on the apical side of principal cells. In MDCK cells, $GABA_B$ receptor R1 and R2 subtype mRNAs were expressed. Furthermore, the cAMP assay revealed that GABA tended to suppress cAMP stimulated by AVP. Additionally, $GABA_B$ receptor agonist baclofen significantly inhibited cAMP production pre-activated by forskolin (n=5, 67±26%, mean±SD, $p<0.05$).

Conclusions: These findings indicate the possibility that GABA signaling via $GABA_B$ receptor may inhibit the production of cAMP in the renal collecting duct and suppress the translocation of AQP2 to apical plasma membrane. This may in part be the mechanism of GABA-induced diuresis. Further analysis of $GABA_B$ and vasopressin receptor crosstalk in AQP2 regulation may elucidate novel regulatory mechanism in the kidney.

TH-PO508

Renal Mechanisms of Clozapine Induced Hypertension in Mice

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Background: Dopamine receptor subtype 4 (D_4R) antagonist clozapine is used in the treatment of schizophrenia. A common side effect of clozapine is hypertension but the mechanism is unknown. D_4R plays an important role in blood pressure (BP) regulation; deletion of the D_4R gene in mice causes hypertension.

Methods: To determine if renal mechanisms are involved in clozapine-induced hypertension, we characterized the distribution of D_4R in mouse kidney and measured BP, water and sodium balance, and renal protein expression of sodium transporters in clozapine- and vehicle-treated mice.

Results: D_4R(immunocytochemistry) was mainly located in thick ascending limb, distal convoluted tubule and cortical and medullary collecting ducts; there is slight staining of D_4R in the proximal convoluted but not straight tubule. Clozapine(20mg/kg/day) or vehicle was delivered subcutaneously to adult male C57BL/6J mice for seven days via osmotic mini-pump(n=5/grp). Inclozapine-treated mice, systolic(110±0.5, mm Hg, under anesthesia) and diastolic(76.6±1) BPs were elevated, relative to vehicle-treated mice(SBP=97.6±1.6, DBP=69.5±2.3). Food and water intake, body weight, serum electrolytes and creatinine, urine volume, and creatinine clearance were similar in the two groups. Thesodium excretion tended to decrease while sodium excretion and BP plot in clozapine-treated mice was shifted to the right of the vehicle-treated mice. Renal protein expressions(immunoblotting) of sodium hydrogen exchanger 3 and sodium phosphate cotransporter 2 were similar in clozapine- and vehicle-treated mice. However, the expressions of sodium potassium 2 chloride cotransporter (180±21, % of control) and sodium chloride cotransporter(171±22%),and the three subunits of epithelial sodium channel, α(140±7%), β(166±21%) and γ(lower band,130±7%), were greater in clozapine- than vehicle-treated mice. α-sodium potassium ATPase expression was slightly increased(118±4%) in clozapine-treated mice.

Conclusions: These findings suggest that inhibition of D_4R by clozapine increases sodium transporters in distal nephron segments. This may cause the impaired sodium excretion that may be responsible for the increased blood pressure in clozapine-treated mice.

Funding: NIDDK Support

TH-PO509

A Role of Renal Megalin in Blood Pressure Regulation

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Background: Megalin, a multi-ligand endocytic receptor abundantly expressed in the apical membrane of proximal tubule cells, is important for reabsorption of filtered proteins and peptides including ANGII which is essential for blood pressure (BP) regulation and sodium balance. Angiotensin type 1 and 2 receptors have been identified in the proximal tubule and have been implicated in regulation of proximal tubule sodium reabsorption. As elevations in the intrarenal content of ANGII have been associated with sodium retention and hypertension, we hypothesized that absence of megalin protects against ANGII-induced hypertension.

Methods: Using conditional megalin knockout (KO) mice and wild type (WT) littermate controls, we examined the effects on BP and renal sodium handling of genetic deletion of megalin in the kidney on a normal, low, and high sodium diet as well as under chronic ANGII infusion using osmotic minipumps. Blood pressure was recorded via telemetric probes and urines were collected in metabolic cages.

Results: At baseline, systolic BPs measured were significantly lower in megalin KOs compared to WT controls (111.8 mmHg versus 117.9 mmHg, n=16; $p<0.05$). The difference was greatest during night (115.8 versus 123.8 mm Hg; $p<0.01$). This was associated with increased urine output (1.9±0.38 versus 0.7±0.07 ml/24h/20gBW; $p<0.01$) and renal sodium excretion (177.5±11.6 versus 113.9±22.1 μmol/24H/20gBW; $p<0.05$) in megalin KO compared to WT. When fed a high salt diet, BP increased in both megalin KO and WT mice with a similar magnitude of BP difference between the groups. Chronic infusion of ANGII increased systolic BP in both groups, however, megalin KOs tended to have lower BP which was significantly lower than controls at several time points.

Conclusions: Loss of megalin in the proximal tubule is associated with lower baseline BP and increased renal sodium and water excretion. This is maintained both during sodium loading and ANGII infusion. Thus, megalin function may play a role in renal regulation of BP and provide a new target for intervention in BP modulation.

TH-PO510

Preserved Expression of IRS2 Mediates the Stimulatory Effect of Insulin on Renal Proximal Tubule Sodium Transport in Insulin Resistance

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Background: Insulin receptor substrate (IRS) 1 and 2 mediate distinct insulin signaling pathways. We have previously reported that while the IRS1-dependent stimulatory effect of insulin on glucose uptake into adipocytes is severely impaired, the IRS2-dependent stimulatory effect of insulin on renal proximal tubule (PT) sodium transport is completely preserved in insulin resistant rats and humans. In liver, transcription factors forkhead box class O (FoxOs) are major contributors to specific control of basal IRS2 gene transcription, whereas hyperinsulinemia in insulin resistance may suppress IRS2 expression by augmenting the expression of sterol regulatory element-binding protein (SREBP) 1. However, little is known about the regulatory mechanism of IRS2 expression in kidney.

Methods: We harvested liver and kidney cortex from Wistar rats that were either starved overnight or allowed to feed ad libitum and intraperitoneally injected with insulin (0.75 mU/g body weight) 2 hr prior to sacrifice, and Western blotting was performed to determine the protein expression levels of IRS2, FoxO1, and SREBP1. Quantitative RT-PCR was performed to determine mRNA expression of SREBP1c and IRS2 in liver and kidney in insulin resistant OLETF ratsand control LETO rats.

Results: In Wistar rats, feeding plus insulin markedly decreased the protein expression levels of IRS2 and FoxO1 by 65% and 62%, respectively, while it induced a massive increase in SREBP1 protein by 123% in liver. By contrast, feeding plus insulin did not affect the protein expression levels of IRS2, FoxO1, or SREBP1 in kidney cortex. Compared to LETO rats, OLETF rats showed enhanced expression of SREBP1c mRNA and decreased expression of IRS2 mRNA expression in liver. However, OLETF and LETO rats showed comparable expression of SREBP1c and IRS2 mRNAs in kidney cortex.

Conclusions: Unlike in liver, hyperinsulinemia failed to suppress IRS2 expression through SREBP1c-dependent negative feedback pathway in kidney cortex. The preserved expression of IRS2 in kidney cortex in insulin resistance may play an important role in hypertension associated with metabolic syndrome.

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TH-PO511

Disruption of Renal ADMA Metabolism Leads to Salt-Sensitive Hypertension

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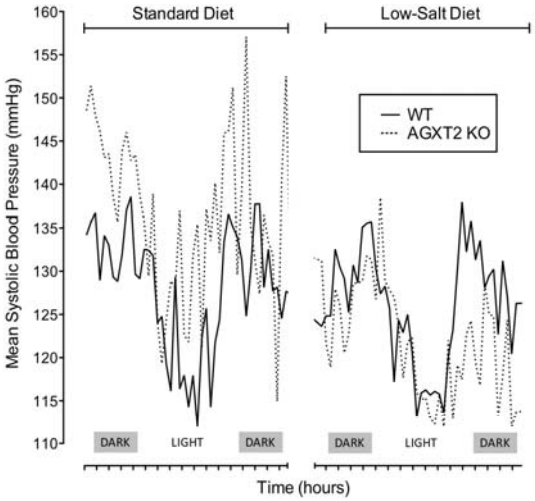
Background: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthesis found at high levels in patients with CKD and is implicated in the associated increase in cardiovascular (CV) risk. Although increases in ADMA have been proposed to cause endothelial dysfunction this has not been demonstrated at the plasma levels of ADMA observed in CKD. As NO also regulates tubular sodium (Na) handling, ADMA could act via this mechanism. We have recently demonstrated that the tubular enzyme alanine-glyoxylate aminotransferase-2 (AGXT2) regulates circulating ADMA concentrations so we examined the detailed CV phenotype in AGXT2 knockout (KO) mice.

Methods: Renal histology was examined in AGXT2 KO and wild-type (WT) mice. *Ex-vivo* endothelial responses were explored. Telemetry probes were implanted in animals at ~12 weeks of age and after recovery continuous BP recordings performed for 48 hours after 2-weeks on a standard (0.3% Na) diet and again following 2-weeks of a low-Na (0.03%) diet.

Results: Serum ADMA levels were 0.2μMol/L ($P<0.05$) higher in AGXT2 KO versus WT mice. AGXT2 KO animals had morphologically normal kidneys and no evidence of impaired renal function. There was no *ex-vivo* evidence of impaired endothelial-dependent dilatation in aortas from AGXT2 KO mice. Mean systolic BP was 8.8mmHg ($P<0.05$) higher in AGXT2 KO versus WT mice at baseline. Overall systolic BP fell by a mean of 2.1mmHg ($P<0.005$) on a low-Na diet but there was an additional 7.3mmHg ($P<0.005$) fall in the AGXT2 KO mice (Figure).



Conclusions: Vessels from AGXT2 KO mice are functionally normal *ex-vivo*. The increase in BP seen with disruption of AGXT2 is entirely abrogated by a low-Na

diet suggesting that increased Na reabsorption may underlie ADMA-induced vascular dysfunction. Increasing renal tubular AGXT2 activity might represent a novel therapeutic strategy in CKD associated vascular disease.

TH-PO512

New Candidate Genes for Salt Sensitive Hypertension Marco Simonini, Chiara Lanzani, Guido Gatti, Nunzia Casamassima, Lorena Citterio, Simona Pozzoli, Stefano Tentori, Paolo Manunta. *San Raffaele Scientific Inst, Milan, Italy.*

Background: Hypertension is an important public health problem affecting more than 60 million individuals in the U.S.A. alone. The most common form, salt sensitive hypertension (SSH), results from the complex interplay between genetic predisposition and environmental influences. Our aim is to identify new genes involved in SSH that explain the different individual variability of to a load/restriction of Na in the everyday diet.

Methods: A GWA study was performed on 321 never treated hypertensive patients, who underwent a Salt-Load test. We performed a case-control analysis by comparing the most salt-sensitive versus most salt-resistent patients. From this analysis, a list of top hits was obtained; we considered top SNPs those with p-value <1-05. The most interesting results were confirmed on a second population (120 pts).

Results: The best result was located on chr. 11 (p=4.38E-07) in the upstream region of DGAT2 gene. In the same region we found, after a process of imputation, 4 different SNPs with p-value <1E-06 not in linkage disequilibirum with the top SNP. Another interesting association was located on chr. 13 (p=5.20E-06). This SNP is on the 5' region of the gene Myosin XVI (MYO-XVI). Also in this region imputation process showed other 3 different SNPs with p-value <1E-04 associated with SSH. Finally, to understand the power on BP regulation, we performed a regression analysis (441 pts) of the difference between diastolic/systolic BP from the baseline to the end of Na-load. This analysis showed a r2 > 4% for both genes.

Conclusions: Our results suggest that DGAT2 and MYO-XVI are involved in regulation of BP after acute saline infusion in primary hypertension. DGAT2 is implicated in the development of Metabolic Syndrome (insulin resistance/diabetes, obesity, increase of blood cholesterol), which makes it a good candidate for SSH. MYO-XVI is a non conventional myosin expressed in many organs. This protein has a structural function that regulates other protein activity. Further investigation will need to understand the gene-gene interaction between these gene and others structural genes responsible of SSH.

TH-PO513

Collectrin in the Kidney Determines Salt-Sensitivity Pei-Lun Chu, Sylvia Cechova, Rosa Chan, Thu H. Le. *Div of Nephrology, Univ of Virginia, Charlottesville, VA.*

Background: Collectrin (*Tmem27*), an ACE-2 homologue and a chaperone of amino acid transporters, is expressed in the endothelium throughout the vasculature and in the renal proximal tubules and collecting duct. Its renal expression is upregulated after reduction of nephron mass and during salt-sensitive hypertension (SSH). We reported that collectrin-deficient (KO) mice have baseline hypertension that is ameliorated by supplementation with L-arginine (L-Arg). Furthermore, they have augmented SSH that is corrected by TEMPOL, a superoxide dismutase mimetic. These phenotypes are associated with impaired cellular uptake of L-Arg, impaired endothelial-dependent relaxation, decreased renal and aortic levels of endothelial nitric oxide synthase (eNOS) dimerization, and a rightward shift of the pressure-natriuresis relationship.

Methods: To begin to delineate the contribution of renal versus extra-renal collectrin on blood pressure (BP) regulation, we next assessed renal neuronal NOS (nNOS) protein levels. Furthermore, we performed renal cross-transplantation (XTP) studies (through a service provided by the Duke O'Brien Center), under normal and high salt conditions, in the following groups: 1) Wild-type (WT) mice with WT kidney (WT->WT), 2) collectrin KO mice with WT kidney (WT->KO), and 3) WT mice with collectrin KO kidney (KO->WT). BP was measured by radiotelemetry.

Results: Similar to that observed for eNOS, the activity or dimerization of neuronal NOS (nNOS) in the renal medulla is also significantly decreased in KO mice. In the renal XTP studies, the collectrin KO->WT group displayed a trend towards higher baseline systolic BP (SBP, mm Hg), but did not reach significance (WT->WT 137.3, WT->KO 133.5, KO->WT 142.4, p = not significant, n = 3-5 per group). By paired analysis, all groups had significantly higher SBP on high salt diet (HSD), compared to normal salt diet. However, collectrin KO->WT group had a statistically significantly higher SBP under HSD (WT->WT 148.9, WT->KO 151.6, KO->WT 168.6, p= 0.037, one way ANOVA).

Conclusions: Collectrin may regulate BP by influencing renal hemodynamics and/or epithelial sodium handling through its central role in mediating cellular L-Arg uptake and NOS dimerization.

TH-PO514

Intrarenal Tumor Necrosis Factor Alpha Contributes to Hypertension in Dahl Salt-Sensitive Rats Baorui Huang,[1,2,3] Kristie Usa,[3] Chun Yang,[3] Yong Liu,[3] Allen W. Cowley,[3] Niansong Wang,[1] Mingyu Liang.[3] *[1]Dept of Nephrology and Rheumatology, Shanghai Jiao Tong Univ Affiliated Sixth People's Hospital, Shanghai, China; [2]Medical College of Soochow Univ, Suzhou, Jiangsu, China; [3]Dept of Physiology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Tumor necrosis factor alpha (TNF-a) is a major proinflammatory cytokine which was reported to be elevated in hypertensive states. Inflammation plays an important role in the development of hypertension and renal injury in Dahl salt-sensitive(SS) rats. We hypothesized that TNF-a specifically in the kidney would contribute to the development of hypertension in SS rats.

Methods: Inbred SS and consomic SS-13BN rats were maintained on a 0.4% NaCl diet until 6 or 7 weeks of age.Then 4%(n=9) or 8%(n=6) NaCl diet were used for 7 days. The expression of TNF-a in the kidney were measured through real-time qPCR. Unilateralnephrectomy were performed in another 2 groups of SS rats when 6 weeks of age. And renal interstitial infusion of TNF-a inhibitor Etanercept were used for 8 or 14 days with 4% NaCl diet. Blood presure and a-SMA, miR-29,collagen genes's expression were measured. Histological sections of kidney were stained with Masson's trichrome.

Results: We found that the expression level of TNF-α in the renal medulla of SS rats was significantly increased when rats had been fed 4%(n=9, p<0.01) or 8%(n=6, p<0.01) high-salt diet for 7days.Renal interstitial infusion of Etanercept, which is an inhibitor of TNF-a,in uninephrectomized SS rats significantly attenuated the increase of blood pressure after 14 days(n=5,p<0.01) on a 4% salt diet.In addition,the Etanercept treatment for 8 days attenuated glomerulosclerosis in cortex (n=5, p<0.05). a-smooth muscle actin(a-SMA) was found significantly lower in cortex after 8 days of treatment with Etanercept (n=5, p<0.05). The miR-29 family, which has anti-fibrotic effects, tended to be increased in the kidney of SS rats after 14 days of treatment with Etanercept.

Conclusions: This study shows that intrarenal TNF-a contributes to the development of hypertension and renal injury in SS rats.

TH-PO515

Mesenchymal Stem Cells-Derived Micro-Particles Attenuate Kidney Injury in Swine Metabolic Syndrome and Renal Artery Stenosis Alfonso Eirin,[1] Xiang-Yang Zhu,[1] Kelly A. Mcgurren,[1] Hui Tang,[1] Andre J. Van Wijnen,[2] Joseph P. Grande,[3] Stephen C. Textor,[1] Amir Lerman,[4] Lilach O. Lerman.[1] *[1]Divs of Nephrology and Hypertension, Mayo Clinic; [2]Orthopedic Surgery, Mayo Clinic; [3]Dept of Pathology, Mayo Clinic; [4]Cardiovascular Diseases, Mayo Clinic, Rochester, MN.*

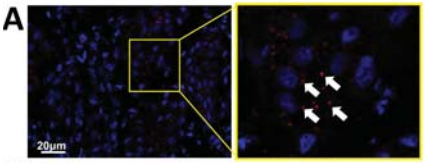
Background: Coexisting metabolic syndrome (MetS) intensifies kidney injury in renal artery stenosis (RAS). Mesenchymal stem cells (MSC) have shown renoprotective properties in experimental RAS, but generate concerns regarding their size and potential replication. MSC release membrane microparticles, including microvesicles and exosomes (Mvex), which express many of their characteristics, but the ability of Mvex to improve kidney viability in pre-clinical models remains unknown. We hypothesized that delivery of MSC-derived Mvex would attenuate kidney injury in swine MetS+RAS.

Methods: MetS pigs were studied after 16 weeks of RAS treated 4 weeks earlier with vehicle or a single intrarenal delivery of pre-labeled Mvex, derived from 2.5x10^5/Kg autologous adipose tissue-MSC; Lean and MetS Sham served as controls (n=7 each). Single-kidney function was assessed by multidetector CT, tubular injury by H&E, and fibrosis by trichrome.

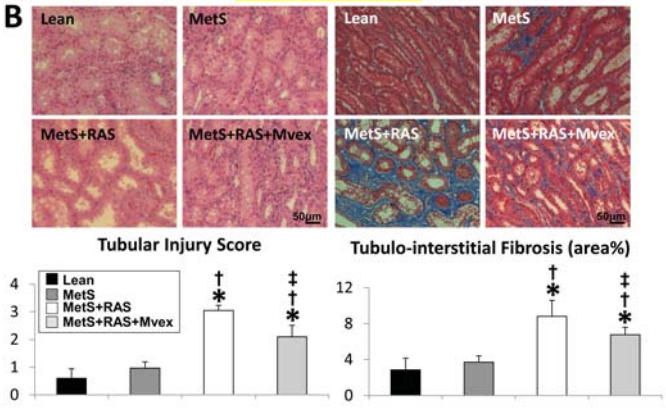
Results: Blood pressure was elevated in all MetS groups (Table) and Mvex were detected in the stenotic-kidney 4 weeks after injection (Figure). Renal blood flow (RBF) and glomerular filtration rate (GFR), which fell in MetS+RAS compared to MetS, improved in MetS+RAS+Mvex, and tubular injury and interstitial fibrosis decreased.

Conclusions: Intrarenal delivery of MSC-derived Mvex attenuates renal damage in MetS+RAS. This study suggests a novel therapeutic potential for MSC-derived Mvex in preserving the kidney in chronic experimental MetS+RAS.

	Lean	MetS	MetS+RAS	MetS+RAS+Mvex
Weight (kg)	71.4±4.0	93.4±0.9*	92.4±2.3*	90.6±3.1*
Blood pressure (mmHg)	102.8±3.6	124.2±4.0*	128.8±6.7*	120.0±3.4*
Degree of stenosis (%)	0	0	60.0±8.4*†	60.0±5.0*†
Volume (ml/min)	137.2±6.4	217.9±7.4*	182.4±8.8*†	207.4±29.1*‡
RBF (ml/min)	502.1±27.4	848.6±76.1*	628.5±27.5*†	866.1±104.1*‡
GFR (ml/min)	76.0±4.0	142.6±7.3*	100.3±7.1*†	128.7±13.6*‡



A: Red fluorescence of chloromethylbenzamido-DiI (arrows, 40X) Mvex in the stenotic kidney 4 weeks after administration. DAPI=blue.



B: Representative staining (top) and quantification (bottom) of H&E (left) and trichrome (right) for tubular injury and interstitial fibrosis. *p<0.05 vs. Lean; †p<0.05 vs. MetS; ‡p<0.05 vs. MetS+RAS .

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TH-PO516

ETS-1 in Salt Sensitive Hypertension: Interactions with the Renin Angiotensin System Wenguang Feng,[1] Phillip H. Chumley,[1] Minolfa C. Prieto,[2] Kayoko Miyata,[2] Gabriel Rezonzew,[1] Edgar A. Jaimes.[3] *[1]Univ of Alabama at Birmingham; [2]Tulane Univ; [3]Memorial Sloan-Kettering Cancer Center.*

Background: The transcription factor ETS-1 regulates the expression of a several growth factors, chemokines and cytokines. We have shown that ETS-1 is a mediator of the proinflammatory effects of Angiotensin II in the kidney (HTN'12). We also showed that ETS-1 is expressed in the cortex of hypertensive Dahl salt sensitive (DS) rats and that ETS-1 blockade reduces the severity of renal injury in DS rats (ASN'13).

Methods: We investigated the effects of ETS-1 blockade and concomitant RAS blockade on blood pressure (radiotelemetry), angiotensinogen (AGT) urinary excretion (ELISA) and protein expression (WB), and prorenin receptor expression (PRR, WB). DS rats (n=6/group) were fed a normal salt diet (0.5% NaCl, NS) or a high salt diet (4% NaCl, HS) for 4 weeks. Four additional groups (n=6) on HS received: ETS-1 dominant negative peptide (HS/DN, 10 mg/kg/day) to block ETS-1, control ETS-1 mutant peptide (HS/MU, 10mg/kg/day), the AT1 receptor blocker Candesartan (HS/ARB. 10 mg/kg/day) or a combination of DN and ARB (HS/DN/ARB).

Results: HS resulted in a significant increase in BP that was not modified by DN or MU but was reduced by ARB and DN/ARB (table). HS resulted in a significant increase in the urinary excretion of AGT that was reduced by DN and DN/ARB but not by MU or ARB. Cortical AGT expression was increased by HS and partially reduced by ARB. HS resulted in non-significant reductions in PRR and not modified by any of the treatments.

	NS	HS	HS/DN	HS/MU	HS/ARB	HS/ARB/DN
MBP (mm Hg)	104±2*	151±12	142±13	151±13	129±6*	108±1*
Urine AGT (ng/mg Creat)	17.6±2*	38.9±3	24.6±6*	54.9±10	30.2±4	12.1±3*
Cortex AGT (Fold change)	1±0.13*	1.8±0.17	1.6±0.14	1.6±0.14	1.3±0.07*	1.4±0.11
Cortex PRR (Fold change)	1±0.37	0.7±0.27	0.5±0.12	0.4±0.14	0.8±0.17	0.5±0.15

* P < 0.05 vs HS

Conclusions: In these studies we have unveiled a novel interaction between ETS-1 and the RAS that may explain at least in part the beneficial effects of ETS-1 blockade on the severity of renal injury in salt sensitive hypertension.

Funding: Veterans Affairs Support, Private Foundation Support

TH-PO517

Involvement of Endoplasmic Reticulum Stress and Autophagy in Hypertensive Kidney Disease Masahiro Takami, Yoshihisa Nakatani, Shuji Arima. *Nephrology, Kinki Univ, Osakasayama, Osaka, Japan.*

Background: Recently, involvement of endoplasmic reticulum stress (ERS) and autophagy in kidney disease such as acute kidney injury and diabetic nephropathy has been reported. However, the relation between hypertensive disease and ERS or autophagy is unclear.

Methods: We investigated whether ERS or autophagy was associated with kidney disease, and whether antihypertensive therapy removed kidney damage by the effect of ERS and autophagy in malignant hypertension model rats (malignant stroke prone spontaneously hypertensive rats: M-SHRSP).First, we examined the kidney of Wister-Kyoto rats (WKY) and M-SHRSP by microarray analysis, western blotting, immunohistochemistry and immunofluorescent staining. Then, M-SHRSP in 5 week age was medicated with hydralazine, azelnidipine, olmesartan for 2 months and were sacrificed.

Results: By microarray analysis, in 5 week's M-SHRSP (early stage), IRE1 alpha increased 2.1 folds compared with 5 week's WKY. In 10 week's M-SHRSP (late stage), LC3B decreased about 1 to 2 folds, and ATG10 increased 1.4 folds compared with 10 week's WKY. By western blotting, ER chaperones such as GRP78, ORP150, CHOP increased in the kidney of 13 week's M-SHRSP compared with WKY. LC3B decreased in the kidney of M-SHRSP. We detected ORP150 was decreased in renal cortex and CHOP was also decreased in medulla as compared with the control group. Furthermore, we confirmed that IRE1 alpha, PERK and JNK pathway were suppressed and Cleaved-caspase 3 was decreased by antihypertensive medication. LC3B-II which associated with activation of macroautophagy was increased by antihypertensive medication. LAMP2a was about 2 folds increased in olmesartan group. HSC70 was almost no change. By immunofluorescent staining, LAMP2a and HSC70, which associated with chaperone-mediated autophagy, were colocalized in the olmesartan group compared with the others.

Conclusions: We could confirm ERS and apoptotic pathway was activated in M-SHRSP, while autophagy was inactivated. By antihypertensive therapy, ERS pathway was down regulation and autophagy was up regulation.Involvement of ERS and autophagy could be considered in pathophysiology of hypertensive kidney disease, ERS and autophagy could be new therapeutic targets.

TH-PO518

Nephron Specific Deletion of Angiotensinogen Modulates Blood Pressure Nirupama Ramkumar,[1] Deborah Stuart,[1] Taiji Matsusaka,[2] Donald E. Kohan.[1] *[1]Nephrology and Hypertension, Univ of Utah, Salt Lake City, UT; [2]Inst of Medical Science, Tokai Univ Sch of Med, Kanagawa, Japan.*

Background: It is unknown if intrarenal generation of angiotensinogen (AGT) is important in blood pressure (BP) regulation. Previous studies showed that proximal tubule-specific overexpression of AGT increases BP, while proximal tubule-specific deletion of AGT using the KAP promoter-Cre transgene did not alter BP. The latter study may not have completely eliminated nephron AGT production; in addition, BP was only assessed on a normal salt diet. To evaluate this issue in greater detail, we developed mice with inducible nephron-wide AGT deletion.

Methods: Mice were generated which were hemizygous for the Pax8-rtTA and LC-1 transgenes and homozygous for loxP flanked AGT alleles to achieve nephron-specific AGT disruption after doxycycline induction. Adult Pax8-rtTA/LC-1/floxed AGT mice at 3 months of age were treated with doxycycline 2 mg/ml in drinking water for 11 days and studied 4 weeks after treatment. Blood pressure (recorded via telemetry) and metabolic balance studies were determined during 5 days of normal, high and low Na diets.

Results: Compared to controls, AGT knockout (KO) mice demonstrated significantly lower systolic, diastolic, and mean BPs on all three diets (N=4 each group). The BP reduction was most evident on a low Na diet (mean BP 107 ± 2 mmHg in controls and 88 ± 13 mmHg in AGT KO). Plasma renin concentration was higher in the AGT KO mice as compared to controls on all three diets. There were no detectable differences in weight, urine volume, urine osmolality or urine Na excretion between the controls and KO mice on all three diets, however due to variability, small differences in these parameters may not have been detected.

Conclusions: Taken together, these data suggest that nephron AGT may contribute to BP regulation and this is most evident during low Na intake.

Funding: Private Foundation Support

TH-PO519

Elastin Insufficiency-Associated Hypertension Is Mediated, at Least in Part, by Angiotensin II Type 2 Receptor Carmen M. Halabi, Beth A. Kozel, Robert P. Mecham. *Washington Univ School of Medicine, Saint Louis, MO.*

Background: Hypertension and vascular stiffness are major consequences of elastin insufficiency, as seen in patients with Williams syndrome and animal models of elastin insufficiency. Altered reactivity of resistance vessels was recently proposed to contribute to hypertension in elastin insufficiency. Specifically, mesenteric arteries of elastin insufficient mice were shown to be hypercontractile to angiotensin II (AngII) *ex vivo*. Interestingly, this hypercontractile response to AngII was mediated, at least in part, by AngII type 2 receptors (AT2R) as blockade of AT2R by PD123319 abrogated the hypercontractile response to AngII. The purpose of this study was to determine whether AT2R contribute to the hypertension seen with elastin insufficiency *in vivo*.

Methods: Elastin haploinsufficient (Eln^{het}) mice were bred to AT2R knock-out ($AT2R^{KO}$) mice. Three month-old male littermate progeny with the following genotypes ($AT2R^{WT}$;Eln^{WT},

AT2R^{KO};Eln^{WT}, AT2R^{WT};Eln^{het}, and AT2R^{KO};Eln^{het}) were used for experimental studies. Arterial blood pressure was measured in anesthetized mice via a catheter introduced in the right common carotid artery. At the end of blood pressure recording, mice were sacrificed and the left common carotid arteries and ascending aortae were isolated for compliance studies.

Results: As expected, compared to wild type (WT) mice, loss of AT2R had no effect on blood pressure or large vessel compliance in the presence of WT elastin (*AT2R^{KO};Eln^{WT}*), while isolated elastin insufficiency resulted in elevated systolic blood pressure, pulse pressure, and reduced large vessel compliance (*AT2R^{WT};Eln^{het}*). Loss of AT2R in elastin insufficient mice (*AT2R^{KO};Eln^{het}*), however, resulted in significant reduction of systolic and diastolic blood pressures, but no change in pulse pressure or large artery compliance.

Conclusions: These results provide *in vivo* evidence for a role of AT2R in mediating elastin insufficiency-associated hypertension. Furthermore, these data suggest distinct mechanisms for the development of hypertension and vascular stiffness in elastin insufficiency, as loss of AT2R reduced blood pressure, but had no effect on large artery stiffness in this mouse model.

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TH-PO520

Renal Medullary (Pro) Renin Receptor Mediates Angiotensin II-Induced Hypertension

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Background: (Pro)renin receptor (PRR) is predominantly expressed in the collecting duct and is stimulated by angiotensin II (AngII) via the COX-2/PGE2/EP4 pathway but the function of PRR is unknown.

Methods: We examined the effect of intramedullary delivery of a novel PRR decoy peptide PRO20 on AngII hypertension via an interstitial catheter chronically placed in the rat medulla and also tested the role of PRR in regulation of ENaC activity in vitro.

Results: Telemetry showed that 1-wk AngII infusion (100 ng/kg/min) elevated MAP from 108 + 5.8 to 164.7 + 6.2 mmHg and this value was lowered to 110.2 + 4.8 mmHg ($p<0.05$) by intramedullary PRO20 infusion (IMPRO) at 120μg/kg/min but was only modestly affected by intravenous PRO20 infusion (IVPRO) (day 7: 151.7 + 4.4 mmHg). Proteinuria (2.7-fold increase in urinary protein), glomerulosclerosis and interstitial fibrosis following AngII infusion were completely normalized by IMPRO so were the polyuria and downregulation of renal AQP2 protein expression. AngII infusion elevated urinary renin activity from 0.17 + 0.013 to 9.3 + 1.7 AngI ng/24h, which was reduced to 0.09 + 0.04 AngI ng/24h by IMPRO. A similar pattern of changes in renin activity was observed in the inner medulla, contrasting to opposite changes in renal cortex and plasma. α-ENaC mRNA and protein were elevated 158% and 180%, respectively, in the inner medulla but not in the cortex or plasma, and the elevations were abolished by IMPRO. By chopstick electrodes, AngII at 500 nM induced a transient 2-fold increase in amiloride-sensitive sodium transport at 5 min, which was completely abolished by PRO20. Similarly, prorenin at 10 nM exhibited a similar stimulatory effect on ENaC activity which was similarly blocked by PRO20 except that prorenin-induced increase in ENaC activity occurred at 1 min, preceding the AngII effect.

Conclusions: Renal medullary PRR mediates AngII-induced hypertension likely via amplifying the local renin response and the activation of ENaC.

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

TH-PO521

Steady-State Profiles of Multiple RAS Peptides in Mouse Plasma and Kidney - New Clues for Enzymatic Processing of Angiotensin Peptides

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Background: A major limitation of research on bioactive peptides, such as angiotensins (Ang), has been the lack of direct methods to simultaneously measure multiple components of this cascade network with enough discriminative power and sensitivity.

Methods: We used liquid chromatography tandem mass spectrometry (LC/MS-MS) to measure concurrently levels of 10 Ang peptides in plasma and kidneys from male C57BL mice (n=5) *in vivo* at a physiologically relevant range (pg/mL and pg/g, respectively).

Results: In plasma, the highest concentrations were for: Ang I (1-10) (1295±313 pg/mL) whereas the levels for the main effector peptide, Ang II (1-8) were very low (7.8±2 pg/mL). Angiotensin (1-7), the peptide formed from the cleavage of Ang II (1-8) by ACE2 was also very low (3.5±0 pg/mL). This is in keeping with low levels of circulating ACE2 activity. In contrast, the levels of plasma Ang (2-10) and Ang III (2-8) (products of Ang I (1-10) and Ang II (1-8) degradation by aminopeptidase A (APA), respectively) were relatively high (657±173 and 1047±178 pg/mL, respectively). In the kidney, the highest Ang concentrations were measured for Ang I (1-10) and Ang II (1-8) (468±96 and 307±37 pg/g, respectively). Ang II (1-8) can be converted by APA to Ang III (2-8) and by ACE2 to Ang (1-7) and the levels of these two Ang II degradation products in the kidney were relatively high (172±12 pg/g and 42±19 pg/g, respectively).

Conclusions: LC/MS-MS measurements of multiple RAS peptides unraveled the prevalent pathways of angiotensins processing which appear to be differentially regulated in kidney and plasma. Under steady state conditions, RAS peptides profiles suggest a major role of aminopeptidase A in the metabolism of angiotensin peptides both in plasma and kidney, whereas ACE2 might play a role in kidney but not in plasma angiotensin II degradation.

Funding: NIDDK Support, Private Foundation Support

TH-PO522

Impact of Renal Mass Reduction on the Effects of Angiotensin II on Renal Hemodynamics and Autoregulation in Conscious Rats

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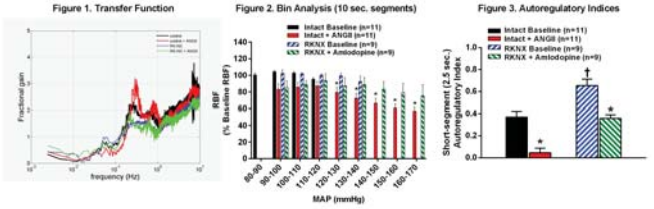
Background: Ang II is widely postulated to play a major role in CKD progression through BP-dependent and independent mechanisms. Although its effects on BP-RBF relationships and autoregulation (AR) are expected to significantly impact the pathogenesis of such injury, only limited data are available as to such effects in conscious animals with and without renal mass reduction (RMR).

Methods: BP and RBF recordings (2-3 at 200Hz for 2-4 hrs on 2-4 separate days) were obtained in conscious male rats with intact renal mass or 3/4 nephrectomy (3/4 NX) before and 48 hrs after Ang II (125ng/kg/min s.c.). The following were assesed (i) transfer functions (Fig 1); (ii) BP-RBF relationships (10 sec segments of BP and corresponding RBF averages separated into 10mmHg BP bins; Fig 2) and (iii) AR indices for adjoining 2.5 sec segments with at least 5mmHg AP difference (>1000/rat; Fig 3).



Results: Transfer function analysis showed that Ang II potentiated the myogenic response (MR) in intact rats. These potentiating effects were blunted in 3/4 NX rats with already attenuated MR. Bin analysis similarly showed that Ang II induced decreases in RBF and the exaggerated vasoconstriction seen with increasing BP in intact rats were greatly attenuated in 3/4 NX rats. AR assessment indicated a significant strengthening of AR by Ang II in both intact and to a lesser degree in the 3/4 NX rats with impaired AR.

Conclusions: These data using 3 different methodologies indicate that in contrast to studies in anesthetized rats, Ang II interacts with and potentiates myogenic AR in conscious rats with intact and to a lesser extent in RMR rats.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO523

The Anti-Hypertensive Effect of Mycophenolate Mofetil Is Mediated By Reducing Vascular Responsiveness to Angiotensin II

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Background: Hypertension after kidney transplantation is associated with poorer graft survival and higher cardiovascular risk. While glucocorticoids and calcineurin inhibitors are hypertensinogenic, mycophenolate mofetil (MMF) has anti-hypertensive properties. The mechanism of the blood pressure lowering effect of MMF is unknown.

Methods: Salt-sensitive hypertension was induced in rats by deoxyocorticosterone acetate (DOCA) and 0.9% NaCl as drinking water. 8 rats were also treated with MMF and 8 with vehicle. Blood pressure was continuously monitored using telemetry. The experiment lasted 4 weeks. Kidney homogenates were differentially centrifuged to analyze sodium transporters in plasma membrane fractions. Vascular reactivity was analyzed with Mulvany myographs, and data are expressed as % of the maximum contraction (E_{max}) to 100 mmol/L KCl.

Results: Mean arterial pressure (MAP) at baseline was identical in both groups (97±6 mmHg). DOCA-salt increased MAP in all rats, but was significantly attenuated in the MMF group (MAP after 4 weeks 107±7 versus 126±14 mmHg; $P<0.001$). MMF did not change abundance or phosphorylation status of sodium transporters. The angiotensin (Ang) II E_{max} in iliac arteries was 15.3±2.4% after MMF versus 47.4±9.6% after vehicle ($P=0.007$). Nitric oxide synthase (NOS) inhibition with L-NAME greatly enhanced ($P=0.0005$) the Ang II response in the MMF-treated animals, but the increase after vehicle was non-significant ($P=0.16$). The Ang II type 1 (AT1) receptor antagonist irbesartan prevented all Ang II induced responses, whereas only in vehicle-treated rats the Ang II type 2 (AT2) receptor antagonist PD123319 blocked the effects of Ang II. In mesenteric arteries, endothelin-1-induced constrictor effects were unaffected by MMF.

Conclusions: MMF blunts the development of salt-sensitive hypertension in experimental animals possibly by inhibiting T-cells. This may involve a selective reduction of Ang II responsiveness due to a preservation of vascular NO generation and downregulation of constrictor AT2 receptors.

TH-PO524

A "Sticky" Tag Delivers Therapeutic Angiotensin-Converting Enzyme 2 to the Kidney Glomeruli: A Novel Approach to Protect Ang II-Induced Podocyte Injury Pan Liu,[1,2] Jan A. Wysocki,[1] Minghao Ye,[1] Daniel Batlle,[1] Jing Jin.[1,2] *[1]Dept of Medicine – Nephrology/Hypertension, Northwestern Univ, Chicago, IL; [2]Feinberg Cardiovascular Research Inst, Northwestern Univ.*

Background: Angiotensin-converting enzyme 2 (ACE2) is a cell surface monocarboxypeptidase that degrades angiotensin II (Ang II) to form Ang1-7. Injection of the recombinant enzymatic ectodomain (soluble ACE2/sACE2) has been proposed as a new therapeutic agent for cardiovascular disease and diabetic nephropathy. However, due to a short serum half-life of sACE2, the benefit from such treatment is limited. More importantly, unlike the naturally produced ACE2, circulating sACE2 does not anchor to the cells or tissues such as the kidney glomeruli. We set out to engineer a version of sACE2 in fusion with a heparin-binding domain (HBD - the "sticky" tag) to target the therapeutic protein to the glomeruli that has high heparin sulfate proteoglycan content, and with an Fc-tag to increase stability.

Methods: We engineered plasmids to produce sACE2-Fc and sACE2-HBD-Fc in HEK293 cells and purified ACE2 proteins by affinity chromatography. The function of HBD to anchor ACE2 to the surface of cultured podocytes was confirmed by western blotting and immunofluorescence. Following i.v. injection of the recombinant proteins to mice, we determined the pharmacokinetics of ACE2 activity in blood. Organs were harvested for anti-Fc detection of sACE2 distribution in tissues.

Results: Both sACE2-Fc and sACE2-HBD-Fc are enzymatically active as determined by their catalysis rate of Ang II. We showed sACE2 with heparin-binding domain readily adhering to the cultured podocytes. Following injection to mice, unlike sACE2-Fc without HBD, sACE2-HBD-Fc dissipates more quickly from blood circulation likely into tissues. This is supported by immunohistochemistry of kidney sections showing sACE2-HBD-Fc being exclusively localized to the glomeruli.

Conclusions: Our results suggest that the addition of HBD and Fc tags together could improve kidney glomerulus anchoring and the overall pharmacokinetics of sACE2, features which would make this hybrid protein an attractive approach for treating kidney disease and hypertension.

TH-PO525

Deficiency of Smad3 Impairs the Renal NLRP3 Signaling Pathway Mohammed Al-Suraih, Stella Hartono, Joseph P. Grande. *Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.*

Background: Renovascular hypertension (RVH) is a major public health problem that affects up to 7% of individuals over 65 years of age. Our laboratory team has previously showed that induction of oxidative stress is an early event in the development of RVH that leads to development of inflammation and renal atrophy. We have also demonstrated that both Smad3 and NLRP3 deficiencies attenuate renal atrophy, inflammation and interstitial fibrosis in the 2K1C murine model. NLRP3 is one of the most well-known sensors of oxidative stress. Smad3 is a key intermediate in TGF-B signaling. However, the relationship between Smad3/TGF-B and NLRP3 in the development of RVH and subsequent end-organ damage is not well understood. We therefore sought to test the hypothesis that Smad3 is essential for NLRP3 activation in the kidneys of mice subjected to RVH.

Methods: Wild type (WT) and Smad3-/- mice (KO) were subjected to RVH for 4, 7, and 14 days. The right kidneys (stenotic) were analyzed by histology (H&E, Trichrome) and by gene expression (qR PCR). Sham surgeries for both groups were carried as a control.

Results: In accordance with our previous observations, wild type (WT) mice subjected to RVH developed significant degree of renal atrophy (20.9% ±7.13) within 2 week following cuff placement. In contrast, Smad3-/- mice (KO) were protected from atrophy (11.8%±6.39) at 2 weeks. NLRP3 expression was increased at 4, 7, and 14 days in WT mice (1.2-fold at day 4, 1.21-fold at day 7, 7.7-fold at day 14). In contrast, NLRP mRNA was slightly elevated at 4 days in Smad3-/- mice, but returned to baseline levels at later time points (3.3-fold at day 4, 0.6-fold at day 7, 0.9-fold at day 14). Expression of IL-1B and TXNIP, components of NAPL3 signaling, paralleled expression of NLRP3 in both WT and Smad3 KO mice.

Conclusions: We propose that the presence of Smad3, and subsequent TGF-B activation plays a critical role in the signaling of NLRP3, and subsequent activation of inflammasome, which can lead to development of renal atrophy in mice subjected to RVH and represent a potential therapeutic target.

Funding: Other NIH Support - AI-100911

TH-PO526

Erythropoiesis and Blood Pressure Are Regulated via AT1 Receptor by Distinctive Pathways Hideki Kato,[1] Taiji Matsusaka,[2] Masaomi Nangaku.[1] *[1]Nephrology and Endocrinology, The Univ of Tokyo Graduate School of Medicine, Hongo, Tokyo, Japan; [2]Inst of Medical Sciences, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

Background: The renin-angiotensin system (RAS) plays a central role in blood pressure regulation. Although clinical and experimental studies suggested that inhibition of the RAS is associated with the progression of anemia, there has been little evidence to support it.

Methods: We generated and analyzed knockout mice which lack angiotensinogen, renin, AT1a, AT1b, AT1a+b, AT2 receptors. Blood pressure was measured by tail-cuff methods. Ang II (0.3 mg/kg/day) was administered by osmotic mini-pump to angiotensinogen knockout mice. Losartan (30 mg/kg/day)was administered in drinking water.

Results: We found that angiotensinogen and renin knockout mice, which lack angiotensin II, displayed anemia. The anemia of angiotensinogen knockout mice were recovered to normal levels by angiotensin II infusion, which was completely blocked by simultaneous AT1 receptor inhibitor administration. We also examined AT1a, AT1b, and AT2 knockout mice, but anemia were not observed in these mice. To examine whether pharmacological AT1 receptor inhibition reproduces anemic phenotype, we administered AT1 receptor antagonist in hypotensive AT1a receptor knockout mice to inhibit the remaining AT1b receptor and found that the hematocrit levels barely decreased, while blood pressure further decreased to the level of angiotensinogen knockout mice. We then generated AT1a and AT1b double knockout mice to completely ablate the AT1 receptors and the mice finally presented anemic phenotype.

Conclusions: Although erythropoiesis and blood pressure are negatively controlled through the AT1 receptor inhibition in vivo, pathways may be complex and distinct, since erythropoiesis is more resistant than blood pressure control to AT1 receptor inhibition.

Funding: Pharmaceutical Company Support - Takeda, Daiichi-Sasnkyo, Government Support - Non-U.S.

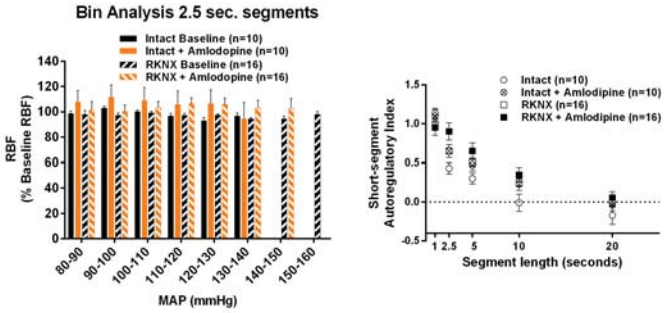
TH-PO527

Renal Autoregulation and Dynamics of Glomerular BP Transmission in Conscious Rats: Novel Analysis Methods and Insights Anil K. Bidani,[1] Aaron J. Polichnowski,[1] Hector Licea-Vargas,[1] Jianrui Long,[2] Geoffrey A. Williamson,[2] Karen A. Griffin.[1] *[1]Medicine, Loyola Univ Med Ctr and Hines VA Hospital, Maywood, IL; [2]Electrical and Computer Engineering, Illinois Inst of Technology, Chicago, IL.*

Background: Renal autoregulation (AR) is believed to be the primary mediator of RBF, GFR and P_{GC} stability with changing BP and to protect against hypertensive damage. Conversely AR impairment, assessed by step BP changes under anesthesia, results in enhanced susceptibility to such damage. However, attempts to demonstrate similar AR patterns in conscious animals with spontaneous BP changes have been unsuccessful.

Methods: BP and RBF recordings at 200Hz for 2-4hrs each on 2-4 separate days were obtained in conscious rats with intact AR (controls, n=10); ~50% AR impairment (controls + amlodipine n=10, or 3/4 nephrectomy, NX n=16); and total AR loss (3/4 NX + amlodipine n=16). Each BP and RBF recording was divided into segments of varying lengths (1, 2.5, 5, 10 and 20 sec). The BP and corresponding RBF averages were separated into 10mmHg BP bins (Fig).

Results: Despite marked differences in step AR, no differences were seen in the ability to maintain stable RBF with spontaneous BP changes regardless of segment length. By contrast, when AR indices were calculated for all adjacent short segments with an AP difference of at least 5mmHg (>4000/rat), significant differences similar to step AR were seen with 2.5 sec segments but which tended to dissipate with increasing segment length (Fig.)



Conclusions: These data indicate that AR protection against glomerular transmission of BP fluctuations occurs over rapid time scales (<5 sec) in conscious animals and that AR-independent and as yet unidentified mechanisms, can maintain RBF (GFR) stability with BP changes. This may explain the fact that acute GFR effects are ususally not seen after antihypertensive agents in CKD with impaired AR.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO528

Urinary Aminopeptidase A Is a Novel Biomarker for Renal Dysfunction in the Two-Kidney, One-Clip Mouse Model of Renovascular Hypertension Nadja Grobe, Laale F. Alawi, Sana Elhagi Emberesh, Aline Lopes Dalmazo, Khalid M. Elased. *Pharmacology and Toxicology, Wright State Univ, Dayton, OH.*

Background: Chronic kidney disease (CKD) and its cardiovascular complications remain one of the leading causes of morbidity and mortality. There is an urgent need to identify early biomarkers for CKD as current diagnostic indicators may not predict disease outcome. Elevated angiotensin (Ang) II contributes to the progression of CKD. Aminopeptidase A (APA) is highly expressed in the brush border of renal tubular cells and metabolizes Ang II to Ang III. We tested the hypothesis that urinary APA is increased in the two kidney, one-clip (2K1C) model of renovascular hypertension.

Methods: 2K1C was induced by constricting the left renal artery using silver clips leaving a 0.12 mm gap for ischemic blood flow to the kidney. We developed a new mass spectrometry-based enzyme assay for measuring APA activity in sham and 2K1C animals

at baseline and 2 weeks after surgery. Samples were incubated with the APA substrate, Ang II (*m/z* 1046). Ang III formation (*m/z* 931) was quantified with stable-isotope labeled peptide standard.

Results: Two weeks after surgery, the weight of the left clipped kidneys was significantly reduced compared with contralateral or sham kidneys ($p<0.01$ or $p<0.05$, respectively). 2K1C animals showed a significant increase of blood pressure, heart rate and urinary albumin-to-creatinine compared with baseline or sham animals ($p<0.01$). Renal APA activity was significantly increased in the clipped kidney compared with the contralateral or sham kidney ($p<0.05$ and $p<0.01$, respectively). Similarly, urinary APA activity was significantly increased in the 2K1C group compared to baseline or sham animals ($p<0.05$). Renal and urinary APA activity was blocked by the specific APA inhibitor 4-aminophosphonobutyric acid.

Conclusions: APA is involved in a feedback mechanism counteracting increased Ang II formation in renovascular hypertension. Urinary APA could be used as biomarker for chronic kidney disease and as an index of intrarenal APA status. Identification of the role of APA in Ang II processing and renal function may have clinical implications in patients with renovascular hypertension.

Funding: NIDDK Support

TH-PO529

Complex Reinnervation Pattern after Unilateral Renal Denervation in Rats

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Background: Renal denervation is a potentially beneficial, but controversial treatment procedure for resistant hypertension. Some reinnervation of efferent sympathetic nerves occurs, but reinnervation of renal afferent nerve fibers has only recently been documented in the renal pelvis of rats after unilateral denervation. We hypthesized, that unilateral renal denervation impacts both ipsilateral and contralateral renal sensory and sympathetic innervation following unilateral denervation.

Methods: Immunoreactivity for tyrosine hydroxylase (TH, i.e. sympathetic efferent), calcitonine gene related peptide (CGRP, i.e., peptidergic afferent) and smooth muscle actin (SMA, i.e. vascular area) were determined in 50µm kidney sections from 12 male SD rats. Denervated left kidneys (L) and non-denervated right kidneys (R) were examined 1, 4 and 12 weeks after left renal denervation. We focused on intrarenal perivascular innervation with special emphasis on the corticomedullary zone.

Results: Morphometric analysis revealed that denervation decreased TH^+ and $CGRP^+$ label by 90 and 95%, respectively ($P<0.05$) within one week. After twelve weeks there was an overshooting reinnervation of TH^+ and $CGRP^+$ nerve fibers ($P<0.05$; L12 *versus* R1) with a shift towards CGRP+ label in the denervated kidneys ($P<0.05$; L12 *versus* L1 and R1). Indeed, even in the non-denervated kidneys there was a doubling of TH^+-label and more than doubling of $CGRP^+$-label ($P<0.05$; R1 *versus* R12).

Conclusions: Neither the functional consequences nor the underlying mechanisms of this complex pattern of enzyme and neurotransmitter expression are clear as yet. However, these findings suggest that complex reinnervation patterns may contribute to the unpredictable clinical outcome observed after interventional renal nerve ablation.

Funding: Pharmaceutical Company Support - Medtronik

TH-PO530

Renal Human Liver-Type Fatty Acid Binding Protein Attenuates against Tubulointerstitial Injury in Aldosterone-Induced Renal Injury

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Background: Liver-type fatty acid binding protein (L-FABP) is expressed in human renal proximal tubules. Because Renal L-FABP is rarely expressed in rodent kidneys, we previously generated human L-FABP (hL-FABP) chromosomal transgenic (Tg) mice and revealed that hL-FABP attenuates tubulointerstitial damage via antioxidant effect in renin angiotensin system (RAS) activated model. Another investigation found that aldosterone (Aldo) activated the intrarenal RAS through positive feedback reactionsand that its activation led to kidney injury via reactive oxidative stress (ROS) generation. The aim of this study is to demonstrate the pathophysiological significance of renal hL-FABP in a systemic Aldo infusion model.

Methods: Tg and wild-type (WT) mice received systemic aldosterone infusions (0.125 µg/kg per minute) and were given 1% NaCl water for 28 days as obstacle model group. Control mice received saline only and normal food in Tg and WT mice.

Results: In this model, Elevation of systolic blood pressure (SBP), urinary albumin, monocyte chemoattractant protein 1 expression, macrophage infiltration in the interstitium, tubulointerstitial damage, and depositions of type I and III collagens were observed. Elevation of SBP, glomerular sclerosis and urinary albumin did not differ in WT-Aldo versus Tg-Aldo, however renal injury was suppressed in Tg-Aldo compared with WT-Aldo mice. ROS was suppressed in Tg-Aldo compared with WT-Aldo mice. Gene expression of angiotensinogen (AGT) in the kidney was up-regulated and excretion of urinary AGT was increased in WT-Aldo mice. This exacerbation was suppressed in Tg-Aldo mice. Expression of hL-FABP was up-regulated in proximal tubules of Tg-Aldo mice. Urinary excretion of L-FABP was significantly greater in Tg-Aldo than in Tg-control mice.

Conclusions: Renal hL-FABP ameliorated the tubulointerstitial damage in Aldo-induced renal injury via ROS and suppressing activation of the intrarenal RAS.

TH-PO531

Mesenchymal Stem Cells Improve the Architecture and Fibrotic Processes in Both Stenotic and Contralateral Kidneys

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Background: Chronic stenosis of renal artery leads to chronic renal hypoxia, renovascular hypertension, renal vascular rarefaction, fibrosis and renal failure. Previous data from our Laboratory showed beneficial effects of mesenchymal stem cells (MSC) in the reconstruction of renal parenchyma of the stenotic kidney, improving the vascular rarefaction and fibrosis. Here we evaluated the effect of MSC on the contralateral kidney.

Methods: It was used the 2 Kidney-1 clip (2K-1C) model in rats. Three weeks after left renal artery occlusion, fluorescently tagged mesenchymal stem cells (MSC) ($2x10^5$ cells/animal) were injected weekly into the tail vein of the 2K-1C rats.

Results: Flow cytometry showed labeled MSC in the cortex and medulla of both ischemic (clipped) and contralateral kidneys (unclipped). MSC prevented further increase in the MAP and significantly reduced proteinuria in 2K-1C rats. Other renal function parameters were unchanged. Contralateral kidney presented altered structure including tubular dilation and glomerular atrophy, cellular infiltration and fibrosis. Similar to the stenotic kidney, MSC improved the morphology and decreased the fibrotic areas in the cortex and medulla of contralateral kidney. Stenotic kidney presented overexpression of the components of the renin angiotensin system (RAS), reversed by MSC. RAS overexpression was not observed in the contralateral kidney, except the AT1 receptor, which was not reversed by MSC.

Conclusions: In conclusion, MSC therapy in the 2K-1C model of chronic renal ischemia prevented the progressive increase of MAP, improved renal morphology and reduced fibrosis in both stenotic and contralateral kidneys. MSC therapy may be a promising strategy to treat renovascular hypertension and its renal consequences.

Funding: Government Support - Non-U.S.

TH-PO532

Cryo-Denervation of the Renal Nerve Alters Na Transporter and Channel Abundance in 2-Kidney 1-Clip (2K1C) Rats

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Background: Renovascular hypertension due to atherosclerosis is increasing in prevalence. The CORAL trial showed angioplasty ± stenting provided no benefit versus medical therapy. Renal denervation has been advocated for hypertension, but it is now less clear who will benefit. Moreover, atherosclerotic renal arteries preclude the use of radiofrequency for denervation.

Methods: We hypothesize that cryo-denervation (cryoDNX) of the renal nerve to the clipped kidney in 2K1C rats will decrease MAP and result in redistribution of Na transporters. Sprague Dawley rats (5 wk) underwent renal artery clipping. MAP was monitored by telemetry. After 6 wk, rats underwent freezing of the renal nerve at -155°C (x3) to the clipped kidney under direct visualization. After 7 days, renal cortex was harvested, homogenized, and fractionated on a 25-66% sucrose gradient: S1-S2 fractions=smooth inner membranes; S3-S4=rough endoplasmic reticulum, trans-golgi, and mitochondria; S5=subapical membrane; S6=plasma membrane. Membrane fractions were subjected to western blot analysis for NHE, NKCC2, NCC, ENaC and the NaK-ATPase.

Results: MAP decreased after cryoDNX of 2K1C rats ($P<0.05$). NHE was most abundant in S5-S6 fractions in both non-clipped and clipped kidneys. After cryoDNX, the S5 fraction in non-clipped kidneys decreased with a shift to S3-S4 in both kidneys. NCC was most abundant in S1-S2 and decreased in S5-S6 after cryoDNX. NKCC2 pattern was similar to NHE. γENaC showed similar abundance across all fractions of the unclipped kidneys with lower abundance in S1 and S6 on the clipped side. After cryoDNX, γENaC from both clipped and non-clipped kidneys was greatest in S3-S4 and S6 fractions. Na,K-ATPase was abundant in all fractions and showed a decrease after cryoDNX in all fractions.

Conclusions: CryoDNX of the clipped kidney in 2K1C rats results in shifts of Na transporters/channels among cellular compartments. NHE and NCC decrease within the plasma membrane and subapical compartments. Modulation of the trafficking of tubular Na transporters by cryoDNX of the renal nerve to the clipped kidney may reduce Na reabsorption and contribute to lower MAP in 2K1C rats.

Funding: Veterans Affairs Support

TH-PO533

Abstract Withdrawn

TH-PO534

Restored Cardiovascular Autonomic Efficiency in Patients after Renal Transplantation Dan Sapoznikov, Rebecca Backenroth, Michal Dranitzki Elhalel, Dvora Rubinger. *Nephrology and Hypertension Services, Hadassah Univ Medical Center, Jerusalem, Israel.*

Background: The present study was undertaken to assess the efficiency of cardiovascular autonomic (CvA) responses during alterations in blood pressure in patients with end-stage renal disease.

Methods: Beat-to-beat systolic blood pressure (SBP) and interbeat interval (IBI) monitoring was performed in non-diabetic patients on chronic hemodialysis (HD, n=73) and after renal transplantation (TX, n=36), and in healthy controls (C, n=28). Differences in variability indices during SBP periods, 10% above (high) or below (low) the mean SBP were cosidered representative of CvA efficiency.

Results: The proportion (%) of baroreflex and nonbaroreflex episodes and SBP and IBI variability in low (LF) or high (HF) frequency ranges (median and interquartile ranges) were:

	C		HD		TX	
	Low-High SBP	p	Low-High SBP	p	Low-High SBP	p
SBP (mmHg)	107(22)-133(21)	0.001	114(24)-155(29)	0.001	109(24)-145(30)	0.001
% Baroreflex episodes	100(8)-100(86)	NS	84(63)-84(68)	NS	32(100)-0(58)*	0.016
sd SBP (mmHg)	5.6(3.6)-6.7(3.1)	0.001	9.2(2.7)-7.0(2.5)	NS	5.1(2.2)-6.8(2.5)	0.001
sd IBI (ms)	42(25)-4(22)	0.009	25(18)-24(19)	NS	21(19)-31(22)	0.001
LF SBP ($mmHg^2$/Hz)	67(91)-139(113)	0.001	119(116)-124(154)	NS	48(65)-103(95)	0.001
HF SBP ($mmHg^2$/Hz)	17(22)-30(28)	0.015	39(42)-41(41)	NS	12(16)-22(20)	0.001
LF IBI (ms^2/Hz)	4192(5744)-5260(5971)	NS	1431(2496)-1360(2896)	NS	1196(2400)-2494(4803)	0.001
HF IBI (ms^2/Hz)	1195(1730)-1860(2682)	NS	508(1023)-590(1063)	NS	360(710)-487(1042)	0.038
LFα (ms/mmHg)	7.8(6.5)-5.8(4.3)	0.001	3.8(2.7)-3.8(2.9)	NS	4.6(5.3)-4.9(4.0)	0.040
HFα (ms/mmHg)	10.2(7.8)-7.5(6.1)	0.002	4.6(3.5)-4.0(3.1)	NS	6.2(5.3)-5.9(3.9)	0.007

sd: standard deviation; α: the square root of the ratio of average power spectral density of IBI and SBI. *p<0.05 vs.C and HD.

Conclusions: 1. Low to high SBP changes are suppressed in HD and enhanced in TX, suggesting restored CvA efficiency in these patients. 2. SBP is predominantly controlled by baroreflex in C and HD, and by nonbaroreflex pathways, most probably sympathetically mediated in TX. Our data may be relevant to the pathogenesis of intradialytic hypotension and post-transplant hypertension.

TH-PO535

Indoxyl Sulfate Suppresses Hepatic Fetuin-A Expression through Aryl Hydrocarbon Receptor Akinobu Ochi, Katsuhito Mori, Shinya Nakatani, Masanori Emoto, Koka Motoyama, Tomoaki Morioka, Shinya Fukumoto, Yasuo Imanishi, Tetsuo Shoji, Eiji Ishimura, Masaaki Inaba. *Osaka City Univ Graduate School of Medicine, Osaka, Japan.*

Background: Fetuin-A (FetA), a circulating glycoprotein mainly synthesized by the liver, has a potent calcification-inhibitory effect. It has been reported that low serum FetA levels were associated with all-cause and cardiovascular mortality in hemodialysis (HD) patients. However, it remains obscure how FetA levels are down-regulated in HD patients.

Methods: We hypothesized that indoxyl sulfate (IS), one of uremic toxins, could suppress hepatic FetA production in HD patients. To investigate this, we examined the direct effects of IS on FetA expression in cultured hepatocytes (HepG2 cells).

Results: FetA mRNA expression was suppressed in treatment with IS (38% decrease by 1 mM IS for 24h). Similarly, IS inhibited FetA protein expression in time- and dose-dependent manner (42% decrease by 1 mM IS for 24h). To clarify intracellular signaling events in IS-induced suppression of FetA, we investigated the involvement of MAPK pathway and oxidative stress. Among p38, ERK and JNK, only p38 was phosphorylated by IS treatment. However, knockdown of p38 didn't alter IS-induced FetA suppression. As previously reported, IS clearly generated reactive oxygen species (ROS). Though IS-induced ROS generation was completely abolished by 10mM N-Acetyl-Cysteine (NAC), no effect of NAC on IS-induced suppression of FetA was found. Finally, we focused on aryl hydrocarbon receptor (AhR) that is known as a dioxin receptor since recent reports suggest that IS is an endogenous agonist for AhR. As expected, knockdown of AhR recovered IS-induced FetA suppression in HepG2 cells.

Conclusions: IS directly inhibited FetA expression through AhR in HepG2 cells. These findings suggest that accumulated IS that is usually unavoidable in HD patients may lead to FetA deficiency, resulting in mortality through cardiovascular calcification.

TH-PO536

Stage 3-5 CKD Patient Coronary Artery Calcification Status Correlates Positively with von Willebrand Factor Platelet Binding Cynthia M. Pruss, Spencer Barr, Angie Tuttle, Julie Grabell, Shawn Tinlin, Michael A. Adams, Wilma M. Hopman, Jocelyn S. Garland, Paula James, Rachel M. Holden. *Queen's Univ, Kingston, ON, Canada.*

Background: Increased Von Willebrand Factor (VWF) levels are linked to cardiovascular disease and poor outcomes and rise over the course of CKD. VWF mediates platelet binding to damaged vessels in primary hemostasis. VWF structure changes conformation when subjected to shear forces, such as by arteries which have become rigid due to calcification. This study examines a stage 3-5 CKD cohort to determine if coronary artery calcification (CAC) correlates with VWF quantity or function.

Methods: VWF antigen (VWF:Ag), propeptide (VWFpp), platelet binding (VWF:RicOF) levels were measured in a stage 3-5 CKD cohort (CKD, N=118, 38% female, eGFR 27.9±10.6 ml/min/1.73m^2) and compared to age matched controls (CON,N=49, 58% female). CAC scores were evaluated via CT, with values >10 considered positive. Statistical analysis reported as mean ±SD, student's t-test. Multivariate logistic regression was used to control for age, gender, diabetes, BMI >30 kg/m2, GFR, VWF:Ag, and VWF:RiCof.

Results: VWF:Ag was significantly increased (P<0.001). There was no significant difference in VWFpp or VWF:Ricof between CON and CKD. VWF:Ricof/VWF:Ag (relative platelet binding), VWFpp/VWF:Ag, (1/VWF circulating half-life), and age were significantly lower in the CKD group. In multivariate analysis, age was highly significant (odds ratio (OR) 2.38 /decade, confidence interval (CI) 1.5-3.7, P<0.001). VWF:Ricof was significant (OR 1.225 per 0.1 U/ml, CI 1-1.4, P=0.011). When VWF:Ricof/VWF:Ag replaced VWF:Ricof in the model, VWF:Ricof/VWF:Ag was also significant (OR 1.23 per 0.1, CI 1.04-1.5, P=0.019).

	CON	CKD
VWF:Ag (U/dL)	1.15 ±0.42	1.48 ±0.72***
VWFpp (U/dL)	1.28 ±0.36	1.34 ±0.43
VWFpp/VWF:Ag	1.16 ±0.23	1.01 ±0.35***
VWF:Rco (U/ml)	1.41 ±0.47	1.40 ±0.67
VWF:Rco/VWF:Ag	1.23 ±0.3	1.02 ±0.4***
Age	67.4 ±7.9	61.5 ±12.9 *

Conclusions: This study finds CAC status correlates with the absolute and relative VWF platelet binding activity. This suggests that early calcification modifies VWF, and could contribute to alterations in hemostasis in early CKD.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO537

Role of Stearoyl-CoA Desaturase in the Regulation Vascular Calcification Makoto Miyazaki. *Medicine, Univ of Colorado Denver.*

Background: Vascular calcification is a common complication in patients with chronic kidney disease. Our recent in vitro study indicates that accumulation of saturated fatty acid (SFA) by inhibiting stearoyl-CoA desaturase (SCD) leads to mineralization of vascular smooth muscle cells (VSMCs) through the activation of endoplasmic reticulum (ER) stress. In this study we determine the function of SCD in VSMCs and SFA-mediated ER stress in regulating vascular calcification *in vivo*.

Methods: SMC-specific SCD knockout mice were generated by breeding SMMHC-Cre^{ERT} mice with SCD1/2 floxed mice. Mice were intraperitoneally injected with tamoxifen (1mg/day) for 5 consecutive days beginning at 5 weeks of age. Mice were sacrificed at 18 weeks of age.

Results: Klotho-deficient mice exhibited a drastic reduction of SCD expression in the medial layer of the aorta, resulting in accumulation of stearate, activation of ER stress and vascular calcification. SMC-specific SCD deletion induced ER stress in the medial layer of the aorta, resulting in severe medial calcification.

Conclusions: SFA-mediated ER stress plays a major role in the pathogenesis of medial calcification.

Funding: NIDDK Support, Other NIH Support - NHLBI, Pharmaceutical Company Support - Merck

TH-PO538

Runx2 Expression in Vascular Smooth Muscle Cells Is Required for Arterial Medial Calcification following Vitamin D Overload in Mice Mu-En Lin, Theodore M. Chen, Elizabeth M. Soberg, Mei Y. Speer, Cecilia M. Giachelli. *Bioengineering, Univ of Washington, Seattle, WA.*

Background: Arterial medial calcification (AMC) is a hallmark of chronic kidney disease-mineral bone disorder (CKD-MBD) and strongly predicts cardiovascular disease mortality. Smooth muscle cell (SMC) transition to an osteochondrogenic (OC) phenotype is a common feature of AMC, and is preceded by expression of Runx2, a master regulator of bone development. The present studies aimed to determine whether Runx2 expression in SMC was required for OC phenotype change and AMC.

Methods: An improved Runx2 targeting construct was used to generate a conditional Runx2 knockout mouse (fl/fl) using Cre-Lox technology. Targeting specificity, efficiency, and compete deletion of Runx2 protein were confirmed by crossing fl/fl mice to Sox2-Cre (global) and SM22a-Cre (ΔSMC) mice. SMC were isolated from mouse aortic media by enzymatic digestion. Vitamin D (VD) overload was used to induce AMC. Serum chemistry, gene expression, and arterial calcium deposition were analyzed.

Results: Global deletion of Runx2 led to severe bone malformation and perinatal death, whereas ΔSMC mice were viable with normal bone and arterial morphology. Following VD overload, arterial SMC in fl/fl mice expressed Runx2, underwent OC phenotype change, and developed severe AMC. In contrast, ΔSMC mice did not express Runx2, maintained SMC phenotype, and did not develop AMC. VD increased serum calcium and FGF23 levels compared to vehicle treated mice, and no differences were observed between fl/fl and ΔSMC. *In vitro*, SMC derived from fl/fl mice calcified to a much greater extent than those derived from ΔSMC mice.

Conclusions: The data indicate that Runx2 expression in SMCs is absolutely required for SMC phenotypic change and subsequent calcification in a mouse model of AMC. Runx2 function in AMC was SMC autonomous, as no differences in systemic mineral homeostasis were observed and the decreased capacity of SMC derived from ΔSMC to calcify was recapitulated *in vitro*. Therefore, Runx2 and downstream signaling pathways that mediate OC phenotypic change may be useful therapeutics targets for treatment of AMC caused by systemic mineral imbalance, such as CKD-MBD.

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TH-PO539

Klotho: Differential Expression and Regulation by Paricalcitol in the Kidney, Parathyroid, and Aorta of Uremic Rats Cynthia S. Ritter, Sarah Zhang, Jane Finch, James A. Delmez, Eduardo Slatopolsky. *Renal Div, Washington Univ School of Medicine, St. Louis, MO.*

Background: Klotho plays an important role in mineral homeostasis and the pathogenesis of cardiovascular disease in CKD. Klotho is highly expressed in the kidney, parathyroid glands (PTGs), and choroid plexus, but its presence in the vasculature has been debated. It is generally accepted that renal Klotho is decreased in CKD; however, the effect of uremia on expression of Klotho in other tissues is not as clearly defined. There are also conflicting reports concerning the effect of vitamin D receptor activator therapy in CKD on expression of Klotho in the various tissues.

Methods: We compared the effect of a 3-month treatment with and without paricalcitol on Klotho in the kidney, PTGs, and aorta in uremic rats (5/6 nephrectomy). One group of uremic rats was treated with paricalcitol 3 x per week (200 ng/rat administered IP). Klotho expression was analyzed by qPCR and immunohistochemical staining (IHC) in the kidney and by IHC in the aorta and PTGs. Samples of archived human PTG tissue from 6 uremic patients with secondary hyperparathyroidism were also analyzed for Klotho by IHC.

Results: With uremia, Klotho expression was unchanged in the PTGs, was significantly decreased in the kidney (65.5%) and the intimal-medial area of the aorta (69.2%), and was significantly increased in the adventitial area of the aorta (67.0%) compared with controls. Paricalcitol treatment of CKD prevented the decrease in Klotho expression in the kidney, increased expression in the PTGs (31.0%), had no effect in the aortic media, but blunted the increase of Klotho in the aortic adventitia. In the human PTGs, Klotho expression was higher in oxyphil cells compared with chief cells in all 6 patients.

Conclusions: This tissue-specific expression and regulation of Klotho in the kidney, PTG, and aorta in uremic rats suggests that Klotho may have different roles in different tissues. In addition, this is the first report of increased expression of Klotho in the oxyphil cells of PTGs from uremic patients. The fact that the function of the parathyroid oxyphil cell is unknown makes the increased expression of Klotho in this cell even more intriguing.

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TH-PO540

Heterogeneous Susceptibility of Various Arterial Beds to Uremic Media Vascular Calcification Alexander H. Kirsch,[1] Andrijana Kozina,[2] Sa?a Frank,[2] Alexander R. Rosenkranz,[1] Kathrin Eller,[1] Philipp Eller.[3] *[1]Clinical Div of Nephrology, Medical Univ of Graz, Graz, Austria; [2]Inst of Molecular Biology and Biochemistry, Medical Univ of Graz, Graz, Austria; [3]Div of Angiology, Medical Univ of Graz, Graz, Austria.*

Background: Cardiovascular morbidity and mortality in CKD are strongly linked with arterial calcifications and sudden cardiac death. Uremic media calcification is not only driven by systemic factors such as hyperphosphatemia, but is also dependent on vascular smooth muscle cells. We hypothesized that different developmental origins of vascular smooth muscle cells might lead to a heterogeneous susceptibility to develop media calcification.

Methods: Female DBA/2 mice were maintained on a 2% high-phosphate diet (HPD) and studied after 14 days. Histological, chemical, functional studies were conducted to study calcification and the response to HPD. Furthermore, we used computer tomography to study calcifications in the corresponding vascular segments in a cohort of ESRD patients (n=36).

Results: Uremic DBA/2 mice did not show any signs of vascular calcification in the thoracic aorta (TA), while there was abundant media calcification in the abdominal aorta (AA). The AA of DBA/2 mice on phosphorus-rich diet displayed significantly higher calcium and phosphorus contents, and an impaired contractility in wire myography measurements, when compared to the TA. Upon high-phosphate diet, the transcriptional profile revealed an induction of an inflammatory phenotype only in the AA, but not in the TA. Arterial calcifications in patients with end-stage renal failure showed a similar pattern with significantly lower calcium scores of the ascending TA when compared to coronary or iliac arteries.

Conclusions: There was a similar heterogeneous pattern of uremic media calcification both in mice and in humans, where mesenchyma-derived arteries were more prone to media calcification when compared to neuronal-crest derived arteries such as the thoracic aorta. Thus, smooth muscle cells from different lineages respond differently to morphogenetic cues *in vivo*.

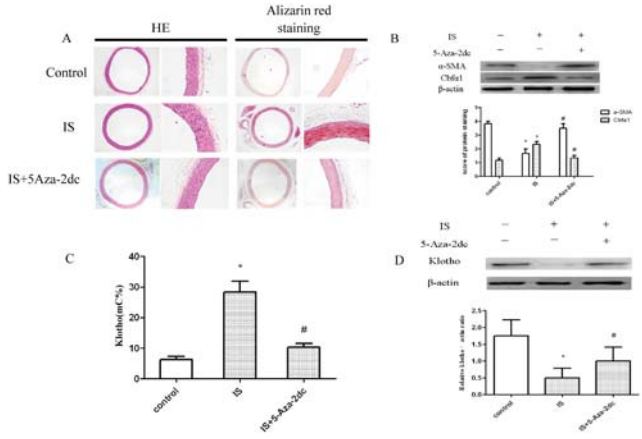
TH-PO541

Vascular Klotho Hypermethylation Potentiates the Development of Vascular Calcification Induced by Indoxyl Sulphate Xiaoyan Zhang, Jing Chen, Yi Fang. *Zhongshan Hospital, Fudan Univ, Shanghai, China.*

Background: Transgenic Klotho overexpressing mice with CKD develop significantly less calcification in comparison with wild-type mice.CKD was a state of vascular Klotho deficiency, a likely explanation for accelerated vascular aging with calcification. It is demonstrated that indoxyl sulfate (IS) can accelerate kidney fibrosis by decreasing Kltho expression via CpG hypermethylation of the Klotho gene. The aim of this study is to investigate whether IS can exacerbates the progression of VC by suppressing vascular Klotho expression by DNA hypermethylation.

Methods: We investigated the effects and possible mechanisms by which Klotho expression is regulated during VC in 5/6 subtotal nephrectomized SD rats (STNx) given IS. Cultured HASMCs treated with IS were used as an in vitro model. 5-aza-2'-deoxycytidine (5Aza-2dc) was used as specific inhibitor of DNA methyltransferase isoform 1.DNA methylation analyses were performed on bisulfite-treated DNA using a quantitative assay based on PCR-pyrosequencing.

Results: Severe vascular calcification was observed by Alizarin red staining in the thoracic aorta of all theSTNx rats given IS, buthardly in the STNx rats. Injections of IS increased CpG methylation level of the Klotho gene and decreased Klotho expression in thoracic aorta. The expression of DNA methyltransferases 1, 3a, and 3b isoforms in HASMCs treated with IS was significantly increase. Specific inhibition of DNA methyltransferase isoform 1 by 5Aza-2dc caused demethylation of the Klotho gene increased Klotho expression and alleviate osteoblast differentiation of HASMCs and vascular calcification.



Conclusions: Inhibition of Klotho gene expression by IS correlates with gene hypermethylation, suggesting that epigenetic modification of specific genes by uremic toxins may be an important pathological mechanism of VC.

TH-PO542

Osteopontin Null Mice on a High Phosphate Diet Develop Vascular Calcification and Elevated FGF-23 Levels Neil Paloian,[1] Elizabeth M. Soberg,[2] Cecilia M. Giachelli.[2] *[1]Nephrology, Seattle Children's Hospital, Seattle, WA; [2]Bioengineering, Univ of Washington, Seattle, WA.*

Background: Chronic kidney disease (CKD) is a common and under recognized disease worldwide. One important cause of morbidity and mortality in this population is vascular calcification (VC). Osteopontin (OPN), a bone protein involved in mineralization regulation, is a known inhibitor of VC and recent clinical studies have demonstrated that FGF-23, a phosphaturic hormone, may also play an important role in VC.

Methods: Wild type (WT) and OPN knockout (KO) mice were placed on normal phosphate (NP) and high phosphate (HP) diets for 11 weeks. At termination serum studies were obtained. Several areas of the aorta were harvested and calcification of the vessels was quantified. Histologic analysis of the vessels and kidneys was performed.

Results: Abundant aortic calcification was observed on histology with alizarin red staining in the HP OPN KO but not the WT group. Total calcium content of the aortic arch + abdominal aorta was 17.3 +/- 4.8 µg Ca/mg dry weight in the HP OPN KO mice compared to 5.1 +/- 2.1 µg Ca/mg dry weight of the WT mice on the HP diet ($p<0.05$). Serum calcium and phosphorus levels were normal for all mice. FGF-23 levels of the HP OPN KO group were 2819 +/- 492 pg/ml compared to 1571 +/- 355 pg/ml in the HP WT mice ($p<0.05$). OPN KO mice placed on the HP diet also developed uremia compared to the WT mice on the HP diet with BUN levels of 36 +/- 4.6 mg/dl and 31 +/- 6.7 mg/dl respectively ($p<0.05$). Renal histology of the HP OPN KO mice with Von Kossa, H+E, and PAS stains demonstrated significant mineral deposits with dilated tubules and increased interstitial inflammation and fibrosis.

Conclusions: We found that the absence of OPN in the setting of a high phosphate diet causes vascular calcification and marked elevation in FGF-23 levels. The VC seen is likely due to the loss of OPN's inhibitory effect on arterial mineralization, the development of kidney disease, and may involve FGF-23. Furthermore, our studies have uncovered a potential novel regulatory role for OPN in relation to serum FGF-23 levels. This has significant therapeutic potential in the prevention and treatment of vascular calcification.

Funding: NIDDK Support

TH-PO543

Effect of Magnesium-Based Phosphate Binder on Cardiac Function in a Rat CKD Model Xoana Barros,[2] Nadine Kaesler,[1] Georg Schlieper,[1] Thilo Krueger,[1] Vincent Brandenburg,[1] Kristina Gundlach,[3] Sonja Steppan,[3] Jutta Passlick-Deetjen,[4] Jürgen Floege.[1] *[1]Uniklinikum RTWH, Aachen, Germany; [2]Hospital Clinic, Barcelona, Spain; [3]Fresenius Medical Care, Bad Homburg, Germany; [4]Univ Düsseldorf, Germany.*

Background: A beneficial effect of treatment with Calcium Acetate-Magnesium Carbonate (CaMg) on vascular calcification (VC) in a rat model of uremia was recently described. Magnesium may also exert pleiotropic effects on cardiovascular function. Our aim was to confirm the beneficial effect of CaMg on VC and in addition to asses CaMg effects on cardiovascular function.

Methods: CKD was induced in male Wistar rats by adenine and high phosphate diet (n=52). Treatment groups included CaMg, magnesium carbonate ($MgCO_3$) and calcium acetate (CaAc). Healthy and CKD animals without treatment served as controls. Blood pressure (BP), serum and urine parameters were measured at baseline and after 5 weeks treatment. Echocardiography and assessment of carotid pulse wave velocity (PWV) were performed before sacrifice. Collagen expression was measured by RT-PCR, Sirius red staining and immunohistochemistry. Aortic calcification (AoC) was assessed with Cresolphthalein method and von Kossa staining.

Results: CKD animals exhibited higher serum levels of creatinine, phosphate, calcium, PTH and FGF23 compared to healthy controls. CaMg reduced phosphaturia compared to CKD-controls (-42%, p<0.001), MgCO3 (-35%, p<0.005) and CaAc (-26%, p=0.06) animals. AoC was significantly increased in the untreated CKD-controls compared to healthy rats whereas no significant differences between treated CKD and healthy rats was observed. No significant differences in BP and PWV were observed between groups. Interestingly, $MgCO_3$ and to a lower degree CaMg treatment led to a higher ejection fraction compared with CKD-control animals (+51%, p<0.05 and +34%, p=0.2, respectively). To investigate potential mechanisms underlying this we performed Sirius red staining and examined the cardiac expression of collagens I and III, which were similar between the groups.

Conclusions: CaMg served as an effective PO_4 binder.The positive effect of magnesium on cardiac function does not seem to be related to altered collagen content and needs further evaluation.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

TH-PO544

TGF-β/Smad3 Signaling Suppresses the Degree of Phosphate-Induced Vascular Smooth Muscle Cell Calcification Aiko Shimokado, Yasuteru Muragaki. *Pathology, Wakayama Medical Univ, Wakayama, Japan.*

Background: The role of transforming growth factor-β (TGF- β) in Pi-induced vascular calcification remains controversial. The aim of this study was to investigate the possible involvement of TGF-β in Pi-induced vascular calcification.

Methods: We compared the degree of Pi-induced vSMC calcification between vSMCs isolated from wild-type ($Smad3^{+/+}$) and Sma3-deficient ($Smad3^{-/-}$) mice.

Results: We found that vSMCs from $Smad3^{+/+}$ mice had less calcium (Ca) than those from $Smad3^{-/-}$ mice when they were exposed to high concentrations of Pi and Ca (Pi + Ca). The phosphorylation of Smad3 was induced in $Smad3^{+/+}$ vSMCsand not in$Smad3^{-/-}$ vSMCs by exposure to Pi + Ca. The concentration of extracellular pyrophosphate (ePPi) was lower in $Smad3^{-/-}$ vSMCs than in $Smad3^{+/+}$ vSMCs and was significantly increased in $Smad3^{+/+}$ vSMCs by treatment with TGF- β1. Also, the addition of a small amount of PPi to culture medium significantly decreased the deposition of Ca in both $Smad3^{+/+}$ and $Smad3^{-/-}$ vSMCs. Extracellular nucleotide phosphatase/phosphodiesterase1 (Enpp1) was decreased at the mRNA, protein, and enzymatic activity levels in $Smad3^{-/-}$ vSMCs compared with $Smad3^{+/+}$ vSMCs. A ChIP assay showed that phosphorylated Smad3 directly binds to the Enpp1 gene. Furthermore, the calcification of aortic segments was attenuated by treatment with TGF- β1 only in $Smad3^{+/+}$ mice.

Conclusions: We conclude that Pi-induced vSMC calcification is suppressed by TGF-β/Smad3 signaling via an increase in ePPi.

Funding: Government Support - Non-U.S.

TH-PO545

Apolipoprotein L1 Gene Associations with Atherosclerosis, Nephropathy, and Mortality in African Americans with Type 2 Diabetes Barry I. Freedman,[1] Carl D. Langefeld,[2] Lingyi Lu,[2] Nicholette D. Palmer,[3] Susan Carrie Smith,[1] Pamela J. Hicks,[3] Jianzhao Xu,[3] Lynne E. Wagenknecht,[2] Laura M. Raffield,[3] Thomas C. Register,[4] Donald W. Bowden,[3] Jasmin Divers.[2] *[1]Internal Medicine/Nephrology, Wake Forest School of Medicine; [2]Center for Public Health Genomics, Wake Forest School of Medicine; [3]Center for Genomics and Personalized Medicine, Wake Forest School of Medicine; [4]Pathology, Wake Forest School of Medicine, Winston-Salem, NC.*

Background: Albuminuria and reduced estimated glomerular filtration rate (eGFR) strongly associate with two apolipoprotein L1 gene (*APOL1*) variants in non-diabetic African Americans. Whether *APOL1* associates with coronary artery calcified atherosclerotic plaque (CAC), carotid artery calcified atherosclerotic plaque (CP), infra-renal aorta CP, and survival remains unclear.

Methods: African American-Diabetes Heart Study participants (n=717) underwent computed tomography to determine CP mass scores in the coronary arteries, carotid arteries and infra-renal aorta; urine albumin:creatinine ratio (UACR), eGFR, and C-reactive protein (CRP) were measured. Associations between CP and *APOL1 G1* and *G2* variants were assessed adjusting for age, sex, African ancestry, body mass index, hemoglobin A1c, smoking, hypertension, use of statins and ACE inhibitors, albuminuria, and eGFR.

Results: Participants were 58.9% female with mean±SD age 56.5±9.6 years, CKD-EPI equation eGFR 89.5±27.7 ml/min/1.73m^2, UACR 169.6±609 mg/g, CAC 610±1412, carotid artery CP 171±531, and aorta CP 5378±10236. In the fully adjusted model, *APOL1* risk variants were associated with significantly lower levels of carotid artery CP (parameter estimate [β] -0.42, SE 0.18, p=0.02 dominant model), with a trend toward lower CAC (β=-0.36, SE 0.21; p=0.08 dominant model), but not with aorta CP, CRP, UACR, or eGFR. After a mean follow-up of 5.0±1.9 years, 89 participants died. *APOL1* nephropathy variants were significantly associated with improved survival (hazard ratio=0.67 for 1 copy; 0.44 for 2 copies, p=0.005).

Conclusions: *APOL1* nephropathy variants were associated with lower levels of subclinical atherosclerosis and reduced risk of death in African Americans with type 2 diabetes.

Funding: NIDDK Support

TH-PO546

Impact of the Stopping of Vitamin K Antagonist Treatment on Concentrations of Dephosphorylated and Uncarboxylated Matrix Gla Protein Pierre Delanaye, Bernard Dubois, Pierre Lukas, Pierre Peters, Jean-Marie H. Krzesinski, Etienne Cavalier. *Univ of Liège, Liège, Belgium.*

Background: Several data suggest that vitamin K antagonist (VKA) therapy is a risk factor for vascular calcifications. Recent data in hemodialysis suggested that fondaparinux, an indirect factor Xa inhibitor, could be safely used in these patients. Matrix Gla protein (MGP) is an 11 kDa protein acting as a potent local inhibitor of vascular calcification. In order to be fully active, MGP must be phosphorylated and carboxylated. This carboxylation is dependent on vitamin K. Inactive dephosphorylated-uncarboxylated (dp-uc) MGP concentrations were higher in hemodialysis patients treated by AVK and could be associated with vascular calcifications. In this study, we measured dp-ucMGP in patients directly after switching from AVK to fondaparinux.

Methods: Switching from AVK (acenocoumarol) to fondaparinux was considered in patients with atrial fibrillation. Seven dialyzed patients were included. Two measurements (T1 and T2) were obtained at the beginning of the dialysis session before stopping AVK. The patients stopped AVK therapy the day before the first dialysis session of the next week. Five measurements were then obtained at the beginning of each dialysis session (T3 to T7). dp-ucMGP was quantified by automated method (IDS, Boldon, UK).

Results: Before switching, median concentrations of dp-ucMGP obtained at two times (T1 and T2) were high but not different: T1 6316 [5485;8693] and T2 6150 [4911;7325] pmol/L. In the first 24 hours following the switch (T3), the median concentration did not change: 5902 [4842;9165] pmol/L. However, all measurements obtained after significantly decreased in comparison to T1: T4 4505 [3295;6791], T5 3810 [2331;4979], T6 3850 [2159;4586], and T7 2948 [1644;3721] pmol/L (p<0.05). Concentrations at T4 were also higher than at T5-T7 but a steady state was then reached and concentrations at T5, T6 and T7 were not different.

Conclusions: Stopping AVK in hemodialysis patients is associated with a rapid reduction of dp-ucMGP concentrations. If this reduction of dp-ucMGP is associated with a better evolution of vascular calcifications remains to be proved.

TH-PO547

Characterization of Circulating Myeloid Calcifying Cells in End-Stage Renal Disease Ryan Gillihan, Cassi Johnson, Shiqin Zhang, Jason R. Stubbs. *The Kidney Inst, Kansas Univ Medical Center, Kansas City, KS.*

Background: Patients with end-stage renal disease (ESRD) exhibit numerous bone and mineral metabolism defects that contribute to vascular calcification and cardiovascular mortality in this population. Recent evidence suggests the presence of osteocalcin-positive (OCN^+) cells in the peripheral circulation of humans that exhibit calcifying abilities both *in vitro* and *in vivo*, raising the possibility that alterations in these circulating OCN^+ cells may contribute to the pathogenesis of vascular calcification.

Methods: The purpose of this study was to characterize levels of circulating OCN^+ cells in healthy volunteers and an ESRD population. Thus, we isolated peripheral blood mononuclear cells (PBMCs) from 16 patients (8 ESRD patients and 8 healthy controls) and evaluated OCN expression in these cells by flow cytometry.

Results: We observed ESRD patients to exhibit a higher percentage of OCN^+ cells from the total PBMC population (6.8% versus 2.7% in controls; P<0.05). Furthermore, in a sub-analysis of myeloid-derived ($CD14^+$) PBMCs, ESRD patients exhibited a higher percentage of $CD14^+OCN^+$ cells compared to controls with normal kidney function (79.2% versus 65.3% in controls; P=NS). Further stratification of the $CD14^+$ cells into $CD16^+$ and $CD16^-$ sub-fractions, revealed ESRD patients to exhibit a higher percentage of OCN^+ cells in the $CD16^+$ subset (39.8% versus 22.3% in controls; *P*<0.05). Culturing isolated $CD16^+$ cells under osteogenic conditions revealed these cells to exhibit calcifying potential *in vitro* as assessed by alizarin red staining. Of note, previously published data from our lab identified this same $CD16^+$ subset of cells to exhibit significantly higher vitamin D receptor expression in ESRD patients following vitamin D therapy, perhaps providing further evidence to support an osteoblast-like phenotype for this cell population.

Conclusions: Patients with ESRD demonstrate higher levels of circulating myeloid-derived, OCN^+ cells, which we propose may contribute to the pathogenesis of vascular calcification. Supplemental functional assays are ongoing to compare the calcifying capacity of OCN^+ cells isolated from ESRD versus control subjects.

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TH-PO548

Intracellular Calcium Signaling Is Altered in Vascular Smooth Muscle Cells (VSMC) in Chronic Kidney Disease Chad A. Zarse,[1] Mikaela Lee Mckenney,[2] Stacey L. Dineen,[2] Michael Sturek,[2] Neal X. Chen,[1] Sharon M. Moe.[1] *[1]Nephrology, IU School of Medicine, Indianapolis, IN; [2]Physiology, IU School of Medicine, Indianapolis, IN.*

Background: Vascular calcification results in morbidity and mortality in CKD. Pathologic intracellular calcium (iCa) signaling in VSMCs may contribute to abnormalities in contractility and/or calcification. This study examined the alteration of iCa in CKD versus normal rats and the effect of common therapies.

Methods: Three groups of slowly progressive CKD Cy/+ rats (untreated, calcitriol given 15 mg/kg IP thrice weekly, and 3% calcium gluconate in drinking water to mimic phosphate binder use; n=6-7 per group) and one group of normal SD rats (n=6) were studied. Treatments began at 25 weeks and rats sacrificed at 34±1 weeks, representing ~15% of normal kidney function. VSMCs were isolated from freshly dissected thoracic aorta and iCa measured using the fluorescent indicator, fura-2 (iCa=ratio at 360/380nm). Basal iCa was measured in the presence of physiologic salt solution. The sarcoplasmic reticulum (SR) Ca store capacity was assessed in the presence and absence of extracellular Ca with caffeine. Aorta calcification was measured biochemically. Results were compared by ANOVA.

Results: Vascular calcification was higher in all CKD groups compared to normal SD rats (p=0.03). Basal VSMC iCa (ratio 360/380) was elevated in all CKD groups compared to normal (p=0.0029, CKD 0.98±0.02 versus Normal 0.81±0.05; no significant differences were observed among CKD treatment groups). SR store capacity was greater in CKD rats (p<0.0001, CKD 0.64±0.04 versus Normal 0.44±0.06). Following SR Ca store release in CKD VSMCs, in the presence and absence of extracellular Ca, there were differences in iCa returning to basal levels, suggesting increased activity of transient receptor potential channels and/or extrusion impairment through sodium-calcium-exchanger. There was a trend toward correlation between basal iCa and aortic calcification (p=0.09, r=0.35).

Conclusions: VSMC in CKD rats have increased basal iCa and sarcoplasmic reticulum store release and an abnormality of calcium extrusion from VSMC. How this relates to the pathogeneisis of vascular calcification remains to be determined.

Funding: Other NIH Support - NIAMS, NHLBI, Veterans Affairs Support, Pharmaceutical Company Support - 2013-2014 Sanofi Nephrology Fellowship Award

TH-PO549

Differential Expression and Vascular Calcification Gene Targeting of microRNA Contained in Matrix Vesicles From CKD Compared to Normal Rats Neal X. Chen,[1] Praneet Chaturvedi,[2] Sarath Chandra Janga,[2] Kalisha O'Neill,[1] Jeanette N. Mcclintick,[1] Sharon M. Moe.[1,3] *[1]Indiana Univ School of Medicine; [2]School of Informatics and Computing, IUPUI; [3]Roudebush VAMC, Indianapolis.*

Background: Matrix vesicles (MV) are involved in calcification of vascular smooth muscle cells (VSMC). The protein make up of MV alters activity and mineralization capability, but the role of microRNA (miRNA) in MV induced calcification has not been evaluated. We hypothesized that the miRNA expression profile in MVs isolated from CKD rats that spontaneously develop arterial calcification would differ from normal (NL) littermates.

Methods: VSMC from NL or CKD rats (n=3) were incubated with calcification media and MV isolated. Total RNA was isolated from MV and VSMC and miRNA quantified using Agilent Bioanalyzer. MiRNA array was performed using GeneChip miRNA 3.0 Array. We analyzed the miRNA that changed at least 2 fold in the comparison groups with a p value of < 0.01 and false discovery rate of < 20%. Target gene prediction was performed using bioinformatics programs Targetscan and Miranda.

Results: We demonstrated that miRNAs were concentrated in MV compared to VSMC, confirmed by real time PCR. Analyses demonstrated 3 miRNAs decreased and 6 miRNAs increased in VSMC from CKD versus NL rats. Six miRNAs were decreased and 9 miRNAs increased in MV from CKD versus NL rats. We then asked if the 'packaging' of miRNA from VSMC to MV would alter calcification and identified 28 dysregulated miRNAs in MV compared to VSMC from CKD rats. Target prediction analysis followed by functional enrichment of the targets revealed the existence of an intricate miRNA-mRNA network across multiple pathways. The primary pathways identified included MAPK signaling, focal adhesion, receptor protein signaling and Wnt signaling. We then determined if the 28 miRNA had the potential to alter 25 known genes involved in vascular calcification (Runx2, ALP, annexin 2, etc.) and found 5 miRNAs alter multiple genes involved in calcification.

Conclusions: MV and VSMC from CKD rats have different patterns of miRNA profiles than those from NL rats. MiRNAs in MV may serve as key regulators of various target genes responsible for vascular calcification in CKD.

Funding: Other NIH Support - NIAMS, Veterans Affairs Support

TH-PO550

Calciprotein Particles Induce Calcification of Vascular Smooth Muscle Cells *In Vitro* Parisa Aghagolzadeh,[1] Rakesh Kumar Bijarnia,[1] Prakash Chandak,[1] Matthias Bachtler,[1] Edward Robert Smith,[2] Andreas Pasch.[1] *[1]Nephrology, Hypertension and Clinical Pharmacology, Univ Hospital Bern, Bern, Switzerland; [2]Nephrology, The Royal Melbourne Hospital, Melbourne, Australia.*

Background: Vascular calcification is prevalent in patients with chronic kidney disease (CKD) and is associated with significant cardiovascular morbidity and mortality. Calciprotein particles (CPP) are calcium phosphate-containing nano-aggregates which have been found in the blood of CKD patients. The effect of CPP on vascular smooth muscle cells (VSMC) mineralization has yet to be evaluated.

Methods: Synthetic primary and secondary CPP were generated using phosphate-enriched culture medium (DMEM/10% FBS) incubated at 37°C for either one day (primary CPP) or seven days (secondary CPP). Human VSMC were cultured with these media and mineralization was assessed qualitatively with Alizarin red staining and quantitatively by measurement of calcium and phosphate content.

Results: The supplementation of culture medium with 3.5 mM phosphate and 1mM calcium resulted in a time- and temperature-dependent generation of primary and secondary CPP, as identified by TEM. Exposure of VSMC to secondary CPP led to a pronounced and consistent dose-related accumulation of calcium and phosphate mineral (i.e. calcification) within 5 days, whereas exposure to primary CPP did not. Furthermore, the amount of FBS used for the generation of morphologically indistinguishable secondary CPP corresponded to the extent of VSMC calcification.

Conclusions: CPP form spontaneously in cell culture medium containing high phosphate. Secondary CPP induce VSMC calcifications in vitro, whereas primary do not. This indicates that controlling CPP particle type and transformation may be an important determinant of VSMC calcification *in vitro*.

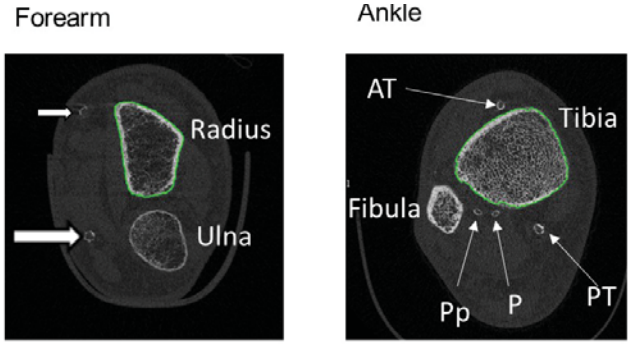
Funding: Government Support - Non-U.S.

TH-PO551

Peripheral Vascular Calcification and Bone Changes in Advanced Chronic Kidney Disease Using High Resolution Peripheral Quantitative Computed Tomography (HR-pQCT) Syazrah Salam,[1,2] Margaret A. Paggiosi,[2] Richard Eastell,[2] Arif Khwaja.[1] *[1]Sheffield Kidney Inst, Northern General Hospital; [2]Academic Unit of Bone Metabolism, Univ of Sheffield, United Kingdom.*

Background: Vascular calcification (VC) in chronic kidney disease (CKD) is linked to abnormal bone mineral metabolism. We aim to evaluate the association between peripheral VC and bone parameters using HR-pQCT in advanced CKD.

Methods: We recruited 31 CKD stages 4-5 (including dialysis) patients and their age- and gender-matched controls with eGFR>60ml/min/1.73m^2. We performed HR-pQCT (XtremeCT, Scanco) at the wrist and ankle to assess bone parameters of distal radius and tibia (82μm resolution, spanning 9.02mm). The same images (figure) were used to quantify peripheral VC in mg hydroxyapatite (mgHA). Abdominal aortic calcification (AAC) was assessed using dual energy X-ray absorptiometry (DXA; Discovery A, Hologic) and AAC-8 score.



Peripheral arteries of the forearm; radial artery (small arrow) and ulnar artery (big arrow). Peripheral arteries of the ankle; anterior tibial artery (AT), posterior tibial artery (PT), peroneal artery (P) and perforating branch of peroneal artery (Pp).

Results: 81% of CKD and 32% of controls had measurable VC. Median VC mass was 0.29 mgHA at the wrist and 0.88 mgHA at the ankle in CKD; whilst controls had median VC mass of 0 mgHA at both sites (p<0.001). Trabecular volumetric bone mineral density (vBMD) was significantly lower in CKD (161.3 versus 184.8 mg/cm^3, p<0.05) at distal radius in addition to lower trabecular bone volume and thickness. At distal tibia, CKD patients had lower total vBMD (277.7 versus 311.8 mg/cm^3, p<0.05) and thinner cortical bone (1.25 versus 1.06 mm, p<0.05). There was a negative correlation between VC and vBMD, especially of cortical bone at distal tibia (r=-0.342, p=0.007). In contrast to VC by HR-pQCT, AAC was only measurable in 48% of CKD and 19% of controls.

Conclusions: CKD was associated with peripheral VC, lower vBMD and thinner cortical and trabecular bone. HR-pQCT is a useful tool for future research in CKD mineral bone disease by allowing simultaneous assessment of peripheral VC, bone density and micro-architecture.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO552

Effect of Rapamycin on High Glucose-Induced Endothelial-To-Chondrocyte Transition in Human Aortic Endothelial Cells

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Background: Vascular calcification is one of the common complications in diabetes mellitus (DM). Studies showed high glucose (HG) caused cardiovascular calcification. Our previous studies showed HG could induce endothelial cells transdifferentiation into chondrocyte via endothelial mesenchymal transition (EndMT), which was involved in medial calcification. However, its underlying mechanism is not understood. Inflammation plays a role in the development of DM. Evidences showed a significant increase in the expressions of IL-1 in DM. Rapamycin reduced inflammation by inhibiting mammalian target of Rapamycin (mTOR). However, whether it prevented the progression of vascular calcification remained unclear. The aim was to explore the influence of IL-1 on the HG-induced endothelial-to-chondrocytes.

Methods: Primary human aortic endothelial cells (HAECs) were treated with normal glucose (NG; 5.5 mM), HG (30 mM D-glucose) and Rapamycin(10ng/ml) (HG+Rapamycin) for 48 h. Immunofluorescence staining was performed to detect the co-expression of CD31 and fibroblast-specific protein 1 (FSP1). The expressions of CD31, FSP1 and mTOR were detected by Western blot. The expression of the Msenchymal Stell Cells markers STRO-1 and the chondrocyte marker SOX9 was detected by immunofluorescence staining and western blots.

Results: The treatment of HAECs in the HG group resulted in increases in the expressions of IL-1 and FSP1 in dose-and time-dependent manners. Double staining of the HAECs indicated a colocalization of CD31 and FSP1 and some cells acquired spindle-shaped morphologies. The cells undergoing EndMT expressed STRO-1 and SOX9. The expressions of FSP1,IL-1,mTOR and SOX9 were increased in the HG group, and these changes were inhibited by Rapamycin. Consistent with SOX9 expression, calcium deposits were enhanced by the HG treatment.

Conclusions: HG-induced vascular calcification via the IL-1 mediation endothelial-to-chondrocyte transition, which was inhibited by Rapamycin.

Funding: Government Support - Non-U.S.

TH-PO553

Telomere Length, Cardiovascular Risk and Arteriosclerosis in Human Kidneys: An Observational Cohort Study

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Background: Replicative senescence, associated with telomere shortening, plays an important role in biological ageing and cardiovascular disease. The relation between cardiovascular risk, telomere length, and kidney histology is unknown.

Methods: Our study consisted of a test cohort of 217 kidney donors for transplantation and a validation cohort of 40 kidney donors. We used quantitative RT-PCR to measure relative telomere length (log T/S ratio) in peripheral blood leucocytes and in kidney biopsies performed prior to implantation. The association between the intrarenal histological lesions, leucocyte and intrarenal telomere length was studied using multiple regression models, adjusted for calendar age, gender and other donor demographics.

Results: Subjects with intrarenal arteriosclerosis had significantly shorter leucocyte telomere length compared with patients without arteriosclerosis (log T/S ratio -0.3 versus 0.1 with versus without arteriosclerosis; p=0.0008). Intrarenal arteriosclerosis was associated with shorter telomere length independent of gender, calendar age, history of hypertension and history of cardiovascular events. For each increase of one standard deviation of the log T/S ratio, the odds for intrarenal arteriosclerosis decreased with 64% (Odds ratio 0.36; 95% CI 0.17-0.77; p=0.02). Leucocyte telomere length was not associated with changes in the tubulo-interstitial and glomerular compartments. In accordance with leucocyte telomere length, shorter intrarenal telomere length associated significantly with the presence of renal arteriosclerosis (log T/S ratio -0.04 versus 0.08 with versus without arteriosclerosis, p=0.007), and not with other histological lesions.

Conclusions: We demonstrate that arteriosclerosis in smaller intrarenal arteries is associated with shorter telomere length, independent of cardiovascular risk factors and calendar age. Our study suggests a central role of replicative senescence in the progression of renovascular disease.

TH-PO554

Intravenous SNF472 Inhibits Vitamin D Induced Cardiovascular Calcification in Rats

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Background: An in vivo study (1) investigated the effects of SNF472, an intravenous (i.v.) formulation of phytate, on vitamin D_3 (VD) induced vascular calcification. Furthermore an in vitro study (2) evaluated the effect of SNF472 on hydroxyapatite (HAP) binding kinetics.

Methods: 1. Four groups of 5-7 male SD rats were studied. Control group received i.v. vehicle daily. Treated groups received 1 mg/kg SNF472 i.v. either daily (o.d) or every other day (e.o.d). Calcification was induced by 5 daily oral administrations of 75 kIU/kg of VD starting on day 3 of treatment. Rats were sacrificed on day 14. 2. SNF472 binding on HAP was studied in vitro by incubation of HAP in the presence of SNF472 for up to 8 hours. SNF472 release was studied by pre-incubation of HAP in the presence of SNF472 and then the pre-incubated HAP was incubated in fresh buffer for up to 7 days.

Results: The administration of VD induced a marked increase in aortic and heart calcium levels. Both o.d and e.o.d. i.v. SN472 at 1 mg/kg resulted in reductions of calcification by 55-60% in aorta and 70-75% in heart. Calcium and phosphorus concentrations in control serums increased compared to the sham group, and were significantly increased by o.d. SNF472 only. In vitro SNF472 was bound to HAP almost immediately, reaching an 80% of maximum adsorption after 5 minutes of incubation, and maximum adsorption (about 5 mg/g) at 60 minutes. This high affinity was confirmed after studying its release from the crystal surfaces and is compatible with the efficacy found in vivo when administered e.o.d.

Conclusions: SNF472 might be used for the prevention of cardiovascular calcification in ESRD patients and calcification-related pathologies as calciphylaxis.

Funding: Pharmaceutical Company Support - Laboratoris Sanifit, S.L., Government Support - Non-U.S.

TH-PO555

Prevention of Vascular Calcification in Uremic Rats by Pharmacological Inhibition of Matrix Metalloproteinases

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Background: We studied the question whether pharmacological inhibition of matrix metalloproteinases (MMPs) can decrease chondro/osteoblastic transition of VMSC and the development of media calcifications in an aggressive model of uremic vascular calcification.

Methods: Uremia was created by 5/6-nephrectomy and all animals were fed a HP diet (1,2% phosphate). Arterial calcifications were induced by treatment with calcitriol (250ng/kg/d). MMPs were inhibited with doxycycline (100mg/kg/d). Five groups of rats were studied: uremic (n=10), shamp-operated (n=10), uremic treated with doxycyclin (n=6), uremic treated with calcitriol (n=14) and uremic treated with calcitriol and doxycycline (n=14). After 5 weeks of calcitriol treatment, animals were sacrificed and media calcification was quantified in aortic sections after von Kossa staining. Osteoblastic transition of VSMC was quantified by RT-PCR for proteins controlling bone mineralization. Aortic content of MMP-2 and MMP-9 was quantified by staining and RT-PCR.

Results: Creatinine, calcium and phosphate levels were significantly higher in groups receiving calcitriol, and not significantly different in the doxycyline-treated group. Systolic blood pressure was similar in all groups. Uremic rats treated with calcitriol, but not rats treated with calcitriol and doxycycline, showed significantly increased gene expression levels of MMP-2 MMP-9, osteopontin, osteocalcin, Runx2, and osterix. Massive calcifications were found in 5/6 nephrectomized rats treated with calcitriol, whereas MMP inhibition with doxycycline resulted in almost complete absence of media calcification.

Conclusions: Phenotypic transition of VSMC and the development of vascular calcification were almost completely prevented by MMP inhibition with doxycycline. This effect was not due to differences in the degree of uremia, calcium or phosphorus levels or systolic blood pressure. MMP inhibition seems a promising strategy in the prevention of uremic vascular calcifications.

Funding: Private Foundation Support, Clinical Revenue Support

TH-PO556

Vascular Calcification in Diabetes - Correlations with Calcification Potential of Serum and with Klotho Expression In Vitro

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Background: Uraemic serum induces calcification of vascular smooth muscle cells outside the disease setting (*in vitro* calcific potential of serum). Vascular calcification (VC) is also prevalent in patients with diabetes. We therefore examined the *in vitro* calcification potential of serum from diabetic subjects. We also investigated the effect of this serum on *klotho* expression as it plays a major role in calcification.

Methods: Serum from 20 healthy controls and 45 age- and sex-matched patients with type 2 diabetes (DM) and MDRD eGFR>60 ml/min were analysed following CT scanning of the femoral arteries. The groups studies were: C- (9 controls without VC), C+ (11 controls with VC), DM- (16 patients with DM without VC) and DM+ (29 patients with DM and VC). Serum-induced calcification in human cultured aortic smooth muscle cells was quantified using the DICA-500 Ca^{+2} assay Kit. *klotho* expression was determined by western blotting.

Results: Diabetes subjects and controls were similar in age, gender, blood pressure, eGFR, serum calcium, phosphate, and albumin. The DM+ group had higher median Agatston score than C+ (785 v 202 Hounsfield units; $p<0.01$). In the whole group, *in vitro* calcification correlated with Agatston score (*rho*=0.445; $p<0.001$), age ($r=0.287$; $p<0.05$), and HbA1c (*rho=0.0432*; $p<0.001$). Induced calcification was higher with serum from DM than from controls (201 ± 12 v 85 ± 12Ca^{+2} nanomoles protein mg^{-1}; $p<0.01$), and higher with DM+ than with DM- (221 ± 12 v 158 ± 23 Ca^{+2} nanomoles protein mg^{-1}; $p<0.05$). Serum from DM- and DM+ groups attenuated *klotho* expression (50% decrease compared to control) while serum from C- and C+ did not change expression. The degree of attenuation correlated negatively with calcification potential (*rho*=-0.320, $p>0.05$) *in vitro* and with Agatston score (*rho*=0.445, $p>0.001$).

Conclusions: Serum from patients with DM possesses enhanced calcific potential which can be demonstrated *in vitro*. These effects are associated with the suppression of *klotho* expression, suggesting a potential mechanism.

Funding: Pharmaceutical Company Support - A unrestricted grant from Genzyme Pharmaceuticals

TH-PO557

Klotho Gene and Transcriptional Profiles of the Uremic, Calcified, and Normal Rat Aorta By RNA-seq

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Background: Development of Vascular Calcification (VC) is a tightly regulated process between factors promoting and inhibiting mineralisation. Klotho has been shown to suppress vascular calcification and maintain cell differentiation. It's unresolved whether this effect is due to circulating Klotho only, or if there is locally produced Klotho in the vasculature. Next generation high-throughput RNA sequencing (RNA-Seq) is a powerful tool for profiling gene expression and the detection of differentially expressed genes and rare transcripts.

Methods: Using RNA-seq we examined the rat aorta transcriptome from long term, 14 weeks, uremic rats (U) kept on a high P diet and given alphacalcidol (vit D), compared to normal control (C) and normal rats given vit D. VC was detected by von Kossa staining. Plasma Ca, P, PTH and FGF23 were measured.

Results: Severe VC was induced in U rat aortas and to less extent in vit D rat aortas. Cufflinks analysis of the RNA-seq data showed 10144 genes to be expressed with a expression level > 1 read per kb transript per 10^6 mapped reads (RPKM) in at least one of the three groups. 2663 genes were differentially expressed with 47% up- and 53% downregulation in uremic rats relative to vit D and control rats. Markers of osteogenesis, fibronectin, osteopontin, periostin, matrix Gla protein (MGP), and LTBP2 were among the top upregulated genes, while a number (e.g. elastin and tropomyosin1a) of aorta related genes were strongly downregulated. A gene ontology analysis showed that significantly deregulated genes were enriched for genes related to extracellular matrix, response to wounding, organic substance, carbohydrate binding and ossification. MGP was highly expressed in normal aorta and significantly upregulated in VC becoming the most expressed gene. The Klotho gene was not expressed in normal or calcified aorta.

Conclusions: Extensive changes in the transcriptional profile were induced in the uremic, calcified aorta. The Klotho gene was not expressed in the rat aorta, when examined by RNA-seq.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO558

Progressive Oxalate Nephropathy Depends on TNFR1/2 Because They Are Needed for CaOx Crystal Adhesion to Tubule Epithelium

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Background: Kidney stones most commonly contain aggregates of calcium oxalate monohydrate (COM). The attachment of COM to epithelial cells is known to be mediated by anionic molecules and urinary macromolecules. We speculated that transmembrane receptors may mediate COM adhesion to epithelial cells.

Methods: All KO mice were generously provided by V. Vielhauer, Germany. All experimental procedures were approved by the local government authorities. Immunostaining and Paraffin Acid Schiff were used for analysing kidney pathology. RTPCR, ELISA, Immunoblot, computed tomography, AFM were used for further data analysis. Human Biopsy samples were generously provided by A. Evan, U.S.A.

Results: We observed that during human and mouse chronic oxalate nephropathy (ON) renal tubular cells surrounding the plaque express TNF receptor (TNFR) 1 and 2. Also western blot analysis confirmed TNFR1/2 induction in murine ON. *Tnfr1*- and/or *Tnfr2*-KO mice were entirely protected from progressive ON as evidenced by plasma creatinine and BUN levels, leukocyte infiltrates and mRNA expression of injury markers and histology. The reason for this protective effect was the complete absence of intrarenal COM crystals, despite unaffected calcium and oxalate excretion, as determined by Pizzolato stains, computed tomography. In vitro studies confirmed the role of TNFR1/2 for COM crystal adhesion. We used atomic force microscopy to measure adhesion forces between COM crystals and primary tubular epithelial cells isolated from wild-type (WT) or *Tnfr1/2*-KO mice. The adhesive forces were almost nil in *Tnfr1/2*-KO cells as compared to WT tubular cells. Moreover, we found that absence of TNFR1/2 blocked the induction of osteopontin, annexin II and CD44, which are genes responsible for crystal adhesion to tubular epithelium after CaOx crystal exposure. Further, the role of TNFRs was independent of TNF as etanercept treatment had no effect on murine ON.

Conclusions: We conclude that TNFR1/2 mediate COM adhesion to tubular epithelium as a starting point of ON. Interfering with this mechanism may provide novel options to prevent kidney disease in patients with hyperoxaluria.

TH-PO559

High Fat Diet Induces Calcification in Nephrectomized Rats

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Background: Vascular calcifications (VC) are very prevalent among patients with chronic renal failure and represent a major cause of morbidity and mortality. High fat diets are associated to metabolic changes (dyslipidemia, chronic inflammation, oxidative stress, etc.) which may impair vascular health. We hypothesized that feeding high fat diets to uremic rats would induce vascular calcification.

Methods: Zucker rats of lean phenotype (n=16) were divided in two groups: one group was fed a diet with 60% energy from fat (HF, n=10) and the second group was fed a standard diet with normal caloric content (NF, n=6). After feeding the diets for two months, all animals were 5/6 nephrectomized and were switched to HF and NF diets with high (1.2%) P content. Rats were maintained on these diets for two additional months. At the end of the experiment, rats were anesthetized with sodium thiopental and sacrificed by exanguination. Blood was collected at sacrifice to measure serum creatinine (Cr), ionized calcium (iCa), phosphate (P) and fibroblastic growth factor 23 (FGF23). Samples of thoracic aorta were obtained and processed for measurement of calcium (CaAo) and phosphate (PAo) content.

Results:

Group	Cr (mg/dL)	iCa (mM)	P (mg/dL)	FGF23 (pg/mL)	CaAo (mg/g)	AoP (mg/g)
HF	1.01±0.10	1.11±0.01	7.8±1.2	11366±1639*	6.3±1.5*	12.4±7.2*
NF	0.91±0.09	1.09±0.03	6.1±1.4	4799±2402	1.4±0.1	0.1±0.1

Values are mean±SE; *P<0.05 vs NF

Conclusions: These data show that feeding a diet with high fat content induces VC in uremic rats. It is interesting to note that in the experimental model of 5/6 nephrectomized rat, treatment with calcitriol is necessary to stimulate VC. However, when fed high fat diets, uremic rats develop VC without calcitriol administration. The significant increases in FGF23 detected in rats fed HF suggest that these diets may influence phosphate handling.

Funding: Government Support - Non-U.S.

TH-PO560

Calcium Sensing Receptor Expression and Activation Protects against Calcification in Human Aortic Smooth Muscle and Arterial Explants

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Background: Vascular calcification (VC) is common in CKD, and contributes to cardiovascular risk. Understanding modulators of VC will facilitate rational treatment design to reduce CV risk in CKD. The Calcium Sensing Receptor (CaSR) is present in human muscular and elastic artery, senses extracellular calcium, and may therefore directly modulate VC. We investigated the role of the CaSR in vascular calcification.

Methods: The effects of CaSR agonists, the calcimimetic R568 on phenotype and calcification were evaluated in human aortic smooth muscle cells (HAoSMC) under static conditions and cyclical strain, and in human arterial explants (9 controls, 11 CKD). The role of the CaSR was further explored by CaSR silencing or over-expression.

Results: In HAoSMC, strain increased CaSR expression by 45% ($p<0.05$). CaSR agonists Ca^{2+} and Gd^{3+} reduced CaSR expression ($p<0.01$), though to a lesser extent under strain. Exposure to high Ca^{2+} increased osteocalcin expression, alkaline phosphatase (ALP) activity and calcification ($p<0.01$), and strain attenuated these responses. R568 limited the reduction in CaSR and the development of calcification, particularly under static conditions. R568 dose-dependently reduced osteocalcin expression, but did not influence ALP activity. Importantly, CaSR silencing greatly enhanced calcification and osteocalcin

expression (p<0.01); conversely, CaSR overexpression reduced calcification, osteocalcin expression and ALP activity. In arterial explants, R568 attenuated the Ca^{2+} induced increase in osteoblast markers (Runx2 and Dmp1) in healthy artery, though this effect was no longer apparent in CKD. R568 did not materially change arterial vessel wall calcium content.

Conclusions: Loss of CaSR expression seen in CKD is permissive to calcification and associated with markers of osteogenic transformation. Calcification is reduced when vascular CaSR expression is preserved or on exposure to R568. These data suggest that the CaSR directly modulates VC.

Funding: Private Foundation Support

TH-PO561

Vascular Calcification Scores Improve Cardiovascular Risk Assessment beyond Traditional Risk Factors in CKD Patients

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Background: Chronic kidney disease (CKD) is associated with an elevated incidence of cardiovascular (CV) events.Although a variety of non-invasive methods for measuring CV risk (such as carotid intima media thickness, pulse wave velocity (PWV), coronary and aortic calcification scores (measured either by CT scan or X-ray) and the ankle brachial index (ABI)) have been evaluated separately in CKD cohorts, few studies have evaluated them simultaneously. The objective of the present study was to determine whether these non-invasive methods ameliorate the risk prediction beyond traditional risk factors (TRF) in patients at different CKD stages.

Methods: We performed a prospective, observational study of the relationship between the outputs of non-invasive measurement methods on one hand and mortality and cardiovascular outcomes in 143 patients at different CKD stages on the other. During the follow-up period (mean duration: 2.5 years), 44 patients died and 30 CV events were recorded. We used a Cox model to calculate the relative risk for outcomes. To assess the putative clinical utility, we also determined the net reclassification improvement (NRI) and the integrated discrimination improvement for each method.

Results: PWV, aortic/coronary calcification and ABI predicted all-cause mortality and cardiovascular events in univariate analysis. However, after adjustment for TRF, only aortic and coronary calcification scores were selected in a multivariate analysis as significant, independent variables. Moreover, coronary and aortic calcification scores allowed significant NRI of 27% and 20% respectively beyond the TRF.

Conclusions: Evaluation of vascular calcification scores appears to improve CV risk assessment (beyond TRF) in a CKD population. Since the radiographic evaluation of aortic calcification is relatively simple, routine use of this technique in CKD populations might improve CV risk stratification.

TH-PO562

Pericardial Fat Is Associated with Coronary Artery Calcification in Non-Dialysis Dependent Chronic Kidney Disease Patients

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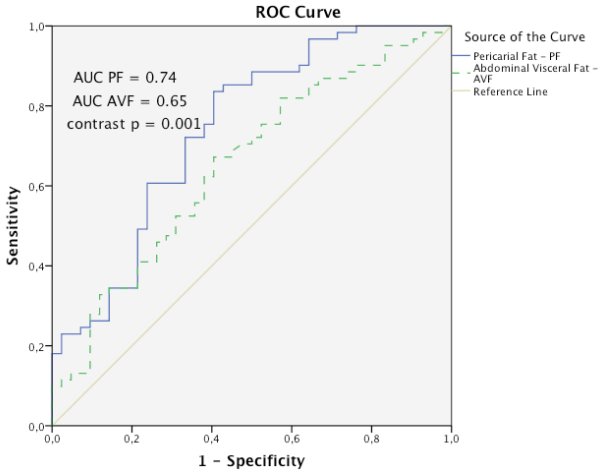
Background: Pericardial fat (PF), a component of visceral adipose tissue has been consistently related to coronary atherosclerosis in the general population. We evaluated the association of PF and coronary artery calcification (CAC) in non-dialysis dependent chronic kidney disease (CKD) patients.

Methods: Cross sectional baseline analysis of a prospective cohort of 117 outward CKD patients without manifest coronary artery disease (age, 56.9±11.0 years, males 64.1%, hypertension 95.1%, diabetics 25.2%, ever smokers 15.5%, CKD stages 2 to 5 and estimated glomerular filtration rate 36.8±18.1ml/m. CAC scores, PF volume and visceral abdominal fat areas were measured by computed tomography. PF association with CAC presence was analyzed by multivariate logistic regression.

Results: 59.2% patients had CAC >0. Those with CAC versus no CAC were 10 years older, had more males (78.7% versus 42.9%,p<0.001), higher waist circumference (95.9±10.7 versus 90.2±13.2cm, p=0.02), higher PF volumes (224.8 ± 107.6 versus 139.1 ± 85.0 cm3, p<0.01) and VAF area (109.2±81.5 versus 70.2±62.9cm², p=0.01). PF was significantly associated with CAC (OR: 1.88 95% CI:1.03-3.43) even after comprehensive multivariable adjustments.

Multivariable Adjusted Associations of Pericardial Fat with Coronary Artery Calcium Presence			
	Hazard Ratio	95% Confidence Interval	p value
Model 1	1.88	1.03 - 3.43	0.04
Model 2	1.89	1.05 - 3.42	0.03
Model 3	1.85	1.00 - 3.42	0.05

HR per one standard deviation
Model 1:age, gender, smoking, diabetes and concentric hypertrophy
Model 2: Model 1 + eGFR
Model 3: Model 1 + phosphate



Conclusions: PF is independently associated with CAC in non-dialysis dependent CKD patients.

TH-PO563

The Role of Calcified Vasculature and Lipids in Chronic Kidney Disease

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Background: Vascular calcification and dyslipidemia may adversely affect kidney function. The aim of this study was to examine the association of dyslipidemia and central vascular calcification (CVC) with kidney function in an aging cohort.

Methods: A cross-sectional analysis of 5,212 participants in the AGES-Reykjavik Study I was performed. Estimated glomerular filtration rate (eGFR) was calculated from standardized serum creatinine using the CKD-EPI equation. CVC was measured by quantitative computed tomography as the sum of calcification of the thoracic aorta. High density lipoprotein cholesterol (HDL) and triglycerides (TG) were measured in serum and non-HDL-cholesterol (NHC) calculated. Linear regression used eGFR as outcome and CVC, NHC, HDL and TG as main variables, adjusting for age, body mass index, diabetes mellitus (DM), hypertension (HTN), albuminuria and history of smoking.

Results: Mean (SD) age was 76.5 (5.5) years, 57% were females, 81% had HTN and 12% DM. Mean eGFR was 63,4 (15,2) ml/min/1.73 m². Median CVC was 2,101 (range 0-50,360) Agatston scores. NHC was not associated with eGFR, whereas lower HDL was associated with lower eGFR in males (p<0.05). Higher TG and CVC levels were strongly associated with lower eGFR (p<0.001 and p<0.05 respectively). Individuals with higher level of CVC had lower eGFR in the setting of high TG, significant in females only (p<0.01; table).

	MALES					FEMALES				
		low TG (60 mg/dL)		high TG (200 mg/dL)			low TG (60 mg/dL)		high TG (200 mg/dL)	
CVC**	N	eGFR*	95% CI	eGFR*	95% CI	N	eGFR*	95% CI	eGFR*	95% CI
very low	457	66.5	64.5-68.5	61.7	58.7-64.8	614	62.6	60.2-65.0	59.5	56.9-62.2
low	437	67.8	65.8-69.9	62.8	59.7-65.8	621	67.6	65.1-70.0	55.7	53.3-58.1
medium	451	67.9	66.0-69.8	62.3	59.2-65.4	565	68.0	65.4-70.7	56.9	54.4-59.4
high	412	67.9	65.9-70.0	61.0	58.0-64.0	553	66.9	64.3-69.4	55.3	52.8-57.8
very high	466	66.7	64.6-68.7	56.4	53.6-59.2	636	65.3	63.0-67.6	52.7	50.1-55.2

* mL/min./1.73 m²
** Very low CVC: 0-600 Agatston scores
Medium CVC: 1,500-3,000 Agatston scores
Very high CVC: 6,000-50,400 Agatston scores

Conclusions: Low fasting TG levels may be important for preserving kidney function in the elderly and may abrogate the effect of vascular calcification on the kidneys, particularly in women. This needs to be confirmed in a longitudinal study.

Funding: Private Foundation Support

TH-PO564

Hyperphosphatemia as an Independent Risk Factor of Coronary Artery Calcification Progression in Peritoneal Dialysis Patients

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Background: Coronary artery calcification (CAC) is associated with cardiovascular mortality in ESRD patients. The present study aimed to identify modifiable risk factors for CAC progression in peritoneal dialysis (PD) patients.

Methods: Adult PD patients who had received regular peritoneal dialysis for more than 6 months and underwent Coronary artery calcification scores (CaCS) measurements by multislice spiral computed tomography for at least 2 times with the interval ≥ 6 months were included in this prospective, observational cohort study. The demographic characteristics and clinical data including laboratory data and adequacy of PD were collected. Binary

Logistic Regression was used to identify the independent risk factors for CAC progression in PD patients and Multivariate Linear Regression was used to identify the associated factors of hyperphosphatemia.

Results: A total of 207 adult PD patients (116 men, 56.0%) with a mean age of 59.8 ± 15.9 years old were recruited to this study and were divided into the slow progression group (n = 131) and the rapid group (n = 76) according to the velocity of CAC progression. The median interval of the first and last CaCS measurement was 24.6 (13.0-37.9) months. Multivariate logistic regression revealed that age (p = 0.039), BMI (p = 0.007) and serum phosphorus (p = 0.013) were independent risk factors of CAC progression after adjusting for gender, basic CaCS, serum calcium, lipoprotein a, albumin, hs-CRP, total Ccr, residual Ccr and D4/P phosphorus. Multivariate linear regression revealed that hyperphosphatemia was associated with higher transferrin (p = 0.007), serum albumin (p = 0.049), and nPCR (p < 0.001), and lower hemoglobin (p = 0.015), residual Ccr (p < 0.001), and PD Ccr (p < 0.001).

Conclusions: Hyperphosphatemia was an independent risk factor of CAC progression and serum phosphorus level was associated with nutritional intake and PD adequacy. These results provide important information for the clinical management of ESRD patients.

Funding: Government Support - Non-U.S.

TH-PO565

Klotho Expression and Circulating Levels in Renal Failure Hannes Olauson,[1] Karin Edvardsson,[1] Karolina Lindberg,[1] Annika Wernerson,[1] Orson W. Moe,[2] Tobias E. Larsson.[1] *[1]CLINTEC, Karolinska Instt, Stockholm, Sweden; [2]Charles and Jane Pak Center for Mineral Metabolism and Clinical Research, Univ of Texas Southwestern Medical Center, Dallas, TX.*

Background: Membrane-bound Klotho (mKL) is predominantly expressed in the renal tubules where it controls trans-cellular flux of calcium and phosphate and functions as a co-receptor for the phosphate-regulating hormone FGF23. mKL can be cleaved from the cell surface and act as a circulating hormone (cKL) by antagonizing numerous signaling pathways including Wnt and TGFβ. Chronic kidney disease (CKD) is ascribed a state of systemic Klotho deficiency and mKL levels are suppressed by uremic factors, including inflammation and oxidative stress. However, data on cKL in CKD are conflicting with reports of decreased, unaltered or even increased levels.

Methods: Herein, we examined mKL and cKL levels in 42 CKD patients using different approaches; we further analyzed dynamic changes of mKL and cKL in a mouse model of adenine-induced renal failure (ARF). To explore shedding of mKL in ARF we employed an *ex vivo* system where kidneys from healthy and ARF mice were removed, sectioned, and placed in cell culture medium for 2 h at ambient room temperature.

Results: In CKD patients, there was no correlation between cKL and eGFR, neither when measured by a commercially available ELISA nor by immunoprecipitation technique. Similarly, mice with ARF had a rapid decline in renal mKL level without a reciprocal reduction in cKL. *Ex vivo* data demonstrated a close correlation between cKL in conditioned media and tissue levels of mKL in all kidneys examined, indicating unaltered mKL shedding mechanisms in ARF.

Conclusions: In sum, we confirm reduced mKL in CKD patients and in a mouse model of ARF, albeit with an apparent uncoupling between mKL and cKL. The reason(s) for this uncoupling could not be substantiated *ex vivo*, but may be related to methodological limitations, interference with uremic factors or compensatory increased shedding from extra-renal sources of Klotho. This issue warrants more attention in clinical studies reporting on cKL and clinical outcomes.

TH-PO566

FGF23 and α-Klotho Acting on Hippocampal Neurons: Impact on Neuronal Morphology and Synapse Formation Ex Vivo Maren Leifheit-Nestler,[1] Anne Schön,[1] Niko Hensel,[2] Timo Konen,[2] Olga Baron,[2] Claudia Grothe,[2] Peter Claus,[2] Dieter Haffner.[1] *[1]Dept of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany; [2]Inst of Neuroanatomy, Hannover Medical School, Hannover, Germany.*

Background: Children with congenital CKD often display impaired cognitive development and memory deficits. Memory formation includes a dynamic control of neuronal morphology and synapse formation in the hippocampus. The circulating levels of the phosphaturic hormone fibroblast growth factor 23 (FGF23) are elevated in CKD while its co-receptor α-klotho decreases with declining renal function. The effects of FGF23 and α-klotho on memory formation are unknown. Here we investigate the impact of FGF23 and α-klotho on morphological outcomes in hippocampal neurons relevant for memory formation.

Methods: Dissociated hippocampal neurons, prepared from postnatal day 5-8 wild-type C57BL/6J mice, were stimulated with FGF23 +/- α-klotho, FGF receptor inhibitor or vehicle. Neuronal morphology was assessed by Sholl analysis including evaluation of neurite length and neurite branching. To analyze whether FGF23 and/or α-klotho affects synaptogenesis, neurons were stained for synaptophysin and post-synaptic density protein - 95 (PSD-95) followed by quantification of morphological synapses. In addition, FGF23/klotho downstream pathways in FGF23 and/or α-klotho treated hippocampal cultures were assessed.

Results: FGF23 increases neurite elongation of hippocampal neurons, and enhances synapse density independent of α-klotho. For FGF23 signaling, FGF receptors as well as the FGF receptor substrate 2 and the FGF23 co-receptor α-klotho with the exception of FGF23 itself are expressed in the hippocampus of neonatal mice. Stimulation of dissociated hippocampal cultures with FGF23 and/or α-klotho differentially induces downstream signaling.

Conclusions: Hippocampal neurons of neonatal mice are a target of FGF23 *ex vivo*. FGF23 on its own increases neurite elongation as well as synapse density without α-klotho. Thus, we hypothesize that excessive FGF23 serum levels might cross the blood-brain barrier and modulate hippocampal function in patients with congenital CKD.

TH-PO567

Delivery of Soluble Klotho Ameliorates Uremic Cardiac Hypertrophy in Mice Jian Xie,[1] Joonho Yoon,[1] Makoto Kuroo,[2] Chou-Long Huang.[1] *[1]UT Southwestern Medical Center, Dallas, TX; [2]Jichi Medical Univ, Tochigi, Japan.*

Background: Klotho (KL) is a membrane protein produced mostly in the kidney; its ectodomain is released as a soluble protein. We have reported that soluble Klotho (sKL) protects the heart against stress-induced cardiac hypertrophy and fibrosis, by inhibiting TRPC6 channel in the heart and independently of serum phosphate (Pi) and FGF23. Here, we examined whether reduced serum sKL contributes to uremic cardiomyopathy and replacement of sKL protects the disease.

Methods: Wild type (WT) and heterozygous (Het) KL-deficient (*kl*) mice (129/SvJ) were rendered CKD by 5/6 nephrectomy (Nx) or sham surgery for 4 wk.

Results: Serum sKL levels (assayed by IP + western blot) were lower in Het versus WT mice, and both were further reduced by 5/6 Nx. CrCl was reduced ~60% by 5/6 Nx, but not different between WT and Het. Heart weight/body weight ratio (HW/BW, mg/g) of WT mice was increased by 5/6 Nx (sham and CKD: 4.1±0.1 and 5.4±0.2, p<0.01); CKD-induced increase was more pronounced in Het (sham and CKD: 4.0±0.1 and 6.2±0.2, p<0.01; also p<0.01, WT/CKD versus Het/CKD). Supporting the HW/BW results, *BNP* expression was increased in hearts of WT CKD versus sham mice, and CKD-induced increase was enhanced in Het *kl* mice. BP, serum Pi and FGF23 levels were not different between WT and Het sham mice, but equally elevated in both CKD mice. Ejection fraction (EF) measured by MRI showed no difference between WT sham and CKD mice (63±3 versus 61±3 %). LV end-diastole volume (LVEDV) was slightly lower and HW/LVEDV ratio increased in WT CKD versus sham, indicating concentric hypertrophy and diastolic dysfunction of WT CKD hearts. In contrast, versus sham, EF was decreased in Het CKD (47±4 versus 62±4 %, p<0.05), LVEDV was markedly increased, and HW/LVEDV unchanged, indicating progression of Het CKD hearts to systolic dysfunction. Tail-vein delivery of cDNA encoding soluble klotho (versus empty vector; weekly x 3 began 5 days post-Nx) to Het CKD mice reduced HW/BW ratio and *BNP* expression without affecting serum Pi and FGF23 levels.

Conclusions: Reduced serum sKlotho in CKD is an important cause of uremic cardiomyopathy, independently of serum Pi and FGF23. Delivery of soluble klotho ameliorates uremic cardiac hypertrophy.

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TH-PO568

Shedding of Klotho by Disintegrin and Metalloproteinase (ADAM10) in the Kidney Ellen P. Van Loon,[1] Wilco P. Pulskens,[1] Marc G. Vervloet,[2] Harry Van Goor,[3] René J. Bindels,[1] Joost Hoenderop.[1] *[1]Physiology, Radboud Univ Medical Center, Nijmegen, Netherlands; [2]Nephrology, VU Univ Medical Center, Amsterdam, Netherlands; [3]Pathology and Medical Biology, Univ Medical Center Groningen, Groningen, Netherlands.*

Background: The anti-aging gene klotho is involved in calcium and phosphate homeostasis. Membrane-bound klotho, consisting of a KL1 and KL2 domain, can be cleaved by a disintegrin and metalloproteinase (ADAM)10 and 17, directly above the plasma membrane (α-cut) or between KL1 and KL2 (β-cut). The aim of the present study was to examine klotho shedding in the kidney.

Methods: A Madin-Darby canine kidney (MDCK)I cell line stably-expressing klotho, was used to investigate cellular localization and klotho shedding. Renal expression of klotho, ADAM10 and ADAM17 were determined by immunofluorescence. Dose-dependent ADAM inhibition on klotho shedding was examined *in vitro* using transiently-transfected human embryonic kidney (HEK)293 klotho-expressing cells. *In vivo*, serum electrolyte concentrations and mRNA expression of renal and duodenal electrolyte transporters were determined 24 hours after intraperitoneal administration of ADAM inhibitor or phosphate-buffered saline in 10 mice per group.

Results: Klotho was expressed on the apical and basolateral membrane of renal cells, with a higher abundance of soluble klotho at the apical side. ADAM10 colocalized with klotho in distal convoluted and connecting tubules, but not with ADAM17. *In vitro*, ADAM inhibition dose-dependently decreased full-length klotho shedding (α-cut), while this inhibitory effect was less specific for the β-cut. Interestingly, ADAM inhibition increased renal and duodenal mRNA expression of phosphate transporters, while serum phosphate levels were significantly decreased in ADAM inhibitor treated mice. In contrast, ADAM inhibition did not change urinary klotho levels.

Conclusions: Our data indicate that renal cells preferentially secrete klotho to the apical side and that sheddases other than ADAMs are likely involved in the cleavage of the β-cut of klotho. Moreover, we suggest that ADAM10 is the primary sheddase responsible for klotho cleavage in the kidney.

Funding: Government Support - Non-U.S.

TH-PO569

Fibroblast Growth Factor 23 but Not Klotho Deficiency Ameliorates Progression of Chronic Kidney Disease in Mice Olena Andrukhova, Svetlana Slavic, Sathish Kumar Murali, Reinhold Erben. *Dept of Biomedical Sciences, Inst of Physiology, Pathophysiology and Biophysics, Vienna, Austria.*

Background: Clinical studies have shown that circulating fibroblast growth factor 23 (FGF23) is associated with disease progression, cardiovascular risk, and mortality in patients with chronic kidney disease (CKD). Here, we sought to elucidate further the vitamin D independent role of FGF23 and its co-receptor Klotho in the pathogenesis of CKD in a mouse model.

Methods: CKD was induced by 5/6 nephrectomy in 3-month-old wild-type (WT) mice, vitamin D receptor (VDR) mutant mice, *Fgf23/VDR*, and *Klotho/VDR* compound mutants. All mice were kept on a rescue diet enriched with calcium, phosphorus, and lactose to prevent hypocalcemia and secondary hyperparathyroidism in VDR mutans.

Results: Sham-operated (SHAM) WT, VDR, Fgf23/VDR, and Klotho/VDR mice served as controls. Both WT and VDR mutants developed progressive CKD by 8 weeks after 5/6 nephrectomy as evidenced by reduced glomerular filtration rate (GFR), increased serum creatinine, increased albuminuria, increased serum FGF23 and parathyroid hormone (PTH) levels, hypertension, impaired left ventricular function, as well as reduced body weight and increased mortality, relative to SHAMs. Fgf23/VDR compound mutant CKD mice lacking Fgf23 showed higher GFR and lower urinary albumin but unchanged serum PTH levels as compared to WT and VDR CKD mice. Furthermore, Fgf23/VDR compound mutants were protected against the CKD-induced weight loss, increase in mortality, volume expansion, hypernatremia, hypercalcemia, hyperphosphatemia, hypertension, left ventricular functional impairment, and increase in heart-to-body weight ratio observed in WT. In contrast, lack of Klotho in Klotho/VDR compound mutant CKD mice did not ameliorate the CKD-induced increase in mortality, volume expansion, GFR reduction, albuminuria, hypertension, or left ventricular functional impairment as compared to WT and VDR CKD mice.

Conclusions: Collectively, our data suggest that elevated Fgf23 contributes to the pathogenesis of CKD in a Klotho and vitamin D-independent manner, and may provide a mechanistic explanation for the association between circulating FGF23 and disease progression in CKD patients.

TH-PO570

Influence of Experimental Renal Failure and FGF23 on Endothelial Function and Myocardial Perfusion Melissa Verkaik,[1,2] Rene Musters,[2] Pieter M. Ter Wee,[1] Etto C. Eringa,[2] Marc G. Vervloet.[1] *[1]Dept of Nephrology, VU Univ Medical Center, Amsterdam, Noord-Holland, Netherlands; [2]Dept of Physiology, Inst of Cardiovascular Research ICaR-VU, VU Univ Medical Center, Amsterdam, Noord-Holland, Netherlands.*

Background: Cardiovascular causes account for approximately 50% of mortality in patients with chronic kidney disease (CKD). Among others, FGF23, a phosphate-lowering protein and elevated in CKD, is associated with cardiovascular mortality. We hypothesized that CKD impairs vascular function and myocardial perfusion and that this can be partly attributed to FGF23.

Methods: Eight week old male wild type C57Bl/6J mice were subjected to partial nephrectomy (5/6Nx) or sham-surgery, and after 6 weeks mice were placed into a metabolic cage and subjected to myocardial contrast echocardiography (MCE) to test myocardial perfusion and a pressure myograph setup to test ex vivo vascular function. A second group received either PBS or FGF23 i.p. injections for 7 consecutive days twice daily.

Results: Plasma FGF23 and urinary phosphate significantly increased after Nx surgery (from 182 to 291 pg/ml (p=0.02) and from 19.2 to 114.9 µmol/24h (p=0.001) respectively). 5/6Nx blunted ex vivo vasodilator responses to acetylcholine (p=0.002), whereas responses to sodium nitroprusside (SNP) or endothelin were normal. FGF23 injections in control mice mimicked these vascular responses. Myocardial blood volume, microvascular filling velocity and perfusion were reduced in 5/6Nx mice upon acetylcholine and SNP. Finally, 5/6Nx affects calcium channels in individual cardiomyocytes for both systolic and diastolic parameters.

Conclusions: Diminished endothelium-dependent vasodilation in renal failure is mimicked by FGF23 injections in non-renal failure mice. Also, renal failure compromises myocardial perfusion increments upon stimulation and disturbs cardiomyocyte calcium handling.

Funding: Government Support - Non-U.S.

TH-PO571

Effect of Dietary Fe on FGF23 Level of Adriamycin-Treated Mice Masanori Takaiwa,[1] Kosei Hasegawa,[2] Hiroyuki Tanaka,[3] Nobuyuki Kodani.[1] *[1]Pediatrics, Matsuyama Red Cross Hospital, Japan; [2]Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan; [3]Okayama Saiseikai General Hospital, Japan.*

Background: Parenteral Fe therapy for CKD patients could increase FGF23 and cause hypophosphatemia. However, Fe deficiency worsens FGF23 elevation in patients with inherited hypophosphatemia. FGF23 elevates during the early CKD, and was documented to be associated with poor prognosis of CKD. Hence, we created an early CKD model by intermittent administration of adriamycin (ADR) to C57BL/6J mice (C57) and examined the effect of dietary Fe on FGF23 during the early CKD.

Methods: Using 6 groups (control and 0.6%-Fe-AN groups – fed a 0.6% Fe diet; 2%-Fe and 2%-Fe-AN groups - fed a 2.0% Fe diet; 0.02%-Fe and 0.02%-Fe-AN groups - fed a 0.02% Fe diet), 10-week-old male C57 were administered serine (the control, 2%-Fe and 0.02%-Fe) or ADR weekly (0.6%-Fe-AN, 2%-Fe-AN and 0.02%-Fe-AN) for 28 days. Data were expressed as the mean±SEM. A value of $p<0.01$ was considered significant (Tukey's test).

Results: After treatment, Fe for 2%-Fe and 2%-Fe-AN (275±9 and 280±13µg/dl) were higher, and that of 0.02%-Fe-AN was lower (115±10µg/dl) than the control (161±6µg/dl). Hb of 0.02% Fe-AN (11.5±0.1 g/dl) was markedly lower than the control (13.5±0.1 g/dl). The kidneys of the ADR treated groups were smaller in size. Although not significant, Cr for 0.6%-Fe-AN, 2%-Fe-AN and 0.02%-Fe-AN were higher (1.8±0.2, 2.3±1.7 and 2.8±0.3mg/dl) than the control (0.9±0.1mg/dl). FGF23 tended to be higher among all ADR treated groups, only 0.02%-Fe-AN showed significant elevation (670±134pg/ml) compared with the control (159±10pg/ml). Ca, Pi and PTH showed no significant difference.

Conclusions: The intermittent ADR injection caused the small kidneys associated with slight Cr increase. The ADR treatment caused mild increase in FGF23 without affecting Pi and PTH, hence serving as a reasonable early CKD model. Only the ADR treatment of the Fe depleted mice increased FGF23, hence the negative regulatory effect of Fe on FGF23 was significant during early CKD. Our results suggest that the initiation of oral Fe during early CKD suppresses FGF23 production and has a favorable effect on progression of CKD.

Funding: Private Foundation Support

TH-PO572

Iron Status Affects FGF23 Levels in Mice with Chronic Kidney Disease Mark Hanudel,[1] Victoria Rivka Gabayan,[2] Elizabeta Nemeth,[2] Katherine Wesseling-Perry,[1] Tomas Ganz,[2] Isidro B. Salusky.[1] *[1]Dept of Pediatrics, UCLA; [2]Center for Iron Disorders, UCLA.*

Background: Iron deficiency upregulates FGF23 expression, resulting in higher circulating FGF23 levels. However, the effects of iron deficiency, the role of hepcidin, and the effects of iron loading on FGF23 levels in CKD are unclear. Our objectives were to measure FGF23 levels in mice, both wild type and with CKD, fed diets with varying iron content, and to assess whether hepcidin-mediated iron sequestration contributes to elevated FGF23 levels.

Methods: Wild type (WT) and hepcidin knockout (HKO) C57BL/6 mice were fed diets that did or did not contain adenine (known to induce CKD), with low iron (4 ppm), standard iron (335 ppm), or high iron (10,000 ppm) concentrations. HKO mice were not fed the high iron diet, given the risk of fatal iron overload. Mice were on the diets for 8 weeks post-weaning, with hematologic parameters and plasma C-terminal FGF23 assessed at 2 weeks (WT controls only, n=4 per group), 4 weeks (n=8-15 per group), and 8 weeks (n=4-12 per group).

Results: Among the WT mice fed control diets, at 2 weeks, cFGF23 did not differ among the three iron groups. However, at 4 and 8 weeks, both the low and high iron groups had higher cFGF23 than the standard iron group. Among the WT-CKD mice, at both 4 and 8 weeks, the high iron group had the lowest cFGF23 levels. Among the CKD mice, the standard iron HKO group had lower cFGF23 than the standard iron WT group at 4 and 8 weeks, and the low iron HKO group had lower cFGF23 than the low iron WT group at 4 weeks. At 8 weeks, the low iron HKO group had higher cFGF23 than the low iron WT group; however, the HKO group was more anemic than the WT group at this time point, suggesting depletion of iron stores in the HKO group.

Conclusions: In CKD, iron status affects cFGF23 levels in a dose-dependent manner. In CKD, hepcidin-mediated iron sequestration may contribute to elevated cFGF23 levels.

4 weeks	WT Control			WT CKD		
Diet	Low Iron	Std Iron	High Iron	Low Iron	Std Iron	High Iron
Hgb (g/dL)	11.1 ± 0.3*	13.5 ± 0.3	12.2 ± 0.6	12.5 ± 0.4	13.5 ± 0.4	14.0 ± 0.4^
MCV (fL)	39.5 ± 0.3*	46.2 ± 0.4	50.1 ± 0.6*^	40.4 ± 0.3*	42.6 ± 0.5	45.9 ± 0.5*^
RDW (%)	23.3 ± 0.5*	17.5 ± 0.3	22.8 ± 0.4*	19.8 ± 0.4	18.8 ± 0.4	17.5 ± 0.3*^
Log_{10}(cFGF23)	3.02 ± 0.07*	2.34 ± 0.03	3.08 ± 0.10*	3.61 ± 0.13	3.33 ± 0.08	3.03 ± 0.03^

4 weeks	HKO Control			HKO CKD		
Diet	Low Iron	Std Iron	High Iron	Low Iron	Std Iron	High Iron
Hgb (g/dL)	11.0 ± 0.5	14.1 ± 0.5	n/a	14.6 ± 0.6†	16.1 ± 0.6†	n/a
MCV (fL)	39.2 ± 0.4	49.5 ± 0.4†	n/a	44.9 ± 0.5†	49.4 ± 0.5†	n/a
RDW (%)	24.5 ± 0.5	17.2 ± 0.4	n/a	18.9 ± 0.8	16.5 ± 0.2†	n/a
Log_{10}(cFGF23)	3.19 ± 0.11	2.30 ± 0.03	n/a	3.00 ± 0.10†	2.88 ± 0.14†	n/a

8 weeks	WT Control			WT CKD		
Diet	Low Iron	Std Iron	High Iron	Low Iron	Std Iron	High Iron
Hgb (g/dL)	7.3 ± 0.2*	14.8 ± 0.2	5.7 ± 0.5*^	9.3 ± 0.6	9.8 ± 1.1	12.6 ± 0.6
MCV (fL)	37.2 ± 0.5*	44.8 ± 0.3	54.9 ± 1.1*^	36.2 ± 0.6	38.6 ± 1.0	44.8 ± 0.4*^
RDW (%)	28.0 ± 1.3*	17.8 ± 0.3	22.2 ± 0.8*^	19.1 ± 0.8	20.9 ± 1.4	19.1 ± 0.4
Log_{10}(cFGF23)	3.83 ± 0.08*	2.42 ± 0.01	4.04 ± 0.02*^	3.70 ± 0.07	3.64 ± 0.13	3.19 ± 0.08*^

8 weeks	HKO Control			HKO CKD		
Diet	Low Iron	Std Iron	High Iron	Low Iron	Std Iron	High Iron
Hgb (g/dL)	4.0 ± 0.5†	14.4 ± 0.3	n/a	7.3 ± 0.6	13.9 ± 0.9†	n/a
MCV (fL)	36.1 ± 0.6	48.9 ± 0.4†	n/a	36.8 ± 0.7	49.8 ± 0.5†	n/a
RDW (%)	25.7 ± 1.1	16.9 ± 0.2†	n/a	21.9 ± 0.8	16.6 ± 0.1†	n/a
Log_{10}(cFGF23)	4.36 ± 0.20	2.17 ± 0.04†	n/a	4.21 ± 0.09†	3.23 ± 0.14	n/a

*p<0.05 vs. Standard Iron (within WT Control and WT CKD groups)
^p<0.05 for Low vs. High Iron (within WT Control and WT CKD groups)
†p<0.05 for HKO vs. WT (within each dietary group)

Funding: Other NIH Support - NIH 2T32DK007789-11 (7/2013-6/2014)

TH-PO573

FGF23 Can Directly Target Hepatocytes and Contributes to Inflammation in CKD Saurav Singh,[1] Alexander Grabner,[1] Alexis J. Sloan,[1] Ansel P. Amaral,[1] Karla J. Schramm,[1] Myles S. Wolf,[2] Christian Faul.[1] *[1]Dept of Medicine, Univ of Miami Miller School of Medicine, Miami, FL; [2]Dept of Medicine, Northwestern Univ Feinberg School of Medicine, Chicago, IL.*

Background: Chronic inflammation is a common feature of chronic kidney disease (CKD), and biomarkers of inflammation are among the strongest predictors of poor clinical outcome. The molecular mechanisms underlying the pathological interrelationship between deterioration of renal function and amplification of the inflammatory state are unknown. Patients with CKD develop marked elevations in circulating levels of fibroblast growth factor (FGF) 23. Recent data from our group indicates that FGF23 directly induces cardiac injury by activating FGF receptor (FGFR) 4 in cardiac myocytes, independent of α-klotho, the FGF23 co-receptor in the kidney. Since hepatocytes express high levels of FGFR4, we postulate that FGF23 can directly target the liver.

Methods: Serum starved HepG2 cells, a hepatocellular carcinoma cell line, and primary mouse hepatocytes were treated with FGF23, in the absence and presence of a pan-FGFR inhibitor followed by the isolation of protein and RNA for expression analyses. Serum FGF23 levels were elevated in wild type mice via administration of a high phosphate diet for 3 months or intravenous delivery of recombinant FGF23 for 5 days, followed by the isolation of serum and liver tissue. Age-matched FGFR4 knockout mice were also administered a high phosphate diet.

Results: FGF23 treatment of cultured hepatocytes increases the expression of pro-inflammatory proteins, including C-reactive protein (CRP), by activating calcineurin/NFAT signaling in an FGFR4-dependent and α-klotho-independent manner. The elevation of serum FGF23 increases CRP levels in the liver and in the circulation of wild-type mice, but not in FGFR4 knockout mice.

Conclusions: Our findings provide a potential causative link between FGF23 elevation and the activation of pro-inflammatory processes in the liver, and they suggest a novel mechanism to explain the development of chronic inflammation in patients with CKD. We postulate that pharmacologic FGFR4 blockade might not only have cardio-protective but also anti-inflammatory effects in CKD.

TH-PO574

Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Inflammatory Mediators Stimulate Fibroblast Growth Factor-23 (FGF23) Production in *In Vitro* Osteocytes Culture Shweta Bansal,[1,2] Khaled Khazim,[3] Chakradhar Velagapudi,[1,2] Basant Bhandari,[1] Sherry L. Werner,[4] Paolo Fanti.[1,2] *[1]Medicine/Renal, Univ of Texas Health Sciences Center at San Antonio, San Antonio, TX; [2]Medicine/Renal, South Texas Veterans Healthcare System, San Antonio, TX; [3]Medicine/Nephrology, Western Galilee Hospital, Nahariya, Israel; [4]Pathology, Univ of Texas Health Sciences Center at San Antonio, San Antonio, TX.*

Background: High levels of FGF23, an exclusive product of osteocytes, are not fully explained by abnormal mineral metabolism in chronic kidney disease (CKD) patients. Recently, we have extended prior observations of correlation between FGF23 and inflammatory mediator levels by reporting an independent association of FGF23 with the acute-phase reactant NGAL in diabetic kidney disease patients. The mechanism supporting association of FGF23 with inflammation, however, remains unknown.

Methods: Hypothesis: The association of NGAL and other inflammatory mediators with FGF23 is the result of direct stimulatory effect of these mediators on the osteocytic synthesis and secretion of FGF23. Methods: Cultures of terminally differentiated IDG-SW3 osteocytes were treated with NGAL (50-200 ng/ml), tumor necrosis factor-α (TNF-α; 1-25 ng/ml), and interleukin-6 (IL-6; 0.1-25 ng/ml) for 24 and 48 hours. 1,25$(OH)_2$vitamin D_3(10^{-9}-10^{-7} M) was used as a positive control. Cell lysate was assessed for FGF23 mRNA and protein expression using RT-PCR and western blot technique, respectively. FGF23 protein was measured in media by ELISA.

Results: Incubation with NGAL and TNF-α significantly increased FGF23 mRNA expression dose-dependently, with maximal increments being 44-fold with NGAL, and 80-fold with TNF-α. IL-6 treatment also increased FGF23 mRNA expression up to 2-fold but insignificantly. In addition, all these mediators increased cytosolic concentration of FGF23 protein significantly up to 3-fold in dose-independent manner.

Conclusions: NGAL, TNF-α, and IL-6 directly stimulate osteocytic synthesis of FGF23. Given that CKD is a state of heightened inflammation, these results suggest a possible new mechanism for increased FGF23 levels in CKD.

Funding: NIDDK Support, Veterans Affairs Support

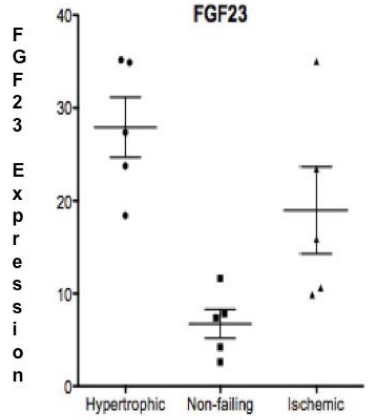
TH-PO575

Cardiac Fibroblast Growth Factor Receptor 1-4 and Fibroblast Growth Factor 23 Expression in Adult Cardiac Patients Michel Chonchol, Amrut V. Ambardekar, Lynn Heasley. *Univ of Colorado Denver.*

Background: Experimental and epidemiological studies have demonstrated a relationship between higher serum fibroblast growth factor 23 (FGF23) concentrations and left ventricular hypertrophy (LVH) in patients with and without chronic kidney disease (CKD). The aim of this study was to measure fibroblast growth factor receptor (FGFR) 1-4 and FGF23 expression in human left ventricular tissue samples from hearts with ischemic cardiomyopathy, hypertrophic cardiomyopathy, and normal function.

Methods: We conducted a retrospective study in patients with normal kidney function and end-stage heart failure from either ischemic cardiomyopathy (n=5) or hypertrophic cardiomyopathy (n=5). Left ventricular tissue was collected and flash frozen at the time of heart transplant. Five donor hearts harvested for transplant, but unused for non-cardiac reasons were utilized as the non-failing control samples. FGFR 1-4 and FGF23 expression was evaluated using quantitative real-time PCR.

Results: Age, serum creatinine, and serum phosphorus were similar for those patients with ischemic and hypertrophic cardiomyopathy at the time of transplant. Cardiac FGFR 1-4 were expressed in all non-failing, ischemic, and hypertrophic hearts; this expression tended to be enhanced in samples with end-stage hypertrophic cardiomyopathy when compared to ischemic cardiomyopathy or non-failing hearts. Cardiac FGF23 mRNA expression was increased in samples from patients with hypertrophic cardiomyopathy when compared to ischemic cardiomyopathy or non-failing hearts.



Conclusions: FGFR 1-4 and FGF23 expression are up-regulated in left ventricular samples of patients with end-stage hypertrophic cardiomyopathy and normal kidney function.

Funding: NIDDK Support

TH-PO576

Role of Fibroblast Growth Factor 23 and Parathyroid Hormone on Renal Phosphate Handling in Chronic Kidney Disease Sinead Kinsella,[1] Ahad Abdalla,[1] Patrick P. O'Connor,[1] Mark T. Kilbane,[2] John N. Holian,[1] Alan J. Watson.[1] *[1]Nephrology, St. Vincent's Univ Hospital, Dublin, Ireland; [2]Metabolism Laboratory, St. Vincent's Univ Hospital, Dublin, Ireland.*

Background: PTH and FGF23 are both potent regulators of renal phosphate excretion, maintaining normal phosphate levels until advanced CKD and ESKD. However, the relative importance of PTH and FGF23 in phosphate homeostasis in earlier stages of CKD is not established.

Methods: We conducted a cross-sectional observational study examining the relationships between FGF23, PTH and phosphate homeostasis in patients with CKD Stage 3-5. PTH, 25-hydroxyvitaminD (25OHD) and 1,25-dihydroxyvitaminD (1,25OH_2D), renal phosphate threshold (TmP/GFR) and markers of bone turnover were measured. Subjects underwent DXA scanning (Lunar IDXA™) of lumbosacral spine, hip and non-dominant forearm.

Results: 30 patients were included, mean age 55.9 years, mean eGFR 29.2mls/min/1.73m^2. Median PTH 90 ng/ml; 60% had elevated PTH (>65ng/l). Median FGF23 264 RU/ml; 90% had elevated FGF23 (>100RU/ml). Mean phosphate 1.1mmol/L; no patients were hyperphosphatemic or taking phosphate binders. eGFR correlated negatively with PTH (r=-0.605, p<0.001), FGF23 (r=-0.635, p<0.001) and markers of bone-turnover. eGFR correlated positively with TmP/GFR (r=0.394, p=0.031), although this relationship was not significant having controlled for FGF23 and PTH. Both PTH and FGF23 correlated negatively with TmP/GFR, (r=-0.647 and r=-0.559 respectively, p<0.001) and 1,25OH Vitamin D (r=-0.391, p=0.036; and r=-0.558, p=0.002 respectively). PTH and FGF23 correlated positively with markers of bone-turnover. PTH was normal up to CKD Stage 4; FGF23 levels were elevated in CKD Stage 3a (eGFR<60ml/min/1.73m^2) and increased progressively with CKD stage. T-score was lowest at the forearm site. DXA measures did not correlate with PTH or FGF23.

Conclusions: Elevations in FGF23 occurred earlier in the progression of CKD than elevations in PTH and correlated with renal phosphate excretion, decreased 1,25OH_2D and increased markers of bone-turnover, even in moderate CKD.

TH-PO577

Determinants of Change in Fibroblast Growth Factor-23 in Maintenance Hemodialysis Patients Sonoo Mizuiri,[1] Yoshiko Nishizawa,[1] Kazuomi Yamashita,[1] Kyoka Ono,[1] Kohji Usui,[2] Kenichiro Shigemoto.[1] *[1]Nephrology, Ichiyokai Harada Hospital, Hiroshima, Japan; [2]Nephrology, Ichiyokai Ichiyokai Clinic, Hiroshima, Japan.*

Background: It has been reported that high serum fibroblast growth factor-23 (FGF-23) levels are associated with increased mortality in maintenance hemodialysis (MHD) patients, but the factors responsible for the change in FGF-23 are largely unknown. The aim of this study was to evaluate the determinants of change in FGF-23 in MHD patients.

Methods: We evaluated serum intact FGF-23, age, dialysis vintage, presence of DM, BMI, blood pressure, urine volume, Ccr+Curea/2, Kt/Vurea, high sensitivity CRP, ß2-MG, cystatin C, serum albumin, nPNA, geriatric nutritional risk index (GNRI), serum phosphate, corrected serum calcium (Ca), iPTH, and dosages of active vitamin D and/or phosphate binders in 332 MHD patients at baseline, and performed a reassessment after 1 year. Statistical analyses were performed with Dr. SPSS II.

Results: During the 1 year, 33 patients died, 16 patients changed hospitals, and 283 patients completed the study. The median age and dialysis vintage were 69 years and 66 months at baseline, respectively. There were no increases in mortality after 1 year according to quartiles of FGF-23 levels at baseline. High sensitivity CRP, age, ß2-MG, and GNRI were significantly associated with death ($p<0.02$), but FGF-23 was not. Rise in FGF-23 after 1 year was observed in 176 patients (62.2%). Patients with rise in FGF-23 showed significantly lower serum phosphate and Ca levels and higher active vitamin D dosage at baseline, while significantly higher serum phosphate, nPNA, nPCR with higher active vitamin D dosage after 1 year compared to patients without rise in FGF-23 ($p<0.05$). Multiple regression analysis showed that serum phosphate ($p<0.01$), Ca ($p<0.05$), and dosage of active vitamin D ($p<0.05$) were significant independent factors for rise in FGF-23. Change in FGF-23 was significantly ($p<0.01$) correlated with changes in serum phosphate ($r=0.60$), Ca ($r=0.65$), albumin ($r=0.17$), GNRI ($r=0.18$) and active vitamin D dosage ($r=0.28$).

Conclusions: Rise in serum FGF-23 is related not only to mineral disorders and active vitamin D dosage, but also to improvement in nutritional status in MHD patients.

Funding: Private Foundation Support

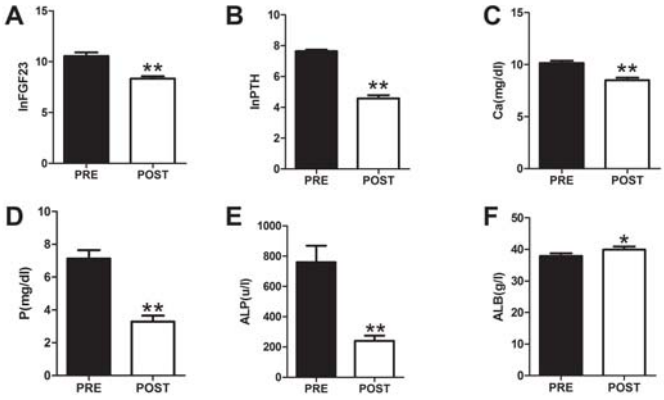
TH-PO578

Fibroblast Growth Factor 23 and Heart Rate Variability in Stage 5 Chronic Kidney Disease Ningning Wang, Lina Zhang. *Nephrology, First Affiliated Hospital of Nanjing Medical Univ, China.*

Background: Lower heart rate variability(HRV) is associated with cardiovascular disease(CVD). We evaluated the relationship between fibroblast growth factor 23(FGF23) and HRV in stage 5 chronic kidney disease(CKD). Their longitudinal changes in parathyroidectomy(PTX) patients were investigated.

Methods: We included 78 controls, 100 CKD patients, and two subgroups classified as successful(n=24) and unsuccessful(n=4) PTX(follow-up for 5 months). Plasma FGF23 was measured by ELISA and Holter were recorded.

Results: In stage 5 CKD, HRV were lower and median plasma FGF23 was higher than controls. FGF23 was correlated with SDNN and SDANN. Serum PTH was correlated with mean NN, SDNN, SDANN, pNN50% and MHR. Successful PTX subgroup had improvements in HRV and laboratory values while unsuccessful PTX not.



	Baseline	After PTX
Successful PTX		
lnFGF23	10.6 ±1.7	8.3±1.2*
Mean 24hHR	87.8±8.1	81.7±8.3*
Mean NN	689.1±60.9	748.1±83.2*
SDNN	66.0±22.5	90.0±25.2*
SDANN	58.8±23.4	81.1±26.6*
rMSSD	16.8±5.9	20.6±8.6#
pNN50 (%)	1.9±2.3	3.9±5.6
lnVLF	5.3±0.7	5.9±0.7*
lnLF	3.9±1.1	3.4±1.6
lnHF	2.7±1.4	1.6±2.5
lnLF/HF	1.2±0.7	1.8±1.2#
Unsuccessful PTX		
lnFGF23	10.8±1.7	8.2±2.0#
Mean 24hHR	76.5±11.4	70.3±10.5
Mean NN	798.3±108.7	871.0±141.0
SDNN	95.5±29.5	116.0±21.6
SDANN	81.5±33.1	102.8±24.3#
rMSSD	23.8±4.6	26.3±14.1
pNN50 (%)	4.4±3.0	6.2±8.4
lnVLF	6.5±0.6	6.6±0.5
lnLF	4.9±1.8	5.0±1.7
lnHF	2.8±2.6	3.5±2.5
lnLF/HF	2.1±0.9	1.4±1.1

Data are mean ± standard deviation (SD). *$P<0.01$ versus baseline; #$P<0.05$ versus baseline.

Conclusions: Higher plasma FGF23 was associated with decreased HRV in stage 5 CKD. Successful PTX reverses these abnormalities and contributes to decrease the risk of CVD.

Funding: Government Support - Non-U.S.

TH-PO579

FGF23 Levels Do Not Differ between Pediatric Pre-Dialysis CKD and Transplant Patients Sabina Susan Thyle, Barbara Gales, Georgina Chow, Isidro B. Salusky, Katherine Wesseling-Perry. *Div of Pediatric Nephrology, UCLA, Los Angeles, CA.*

Background: Fibroblast growth factor 23 (FGF23) excess plays a key role in disordered mineral bone metabolism in patients with chronic kidney disease (CKD). Impaired renal function is prevalent in pediatric renal transplant recipients. Some data suggest that immunosuppressive agents may affect circulating FGF23 values but there are conflicting data as to the effect of transplant status on FGF23 values.

Methods: We performed a cross-sectional study in 30 pediatric, pre-dialysis CKD patients and 61 pediatric renal transplant recipients. Patients with an estimated GFR of 15-89 mL/min/1.73m^2 were included in the study. Biochemical parameters of calcium, phosphorus, PTH, alkaline phosphatase, creatinine and FGF23, along with patient demographics including age, gender, height and time since transplantation were evaluated in all subjects. GFR was estimated according to the Schwartz formula. Values were expressed as means ± SEM or medians (IQ ranges) (Table).

	CKD Stage					
	2		3		4	
Parameter	Pre-dialysis (n=2)	Transplant (n=25)	Predialysis (n=16)	Transplant (n=32)	Predialysis (n=12)	Transplant (n=4)
Age (years)	9.0±2.4	14.4±1.0	14.1±1.1	16.5±0.6*	14.8±0.9	19.5±0.6*
Calcium (mg/dL)	10.0±0.3	10.0±0.1	9.3±0.1	9.7±0.1*	9.5±0.2	9.7±0.2
Phosphorus (mg/dL)	5.0±0.2	4.4±0.2	4.7±0.2	4.0±0.1*	4.6±0.2	5.0±0.4
Time since transplant (years)	-	2.6±0.5	-	5.6±0.7	-	15.7±1.1
iPTH (pg/mL)	55.0(12.0,98.0)	77.0(46.0,143.0)	69.0(50.0,120.0)	65.0(53,110)	121.5(58.5,196.0)	160.0(65.5,303.5)
FGF-23 (RU/mL)	376.5(291.0,462.0)	95.0(43.0,115.0)	142.0(84.0,278.0)	148.0(64.0,196.0)	208.5(137.5,414.5)	369.0(78.5,1267.0)
Alkaline phosphatase (U/L)	259.0(253.0,265.0)	158.0(94.0,232.0)	213.5(114.0,312.0)	104.0(72.5,177.0)	169.5(115.5,220.0)	119.5(98.5,132.0)
Steroid use (%)	0	60.0*	0	87.5*	0	100*
1,25 Vitamin D use (%)	100	0	18.8	0*	75.0	0*

* Indicates a difference ($p<0.05$) between pre-dialysis CKD and transplant patients.

Results: Transplant patients were older, had higher serum calcium levels, and had lower serum phosphorus levels than their pre-dialysis counterparts, and patients with more advanced CKD were older than those with earlier CKD stages. Linear regression analysis showed no difference in FGF23 levels between CKD and transplant patients after controlling for serum calcium, phosphorus, PTH, and estimated GFR. Serum phosphorus ($p<0.05$) and estimated GFR ($p<0.001$) alone were significant predictors of circulating FGF23 values.

Conclusions: These data confirm previous findings from smaller studies suggesting that FGF23 levels rise with decreasing GFR irrespective of transplant status. GFR and phosphorus levels predict FGF23 levels in children with CKD stages 2-4 independent of transplant status, calcium levels, and intact PTH.

TH-PO580

Calcium Carbonate Is Effective for Decreasing Phosphate Loading and FGF-23 in Stage 3 CKD Kraiwiporn Kiattisunthorn, Chanchira Janwijit, Krongkarn Klayklung, Kriengsak Vareesangthip. *Medicine, Siriraj Hospital, Bangkok-noi, Bangkok, Thailand.*

Background: Fibroblast growth factor-23 (FGF23) is driven against phosphate loading to prevent toxicity. FGF-23 is expected to be a superior biomarker to blood phosphate levels to predict phosphate loading in non-dialysis CKD. However, there is no consensus supporting efficacy of phosphate binders to suppress FGF23. The study is conducted to present the efficacy of calcium carbonate ($CaCO_3$) on reduction of phosphate loading and FGF23 in CKD stage 3.

Methods: Stage 3 CKD patients (n=27) were assigned to limit dietary phosphate (PO_4) intake 800-1000 mg/day, then randomized to group A ($CaCO_3$350 mg or 700 mg thrice a day with meal) or group B (placebo), for 9 months. Serum calcium, PO_4, GFR, intact PTH, FGF23, dietary protein and phosphate intake were monitored every 12 weeks. Plasma c-terminal FGF23 was measured by using human FGF23 ELISA kit (Immunotopics, Inc., CA, U.S.A.) [intra- and inter-assay CV 1.4-3% and 2.4-5.1%, respectively]. Estimated GFRs were calculated by CKD-EPI formula using serum creatinine measured by enzymatic assays.

Results: Baseline characteristics were presented in Table 1.

Parameter	Placebo (n=9)	$CaCO_3$ (n=18)
Age (years)	71±10	61±12
Male(%)	67	78
GFR (ml/min/1.73m^2)	40.7±9.8	43.0±7.1
Calcium (mg/dL)	9.5±0.2*	9.1±0.3
PO_4 (mg/dL)	3.2±0.5	3.1±0.5
iPTH (pg/mL)	53.4±24.5	79.9±37.2
FGF-23 (pg/mL)	124.8±114.4	131.4±77.2
nPNA (g/kg/day)	0.9±0.2	1.0±0.3
Urine PO_4 (mg/day)	390+174	369+112
$FePO_4$(%)	18.6±5.5	19.2±10.2
*p<0.05; nPNA=normalized protein nitrogen appearance; $FePO_4$=fractional excretion of phosphate		

There was no statistical significance in changes of serum calcium and PO_4, PTH, GFR, albumin, protein intake, urinary calcium and body weight over 36 weeks. The FGF23 level of gr.A was significantly decreased compared to those of gr.B (-24.7+ 24.7% versus 2.2+28.9%, p=0.045). Sixty percent of the patients in gr.A could achieve a >30% decrease of FGF23 level compared to 20% of those in gr.B (p=0.04). Urinary PO_4 was significantly decreased in gr.A (-25.7+22.0% versus 33.6+61.2%, p=0.02).

Conclusions: Calcium carbonate is an effective phosphate binder for stage 3 CKD without a significant hypercalcemia and hypercalciuria over the 36-week treatment.

Funding: Government Support - Non-U.S.

TH-PO581

Phosphorus Removal and Serum FGF23 Levels in Chronic Dialysis Patients E. Constantz,[2] T. Alp Ikizler,[3] G. Wei,[2] Xiaorui Chen,[2] R. Boucher,[2] Yufeng Huang,[2] Bin Wang,[2] Tom Greene,[2] Srini Beddhu.[1,2] *[1]SLC VAMC; [2]Univ Utah; [3]Vanderbilt Univ.*

Background: Serum phosphorous (P) affects serum fibroblast growth factor 23(FGF-23) levels. We examined the hypothesis that hemodialysis (HD) P removal as estimated from P reduction ratio (PRR) affects serum P and FGF23 levels.

Methods: Midweek pre and post HD serum P levels were measured at baseline (BL) in 117 participants with 6-month (6m) follow-up measurements in 96. PRR was calculated as (pre-post)/pre serum P. 44h urinary P excretion (UPE_{44}) was measured from 44h urine samples in those with residual renal function (RRF). UPE_{44} was considered 0 in anuric pts. Serum FGF23 levels were measured with ELISA. Baseline PRR was related to the average of baseline and 6 month FGF23 levels in mixed effects models.

Results: Mean BL PRR was 57± 15 %. Median FGF23 levels at BL and 6m were 6831 (1718, 16074) RU/ml and 4062 (1048, 12387) RU/ml, respectively. BL characteristics are summarized in the table.

	PRR<52.8%	PRR 52.8 to 65.1%	PRR≥65.1%
	N=39	N=39	N=39
PRR	0.41±0.13	0.59±0.04	0.72±0.06
Age(yr)	54±16	52±16	48±17
Female (%)	30.8	31.6	53.8
Black(%)	7.7	15.4	10.3
Vintage(yr)	2.9(0.8,4.9)	2.6(0.8,4.7)	1.7(0.7,3.2)
Cardiovascular Dis(%)	56.4	53.9	33.3
Diabetes(%)	51.3	42.1	47.4
PreHD Ca(mg/dl)	9.0±0.7	8.9±0.6	9.0±0.7
PreHD P(mg/dl)	5.1±1.3	6.4±1.4	6.3±1.8
PreHD PTH(ng/L)	284.0(206.0,459.0)	469.0(216.0,736.0)	392.0(231.0,557.0)
FGF23(ru/mL)	6950.6 (2165.0,16074.4)	9973.8 (1981.3,16074.4)	5149.9 (1772.8,19738.2)
Anuria(%)	59.0	59.0	48.7
Median UPE_{44} in non-anuric	262.2(100.7,417.5)	259.5(192.5,313.3)	206.7(100.1,458.5)

In mixed effects models, adjusted for demographics, clinical characteristics and UPE_{44}, each SD (12.9%) ↑ in PRR was associated with a 0.76 (95% CI 0.48 to 1.04) mg/dl higher average (of BL and 6m) preHD P and statistically non-significant increase (11.4%, 95% CI -13.0% to 42.6%) in serum FGF-23.

Conclusions: The counter-intuitive association of ↑ PRR with ↑ preHD P levels probably reflects the greater ↓ in serum P when preHD P is higher. As post-HD P levels are affected by equilibration from intracellular compartment, longer HD sessions might result in ↑ P removal and affect FGF23 levels.

Funding: NIDDK Support

TH-PO582

Protein Intake, Serum Phosphorous, and FGF23 Levels in Dialysis Patients Xiaorui Chen,[2] T. Alp Ikizler,[3] G. Wei,[2] E. Constantz,[2] R. Boucher,[2] Yufeng Huang,[2] Bin Wang,[2] T. S. Bjordahl,[2] Tom Greene,[2] Srini Beddhu.[1,2] *[1]SLC VA; [2]Univ Utah; [3]Vanderbilt Univ.*

Background: We examined the hypothesis that as high protein intake (PI) could ↑ serum P, it might also affect FGF23 levels.

Methods: 144 hemodialysis (HD) patients (pts) from the Protein Intake, Cardiovascular Disease and Nutrition in CKD Stage V (PICNIC) study were included. PI was estimated from protein nitrogen appearance (PNA), calculated from mid-week pre and post HD BUN. PNA was adjusted for measured 44h urine urea nitrogen in those with residual renal function. PreHD serum P and FGF23 levels were measured. Baseline (BL) and 6 month (6m) PNA were related to the average of BL and 6m serum P and FGF23 levels in mixed effects models.

Results: Of the 144 pts, 121 completed the 6m visit. Mean PNA was 1.00± 0.32 g/kg/d. Mean BL and 6m serum P levels were 5.8 ±1.6 and 6.0 ±2.0 mg/dl, respectively. Mean serum FGF23 levels were 10347± 10153 and 6380± 5722 RU/ml, respectively. BL characteristics of the study population by FGF23 tertiles are summarized in Table 1.

	FGF23<2500 RU/ml	2500≤FGF23 <12500 RU/ml	12500≤FGF23 RU/ml
Median Serum FGF23(ru/ml)	945(470,1719)	6831(4559,10569)	24622(16074,29724)
Age(yr)	52±17	53±16	48±16
Female(%)	39.6	46.8	33.3
AA race(%)	27.1	20.8	10.4
Vintage(yr)*	1.8(0.7,3.1)	2.6(0.9,4.6)	3.1(2.0,5.3)
Cardiovascular Dis(%)	41.7	50.0	39.6
DM (%)	47.9	39.1	41.7
Serum Ca(mg/dl)	8.9±0.7	8.9 ± 0.9	9.2±0.6
Serum P(mg/dl)*	5.0±1.4	6.0 ± 1.5	6.6±1.6
PTH(ng/l)	333 (226,504)	278(185, 625)	468 (280 ,674)
PNA(g/kg/day)	0.94±0.26	1.01±0.33	1.03±0.31
* P value≤0.05			

In a mixed effects model, adjusted for demographics and clinical characteristics, each SD (0.31 g/kg/d) ↑ in PNA was associated with 0.62 (95% CI 0.36,0.88) mg/dl ↑ in the average of BL and 6m serum P. In similar models, each SD ↑ in PNA was associated with 14.9% (95% CI -8.6%, 44.4%) ↑ in serum FGF23 levels. Each SD (1.74 mg/dl) ↑ in serum P was associated 61.2% (95% CI 32.7%,95.8%) ↑ in serum FGF23 levels.

Conclusions: ↑ PI was associated with significant ↑ in serum P but a statistically non-significant ↑ in serum FGF-23. Larger studies are warranted to determine the association of PI with FGF23 levels.

Funding: NIDDK Support

TH-PO583

Effect of a Predominantly Plant Based Diet on Mineral Metabolism and Skeletal Muscle in Chronic Kidney Disease Ranjani N. Moorthi,[1] Kevin M. Janda,[1] Cheryl L.H. Armstrong,[2] Sharon M. Moe.[1] *[1]Medicine, Indiana Univ/ Indiana Univ Health, Indpls, IN; [2]Purdue Univ, West Lafayette, IN.*

Background: CKD is associated with alterations in phosphate (P) excretion, increases in fibroblast growth factor (FGF23) and parathyroid hormone (PTH). Previous short terms studies of a 100% vegetarian diet showed reduction in urinary P compared to a meat diet. For the average American CKD patient, a 70% vegetarian diet is more feasible than a completely vegetarian one. We tested the hypothesis that 4 weeks on a 70% plant based diet would reduce urinary P and improve markers of mineral metabolism without adverse biochemical changes or loss of muscle mass.

Methods: Thirteen subjects with CKD 3-4 underwent an intervention trial of 4 weeks of a study diet. The study diet provided an average of 2300 kcals/d, 0.8-1 mg/kg protein, 3g sodium and 70% of the protein came from plant sources. The primary outcome was change in 24 hour urine P. Two pre-study urine collections and blood draws were performed. All these were repeated at week 2 and week 4 while on the study diet. Body fat content by bioelectrical impedance and hand-grip strength were serially measured. Paired T-tests compared differences between baseline and on-diet. For non-normally distributed variables, Wilcoxon signed rank tests were used.

Results: Mean age of subjects in the study was 54.8 years and mean eGFR was 27.2+11.5ml/min. Urine P significantly decreased by 231 +131mg/d on the study diet (p<0.01). There were no significant changes in serum FGF23, P, PTH, C02, or potassium on the study diet although reductions were seen in some patients. Urine sodium decreased by 32%, and urine pH increased significantly from 6.1 to 6.2.on the 70% plant based diet (p<0.01). Urine creatinine was reduced but 24 hour creatinine clearance was unchanged. Hand grip strength and fat mass% did not change on study diet over the 4 week period.

Conclusions: A 70% plant based diet is efficacious in lowering urine P and sodium excretion, was feasible, and well tolerated. No adverse effects were observed on muscle strength, and there was an overall feeling of improved well-being reported by participants. Long term studies are required to determine if these effects are sustained.

Funding: Private Foundation Support

TH-PO584

Effect of Alkali Therapy on Fibroblast Growth Factor-23 in Chronic Kidney Disease Patients Wei Chen,[1] Michal L. Melamed,[1,2] Carolyn A. Bauer,[1] Amanda C. Raff,[1] Thomas H. Hostetter,[3] David A. Bushinsky,[4] Matthew K. Abramowitz.[1,2] *[1]Medicine, Albert Einstein College of Medicine, Bronx, NY; [2]Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY; [3]Internal Medicine, Case Western Reserve Univ School of Medicine, Cleveland, OH; [4]Internal Medicine, Univ of Rochester, Rochester, NY.*

Background: Metabolic acidosis has been demonstrated to increase FGF-23 levels in neonatal mouse bone. This study examines the effect of alkali therapy on FGF-23 levels in patients with chronic kidney disease (CKD).

Methods: In this single-blinded pilot study, 20 adults with eGFR 15-45 ml/min/1.73m^2 and serum bicarbonate 20-24 mEq/L were treated during successive 2-week periods with placebo followed by escalating oral sodium bicarbonate doses (0.3, 0.6 and 1.0 mEq/d/kg ideal body weight). C-terminal FGF-23 were measured at the initial visit, after 2-weeks of placebo and at the end of the study. Values from the initial and post-placebo visits were averaged to determine each individual's baseline. Paired t-test was used to compare serum bicarbonate levels, and Wilcoxon matched-pair signed rank sum test was used to compare FGF-23 levels before and after the intervention.

Results: No correlation was observed between serum bicarbonate and FGF-23 levels at baseline (p=0.37). After 6 weeks of oral sodium bicarbonate supplementation, serum bicarbonate levels increased from 22.6±2.3 to 25.5±2.2 mEq/L (p<0.001), and FGF-23 levels increased from 150.9 (100.8, 267.4) to 191.4 (132.6, 316.9) RU/mL (p=0.048).

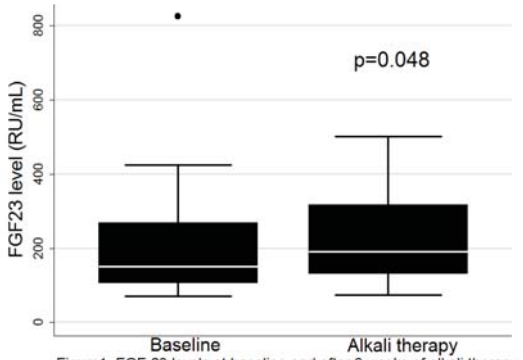


Figure1. FGF-23 levels at baseline and after 6 weeks of alkali therapy

Conclusions: Sodium bicarbonate supplementation increased FGF-23 levels after a short-term intervention in CKD patients with mild acidosis. The study is limited by small sample size and lack of control group. As FGF-23 levels are expected to increase with time in CKD patients, long-term placebo-controlled studies are needed to substantiate these findings.

Funding: Other NIH Support - U01 087783, T32 DK007110,1UL1 TR001073-01, 1TL1 TR001072-01, 1KL2 TR001071-01

TH-PO585

Are Blood Pressure and Plasma Fibroblast Growth Factor-23 Concentration Changes Interrelated after Cinacalcet Treatment in Hemodialysed Patients with Chronic Kidney Disease and Secondary Hyperparathyroidism? Marcin Adamczak, Piotr Kuczera, Andrzej Wiecek. *Dept of Nephrology, Endocrinology and Metabolic Diseases, Medical Univ of Silesia, Medical School in Katowice, Katowice, Poland.*

Background: It is well known that parathyroid hormone (PTH) plays a role in the pathogenesis of arterial hypertension. Treatment with cinacalcet decreases serum PTH concentration in hemodialysed patients with chronic kidney disease (HDP) and secondary hyperparathyroidism (sHPT). The aim of this study was therefore to assess the influence of 6 month treatment with cinacalcet on blood pressure (BP) in HDP with sHPT.

Methods: In 58 HDP with sHPTserum PTH, fibroblast growth factor-23 (FGF-23), calcium and phosphate concentrations were assessed before the first dose of cinacalcet and after 3 and 6 months of treatment. BP was measured before hemodialysis sessions. The results were shown as means and 95% CI.

Results: Serum PTH concentration decreased significantly after 3 and 6 month of cinacalcet treatment from 1138 (931-1345) to 772 (551-992); p<0.0001 and to 635 (430-839)pg/ml; p<0.0001, respectively. Plasma FGF23 concentration decreased after 3 and 6 months of treatment from 593 (457-730) to 513 (380-645)pg/ml; p=0,099 and to 433 (304-561)pg/ml; p=0.015 respectively. Mean serum calcium and phosphate concentrations remained stable. Systolic BP decreased after 3 and 6 month of treatment from 128 (123-133), to 125 (120-131); p=0.1 and to 121 (115-127)mmHg; p=0.005, respectively. Diastolic BP did not change significantly. There were no significant differences in the number of antihypertensive drugs, alphacalcidol dose and patients' body weight during the treatment period. No significant correlation was found between changes of systolic BP and changes of serum PTH, FGF-23, calcium and phosphate concentration and both cinacalcet and alphacalcidol dose, respectively.

Conclusions: 1. Six-month cinacalcet treatment decreases systolic BP in hemodialysed patients with chronic kidney disease and secondary hyperparathyroidism. 2. Such a decrease of systolic BP might be related to the decrease of plasma FGF-23 concentration, however such a potential relation needs further elucidation.

Funding: Government Support - Non-U.S.

TH-PO586

Fibroblast Growth Factor 23 and Risk of Incident Stroke in Community-Living Adults Bhupesh Panwar,[1] Nancy Jenny,[2] Virginia J. Howard,[1] Virginia G. Wadley,[1] Paul Muntner,[1] Brett Kissela,[3] Suzanne E. Judd,[1] Orlando M. Gutierrez.[1] *[1]Univ of Alabama at Birmingham; [2]Univ of Vermont; [3]Univ of Cincinnati.*

Background: Elevated fibroblast growth factor 23(FGF23) levels are associated with increased risk of cardiovascular disease events, particularly those linked to heart failure. Associations of FGF23 with stroke outcomes are less clear.

Methods: The association of plasma FGF23 levels with incident stroke in the Reasons for Geographic and Racial Differences in Stroke(REGARDS) study, a cohort of black and white adults ≥45 years of age, was examined. FGF23 was measured in 615 participants who developed incident stroke(cases) and in 936 participants randomly selected from the REGARDS cohort(comparison cohort).

Results: In multivariable-adjusted models, higher calcium and phosphorus levels, lower estimated glomerular filtration rate and higher urine albumin excretion were independently associated with higher FGF23. There was no statistically significant association of FGF23 with risk of all-cause stroke in models adjusted for demographic factors and known stroke risk factors (hazard ratio [HR] comparing fourth to first quartile 1.19, 95%CI 0.78, 1.82). When stroke subtypes were investigated, there was a graded association of FGF23 with risk of cardioembolic stroke in fully adjusted models(table). There were no statistically significant associations of FGF23 with other stroke subtypes.

Table. HR (95% CI) of stroke subtypes by quartiles of FGF23

	Events	FGF23 Quartile 1 (<53 RU/ml)	FGF23 Quartile 2 (53-70 RU/ml)	FGF23 Quartile 3 (70.5-100 RU/ml)	FGF23 Quartile 4 (>100 RU/ml)	P_{trend}
All Cause	615	ref	1.34 (0.91-1.99)	1.09 (0.74-1.63)	1.19 (0.78-1.82)	0.77
Ischemic	540	ref	1.33 (0.89-2.01)	1.23 (0.81-1.85)	1.28 (0.81-2.01)	0.56
Cardio-embolic	136	ref	1.48 (0.63-3.47)	1.99 (0.89-4.44)	2.52 (1.08-5.91)	0.04
Large Vessel Athero-sclerosis	85	ref	1.77 (0.74-4.19)	1.26 (0.51-3.12)	1.53 (0.58-4.02)	0.63
Small Vessel Occlusion	104	ref	1.04 (0.49-2.12)	1.15 (0.57-2.32)	0.80 (0.36-1.79)	0.45
Un-classified	245	ref	1.35 (0.79-2.27)	1.23 (0.72-2.09)	1.23 (0.68-2.23)	0.79

Conclusions: Higher FGF23 was an independent risk factor for cardioembolic strokes but not other stroke subtypes in community-dwelling adults.

Funding: Other NIH Support - This study was supported by a cooperative agreement U01 NS041588 and by R01NS080850 from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Pharmaceutical Company Support - Additional funding was provided by an investigator-initiated grant-in-aid from Amgen Corporation

TH-PO587

Association of Mineral Metabolism Biomarkers with Risk of End-Stage Renal Disease: Chronic Kidney Disease Biomarkers Consortium and the Atherosclerosis Risk in Communities Study C. Rebholz, M. Grams, Pamela L. Lutsey, Lesley Inker, Andrew S. Levey, Elizabeth Selvin, Chi-Yuan Hsu, Paul L. Kimmel, Vasan S. Ramachandran, John H. Eckfeldt, Josef Coresh. *Chronic Kidney Disease Biomarkers Consortium.*

Background: Disordered mineral metabolism is characteristic of impaired kidney function. However, little is known about the associations between circulating levels of fibroblast growth factor-23 (FGF-23), vitamin D binding protein (VDBP), and 25-hydroxyvitamin D [25(OH)D] and end-stage renal disease (ESRD).

Methods: In a nested case-control study within the ARIC Study, incident ESRD cases (n=184) were identified through hospitalization surveillance from 1996-2008. Cases were frequency matched to controls (n=251) on estimated glomerular filtration rate (eGFR) category, urinary albumin-to-creatinine ratio (ACR) category, diabetes status, sex, and race. Baseline serum levels of FGF-23, VDBP, and 25(OH)D were measured. Logistic regression was used to estimate the association between mineral metabolism biomarkers and incident ESRD, adjusting for matching factors, age, and hypertension status. Analyses were repeated after stratifying by race and VDBP SNPs (rs4588, rs7041).

Results: In this case-control study, 57% of participants had diabetes, 48% were women, and 44% were black. After adjustment, one interquartile range increases in FGF-23

and VDBP were associated with incident ESRD (FGF-23 OR: 1.42, 95% CI: 1.06, 1.92; VDBP OR: 1.48, 95% CI: 1.02, 2.14). Overall, 25(OH)D was not statistically significantly associated with ESRD (OR: 0.80, 95% CI: 0.60, 1.07, p=0.14). However, 25(OH)D was more strongly associated with ESRD within racial and VDBP genotype subgroups (blacks OR: 0.48, 95% CI: 0.28, 0.85; rs4588 CC allele OR: 0.65, 95% CI: 0.42, 1.00).

Conclusions: In the general population, serum levels of FGF-23 and VDBP, but not 25(OH)D, were positively associated with new-onset ESRD during follow-up. Further research is warranted to explore the role of free and bioavailable vitamin D in predicting future ESRD risk.

Funding: NIDDK Support, Other NIH Support - NHLBI

TH-PO588

A Test of the Hypothesis That Calcitriol Deficiency Mediates Secondary Hyperparathyroidism of Chronic Kidney Disease Jennifer L. Ennis,[1] John R. Asplin,[1] Fredric L. Coe.[2] *[1]Litholink Corporation, a LabCorp Company, Chicago, IL; [2]Medicine, Univ of Chicago, Chicago, IL.*

Background: Calcitriol down-regulates parathyroid hormone (PTH). Chronic kidney disease (CKD) is known to be a state of calcitriol deficiency and secondary hyperparathyroidism. Calcitriol deficiency is presumed to be a factor mediating the secondary hyperparathyroidism. If this presumption were true, then PTH should be an inverse function of calcitriol in patients with CKD. In order to test this prediction, we analyzed a large dataset from a national clinical laboratory. Our data were not compatible with the hypothesis.

Methods: We performed a cross-sectional analysis of 722 laboratory encounters collected on 437 stage 2-5 U.S. CKD patients from a large national laboratory (LabCorp®) from 2009-2013. Estimated glomerular filtration rate (eGFR), serum calcium (Ca), phosphorus (P), 25-hydroxy vitamin D (25-D), calcitriol and plasma PTH levels were analyzed. Labs were measured as a part of routine clinical care.

Results: By ANOVA with trend analysis,mean PTH values adjusted for Ca, P, 25-D, and calcitriol were higher with progressive CKD stage. In a similar analysis, calcitriol values adjusted for Ca, P, 25-D, and PTH were lower with progressive CKD stage. In the PTH model, Ca (F-ratio=31.5, p<0.001), 25-D (F-ratio=22.4, p<0.001), and CKD stage (F-ratio=15.9, p<0.001) were significant covariates, whereas calcitriol had no effect (F-ratio=0.02, p =0.88). In the calcitriol model, 25-D (F-ratio=74.8, p<0.001), CKD stage (F-ratio=10.1, p<0.001), and phosphorus (F=3.9, p=0.048) were significant covariates, although the effect of phosphorus was minimal. PTH had no effect.

Conclusions: Despite the large data set, we were unable to detect any regression relationship between PTH and calcitriol once adjustments were made for other confounders: Ca, P, 25-D, and CKD stage. Of great interest, we detected a very large effect of 25-D on PTH independent of calcitriol, suggesting an alternative relationship between the vitamin D hormones and PTH in CKD. The simple hypothesis of calcitriol deficiency as an important contributor to secondary hyperparathyroidism of CKD does not seem to have withstood a fairly straightforward test.

Funding: Pharmaceutical Company Support - LabCorp

TH-PO589

Serum 1,25-Dihydroxyvitamin D Level Is Independently Associated with Endogenous Erythropoietin Resistance in Patient with Chronic Kidney Disease Il Young Kim,[1] Min Jung Kim,[1] Dong Won Lee,[1] Soo Bong Lee,[1] Byeong Yun Yang,[2] Eun Young Seong,[2] Sang Heon Song,[2] Ihm Soo Kwak.[2] *[1]Internal Medicine, Pusan National Univ Yangsan Hospital, Yangsan, Korea; [2]Internal Medicine, Pusan National Univ Hospital, Busan, Korea.*

Background: Resistance to exogenous erythropoietin (EPO) stimulating agent (ESA) has been known to be associated with mortality in patients with chronic kidney disease (CKD). Recent studies have also demonstrated an association between resistance to endogenous EPO and mortality in CKD patients. We aimed to identify factors associated with endogenous EPO resistance, represented by serum EPO concentration and its ratio to hemoglobin (EPO/Hb ratio), with a focus on serum 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D].

Methods: This study included 210 CKD patients [estimated glomerular filtration rate < 60 ml/min/1.73m^2] who were not on dialysis therapy from Pusan National University Yangsan Hospital between 2008 and 2013. The patients were excluded if they were on ESA therapy or had iron deficiency (Transferrin saturation < 20% or ferritin < 200 ng/ml) at the time of study enroll. The association of endogenous EPO resistance (EPO/Hb ratio) with clinical and laboratory variables was investigated by univariate and multivariate linear regression analysis.

Results: In the overall study sample, mean erythropoietin level was 16.4 ± 6.8 mU/ml and mean 1,25 $(OH)_2$D level was 15.5 ± 10.0 pg/ml. In univariate analysis, elevated EPO/Hb ratio was associated with decreased eGFR (β = -0.166, P < 0.05), albumin (β = -0.268, P < 0.001), 1,25-$(OH)_2$D (β = -0.536, P < 0.001) and elevated ferritin (β = 0.677, P< 0.001), CRP (β = 0.663, P < 0.001). In mutivariate analysis, elevated EPO/Hb ratio was independently associated with decreased 1,25-$(OH)_2$D (β = -0.297, P < 0.001) and elevated ferritin (β = 0.376, P < 0.001), CRP (β = 0.345, P < 0.001).

Conclusions: In CKD patients, endogenous EPO resistance was independently associated with not only inflammatory marker such as ferritin, CRP but also 1,25-$(OH)_2$D. This study suggests deficiency of biologically active 1,25-$(OH)_2$D, which has been known to have anti-inflammatory effect, in CKD patients might increase endogenous EPO resistance via augmentation of inflammatory process.

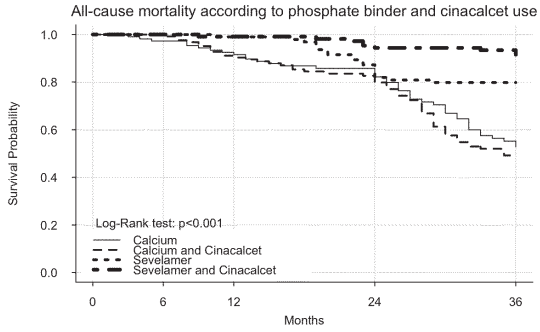
TH-PO590

Cinacalcet but Not Vitamin D Use Modulates the Survival Benefit Associated with Sevelamer in the Independent Study Antonio Bellasi,[1] Mario Cozzolino,[2] Domenico Russo,[3] Donald A. Molony,[4] Biagio Raffaele Di Iorio.[5] *[1]Ospedale Sant'Anna-Como; [2]Univ of Milan; [3]Univ "FEDERICO II" Napoli; [4]Univ of Texas-Houston; [5]PO "A Landolfi" – Solofra.*

Background: Whether the concomitant use of calcium sensing modulator or vitamin D with either a calcium free or calcium containing phosphate binder impacts patient-centered outcomes remains to be elucidated.

Methods: Post hoc analysis of an open label, randomized, controlled trial designed to evaluate the impact of sevelamer (SV) versus calcium salts (CS) on survival in incident dialysis patients. All individuals were followed until study completion or death. Cinacalcet and vitamin D were administered to a portion of patients as part of routine care (53% and 42%, respectively). We tested the impact of cinacalcet and vitamin D on survival for the overall study cohort and in both treatment arms of the original study.

Results: We recruited 466 middle-age (65 years) men and women. After a mean follow-up of 28(10) months, SV but not cinacalcet administration was associated with a survival benefit. However, a significant (p=0.003) interaction of phosphate binder and cinacalcet use on mortality was observed.



Calcium	108	105	98	73	47
Calcium and Cinacalcet	126	126	113	89	53
Sevelamer	110	106	95	82	74
Sevelamer and Cinacalcet	122	118	107	101	99

A synergistic survival benefit was noted between SV and cinacalcet (HR 0.41, 95%CI 0.17-1.01, p=0.054 for SV treated subjects receiving versus not receiving cincalcet). In contrast a trend toward an increased mortality was observed for patients receiving CS and cinacalcet concomitantly (HR 1.5, 95%CI 0.96-2.34; p=0.07 for CS treated subjects receiving versus not receiving cincalcet). No effect on mortality or interaction with phosphate binder use was noted with vitamin D.

Conclusions: Though hypothesis generating, these results lend support to a hypothesis that use of a calcium-free versus -containing phosphate binder may increase survival in incident hemodialysis patients, in particular, when patient are treated concurrently with cinacalcet.

TH-PO591

Effects of Soluble Inhibitors of Wnt-Beta Catenine Signaling (Sclerostin and Dkk-1) on Mineral and Bone Metabolism in Patients on Peritoneal Dialysis Shunsuke Yamada,[1] Kazuhiko Tsuruya,[3] Masanori Tokumoto,[1] Hiroaki Ooboshi,[1] Takanari Kitazono.[2] *[1]Dept of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan; [2]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [3]Dept of Integrated Therapy for Chronic Kidney Disease, Kyushu Univ, Fukuoka, Japan.*

Background: Sclerostin and Dkk-1, soluble inhibitors of canonical Wnt-β catenine signaling, negatively regulate bone volume. Studies have shown that these inhibitors are involved in the development of CKD-mineral and bone disorder (MBD). However, it remains unclear how these soluble inhibitors are regulated and whether these inhibitors play roles in the regulation of MBD in patients undergoing peritoneal dialysis (PD).

Methods: The present study was a cross-sectional study consisting of 74 outpatients who received PD therapy during 2010-2013 at Kyushu University hospital. Sclerostin and Dkk-1 levels in the serum, urine, and dialysate were determined by ELISA kits, and we examined the associations with clinical and biochemical parameters. The associations between the serum soluble inhibitors and serum bone metabolic marker levels were also examined.

Results: The mean patient age was 55 years. The subjects included 49 male patients, and the dialysis vintage was 359 (median) days. The serum sclerostin level was 342 (median) pg/mL and the serum Dkk-1 level was 980 (median) pg/mL, which were higher than the values in the general population. The sclerostin levels in the urine and dialysate were positively associated with serum sclerostin level. A multivariable analysis showed that the serum sclerostin level was significantly and positively correlated with age and male gender and significantly negatively correlated with the serum PTH level and the renal Kt/V, while Dkk-1 was correlated with platelet count. The serum sclerostin level was significantly and negatively associated with the serum levels of bone metabolic markers, even after adjusting for confounders, while Dkk-1 was not.

Conclusions: The metabolic regulation of sclerostin and Dkk-1 and the effects of these inhibitors on CKD-MBD might be different in PD patients. The clinical significance of measuring these inhibitors needs further investigations.

TH-PO592

Increased Proliferation and Delayed Mineralization *In Vitro* Suggest Intrinsic Abnormalities in Osteoblast Growth and Differentiation in the Context of CKD

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Background: Abnormalities in bone remodeling and skeletal mineralization are commonly observed in pts with CKD. Osteocytes are terminally differentiated cells derived from osteoblasts which play an important role in bone and mineral homeostasis. Alterations in osteoblasts or osteocytes may contribute to the skeletal abnormities observed in CKD.

Methods: Thus, proliferation and mineralization characteristics of osteoprogenitors isolated from bone chips of 9 pts with ESKD (3 with adynamic bone (AD), 3 with normal bone turnover (N), and 3 with osteitis fibrosa (OF)) and from 3 healthy controls were evaluated.

Results: Proliferation rate, determined by MTS assay over a 4 day period, was higher in CKD cells than in cells from healthy controls ($p<0.01$ between CKD and controls, NS between subtypes of renal osteodystrophy (ROD)). Mineralized matrix formation, determined by Alizarin Red staining and normalized for cell number as assessed by Crystal Violet staining, was evaluated over a 4 week culture period. In contrast to cell proliferation, mineralization was delayed in CKD cells as compared with controls ($p<0.01$). In this small sample, differences in proliferation and mineralization rates between subtypes of ROD did not reach significance, although mineral content of cells from pts with AD tended to be higher at 2 weeks than in their counterparts with OF ($p=0.19$).

Conclusions: Increased proliferation rates and decreased mineralization in cells obtained from CKD pts suggest that these osteoprogenitors retain an abnormal phenotype in vitro. Thus, this in vitro model may be helpful in better understanding the abnormal bone biology observed in patients with CKD. The role of different subtypes of ROD on proliferation and mineralization warrants further investigation.

TH-PO593

Cultured Osteoprogenitors From Patients with CKD Provide a Novel Model for Studying Abnormal Bone Biology Associated with Kidney Disease

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Background: Abnormalities in bone remodeling and skeletal mineralization are commonly observed across the spectrum of CKD. Osteocytes, terminally differentiated cells derived from osteoblasts, play a critical in bone and mineral homeostasis. Alterations in osteoblast differentiation, maturation, and/or osteocytic transition could contribute to the skeletal abnormities observed in CKD. However, model systems for studying the mechanisms regulating these processes, in the context of CKD, are lacking.

Methods: Thus, a model system was established from osteoprogenitors isolated from patient bone biopsies. Iliac crest bone tissue (2 mm in diameter) was obtained from 24 dialysis pts (14M,10F) aged 17.1 + 0.7 (SE)yrs. and 4 healthy controls (median age 16 yrs.), RNA was isolated from one half of each core; the other half was minced, the fragments plated in DMEM, and the emerging cells grown to confluence. qRT-PCR was performed on RNA from both cells and bone cores to evaluate expression of osteoblastic and osteocytic markers and signaling genes.

Results: Expression of analyzed genes was normalized by GAPDH and expression in ESKD samples was normalized by expression in healthy controls.

	Bone expression	Cell expression	Correlation between bone and cell expression
Osteoblast markers			
OSX	8.84 (4.74, 13.84) *	2.93 (1.01, 5.20) *	r=0.30, p=0.18
Runx2	3.36 (2.53, 5.49) *	2.61 (1.79, 3.40) *	r=0.30, p=0.18
Col1A1	3.83 (3.28, 4.37) *	1.37 (1.10, 1.70) *	r=0.34, p=0.12
BGLAP	7.91 (3.92, 10.44) *	1.13 (0.56, 2.03)	r=0.19, p=0.39
OPG	**4.23 (3.11, 7.08) ***	**1.38 (0.64, 1.92) ***	**r=0.44, p=0.04**
ALPL	5.15 (3.21, 6.41) *	2.96 (1.79, 5.59) *	r=0.03, p=0.90
MGP	3.23 (1.69, 4.07) *	2.35 (1.24, 3.65) *	r=0.18, p=0.43
BMP2	2.93 (1.81, 5.14) *	2.46 (1.74, 4.22) *	r= - 0.06, p=0.80
Cell signaling			
Cyp24A1	**0.04 (0.01, 0.43) ***	**1.28 (0.45, 3.69)**	**r=0.42, p=0.05**
Cyp27B1	**2.45 (1.66, 4.13) ***	**0.96 (0.77, 1.75)**	**r=0.73, p=0.0001**
VDR	**2.49 (2.07, 3.88) ***	**2.79 (1.98, 3.97) ***	**r=0.45, p=0.04**
FGFR1	**4.33 (2.52, 6.05) ***	**1.68 (1.13, 2.09) ***	**r=0.49, p=0.02**
PHEX	6.46 (4.42, 12.48) *	0.99 (0.70, 1.60)	r=0.36, p=0.10
RANKL	6.50 (4.46, 12.00) *	1.63 (0.24, 2.90) *	r= - 0.07, p=0.75
NHERF1	**1.04 (0.82, 1.65)**	**2.42 (1.83, 3.19) ***	**r=0.64, p=0.001**
PTHR1	8.25 (4.91, 11.08) *	4.77 (1.83, 6.73) *	r= - 0.18, p=0.43
IGF1	**1.60 (1.19, 2.51) ***	**4.14 (1.96, 9.15) ***	**r=0.47, p=0.03**
Osteocyte markers			
DMP 1	10.06 (5.21, 16.32) *	0.96 (0.53, 1.04)	r=0.17, p=0.46
FGF23	3.88 (1.55, 9.95) *	1.10 (0.89, 1.22)	r= - 0.06, p=0.80
MEPE	6.29 (4.38, 11.62) *	3.82 (2.22, 9.86) *	r=0.04, p=0.85
SOST	4.15 (2.77, 9.02) *	Not detected	NA

* different from healthy controls, $p<0.05$

Expression of osteoblast markers was increased in CKD bone and cells and expression of genes critical to PTH, FGF, and vitamin D signaling correlated between cells and bone core itself, suggesting that these cells maintain an abnormal phenotype in vitro.

Conclusions: Hormone signaling mechanisms appear to be preserved in this novel model, providing a potentially useful characteristic by which to evaluate the abnormal bone biology associated with CKD.

TH-PO594

The Importance of Serum TRAP-5b and Sclerostin in Osteocytic Perilacunar/Pericanalicular Remodeling in Dialysis Patients

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Background: Sclerostin (Scl) inhibts osteoblastic bone formation and accelerates osteocytic osteolysis and parathyroid hormone (PTH) also accelerates osteoclastic bone resorption and osteocytic osteolysis. However, chronic PTH elevation inhibits Scl, resulting in prevention of severe reduction of bone volume (BV). We investigated the mechanism to prevent an acute and severe reduction of BV and tartrate resistant acid phosphatase-5b (TRAP-5b) derived from the osteocyte in dialysis patients.

Methods: Serum parameters, including TRAP-5b, Scl and intact PTH (iPTH) levels were measured in patients with hypoparathyroidism (Hypo) (N=13) and subjects without hypoparathyroidism (N=23). Histomorphometric parameters, including the number of osteocytes inside resorption predominant osteocyte lacunae (N.Ot.ES/Ct.Ar;N/mm^2) and poorly mineralized BV (PMBV/Ct.Ar;%) were measured in the cortex. The relationships between TRAP-5b and both N.Ot.ES/Ct.Ar and PMBV/Ct.Ar were investigated in Hypo whose osteoclast surface was below 0.3 % in cortex to evaluate TRAP derived from osteocyte. The 25 cortices obtained from 13 Hypo were divided into Group I (N=13) and Group II (N=12) by N.Ot.ES/Ct.Ar and PMBV/Ct.Ar to compare TRAP-5b between these two groups (Nonparametric statics). Both N.Ot.ES/Ct.Ar and PMBV/Ct.Ar values of Group I were greater than Group II. And the relationships between serum parameters were investigated in 36 patients (Linear regression analysis).

Results: Serum TRAP-5b was greater in Group II than Group I when these cortices were divided by N.Ot.ES/Ct.Ar ($P=0.041$) and PMBV/Ct.Ar ($P=0.005$) in Hypo. Scl was negatively associated with iPTH ($P<0.001$, $R=-0.67$) and TRAP-5b ($P<0.05$, $R=-0.41$) in all patients.

Conclusions: Chronic elevation of PTH seems to suppress Scl in dialysis patients. Increased osteocytic osteolysis constitutes a negative-feedback mechanism for lowering TRAP activity.

TH-PO595

The Contribution of Osteocytes to Minimodeling Formation on the Endocortical Surface after Parathyroidectomy for Secondary Hyperparathyroidism

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Background: Osteocytes in minimodeling area on the endocortical surface have an important role to increase cortical bone volume after parathyroidectomy (PTX) for secondary hyperparathyroidism (II HPT) because mechanical loading transmits to the cortex and osteocyte is the important mechanical sensor to mechanotransduction. The importance of osteocyte in minimodeling formation was investigated because a significant increase of minimodeling following PTX was reported.

Methods: We measured minimodeling volume (Ml.BV)-referent osteocyte number (N.Ot/Ml.BV; N/mm^2) at the endocortical surface and cortical area-referent N.Ot (N.Ot/Ct.Ar; N/mm^2) in transiliac bone biopsy specimens taken at 2 to 4 (mean; 3.6 ± 0.7) weeks after PTX for IIHPT (n=17, 57.9 ± 9.0 years old, with a HD duration of 13.4 ± 7.2 years). As a result, 34 cortices were obtained and equally divided into the two groups. The relationship between N.Ot/Ml.BV and changes of Ml.BV-referent minimodeling osteoid volume (Δ Ml.OV/Ml.BV; %) after PTX was investigated in specimens from Group I, in which cortical width (Ct.Wi; μm) was over 700 μm, and subjects of Group II, in which with Ct.Wi was below 700 μm (n=17 each).

Results: N.Ot/Ml.BV was greater than N.Ot/Ct.Ar in both Group I (427.0 ± 211.4 versus 151.2 ± 53.6 N/mm^2, $P = 0.004$) and Group II (456.0 ± 205.4 versus 138.2 ± 70.6 N/mm^2, $P = 0.005$). Δ Ml.OV/Ml.BV was associated with N.Ot/Ml.BV in Group I ($P = 0.004$, $R = 0.650$), but not in Group II.

Conclusions: The Osteocyte seems to play an important role in minimodeling formation because of the abundance of osteocytes in minimodeling area. Mechanical loading effectively influences minimodeling formation in Group II patients with severe cortical thinning independently of N.Ot. And N.Ot is so much important in minimodeling formation in Group I.

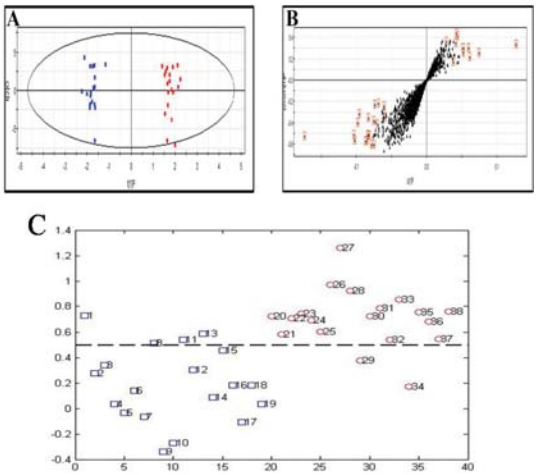
TH-PO596

Metabolic Pattern Shift in Chronic Kidney Disease-Mineral and Bone Disorder by Mass Spectrometry-Based Metabolomics Xueli Lai, Zhiyong Guo. *Dept of Nephrology, Changhai Hospital, Shanghai, China.*

Background: Abnormalities in serum calcium, phosphorus, and parathyroid hormone (PTH) levels are common in patient with CKD, and are the classical markers using in predicting the onset of CKD-MBD. Therefore, CKD-MBD is associated with metabolic disturbances related to its classical markers. Identifying metabolite biomarkers associated with CKD-MBD may have important value for better prognosis, risk assessment and potential target screening. The objective of the study was to find the endogenous metabolic difference associated with iPTH between secondary hyperparathyroidism patients and disease controls.

Methods: This study was a cross-sectional study. Metabolic profiling analysis of serum was performed by ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS) in 38 PD patients including 19 disease controls (DC) (iPTH: 150-300 pg/ml) and 19 secondary hyperparathyroidism (SHPT) patients (iPTH > 300 pg/ml).

Results: Metabolic profiling analysis revealed different patterns of endogenous metabolites between SHPT and DC groups, The present study identified total 28 metabolites that performed best in differentiating SHPT group from DC group. The OPLS-DA model obtained revealed a good explained variance and predictability,(1A and 1B). and was assessed by leave-one-out analysis, exhibiting 89.5% sensitivity and 78.9% specificity.



(1C)

Conclusions: Metabolomic analysis is an alternative tool for discriminating SHPT patient from DC. And with this way in this study, we find that uremic toxins, steroid hormones and TCA cycle intermediates are mainly involved in the onset of CKD-MBD, with obvious promise for better prognosis and new target screening.

Funding: Government Support - Non-U.S.

TH-PO597

Looking inside the Skeleton: Bone Protein Quantification in CKD Patients Patricia T. Goldenstein, Fabiana Graciolli, Rosilene M. Elias, Gisele Antunes Lins, Wagner Dominguez, Luciene dos Reis, Vanda Jorgetti, Rosa M.A. Moyses. *Nephrology, Univ de Sao Paulo, Brazil.*

Background: CKD-MBD is a complex disorder, with several unsolved issues regarding its pathophysiology. Therefore, new study techniques that could help us understand it are welcome. Previous studies have shown high serum concentration of Wnt pathway inhibitors such as Sclerostin (Scl) and Dickkopf 1 (DKK1) in CKD. In addition, high bone Scl expression by immunohistochemistry has already been demonstrated in CKD patients. However, the quantification of this and other CKD-MDB related proteins in bones is still lacking.

Methods: Patients with severe secondary hyperparathyroidism (SHPT) who were referred to parathyroidectomy underwent a transiliac bone biopsy with histomorphometric analysis. A total bone protein lysate was obtained and Scl, DKK1, Osteocalcin (OC) and Osteoprotegerin (OPG) were quantified by MILLIPLEX®, which was also used to measure serum concentration of the same proteins.

Results: Eighteen patients aged 44 ± 13 years; on dialysis for a median time of 60 months; with PTH ranging from 631 to 5418 pg/ml (median = 2124) were included. All patients had histomorphometric pattern of high turnover bone disease. There was no correlation of the same protein in bone and serum for any evaluated protein. The highest bone concentration was found for OC (median = 94 mg/g protein), followed by Scl (median = 0.8 mg/g protein). Patients with higher bone concentration of DKK1 were on dialysis for a longer time, whereas a higher bone Scl was associated with higher serum phosphate, bone eroded surface, and bone OC. Higher serum Scl was found in patients with lower bone OPG.

Conclusions: By using this new technique, we have demonstrated that there is no association between bone and serum protein concentration. Our findings suggest that serum phosphate could stimulate bone Scl, which, in turn, would increase bone resorption. In addition, a higher serum Scl would inhibit bone OPG, also favoring the resorptive process. However, as only patients with severe SHPT were included, further studies are needed. Given these promising preliminary findings, this innovative technique has the potential to help elucidate renal CKD-MBD pathophysiology.

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TH-PO598

Hyponatremia and Osteoporosis: Perspectives from a Single Academic Center Farsad Afshinnia,[1] Baskaran Sundaram,[2] Robert J. Ackermann,[1] Ka Kit Wong.[1,3] *[1]Univ of Michigan; [2]Thomas Jefferson Univ; [3]Ann Arobor VA Medical Center.*

Background: Independent association of hyponatremia with osteoporosis (OP) is controversial, with few small studies having contradictory results. We aimed at testing for a relationship between hyponatremia with OP in a large cohort of patients undergoing bone densitometry.

Methods: This is a cross-sectional observation of patients who have undergone bone densitometry at the outpatient units of the University of Michigan from 2001 to 2013. Consecutive patients with available demographic, clinical and laboratory data were included. OP was defined as a bone mineral density of ≤2.5 standard deviations (SD) below the mean peak bone mass of young, healthy adults. Hyponatremia was defined as serum sodium ≤135 meq/L, based on time-averaged serum sodium over the past 2 years prior to the date of densitometry. General Electric Lunar iDXA densitometers were used for densitometry. Multiple logistic regressions were used to calculate adjusted Odds Ratio (OR).

Results: Overall 25,117 patients were included. Mean age was 61 years (SD=14). There were 4701 males (18.7%) and 1895 black patients (7.5%). There were 715 patients (2.8%) with hyponatremia, 2672 (10.7%) with neck OP, 1885 (7.5%) with total hip OP, and 4880 (19.4%) with lumbar OP. Using a case mix model OR of OP associated with hyponatremia was 1.6 (95% CI: 1.4 – 1.9) at lumbar spine, 2.2 (95% CI, 1.8 – 2.7) at femoral neck, and 3.0 (95% CI, 2.5 – 3.8) at total hip. After adjusting for age, sex, race, weight, GFR, and use of medications, the OR was attenuated to 1.2 (95% CI, 1.0 – 1.5) at lumbar spine, 1.3 (1.1 – 1.7) at femoral neck, and to 1.9 (1.5 – 2.3) at the total hip site. Subgroup analysis revealed a significantly higher adjusted OR in patients aged ≤ 55 years, and BMI of 25-29 kg/m2 at all anatomical sites as compared to other subgroups. Males had a significantly higher OR at the lumbar site while females had a higher OR at the femoral neck. Similarly, black race had significantly higher OR at all hip anatomical sites.

Conclusions: Hyponatremia was independently associated with OP. This association persisted, though was significantly attenuated after multiple adjustments. It varied by age, gender, race, and BMI.

TH-PO599

Bone Microstructure Evaluated by High-Resolution Peripheral Quantitative Computed Tomography and Low-Impact Fractures in Men with Predialysis Chronic Kidney Disease Maurilo Leite,[1] Francisco Paranhos-Neto,[2] Guilherme Cunha Lima,[2] Alvimar Delgado,[1] Carlos Perez Gomes,[1] Maria Lucia Fleiuss Farias.[2] *[1]Nephrology, Univ Federal do Rio de Janeiro, Brazil; [2]Endocrinology, Univ Federal do Rio de Janeiro, Brazil.*

Background: CKD is associated with increased risk of fractures, not well correlated with findings of dual energy X-ray absorptiometry (DXA). High-resolution peripheral quantitative computed tomography(HR-pQCT) assesses bone microarchitecture, providing information on bone quality. This study aimed to evaluate bone structure by HR-pQCT in male predialysis patients, and correlate these alterations with metabolic parameters and low-impact fractures (LIF).

Methods: Forty-five hypertensive men, between 50-75 years of age, identified as CKD stages 3 or 4, were selected. Men were chosen to avoid the effect of menopause on BMD. Mean values of biochemical parameters, collected from the previous 6 months, as well as serum bicarbonate, iPTH, 25(OH)vitD, and FGF-23 were analyzed. Fifteen patients reported at least one LIF. The remaining group had no history of fractures. Both groups were assessed by DXA (femoral neck, total femur and radius) and HrPQCT (distal radius and distal tibia).

Results: BMD T-scores were lower in the stage 4 compared to 3 CKD patients and were positively correlated with serum bicarbonate levels (P=0.005). There was no correlation between T-scores results and LIF. Regression analysis of the radius HR-pQCT found a negative correlation between low-impact fractures and cortical thickness (P=0.016), cortical perimeter (P=0.036) and cortical density (p=0.029), as well as positive correlation with cortical surface (P=0.014). Serum bicarbonate and FGF-23 were negatively and positively correlated with trabecular area (r=-0.5, P=0.008 and r=0.6, P=0.009, respectively).

Conclusions: These results confirm that DXA is not a reliable tool to investigate CKD patients under the risk of low impact fractures. On the other hand, HR-pQCT can be of significant value. It is suggested that cortical bone trabecularization occurs early in CKD and results in cortical bone fragility. Finally, serum levels of FGF-23 and the degree of metabolic acidosis are crucial determinants of bone fragility.

Funding: Government Support - Non-U.S.

TH-PO600

Evaluation of Bone Microarchitecture by High-Resolution Peripheral Quantitative Computed Tomography in Patients with Chronic Kidney Disease: Comparison with Transiliac Bone Biopsy Igor Marques,[1] Maria Julia C.L.N. Araujo,[1] Fabiana Graciolli,[1] Luciene dos Reis,[1] Melani R. Custódio,[1] Rosa M. Pereira,[3] Sophie Jamal,[2] Vanda Jorgetti,[1] Elias David-Neto,[1] Rosa M.A. Moyses.[1] *[1]Nephrology Div, Univ de Sao Paulo; [2]Univ of Toronto; [3]Reumathology Div, Univ de Sao Paulo.*

Background: High-resolution peripheral quantitative computed tomography (HR-pQCT) is a noninvasive imaging technique that assesses trabecular and cortical bone microarchitecture in vivo. The purpose of our study is to evaluate, for the first time, the correlation between HR-pQCT measures and transiliac bone biopsy (Bx) in dialysis patients.

Methods: We measured BMD by HR-pQCT and dual energy x-ray absorptiometry (DXA) in 31 CKD stage 5D patients. Bx were analyzed by 2D quantitative histomorphometry; and measures of total and mineralized trabecular bone volume (2D BV/TV and 2D Md.V/TV, respectively), as well as cortical porosity (2D Co.Pt) were obtained.

Results: Patients (19 males) were 41±11 years old, with a dialysis vintage of 28 months, BMI of 24kg/m², serum Ca of 8.4±0.6 mg/dl; P of 3.4±1.5 mg/dl; alkaline phosphatase 93(71-145) U/L; PTH 688±489 pg/ml; 25-vitamin D 22(15-34) ng/ml and sclerostin 1.03(0.51-1.83) ng/ml. 2D BV/TV correlated significantly with height; lumbar spine (r =0.70) and total femur (r = 0.59) DXA; and modestly with HR-pQCT BV/TV at the radius (r = 0.42; p< 0.05) but not at the tibia. 2D Md.V/TV correlated significantly with age; height; lumbar spine (r = 0.67) and total femur (r = 0.63) DXA; and HR-pQCT BV/TV (r = 0.50; p<0.05) only at the radius. Conversely, a strong correlation was found between 2D Co.Pt and HR-pQCT cortical bone density (DComp) both at radius and tibia (r = -0.60 and -0.64, respectively; p <0.05).

Conclusions: There was a good agreement between HRpQCT and 2D BV/TV regarding cortical compartment. On the other hand, in trabecular bone, despite being statistically significant and greater than that described for the general population, the correlations between both methods were modest, indicating that HRpQCT cannot be used to predict 2D BV/TV. Further, larger studies are needed as well as studies to determine if these noninvasive measures can predict fracture.

Funding: Government Support - Non-U.S.

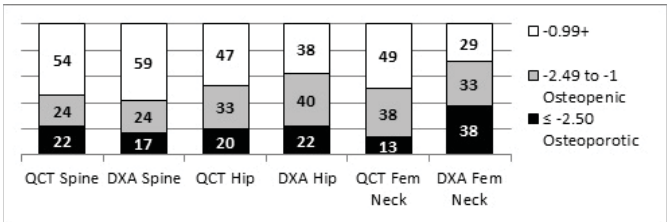
TH-PO601

Determination of Bone Mass By Invasive and Non-Invasive Methods: Evidence for Usefulness of DXA in CKD-5D Gustav A. Blomquist,[1] Daniel Davenport,[2] Hanna W. Mawad,[2] Marie-Claude M. Faugere,[2] Hartmut H. Malluche.[2] *[1]Radiology, Univ of Kentucky, Lexington, KY; [2]Internal Medicine, Div of Nephrology, Univ of Kentucky, Lexington, KY.*

Background: Low bone mass is an integral component of renal osteodystrophy and is seen in virtually all patients with CKD-5D. Bone mass is measured noninvasively by dual-energy x-ray absorptiometry(DXA) and quantitative computed tomography(QCT), or invasively by bone biopsies with histology. DXA is readily available. QCT is less available, higher radiation, and more expensive. Histomorphometric evaluation is considered the gold standard but rarely used because of the bone biopsies. This study evaluates the utility of DXA and QCT for screening of osteoporosis with validation by histology.

Methods: A total of 46 patients(mean age 51yrs, 52% women, median dialysis 46 mos.) had bone biopsies and bone mineral density(BMD) measured by DXA and QCT.

Results: QCT and DXA absolute BMD values(g/cm²) were highly correlated at the femoral neck (rho=0.968), total hip (rho=0.961) and to a lesser degree at the spine (rho=0.612). Both DXA and QCT t-scores were highly correlated, but QCT t-scores were up to 1 S.D. greater than DXA t-scores leading to less recognition of low BMD by QCT(Figure: (%) of BMD t-scores by method and site)



DXA and QCT measurements correlated with histologic cancellous bone volume(BV/TV) and negatively with trabecular separation(TbSp), p<.001.

	DXA Spine	QCT Spine	DXA Fem Neck	QCT Fem Neck	DXA Hip	QCT Hip
BV/TV	.538	.565	.567	.616	.644	.663
TbSp	-.506	-.570	-.577	-.637	-.633	-.653

Conclusions: Current nephrologic and radiologic guidelines were formulated with limited available DXA and QCT data and thus recommend limited use of DXA in CKD-5D. The presented results show DXA is a useful tool for assessment of bone mass in CKD-5D patients and warrants reconsideration of the current guidelines.

Funding: NIDDK Support, Private Foundation Support

TH-PO602

Sotatercept: Initial Signal-Seeking Quantitative Computed Tomography Results for Bone Mass in Hemodialysis Subjects Treated with Escalating Doses: Interim Analysis of ACE-011–REN-001 Hartmut H. Malluche,[1] Keith A. Hruska,[2] Hem N. Singh,[3] William T. Smith.[3] *[1]Univ of Kentucky, Lexington, KY; [2]Washington Univ St. Louis, St. Louis, MO; [3]Celgene Corporation, Warren, NJ.*

Background: High turnover renal osteodystrophy (ROD) is marked by increased cortical porosity, higher trabecular bone mass, and increased risk of fracture. Sotatercept, an activin A RIIA-IgG1 fusion protein ligand trap under study for the correction of anemia in hemodialysis (HD) subjects, blocks activin A signaling and may reduce osteoclastogenesis and promote osteoblast maturation in bone. The current analysis in HD subjects evaluated the effect of sotatercept on bone mineral density (BMD) and vascular calcification using quantitative computed-tomography (QCT).

Methods: In an ongoing studyof sotaterceptin HD subjects for the correction of anemia, subjects who were erythropoietin-stimulating agent (ESA)-responsive were washed out of their ESA effect until Hb was <10 g/dL, then randomized to sotatercept 0.3 mg/kg (n=9), 0.5 mg/kg (n=8), or placebo (PBO; n=7) SC every 28 days for up to 8 dose cycles; a 0.7 mg/kg dose group (n=6) is ongoing. Subjects were assessed for effects on Hb, BMD, and biomarkers of bone turnover. Treatment failures (Hb <9 g/dL) were rescued with ESA/transfusion. QCT of the hip, and lumbar spine, was obtained at baseline and after the 225-day treatment phase. Biomarkers, BSAP and CTX, were measured at baseline and after dose cycles 3, 5, and 7.

Results: Of the 30 randomized subjects, 13 had paired QCT assessments (n=3, 6, and 4 for PBO, 0.3 mg/kg, and 0.5 mg/kg, respectively). Relative BMD changes from baseline for PBO, 0.3 mg/kg, and 0.5 mg/kg were -0.9%, -1.4%, and +1.9% of the femoral neck cortical, and +12.6%, +8.0%, and -1.9% of the lumbar spine, respectively. Biomarker changes with 0.5 mg/kg suggest a beneficial antiresorptive effect.

Conclusions: These interim data suggest an emerging dose effect with sotatercept 0.5 mg/kg, which appeared to reverse the effects of high turnover ROD on cortical and cancellous bone. PBO results were as expected. We anticipate a complete data set to further substantiate these effects.

Funding: Pharmaceutical Company Support - Celgene Corporation; Acceleron Pharma, Inc.

TH-PO603

Risedronate Combined with Menatetrenone May Prevent Glucocorticoid-Induced Osteoporosis in Patients with Chronic Glomerulonephritis Yuko Makita, Hitoshi Suzuki, Masao Kihara, Takashi Kobayashi, Yasuhiko Kanaguchi, Satoshi Mano, Teruo Hidaka, Yasuhiko Tomino. *Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan.*

Background: Glucocorticoid therapy may induce secondary osteoporosis, although glucocorticoid is useful for the treatment of chronic glomerulonephritis (CGN). Bone loss is observed to begin developing just after the administration of glucocorticoid, and the degree of osteoporosis depends on the cumulative doses of glucocorticoid. Although bisphosphonate treatment is well known to improve bone quality and reduce the risk of bone fractures, recent studies have shown that vitaminK2 also stabilizes bone mineral density (BMD). Furthermore, vitamin K2 works with osteocalcin for bone formation. Thus, we examined the clinical efficacy of bisphosphonate alone and bisphosphonate combined with vitamin K2 for the prevention of glucocorticoid-induced bone loss in CGN patients using serum levels of N-terminal telopeptide of type I collagen (NTx) and uncarboxylated osteocalcin (ucOC) with BMD.

Methods: We recruited 62 patients (mean age 42.4 ± 16.9) with CGN who were treated with prednisolone from 2011 to 2013 at Juntendo University Hospital. six-month prospective randomized study was conducted. These patients were randomly assigned to either Risedronate (17.5 mg/week) alone (Risedronate group, n=30) or Risedronate with Menatetrenone (45 mg/day) (Combined group, n=32) treatment groups. Serum levels of NTx and ucOC as well as BMD were measured before and after 6 months of commencing treatment with prednisolone.

Results: In the Risedronate alone group, the percent change of serum levels of NTx was -9.8 % after 6 months, whereas the Combined group observed changes of -27.8 % (P<0.05). Serum levels of ucOC were decent decreased in the Combined group, compared with the Risedronate alone group. Consequently, increased levels of BMD in spine was higher in the Combined group (+15.6%) than in the Risedronate alone group (+4.5%). Notably, the effect of bone formation by combination therapy was remarkable in female patients (P<0.05).

Conclusions: The combination therapy of Risedronate with Menatetrenone may have a synergistic effect to prevent glucocorticoid-induced osteoporosis in patients with CGN.

TH-PO604

Effects of Muscle Strengthening on Bone Mass in Hemodialysis Patients Kei Yoneki,[1] Atsuhiko Matsunaga,[1] Jun Kitagawa,[1] Yoshifumi Abe,[1] Manae Harada,[1] Ryoma Ishikawa,[1] Takaaki Watanabe,[1] Ryota Matsuzawa,[1] Atsushi Yoshida,[2] Kouju Kamata.[1] *[1]Kitasato Univ, Sagamihara, Japan; [2]Sagami Junkanki Clinic, Sagamihara, Japan.*

Background: Muscle strengthening is a recommended preventive strategy against bone loss in the general population. However, how muscle strength affects bone mass in hemodialysis (HD) patients is unclear. We performed cross-sectional (Study 1) and longitudinal (Study 2) studies to examine the association between muscle strength and bone mass in HD patients.

Methods: In Study 1, 172 outpatients with HD (79 men and 83 postmenopausal women; mean age, 66 ± 8 years) were recruited. Clinical characteristics (age, sex, body mass index, HD vintage, and levels of serum albumin, serum phosphorus, corrected calcium, and intact parathyroid hormone [iPTH]) were obtained from clinical records. Bone mass was assessed by quantitative ultrasound measurement (stiffness index) at the calcaneus. Grip strength was assessed as an indicator of general muscle strength. The association between grip strength and stiffness index was assessed by multiple regression analysis adjusted for clinical characteristics for each sex. In Study 2, a 2-year follow-up of subjects of Study 1 was performed in terms of clinical characteristics, stiffness index, and grip strength. Subjects were divided into two groups by the median grip strength both at baseline and endpoint. Multiple regression analysis, adjusted for baseline clinical characteristics, baseline stiffness index, and changes in iPTH, was performed to examine the association between grip strength during the study period and changes in stiffness index for each sex.

Results: In Study 1, adjusted multiple regression analysis revealed a significant association between grip strength and stiffness index for each sex (P<0.05 for both). In Study 2, follow-up of 97 HD patients and subsequent adjusted multiple regression analysis revealed a significant association between grip strength during the study period and changes in stiffness index for each sex (P<0.05 for both).

Conclusions: Our findings suggest that maintaining or improving muscle strength may contribute to the effective prevention of bone loss in HD patients.

TH-PO605

Effects of Acidosis Correction on Chronic Kidney Disease - Mineral Bone Disorder Parameters in Patients on Maintenance Hemodialysis

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Background: Metabolic acidosis (MA) is common among patients with end-stage renal disease, and is not completely corrected by hemodialysis. Acidosis can cause impairment in nutritional status and worse uremic bone disease. However, few studies evaluated the effects of MA correction on CKD-MBD laboratorial parameters. **Objective:** to investigate the impact of MA correction on CKD-MBD.

Methods: We prospectively studied 48 patients on hemodialysis with age 43 ± 19 years. Individual adjustments in dialysate bicarbonate concentration (dBic) were made. Blood gas analysis was done monthly for 4 months (M1 to M4). Biochemical variables were accessed at M0 and M4.

Results: Bic and pH increased and maintained in the target range during the study (p<0.05). Although there was an increase in sodium, no changes were observed in the interdialytic weight gain and also in the hypotensive drugs need. From M0 to M4, there was an increase in PTH (parathyroid hormone) [from 191 (85, 459) to 446pg/ml (212, 983), p<0.0001], in serum phosphate (from 5.4±1.4 to 5.8±1.1mg/dl, p=0.048), and a decrease in ionized serum calcium (from 5.0±0.5 to 4.7±0.5mg/dl, p=0.002). Serum albumin increased (from 3.5±0.3 to 4.0±0.3g/L, p<0.0001) while $ß_2$ microglobulin decreased (from 27.6±8.3 to 25.8±6.8 µg/ml, p=0.025). Serum leptin decreased in all but 3 patients (p<0.0001). Thirty-one out of 48 patients (64.6%) had PTH <300pg/ml at baseline. Despite the same degree of MA correction and decrease in serum calcium, these patients had much higher percentage increase rate in PTH when comparing to those with PTH >300pg/ml at baseline [250 (62, 330) versus 75 (-2.2, 145), p=0.002]. If we consider the KDIGO recommendation (PTH 2-9 times the upper normal limit of the assay), the percentage increase rate of PTH was higher in those patients with PTH in the lower limit (p=0.008).

Conclusions: MA correction by increasing dBic is safe. This approach might improve nutritional status and decrease inflammation. The benefit appears to be much greater for patients with low PTH, and caution is advised in patients with high PTH.

TH-PO606

Effecst of Parathyroid Function and Bone Turnover on Bone Structural Properties in Dialysis Patients

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Background: Although abnormalities in parathyroid function and bone turnover are commonly found among individuals with chronic kidney disease (CKD), the influence of these abnormalities on bone microscopic structural properties remains obscure. Conventional two-dimensional (2D) bone histomorphometry has a limitation in assessing cancellous bone structure.

Methods: Biopsied iliac bone samples from 48 stable dialysis patients were used for the analyses. Quantitative histomorphometric studies were performed in the virtual 3D space generated from the serial micro-computed tomography sections of the samples. These data were compared with those obtained by biochemical and tetracycline labeling–based 2D histomorphometric results.

Results: Neither cancellous bone volume nor trabecular thickness showed significant correlations with the bone formation rate (BFR/BS) at the 3D level. Both the trabecular pattern factor (TBPf) and the structural model index showed significant inverse correlations with the BFR/BS, whereas the marrow cavity star volume, the connection density, and the number of nodes did not. Circulating intact parathyroid hormone (PTH) levels showed a significant inverse correlation with cortical bone thickness, whereas none of the above five parameters showed a correlation with PTH. In the subgroup of 33 patients with osteoid volume>3.0%, those with a mineral apposition rate (MAR) of 0 showed significantly higher levels of TBPf than those with a MAR>0.

Conclusions: In iliac bone samples obtained from dialysis patients, (1) increased bone turnover drove cancelluos bones into more like a plate-shaped pattern with an uneven surface, (2) these morphological changes were not linked to altered trabecular bone connectivity or trabecular bone mass, (3) the bone formation/mineralization step was responsible for the morphological changes, and (4) cortical bone thinning was the only likely findings related to parathyroid function, which would potentially increase the fracture risk.

Funding: Government Support - Non-U.S.

TH-PO607

The Pitfall of Treating Low Bone Turnover Through the Stimulation of PTH: Effects on Cortical Porosity

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Background: Although it is recognized that cortical bone contributes significantly to the mechanical strength of the skeleton, little is known about this compartment from bone biopsy studies. Recently, it has been described that an increase in cortical porosity (Ct.Po) is a common finding in CKD patients, especially in black patients. However, there is no prospective data of the effects of CKD-MBD therapy on Ct.Po.

Methods: We measured Ct.Po in bone biopsies (Bx) obtained from dialysis patients who have undertaken a prospective study (BRIC). CKD-MBD therapy was adjusted according to the first Bx, trying to normalize bone turnover rate, regardless of baseline serum PTH. After one year of follow-up, a second Bx was obtained and changes in Ct.Po were the variable of interest.

Results: We evaluated 52 patients with baseline PTH 265 pg/ml (124-633). According to TMV, 44% had low turnover disease, 50% had low cancellous volume and 54% abnormal mineralization. High initial Ct.Po was found [29.5% (16.7, 46)], and after one year, increased in 59.6% of patients, with a delta Ct.Po of 6.1% (-11, 23). PTH increased in 30 patients as a result of CKD-MBD therapy. The change in PTH correlated with a change in bone formation rate (r=0.28; p<0.05), but also with the delta of Ct.Po (r=0.30, p<0.05). Delta of Ct.Po was higher in non-white than in white patients (16.6 versus -1.1%, respectively) as well as in patients with baseline PTH <300 pg/ml (p<0.05). Multiple regression analysis showed that the delta of Ct.Po was dependent on delta PTH and race, in a model adjusted for age, gender and therapy.

Conclusions: In this prospective study, we showed that there was an increase in Co.Pt in dialysis patients, which was more pronounced in black patients, despite the continue raise of PTH. Therefore, elevation of PTH as an attempt to normalize cancellous bone formation rate may cause a higher risk of cortical bone loss and probably of fractures.

Funding: Government Support - Non-U.S.

TH-PO608

Prolonged Hyperparathyroidism May Cause Subendocardial Ischemia Risk That Remains after Parathyroidectomy

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Background: Secondary hyperparathyroidism (SHPT) in patients on hemodialysis is associated with increased risk for myocardial ischemia and endothelial dysfunction. Parathyroidectomy (PTx) can reduce the cardiovascular risk in these patients, but whether PTx improves these conditions is still unknown. The pupose of this study was to investigate the risk of myocardial ischemia and endothelial dysfunction after long term PTx.

Methods: The propensity to myocardial ischemia (Buckberg index) was calculated as the ratio of the area of the diastolic phase to that of the systolic phase in the central aortic profile. This ratio was designated as the subendocardial viability ratio (SEVR), measured by SphygmoCor (AtCor Medical Ltd.). Endothelial dysfunction (accessed as flow mediated dilation – FMD) was done through the reactive hyperemia. Echocardiography was also performed.

Results: 27 patients (41% male) with age 48 ± 13 years, on dialysis for 95 (21, 181) monthswere included. SHPT was seen in 17 (63%) patients in whom PTx was performed in 14. Three patients were still with severe SHPT, defined as PTH >500pg/ml. The median time patients were exposed to severe SHPT was 1049 days. This period corresponds from the first PTH>500pg/ml until the day of PTx (PTx group) or the day of study assessment (including patients with current severe SHPT). FMD correlated to septum thickness (r=-0.715, p=0.0004), and was not different between patients who underwent PTx or who had SHPT. SEVR correlated to 25-vitamin D (r=0.506, p=0.022), and was higher in both group of patients, those submitted to PTx (p=0.033) and those with SHPT (p=0.0003). There was a correlation between the exposure time to SHPT and SEVR (r=-0.612, p=0.003). Multiple linear regression revealed, in a model adjusted for hemoglobin, age and creatinin, that 25-vitamin D (p=0.040), time exposed to SHPT (p=0.001) and gender (p=0.015) were independent associated to SEVR, explaining 57% of its variability.

Conclusions: We demonstrated for the first time that severe SHPT might cause functional myocardial abnormalities, associated to higher myocardial ischemia risk, which remains even after PTx.

TH-PO609

22-Oxacalcitriol Ameliorates Diabetic Bone Disease in Diabetic Model Rats through Antioxidative Effects and Increase in IGF-1
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Background: Bone disease is an important complication in patients with chronic kidney disease (CKD). The bone fragility in these patients is caused by not only kidney dysfunction but also concomitant disease. Diabetes is a major cause of CKD and a risk factor for fracture. Although some studies have reported that vitamin D improves bone mineral density and bone formation in diabetes, the mechanisms remains unclear. Our study investigated the effect of 22-oxacalcitriol (OCT) on diabetic bone disease. In addition, we also investigated the effect of OCT on oxidative stress and insulin growth factor-1 (IGF-1).

Methods: We used Spontaneously Diabetic Torii (SDT) rats, which are newly established models of non-obese type 2 diabetes. Sprague-Dawley rats were used as a control group. SDT rats were divided into three groups: the diabetic (DM) group, the insulin (INS) group, and the OCT group at 20 weeks of age. OCT was administered at a dose of 0.2 mg/kg three times per week. We evaluated blood and urine analyses, bone mineral density (BMD), histomorphometry, oxidative stress marker 8-OHdG, and serum IGF-1 at 30 weeks of age.

Results: HbA1c and serum calcium levels were comparable between the OCT and DM groups. BMD, mineral apposition rate, and bone formation rate per bone surface were higher in the OCT group than those in the DM group. Urinary 8-OHdG levels and 8-OHdG positive cells in bone were lower in the OCT group than those in the DM group. Serum IGF-1 levels were higher in the OCT group than those in the DM group.

Conclusions: Our data suggested that OCT ameliorated diabetic bone disease in diabetic model rats partially through its antioxidative effects and increase in IGF-1.

Funding: Pharmaceutical Company Support - Chugai Pharmaceutical Co., Ltd.

TH-PO610

Differential Effects of the Calcimimetic R-568 and Calcitriol on the FGF23-Vitamin D-PTH Axis in the Hyp Mouse as an Animal Model of Post-Transplant Hypophosphatemia
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Background: Post-transplant hypophosphatemia is due to persistent secondary hyperparathyroidism, $1,25(OH)_2D_3$ deficiency, and elevated levels of the phosphaturic hormone fibroblast growth factor 23 (FGF23) resulting in post renal transplant (PRT) osteodystrophy. The Hyp mouse is a murine homolog to the human X-linked hypophosphatemia, displaying all features of PRT hypophosphatemia, and thus is excellently suited to investigate potential new measures.

Methods: The effects of a 7 day treatment with the calcimimetic R-568 (1-30 mg/kg BW/day), calcitriol (150-450 ng/kg BW/day), and vehicle on the FGF23-vitamin D-PTH axis was investigated in male Hyp mice. Wild type mice served as controls. Serum levels of FGF23, PTH, calcium, and phosphate were evaluated. The kidneys were assessed with respect to Cyp27b1, Cyp24 and klotho mRNA and/or protein expression.

Results: Hyp mice showed reduced weight, shorter tibia and femur lengths, reduced serum phosphate levels, and increased FGF23 and PTH serum levels compared to WT animals (each $P<0.01$). Both, treatment with R-568 and calcitriol increased serum phosphate levels, whereas no significant changes in serum calcium levels were observed. FGF23 serum levels were significantly increased by calcitriol in a dose dependent fashion, but decreased by R-568 treatment. PTH serum levels were reduced by low dose calcitriol, stimulated by high dose calcitriol, and generally reduced by R-568 treatment. Renal Cyp24 mRNA and protein expression were stimulated by calcitriol, whereas the corresponding mRNA and protein levels of Cyp27b1 were reduced. Renal *Klotho* mRNA expression was reduced in Hyp mice compared to WT animals, and normalized by calcitriol treatment.

Conclusions: Both, treatment with the calcimimetic R-568 and calcitriol increased serum phosphate levels in Hyp mice. Whereas R-568 decreased FGF23 and PTH serum levels, with calcitriol a dose dependent stimulation of FGF23 and PTH levels was observed.

TH-PO611

A New Early Start Cinacalcet Protocol Demonstrates a Sustained Improvement in Bone Mineral Outcomes in Prevalent Dialysis Patients; a 2-Year Retrospective Study
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Background: End stage renal disease patients are characterized by hypocalcemia and hyperphosphatemia with secondary hyperparathyroidism which impact morbidity and mortality when analyzed as time-dependent covariates. The 2013 United States Renal Data System reported that guidelines for mineral bone disorders (MBD) were poorly achieved (> 30% phosphorous (P) > 5.5 mg/dL). Studies indicate the efficacy of cinacalcet in decreasing serum levels of intact parathyroid hormone (iPTH) and controlling serum P and calcium (Ca) levels when compared to more traditional therapies. We propose a new combination therapy protocol of; (1) early cinacalcet administration, (2) low vitamin D analogue (VitD) dose and (3) oral Ergocalciferol; all in accordance with KDOQI guidelines.

Methods: For initiation, levels of serum iPTH, Ca, P and VitD25 levels were assessed. A starting dose of 30 mg cinacalcet was given if iPTH > 300 pg/mL and Ca > 8.4 mg/dL. If iPTH was 300-600 pg/mL, VitD dose was decreased by 50%. Serum Ca and P were monitored fortnightly and iPTH monthly. Cinacalcet dose was then titrated in 30 mg increments if iPTH > 300 pg/mL, Ca > 8.4 mg/dL and P > 3.5 mg/dL. If Ca < 7.5 mg/dL or symptoms of hypercalcemia persist VitD was increased by 25% if P < 5.5 mg/dL but cinacalcet was withheld if VitD could not be increased.

Results: We report that our patients show annual average levels of {compared to regional and national levels respectively}; (1) 15.3% serum P levels of > 5.5 mg/dL {30% and 29.3%}, (2) 0.0% serum Ca level of > 10.2 mg/dL {3.3% and 2.4%} (3) and VitD dose of 2 mcg per administration {4 mcg and 4 mcg}.

Conclusions: This 2-year retrospective study suggests our newly early start cinacalcet protocol demonstrates sustained improvements in MBD outcomes in dialysis patients; potentially attributed to; (1) higher (50-80%) than national (21-25%) average cinacalcet utilization, (2) lower VitD dose and (3) close dose monitoring (with consequent reduction in side effects) enabling the retaining of patient numbers, unveiling the practical nature of this protocol.

TH-PO612

AMG 416, a Peptide Agonist of the Calcium-Sensing Receptor, Preserved Cortical Bone Structure and Bone Strength in 5/6 Nephrectomized Rats with Established Secondary Hyperparathyroidism
Xiaodong Li, Longchuan Yu, Frank Asuncion, Mario Grisanti, Shawn T. Alexander, Kelly Sue Warmington, Kelly M. Hensley, Chun-Ya Han, Qing-Tian Niu, Denise C. Dwyer, Marina Stolina, Charles E. Dean, William G. Richards, Michael S. Ominsky, Hua Zhu "David" Ke, James Tomlinson. *Metabolic Disorders and Pathology, Amgen Inc.*

Background: Continuous elevation of parathyroid hormone (PTH) is catabolic to cortical bone, causing deterioration in cortical bone structure (eg, cortical porosity), and is a major factor for increased fracture risk in chronic kidney disease. AMG 416 reduces PTH levels in patients on hemodialysis with secondary hyperparathyroidism (SHPT). We hypothesized that AMG 416 would prevent the cortical bone deterioration in a rat model of established SHPT.

Methods: Twelve-week-old male 5/6 nephrectomized (5/6 Nx) rats were fed a special diet (0.9% phosphate and 0.6% calcium) to induce SHPT. Sham-operated rats were used as non-SHPT controls. Following 8 weeks on the special diet, rats were chosen with a baseline PTH level > 750 pg/mL (SHPT rats) and were subcutaneously injected daily with vehicle or AMG 416 for 6 weeks.

Results: Prior to treatment, SHPT rats had significant increases in serum creatinine (2-fold), blood urea nitrogen (BUN, 3-fold), PTH (5-fold), FGF-23 (13-fold) and osteocalcin (12-fold, a marker of bone turnover) compared with the non-SHPT controls. Serum creatinine and BUN continued to increase in both vehicle- and AMG 416-treated SHPT rats during the treatment period. PTH, FGF-23 and osteocalcin continued to increase in vehicle-treated SHPT rats, but were significantly reduced in AMG 416-treated SHPT rats. At the end of treatment, vehicle-treated SHPT rats had deteriorated cortical bone structure (increased cortical porosity: 8.3% in SHPT versus 0.07% in non-SHPT) and lower energy to failure (-30%), a bone strength endpoint, compared with non-SHPT controls. In contrast, cortical bone structure was improved (-72% in cortical porosity) and energy to failure was significantly greater (+54%) in the AMG 416-treated SHPT rats than in vehicle-treated SHPT rats.

Conclusions: AMG 416 preserved cortical bone structure and bone strength in a 5/6 Nx rat model of established SHPT.

Funding: Pharmaceutical Company Support - This study was supported by Amgen Inc.

TH-PO613

Effect of RANK Ligand Inhibition with Denosumab on CKD-MBD Parameters in De Novo Renal Transplant Recipients
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Background: De novo renal transplant recipients (RTXR) are at risk to lose bone mineral density (BMD) in the 1st year after transplantation, heightening the risk for fractures. Denosumab targets RANK ligand, inhibits bone resorption and may be preferred over bisphosphonates due to its lack of nephrotoxicity. We studied selected CKD-MBD parameters in RTXR which correlate with denosumab's therapeutic effect.

Methods: RTXR (n=50; age 48±15 years; 64% males; eGFR 55±17 ml/min/1.73 m^2; 33% osteopenic and 12% osteoporotic by DXA [lumbar spine]) were randomized 1:1 to denosumab (60 mg sc at baseline and 6 months) or no treatment (NCT01377467). All patients were prescribed daily vitamin D (800 IE) and calcium (1000 mg), and were followed at defined visits in the 1st year post-transplant.

Results: RTXR improved or normalized their blood levels of calcium (2.30±0.19 to 2.52±0.15 mmol/L), phosphate (0.62±0.35 to 0.86±0.24 mmol/L), PTH (171.5±174.8 to 105±81.0 ng/L), 25 (OH) vitamin D_3 (17.2±9.0 to 25.9±8.0 µg/L) and 1,25 $(OH)_2$ vitamin D_3 (29.9±20.0 to 51.8 ± 22.1 ng/L) between baseline and 12 months after transplantation (all $p<0.001$), without difference if denosumab-treated or not. Compared with controls, patients treated with denosumab had significantly decreased plasma levels of the bone resorption marker β-CTX (0.79±0.51 versus 0.22±0.20 µg/L; $p<0.001$) and the bone formation markers P1NP (150±93 versus 55±69 µg/L; $p<0.001$) and BSAP (20.5±11.5 versus 10.7±8.9 µg/L; $p<0.02$) at 12 months. The suppression of β-CTX did not correlate with changes in BMD at 12 months, whereas the change in P1NP correlated with the change in BMD at the lumbar

spine (p=0.04) but not at the hip. Denosumab treatment was well tolerated, except for a higher percentage of RTXR with urinary tract infections (60% versus 32%, p=0.047).

Conclusions: The significant decrease of β-CTX, P1NP and BSAP upon treatment with denosumab may be used to monitor the effect of denosumab treatment in de novo RTXR. Further analyses are needed to correlate the change of these biomarkers with the effect of denosumab on BMD.

Funding: Government Support - Non-U.S.

TH-PO614

Denosumab Induced Severe Hypocalcemia in Patients with Advanced Chronic Kidney Disease Vatsa Dave,[1] Cherie Y. Chiang,[2] Jane Booth,[3] Peter F. Mount.[1] *[1]Dept of Nephrology, Austin Health, Melbourne, Victoria, Australia; [2]Dept of Endocrinology, Austin Health, Melbourne, Victoria, Australia; [3]Dept of Pharmacy, Austin Health, Melbourne, Victoria, Australia.*

Background: Patients with severe CKD frequently have low bone mineral density and are at high risk of fracture but treatment options are limited. The RANK-ligand inhibitor denosumab is a potential treatment option for osteoporosis in patients who cannot have bisphosphonates due to severe CKD (eGFR <30 ml/min). Whilst denosumab is not renally cleared, little is known about its effects and safety in patients with severe CKD.

Methods: We retrospectively extracted all patients with CKD stage IV or V who received denosumab since 1/1/2010 at Austin Health. Patients were identified by cross-referencing pharmacy administration records with patient's renal function prior to drug administration. Chart reviews were conducted for clinical parameters, including calcium levels prior to and following administration of denosumab.

Results: 8 patients with stage V and 5 patients with stage IV CKD were identified (all female). Baseline data showed mean(SD) values for calcium of 2.40(0.13) mmol/L, PTH 19.8(15.2) pmol/L and 25-OH vitamin D 71.3(31.7) nmolL. Only one patient had a baseline 25-OH vitamin D level below 50 nmol/L. After denosumab 6 of 8 patients with CKD V, and 2 of 5 patients with CKD IV developed significant hypocalcaemia, (corrected calcium <2.0 mmol/L), with the lowest corrected calcium being 1.18 mmol/L. Of these 8 patients, 3 patients had significant life-threatening complications requiring intensive monitoring. For patients who developed hypocalcaemia, the median time to serum calcium nadir was 26 days and the median time to achieve correction of hypocalcaemia was 71 days. Treatment of hypocalcaemia required large doses of calcium and vitamin D as well as increases to dialysate calcium, consistent with a pattern of hungry bone syndrome.

Conclusions: A high rate of severe hypocalemia was observed in patients with advanced CKD who were given denosumab, despite adequate levels of 25-OH vitamin D. Denosumab is best avoided in patients with advanced CKD but if used very close monitoring is recommended.

TH-PO615

Abstract Withdrawn

TH-PO616

Calciuria May Not Fully Explain the Thiazide-Induced Serum Calcium Elevation on Chronic Kidney Disease Patients Raquel F.V. Vasco, Joice Manes, Gisele G. Maciel, Rosa M.A. Moyses, Rosilene M. Elias. *Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil.*

Background: Thiazide therapy is not only useful for blood pressure control but also has an effect on CKD-MBD as can raise serum calcium (Ca) and downregulate PTH. Calciuria is the main mechanism proposed to explain the decrease in Ca. However, other authors described an increase in bone resorption on hemodialysis patients mediated by thiazide. Therefore, we challenged the concept that calciuria is the main accountable factor to induce the increase in Ca and consequent decrease in PTH among CKD patients.

Methods: Electronic charts of all nephrology outpatients clinic, who were given hydrochlothiazide (Hydro) or furosemide (Furo) were included. Estimated glomerular filtration rate (eGFR) based on CKD-EPI equation, biochemical parameters and VitD and calcium supplementation were assessed.

Results: Out of 542, 357 (66%) were taking Furo and 184 (34%) Hydro. Patients on Hydro group presented lower age, alkaline phosphatase, parathyroid hormone (PTH), uric acid, and phosphate. Also, they presented higher eGFR, Ca, bicarbonate, hemoglobin and serum albumin (all p values <0.005). Hypocalcaemia was observed in 50 patients (9%) and hypercalcaemia in 44 patients (8%), which was more frequent in Furo and Hydro groups, respectively (p<0.0001). Dietary VitD and calcium supplementation were prescribed to 37% and 7% of patients, respectively. The median calciuria was 83 (36,161) mg/24 hours. There was a weak correlation between Ca and calciuria (r=0.129, p=0.003). Logistic regression shows that age (RR 1.02), hydrochlorothiazide (RR 1.69) and dietary VitD supplementation (RR 1.62) were independent associated to Ca in a model adjusted for eGFR, calcium supplementation, calcitriol use, VitD levels, and calciuria. Secondary hyperparathyroidism was dependent on age (RR 1.02), Furo (RR 1.77) and Ca (RR 0.75), in a fully adjusted model. Neither Ca nor hyperparathyroidism were dependent on calciuria.

Conclusions: Thiazide effect on calciuria did not fully explain serum Ca levels and PTH among CKD patients. Therefore, the mechanisms by which thiazide may raise Ca and decrease PTH on CKD patients are complex, and warrant further investigation.

TH-PO617

Bone Erythropoietin Receptor Is Depressed in Anemia of Chronic Kidney Disease Daniel Landau,[1,3] Lital London,[2] Yael Segev.[2] *[1]Pediatrics, Ben Gurion Univ, Beer Sheva, Israel; [2]Microbiology and Immunology, Ben Gurion Univ, Beer Sheva, Israel; [3]Pediatrics, Soroka Univ Medical Center, Beer Sheva, Israel.*

Background: Anemia contributes to growth retardation in children with CKD and is due to impaired renal erythropoietin (EPO) synthesis or EPO receptor (EPOR) signaling. We have previously shown reduced epiphyseal growth plate (EGP) GH receptor (GHR) signaling as well as vascularization in growth impaired CKD rats. Both GHR and EPOR signals are transduced through the JAK2-STAT5 pathway. In this study we characterized the expression and signaling pathway of EPO and EPOR in kidney, EGP and bone marrow in juvenile CKD rats.

Methods: We divided young Sprague-Dawley rats (20 days old) into two groups: C versus CKD. CKD was induced by 5/6 nephrectomy while C group was sham operated. Somatic growth was followed throughout the experiment. Rats were sacrificed after 4 weeks. Blood, kidney, bone marrow and proximal tibia EGP tissue were isolated for protein and mRNA analysis and immunohistochemistry.

Results: Hemoglobin levels were significantly reduced after 4 weeks of CKD (14.3 ± 0.2 versus 11.7 ± 0.4 g/dL, p<0.0001). Serum iron and transferrin levels were unchanged. There was a significant decrease in weight gain and longitudinal growth in CKD rats compared to C. Remnant kidney EPO and EPOR protein levels were unchanged. Bone marrow aspirate EPO was also unchanged but EPOR was decreased in CKD. EGP EPO protein levels were unchanged while EPOR levels were significantly reduced in CKD. Immunohistochemicalstaining of the EGP showed decreased EPOR levels in CKD rats in the EGP chondrocytes. IV injection of erythropoietin-alpha (25 U/kg) 5 min prior to sacrifice induced a significant increase in EGP phsopho-STAT5 levels in both C and CKD.

Conclusions: Anemia in rats with early CKD is associated with normal EPO levels in the remnant kidney. However given the loss of kidney tissue in this model, it is likely that total renal EPO production is reduced, thus contributing to the anemia of CKD. Of note bone marrow and EGP EPOR levels were significantly reduced in CKD and this may well be a cause of uremic EPO resistance. Whether downstream defects in EPO signal transduction also exist requires further investigation.

TH-PO618

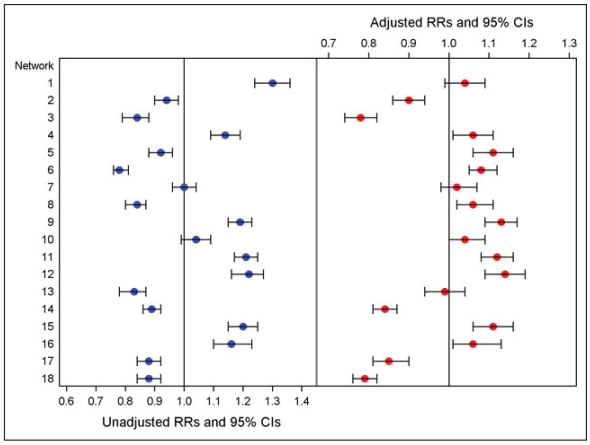
Geographic Variation in Fracture Incidence in U.S. Patients Receiving Hemodialysis James B. Wetmore,[1] Jiannong Liu,[1] Heidi S. Wirtz,[2] Thy P. Do,[2] David T. Gilbertson,[1] Brian D. Bradbury,[2] Allan J. Collins.[1] *[1]Chronic Disease Research Group, Minneapolis, MN; [2]Center for Observational Research, Amgen, Inc., Thousand Oaks, CA.*

Background: Fracture burden is high in patients on hemodialysis, but whether fracture incidence varies geographically is unknown.

Methods: The United States Renal Data System ESRD database was used to study all U.S. prevalent adult patients receiving hemodialysis from 2007-10, with Medicare as primary payer for ≥ 1 yr. Patients were followed from January 1, 2007, or January 1 of the first calendar year to the earliest date of death, transplant, modality switch, or loss of Medicare coverage. Fractures were identified using ICD-9-CM diagnosis codes and HCPCS procedure codes from inpatient and physician claims. Fractures of the pelvis/hip, femur, lower leg, rib/sternum, shoulder/upper arm, and forearm/wrist were studied. Geographic region was defined by ESRD network. Poisson models were used to calculate rate ratios (RRs), unadjusted and adjusted between each network and the national rate. Adjustment factors were age, race, sex, primary ESRD cause, body mass index, dialysis vintage, and numerous comorbid conditions. Variation of RRs was used to illustrate the disparity in fracture rates among networks.

Results: The national fracture rate was 6.11 per 100 patient-years. Unadjusted RRs (Figure 1, left panel) varied from a low of 0.78 (network 6) to a high of 1.30 (network 1). The variation in RRs across networks did not change materially after adjustment for patient characteristics (Figure 1, right panel), varying roughly 1.5-fold across regions.

Conclusions: Fracture rates among patients on hemodialysis vary substantially by geographic region and are not explained by case mix, suggesting a potential etiologic role for regional differences in the management of chronic kidney disease-mineral and bone disorder.



Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO619

Predictors of Hip Fracture in Incident Hemodialysis Patients: Results from the MONDO Consortium Fellype C. Barreto,[1] Viviane Calice-Silva,[1,2] Aileen Grassmann,[3] Bernard J. Canaud,[3] Daniele Marcelli,[3] Peter Kotanko,[2] Len A. Usvyat,[2,3] Roberto Pecoits-Filho.[1] *[1]Pontificia Univ Catolica do Parana, Curitiba, Brazil; [2]Renal Research Inst, New York; [3]Fresenius Medical Care North America.*

Background: Hip fracture (HipF) is a hazardous condition associated with reduced survival in chronic kidney disease patients. HipF predictors are not well established in this population. Our aim was to evaluate the predictors of HipF in incident hemodialysis (HD) patients in MONDO database.

Methods: This analysis included incident HD patients from the MONDO database with in-center treatments between 1/2006 and 12/2012 who survived at least six months. Clinical and laboratory parameters were ascertained for the first 6 months (baseline). HipF were recorded in months 7 to 18 (follow-up). HipF were ascertained by ICD-9, indicating cervical, intertrochantecric, or subtrocanchanteric fractures. Poisson regression models were constructed to explore associations between baseline parameters and the number of HipF during the follow-up.

Results: We studied 18,955 patients (59% male, age 64±15 yrs, diabetics 37%). Baseline levels of calcium (Ca), phosphate (P) and iPTH were 8.8±0.6 mg/dl, 4.8±1.1 md/dl, 334±286 pg/ml, respectively.47 HipF were registered during follow-up. Results of Poisson analysis are shown in table 1. Table 1: Predictors of hip fractures in incident HD patients (significant in bold).

	Estimate	Wald 95% CI	P
Age	**0.067**	**0.027 – 0.106**	**0.001**
Gender (Male)	-0.250	-1.130 – 0.629	0.57
BMI	-0.051	- 0.146 – 0.042	0.28
Catheter	**0.996**	**0.166 – 1.826**	**0.02**
Daibetes	0.550	-0.315 - 1.415	0.21
PreSBP > 140 mmHg	**-1.059**	**-1.969 - -0.149**	**0.02**
IDWG	**0.611**	**0.223 - 0.998**	**0.002**
Ca - KDOQI target	0.655	-0.350 - 1.661	0.20
P - KDOQI target	0.290	-0.638 - 1.219	0.54
iPTH - KDOQ target	-0.300	-1.159 - 0.559	0.49
iPTH standard deviation	**-0.008**	**-0.014 - -0.001**	**0.01**

Conclusions: Since HipF are in most cases the consequence of falls, it is conceivable that higher blood pressure (SBP > 140 mmHg), lower IDWG and younger age are associated with a lower risk. The associations with IDWG and iPTH variability (expressed as SD), but not Ca, P and PTH within target, are novel and call for further studies.

TH-PO620

Prognostic Significance of the Presence of Vertebral Fractures in the Survival of Chronic Kidney Disease Patients Stages 3-5 Not on Dialysis Jose L. Gorriz,[1] Jaume Pomes-Tallo,[2] Cristina Castro,[1] Montserrat del Amo,[2] Ana Isabel Garcia,[2] Pablo Molina,[1] Belen Vizcaino,[1] Marco Montomoli,[1] Daniel A. Molina,[1] Secundino Cigarran,[1] Javier Nieto,[1] Sophie Jamal,[3] Luis M. Pallardo.[1] *[1]On Behalf of the Investigators of OSERCE II Study, Spain; [2]Musculoskeletal Radiology Dep. Hospital Clinic Barcelona; [3]Univ of Toronto, Women's College Hospital.*

Background: There are little data concerning the prevalence of vertebral fractures (VF), risk factors for fracture and relationship between fractures and mortality in chronic kidney disease (CKD) patients. To address this knowledge gap we conducted a "post hoc" analysis using data from the OSERCE II study (a 3-year follow-up prospective multicenter study which included 742 CKD patients stages 3-5 not on dialysis from 39 centres in Spain).

Methods: The OSERCE II study enrolled 742 subjects. Of these 612 had thoracolumbar xrays available for review. Xrays were reviewed by 3 musculoskeletal radiologists and VF identified using Genant semi-quantitavie morphometry. Mean age: 66±12 years, about 1/3 were female, and 1/3 had diabetes. The mean eGFR by MDRD was 27±12 ml/min/1,73m^2).

Results: VF were identified in 110 (18%) subjects. Factors associated with VF were phosphoremia (OR 0.719, P = 0.032, Ankle Brachial Index<0.9 (OR 1.694,P = 0.029) and treatment with biphosphonates (OR 5.636, p = 0.002). Over the 3-year follow-up, 62 subjects(10%) died. The most common causes of mortality were cardiovascular (n = 21, 34%). In the crude analysis, the group of VF patients had poorer survival (p=0.02). Multivariate analysis, (adjusting for age, MDRD, albumin, DM, comorbidity, Adragao Score> 3 and phosphorus), showed age (HR 1.074, 95%, p <0.001), phosphoremia (HR 1.699, 95%, p = 0.005), presence of VF (HR 1.983, P = 0.047) and vascular calcification by Adragao Score> 3 (HR 2.487, P = 0.004) as independent predictors of mortality.

Conclusions: About 20% of subjects in our study had VF. Factors associated with VF were age, low levels of phosphorus and peripheral vascular disease. The presence of VF is an independent risk factor for mortality in patients with CKD stages 3-5 not on dialysis. Clinical trials are needed to confirm whether this relationship is causal and reversible.

TH-PO621

Post-Transplant Bone Disease: A Prospective Bone Biopsy Study Catarina Carvalho, Juliana Magalhães, Luciano Pereira, Liliana Simoes-Silva, Inês Castro Ferreira, Joao M. Frazao. *Nephrology and Infectiology Research and Development Group, INEB, School of Medicine, Porto Univ, Porto, Portugal.*

Background: Post-transplant bone disease results from a combination of factors, which include previous bone and mineral metabolism disturbances, and effects from transplant-related medications. Bone biopsy (BB) remains the gold-standard diagnostic tool.

Methods: 6 patients (5M/1F, age 52.5±9.7, on RRT for 56.5±10.2 months) were prospectively studied after renal transplantation (RT). Dual-X-ray absorptiometry (DXA) and trans-iliac BB after double tetracycline labeling were performed at baseline (BL) in the first 2 months after RT and after 2 yrs of follow-up (1 pt after 5 yrs). We used Paired t-tests and Pearson correlation in statistical analysis.

Results: Comparing to BL biopsy, 2nd biopsy revealed a significant decrease in Ob.S/BS (0.65±0.47 to 0.06±0.13%, p=0.035), ES/BS (3.72±2.21 to 1.87±1.10%, p=0.025), Oc.S/BS (0.63±0.34 to 0.40±0.62%, p=0.035), N.Ob/BS (0.46±0.29 to 0.07±0.18/mm^2, p=0.043). Although a decrease in BV/TV did not reach significance, a significant decrease in TbN (2.29±0.42 to 1.68±0.46/mm, p=0.008) and increase in TbSp (390.53±95.84 to 530.87±163.37µm, p=0.029) in the 2nd biopsy suggest loss in bone quantity. We also did not find significant differences between OV/BV, OS/BS and Oth. All BB demonstrated a marked reduction in BFR, and so no difference was seen between the 1st and 2nd. At T0 3/6 patients had PTHi>300pg. The scarce tetra labeling in the 1st BB was probably a result of high dose corticosteroids in the first weeks after RT, which can lead to miss-interpreted results. Mlt was 4 times higher in the 2nd BB, although not significant, possibly due to small number of patients. At BL, DXA lombar parameters strongly correlated with OV/BV (DMO r=0.822, p<0.05; T score r=0.853, p<0.05; Z score r=0.942, p<0.01). At follow-up BB, this correlation is lost, possibly due to effects of transplant medications on bone density. We found no correlation with femoral site.

Conclusions: The results observed in this population show a reduction in bone activity, namely at cellular level, suggesting increased risk of adynamic bone and loss of bone volume.

TH-PO622

Bisphosphonates and Risk of Fractures after Renal Transplantation: A Unicenter Retrospective Study Ioan-Andrei Iliuta, Sacha A. De Serres, Isabelle Houde, Mohsen Agharazii, Fabrice Mac-Way. *Nephrology, L'Hôtel-Dieu de Québec Hospital, Quebec, QC, Canada.*

Background: Renal transplanted patients have higher risk of fractures, mainly related to the rapid loss of bone mass after transplantation. Bisphosphonates (Bps) have been shown to reduce the bone mass loss and are commonly used to prevent bone fractures. However, it is still unknown whether the use of Bps is associated with reduced fracture risk or not in this population. In this context, the objective of this study was to evaluate the effect of oral Bps on fractures in a population of renal graft recipients.

Methods: A retrospective and comparative study between renal transplant recipients treated with Bps (2001 to 2013) versus no Bps (1987 to 1999) was conducted on patients transplanted at l'Hôtel-Dieu de Québec hospital (Quebec, Canada). Since 2001, all patients in our center are put on Bps at the time of transplantation. The study length time was restricted to 12 years to have comparable exposition time from transplantation between groups. The patient records were reviewed for biochemical, demographic data and comorbidities and the occurrence of fractures was analyzed from time of transplantation to first fracture. Adjusted multivariate regression analysis for predetermined covariables and a propensity score analysis were performed.

Results: 472 patients with mean age of 47±14 (Bps) and 40±12 (no Bps) were included. There were 57 fractures in the Bps group (36.3 %) versus 157 fractures (49.8%) in the no Bps group (p=0.005). When adjusted for age, sex, weight, cause of renal failure, dialysis vintage, presence of diabetes, donor characteristics and previous dialysis modality, the use of Bps was not associated with a reduced risk of fractures. Only female gender and a marginal donor were positively associated with fractures after adjusted multivariate analysis. Propensity score analysis by matching, stratification by quintiles and logistic regression confirmed that Bps use was not associated with reduced fracture risk.

Conclusions: The use of Bps seems not to be associated with reduced risk of first fractures in renal transplanted patients. This is however a retrospective study and does not preclude the need to perform randomized controlled trials.

TH-PO623

Pregnancy Outcomes in Women with Primary Glomerular-Based Disease: A Systematic Review Ayodele Odutayo, Arti Bhasin, Michelle A. Hladunewich. *Nephrology, Sunnybrook Health Sciences Centre, Univ of Toronto, Toronto, ON, Canada.*

Background: Patients with glomerular-based kidney disease are at increased maternal and fetal risk during pregnancy. Divergent opinions exist regarding prognostication of pregnancy risk and individual studies are often small and incompletely reported. We conducted a systematic review to evaluate the quality of reporting of baseline characteristics and to assess disease-specific variations in pregnancy outcomes.

Methods: We searched MEDLINE and EMBASE with no limits on the search strategy. We included studies with at least 5 patients that provided a biopsy-based diagnosis of any of four disease – IgA Nephropathy (IgA), Focal Segmental Glomerulosclerosis (FSGS), Minimal Change Disease (MCD) and Membranoproliferative Glomerulonephritis (MPGN) – and reported on the number of live births in the given disease category. Review of abstracts,

full text articles and extraction of data were performed in single (AO), with a review of 25% of full text articles by a second reviewer (MH). Four baseline characteristics were assessed for frequency of reporting: age, blood pressure, creatinine and proteinuria. The frequency of live births in disease categories was compared with a chi-squared test.

Results: 1272 abstracts were reviewed, 133 full texts were identified and 15 primary reports met the inclusion criteria. The median year in which articles were published was 1989 (inter-quartile range: 1986-1996). Thirteen articles reported on IgA (672 women) whereas only 4 (55), 3 (29) and 5 (170) articles reported on FSGS, MCD and MPGN respectively. For all baseline characteristics, frequency of reporting was poor across disease categories: average age (0%-31% of articles in a disease category), blood pressure (0-39%), creatinine (0-46%) and proteinuria (0-31%). The proportion of live births varied by disease category ($p<0.001$): IgA (99%), FSGS (71%), MCD (74%) and MPGN (84%).

Conclusions: Pregnancy outcomes vary significantly by the type of glomerular-based disease. However, the current literature if out of date and reporting of baseline characteristics is poor. More contemporary and completely reported studies are required to facilitate prognostication of pregnancy-associated risk.

TH-PO624

Pediatric Chronic Kidney Disease (CKD) Treatment Burden and Quality of Life in the CKiD Cohort Maria E. Ferris,[1] Susan L. Furth,[2] Bradley A. Warady,[3] Kelly C. McDermott,[4] Stephen R. Hooper.[1] *[1]Nephrology, Univ of North Carolina, Chapel Hill, NC; [2]Pediatric Nephrology, Childrens Hospital of Philadelphia, Philadelphia, PA; [3]Pediatric Nephrology, Univ of Kansas, Kansas City, MO; [4]Epidemiology, Johns Hopkins, Baltimore, MD.*

Background: The relationship of CKD treatment burden and quality of life has not been characterized.

Methods: In a cross-sectional study, we determined treatment burden and its relationship to health-related quality of life in the CKiD cohort at study initiation. Frequency and administration route of CKD- and non-CKD-related medications were correlated with disease severity based on glomerular filtration rate. Linear regression was used to determine the relationship between treatment burden, adjusted for other covariates, and overall health-related quality of life (PedsQL™) scores, as reported by participants and their parents.

Results: In 699 patients ages 8-19 years, 504 (72%) had child and parent proxy PedsQL™ scores. The sex distribution was similar in all CKD stage with ≥ 56% males. In the CKD 4 group there was significantly more short stature and anemia. The number of CKD related medications increased as GFR decreased ($p< 0.001$). Likewise, the proportions of children on at least one CKD related oral ($p= 0.003$) and at least one CKD related injectable ($p< 0.001$) increased as GFR decreased. Tube feeding was more common among children with lower GFR ($p= 0.027$), and total weekly instances of medication use increased as GFR decreased ($p< 0.001$). As the number of CKD related medications increased, median overall parent proxy QOL scores decreased from 83 (interquartile range (IQR): 70 to 89) for children on zero CKD related medications to 75 (IQR: 59 to 88) for children on ≥ 5 CKD related medications. Overall child QOL scores did not differ by CKD related oral medication use. We observed no differences in parent proxy or child overall QOL scores by CKD related injectables. Comorbidities such as depression, hypertension, ADHD and/or learning disabilities were similar across all CKD Stages.

Conclusions: The number of CKD-related oral/injectable medications, feeding tube nutrition and treatments were directly associated with disease stage.

Funding: NIDDK Support

TH-PO625

The Association of Kidney and Brain Disease in the Elderly Katherine H. Michener,[1] Lenore J. Launer,[2] Farzad Noubary,[1] Gary F. Mitchell,[3] Hrefna Gudmundsdottir,[4] Olafur S. Indridason,[4] Vilmundur Gudnason,[5,6] Mark J. Sarnak,[1] Lesley Inker.[1] *[1]Tufts Medical Center, Boston, MA; [2]The National Inst on Aging, Bethesda, MD; [3]Cardiovascular Engineering, Inc., Norwood, MA; [4]Landspitali- The National Univ Hospital of Iceland, Reykjavik; [5]Univ of Iceland, Reykjavik; [6]Icelandic Heart Association, Kopavogur.*

Background: Due to similarities in the high flow nature of the vasculature of the kidney and brain, we hypothesized that lower kidney function may be associated with brain pathology by MRI and cognitive impairment.

Methods: We utilized data from 804 participants from the Age, Gene/Environment, Susceptibility-Reykjavik Study, a community-based prospective cohort study of cardiovascular disease (CVD) in Iceland. MRI was performed to measure segmental brain matter and white matter hyperintensity (WMH) volumes as well as presence of subcortical, cortical and cerebellar infarcts. Associations of estimated glomerular filtration rate based on creatinine and cystatin C (eGFR) and urine albumin-creatinine ratio (ACR) with these MRI findings as well as cognitive scores on executive function, memory and speed were assessed using linear or logistic regression, adjusting for demographics and standard CVD risk factors.

Results: Mean age was 76 years, mean (SD) eGFR was 69 (15) ml/min/1.73m^2, and median (IQR) ACR was 3 (2-6) mg/g. In adjusted analyses, higher ACR was significantly associated with lower volumes of total brain parenchyma, white and gray matter, and increased WMH. Lower eGFR was only significantly associated with lower white matter and total brain parenchyma volume. ACR and eGFR were not significantly associated with infarcts or cognitive scores.

Relationship of kidney measures and brain structure

	eGFR	ACR
Total brain parenchyma	**0.34 (0.10, 0.59)**	**-0.36 (-0.60, -0.12)**
Gray matter	0.18 (-0.03, 0.38)	**-0.34 (-0.54, -0.14)**
White matter	**0.17 (0.04, 0.30)**	**-0.18 (-0.31, -0.06)**
WMH	0.02 (-0.04, 0.09)	**0.10 (0.04, 0.16)**

Results are % intracranial volume (95% CI) per SD higher eGFR or ln ACR, adjusted for sex, age, MAP, HDL, HbA1c and CRP.
Bold results are statistically significant.

Conclusions: Higher ACR and lower eGFR were associated with smaller brain parenchymal volume but not infarcts or cognition. Higher ACR was associated with greater WMH volume. Further studies are needed to explore disease mechanisms.

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TH-PO626

Depression in Late Stage Kidney Disease Rimda Wanchoo, Azzour Hazzan, Vipulbhai Sakhiya, Candice Halinski, Anna Mathew, Steven Fishbane. *Nephrology, Hoffstra NSLIJ School of Medicine, Great Neck, NY.*

Background: Patients with CKD experience a high symptom burden with a sub optimal quality of life. The overall prevalence of depression in CKD is varied depending on the stage of kidney disease as well as the diagnostic tool used for identifying it. Prevalence of depression based on patient or clinician administered questionnaire is suggested to be approximately 26 % and is associated with increased hospitalization and mortality. Because psychosocial stressors are likely to be increased in late stage CKD, we studied depression in these patients.

Methods: A cohort of 203 patients with stage 4 or 5 CKD were evaluated for depression using the PHQ-9 (patient health questionnaire) at their first clinic visit. The PHQ-9 is a validated questionnaire that is interpreted based on overall score. Minimal, mild, moderate and severe were defined by total scores of (1-4), (5-9), (10-19) and (20-27) respectively. Patients were divided into two groups based on PHQ-9 score. One group comprised of patients that had none or minimal depression (score 0-4) while the second group comprised of the mild, moderate or severe depression (score >5). Mean age and eGFR were compared between the two groups using a Student's t-test. Proportion of diabetes and congestive heart failure was compared between the two groups using Fishers Exact test.

Results: There were a total of 203 patients, 53 (26%) of which had mild or moderate to severe depression. There was no significant difference among the two groups in the mean age and the presence of diabetes. The mean estimated GFR was 22 ml/min in the group with none or minimal depression and was 16.8 ml/min in the mild/moderate/severe group ($p=0.0001$). Patients with mild, moderate or severe depression were more likely to have congestive heart failure (26% versus 15%) as compared to the group with none or minimal depression; ($p=0.05$).

Conclusions: Our study demonstrates that depression is common in late stage CKD. Lower eGFR and congestive heart failure are correlated with a higher prevalence of depression. Since depression is associated with increased mortality and hospitalization, clinicians should be vigilant in screening for symptoms in this patient population to initiate treatment.

TH-PO627

Patient-Reported Symptoms in Chronic Kidney Disease Stephanie Amy Brown,[1] Amy L. Clarke,[1] Balraj Singh Jagdev,[2] Laetitia H. Lloyd-Davies,[1] Katherine Leigh Hull,[1] Andrew G. Stein,[2] James O. Burton,[1] Carolyn Tarrant,[3] Alice C. Smith.[1] *[1]Leicester Kidney Exercise Team, Univ of Leicester, United Kingdom; [2]Univ Hospitals of Coventry and Warwickshire, United Kingdom; [3]Dept of Health Sciences, Univ of Leicester, United Kingdom.*

Background: Chronic Kidney Disease (CKD) is associated with a range of biochemical abnormalities, precipitates a pro-inflammatory state, and patients experience debilitating symptomatology and reduced quality of life. HMG-CoA reductase inhibitors (statins) are increasingly used for lipid management in these patients. This study aimed to explore the patient-reported symptom experiences in CKD patients not on dialysis, and relationships with biochemical parameters including inflammation, and statin treatment.

Methods: 179 patients completed a symptom questionnaire in which 11 common renal symptoms are rated for frequency and intrusiveness on a scale of 0-8. Additional information was extracted from clinical records (102 male; median age 61 [range 21-93 years]; median eGFR 27ml/min/1.73m^2 [range 5-90]). 92 patients (51%) were receiving statins.

Results: Patients reported a median of 6 symptoms. The most common symptoms were "excessive tiredness" (76.5%), "sleep disturbance" (65.9%) and "pain in bones/joints" (62.6%). The number of symptoms reported correlated with C-Reactive Protein (CRP) levels (rho=.255, p=.029), and patient-rated symptom frequency and intrusiveness both also positively correlated with CRP (rho=.324 and .322, respectively with p=.005). No other biochemical marker was found to relate to the frequency and intrusiveness of the reported symptoms. Statin use was associated with significantly higher muscle symptom scores (median symptom score 3 (IQR 0-6) versus 1 (IQR 0-3.5), $p<0.05$), even after correction for age and eGFR.

Conclusions: Although no direct relationship was found between declining renal function and symptom burden, inflammation in CKD patients appears related to patient symptom experience. Statin use may contribute to muscular symptoms and may require greater consideration when prescribing to minimise patient symptom burden.

Funding: Private Foundation Support

TH-PO628

Association of Fronto-Temporal Gray Matter Volume with Executive Function in Patients with Non-Dialysis Dependent Chronic Kidney Disease
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Background: It is well known that cognitive impairment (CI) in patients with chronic kidney disease (CKD) is characterized by executive dysfunction rather than memory dysfunction, although the precise mechanism remains to be elucidated. The purpose of the present study is to examine the association of gray matter volume (GMV) with executive function in CKD patients.

Methods: Ninety-five patients with non-dialysis dependent CKD (NDD-CKD) without overt CI and history of cerebrovascular disease were recruited. The subjects underwent brain MRI and cognitive test of mini mental state examination (MMSE), trail making test part A (TMT-A) and part B (TMT-B). The segmentation algorithm from Statistical Parametric Mapping 8 software was applied to every T1-weighted MRI scan to extract tissue maps corresponding to gray matter, white matter, and cerebrospinal fluid. To normalize for head size variability, GMV ratio (GMR) was calculated as a ratio of GMV to the total intracranial volume, calculated by adding GMV, WMV, and CSFV. Then, GMR was divided into four categories of the brain lobes; frontal lobe (Fr-GMR), parietal lobe (Par-GMR), temporal lobe (Tem-GMR), and occipital lobe (Occ-GMR). We assessed the association of GMR with the scores of these cognitive tests using multivariable regression analysis.

Results: GMR significantly positively associated with the score of MMSE and significantly negatively associated with the scores of TMT-A, TMT-B, and ΔTMT (TMT-B minus TMT-A). These associations were remained significant even after adjustment for the relevant confounding factors. Fr- and Tem-GMR, but not Par- and Occ-GMR, were significantly negatively associated with the scores of TMT-A, TMT-B, and ΔTMT in the multivariable regression analysis.

Conclusions: The present study demonstrates the association of GMR, especially frontal and temporal gray matter, with executive function, suggesting that fronto-temporal brain atrophy might contribute to executive dysfunction in NDD-CKD.

TH-PO629

The Link Between eGFR and Neuropsychological Functioning Among Mexican-Americans
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Background: Although CKD has been linked to cognitive dysfunction, little research has examined the CKD-cognition link among Mexican-Americans. Because prior studies suggest that "established" risk factors for cognitive dysfunction do not apply to Mexican-Americans (hypertension, dyslipidemia, APOE4 genotype), there is a need to understand factors related to cognitive decline among this underserved group. The current study examines the link between eGFR and risk of mild cognitive impairment among Mexican-Americans.

Methods: Data were examined from the Health and Aging Brain among Latino Elders study. The cohort consists of over 500 Mexican-Americans, 377 (292 women, 85 men) whom had calculated eGFR. Each participant underwent an interview, physical, neuropsychological testing and blood draw. The link between eGFR and neuropsychological outcomes was assessed. eGFR categories were broken down as follows: <45 (n=11), 45-59 (n=19) and >=60 (n=347).

Results: In the unadjusted models, lower eGFR was associated with significantly poorer performance in the domains of global cognition, memory, executive functioning, processing speed, visuospatial skills, and language. In the adjusted models, the <45 group performed significantly worse than the 45-59 and >=60 groups in the following domains: memory (WMS-III Logical Memory 1, F=3.4, p=0.03), processing speed (Trail Making Test part A, F=13.5, p<0.001), executive functioning (CLOX1, F=5.8, p=0.003), visuospatial skills (CLOX2, F=16.6, p<0.001) and global cognitive functioning (MMSE, F=7.0, p=0.001). In logistic regression model, eGFR<60 was associated with significant increased risk for MCI among Mexican Americans (OR=2.9, 95% CI= 1.003-8.7, p=0.04).

Conclusions: In Mexican-American adults, eGFR < 60 was associated with significantly poorer neuropsychological functioning. Those with eGFR <45 performed more poorly than all others on cognitive tests of memory, executive functioning, processing speed, visuospatial skills and global cognition. eGFR <60 was also associated with increased risk for MCI diagnosis. Early detection of CKD is important in order to prevent MCI risk. Further research is needed to evaluate other biomarkers to delay cognitive decline.

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TH-PO630

A Computerized Neurocognitive Battery Reveals Deficits in Executive Control and Complex Cognition in Children and Young Adults with Chronic Kidney Disease
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Background: Neurocognitive (NC) deficits in chronic kidney disease (CKD) patients are well documented, but traditional tests are time-consuming. The 1-hour Penn Computerized NC Battery (CNB) offers a more efficient assessment method; this is the first systematic study of the CNB in youth with CKD.

Methods: The CNB was administered to subjects aged 8-25 yrs with Stage 2-5 CKD, and age-matched healthy controls. We analyzed CNB results from 12 tests in 4 domains: executive control, episodic memory, complex cognition, and social cognition. All tests measure correct responses (CR) and response time (RT). Raw CR and RT results were converted to age-specific z-scores based on norms from the Philadelphia Neurodevelopmental Cohort (n=1790). Each test was analyzed in a linear regression with CR z-score as the dependent variable and CKD versus control as the explanatory variable, adjusted for RT ($z \geq 0$, $z<0$), race, sex, and maternal education.

Results: 74 CKD subjects [mean(SD) eGFR 49(24) mL/min/1.73m^2, age 16.0(4.0) yrs] and 48 controls [mean(SD) eGFR 100(20) mL/min/1.73m^2, age 15.5(4.1) yrs] were studied. CKD subjects had significant deficits versus controls in executive control (attention) and complex cognition (verbal and non-verbal reasoning, and spatial processing). CKD versus control was not associated with performance differences in episodic memory or social cognition.

Domain	Test	Control vs. CKD diff. in CR z-score*	p
Executive Control	Attention	0.43	0.03
Complex Cognition	Verbal reasoning	0.69	<0.001
	Non-verbal reasoning	0.39	<0.01
	Spatial processing	0.66	<0.01

*adj. for RT ($z \geq 0$, $z<0$), race, sex, & maternal education

Conclusions: In youth with CKD, the Penn CNB shows deficits in executive control and complex cognition, with moderate effect sizes. These results are consistent with traditional NC testing in previous studies and in this cohort. The CNB's 1-hour administration time may facilitate NC assessment of CKD patients. Future analyses will include correlating CNB results with structural/functional brain imaging.

Funding: Other U.S. Government Support

TH-PO631

Prevalence and Severity of Sleep Apnea in Different Stages of Chronic Kidney Disease
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Background: The prevalence and severity of sleep apnea in the non-dialysis chronic kidney disease (CKD) population have not been well characterized. A handful of studies performed to date have yielded highly variable prevalence rates due to cohort heterogeneity and inter-study inconsistencies in sleep apnea definition. This study sought to determine the association of sleep apnea with non-dialysis CKD by recruiting a uniform cohort to undertake overnight polysomnography (PSG).

Methods: 141 male Chinese CKD patients, aged 40 to 60 years old, were recruited to undergo overnight PSG. Height, weight, neck girth, estimated glomerular filtration rate, spot urinary protein excretion and Epworth sleepiness scale (ESS) score were collected at baseline. The prevalence and severity of both sleep apnea and associated nocturnal hypoxemia (NH) were determined across the full spectrum of non-dialysis CKD.

Results: The prevalence of sleep apnea (apnea-hypopnea index [AHI] ≥ 15) and NH (Sleep Heart Health Study arbitrary definition) was 35.5% and 10.6% respectively in this study population, which had a mean (± SD) age and BMI of 51.44 ± 6.05 y and 26.05 ± 4.22 kg/m^2. The adjusted odds ratio (OR) for sleep apnea by body mass index (BMI) and proteinuria were 1.18 (95% confidence interval [CI] 1.02 - 1.37; P ≤ 0.05) and 2.60 (95% CI 2.56 - 2.61; P ≤ 0.05) respectively. The adjusted OR for median cohort oxygen desaturation index (ODI) by BMI and proteinuria were 1.23 (95% CI 1.05 - 1.45; P ≤ 0.05) and 2.60 (95% 2.56 - 2.61; P ≤ 0.05). However, no significant correlation between prevalence and severity of sleep apnea and NH with progressive renal deterioration was observed. Furthermore, an ESS score above 10 showed no significant mean difference in AHI and ODI when compared to a score below 10.

Conclusions: Sleep apnea is prevalent in the Chinese non-dialysis CKD population and strongly correlated with BMI and proteinuria, but not renal function. The study results also indicate that ESS is an investigative tool that lacks discriminatory power in patients with renal insufficiency. This study supports the need to maintain high clinical vigilance for sleep apnea when attending to CKD patients with significant proteinuria.

TH-PO632

Prevalence of Chronic Kidney Disease in Patient with HIV in Ilorin, Nigeria
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Background: HIV/AIDS is an important cause of renal diseases in sub-Saharan Africa. There is paucity of studies on the burden of chronic kidney disease among the HIV/AIDS patients in the North-Central zone of Nigeria.

Methods: A cross-sectional study of 227 newly-diagnosed, HAART-naïve patients with HIV/AIDS seen at the HIV clinic of the medical out-patient department of University of Ilorin Teaching Hospital (UITH). They were matched with 108 control group. Biochemical, heamatological and urinary tests were performed for all the participants. CKD was defined as eGFR ≤60 ml/min/1.73m^2 and albumin creatinine ratio ≥30mg/g.

Results: There were 100(44%) males among the patients and 47(43.5%) among the control group. The mean age of the patients and controls were 40.3 ± 10.3 years and 41.8 ± 9.5years respectively. CKD was observed in 108(47.6%) among the patients and 18(16.7%) of the controls p= 0.01. About half of the patients were in stage 1 CKD The median CD4 counts was significantly lower in patients with CKD (164.5{6-947} cell/µl) than patients

without CKD (247{8-1202} cell/µl) (p=0.01). Ninety-three (41.0%) of the patients had dipstick proteinuria of ≥2+. The median albumin creatinine ratio (ACR) was significantly higher among the HIV-positive patients (272.3mg/g) compared with the HIV-negative controls (27.22mg/g) p=0.01. The CD4+ count correlates positively with eGFR (r =0.463, p =0.001) and negatively with ACR (r = -0.806, p=0.001).

Conclusions: CKD is very common among patients with HIV/AIDS in Ilorin. Screening and early intervention for CKD should be part of protocols in the management of patients with HIV/AIDS.

TH-PO633

Patient Profiles in Calciphylaxis - Data From the German Calciphylaxis Registry Vincent Brandenburg,[1] Jürgen Floege,[2] Markus Ketteler.[3] *[1]Dept of Cardiology, Univ Hospital RWTH Aachen; [2]Dept of Nephrology, Univ Hospital RWTH Aachen; [3]Dept of Nephrology, Klinikum Coburg.*

Background: Calciphylaxis (CUA) is a rare disease and a clinical challenge. For patients, CUA is a devastating condition associated with high mortality. CUA is characterised by painful, ischemic, partly necrotic skin ulcerations. Pathomorphologically, media calcification of cutaneous arterioles and extracellular matrix remodelling are the hallmarks of CUA. Epidemiology and risk factors are incompletely understood.

Methods: We established an international internet-based registry in 2006 (www.calciphylaxie.de) to allow online notification for pts with established or suspected CUA. The registry includes a comprehensive data base with 71 parameters concerning patient and laboratory data, clinical background and presentation as well as therapeutic strategies. The diagnosis of CUA is made on clinical and/or histological grounds by the referring physician.

Results: Until May 2014 n=219 patients with CUA have been documented in 7.5 years: 62% females; 89% dialysis (HD and PD) patients, median age 66 (21-88) years. Stored serum samples were used for central laboratory analysis in core facility in n = 92 dialysis patients: PTH levels varied significantly between undetectably low and > 1100 pg/ml, mean 191 +/-176 pg/mL; fetuin-A: 0.21+/-0.01 g/L. Fetuin-A levels in control HD pts without CUA were significantly higher (n=65; 0.46+/-0.1 g/L, p < 0.01). Oral anticoagulation with Vit K antagonists was common in ESRD pts (53%) prior to CUA diagnosis. Cutaneous lesions were localized in 80% at the lower extremities or gluteal and abdominal region. Among the most frequently recorded therapeutic procedures were: surgical necrosectomy, intensifying dialysis modality, reduction of calcium supply, i.v. sodium-thiosulfat application. The median survival time was 516 days after online notification.

Conclusions: CUA is a rare disease among ESRD pts with increased mortality. Therapeutic strategies vary significantly among centers. The German CUA registry is a valuable tool to collect data and may become a basis for a European registry (EuCalNet, www.calciphylaxis.net) and for prospective controlled trials in the near future.

Funding: Pharmaceutical Company Support - Amgen, Sanofi

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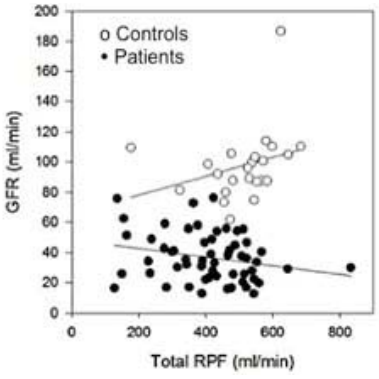
Renal Artery Blood Flow and Oxygenation in Chronic Kidney Disease Determined by Magnetic Resonance Imaging Dinah Sherzad Khatir,[1] Michael Pedersen,[2] Bente Jespersen,[1] Niels Henrik Buus.[3] *[1]Dept of Renal Medicine, Aarhus Univ Hospital, Aarhus N, Aarhus, Denmark; [2]Dept, Dept of Diagnostic Imaging, Aarhus N, Aarhus, Denmark; [3]Dept of Renal Medicine, Aalborg Univ Hospital, Aalborg, Denmark.*

Background: Animal studies suggest that progression of chronic kidney disease (CKD) is related to hypoxia of the renal tissue. Renal blood supply determines oxygen delivery and sodium absorption is the main contributor to oxygen consumption. We aim to describe the relationship between renal oxygenation and renal artery blood flow (RABF) and sodium absorption in patients with CKD and healthy controls.

Methods: 64 stable stage 3-4 CKD patients (61±13 years) were compared with 24 age and sex-matched controls. Glomerular filtration rate was determined by ^{51}Cr-EDTA clearance and sodium excretion was measured in a 24-hour urine collection. Hematocrit was measured to calculate renal plasma flow (RPF). RABF and renal tissue oxygenation was determined by magnetic resonance imaging using cine phase-contrast and blood oxygen level dependent (BOLD) measurements of deoxyhemoglobin relaxation time (R_2*) respectively.

Results: GFR in patients was 37% of controls (36±15 versus 97±23 ml/min/1.73 m^2, P<0.001) and the amount of reabsorbed sodium was 37% of controls (6.9 versus 18.8 mol/24 hours, P<0.001). Single-kidney RABF of patients was 72% of controls (319 versus 443 ml/min, P<0.001). Likewise, RPF was decreased in patients compared with controls (P< 0.05). Glomerular filtration fraction (FF) was 9% in patients and 18% in controls (P<0.001). Patients and controls had similar cortical R_2* (13.4 versus 13.3 s^{-1}, P=NS) and medullary R_2* (26.4 versus 26.5 s^{-1}, P=NS) values.



Conclusions: In CKD, GFR and reabsorbed sodium are reduced more than twice that of RABF while cortical and medullary oxygenation is within the range of healthy persons. Reduction in glomerular FF may prevent renal hypoxia in CKD.

Funding: Private Foundation Support, Government Support - Non-U.S.

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Effect of Oral Supplementation with Curcumin in Patients with Proteinuric Chronic Kidney Disease Jonathan Salazar,[1] Edilia Tapia,[1] Armando Vazquez-Rangel,[1] L. Gabriela Sanchez-Lozada,[1] Jose Pedraza-Chaverri,[2] Susana Gonzalez Reyes,[2] Susana Gonzalez Reyes,[2] Alejandra Elizabeth Álvarez-Mejía,[2] Sebastián Guerra-León,[2] Magdalena Madero.[1] *[1]National Heart Inst, Mexico; [2]UNAM.*

Background: Proteinuria is the most powerful risk factor for progression of CKD. Despite the use of RAAS blockade, a large percentage of proteinuric CKD patients progress to kidney failure. Curcumin has potent antioxidant and antiinflamatory effects and in animal models and small clinical trials has been associated with proteinuria reduction and attenuation of CKD progression. Our objective was to evaluate the effect of curcumin on proteinuria and kidney disease progression in a randomized trial.

Methods: The study was designed as a randomized double blinded clinical trial conducted at the National Heart Institute in Mexico. Patients were included if they were 18 years or older, had a prot/creat (P/C) > 1 gram in 24 hrs, and were on RAAS blockade. Four grams of curcuma versus placebo were given daily for 8 weeks. The primary outcome was change in proteinuria and secondary outcomes included change in blood pressure (BP) and eGFR.

Results: 99 patients were randomized. 11 patients did not have P/C >1 at baseline and were excluded while 10 participants were lost to follow up leaving 78 patients for the analysis. The median (IQR) age was 52 (34-59) years, 50% had diabetes, mean SBP and DBP were 126.7 mm Hg and 77.9 mmHg, respectively and median(IQR) eGFR and P/C were 28 (19-56) ml/min /1.73 m2 and 3.5 (2.2-6.6) g, respectively. Baseline characteristics were similar between groups except SBP which was higher in the curcumin group (p=0.002) and hemoglobin which has higher in the placebo arm (p=0.02). At the end of follow up the delta P/C change between groups was not significant (p=0.29) for the curcumin -0.04 IQR (-1.20- 1.07) and placebo -0.19 IQR (-2.18- 0.66) units. There was a trend towards less eGFR loss in the curcumin group -0.5 IQR (-2.8- 1.2) versus -2.0 IQR (-6.1- -0.1), p = 0.06. Change in BP was not different between groups (p=0.65).

Conclusions: Curcumin was not associated with proteinuria reduction but was associated with a trend towards less eGFR loss. Our results need to be confirmed in larger trials.

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Vascular Effects of Exercise Training in Chronic Kidney Disease: A Randomized Controlled Trial Amaryllis H. Van Craenenbroeck,[1,3,4] Emeline M. Van Craenenbroeck,[2,3] Katrijn Van Ackeren,[3] Jean-Louis Bosmans,[1,4] Vicky Y. Hoymans,[3] Christiaan J. Vrints,[2,3] Marie M. Couttenye.[1] *[1]Dept of Nephrology, Antwerp Univ Hospital, Belgium; [2]Dept of Cardiology, Antwerp Univ Hospital, Belgium; [3]Laboratory of Cellular and Molecular Cardiology, Antwerp Univ Hospital, Belgium; [4]Laboratory of Experimental Medicine and Pediatrics, Univ of Antwerp, Belgium.*

Background: Endothelial dysfunction is an independent marker of increased cardiovascular (CV) risk and contributes to decreased aerobic capacity in patients with CKD. The aim of this study is to investigate whether a formal ET programme corrects endothelial dysfunction in CKD. Secondary endpoints were aerobic capacity (VO_2peak), arterial stiffness (carotid-femoral pulse wave velocity, PWV) and intima-media thickness (IMT).

Methods: Forty CKD patients in stage 3-4 (age 53 ± 13 yrs, eGFR 38.5 ± 12.9 ml/min/1.73 m^2) without documented CV disease were randomized to home-based ET consisting of daily cycling sessions (4x10 minutes) at moderate intensity (n=19) or usual care (UC, n=21). Aerobic capacity (VO_2peak), circulatory power (VO_2peak × peak systolic BP), endothelial function (flow mediated dilation, FMD), PWV (Sphygmocor) and carotid IMT (high-resolution ultrasound) were measured baseline and at 12 weeks follow-up.

Results: Groups were age- and gender matched. After 12 weeks of ET, VO_2peak increased by 21% (26.6 ± 5.6 to 32.1 ± 6.9 ml/kg/min), whereas no change was observed in the UC group (p<0.001). Circulatory power increased significantly in the ET group (19.6%), but remained unchanged in UC (p<0.001). Whereas FMD was clearly impaired in CKD (ET 4.0 ± 1.9%, UC 5.2 ± 3.4%), no significant changes were observed after follow-up (p=0.7). In addition, PWV and IMT were not significantly altered after follow-up in the 2 groups (all p<0.05). In trained subjects, change in PWV was related to the change in systolic BP (Pearson r=0.603, p=0.01).

Conclusions: In CKD patients stage 3-4 without overt CV disease, 12 weeks of ET at moderate intensity improved VO_2peak, without altering peripheral endothelial function or arterial stiffness, suggesting that other mechanisms may be responsible for the training-induced benefits on aerobic capacity.

TH-PO637

Gadolinium Exposure Induced Systemic Inflammatory Response (GEISIR) Is due to Activation of the Calcium-Sensing Receptor

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Background: Two clinical syndromes resulting from the administration of gadolinium (Gd)-containing MRI contrast agents to patients with advanced or end-stage kidney disease (ESKD) have been described: nephrogenic systemic fibrosis (NSF) and Gd exposure induced systemic inflammatory response (GEISIR). GEISIR is a peracute event in reaction to Gd-DTPA. We argue that activation of the calcium-sensing receptor (CaSR) by Gd ions causes GEISIR in ESKD patients.

Methods: cell culture – human macrophages, known to trigger early stages of NSF, were cultured and pro-inflammatory cytokines mRNA expression in response to Gd and other agents measured; clinical - 13 ESKD patients who received Gd-DTPA and subsequently developed either GEISIR or NSF were observed for up to 9 years.

Results: Cultured macrophages release Il-6, IL-1b, CCl2 and TNF-alpha in response to Gd, mRNA expression (normalized to vehicle-treated controls) increased by >50% after incubation with 5µM gd chloride for 72h. This response is enhanced by cinacalcet and attenuated by the calcilytic agent NPS, the NPS effect was significant for Il-6 and IL-1b ($p<0,05$ in Wilcoxon mathed pairs test) with a trend for CCl2 and TNF-alpha (p between 0,05 and 0,2). Two patients developed NSF, both had raised CRP levels but lacked the SIRS-like clinical features of GEISIR in the early phase. None of the 11 patients with GEISIR went on to develop NSF during the follow-up. ***Discussion:*** Our findings strongly suggest a causal involvement of the CaSR. GEISIR does not necessarily develop into NSF even after several years. A second event and/or genetic disposition may be required. This and the possible role of uremic toxins such as phenylacetic acid require more studies.

Conclusions: If our hypothesis were true, calcilytics could be beneficial to prevent adverse effects of Gd containing contrast agents in kidney disease patients.

Funding: Pharmaceutical Company Support - AMGEN

TH-PO638

Sub Acute Kidney Injury (sAKI) Induced by Intravitreal AVASTIN Injection: Time for Concern

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Background: Systemic injection of AVASTIN (Bevacizumab), an anti Vascular Endothelial Growth Factor Ab, is associated with adverse renal events; hypertension and proteinuria. Intravitreal AVASTIN injections are currently used for ophthalmic indication such as age-related macular degeneration and diabetic retinopathy. The aim of this study was to investigate renal side effects of repeated low dose intravitreal Avastin.

Methods: 37 patients (70% diabetic, 81% hypertensive, 27% CKD (eGFR<60 ml/min)) received 3 injections of 1.25 mg/dose, once monthly. Blood and urine were drawn before each injection and one month (1m) following third injection for: Serum creatinine (Scr), eGFR and urine for ACR and PCR. sAKI was defined as Scr rise of > 0.3 mg% over 7-30 days after injection.

Results: Scr increased and GFR decreased each month after injection, as compared to baseline (Bsln).

Renal function changes	N	Mean± Std	p
Scr mg% Bsln	37	1.125 ± 0.74	0.003 *
1m post 1 inj		1.28 ± 1.00	
Scr mg% Bsln	31	1.16 ± 0.81	0.0015 *
1m post 2 inj		1.32 ± 1.00	
Scr mg% Bsln	26	1.19 ± 0.86	0.020 **
1m post 3 inj		1.35 ± 1.31	
GFR ml/min Bsln	37	70.46 ± 23.55	p<0.001 *
1m post 1 inj		65.03 ± 24.01	
GFR ml/min Bsln	37	70.99 ± 25.14	p<0.001 *
1m post 2 inj		64.43 ± 25.44	
GFR ml/min Bsln	26	71.44 ± 26.41	0.0255 *
1m post 3 inj		68.94 ± 26.91	

* Paired sample t-test

** Wilcoxon signed rank test

sAKI occurred in 18.5% (5/37) of patients 1 month after first injection, with Scr rise from 2.41 to 3.2 mg % (p=0.031) and eGFR decreased by mean of 33.50%. In diabetic patients with GFR<60 ml/min, sAKI occured in 33.3% (3/9) after 1 injection compared to 5.9% (1/17) in diabetic patients with GFR>60mll/min. No statistically significant changes were found in blood pressure, albuminuria and proteinuria.

Conclusions: sAKI can occur after a single low dose of intravitreal Avastin. Diabetic patients with GFR<60mll/min are at higher risk for sAKI. More trials are required to investigate long term renal effects of this therapy. Nephrologist follow up should be recommended.

Funding: Government Support - Non-U.S.

TH-PO639

Acute Weight Change prior to Hospitalization in Late Stage Chronic Kidney Disease

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Background: There is a high rate of hospitalizations in patients with late stage chronic kidney disease (LS-CKD). Because of reduced salt and water excretion and a high prevalence of left ventricular hypertrophy and congestive heart failure (CHF) in these patients there is a great vulnerability to acute volume changes. Accordingly, we studied the relationship between acute weight change and risk for hospitalizations in LS-CKD.

Methods: 203 patients with stage 4/5 CKD were studied for between 4-16 months. All patients used a telephonic informatics system for capture of daily weights. A significant change in weight was considered 1.7% of body weight in any two days compared to the mean of the 5 previous days. Hospitalization data was collected by monthly review and interview of patients and the reason for all hospitalizations was determined. Patients' weights for the 14 days prior to each hospitalization were analyzed. If less than 10 days of weights were available or if the weight on the day prior to hospitalization was missing then that hospitalization was excluded from analysis.

Results: There were a total of 189 hospitalizations occurring in 93 patients. 64 of the hospitalizations were excluded due to missing weight data, leaving 125 hospitalizations analyzed. The leading causes of hospitalization were cardiovascular 30.4%, infection 17.3%, gastrointestinal bleeding 10.0%, syncope 3.6%, CVA 2.2%. There was no significant relationship between weight change in either direction with all cause hospitalization. There was, however, significant weight gain prior to CHF/volume overload admissions in 64% of cases and CV admissions in 31.6% of cases ($p <0.05$ for both compared to all cause admissions). There was significant weight loss prior to syncope in 40% of cases ($p<0.05$).

Conclusions: Weight often acutely increases prior to CHF/volume overload and cardiovascular hospitalizations and declines prior to syncope admissions in late stage CKD patients. Because CHF/volume overload hospitalizations are frequent in this population, weight monitoring and intervention for weight gain might be beneficial.

Funding: Clinical Revenue Support

TH-PO640

Urine HBV DNA in the Diagnosis of Hepatitis B Virus-Associated Glomerulonephritis

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Background: Serum hepatitis B virus (HBV) DNA is one of the diagnostic criteria for HBV-associated glomerulonephritis (HBV-GN). However, HBV DNA has been detectedin the urine of patients with HBV-GN, which may have implications for pathogenesis of this disease. This study was conducted to investigate whether urine HBV DNA could be a new diagnostic criterion for HBV-GN.

Methods: A total of 152 patients were enrolled in this study. The patients were divided into the following groups: HBV-GN (66 patients), non-HBV-GN (66 patients), and chronic hepatitis B (CHB) without renal disease (20 patients). Serum and urine HBV DNA levels were determined using polymerase chain reaction (PCR). Other serum HBV markers were detected using enzyme-linked immunosorbent assays.

Results: In the diagnosis of HBV-GN, urine HBV DNA had a high specificity (0.96), a good positive predictive value (PPV, 0.96), and a modest negative predictive value (NPV, 0.60). The combination of urine HBV DNA with serum HBV DNA, the hepatitis B surface antigen (HBsAg) or the hepatitis B e antigen (HBeAg) was inferior to urine HBV DNA in the diagnosis of HBV-GN.

Conclusions: Urine HBV DNA may be a new noninvasive diagnostic criterion for HBV-GN.

Funding: Government Support - Non-U.S.

TH-PO641

Urine MMP-7 Is Associated with Mortality in People with Type 2 Diabetes and Kidney Disease

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Background: The renin angiotensin system (RAS), bone morphogenetic protein (BMP) and WNT pathways are involved in pathogenesis of experimental diabetic kidney disease (DKD). Urine excretion of angiotensinogen, gremlin-1 and matrix metalloproteinase-7 (MMP-7), components of the RAS, BMP and WNT pathways, are markedly increased in people with diabetic kidney disease. It is not known whether this increase is associated with subsequent progression to end-stage renal disease (ESRD) or death.

Methods: We measured urine concentration of angiotensinogen, gremlin-1 and MMP-7 in baseline samples from a cohort with type 2 diabetes and kidney disease (n=141). Using time-to-event analyses, we examined the association between creatinine-adjusted urine concentration of these proteins and progression to ESRD or death.

Results: Among the cohort, 38 progressed to ESRD over a mean (standard deviation, SD) follow-up of 3.2 (1.9) years and 39 died over a mean (SD) follow-up of 3.7 (1.9) years. All three proteins were associated with ESRD and death after adjustment for age, sex, race/ethnicity, smoking, hemoglobin A1c, LDL and RAS inhibitor use. After additional adjustment for baseline glomerular filtration rate and albuminuria, only urine MMP-7/

Creatinine remained associated with death (Hazard ratio 3.59 for highest versus lowest tertile), but not ESRD. Serum MMP-7 was not associated with death and did not attenuate the association of urine MMP-7 with death in this cohort (HR 4.03 for highest versus lowest urine MMP-7/Cr tertile, after adjustment for serum MMP-7).

Conclusions: Among people with type 2 diabetes and kidney disease, urine MMP-7/Cr concentration was strongly associated with death, but not progression to ESRD, during a 3.7 year follow-up. Adjusting for serum MMP-7 did not attenuate this association.

Funding: NIDDK Support, Private Foundation Support

TH-PO642

Diagnostic Utility of Immunochemical Fecal Occult Blood Tests to Detect Lower Gastrointestinal Lesions in Patients with Chronic Kidney Disease

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Background: The patients with chronic kidney disease (CKD) have an increased risk for gastrointestinal (GI) bleeding. However, the diagnostic utility of immunochemical fecal occult blood test (iFOBT) to detect bleeding-related lower GI lesions is undetermined in CKD patients.

Methods: We included a total 783 patients with nondialysis-dependent CKD stages 2–5 or receiving dialysis. The specific type of bleeding-related lower GI lesions was identified by colonoscopy, and the diagnostic utility of iFOBT was evaluated for these lesions.

Results: Bleeding-related lower GI lesions were found in 14.3% of 617 patients with CKD stage 2/3, 20.0% of 70 patients with CKD stage 4/5, and 32.3% of 96 patients receiving dialysis (P< 0.001). Compared to CKD 2/3 patients, dialysis patients had an independent risk for bleeding-related lower GI lesions (adjusted odds ratio, 2.25; P= 0.014). The iFOBT was positive in 125 (16.0%) patients, and the sensitivity of iFOBT for the detection of bleeding-related lower GI lesions was significantly increased as CKD stage worsened (29.5% versus 50.0% versus 58.1%; P = 0.012). However, the specificity of iFOBT was decreased with the severity of CKD stage (92.6% versus 75.0% versus 67.7%; P < 0.001). The negative predictive value was also decreased in a similar pattern (88.8% versus 85.7% versus 77.2%; P = 0.039), and the positive predictive value did not differ between different CKD stages (P = 0.617).

Conclusions: The prevalence of bleeding-related lower GI lesions was increased as CKD stage worsened, and the sensitivity of iFOBT to detect these GI lesions is also increased with the severity of CKD stage. However, iFOBT should be used with caution in patients with higher stage of CKD, because its specificity and negative predictive value is decreased.

TH-PO643

Salviae Miltiorrhizae Liguspyragine Hydrochloride Improved eGFR in Patients with Chronic Kidney Diseases

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Background: The progressive decline of glomerular filtration rate (GFR) ultimately resulted in end stage renal disease (ESRD) in patients with chronic kidney diseases (CKD). Current ways to slow down progression of CKD to ESRD are to control hypertension, proteinuria, anemia, and unbalance of electrolytes and acid-base. Few medicines have shown effects on maintaining GFR in CKD patients. Salviae Miltiorrhizae Liguspyragine Hydrochloride (SMLH) has been demonstrated an improvement of tissue microcirculation in ischemic heart and brain in patients. The effect of SMLH on renal function in CKD patients remains unclear. We aim to study if SMLH can attenuate GFR in patients.

Methods: 83 patients with CKD (G2~5) were treated with intravenous infusion of 200ml SMLH per day for 1week (n=43) or 2 weeks (n=40). The estimated GFR (eGFR) by CKD-EPI equation and the levels of serum creatinine (SCr), blood urea nitrogen (BUN), serum cystatin C (SCysC), and serum uric acid (SUA) were examined before and after the treatment.

Results: eGFR increased from 30 ± 3 to 45 ±3 ml/min (p=0.0006), SCr decreased from 304 ± 27 to 209 ± 18 umol/L (p=0.004), SUA also decreased from 449 ± 19 to 390 + 18.39 (p=0.032) in all patients after SMLH treatment. With further stratification by 1 week and 2 weeks of SMLH treatment, we found that 2 weeks treatment brought similar increase of eGFR (15 ± 6 versus 14.0 ± 6, p=0.93), but significant large reduction of SCr and SUA level compared to 1week treatment (138 ± 56 versus 66 ± 38, p=0.028 in SCr and 110 ± 46 versus 61 ± 43, p=0.046 in SUA). However, BUN and SCysC were reduced after the treatment but did not show significant difference.The 3 and 6 month follow up study is on the way.

Conclusions: SMLH significantly improved eGFR and decreased the levels of SCr and SUA but not SCysC and BUN in patients with CKD (G2~G5) in this study. The effect of repeated SMLH administration and long-term effects of SMLH need to be evaluated in a longitudinal study in large cohort of CKD patients. The mechanism of SMLH renal function improvement needs to be investigated in CKD animal models. SMLH might be a useful medicine to slow down the decline of eGFR in CKD patients.

Funding: Government Support - Non-U.S.

TH-PO644

Renal Artery Diameter Is a Predictor of Cardiovascular Events in Subjects with Ischemic Heart Disease and Low-to-Moderate Renal Artery Stenosis

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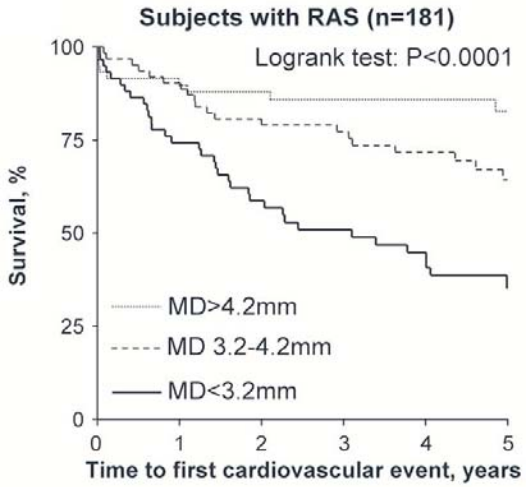
Background: Several studies report that small renal arteries defined by a low reference diameter(RD)or minimal luminal diameter(MD),are independently associated with low GFR,resistant hypertension and onset of contrast-induced nephropathy and suggested a post-hoc analysis of CORAL trial based on RD categories.We hypothesized that RD and MD are markers of non-traditional cardiovascular risk factors.Aim:To look whether low RD and MD could impact the prognosis of patients with ischemic heart disease.

Methods: Prospective cohort study.We used proportional hazards models to analyse the first-onset of cardiovascular events(CVE) [myocardial infarction,coronary stent implantation,heart failure,stroke and cardiac death]in relation with RD,MD % of RAS in those with low-to-moderate RAS(10-70%)(n=181).

Results: During median follow-up of 4.5yrs(range,0.1-5),27.8% participants(n=623;mean age,64yrs;29%women)experienced a CVE(35.4% in those with RAS10-70%).The presence of low-to-moderate RAS was associated with CVE.In these subjects,those with low MD were associated with higher risk of CVE(MD>4.2mm,HR:1;MD3.2-4.2mm,HR:1.67,95%CI:0.75-3.74,p=0.21;MD<3.2mm,HR 3.76,95%CI:1.67-8.44,p=0.001).When MD was added to a standard risk factor model,risk prediction improvement was4.6%.Results were qualitatively similar if MD was replaced by RD or % of stenosis, but with smaller improvement of risk prediction and model fit.



Conclusions: In patients with ischemic heart disease and low-to-moderate RAS,MD was a significant predictor of CVE, improves risk prediction and may represent a valuable biomarker of cardiovascular disease risk.

TH-PO645

Evaluation of Retinal Microvascular Variation in Hypertension and Chronic Kidney Disease

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Background: The retinal vasculature is accessible to repeated non-invasive assessment, enabling detection of early microvascular changes prior to clinically significant events. Previous studies have associated variation in retinal vessel caliber (RVC) with both chronic kidney disease (CKD) and hypertension. We seek to evaluate RVC with both hypertension and CKD status in elderly white Irish nuns.

Methods: The Irish Nun Eye Study was a cross-sectional analysis of 1242 individuals. Retinal status was assessed by stereo retinal photography in 1122 participants. Retinal arteriolar and venular caliber (central retinal arteriolar [CRAE] and central retinal venular [CRVE] equivalents) were measured from retinal photographs by a standardized computer-assisted method (IVAN). Multiple linear regression was used to compare mean CRAE and CRVE by CKD (eGFR<60 mL/min/1.73 m^2) and hypertension status following adjustment for the potential confounders age, BMI, mean arterial blood pressure, refraction, hypertension, diabetes, CKD and smoking status.

Results: In an adjusted analysis, no significant variation in CRVE was detected in association with hypertension. However, a significant decrease in CRAE was reported in hypertensive individuals (P=0.02; effect size = -1.8; 95%CI: -3.32, -0.27 um). In an adjusted analysis, no significant variation was found in either CRAE or CRVE with CKD (P=0.07; effect size = 2.1; 95%CI: -0.16, 4.34 um).

Conclusions: Variation in retinal vessel caliber was identified in association with hypertension. Hypertensive individuals have significantly narrower retinal arterioles. Several pathological processes including inflammation, hyperglycemia, endothelial dysfunction

and cerebral hypoxia have been implicated in the dilation of retinal venules. Hypertension associated retinal arteriolar narrowing has been linked to increased peripheral resistance through elevated blood pressure. Whether these measurable changes in the microvasculature offer prognostic value remains to be determined.

TH-PO646

C-Reactive Protein Modifies the Association Between Metabolic Syndrome Components, Microalbuminuria, and Race/Ethnicity Satyesh K. Sinha,[1] Magda Shaheen,[1,2] Deyu Pan,[1] Susanne B. Nicholas.[1,2] *[1]Research, Charles R Drew Univ of Medicine and Science, Los Angeles, CA; [2]David Geffen School of Medicine, Univ of California Los Angeles, Los Angeles, CA.*

Background: C-reactive protein (CRP) has been shown to be associated with both the metabolic syndrome (MetS) and chronic kidney disease (CKD). However, little is known about the association of CRP and individual components of the MetS and microalbuminuria, as a marker for CKD.

Methods: We analyzed data from the National Health and Nutrition Examination Surveys 1999-2008 of adults aged ≥20 years with the MetS to study the effect of CRP on the association between microalbuminuria (30-300µg/ml) and the individual components of the MetS and whether this effect is further modified by race/ethnicity. Multivariate analyses were performed to test the association between microalbuminuriaand components of the MetS. We further performed stratified analysis by CRP levels (cutoff ≥0.5mg/dL) to find out if CRP modifies the independent adjusted associations between MetS components and microalbuminuria. Analysis was also done to determine racial/ethnic variation in this association.

Results: In the adjusted multivariate model, microalbuminuria was independently associated with central obesity, pre-diabetes, and low-HDL, p<0.005. In the stratified analysis, CRP levels modified this association. Among those with CRP≥0.5mg/dL, microalbuminuria was independently associated with central obesity (AOR=2.26, 95% CI 1.16-4.43), pre-hypertension (AOR=2.06, 95% CI 1.20-3.56), and low HDL (AOR=1.58, 95% CI 1.15-2.18), p<0.01. Among those with CRP<0.5mg/dL, microalbuminuria was independently associated only with pre-diabetes (AOR=1.59, CI 1.14-2.23), p<0.007. These associations were further varied by race/ethnicity. Among whites, blacks and Hispanics with CRP≥0.5mg/dL microalbuminuria was independently associated with low HDL (AOR=2.34, CI 1.55-3.54), pre-hypertension (AOR=2.63, CI 1.16-5.96), and central obesity (AOR=7.78, CI 1.63-37.13) respectively (p<0.03).

Conclusions: We conclude that CRP and race/ethnicity both modify the independent association between microalbuminuria and components of the MetS.

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TH-PO647

Dietary Acid Reduction Provides Adjunctive Kidney Protection to Angiotensin Converting Enzyme Inhibition in Patients with Stage 1 CKD through Amelioration of Tubulo-Interstitial Injury Nimrit Goraya,[1,2] Jan Simoni,[3] Chanhee Jo,[4] Donald E. Wesson.[1,2] *[1]Texas A&M College of Medicine, Medicine, TX; [2]Internal Medicine, Baylor Scott & White Health, Temple, TX; [3]Surgery, Texas Tech Univ Health Sciences Center, Lubbock, TX; [4]Biostatistics, Baylor Scott & White Health, Temple, TX.*

Background: Tubule-interstitial injury (TII) mediates GFR decline in animal models of CKD and is ameliorated by dietary acid reduction. As patients with hypertension-associated nephropathy (HAN) and macroalbuminuria (mg albumin-to-g creatinine ratio [Ualb] > 200) compared to normoalbuminuria (Ualb<20) have higher risk for GFR decline, we examined if 1) macroalbuminuric HAN patients had TII; and 2) adding dietary acid reduction to angiotensin converting inhibition (ACEI) stopped progression of kidney injury.

Methods: We compared urine N-acetyl-D-glucosaminidase (U)-to creatinine (g) ratio (UNAG), an index of TII, in hypertensives with CKD Stage 1 eGFR with macroalbuminuria and with normoalbuminuria to normotensives with normoalbuminuria. We measured UNAG and Ualb after 2 years in CKD Stage 1 macroalbuminuric HAN patients on ACEI, randomized to Usual Care (n=25) or to dietary acid reduction with either fruits and vegetables (F+V, n=23) or oral $NaHCO_3$ (HCO_3, n=23), 0.4 meq/Kg bw/d, each to reduce dietary acid 50%.

Results: Of 281 hypertensives, UNAG was higher in those with macroalbuminuria (n=8) than normoalbuminuria (n=218) (2.5±0.6 versus 1.6±0.5 U/g, p<0.01) and higher than normotensives with normoalbuminuria (1.6±0.9 U, p<0.01, n=10), supporting TII in macroalbuminuria but not normoalbuminuria. At 2 years, UNAG (2.5±0.5 to 2.7±0.4 U/g cr, p<0.01) and Ualb (347±82 to 407±61 mg/g, p<0.01) were higher in Usual Care, consistent with worsening TII and overall kidney injury. By contrast, they were not different at 2 years in F+V (UNAG: 2.5± 0.4 to 2.4±0.4 U/g, p=0.22; Ualb: 350±75 to 341±59 mg/g, p=0.34) or HCO_3 (UNAG: 2.5±0.3 to 2.5±0.3 U/g, p=0.43; Ualb: 341±83 to 330±67 mg/g, p=0.32), consistent with stable TII and stable kidney injury.

Conclusions: Dietary acid reduction in Stage 1 CKD due to HAN appears to provide kidney protection through amelioration of TII that is adjunctive to ACEI.

TH-PO648

Low Fetuin-a and 25(OH) Vitamin D along with High Pentraxin-3 and IL-1ß Levels Are Linked to Increased Left Ventricular Mass Index in Diabetic and Non-Diabetic Chronic Kidney Disease Patients Belda Dursun,[1] Sahan Yasin,[1] Halil Tanriverdi,[2] Rota Simin,[3] Sukriye Uslu.[2] *[1]Nephrology, Pamukkale Univ, Medical School, Denizli, Turkey; [2]Cardiology, Pamukkale Univ, Medical School, Denizli, Turkey; [3]Biochemistry, Pamukkale Univ, Medical School, Denizli, Turkey.*

Background: Increased ventricular stiffness leads to left ventricular hypertrophy (LVH) in chronic kidney disease (CKD) patients. Fetuin-A is involved in mineral metabolism and acts as inhibitor of calcification. Pentraxin-3 (PTX3) and IL-1ß are reliable markers of inflammation. Aim was to investigate relationships between fetuin-A, 25(OH) vitamin D, pentraxin3, IL-1ß and left ventricular mass index (LVMI) in diabetic and non-diabetic CKD patients.

Methods: Stage 3-5 CKD patients (40 diabetic, 40 non-diabetic) and 40 controls were included. Fetuin-a, 25(OH)D, pentraxin-3, IL-1ß and other biochemical parameters were determined. Echocardiographically LVMI was calculated.

Results: LVMI (g/m^2) was higher in diabetic (116.8±20.5) and non-diabetic CKD (112.6±26.9) than controls (78.8±9.4) (p=0,0001). Fetuin-A levels (ng/ml) were lower in diabetic (17.4±10.4) and non-diabetic CKD (18.1±10.5) than controls (42.3±16.8); p=0,0001. 25(OH)D (pg/ml) were lower in diabetic (25.7±9.3) and non-diabetic CKD (26.1±10.9) than controls (42.4±9.8); p=0.001. Ptx-3 level (ng/ml) were higher in diabetic (1.13±0.2) and non-diabetic CKD group (1.04 ±0.24) than controls (0.33 ±0.39), p=0,0001; diabetics had higher levels of PTX3 than non-diabetics (p=0.022). IL-1 ß (pg/mL) was higher in diabetic (7.3±12.9) and non-diabetic CKD (7.9±12.9) than controls (1.82±9.11) (p=0,001).IL-1 ß (pg/mL) was higher in diabetic (7.3±12.9) and non-diabetic CKD (7.9±12.9) than controls (1.82±9.11) (p=0,001). Fetuin-A was positively correlated with 25(OH)D. Pentraxin-3 and IL-1ß showed positive correlations with LVMI (r=0.253, P=0.005 and r=0.243, p= 0.007). Fetuin-A and 25(OH)D showed negative correlations with LVMI (r=-0.229, P=0.012 and r=0.276, p= 0.002).

Conclusions: Based on our results, lower fetuin-a and 25(OH) vitamin D along with higher pentraxin-3 and IL-1ß levels could mediate the progression of left ventricular hypertrophy in diabetic and non-diabetic CKD patients.

TH-PO649

Importance of Assessing the Cell Mass Index (BCMI) Measured By Bioelectrical Impedance Vector (BIVA) as a Marker of Muscle Mass in 240 CKD Patients Guillermina Barril,[1] Angel Nogueira Perez,[1] Maria Bernardita Puchulu,[1] Secundino Cigarran,[2] Jose-Antonio Sanchez-Tomero.[1] *[1]Nephrology, Hospital U. de la Princesa, Madrid, Spain; [2]Nephrology, Hospital Da Costa, Burela, Lugo, Spain.*

Background: BIVA is a noninvasive method for determining body composition. Phase angle (PA) and body cell mass (BCM) are considered as nutritional markers. Body cell mass index (BCMI) may be a marker of muscle mass (MM) AIM:To determine the usefulness of BCMI as MM marker and its relationship to body composition,total body potassium,visceral proteins, inflammation, and dynamometry values with age and sex changes.

Methods: Cross-sectional study of 240 advanced CKD patients xage 71.33±13y (33-96). Diabetics 35.3%. Body composition was performed with BIVA model BIA 101 (Akern,Italy) evaluating: total body water (TBW), BCM, PA, ICW, Na/K and BCMI. Also albumin, prealbumin, CRP,Hb, total lymphocytes, GFR(Cr clearance and MDRD), tricipital skin fold,arm muscle circumference and abdominal circumference were measured. Patients were classified in two groups according BCMI: G1 BCMI<8, 183 patients (73% male) and G2 BCMI>8, 57 patients 81.9% male.

Results: TBW was higher in G2 (p<0.005). We found no significant differences between groups for triceps skinfold and arm muscle circumference, NPNA, Hb, total lymphocytes, GFR and TBW. Regarding BMI no sig. differences between men and women in both groups. In G2 BCMI shows higher sig.values than G1 in: PA, ICW, FFM, muscle mass %, dynamometry, albumin and prealbumin. No differences in MDRD, NPNA or creatinine clearance, lymphocytes or TBW. Patients with BCMI> 8 (G2) showed lower CRP level than G1. Globally significant direct correlation was found between dynamometry (0,000) and BCMI (0.000), %TBW (0.045), albumin and prealbumin (0.000), MDRD (0.05), Hb (0.02), total lymphocyte (0.006), AIC (0,000).and reverse with MIS (0,000) and ECW (0.000).

Conclusions: Our findings suggest that BCMI>8 together with an adequate ICW and PA were correlated with dynamometry and can be considered as parameters of muscle mass and muscle strength.These parameters can be considered as representative as better body composition and muscle strength interrelated with visceral proteins, inflammation,age and sex.

TH-PO650

Bioimpedance Assessment of Volume Status in Referred Patients with Chronic Kidney Disease: Baseline Data from BIO-CANPREDDICT

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Background: Volume status in patients with chronic kidney disease is difficult to assess. Establishing construct validity for new methods of assessment is challenging because no criterion measure exists. A modest correlation with other methods of assessment would suggest that the methods assess the same underlying construct, and confirm the potential for bioimpedance to provide more accurate information than the comparator studied.

Methods: We measured bioimpedance, in triplicate, in patients with CKD who were already participating in CANPREDDICT. We analyzed the data according to the method of Piccoli, classifying patients based on the resistance (R) – reactance (Xc) graph.

Results: We recruited 416 patients, median age was 70 (IQR 17) and 61 % were male. Median MDRD GFR was 28 mL/min/1.73 m^2 (IQR 16), median urine albumin-creatinine ratio was 107 mg/L (IQR 391), 44% had diabetes, 26% ischaemic heart disease and 10% a history of congestive heart failure. Median physician-assessed volume status was 1 on a 1-to-7 scale (IQR 1) and volume status by bioimpedance was 1 on a 0-to-3 scale (IQR 2). For both scales higher values reflect worse volume overload. By bioimpedance, 179 (44%) of patients had normal volume status (0), 91 (22%) status of 1, 108 (26%) status of 2 and 33 (8%) status of 3. Correlation between physician-assessed and bioimpedance- measured volume status was 0.31 (p< 0.001). In multivariate analysis, volume status by bioimpedance was associated with age (OR 1.07 per year, 95% CI 1.05-1.10), gender (OR 0.56 for women, 95% CI 0.34-0.92) and diabetes (OR 2.3, 95% CI 1.26-4.09).

Conclusions: Volume overload by bioimpedance is prevalent in referred patients with CKD. The moderate correlation with physician-assessed volume overload establishes construct validity for this method.

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TH-PO651

Bioelectrical Impedance Vector Analysis (BIVA) in the Assessment of Volume and Nutrition Status in Hemodialysis Patients

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Background: Determining fluid status in patients with end stage renal disease represents a diagnostic challenge. Bioelectric impedance analysis detects hydration status in the soft tissues and ElectroFluidGraph uses the data generated by an impedance device to generate a Bioelectrical Impedance Vector Analysis (BIVA). We hypothesized that volume overloaded patients, as assessed by BIVA before and after HD would have increased hospitalizations, ER visits, and death over 90 days.

Methods: This was a single-center observational study involving 58 patients receiving chronic hemodialysis at the VA Hospital. BIVA was performed immediately before and after dialysis to assess hydration status. One standard deviation above and below the mean was defined as "normal" hydration. Greater than two standard deviations above or below the mean were designated "over-hydrated" and "under-hydrated," respectively. 90 day outcomes including ER visits, hospitalizations, cardiac events, and death were ascertained via chart review.

Results: Mean age of the cohort was 65.1 ± 9.6 yrs and 93% were male. The mean pre-hydration was 78.56% ±4.66. The mean pre-dialysis hydrations were 72.3 ± 2.1%, 78.5 ± 3.0%, and 85.5 ± 1.5% in the under-, normal, and over-hydrated groups, respectively. The average pre-dialysis weights were 70.1 ± 13.6, 90.4 ± 20.9, and 83.7 ± 30.0 kg respectively. Among the under-hydrated, 50% had ER visits compared to 46% in the normal hydration and 22% in the overhydrated group (p=0.379) Similarly, the under-hydrated group had a 50% hospitalization rate as compared to 41% in the normal hydration group and 33% in the over-hydrated group at 90 day follow up (p = 0.760 for all three groups).

Conclusions: Among elderly hemodialysis patients, BIVA measurements correlating with under-hydration prior to HD were associated with a trend toward higher rates of adverse events and statistical significance may have been limited by small sample size. One possible explanation is that under-hydration may be a surrogate marker for poor nutritional status, a finding which should be explored in larger cohorts.

Funding: Veterans Affairs Support

TH-PO652

Urinary Sodium Excretion Has Positive Correlation with Activation of Urinary Renin Angiotensin System and Reactive Oxygen Species in Hypertensive Chronic Kidney Disease

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Background: High sodium intake affects blood pressure, proteinuria, and glomerular hemodynamic and causes renal damage. Although the pathophysiology of the harmful effects of high salt intake seems to have been well described in animal studies, it is not well described in human studies.We aim to determine the relationship between salt intake—estimated by measuring 24-hour urine sodium—and urinary biomarkers of the RAS, inflammation, and ROS.

Methods: We analyzed the relationship between the 24-hour urine sodium-to-creatinine ratio (24HUna/cr) and urine angiotensinogen, monocyte chemoattractant peptide-1, and malondialdehyde-to-creatinine ratio (uAGT/cr, uMCP1/cr, and uMDA/cr) by using the data derived from 226 hypertensive CKD patients, followed for 16 weeks, prospectively.

Results: At baseline, the 24HUna/cr group or levels had a positive correlation with uAGT/cr and uMDA/cr adjusted for related factors (p < 0.005 for each analysis). When we estimate uAGT/cr in the 24HUna/cr groups by ANCOVA, the uAGT/cr in patients with ≥200 mEq/g cr was higher than in patients with <100 mEq/g cr [708 (95% CI: 448–967) versus 334 (95% CI: 184–483) pg/mg cr, p = 0.014]. Similarly, uMDA/cr was estimated as 0.17 (95% CI: 0.14–0.21) pM/mg cr in patients with <100 mEq/g cr and 0.27 (95% CI: 0.20–0.33) pM/mg cr in patients with ≥200 mEq/g cr (p = 0.016). During the 16-week follow-up period, an increase in urinary sodium excretion predicted an increase in urinary angiotensinogen excretion.

Conclusions: High salt intake increases renal RAS activation, primarily, and directly or indirectly affects the production of ROS through renal RAS activation.

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TH-PO653

Relationship between Aortic Stiffness and Renal Small Artery Sclerosis in Patients with Chronic Kidney Disease

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Background: Arteriosclerosis of medium-to-large arteries was suggested to be linked to small artery sclerosis. We investigated a possible relationship between aortic stiffness and renal small artery sclerosis in patients with chronic kidney disease (CKD).

Methods: A total of 172 CKD patients underwent renal biopsy at our department between 2010 and 2013. We excluded patients receiving calcineurin inhibitors etc and those with diseases such as vasculitis. We then selected patients whose pulse wave velocity between the brachial and ankle (baPWV) had been measured, leaving us with 102 patients for analysis. Small artery sclerosis was analyzed according to mean scores (small artery intimal thickening grade) converted from semi-quantitative evaluations of the degree of intimal thickening of arcuate arteries and relatively thick interlobular arteries (diameter: ≥200 μm).

Results: The mean values for patients' age, body mass index, blood pressure, estimated glomerular filtration rate (eGFR), HbA1c, and baPWV were as follows: 44 years, 24 kg/m^2, 120/74 mmHg, 73 ml/min/1.73 m^2, 5.6%, and 1393 cm/s, respectively.Positive correlations were observed for baPWV with age (r=0.69, P<0.001), small artery intimal thickening grade (r=0.63, P<0.001), pulse pressure (r=0.49, P<0.0001), and HbA1c (r=0.30, P=0.0008). A negative correlation was observed between baPWV and eGFR (r=-0.57, P<0.0001). Multiple logistic analysis of age, sex, hypertension, HbA1c, eGFR, smoking habit, LDL cholesterol, and high baPWV level (mean level or higher) was performed to investigate determination factors for severe small artery sclerosis (median or higher small artery intimal thickening grade). This revealed that high baPWV level was a significant factor (OR=3.8, P=0.03).

Conclusions: Results indicated that aortic stiffness was related to renal small artery sclerosis in CKD patients. Aortic stiffness may be involved in promoting the progression of organ dysfunction via small artery sclerosis in hypertensive target organs.

TH-PO654

The Amount of Albuminuria Does Not Contribute to the Development of Cardiovascular Disease in Diabetic Patients with Renal Insufficiency

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Background: Diabetic patients are likely to progress from normoalbuminuria (NA) to microalbuminuria (MA), and eventually to overt proteinuria (P) coincided with renal insufficiency. Recently, however, several studies have demonstrated that diabetic patients show diverse renal presentations and that 20-40% of patients are normo- to micro-albuminuric despite decreased renal function. In this study, the renal and cardiovascular (CV) outcomes in normo-, micro-, or macro-albuminuric diabetic patients with renal insufficiency were investigated.

Methods: Data from 1,136 diabetic patients with chronic kidney disease stage III or IV between 2007 and 2009 were retrospectively collected. Subjects were divided into NA, MA, and P groups. The primary outcome was new-onset CV events or death, and the secondary outcome was the onset of end-stage renal disease (ESRD). Cox proportional hazard analysis was performed to evaluate the association of albuminuria with the clinical outcomes.

Results: The mean age was 61.7±10.1 years and the mean estimated glomerular filtration rate was 38.9 ml/min/1.73m^2. Among 1,136 patients, 255 patients (22.4%) were NA, 275 patients (24.2%) were MA, and 606 patients (53.3%) were P group. During a mean follow-up duration of 44 months, the development of CV disease was not different among the three groups (P=0.67). However, progression to ESRD was significantly more observed in the P group compared to the NA and MA groups (P<0.001). Multivariate Cox analysis revealed that P was an independent predictor of progression to ESRD (odds ratio=2.414, 95% confidence interval=1.730-3.369, P<0.001), whereas the amount of albuminuria was not a risk factor for the development of CV disease.

Conclusions: There are significant and substantial differences in the clinical outcomes of diabetic patients with renal dysfunction according to the degree of albuminuria. In particular, CV outcome was comparable, while renal outcome was significantly different according to the amount of albuminuria in diabetic patients with renal insufficiency.

TH-PO655

Crossover Randomised Clinical Trial to Evaluate the Antialbuminuric Effect of Three Different Types of Diuretics (Spironolactone, Hydrochlorothiazide and Hydrochlorothiazide + Amiloride) on Top of RAAS Blockade in Proteinuric Nephropathies

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Background: Addition of diuretics enhances the effects of RAAS blockade on residual albuminuria in patients with chronic proteinuric nephropathies. However, comparative studies to evaluate the antialbuminuric effect of different diuretics are lacking. We designed a prospective crossover study to compare the effects of spironolactone (SR), hydrochorothiazide (HCT), or HCT plus amiloride (A) administered on top of RAAS blockade in patients with proteinuric nephropathies.

Methods: Patients with residual urine albumin-to-creatinine ratio (ACR)> 300 mg/g on top of enalapril therapy (40 mg daily) and CKD stages 1-3 were selected for the study. Patients were given SR (25 mg/day), HCT (50 mg/day) or HCT+A (50 mg+5mg /day), respectively during three treatment periods of 4 weeks in a ramdom order. Treatment periods were separated by a washout period of one month. Enalapril (40 mg/day) was maintained throughout treatment.

Results: Twenty nine patients (54 ± 12 years) were included. 15 patients (52%) were diabetics. ACR significantly decreased in the three groups

% (95% CI)	SR	HCT	HCT+A
ACR (mg/g)	-31.1*(-7.7,-54.4)	-52.2*(-30.3,-64.7)	-63.1*(-34.4,-78.6)
eGFR (ml/min/1.73m2)	-5.4*(1.6,-9.2)	-11.6*(-2,-18.4)	-10.4*(2.1,-5.9)
Mean arterial pressure (mmHg)	-0.7(0.6,-4.6)	-0.6(0.6,-3.8)	-2.4*(-0.2,-5.9)
Body weight (kg)	-1.2*(0.2,-2.2)	-0.5(0.4,-1.7)	-0.9*(0.2,-2.2)

*p<0.05 (intra-group analysis)

: -31.1% (95% CI –7.7%, -54%, p<0.01) in the group assigned to SR, -52.2% (95% CI -30.3%, -64.7%, p<0.01) in the group assigned to HCT and -63.1% (95% CI -34.4%, -78.6%, p<0.01) in the group assigned to HCT+A. Body weight, blood pressure (BP) and estimated GFR (eGFR) decreased in the three groups, but albuminuria reduction was independent of these changes.

Conclusions: SR, HCT and the combination of HCT+A induced a significant and remarkable reduction in albuminuria. These results are important for the design of clinical trials. The possible renoprotective consequences of diuretic-induced albuminuria reduction should be evaluated by long-term prospective trials.

TH-PO656

The Risk of Cardiovascular Events in Patients with Advanced Glomerulonephritis Is Not Higher Than Those with Other Causes of Chronic Kidney Disease

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Background: The risk of cardiovascular disease (CVD) in glomerulonephritis (GN) is poorly understood but has been suggested to be high, possibly due to disease-related inflammation promoting atherosclerosis. Therefore, we hypothesize that the risk of CVD in CKD patients with GN should be higher than other CKD patients with similar CV risk factors.

Methods: 2197 patients from CANPREDDICT (N=2544), a prospective cohort study of patients with stages 2-4 CKD with 3 years of follow-up and centrally adjudicated CVD events, were eligible for analysis. GN patients (N=272) were 1:1 direct and propensity score matched to non-GN patients based on age, eGFR, diabetes, prior CVD and sex. Time from cohort entry to CVD events was compared using a shared frailty Cox survival model.

Results: Pre and post matching data is shown in Table 1.

	Pre matching		Post matching	
	GN (288)	Non GN (1899)	GN (272)	Non GN (272)
Age (mean; yrs)	58.9	69.7	60.5	60.4
eGFR (mean; mls/min)	27.7	27.0	27.0	27.1
Caucasian (no, %)	241, 83.7	1703, 89.7	236, 86.8	243, 89.3
Female (no, %)	99, 34.4	715, 27.7	95, 34.9	95, 34.9
Diabetes (no, %)	58, 20.1	1018, 53.6	57, 21.0	61, 22.4
IHD/heart failure (no, %)	82, 28.5	911, 48.0	79, 29.0	72, 26.5

CVD events occurred in 8.7% (N=25) versus 17.8% (N=388) of GN compared to non-GN CKD patients. After matching, CVD events occurred in 9.2% (N=25) of both GN and non-GN CKD patients (HR 1.01 95%CI 0.58-1.78 p=0.96). In sensitivity analyses, GN and non-GN CKD patients had similar CVD risk in subgroups with diabetes (21.4% versus 21.4%, HR 1.03 95%CI 0.46-2.29 p=0.94) or without diabetes (6.1% versus 8.2%, HR 0.75 95%CI 0.36-1.59 p=0.45).

Conclusions: Patients with advanced CKD due to GN do not have a higher risk of CVD compared to otherwise similar CKD patients without GN. These novel findings question the belief that GN contributes directly to CV risk, and emphasizes the need to further prospectively study CVD at earlier stages of GN.

TH-PO657

Increased Malignancy Risk for Patients with ANCA-Associated Vasculitis

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Background: Recent studies showed a relatively high incidence of malignancies during long term follow-up of patients with ANCA-associated vasculitis (AAV). In this study, we assessed the risk of developing a malignancy for patients with AAV in comparison to the risk for the general population and investigated whether duration of immunosuppressive therapy was related to the development of malignancies.

Methods: 143 patients with biopsy proven AAV diagnosed between 1991 and 2013 at LUMC, The Netherlands, are included in this study. The development of a malignancy after AAV diagnosis was assessed using PALGA, a Dutch national pathology database. To compare the incidence of malignancies in our AAV cohort with that of the general population, Standardized Incidence Ratios (SIRs) were calculated using the Netherlands Cancer Registry incidence rates. To investigate an association between the duration of immunosuppressive therapy and the development of malignancy, the duration of therapy was assessed in 39 patients.

Results: 37 patients developed 82 malignancies during a mean follow-up of 9 years. These comprised 63 skin cancers (42 basal cell carcinomas, 20 squamous cell carcinomas and 1 melanoma), 3 breast cancers, 3 prostate cancers, 3 colon cancers, 2 lung cancers, and a number of infrequently occurring malignancies. Compared to the general population, patients with AAV had a 2.9 (95% CI = 2.2 – 3.8, p=0.01) times increased risk to develop a malignancy. 13 of 39 patients of whom theraphy data were available developed a malignancy during follow-up. Cox-regression analysis did not show a significant association between the duration of cyclophosphamide or azathioprine treatment in patients with and without a malignancy.

Conclusions: AAV patients have a significantly higher risk to develop malignancies compared to the general population. It is generally assumed that this finding could be related to the immunosuppressants these patients receive. However, in the current study we found no association between the duration of immunosuppressive therapy and the development of malignancies in AAV.

TH-PO658

Predictors of Hyperkalemia Risk following Hypertension Control with Aldosterone Blockade According to the Presence or Absence of Chronic Kidney Disease

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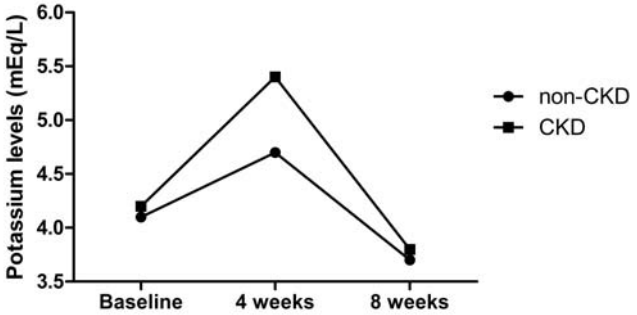
Background: Aldosterone antagonists have proven efficacy for management of hypertension and proteinuria reduction; however, they are not widely used due to risk of hyperkalemia. This study assesses predictors of hyperkalemia risk following hypertension control with aldosterone blockade according to the presence or absence of chronic kidney disease.

Methods: Patients used in the analysis were seen between January 1, 2000 through November 30, 2012. 6575 patients with hypertension were evaluated for safety of aldosterone blockade added to preexisting BP-lowering regimens. Hyperkalemia was defined more than serum K 5.0 mEq/L. All patients were on three mechanistically complementary antihypertensive agents including a diuretic and a renin-angiotensin system blocker. Patients were evaluated after 4 and 8 treatment weeks. Incidence of hyperkalemia, significant renal dysfunction (reduction eGFR≥30%) and adverse effects according to the presence or absence of chronic kidney disease was assessed.

Results: After 8 weeks of treatment, the portion of hyperkalemia (serum K ≥ 5.0 mEq/L) according to the presence or absence of chronic kidney disease was 0.5 %, 2.6% respectively.



In logistic regression for predicting hyperkalemia following aldosterone antagonism, predictors of hyperkalemia risk were old age, CKD, male, basal hyperkalemia and reduction in eGFR.

Conclusions: Spironolactone was well tolerated in selected patients with CKD. The risks of serious hyperkalemia or significant renal deterioration appear to be low, particularly after the 2nd month of treatment. Strict monitoring over the first month of treatment followed by standard surveillance as for ACE inhibitors and ARBs is suggested.

TH-PO659

The Relationship Between Red Blood Cell Distribution Width (RDW) and the Resistance to Erythropoiesis-Stimulating Agents (ESA)

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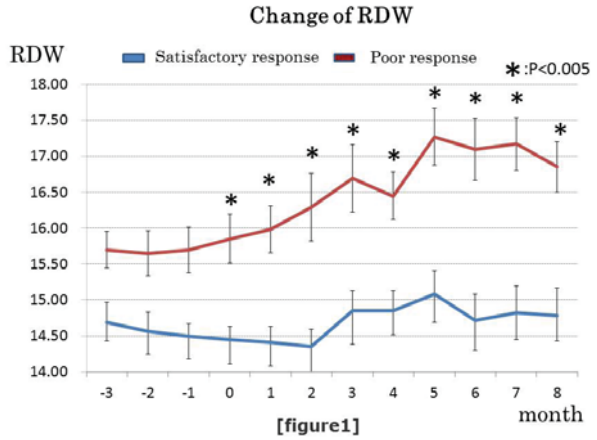
Background: There are numerous reports on the relationship between high values of RDW and the onset of cardiovascular events, but there are very few reports on RDW in the renal anemia and ESA-resistance. In the present study, we evaluated patients with chronic kidney disease(CKD) and stratified them according to their responses to ESA.We report the treatment status of patients with renal anemia and the changes in RDW in these patients.

Methods: We studied 40 patients with CKD undergoing treatment for renal anemia who regularly visited our hospital. All of these patients had switched treatment drugs from darbepoetin to epoetin beta pegol. Using the following formula, response to ESA was determined by ESA doses given and Hb levels measured after switching drugs: (mean ESA dose during the 8-month period)/(mean Hb level during the 8-month period)/body weight. Patients with responses of <0.2 were assigned to the satisfactory response group, and those with ≥0.2 were assigned to the poor response group. Changes in Hb and RDW during the 8-month period after switching treatment drugs were the primary focus of this study.

Results: Hb levels increased in both the satisfactory response group (10.26 ± 0.22 g/dl→10.85 ± 0.24 g/dl) and the poor response group (9.05 ± 0.22 g/dl→9.88 ± 0.30 g/dl). RDW after switching drugs was significantly elevated in the poor response group.There was no significant difference in serum ferritin of both groups.



[figure1]

Conclusions: In the group of patients with poor response to ESA, a greater dose of ESA was given in order to correct anemia, and RDW significantly increased. It was suggested that this increase in RDW may be a factor that worsens mortality in patients with high resistance to ESA in the group that received greater doses of ESA.

TH-PO660

Association between Muscle Mass Estimates, Strength, and Physical Performance among Patients with Advanced Chronic Kidney Disease

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Background: Chronic Kidney Disease may be associated with deficits in muscle mass and function. Few studies have rigorously evaluated the association between muscle mass estimates and muscle function in patients with advanced CKD.

Methods: We performed dual-energy X-ray absorptiometry (DXA) assessment of appendicular lean mass (ALM), bio-electrical impedance analysis (BIA) of fat free mass (FFM), and 24-hr urine creatinine collection (UCr) in individuals of age >=45 and <80 years and eGFR >=15 and <=45 ml/min/1.73m^2. Lower extremity strength was assessed via 1 repetition-maximum (1RM) testing using a Life Fitness seated leg-press machine (Rosemont, Il). The short-physical performance battery (SPPB) was used to assess functional status. We used Spearman correlation coefficients to compare the muscle mass and function measures, and Tobit regression to analyze associations with SPPB.

Results: At the time of this writing, 21 participants have undergone all relevant measurements. The median (IQR) age was 63.5 (56.5 - 71) years and 67% were male. Median (IQR) eGFR was 25 (20 - 37) ml/min/1.73m^2. The median (IQR) BMI was 28.4 (27.6 - 30.8) kg/m^2. 1RM capability ranged from 100 to 410 pounds. There were strong correlations between DXA assessment of appendicular lean mass and BIA estimates of FFM (rho=0.97, p<0.001). ALM, FFM, and UCr were correlated with 1RM (rho=0.60, 0.63, and 0.54 respectively, p<0.05). Only 1RM was associated with SPPB score (p=0.04).

Conclusions: In this small population of patients with advanced CKD, BIA, DXA, and UCr excretion all demonstrated significant associations with quadriceps strength but not functional scores. Further studies correlating lower extremity strength with functional status, risk of falls, and other morbidities are necessary.

Funding: NIDDK Support

TH-PO661

Failure to Reach Target Blood Pressure Is Related to Fluid Overload in CKD Patients Treated for Hypertension

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Background: It is well recognized that hypertension is highly prevalent in patients with CKD. Despite effective medication, target blood pressure is often not achieved. In this study, we tested the hypothesis that not reaching target blood pressure is related to extracellular fluid volume (ECFV) expansion. Moreover, we related fluid overload to arterial stiffness.

Methods: In 26 subsequent hypertensive CKD patients on their routine visit to our outpatient clinic, fluid volume status was assessed using multifrequency bio-impedance (Body Composition Monitor, Fresenius). Fluid overload was defined as exceeding the predicted normal ECFV by >1.1 L. Blood pressure was assessed using an automated device, augmentation index and pressure using a planometry (SphygmoCor, Atcor). Clinical and laboratory data were obtained from the charts and the EMR.

Results: Of the 26 patients, 9 were fluid overloaded; all of these patients were not treated to target (<140/90 for CKD; <130/80 for CKD and DM). Only 2 patients that were not treated to target were normovolemic. Clinical characteristics of both groups are in table 1. Augmentation index was significantly elevated hypertensive patients.

	Fluid expansion (n=9)	No fluid expansion (n=17)	P-value
Age, years	65±11	53±19	NS
Gender, M/F	7 / 2	10 / 7	NS
BMI, kg/m^2	27.0±1.6	32.2±4.5	NS
BP, mmHg	153±9 / 81±13	126±14 / 77±8	<0.0001 / NS
eGFR	43±10	57±26	NS
Fluid status, Δ from normal ECFV, L	1.5±0.4	-0.8±1.3	<0.001
On antihypertensive medication (not diuretic)	9/9	15/17	NS
Treated with diuretic	9/9	6/17	NS
Augmentation index, %	32±11	15±20	<0.05

Conclusions: This study indicates that fluid overload is likely an important but treatable cause for not achieving target blood pressure in CKD patients. The data also indicate that such fluid overload can not be readily appreciated from clinical parameters. Finally, this fluid overload was associated with increased augmentation index.

Funding: Pharmaceutical Company Support - The BCM was borrowed from Fresenius

TH-PO662

Uric Acid Associated with Decline of GFR in Diabetic Nephropathy

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Background: Diabetes is the major cause of chronic kidney disease (CKD). Evidence from epidemiological and prospective studies indicates that serum uric acid (UA) is a risk factor for development and progression of CKD and loss of kidney function. We evaluated the effect of serum UA on change in GFR in patients with type 1 diabetes (T1DM) in a previous conducted clinical trial.

Methods: Post hoc analysis of a prospective, double-blinded, clinical intervention trial of the long-term renoprotective effect of AT1 receptor blockade (losartan 100 mg) on progression of diabetic nephropathy in T1DM homozygous for the I (n=26) or D (n=27) allele of the ACE/ID polymorphisms. Mean follow-up time was 3 years (range 1.5 – 3.5). Serum UA was measured at baseline. Primary end-point was GFR measured with Cr51-clearance every 6 month. Effect of UA was tested in a linear regression model with and without adjustment for known progression factors (Gender, HbA1c, systolic blood pressure, cholesterol, baseline GFR and baseline urinary albumin excretion rate (UAER)).

Results: Mean baseline UA was 5.7 mg/dL (SD ±1.8), GFR 87 mL/ min/ 1.73m2 (SD ±23), geometric mean of UAER 1023 mg/ 24h (IQR, 631 – 1995). Similar association between UA and change in GFR was present for the two ACE/ID polymorphisms. In an unadjusted linear model UA was positively associated with decline in GFR (r^2 = 0.06, p = 0.09). After adjustment for known progression factors the association increased to a significant level (r^2 = 0.35, p = 0.011). In the backward elimination UA remained in the model, together with baseline UAER and baseline GFR (r^2 = 0.26, p = 0.0031).

Conclusions: Uric acid was positively associated with decline in GFR in type 1 diabetic patients with nephropathy. UA was a significant predictor together with UAER and baseline GFR. The clinical significance of UA is currently investigated in a multicenter clinical trial (PERL Study).

Funding: Private Foundation Support

TH-PO663

Effect of Ascorbic Acid on Endothelial Dysfunction and Oxidative Stress in Chronic Kidney Disease Keith Gillis,[1] Kathryn K. Stevens,[1] Scott Morris,[2] Christian Delles,[1] Patrick B. Mark.[1] *[1]Inst of Cardiovascular and Medical Science, Univ of Glasgow, United Kingdom; [2]Renal and Transplant Unit, Western Infirmary Glasgow, United Kingdom.*

Background: The cardiovascular risk associated with chronic kidney disease (CKD) is not fully explained by conventional factors. Oxidative stress is a hallmark of CKD. We investigated the use of ascorbic acid to reduce oxidative stress and endothelial dysfunction (ED) in CKD.

Methods: We performed a crossover study of normal saline (NS) and ascorbic acid (AA) in patients with CKD and matched hypertensive patients with normal renal function (HTN). Biomarkers of oxidative stress were measured after NS, at 10 and 60 minutes after AA (superoxide (O_2^-) production, antioxidant capacity (TAC), isoprostanes and glutathione oxidation ratio (GSH:GSSG), plus asymmetric dimethylarginine (ADMA) and vitamin C). ED was measured as flow mediated dilatation (FMD) using brachial artery ultrasound.

Results: Forty patients were studied (aged 58±11 years; mean blood pressure 145/88±15/12mmHg). eGFR was lower in the CKD group (28±9ml/min/1.73m^2 versus 95±12ml/min/1.73m^2,p<0.001). The CKD group had lower vitamin C (p=0.003), higher ADMA (p=0.001), with higher TAC (p=0.03) and numerically higher O_2^- production (0.514 versus 0.371 AU; p=NS). eGFR was found to be closely associated with ADMA (r=0.59 p< 0.001), ROS (r=0.33 p<0.05), and TAC (r=0.35 p<0.05). No difference was observed in GSH:GSSG or isoprostanes. Baseline FMD was 3.9±2.8% in CKD and 4.1±2.0% in HTN (p=NS), and did not significantly improve after AA, in either CKD (5.1±2.9%) or HTN (5.0±2.9%). In both groups there was a significant rise in both O_2^- production (CKD p<0.05, HTN p<0.05) and TAC (CKD p<0.001 HTN p<0.05) following AA, but no change in GSH:GSSG. In the CKD group there was a significant reduction in ADMA post AA (p=0.03). This was not observed in HTN group.

Conclusions: CKD patients are vitamin C deplete and have elevated ROS production, but markers of OS are otherwise not different to matched patients with HTN. No significant difference in FMD was seen in either group following administration of ascorbic acid but reduction in ADMA levels in the CKD group suggests a mechanism by which antioxidants may have beneficial effects.

Funding: Private Foundation Support

TH-PO664

The Associatoin between Anemia and Abnormal Bleeding Times in Patients with Chronic Kidney Disease Ha Yeon Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Mediciney, Chonnam National Univ Medical School, Gwangju, Korea.*

Background: Platelet dysfunction associated hemorrhagic complications and chronic anemia are often encountered in patients with chronic kidney disease. Decreased hemoglobin (Hgb)level reduces the rheologic interactions between vessel walls and platelets and weakens the ability of red blood cells to scavenge nitric oxide. The present study aimed to evaluate the association of anemia and abnormal bleeding time in patients with chronic kidney disease.

Methods: We retrospectively analyzed the data of 1022 (60.24 ± 15.89 years, men 58.7%). patients with chronic kidney disease, which was estimated glomerular filtration rates (eGFR) < 60 mL/min/1.73 m^2. Age, gender, Hgb, hematocrit (Hct), platelet (PLT), blood urea nitrogen (BUN), creatinine (Cr) levels were determined. Bleeding times were estimated using a platelet function analyzer 100 (PFA-100).

Results: Patients with prolonged bleeding time (bleeding time > 182 sec) were older age and lower eGFR, Hgb, Hct and PLT, and higher BUN and Cr levels compared to those with normal bleeding time.The prevalence of abnormal bleeding time was found to be higher as Hgb declined (25.0% for patients with Hgb ≥ 10 mg/dl (n= 595), 31.9 % for patients with 8 ≤Hgb < 10 mg/dl (n= 343), and 43.4 % for patients with Hgb <8 mg/dl (n= 84), P < 0.001, respectively). eGFR was decreased as a Hgb declined (29.8 ± 19.0 mL/min/1.73m^2 in patients with Hgb ≥ 10 mg/dl, 16.0 ± 13.0 mL/min/1.73m^2 in patients with 8 ≤Hgb < 10 mg/dl, and 10.3 ± 8.61 mL/min/1.73m^2 in patients with Hgb < 8 mg/dl, P < 0.001, respectively). Multivariate analysis revealed that age (OR,1.019; CI, 1.010 – 1.029), 8 mg/dl ≤Hgb < 10 mg/dl (OR,1.214; CI, 0.873 – 1.686), Hg < 8 mg/dl (OR,1.820; CI, 1.084 – 3.055), 15 mL/min/1.73m^2≤ eGFR < 30 mL/min/1.73m^2 (OR,1.616; CI, 1.053 – 2.481), eGFR < 15 mL/min/1.73m^2 (OR,1.668; CI, 1.160 – 2.397), thrombocytopenia (PLT < 150 × 10^9/L) (OR, 2.659; 95% CI, 1.981–3.569) were independently associated with prolonged bleeding time.

Conclusions: Severe anemia (Hgb < 8mg/dl) is independently associated with prolonged bleeding time in chronic kidney disease, even after adjusting for eGFR and other potential confounders.

Funding: Clinical Revenue Support

TH-PO665

Effect of Ferumoxytol on Fatigue in Patients with Iron Deficiency Anemia and Kidney Disease Naomi V. Dahl, William Strauss, Kristine Bernard. *AMAG Pharmaceuticals, Inc.*

Background: Many patients with IDA do not tolerate or adequately respond to oral iron and live with chronic anemia and related negative effects on health-related quality of life (HRQL). While the effect of anemia on HRQL in patients with CKD has been studied, data from double-blind placebo-controlled trials are lacking. The objective of this analysis was to explore the impact of IV iron on patient reported outcomes in IDA patients with CKD and a history of unsatisfactory oral iron therapy or in whom oral iron cannot be used.

Methods: This is a subgroup analysis of a randomized, double- blind, placebo-controlled, Phase 3 study (NCT01114139) of 808 patients (Hgb <10 and >7g/dL, TSAT<20%, and GFR>30 mL/min/1.73m^2), randomized 3:1 to either 2 injections of 510 mg of ferumoxytol (FER) 5±3 days apart or normal saline placebo (PL). Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), and Linear Analog Scale Assessment -Energy (LASA-E) were assessed from Baseline (BL) to Week 5. Patients with evidence of probable CKD were identified by one or more of: GFR<60mL/min/1.73m^2, evidence of kidney damage from medical history, proteinuria >30mg.

Results: 132 patients with a variety of underlying conditions (GI disorders, 39; abnormal uterine bleeding, 36; cancer, 9; other, 48), met criteria for probable CKD. Their BL scores (FACIT-F; FER, n=95, 24.0; PL, n=37, 27.2) were much lower than general U.S. population norms (40.1) and close to those reported in anemic cancer patients (23.9 for FACIT-F, 37.2 for LASA-E). By Week 5, in parallel to a significant increase in mean Hgb (FER 2.2 versus PL -0.1, p<0.0001), FER-treated patients demonstrated significantly greater improvements in mean FACIT-F score than PL, (11.1 versus 6.8, p=0.039), exceeding the minimum important difference (MID=3) and approaching population norms in the FER group. Improvement in LASA-E from BL (35.9) to week 5 in FER-treated patients (16.0, 95% CI 10 to 22) also exceeded the MID (9.61).

Conclusions: This analysis found that patients with IDA and evidence of probable CKD, unsuccessfully treated with oral iron, had very poor baseline HRQL scores and that in concert with increased Hgb, FER treatment resulted in significant improvements in measures of fatigue and energy.

Funding: Pharmaceutical Company Support - AMAG Pharmaceuticals, Inc.

TH-PO666

Benefits of Early Intervention with ESA Therapy on Renal Survival in Patients with CKD Tadao Akizawa,[1] Michiko Kumagai,[2] Yasuo Ohashi.[3] *[1]Showa Univ School of Medicine; [2]Chugai Pharmaceutical Co., Ltd.; [3]Chuo Univ.*

Background: To investigate the relationship between Hb levels of CKD patients at the initiation of ESA therapy and renal outcome in Japanese non-dialysis CKD patients with anemia, the Japan Erythropoietin Treatment survey for STarting hemoglobin level in REnal Anemia Management (JET-STREAM) study was conducted.

Methods: In this prospective, multi-center, observational study, non-dialysis CKD patients with anemia who had never been treated with ESAs were included. Eligible patients were divided into three groups depending on their Hb levels at initiation of epoetin beta therapy (Group I: 10 ≤ Hb < 11 g/dL, Group II: 9 ≤ Hb < 10 g/dL, and Group III: Hb < 9 g/dL). The primary endpoint was time to first occurrence of any renal event (RRT, Cr doubling or eGFR < 6.0 mL/min/1.73 m^2). To account for lead time bias, the primary endpoint was analyzed from the time when the Hb level dropped below 11 g/dL for the first time. Dynamic treatment regime was applied for group comparisons using the IPW Cox regression model to adjust the baseline and time-dependent selection bias in the artificially censored data.

Results: A total of 1113 patients were eligible for primary endpoint analysis. Since there were patients with extreme weights when comparing Groups I and II, the weights larger than 99th were set to the value of 99th percentile. After adjusting the artificial censoring using the IPW methods, the risk of renal events was significantly higher in Group III compared with Group I (HR, 2.52; 95% CI, 1.98 to 3.21; P < 0.0001); the risk was also higher in Group II compared to Group I (HR, 1.48; 95% CI, 0.91 to 2.40, *P = 0.11). When we used a weight truncated at the 98th percentile for the comparison between Groups I and II in the sensitivity analysis, the risk of renal events was significantly higher in Group II compared with Group I (HR, 1.29; 95% CI, 1.02 to 1.64, P* = 0.033).

Conclusions: The JET-STREAM study prospectively demonstrated that initiation of ESA therapy at an early stage when Hb levels have declined below 11 g/dL reduces the risk of renal events in Japanese CKD patients with anemia.

Funding: Pharmaceutical Company Support - Chugai Pharmaceutical Co., Ltd

TH-PO667

Mortality Risk of Darbepoetin Alfa versus Epoetin Alfa in Patients with Chronic Kidney Disease: Systematic Review and Meta-Analysis of Randomized Trials Emilee R. Wilhelm-Leen, Wolfgang C. Winkelmayer. *Stanford Univ School of Medicine, Palo Alto, CA.*

Background: Darbepoetin alfa (DPO) has been shown to possess similar efficacy compared with epoetin alfa (EPO), yet little is known about the comparative safety of these agents. We conducted a systematic review of randomized trials comparing DPO *versus* EPO in patients with chronic kidney disease (CKD) or end-stage renal disease (ESRD) and tested for differences in mortality in meta-analysis.

Methods: We systematically searched Medline (all years) and documents submitted by Amgen to the U.S. Food and Drug Administration. Two reviewers identified suitable reports and independently abstracted data with resolution *via* conference. We used random effects meta-analysis to estimate the summary odds ratio for mortality.

Results: From 155 search hits, we identified 5 trials; 2 additional unpublished trials were identified from FDA documents. Cumulatively, 1808 (820 EPO, 988 DPO) patients received study drug (modified intent-to-treat) and were followed for up to 52 weeks (wks). The summary odds ratio was 1.34 (95% CI: 0.87-2.04). No treatment heterogeneity across studies was detected (Q-statistic=4.3; P=0.64).

	Population	Duration	Blinding	Mortality (DPO)	Mortality (EPO)	Odds Ratio
Locatelli, et al.	CKD	24 wks	Open Label	5/129 (3.9%)	1/37 (2.7%)	1.45
Allon, et al.	ESRD (HD)	52 wks	Open Label	4/32 (12.5%)	2/15 (13.3%)	0.93
Nissenson, et al.	ESRD (HD)	28 wks	Double-blind	9/169 (5.3%)	23/335 (6.9%)	0.76
Vanrenterghem, et al.	ESRD (HD/PD)	52 wks	Open Label	41/346 (11.9%)	11/173 (6.4%)	1.98
Li, et al.	ESRD (HD)	24 wks	Open Label	0/22 (0%)	1/23 (4.4%)	0
Amgen #20010125	ESRD (HD)	28 wks	Double-blind	11/200 (5.5%)	7/206 (3.4%)	1.65
Amgen #980211	ESRD (HD)	20 wks	Open Label	5/90 (5.6%)	1/31 (3.2%)	1.76
Summary Odds Ratio (95% CI; DPO vs. EPO)	-	-	-	-	-	**1.34 (0.87-2.04)**

Conclusions: Only few studies have randomized patients with CKD or ESRD to DPO *versus* EPO. While no significant difference in mortality was detected between DPO and EPO, the confidence interval was wide and remained compatible with substantial harm in patients randomized to DPO. Further comparative effectiveness studies using observational data are warranted to better delineate the safety profiles of DPO *versus* EPO.

Funding: NIDDK Support

TH-PO668

Novel Side Effect Profiles of Sodium Ferric Gluconate IV Injection (Ferrlecit®) in CKD Patients Not on Dialysis

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Background: Iron deficiency is a common sequelae of CKD, with an increase in prevalence and severity with gradual loss of kidney function. Ferrlecit®(sodium ferric gluconate complex in sucrose injection) is an IV iron product indicated for the treatment of iron deficiency anemia in patients undergoing chronic HD who are receiving supplemental EPO therapy. Existing studies have suggested that 500mg of Ferrlecit® is well-tolerated when administered over four hours and to date, no study has reported any episodes of unmanageable life threatening adverse events.

Methods: This study was approved by the Research Ethics Board of our Institution. This study was designed as an open-label, dose escalation pilot study to assess the safety and efficacy of Ferrlecit®. Six pre-dialysis patients with anemia were treated with intravenous Ferrlecit® administered at 250mg in 100mL normal saline over 2 hours following the pre-test dose. Patient demographic, clinical, and biochemical parameters were assessed at baseline. Post-infusion serious adverse events were reported on a severity of index scale.

Results: GI symptoms including abdominal pain, cramping or diarrhea were the most commonly reported complaints and all patients experienced these symptoms. Three patients had hemodynamically significant adverse events by the end of infusion. One of the patients experienced hemodynamic adverse events characterized by bradycardia and severe hypotension. One responded to fluid volume, anti-histamine and hydrocortisone. The other required, in addition, temporary inotropic support. The study was terminated due to the severity of adverse effects.

Conclusions: The milder reaction occurred at a dose of 4mg/kgBW, the moderate reaction occurred at a dose of approximately 4.5mg/kg BW, and the severe reaction occurred at a dose of 5.1mg/kg BW. In our study, since there was no difference in the peak post administration drug levels as a result of the constant rate of administration, the data suggests a hypothesis that there may be a body weight limitation on daily doses of 3.5mg/kg/day.

TH-PO669

Altered Clot Structure Predicts Mortality in Hemodialysis Patients

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Background: Chronic kidney disease (CKD) is associated with both increased cardiovascular mortality and coagulopathy. Patients with CKD are known to have a prothrombotic clot structure characterized by small pores and resistance to fibrinolysis. However, the impact of altered clot structure on outcome in CKD patients is unknown. Therefore, the aim of the current work was to investigate whether clot structure is associated with outcome in CKD patients.

Methods: Fibrin clot structure and lysis were determined in plasma of 172 hemodialysis patients (59±11y, 54% male) using a validated turbidimetric assay. Plasma levels of fibrinogen were analysed by ELISA. Fibrinogen was purified from 6 healthy control individuals and 6 patients with CKD stage IV using a calcium dependent IF-1 monoclonal antibody.

Results: Plasma of CKD patients exhibited a denser clot structure when compared to controls with normal renal function (0.44±0.04 (CKD) versus 0.26±0.01 (control) AU, respectively; p<0.01) and prolonged clot lysis (1274±134 versus 468±38 sec respectively; p<0.01). Kaplan Meier analysis revealed that CKD patients with a denser clot structure had an increased mortality risk (log-rank p=0.009). Multivariate Cox regression model (adjusted for age, diabetes, gender, duration of dialysis and fibrinogen) confirmed the massive, independent risk prediction of denser clots (hazard ratio 76, confidence interval= 3 – 1800; p=0.007). Fibrinogen levels were not significantly related to outcome. To further analyze whether the effect of clot structure on mortality was dependent on fibrinogen we purified fibrinogen from healthy controls and CKD patients. Clot density of purified fibrinogen did not differ between both groups (0.15±0.01 and 0.16±0.01 AU, respectively; p=0.6) as did lysis time (3294±182 sec and 3290±209 sec, respectively; p=0.9).

Conclusions: A denser clot structure is a potent, independent predictor of mortality in hemodialysis patients. Using purified fibrinogen clot structure did not differ between CKD patients and controls, suggesting alterations of plasma and not fibrinogen as the driving factors for uremic coagulopathy.

TH-PO670

Post Hoc Analysis of Iron Indices in Dialysis Patients with Lower versus Higher Baseline Ferritin in a Sucroferric Oxyhydroxide Study

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Background: This *post hoc* analysis evaluated the effect of the iron-based phosphate binder, sucroferric oxyhydroxide (SFO; VELPHORO®/PA21) versus sevelamer carbonate (SEV) on iron indices by baseline ferritin levels in dialysis patients.

Methods: 1,059 patients were randomized to SFO (1.0–3.0 g/day) or SEV (2.4–14.4 g/day) for 12 weeks' dose titration plus 12 weeks' maintenance. Eligible patients enrolled in a 28-week extension study.

Results: 549 patients completed the extension study. Changes in mean iron indices stratified by median baseline ferritin levels are shown in the Table. More patients with higher (SFO: 81%; SEV: 88%) versus lower (SFO: 67%; SEV: 72%) baseline ferritin received concomitant intravenous iron.

Table: Mean values at baseline and mean changes at Week 52 endpoint in iron indices by baseline ferritin levels (< or ≥ median 604 ng/mL).

		SFO N=322		SEV N=227	
		< median n=172	≥ median n=150	< median n=102	≥ median n=125
Ferritin, ng/mL	BL	304.4	985.1	301.7	1043.4
	Δ BL to endpoint	211.0	88.2	144.3	13.7
	P-value for Δ from BL	<0.0001	0.0119	<0.0001	0.7465
TSAT, %	BL	22.5	31.0*	23.5	30.5
	Δ BL to endpoint†	6.2	2.9*	2.5	0.17
	P-value for Δ from BL	<0.0001	0.0778	0.0382	0.9120
Hb, g/L	BL	110.6	114.6	113.8	113.4
	Δ BL to endpoint‡	4.8	−2.4	0.06	−1.8
	P-value for Δ from BL	0.0002	0.0485	0.9667	0.0786

Endpoint is last post-baseline non-missing value across both studies. *n=149.
†P=0.0483 and ‡P0.0153 for SFO vs SEV in < median group.
BL, baseline; Hb, hemoglobin; TSAT, transferrin saturation.

Conclusions: Changes in iron indices in patients with lower baseline ferritin levels were more pronounced with SFO versus SEV, likely due to low-grade iron absorption with SFO. In patients with a higher baseline ferritin level, no such differences were noted. There was no indication for a risk of iron overload with SFO in either of the subgroups.

Funding: Pharmaceutical Company Support - Vifor Pharma

TH-PO671

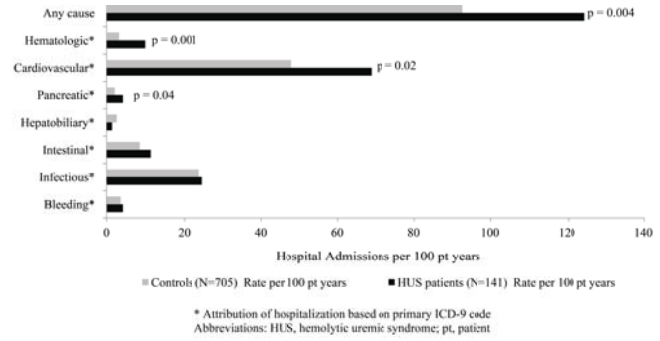
Consequences of Hemolytic Uremic Syndrome Among Dialysis Patients

Steven M. Brunelli,[1] Ami Claxton,[1] Sunil Mehta,[2] Emmanuel A. Anum.[1] *[1]DaVita Clinical Research, Minneapolis, MN; [2]Alexion Pharmaceuticals, Cheshire, CT.*

Background: Hemolytic uremic syndrome (HUS) is described by hemolytic anemia, low platelets, and renal impairment. Resultant thrombotic microangiopathy (TMA), affects nearly every organ system. Most HUS is bacterial in origin, but ~10% (including most patients who progress to end-stage renal disease [ESRD]) are due to genetic complement disease (atypical HUS). We conducted this analysis to better understand the potential consequences of HUS in ESRD patients.

Methods: We identified new dialysis patients at a single provider organization for ESRD due to HUS (ICD-9 code 283.11; n=217). These were propensity-score matched 1:5 to controls (n=1,085) on dialysis for ESRD from any other cause for age, gender, race, dry weight, insurance, access, comorbidities, and Charlson comorbidity index. Mortality and laboratory data were from health records; hospitalization data were from Medicare claims. Comparisons were made using Cox models and linear mixed models. Patients considered at risk until death, censoring, or end-of-study period (Mar 2013; Dec 2010 for hospitalizations).

Results: Compared to controls, HUS patients had significantly greater risk for hospitalizations overall (RR=2.3 [1.3-4.1]) and hospitalization for hematologic (RR=5.8 [1.9-15.9]), CV (RR=2.1 [1.1-4.0]), and pancreatic (RR=7.9 [1.1-59.8]) causes (Figure). Cases also had evidence of ongoing TMA: higher LDH (215.9 versus 193.9 U/L) and RDW (15.6% versus 15.3%), lower platelets (240.1 versus 248.1/mcL) and Hb (11.1 versus 11.3 g/dL), and more frequent spikes in LDH (rise in LDH > 100).



Conclusions: Dialysis patients with HUS had laboratory evidence consistent with ongoing TMA and were at significantly higher risk than matched controls for hospitalizations, particularly those due to CV, hematologic, and pancreatic disease. Trials are needed to see whether targeted therapy for HUS reduces hospitalizations.

Funding: Pharmaceutical Company Support - Alexion Pharmaceuticals

TH-PO672

Prevalence of Periodic Limb Movement Disorder (PLMD) in Dialysis Patients Seerapani Gopaluni, Mohamed S. Sherif, Sheera Sutherland, Kristine Ventura, David J. Meredith, Philip David Mason. *Renal Unit, Churchill Hospital, Oxford, United Kingdom.*

Background: Abnormal limb movement disorders (PLMD and Restless Legs Syndrome, RLS) are commonly reported in dialysis patients, causing sleep disruption, poor quality of life and increased cardiovascular morbidity. PLMD is marked by episodic jerking limb movements during sleep. Polysomnography establishes the diagnosis but is expensive. Leg worn accelerometers are validated to detect periodic limb movements (PLM) in the general population. We used these to establish the prevalence of PLMD in haemodialysis patients and correlated the PLM index-PLMI(PLM/hr) with sleep quality and overall quality of life measures. We performed simultaneous pulse oximetry to exclude movements occurring during hypoxic dips as the accelerometers pick up non-PLM movements associated with sleep apnoea.

Methods: Participants completed validated questionnaires (Pittsburgh sleep quality index (PSQI), International Restless Legs Syndrome Group Questionnaire, KDQOL-36) and wore an accelerometer on each leg and a wrist pulse-oximeter during sleep for 3 nights.

Results:

	n=35	p
Age Mean	59yr	
M:F	24:11	
Mean PLMI	64.26*	
Mean PSQI	8.07 (n=28)†	
Prevalence of high PLMI (>15)	74.29%	
Prevalence of PLMD (PLM affecting sleep)	14.28%	
Prevalence of RLS	42.85%	
PLMI in good sleepers vs poor sleepers	52 vs 72	0.34
PLMI in patients with RLS vs no RLS	89 vs 46	0.05
SF-12 MCS¶ scores in PLMD vs non-PLMD patients	31.8% vs 53%	0.0005
SF-12 PCS# scores in PLMD vs non-PLMD patients	46% vs 53%	0.08
Correlation between PLMI & RLS	0.31	0.09
Correlation between PLMI & PSQI	0.13	ns
Correlation between RLS & PSQI	0.36	0.06
* >15 movements/hr is considered significant, † >5-associated with bad sleep quality		
¶ Mental & # Physical Component Summary (sub components of KDQOL-36)		

Conclusions: This is the only study of PLMD in dialysis patients using accelerometers. Although the majority had a very high PLMI, sleep quality was not always affected. PLMI was higher in patients with RLS and those with PLMD perceived themselves to have relatively poor mental and physical health. These findings will form the basis of an intervention study to reduce PLMD in symptomatic patients and hopefully improve QOL.

Funding: Private Foundation Support

TH-PO673

Clinical Correlates of Urinary KIM-1 in Three Cohorts Sushrut S. Waikar, Meredith C. Foster, Gudeta D. Fufaa, Robert G. Nelson, Venkata Sabbisetti, Theodore E. Mifflin, Xiaoming Zhang, Dawei Xie, Harold I. Feldman, Josef Coresh, Vasan S. Ramachandran, Paul L. Kimmel, Chi-Yuan Hsu, Kathleen D. Liu, Joseph V. Bonventre. *CKD Biomarkers Consortium.*

Background: Kidney injury molecule-1 is expressed in proximal tubules following injury. Urinary KIM-1 (uKIM-1) is an AKI biomarker, but is also detectable in CKD. To better understand KIM-1 in CKD, we measured urinary levels in cohorts with a wide range of kidney function.

Methods: We measured uKIM-1/creatinine (uKIM-1/Cr) in 260 Pima Indians with type 2 DM, 361 participants from a nested case-control study of ESRD in the Atherosclerosis Risk in Communities (ARIC) study, and 2320 participants in the Chronic Renal Insufficiency Cohort (CRIC). We compared uKIM-1/Cr across cohorts and examined associations with demographic and clinical variables in each cohort and in a pooled analysis using linear regression.

Results: Median (10^{th} to 90^{th} %ile) uKIM-1/Cr (pg/mg) were 673 (130-2448) in Pima Indians, 795 (267-2622) in ARIC, and 1387 (396-4613) in CRIC. Levels above 2000 pg/mg, typically observed in established AKI, were present in 13.8% of Pima, 17.2% of ARIC and 35.6% of CRIC participants. The direction and statistical significance of associations were consistent across cohorts (Table 1): uKIM-1/Cr was higher with lower eGFR, higher ACR or PCR, older age, non-black race, higher SBP, and in subjects with DM. In multivariable adjusted analyses, DM was no longer associated with uKIM-1/Cr, whereas female sex was associated with higher levels.

Table 1. Associations of log (uKIM-1/Cr)	Unadjusted			Multivariable-adjusted
	Pima	ARIC	CRIC	All three cohorts
Median GFR ml/min/1.73m^2	129.7	67.9	41.5	
	β	β	β	β
GFR (per 10 ml/min/1.73m^2)	**-0.04**	**-0.20**	**-0.09**	**-0.03**
logACR	**0.21**	**0.20**	**0.14**	**0.18**
Age (per 10y)	**0.22**	**0.07**	**0.23**	**0.13**
Black race (vs. other)	-	**-0.31**	**-0.23**	**-0.38**
Women (vs. men)	0.09	-0.02	0.11	**0.14**
DM (yes vs. no)	-	**0.32**	**0.36**	0.01
SBP (per 10mm Hg)	**0.14**	**0.13**	**0.06**	**0.03**
BMI (per 5kg/m^2)	0.06	0.00	0.00	0.01

Bold font: P < 0.

Conclusions: uKIM-1/Cr reflects underlying kidney disease (lower eGFR and higher ACR and/or PCR) in the chronic setting across three diverse cohorts.

Funding: NIDDK Support

TH-PO674

GDF-15, Soluble ST2, and Galectin-3 and Risk of Cardiovascular Events, Decline in Kidney Function and Death in Chronic Kidney Disease Cassianne Robinson-Cohen,[1] Bryan R. Kestenbaum,[1] Ronit Katz,[1] Alan S. Go,[2] Jonathan Himmelfarb,[1] Ian H. de Boer,[1] Nisha Bansal.[1] *[1]UW; [2]KPNC.*

Background: Growth differentiation factor (GDF)-15, soluble ST2 (sST2) and galectin-3 are novel biomarkers that may be involved in cardiac inflammation, remodeling and fibrosis. These biomarkers predict cardiovascular events, kidney disease and death in the general population. We studied the association of these biomarkers with cardiovascular and kidney outcomes in a prospective cohort study of chronic kidney disease (CKD).

Methods: Baseline serum concentrations of GDF-15, sST2 and galectin-3 were measured in 297 participants with non-dialysis requiring CKD from the Seattle Kidney Study. We followed participants for a composite cardiovascular event (atherosclerotic events, heart failure events, and death) and a composite kidney event (progression to ESRD or ≥30% loss of eGFR). We used Cox's proportional hazards model to test associations of each biomarker with the study outcomes.

Results: The mean age was 62 years, 18% were women, 24% were Black and mean eGFR was 39.6 ml/min/1.73m^2. During a median follow-up of 2.7 years (IQR 1.5 – 3.8 years), 104 participants died or had a cardiovascular event and 79 progressed to ESRD or lost >30% eGFR. After adjustment for demographics, co-morbidity, and baseline eGFR, high serum concentrations of GDF-15 and sST2, but not galectin-3, were associated with greater risks of the composite cardiovascular outcome and the composite kidney outcome (**Table**).

Conclusions: Higher serum concentrations of GDF-15 and sST2 were significantly associated with increased risk of cardiovascular events, death and progression of kidney disease among patients with CKD. These biomarkers may highlight novel pathways of cardiovascular and kidney disease in the setting of CKD.

Biomarker	Median (IQR)	Cardiovascular events and death* HR (95% CI) per doubling of biomarker	30% decline in eGFR or ESRD** HR (95% CI) per doubling of biomarker
GDF-15 (pg/mL)	2337.8 (1624.8,3498.7)	**2.51 (1.73, 3.65)**	**2.22 (1.51, 3.27)**
sST2 (ng/mL)	32.6 (25.0, 41.0)	**1.68 (1.15, 2.45)**	**1.51 (1.02, 3.97)**
Galectin-3 (ng/mL)	13.5 (9.9, 17.0)	1.23 (0.79, 1.91)	1.07 (0.72, 1.59)

*Adjusted for age, race, gender, eGFR, urine ACR, educational attainment, current smoking status, physical activity, diabetes, cardiovascular disease, systolic blood pressure, anti-hypertensive medication use, diuretic use, CRP
** Adjusted for age, race, gender, eGFR, eGFR-squared, urine ACR, educational attainment, cardiovascular disease, physical activity, diabetes, systolic blood pressure, anti-hypertensive medication use and diuretic use

Funding: NIDDK Support

TH-PO675

Longitudinal Association of Inflammation Markers and Endothelial Dysfunction Markers with Left Ventricular Mass in Hypertensive Predialysischronic Kidney Disease Patients Vianda S. Stel,[1] Kyriakos Ioannou,[2] Evangelia Dounousi,[3] Kitty J. Jager,[1] Aikaterini A. Papagianni,[4] Konstantinos D. Pappas,[5] Kostas C. Siamopoulos,[3] Carmine Zoccali,[6] Dimitrios Tsakiris.[7] *[1]ERA-EDTA Registry, Netherlands; [2]Nicosia General Hospital, Cyprus; [3]Univ of Ioannina, Greece; [4]Hippokration General Hospital, Greece; [5]Univ Hospital of Ioannina, Greece; [6]Ospedali Riuniti, reggio Cal, Italy; [7]Papageorgiou General Hospital of Thessaloniki, Greece.*

Background: This is the first longitudinal study investigating the association of inflammation markers C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) and endothelial dysfunction markers intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) with left ventricular mass indexed for height2·71 (LVMI) in hypertensive predialysis CKD patients, trying to better understand early mechanisms of increased LVMI in this patient group.

Methods: From 2004 to 2005, 206 incident consecutive adult patients from the outpatient CKD clinics of two hospitals in Greece with CKD and hypertension, were included. CRP (mg/l) and LVMI (g/height2·71) were assessed annually for three years.

Results: The median LVMI was 69 g/m2·71. Using linear mixed modeling we found that a higher IL-6 (ß=2.0 (95%ci:0.30;3.7), inflammation score based on CRP, IL-6 and TNFα (ß=6.7 (95%ci:2.3;11.1) and VCAM-1 (ß=0.014 (95%ci:0.051;0.023) were associated with higher LVMI, after adjustment for age, sex, primary renal disease, smoking, history of cardiovascular disease, HDL/LDL ratio, and the use of ace-inhibitor/arbs, fibrates, diuretics, aspirin, and statins. The association of the inflammation score and LVMI was pronounced in CKD stages 1-3, but absent in CKD stages 4-5.

Conclusions: This study for the first time demonstrates that in hypertensive predialysis CKD patients, inflammation may play a more important role towards the increase in LVMI in the early than in the later CKD stages. The results further suggest that VCAM-1 may have a more crucial role than ICAM-1 in both atherosclerosis and hypertension which contribute to the increased LVMI.

Funding: Government Support - Non-U.S.

TH-PO676

Soluble Tumor Necrosis Factor Receptors 1 and 2 Predict Outcomes in Advanced Chronic Kidney Disease: A Prospective Cohort Study Nathalie Neirynck, Griet Lrl Glorieux, Eva Schepers, Francis Verbeke, Raymond C. Vanholder. *Nephrology Div, Dept of Internal Medicine, Ghent Univ Hospital, Gent, Belgium.*

Background: Soluble tumor necrosis factor receptors 1 (sTNFR1) and 2 (sTNFR2) have been associated to progression of renal failure, end stage renal disease and mortality in early stages of chronic kidney disease (CKD), mostly in the context of early diabetic nephropathy. The predictive value of these markers in advanced stages of CKD irrespective of the specific cause of kidney disease has not yet been defined. In this study, the relationship between sTNFR1 and sTNFR2 and the risk for cardiovascular events (CVE) and all-cause mortality was investigated in a population with CKD stage 4-5, not yet on dialysis, to minimize confounding by renal function.

Methods: In 131 patients, CKD stage 4-5, sTNFR1, sTNFR2 were analysed for their association to a composite endpoint of all-cause mortality or first non-fatal CVE by univariate and multivariate Cox proportional hazards models. In the multivariate models, age, gender, CRP, eGFR and significant comorbidities were included as covariates.

Results: During a median follow-up of 31 months, 42 events (32.1%) occurred of which 22 deaths (16.8%) and 20 (15.3%) first non-fatal CVE. In univariate analysis, the hazard ratios (HR) of sTNFR1 and sTNFR2 for negative outcome were 1.428 (p<0.001) and 1.127 (p<0.001) respectively. After adjustment for age, gender, CRP, eGFR, diabetes and a history of cardiovascular disease both sTNFR remained independently associated to outcomes (HR: sTNFR1: 1.512, p<0.001; sTNFR2: 1.125, p<0.001). A subanalysis of non-diabetic patients in the group confirmed the same findings.

Conclusions: sTNFR1 and sTNFR2 are independently associated with all-cause mortality or an increased risk for cardiovascular events in advanced CKD. Whether this association is causal and linked to biological functions of these receptors needs further investigation.

Funding: Government Support - Non-U.S.

TH-PO677

The Association between Postoperative Troponin and Mortality in Patients with and without Impaired Kidney Function Michael Walsh,[1] Chew Yin Wang,[2] Amit X. Garg,[3] Philip J. Devereaux.[1] *[1]McMaster Univ/Population Health Research Inst; [2]Univ of Malaysia; [3]Western Univ.*

Background: Postoperative cardiac troponin T (cTnT) is a risk factor for 30-day mortality in patients undergoing noncardiac surgery but there is uncertainty as to whether the risk associated with cTnT is maintained when kidney function is reduced. We evaluated the association between cTnT after noncardiac surgery at different levels of kidney function.

Methods: We performed a p*ost-hoc* analysis of a prospective cohort study of patients at least 45 years old, undergoing noncardiac surgery that required an overnight hospital admission. cTnT was measured for three days after surgery and considered abnormal if the peak was ≥0.02 ng/mL. We examined the interaction between an abnormal cTnT and estimated glomerular filtration rate (eGFR) strata for the outcome of 30 day mortality.

Results: We studied 14,037 patients of which 267 (1.9%) died within 30 days of surgery. The adjusted hazard ratio (aHR) for death with an abnormal cTnT was 4.37, 6.15, 6.30, 1.33 and 1.46 for an eGFR ≥60, 45 to <60, 30 to <45, 15 to <30, and <15 ml/min/1.73m^2 or on dialysis respectively. Compared to the ≥60 ml/min/1.73 m^2 eGFR group, the aHR was significantly lower for the 15 to <30 eGFR group (interaction p-value=0.02). Redefining an abnormal cTnT as ≥0.03 ng/mL or as a change of at least 0.02 ng/mL did not substantially alter results.

Conclusions: The risk associated with postoperative cTnT may be different for patients with an eGFR <30 ml/min/1.73 m^2. Further research is required to determine how to interpret perioperative cTnT values for patients with low eGFR.

Funding: Pharmaceutical Company Support - Roche Diagnostics, Government Support - Non-U.S.

TH-PO678

NTpro-BNP and Troponin T Is a Novel Valuable Biomarker for Progression of CKD Patients: A Longitudinal Follow-Up Study Yoshiko Shimamura,[1] Tatsuki Matsumoto,[1] Kazu Hamada,[1] Koji Ogata,[1] Kosuke Inoue,[1] Yoshinori Taniguchi,[1] Taro Horino,[1] Kenji Yuasa,[2] Tetsuro Sugiura,[1] Yoshio Terada.[1] *[1]Kochi Univ, Nankoku, Japan; [2]Kochi-Takasu-Hospital, Kochi, Japan.*

Background: NTpro-BNP and Troponin T(TNT) is known as diagnostic and prognostic biomarker of cardiac events. Recent report showed that cardio-renal interaction is an important problem in CKD patients. Furthermore, FGF23 was reported to injure cardiomyocyte directly in mice and high FGF23 is associated with cardiovascular risk factor. However, only few longitudinal follow-up studies have been reported to evaluate NTpro-BNP/TNT as a biomarker for renal prognosis. Accordingly, we elucidated the relation between NTpro-BNP/ TNT and renal function in CKD patients.

Methods: Alongitudinal follow-up study for 24 months was performed in 396 consecutive CKD patients in Kochi prefecture. NTpro-BNP, TNT, FGF23, serum creatinine, hemoglobin, albumin, calcium, phosphate and urine protein amount were measured. This study was approved by the Kochi Medical School review board. All patients provided written informed consent.

Results: The serum level of NTpro-BNP was positively correlated to creatinine (P<0.0001: r=0.442), FGF23 (P<0.0001: r=0.480), TNT (P<0.0001: r =0.665), phosphate (P<0.0001: r =0.262), PTH (P<0.0001: r =0.412) and urine protein amount (P<0.05:r=0.165) and negatively to eGFR (P<0.0001: r=-0.300), hemoglobin (P<0.0001: r=-0.323) and albumin (P<0.0001: r=-0.262). NTpro-BNP level in patients with heart failure was higher than in those without (P<0.0001). Furthermore, NTpro-BNP level elevated significantly according to the progression of CKD stage. However, TNT level elevated slightly according to the progression of CKD stage. Interestingly, NTpro-BNP and TNT level elevated early phase of CKD (stage 3) as compared with stage2 (P<0.01/ P<0.01). During the study period of 24 months observation, NTpro-BNP/TNT values inversely correlated with ΔeGFR in all the patients (P<0.01: r =-0.210/ P<0.01: r =-0.226).

Conclusions: In CKD patients, serum level of NT-proBNP/TNT inversely correlated with ΔeGFR. Thus, NT-proBNP/TNT might be an novel valuable biomarker to predict the prognosis of CKD patients.

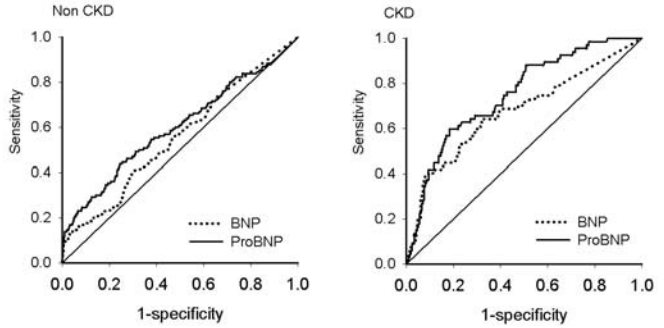
TH-PO679

CKD Modifies the Association of Brain Natriuretic Peptide (BNP) and N-terminal-pro-BNP (NT-pro-BNP) with Death Gates Colbert, Beverley Adams-Huet, Xilong Li, James Delemos, Susan Hedayati. *UT Southwestern, Dallas.*

Background: NT-pro-BNP and BNP are elevated in >50% of asymptomatic CKD patients. NT-pro-BNP has a longer $t_{1/2}$ and may be a better biomarker than BNP for risk assessment. We investigated if the association between BNP and NT-pro-BNP with death was modified by CKD in 3,303 Dallas Heart Study subjects free of CV disease and which natriuretic peptide was a better marker for death in CKD.

Methods: CKD was defined as eGFR<60 mL/min/1.73m^2 by MDRD or ACR ≥17 mg/g in men or ≥25 in women. Logistic regression determined the association between BNP and NT-pro-BNP with all-cause death at 7 years, adjusted for age, gender, race, diabetes, and hypertension. The interaction of CKD with biomarkers was tested. The area under the ROC curve (AUC) for each biomarker was compared for CKD versus non-CKD groups.

Results: Mean age was 44±10 years. 55% were female, 50% African American, 31% Caucasian, 17% Hispanic. 289 had CKD. 201 total deaths occurred. Proportion of deaths was higher if CKD versus no CKD, 23.5 versus 4.4%, p<.0001. Both markers were associated with death, main effect p<.0001; there was an interaction between BNP and CKD, p=.08, such that the OR for death per $\log_e$ unit BNP increase was significant for CKD, aOR 1.36 (1.15, 1.61), p=.0004, but not if no CKD, p=.07. The aOR of NT-pro-BNP per $\log_e$ unit increase for death were 1.40 (1.21, 1.63) if no CKD and 1.66 (1.36, 2.04) if CKD, interaction p>.1. The optimal NT-pro-BNP cutoff for death was 123 pg/mL if CKD versus 56 if no CKD. NT-pro-BNP was a superior marker for death than BNP in CKD (aAUC=0.81 versus 0.75, p=.008). The AUCs for BNP and NT-pro-BNP were not different if no CKD, p=.16.



Conclusions: CKD modifies the association of BNP with death. NT-pro-BNP is a superior marker than BNP for death in asymptomatic CKD patients. Future studies should confirm if NT-pro-BNP can be used as an early biomarker for risk stratification in CKD.

Funding: Other NIH Support - USPHS GCRC grant #M01-RR00633 from NIH/NCRR-CR and UL1TR001105 from the National Center for Advancing Translational Sciences, Veterans Affairs Support, Private Foundation Support

TH-PO680

Serum High-Sensitivity Troponin Concentrations in a Multi-Ethnic Asian Population of Stable Chronic Kidney Disease Patients Boon Wee Teo,[1] Titus W. Lau,[1] Qi Chun Toh,[1] Weng Kin Wong,[1] Sabrina Haroon,[1] Srinivas Subramanian,[1] Sunil Sethi.[2] *[1]Medicine, National Univ Health System, Singapore, Singapore; [2]Pathology, National Univ Health System, Singapore, Singapore.*

Background: Serum troponins may be elevated because of reduced glomerular filtration, reduced renal catabolism, and possibly subclinical myocardial injury as a result of volume overload in chronic kidney disease (CKD) patients. The statistical normal of new troponin assays in asymptomatic CKD patients is unknown. We determined levels of troponin-I (hsTnI) in stable CKD patients in a multi-ethnic Asian population using a high-sensitivity assay.

Methods: This is a prospective, observational, cohort study which recruited 465 stable CKD patients with >125 participants per estimated glomerular filtration rate (GFR) category (<30, 30 to 60, >60 mL/min/1.73m^2). Serum troponin I was measured using a high-sensitivity assay (Abbott Diagnostics). Analyses were performed using JMP.

Results: The median serum creatinine concentration was 137 µmol/L (IQR: 89 to 208), with an estimated GFR of 42 (25 to 73) mL/min/1.73m^2. The median serum hsTnI concentration was 5.5 ng/L (IQR: 3.46 to 9.18). The 90th, 97.5th, and 99.5th percentile observed values were 16.3, 40.1, and 119.8 ng/L, respectively. Serum hsTnI was lower in women than men (p <0.001). Seven patients were admitted to the hospital within 30 days after study recruitment (mean serum hsTnI = 10.1 ± 8.25 ng/L). Only 1 admission is cardiac-related. Reviewing the distribution of data and prior literature, the proposed serum hsTnI upper threshold should be taken at the 90th percentile level in CKD patients by GFR categories.

Table Quantiles of high-sensitivity troponin I.

GFR	Minimum	10%	25%	Median	75%	90%	Maximum
>60	1.1	2.1	2.6	3.5	5.1	7.2	25.7
30-60	1.1	2.9	3.9	5.9	8.9	16.2	140.4
<30	1.3	2.3	3.7	8.7	14.1	29.2	176.1

Conclusions: Patients with CKD represent a population with one of the highest risk for adverse cardiovascular events. Kidney dysfunction confounds the interpretation of serum troponin concentrations. To achieve clinical decision equipoise, we suggest taking the 90th percentile threshold for further evaluation of CKD patients presenting with possible acute coronary syndromes.

Funding: Pharmaceutical Company Support - Abbott Diagnostics, Private Foundation Support

TH-PO681

The Association Between Uric Acid and the Rate of Kidney Function Decline in Stage III, IV, and V Chronic Kidney Disease Patients from the Swedish Renal Registry Hakan Nacak,[1] Merel Van Diepen,[1] Friedo W. Dekker,[1] Marie Evans,[2] Juan Jesus Carrero.[2] *[1]Dep. of Clinical Epidemiology, Leiden Univ Medical Center, Leiden, Zuid-Holland, Netherlands; [2]Div. of Renal Medicine, Karolinska Instt, Stockholm, Sweden.*

Background: Although many studies have shown an association between higher uric acid (UA) and both development of CKD and decline in renal function it is not clear whether this effect is consistent through the CKD stages. The aim of this study was to investigate the association between baseline UA and the rate of decline in renal function in stage III-V CKD patients as well as in stage III, IV, and V CKD patients, separately.

Methods: We analyzed data in the Swedish Renal Registry – Chronic Kidney Disease (SRR-CKD) which is a nationwide registry of referred CKD patients. Patients with a visit between January 1, 2005 and December 31, 2011 were followed until initiation of renal replacement therapy, death, or censoring. Change in renal function was assessed with a linear mixed model using all estimated glomerular filtration rate measurements (eGFR) recorded during median 28 months of follow-up, adjusting for important confounders such as demographic factors, primary renal disease, medication, diet, blood pressure, and body mass index.

Results: There were 2094 patients with a baseline UA measurement (mean (standard deviation (SD)) of 7.75 (1.93) mg/dl). The mean decline in renal function was -1.87 (95% CI -2.08; -1.67) ml/min/1.73m^2 per year. The overall adjusted change in the rate of decline in renal function associated with a unit increase in baseline UA was 0.10 (95% CI -0.03;0.22) ml/min/1.73m^2 per year, which corresponds to less decline. In stage III, IV, and V CKD patients the mean decline in renal function was -1.63 (95% CI -2.30; -0.96), -1.89 (95% CI -2.13;-1.66), and -1.57 (95% CI -1.91; -1.22) ml/min/1.73m^2 per year respectively. The adjusted change in the rate of decline in renal function associated with a unit increase in baseline UA was -0.11 (95% CI -0.4;0.19) in stage III CKD, 0.13 (95% CI 0.00; 0.26) in stage IV CKD, and -0.01 (95% CI -0.31; 0.29) in stage V CKD.

Conclusions: Uric acid is not associated with the rate of decline in renal function in stage III, IV, and/or V CKD patients.

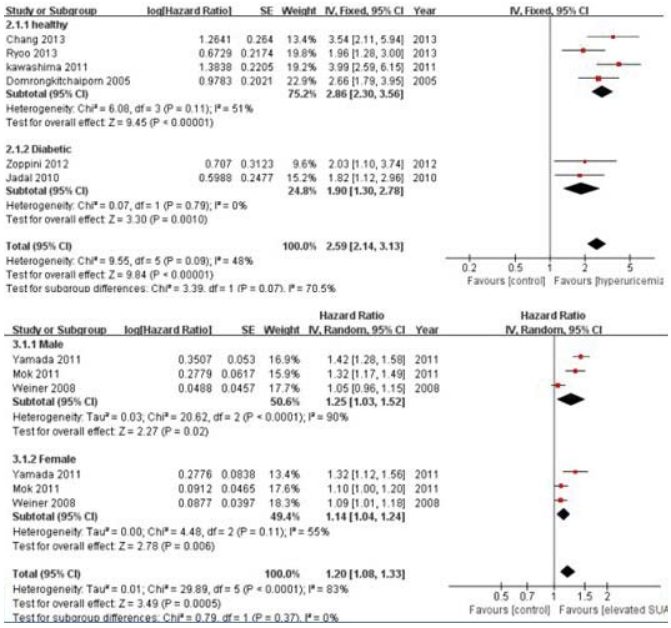
TH-PO682

Is Hyperuricemia an Independent Risk Factor for New-Onset Chronic Kidney Disease? A Systematic Review and Meta-Analysis Based on Observational Cohort Studies Ling Li, Fang Liu, Ping Fu. *Div of Nephrology Dept of Internal Medicine, West China Hospital of Sichuan Univ, China.*

Background: To assess whether an elevated serum uric acid level is an independent risk factor for new-onset chronic kidney disease (CKD).

Methods: A systematic review and meta-analysis using a literature search of online databases including PubMed, Embase, Ovid and ISI Web Web of Science was conducted. Summary adjusted odds ratios with corresponding 95% confidence intervals (95% CI) were calculated to evaluate the risk estimates of hyperuricemia for new-onset CKD.

Results: Thirteen studies containing 190,718 participants were included. A significant positive association was found between elevated serum uric acid levels and new-onset CKD at follow-up (summary OR, 1.15; 95% CI, 1.05-1.25). Hyperuricemia was found be an independent predictor for the development of newly diagnosed CKD in non-CKD patients (summary OR, 2.35; 95% CI, 1.59-3.46). This association increased with increasing length of follow-up. No significant differences were found for risk estimates of the associations between elevated serum uric acid levels and developing CKD between males and females.



Conclusions: With long-term follow-up of non-CKD individuals, elevated serum uric acid levels showed an increased risk for the development of chronic renal dysfunction.

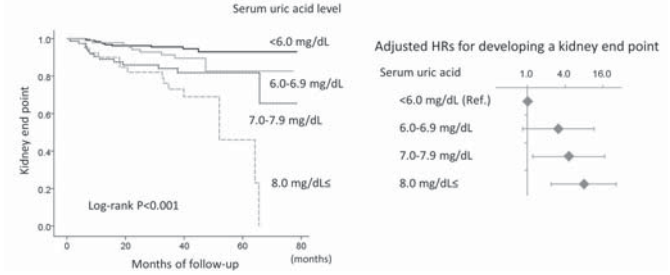
TH-PO683

Relationship between the Serum Uric Acid Level and Renal Progression in Patients with Chronic Kidney Disease: A Prospective, Observational Study Hiroshi Kimura, Kenichi Tanaka, Makoto Kanno, Kimio Watanabe, Yoshimitsu Hayashi, Hiroyuki Terawaki, Koichi Asahi, Masaaki Nakayama, Tsuyoshi Watanabe. *Nephrology and Hypertension, Fukushima Medical Univ, Fukushima, Japan.*

Background: Hyperuricemia is associated with the onset of chronic kidney disease (CKD) and is a risk factor for end-stage renal disease in the general population. However, data on the relationship between serum uric acid (UA) levels and the progression of renal disease in the CKD patient population are limited.

Methods: A prospective, observational study of 409 pre-dialysis patients with CKD was conducted to examine the association between the serum UA level and the progression of renal disease. The kidney end point was defined as a combination of doubling of baseline serum creatinine and end-stage kidney disease requiring kidney replacement therapy.

Results: The median eGFR was 55.8 (40.8-72.4) mL/min per 1.73 m^2, and the median serum UA was 6.0 (5.0-7.2) mg/dL. During a median follow-up period of 3.2 years, 46 (11%) participants reached the kidney end point.Kaplan-Meier curve analysis revealed that patients with a higher serum UA level reached the kidney end point more frequently than those with a serum UA level below 6.0 mg/dL. After adjustment for age, sex, body mass index, smoking history, diabetes, kidney function, hemoglobin, serum albumin, and proteinuria, the risk of developing a kidney end point was 24% higher per 1 mg/dL increase in the serum UA level (HR 1.24, 95% Confidence Interval 1.03-1.49, P=0.02). The risk was significant in patients with a serum UA more than 7 mg/dL in the Cox regression model using patients with serum UAless than 6.0 mg/dL as the reference group.



Conclusions: An independent, graded association was observed between an increased serum UA level and the risk of renal disease progression in pre-dialysis patients with CKD.

TH-PO684

Higher Uric Acid Is a Risk Factor for the Progression of IgA Nephropathy with CKD G3a Takahito Moriyama, Keiko Uchida, Kosaku Nitta. *Dept of Medicine, Kidney Center, Tokyo Women's MEdical Univ, Tokyo, Japan.*

Background: We have been reported 30 year prognosis of IgA nephropathy (IgAN) and higher uric acid (UA), lower estimated glomerular filtration rate (eGFR), and higher amount of proteinuria as independent risk factors to progress to end stage renal disease (ESRD) (ASN 2013, Plos one 2014; 9: e29976). However, it is still unclear the influence of higher UA in IgA nephropathy. Therefore, in this study, we investigated the details of influence of high UA in IgAN.

Methods: In 611 adult IgAN patients diagnosed at our institution between 1974 and 2005, we divided them into high UA group (HUA) and normal UA group (NUA) in each CKD stage (G1, G2, G3a, G3b and G4), and compared the clinical findings at renal biopsy, the histological findings according to the Oxford classification, and the renal survival rate.

Results: The ratio of HUA was increased with the progression of CKD stage [12.3% (HUA versus NUA=23 versus164) in CKD G1, 19.0% (52 versus 222) in G2, 43.7% (45 versus 58) in G3a, 69.0% (29 versus 13) in G3b+4]. Renal survival rate was similar in CKD G1, 2, and G3b+4. However, in CKD G3a, it was significantly lower in HUA than NUA (67.9 versus 89.7 % in 10 years, 49.5 versus 69.3 years in 20 years, and 24.7 versus 51.9 % in 30 years, P=0.0205). In CKD G3a, clinical findings without UA were similar between HUA and NUA, however the ratio of global sclerosis was significantly higher in HUA than NUA (33.3 versus 11.4 %, P=0.0005), though Oxford classification was similar between both groups. Multivariate Cox regression analysis indicated that higher UA (HR 1.36, 95%CI 1.07-1.72, P=0.011) and higher amount of proteinuria (HR 1.38, 95%CI: 1.09-1.74, P=0.0084) were independent risk factors for progression to ESRD in CKD G3a. These results indicated that higher UA induced glomerular impairment in addition to the renal impairment according to IgAN, and accelerated the progression to ESRD.

Conclusions: In IgAN patients with CKD G3a, glomerular impairment was resulted from not only IgA nephropathy but also high UA, and higher UA increased the risk to deteriorate renal function and progress to ESRD. In IgAN patients complicated with high UA, it may be important to control high UA before progress to CKD G3a.

TH-PO685

The Association between Serum Uric Acid, Genotypes of Uric Acid-Related Genes, and Mortality in the Community-Based Population: The Takahata Study Keita Kamei, Hiroko Sato, Atsushi Hirayama, Kazuko Suzuki, Kazunobu Ichikawa, Tsuneo Konta, Isao Kubota. *Dept of Cardiology, Pulmonology, and Nephrology, Yamagata Univ School of Medicine, Yamagata, Japan.*

Background: Serum uric acid is regulated by gender, dietary habit, genetic predisposition and renal function, and is associated with renal and cardiovascular outcomes. This study prospectively investigated the association between serum uric acid, genotypes of uric acid-related genes, and mortality in the community-based population.

Methods: The participants of this study were 3479 subjects (male 45%, mean age 63 years) in Takahata town, Japan, that were registered and followed up for 7 years (median 6.7 years). The single nucleotide polymorphisms of uric acid-related genes including URAT1 (rs505802), ABCG2 (rs2231142), UMOD (4293393) and SLC2A9 (rs16890979) were genotyped.

Results: During the follow-up 142 subjects died, including 30 cardiovascular deaths. Kaplan-Meier analysis showed that all-cause mortality was significantly increased along with the increase in serum uric acid at baseline in females (Log-rank P <0.001), but not in males (P = 0.774). Cox proportional hazard model analysis with the adjustment for possible confounders including age, renal function and comorbidities showed that hyperuricemia (uric acid ≥ 7.0 mg/dL) was an independent risk factor for all-cause and cardiovascular mortalities in females (hazard ratio [HR] 5.56, 95% confidence interval [CI] 1.89-16.3 for all-cause mortality, and HR 18.4, 95%CI 1.68-201.3 for cardiovascular mortality), but not in males. The genotypes of URAT1, ABCG2, and UMOD that modulate urinary excretion of uric acid were independently associated with serum uric acid levels and all-cause mortality, after the adjustment for confounders.

Conclusions: Hyperuricemia was an independent risk for all-cause and cardiovascular mortalities in females in community-based population and the genetic variants of uric acid-related genes were significantly associated with serum uric acid levels and mortality.

Funding: Government Support - Non-U.S.

TH-PO686

Urinary iC3b Detected at Baseline Independently Predicts Loss of Kidney Function in Stage 3 Chronic Kidney Disease Girish N. Nadkarni,[1,2] Avelino Teixeira,[1,2] Stefano Malerba,[1] Josef Coresh,[2] Chi-Yuan Hsu,[2] Kathleen D. Liu,[2] Joseph V. Bonventre,[2] Harold I. Feldman,[2] Vasan S. Ramachandran,[2] John W. Kusek,[2] Paul L. Kimmel,[2] Emilia Bagiella,[1,2] Erwin P. Bottinger.[1,2] *[1]Icahn School of Medicine at Mount Sinai, New York, NY; [2]CKD Biomarker Consortium, NIDDK, Bethesda, MD.*

Background: We showed significant inverse associations between urine complement fragments C3a, C5b-9 and iC3b and eGFR cross-sectionally (Abstract#1512, ASN 2013). We examined whether these predict CKD progression.

Methods: The three urinary markers were measured in anonymized residual urine specimens from tests ordered in routine clinical care using modified ELISA assays (Quidel® Corp, San Diego, CA). We studied 151 stage 3 CKD patients whose CKD-EPI eGFR was measured at baseline and at six-months intervals over 18 months.

Results: Mean age: 65 years, female: 52%, diabetic: 54% and hypertensive: 88% of patients. Baseline eGFR: 44 ml/min/1.73m^2. Mean (SD) loss of eGFR (ΔeGFR) during follow-up remained significantly increased in patients with detectable markers after adjustment for age, gender, race/ethnicity, diabetes, and hypertension.

Mean (SD) ΔeGFR ml/min/ 1.73m²	Det C3a 76 (51%)	No C3a 58(38%)	Det C5b-9 58 (38%)	No C5b-9 93(62%)	Det ic3b 80 (53%)	No ic3b 71(47%)
6 months	-3.6(6)	-0.1(4.5)*	-2.95(6.02)	1.28(5.28)	-3.83(5.53)	0.36(4.90)*
12 months	-5(7.9)	-1.5(7.9)*	-5.38(7.77)	-2.51(6.81)^	-6.21(7.44)	-0.68(5.97)*
18 months	-8.1(10.1)	-1.5(7.1)*	-7.94(1.42)	-2.5(8.16)^	-8.79(9.77)	-0.40(6.31)*

Table 1: eGFR change at 6,12 and 18 months after sample collection in patients with detectable(det) and undetectable(no) urinary C3a, C5b-9, or iC3b(n=151) ^P value<0.05; * P value<0.01

Baseline urine protein creatinine ratio attenuated C3a and C5b-9, but iC3b marker remained significant (coefficient (95% CI) of -2.78(-4.5 to -1.0), -3.89(-6.2 to -1.6), -6.41(-9.3 to -3.5) at 6,12,18 months). eGFR decline over 18 months was significantly higher in patients with detectable ic3b compared to undetectable (-8.7 versus-1 ml/min/1.73m²).

Conclusions: Presence of complement fragment iC3b in urine of stage 3 CKD patients predicts loss of eGFR independent of proteinuria.

Funding: NIDDK Support

TH-PO687

A Novel Urinary Epigenetic Marker 5'-Methyl-2'-Deoxycytidine in Combination with Albuminuria Significantly Predicts the Renal Outcome in Patients with Chronic Kidney Disease

Akifumi Onishi, Hitoshi Sugiyama, Toshio Yamanari, Ayu Akiyama, Masashi Kitagawa, Hiroshi Morinaga, Keiko Tanaka, Yoko Kikumoto, Tatsuyuki Inoue, Yohei Maeshima, Jun Wada. *Dept of Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Epigenetic risk factors for chronic kidney disease (CKD) have only recently been investigated (Epigenetics 2014), and the DNA methylation profile in the blood may be associated with a rapid decline in the renal function (Nephrol Dial Transplant 2014). However, there is no current information available regarding the urinary levels of 5'-methyl-2'-deoxycytidine (5MedCyd), a marker of DNA methylation, in patients with CKD.

Methods: We determined the levels of urinary 5MedCyd in spot urine samples from 512 patients with CKD (median age: 52.4 years, male: 54.0%, glomerulonephritis: 50.0%) using a competitive ELISA and investigated the relationships among 5MedCyd, eGFR, albuminuria, urinary α1-microglobulin (α1MG) and urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative stress. The patients were prospectively followed for three years for doubling of the baseline serum creatinine level and the initiation of renal replacement therapy.

Results: The urinary 5MedCyd levels were significantly decreased in the patients with CKD stage G3 (p=0.031), and this parameter was found to be a significant predictor of CKD G3 in a multiple logistic regression analysis (P = 0.019). The urinary 5MedCyd level significantly correlated with the urinary 8-OHdG level (P < 0.0001), but not albuminuria or the urinary α1MG level. A Kaplan-Meier analysis demonstrated that the patients with a higher urinary 5MedCyd level in combination with macroalbuminuria (log-rank, P < 0.001) or a high α1MG level (log-rank, P < 0.001), had a significantly worse renal prognosis. In addition, the urinary 5MedCyd level was found to be significantly associated with renal endpoints in a multivariate Cox proportional hazard model (P = 0.027).

Conclusions: The present data indicate that the urinary 5MedCyd level in combination with albuminuria or the α1MG level can serve as a novel biomarker to predict the renal outcome in CKD patients.

TH-PO688

Shear Wave Elastography in Chronic Kidney Disease: A Novel Noninvasive Ultrasound Technique as a Marker for Renal Fibrosis

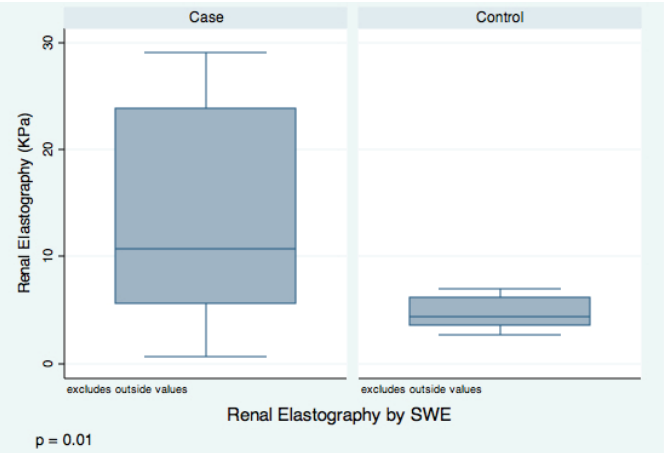
Andrew S. Allegretti,[1] Anthony Samir,[2] Qingli Zhu,[2] Manish Dhyani,[2] Dorothy A. Dobens,[1] Caitlin A. Trottier,[1] Winfred W. Williams,[1] Jodie L. Babitt,[1] Ravi I. Thadhani,[1] Herbert Y. Lin.[1] *[1]Div of Nephrology, Massachusetts General Hospital, Boston, MA; [2]Dept of Radiology, Massachusetts General Hospital, Boston, MA.*

Background: Intrarenal fibrosis is a final common pathway for all chronic kidney disease (CKD). Currently, invasive renal biopsy is the only method to quantify degree of fibrosis. Shear wave elastography (SWE) uses focused acoustic energy pulses to quantify fibrosis via microscopic tissue displacement. SWE is approved for use in staging liver fibrosis and may have a role in measuring fibrosis in CKD.

Methods: Participants with CKD and healthy volunteers underwent SWE imaging. Renal elasticity was estimated as Young's modulus in kilopascals (kPa), where higher values represent stiffer tissue.

Results: Fourteen participants with CKD (median GFR 41.5 mL/min, IQR 38-54) and 19 controls without CKD underwent SWE performed by a single radiologist. Presence of CKD was associated with higher renal elasticity (10.70 KPa, IQR 9.27-11.14 versus 4.40 KPa, IQR 3.55-6.20; p = 0.01).

	Case (n = 14)	Control (n = 19)
Age (years)	61.44 (56-71.6)	33.96 (29.9-49.2)
Male	8 (57%)	5 (26%)
Non-Hispanic	10 (71%)	18 (95%)
Hispanic	4 (29%)	1 (5%)
White	12 (86%)	14 (74%)
Other Race	2 (14%)	5 (26%)
BMI (kg/m²)	25.3 (24.2-25.6)	23.3 (21.8-23.9)
GFR (mL/min) by MDRD	41.5 (38-54)	
Kidney Size (cm)	10.48 (9.27-11.14)	10.43 (10.1-11)
CKD Stage 2	1 (7%)	
CKD Stage 3	11 (79%)	
CKD Stage 4-5	2 (14%)	



Conclusions: SWE estimates of renal stiffness are higher in patients with CKD than in healthy controls. Preliminary data suggests SWE measurement of renal fibrosis is a promising new non-invasive tool for the detection and staging of CKD. Further study is needed to delineate the correlation between this imaging technique and CKD stage measured by biopsy or serum fibrosis markers.

Funding: Clinical Revenue Support

TH-PO689

Association between Endothelin-1 Levels and Reduced Kidney Function among African-Americans: Jackson Heart Study

C. Rebholz,[1] Jane L. Harman,[2] M. Grams,[1] Daichi Shimbo,[3] Bessie A. Young,[4] Josef Coresh.[1] *[1]Johns Hopkins Univ; [2]National Heart, Lung, and Blood Inst; [3]Columbia Univ Medical Center; [4]Univ of Washington.*

Background: Endothelin-1, a marker of endothelial dysfunction, is a potent vasoconstrictor released by endothelial cells and is an important regulator of renal physiology. However, the prospective relation between endothelin-1 and kidney function is not well characterized in the general population.

Methods: Jackson Heart Study is a community-based observational study of African-American adults in the Jackson, MS metropolitan area. Serum endothelin-1 was measured at baseline (2000-04; N=5,200). CKD-EPI estimated glomerular filtration rate (eGFR) was calculated at baseline and visit 3 (2009-13). Among those with baseline eGFR≥60 mL/min/1.73 m², incident chronic kidney disease (CKD) was defined conservatively as the development of eGFR<60 mL/min/1.73 m² accompanied by 25% eGFR decline from baseline. Logistic regression were used to estimate the relation between endothelin-1 and kidney function, adjusting for age, sex, body mass index, smoking, hypertension, diabetes, high density and total cholesterol, history of cardiovascular disease, hematocrit, and C-reactive protein.

Results: Among study participants at baseline, mean age was 55 years, 37% were male, and median eGFR was 96 mL/min/1.73 m². At baseline, reduced eGFR (<60 mL/min/1.73 m²) was more prevalent among participants with higher quartiles of endothelin-1 (3.8%, 5.5%, 5.9%, 10.9%, for quartiles 1-4, respectively, p<0.001). This association persisted after adjusting for multiple covariates. During a median follow-up of 12 years, there were 266 incident CKD cases (7.5%). CKD incidence was higher among those with higher quartiles of baseline endothelin-1 (quartile 4 versus 1, OR: 1.83, 95% CI: 1.19, 2.82). After adjusting for multiple covariates and baseline eGFR, one standard deviation higher baseline endothelin-1 was associated with elevated risk of incident CKD (OR: 1.20, 95% CI: 1.06, 1.36).

Conclusions: Baseline serum endothelin-1 is independently associated with contemporaneous kidney function as well as kidney disease progression, which has not been previously reported in the general population.

Funding: Other NIH Support - NHLBI

TH-PO690

Change in Novel Kidney Filtration Markers and Risk of Cardiovascular Disease and Mortality C. Rebholz, M. Grams, Kunihiro Matsushita, Elizabeth Selvin, Josef Coresh. *Johns Hopkins Univ, Baltimore, MD.*

Background: Kidney disease progression, assessed by change in estimated glomerular filtration rate based on creatinine (eGFR-Cr), is a strong risk factor for cardiovascular disease and death. The associations of change in novel filtration markers with these outcomes are uncharacterized.

Methods: Creatinine, cystatin C, and β_2-microglobulin were measured among 9,716 ARIC Study participants in 1990-92 and 1996-98. Cardiovascular events (coronary heart disease, stroke, heart failure) and deaths were ascertained from 1996-98 through 2011.

Results: During a median follow-up of 14 years, there were 1,922 incident cardiovascular events and 2,285 deaths. Substantial decline in filtration markers (<-30%) was significantly associated with two-fold increased risks of cardiovascular disease and all-cause mortality.

Table. Adjusted* Hazard Ratios (95% Confidence Intervals) for Cardiovascular Disease and All-Cause Mortality by Change in Filtration Markers

	Category of Percent Change in Filtration Marker			
Filtration Marker	< -30%	-30% to -10%	-9% to +9%	≥ +10%
Cardiovascular Disease (1,922/9,712)				
eGFR-Cr	**2.02 (1.75, 2.33)**	1.07 (0.96, 1.18)	1 [Ref]	1.15 (0.92, 1.43)
eGFR-Cystatin C	**1.75 (1.48, 2.06)**	**1.16 (1.05, 1.28)**	1 [Ref]	1.18 (0.99, 1.40)
1/β_2-Microglobulin	**2.13 (1.80, 2.51)**	**1.31 (1.19, 1.45)**	1 [Ref]	1.14 (0.98, 1.33)
All-Cause Mortality (2,285/9,716)				
eGFR-Cr	**1.92 (1.68, 2.19)**	1.05 (0.96, 1.15)	1 [Ref]	1.03 (0.84, 1.28)
eGFR-Cystatin C	**2.33 (2.03, 2.68)**	**1.17 (1.07, 1.29)**	1 [Ref]	**1.30 (1.11, 1.52)**
1/β_2-Microglobulin	**2.56 (2.20, 2.93)**	**1.28 (1.16, 1.40)**	1 [Ref]	1.03 (0.88, 1.20)

Bold font indicates statistical significance.
*Adjusted for age, sex, race, body mass index, systolic blood pressure, anti-hypertensive medication use, diabetes status, total and high-density lipoprotein cholesterol, smoking status, 1st eGFR-Cr measurement

Conclusions: Kidney disease progression assessed using change in novel filtration markers is independently associated with risk of cardiovascular disease and death, as we have previously shown for risk of end-stage renal disease. Interventions aimed at stabilizing kidney function may reduce future risk of cardiovascular morbidity and mortality.

Funding: NIDDK Support, Other NIH Support - National Health, Lung and Blood Institute

TH-PO691

Thyroid Hormones and Kidney Function in a Community-Based Population Ulla T. Schultheiss,[1] M. Grams,[2,3] Josef Coresh,[3] Michael Steffes,[4] Elizabeth Selvin,[3] Anna Kottgen.[1,3] *[1]Dept of Medicine IV, Nephrology and Primary Care, Medical Center - Univ of Freiburg, Freiburg, Baden-Württemberg, Germany; [2]Div of Nephrology, Johns Hopkins Univ, Baltimore, MD; [3]Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [4]Dept of Laboratory Medicine and Pathology, Univ of Minnesota Medical School, Minneapolis, MN.*

Background: Patients with reduced estimated glomerular filtration rate (eGFR) have higher prevalence of subclinical/overt hypothyroidism. Little is known about the cross-sectional association of kidney function with individual markers of thyroid function: thyrotropine(TSH), free thyroxine (FT4), thyroid peroxidase antibodies (TPOAb), and, in particular, triiodothyronine (T3).

Methods: We tested the association of thyroid markers and eGFR <60 ml/min/1.73 m^2 at visit 2 (1990-92) in the Atherosclerosis Risk In Communities (ARIC) study, a prospective cohort study in four U.S. communities. Multiple logistic regression models adjusting for age, sex, race-site, CRP, smoking, serum albumin and BMI were run for each thyroid marker individually and in combination.

Results: Median and IQR(p75-p25) were: TSH (mIU/L 1.8, 1.44), FT4 (ng/dL 1.11, 0.21), T3 (ng/dL 126.2, 27.7), TPOAb (IU/mL 10.24, 8.38). Higher TSH, FT4 and lower T3 were significantly associated with eGFR<60 ml/min/1.73m^2 individually and in combination, with little variation in the effect size.

OR for eGFR<60 (95% CI)**	Q1 (lowest)	Q2	Q3	Q4 (highest)	p-trend
TSH	1 (ref)	1.61 (1.06-2.45)*	1.60 (1.06-2.43)*	2.52 (1.68-3.78)*	p<0.001
FT4	1 (ref)	1.00 (0.67-1.48)	1.04 (0.70-1.56)	2.54 (1.79-3.63)*	p<0.001
T3	1 (ref)	0.48 (0.35-0.67)*	0.38 (0.26-0.54)*	0.17 (0.11-0.27)*	p<0.001
TPOAb	1 (ref)	1.15 (0.79-1.67)	1.19 (0.81-1.75)	1.11 (0.75-1.63)	0.47
*p<0.05** Multiple logistic regression including all thyroid markers					

Conclusions: Higher TSH, FT4 and T3 were strongly and independently associated with reduced kidney function in this cross-sectional, population-based study. Our results warrant the evaluation of a full panel of thyroid markers as risk factors for incident kidney disease.

Funding: NIDDK Support, Other NIH Support - NHLBI, Pharmaceutical Company Support - Reagents for the thyroid assays were donated by Roche Diagnostics

TH-PO692

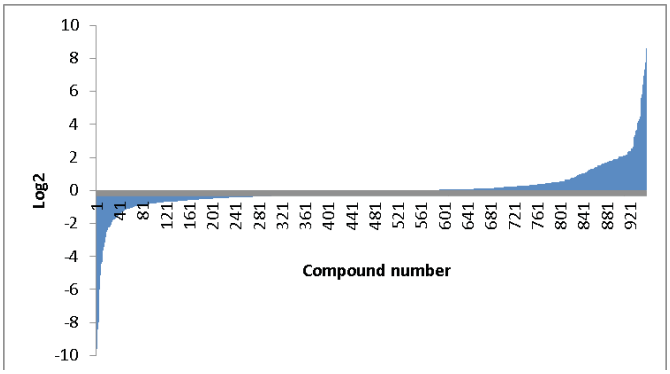
Urine Metabolomics in Patients with Nephrosclerosis Marius Altern Øvrehus,[1,2] Stein I. Hallan.[1,2] *[1]Dept of Cancer Research and Molecular Medicine, Norwegian Univ of Science and Technology, Trondheim, Norway; [2]Dept of Nephrology, St. Olavs Hospital, Trondheim Univ Hospital, Trondheim, Norway; [3]Dept of Biotechnology, Norwegian Univ of Science and Technology, Trondheim, Norway.*

Background: Nephrosclerosis (NS) is one of the most common causes of end stage renal disease but still one of the least studied. Urine metabolomics could potentially be useful to characterize the pathophysiology and prognosis in NS.

Methods: Frozen urine samples from 150 NS patients and 70 healthy controls (mean age 67.5 versus 59.5 years, mean eGFR 64.9 versus 83.2 mL/min/1.73m^2) from the HUNT 3 Study (2006-08, Norway) were analyzed. Nephrosclerosis was defined as eGFR <60 or a loss of >20 mL/min/1.73m^2 over 10 years, with at least 10 years of hypertension, and no diabetes, hematuria or proteinuria. Samples were diluted 1:3 with water, spun through a 3K Da filter, and analyzed non-targeted with liquid chromatography-mass spectrometry (LC-MS) in negative and positive electrospray ionization (ESI) mode.

Results: Raw LC-MS data were processed using Transomics software. 953 compounds were retrieved for analysis. Some metabolites in NS patients with eGFR <60 were over-expressed (47 compounds over 4-fold increased) and some were under-expressed (25 compounds over 4-fold decreased) compared to controls (figure 1). Metabolite identities were not ascertained. Principal component analysis explained only 34% of the total variance. In NS patients with rapid versus slow decline in kidney function (more versus less than 25 mL/min/1.73m2 over 10 years), 7 and 32 compounds were over 4-fold increased and decreased, respectively.

Conclusions: Non-targeted urine metabolomics analysis showed no global metabolite differences in nephrosclerosis patients versus healthy controls. However, large abundance differences were found in some metabolites, indicating a possibility for further characterization of nephrosclerosis.



Funding: Government Support - Non-U.S.

TH-PO693

Symmetric Dimethylarginine's Predictive Role in Chronic Kidney Disease Progression in Children Ellen Brooks,[1,2] Shannon Haymond,[1,2] Rod Passman,[1] Alfred Rademaker,[1] Irene Helenowski,[1] Susan L. Furth,[3] Bradley A. Warady,[4] Craig B. Langman.[1,2] *[1]Feinberg School of Medicine, Northwestern Univ, Chicago, IL; [2]Ann & Robert H Lurie Children's Hospital of Chicago, Chicago, IL; [3]Children's Hospital of Philadelphia, Philadelphia, PA; [4]The Children's Mercy Hospital, Kansas City, MO.*

Background: The rate of CKD progression in children is not fully explained by traditional markers. Methylated arginine (Arg) derivatives (MADs), non-traditional biomarkers, may provide such data in children due to MADs relationship with inhibition of eNOS and NO synthesis.

Methods: Plasma specimens from the baseline (BL) visit were provided by the "Chronic Kidney Disease in Children" (CKiD), an observational cohort study of children with CKD, and analyzed for asymmetric dimethylarginine (ADMA), symmetric DMA (SDMA), and Arg using HPLC-Tandem Mass Spectrometry. We used CKiD data, including directly measured GFR (mGFR) by plasma iohexol clearance (iCL) to assess the contribution of individual MADs as independent predictors and/or co-contributors with traditional markers for rapid CKD progression in multivariate models.

Results: Multivariate modeling was performed in N=288, with mGFR=46.9±18.2 ml/min/1.73m^2 and CKD vintage=3.6±4.7 yrs. SDMA and its ratios were strong predictors for mGFR change (Δ), obtained by iCL at BL and 6-12 mos. later. Partial correlations (ρ) were seen between SDMA, SDMA/ADMA, SDMA/Arg and mGFR Δ, after adjusting for BL mGFR: ρ=-0.24; p<0.001; ρ=-0.28; p<0.001; and ρ=-0.16; p=0.007, respectively. However, inclusion of other covariates into the model: BP index, CKD vintage, Tanner, Ht. and Wt. did not result in improvement of the prediction model beyond BL mGFR.

Conclusions: SDMA and its ratios were predictive of mGFR declines over 6-12 mos. in children and adolescents with CKD. Prospective analyses to define its significance in rapid CKD progression continue.

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TH-PO694

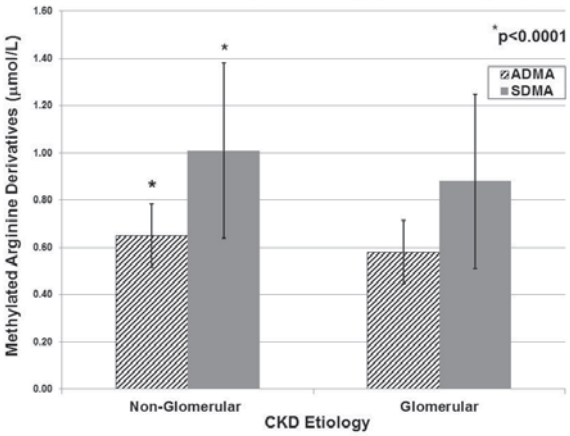
Methylated Arginine Derivative Concentrations Differ Between Non-Glomerular and Glomerular Chronic Kidney Disease in Children Ellen Brooks,[1,2] Shannon Haymond,[1,2] Rod Passman,[1] Irene Helenowski,[1] Alfred Rademaker,[1] Susan L. Furth,[3] Bradley A. Warady,[4] Craig B. Langman.[1,2] *[1]Feinberg Medical School, Northwestern Univ, Chicago, IL; [2]Ann & Robert H Lurie Children's Hosp. of Chicago, Chicago, IL; [3]Children's Hosp. of Philadelphia, Philadelphia, PA; [4]Children's Mercy Hosp., Kansas City, MO.*

Background: CKD GFR decline contributes to an imbalance of circulating methylated arginine (Arg) derivatives (MADs). Decreased asymmetric dimethylarginine (ADMA) catabolism is coupled to renovascular resistance and other pathology, denoting risk for end-organ damage, while symmetric DMA (SDMA) may augment inflammation as GFR declines. Our goal was to establish whether MADs concentrations differ in children and adolescents by their CKD etiology.

Methods: Baseline (BL) visit plasma was provided by the "Chronic Kidney Disease in Children" (CKiD) observational cohort study of children with CKD. Cohort etiology was categorized as non-glomerular (NG) versus glomerular (G). LC-MS/MS analyses for ADMA, SDMA, and Arg and comparison between cohorts using a Wilcoxon Rank-Sum test were completed. Directly measured GFR data (mGFR by plasma iohexol clearance) were similarly tested.

Results: 573 specimen results established that NG subjects had higher ADMA, SDMA, Arg/ADMA (p=0.003) and Arg/SDMA (p=0.002) ratios versus G (figure 1), whereas no difference was found between their BL mGFR (NG=48.8±18.4; G=53.7±25.7 ml/min/1.73m^2). However, the mGFR of NG and G subjects each had large standard deviations.

Figure 1. ADMA and SDMA By CKD Etiology (N=573; Mean±S.D.).



Conclusions: NG related CKD is mainly associated with congenital anomalies and reflux related infections, inflammation and renal scarring. Although, no BL mGFR cohort difference was found, it is possible that protracted infection from chronic bacterial colonization, monocyte migration, cytokine/chemokine upregulation, and oxidative stress may lead to higher MADs levels in NG versus G subjects.

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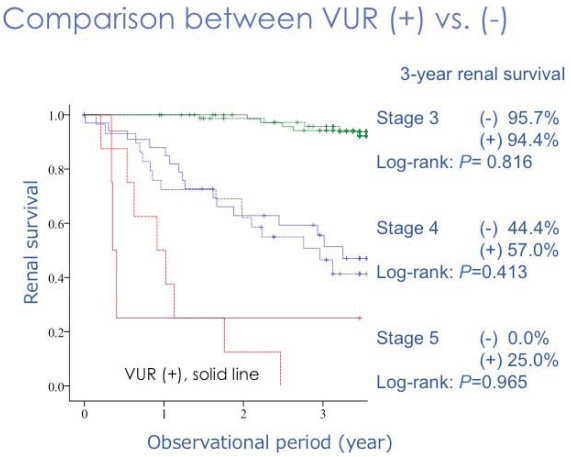
TH-PO695

Impact of Vesicoureteral Reflux on the Progression of Chronic Kidney Disease in Children: Results of a Nationwide Prospective Cohort Study in Japan Kenji Ishikura, Osamu Uemura, Yuko Hamasaki, Hideo Nakai, Shuichi Ito, Motoshi Hattori, Yasuo Ohashi, Ryojiro Tanaka, Koichi Nakanishi, Ryoko Harada, Tetsuji Kaneko, Masataka Honda. *The Pediatric CKD Study Group in Japan.*

Background: Congenital anomalies of the kidney and urinary tract (CAKUT), particularly hypoplastic and dysplastic kidneys, are frequent in children with advanced chronic kidney disease (CKD). However, little is known about the effects of vesicoureteral reflux (VUR), a frequent complication of hypoplastic and dysplastic kidneys, on the progression to end-stage kidney disease (ESKD) in such children.

Methods: In 2,010, we started a nationwide prospective study of our established cohort of 447 Japanese children (aged 3 months to 15 years) with stage 3–5 CKD. In the present study, 278 children with CAKUT as a primary etiology of CKD were divided into those with or without VUR. We compared the progression to ESKD between these groups.

Results: VUR was present in 117/278 children, of which 59 had bilateral severe (grade III or worse) VUR. The distributions of CKD stages and age were similar in both groups. The proportion of boys was higher, and more patients had hydronephrosis, megaureter, bladder dysfunction, or posterior urethral valves in the VUR-positive group (all, p<0.001; χ^2 test). However, the 3-year survival rates in each CKD stage did not differ between the two groups.



Multivariate analysis using Cox's proportional hazards model revealed that higher age and greater CKD stage were the only risk factors for progression to ESKD, and that VUR did not contribute to the progression to ESKD.

Conclusions: This 3-year prospective cohort study of children with CKD caused by CAKUT revealed significant differences in the sex distribution and rates of complicated urinary abnormalities between children with or without VUR. However, the presence of VUR did not influence progression to ESKD in these children.

Funding: Government Support - Non-U.S.

TH-PO696

Mortality and Renal Insufficiency among Children with Posterior Urethral Valve, a Longitudinal PHIS Study Vaka Kristin Sigurjonsdottir,[1] Katherine W. Herbst,[2] Cynthia J. D'Alessandri,[3] John H. Makari,[2] Vaka Kristin Sigurjonsdottir.[3] *[1]Univ of Connecticut; [2]Div of Urology; [3]Div of Nephrology, Connecticut Children´s Medical Center, Hartford, CT.*

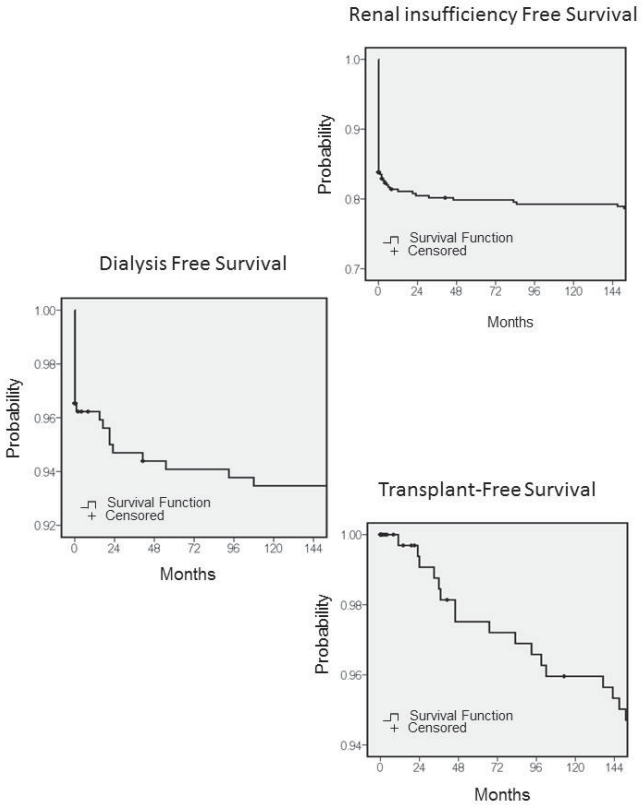
Background: The aim of this study was to determine mortality and estimated time to renal insufficiency, dialysis, or kidney transplant among a large cohort diagnosed with PUV in the neonatal period.

Methods: The Pediatric Health Information System (PHIS) database was searched for inpatient records of neonates admitted ≤30 days of life between 1/1/1992 - 12/31/1999 with an ICD-9 Diagnosis code for PUV (753.6). The database was then searched forward to 12/31/2013, and mortality based on discharge disposition, and all events based on ICD-9 code identified. Events were; renal insufficiency defined as Diagnostic codes 584.8, 584.9, 585, 585.5, 585.6, 585.9, 586; dialysis defined as Procedure codes 38.95, 39.95, 54.98, and transplant defined as Procedure codes 55.6, 55.61, 55.69. Kaplan-Meyer survival analysis was performed on time to first event using a proxy for follow-up of date of birth to study end date.

Results: A total of 346 neonates were identified, with median age at admittance of 2 days. Proxy follow-up was 14 – 22 years. Twenty (5.8%) expired during their neonatal hospitalization at a median age of 2.5 days (range 0-259). Three children expired after their neonatal admission (0.8%) due to renal failure at a median age of 4.12 years (range 14-113

mos). Seventy-eight (22.5%) reported renal insufficiency, 26 (7.5%) underwent dialysis, and 22 (6.4%) underwent transplant. Survival curves show most events, except transplant, occurred within the first 2 years of life (Fig 1).

Fig 1. 12 Year Event-Free Survival from Date of Birth to First Event (Censored for Mortality and Follow-up Duration)



Conclusions: In this cohort, incidence of renal insufficiency was substantial among patients with PUV despite early diagnosis and management. Transplant within this cohort appeared to be a delayed occurrence.

TH-PO697

The Associations of Perinatal Maternal and Offspring Health with Kidney Function in Young Adult Offspring Christine W. Hsu,[1] Jonathan Himmelfarb,[1] Bryan R. Kestenbaum,[1] Hagit Hochner,[2] Yechiel Friedlander,[2] David Siscovick.[3] *[1]Medicine/Nephrology - Kidney Research Inst, Univ of Washington, Seattle, WA; [2]Braun School of Public Health, Hebrew Univ, Jerusalem, Israel; [3]New York Academy of Medicine, New York, NY.*

Background: Prenatal factors including maternity obesity and low birth weight are associated with chronic kidney disease (CKD) in children. The extent to which prenatal risk factors impact adult kidney function is unclear.

Methods: We studied 1376 offspring who enrolled in the Jerusalem Perinatal Study between 1974-1976 and returned for a follow-up examination at a mean age of 32 years. Offspring were all born after a full term pregnancy (>36 weeks) and were free of congenital malformations. We measured serum creatinine and cystatin C in adult offspring and estimated the glomerular filtration rate (eGFR) using the combined CKD Epidemiology-Collaboration (CKD-EPI) equation. We used linear regression to determine associations of maternal pre-pregnancy body mass index (ppBMI), gestational weight gain (GWG), and birth weight (BW) with young adult offspring eGFR, adjusting for potential confounders.

Results: Mean eGFR in young adult offspring was 114.3 ml/min/1.73m^2 (interquartile range 107.2, 122.0 ml/min/1.73m^2). The lowest eGFR was 65.4 ml/min/1.73m^2 After adjustment for offspring gender, ethnicity, parental smoking status, socioeconomic status, maternal comorbidities, maternal age, and parity each 5 kg/m^2 higher maternal ppBMI was associated with an estimated 0.45 ml/mi/1.73m^2 greater eGFR (95% CI -0.87, 1.77). Each 5 kg greater maternal GWG and 500 g greater BW were associated with an estimated 0.25 ml/min/1.73m^2 lower (95% CI -1.38, 0.89) and 0.06 ml/min/1.73m^2 greater (95% CI -0.85, 0.96) eGFR in adult offspring, respectively. Null associations were similar for eGFR based on only creatinine or only cystatin C. Associations did not differ by strata of offspring BMI.

Conclusions: Maternal ppBMI, GWG, and birth weight are not associated with estimated GFR in young adult offspring with a history of full-term gestation. Confidence intervals from these analyses exclude large effects of maternal pregnancy factors on estimated kidney function in young adult offspring.

Funding: NIDDK Support, Private Foundation Support

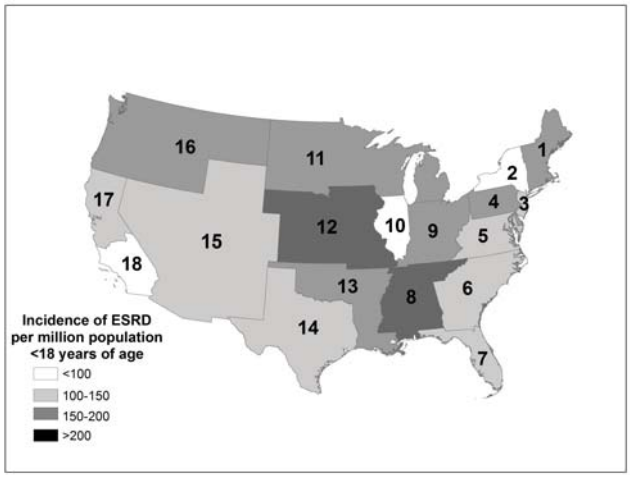
TH-PO698

Geographic Variation in Pediatric End-Stage Renal Disease Incidence Sandra Amaral,[1] Laura Plantinga,[2] Rachel E. Patzer.[3] *[1]Pediatrics, The Children's Hosp of Phila, Phila, PA; [2]Rollins School of Public Health, Emory Univ, Atlanta, GA; [3]Emory Transplant Center, Emory Univ, Atlanta, GA.*

Background: Geographic variation in end-stage renal disease (ESRD) is well-recognized in adults, with the highest rates in the southern and middle Atlantic United States (U.S.). The causes of renal disease differ substantially in children versus adults, with a significant proportion of children having congenital anomalies of the kidneys and urinary tract (CAKUT). We explored whether incidence and etiology of pediatric ESRD vary by U.S. region.

Methods: We identified all incident ESRD patients (2000-2011) <18 years of age in the United States Renal Data System (USRDS) data. We calculated pediatric ESRD incidence by dividing the number of pediatric ESRD patients across the 18 ESRD Network regions by the number of children <18 years within those regions (using census data). We examined geographic variation across the Network regions by etiology of pediatric ESRD (CAKUT, glomerulonephritis, FSGS, lupus, other).

Results: Pediatric ESRD incidence varies across ESRD Network regions, from 71.6 cases per million population (pmp) in Network 18 (S. CA) to 223.3 cases pmp in Network 12 (IA, KS, MO, NE).



ESRD Incidence was highest overall for CAKUT (133.3 pmp) and lowest for lupus (107.6 pmp). Significant geographic variation in disease etiology is observed across Networks (p<0.001), with pediatric ESRD incidence due to CAKUT being highest in Network 8 (AL, MS, TN) and lowest in Network 18 (92.5 versus 24.0 pmp), and incidence due to lupus highest in Network 7 (FL) and lowest in Network 16 (AK, WA, OR, ID, MT) (10.7 versus 1.4 pmp).

Conclusions: There is substantial geographic variation in ESRD incidence and etiology in the U.S. Further studies are needed to explain whether these findings are due to variations in demographics (such as race/ethnicity) or environmental exposures.

Funding: NIDDK Support, Other NIH Support - NIMHD, Other U.S. Government Support

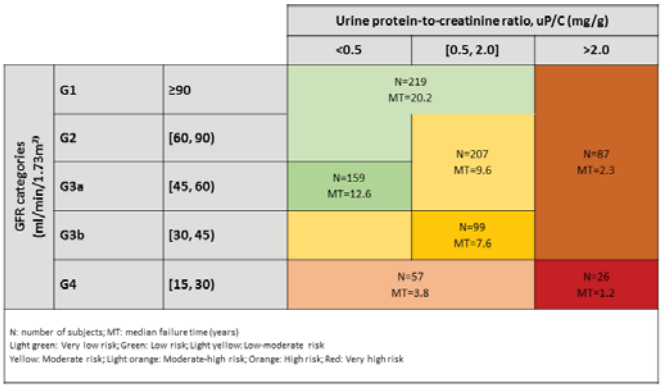
TH-PO699

KDIGO eGFR/Proteinuria Category and Risk in the Chronic Kidney Disease in Children (CKiD) Study Wun Fung Hui,[1,2] Christopher B. Pierce,[2] Alison G. Abraham,[2] Bradley A. Warady,[2] Colin T. White,[2] Susan L. Furth.[1,2] *[1]Dept of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA; [2]The Chronic Kidney Disease in Children (CKiD) Study Investigators.*

Background: Current KDIGO guidelines suggest incorporating albuminuria/proteinuria with eGFR and underlying disease in classifying and prognosticating patient outcome(s). Using CKiD study data we assessed risk of chronic kidney disease (CKD) progression in children.

Methods: Subjects were classified into 5 categories of eGFR and 3 ranges of proteinuria (table). Prognosis was determined by time (from baseline) to development of (i) a 50% reduction of baseline eGFR (ii) initiation of RRT or (iii) death. Parametric accelerated failure time models were used to model risk for the composite event by baseline eGFR/proteinuria categories. Akaike Information Criteria (AIC) of nested models were used to amalgamate categories of similar risk.

Results: We included 854 children (71% non-glomerular and 29% glomerular) with a median (IQR) age of 11 (8, 15) yr and 63% male. The distribution across the 5 GFR categories was: G1: 4%; G2: 30%; G3a: 29%; G3b: 27% and G4: 10%. The distribution of uP/C across the 3 ranges was <0.5: 57%, 0.5-2.0: 30% and >2.0: 13%. 219 events occurred over a median (IQR) follow-up of 2.8 (1.2, 5.7) yrs. Median time to the composite outcome was best described by 7 stages (table) and was observed to decrease when either a decline in eGFR category or increase in the range of proteinuria occurred. Patients with uP/C>2.0 and eGFR category of G4 carried a nearly 20-fold increased risk in comparison to those with eGFR categories of G1/G2 and uP/C<2.0.



Conclusions: A modified KDIGO classification in pediatric CKD provides valuable prognostic information regarding time to 50% reduction in eGFR, need for RRT or death, and should be implemented across pediatric CKD practice.

Funding: NIDDK Support

TH-PO700

Dyslipidemia Is Common and Persistent in Children with CKD Jeffrey M. Saland,[1] Christopher B. Pierce,[2] Mark Mitsnefes,[3] Joseph T. Flynn,[4] Juan C. Kupferman,[5] Bradley A. Warady,[6] Susan L. Furth.[7] *[1]Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Bloomberg School of Public Health, Johns Hopkins Univ, Baltimore, MD; [3]Pediatrics, Cincinnati Children's Hospital, Cincinnati, OH; [4]Pediatrics, Seattle Children's Hospital, Seattle, WA; [5]Pediatrics, Maimonides Children's Hospital, Brooklyn, NY; [6]Pediatrics, Children's Mercy Hospital, Kansas City, MO; [7]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: Dyslipidemia, a risk factor for CV disease, is common in pediatric CKD but its stability over time is not well-described.

Methods: CKiD is a prospective longitudinal cohort study of children aged 1-16 with CKD. Data from 338 subjects with at least 2 fasting lipid measures from year (yr) 2 (baseline), 4, 6, or 8 study visits were analyzed. 199 subjects had ≥3 measures. Total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) were measured; non-HDL-C was calculated.

Results: Dyslipidemia was found in 48% and 53% of children at baseline and after 6 yr of follow-up, respectively; only modest changes in lipids were seen. Specifically, there was no consistent change in average non-HDL-C after 2, 4, or 6 yr. During the same period there were small increases in HDL-C (+2, +3, and +3 mg/dl respectively) and also TG (+5%, +16%, and +12% respectively). For the latter trends, 2 and 4 yr change was statistically significant. Expected pubertal effects occurred: TC declined more in males than females, driven mainly by declines in HDL-C and less by non-HDL-C. These differences were larger with longer follow-up (6 yr> 4 yr > 2 yr). Non-HDL-C trended higher in African American (AA) children over time with statistical significance noted at the 4 yr follow-up. Among AA children with 4-6 yr of follow-up, prevalence of non-HDL>160 mg/dL rose from 10% to 25%, a trend (p=0.04) not seen in non-AA children. Other Factors: Change in HDL-C, non-HDL-C, and TG over follow-up was not associated with age, GFR, proteinuria, or glomerular CKD.

Conclusions: Dyslipidemia is a common and persistent exposure in children with CKD. African American children may be at increased risk of developing elevated non-HDL-C levels.

Funding: NIDDK Support

TH-PO701

Apolipoprotein L1, Income, and Early Kidney Damage Ruth Tamrat,[1] Carmen A. Peralta,[2] Salman Tajuddin,[3] Michele Kim Evans,[3] Alan B. Zonderman,[3] Deidra C. Crews.[1] *[1]Dept of Medicine, Johns Hopkins U., Baltimore, MD; [2]Dept of Medicine, U. of California-San Francisco, San Francisco, CA; [3]National Inst on Aging, NIH, Bethesda, MD.*

Background: High-risk Apolipoprotein L1 (*APOL1*) variants are associated with CKD/ESRD among African Americans (AAs). However, the degree to which genetic or environmental factors explain the risk of early kidney damage among AAs is unknown.

Methods: Among AAs in the community-based Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) cohort (Baltimore, MD), we examined the cross-sectional association of *APOL1* and income with: 1) high urine albumin to creatinine ratio (ACR), defined as ACR >17mg/g in men and ACR >25mg/g in women, 2) continuous eGFR (creatinine-cystatin C equation), and 3) reduced eGFR (<75 mL/min/1.73m²). *APOL1* status was considered 'high risk' if 2 copies of high-risk variants were present or 'low risk' if 0 or 1 copy was present. Income groups were dichotomized as annual household income <$14,000/year (lowest income group) or >=$14,000/year (higher income group). Multivariable logistic regression models included adjustment for age, sex, and % European ancestry.

Results: Among 462 participants (mean age 47y), 72% lived in poverty, and 16% had high risk *APOL1* status. High-risk *APOL1*, but not income, was associated with high ACR [Odds Ratio (OR) 3.8, 95% CI 2.0-7.3]. Neither *APOL1* nor income was associated with continuous eGFR. Lower income, but not *APOL1*, was associated with eGFR <75 (OR 1.8, 95% CI 1.2-2.7). Of note, low-risk *APOL1* participants accounted for the majority of high ACR (71%), and reduced eGFR (78%) in the cohort. Additionally, the lowest income group accounted for a substantial proportion of high ACR (43%) and eGFR<75 (53%). No statistically significant interactions between *APOL1* and income were noted.

Conclusions: High risk *APOL1* is associated with risk of albuminuria, but the polymorphism does not explain the majority of early kidney damage in this urban AA population. Other factors, including those related to socioeconomic status, likely play a greater role in early kidney disease among AAs.

Funding: NIDDK Support, Other NIH Support - National Institute on Aging, Private Foundation Support

TH-PO702

HDL-C Levels Are Not Associated with MACE in Patients with Advanced Reduced Kidney Function Jiyun Park, Kyung-Hwan Jeong, Chun-Gyoo Ihm, Tae Won Lee, Yang Gyun Kim, Ju-Young Moon, Tae Won Lee, Jong Shin Woo, Weon Kim. *Internal Medicine, Kyung Hee Univ School of Medicine, Seoul, Korea.*

Background: Recent data suggested that HDL-C levels did not associate with coronary artery disease severity in patients with reduced kidney function. But the impact of HDL-C levels on MACEs(Major Adverse Cardiac Events) during 1-year follow-up after AMI in patients with kidney dysfunction has not been defined. This study was based on a retrospective cohort, the Korean Acute Myocardial Infarction Registry (KAMIR) database.

Methods: Among 13897 patients who diagnosed AMI from November 2005 to July 2008, 1165 patients (male : 519, female : 646) with advanced reduced kidney function (eGFR < 30 ml/min per 1.73 m²) were included. We examined the association of pretreatment lipid profiles including HDL-C, total cholesterol, triglyceride, and LDL-C levels with MACEs. MACEs include including cardiac death, recurrent MI, or coronary artery bypass graft (CABG) surgery. Univariate and multivariate analyses were utilized for identifying clinicopathologic factors including lipid profiles predictive of MACEs.

Results: Total 248 patients experienced MACEs.On the Multivariate analyses, lower HDL-C (<40 mg/dl) was not associated with increased risk of MACEs (hazard ratio [HR] of 1.38, 95% confidence interval [95% CI], 0.98–1.94, [P = 0.06]). There was not graded interaction between reduced kidney function with MACEs (hazard ratio [HR] of 1.04, 95% confidence interval [95% CI], 0.69–1.54, [P = 0.83]). Other lipid profiles including total cholesterol, triglyceride, and LDL-C levels, diabetes, cardiovascular disease history, smoking history, age, and BMI were not also associated with MACEs [P > 0.05] in patients with advanced reduced kidney function. Only statin therapy after MI was associated with reduced MACEs (hazard ratio [HR] of 0.71, 95% confidence interval [95% CI], 0.51–1.10, [P = 0.05]).

Conclusions: In conclusion, HDL-C levels are not associated with MACEs and statin therapy could be beneficial after MI in patients with advanced reduced kidney function.

TH-PO703

Low High-Density Lipoprotein Cholesterol Is Associated with All-Cause Mortality in People with Chronic Kidney Disease: Pooled Analysis of 6 Cohort Studies in Japan (EPOCH-JAPAN) Masaharu Nagata,[1] Toshiharu Ninomiya,[2] Yutaka Kiyohara,[1] Yoshitaka Murakami,[3] Yoshihiro Miyamoto,[4] Hiroyasu Iso,[5] Yutaka Imai,[6] Katsuyuki Miura,[7] Hirotsugu Ueshima,[7] Tomonori Okamura.[8] *[1]Dept of Environmental Medicine, Kyushu Univ; [2]Center for Cohort Studies, Kyushu Univ; [3]Dept of Medical Statistics, Toho Univ; [4]Dept of Preventive Cardiology, National Cerebral and Cardiovascular Center; [5]Public Health, Dept of Social Medicine, Osaka Univ Graduate School of Medicine; [6]Dept of Planning for Drug Development and Clinical Evaluation, Tohoku Univ; [7]Dept of Public Health, Shiga Univ of Medical Science; [8]Dept of Preventive Medicine and Public Health, Keio Univ.*

Background: Serum high-density lipoprotein cholesterol (HDLC) has been reported to be inversely associated with mortality in the general population. However, this issue remains unclear in people with chronic kidney disease (CKD).

Methods: Using individual pooled data of 33,180 subjects aged 40 to 89 years participated in 6 prospective cohort studies in Japan, we investigated the association between low HDLC levels defined as HDLC <40 mg/dL and all-cause mortality by CKD status. CKD was defined as eGFR <60 ml/min/1.73 m². The influence of HDLC on the risk of all-cause mortality was estimated by using a stratified Cox proportional hazards model.

Results: During an average of 12.5-year follow-up period, a total of 4,273 subjects died. In the multivariable analysis, low HDLC was associated with 37% (95% confidence interval 3-83%) greater risk of all-cause mortality in subjects with CKD and 17% (5-30%) greater risk in those without CKD. There was no evidence of heterogeneity in the association between subgroups of CKD status (p for heterogeneity=0.58). Subjects with both low HDLC levels and CKD had a 1.83-fold (1.50-2.23) higher risk of all-cause mortality than those with neither of these risk factors.

Conclusions: Our findings suggest that low HDLC levels are a significant risk factor for all-cause mortality in people with CKD, as well as in those without CKD.

Funding: Government Support - Non-U.S.

TH-PO704

Association Between Non-High-Density Lipoprotein Cholesterol and Coronary Heart Disease According to Chronic Kidney Disease Status: The Hisayama Study Tomoko Usui,[1,2] Masaharu Nagata,[1,2] Toshiharu Ninomiya,[2,3] Kensuke Izumaru,[1,2] Takanari Kitazono,[2,3] Yutaka Kiyohara.[1,3] *[1]Dept of Environmental Medicine; [2]Dept of Medicine and Clinical Science; [3]Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: Growing evidence suggests that elevated non-high-density lipoprotein cholesterol (non-HDLC) levels are causally related to an increased risk of coronary heart disease (CHD) events in the general population. However, it is not clear whether the association is similar for those with and without chronic kidney disease (CKD).

Methods: We evaluated the association between non-HDLC levels and the risk of CHD in a prospective cohort study in Japan. A total of 2,630 community-dwelling subjects (1,107 men and 1,523 women) aged ≥40 years were followed for 19 years. Non-HDLC levels were divided into <150 mg/dL, 150-189 mg/dL, and ≥190 mg/dL. CKD was defined as eGFR <60 ml/min/1.73m² or proteinuria (≥1+ on dipstick). The multivariable-adjusted hazard ratio (HR) and 95% confidence interval (CI) were estimated using a Cox proportional hazards model.

Results: Among all subjects, 357 (13.6%) had CKD. During the follow-up period, 186 CHD events occurred. The age- and sex-adjusted incidence of CHD significantly increased with elevating non-HDLC levels in subjects with or without CKD (p for trend=0.002 in subjects without CKD, 0.019 in subjects with CKD). In subjects without CKD, those with non-HDLC ≥190 mg/dL had a 2.12-fold (95% CI: 1.32-3.38) greater risk of CHD than those with non-HDLC <150 mg/dL after adjustment for potential confounding risk factors. Similarly, in CKD subjects, the risk of CHD was significantly higher in non-HDLC 150-189 mg/dL level and more (adjusted HRs [95% CI]: 1.00 [reference] in non-HDLC <150 mg/dL, 2.23 [1.04-4.77] in 150-189 mg/dL, and 3.20 [1.46-7.03] in ≥190 mg/dL). There was no evidence of interaction in the association for CHD between non-HDLC levels and CKD status (p for interaction=0.61).

Conclusions: Elevated non-HDLC levels are a significant risk factor for the development of CHD not only among people without CKD, but also with CKD.

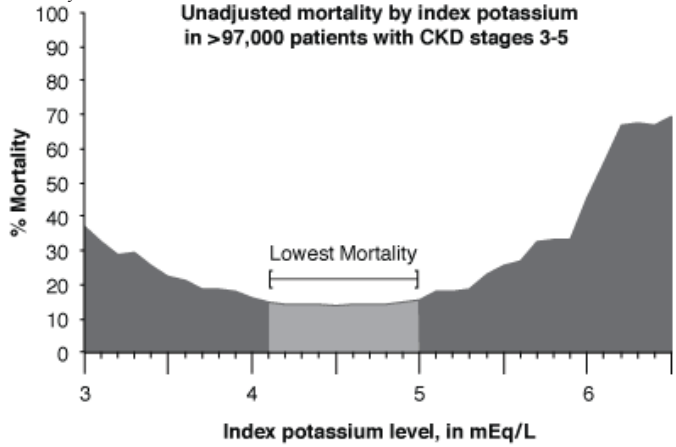
TH-PO705

Potassium Levels and Mortality in Patients with CKD Allan J. Collins,[1] Nancy Reaven,[2] Susan Funk,[2] George L. Bakris,[3] Bertram Pitt,[4] David A. Bushinsky.[5] *[1]Univ of Minnesota, Minneapolis, MN; [2]Strategic Health Resources, La Canada, CA; [3]Univ of Chicago, Chicago, IL; [4]Univ of Michigan, Ann Arbor, MI; [5]Univ of Rochester, Rochester, NY.*

Background: Abnormal serum potassium levels are common in patients with advanced chronic kidney disease (CKD), yet the degree of mortality risk at different levels of potassium is not clear. We evaluated the odds of death in patients with CKD stages 3-5, stratified by potassium level.

Methods: De-identified medical records (2007-2012) from a large U.S. population of individuals ≥ 5 years of age with at least 2 potassium readings were evaluated. Patients with CKD stages 3-5 (n=97,415) were identified from ICD-9 codes and biochemical data, excluding those with acute kidney injury or end stage renal disease. Index potassium value was defined as the last reported value prior to pre-determined cut-off date. Mortality was evaluated through hospital discharge records and Social Security registry information.

Results: Unadjusted mortality rates are shown in the Figure. Patients with index potassium levels below 4.1 mEq/L and above 5.0 mEq/L show a significant increase in mortality, even at levels within the usual normal laboratory range. The increased mortality remained after adjustments for demographic characteristics (sex, age, race) and comorbidities, including heart failure, diabetes, hypertension, cardiovascular disease and acute myocardial infarction.



Conclusions: These findings suggest that there is a significant increase in mortality at serum potassium levels below 4.1 mEq/L and above 5.0 mEq/L in patients with CKD stages 3-5.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

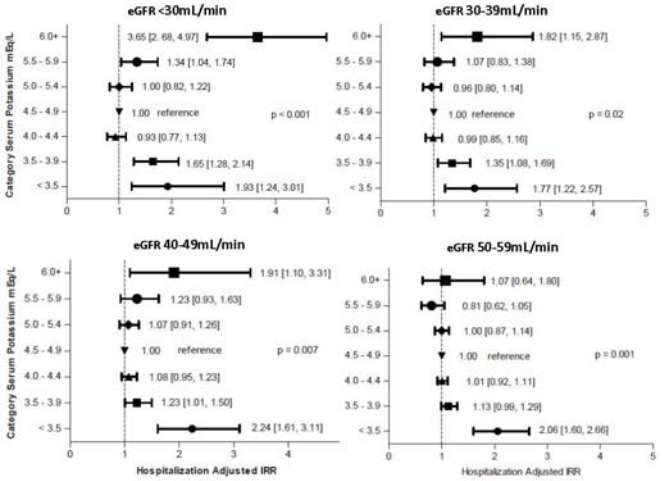
TH-PO706

The Association Between Serum Potassium and Hospitalization in Patients with Chronic Kidney Disease Jiacong Luo,[1] Steven M. Brunelli,[1] Donna E. Jensen,[1] Alex Yang.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]ZS Pharma, Inc, Menlo Park, CA.*

Background: In states of health, serum potassium (K) is tightly regulated, typically between 3.5 and 5.0mEq/L. The resultant transmembrane electrochemical potential is essential to normal physiologic function. Due to impaired excretory capacity, patients with chronic kidney disease (CKD) accumulate K in the serum (hyperkalemia). We studied the association of serum K with hospitalization rates, within narrow estimated glomerular filtration rate (eGFR) strata to understand the potential burden of hyperkalemia and how this may vary by underlying CKD severity.

Methods: We assembled a retrospective cohort of patients who had eGFR <60 mL/min/1.73 m² and available serum K data between Jan-2009 and Jun-2013 (N=55,266). Patients were followed until study end (30-Jun-2013), death, end-stage disease, transplant, or transfer of care. Serum K, eGFR, and covariates, which included 13 demographic, comorbid, and medication utilization characteristics, were considered on a time-varying basis and updated at each K measurement. Hospitalization was considered as events per time at-risk.

Results: At baseline, serum K was <3.5 and ≥5.0mEq/L in 1.6% and 20% of patients, respectively. Graphical representation of the adjusted K-hospitalization association was U-shaped.



Risk associated with higher K was greater at lower levels of eGFR. Compared to K 4.5-4.9mEq/L, K ≥6.0mEq/L was significantly associated with higher adjusted hospitalization rate for eGFR <49 mL/min/1.73m²; K 5.5-5.9mEq/L was significantly associated with higher adjusted hospitalization rate for eGFR <30 mL/min/1.73m².

Conclusions: Higher serum potassium is associated with greater adjusted rates of hospitalization. Hospitalization risk is greater with higher serum K and lower eGFR with almost 4-fold greater risk in CKD stage 4/5 patients with a K ≥6.0 mEq/L.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

TH-PO707

The Acute Effect of Red Blood Cell (RBC) Transfusion on Hyperkalemia (HK) in Advanced Chronic Kidney Disease (CKD) Keri Monda,[1] Jeffrey C. Fink,[2] David T. Gilbertson,[3] Karminder S. Gill,[4] Paul Muntner,[5] Richard A. Lafayette,[6] Jeffrey Petersen,[1] Glenn M. Chertow,[6] Brian D. Bradbury.[1] *[1]Center for Observational Research, Amgen, Inc, Thousand Oaks, CA; [2]Dept of Medicine, U Maryland, Baltimore, MD; [3]Chronic Disease Research Group, Minneapolis, MN; [4]Ascentiant Int, Carlsbad, CA; [5]Dept of Epidemiology, U of Alabama, Birmingham, Birmingham, AB; [6]Div of Nephrology, Stanford U School of Medicine, Palo Alto, CA.*

Background: Transfusion may contribute to the development of HK in patients with advanced CKD due to their impaired response to an expanded potassium load. The risk for HK associated with transfusion has not been quantified previously.

Methods: Persons 18-64 years of age with diagnosed stage 4 or 5 CKD (not requiring dialysis) between 2006 and 2010 were followed until their first hospitalization with a diagnosis of HK, termination of insurance coverage, or death, using the OptumInsight claims database. We estimated incidence rates (IRs) and 95% confidence intervals (CIs) for HK using Poisson regression. We estimated the rate ratio (RR) and 95% CI for the association between transfusion and the risk of HK using a case-only design, matching each HK case to multiple (1:m) weekly control periods with no HK, and adjusted for the following time-varying confounders: acute kidney injury, hospitalization, anemia, and GI bleeding. Exposure was assessed in the 3 days immediately preceding the HK or control date.

Results: Overall, 7,829 individuals with stage 4 or 5 CKD met our inclusion criteria; 68% were age 50 or older; 43% were female; 51% had diabetes. During follow-up 711 patients were hospitalized with a diagnosis of HK (IR: 7.9 per 100 PYs [95% CI: 7.3-8.5]). For the 575 cases with at least one week of follow-up there were 24,769 control periods;

2.3% of cases and 0.1% of controls were exposed to transfusion. This corresponded to a crude RR of 21.6 (95% CI: 10.1-46.5), and 6.1 (95% CI: 2.5-15.1) after adjustment for time-varying confounders.

Conclusions: These data provide evidence of a high burden of hyperkalemia in patients with advanced CKD and show a meaningful elevation in risk for hyperkalemia following transfusion.

Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO708

The Association of BUN to Creatinine Ratio with Pre-ESRD Mortality and ESRD in Chronic Kidney Disease Georges Saab,[1] Jesse D. Schold,[2] Susana Arrigain,[2] Joseph V. Nally,[2] Robert J. Heyka,[2] Sankar D. Navaneethan.[2] *[1]MetroHealth Medical Center, Cleveland, OH; [2]Cleveland Clinic Foundation, Cleveland, OH.*

Background: An elevated blood urea nitrogen to creatinine ratio (BUN/Cr) has been suggested to be a renal manifestation of neuro-hormonal activation (NHA) in congestive heart failure (CHF). The mediators of NHA may also lead to progressive renal function decline and increased mortality in chronic kidney disease (CKD). Whether an elevated BUN/Cr may be associated with these outcomes in CKD is unknown.

Methods: Patients with stages 3 and 4 CKD with at least 2 measurements of estimated glomerular filtration rate (eGFR) 15-60 at least 90 days apart between January 2005 and September 2009 were examined. For primary analysis, we calculated BUN/Cr from the 2nd eGFR < 60. For sensitivity analysis, we selected patients that had at least one other BUN/Cr ratio drawn in the 4 months prior to 2nd eGFR, and took the average of all the ratios available in that period. Gender specific BUN/Cr quartiles were generated and then combined. We used Cox proportional hazards models to evaluate the association between BUN/Cr quartiles and ESRD, and pre-ESRD mortality.

Results: A total of 38,927 and 13,173 patients were included in the primary and sensitivity analyses, respectively. Higher BUN/Cr quartiles were associated with increasing age and a lower percentage of African Americans. Patients with higher BUN/Cr had an increasing prevalence of diabetes, hypertension, coronary artery disease, CHF, and cerebrovascular disease and more likely to be treated with diuretics, statins, and angiotensin converting enzyme inhibitors or angiotensin receptor blockers. After multivariate adjustment, patients in the 4th quartile had a higher risk of ESRD (HR: 1.40, 95% CI 1.14, 1.72) and pre-ESRD mortality (HR: 1.34, 95% CI 1.25, 1.44) as compared to the 1st quartile. In the sensitivity analysis, the results were similar for ESRD (HR: 1.34, 95% CI 1.00, 1.78) and pre-ESRD mortality (HR: 1.29, 95% CI 1.16, 1.43). These results were not modified by gender nor eGFR.

Conclusions: Patients in the highest BUN/Cr quartile have an increased risk for ESRD and pre-ESRD mortality. The mechanisms behind this require further study.

Funding: Pharmaceutical Company Support - Amgen

TH-PO709

Serum Phosphorus Levels Predict All-Cause Mortality without Evidence of Chronic Kidney Disease Kyung Don Yoo, Nam Ju Heo, Dong Ki Kim, Ho Jun Chin, Kwon Wook Joo, Yon Su Kim, Hajeong Lee. *Internal Medicine, Seoul National Univ College of Medicine, Korea.*

Background: Higher serum phosphorus levels were significantly associated with cardiovascular morbidity and mortality in both chronic kidney disease (CKD) and end-stage renal disease patients. However, whether serum phosphorus levels have an influence on mortality in general population without chronic kidney disease remains to be clarified.

Methods: Data had been collected from 136,235 individuals who received routine health check-ups between 1995 and 2009. Among them, participants who were undergone a colonoscopy in same date was excluded. Subjects with possible CKD such as estimated glomerular filtration rate under 60 mL/min/1.73m^2 and urine dipstick albumin more than 1+ were also excluded. Outcome was all-cause mortality extracted from Statistics Korea at December 2011.

Results: A total of 89,193 individuals were included. The mean age was 53.3 years and male participants were 49,078 (55.0 %). Because serum phosphorus levels were distributed differently according to gender, they were analyzed separately. Serum phosphorus levels were categorized by quartile. In men, highest phosphorus quartile group was younger, had higher serum albumin levels, and included more current smoker. In contrast, highest phosphorus quartile group was older and included more diabetes patients in women. In survival analysis, serum phosphorus levels were not associated with mortality in univariate cox-regression analysis in men. Interestingly however, multivariate analysis revealed that higher serum phosphorus levels were independent predictor for all-cause mortality after adjustment for age, diabetes, hypertension, body mass index, calcium, albumin, hemoglobin, glucose, systolic blood pressure, dipstick albuminuria, smoking history (HR 1.27, 95% CI 1.102–1.484, p=0.0012). In case of women, these associations were not observed (HR 1.094, 95% CI 0.837–1.430, p=0.5114).

Conclusions: In this study, we demonstrated that serum phosphorus levels were associated with all-cause mortality in general men without evidence of CKD. Further prospective interventional studies are warranted to elucidate different gender effect to serum phosphorus levels.

TH-PO710

Association of High and Low Serum Bicarbonate with Left Ventricular (LV) Structure and Function in CKD –A Report from the Chronic Renal Insufficiency Cohort (CRIC) Study Mirela A. Dobre,[1] Amanda Hyre Anderson,[2] Nisha Bansal,[2] Jing Chen,[2] Rajat Deo,[2] Paul E. Drawz,[2] Harold I. Feldman,[2] L. Lee Hamm,[2] Thomas H. Hostetter,[1] John W. Kusek,[2] Claudia M. Lora,[2] Akinlolu O. Ojo,[2] Kumar Sharma,[2] Mahboob Rahman.[1] *[1]Case Western Reserve Univ; [2]CRIC Study.*

Background: Heart failure (HF) is a common occurrence in patients with CKD. High bicarbonate levels have been associated with increased rates of HF; however, the mechanisms mediating this association are incompletely known. We aim to evaluate whether bicarbonate levels are also associated with subclinical measures of HF.

Methods: We performed a cross-sectional analysis of the association between serum bicarbonate and echocardiographic measures of cardiac structure and function, including LV hypertrophy, LV mass indexed to height$^{2.7}$, relative wall thickness, LV geometry, ejection fraction and diastolic dysfunction in CRIC. Study participants (n=3483) who did not have HF class III/IV were stratified into 3 groups by levels of serum bicarbonate: Low (<22mEq/L), n=614; Normal [22-26mEq/L], n=2001; and High (>26mEq/L), n=868.

Results: The mean age was 58.9 (10.8) years and baseline eGFR was 42.5±17 ml/min per 1.73m^2. The prevalence of LV hypertrophy (LVH) was 64%, 56% and 53% for serum bicarbonate categories < 22, 22-26 and > 26 mEq/L, respectively. In multivariable analyses adjusted for demographic characteristics, kidney function, traditional cardiovascular risk factors and medications, there was no difference in prevalence of LVH between the low (OR 1.17, 95%CI 0.88-1.55, p = 0.29) and high bicarbonate groups (OR 0.97, 95%CI 0.77 - 1.22, p = 0.79) compared to the normal group. Similarly, there were no differences in the mean left ventricular mass, relative wall thickness, LV geometry, ejection fraction and prevalence of diastolic dysfunction when comparing the low and high to the normal bicarbonate group.

Conclusions: In this large cohort of men and women with CKD, serum bicarbonate was not associated in cross-sectional analyses with measures of abnormal cardiac structure and function. Whether changes in serum bicarbonate over time are associated with changes in cardiac structure and function, or risk of heart failure should be investigated.

Funding: NIDDK Support, Private Foundation Support

TH-PO711

Urinary Ammonia and Long Term Outcomes in Chronic Kidney Disease Pascal Houillier,[1,2] Marion Vallet,[1] Marie Metzger,[3] Jean-Philippe Haymann,[1,2] Martin Flamant,[1,2] Benedicte Stengel.[3] *[1]Assistance-Publique-Hopitaux de Paris, Paris, France; [2]INSERM, Paris, France; [3]CESP, INSERM, Villejuif, France.*

Background: Recent studies suggest that alkalinizing treatments improve the course of chronic kidney disease (CKD), even in patients without overt metabolic acidosis. We hypothesized that a decreased ability in excreting urinary acid rather than overt metabolic acidosis may be deleterious to the course of CKD.

Methods: We studied the associations between baseline plasma total CO_2 level or urinary ammonia excretion and long-term CKD outcomes in 1,065 patients with CKD stage 1 to 4 from the NephroTest cohort. All patients had glomerular filtration rate measured (mGFR) by ^{51}Cr-EDTA renal clearance. Crude and adjusted-hazard ratios (HR) of mortality and end stage renal disease (ESRD) were estimated using Cox models. Crude and adjusted odd ratios of fast decline in GFR (>10 %/year) were estimated by logistic regression.

Results: Median mGFR at baseline was 37.6 mL/min/1.73 m^2. Urinary ammonia excretion decreased with GFR, while net endogenous acid production did not. After a mean follow-up of 4.3 years, 201 patients reached end-stage renal disease (ESRD) and 114 died before ESRD. Twenty-six percent of the patients had mGFR decline rate greater than 10% per year. Hazard ratio for ESRD was significantly and independently increased in those in the lowest tertile of urinary ammonia excretion compared to those in the highest tertile (HR: 1.82, 95% CI 1.06-3.13). Likewise, the odds ratio of fast mGFR decline was borderline significant (OR: 1.84, 95% CI 0.98-3.48). Neither a low plasma total CO_2 level nor metabolic acidosis were associated with ESRD risk or fast renal function decline. None of these biomarkers was associated with mortality.

Conclusions: These results suggest that the inability to excrete the daily acid load is deleterious to renal outcomes, but not to mortality.

Funding: Government Support - Non-U.S.

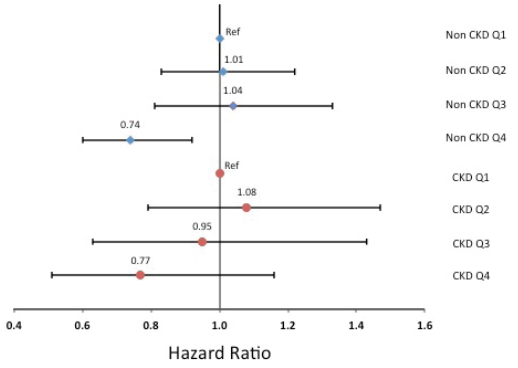
TH-PO712

Net Endogenous Acid Production and Mortality in Chronic Kidney Disease Hunter K. Huston,[1] Matthew K. Abramowitz,[2] Y. Zhang,[1] Tom Greene,[1] Kalani L. Raphael.[1] *[1]Medicine, Univ of Utah School of Medicine; [2]Medicine, Albert Einstein College of Medicine.*

Background: Metabolic acidosis increases mortality risk in chronic kidney disease (CKD). High dietary acid intake may lower serum [HCO_3^-] and influence this relationship. This study investigated whether higher net endogenous acid production (NEAP), an estimate of dietary acid intake, is a mortality risk factor independent of serum [HCO_3^-] in CKD.

Methods: Data from the Third National Health and Nutrition Examination Survey were used in this analysis. Daily NEAP (mEq) was calculated as -10.2 + 54.5 x [protein intake (grams)/potassium intake (mEq)]. Protein and potassium intakes were obtained from dietary recall. Cox models were performed in CKD and non-CKD subgroups and were adjusted for demographic factors, estimated glomerular filtration rate, serum [HCO_3^-], urinary albumin:creatinine, comorbidities, and diuretics. Additional analyses were performed using protein intake and potassium intake as predictor variables in separate Cox models.

Results: In participants without CKD, mortality risk was significantly lower for those in the highest NEAP quartile compared to the lowest. In participants with CKD, there was a trend towards lower mortality risk for those in the highest NEAP quartile, however, this was not statistically significant.



Higher protein intake associated with a trend towards lower mortality risk, whereas potassium intake did not associate with mortality.

Conclusions: Higher NEAP associates with lower mortality risk in non-CKD independent of serum [HCO_3^-]. A similar pattern was observed for those with CKD, but this was not statistically significant, contrary to our hypothesis. Higher protein intake may explain the relationship between lower mortality risk and higher NEAP in NHANES III participants.

Funding: Other NIH Support - National Center for Research Resources and the National Center for Advancing Translational Sciences, Veterans Affairs Support, Private Foundation Support

TH-PO713

Dietary Habits and Risk of Incident Chronic Kidney Disease in an Urban Population Yang Liu,[1] Marie Kuczmarski,[2] Edgar R. Miller,[1] Alan B. Zonderman,[3] Michele Kim Evans,[3] Neil R. Powe,[4] Deidra C. Crews.[1] *[1]Johns Hopkins U, Baltimore, MD; [2]U of Delaware, Newark, DE; [3]National Inst of Aging, NIH, Bethesda, MD; [4]U of California, San Francisco, CA.*

Background: Adherence to a Dietary Approaches to Stop Hypertension (DASH) –type diet has been shown to lower blood pressure, however, little is known about its potential relation with risk of chronic kidney disease (CKD).

Methods: Among 1,339 community dwelling participants free of CKD at baseline in the Baltimore, MD based Healthy Aging in Neighborhoods of Diversity across the Life Span study, a DASH diet adherence score based on 9 target nutrients (total fat, saturated fat, protein, fiber, cholesterol, calcium, magnesium, sodium, and potassium) was examined for its association with significant decline in kidney function over an average of 5 years of follow up. Cox models were used to adjust for potential confounders.

Results: Participants' mean age was 47 years with the majority being either female (65%) or African American (AA) (59%). Low DASH diet adherence (lowest tertile of DASH score), when compared to higher adherence, was associated with being male, AA, poor, and/or less educated. Low DASH adherence was significantly associated with the composite measure of eGFR decline below 60 ml/min/1.73m^2 and greater than 25% during follow-up.

		eGFR<60 at follow up (N events = 67)		eGFR decline >=25% (N events = 81)		BOTH eGFR decline >=25% & eGFR < 60 at follow up (N events= 35)	
Model	Adjusted for:	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
1	Crude	1.4 (0.9,2.2)	0.19	1.4 (0.9,2.2)	0.10	2.0 (1.0,4.0)	0.04
2	+ Age, Sex, Race	1.4 (0.9,2.3)	0.19	1.5 (0.9,2.3)	0.09	2.1 (1.1,4.3)	0.03
3	+ Poverty	1.4 (0.8,2.2)	0.21	1.5 (1.0,2.3)	0.08	2.1 (1.1,4.2)	0.03

Further adjustment for albuminuria among 892 participants with available urinary albumin-to-creatinine measures attenuated the association towards the null [HR 0.9; 95% CI 0.4–2.3]. No effect modification by race, poverty status, hypertension or diabetes was noted.

Conclusions: Low DASH diet adherence is associated with higher risk of incident CKD among urban-dwelling adults, and albuminuria may mediate this association.

Funding: Other NIH Support - National Institute on Aging, NIH, Other U.S. Government Support

TH-PO714

The Views of Patients on Dietary and Fluid Restrictions in CKD: A Thematic Synthesis of Qualitative Studies Suetonia Palmer,[1] Camilla Sara Hanson,[2] Jonathan C. Craig,[2] Giovanni F.M. Strippoli,[3,4] Marinella Ruospo,[3,4] Katrina L. Campbell,[5] David W. Johnson,[5] Allison Tong.[2] *[1]Univ of Otago Christchurch; [2]Univ of Sydney; [3]Fondazione Mario Negri Sud; [4]Amedeo Avogadro Univ of Eastern Piedmont; [5]Univ of Queensland.*

Background: Negotiating complex fluid and dietary recommendations in chronic kidney disease is very challenging for patients. We aimed to describe patients' perspectives of dietary and fluid management in CKD to inform clinical practice that can incorporate patient experiences as well as to understand and explain patient choices.

Methods: We did a systematic review of qualitative studies in adults with CKD that reported experiences and beliefs about dietary and fluid management. MEDLINE, Embase, PsycInfo, CINAHL, Google Scholar and PhD dissertations were searched. Thematic synthesis was used to analyze findings.

Results: We included 46 studies involving 816 patients living in middle- to high-income countries. Five major themes were identified: preserving relationships (interference with roles, social limitations, and being a burden), navigating change (feeling deprived, disrupting held truths, breaking habits and norms, overwhelmed by information, questioning efficacy, and negotiating priorities), fighting temptation (resisting impositions, mental invasion, and withstanding physiological needs), optimizing health (accepting responsibility, valuing self-management, preventing disease progression, preparing for and protecting a transplant), and becoming empowered (comprehending paradoxes, finding solutions, and mastering change and demands). Limited data were available for low income settings and for adults who had CKD not treated with dialysis or transplantation.

Conclusions: Dietary and fluid restrictions are disorienting and an intense burden for patients with CKD. Patient prioritized education strategies, harnessing patients' motivation to stay well for a transplant or to avoid dialysis, and viewing adaptation to restrictions as a collaborative journey are suggested strategies to help patients adjust to dietary regimens in order to reduce their impact on quality of life.

TH-PO715

The Relationship between Poor Appetite and Clinical Outcomes in Pediatric Patients with Chronic Kidney Disease Frank Ayestaran,[1] Michael F. Schneider,[2] Frederick J. Kaskel,[2] Poyyapakkam Srivaths,[2] Patricia Seo-Mayer,[2] Marva M. Moxey-Mims,[2] Susan L. Furth,[2] Bradley A. Warady,[2] Larry A. Greenbaum.[1,2] *[1]Emory Univ, Atlanta, GA; [2]Chronic Kidney Disease in Children Study (CKiD).*

Background: Poor appetite increases the risk for malnutrition in children with chronic kidney disease (CKD). In adult dialysis patients, self-reported poor appetite is related to lower quality of life (QoL), higher inflammatory markers, hospitalization rates and risk of death. The relationship between self-reported appetite and clinical outcomes hasn't been reported in children with CKD. We plan to describe the relationship between self-reported appetite and GFR, number of ER visits, hospitalizations and QoL in pediatric patients with CKD.

Methods: 802 participants in the Chronic Kidney in Children (CKiD) study, a longitudinal cohort study of children with CKD, contributed 2918 person-visits to analyses. Appetite in the week prior to each annual visit was characterized as very good, good, fair, or poor/very poor. Mixed effects ordinal logistic regression was used to quantify the degree of association that CKD stage had with appetite. Linear regression was used to assess the relationship between QoL and appetite while Poisson regression was used to assess the relationship between both ER visits and hospitalizations and appetite.

Results: Decreasing GFR was associated with a worse appetite; those with GFR < 30 ml/min per 1.73m^2 had a 6.08 greater odds (95% CI: 3.48, 10.63) of reporting a worse appetite than those with GFR > 90. Compared to a report of a very good appetite, a poor or very poor appetite was associated with a higher number of ER visits [Rate Ratio: 1.92 (CI: 1.19, 3.09)], lower parental reported QoL [mean difference: -7.7(CI: -11.5, -3.9)] and lower child reported QoL [mean difference: -6.2 (CI: -10.1, -2.3)].

Conclusions: Self-reported appetite worsens with declining GFR in children with CKD. Poor appetite is associated with worse outcomes in children with CKD, including higher risk for ER visits in the following year and poorer QoL.

TH-PO716

Quality of Life and Depression in Patients with Chronic Kidney Disease: The Systolic Blood Pressure Intervention Trial Suzanne Watnick,[1] Jill C. Newman,[2] Gregory W. Evans,[2] Joni Snyder,[3] Capri G. Foy,[3] Dan Berlowitz,[5] Paul L. Kimmel.[4] *[1]Dept of Veterans Affairs, Portland, OR; [2]Wake Forest School of Medicine, Winston-Salem, NC; [3]NHLBI, Bethesda, MD; [4]NIDDK, Bethesda, MD; [5]Dept of Veterans Affairs, Bedford, MA.*

Background: Depression and diminished quality of life (QOL) are strongly associated with chronic kidney disease (CKD). However, no data exist comparing depression and QOL in CKD to a control group. The Systolic Blood Pressure Intervention Trial (SPRINT) is a unique opportunity to evaluate QOL and depression in a large community-based cohort of hypertensive patients, with and without CKD.

Methods: We performed a cross sectional study of patients in SPRINT, a randomized trial of 9361 hypertensive subjects, including 2648 with CKD stage 3-4. We compared QOL variables at baseline by CKD status, and correlations between QOL parameters, including

mental (MCS) and physical (PCS) scores on the VR-12 instrument, pain perception, and depressive symptoms on the PHQ-9. We performed multivariable logistic regression analyses to examine associations between CKD and the four QOL parameters.

Results: Mean eGFR was 47.9 + 9.5 in CKD and 81.3 + 15.5 ml/min/1.73m2 in non-CKD hypertensive patients. CKD patients had, as expected, worse PCS (43.2 + 10.5 versus 45.3 + 10.1), but unexpectedly, better MCS (53.9 + 9.1 versus 52.8 + 9.7) than those without CKD ($p < 0.0001$ for both). In all patients, pain scores correlated with PHQ-9, MCS and PCS scores. MCS correlated with PHQ-9 scores, and PHQ-9 scores correlated with pain, MCS and PCS scores (all $r>0.3$, $p<0.0001$). In bivariate analyses, CKD was not associated with PHQ-9, but was associated with better MCS and worse PCS. After multivariable adjustment for demographic/personal characteristics, CKD remained associated with worse PCS scores, but not with mental health scores.

Conclusions: We conclude that QOL and depression are related to pain in patients with hypertension, with and without CKD. Although CKD patients in SPRINT had worse physical health scores than non-CKD subjects, unexpectedly depression is not characteristic of hypertensive CKD subjects with early stage CKD. This may be because CKD is usually symptomless at this stage.

Funding: Other NIH Support - NHLBI, Veterans Affairs Support

TH-PO717

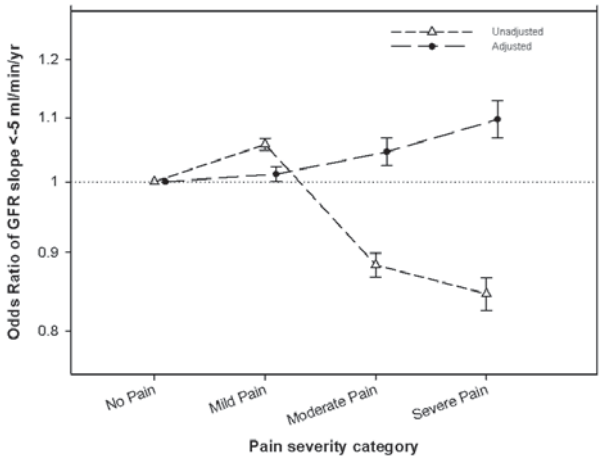
Association of Pain with Progressive Chronic Kidney Disease in over 1.5 Million U.S. Veterans

Joline L.T. Chen,[1] Elani Streja,[2] Vanessa A. Ravel,[2] Miklos Zsolt Molnar,[3] Jun Ling Lu,[3] Kamyar Kalantar-Zadeh,[2] Csaba P. Kovesdy.[3] *[1]Long Beach VAMC, Long Beach, CA; [2]Harold Simmons, UC Irvine MC, Orange, CA; [3]Memphis VAMC, Memphis, TN.*

Background: Chronic non-malignant pain is prevalent among U.S. veterans. Pain activates the sympathetic nervous system, which may lead to hypertension. It is also associated with higher analgesic and antidepressant use. These may lead to increased risk for CKD and ESRD, which are highly prevalent among U.S. veterans. The risk of incident CKD or worsening kidney function associated with pain in patients with normal kidney function is unclear.

Methods: In a cohort of 1,585,051 Veterans with normal baseline eGFR in 2005-2006, we examined the association of reported pain with incidence of CKD stages 3a, 3b, 4, 5, and slopes of eGFR over median follow up of 7.3 years (IQR: 4.1-8.3). Pain scores reported with the Brief Pain Index were categorized into none, mild, moderate, or severe pain. Associations were examined in crude and adjusted Cox models and logistic regression models (for slopes ≤-5ml/min/1.73m^2/year), with adjustments for demographics, comorbidities, cardiovascular risk factors, and depression.

Results: At baseline, patients were 60±14 yrs, 16.8% African American, 93.6% male with mean baseline eGFR was 84±16 ml/min/1.73m^2. 15.3%, 3.7%, 0.7%, and 0.3% of patients developed CKD stage 3a,3b,4,and 5 respectively. 9.1% of veterans had GFR decline greater than 5 ml/min/1.73m^2/year. Pain severity was associated with higher baseline eGFR, younger age, fewer cardiovascular and other comorbidities. In multivariable adjusted logistic regression models worse pain was associated with incrementally higher odds ratios of kidney function loss greater than 5 ml/min/1.73m^2/yr.



Conclusions: Pain is incrementally associated with faster progression of CKD. Studies to evaluate whether pain management can have a bearing on development and progression of CKD and poor outcomes are warranted.

Funding: NIDDK Support

TH-PO718

Cognitive Impairment in Chronic Kidney Disease: The Brain IN Kidney Disease (BRINK) Study

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Background: The BRain IN Kidney disease (BRINK) study aims to characterize the burden of and risk factors for cognitive impairment (CI) in 400 CKD and 130 non-CKD subjects. We describe the frequency of domain-specific CI in both cohorts thus far.

Methods: Participants (ppts) with CKD stages 3-5 (eGFR < 60 mL/min/1.73m^2, not on dialysis) and age- and race-matched non-CKD controls, (eGFR ≥ mL/min) were recruited from 4 Minneapolis/St. Paul health systems. Domain-specific neuropsychological testing was performed at baseline using the following tests: Modified Mini-Mental State Exam (3MS), global cognition; Hopkins Verbal Learning Test-Revised (HVLT-R, Immediate and Delayed), verbal memory; Controlled Oral Word Association Test (COWAT), language; Color Trails Test 2 (CTT2), executive function; Symbol-Digit Modality Test (SDMT), executive function; Digit Span, attention; Brief Visuospatial Memory Test-Revised (BVMT-R Immediate and Delayed), visual spatial/memory; and the PHQ Depression Scale. We compared raw scores and T-scores using published norms.

Results: We recruited 423 ppts (CKD [eGFR < 45] N=265, mild CKD (eGFR 45-59) N=76, non-CKD N=82). Mean ages were 70±9.6 (CKD) and 66±10.5 (non-CKD) yrs ($p<0.01$). Baseline eGFR (CKD) was 30±9.1 mL/min. Prevalence of HTN was higher in CKD ppts than in controls (94% versus 65%, $p<0.01$) but was similar for prior stroke (17% versus 15%, $p=0.56$) and diabetes (53% versus 59%, $p=0.35$). CKD ppts scored worse on the 3MS (93±5.9 versus 95±5.6, $p=0.01$), BVMT-R immediate (16±7.0 versus 20±7.9, $p<0.01$), COWAT (35±12.0 versus 40±12.5, $p<0.01$), and SDMT (36±11.3 versus 43±11.8, $p<0.01$); associated T-scores also differed significantly. Scores on the HVLT-R delayed, CTT2, Digit Span, BVMT-R delayed, or PHQ9 depression scale did not differ significantly.

Conclusions: CKD ppts had significantly lower levels of global cognitive function, visual spatial/memory, and executive function/processing speed, consistent with possible mixed neurodegenerative and vascular cognitive impairment. Further analyses will measure factors associated with global and domain-specific CI.

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TH-PO719

Associations Between Chronic Kidney Disease, Depression, and Incident Dementia in a Diabetic Cohort

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Background: Chronic kidney disease (CKD) is a risk factor for dementia in the elderly. Whether CKD is associated with dementia in a primary care population with diabetes is not known. Furthermore, whether the association between CKD and dementia is modified by depression, is not known.

Methods: The Pathways Study is a prospective, observational cohort of ambulatory, diabetic patients from a large managed care population in Seattle, WA. Subjects without baseline dementia were followed for up to 10 years for incident dementia based on ICD-9 codes. Baseline estimated glomerular filtration rate (eGFR) (CKD-EPI) and microalbuminuria were used to categorize patients into early (CKD stage 1-2), moderate (CKD stage 3), or advanced (CKD stage 4-5) stage CKD. Cox proportional hazards regression was used to analyze the associations between CKD and incident dementia after adjusting for age, race/ethnicity, marital status, education, smoking, hypertension, low-density lipoprotein (LDL), body mass index (BMI), hemoglobin A1c, diabetes duration, and major depression.

Results: Of the 3,960 total subjects, 331 developed dementia over a median follow up of 8.7 years (IQR 3.8-10). Compared to subjects with normal renal function, CKD was progressively associated with an increased risk of dementia (adjusted hazard ratio (AHR) 1.65, 95% CI 1.00-2.71 for early CKD; AHR 1.97, 95% CI 1.17-3.31 for moderate CKD; AHR 3.78, 95% CI 1.68-8.50 for advanced CKD). Major depression was associated with a 3.48-fold increased risk of dementia (95% CI 2.42-5.01). There was no evidence for an interaction between CKD stage and major depression. Additional risk factors for dementia included older age and smoking (AHR 1.83, 95% CI 1.02-3.28), but not duration of diabetes or hemoglobin A1c. Moderate obesity and higher LDL were associated with a lower risk of dementia.

Conclusions: All stages of CKD were risk factors for incident dementia in a primary care diabetic population, and these associations were not modified by major depression. Advanced stage CKD poses a similar risk for dementia that is comparable to major depression.

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TH-PO720

Association of Systolic Blood Pressure Level with Mortality, ESRD, and Cardiovascular Events in Elderly Patients with CKD

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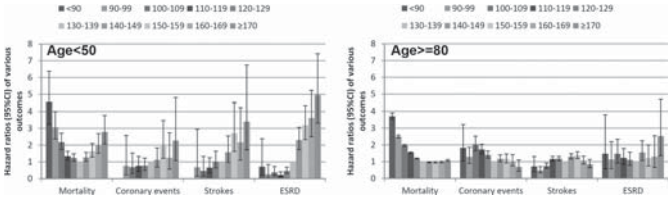
Background: Hypertension is considered an important treatable risk factor for vascular and renal outcomes. A large proportion of patients with CKD are elderly, but the ideal SBP levels in these individuals are unknown.

Methods: From among 3,285,684 U.S. veterans with normal eGFR during 2005-2006, we identified 690,380 patients who developed CKD (2 consecutive eGFR values <60, >=90 days apart). Associations of SBP with subsequent clinical end points (all-cause mortality, incident coronary events (MI or CABG), strokes, and ESRD) were examined in time-dependent Cox models adjusted for gender, baseline eGFR, comorbidities, and time dependent DBP and antihypertensive medication use. The effect of age was studied by examining outcomes in patients categorized by age (<50 through >=80 years old, in 10-year increments).

Results: Compared to young patients, older patients showed markedly different associations with all studied outcomes. The lowest all-cause mortality was associated with

SBP 130-140 mmHg in the <50 group, and with SBP 140-160 mmHg in the >=80 group. The risk of coronary events, strokes and ESRD increased linearly with higher SBP in the <50 group, but showed an inverse association for coronary events, and no associations with strokes or with ESRD in the >=80 group (Figure). The transition in risk patterns was gradual, with more marked changes observed above age 70.



Conclusions: SBP shows markedly different associations in young versus old patients with CKD. High SBP shows significant associations with poor vascular outcomes in young patients, but no, or inverse associations in older patients. Until randomized clinical trials become available, caution is warranted when treating hypertension in elderly patients with CKD.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO721

Swimming Exercise Training (EXE) Decrease the Mortality Rate in Rats with Chronic Kidney Disease (CKD) by 5/6 Nephrectomy (5/6Nx)

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Background: The aim of this study was to evaluate the EXE effects on renal function and mortality rate in rats with 5/6Nx.

Methods: Adult Wistar rats were divided in groups (n=8): Control (CTL), Control + Exercise (CTL+EXE), Sedentary 5/6Nx (Nx+SED) and 5/6Nx + Exercise (Nx+EXE). The protocol was employed in 5/6Nx rats after 7 days from the surgical procedures. Swimming periods were 60min/day, 5 days a week during 8 weeks. It was evaluated arterial pressure (AP), maximal exercise test (MEtest), creatinine *clearance* (CrCl), proteinuria (uProt), as well mortality rate.

Results: EXE did not modify the increment in MAP but prevent, at least in part, a lower decline in the MEtest caused by 5/6Nx (29±1 versus 16±2 m/min, p<0.05), Table. A higher CrCl in Nx+EXE was observed compared with Nx+SED, 2.27±0.33 versus 0.96±0..20 ml/min, respectively (p<0.05). Proteinúria was significantly lower in Nx+EXE versus NxS group (42.73 ± 1.50 versus 60.60 ± 2.00 mg/24h, p<0.05). A higher mortality rate was observed in Nx+SED (70%) versus Nx+EXE group (38%, p<0.05).

	C	E	NxS	NxE
AP (mmHg)	125 ± 1	128 ± 2	220 ± 16	210 ± 6
MEtest (m/min)	26 ± 2	36 ± 1	16 ± 2 *#	29 ± 1 #&
CrCL/ (ml/min/BW)	1.77 ± 0.18	1.52 ± 0.21	0.96 ± 0.20	2.27 ± 0.33 &
uProt (mg/24h)	12.00 ± 1.12	17.70 ± 1.38	60.60 ± 2.00 *#	42.73 ± 1.50 *#&
Mortality Rate (%)	0	0	70 *#	39 *#&

* vs C; # vs E; & vs NxS , p<0.05

Conclusions: Results suggested that the EXE minimize the impact of 5/6Nx, by lower decline in the CrCl (42%) and an important reduction in the increment of proteinuria that occurs with 5/6Nx by 30%. Finally, the lower mortality rate in Nx+EXE versus Nx+SED, indicate exercise protection especially in renal function that under this experimental protocol, prevent a potential decline in the progression of renal disease. Thus, it is reasonable to suggest that EXE could be an additional strategy to be employed in CKD.

TH-PO722

Exercise Training Improves Kidney Function, Cardiovascular Health, and Cardio-Respiratory Fitness in Patients with Progressive Stage 3-4 Chronic Kidney Disease: A Randomised Controlled Study

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Background: The leading cause of death in patients with chronic kidney disease (CKD) is cardiovascular disease (CVD). Exercise capacity is significantly reduced in pre-dialysis patients.This pilot study examined the effect of 12-months of exercise training on kidney function and indices of CVD risk in patients with progressive stages 3-4 CKD.

Methods: 20 patients were randomised to a rehabilitation group (REHAB, n = 10) or usual care (UC, n = 10) for 12 months. The REHAB group received exercise training (3x/week) and the UC group received standard care. Kidney function was assessed by comparing individual rate of change slopes in eGFRcr for each participant for a 12 months pre-intervention period, and for the 12-month intervention period using ANCOVA. Pulse Wave Velocity (PWV), VO_2peak, waist circumference (WC), and weight were assessed at 0, 6 and 12 months.

Results: 18 participants completed the study (REHAB, n=8; UC n=10). Following the intervention, a significant mean difference in eGFRcr (+7.8±3.0 ml/min/1.73m^2/year (p=0.03, 95% CI 0.53, 9.79) was observed between REHAB and UC groups. There were significant between group mean differences,after adjustment for baseline values, in PWV (-2.30 m/sec; 95%CI -3.02, -1.59), BMI (-3.3 kg/m^2; 95%CI -5.7, -0.9), weight (-5.6 kg p=0.02, 95%CI -10.1, -1.2), WC (-7.1±2.18 cm; 95%CI -12.4, -3.2), and relative VO_2peak (5.7 ml/kg/min; 95%CI 1.34, 10.10). Change in eGFRcr was inversely correlated with PWV (r = -0.5, p= 0.04), and the change in WC was associated with change in eGFRcr (r = -0.6, p= 0.004) in all patients at 12 months.

Conclusions: The intervention has the potential to improve kidney function, VO2peak, PWV, waist circumference and quality of life in patients with progressing stages 3–4 CKD. This small study suggests that this is a feasible approach for exercising this patient population and sets the scene for a large multi-centre study.

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TH-PO723

Renal Function Reserve in Healthy Subjects by Two Different Oral Protein Load to Evaluate a Standard Method for 'Renal Stress Test'

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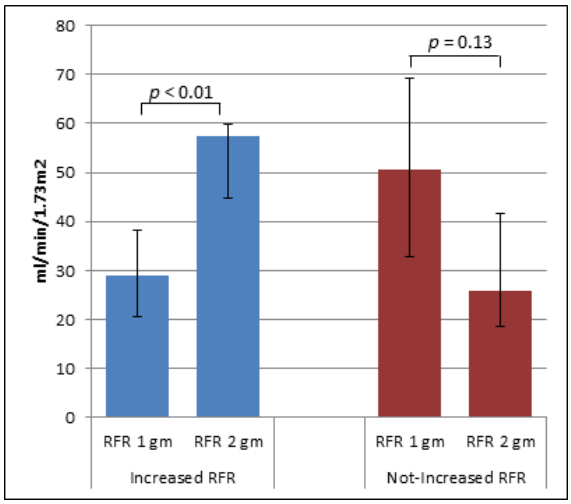
Background: Renal Function Reserve (RFR) represents the capacity of the kidney to increase glomerular filtration rate (GFR) in response to certain physiological or pathological stimuli.

Methods: An observational study among 18 young healthy individuals for the evaluation of a standard protocol for renal stress test with a protein load of 1 and 2 gram/kg body weight. To evaluate the differences of GFR observed before and after protein load (PL) a t-test was performed in all subjects. Data are expressed as ml/min/1.73m2, median [I-III interquartile] and *p* value. A *p* value <0.01 was considered statistically significant.

Results: Median basal GFR for all subject was 99.7[99.4-106.8]. The maximal GFR after 1 gm PL (139.64[123.2-165.7]) was not statistically different than 2 gm PL (147.2[130.5-155.0]). 44.4% of subject had a statistically significant higher RFR with 2gm than 1 gm (respectively 57.4[44.6-59.8] versus 28.9[20.4-38.2], p<0.01) while 55.6% did not (50.5[32.7-69.2] versus 25.7[18.6-41.5], p>0.01).

Basal GFR 99.7[99.4-106.8]	**Stress GFR 1gm**	**Stress GFR 2gm**	***p***
	139.6[123.2-165.7]	147.2[130.5-155.0]	N.S.
	RFR 1gm	**RFR 2gm**	***p***
Increased RFR (44.4%)	28.9[20.4-38.2]	57.4[44.6-59.8]	<0.01
Not Increased RFR(55.6%)	50.5[32.7-69.2]	25.7[18.6-41.5]	N.S.



Conclusions: With this study we found large variation in the stress GFR and the renal function reserve among individuals. These variations in the stress GFR and RFR can be used to make an ideal standard stress test to predict risk of acute kidney injury and future risk for chronic kidney disease, and to uncover possible loss of renal functional mass.

TH-PO724

Accuracy for Creatinine Test on Dry Blood Drop Sample on Filter Paper for Chronic Kidney Disease Screening

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Background: CKD screening is advisable due to the high morbidity and mortality and is usually performed by sampling blood and urine.

Methods: Here we present an innovative and simpler means, by measuring creatinine in a drop of dry blood on filter paper. 106 individuals at high risk for CKD were enrolled.

Results: Mean age 57 ± 12 years, 74% were female, 40% white, 34% mullatos and 25% black. 76% were hypertensive, 30% diabetic, 37% had family history for CKD, and 22% smoked. The BMI was 29,5 ± 6,9 kg/m^2, systolic blood pressure was 125 mmHg (IQR 120-140 mmHg) and diastolic blood pressure was 80 mmHg (IQR 70-80 mmHg). The validity of the filter paper test was calculated considering CKD as eGFR<60/ml/min. According to MDRD equation, sensitivity was 96%, specificity 55%, predictive positive value was 96%, predictive negative value was 55%, and accuracy was 92%. Results for Cockcroft-Gault and CKD-EPI were similar. A Bland and Altman analysis showed a relatively narrow range of creatinine values differences (+0,68mg/dl to -0,55mg/dl) between ±1,96 SD, without systematic differences.

Conclusions: Accordingly, creatinine measured on dry blood samples is minimally invasive, disclosed excellent accuracy, and may be useful for screening CKD.

TH-PO725

Albumin Creatinine Ratio versus Protein Creatinine Ratio for Nephrology Referral in Primary Care Sohan Shah,[1] Bhavna Pandya.[1,2] *[1]Faculty of Health and Life Sciences, Univ of Liverpool, Liverpool, United Kingdom; [2]Nephrology Dept, Aintree Univ Hospitals, Liverpool, United Kingdom.*

Background: It is well established that spot-urine tests like albumin:creatinine ratio (ACR) and protein:creatinine ratio (PCR) are comparable with values from 24-hour urinary protein collection (24h UPC) within certain ranges of proteinuria. ACR, in particular, has shown to be more sensitive and useful in diabetic patients. Current UK guidelines suggest referral of patients at ACR >70 or PCR >100 mg/mmol. We aimed to compare both tests for referral of nephrology patients from primary care.

Methods: Retrospective analysis of 502 urine samples with data for ACR, PCR and 24h UPC between 2006 and 2013 was conducted. Samples were assessed for corresponding ACR and PCR values for 24h UP >1g/day in order to determine cut-off values for significant proteinuria. Cost analysis was also performed.

Results: ACR [Spearman's $\rho = 0.813$ ($p<0.001$), $R^2 = 0.312$] and PCR [$\rho = 0.918$ ($p<0.001$), $R^2 = 0.845$] correlate well with 24h UP. The correlation between ACR and PCR was also strong [$\rho = 0.822$ ($p<0.001$), $R^2 = 0.276$]. ROC curves were used to analyse the performance of ACR and PCR in predicting significant proteinuria. The area under the curve (AUC) for ACR was 0.953 and 0.978 for PCR. An ACR of 70 mg/mmol had a much lower sensitivity (67.9% versus 82.4%) but marginally higher specificity (98.1% versus 96.0%) than a PCR of 100 mg/mmol in detecting significant proteinuria. The optimal cut-off points for ACR (27.11 mg/mmol) and PCR (69.55 mg/mmol) were determined to maximise the discriminatory ability of each test for significant proteinuria (>1g/day). The average cost per sample for ACR and PCR was ≤0.76 and ≤0.60 respectively.

Conclusions: ACR and PCR spot-urine tests are both suitable for the detection of significant proteinuria in an unselected cohort of patients. PCR is more sensitive and correlates better with 24h UP at lower and higher thresholds; however, the variable proportion of non-albumin proteins contributing to PCR must be accounted for. Still, ACR is the more specific test over different age groups and in the presence of comorbidities. We recommend lower referral cut-off criteria for ACR and PCR.

TH-PO726

Glycated Albumin as an Indicator of Glycemic Control in Pre-Dialysis Diabetic Chronic Kidney Disease Patients Sung Chang Bae, Hyeong Cheon Park, Hoon Young Choi, Sung-Kyu Ha, Ah Ran Choi, Tae Hoon Kim, Miok Cho. *Nephrology Div of Internal Medicine, Gangnam Severance Hospital, Yonsei Univ College of Medicine, Seoul, Korea.*

Background: Good glycemic control is important to prevent progression of diabetic nephropathy and reduce cardiovascular complications. Recently, several studies suggested that hemoglobin A1c (HbA1c), a widely used glycemic control marker, has serious limitations by falsely underestimating glycemic control in diabetic patients on hemodialysis. In contrast, glycated albumin (GA), which is not influenced by RBC survival time, is thought to more accurately reflect glycemic control in ESRD patients. Study aim was to validate this finding in pre-dialysis diabetic chronic kidney disease (CKD) patients.

Methods: Clinically stable pre-dialysis type 2 diabetic patients were enrolled between March 2009 and August 2012. A total of 497 patients were enrolled and stratified into 6 groups using newly revised KDIGO CKD staging that subdivided stage 3 into 3a and 3b stages. Parameters of glycemic control were investigated along with other biochemical and clinical parameters.

Results: The numbers of patients according to CKD stages 1 to 4-5 were consisted of 168, 151, 76, 47, and 55 subjects, respectively. The HbA1c and GA showed positive correlation at all CKD stages, however, the slope of regression line between GA and HbA1c was significantly steeper (control group versus other CKD stages: $p<0.05$) with decrease in renal function. The GA/HbA1c and serum glucose/HbA1c in CKD stage 3b and 4-5 were significantly higher than in controls, and the GA/HbA1c increased ($r=0.22$, $p<0.001$) with progression of renal failure. In contrast, the glucose/GA remained constant throughout the all CKD stages ($r=0.07$, $p=0.06$). In multivariate analysis, weekly erythropoietin (EPO) dose ($p=0.02$) was associated with HbA1c. The cut off value in ROC curve of weekly EPO dose affecting HbA1c was 6000 U/week (AUC 0.91, 95% CI 0.78–1.00).

Conclusions: Our results indicate that HbA1c may underestimate glycemic control state even in pre-dialysis diabetic CKD patients, especially those who are on EPO treatment. The GA might be an useful indicator of glycemic control in pre-dialysis diabetic CKD patients.

TH-PO727

An Increase in the EPA/AA Ratio Is Associated with Reduction of Proteinuria in CKD Patients Hitoshi Suzuki, Chieko Nogi, Hiroaki Io, Yusuke Suzuki, Yasuhiko Tomino. *Nephrology, Juntendo Univ Faculty of Medicine, Tokyo, Japan.*

Background: Previous epidemiological studies demonstrated that the ratio of n-6 to n-3 polyunsaturated fatty acids is associated with cardiovascular diseases. Recently, there is increasing evidences that dyslipidemia contribute to progression of CKD. We herein investigated whether the beneficial effect of highly purified eicosapentaenoic acid (EPA) on progression of CKD is associated with changes in the ratio of EPA relative to arachidonic acid (AA), in patients with dyslipidemia.

Methods: We measured serum levels of EPA/AA ratio, oxidized-LDL, high sensitive CRP (hs-CRP), Asymmetric Dimethylarginine (ADMA), and urinary albumin and 8-Hydroxydeoxyguanosine (8-OHDG) before and after treatment with highly purified EPA for six months (1.8g daily, n=71). Basic therapy, such as, statin, angiotensin receptor blocker and angiotensin converting enzyme inhibitor were not changed during the clinical study.

Results: Before treatment with EPA, the EPA/AA ratio in CKD patients is lower than those in non-CKD patients ($P<0.05$). Especially, in patients with CKD G4 and G5, the EPA/AA ratio were low, compared to patients with early stage of CKD ($P<0.05$). EPA significantly increased the EPA/AA ratio and decreased serum level of triglyceride ($P<0.05$). Moreover, the levels of urinary albumin significantly decreased at six months (-14.5 %, $P<0.01$). Importantly, a regression analysis revealed that an increase in the EPA/AA ratio significantly determinants of a reduction of proteinuria, but not a decrease of serum triglyceride by EPA($P<0.05$). Highly purified EPA also has effects to reduce serum levels of oxidized-LDL, hs-CRP, and urinary 8-OHDG ($P<0.01$, respectively).

Conclusions: EPA improves the urinary protein in association with an increase in the EPA/AA ratio in CKD patients with dyslipidemia. EPA may have renoprotective role by the anti-inflammatic effect due to the reduction of oxidative stress.

TH-PO728

Dietary and Herbal Supplement in CKD and ESRD on the Internet: Truth versus Myth Helga B. Vamenta-Morris, Emaad M. Abdel-Rahman. *Nephrology, UVA, Charlottesville, VA.*

Background: Interest and use of complementary and alternative medicine have garnered rising interest recently. Natural products including herbs, vitamins, and minerals are the most popularly consumed. The Internet is a ubiquitous source of information and a market for herbal and dietary supplements. Many of these substances have not been validated scientifically. Production and sale are not strictly regulated. Studies showed that the use of some of these supplements may be potentially harmful to CKD patients. **Aim:** Evaluate dietary and herbal supplement product advertisement claims on CKD/ESRD benefit on the Internet and determine if these claims are scientifically validated.

Methods: A questionnaire assessing each website was formulated. Each product ingredient was recorded onto the questionnaire by two independent reviewers. Statistical analysis was performed.

Results: Of the 184 websites reviewed, 9.2 % and 28.7% claimed to have products that are alternative to renal replacement therapy and decrease CKD progression, respectively. Only 39.4% of the sites recommended consulting a doctor. Over 90 % did not caution against potential drug- and disease-interaction and in the use during pregnancy or in children. The ten common plant ingredients found with claims to be beneficial for kidney patients were uva ursi, dandelion, parsley, corn silk, juniper, celery, buchu, horsetail, marshmallow, and stinging nettle. In contrast to their claims, the effects of these substances on kidneys were not validated in humans. The available animal studies showed detrimental effects and potential drug interactions with commonly used medications in the CKD/ESRD population.

Conclusions: Most sites do not offer sufficient cautionary guidelines, and their product claims of renal benefit could not be verified. Nephrologists need to be cognizant of the lack of substantiated proof of benefits of these substances and the potential adverse effects in the animal models that may translate to patients. Most importantly the policy needs to change regarding regulation of manufacture, advertisement, and sale of these products to prevent patient harm and misinformation.

TH-PO729

Treatment of IgANephropathy with Renal Insufficiency Using a Chinese Herbal Medicine Prescription Lin Wang,[1] Pan Gan,[2] Xianwen Zhang,[3] Yueyi Deng,[4] Zhi Qiang Huang,[5] Yiping Chen.[6] *[1]Long Hua Hospital, China; [2]Shanghai Univ of Traditional Chinese Medicine, China; [3]Long Hua Hospital, China; [4]Long Hua Hospital, Shanghai, China; [5]Univ of Alabama at Birmingham, Birmingham, AL, American Samoa; [6]Long Hua Hospital, China.*

Background: Shen'An Decoction (SA) is a Chinese herbal medicine prescription used to treat IgA nephropathy (IgAN) with renal insufficiency in China for decades. To evaluate its efficacy, we compared therapeutic responses to SA with those to SA plus steroids, cytotoxic agents with or without ARB (SA+).

Methods: Seventy-one patients with biopsy-proven IgAN with renal insufficiency were studied. All the pathological findings were at or above Lee Grade III. 32 patients were treated with SA, and 39 patients with SA+. Median follow-up time was 38.5 mos. Average age was 38±10 and 36±8 yrs, respectively. Estimated glomerular filtration rate (eGFR) ranged from 15 to 75 ml/min/1.73m^2 and urinary protein (UP)≥1.0 g in both groups.

Results: In SA group, eGFR improved from 44.81±20.5 to 53.4±22.9, Serum creatinine (Scr) dropped from 2.0±1.13 to 1.72±0.93 mg/dl and UP dropped from 2.60±0.35 to 1.11±0.26 g/24 h after the treatment; in SA+ group, eGFR improved from 56.1±15.2 to

67.8±19.2, Scr dropped from 1.58±0.49 to 1.36±0.44 mg/dl and UP dropped from 1.65±0.19 to 1.27±0.20 g/24 h after the treatment. There was no statistically significant difference between SA *versus SA+ in* ΔeGFR and ΔScr. ΔUP *of SA group was significant greater than that of SA+*. There were no differences in blood pressure among the groups with different treatments. *Among patients treated with SA+, 5 patients developed herpes zoster 12 months after treatment and 6 patients developed upper-respiratory-tract infection followed by exacerbated proteinuria, 3 female patients developed cessation of menstruation and 2 showed increased GPT. No side effects were reported for patients treated with SA only.*

Conclusions: SA improved renal function and proteinuria in severe IgAN with similar efficacy as SA+. Significant side effects were found in SA+ group but not in SA. These data justify future randomized clinical trials to assess the clinical impact of SA in severe IgAN treatment.

TH-PO730

Exploring Clinical and Pharmacological Effects of Nalbuphine HCl Oral Tablets in Hemodialysis Subjects with Pruritus

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Background: Nalbuphine is a mixed mu-antagonist/kappa agonist opioid marketed as a parenteral solution indicated for moderate to severe pain. It has been shown to reduce itch in morphine induced pruritus and Substance-P induced itch mouse model. An extended release (ER) nalbuphine oral dosage form was developed. The literature is sparse on opioid drug class studies in hemodialysis (HD) subjects and there is no literature on the use of nalbuphine in this population. There is no currently approved treatment of uremic pruritus and the literature reports increased morbidity and mortality in the moderate to severe pruritic HD population.

Methods: Nalbuphine ER tablets were studied up to 17 days in an open label multiple escalating dose study in 14 HD subjects with pruritus to assess safety, PK and explore its impact on itch as measured by self-report itch-VAS (visual analog scale) ranging from 0 (none) to 10 (worst possible). Nalbuphine was safely titrated over a wide range (30 mg-240 mg BID).

Results: Safety data included extensive monitoring of EKG, blood pressure, pulse oximetry during waking hours and continuous pulse oximetry at nighttime with no significant findings. The drug was clinically well tolerated over the entire dose range. PK analysis indicated that nalbuphine does not accumulate upon repeated doses and is not dialyzed by HD. Despite the extensive use of concomitant medications, there was no obvious findings indicating safety, PK or pharmacodynamic adverse drug interactions. Nalbuphine suppressed itch in a dose dependent manner in 12/14 subjects from a mean VAS 4.0 (1.3-6.6) to 1.2 at 180 mg BID. Subgroup analysis of 8/14 with moderate to severe pruritus (mean VAS 5.1 (4.2-6.6) showed a pronounced decrease with mean change -1.2, -2.2, -3.4, -3.6, -4.9 at the 30, 60,120, 180 and 240 mg BID doses respectively.The greatest percentage decrements occurred at 60 and 120 mg BID doses.

Conclusions: Nalbuphine ER tablets can be safely administered to HD subjects over a wide dose range and shows promise as an anti-pruritic.

Funding: Pharmaceutical Company Support - Trevi Therapeutics

TH-PO731

Lifetime Benefits and Cost-Effectiveness of LDL-Cholesterol Lowering in Chronic Kidney Disease: Results From the Study of Heart and Renal Protection (SHARP)

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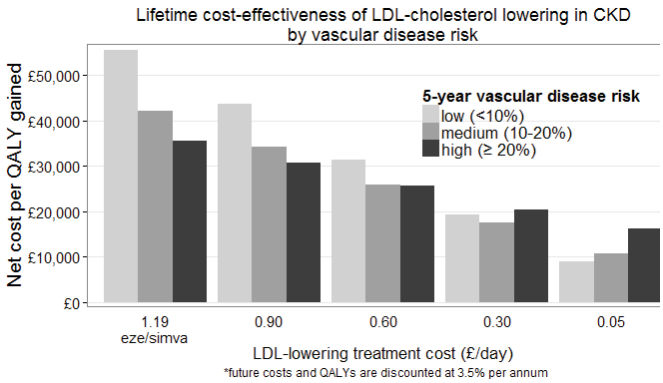
Background: The SHARP trial showed that lowering LDL-cholesterol (LDL-C) with simvastatin 20 mg plus ezetimibe 10mg daily (Eze/Simva) safely reduces major atherosclerotic events (rate ratio 0.83, 95% CI 0.74-0.94) in moderate-to-severe chronic kidney disease (CKD). We report lifetime benefits and UK cost-effectiveness of the intervention in categories of CKD patients.

Methods: Vascular disease risks, CKD progression, health-related quality of life and UK hospital costs were estimated using SHARP data and combined into a lifetime CKD model that was externally validated. Cost of LDL-lowering in the analysis was varied from UK ≤1.19/day (Eze/Simva) to ≤0.05/day (equivalent to the cost of a generic statin regimen in the UK). Net healthcare costs (intervention cost minus hospital cost savings) per quality-adjusted life-year (QALY) gained with Eze/Simva are reported for categories of CKD patients subdivided by predicted 5-year vascular disease risk at randomisation.

Results: In SHARP, lifetime use of Eze/Simva compared to no statin treatment increased quality-adjusted life years (QALYs) by 0.22 in high-risk participants with net cost per QALY ≤35,700, and by 0.18 in low-risk participants with net cost per QALY ≤55,500 (Figure).



Conclusions: At current prices, Eze/Simva is unlikely to be judged cost-effective in CKD in UK compared to no treatment. Less costly regimens (e.g. atorvastatin 20mg daily, which lowers LDL cholesterol by slightly less than Eze/Simva), although not assessed directly in this population, may be a more cost-effective means of reducing cardiovascular risk in patients with CKD.

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TH-PO732

Designing a Patient-Centered Medical Neighborhood (PCMN) for CKD: Identifying a High-Risk, High-Cost Population

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Background: The PCMN expands the patient-centered medical home by combining primary care and specialty physicians, nurses, pharmacists, and others to provide coordinated, high-quality care. We developed a nephrology PCMN for patients with CKD with 5 academic primary care practices. We first identified a target population.

Methods: We mined an electronic medical record (EMR) to identify non-dialysis patients with CKD 3 or greater using the CKD-EPI formula and 2 serum creatinines > 30 days apart over 2 years. Additional comorbidities including proteinuria (dipstick positivity, protein/creatinine >0.2 g/g or microalbumin/creatinine >30mg/g); hyperkalemia (K.>5.5 mEq/L); heart failure; diabetes; or uncontrolled hypertension (12-month average systolic BP above goal) were identified from EMR data or coding. Claims data from BCBSRI were cross-referenced. A nephrology nurse care manager (NCM) completed screening using a novel tool to stratify risk.

Results: Out of 27,253 patients, 731 had CKD 3A or above. Of 731 with CKD 35% had diabetes, 16% hypertension not at goal, 18% proteinuria, 8% CHF, and 4% hyperkalemia; 71% had never seen a nephrologist. More than 50% of 104 stage 4 and 5 patients had >2 co-morbidities. BCBSRI claims data revealed annual per-patient costs of $22,647 compared with $3,829 in non-CKD patients. (Comparable USRDS data: $23,128/patient-year). Inpatient stays and pharmacy accounted for 38% and 20% of the cost respectively. NCM screening was completed for 95% of the 731 people; 35% were felt to merit nephrology involvement based on CKD stage and/or comorbidities.

Conclusions: EMR based screening of patients in primary care practices using creatinine-based eGFR and other clinical measures defines a validated high-risk, high-cost population, the majority of whom have not seen a nephrologist and might benefit from collaborative care. Whether the PCMN model will improve patient outcomes and/or control costs is being evaluated.

TH-PO733

A Patient-Centered Medical Neighborhood (PCMN) for CKD

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Background: The PCMN expands the patient-centered medical home (PCMH) by combining primary care, specialty physicians, and others to provide coordinated, high-quality cost-effective care. With support from BCBSRI, we designed a PCMN for patients with CKD with 5, PCMH practices and one nephrology practice. Here we describe our PCMN intervention and model.

Methods: Nephrologists, primary care Physicians, geriatricians, analysts, practice management professionals, nurse care managers (NCM), and medical informatics experts designed the program. The care teams include PCP physicians and NCMs, nephrologists, a nephrology NCM, pharmacist, social worker and office staff.

Results: After screening over 27,000 primary care patients for ≥ CKD 3B or 3A with co-morbidities, 330 patients were identified as requiring intervention. Multiple levels of interaction between Primary Care and Nephrology were planned based on CKD stage and co-morbidities. This includes no interaction, pre-consultation (recommendations without referral) with the nephrology NCM (n=64), pre-consultation with nephrology (n=240),

referral for diagnosis and renal/PCP co-management (n=26), or PCMH within nephrology (n=30). A compact was executed between PCMH and Nephrology defining expectations. A care plan created for each patient sets patient specific goals and clinical responsibilities for each physician/practice. All patient encounters and formal communication are documented in and conducted via a shared EMR. Data on quality outcome measures including blood pressure control, metabolic bone disease screening, anemia management, hospitalizations, ER visits, and overall cost are being tracked.

Conclusions: EMR based screening of patients in several PCMH practices identified a high-risk, high-cost population with CKD for inclusion in a collaborative, team care model. Eventually, we plan to include other specialists in the care team. The impact of the PCMN model on clinical outcomes and costs is being evaluated.

Funding: Pharmaceutical Company Support - Blue Cross Blue Shield of Rhode Island

TH-PO734

Not Only Hyperuricemia but also Hypouricemia Is Associated with Glomerular Filtration Rate Decline in Healthy People

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Background: The relationship between hyperuricemia and chronic kidney disease (CKD) has been found in various observational studies. Although hypouricemia is associated with cardiovascular events, it was not established as a risk factor for CKD. In 8-year longitudinal study, we investigated the relationship between serum uric acid level (UA) and estimated glomerular filtration rate (eGFR) decline in healthy people.

Methods: 12,819 healthy people were enrolled as subjects in this analysis as part of the Saitama Cardiometabolic Disease and Organ Impairment Study, Japan. Their laboratory data including eGFR-decline speed were examined every three years. The outcome event was the decrease in eGFR by >25%. The subjects were divided into 5 groups on the basis of quintiles of UA at baseline. Then, the group 1 (UA<4mg/dl, n=2365) was further divided into 4 groups on the basis of quartiles of UA.

Results: Mean(SD) age was 42.1(10.8)years; eGFR, 75.7(19.9)ml/min/1.73m^2; and UA, 5.3(1.4)mg/dl. In analysis 1 (all participants), multiple regression analysis showed that eGFR-decline speed was negatively associated with UA (p=0.0001). Logistic regression analysis adjusted for baseline patients' characteristics showed that high-UA groups had a higher risk of the outcome event compared to the group 1; group 3 (5.7<=UA<6.5mg/dl), adjusted odds ratio (aOR) 1.99 (95%CI 1.35-2.92); group 4 (6.5mg/dl<=UA), aOR 2.14 (1.43, 3.20). In analysis 2 (subjects without CKD in group 1), UA positively correlated with eGFR-decline speed (p=0.01). Among the subjects, those with high or very low UA had a higher risk of the outcome event than group B (3.2<=UA<3.5mg/dl); group A (UA<3.2mg/dl), aOR 3.55 (1.45, 8.69); group D (3.8<=UA<4mg/dl), aOR 2.48 (1.10, 5.59). Generalized estimating equation models adjusted for baseline patients' characteristics showed that as increase of 1 in UA decreases the risk of the outcome event; aOR 0.488 (0.454, 0.522).

Conclusions: This study showed that not only high but also very low UA was associated with eGFR decline. Hypouricemia may be also a predictor of kidney function decline in healthy people.

TH-PO735

Uric Acid Control in Stage 1-3 CKD in a Southeast Urban Cohort

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Background: Uric acid is an emerging risk factor for Chronic Kidney Disease (CKD). The effectiveness of uric acid control may have an impact on the risk of future CKD. The effectiveness of current allopurinol dosing has not been well studied in populations affected by multiple risk factors for hyperuricemia.

Methods: We have collected data on multiple clinical and laboratory parameters for 73 consecutive CKD clinic patients with stage 1-3 CKD (68.5% stage CKD), representing a local urban cohort from the Southeast U.S. Data have analysis with SPSS v19 and expressed with means (SD) and percent (%).

Results: Mean age for the was 61.9 (12) years, weight 107.6 (29.9) kg with BMI 37.4 (16.7) kg/m2; 42.5% was female, 68.5% African-American, 53.4% had diabetes and 48% had gout. Aspirin was used in 52% of participants. Overall metabolic control was acceptable: potassium 4.1 (0.5) mEq/l; HCO3 25.4 (3.2)mEq/L, phosphorus 3.2 (0.5) mg/dL, hemoglobin 12.3 (1.9) g/dl and albumin of 4 (0.4) g/dl. Mean allopurinol dose was 195 (100) mg and serum uric acid 7.7 (2.5) mg/dL in the entire cohort. Uric acid correlated significantly with both weight (r: 0.255, p=0.03) and BMI (r: 0.250, p=0.04) but not with allopurinol dosing. Among patient with stage 3 CKD (mean race-adjusted MDRD eGFR: 42.9 (8.1) ml/min/1.73m2), allopurinol dose was 195 (96) mg/day and achieved uric acid was 8.1 (2.6) mg/dL.

Conclusions: Conventional and conservative allopurinol dosing guidelines may not achieve normalization of serum uric acid in the current era of obesity epidemics in CKD.

TH-PO736

Effect of Mycophenolate Mofetil on Expression of Serum and Urine TGF-β1 in Immunoglobulin A Nephropathy

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Background: IgAN is the most common form of glomerulonephritis in the world .TGF-β1 stimulates renal fibrosis in various renal diseases including IgA nephropathy. Previous studies showed that TGF-β1 over expressed in the kidney tissue, serum and urine of IgAN patient.There is few study explore the effect of MMF on the expression of TGF-β1.In this study,we aim to explore the serum and urine level of TGF-β1 and the effect of MMF on it in IgAN patients.

Methods: Samples were divided into three groups: Group C was composed of 19 healthy volunteers, Group MMF included 48 patients treated with MMF and Group IgAN formed by 39 IgAN patients which matched with MMF treatment group .13 patients in Group MMF had been treated with MMF for 3 months after kiney puncture biopsy and finished the follow-up visits.The level of TGF-β1 was detected by ELISA(R&D system).

Results: Comparing with Group IgAN, lower level of TGF-β1 in serum (*P=0.036, P=0.0004*) and in urine (*P<0.001*, *P =0.0001*) were found both in Group C and Group MMF. The level of TGF-β1 in Serum was positively correlated with pathological grading and urinary protein excretion (*p=0.044, 0.001*), while negatively correlated with eGFR (*p=0.012*)and positively correlated pathological grading,and urinary protein excretion(*p*=0.01,*P<0.001*)in urine. The level of TGF-β1 in both serum (*p=0.049*)and urine (*p=0.028*) had significantly difference between the group (n=18) with the LEE clasification>3 and the group (n= 21) with the LEE clasification≤3 as well as between the group with treatment for 3 to 6 months and the group with treatment > 6 months.The level of TGF-β1 in 13 patients that finished prospective follow-up visits showed obvious difference before and after treatment in urine (*p=0.002*) and a downward trend after treatment in serum.

Conclusions: The level of TGF-β1 in serum and urine was significantly elevated in IgAN patients,and MMF could reduce the level of TGF-β1 in serum and urine in IgAN significantly,especially in the treatment > 6 months. Therefore, TGF-β1 can be considered as an important indicator not only to predict the progression of IgAN, but also to assess the therapeutic effect of MMF in IgAN.

TH-PO737

Prediction of 30-Day Readmission after Percutaneous Coronary Intervention: Effect of Post Intervention Glomerular Filtration Rate

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Background: Chronic kidney disease is a risk factor for 30-day readmission in patients who undergo percutaneous coronary intervention (PCI). We investigated if adding the post-PCI Glomerular filtration rate (GFR) or the % GFR difference pre-post PCI improved the prediction of 30-day readmission.

Methods: We conducted a retrospective study of patients admitted for PCI in 2010-2014. GFR was estimated using CKD-Epi. Multilevel logistic regression was used to assess the impact of post-PCI GFR and % GFR difference on 30-day readmission after adjusting for age, sex, race, diabetes, congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), elective procedure, previous 6-month hospitalization, past PCI. Analyses were stratified by pre-PCI GFR (GFR≥60, GFR30-59, GFR<30 mL/min/1.73m^2).

Results: A total of 6,296 patients had PCI; 5,066 had pre-post PCI GFRs. Patients characteristics are listed in table 1; 30-day readmission rate was 6.5%, 12.3%, 14.5% respectively for patients with pre-PCI GFR≥60, 30-59, <30. In patients with pre-PCI GFR≥60, adding the post-PCI GFR improved the c-index (0.76 to 0.78) and the risk of readmission decreased by 12% for each 10 mL/min raise in post-PCI GFR (OR=0.88;95%CI 0.81-0.96). In patients with pre-PCI GFR30-59, the decrease in risk was not significant (OR=0.87;95%CI 0.76-1.01) and the c-index did not change. In those with pre-PCI GFR<30, post-PCI GFR did not impact the readmission or the c-index. Replacing post-PCI GFR with the % GFR difference pre-post PCI did not improve the prediction models.

Conclusions: Adding post-PCI GFR improves the prediction of 30-day readmission in patients with pre-PCI GFR≥60. Adding post-PCI GFR into a risk stratification strategy may be helpful to identify patients at higher risk despite normal baseline kidney function.

	GFR≥60 n=3680	GFR30-59 n=1083	GFR<30 n=303	p
Age	61±11	72±10	70±12	<0.01
Male (%)	71.1	59.0	54.8	<0.01
Black (%)	12.5	11.0	23.1	<0.01
Diabetes (%)	23.8	33.1	59.1	<0.01
CHF (%)	11.5	26.3	52.5	<0.01
COPD (%)	17.0	22.5	34.0	<0.01
Elective PCI (%)	34.0	33.0	22.4	<0.01
Past hospitalization (%)	27.2	30.7	53.8	<0.01

Funding: Other U.S. Government Support

TH-PO738

Variation in Cost and Quality in Kidney Transplantation Carl E. Dean,[1] Suying Li,[2] Nicholas Salkowski,[3] Craig Solid,[2] Mark Schnitzler,[4] Jon J. Snyder,[3,5] Bertram L. Kasiske,[3,7] Ajay K. Israni,[3,5,7] S.J. Kim. *[1]Medicine, Univ of Minnesota, Minneapolis, MN; [2]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [3]Scientific Registry of Transplant Recipients, Minneapolis Medical Research Foundation, Minneapolis, MN; [4]Medicine, St. Louis Univ, St. Louis, MO; [5]Epidemiology, Univ of Minnesota, Minneapolis, MN; [6]Medicine, Univ of Toronto, Toronto, ON, Canada; [7]Medicine, Hennepin County Medical Center, Minneapolis, MN.*

Background: The need to maintain quality while containing costs remains at the forefront of health care policy discussions. The relationship between cost and kidney allograft failure has not been described.

Methods: We compared Medicare claims from the United States Renal Data System for all adult kidney recipients who underwent transplantation between January 1, 2007 and June 30, 2009. Adjusting for recipient, donor and transplant characteristics; region; and local wage index, we calculated and compared the relative cost (observed/expected cost) for the first year post-transplant for all kidney transplant centers. We then calculated the ratio of observed/expected kidney allograft failure over the same time period for each center using the program-specific reports from the Scientific Registry of Transplant Recipients.

Results: Overall, there was no correlation between relative cost and observed/expected allograft failure (r = 0.096, P = 0.22) (Figure 1). Centers with costs and allograft failure rates lower than expected (higher-performing centers) varied significantly from centers with costs and allograft failure rates higher than expected (lower-performing centers) by donor and recipient characteristics and region (Table 1).

Conclusions: Further investigations are needed to determine the specific cost-effective practices of higher- and lower-performing centers to reduce both cost and incidence of allograft failure.

Figure 1: Variation in Cost of Transplantation & Allograft Failure Outcomes

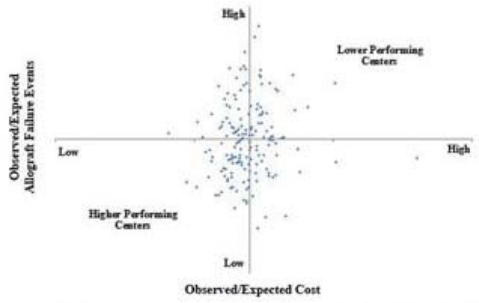

Table 1: Characteristics of Higher vs Lower Performing Centers

Variable	Higher Performing Centers*	Lower Performing Centers**	P-value
Total Centers	53	38	
Total Recipients	6839	4635	
Deceased donor (%)	72.9	77.8	< .0001
Recipient age in years (mean ± std)	52.6 ± 13.9	51.8 ± 13.9	0.0042
Race (%)			<.0001
White	63.0	59.4	
Black	29.1	33.9	
Other race	7.9	6.7	
Primary cause of ESRD (%)			0.0094
DM	28.2	29.3	
HTN	23.7	25.7	
GN	22.3	20.7	
Other	25.9	24.3	
BMI group (%)			< .0001
< 10 or missing	7.7	1.7	
10-<25	30.8	33.6	
25-<30	32.1	34.2	
30+	29.4	30.5	
Previous organ transplant: yes (%)	13.4	14.8	0.0454
Preemptive: yes (%)	7.5	6.1	0.0024
HCV positive (%)	6.0	4.4	0.0002
Length of stay in days 6 months prior transplant (mean ± std)	2.72 ± 5.23	3.00 ± 6.06	0.009
Comorbidites in 6 months prior to transplant (%)			
CHF	23.4	26.7	<.0001
Peripheral Vascular Disease	23.0	24.5	0.0583
Other Cardiac Disease	20.9	23.9	0.0001
Liver disease	7.4	8.9	0.0041
Rural resident (%)	0.0	1.0	< .0001
Region of Country			< .0001

***Higher Performing Centers had observed/expected cost <1 and observed/expected allograft failure <1.**

****Lower Performing Centers had observed/expected cost >1 and observed/expected allograft failure >1.**

Funding: Other U.S. Government Support

TH-PO739

Choice to Accrual for Home Dialysis Ashutosh M. Shukla,[1] Rebecca Hubbard,[2] Michelle Thomas,[2] Teri B. Martinez.[1] *[1]NFSGVHS & Univ of Florida; [2]Dialysis Clinic Inc., Gainesville, FL.*

Background: Comprehensive CKD education (CPE) improves patient awareness, and choice of home dialysis therapies (HoD). However, their impact on patients' eventual renal replacement therapy (RRT), (i.e. Choice to Accrual) has not been well reported.

Methods: We performed a retrospective analysis of the first 14 months of our recently established CPE clinic at University of Florida (UF) aimed to examine this issue. All predialysis patients with stage IV/V CKD followed at UF, were eligible for referral. All patients were advised for a minimum of 3 CPE sessions with each comprised of group education followed by individual counseling, and lasting > 2 hours.

Results: Seventy one, stage IV/V CKD patients, aged 60.2 ±14 years, with a mean follow up of 5.5±4 months, and referral eGFR of 16.3±5.6 ml/min were seen over the study period. Despite a universal mandate within an academic set up, 50% of patients were referred with stage V CKD. The remaining half were distributed between early(eGFR 15-20: 27%) and late(eGFR 21-29: 22%) stage IV CKD. 70% attended only one CPE session, and 30% returned for a second session. None attended the third session. After CPE, Peritoneal Dialysis(PD) was the preferred 'choice' for RRT for 68%, with In-center dialysis(IHD), Home Hemodialysis(HHD) and no dialysis chosen by 16%, 7% and 2% respectively. 4 patients(7%) remained undecided after a single session of CPE and did not return. 25(35%) patients initiated RRT with mean follow-up from the first session to dialysis being 5.2±3.7 months. Overall, 76% of those educated, initiated with RRT of choice. Choice to accrual rate for HoD was 71%; and for IHD was 250% with 10 patients resulting on IHD even though only 4 of them chose IHD. The choice to accrual rate for PD was 76% and HHD was 50%. Unplanned initiation of RRT, and subsequent placement of patients to alternate dialysis facilities, was the primary cause for loss from HoD.

Conclusions: CPE is effective in improving the 'choice' for HoD. However, there is a significant difference between choice to accrual for HoD in favor of IHD. Further objective studies are needed with a larger, and longer followed CPE population to understand the true impact of this, and identify the causes of this loss for HoD.

TH-PO740

A Quantitative Examination Evaluating the Impact of Implementation of Individualized Queuing in an Outpatient Hemodialysis Clinic Setting Robert W. Nappo,[1] Rita E. Alza,[1] Michael C. Blythe,[1] Edward A. Ross,[1] Jean Gordon. *[1]Univ of FL- Shands Healthcare, Gainesville, FL; [2]American Sentinel Univ, Aurora, CO.*

Background: The current model for scheduling a patient's HD therapy is often a group modality, with all patients scheduled to arrive for each shift simultaneously. Due to the lack of a well-developed scheduling model, patients were routinely left in the waiting room for extended periods of time. Patients often arrive hours early in an attempt to secure the first available chair. A strict scheduling model was implemented with disincentives for tardiness. This model eliminated the need for patient's to wait beyond their scheduled treatment times. This defined scheduling system was studied for its effectiveness on HD dose delivery.

Methods: A quantitative methodology was chosen to evaluate the relationship between the means of the control and impact group. The scheduling model was changed to align with clinic staffing and resource availability from a mass schedule to an individualized schedule. Patients are now scheduled to arrive in groups of 8 spaced in twenty-minute intervals; any patient that arrives late is bumped to the end of the shift. The research evaluated the impact of the new scheduling program on patient URR results.

Results: The two tailed matched pairs t-test results demonstrated a (t = 5.6597) with a P < 0.0001 which indicates there is a statistical significance between the mean of the impact group and control group. The data also reveal a decrease in the number of treatments that fall below the (65%) minimum URR threshold from 8 down to 3 failed treatments. The impact group displayed an overall increase in the mean URR from (72.789%) to (75.130%). The standard deviation dropped from 5.9486 to 5.1628 highlighting the increased consistency between individual treatments. Additionally, the minimum URR increased from (51.7%) to (56.1%) and in the maximum URR increased from (83%) to (85.3).

Conclusions: Providing high quality patient care is contingent on the coordination of departmental resources. Placing patients where they need to be at a precise moment in time streamlines the treatment process, improves HD delivery, and maximizes departmental productivity.

TH-PO741

Trends in Timing of Dialysis Initiation in the Veterans Health Administration Margaret K. Yu,[1,2] Adam J. Batten,[1] Ann M. O'Hare,[1,2] Paul L. Hebert. *[1]HSR&D, VA Puget Sound Health Care System, Seattle, WA; [2]Nephrology, Univ of Washington, Seattle, WA.*

Background: Over the last several decades U.S. adults are initiating dialysis at progressively higher levels of estimated glomerular filtration rate (eGFR), but to what extent this trend is occurring in the Department of Veterans Affairs (VA), the largest single integrated non fee-for-service health system in the U.S., is not known. Our objective was to compare temporal trends of eGFR at dialysis initiation within the VA with those in the wider U.S. dialysis population.

Methods: This was a retrospective analysis using data from the U.S. Renal Data System, a comprehensive national registry of end-stage renal disease. Study participants were adults who initiated chronic dialysis within the U.S. between 2000-2009 and had

information on eGFR at initiation (N=971,543). The primary exposure was veteran status and site of dialysis initiation (within versus outside the VA). The main outcome was an eGFR ≥10 mL/min/1.73 m^2 at initiation. Logistic regression was used to estimate the probability of starting dialysis with a high eGFR after adjustment for year, demographics, dialysis characteristics, and comorbidities.

Results: The adjusted probability of starting dialysis at an eGFR ≥10 mL/min/1.73 m^2 increased throughout the study period in all groups and was higher for patients who started outside the VA (39.69%, 95% CI 39.40-39.98 for veterans and 38.88%, 95% CI 38.78-38.98 for nonveterans) compared with patients who started within the VA (30.56%, 95% CI 29.91-32.21). Differences in eGFR at initiation by site were consistent across time periods and in all subgroups, but most pronounced among older cohort members and those with more limited life expectancy.

Conclusions: From 2000-2009, trends in eGFR at initiation within the VA mirrored those in the wider U.S. dialysis population, but eGFR at initiation was consistently lowest for veterans initiating dialysis within the VA. The most pronounced differences in eGFR at initiation within versus outside the VA were among older patients and those with more limited life expectancy, populations for whom the benefits of dialysis are least certain.

Funding: Veterans Affairs Support

TH-PO742

Chronic Kidney Disease Stage 5. Conservative Therapy versus Dialysis: What's the Money Saving?

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Background: In previous studies (Dattolo et al, ERA- EDTA, 2013) we have shown that a close clinical follow-up in outpatient dedicated clinics can safely delay the start of dialysis until eGFR values much lower than 10 ml/min, values recommended by current guidelines. In this study we wanted to calculate the hypothetical cost saving achieved with such a conservative attitude.

Methods: We assume 10.766 € the yearly cost of CKD5 patient, according to the S. Anna Institute study conducted on Tuscany CKD 5 population. For CKD 5D, the annual cost/patient of 71.666 € in HD and 23.926 € in PD was derived from an European study (Durand- Zaleski, Int J Health Care Finance Econ 2007). We here analyzed 1 year cost of the 340 patients followed in our dedicated clinics with eGFR≤10 ml/min; 188 of them (55%) remained in conservative follow-up, while the remaining started dialysis earlier, 49 patients (15%) with PD and 103 patients (30%) with HD.

Results: This case mix resulted in an overall cost of 10.577.980 €, e.g. 31.100 € patient/year, the cost would have been 20.308.540 €, e.g. 59.731 € patient/year if all 340 patients were immediately started on dialysis (25% DP).

Conclusions: Our model, compared with guidelines recommendations, implies the yearly saving of about 29.000 € per incident patient with eGFR≤10 ml/min/1,73m2.

TH-PO743

Cost of Chronic Kidney Disease in Adults with Type II Diabetes Mellitus (T2DM) - United States 2011

Mukoso N. Ozieh,[1] Leonard Egede.[1] *[1]Nephrology, MUSC, Charleston, SC; [2]Internal Medicine, MUSC.*

Background: The United States Renal Data System reports 44percent of diagnosed Chronic Kidney Disease (CKD) in 2011 were secondary to diabetes. CKD has impact on utilization and cost of healthcare however there are no recent national estimates on the cost of CKD in the U.S. population. This study aims to assess the cost of CKD in adults with T2DM in the U.S. population using novel cost estimation methodology.

Methods: Data on 2,053 adults with T2DM in 2011 Medical Expenditure Panel Survey (MEPS) was analyzed. Individuals with CKD were identified based on self-report. Mean total expenditure was estimated for the overall sample and by CKD status. We used a two-part model to estimate overall adjusted expenditure and by CKD status. The first part of the model was a probit model to estimate the probability of having expenditures and the second part was a generalized linear model with gamma distribution to estimate total expenditure accounting for the probability of having expenditure. Covariates in both models include age, race, gender, marital status, education, insurance, Metropolitan Statistical Area, region, poverty status and comorbidities. STATA version 13 was used to account for the complex design of MEPS.

Results: Of the 2,053 individuals with T2DM, 9.7percent had self-reported CKD. Those with CKD have lower income, were more likely to be depressed, have hypertension, cardiovascular disease, high cholesterol, arthritis and asthma. These were the only significant difference among those with CKD compared to those without CKD. In the unadjusted analysis, mean expenditure for those without CKD were $9,689.49 (95% CI $8,871.18-$10,507.81) while those with CKD were $20,726.32 (95% CI $16,322.42-$25,130.22). Extrapolating to the U.S. population in 2011, the mean expenditure for those with CKD was over $43 billion. In the adjusted analysis using the two-part model, those with CKD had $8,473 (95% CI $4,957.75-$11,989.28) more expenditure compared to those without CKD.

Conclusions: DM is associated with significant healthcare expenditure but a significant proportion is due to CKD. This highlights the importance of aggressive strategies for prevention, early recognition and treatment of CKD in patients with DM.

TH-PO744

Screening for Down's Syndrome in Women with Renal Disease

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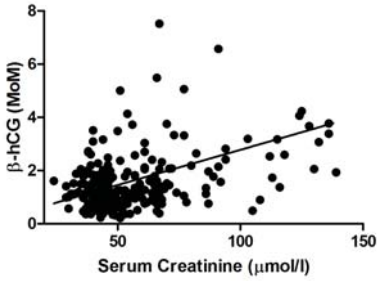
Background: Screening for Down's syndrome (DS) routinely involves measuring maternal concentrations of a combination of serum markers including beta-human chorionic gonadotrophin (β-hCG) and ultrasound measurement of nuchal translucency. Certain confounding risk factors such as maternal age are corrected for. There is limited data reporting raised β-hCG levels in women with chronic kidney disease (CKD). Our aim was to establish whether current DS screening practices in the United Kingdom are appropriate for pregnant women with CKD.

Methods: We performed a retrospective study identifying all women who had attended our regional renal obstetric clinic and undergone DS screening during 2008-2013. Those patients with a serum creatinine (sCr) or urinary albumin creatinine ratio measured at the time of screening were included and pregnancy outcome followed up.

Results: A total of 240 women were identified of which 32% had a sCr of ≥60 µmol/L (upper limit of normal in pregnancy). There were a higher number of false positive screens in our renal obstetric cohort compared to the general population (adjusted for age) both at first (5.7% versus 3.65%) and second trimester (9% versus 3.52%). We found a significant correlation between sCr and free and total β-hCG (p<0.01) and with DS risk score (p<0.01).



Increased proteinuria also correlated with free β-hCG (p<0.01) and risk score (p<0.02). There were no cases of DS in the renal group although 10% of those with a high risk score had a pre-term birth and 56% delivered a small baby.

Conclusions: Renal impairment appears to affect levels of β-hCG and falsely increase the risk score in DS screening. Alternative screening may need to be considered in this group or renal impairment corrected for.

TH-PO745

Validity of the Berlin Questionnaire to Predict Sleep Apnea in CKD

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Background: The Berlin Questionnaire (BQ) is a validated screening tool for sleep apnea (SA) in the general population. However, whether it is also valid in individuals with CKD is unknown.

Methods: We conducted a cross-sectional interim analysis of 73 veterans aged 18-89 with eGFR of 15-44 ml/min/1.73m^2 enrolled in the SNORE Study. At baseline, subjects completed BQ and Epworth Sleepiness Scale (ESS) questionnaires and underwent complete overnight sleep study. SA was defined using the apnea-hypopnea-index(AHI). We assessed the validity of BQ at a standard cut-off ≥2 and the ESS>10 to predict SA (at cut-points of ≥5, 15, and 30) using sensitivity, specificity, positive predictive value (PPV), negative predictive value(NPV), and area under the receiver operating characteristics curve(AUC).

Results: Baseline characteristics of enrolled subjects: mean (±SD) age, 75±10 years; 98% male; 82% Caucasian; body-mass-index (BMI), 30.2±4.7 kg/m^2; 52% had BMI≥30 kg/m^2; 95% had hypertension.BQ score ≥2 was 68%. Median (IQR) of ESS was 8(5-13); 33% had ESS>10. Median (IQR) AHI was 11 (4.1-21.7). The proportion with AHI≥5, 15, and 30 was 70%, 41%, and 16%, respectively.

Table 1. Predictive validity of BQ with or without ESS to detect SA.

	Sensitivity	Specificity	PPV	NPV	AUC (95%CI)
AHI ≥ 5					
BQ ≥ 2	75%	45%	76%	43%	0.600(0.478-0.722)
BQ ≥ 2 + ESS>10	33%	86%	85%	36%	0.598(0.5-0.697)
AHI ≥ 15					
BQ ≥ 2	80%	39%	48%	74%	0.598(0.494-0.701)
BQ ≥ 2 + ESS>10	43%	84%	65%	68%	0.635(0.529-0.741)
AHI ≥ 30					
BQ ≥ 2	83%	34%	20%	91%	0.589(0.463-0.714)
BQ ≥ 2 + ESS>10	50%	77%	30%	89%	0.635(0.478-0.792)

Differences between AUC values for BQ with and without ESS>10 were not statistically significant (for AHI≥5, ≥15, and ≥30 p for difference=0.8, 0.4, and 0.6).

Conclusions: Among veterans with CKD, a BQ ≥2 was a sensitive but not specific predictor of SA across various cutpoints for SA severity. Addition of ESS to BQ score in screening improved specificity but reduced sensitivity for SA.

Funding: Veterans Affairs Support

TH-PO746

Psychological Characteristics of Adults with Pediatric-Onset End-Stage Renal Disease: Personality and Attachment Scott Mullaney,[1] Mojgan Khademi,[2] Tovah Sirkin,[2] Luciano Giromini,[2] Heidi Miller.[2] *[1]UC San Diego, San Diego, CA; [2]Clinical Psychology, Alliant International Univ, San Diego, CA.*

Background: The role of psychological variables in chronic illness has been investigated with a focus on health related behaviors, but there is limited understanding of possible influences of pediatric-onset chronic illnesses such as End-Stage Renal Disease (ESRD) on personality and attachment style (interpersonal functioning based on the quality of early childhood experiences with caregivers).

Methods: We studied eight adults (six women; two men) who developed ESRD before age 13 using semi-structured interviews (focused on illness and personal/social functioning), the Relationship Scales Questionnaire (which divides respondents into categories of securely or insecurely attached) and the Rorschach Inkblot Method (a performance-based personality test). Statistical analyses focused on seven empirically supported Rorschach variables related to bodily preoccupation and psychosomatic complaints.

Results: Results of t-test comparisons with the international reference normative data were statistically significant for five of the variables at $p < .05$ and in the expected direction, indicating alexithymia (characterized by difficulty identifying and describing feelings) with large effect sizes. The qualitative findings indicated coping through avoidance and rational acceptance of their illness. Participants reported longstanding interpersonal difficulties and limited plutonic and romantic relationships as adults. Majority of the participants were insecurely attached (62% versus 41% in the general population), which is associated with being perceived negatively by others, and among chronically ill patients correlates with fragility and overreacting to stressors.

Conclusions: Alexithymia among medical patients is associated with cold and distant relations, vindictiveness, self-centeredness and with reporting more symptoms in the absence of greater somatic disease. Awareness of and interventions focused on these psychological variables could enhance medical interactions and patient care. Future studies with a larger pediatric-onset sample as well as adult-onset ESRD patients should be considered.

TH-PO747

Prevention of CKD Through Educational Programs and Screening Camps Priyanka Govindan, Arjun V. Sharma. *Nephrology, Univ of Washington, Seattle, WA.*

Background: There are an estimated 55,000 patients on dialysis in India with 44% caused by Hypertension and/or Diabetes Mellitus. Early detection of DM, hypertension, with subsequent control of blood pressure and blood sugars have shown to cause delay in progression of kidney disease. Therefore early screening will take an important role in preventing dialysis rates from continuing to rise. Here we note the efforts of one program aimed at education and prevention of kidney disease.

Methods: The Tamil Nadu Kidney Research Foundation conducted a series of 584 awareness programs and 71 screening camps in Tamil Nadu, India with the goal of educating the general populace and screening for early risk factors for kidney disease. 97,860 people attended the awareness programs which consisted of a presentation with the aim of educating on basic physiology of kidneys, risk factors for CKD, and an overview of Renal Replacement Therapy in India. The talk was on symptoms and warning signs and the importance of adopting a healthy diet and lifestyle modification. 6,415 people attended the screening camps which were designed to screen for hypertension, elevation in creatinine, glucosuria or proteinuria.

Results: Of the 6,415 people screened, 727 had newly detected diabetes, 1170 of those screened had newly detected BP of over 130/90 mmHg and 953 had proteinuria. All people with risk factors were told to follow up with a doctor, referred to the awareness program, and given specific advice depending on their comorbidity.

Conclusions: The screening camps that were initiated by the TANKER foundation were able to diagnose thousands of otherwise seemingly healthy people with risk factors for CKD. The screening methods were not ideal as a one time measurement of glucosuria, hypertension, and proteinuria may have myriad causes which may not be representative of pathology. However, with the knowledge that they are significantly at risk for kidney disease, our hope is that patients with risk factors would be more likely to seek medical attention and pursue lifestyle modifications with an end result of decreasing their risk of developing kidney disease. Thus far there has been no objective evaluation of the program but one is currently being developed.

TH-PO748

Muscle-Kidney Crosstalk via a Myokine, Irisin, Can Suppress Progression of CKD in Mice Hui Peng,[1,2] William E. Mitch,[2] Zhaoyong Hu.[2] *[1]Div of Nephrology, Dept of Medicine, The third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Nephrology, Dept of Medicne, Baylor College of Medicine, Houston, TX.*

Background: beneficial effects of exercise on muscle metabolism are associated with improved survival of patients with ESRD but the mechanisms underlying this observation are unknown. A potential mediator is irisin which is released from muscle by exercise and can counteract obesity and increase the accumulation of brown fat. We hypothesized that irisin could mediate muscle-kidney crosstalk to slow the progression of CKD.

Methods: we created mice with skeletal muscle-specific, forced expression of PGC-1α because this transgene increases circulating irisin. Transgenic and control mice underwent subtotal nephrectomy to create CKD and we evaluated the function and degree of fibrosis in the remnant kidney plus irisin-induced responses in cultured tubular cells.

Results: after 1 month of CKD, serum irisin was 2.8-fold higher in PGC1-α versus wild type (WT) mice. CKD mice expressing PGC-1α had lower values of BUN and serum creatinine ($P<0.05$). In kidneys of WT mice with CKD, there was increased interstitial fibrosis plus tubular cell apoptosis. In contrast, remnant kidneys of PGC-1α mice that were expressing irisin had a 40% lower degree of interstitial fibrosis ($P<0.05$) and 36% less tubular cell apoptosis ($p<0.05$). Cultured tubular cells treated with irisin exhibited less apoptosis plus reduced expression of collagen-1A (31%) and fibronectin (39%; $P<0.05$ for both). Because UCP2 down-regulation is linked to increased reactive oxygen species (ROS) production, it contributes to the development of fibrosis and kidney cell apoptosis. We found that irisin stimulates UCP2 in cultured tubule cells and lowers ROS production, providing a potential mechanism for the protection of the kidney.

Conclusions: Activation of muscle-specific PGC-1α stimulates the release of irisin from muscle. In mice, we found that irisin up-regulation reduces CKD-induced renal tubular cell apoptosis and fibrosis. We conclude that irisin may be a mediator of the beneficial influence of muscle exercise in CKD.

Funding: Other NIH Support - NIAMS

TH-PO749

Urinary Metabonomic Study of Rhubarb as an Effective Treatment for Chronic Renal Injury in Rats Ying-Yong Zhao,[1,2] Ya-Long Feng,[1] Hua Chen,[1] Shuman Liu,[2] Ruichao Lin.[1] *[1]Dept of Traditional Chinese Medicine, Northwest Univ, Xi'an, Shaanxi, China; [2]Div of Nephrology and Hypertension, Univ of California, Irvine, Irvine, CA.*

Background: Chronic kidney diseases (CKD) are a major challenge for the public healthcare, and there is still a significant need for strategies for the treatment of CKD. Rhubarb, a well known worldwide herbal medicine, has long been used for the treatment of CKD.

Methods: Urinary metabolomics based on the ultra-performance liquid chromatography/SYNAPT high-definition mass spectrometry was undertaken to explore the pattern of low molecular mass metabolites in the rat model of CKD induced from adenine excess. Fifty rats were randomized into control, CKD and rhubarb-treated CKD groups. The CKD and rhubarb-treated CKD groups were given 200 mg/kg/d of adenine for 3 weeks. During the adenine gastric gavage after 3 h, rhubard-treated groups were given the petroleum ether, ethyl acetate and n-butanol extracts of rhubarb respectively for 6 weeks. Urine biochemistry, and Masson's trichrome staining and Western blot analysis of kidney tissues were performed. Data were analyzed using principal component analysis, partial least squares-discriminant analysis, heatmap analysis and metabolic pathways analysis.

Results: After treatment with rhubarb, the kidney pathological abnormalities were gradually ameliorated compared to CKD group. This was coupled with a significant change in the global urinary metabolites from rhubarb-treated rats compared to CKD rats at weeks 3 and 6. Among these metabolites, 15 of them including adenine, creatinine, tryptophan, phenylalanine, taurine, adenosine and uric acid were reversed to the control group level in rhubarb-treated group. Combined with biochemistry and histopathology result, the changes in urine metabolites indicate that the perturbations of amino acid and purine metabolisms are related to adenine-induced CKD and to the interventions of rhubarb on these metabolic pathways.

Conclusions: This study not only supplied a systematic view of the development and progression of CKD and the mechanism studies of rhubarb but also provided the theoretical basis for the prevention or treatment of CKD.

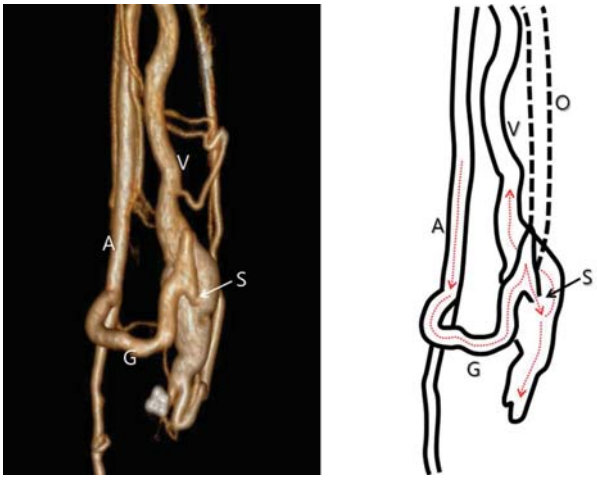
TH-PO750

A Complication of Shunt Formation between Arteriovenous Graft and Cephalic Vein Youngsub Kim, Jae Seok Kim, Jae Won Yang, Byoung Geun Han, Seung-Ok Choi. *Div of Nephrology, Yonsei Univ Wonju College of Medicine, Wonju, Republic of Korea.*

Introduction: Thrombosis and infection are common complications of arteriovenous graft (AVG). But the complication of shunt formation between AVG and natural vein is rare. Here we present a case of AVG - cephalic vein shunt.

Case Description: 63-years old male on hemodialysis(HD) using AVG(Left, brachial artery-axillary vein) presented with fever. He was diagnosed with sepsis and an abscess around AVG was shown. Methicillin-resistant staphylococcus aureus was documented in blood and pus from the abscess. Therefore we believed that sepsis was caused by AVG infection. During admission, bruit and thrill of the AVG were lost and AVG dysfunction developed.Doppler ultrasound(US) showed the shunt flow from the AVG to a near cephalic

vein and luminal thrombosis above the shunt was seen. Computed tomography(CT) angiography showed clearly the cephalic vein was dilated and the AVG was occluded by thrombus.



CT angiography and schema of shunt. (A, brachial artery; G, AVG; S, shunt; V, cephalic vein; O, occluded part of AVG; dotted line, direction of blood flow)

In surgery, we could find the connecting tract between the AVG posterior wall and a near cephalic vein. The AVG was partially removed due to infection and dysfunction. We created a new arterio-venous fistula(AVF) by connecting the dilated cephalic vein to a brachial artery. We expect the new AVF will be usable soon, because the flow of AVF is more than 1400ml/min in doppler US at post-op 7th day.

Discussion: We believe that this process has been progressed from several weeks ago and also may be related to needling during HD. The diameter of connecting tract was similar to the one of needle used in HD. Our hypothesis is that blood flow was divided into the two pathways and the AVG decreased in flow with growing of the cephalic vein. As a result, occlusion with thrombosis occurred.

TH-PO751

Colchicine as Treatment for Culture Negative Peritonitis in Patient with Behcet's Disease on Peritoneal Dialysis Ardeshir Khosraviani, Scott A. Rasgon, Nitin Bhasin. *Nephrology, Kaiser Permanente - Los Angeles Medical Center, CA.*

Introduction: Behçet's disease (BD) is a rare disease characterized by recurrent oral aphthae and several systemic manifestations. Most clinical manifestations of BD are due to the vasculitic nature of the disease. In patients with BD and ESRD, Peritoneal Dialysis (PD) is a favored modality for providing renal replacement therapy. Here we present a patient with BD on PD with culture negative peritonitis.

Case Description: A 61 year old female with history of BD, Diabetes Mellitus (DM) and ESRD receiving PD for five years, presented with abdominal pain and tenderness around her PD catheter insertion site. Patient was afebrile and her vital signs were normal. Her physical examination was unremarkable except for tenderness on palpation around the PD catheter insertion site. The abdomen was otherwise soft and rebound tenderness was absent. The patient was empirically started on intraperitoneal (IP) Ceftazidime and Cefazolin. Analysis of peritoneal fluid revealed 150 WBC per hpf with lymphocytic predominance. The gram stain and cultures for bacterial (including Mycobacterium) and fungal organisms were negative. Patient returned to clinic after two weeks with the same presentation. Peritoneal fluid analysis now revealed 730 WBC per hpf with a similar lymphocytic predominance with negative gram stain and culture. Oral Ciprofloxacin was added to previous regimen. She returned with persistent symptoms and was started on Colchicine due to concern for peritoneal irritation. Her symptoms resolved within twenty-four hours of taking Colchicine and a repeat analysis of her peritoneal fluid revealed no WBC. Cultures remained negative.

Discussion: The incidence of ESRD in patients with BD is very low. PD is the favored renal replacement modality due to the high risk for vascular thrombosis in these patients. PD patients with BD may develop peritoneal irritation from their catheter. Their clinical presentation may be similar to that of peritonitis. It is important that the diagnosis of peritonitis is excluded in these patients. Treatment with anti-inflammatory agents such as Colchicine may result in resolution of symptoms and abnormal laboratory findings.

TH-PO752

***Helicobacter cinaedi* Infection in a Polycystic Kidney Disease Patient Receiving Hemodialysis** Yuka Hibino, Tomoka Nango, Maoto Negishi, Kanna Watanabe, Yuki Fukuhara, Toshikazu Wada, Yume Nagaoka, Yoshihiko Kanno. *Dept of Nephrology, Tokyo Medical Univ, Japan, Tokyo, Japan.*

Introduction: *Helicobacter cinaediis* a bacterium in the family *Helicobacteraceae* that causes mainly enteric and bloodstream infections. A relatively large proportion of patients with chronic kidney disease have *H. cinaedi* bacteremia, as they are immunocompromised. However, there are few reports of cyst infection and bacteremia by *H. cinaediin* patients with polycystic kidney disease (PKD).

Case Description: A 51-year-old male patient with PKD who was receiving hemodialysis developed a high fever and abdominal pain. He had experienced a similar episode 2 years previous to the current admission, and levofloxacin had effectively improved his symptoms. This time, fluoroquinolone antibiotics did not improve his symptoms, and *H. cinaedi* was detected from his venous blood after 14 days of culture. Meropenem controlled his infection until neutropenia was induced by it. After other antibiotics failed to show a clear effect, the patient was examined by CT, diffusion-weighted MRI, and Ga-67 scintigraphy; however, no infected cysts could be identified as targets for injection with antibiotics. After drainage, minocycline was percutaneously injected to 3 randomly selected cysts for 1 week, and the symptoms remitted.

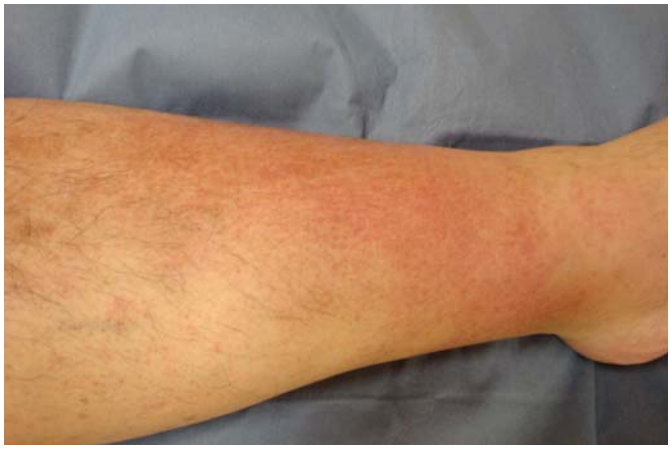
Discussion: The characteristics of *H. cinaedi* include its poor culturability, the need for more than 10 days for its detection, and its tolerance to antibiotics. Primary treatment by fluoroquinolones according to the guidelines from the Japanese Society of Nephrology may not necessarily lead to a favorable response. In the case of refractory cyst infections, *H. cinaedi* should be considered as a causative pathogen, and a long observation period should be expected for its detection. Furthermore, not only antibiotic therapy, but also interventional treatment should be considered in the early phase of its clinical course.

TH-PO753

Atypical Cellulitis in a Hemodialysis Patient Caused by Helicobacter cinaedi Toko Hashimoto, Naomi Sasaki, Nobuo Hashimoto. *H.N.Medic, Sapporo, Hokkaido, Japan.*

Introduction: Helicobacter cinaedi is known to cause diarrhea and bacteremia in immunocompromised hosts, however, few cases are reported in dialysis patients. Our case presented atypical skin manifestation which originally suspected as nephrogenic fibrosing dermopathy in pathological assessment. Difficulty of its diagnosis is due to the fastidiousness of this pathogen requiring specific culture condition.

Case Description: Case Description: A 40-year-old man who had been receiving hemodialysis 3 times per week for a year presented skin redness and swelling with painful induration on his lower limbs for 2 weeks. He was clinically diagnosed idiopathic erythema nodosum which improved with 15 mg of prednisone per day for 2 weeks. Whist ceasing prednisone, he developed spike fever (>38°C) and redeveloped red papular eruptions on extremities.



Systemic autoimmune diseases, malignancy and HIV infection were excluded and nephrogenic fibrosing dermopathy was suspected by skin biopsy. Although the initial blood culture was negative, H. cinaedi was detected from the following sample with the condition of long-term-culture for 9 days. The culture of dialysis fluid from the circuit was negative. He was diagnosed cellulitis caused by H. cinaedi and treated with 100mg of cefcapene per day for 4 weeks. Rapid improvement of the skin rush and decline of fever was observed, and there was no recurrence of any symptoms after the followup of antibiotic treatment.

Discussion: H. cinaedi is one of the most difficult bacteria to detect and its clinical manifestation varies. Thus, the prevalence of this infectious disease might be under estimated. In immunocompromised hosts including hemodialytic patients, skin lesion can be the earliest symptom in presence of H. cinaedi bacteremia. Repeated and long term culture of blood sample is helpful clue for the diagnosis.

Funding: Clinical Revenue Support

TH-PO754

BJP Lambda-Type Multiple Myeloma Successfully Withdrawn from Maintenance Hemodialysis after Long-Term Continuous Bortezomib and Dexamethasone Therapy Wakaba Yamaguchi,[1] Naofumi Yui,[1] Toshikage Nagao,[2] Haruna Azetsu,[1] Soichiro Iimori,[1] Eisei Sohara,[1] Tomokazu Okado,[1] Tatemitsu Rai,[1] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan; [2]Hematology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

Introduction: The effect of bortezomib for multiple myeloma (MM) has been well established, however, how to continue maintenance therapy with this agent on patients requiring regular hemodialysis (HD) due to MM is undetermined. Here, we report a rare

case of cast nephropathy caused by BJPλ-type MM that developed severe renal failure and finally became independent of hemodialysis after long-term bortezomib and dexamethasone (BD) therapy.

Case Description: A 61-year-old Japanese woman with no medical history of kidney disease was referred to our hospital for severe renal dysfunction. On admission, she presented oliguria, and her serum creatinine (sCr) level was at 15.5 mg/dL, requiring HD. BJPλ was detected in her urine. Her serum β2 microglobulin level was increased at 30.8 mg/L. Bone marrow aspiration showed an increase of atypical plasma cells up to 15%, that were λ-positive in immunostaining. Renal biopsy revealed λ-positive cast in tubules with no pathological signs of amyloidosis. We diagnosed that the cause of her renal failure is BJPλ-type MM staged at ISS 3, and started BD therapy. During four cycles of BD therapy, BJP disappeared from her urine, and her urine volume was gradually increased. In addition, no clinical signs of adverse effect were observed. We decided to continue BD therapy for her renal recovery. After eight cycles of the treatment, her pre-dialysis sCr level was decreased to 6.5 mg/dL. We continued monthly BD therapy. During two years of monthly BD therapy, her renal recovery continued, and frequency of HD was successfully decreased to once weekly. Finally, her pre-dialysis sCr level was decreased to 3.7 mg/dL, therefore, we decided to discontinue HD and BD therapy. After 1 year from the discontinuation, her sCr level was at 2.9 mg/dL, and her MM was maintained in complete remission.

Discussion: Our present report supports the usefulness of long-term continuous BD therapy for renal recovery in MM-caused cast nephropathy.

Funding: Government Support - Non-U.S.

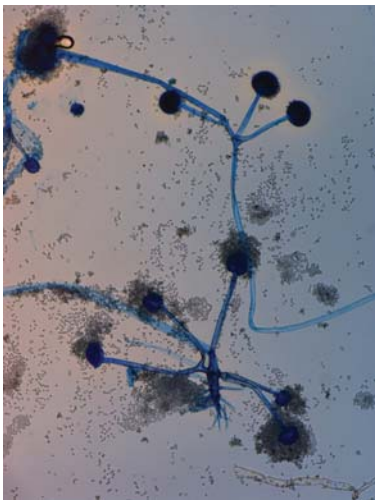
TH-PO755

Simultaneous Mucormycosis of Arteriovenous Fistula and Candidial Peritonitis in a Dialysis Patient

Jobert Anjelo,[1] Nitesh Nikilesh Rao,[1] Stephen M. Hogg,[1] Sarah E. Kidd,[2] Tony J. Elias.[1] *[1]Nephrology, Royal Adelaide Hospital, Adelaide, South Australia, Australia; [2]SA Pathology, Royal Adelaide Hospital, Adelaide, South Australia, Australia.*

Introduction: Dialysis access is considered the "lifeline" for patients with end stage renal failure. Fungal infections are relatively uncommon as compared to bacterial infections and therefore access infections are usually treated with empirical antibiotics. This case report describes a potentially life threatening infection of an arteriovenous fistula by mucormycosis masquerading as a persistent bacterial infection. This patient also simultaneously developed candida peritonitis.

Case Description: A 58 year old Caucasian woman with end stage kidney disease secondary to type 1 diabetes mellitus, on peritoneal dialysis, presented with erythema and pain in her left brachiocephalic AV fistula. She had undergone a DRIL procedure for "steal syndrome" about 4 weeks ago in preparation for an elective transition to haemodialysis. She received two weeks of antibiotic therapy which did not result in improvement of the infection. A repeat swab from the wound over the AV fistula demonstrated rhizopus microspores.



At around the same time, the peritoneal fluid became cloudy and the causative organism was found to be candida albicans, mandating tenckhoff catheter removal and transfer to haemodialysis. A CT scan of the head, chest and abdomen did not demonstrate fungal dissemination. She was treated with a prolonged course of antifungals which included IV liposomal amphotericin B and posaconazole. She also required extensive multiple surgical debridement over the arteriovenous fistula. She responded to this therapy after 3 weeks.

Discussion: To our knowledge, this is the first case of mucormycosis infection in an arteriovenous fistula. This case also reinforces the importance of antifungal prophylaxis in patients on peritoneal dialysis, who require long term antibiotics.

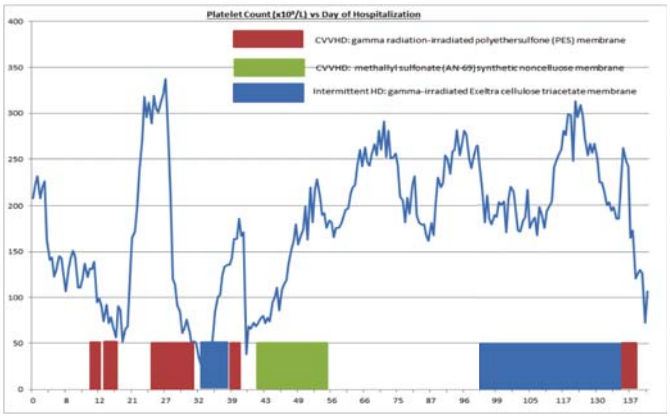
TH-PO756

Dialysis Associated Thrombocytopenia in a Patient with AKI on Continuous Venovenous Hemodialysis

Eugene K. Essandoh, Jonathan J. Hogan. *Nephrology, Columbia Medical Univ Hospital, New York, NY.*

Introduction: Dialyzer-associated thrombocytopenia (DAT) has been observed in case reports of patients with ESRD on HD, with many recently published cases occurring with the use of electron beam-sterilized polysulfone membranes. Here, we report a case of acute, severe and recurrent DAT in a patient on continuous replacement therapy (CRRT) for AKI.

Case Description: An 80 yo woman with a PMH of CHF, CKD and pulmonary HTN was admitted for volume overload. A LVAD was placed after failure of inotrope-assisted diuresis. Her hospital course was complicated by cardiogenic shock and an arterial thrombus requiring heparin. In this setting, she developed AKI for which CVVHD was initiated using a gamma radiation-sterilized polyethersulfone (PES) membrane. Prior to admission, her platelet count was 160 $X10^9$/L, was 143 $X10^9$/L on admission and 92 $X10^9$/L when she returned to OR for a chest washout. Over the next week, her thrombocytopenia worsened with PES membrane exposure and improved with stopping CVVHD [Figure 1]. This temporal relationship of acute and severe thrombocytopenia with PES membrane exposure occurred multiple times, with a platelet nadir of 26 $X10^9$/L and rapid resolution after stopping PES membrane exposure [Fig 1]. A workup for other causes of thrombocytopenia (including peripheral blood smear, SRA, fibrinogen, INR, aPTT, evaluation of medication-related thrombocytopenia) was negative. Given the concern DAT due to PES membrane, CVVHD was re-initiated using a methallyl sulfonate (AN-69) synthetic noncelluose membrane, during which time her plts normalized, and DAT did not recur on intermittent HD with a gamma-irradiated cellulose triacetate membrane.



Discussion: Nephrologists should be aware that acute and severe DAT may occur with CRRT, and in such cases, using a different dialyzer membrane may lead to resolution of thrombocytopenia.

TH-PO757

Post-Dialysis Anaphylactoid Reactions from Alginate-Containing Compression Dressing (ACCD)

Pratima Ghimire,[1] Philip Goldwasser,[1,2] Robert H. Barth.[1,2] *[1]Renal Div, SUNY Downstate College of Medicine, Brooklyn, NY; [2]Nephrology Section, VA NY Harbor Healthcare System, Brooklyn, NY.*

Introduction: Alginates are linear polysaccharides derived from marine brown algae, which have long been used in wound dressings for their non-adherent and hemostasis-inducing properties. This report describes a case of frequent post-hemodialysis generalized anaphylactoid reactions apparently caused by an ACCD, the Gambro TipStop [TM].

Case Description: A 31-year-old woman with ESRD attributed to chronic glomerulonephritis, on hemodialysis (HD) for 8 years, with a history of allergy to isoniazid and radiographic contrast media, began having intermittent severe anaphylactoid reactions after HD, usually 5-10 min after completion of the treatment, occurring 3-5 times a month. Reactions were characterized by generalized flushing and pruritus, with tachycardia, dyspnea, and throat and chest tightness, and responded to antihistamines, steroids, and epinephrine. They were variously ascribed to paricalcitol, iron sucrose, and erythropoietin, but as each medication was discontinued, reactions did not abate, and continued even when no medications at all were given with HD. To test the hypothesis of reaction to backfiltrate from dialysate at the end of the treatment, the last 20 min of each session were limited to isolated ultrafiltration without dialysate, but reactions continued. Finally, it was observed that reactions occurred only after application of the ACCD to cannulation sites, and had begun to occur 4-6 months after the initiation of ACCD use. Skin tests were performed in an Allergy Clinic for hypersensitivity to paricalcitol, iron sucrose, and erythropoietin and all were negative, while a test using a saline extract of the ACCD was positive. Use of the ACCD was discontinued and since that time no reactions have occurred, despite gradual reintroduction of all IV medications.

Discussion: ACCDs have been used extensively with few reported cases of allergy. Nonetheless, fatal anaphylactic shock due to an alginate-containing dental impression material has recently been reported, and this case illustrates that alginate-containing dressings may be associated with severe post-dialysis anaphylactoid reactions.

TH-PO758

Hemodialysis Followed By CVVHDF for Dabigatran Clearance in Acute Subdural Hematoma Salem Almaani, Shashikant Patel, Asish Thakkar, Udayan Y. Bhatt, Jason Prosek. *Dept of Nephrology, The Ohio State Univ, Columbus, OH.*

Introduction: Dabigatran is a direct thrombin inhibitor that is FDA approved for the prevention of stroke in patients with non-valvular atrial fibrillation. It has some advantages over warfarin, such as predictable levels of anticoagulation without the need for monitoring. Despite this, no reliable antidote exists when the need to reverse its anticoagulant effect is needed. Dialysis has been used for drug clearance in a few case reports, but there is concern of a rebound in the drug level after cessation of dialysis because its lipophilic structure and protein binding properties are the basis for a large volume of distribution.

Case Description: A 75 year old male with a past medical history of atrial fibrillation, on dabigatran 150mg b.i.d for stroke prevention, presented to the emergency department after he sustained a fall from a chair. A head CT scan revealed a 1.5cm subdural hematoma. The patient's mental status deteriorated and repeat imaging showed an increase in the size of his hematoma as well as new uncal and transtentorial herniations necessitating open evacuation. The patient's thrombin time (TT), which was used to assess the degree of his ongoing anticoagulation, was found to be elevated to 84.7 sec (normal 13-20 sec). He had a normal renal function when he presented with a serum creatinine of 0.67mg/dl. Hemodialysis was initiated to aid in drug clearance, and serial TTs were obtained. The patient's TT dropped to 75.0 sec after a 4 hour dialysis session. He was switched to CVVHDF for a total of 30 hours. A gradual decrease in TT was observed, and no rebound was noted. The TT at the time of cessation of CVVHDF was 22.4 sec.

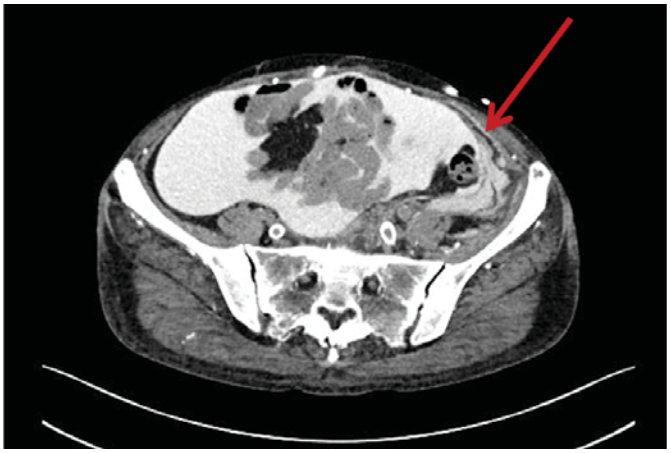
Discussion: Direct dabigatran removal by extracorporeal methods has been described. Most authors reported a rebound in drug levels after cessation of dialysis. A rebound in patients with closed-space bleeding may be catastrophic and the use of continued clearance should be considered to address the anticipated rebound in TT levels as the drug returns to the plasma. The kinetics of dabigatran clearance with CRRT has yet to be defined, but it is clear that it is adequate to prevent the anticipated rebound of this drug.

TH-PO759

Dialysate Leakage From Retroperitoneum in a Long-Term Peritoneal Dialysis (PD) Patients Takafumi Yamakawa, Mao Watanabe, Nanae Matsuo, Yasuyuki Nakada, Izumi Yamamoto, Yudo Tanno, Ichiro Ohkido, Keitaro Yokoyama, Takashi Yokoo. *Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

Introduction: Dialysate leakage can be a common problem in peritoneal dialysis (PD) patients. PD catheter exit sites and less commonly inguinal areas are usual sites of leakage. To this date, retroperitoneal leakage has not yet been reported as a leakage site. Here, we report a case of retroperitoneal leakage of dialysate fluid in a long-term PD patient.

Case Description: A 52 year old woman with end-stage renal disease due to lupus nephritis had begun PD at the age of 26. Because of difficulties to maintaining her vascular access due to prothrombotic nature of Antiphospholipid antibody syndrome, she had to continue with PD ever since. In January 2014, this patient presented with fluid overload owing to poor fluid removal. On CT peritoneography and 99mTc-MAA peritoneoscintigraphy, retroperitoneal leakage was confirmed by observation of increasing edema in the subcutaneous and muscular tissues of the left hip as dialysate fluid was being inserted into the peritoneal cavity. As for treatment, fragility of the peritoneal membrane due to long term steroid therapy and PD was a concern when considering surgical repair. Therefore, a decision was made to change the modality of her PD from continuous ambulatory PD to automated PD which would result in decreased intra-abdominal pressure thus minimizing retroperitoneal leakage. This change resulted in a substantial improvement in her fluid status.



Discussion: Retroperitoneal leakage is a rare presentation, and is likely to be the result of frail peritoneal membrane caused by long history of PD, in this case 26 years, and long term steroid therapy. In such a case, surgical repair is often difficult, and modification of PD modality may be a beneficial solution.

TH-PO760

Dialysis-Related Amyloidosis: It Is Just a Matter of Time Mana Dissadee,[1] Subhash Popli.[2] [1]*Nephrology, Loyola Univ Medical Center, Maywood, IL;* [2]*Nephrology, Edward Hines Jr VA Hospital, Hines, IL.*

Introduction: Dialysis-Related Amyloidosis is a significant disease that affects patients who are on long-term dialysis. It involves predominantly the musculoskeletal and gastrointestinal system. We are presenting a case of peritoneal dialysis patient with full-blown manifestations.

Case Description: A 66-year-old male with ESRD secondary to hypertension was hospitalized with 2-week history of diffuse joint pain, abdominal pain. He had been on hemodialysis for 12 years and changed to peritoneal dialysis 2 years ago due to multiple episodes of vascular access malfunction. He had chronic intermittent shoulders and bilateral hip pain. He had bilateral carpal tunnel decompression 3 years ago. Physical exam revealed macroglossia. Peritoneal fluid analysis showed no leukocytosis. CT scan of the abdomen and pelvis was negative for intra-abdominal processes. Enlarged cystic lesions on left superior acetabulum and femoral head were found. Bone survey revealed multiple cystic lesions on shoulders and hips. Bone marrow aspiration and biopsy were consistent with MGUS. The patient underwent a prophylactic intra-medullary nailing of the left femur. The bone biopsy of the lesion showed amorphous eosinophilic depositions, positive staining with Congo red dye and characteristic apple-green birefringence on polarized light. High performance liquid chromatography tandem mass spectrometry detected a peptide profile consistent with beta 2-microglobulin. His hospitalization was complicated by bacterial peritonitis, C. Difficile infection and intestinal pseudo-obstruction. He expired on 120 days of admission.

Discussion: This case demonstrates the typical presentation of dialysis-related amyloidosis (DRA). Although the incidence of DRA is decreasing with use of high flux dialysis in the last two decades, there is no cure. Findings of carpal tunnel syndrome or cystic bone lesions in dialysis patients warrant physicians of DRA. Definitive diagnosis requires tissue biopsy. Kidney transplantation is the treatment of choice. There are limited studies to compare the efficacy of dialysis modalities. Early diagnosis and effective renal replacement therapy are keys to slowing the occurrence of DRA.

TH-PO761

Successful Treatment of Rare Peritoneal Tunnel Infection with Nocardia Asteroides Rajat Lamba, Savneek S. Chugh, Aromma Kapoor, Anjani K. Dubey, Anita Kaul, Rishikesh Morey. *Nephrology, Westchester Medical Center, Valhalla, NY.*

Introduction: Nocardia is a partially acid fast aerobic bacteria that usually grows in soil rich in organic matter, fresh and salt water. Human infections with Nocardia asteroides manifest as cutaneous, pulmonary and disseminated forms, the most common form being a slowly progressive pneumonia in immunocompromised host. Nocardia asteroides rarely cause peritonitis in patients on Peritoneal Dialysis (PD). Less than 10 cases have been reported, half of which required catheter removal. We are reporting the first case of PD tunnel infection with Nocardia asteroides which was successfully treated with externalization of the catheter, oral Trimethoprim/sulfamethoxazole and topical sulfacetamide.

Case Description: Patient with diabetes and ESRD from FSGS on PD for 5 months, presented to the clinic with reddish brown discharge from PD catheter exit site. He reported to have returned from a recent trip to Poconos where he had a hot tub bath in Jacuzzi. He was on Continuous Cyclic PD with 4 exchanges each of 2.5 liters of 2.5% dextrose and 3.5 meq/l calcium solution overnight and a day fill of icodextrin. He denied any fever, chills, abdominal pain or cloudy effluent. He was given a dose of intravenous vancomycin, gentamycin and oral amoxicillin/clavulanic acid. Cultures were sent from the exit site, blood and PD effluent. Exit site cultures came back positive for Nocardia Asteroides. Patient was subsequently hospitalized for externalization of the PD catheter from subcutaneous tunnel. Antibiotics were switched to oral Trimethoprim/Sulfamethoxazole and topical 10% sulfacetamide ophthalmic solution. Patient was noted to have complete resolution of the exit site infection at 2 week follow up.

Discussion: Nocardia asteroides is a rare cause of peritonitis in patients on peritoneal dialysis. Our patient likely acquired Nocardia from hot tub bath. Nocardia infection, although rare, should be considered as a possible cause of peritonitis and catheter infection especially in cases that do not respond to standard antibiotic regimen. Patients should be instructed to avoid improperly sterilized pools, while on peritoneal dialysis.

TH-PO762

Myoclonic Jerks and Generalized Epilepsy in End Stage Renal Disease due to Gabapentin Sandesh Joshi, William DiFilippo. *Nephrology, Geisinger Medical Center, Danville, PA.*

Introduction: Gabapentin is widely used in our clinical practice. Gabapentin induced myoclonic jerks and epilepsy is a rare and debilitating condition. Appropriate dose reduction or discontinuation of this drug should be considered in patient with chronic kidney disease and end stage renal disease on dialysis.

Case Description: A 66 year old female with end stage renal disease on chronic hemodialysis, seizures on Ethosuximide, restless leg syndrome was admitted to hospital due to dizziness, tremors and a fall. One day prior to admission, pt experienced dizziness for a brief period of time while driving. Later she noticed twitching of her right arm which gradually got worse. Also started to have jerky movement of the same arm and right leg following which she sustained a fall hitting her head. She did not have loss of consciousness and no injuries. Symptoms went away on its own. Later during the night, she woke up with some tremors in her both arms which gradually worsened, started to have jerky movement involving all four extremities. These symptoms persisted throughout the night and during the

day as well which prompted her to come to emergency department for further evaluation. She was hemodynamically stable and on examination was found to have myoclonic jerks of all four extremities. Her blood work was within normal limits and a computed tomography of head was done which was negative for any acute findings. An Electro-enceplalogram was done which was consistent with primary generalized epilepsy corelating with myoclonus. Upon further interviewing, patient revealed that she was started on Gabapentin(400 mg twice daily) a day prior to her symptoms. Neurology was consulted who increased her ethuosiximide. She was dialyzed next day after which her myoclonic jerk was relieved. She was place in continuous EEG monitoring for next few days until elipsy discharge went away. Her Gabapentin was discontinued at the time of discharge.

Discussion: Gabapentin induced myoclonic jerks and epilepsy is a rare and debilitating condition which we should be aware of. Appropriate dose reduction or discontinuation of this medications should be considered in patient with chronic kidney disease and end stage renal disease on dialysis.

TH-PO763

Cefepime Related Neurotoxicity in a Peritoneal Dialysis Patient Veni J. Peram, Sandeep S. Soman, Jerry Yee. *Nephrology, Henry Ford Hospital, Detroit, MI.*

Introduction: Incidence of Cefepime-related neurotoxicity (CRN) has been reported in 4–16% and is associated with impaired renal clearance. Very few cases of cefepime related neurotoxicity have been reported in continuous ambulatory peritoneal dialysis (CAPD) patients, and peritoneal dialysis (PD) does not clear cefepime efficiently. We present a case of CRN in a patient on CAPD, which resolved rapidly after hemodialysis (HD).

Case Description: Case Description:A 60 year-old man with type 2 diabetes and end-stage renal disease (ESRD), underwent right nephrectomy for renal cell carcinoma. Post-operatively, the patient had hypotension and received empiric cefepime therapy for suspected infection: total dose of 9 gms over 72 hours. Forty-eight hours after cefepime initiation the patient developed disorientation and intermittent generalized tonic-clonic jerking, subsequently followed by aphasia. Moderate encephalopathy was demonstrated by an electroencephalogram and an extensive infection workup was negative (CSF, blood, peritoneal fluid and urine cultures). CRN has been reported at variable serum levels (14–716 mcg/ml) and may be mediated by competitive inhibition of the brain's principal inhibitory neurotransmitter, gamma aminobutyric acid (GABA), which could lower the neuronal excitation threshold. Cefepime level which is shown in the table was calculated based on a study by Barbhaiya et al in CAPD patients.

Volume Of distribution (Vd) over 72 hrs	22 L
Percent dose excretion in PD fluid, Urine	26%, <5%
Calculated drug level: 74% total dose/Vd	302 mcg/ml
Estimated drug level for 2 times the Vd	151 mcg/ml (which is still in the toxic range)

The patient remained disoriented for 40 hrs after cessation of cefepime. Neurologic symptoms improved drastically and the patient's mental status returned to baseline after the third session of HD.

Discussion: ESRD patients are vulnerable to CRN from impaired renal clearance. We recommend a substantially lower dose in CAPD patients as treatment of infection(s): 1–2 g per 48 h. The development of untoward neurological symptoms during cefepime administration mandates early recognition, drug discontinuation and possibly, HD for rapid drug clearance, especially in PD patients.

TH-PO764

Intradialytic Abdominal Pain Piangwarin Phaosawasdi, Billy T. Hour, Jane Y. Yeun. *Internal Medicine - Div of Nephrology, Univ of California, Davis, Sacramento, CA.*

Introduction: Abdominal pain occurring only during hemodialysis (HD) is uncommon. The underlying etiology may remain elusive, leading to a significant delay in diagnosis and treatment.

Case Description: 57 year old man with hepatitis C cirrhosis, orthotopic liver transplantation in 2000, and calcineurin inhibitor nephrotoxicity on HD since 2005, developed recurrent severe abdominal pain after 1 hour of HD, necessitating termination of treatment. Pain gradually resolved in an hour. He described intermittent abdominal bloating relieved with belching without other symptoms. Past medical history was remarkable for coronary artery disease and diabetes mellitus. Medications included lanthanum, lisinopril, metoprolol, and tacrolimus. On exam, blood pressure was 196/111 and pulse 87. Abdomen was soft, nontender, without masses or heptosplenomegaly, and bowel sounds were present. Liver tests were normal. Computed tomography (CT) of the abdomen revealed stable mild intrahepatic ductal dilatation, CT angiogram and Doppler study without evidence of mesenteric ischemia. Upper endoscopy and colonoscopy were unremarkable. Reducing the ultrafiltration (UF) rate, changing the dialyzer membrane, pre-treating with antihistamine and steroid, discontinuing lisinopril, and relieving constipation were ineffective. Abdominal pain improved, but patient developed intradialytic hypotension despite minimal UF, transaminitis, marked jaundice, and elevated lipase and amylase. Repeat CT abdomen revealed pancreatic pseudocysts and a large biloma; CT angiogram demonstrated extensive hepatic artery calcification with occlusion, splenic artery calcification, and splenic infarction. Transaminitis and jaundice resolved after percutaneous drainage of the biloma. Intradialytic abdominal pain has not recurred.

Discussion: Intradialytic abdominal pain was due to recurrent liver ischemia from extensive hepatic artery calcification, resulting eventually in liver infarction, disruption of bile drainage, biloma compression of the pancreatic duct, and pancreatitis. The initial focus on mesenteric ischemia and HD reactions delayed the diagnosis. Intra-abdominal vascular catastrophes must be considered when evaluating intradialytic abdominal pain.

TH-PO765

Spontaneous Healing of Acquired Pleuroperitoneal Leak in a Peritoneal Dialysis Patient after Coronary Artery Bypass Grafting Surachit Kumar, Fahd Syed, Dumitru Rotaru. *Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

Introduction: Pleuroperitoneal leak is caused by an abnormal communication between peritoneal and pleural spaces. It can be due to a congenital or acquired defect in the diaphragmatic muscle fibers or connective tissue. In patients on peritoneal dialysis (PD) the increased intraabdominal pressure and negative intrathoracic pressure can cause fluid accumulation in the pleural cavity.

Case Description: A 65 year old male was on Continuous Ambulatory Peritoneal Dialysis (CAPD) for about a year. His PD prescription was 4 exchanges of 2L of 1.5 percent dianeal and 2L of icodextrin overnight. He underwent Coronary Artery Bypass Grafting (CABG) for chest pain. He was on CVVH post operatively for 7 days and resumed PD on discharge. A month later the patient presented in the clinic with increased shortness of breath. A large right sided pleural effusion was diagnosed. Thoracocentesis showed transudative fluid with pleural fluid glucose greater than serum glucose and low pleural fluid LDH consistent with hydrothorax associated with PD. CABG operative report showed no breach of peritoneum during surgery. He had peritoneal scintigraphy done with Technetium (Tc) 99 which showed a communication between the peritoneal and right pleural cavity. He was then switched to Hemodialysis. Cardiothoracic surgery decided to conservatively observe the patient. Approximately 2 months later the patient was restarted on CAPD with 4 exchanges of 1.5 L of 1.5 percent dianeal with 2 liter of overnight last fill with icodextrin. He tolerated PD comfortably with no recurrence of the pleuroperitoneal leak.

Discussion: In this case the etiology of the pleuroperitoneal leak is probably due to an acquired defect post surgery as there was no hydrothorax while the patient was on PD for a year prior to surgery. Although no breach of the peritoneum was reported, the leak is believed to have developed due to mechanical stretch or trauma to diaphragmatic fibers or connective tissue. This case demonstrates a spontaneous healing of acquired pleuroperitoneal leak and the ability of PD to be resumed in such a scenario.

TH-PO766

Unusual Cause of Intra-Abdominal Air in a Patient on Peritoneal Dialysis Waleed W. Siddiqi, Jason M. Kidd, Todd W. Gehr. *Nephrology, Virginia Commonwealth Univ, Richmond, VA.*

Introduction: Volume overload is a common problem in patients on peritoneal dialysis. Failure of ultrafiltration can lead to changes in prescription, tonicity of dialysate and even dialysis modality. This case describes an unusual presentation of ultrafiltration failure due to a mechanical problem.

Case Description: A 69 years old female with end stage kidney disease on peritoneal dialysis was admitted with shortness of breath, abdominal distension and swelling. She had no abdominal pain or cloudy dialysate. On presentation to the emergency department, she was dyspneic and had 3+ lower extremity edema. Lung exam was significant for bibasilar crackles. Abdomen was distended, but soft. Significant intra-peritoneal air was seen on abdominal x-ray. Surgery was consulted and acute surgical etiologies were ruled out. Further investigation revealed the patient had recently increased the length of tubing that was being used to fill her peritoneal cavity, leading to her symptoms. Intra-abdominal air was removed by placing patient in prone trendelenburg position and using a large syringe attached to peritoneal dialysis catheter, leading to resolution of her symptoms.

Discussion: Common causes of volume overload in a peritoneal dialysis patient include dietary indiscretion, inappropriate prescription, and mechanical problems including leaks, obstruction, entrapment and malposition.[1] This case describes an uncommon mechanical problem leading to volume overload and a novel approach to correct this issue. It also illustrates the importance of a thorough history in patients on home dialysis therapies, including queries regarding their equipment. [1]Mujais S et al. Evaluation and management of ultrafiltration problems in peritoneal dialysis. Peritoneal Dialysis International, Vol 20, Suppl. 4. 2000.

TH-PO767

Hypertonic Dialysate to Manage Elevated Intracranial Pressure During Intermittent Hemodialysis Lee M. Ferguson, Divya Monga, Kenneth E. Kokko. *Dept of Medicine, Div of Nephrology, Univ of Mississippi Medical Center, Jackson, MS.*

Introduction: Standard intermittent hemodialysis (HD) is traditionally avoided in patients with elevated intracranial pressures (ICP), as it can lower cerebral perfusion pressure (CPP) by either decreasing the mean arterial pressure (MAP) or increasing the intracranial pressure (ICP). At-risk patients are usually placed on a continuous form of renal replacement therapy (RRT), as this modality provides a slower solute clearance and increased hemodynamic stability, leading to a more stable CPP. We describe the case of a 28-year-old male with end-stage renal disease on intermittent HD, who presented post-motor vehicle collision with a C3 fracture and small brain hemorrhages without midline shift. An intraventricular drain was placed and demonstrated an ICP of 20 mmHg and a CPP of 69 mmHg. The patient was then started on conventional intermittent HD for worsening hyperkalemia via an AV fistula. After one hour of HD, the patient's MAP dropped to 81 mmHg, his ICP increased to 28 mmHg, and his CPP dropped to 53 mmHg. In response to the lower CPP, ultrafiltration was turned off, dialysate sodium was increased to 145 mEq/L, and the patient was given a 500 cc intravenous bolus of normal saline. As a result of these changes, the MAP increased to 86 mmHg, the ICP decreased to 17 mmHg, and his CPP increased to 69 mmHg. He was then able to complete a three hour session of HD. Little is

known about the timing of changes in CPP in patients on conventional intermittent HD. This case illustrates that a drop in CPP can be a late occurrence of conventional hemodialysis, and that it can be corrected with a hypernatremic dialysate. More research should be done to determine how often CPP drops during conventional HD and whether or not there are patients that may have an adverse outcome as a result of a low CPP.

TH-PO768

Hyperlactatemia due to Thiamine Deficiency in a Peritoneal Dialysis Patient
Santhi Voora, Todd Ing, Anuradha Wadhwa. *Div of Nephrology, Loyola Univ Medical Center, Maywood, IL.*

Introduction: Lactic acidosis commonly occurs because of diminished tissue oxygenation leading to anaerobic metabolism and increased lactate production. Here we report an interesting case of persistently elevated serum lactate levels in a peritoneal dialysis (PD) patient, raising concerns about the PD fluid lactate content as a possible cause.

Case Description: A 44-year-old female with end-stage renal disease maintained on PD was admitted with generalized weakness and poor oral intake. She was hypotensive and tachycardic with lab findings notable for leukocytosis, acidosis (CO2 17 mmol/L), anion gap 19 mEq/L and lactate 6 mmol/L (normal 0.9-1.7 mmol/L). Further workup revealed fungal peritonitis which was treated with fluconazole. Patient refused PD catheter removal but all subsequent cultures were negative. Despite improvement in blood pressure and resolution of infection, serum lactate levels remained elevated between 4-7 mmol/L. Her liver function tests were normal except for low serum albumin. There was concern about lactate content of PD fluid as the source of elevated serum lactate. Blood lactate concentrations reflect the balance between lactate production and clearance. Thiamine deficiency inhibits the oxidative decarboxylation of pyruvate, which accumulates and is converted to lactate by lactate dehydrogenase. In our patient, thiamine was low at 59 nmol/L (normal 78-185 nmol/L) and pyruvate was elevated at 2.08 mg/dL (normal 0.3-1.5 mg/dL). Fifty mg of oral thiamine was started daily and the serum lactate normalized to 1.6 mmol/L within days after initiation of therapy.

Discussion: The temporal relationship between thiamine supplementation and lactate normalization supports thiamine deficiency as the cause of this patient's elevated lactate level *rather than lactate from the PD fluid* (which could have contributed as a substrate). While thiamine deficiency-related lactic acidosis has been described in the setting of parenteral nutrition, to our knowledge, our case is the only description of thiamine deficiency-related elevated serum lactate value in the setting of PD. Thiamine deficiency should be included in the differential diagnosis of lactic acidosis, especially in PD patients.

TH-PO769

A Rare Cause of Wide Anion Gap Metabolic Acidosis in Uncommon Calcific Uremic Arteriolopathy
Sahil Garg, Nader S. Bahri, Leighton R. James. *Nephrology, UF Health Jacksonville.*

Introduction: Calcific uremic arteriolopathy (CUA) has high mortality despite treatment. This case highlights the importance of multi-pronged approach to treat CUA. Metabolic acidosis due to STS poses a significant problem, requiring use of high bicarbonate bath during hemodialysis or even reduction in STS dose.

Case Description: A 57 year old morbidly obese caucasian male with history of hypertension, type 2 Diabetes Mellitus, End stage renal disease (on Hemodialysis for 3 years) and atrial fibrillation (on coumadin for 4 months) was admitted to the hospital with complaint of bilateral thigh and calf pain for 1-2 months. Pain was present at rest and worsened on exertion. He admitted being non-compliant with phosphate binders. Physical examination revealed painful, indurated subcutaneous plaques in infra-umbilical region, medial part of bilateral thighs and calves. Subsequently over the next 3-4 days lesions evolved into ulcerations with surrounding violaceous discoloration and erythema. Serological work up for vasculitis including ANA, ANCA, cryoglobulin, anticardiolipin Ab was negative. Complement C3 (174 mg/dl) was normal while C4 (60.9 mg/dl) was elevated. Review of laboratory data revealed phosphorus levels ranging from 7-8 mg/dl, PTH 325-750 pg/ml, and serum calcium 8.8-9 mg/dl in prior 12 months. He was diagnosed with CUA. He was started on Sodium thiosulphate (STS) 25 gm intravenously after each Hemodialysis session, daily hemodialysis to improve clearance, a non-calcium based phosphate binder and cinacalcet. Coumadin was switched to aspirin and plavix. Skin biopsy was not done because of patient's refusal. Skin lesions and pain exhibited substantial improvement in following 2.5-3 months with above-mentioned strategies, but surprisingly he developed wide anion gap metabolic acidosis despite daily hemodialysis. Anion gap increased from baseline of 17-18 to 25-30 after starting STS. Acidosis improved when hemodialysis was performed against higher Bicarbonate bath.

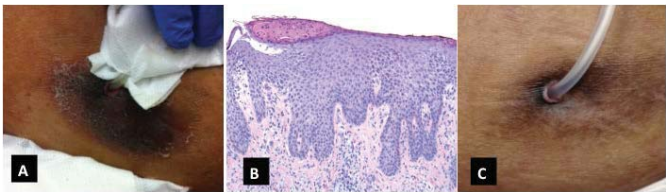
Discussion: CUA can be diagnosed early if there is high index of suspicion. Medical therapy when started in timely manner can improve overall moratlity and morbidity in CUA. STS is a strong acid causing metabolic acidosis as reported in many case reports.

TH-PO770

Gentamicin-Induced Contact Dermatitis Mimicking Peritoneal Dialysis Catheter Exit Site Infection
Ike Ezumba, Kristopher R. Fisher, Elvira Gosmanova. *Nephrology, UTHSC, Memphis, TN.*

Introduction: Topical gentamicin is an effective and widely used antibiotic for the prophylaxis of peritoneal catheter exit site infection in patients undergoing peritoneal dialysis (PD). We describe a rare case of contact dermatitis induced by gentamicin cream that mimicked peritoneal catheter exit site infection.

Case Description: A 54-year old female undergoing PD presented to a dialysis clinic with 2 weeks history of progressively worsening itchy lesion around her peritoneal catheter exit site. 3 weeks ago the patient started topical gentamicin cream for the prophylaxis of exit site infection. On examination, peritoneal catheter exit site had an 8.5 x 4.5 cm nummular plaque, violaceous in color with peripheral rim of erythema with no granulation tissue or discharge (figure 1A). Clinical diagnosis of allergic contact dermatitis due to gentamicin was made and gentamicin cream was discontinued. Patient underwent a skin biopsy that showed psoriasiform spongiotic dermatitis with eosinophils, consistent with contact dermatitis (figure 1B). Exit site lesion slowly resolved over 4 weeks after stopping gentamicin and initiation of 2.5% hydrocortisone cream (figure 1C).



Discussion: Peritoneal catheter associated exit site infection requires a prompt treatment to avoid the development of peritonitis. However, not all peritoneal catheter exit site lesions are due to microbial infection. Allergic contact dermatitis due to topical gentamicin is rare. It is important to be aware of this association to avoid incorrect diagnosis of peritoneal catheter exit site infection and inappropriate antibiotic use in patients undergoing peritoneal dialysis.

TH-PO771

A Case of Successful Conservative Management of End Stage Renal Disease
Phillip Madonia,[1] Mohana B. Karlekar,[2] Rachel B. Fissell.[1] *[1]Dept of Nephrology, Vanderbilt Univ, Nashville, TN; [2]Dept of Internal Medicine, Vanderbilt Univ, Nashville, TN.*

Introduction: End stage renal disease (ESRD) patients often present with multiple comorbid conditions, advanced age, and an increased risk of death. While renal replacement therapy extends life, it does not restore kidney function. This case illustrates successful conservative management of renal failure, achieving expectations of both the patient and the family.

Case Description: The patient is a 95 year old man who was evaluated for stage V chronic kidney disease due to hypertension. Other history included prior cerebrovascular accident,depression, and remote prostate cancer. The patient lived at home with his daughter and her husband, both accompanying him to clinic visits. On initial evaluation, his glomerular filtration rate was 15 ml/min, and he complained of poor energy and occasional nausea. He exercised at home several times weekly. He had fair blood pressure control on three anti-hypertensive medications. After discussions with the patient and his family, the team was able to clearly define the patient's overall goals of care to be maximizing his quality of life and remaining at home with his family. It was agreed that when dialysis became necessary, it would not be initiated. Over the next few months, his renal function slowly declined, and management of the sequelae of kidney disease were managed conservatively. The family voiced satisfaction with his care during this period until he passed away peacefully at home.

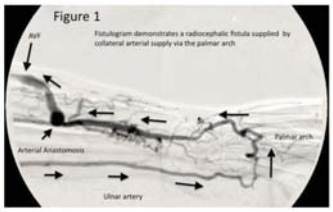
Discussion: The fastest growing demographic among new dialysis patients is people older than 75 years. Given their advanced age and significant comorbid conditions, it is no surprise that dialysis patients have a mortality rate significantly higher than the general population. Dialysis patients who complete an advance directive are more likely to receive palliative care prior to dying than cancer or heart failure patients. Unfortunately, fewer than half of all dialysis patients in the United States complete an AD prior to their death. More work is needed to increase awareness of palliative care resources, and to encourage end of life conversations between ESRD patients, families, and caregivers.

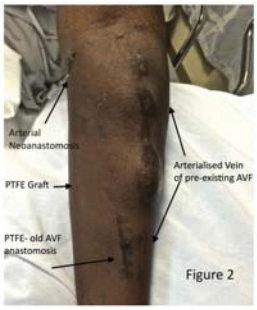
TH-PO772

Operative Salvage of Radiocephalic AVF with Poor Inflows By Formation of a Neoanastomosis to the Proximal Artery Using Tapered PTFE Graft to Complete a Composite Forearm Loop Configuration
Siddiq Anwar, Ravkiran Kaur Khurana, Mansumeet Singh, Daniel C. Brennan. *Renal Div, Washington Univ School of Medicine, St. Louis, MO.*

Introduction: Loss of arteriovenous fistula as access for hemodialysis (HD) leads to significant morbidity and increased mortality risk. Forearm HD access are associated with less cardiovascular remodeling and less steal.

Case Description: We describe a case of a 34-year-old, African-American man with a poorly functioning radio-cephalic fistula (RCF). Venography suggested that the patient's flow into the fistula was through the palmar arch and the patient had a diseased radial artery .A tapered polytetrafluoroethylene graft (4 mm to 7 mm Propaten graft) was inserted to connect brachial artery to distal end of the RCF fistula. This completed a medial segment for a loop graft which would be composed of his old fistula with the arterialized vein on the lateral segment and graft on the medial segment.





Discussion: Operative salvage of radiocephalic AVF with poor inflows by formation of a neoanastomosis to the proximal artery using PTFE graft can be performed with good results. The arterialized vein provides immediate HD access and saves proceeding to elbow fistula formation.

TH-PO773

Massive Pneumoperitoneum in Patients on Chronic Automated Peritoneal Dialysis Vanya Grover,[1] Anu Neerukonda,[2] Cheryl Laveglia,[3] Reem Miller,[3] Heesuck Suh,[1] Nand K. Wadhwa.[1] *[1]Nephrology, Stony Brook Hospital; [2]Medicine, Stony Brook; [3]Stony Brook Kidney Center, DCI, Stony Brook, NY.*

Introduction: A massive pneumoperitoneum (PP) is a rare complication in patients on automated peritoneal dialysis (APD). Infusion of air can occur with catheter insertion or manipulation or faulty PD techniques. We describe two cases of huge pneumoperitoneum due to a faulty technique in setting up of APD.

Case Description: A 68 year-old woman with ESRD due to hemolytic uremic syndrome on APD was admitted with diffuse abdominal pain. She was afebrile and her abdomen was soft, mildly distended, and diffusely tender. She was thought to have peritonitis and was empirically treated with antibiotics. The peritoneal fluid was hazy with a cell count of 411 per mcL and 2% neutrophils. Cultures were negative. APD was resumed and within 24 hours her abdominal pain resolved. She was discharged home and antibiotics were discontinued. Ten days later she developed similar abdominal pain. She was again afebrile and her abdomen was mildly distended. A computed tomography (CT) of her abdomen revealed massive free intraperitoneal air. APD was resumed and her abdominal pain subsided. Repeat peritoneal fluid revealed a cell count of 151 per mcL and 23% neutrophils. Cultures were negative. The second patient is a 48 year-old man with ESRD due to diabetic nephropathy on APD who was admitted with a distended abdomen for one week. He was afebrile. His abdomen was mildly distended, and not tender. The peritoneal fluid was clear with a cell count of 69 per mcL and 9% neutrophils. CT of the abdomen revealed moderate PP. Cultures were negative. APD was resumed and his abdominal pain subsided.

Discussion: Pneumoperitoneum in both cases was due to a faulty technique, as both patients left the fill line clamped during priming. The clinical picture was due to a significant intraperitoneal free air from air entry when initiating APD. The patient's symptoms resolved with the proper technique. These index cases prompted evaluation of all APD patients' technique of APD. All patients underwent retraining of the APD procedure. No further case of pneumoperitoneum was observed after training over a follow up period of 903 patient-months in our program.

TH-PO774

Pre-Emptive Continuous Renal Replacement Therapy in a Patient at High Risk for Tumor Lysis Syndrome Neha Garg, Parth Rao, Miguel Conde, Neil W. Lyman. *Saint Barnabas Medical Center, Livingston, NJ.*

Introduction: Tumor lysis syndrome (TLS) is a life threatening complication occurring either spontaneously or after chemotherapy for hematological tumors. It is characterized by acute kidney injury (AKI) and electrolyte disturbances. Prophylaxis of TLS includes vigorous hydration, control of hyperuricemia, managing electrolyte disorders, and renal replacement therapy (RRT) for patients who develop AKI. We report a case of pre-emptive RRT in a patient at high risk for TLS.

Case Description: A 67 year old African American male with a 10 year history of chronic lymphocytic leukemia was admitted to our hospital with a 50 lbs. weight loss over 1 year, moderate splenomegaly, marked lymphadenopathy and a leukocyte count of 204,000/ µL. He also had dilated cardiomyopathy with ejection fraction of 15-20%, cardio-renal syndrome, diabetes mellitus, chronic kidney disease (CKD) stage 4 with a baseline creatinine (Cr) of 2.2-2.7mg/dl. He received rasburicase and was started on bendamustine and decadron. Continuous RRT was initiated pre-emptively 2 hrs. post chemotherapy. Bendamustine is highly protein bound hence CRRT didn't interfere with its excretion. Uric acid level on admission was 8.4mg/dl and remained low during RRT, however peaked to 11mg/dl after discontinuation of RRT. His hospital course remained stable. Leukocyte count trended down to a nadir of 32,000/ µL. CRRT was discontinued on day8 and patient was discharged on Day12 with a Cr of 2.11mg/dl. Patient's Cr remained stable at 2.23 mg/dl two months post discharge.

Discussion: Our patient had cardiorenal syndrome with CKD stage 4 and congestive heart failure (CHF) which precluded the use of vigorous hydration. We successfully used pre-emptive CRRT to prevent development of AKI and delay the need of chronic dialysis for our patient. Saccente et al (*pediatric nephrology, 1995, 9; 569-573*) had used similar strategy in children with burkitt's lymphoma. Pre-emptive RRT may be used in patients with underlying advanced renal disease with CHF to prevent severe electrolyte imbalance. It may also delay the progression of CKD and the need of chronic dialysis. Further guidelines are required to initiate RRT pre-emptively in high risk patients.

TH-PO775

Intermittent Hemodialysis Prescription with Intracerebral Hemorrhage: Practical Approach Eric Loman, Gaurav Alreja, Kelly Mazurek, Ruchir D. Trivedi. *Internal Medicine and Nephrology, Univ of Connecticut, Farmington, CT.*

Introduction: Patients with intracerebral hemorrhage (ICH) have cerebral edema and increased intracranial pressure (ICP). Intermittent hemodialysis (IHD) potentiates brain injury by increasing water content (cerebral edema) and ICP. The mechanisms involved are "Cerebral Intracellular Urea Trapping" with a rapid fall in serum urea/sodium/osmolality and osmotic water shift across the gradient. Although CRRT is the preferred modality to prevent rapid lowering of urea and slow volume removal to maintain cerebral perfusion pressure, it is not tenable in all cases both due to logistics and need for additional catheter placement. Our case highlights challenges with IHD and suggests practical prescription changes.

Case Description: 68 year old female on chronic IHD presented with altered mental status, uremia (BUN 90 mg/dl) and a large subarachnoid hemorrhage. On her regular prescription (Na-140, Ca-2.0, HCO3-35, BFR-400cc/min, DFR-800cc/min, time-240 min), she became non-responsive with stable hemodynamics. Her dialysis prescription was then altered (raised dialysate Na 5 meq/dl above serum, reduced BFR-250 cc/min, decreased DFR-500cc/min, increased Ca-2.5meq/dL, shortened dialysis time-150 min, limited volume removal-1 L/treatment, cooled dialysate temp: 35.5 C and dialyzed 5-6days/week). Over the duration of the next 2 weeks, the dialysis prescription was changed every third day (decreased Na-2 meq/dl, increased BFR-50 cc/min, increased duration-30 min, increased temp-0.5 C and increased HCO3-1meq/dl) to transition the patient to her routine prescription.

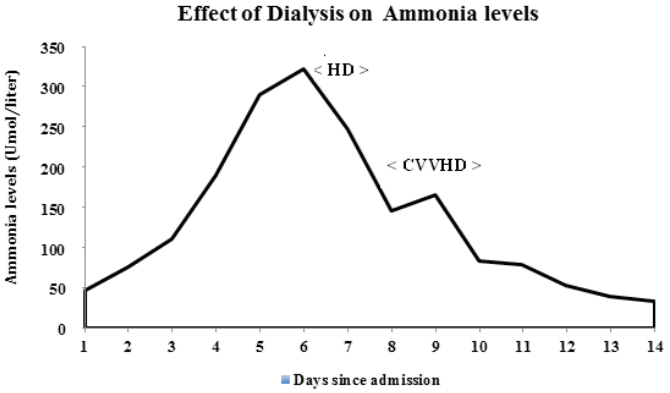
Discussion: IHD lowers serum osmolality rapidly and urea in brain cells is trapped as it moves through slow urea transporters, setting up a large osmotic gradient, resulting in worsening edema and ICP. Slowing the rate of urea reduction, by decreasing BFR and dialysis duration, prevents rapid osmotic shifts. More frequent dialysis provides control over volume (high Na will predispose to volume overload), rapid reduction in urea and hemodynamic stability. Higher Na baths may also partially offset the fall in serum osmolality. Lowering bicarbonate prevents paradoxical intracellular acidosis and high osmolality.

TH-PO776

Role of Dialysis in Multifactorial Hyperammonemic Encephalopathy Prasanna Sujaritha Durairaj, Amanda Fernandes, Ashish Verma. *Internal Medicine, Baystate Medical Center, Springfield, MA.*

Introduction: Hyperammonemia (HA) in adults without liver disease is responsive to dialysis when optimal medical management fails.

Case Description: A 48 year-old white female with history of alcohol abuse, gastric bypass, and bipolar disorder on Valproate for 4 years, presented with altered mental status, non-convulsive status epilepticus, and rising serum ammonia levels. This persisted a week after discontinuation of Valproate and initiation of lactulose, rifaximin, levocarnitine, and sodium benzoate. Valproate levels were initially low normal and then undetectable. Kidney and liver function were normal. No acid-base disorders were evident. Urine toxins screen and lumbar puncture were negative. Brain imaging showed nonspecific changes. Cirrhosis and porto-systemic shunt were absent on imaging. Serum glutamine and lysine were elevated, consistent with **HA** of any etiology. Other amino acids were below normal range. Urine organic acid screen suggested glutathione depletion and ruled out orotic aciduria or other urea cycle defects. Zinc and Vitamin B12 were low and repleted. Pseudomonas pneumonia was diagnosed and treated. She had 2 sessions of intermittent hemodialysis followed by 48 hours of continuous veno-venous hemodialysis. Ammonia levels trended to normal without significant improvement in mental status.



Discussion: Causes for **HA** in this patient included Valproate and its metabolites inhibiting mitochondrial carbamyl phosphate synthetase in the setting of altered gut function, severe catabolic state and past alcohol abuse. Zinc and vitamin B12 deficiencies, Pseudomonas pneumonia and a partial, late-onset urea cycle disorder were also contributory

to her multifactorial **HA** encephalopathy. Ammonia is 17 Daltons in molecular weight and at pH of 7.3, over 90% is in ionized form. Hence, it is successfully dialyzable with clearance similar to that of urea.

TH-PO777

Role of Renal Replacement Therapy in Fioricet® Overdose Disha Narula, Amit A. Deshpande, Shabnum Haleem, Hasan Arif. *Drexel Univ.*

Introduction: Caffeine has a low volume of distribution and is responsive to extracorporeal methods of elimination. Charcoal hemoperfusion has been the conventional method for enhanced elimination of methylxanthines, particularly theophylline. This case is an example of the challenges surrounding data available for role of different hemodialysis modalities in cases of caffeine toxicity.

Case Description: 58 year old F with PMH of Depression and RSD presented with Fioricet® overdose. Patient took 150 pills of Fiorcet® as a suicide attempt .In the ED, the patient was found to be lethargic and hypotensive with a BP of 75/49, pulse of 69 RR of 8, and O2 sat of 99% on RA. The patient was given 3 L of NS, but she remained hypotensive and was started on a norepinephrine drip. Patient was also intubated for airway protection. Labs: Cr 0.28, HCO3 18, Acetaminophen level was 57. Her ALT 19 AST 14 Alk Phos 36. Salicylate level was negative. Ethanol level <5.0. ABG was pH of 7.45, PCO2 33, PO2 135 HCO3 22, and O2 sat 98. Patient was started on NAC for her elevated Tylenol levels. Nephrology was consulted for the role of HD in Fioricet® overdose for the caffeine and barbiturate component.The patient was initiated on hemodialysis and was dialyzed for 6 hours with a high flux dialyzer.

Discussion: Fioricet® is composed of acetaminophen, butalbital, and caffeine. Fioricet® overdose can lead to renal tubular necrosis. Dialysis has been recommended in cases of severe toxicity. Caffeine has a low volume of distribution and is responsive to extracorporeal methods of elimination. Charcoal hemoperfusion has been the conventional method for enhanced elimination of methylxanthines, particularly theophylline. The endogenous rate of theophylline clearance has been shown to be a 50 mL/min, while hemoperfusion rates have been reported up to 4 to 6 times higher. HD has lower clearance rates (100 mL/min) compared with hemoperfusion.The challenge in these cases is to monitor the need for continued HD without butalbital or caffeine levels to trend. Caffeine ingested in large amounts can lead to toxic theophylline levels in some instances,which can be trended to assess the need for further HD. We, however, used her hypotension and pressor requirement as a surrogate for continued HD needs.

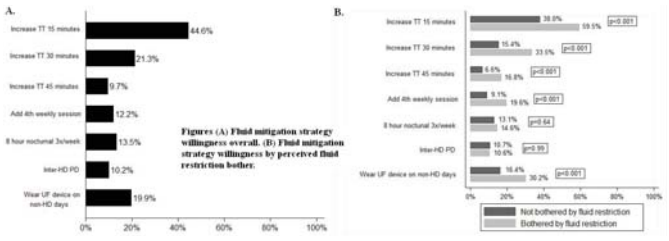
TH-PO778

Patient-Stated Preferences in Fluid Management on Hemodialysis Jennifer E. Flythe,[1,2] Steven M. Brunelli,[2,3] Gary C. Curhan.[2] *[1]Univ of North Carolina Kidney Center; [2]Brigham and Women's Hospital; [3]DaVita Clinical Research.*

Background: Larger interdialytic weight gain (IDWG) and higher ultrafiltration (UF) rates are associated with poorer outcomes among hemodialysis (HD) patients. Dietary restrictions may reduce fluid-related risk; however, adherence is difficult. No data regarding patient preferences for fluid management strategies such as treatment time (TT) extension, more frequent HD, adjunct peritoneal dialysis (PD), and wearable UF devices exist. We designed, tested, and administered a survey to assess patient preferences for fluid management.

Methods: A written survey concerning fluid-related symptoms, patient and HD characteristics, and fluid management preferences was developed. The survey was completed by 600 HD patients at 18 geographically diverse HD units. Comparisons of patient willingness to engage in volume mitigation strategies across patient and fluid-related characteristics were performed.

Results: Survey respondents were 57.6% male, 40.0% age 40-59 years, and 47.6% black. The mean self-reported IDWG was 2.8 ± 1.1 kg and mean TT was 229.7 ± 45.6 minutes. Prescribed fluid restrictions were reported by 74.2% of patients. Overall, if allowed to liberalize fluid intake, >40% of patients were willing to extend TT 15 minutes. Respondents were less willing to engage in other strategies (Figure A). Patients more bothered by their fluid restrictions (versus less bothered) were more willing to engage in fluid mitigation strategies (Figure B). Demographics and symptoms (cramping, dyspnea) were not consistently associated with willingness to engage in the proposed strategies (not shown). >25% of patients were unsure of their dry weights and typical IDWGs.



Figures (A) Fluid mitigation strategy willingness overall. (B) Fluid mitigation strategy willingness by perceived fluid restriction bother.

Conclusions: Patients are generally averse to TT extension greater than 15 minutes and to the other interventions studied. Further study of patient-stated preferences in HD treatment practices is needed to guide patient care.

Funding: NIDDK Support, Other NIH Support - National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award

TH-PO779

Clinical Assessment Cannot Identify Hypovolemia in Hemodialysis Patients Sylvia Kalainy, Kailash K. Jindal, Branko Braam. *Medicine/Nephrology, Univ of Alberta, Canada.*

Background: Determining dry weight of haemodialysis (HD) patients is a challenge. Both underhydration and hypervolemia are associated with HD complications. Therefore, determination of fluid balance to prevent both underhydration and overhydration is an important issue in HD patients. We previously reported that clinical parameters cannot identify HD patients with fluid overload. Using Bio-impedance to assess fluid volume, we hypothesized that a significant number of HD patients could have unidentified hypovolemia.

Methods: Multifrequency Bio-impedance spectroscopy (BCM- Fresenius Canada) measurement was performed before the start of a mid-week dialysis session in 175 patients to assess extracellular fluid volume (ECFV) and overhydration (OH). Physical signs (BP, edema), plasma Na, K and albumin, urea reduction ratio (URR), and a recent CXR (cor-thorax ratio) were investigated as potential markers for volume assessment.

Results: Patients (age 61 (18-91) y, 58% males, 42% diabetic) were divided into 3 groups: normovolemia (OH=>0<1.1L), hypervolemia (OH>1.1L), hypovolemia (OH<0L). BP was significantly lower in hypovolemic patients. Interdialytic weight gain (IDWG) was high in some of the hypovolemic patients. When the entire group was evaluated, no significant correlations between continuous variables and OH was detected except with a weak correlation with pre-dialysis SBP.

	Total (n=175)	Hypovolemic (n=44)	Normovolemic (n=53)	Hypervolemic (n=78)
Gender,M/F	102/73	23/21	30/23	49/29
Age, years	61±15	61±15	60±18	61±13
Hydration status, L.	1±1.8	-1.1±0.8*	0.5±0.3*	2.6±1.4*
Diabetes, %	42	41	37	51
Pre-HD-SBP, mmHg	130±24	125±20*	131±30	138±22*
Pre-HD-DBP, mmHg	73±17	70±15	73±20	73±16
Pre-HD-PP, mmHg	60±20	55±20*	58±23	65±18*
Hypertension, %	32	25	35	41
IDWG,kg	1.6±1.2	1.4±0.9	1.5±1	1.8±1.4

Conclusions: A significant number of our patients were hypovolemic and that could not be predicted by clinical parameters alone. Some of these patients had high IDWG despite hypovolemia. This suggests that some patients purposely have high sodium and fluid intake to correct their own hypovolemia.

Funding: Pharmaceutical Company Support - The BCM was borrowed from Fresenius, Government Support - Non-U.S.

TH-PO780

Blood Volume Monitoring During Hemodialysis Identifies Fluid Overload Not Recognized by Clinical Assessment Wael F. Hussein,[1,2] Rohini Arramreddy,[2] Sheila Doss-McQuitty,[2] Sumi J. Sun,[2] Brigitte Schiller.[1,2] *[1]Div of Nephrology, Dept of Medicine, Stanford Univ School of Medicine, Palo Alto, CA; [2]Satellite Healthcare, San Jose, CA.*

Background: Assessment of dry weight (DW) is critical for fluid management in HD patients, but is a subjective process with several limitations. Blood volume monitoring (BVM) is considered an objective tool that can improve fluid management. We evaluated the performance of BVM for identification of fluid overload in comparison to standard clinical evaluation.

Methods: In this cross-sectional observational study in 5 dialysis centers, BVM using Crit-line was performed once on 213 randomly selected patients during one mid-week HD session. Vascular refill was assessed by stopping ultrafiltration in the last 10 minutes of HD. Patients were considered to have fluid overload at the end of the session when blood volume change (ΔBV) did not fall below -5% during treatment, or if ΔBV increased by more than 1.5% during refill time. Only BVM studies that included a refill test were used in this analysis (n=169). Results of BVM, blinded to staff, were compared to achievement of DW and to fluid status assessment by nurses.

Results: Fluid overload post dialysis was identified in 35 (21%), 65 (40%) and 73 (43%) patients by non-achievement of DW, nurses evaluation, and BVM criteria respectively. There was poor agreement between DW achievement and nurses assessment (kappa 0.28 (95% CI 0.14 to 0.42)). There was no agreement between BVM evaluation and either DW achievement (k 0.10 (-0.03 to +0.23)) or nurses evaluation (k 0.07 (-0.08 to +0.22)). Forty four patients (26%) had volume overload by BVM while having no intradialytic symptoms or interventions, and may be candidates for dry weight reduction.

Conclusions: A substantial proportion of patients have inadequate fluid removal identified by BVM and undetected by clinical assessment in routine outpatient HD. The response to DW reduction in patients identified by BVM to have volume overload remains to be tested. Validated algorithms for BVM in both hemodynamically stable and unstable patients need to be developed to optimize fluid management in HD patients.

Funding: Pharmaceutical Company Support - Satellite Healthcare

TH-PO781

Bioimpedance Fluid Analysis in Prevalent Hypertensive Hemodialysis Patients with Different Blood Pressure Patterns

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Background: Extracellular volume overload is common in hypertensive hemodialysis (HD) patients. We hypothesized that patients with different systolic blood pressure (SBP) patterns during HD would have different distribution of fluids from pre to post-HD.

Methods: As part of a case-control study, we used whole body multifrequency bioimpedance spectroscopy to measure intracellular fluid (ICF), extracellular fluid (ECF), total body water (TBW) and the ECF/TBW ratio before and after HD in prevalent hypertensive hemodialysis patients with SBP increases and decreases from pre to post-HD. We compared the percentage change of these variables between groups using T-tests.

Results: We analyzed data from 9 subjects (90% men, 70% African-American) with increased SBP (+21 ± 15 mmHg) after HD and 10 (80% men, 40% African-Americans) with decreased SBP after HD (-37 ± 29 mmHg). The mean age was 47.6 ± 12 and 51 ± 12 years, respectively. The changes in volume compartments from pre to post-HD are presented in Table 1. There was a significantly greater reduction in the ECF/TBW ratio in subjects in whom SBP decreased after HD compared to those in whom it increased. Contrary, there was a greater reduction in ICF in those in whom SBP increased after HD when compared to their counterparts.

Table 1	**SBP Decrease**	**SBP Increase**	**P-value**
% Decrease ECF	12.6 (4.2)	10.7 (3.9)	0.3
% Decrease ICF	0.2 (5.7)	8.0 (6.0)	0.01
% Decrease TBW	6.1 (1.9)	9.2 (4.1)	0.05
% Decrease ECF/TBW	7.0 (4.4)	1.7 (3.7)	0.01
% Volume Removal	3.5 (1.3)	3.1 (1.6)	0.5

Conclusions: In hypertensive hemodialysis patients, heterogeneous changes in body fluid compartments were identified in relation to different SBP patterns during HD and despite similar percentage of volume removal. Identifying the factors governing fluid redistribution may provide further insight how fluids and blood pressure are related.

Funding: NIDDK Support

TH-PO782

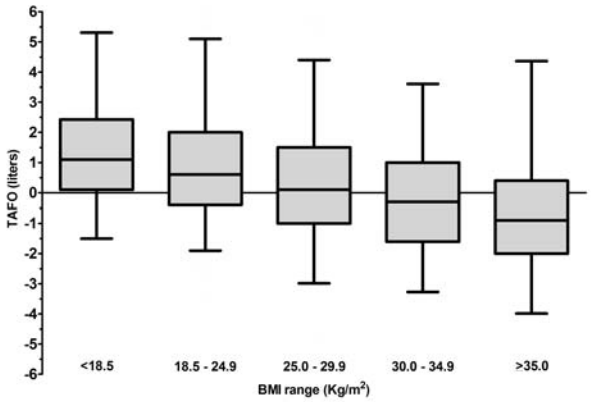
The Potential Bias of Hydration Status Evaluation by Bioimpedance Spectroscopy in Hemodialysis Patients: A Role for the Body Mass Index

Amanda Bedran, Jocemir R. Lugon, Jorge P. Strogoff-de-Matos. *Div of Nephrology, Univ Federal Fluminense, Niteroi, Rio de Janeiro, Brazil.*

Background: Body composition by bioimpedance spectroscopy (BIS) analysis was previously validated by deuterium and bromide dilution methods, but a small number of obese subjects or hemodialysis (HD) patients were included in those studies. We aimed to identify potential bias in the analysis of body composition by BIS in the HD population.

Methods: Prevalent patients on HD of 31 facilities were studied at the time they first underwent BIS (BCM®, Fresenius Medical Care) assessment. Demographic and anthropometric variables were analyzed looking for systematic errors in the evaluation of hydration status. Fluid excess was defined by timed-average fluid overload (TAFO, average pre and post dialysis weight – normohydration weight defined by the BIS device).

Results: A total of 3,358 patients were included in this study (59.7% males, 22.7% diabetics, 54±15years old, 45[3-472] months on HD, body mass index [BMI] 24.9±5.1 Kg/m^2). TAFO (median, interquartile range) was higher in males than females (0.7 [-0.5 to 2.2] and 0.0 [-1.1 to 1.1], respectively; P<0.001). No significant correlation between TAFO and age was found. However, there was an inverse correlation with BMI (r= -0.184, p<0.0001). TAFO significantly varied, according to BMI ranges, from 1.1[0.1 to 2.4] to -0.9 [-2.0 to 0.4] in patients whose BMI were <18.5 and >35 Kg/m^2, respectively.



Despite differences in TAFO, intradialytic changes in blood pressure and the number of hypotensive drugs did not differ between BMI ranges.

Conclusions: The unexpected inverse correlation between BMI and hydration status could be attributed to a bias in the estimation of the normohydration weight by the BIS device. Further studies are desired in order to improve the use of BIS for patients on HD with extreme values of BMI.

TH-PO783

Inaccuracy of Plasma Na Measurement in Hemodialysis (HD) Patients

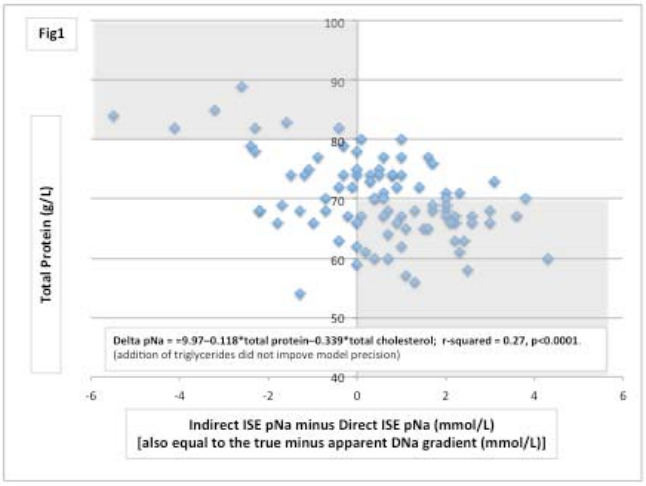
Gabrielle Guilbert- Vandal, Rita Suri. *Univ of Montreal, Montreal, QC, Canada.*

Background: Matching dialysate (D) to plasma (p) Na has been advocated to reduce interdialytic weight gain (IDWG) in HD patients, and requires precise measurement of pNa. Standard indirect ion-selective electrode (ISE) methods normalize serum to pNa by mathematically correcting for plasma protein and lipid fractions. Direct ISE requires no correction. As protein and lipids are often abnormal in HD patients, we compared indirect with direct ISE pNa in this population, and determined if differences could be predicted by a regression equation.

Methods: Pre-dialysis pNa was measured by indirect and direct ISE in all consenting patients at a single HD unit (n=94). All patients had DNa=140mmol/L. Delta pNa = indirect ISE pNa – direct ISE pNa; apparent DNa gradient = DNa – indirect ISE pNa; true DNa gradient = DNa – direct ISE pNa.

Results: Mean age was 64±15yrs, mean vintage was 24 ms, 89% had hypertension, 50% had diabetes, 51% had total protein<70g/L. Delta pNa ranged -5 to 4.3 mmol/L, and was significantly different between the 2 methods (absolute mean delta 1.4±1.1mmol/L, p=0.013); 27% of patients had absolute delta pNa>2mmol/L. IDWG was significantly (albeit weakly) correlated with true (r^2=8%, p=0.0007) but not with apparent DNa gradient (r^2=3%, p=0.19). Whereas the final equation (Fig 1) only weakly predicted delta pNa (r^2=27%, p<0.0001), total protein <70g/L had sensitivity of 80% to detect a true DNa gradient that was at least 2mmol higher than the apparent gradient (NPV=86%). Total protein >80g/L had specificity of 100% to detect a true gradient that was at least 2mmol/L lower than the apparent gradient (PPV=100%).

Conclusions: Indirect ISE pNa may be inaccurate in HD patients with abnormal protein levels. We found total protein of <70 or >80g/L is predictive of a >2 mmol/L error in pNa by indirect ISE. These levels should prompt direct ISE pNa measurement before an individualized dialysate Na is prescribed.



TH-PO784

Assessment of Intradialytic Calcium Balance Based on a Single Pool Variable-Volume Calcium Kinetic Model

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Background: Low dialysate calcium may cause hyperparathyroidism and acute arrhythmias whereas high dialysate calcium may cause soft tissue calcification. We describe the results of a single-pool kinetic model for the quantitative assessment and of intradialytic calcium balance.

Methods: Twenty-one patients on chronic bicarbonate high-flux HD and a total of 97 dialysis sessions performed using a nominal total calcium concentration in the dialysate of 1.5 mM/L were examined. The calcium distribution volume (V_{Ca}) was estimated 1/3 of urea distribution volume; ionized calcium concentration in the dialysate (Cd_{Ca}) and in plasma water (Cp_{Ca}) were measured using direct ionometry; calcium dialysance (D_{Ca}) was estimated from conductivity dialysance; the Donnan factor (α) was rated as equal to 0.938. The accuracy of the model was evaluated by comparing the end-dialysis ionized plasma water calcium concentration predicted by the model, $Cp_{Ca}(t)P$ and the measured values normalized to pH 7.40, $nCp_{Ca}(t)M$. Predicted values were calculated according to the equation: $Cp_{Ca}(t)P=1/\alpha\bullet[Cd_{Ca}-(Cd_{Ca}-\alpha\bullet Cp_{Ca}(0)\bullet(V_{Ca}(t)/V_{Ca}(0))^{DCa\bullet\alpha\bullet(1/QF-1/Qpi)}]$.

Results: Mean $V_{Ca}(0)$ and $V_{Ca}(t)$ were respectively 13.6 ± 2.2 L and 10.9 ± 2.2 L; Cd_{Ca} and $Cp_{Ca}(0)$ were 1.27 ± 0.03 and 1.19 ± 0.06 mM/L; D_{Ca} was 0.156 ± 0.023 L/min; mean plasma water flow (Qpi) was 0.179 ± 0.015 L/min and mean ultrafiltration flow (Qf) was 0.009 ± 0.003 L/min.

	Predicted	Measured	Difference	p value
$nCp_{Ca}(t)$ (mM/L)	1.34 ± 0.04	1.33 ± 0.04	0.00 ± 0.04	NS
Balance (mM/session)	-1.17 ± 1.44	-1.20 ± 1.44	0.03 ± 0.21	NS

Mean calcium balance resulted negative and was not statistically different from the balance calculated using the predicted value. Finally PTH reduction was not statistically significant.

Conclusions: Our calcium kinetic model allows an accurate prediction of calcium exchange during hemodialysis and can be used to select the dialysate calcium concentration necessary to obtain the desired calcium balance.

Funding: Government Support - Non-U.S.

TH-PO785

The Association between Incidence of Cardiovascular Events, Hip Fractures, and Dialysate Calcium Concentration, Stratified by Pre-Dialysis Serum Calcium Concentration in Japanese Hemodialysis Patients

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Background: Previous studies showed that hypercalcemia and higher dialysate Ca concentration were associated with increased mortality. Whether the associations between cardiovascular events, hip fractures and dialysate Ca concentration differ depending on predialysis serum Ca concentration has not been studied.

Methods: This was a longitudinal study based on the Japan Renal Data Registry (JRDR) from 2008 to 2009. Data were divided into three groups by tertiles of predialysis albumin corrected Ca (corrected Ca8.9mg/dl, 9.0-9.5, and 9.6mg/dl). Predictor variable was dialysate Ca concentration (dialysate Ca 2.5 mEq/L versus 3.0 mEq/L). Outcome variables were incidence of myocardial infarction (MI), cerebral hemorrhage (CH), cerebral infarction (CI), amputation, and hip fracture during 1 year observation period. Statistical analyses were performed using multivariable logistic regression model, adjusted for potential confounders.

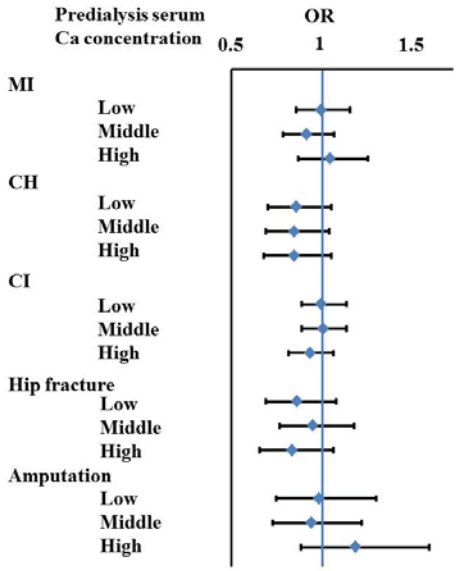

Figure. Odds (95% CI) of incident events for higher dialysate Ca users compared with lower dialysate Ca users

Results: There were data on 263,225 patients on JRDR database. After excluding patients with missing data, data for 76,820 patients were available for analysis.

Conclusions: There were no significant associations between dialysate Ca concentration and cardiovascular events and hip fracture in all tertiles of pre-dialysis serum Ca concentration. Adjusting dialysate Ca concentration depending on serum Ca concentration might not affect clinical outcomes.

TH-PO786

Conversion From Parenteral Paricalcitol to Oral Calcitriol for the Management of Hyperparathyroidism in Hemodialysis

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Background: The management of hyperparathyroidism in hemodialysis (HD) patients involves the administration of phosphate binders, vitamin D analogs and calcimimetics. IV paricalcitol has been preferred over oral calcitriol as it may cause less hypercalcemia and hyperphosphatemia. However, there is little data looking at the efficacy and tolerability of oral calcitriol in the calcimimetic era particularly in a real practice-based experience. Our free-standing Dialysis Center converted from routine IV paricalcitol to oral calcitriol because of pharmacy purchasing preferences. We report the feasibility, efficacy, safety and cost of such a change.

Methods: Of the 139 ESRD HD patients (age 54±16 yrs, 53% female, 57% DM) on dialysis, 83 were receiving IV paricalcitol. All were converted to in-center, oral calcitriol (0.25 mcg =1 mcg paricalcitol) 3 times a week. Additional dose adjustments were made by the nephrologists based on clinical indications. We compared data 5 months pre and post conversion.

Results: At the end of the 5 month observation period 69 of those converted were still on oral agent and 25 new patients had initiated calcitriol such that 94 patients were on oral therapy. Laboratory values 5 months before and after conversion reported as mean±SD and as % within KDOQI guidelines are shown in Table 1. No adverse events were reported with use of oral calcitriol. Estimated vitamin D cost savings were $564 per person/year. Cinacalcet use rose from 47 patients pre- to 57 post-conversion. Sevelamer use dropped from 98 patients pre- to 68 post, while calcium acetate use rose from 57 patients pre- to 73 post conversion. No change in the incidence of cardiovascular events was observed.

Table 1.	Calcium mg/dl	Phosphorous mg/dl	iPTH pg/ml
IV paricalcitol	8.9±1.4	5.2±1.6	423.1±374.2
% within KDOQI guidelines	85	52	58
PO calcitriol	8.9±1.5	5.15±1.76	434.5±508.6
% within KDOQI guidelines	88	51	64

Conclusions: We conclude that in-center distributed pulse oral calcitriol is an effective, safe and economical treatment option for the management of hyperparathyroidism in HD patients.

TH-PO787

Intradialytic Calcium Kinetic Reveals High Calcium Burden with Standard Dialysate Calcium Concentrations in Chronic Hemodialysis Patients

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Background: Currently most hemodialysis (HD) patients are treated with a dialysate calcium concentration (dCa) of 1.25 mmol/l. In this setting, intradialytic calcium mass balance (Ca_{MB}) is expected to be close to neutral. However, this assumption is based on the stability of serum calcium (Ca) levels. We evaluated intradialytic Ca_{MB} in chronic HD patients to account for possible Ca storage in acutely accessible buffers.

Methods: Dialysate-sided Ca_{MB} was measured during 2 HD sessions in chronic HD patients (10 patients using 1,25 mmol/l dCa and 28 patients using 1,75 mmol/l dCa). Change in extracellular fluid Ca (ΔCa_{ECF}) and $\Delta Ca_{ECF}/Ca_{MB}$ (reflecting Ca buffer capacity) were calculated. Measurements were based on ionized Ca concentrations.

Results: Prescribed dCa values significantly differed from measured precapillary dCa (1,09 ± 0,04 and 1,45 ± 0,05 versus 1,25 and 1,75 dCa, respectively), the difference being mostly explained by a linear influence of HCO3 levels on dCa. Consequently, individual pre- to postcapillary HCO3 decline (leading to a diffusion-independent postcapillary dCa increase) was considered for dialysate-sided Ca_{MB} calculations: Ca_{MB} was invariably positive for both 1,25 and 1,75 dCa, with a median of 465 and 721 mg/HD, respectively. At both dCa used, Ca_{MB} showed interindividual differences, which were highest in the first 30min of HD and determined by the dialysate to blood Ca gradient. Ca_{MB} - ΔCa_{ECF} was consistently positive with a median of 425 mg at 1,25 dCa and 539 mg at 1,75 dCa. Acute Ca buffer capacity was 97% and 80% (median at 1,25 and 1,75 dCa). Using 1,75 dCa, Ca_{MB} significantly correlated with ΔCa_{ECF} (r=0,69, p<0,01) and predialysis Ca gradient (r=0,59, p<0,01) but not with Ca buffer capacity. Multivariate regression analysis showed significant association of Ca_{MB} with LDL levels (β= 0,497, p<0,003) and male sex (β= 0,382, p<0,017) and of Ca buffer capacity (at 30 min) with LDL levels (β= -0,544, p<0,000).

Conclusions: Our studies of intradialytic Ca kinetic reveal high Ca burden with standard dCa. Our results also provide strong evidence for the existence of a rapidly accessible Ca storage pool that counteracts acute Ca deviations.

TH-PO788

Two-Pool Model for Phosphorus Removal During Hemodialysis Using an Intercompartmental Clearance That Rises Exponentially as Serum Phosphorus Falls

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Background: Previous studies have suggested that a conventional 2-pool model cannot be used to predict intradialytic and postdialysis phosphorus (P) concentrations.

Methods: A conventional 2-pool urea model was modified by increasing distal compartment volume from 0.67 to 2-3 times the total body water and by using a exponentially increasing intercompartmental P clearance that ranged from 90 to 2000 ml/min as the serum phosphorus level ranged from greater than 6.0 down to below 1.5 mg/dl. Daily absorbed P amount was estimated as a multiple of protein nitrogen appearance rate minus an adjusted phosphorus-binder equivalent dose. After calculating a weekly serum P profile, the absorbed amount was iteratively adjusted until predicted predialysis serum P level matched the measured value. Predicted intradialytic postdialysis, and early (30 min) postdialysis serum P values then were compared to measured values in 450 patients who had such measurements made in the HEMO Study.

Results: The model predicted median measured P levels at various time points well, at both the highest and lowest deciles of predialysis serum P. Prediction accuracy was not affected by alkaline phosphatase or serum PTH levels but was slightly affected by sex and body size. The model predicted higher predialysis serum P levels on Monday versus Wednesday versus Friday. In further, non-HEMO, simulations the model predicted hypophosphatemia with intensive frequent dialysis, and predicted observed changes in

predialysis serum P after applying different schedules of dialysis; however in non-HEMO data, the model underestimated intradialytic P values late in dialysis with session lengths longer than 5 hrs and was unable to model a subset of patients in whom serum P decreased very little during a hemodialysis treatment, including many patients on long nocturnal dialysis.

Conclusions: A modified 2-pool diffusion-based model using an expanded distal compartment and a serum P-dependent intercompartmental P clearance, while far from perfect, can be used to predict serum P level during and shortly after conventional dialysis and might be useful to predict the impact of changes in diet, binder dose, or dialysis schedule on predialysis serum P.

Funding: NIDDK Support

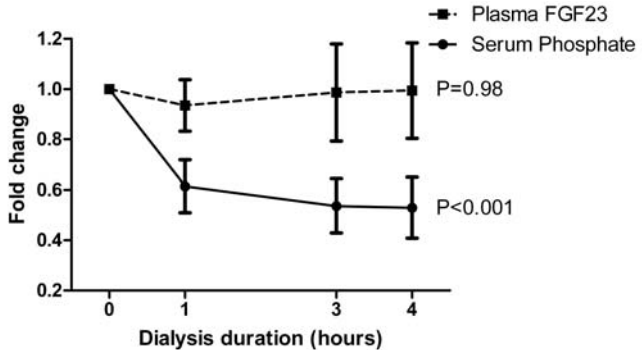
TH-PO789

Discordant Courses of Plasma Fibroblast Growth Factor 23 and Serum Phosphate During Hemodialysis Jelmer K. Humalda,[1] Ineke J. Riphagen,[1] Solmaz Assa,[1] Marc G. Vervloet,[2] Gerjan Navis,[1] Casper F.M. Franssen,[1] Martin H. De Borst.[1] *[1]Nephrology, Univ Medical Center Groningen, Netherlands; [2]Nephrology, VU Medical Center, Amsterdam, for NiGrAm.*

Background: A high fibroblast growth factor 23 (FGF23) level is a risk factor for mortality in hemodialysis (HD) patients. Phosphate binders require days to lower FGF23 levels, however the effect of acute phosphate reduction in HD on FGF23 is unknown. We aimed to assess intradialytic changes in FGF23 in relation to phosphate levels.

Methods: Plasma C-terminal FGF23 levels were determined by ELISA at 0, 1, 3, and 4 hrs after onset of a standardized low-flux HD session in 109 HD patients. We corrected plasma FGF23 for hemoconcentration (Schneditz, ASAIO Journal, 2012). The intradialytic courses of FGF23 and serum phosphate were analyzed with linear mixed models, and correlations between FGF23 and phosphate changes with Pearson's test. In a subgroup (n=10), FGF23 was measured in samples before and after the dialyzer and in the dialysate.

Results: Median age was 66 [IQR 51-75] yrs, dialysis vintage 25.4 [8.5-52.5] mo, Kt/V 4.31±0.71, 65% was male. FGF23 did not change during dialysis (Figure; t=0h: 7627 [3300-13514] RU/mL; t=4h: 7503 [3109-14433] RU/mL; P=0.98). Conversely, serum phosphate decreased from 5.14 [4.06-6.02] mg/dL to 2.60 [2.21-3.10] mg/dL, respectively (P<0.001). Changes in FGF23 and phosphate did not correlate at any timepoint. We could not detect FGF23 in the dialysate (<85 RU/mL), and FGF23 levels were similar before (3472 [2657-9626] RU/mL) and after (3564 [2725-10346] RU/mL) the dialyzer.



Conclusions: The discordant courses of phosphate and FGF23 during HD are in line with insights pointing to additional triggers that up-regulate FGF23 in advanced CKD. The absent effect of HD on FGF23 may partly explain the extremely high FGF23 levels in HD patients. Whether intensified or high-flux HD may lower FGF23 should be addressed in future studies.

Funding: Government Support - Non-U.S.

TH-PO790

Impact on Serum Magnesium Normalization and Blood Pressure By Different Dialysate Magnesium Concentrations in Hemodialysis Patients Li Wang, Daqing Hong. *Renal Dept, Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: Magnesium ion plays an important role in maintenance dialysis patients. Abnormal magnesium ion concentration during hemodialysis is associated with fluctuations in blood pressure, weakness, arrhythmia and other complications. The objective of this study was to compare the effect on serum magnesium normalization and blood pressure by using dialysates of 0.5mmol/L and 1.0mmol/L of magnesium ion concentrations.

Methods: 168 maintenance hemodialysis patients were enrolled and randomized to group A (dialysate with 0.5 mmol/L magnesium concentration) or group B(dialysate with 1.0 mmol/L magnesium concentration). Serum concentration of magnesium and blood pressure was measured before and after dialysis. The rate of magnesium normalization and blood pressure was compared between two groups.

Results: The concentrations of serum magnesium were abnormal in 72 patients, and were normal in 10 patients before hemodialysis in group A. After hemodialysis, they were abnormal in 7 patients and normal in 75 patients. The concentrations of serum magnesium were abnormal in 56 patients, and were normal in 27 patients before hemodialysis in group B. After hemodialysis, they were abnormal in 79 patients and normal in 4 patients. Serum magnesium concentrations were significantly lower in group A compared to group B (0.9 ± 0.2mmol/L *versus* 1.4 ± 0.2mmol/L; *P*<0.05). Serum magnesium normalization rate after dialysis was higher in group A (91.4%) than that in group B (4.8%), (*P*<0.01). There was no significant difference in blood pressure before and after dialysis between two groups. 1 case of hypotension occured during hemodialysis in each group.

Conclusions: Hemodialysis with dialysate of 0.5mmol/L magnesium ion concentration is associated with lower serum magnesium concentrations and higher serum magnesium concentration normalization rate, compared to hemodialysis with dialysate of 1.0 mmol/L magnesium ion concentration. There is no obvious adverse effect between two dialysate groups. These findings require longer follow up and confirmation in further study.

TH-PO791

Microcirculatory Changes Observed on Hemodialysis Using Novel Incident Darkfield Imaging Hui Xue,[1] Marcela Zhou Huang,[1] Anderson Paz,[2] Frank Messie,[3] Can Ince,[4] Ravindra L. Mehta.[1] *[1]Nephrology and Hypertension, Univ of California San Diego, CA; [2]Univ Federal do Amazonas, Brazil; [3]Braedius Medical V; [4]Dept of Intensive Care, Erasmus MC Univ Hospital Rotterdam, Netherlands.*

Background: Rapid fluid removal during hemodialysis (HD) has been shown to be associated with a greater risk of all-cause and cardiovascular death. Blood pressure (BP) and symptoms are used to guide fluid removal to dry weight, but may be insensitive to changes in the microcirculation. We hypothesized that fluid removal during HD would be associated with changes in the microcirculation measured sublingually.

Methods: Patients undergoing chronic HD at an academic medical center were evaluated for changes in their sublingual microcirculation using a novel hand-held image-sensor, CytoCam, based on IDF(incident darkfield imaging). Measurements were performed at the start, mid-point, and at the end of HD. At each time, microscopy videos of capillary (diameter <25µm) flow were recorded from at least three locations in sitting and in Trendelenburg(Trend) position with results averaged. Sublingual microcirculatory function was evaluated as Functional Capillary Density(FCD), the total number of functional capillaries (eg capillaries with flow) per unit field of view. The difference in capillaries FCD at the start, mid, and end of HD was compared in the sitting versus Trend (delta FCD= FCD Trend-FCD Sit). BP, heart rate, and volume removed were recorded at each time point and fluid removal rates were correlated with FCD.

Results: 12 HD sessions in 7 patients were recorded. Mean FCD Sit at start, mid point and end of HD were 1.629, 1.580, and 1.619 mm vessels/mm2 respectively. Delta FCD was greater for start to mid-point (0.06031mm vessels/mm2 (95% CI, 0.0083, 0.1124), than from mid-point to end of HD (-0.05813 mm vessels/mm2 (95% CI, -0.1102, -0.0061; P =<0.01). Fluid removal rates correlated with Delta FCD by Delta FCD=-0.04+6.075*L removed/kg/hr (R2=0.258, p<0.01).

Conclusions: Fluid removal during HD is associated with changes in microcirculation and could be utilized to monitor tissue perfusion during HD. Future studies will develop this technique further to guide fluid management in HD.

Funding: NIDDK Support

TH-PO792

Myocardial Ischemia and Sympathetic Innervation Abnormalities Coincide during Renal Replacement Therapy Akin Ozyilmaz,[1,4] Walter Noordzij,[2] Esther Goet,[1,4] Casper F.M. Franssen,[1] Rene A. Tio,[3] Riemer Hja Slart.[2] *[1]Dept of Nephrology, Univ Medical Center Groningen, Groningen, Netherlands; [2]Dept of Nuclear Medicine and Molecular Imaging, Univ Medical Center Groningen, Groningen, Netherlands; [3]Dept of Cardiology, Univ Medical Center Groningen, Groningen, Netherlands; [4]Dialysis Center Groningen, Groningen, Netherlands.*

Background: Patients with chronic kidney disease (CKD) undergoing hemodialysis (HD) show altered sympathetic tone, increased norepinephrine levels and decreased global myocardial perfusion, which are related to higher cardiovascular mortality. Impaired cardiac sympathetic innervation recovers after renal transplantation. However, the effect of transition from pre-dialysis to HD has not been investigated before.

Methods: Patients with CKD were included in this prospective study between January 2008 and December 2013. All patients underwent technetium-99m labelled tetrofosmin and iodine-123 labelled meta-iodobenzylguanidine (^{123}I-MIBG) scintigraphy prior to (baseline) and six months after the start of HD (follow-up). Early (15 min) and late (4 hrs) heart-to-mediastinum ratio (HMR) and wash-out rate were determined after administration of ^{123}I-MIBG. Results of ^{123}I-MIBG scans were compared to healthy control (HC) subjects.

Results: 17 patients and 9 HC subjects, mean age 58±18 and 52±17 years (ns) respectively, were included. Between baseline and follow-up, two patients developed myocardial ischemia, and two other patients developed myocardial infarction from ischemia at baseline. Late HMR did not differ between baseline and follow-up. There was also no difference between patients and HC. However, wash-out rate was lower in HC than in patients at follow-up (median 1.0 (range -22-12) versus 3.0 (-2-41), p = 0.018). At follow-up, four patients showed sympathetic denervation. Two patients already had denervation at baseline, of which one developed myocardial ischemia, whereas in the other patient ischemia did not change. Of two other patients one developed infarction out of pre-existing ischemia, whereas the last patient did not show perfusion defects.

Conclusions: Shortly after the start of HD cardiac sympathetic denervation seems to occur with coexisting perfusion abnormalities.

TH-PO793

Angiopoietin-2 Associates with Markers of Inflammation, Fluid Overload, and Cardiac Damage in Dialysis Patients and Predicts Cardiovascular Events Welmoet H. Westendorp,[1] Solmaz Assa,[2] Harry Van Goor,[3] Ralf Westerhuis,[2] Casper F.M. Franssen,[2] Henri G.D. Leuvenink.[1] *[1]Surgery, Groningen Transplant Center, Univ Medical Center Groningen, Groningen, Netherlands; [2]Nephrology, Univ Medical Center Groningen, Groningen, Netherlands; [3]Pathology, Univ Medical Center Groningen, Groningen, Netherlands.*

Background: Ang2 plays an important role in vascular stability, permeability and inflammatory balance through binding to Tie2. Its pro-inflammatory role is shown in chronic kidney disease and cardiovascular disease. We investigated the effect of dialysis on Ang2 levels and studied its potential predictive value.

Methods: In 100 HD patients with a mean age (±SD) of 63±16 and median (IQR) dialysis vintage of 1.8 (0.7-4) years, plasma Ang2 levels were measured pre-HD, at 60 and 180 min intra-HD and post-HD (240 min), next to markers of inflammation, endothelial function, and cardiac damage. Associations with log-transformed Ang2 were tested with multivariate analyses. Follow-up for all-cause mortality and cardiovascular events was 2 years.

Results: Ang2 levels increased significantly during HD and peaked at 60 min intra-HD (pre-HD: 3331±2380; 60 min intra-HD: 4362±3635 pg/ml; $p<0.05$) and subsequently decreased to 3447±2557 at 180 min intra-HD and 3060±2291 pg/ml post-HD. Pre-HD Ang2 was significantly associated with pre-HD levels of pentraxin-3, IL-6, pro-endothelin, NT-pro-BNP, and cardiac troponin T ($p<0.05$).Pre-HD Ang-2 levels were associated with a higher incidence of cardiovascular events after correction for age, sex, diabetes, and dialysis vintage (HR 2.1; CI 1.2-3.6; p=0.007).

Conclusions: Ang2 levels associate with markers of inflammation, fluid overload, and cardiac damage and higher levels are associated with worse outcome. Together these findings suggest that Ang2 levels are a marker of endothelial dysfunction that contributes to the dismal prognosis of dialysis patients.

TH-PO794

Does Hemodialysis Create the Potential for a Perfect Storm? A. Harford,[1,2] Philip Zager,[1,2] S. Paine.[2] *[1]UNM, Albuquerque, NM; [2]DCI, Nashville, TN.*

Background: There is increasing awareness of the preventable complication of sudden death occurring during or shortly after hemodialysis (HD). Allan Collins, past Director of the USRDS, has suggested that dialysis may create the "perfect storm".

Methods: To explore the hypothesis that HD may lead to life threatening electrolyte abnormalities we measured serum sodium (Na), potassium (K), magnesium (Mg) and bicarbonate (CO_2) during the last 10 minutes of the procedure or immediately post-dialysis. The blood samples were drawn prior to equilibration since this may be the period of highest risk. The study was done in 340 patients treated at five facilities operated by DCI in Albuquerque, Stony Brook and Syracuse. All values are expressed as mEq/L.

Results: Although the mean values of Na (138.7), K (3.3), Mg (1.5) and CO_2 (25.5) were not unexpected some results were disturbing. The distributions of the postdialysis values are shown.

Distribution of Postdialysis Lab Values							
Percentile	**Na**	**K all**	**K_1**	**K_2**	**K_3**	**Mg**	**CO_2**
1st	131	2.4	2.0	2.6	3.0	1.2	20
5th	133	2.7	2.2	2.8	3.2	1.2	21
10th	134	2.9	2.2	2.9	3.3	1.2	22
25th	136	3.1	2.5	3.1	3.5	1.3	24
50th	139	3.3	2.8	3.2	3.6	1.5	25
75th	141	3.6	3.3	3.5	3.9	1.7	27
90th	142	3.9	3.5	3.8	4.1	1.8	29
95th	144	4.1	3.5	3.9	4.2	1.8	30
99th	145	4.4	3.9	4.3	4.5	1.9	32
K all: All K baths. K_1, K_2, K_3 refer to K baths of 1, 2, 3 mEq/L.							

Median values for each variable, except K (3.3) were within normal range. The 25th percentiles for K (3.1) and Mg (1.3) were significantly below the normal range. The 90th percentile for CO_2 (29) was elevated. The mean predialysis K was 5.1, 5.0, and 4.6 among patients dialyzed on 1.0, 2.0, and 3.0 K dialysate, respectively. The mean postdialysis K was 2.9. 3.3, and 3.7 among patients dialyzed on 1.0, 2.0, and 3.0 K dialysate, respectively. The median postdialysis serum K, stratified by dialysate K, was 2.8, 3.2, and 3.6 among patients dialyzed on 1.0, 2.0, and 3.0 K dialysate, respectively. Among patients dialyzed with ordered CO_2 36-37 and 38-40, 25% and 50%, respectively, had a postdialysis $CO_2 \geq 28$. A combination of postdialysis $K < 3.5$ and $CO_2 > 27$ was present in 43 patients (12.8%).

Conclusions: The use of a dialysate with a low K and high CO_2 has the potential to develop into a "Perfect Storm".

Funding: Clinical Revenue Support

TH-PO795

High Convection Volumes in On-Line Hemodiafiltration - Do We Know What We Prescribe? Angel Argiles,[1,2] Nathalie Gayrard,[1] Flore Duranton,[1] Caroline Guzman,[1] Ilan Szwarc,[2] Philippe Brunet,[3] Alain Ficheux.[1] *[1]RD - Néphrologie and EA7288, Univ Montpellier 1; [2]Néphrologie Dialyse St. Guilhem, Sète; [3]Hôpital de la Conception, Univ Aix-Marseille.*

Background: Although rarely used in U.S.A., on-line hemodiafiltration with high convective volumes could improve survival. However, there is scant information on the feasibility and immediate consequences of prescribing high convection volumes.

Methods: Twelve dialysis patients were sequentially treated with different dialyzers (Xevonta 1.8 and 2.3m²) and convection settings: hemodialysis (HD), maximal global ultrafiltration coefficient of the dialysis setting ($_GK_{D\text{-}UF}$-MAX), 40% UNDER and 40% OVER $_GK_{D\text{-}UF}$-MAX. Dialysis feasibility and efficacy were assessed in terms of TMP, convection volumes, and middle molecule removal.

Results: With 1.8 m² dialyzers, obtained convection volumes were 3.1L in HD (weight loss) and 12.9, 20.6 and 24.5L with UNDER, $_GK_{D\text{-}UF}$-MAX and OVER conditions respectively. Mean TMP significantly increased with prescribed convection (from 79 in HD to 242mmHg in OVER). TMP alarms occurred in 9 and 83% of $_GK_{D\text{-}UF}$-MAX and OVER sessions, leading to infusion reduction and fewer convection volume achievements (94 and only 33% of sessions, respectively). Using a larger dialyzer reduced TMP and TMP alarms and enhanced achievement of prescribed convection volume. Convection increased high mol wt-compound removal, particularly with the larger surface (figure 1).

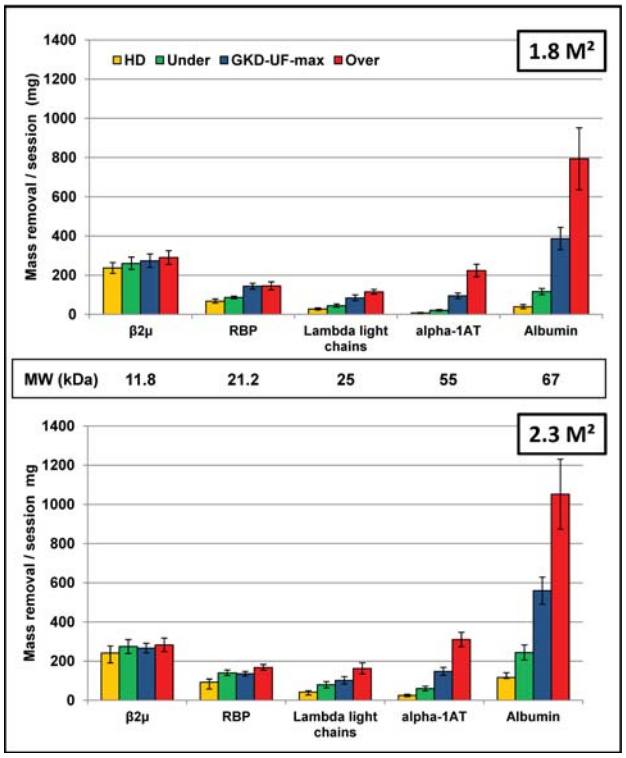


Figure 1: Dialysate removal of 5 middle molecules with different convection settings.

Conclusions: Maximal convection in OL-HDF increases TMP instability, prevents from achieving the prescription and increases albumin leakage. Larger surface improved system stability and reduced alarms but increased albumin loss. Setting the Quf at $_GK_{D\text{-}UF}$-MAX resulted in high convection volumes, with little risk of alarms, deviation from prescribed convection or albumin loss.

Funding: Pharmaceutical Company Support - BBraun Avitum

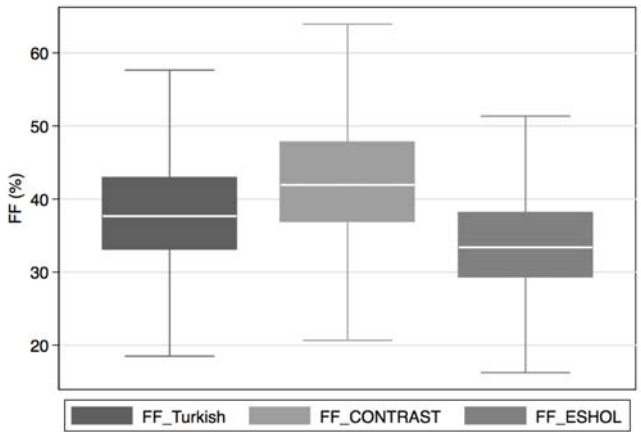
TH-PO796

What Proportion of Patients in the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) Can Practically Achieve High-Volume Hemodiafiltration (hvHDF)? Mark R. Marshall,[1] Kevan Polkinghorne,[2] Stephen P. McDonald.[3] *[1]Faculty of Medicine and Health Sciences, Univ of Auckland, Auckland, AKL, New Zealand; [2]Dept of Nephrology, Monash Medical Centre, Clayton, VIC, Australia; [3]Australia and New Zealand Dialysis and Transplant Registry, The Royal Adelaide Hospital, Adelaide, SA, Australia.*

Background: hvHDF may have mortality benefits compared to conventional thrice-weekly HD. hvHDF is defined by outcomes in various studies - in the Turkish HDF study, it is defined by a filtration volume (FV) ≥20 L/treatment (Rx) in post-dilution mode, in CONTRAST ≥22 L/Rx, and in ESHOL ≥18 L/Rx. To minimize the procoagulant effects of hemoconcentration, it is generally recommended to keep the filtration fraction (FF, the ratio of ultrafiltrate flow (QF) to filter plasma water flow rate (QP), both mL/min) to <30% during post-dilution hvHDF. This may limit achievable FV per Rx, and ability to achieve hvHDF.

Methods: We examined all patients ≥40kg in ANZDATA receiving conventional HD (thrice-weekly, <=6 hours/Rx) as of 31st Dec 2011. We calculated the FF for each patient had they been receiving hvHDF with targets for FV specified by the above studies, under the assumption of routine ultrafiltration of 3% of their dry weight to achieve target. FF(%) = (QF×100)/QP, where QP = QB×(1-hct)×0.93, where QB is blood flow rate and hct hematocrit.

Results: n=10,250. Median (IQR) QB was 300 mL/min (300-330), weight was 74 kg (63-88), duration/Rx was 4.5 hours (4-5), hct was 0.34 (.37-.40). 76% had AVFs, 7% AVGs, and the rest CVLs. FF by study target is shown below



Conclusions: Using the Turkish target, 12% of patients were able to achieve post-dilution hvHDF using their current operating parameters, with CONTRAST 4%, and with ESHOL 28%. Increasing the FF partition value to 40% increases these proportions, but may not be clinically workable. In everyday practice, hvHDF is likely to be unachievable for many patients.

Funding: Government Support - Non-U.S.

TH-PO797

Energetic Expenditure during Hemodiafiltration with and without Exercise

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Background: Dialysis patients are at risk for malnutrition and this is a major risk factor for mortality. Higher resting energy expenditure in dialysis has been associated with adverse outcomes. To our knowledge the caloric expenditure (CE) during hemodiafiltration (HDF) and the impact of exercise in CE has not been described. Our aim was to determine by indirect calorimetry the CE and the carbon/oxygen quotient to determine substrates consumed before dialysis and during HDF with and without exercise.

Methods: Twenty four determinations were performed on 6 individuals on chronic HDF. At their arrival to their HDF unit a baseline calorimetry was performed. Subjects where then prescribed a 3 hour HDF session and 90 minutes into the treatment another calorimetry was performed. This process was repeated in 4 different HDF sessions for each patient, 2 of them without exercise and 2 with aerobic exercise (stationary bicycle). Energetic expenditure (EE) and carbon/oxygen quotient were determined with calorimeter MGH3 (UAM-INCICh).

Results: The mean age of the population was 29±8, 50 % were male and none of the subjects were diabetic. EE during HDF was 1600±301 Kcal before dialysis, 1752±280 Kcal (*P*= 0.02) for HDF without exercise and 1784±360 Kcal (*P*=0.002) for HDF with exercise. No significant difference was found between EE with or without exercise (*P*=0.29). Substrates consumed during the 3 phases are shown in table.

	Carbohydrate(%)	Fat(%)	Protein(%)	*P* value
Before Dialysis	26±16	53±17	21±4	
HDF without exercise	42±7	39±7	19±3	<0.001
HDF with exercise	48±16	34±15	18±3	<0.001

No significant difference was found between substrates consumed during HDF with or without exercise (P=0.45). CE was 214 ± 44 Kcal and 213 ± 39 Kcal (P=0.95) for HDF without and with exercise, respectively.

Conclusions: HDF produces a raise in CE and carbohydrate consumption that does not appear to be modified by exercise. This may be explained by a physiologic stress response in increase CE caused by HDF. Our data reflects that HDF patients have high CE and this may explain in part the risk for protein malnutrition syndrome.

TH-PO798

The Prescribed Dose of Anticoagulation Increases After Assigning Patients to Hemodiafiltration (HDF) Treatment

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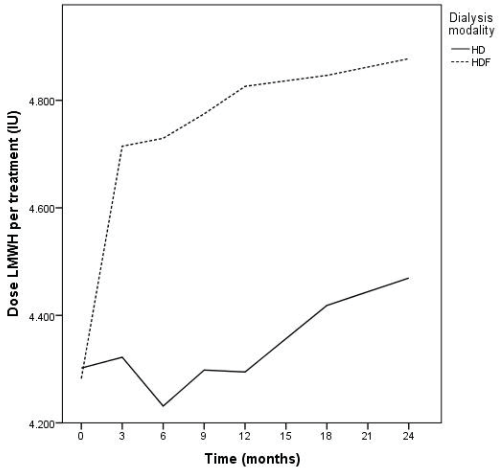
Background: to prevent coagulation in the dialyzer during hemodialysis (HD), anticoagulation - in Europe most often low molecular weight heparin (LMWH) - is administered. Both the intradialyzer hemoconcentration and trans membrane pressure are higher during HDF compared to HD. Therefore, we investigated if the prescribed dose of LMWH differs between HD and HDF patients.

Methods: a cohort study was performed using data from the CONvective TRAnsport STudy (CONTRAST, NCT 00205556), a RCT evaluating the survival effect of HDF compared to HD (mean follow-up 3.04 years). At baseline (M0) and at 3 (M3), 6, 9, 12, 18, 24 months, LMWH type, dose and frequency were registered and standardized to number of anti Xa international units (IU) per treatment. The LMWH dose over time was analyzed with linear mixed models.

Results: patients were assigned to HD (n=356) or HDF (n=358). Baseline characteristics did not differ between groups. Baseline LMWH dose was 4288 IU for patients randomized to HDF and 4279 IU for HD patients. Mean LMWH dose was 4300 IU for HD and 4699 IU for HDF at M3. The slope from M0 to M3 differed significantly between the groups (*p* interaction 0.002). From M3 onwards, LMWH dose increased over time in both groups, but the slope was not different (*p* interaction 0.123).



Conclusions: At M3, the prescribed dose of LMWH was ±10% higher in HDF patients compared to HD. Thereafter, the LMWH dose increased in both groups with a similar slope. Whether these findings are clinically relevant remains to be determined.

TH-PO799

Change in Convection Volume Over Time in Online Post-Dilution Hemodiafiltration Patients. Results From the CONvective TRAnsport STudy (CONTRAST)

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Background: Recent RCTs showed a relation between a high convection volume (CV) and a low mortality risk. It is unclear whether an individuals CV remains stable over time. If so, that would argue in factor of hospital characteristics as the main drivers for the level of received CV, since patient characteristics do tend to change over time. Therefore, this study examines the rate of change of CV in patients undergoing online post-dilution hemodiafiltration (HDF).

Methods: Data from 339 HDF patients from CONTRAST (NCT 00205556) were used for the present analysis. CV was measured every 3 months up to 6 years. The rate of change over time in CV and its components were estimated using linear mixed effects models.

Results: At baseline, mean age was 64±14.9 years and 56% were male. Baseline CV was 19.8±4.6 L/treatment. CV increased significantly over time by 0.34 L/treatment/year (95%CI 0.14-0.54, *p*=0.001). Intradialytic weight change remained stable over time (Δ=0.01L/treatment/year, *p*=0.83), while the amount of substitution volume increased

(Δ=0.33L/treatment/year, 95%CI 0.13-0.53, p=0.001). Rate of change of CV did not differ in subgroups of age, gender, body surface area and medical history. Large differences were observed in change of CV between treatment facilities.

Conclusions: Achieved CV tends to increase over time, although this may not be clinically relevant. As patient characteristics were not related to rate of change and large differences in rate of change exist between dialysis centers, this supports the hypothesis that mainly treatment characteristics determine the volume of convection.

TH-PO800

Modified Ionic Strength Hemodiafiltration, a Novel Dialysis Technique for Increased Protein Bound Toxin Removal Detlef H. Krieter,[1] Thomas Koerner,[1] Eric Devine,[2] Marieke Rueth,[2] Joachim Jankowski,[3] Christoph Wanner,[1] Horst-Dieter Lemke.[2] *[1]Nephrology, Univ Hospital, Würzburg, Germany; [2]EXcorLab GmbH, Obernburg, Germany; [3]Medicine IV, Charité-Universitätsmedizin, Berlin, Germany.*

Background: Protein bound uremic toxins (PBT) are involved in dialysis associated morbidity, but are inadequately removed by hemodialysis (HD). Purpose of the present pilot trial was to demonstrate the clinical feasibility of a modified hemodiafiltration technique, which enhances PBT removal based on modification of the ionic strength in plasma.

Methods: In a prospective, randomized, controlled trial enrolling 8 maintenance dialysis patients (*NCT01923961*), HD was compared with online predilution hemodiafiltration (HDF) and predilution HDF using an infusion fluid loaded with NaCl (HDF_{NaCl}). Blood and dialysate flow rate (250 and 575 mL/min, resp.), treatment time (240 min), and high-flux dialyzer (PUREMA® H, 2.1 m²) were always identical. In HDF, the infusion flow rate was 125 mL/min. In HDF_{NaCl}, the infusate Na^+ was adjusted at 240 mmol/L in blood entering the dialyzer and dialysate Na^+ was set at the given minimum of 130 mmol/L. Removal of free and total para-cresyl sulfate (pCS) and indoxyl sulfate (IS) was determined. Hemocompatibility was assessed by the courses of white blood cells, platelets, hemolysis (free hemoglobin, LDH), complement C5a, and thrombin-antithrombin III (TAT). Na^+ was monitored in blood.

Results: All treatments were well tolerated without adverse events. Compared to HD and HDF, the reduction ratios of free and total PBT were highest in HDF_{NaCl} (free pCS, 66.5±14.4, 72.0±14.6 versus 74.0±13.8 %; total pCS, 43.0±9.4, 42.9±14.3 versus 46.0±12.9 %; free IS, 60.4±16.5, 68.4±8.2 versus 72.6±6.1 %, (P=0.026); total IS, 47.8±10.3, 45.2±7.7 versus 52.0±12.9 %). Between treatments no differences in hemocompatibility were observed. In HDF_{NaCl}, Na^+ slightly increased over time (P<0.001) in arterial (0 and 240 min, 132±2 versus 136±3 mmol/L) and venous blood (132±2 versus 140±3 mmol/L).

Conclusions: HDF_{NaCl} is technically feasible and may enhance the removal of PBT without adverse effects. More effective HDF_{NaCl} will require higher Na^+ in blood. To avoid Na^+ accumulation, reduction of the minimum dialysate Na^+ is indispensable.

Funding: Pharmaceutical Company Support - eXcorLab GmbH

TH-PO801

Differences in Bisphenol A (BPA) Serum Levels in Hemodialysis (HD) Patients with Two Different Membranes Emilio E. Gonzalez-Parra,[1] Vanesa Camarero,[2] Didier Sanchez-Ospina,[1] Enrique Bosch,[1] Sebastian Mas,[1] Maria Vanessa Perez Gomez,[1] Pedro Abaigar,[2] Jesus Egido.[1] *[1]Nephrology, Fundación Jimenez Diaz, Madrid, Spain; [2]Nephrology, Hospital General, Burgos, Spain.*

Background: FDA and EU Health Programme are studying the BPA in medical devices, especially in CKD patients. BPA is an industrial chemical used in dialyzers. BPA is excreted by kidney. Its cytotoxic and mutagenic. The potential healthy implications of blood BPA accumulation in ´HD patients using different dialyzer membranes is not well established. We have studied BPA and its effects in a group of patients using two dialyzers with or wlthout BPA in its composition. Objectives: 1.- To determine whether there are changes in BPA serum levels in HD patients underthe two different dialyzers. 2.- To examine the factors that influence those potential differences.

Methods: Crossover trial we compared Polinephron (PN), without BPA versus polysulphone (PS) with BPA in membrane and housing composition. Patients were dialyzed for 3 months with either dialyzers. For all basal BPA values, 3 and 6 months were done. The BPA was determined by high-sensitive comercial ELISA and HPLC with fluorescence detection. General data (age, sex, months in HD, TA), dialysis KTV, conventional analysis (Ca, P, PTH, vitamin D, Col, Tg, PCR) were collected. Intracelular BPA were messured (lymphocytes), and proinflammatory cytokines (Tweak, IL1, IL6).

Results: 69 HD patients, mean age 65.06±13.2y, with 63.28±92.04 months HD, 40 males. 41 patients baseline trated with PS, basal BPA 0.091 ng/ml versus PN 0.082 ng/ml. 30 normal renal function control BPA was 0.00318 (p.0001). After 3 months PS group incresed 0.023 (p0.0161), and PN decreased 0.016 (p 0.1527). When percentages are compared PS increases 80.35%, PN decreced 53.7% (p0.021). Intracelular BP in 10 patients with both dializers was 4.4 x 10^{-6}, and It did´t change in 3 months with both diailizers. BPA does not correlate with time in dialysis (p 0.1447).

Conclusions: The baseline BPA levels are greater than control group, but are not different in both groups. In PN group BPA decrease in 3 months, and PS group increase being these difference significant. BPA not correlate with months on HD. We are analyzing their clinical implications.

Funding: Pharmaceutical Company Support - Nipro

TH-PO802

Prominent Accumulation in Hemodialysis Patients of Numerous Solutes Cleared by Secretion in the Native Kidney Tammy L. Sirich, Natalie Plummer, Timothy W. Meyer. *Medicine, Stanford & VA Palo Alto, Palo Alto, CA.*

Background: We previously demonstrated that high plasma levels of four organic anions in hemodialysis (HD) patients can be accounted for by the inability of hemodialysis to replicate the native kidney's tubular secretory function (JASN 25:3; 2014). This study employed untargeted high resolution mass spectrometry to test whether other normally secreted solutes accumulate to similar high levels in hemodialysis patients.

Methods: Plasma solute concentrations and clearances in 6 patients maintained on HD and in 6 normal subjects were compared.

Results: A total of 740 solutes were detected in the plasma of HD patients. Of these, 153 solutes were classified as normally secreted based on the finding of a native kidney clearance that was at least twice that of creatinine. For these normally secreted solutes, the ratio of the average pre-treatment plasma level in HD patients to the average plasma level in normal subjects ([HD]/[NL]) was 139±69 and the ratio of the average dialytic clearance to the average normal kidney clearance (K_{HD}/K_{NL}) was 0.48±0.47. For 70 of the normally secreted solutes, the [HD]/[NL] concentration ratio was greater than 100. For these solutes, the average [HD]/[NL] concentration ratio was 254±193 and the average K_{HD}/K_{NL} was only 0.31±0.17. In contrast, the standard dialysis index solute urea had an [HD]/[NL] concentration ratio of 3.6 and a K_{HD}/K_{NL} of 3.9. Mathematical modeling showed that the low K_{HD}/K_{NL} combined with the intermittency of treatment accounted for the high [HD]/[NL] concentration ratios of the normally secreted solutes.

Conclusions: Hemodialysis patients have high plasma levels of a large number of solutes secreted by the native kidney because dialysis fails to replicate the tubular secretory function and the dialytic clearance of these solutes is therefore low relative to the native kidney clearance. Adoption of urea as our index solute for dialysis efficacy has had the unintended effect of concealing how poorly conventional treatment controls the levels of these solutes.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO803

Trimethylamine Oxide (TMAO) Circulates at Very High Levels in Hemodialysis Patients Xin Hai,[1] Veeda O. Landeras,[1] Mirela A. Dobre,[1] Peter B. De Oreo,[1] Timothy W. Meyer,[2] Thomas H. Hostetter.[1] *[1]Medicine, Case Western Reserve Univ, Cleveland, OH; [2]Medicine, Stanford Univ, Palo Alto, CA.*

Background: TMAO has been identified as a cardiovascular risk factor in the general population (Tang et al, NEJM 368:1575, 2013). People with end stage renal disease (ESRD) have a very high incidence of cardiovascular disease.

Methods: We measured TMAO levels and its handling by hemodialysis and the normal kidney in subjects on chronic hemodialysis (ESRD, n= [6- 10]) and in normal controls (NLS, n=6).

Results: The ESRD patients had much higher plasma levels than NLS (118±100 versus 2±1 μM, respectively, mean ± SD, p<0.05). For comparison, predialysis BUN levels in ESRD subjects were 45±11 mg/dl and 15±3 mg/dl in NLS. Thus TMAO levels in ESRD average about 50 fold those in NLS while BUN is 3 fold NLS. However, the fractional reduction of TMAO concentration during dialysis, was in fact greater than that of urea (83±4 versus 77±4 %, TMAO versus urea, p < 0.05) but with smaller volumes of distribution for TMAO compared to urea (23±6 % versus 42±11 % body weight, respectively, p<.05). Also production rates were similar (581±210 versus 533±248 μ moles/day, ESRD versus NLS, p>0.05). In NLS the urinary clearance of TMAO was high (219±78 ml/min) compared to their urinary urea and creatinine clearances (55±14 and 119±21 ml/min, respectively).

Conclusions: Thus, TMAO levels are especially high in ESRD patients due at least in part to 1) its relatively high secretory clearance by the normal kidney and 2) its small volume of distribution, which renders standard intermittent hemodialysis less efficient than for urea.

Funding: NIDDK Support, Private Foundation Support

TH-PO804

An Economic Assessment Model for Remote Rural Satellite Hemodialysis Units Thomas W. Ferguson,[1] James M. Zacharias,[1] David Thomas Collister,[2] Simon Richard Walker,[1] Navdeep Tangri,[1] Claudio Rigatto,[1] Paul Komenda.[1] *[1]Medicine, Univ of Manitoba, Winnipeg, MB, Canada; [2]Medicine, Univ of Alberta, Edmonton, AB, Canada.*

Background: CKD and kidney failure are epidemic in Manitoba, Canada's remote rural communities. In-centre hemodialysis is provided for these settings in satellite hemodialysis units termed Local Centre Dialysis Units (LCDUs). The total costs and key cost drivers of this program have not been fully described. Such information is crucial in informing the design of future programs aimed at delivering dialysis care in remote communities.

Methods: We constructed a cost model based on data from 16 of Manitoba's rural and remote LCDUs. We included all costs determined on an annual, per-patient basis for operation of the unit, transportation to dialysis or for medical care in Winnipeg, treatment expenses, and capital costs. All costs were inflated and exchanged to 2013 U.S. dollars. Information on costs was obtained from a search of the available literature, review of financial statements, and consultation with service providers.

Results: The annual per-patient cost of providing hemodialysis in the LCDUs ranged from $80,450 to $226,940 per patient, per year. The median per patient, per year cost of LCDU care was $105,450 with an interquartile range of $88,400 - $130,270. The primary factors that drove the high cost estimates were the capital costs of the LCDU construction, human resource expenses, and expenses for return to tertiary care centers for health care.

Costs related to transport increased estimates in units that required plane or helicopter transport for both returns to tertiary centers for emergency medical care and during regular dialysis treatment. Smaller and more remote units tended to be more costly to operate.

Conclusions: LCDUs in rural and remote areas of Manitoba are considerably more expensive on a per-patient basis than in-centre hemodialysis provided in larger urban areas. The differences in these costs are driven by the difficult nature of providing care in sparsely populated, remote regions with limited and expensive transportation. These findings support more aggressive measures to improve home dialysis utilization, particularly in rural and remote regions.

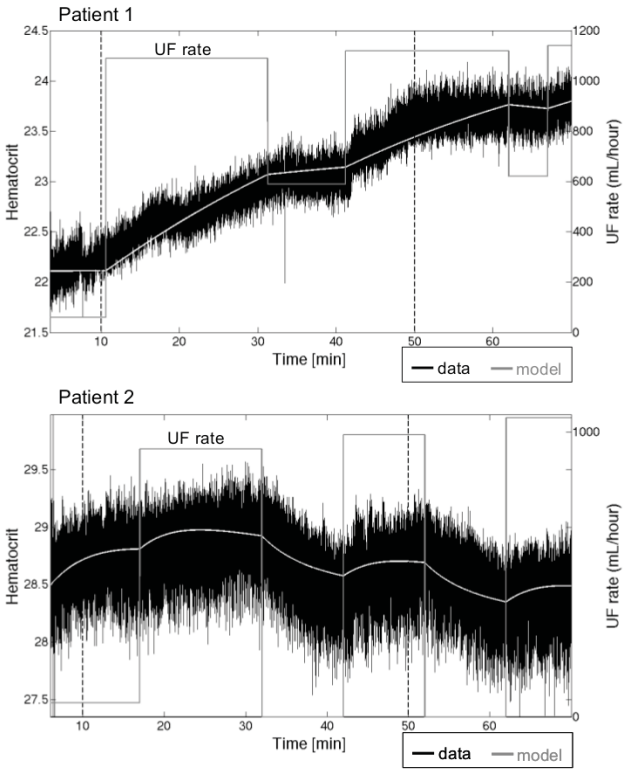
TH-PO805

Mathematical Model Providing New Insights into Vascular Refilling During Dialysis Aurelio A. de los Reyes V,[1] Doris Helene Fuertinger,[2] Franz Kappel,[3] Anna Meyring- Wosten,[2] Stephan Thijssen,[2] Peter Kotanko.[2] *[1]Univ of the Philippines Diliman; [2]Renal Research Inst, NY; [3]Univ of Graz, Austria.*

Background: Intradialytic monitoring of relative blood volume (RBV) can be applied to assess fluid status and aid the prescription of ultrafiltration targets. RBV dynamics are clinically relevant and determined by 2 components: fluid removal from the intravascular compartment by ultrafiltration (UF) and vascular refill from the interstitium. We aimed to characterize these dynamics by development of a 2-compartment mathematical model describing the short-term dynamics of vascular refilling and UF.

Methods: Fluid flow is set as lymphatic and microvascular fluid shifts; protein flux is described by convection and diffusion in the model. Patient specific parameters are identified based on hematocrit (Hct) measurements by the Crit-Line® monitor, including Lp, which is hydraulic conductivity x surface area, the hydrostatic capillary pressure (Pc) and the lymphatic flow (κ).

Results: The estimated parameters are well identifiable using the Hct data between 10 to 50 min. Further, the model shows a good prediction for the following 20 min (Fig.1). Patient 1 shows values within the normal range (Lp=2.89 mL/mmHg/min, Pc=20.42 mmHg), whereas patient 2 has elevated values for Lp and κ (Lp=30.3 mL/mmHg/min, Pc=21.2 mmHg, κ=10.3 mL/min).



Conclusions: This novel mathematical model provides insights into the qualitative and quantitative characteristics of intradialytic vascular refill. Parameters reflecting patients' (patho)physiological characteristics can be identified and the short-term dynamics of vascular refilling predicted. The parameter estimates can be used to assess fluid and inflammatory status. High values of Lp suggest leakiness of capillary wall, which may indicate inflammatory processes, and large κ values indicate a strong lymphatic flow, possibly due to fluid overload.

TH-PO806

A Comparison of Dynamic Blood Pressure Variability between Haemo and Non-Haemodialytic Blood Purification Therapies Scott Wilson, Stephen Harrap. *Univ of Melbourne.*

Background: The harmful effect of significant intradialytic blood-pressure (BP) variability during haemodialysis (HD) is well established. It remains unclear if such volatility is a function of the dialysis or the extracorporeal circulation. To refine our understanding we studied BP variability in patients undergoing peritoneal dialysis (PD) or isovolaemic plasma exchange (PE).

Methods: We measured continuous beat-to-beat systolic BP by the Finometer Midi in 5 PD patients undergoing peritoneal equilibration testing, 5 non-dialysis requiring patients undergoing PE and 46 standard outpatient HD treatments. Variability was assessed across the continuous BP time-series by standard deviation per-treatment and rate-of-change momentum analyses where threshold BP changes were defined by an absolute (+20mmHg) or relative (+15%)over continuously moving 500 heartbeat intervals.

Results: Mean SD (+SEM) of the continuous BP record was 4.2+0.61mmHg, 10.4+0.76mmHg and 15.9+1.18mmHg on a per-treatment basis across PD, PE and HD respectively. No PD patient recorded significant short-term fluctuation in BP in either absolute or relative terms however the mean number (+SEM) of absolute BP swings >20mmHg was 1.6+0.81 and 9.71+0.98 episodes per-treatment for PD and HD respectively. Using a relative definition of BP change saw no events captured during PD but 0.2+0.2, and 7.68+0.9 per PE and HD respectively. All BP fluctuations were asymptomatic. Irrespective of the comparison metric, BP variability was significantly higher in the HD cohort than PD ($p<0.01$). When compared with PE the frequency of absolute or relative threshold changes in BP was significantly greater in the HD group ($p<0.01$) however the difference in SD did not reach significance ($p=0.1$).

Conclusions: This is the first comparative study of continouously monitored haemodynamic stability between blood purification techniques. PD demonstrated a significantly lower degree of BP variability per-treatment than both extracorporeal modalities, with patients undergoing HD being at greatest risk of cardiovascular instability.

TH-PO807

Sodium-Based Potassium Resin and Inter Dialytic Weight Gain (IDWG), Is There a Relevant Link? A Case Report Mahen Al Badawy, B. Coevoet, David Attaf. *CH Saint-Quentint, France; SANTELYS Saint-Quentin, France; Fresenius Medical Care, France.*

Background: Regarding hyperkalemia, studies show equivalent efficacy of both Potassium-Binding Resins (PBR) available : Na^+ and Ca^{++}-based PBR. Nevertheless, Na^+-PBR provide 60 mmol of Na^+ i.e. 1650 mg of NaCl. A DOPPS's study indicates a positive correlation between IDWG and Na^+-PBR. This mechanism should be mediated by salt intake. To illustrate this hypothesis we report a case report of a hemodialysis patient.

Methods: A dialysis patient clinicaly stable, without change in his diet, with a good adherence with his Ca^{++}-PBR to allow a fair control of Kaliema. At D_0 his Ca^{++}-PBR was switched to a Na^+-PBR in order to compare 6 dialysis sessions before and after D_0. 2 consecutive periods of one week were compared: P1 (Ca^{++}-PBR) / P2 (Na^+-PBR). Dry Weight, dialysis prescription, medication and diet were not modified during P1 and P2. To assess impact of Na^+-PBR we estimate (1) dialytic (IMT, dNa^+, eKtV), (2) biologic (Na^+, K^+), and (3) clinical parameters (BLood Pressure (BP), IDWG) at P1 and P2 (unpaired t-test).

Results: Immediately after switching Ca^{++}-PBR to a Na^+-PBR mean dialysate conductivity increase (14,08 (P1)-14,34 mS/cm2 (P2), p=0,05). At the same time mean pre dialysis plasma Na^+increased (134 (P1)-140 mmol/l (P2), p=0,04). Post dialysis plasma Na+ P1 and P2 are equivalent (p=0,7). Mean Ionic Mass Transfer increases from 357 +/- 76 (P1) to 504 +/- 42 mmol/l (P2), p<0,001). Average pre-Dialysis K+ level decreases from 6,82 (P1) to 6,47 mmol/l (P2), as for post-Dialysis K+ level from 4,42 (P1) to 4,18 mmol/l (P2). Average BP (SBP/DBP) evolves from 150 /91 (P1) to 161/101mmHg (P2), p=0,001. IDWG increases significantly from 3,07 +/- 0,4 (P1) to 3,6 +/- 0,3 kg (P2), p<0,03. Prédialysis K+ was superior at the start of the period P1 under Ca^{++}-PBR (difference linked to a higher K^+ intake?). eKt/V was higher during P2.

Conclusions: A Na^+-PBR is associated with an increase of IDWG and BP in this case report. Several case reports show equivalent iatrogenic effect. It should be keep in mind in case of unexplained rise of IDWG. Long-term studies are needed to confirm this effect.

TH-PO808

Activation of the Contact System in Blood Plasma by Hemodialysis Membrane Michael E. Henrie,[1] George M. Rodgers,[2] Nancy J. Brown,[3] Chih-Hu Ho.[1] *[1]Fresenius Medical Care North America, Ogden, UT; [2]Div of Hematology, Univ of Utah Health Science, Salt Lake City, UT; [3]Vanderbilt Univ School of Medicine, Nashville, TN.*

Background: Negatively-charged biomaterial surfaces have been shown to activate the contact system of intrinsic coagulation using *in vitro* models. Reports have shown angiotensin-converting enzyme inhibitors (ACEi) used with negative charged dialysis membranes may lead to bradykinin accumulation within hemodialysis (HD) patients, potentially resulting in anaphylactoid reactions.

Methods: This study compared the effect of different HD membranes on contact system activation. *In vitro* generation of bradykinin (BK) and kallikrein (KLK) was measured from HD membrane exposure to plasma incubated with ACEi. Extracorporeal test circuits were comprised of miniature dialyzers manufactured with various membranes [polysulfone

(PS); polyacrylonitrile (PAN); experimental PS membrane] attached to custom bloodlines. Citrated plasma was used to test for BK generation, while diluted citrated plasma was used to test for KLK generation.

Results: Test results show substantial generation of BK was associated with AN69 membranes (70.1 ± 32.6 ng). All other tested membranes generated BK slightly higher than native levels (527 – 712 pg) measured within the plasma. AN69 membranes were also associated with a substantial generation of KLK (7.5 ± 1.0 units); all other membranes exhibited KLK concentrations closer to native levels (1.3 – 1.7 units). These results were compared with membrane surface electronegativity (zeta potential) to determine if a correlation exists between contact system activation and zeta potential, however a relationship was not found. No significant difference in BK or KLK generation was observed for similar PS membranes using different sterilization methods.

Conclusions: Overall, the results suggest contact system activation is not governed exclusively by specific membrane characteristics. The results demonstrate that dialysis membrane interaction with blood/plasma leading to activation of the contact system is likely multifactorial – a function of membrane materials, membrane surface characteristics, and fluid dynamics.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

TH-PO809

Agreement Between Medicare Claims and CrownWeb Kt/V Data

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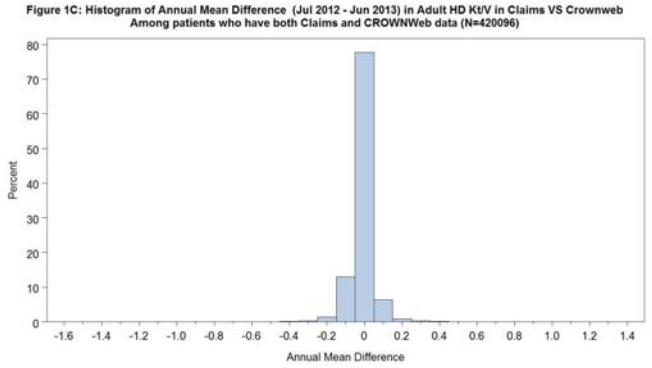
Background: Dialysis facilities in the U.S. are currently evaluated through analyses based on CMS Medicare Claims data. It has been proposed to instead use the CROWNWeb (CW) database for this purpose. Therefore, it is of great interest to evaluate the level of consistency between CROWNWeb and Claims data with respect to laboratory parameters used to construct the measures through which facility performance is assessed. One key component in this regard is Kt/V, a marker of small solute (urea) clearance.

Methods: Our analysis was based on the 251,641 unique hemodialysis patients and 1,995,404 patient months from July 2012 to June 2013 for which Kt/V values were available from both CW and Claims. We computed sample correlations for the paired Kt/V values, as well as 2x2 tables based on Kt/V>1.2 (yes, no). Regression models based on generalized estimating equations were used to determine patient- and facility-level characteristics associated with degree of difference between CW and Claims Kt/V values (linear regression) and agreement (yes, no) with respect to Kt/V>1.2 (logistic regression). Agreement with respect to facility-level percentage of patients with Kt/V>1.2 was also evaluated.

Results: Histograms for Kt/V based on CW and Claims data were virtually indistinguishable. 91.0% of the differences in paired (by patient-month) Kt/V values were less than 0.05.



The correlation between CW and Claims Kt/V values was 0.94. For 98.9% of pairings, CW and Claims data were consistent in terms of Kt/V>1.2 (yes, no). The average correlation between CW and Claims data with respect to facility-level of percentage of patients with Kt/V>1.2 was 0.82.

Conclusions: Overall, there is very strong agreement between CROWNWeb and Medicare Claims Kt/V values, both at the patient- and facility-level. This is reassuring with respect to reliability of CROWNWeb as a data source for evaluation of the Kt/V measure for dialysis facilities in the United States.

Funding: Other U.S. Government Support

TH-PO810

Gamma-Glutamyltransferase Levels and Patient Mortality in Hemodialysis Patients

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Background: Serum gamma-glutamyltransferase (GGT) level has been reported associated with oxidative stress and elevated serum GGT levels are associated with mortality the general population. However, the association between serum GGT levels and mortality in hemodialysis (HD) patients is uncertain. Our aim is to investigate the relationship between serum GGT levels and mortality in HD patients.

Methods: HD patients were selected from Clinical Research Center registry for End Stage Renal Disease cohort in Korea. Patients were categorized into three groups by tertiles of serum GGT levels as follows: Tertile 1, GGT < 14 U/L ; Tertile 2, GGT = 14-25 U/L; Tertile 3, GGT > 25 U/L. Cox regression analysis was used to calculate the adjusted hazard ratio (HR) of mortality with a serum GGT levels of tertile 1 as the reference.

Results: A total of 1,129 HD patients were included. The median follow-up period was 28 months.The multivariate Cox proportional hazard model showed that the highest tertile of serum GGT levels were associated with highermortality with tertile 1 as the reference (HR 1.81, 95% CI, 1.05-3.13, p=0.033) after adjustment for clinical variables, while there was no significant difference in mortality between tertile 1 and tertlie 2 (HR 1.15, 95% CI, 0.62-2.13, p=0.655).

Conclusions: Our data showed that the highest serum GGT levels were significantly associated with increased mortality in HD patients. These findings suggest that serum GGT levels are useful predictor for mortality in HD patients.

TH-PO811

A Reliable Method to Assess the Water Permeability of a Dialysis System: The Global Ultrafiltration Coefficient

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Background: Recent RCTs on hemodiafiltration (HDF) suggest that high convection improves survival in dialysis patients. When convection flow (Q_{UF}) is high, ultrafiltration coefficient required by FDA does not reflect system permeability. The global ultrafiltration coefficient of the dialysis system ($_GK_{D\text{-}UF}$ defined as Q_{UF}/TMP) is better adapted. $_GK_{D\text{-}UF}$ over Q_{UF} follows a parabolic function and its maximum ($_GK_{D\text{-}UF}$-max) is the highest water permeability which occurs at a specific Q_{UF}. The present study analyses in vivo the factors influencing $_GK_{D\text{-}UF}$-max and its Q_{UF}.

Methods: 12 stable dialysis patients were closely monitored as for the $_GK_{D\text{-}UF}$ in HDF setting for 2 months. Blood and dialysate flow was respectively 368±9 and 602±1 mL/min. Weight loss was 2.9±0.1 kg/session. $_GK_{D\text{-}UF}$ was weekly determined at the start of session, and could be repeated during treatment.

Results: $_GK_{D\text{-}UF}$ always followed a parabolic function (least R^2=0.96 of 150 determinations). For every patient, initial $_GK_{D\text{-}UF}$-max and related Q_{UF} were very reproducible (VC, 4.7%±0.5%, max 11%; 3.9±0.3%, max 7.8%). $_GK_{D\text{-}UF}$-max greatly varied across patients (from 31 to 45 mL.h^{-1}.mmHg^{-1}), and associated Q_{UF} changed from 82 to 99 mL/min. Multivariate analysis showed that $_GK_{D\text{-}UF}$-max and Q_{UF} at $_GK_{D\text{-}UF}$ max were influenced by patient, dialyzer and blood flow (all p≤0.01). The $_GK_{D\text{-}UF}$-max and associated Q_{UF}decreased during dialysis treatment. Decrease in $_GK_{D\text{-}UF}$-max was not related to convection, but to patients and associated with weight loss (R^2=0.66;p<0.001).

Conclusions: $_GK_{D\text{-}UF}$-max translates the convection ability of the whole system including the patient and dialyzer. It decreases during dialysis, in association with weight loss, suggesting that hemoconcentration and refilling capacity could be involved. $_GK_{D\text{-}UF}$ is a reliable method to assess the water permeability of a system, more adapted to modern dialysis with high convection than current methods requested by National Authorities. Monitoring $_GK_{D\text{-}UF}$ may help prescribe and deliver high convection volume therapies.

TH-PO812

Safety, Efficacy, and Dose Adjustment of Chemotherapy in Lymphoma Patients Requiring Hemodialysis - A Case Series

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Background: Cancer, including hematologic malignancies, is the third leading cause of death in hemodialysis (HD) patients and is associated with increased mortality in this population. A paucity of data, however, exists in the literature to guide the timing and dose adjustment of chemotherapy in HD patients.

Methods: We present a case series of 9 patients who underwent chemotherapy for lymphoma while on HD between 2007 and 2014.

Results: There were 5 men and 4 women with the following diagnoses: 5 diffuse large B-cell lymphoma, 1 high grade B-cell lymphoma and 3 T-cell lymphoma. Median age was 66 years (range 46-83) and median follow-up was 7 months (range 1-79 months). Of the 9 patients, one patient had 6 cycles of chemotherapy, another had 3 cycles, and the rest received a single cycle each for a total of 16 cycles of chemotherapy. The majority of the cycles (n=15) were CHOP (Cyclophosphamide (CTX), doxorubicin, vincristine, and prednisone), either alone (2), or with rituximab (4) or with etoposide (2). Of the chemotherapy drugs, only CTX was dose reduced due to HD: 20-25% in 6 patients (13 out of 16 chemotherapy cycles), 50% in 1 patient, and no reduction in 2 patients. CTX was given 7-12 hours before HD. Good tolerability with no significant adverse reactions was noted in 8 of the 16 cycles, and events in the other cycles were: febrile neutropenia/ pneumonia (3), blood stream infection (1), tumor lysis syndrome (2), and altered mental status of unknown etiology (2). Six of the patients (representing 13/16 cycles) did well long term and 5 continue to be in remission, while 1 died in remission of unrelated causes 6 years post-chemotherapy. Two patients died within 4 weeks of regimen due to altered mental status of unknown etiology and transitioned to comfort care. Another patient died of infectious complications of subsequent peripheral blood stem cell transplant.

Conclusions: Chemotherapy for lymphoma while undergoing HD with 20-25% dose reduction of CTX was fairly well tolerated in this small group. Timing of HD at 7 to 12 hours after CTX administration allows for therapeutic effect while minimizing toxicity.

TH-PO813

Impact of a Quality Management System (QMS) on Key Performance Indicators (KPIs) in a Belgian Predialysis Clinic and Dialysis Unit

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Background: We evaluated the impact of a QMS (ISO 9001:2008) on KPIs in a predialysis kidney clinic and dialysis unit.

Methods: The approach in the multidisciplinary predialysis clinic (MPC; n=69) was compared with standard care (n=62). Initiatives to improve quality were taken and evaluated in PDCA-cycles (Plan-Do-Check-Act). After 2 years an analysis of the KPIs in our balanced score card was made. A survey of patients and co-workers of our unit was performed in 2011 (before acquisition of an ISO certification) and repeated in 2013, based on standardized and validated questionnaires: CQ-Index (Consumer Quality Index) for patients and a combination of JCQ (Job Content Questionnaire) and COPSOQ (Copenhagen Psychosocial Questionnaire) for co-workers.

Results: Patients in the MPC: - needed significantly less urgent start of dialysis (23.5 % versus 43.5 %, p <0.015) - more frequently had an AV fistula present at the start of dialysis (38.2 % versus 19.4 %, p <0.018) - more frequently were dialysed with an AV-fistula after 1 year (58.8 % versus 23.8%, p=0.002) Mortality trended to be lower after 1 year in the MPC group (30.6 % versus 41.9 %, p = 0.171). We noticed an increase in the proportion of patients with a Kt/V > 1.2 of almost 10% over 2 years. Patient satisfaction (48 % of our patients returned the questionnaire) increased from 87 % to 90 %. Net Promotor Score (NPS) increased from 47 to 51 (+ 4 %). Satisfaction of co-workers (60 % of co-workers returned the questionnaire) decreased from 77 % to 68 % due to 'too much ISO' (too much change, imperfect communication and increased work load). Job typology showed that 46 % experienced their work as a 'high risk job' and 21 % as a 'high strain job'. Systematic appraisal of co-workers resulted in a decrease of absence through illness with 37 %.

Conclusions: Implementation of a QMS (ISO 9001:2008) in a predialysis kidney clinic and dialysis unit resulted in improvement of several KPIs (less urgent start of dialysis, presence of AV fistula, hemodialysis.

TH-PO814

Large Variation among U.S. Facilities in Red Blood Cell Transfusion Frequency, 2010-2012

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Background: The % of U.S. Medicare-insured dialysis patients (pts) receiving a red blood cell transfusion (RBCT) rose in 2011-2012, coincident with the start of the CMS Prospective Payment System (PPS). Pt characteristics and facility (FAC) practice variation likely affect RBCT rates. We describe U.S. dialysis FAC variation in RBCTs from 2010 to 2012.

Methods: Pts w/8+ CMS Medicare outpatient claims for hemodialysis (HD) in a month during 2010-2012 were assigned each month to the FAC with the most HD claims. Analyses were restricted to FACs with 20+ pts (FAC 20+) or 60+ pts (FAC 60+). RBCT claims were identified by revenue center and other claim codes. The % of pts in each FAC receiving 1+ RBCT in each 1-, 3-, or 6-mo period (FAC %RBCT) was estimated overall and within strata of FAC geographic region, for-profit status, affiliated chain size, and rural-urban commuting area score.

Results: Median: From Jan 2010 to Dec 2012, 1-mo FAC %RBCT rose from 2.4% to 2.7% (FAC 60+) and from 2.4 to 2.9% (FAC 20+); 6-mo FAC %RBCT rose from 13.0% to 13.8% (FAC 60+) and from 13.0 to 14.3% (FAC 20+). Range: In Dec 2012, 5th-95th %iles for 1-mo FAC %RBCT were 0-9% (FAC 60+) and 0-7% (FAC 20+); 5th-95th %iles for 6-mo FAC %RBCT were 6-24% (FAC 60+) and 5-28% (FAC 20+). FAC comparisons: No clear differences were seen by rurality or for-profit status. Median FAC %RBCT was lower in large-chain versus small-chain/independent free-standing FACs (2.7% versus 3.4% in Dec 2012, p<0.001). Median FAC %RBCT was slightly lower in West/East versus Central/South regions (2.7% versus 2.9%, p=0.04).

Conclusions: The effect of the 2011 payment and regulatory changes on RBCT was small compared to overall variation in FAC %RBCT (>4-fold for 6-mo %RBCT; some FACs >20%). While effect of case-mix needs to be considered, efforts to limit RBCT should focus on FAC performance and specific RBCT-related FAC practices.

Funding: Pharmaceutical Company Support - The DOPPS program is supported by grants to Arbor Research from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, Ltd. Additional support for specific projects is provided in Canada by Amgen, BHC Medical, Janssen, Takeda, Kidney Foundation of Canada (for logistics support); in Germany by Hexal, DGfN, Shire, WiNe Institute; for PDOPPS in Japan by the Japanese Society for Peritoneal Dialysis; for PDOPPS by Fresenius Medical Care

TH-PO815

Transfusion Events in Hemodialysis Patients by Hemoglobin Level, ESA Users versus Non-ESA Users

Allan J. Collins, Thomas Matlon, Yi Peng, David T. Gilbertson. *Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN.*

Background: Assessing Hb levels and transfusion use had been possible only for ESA-treated dialysis patients due to Medicare reporting requirements. In 2012, CMS required Hb levels to be reported for all dialysis patients. Hb levels from the prior month are reported on the current month's claim. We assessed differences in transfusion use by prior Hb level among ESA users versus non-ESA users.

Methods: Monthly cohorts, April-December 2012 (first complete reporting), were created from Medicare ESRD standard analysis files. Patients receiving hemodialysis (HD) as of the first day of the month were included and followed from that day until death, kidney transplant, loss to follow-up, or the last day of the month. ESA use and transfusion events were evaluated during the follow-up period.

Results: In April 2012, for HD patients with Hb ≥ 13 g/dL, 2.84% of ESA users and 0.45% of non-ESA users received transfusions. For patients with Hb at 12 g/dL, 1.45% of ESA users and 0.85% of non-ESA users received transfusions. Transfusion use was similar for ESA users and non-ESA users when Hb was 10 to < 12 g/dL. Transfusion use was 5.41% and 16.03% for ESA users with Hb at 9-< 10 g/dL and < 9g/dL; corresponding percentages were 16.10% and 27.60% for non-ESA users. Similar patterns in monthly transfusion use were observed for the May-December cohorts (data for July-Dec not shown).

Hb levels	ESA use	Apr	May	Jun
<9 g/dL	Non-ESA users	27.60	27.02	32.98
	ESA users	16.03	16.90	16.89
9-<10 g/dL	Non-ESA users	16.10	15.34	11.22
	ESA users	5.41	5.49	5.30
10-<11 g/dL	Non-ESA users	2.93	2.88	3.10
	ESA users	2.26	2.32	2.15
11-<12 g/dL	Non-ESA users	1.22	1.01	0.98
	ESA users	1.58	1.60	1.45
12-<13 g/dL	Non-ESA users	0.85	0.85	0.47
	ESA users	1.45	1.82	1.71
13+ g/dL	Non-ESA users	0.45	0.42	0.36
	ESA users	2.84	2.50	2.62

Conclusions: For patients with low Hb levels (< 10g/dL), transfusion risk is higher among non-ESA users than among ESA users in the next month. Longer-term assessment is needed to determine the sustained risk of transfusions when Hb falls below 10g/dL.

Funding: Pharmaceutical Company Support - Amgen

TH-PO816

Standardized Transfusion Ratios and Standardized Hospitalization Ratios Are Positively Correlated among Dialysis Facilities

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Background: As Medicare beneficiaries undergoing maintenance dialysis are hospitalized 1.8 times per person-year and experience most blood transfusions in inpatient settings, dialysis facility standardized transfusion ratios may reflect the incidence of hospitalization more than the adequacy of anemia management. We aimed to estimate correlation of standardized transfusion ratios with facility-level metrics regarding hospitalization and anemia management.

Methods: We analyzed data from Dialysis Facility Compare. For each included provider, we extracted the standardized transfusion ratio (STrR), the standardized hospitalization ratio (SHR), and the percentage of patients with hemoglobin less than 10 g/dL (% patients with low Hb), each measured from January 1, 2012, to December 31, 2012. We retained providers with recorded data for all of these metrics. We fit linear regression models of STrR as a function of SHR and % patients with low Hb.

Results: We identified 5172 providers. Median STrR was 0.92, with 10th and 90th percentiles 0.46 and 1.68; median SHR was 0.96, with 10th and 90th percentiles 0.65 and 1.35; and median % patients with low Hb was 8%, with 10th and 90th percentiles 2% and 21%. In univariate models, the proportion of explained variation (R^2) in STrR was 18.9% for SHR and 5.4% for % patients with low Hb. In a multivariate model of STrR with SHR and % patients with low Hb, R^2 was 22.9%; each standard deviation increment in SHR and % patients with low Hb associated with increments in STrR equal to 0.22 and 0.09, respectively ($P < 0.01$ for each).

Conclusions: Standardized transfusion ratios are more strongly correlated with standardized hospitalization ratios than with facility-level percentages of patients with low hemoglobin. Common risk factors for blood transfusion and hospitalization may be currently omitted from risk adjustment schema. Alternatively, hospitalists may tend to transfuse dialysis patients at relatively high hemoglobin concentrations. The role of hospitals in the decision to transfuse blood merits further scrutiny.

TH-PO817

Dialysis Facility-Level Transfusion Rates Can Be Unreliable due to Variability in Hospital-Level Billing Patterns for Blood Eric D. Weinhandl,[1] David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]School of Medicine, Univ of Minnesota, Minneapolis, MN.*

Background: Methods to estimate standardized transfusion ratios from Medicare claims depend partially on accurate identification of transfusion incidence during hospitalizations. We aimed to describe variability of billing patterns for blood among hospitalized dialysis patients.

Methods: For Medicare beneficiaries undergoing dialysis in 2011 or 2012, we searched Medicare claims for hospital admissions with any billing for blood (ICD-9-CM procedure codes 99.0x, revenue center codes 038x-039x, and value code 37). We classified stays into 4 mutually exclusive categories: whole blood (WB)/red blood cell (RBC) transfusion, non-WB/RBC transfusion, transfusion administration (without explicit coding for components transfused), and blood processing alone (without explicit coding for transfusion administration). We examined variability of the distribution of categories across states and hospitals.

Results: We identified 309,650 admissions with billing for blood (ABB) between Jan 1, 2011, and Dec 31, 2012; 63% were for WB/RBC transfusion, 4% for non-WB/RBC transfusion, 12% for transfusion administration, and 21% for blood processing alone. In states (n = 34) with >1000 ABB, percentages of ABB with WB/RBC transfusion ranged from 47% to 79%; percentages with blood processing alone ranged from 7% to 36%. In hospitals (n = 1041) with >100 ABB, the 10th and 90th percentiles of the percentages of ABB with WB/RBC transfusion were 2% and 88%, respectively; corresponding percentiles with blood processing alone ranged from 0% to 63%.

Conclusions: The incidence of WB/RBC transfusion among hospitalized dialysis patients is uncertain, primarily because of frequent coding of blood processing without evidence of transfusion. Between-hospital variability in billing patterns for blood was large. Thus, for methods relying on specific definitions of WB/RBC transfusion, dialysis facility-level transfusion rates are likely to be highly influenced by billing patterns of nearby hospitals. Multicenter validation studies of hospital billing for blood are needed.

TH-PO818

Comparison Between CMS CROWNWeb and Medicare Claims Data with Respect to Hemoglobin Douglas E. Schaubel, Lan Tong, Valarie B. Ashby, Debabrata Ray, Rajiv Saran. *Univ of Michigan.*

Background: CROWNWeb (CW) is intended to replace Medicare Claims as the primary CMS data source for evaluating dialysis facilities' performance. Thus, it is of interest to quantify the consistency between the two sources.

Methods: The study population included adult (age ≥18) end-stage renal disease patients treated during 7/2012 to 6/2013 who, according to both sources (CW and Claims), were on dialysis ≥ 90 days, were prescribed erythropoietin, and had a valid hemoglobin (HB) value (5≤HB≤20). A total of 270,561 patients, 2,786,075 patient-months in Claims, 3,199,149 patient-months in CW, and 5,216 dialysis facilities were included. The analyses consisted of patient-, center-, and population-level comparisons between CW and Claims HB records. Means, standard deviations and histograms were compared at the population-level. Center- and patient-level analyses consisted of 2 x 2 tables, correlations, and linear and logistic regression modeling.

Results: At the population level, HB distributions were very similar. A subset of the patient- and center-level findings are summarized in the Table:

Quantity	Restrictions	Level	N	Value
Mean annual HB	None	Patient	289,961	Correlation=0.77
Mean annual HB	CW: HB≤12	Patient	284,303	Correlation=0.77
Mean annual HB	CW: HB>12	Patient	5,658	Correlation=0.13
Mean annual HB>12 (yes, no)	None	Patient	289,961	Agreement=97.9%
HB>12 (yes, no)	Jan 2013 only	Patient	179,824	Agreement=90.7%
% patients with annual mean HB>12	None	Center	5,216	Correlation=0.44
Mean annual HB	None	Center	5,216	Correlation=0.79

Linear regression modeling of the patient-level difference in HB values (CW minus Claims) revealed age, sex, race, diabetes, region, facility size, large dialysis organization, and free-standing facilities as significant predictors. Logistic models of patient-level agreement (HB>12: yes, no) indicated generally similar patterns except that ethnicity was also significant.

Conclusions: Agreement between CW and Claims HB data ranged from moderate to very good depending on the degree of averaging. Future work should investigate why (i) the correlation is lower for higher HB values and (ii) the level of disagreement depends on patient- and center-level characteristics.

Funding: Other U.S. Government Support

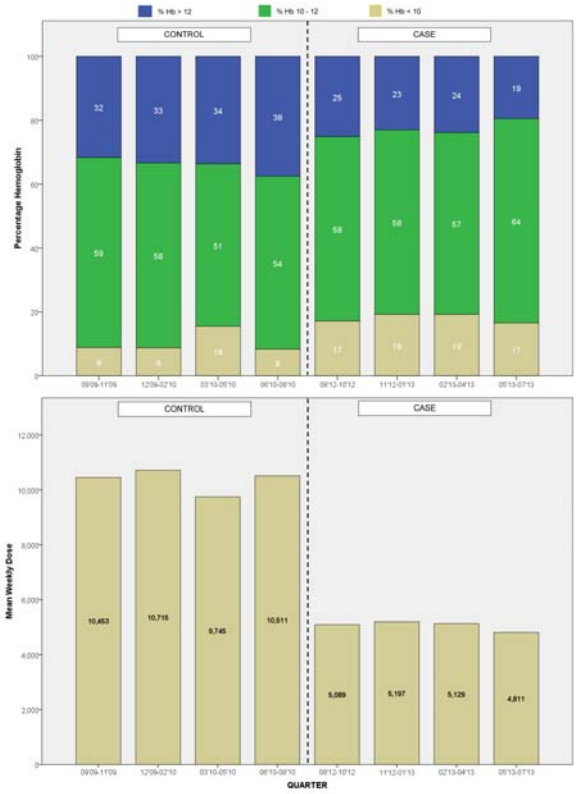
TH-PO819

Individualized Anemia Management: Pragmatic Study Adam E. Gaweda,[1] Michael E. Brier,[1] Alfred A. Jacobs,[1] George R. Aronoff.[1,2] *[1]Dept of Medicine, Univ of Louisville, Louisville, KY; [2]Renal Ventures Management, LLC, Denver, CO.*

Background: Current product label for Erythropoiesis Stimulating Agents (ESA) stipulates individualization of anemia treatment in End Stage Renal Disease (ESRD). We developed a computer-assisted approach to individualized ESA dosing and recently demonstrated its efficacy through a randomized controlled clinical trial. We now present the results of a pragmatic study showing the utility of this approach in a daily clinical practice.

Methods: We began implementing our approach at University Kidney Center (UKC), Louisville, KY in 09/10 and introduced it as a standard of care in 04/12. We performed a case-control study of all ESRD patients undergoing uninterrupted treatment at our facility between 09/10 and 09/13. 80 patients satisfied this criterion. We compared Hemoglobin (Hb) percentages below, within, and above the target range 10-12 g/dL, as well as ESA utilization over 12-month periods: 09/09-09/10 (control) and 08/12-08/13 (case). We excluded the washout period 04/12-08/12 from analysis. At all times, anemia managers were allowed to modify the recommended dose based on their judgment.

Results: Individualized ESA dosing resulted in a modest but consistent improvement of Hb distribution within and above the target range and a two-fold decrease in ESA utilization, when compared to dosing driven by a standard population-oriented protocol.



Outcome	Control	Case	P-value
%Hb<10	10.4	18.0	0.04
%Hb 10-12	55.6	59.1	0.04
%Hb>12	34.0	22.9	0.04
Mean Hb (± Std)	11.6 ± 1.4	11.1 ± 1.6	<0.001
Median ESA dose per week (min, max)	4,500 (0 ... 97,500)	2,000 (0 ... 63,400)	<0.001
Mean ESA dose per week (± Std)	10,356 ± 15,233	5,083 ± 7,904	<0.001

Conclusions: Individualized ESA dosing such as the one implemented at our facility improves cost-effectiveness of anemia management in daily clinical practice.

Funding: NIDDK Support

TH-PO820

Study of an ESA Treatment Algorithm for Treatment of Renal Anemia – Stable Hb Value Obtained with CERA Administered Once Every 2 Weeks

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Background: When treating renal anemia in hemodialysis patients, it is necessary to maintain a stable Hb value and to administer a correct ESA dose. In Japan, twice monthly measurement of the Hb value by blood sampling is recommended, and the ESA dose is decided based on the results of this measurement. We have created an algorithm for the use of ESA (CERA: Continuous Erythropoietin Receptor Activator) with hemodialysis patients.

Methods: We administered CERA based on the algorithm to 102 hemodialysis patients who were being treated with rHuEPO and observed them for 1 year. When we reviewed the algorithm after 1 year, we found cases in which the Hb value fluctuated as a result of suspending and restarting ESA treatment. We therefore revised the algorithm and observed the patients for an additional year. The target Hb value was 10.5 – 11.0 g/dl, and we observed the Hb value, CERA dosage, ERI (Erythropoietin Resistance Index), and iron dosage. CERA was administered once every 2 weeks, and the dosage was increased or decreased within a range of 25 mg per dose according to the measured Hb values. The standard for iron dosage was not changed during the evaluation period.

Results: The Hb values at the start of evaluation, after 1 year, and after 2 years were respectively 10.9 g/dl, 11.1 g/dl, and 11.2 g/dl. The corresponding CERA dosages were 62±16 mg/2w, 37.5±25.4 mg/2w, and 35.9±22.7 mg/2w. The corresponding ERI were 0.11±0.04, 0.06±0.05, and 0.06±0.04. Compared with the evaluation start, there were significant decreases in both CERA dosage and ERI after 1 year and after 2 years. Hb fluctuation was reduced by minimizing the suspension of CERA treatment to the extent possible. There was no change in the serum level of ferritin during the evaluation period, and the iron dosage decreased significantly from 13.0±1.5 mg/w/patient before the start of the evaluation to 10.9±2.6 mg/w/patient.

Conclusions: CERA administered once every 2 weeks based on an ESA treatment algorithm can maintain a stable Hb value and improve ERI, and can also reduce the dosage of iron.

TH-PO821

Clinical Assessment of Pleiotropic Effects of Erythroid-Stimulating Agents in End-Stage Renal Disease

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Background: Several lines of evidence have shown that circulating endothelial progenitor cells (EPCs) play a pivotal role in vasculogenesis and that they promote angiogenesis by secreting growth factors. Recent studies have suggested that erythropoietin (EPO) may accelerate not only angiogenesis but also vasculogenesis, beyond erythropoiesis. In this study we investigated whether two erythroid-stimulating agents (ESAs) modulate vascular-related factors, including the mobilization of EPCs, in patients with end-stage renal disease (ESRD).

Methods: We conducted a 12-week prospective study on 55 patients with ESRD. We treated 21 patients with recombinant human EPO (rhEPO) (EPO group, 4565.5 ± 435.2 IU/week) and 19 patients with darbepoetin (DA) (DA group, 39.2 ± 14.2 μg/week). The other 15 patients did not receive ESA therapy (no-ESA group). Vascular mediators such as EPCs, vascular endothelial growth factor, matrix metalloproteinase-2 (MMP-2), high-sensitivity C-reactive protein, and asymmetric dimethyl arginine were measured at 0 and 12 weeks. EPCs were measured by flow cytometry as $CD45^{low}CD34+CD133+$ cells.

Results: The change in EPC count from 0 to 12 weeks increased significantly in a dose-dependent manner in the EPO group ($p<0.01$). In the DA group, the change in EPC count was not dose-related, but the change in serum MMP-2 concentration from 0 to 12 weeks decreased significantly in a dose-dependent manner ($p<0.01$). Meta regression analysis showed the same results as above.

Conclusions: EPC mobilization and modulation of circulating MMPs in ESRD patients differ depending on the ESA used in treatment.

Funding: Clinical Revenue Support

TH-PO822

Longer-Term Outcomes of Darbepoetin Alfa *versus* Epoetin Alfa in Patients with End-Stage Renal Disease Initiating Hemodialysis: A Quasi-Experimental Study

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Background: Epoetin alfa (EPO) and darbepoetin alfa (DPO) are erythropoiesis-stimulating agents (ESA) used for anemia treatment in patients undergoing dialysis. We compared rates of mortality and cardiovascular events in patients treated with EPO *versus* DPO in incident hemodialysis patients akin to a cluster-randomized trial by exploiting the natural experiment that occurs when facilities make a formulary decision to provide one or the other drug to all their patients.

Methods: From the USRDS, we identified all facilities that switched from EPO to DPO (2003-2010). We matched each DPO facility with one EPO facility on geographic location, profit status, and facility type (free-standing *versus* hospital-based). Patients subsequently initiating hemodialysis were assigned their facility-level ESA exposure regardless of their actual treatment received. Patients were followed for mortality and cardiovascular mortality. Among patients covered by fee-for-service Medicare, we evaluated the composite of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke.

Results: Of 508 dialysis facilities that switched to DPO, 492 were matched with a similar EPO facility; 19,932 (EPO: 10,467; DPO: 9465) incident hemodialysis patients were followed for 21,918 person-years during which 5550 deaths occurred. Most baseline characteristics were balanced; however, fewer patients in DPO facilities were Hispanic or Asian, and serum albumin was slightly lower. The demographics-adjusted mortality hazard ratio (HR) for DPO (*versus* EPO) was 1.06 (95% confidence interval [CI]: 0.99-1.13) and remained materially unchanged after adjustment for other baseline characteristics. Among 9455 patients with fee-for-service Medicare, there was no significant difference in the demographics-adjusted composite cardiovascular endpoint (HR=1.09; 95%CI: 0.96-1.24) or any individual event type.

Conclusions: In incident hemodialysis patients, rates of mortality and cardiovascular events did not differ among patients treated at facilities predominantly using DPO *versus* EPO.

Funding: NIDDK Support

TH-PO823

Use of the Darbopoietin Resistance Index to Optimize Anemia Management

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Background: Guidelines that minimize ESA usage in ESRD reduce financial burden and possible dose-related adverse effects. Instead of absolute dose or dose/kg body weight, adjustment for hemoglobin has been suggested as the Erythropoietin or Darbopoietin Resistance Index (ERI, DRI): dose/kg wt/g/dl hgb. Unlike ERI, DRI data are lacking so we studied it in all our pts and in a hematologically healthy subset.

Methods: We retrospectively reviewed 94 clinically stable chronic HD subjects for darbopoietin (D) dose, weight, DRI, chemistry, hematology and iron studies, URR, iPTH, catheter usage, medications and diseases with hematologic effects, and primary heme disorders. Analyses were repeated for the subset of pts deemed hematologically healthy. Based on those "normal" ESA dosing ranges, we identified pts from the entire group in whom high or low doses/kg were out of synch with their DRI values.

Results: For all 94 pts: 54% male, age 52.5 ± 1.6 yrs (mean ± SEM), hgb 10.0 ± 0.1 g/dl, Fe sat 29.1 ± 1.25%, ferritin 553 ± 100 ng/ml, URR 73.5 ± 0.8%, iPTH 386 ± 35 pg/ml, and 46 had heme-active factors. The D dose was 46.9 ± 6.3 mcg/wk (range 6.3-200), 0.61 ± 0.06 mcg/kg (0.06-2.55), DRI 0.06 ± 0.01 (0.01-0.27). For the 48 heme-healthy pts: 44% male, 57.1 ± 1.9 yrs, hgb 10.4 ± 0.16 g/dl, Fe sat 29.6 ± 1.6%, ferritin 434 ± 52 ng/ml, URR 74.3 ± 1.0%, iPTH 354 ± 45 pg/ml. The D dose was 38.5 ± 3.5 mcg/wk (6.3-120), 0.50 ± 0.05 mcg/kg (0.06-1.60), DRI 0.05 ± 0.01 (0.01-0.19). Using target D/kg of 0.45-0.55 and DRI of 0.04-0.06, the two indices were out of synch in 16/94 pts (17%) despite hgb at goal. With DRI within range, 9 had low D/kg, 6 high D/kg. With D/kg within range, 1 had high DRI.

Conclusions: ESA management protocols based on D doses adjusted for both body weight and hemoglobin are promising for improved anemia control and provide better guidelines than when using weight alone. We suggest that a high DRI is particularly useful for identifying pts reaching hgb targets but who nevertheless may have remediable causes of ESA hyporesponsiveness (e.g. functional iron deficiency). Larger studies are needed with the goal that pts with elevated DRI levels undergo protocolized evaluation for entities that cause ESA resistance.

TH-PO824

The Use of Epogen via Intravenous and Subcutaneous Routes

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Background: In the new era of payment bundling, dialysis centers are incentivized to be more efficient in delivery of health services including ESA administration. We investigated the use of intravenous(IV) Epogen versus subcutaneous(SQ) Epogen.

Methods: Medical records of all hemodialysis(HD) patients that received Epogen during the study periods were reviewed. Study period for IV Epogen and SQ Epogen was May to Jul 2010 and May to Jul 2011, respectively. Patients who received darbepoietin within 6 months; were hospitalized for > 1 day; or were not registered for HD for at least 3 months prior to and during both study periods were excluded. Patients who continued to receive IV Epogen beyond Mar 2011 were also excluded. Paired t-test was used for analysis.

Results: Mean age of patients was 67±14 years that included 23 females and 33 males with estimated dry weight of 71±22Kg. During the 3-month study period, mean IV Epogen administered was significantly higher compared to mean SQ Epogen (106677±113331 IU versus 85228±83381 IU;p=0.024). Mean hemoglobin and TSAT were similar during the two study periods. However, mean ferritin was significantly increased during SQ Epogen study period compared to IV Epogen study period (457±243 versus 650±321, $p<0.0001$) in spite of increased iron supplementation during IV Epogen study period compared to SQ Epogen study period (731±798 versus 445±506; p=0.014).

Conclusions: SQ administration of Epogen resulted in a 20% reduced Epogen dose in 56 HD patients during May 2011 to July 2011 compared to IV Epogen during May 2010 to July 2010 in spite of increased iron supplementation during IV Epogen study period.

TH-PO825

Epoetin Beta Pegol Promotes Utilization of Iron for Erythropoiesis through Intensive Suppression of Serum Hepcidin Levels in Inherited Super-Anemic Mice Yusuke Sasaki,[1] Yasushi Shimonaka,[1] Mitsue Kurasawa,[1] Keigo Yorozu,[1] Norio Suzuki,[2] Masayuki Yamamoto.[2] *[1]Chugai Pharmaceutical Co., Ltd., Kamakura, Japan; [2]Tohoku Univ Graduate School of Medicine, Sendai, Japan.*

Background: Epoetin beta pegol (C.E.R.A.) is a novel, long-acting erythropoiesis-stimulating agent (ESA). We previously reported that C.E.R.A. has the potential to induce sustained reduction of serum hepcidin levels in normal mice. In the present study, we investigated how long-term suppression of hepcidin following C.E.R.A. administration affects iron utilization associated with correction of anemia in inherited super-anemic mice (ISAM) as a mouse model of adult-onset anemia caused by erythropoietin deficiency.

Methods: Hemoglobin (Hb) levels, cardiac weight, and iron indices (serum hepcidin levels, serum iron levels, and hepatic and splenic iron contents) were analyzed in ISAM and control mice. ISAM were treated with a single subcutaneous injection of C.E.R.A. (3 μg/kg), recombinant human epoetin beta (rhEPO) (3 μg/kg), or vehicle.

Results: Hb levels were lower and the ratios of cardiac weight to body weight were higher in ISAM than in control mice. ISAM showed higher serum hepcidin levels and hepatic iron contents than did control mice. Serum hepcidin levels were significantly suppressed on Day 3 after both C.E.R.A. and rhEPO treatment, but only C.E.R.A.-treated ISAM showed significantly lower serum hepcidin levels than vehicle-treated ISAM on Day 7. High Hb levels were sustained for 14 days and the cardiac hypertrophy was improved following C.E.R.A. treatment. Serum hepcidin levels, serum iron levels, and hepatic and splenic iron contents were significantly lower in the C.E.R.A.-treated ISAM than in vehicle-treated ISAM over 14 days.

Conclusions: ISAM developed anemia associated with a decrease in utilization of iron for erythropoiesis. Our results indicated that the novel long-acting ESA C.E.R.A. has the potential not only to correct anemia but also to improve utilization of iron for erythropoiesis by mobilizing stored iron through the intensive suppression of serum hepcidin levels.

TH-PO826

Peroxynitrite Scavenger Ebselen Modulates Erythrocyte Intracellular ROS and Eryptosis *In-Vitro* Viktoriya Kuntsevich,[1] Nijal R. Sheth,[1] Nikolas B. Harbord,[1] James F. Winchester,[1] Andrea Novais Moreno-Amaral,[2] Roberto Pecoits-Filho,[2] Stephan Thijssen,[3] George A. Kaysen,[3] Peter Kotanko.[3] *[1]MSBI, New York, NY; [2]PUCPR, Curitiba, Brazil; [3]RRI, New York, NY.*

Background: Multiple factors contribute to anemia in hemodialysis (HD) patients. We recently reported that levels of total intracellular ROS (IC-ROS) in red blood cells (RBC) from HD patients are significantly higher compared to RBCs from healthy controls (HC). To further explore contribution of specific ROS to RBCs redox balance we investigated if short-term incubation of RBCs with hydrogen peroxide scavenger pyruvate (PYR), hydroxyl radical scavenger mannitol (MANN), peroxynitrite scavenger ebselen (EBS) and uremic toxin indoxyl sulfate (IS) can modulate IC-ROS and influence eryptosis.

Methods: RBCs from HC Li heparin blood were treated for 30 min at 37°C with PYR (5mM), MANN (20mM), EBS (20uM) or IS (50ug/ml) alone or were pretreated with PYR, MANN, EBS or ROS inhibitor N-acetyl-L-cysteine (NAC, 5mM) following by 30 min incubation with IS. Levels of eryptosis and RBCs IC-ROS were assessed using flow cytometry with Annexin-V-FLUOS (Roche) and Total ROS/SO Kit (Enzo). Statistical analysis was done with K-S statistics, P<0.05 was considered significant; results presented as mean±SD.

Results: Incubation of RBCs with IS resulted in significantly increased IC-ROS (54.38±3.59% versus 32.22±7.28%) and elevated eryptosis (1.84±0.42% versus 1.06±0.38%) compared with RBCs incubated in Ringer. Pretreatment of RBCs with MANN orPYR did not affect fluorescent signals triggered by subsequent incubation with IS (53.16±5.55% or 45.70±7.11%). Pretreatment of RBCs with EBS significantly decreased ROS signal before and after incubation with IS (13.52±14.24% and 19.03±15.27%), but resulted in massive eryptosis (22.04±7.16% and 28.58±5.9%). NAC abolished IS triggered ROS production (30.61±13.41%) and eryptosis (1.64±0.82%).

Conclusions: Short-term incubation of healthy RBCs with IS resulted in significant increase in intracellular ROS and eryptosis, suggesting additional mechanism of IS uremic toxicity. Modulation of ROS and eryptosis by scavenging of peroxynitrite indicates its significance in redox balance and RBCs lifespan and warrants further investigation.

TH-PO827

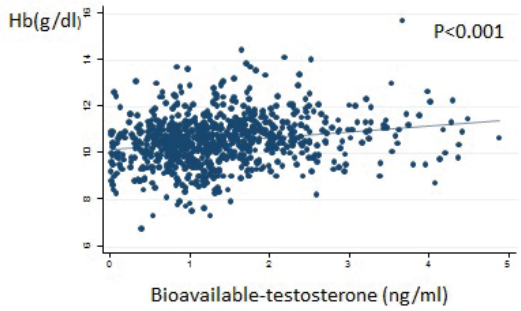
Bioavailable-Testosterone Affects Renal Anemia in Male Hemodialysis Patients Akio Nakashima,[1] Ichiro Ohkido,[1] Keitaro Yokoyama,[1] Mitsuyoshi Urashima,[2] Takashi Yokoo.[1] *[1]Div of Nephrology and Hypertension, Dept of Internal Medicine, Jikei Univ School of Medicine, Tokyo, Japan; [2]Div of Molecular Epidemiology, Jikei Univ School of Medicine, Tokyo, Japan.*

Background: Among hemodialysis patients, low serum testosterone was reported to associate with high mortality. Recently, bioavailable-testosterone (BT) has been suggested more accurate than total-testosterone (TT) to know testosterone deficiency. However, there are no reports to study relationship between BT and renal anemia. Therefore, we aimed to investigate the relationship between serum BT levels and anemia in hemodialysis patients. We also analyzed the copy number variants (CNVs) of *UGT2B17* gene, of which product metabolizes testosterone. Deletion of *UGT2B17* gene, which may increase the serum levels of testosterone, is known to highly prevalent in Japanese.

Methods: The prospective cohort study was carried out at 17 dialysis units in Tokyo Japan. We included 820 male hemodialysis patients who were older than 20 years and agreed to participate in this study. DNA was isolated from leukocytes from peripheral blood. We used real-time polymerase chain reaction to detect CNVs of *UGT2B17*.

Results: Median age was 63.4 years, and average duration of dialysis was 8.75 years. Mean BT was 1.37ng/ml and mean hemoglobin was 10.5g/dl. CNVs of *UGT2B17* gene were as follows: 0-copy: 645; 1-copy: 173; 2-copies: 2. Although TT did not show significant associations with hemoglobin levels (r=0.038 P=0.144), BT strongly correlated with it (r=0.2, P<0.001) in both single and multivariate analysis using age, PTH, Alb, TSAT and CRP.There was no relationship between the levels of BT and *UGT2B17* CNVs.

【The relationship between Bioavailable-testosterone and hemoglobin】



Conclusions: BT may associate with anemia in male hemodialysis patients, which imply testosterone as a novel therapeutic use for anemia in dialysis male patients with low levels of BT.

TH-PO828

Ergocalciferol Supplementation Does Not Reduce the Requirement of Erythropoietin (EPO) Stimulating Agent in Hemodialysis (HD) Patients Gaurav Agarwal, Padam Hirachan, Paolo Fanti, Shweta Bansal. *Div of Nephrology, Univ of Texas Health Science Center, San Antonio, TX.*

Background: Vitamin D deficiency (VDD) is highly prevalent in dialysis patients and has been associated with anemia and EPO resistance. The aim of this study was to evaluate the effect of ergocalciferol supplementation on EPO dose.

Methods: We conducted a case-control study of 186 patients, who were on HD for at least 3 months and had 25-hydroxyvitamin D (25OHD) level < 30 ng/ml. Over a period of one year, 107 patients were treated with protocol based ergocalciferol (case) and 79 were not (control). Parameters of erythropoiesis, mineral metabolism and monthly doses of EPO, intravenous iron and paricalcitol were assessed at 6- and 12- months of ergocalciferol treatment.

Results: The baseline characteristics including demographics and clinical parameters were similar in the two groups except for serum ferritin and transferrin saturation levels which were significantly higher in cases. At 12 months, mean 25OHD level increased from 16.2±7.5 to 30.5±11.7 ng/ml in cases compared with 19.2±6.8 to 14.2±9.3 ng/ml in the control group (p<0.001). However, there was no significant difference between the two groups in EPO dose requirements (28401±27203 versus 25758±32882 U/month, p=ns). Hemoglobin level and other iron parameters were similiar by the end of the study between 2 groups. There was a significant reduction in the paricalcitol dose in the cases compared to the controls (23.7±19.7 versus 38.1±28.2 μg/month, p<0.001); however, the cases had higher parathyroid hormone (452±547 versus 328±220 pg/ml, p=0.059) and phosphorus levels (5.7±1.8 versus 5±1.3 mg/dl, p=0.01). On multivariate regression analysis there was consistently no effect of ergocalciferol treatment on EPO dose (p=ns). Over an extended observation of 30 months, when some of the patients in the control group received ergocalciferol, we found no difference in EPO dose according to different levels of 25OHD (p=ns).

Conclusions: Treatment of VDD with one year of ergocalciferol was not associated with reduction in EPO dose in HD patients. Further studies are warranted to determine definitive role of nutritional vitamin D in HD patients.

TH-PO829

Vitamin B6 Supplementation Increases Resistance to Erythropoiesis-Stimulating Agents in Prevalent Hemodialysis Patients: A Bicenter, Open-Label, Randomized Controlled Trial Yoshitsugu Obi,[1] Takayuki Hamano,[2] Daisuke Mori,[1] Yasuo Kusunoki,[1] Akihiro Shimomura,[1] Isao Matsui,[1] Hiromi Rakugi,[1] Yoshitaka Isaka,[1] Yoshiharu Tsubakihara.[2] *[1]Dept of Geriatric Medicine & Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Dept of Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Resistance to erythropoiesis-stimulating agents (ESA) is a risk factor of cardiovascular events and death in hemodialysis patients. Vitamin B6 deficiency, which may cause sideroblastic anemia, is common in this population. We conducted this open-label, randomized controlled trial to determine whether vitamin B6 supplementation could reduce ESA resistance index (ERI).

Methods: We screened 231 prevalent hemodialysis patients from 2 dialysis facilities in Japan. ERIs were calculated for all patients in July 2013. After excluding patients with iron

deficiency, 60 prevalent patients with ERI above the median were recruited. Participants were randomly assigned to a vitamin B6 group (60 mg of intravenous pyridoxal after each thrice-weekly hemodialysis session) or a control group. The primary outcome was change in ERI between baseline and the 12th week. We employed intention-to-treat approach and last observation carried forward imputation for missing values at 12th week.This trial is registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR), number UMIN000011786.

Results: The mean baseline ERI was 9.5 (SD, 5.3) and 10.1 (SD, 5.6) IU/kg per g/dL in the control group and the vitamin B6 group, respectively. None was lost to follow-up and 53 patients completed the study. Contrary to our hypothesis, the vitamin B6 group showed significantly increased ERI at 12th week compared with the control group by 2.4 (95% CI 0.3–4.5) IU/kg per g/dL after adjustment for baseline ERI and type of ESA (p=0.028). There were no severe or moderate adverse events associated with vitamin B6 supplementation.

Conclusions: Vitamin B6 supplementation with 60 mg of intravenous pyridoxal phosphate thrice-weekly does not improve the response to ESA in hemodialysis patients. Rather, it does increase resistance to ESA.

Funding: Private Foundation Support

TH-PO830

The Role of Convection on the Long-Term Variations (Δ) of Serum Beta 2 Mycroglobulin (B_2M), C-Reactive Protein (hsCRP) Concentrations, and ESA Requirement (Δ ESA) in Uremic Patients Treated By Post Dilutional On-Line HDF

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Background: Inflammation and increased ESA requirement are frequently associated in patients on dialysis. On-line Hemodiafiltration (Ol-HDF), putting together high levels of diffusion and convection could improve both conditions. However, it is still not known which depurative component predominate in determining this result. Aim of the study**:** to evaluate the role of convection and diffusion on ΔB_2M, ΔhsCRP), and ΔESA in Ol-HDF.

Methods: 30 patients, 26 men, age 57±13 years, dialytic vintage 12-108 months, were switched from conventional HD to Ol-HDF**.** At 12 months the effect on ΔhsCRP, ΔB_2M, and ΔESA (U/Kg/sett) were evaluated. Other variables considered: Body weight (BW), serum albumin (sAlb), Hemoglobin (Hb), Kt/V. Iron therapy and ESA were administered IV according to the K/DOQI guidelines. Qb, treatment time and Qd remained constant. Ol-HDF was performed utilizing High-flux membranes 1.9-2.1 sqm. Ultrapure dialysate was employed in both HDF and HD treatments. Data are expressed as mean±SD. Paired t test, Mann-Whitney U test, simple and multiple regression analysis were employed for statistical evaluation.

Results: Total convective volume (TCV) was 21.8±1.7 l/session. Significant reduction of hsCRP: (from 5.3±7.5 to 2.1±2.7 mg/dl; p<0.01), B_2M (from 29.0±14.4 to 21.3±12.3 mg/dl; p<0.0001) and ESA (from 92±6 to 57±35 U/Kg/week; p<0.008). No significant variations of Kt/V, Hb, BW, sAlb. A significant inverse correlation was found betweenTCV and ΔB_2M (r: 0.74; p<0.0001), and TCV and ΔhsCRP (r: 0.41; p<0.02), no correlation between TCV and ΔEPO. No correlation was found between Kt/V and ΔB_2M, ΔhsCRP, and ΔESA. Multiple **r**egression analysis with ΔEPO as dependent variable showed ΔhsCRP as the only significantly associated independent factor (p<0.008).

Conclusions: Ol-HDF induces a long-term significant reduction of B_2M and hsCRP concentrations. This reduction is directly correlated to the amount of TCV. The observed reduction in ESA requirement is associated to decrease of inflammation and is independent from convection.

TH-PO831

Efficacy Comparison of HX575 (Biosimilar Epoetin Alpha, Binocrit) with Epoetin Alpha (Eprex) and Darbepoetin Alpha (Aranesp)

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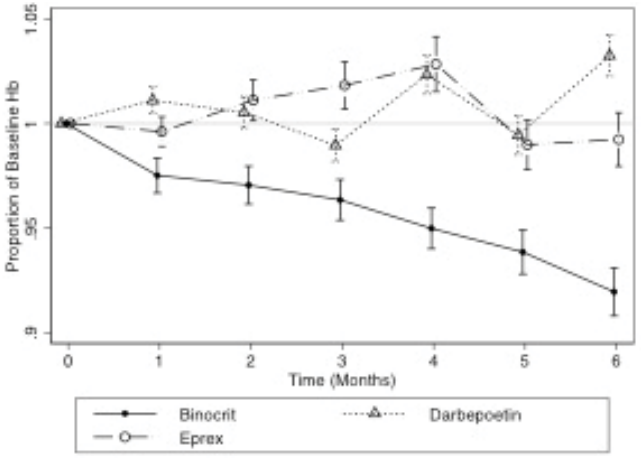
Background: Biosimilar erythropoiesis stimulating agents (ESAs) hold promise of effective anaemia treatment at reduced cost. In a climate of austerity and escalating healthcare cost, many healthcare providers have adopted their use. However, real-world evidence of relative efficacy is limited.

Methods: In a retrospective cohort study, we identified stable dialysis patients receiving ESA treatment and converting to a different agent. We abstracted demographics, haemoglobin (Hb), CRP, PTH, phosphate, ESA and drug dose from an electronic database for the period January 2002 to December 2013, and compared Hb response after conversion between ESAs using response feature analysis and multilevel mixed effects regression.

Results: During the study period, 1545 patients receiving ESA had 109,334 Hb measurements. We identified 513 conversions, from existing treatment onto Darbepoetin (179), Binocrit (89) and Eprex (85). Baseline characteristics and starting Hb did not differ between groups. Using AUC for change in Hb over the first 6 months for each treatment as response feature, Binocrit resulted in a lower Hb over time than Darbepoetin (p=0.01), and non-significantly lower than Eprex (p=0.08)



Hb on Binocrit declined over time independent of dose, but was maintained on Eprex or Darbepoetin. In mixed effects linear regression models for repeated measures of Hb, only treatment with Binocrit (-3.2, 95% CI -5.6 to -0.7 g/L, p=0.01) and Darbepoetin (3.6, 95% CI 1.6 to 5.5 g/L, p<0.001) were associated with haemoglobin level.

Conclusions: We identified an inferior Hb response in patients changing from any ESA to Binocrit compared to those changing to Darbepoetin or Eprex. While the cost of biosimilar ESA may be lower, conversion to biosimilar ESA may result in inferior Hb responses.

Funding: Private Foundation Support

TH-PO832

Methods to Detect Candidate Serum Biomarkers of ESA Resistance

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Background: Approaches to managing anemia in ESRD patient receiving dialysis include: transfusions, iron supplementation, and erythropoiesis stimulating agents (ESA). Substantial difficulties exist for predicting each patient's response to ESA dosing. We hypothesized those surrogate serum biomarkers reflect the individual's response to ESA's exist in the low abundant serum proteome (LASP).

Methods: We developed a discovery strategy using hexapeptide-bead library (Proteominer, BioRad) enrichment of LASP and isobaric tagging reagents (TMT, ThermoFisher) allow semi-quantitative LC-MS methods to establish candidate biomarkers. LASP were isolated from the serum of individuals with high (n_1=3) and low (n_2=3) erythropoietic response indexes. Enriched proteins were eluted and digested according to the FASP protocol. Samples (100ug) were labeled with TMT 6-plex reagents, admixed and analyzed using 2DLCM methods comparing 1st dimension separation with strong cation exchange (SCX) and high pH reverse phase (>pH-RP) chromatography then by 1D-LCMS analysis on an Orbitrap ELITE. Data were searched using Mascot and PD 1.4 using decoy database and PeptideProphet strategies to control FDR levels <0.1%. There were 206 proteins commonly identified within the two groups. ANOVA with an interaction was performed on the identified proteins to determine if it is differentially abundant between the two groups of ESA responders.

Results: 700+ serum proteins were identified and 206 proteins were common to the SCX and high pH RP experiments. 53 proteins were differentially abundant (FDR<0.10) including three proteins mainly expressed by the liver: haptoglobin, hepatocyte growth factor activator, and mannan-binding lectin serine protease 1. Neither the SCX or >pH-RP methods show a significant benefit based on observed protein identifications.

Conclusions: Differentially abundant proteins from these data suggest that the LASP can be used to define the response to ESA dosing.

Funding: NIDDK Support, Veterans Affairs Support

TH-PO833

Isocitrate Ameliorates Anemia of Inflammation in Mice Injected with Heat-Killed *Brucella abortus*

Airie Kim, Eileen Fung, Victoria Rivka Gabayan, Elizabeta Nemeth, Tomas Ganz. *Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA.*

Background: Anemia of inflammation (AI) is a multifactorial disorder whose effects are mediated by hepcidin-induced iron restriction, interferon-γ (IFN-γ)-induced erythropoietic suppression and shortened erythrocyte lifespan. Hepcidin and IFN-γ may also act synergistically to switch the hematopoietic program from erythropoiesis to monocytopoiesis through the transcription factor PU.1. The iron-sensitive enzyme aconitase, which acts in the Krebs cycle to convert citrate to isocitrate, is thought to sense iron levels in erythroblasts and together with IFN-γ influence erythropoietin (EPO) signaling through an incompletely-defined mechanism that converges on PU.1 (Richardson, et al. JCI 2013). We explored the effects of isocitrate in our recently established mouse model of AI induced by heat-killed *Brucella abortus* (HKBA).

Methods: After two weeks on an iron-adequate diet, the mice were injected with HKBA or saline on day 0, and with isocitrate 1000 μg/g/day or saline from days -3 to 5. The mice were analyzed on day 7, 14, and 21 for iron, hematological, and inflammatory parameters.

Results: On day 7, the isocitrate-treated mice had a dramatically attenuated anemia (Hb 11.7 versus 6.3, p<0.001), but this difference was no longer evident on days 14 and 21. Reticulocyte product indices showed increased erythropoiesis of the treated mice on days 7 (7.6 versus 3.0, p<0.05) and 14 (15.1 versus 10.2, p<0.05), despite similar serum EPO levels in isocitrate and control mice. SAA1 mRNA, regulated by IL-6, and hepcidin mRNA, which causes iron restriction, were both unchanged or increased in treated mice (SAA1 day 7: 0.018 versus 0.002; hepcidin day 14: 0.158 versus 0.103; p<0.05, the rest unchanged). White blood cell count (day 7: 19.9 versus 9.7, p<0.001) and platelet count (day 7: 538 versus 238, p<0.05) were also increased in the isocitrate-treated mice.

Conclusions: Isocitrate treatment in our mouse model of acute and severe AI results in a dramatic but transient improvement in anemia. The treated mice have increased erythropoiesis despite similar EPO levels and increased inflammation. The mechanism of these effects remains to be elucidated.

Funding: Pharmaceutical Company Support - Keryx Biopharmaceuticals

TH-PO834

Erythropoietin Resistance Index Does Not Represent Physiological Resistance

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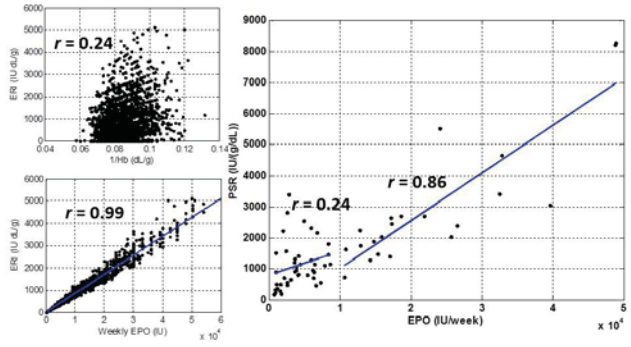
Background: The Erythropoietin (EPO) resistance index (ERI) is commonly used to study the association between EPO and all-cause and cardiovascular disease morbidity and mortality. Our objectives were to show that ERI is a poor measure of the physiological notion of resistance, and to propose a new measure, the patient-specific resistance (PSR).

Methods: ERI was calculated using retrospective data on 43 ESRD patients receiving EPO. A linear relation between ERI and corresponding EPO doses was used to predict ERI based only on EPO. Patient-specific resistances, the inverse of the sensitivity of Hb to changes in EPO dose, were computed from patient-specific parameters in a validated erythropoiesis model.

Results: ERI was weakly correlated with Hb (Figure, left top) and strongly linearly related with EPO doses based on ERI = (population mean of 1/Hb)×EPO (left bottom). This relation predicted 86% of weekly ERIs within 15% of actual values. Results for ERI computed from monthly and 3-month EPO and Hb averages were similar. For 20 patients, a single, individualized model was valid over the 16-month study period and the corresponding PSR remained constant over that time. For the others, PSR remained constant for periods ≥ 90 days, changing once for 20 patients and twice for 1 patient. Correlation between EPO and PSR was low for < 8600 IU/week (right).

Conclusions: ERI is strongly linearly related to, and thus closely tracks EPO doses. Hence it is a surrogate of EPO dose and does not reflect physiological resistance. In contrast, PSR accurately reflects resistance since it is computed from an individualized and physiologically-motivated erythropoiesis model.



Funding: NIDDK Support

TH-PO835

Treatment with High Dose of Erythropoiesis-Stimulating Agents and Mortality: A Sequential Cox Approach and Marginal Structural Model

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Background: Anemia-correction trials in chronic kidney disease patients indicated higher mortality rates in patients assigned to higher hemoglobin targets. The safety of the high erythropoiesis-stimulating agent (ESA) doses that these patients received was therefore questioned. Thus we aimed to estimate the effect of high ESA dose on mortality in a cohort of incident dialysis patients.

Methods: NECOSAD is a Dutch cohort study of incident dialysis patients in which ESA dose, co-morbidities and laboratory parameters were collected every 6 months. Mortality in patients with a high ESA dose (above median 6000 units/week) was compared to patients with a low ESA dose with Cox regression analyses. To handle time-dependent confounding, a sequential Cox approach was used conditional on baseline covariates, with inverse probability of censoring weights (IPCW) for any dependent censoring, including ESA treatment switch and kidney transplant. Analyses were repeated with a Marginal Structural Model (MSM) with inverse probability weights for ESA treatment and IPCW for ESA treatment switch and kidney transplant. Weights were stabilized and based on age, gender, primary kidney disease, co-morbidities, nutritional status, hemoglobin, ferritin, albumin, residual renal function and dialysis modality.

Results: Hazard Ratio (HR) for high ESA dose was 1.20 (95% CI 0.83-1.73) with a sequential Cox and 1.54 (95% CI 1.08-2.18) with a MSM. Truncation of weights in the MSM did not affect estimates. To compare: conventional Cox analyses indicated a baseline adjusted HR of 1.66 (95% CI 1.20-2.31).

Conclusions: Patients treated with high ESA dose have a 1.2-1.5 increased risk of mortality. Our analyses support guidelines advising a conservative ESA dosing regimen, which carefully weighs the patients benefits and risks.

Funding: Government Support - Non-U.S.

TH-PO836

Hemoglobin Outcomes During Routine Use of Continuous Erythropoietin Receptor Activator (C.E.R.A.)

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Background: Continuous erythropoietin receptor activator (C.E.R.A.) permits once-monthly dosing of ESA therapy in the maintenance phase compared to up to thrice weekly dosing with shorter-acting agents. The aim of the current study was to evaluate hemoglobin (Hb) outcomes associated with routine C.E.R.A. use in CKD patients at German nephrology centers, and compare outcomes by center size.

Methods: This was a 12-month prospective, observational study of adult patients with dialysis-dependent or non-dialysis-dependent CKD receiving *de novo* or ongoing C.E.R.A.

Results: During December 2009 to February 2013, 1,510 evaluable patients (78.4% dialysis-dependent, 21.6% non-dialysis-dependent) were recruited at 33 centers: 14 large centers (>100 patients), 19 small centers (≤100 patients). In the 16 weeks prior to study entry, 1,061 patients (70.3%) had received ESA therapy, including C.E.R.A. (n=482), darbepoetin alfa (n=257), epoetin beta (n=235) and epoetin alpha (n=131). Overall, C.E.R.A. dose (mean±SD) was 109±76μg at study entry; final dose was 121±99μg. The median number of dose changes was 3.0 (range 0–14) in large centers versus 1.0 (0–12) in small centers. Mean Hb was 11.2±1.3g/dL at study entry versus 11.6±1.2 at month 12. Mean intra-individual Hb fluctuation during the 12-month study was 1.5±0.8g/dL. Patients at larger centers maintained stable Hb values within 10–13g/dL more frequently than patients at smaller centers. Five patients discontinued C.E.R.A. due to adverse events (0.3%). At the final study visit, 45.4%, 39.9%, 9.0% and 2.1% of physicians were very satisfied, satisfied, undecided or dissatisfied with C.E.R.A. therapy; for patients, the proportions were 39.3%, 47.2%, 7.5 and 1.1% of patients.

Conclusions: Hb level showed a small increase during this 12-month observational study, accounted for by C.E.R.A. initiation in ESA-naïve patients. There was a low rate of Hb fluctuation during C.E.R.A. therapy. More frequent C.E.R.A. dose adjustments at larger centers were associated with more frequent achievement of Hb target range (10–13g/dL). Over 85% of physicians and patients were very satisfied/satisfied with C.E.R.A. therapy.

Funding: Pharmaceutical Company Support - Roche

TH-PO837

Responsiveness to Erythropoiesis-Stimulating Agent and Long-Term Outcomes in Chronic Hemodialysis Patients

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Background: Responsiveness to erythropoietin-stimulating agent (ESA) may be associated with mortality risk in hemodialysis (HD) patients. The aim of the present study was to assess the relationship between responsiveness to ESA and long-term outcome in chronic hemodialysis (HD) patients.

Methods: Patients on HD therapy for more than 6 months were enrolled in this cohort study. The first year was used to assess the longitudinal dialysis status of patients; the subsequent years were used to assess the time-dependent risk of all-cause mortality. Hazard ratios were estimated using a Cox proportional model for the association between ESA dose and hemoglobin (Hb) level and mortality, adjusting for potential confounders. The ESA resistance index (ERI) was determined as the weekly weight-adjusted dose of ESA divided by Hb concentration. Patients were divided into three groups by tertiles of ERI.

Results: Of the 320 subjects enrolled, 105 died during the follow-up period of 70.4 ± 29.0 months. When subjects were stratified by epoetin dose and Hb level into four groups, those who had low Hb despite a high dose of epoetin were associated with the highest risk of mortality among the four groups (adjusted hazard ratio 1.86; 95 % confidence interval 1.25–2.75). These highest-risk subjects had older age, lower body mass index, and lower serum levels of albumin, triglyceride and transferring saturation. The impact of serum albumin and serum ferritin on mortality risk in an adjusted Cox proportional hazards model was in accordance with low Hb and higher ESA. There was no significant difference between the mortality risk and tertile of ERI.

Conclusions: High ESA dose and low Hb level were associated with an increased risk of all-cause mortality. However, the responsiveness to ESA estimated by ERI was not related to mortality risk. These findings suggest that the responsiveness to ESA should be evaluated by different methods in HD patients.

TH-PO838

Are Hemodialysis (HD) Patients (Pts) Treated by Antiplatelet Agents (CA) and/or Vitamin K Antagonists (VKA) Really Bleeding: An Evidence by the Anemia Management (AM) Jacques B. Rottembourg. *Hemodialysis Unit, Diaverum, Paris, France.*

Background: HD Pts required frequently treatments by CA (Clopidrogel+Aspirin) and/or VKA, for atrial fibrillation (AF), cardiovascular diseases (CVD), deep-vein thrombosis (DVT), pulmonary embolism (PE), or arteriovenous fistula problems (AVF). The conséquences on AM in these Pts were never investigated.

Methods: Out of 300 Pts, 60 of them (Gr2), received CA and/or VKA and 240 did not (Gr1).We matched Gr2, with 60 Pts, included in Gr1, for age, sex, renal disease and co-morbidity (Gr3). Pts received ESA, darbepoetin alfa (DA), every 2 weeks (Q2W) and IV iron (IS). Hb level was assessed Q2W and iron parameters (ferritin [F], and TSAT), albumin level and CRP every 3 months; hospitalizations (H) were recorded.

Results: Gr1 included 240 Pts, male 65%, mean age (SD) 54.2 (16.7) years, diabetes (25%), Charlson index 7.9 (3.7); Gr2 included 60 Pts, male 75%, mean age 62.1 (12.1), diabetes in 35%, Charlson index 9.7(3.8); reasons for CA and/or VKA were AF for 18, DVT for 8, CVD for 15, PE for 8, and AVF for 11 Pts respectively. Gr3 was strictly matched with Gr2. Main results were:

Parameters	Gr1: 240 Pts	Gr2: 60 Pts	p: G1 vs Gr2	Gr3: match 60 Pts
Hb g/dl mean (SD)	11.64 (0.75)	11.20 (0.75)	p<0.0001	11.64 (0.63)
F µg/L mean (SD)	562 (341)	562 (235)	NS	588 (422)
TSAT % mean (SD)	37.5 (8.9)	35.4 (6.2)	p<0.04	39.3 (8.4)
CRP mg/L mean (SD)	9.52 (9.46)	15.9 (14.5)	p<0.001	10.3 (9.5)
Albumin g/L mean (SD)	39.9 (3.1)	38.1 (3.0)	p<0.0001	39.8 (3.3)
DA µg/kg/week mean (SD)	0.53 (0.37)	0.79 (0.47)	p<0.0001	0.48 (0.21)
IS mg/week mean (SD)	77.2 (26.3)	81.4 (28.4	NS	78.5 (26.4)
H days/pts/year mean	2.41	6.46	p<0.01	2.1

HD Pts receiving CA and/or VKA present a lower Hb level and required 1/3 more ESA; they have more co-morbidities, are more hospitalized, and present more severe biological values for CRP and albumin. Matched Pts (Gr3) have same results than Gr1. Bleeding (minimal chronic or severe) is the most common adverse event associated with CA and/or VKA.

Conclusions: HD Pts receiving for any reason CA and/or VKA, are particularly at risk, and the decision to recommend them in any clinical condition should be based on an individualized risk-benefit approach.

TH-PO839

The Impact of Erythropoietin Stimulating Agents on Quality of Life in Patients with CKD: A Systematic Review and Meta-Analysis Ravindi M. Gunasekara,[1] Yang Xu,[1] Brett M. Hiebert,[1] Blake R. Lerner,[1] Frederick Eng,[1] Kerry Macdonald,[2] Claudio Rigatto,[1] Paul Komenda,[1] Navdeep Tangri.[1] *[1]Medicine, Univ of Manitoba, Winnipeg, MB, Canada; [2]Library Services, Univ of Manitoba, Winnipeg, MB, Canada.*

Background: Anemia is common in patients with chronic kidney disease (CKD) and those on dialysis. The use of erythropoietin stimulating agents (ESAs) has reduced the need for transfusions in CKD, but at the expense of increased cardiovascular risks and health care costs. Previous systematic reviews have suggested that ESAs have a small positive impact on quality of life (QoL), but have failed to include several recently published randomized trials. Our objective was to evaluate published randomized controlled trials examining the effect of lower versus higher doses of ESA or different hemoglobin targets on QoL in patients with CKD and those on dialysis.

Methods: We performed a systematic review and meta-analysis of studies evaluating the impact of ESAs have on all aspects of QoL in dialysis and non-dialysis CKD WE searched PubMed, EMBASE, and Cochrane Library ranged from their establishment until June 2013. The primary outcome was the change in baseline and follow up scores of two QoL instruments: Short Form (36) Health Survey (SF-36) and Kidney Disease Questionnaire (KDQ).

Results: Sixteen studies including 9842 patients met our inclusion criteria. Data from eight domains of the SF-36 and three domains of the KDQ were meta- analyzed using a random effects model. Difference in each SF-36 domain and KDQ domain were summarized. For the SF-36 domains, small clinically non-significant changes in the physical function (-1.8; 95% CI, -2.9 to -0.7) and physical role (-2.4; 95% CI, -4.0 to -0.7) domains were noted. Findings from the KDQ were similar in magnitude (Physical Domain (-0.6; 95% CI, -1.0 to -0.1), Fatigue (-0.5; 95% CI, -0.8 to -0.1).

Conclusions: In this contemporary systematic review and meta-analysis, treatment with ESA to a higher hemoglobin target did not result in any meaningful improvements in health related quality of life. Our findings support lower targets for anemia management in CKD, and recommend revisions of cost-effectiveness analyses of ESA use.

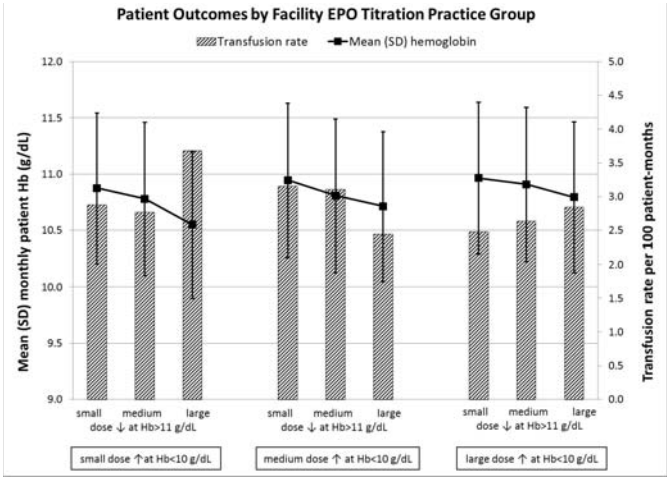
TH-PO840

Facility EPO Titration Practices, Hb Levels, and Transfusion Use Julia T. Molony,[1] Keri Monda,[2] Suying Li,[1] Anne C. Beaubrun,[2] David T. Gilbertson,[1] Brian D. Bradbury,[2] Allan J. Collins.[1] *[1]Chronic Disease Research Group, Minneapolis, MN; [2]Amgen, Inc, Thousand Oaks, CA.*

Background: Epoetin alfa (EPO) doses and Hb levels have declined and RBC transfusion rates have risen following implementation of Medicare's prospective payment system (Jan 2011) and EPO labeling changes (June 2011) advising initiating at Hb<10 g/dL and reducing or interrupting at Hb>11 g/dL. We investigated facility-level EPO dosing practices in 2012 and their effects on Hb levels and RBC transfusion use.

Methods: Adult (≥18) patients with Medicare Parts A and B as primary payer undergoing hemodialysis in 2012 were included. From Jan-June, the month-to-month EPO dose titrations over 5 consecutive 2-month intervals were assessed. Then, for each facility, the median EPO dose titrations when Hb<10 g/dL and Hb>11 g/dL were calculated; these summary values were used to classify each facility into 1 of 9 titration practice groups. Dose escalations and reductions when Hb<10 g/dL and Hb>11 g/dL, respectively, were categorized as small (<20%), medium (20-30%), and large (>30%). The mean (SD) monthly patient Hb and RBC transfusion rate over the next 6 months was assessed by facility titration group.

Results: Each titration group included on average 7271 patients at 147 facilities. The mean and SD for monthly patient Hb was similar across all facility titration groups. Titration patterns that involved both greater dose reduction at Hb>11 g/dL and smaller dose escalation at Hb<10 g/dL produced more Hb measurements below 10 g/dL and more transfusion use. Conversely, titration patterns with both smaller dose reductions at Hb>11 g/dL and greater dose escalations at Hb<10 g/dL were associated with fewer transfusions.



Conclusions: FacilityEPO titration practices that minimize Hb<10 and Hb>11 g/dL and limit RBC transfusion may help achieve the appropriate balance of maximizing benefits while minimizing risks in accordance with product labeling.

Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO841

Centralized Anemia Case Management Program Is Associated with Improved Anemia Outcomes and More Efficient Medication Delivery Len A. Usvyat, Phyllis Brenda Berggren, Fern Parlier, Debra Marshall, Cindy Allegretti, Barbara Williams, John W. Larkin, Kim L. Sonnen, Jeffrey L. Hymes, Franklin W. Maddux. *Fresenius Medical Care, Waltham, MA.*

Background: FMCNA utilizes a remote centralized anemia case management (CACM) program in collaboration with clinic-based staff and physicians to improve outcomes in clinics where clinical leadership is missing, or in transition (i.e. clinic manager (CM) position is temporarily vacant). During CACM, hemodialysis (HD) patient (Pt) nurse case managers, provide comprehensive review of laboratory findings and root cause analysis for Pts with anemia issues. We investigated whether clinics enrolled in CACM experienced improvements in anemia management.

Methods: We studied all clinics enrolled in CACM from Jan 1, 2013 to Dec 31, 2013; clinics were enrolled proximal to the CM's position becoming available. Endpoints were hemoglobin (Hgb), transferrin saturation (TSAT), Epogen® (EPO) dose/HD treatment (tx) and IV iron dose/HD tx for 90 days before and after enrollment in CACM (or sooner if CM's position was filled). Paired t-tests were used to compare results.

Results: Overall, 119 clinics and 9,421 Pts in the CACM program were studied. During CACM there was reductions in both Hgb <10g/dL (p<0.0001) and >12g/dL (p=0.008), as well as, increases in Hgb in the 10-11g/dL target range (p=0.012). EPO dosing requirements did not have any significant change during CACM, although decreased by 3.7% (NS). Iron dosing had risen by 11.9% (p<0.0001) during CACM and was correlated with increases of 2.9% in TSAT ≥30% (p<0.0001).

Figure 1:	90 days before	90 days after	% change	p-value
EPO dose per HD tx	4166 (SD±4925)	4011 (SD±4726)	-3.7%	NS
% of pts with Hgb<10 g/dL	26.4%	25.2%	-4.6%	<.0001
% of pts with Hgb 10-11 g/dL	37.4%	37.8%	1.1%	0.012
% of pts with Hgb>12 g/dL	11.8%	11.1%	-5.8%	0.008
Iron dose per HD tx	19.1 (SD±21.1)	21.4 (SD±21.8)	11.9%	<.0001
% of pts with TSAT>=30%	49.4%	50.8%	2.9%	<.0001

Conclusions: This analysis identified that CACM is associated with significant improvements in meeting anemia management target outcomes without changes in EPO dosing requirements. The CACM program may be an effective tool for helping clinics with missing clinic leadership or anemia case managers.

TH-PO842

Trends in Anemia Management in U.S. Patients on Dialysis, 2005-2011

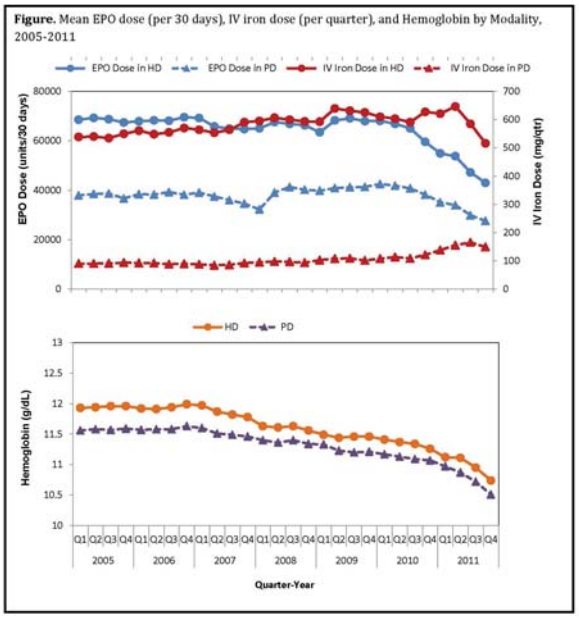
David T. Gilbertson,[1] Anne C. Beaubrun,[2] Jiannong Liu,[1] Akhtar Ashfaq,[2] Wolfgang C. Winkelmayer,[3] Glenn M. Chertow,[3] Allan J. Collins.[1] *[1]Chronic Disease Research Group, Minneapolis, MN; [2]The Center for Observational Research, Amgen, Inc., Thousand Oaks, CA; [3]Stanford Univ, Palo Alto, CA.*

Background: The landscape of anemia management practice changed with clinical trials in the 2000s showing increased risk of adverse events with higher targeted Hb levels using ESAs, a new bundled payment system, and an ESA label revision in 2011. We investigated the impact of the most recent policy and clinical events on anemia management in dialysis patients.

Methods: We used the CMS ESRD database and included all adult (≥18) patients on hemodialysis (HD) or peritoneal dialysis (PD) for ≥9 months, 2005-2011, with Medicare as primary payer for ≥6 months. By modality, we summarized quarterly Hb levels and EPO and IV iron use and dose. As almost all patients on dialysis receive EPO, patients receiving other ESAs were excluded.

Results: About 90% of HD patients used EPO from 2005-2010; this fell to 85% in 2011. EPO use remained constant at about 70% in PD patients. Between 2005-2011, the mean monthly EPO dose in HD patients fell from ~69000 to 43000 units; the dose was more consistent for PD patients but began to decline in 2010, reaching a low of 28000 units in 2011 from a peak of 41000 in 2008. In HD and PD patients, respectively, quarterly iron use rose from 69% and 21% in 2005 to 75% and 39% in 2011. Iron dose, in contrast, rose from 2005-2010 before declining in 2011 in both groups. Hb levels, higher in HD patients, fell from 11.9 and 11.6 g/dL in 2005 to 10.7 and 10.5 g/dL in 2011 in HD and PD patients, respectively.

Figure. Mean EPO dose (per 30 days), IV iron dose (per quarter), and Hemoglobin by Modality, 2005-2011



Conclusions: Anemia management practices shifted in response to regulatory and reimbursement events, particularly recently. The clinical consequences for anemia-related outcomes warrant further study.

Funding: Pharmaceutical Company Support - Amgen, Inc.

TH-PO843

Intravenous Iron Reverses Anemia-Induced Inflammation and Improves Cardiac Function in Anemic Rats

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Background: Iron deficiency anemia (IDA) is very common in CKD patients greatly contributing to patient outcomes. Recent trial data with intravenous (IV) iron indicate favorable outcomes on cardiac function in iron deficient patients. This study evaluates whether IV iron therapy modifies oxidative-nitrosative stress and proinflammatory markers in the heart together with the left ventricular performance in anemic rats.

Methods: Sprague-Dawley rats, Group A: Control; Group B: IDA; Group C: IDA+IV iron. Group A received standard diet, Group B and C low iron diet for 16 weeks, after which hematology variables were evaluated (baseline). Groups continued with their corresponding diet for 4 more weeks, during which Group C received weekly administrations of IV iron sucrose (10mg Fe/kg bw) and Group A and B saline solution. Malondialdehyde (MDA) and GSH:GSSG were assessed in heart homogenates. Nitrotyrosine, heme oxygenase-1 (HO-1), NFkB(p65), TNF-a and heat shock proteins (HSP) in the heart were evaluated. Echocardiogram and tissue Doppler were also performed.

Results:

Mean ± SD	**Group A** Control	**Group B** IDA	**Group C** IDA+IV iron
Hb (g/dl)	14.6±0.5*	7.8±0.5#	11.7±0.7
Serum Iron (mg/dl)	225.9±21.7*	36.0 ±14.5#	179.6±19.9
TSAT (%)	42.2 ±2.9*	9.2 ± 2.0#	25.6 ± 4.7
MDA (nmol/mg prot.)	0.8±0.2	2.5±0.6*	1.0±0.3
GSH:GSSG (ratio)	6.5±0.9*	3.3 ± 0.6#	5.1±0.5
Heart weight to BW ratio (g/kg)	2.6±0.2*	4.1±0.4#	3.1±0.1
Cardiomyocyte size (μm)	20.7±1.0*	28.5±2.2#	23.2±1.3
Nitrotyrosine (%/mm^2)	1.9±0.4	16.1±2.7*	2.3±0.6
HO-1(%/mm^2)	2.9±0.6	21.5±1.8*	3.4±0.9
$NFkB_{65}$ (positive cells/mm^2)	2.8±0.8	15.1±2.0*	3.2±0.9
TNF-a (%/ mm^2)	1.6±0.4	10.5±1.6*	2.0±0.4
HSP27 (%/mm^2)	2.6±0.5	18.9±2.1*	3.5±0.8
HSP70 (%/mm^2)	0.9±0.2	9.8±1.9*	1.0±0.2
Fractional shortening (%)	54.8±1.5†	37.0±1.2*	49.7±1.6

*p<0.01 versus all groups #p<0.01 versus C †p<0.05 versus C

Conclusions: IV iron significantly reduced oxidative-nitrosative stress, modified favorably the inflammatory response, and improved cardiac function in anemic rats.

TH-PO844

Heme Precursor, 5-Aminolevulinic Acid (ALA), Decreases Fataxin Expression in Polymorphonuclear Leukocytes (PMNLs)

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Background: Frataxin is a mitochondrial protein and essential for utilization of iron as the synthesis of iron-sulfur clusters and heme. We have already demonstrated that mRNA levels of frataxin is significantly decreased in polymorphonuclear leukocytes (PMNLs) from the patients on maintenance hemodialysis (MHD) (Clin Exp Nephrol 2013). However, the factors affecting frataxin in MHD has not been determined. In the present study, we screened the causative substances, which could be accumulated in uremic serum.

Methods: PMNLs were obtained by differential centrifugation from healthy volunteers. PMNLs were cultured in the media added with several candidate substances including ALA, LPS, TNFα, IL-6, cysteine, β2-microglobulin for 2 hours. For determining PMNL frataxin mRNA, the quantitative PCR was performed using TaqMan polymerase. FRX mRNA was normalized using the expression of GAPDH. For the analysis of PMNL FRX protein, Western blot analysis was performed using anti-FRX polyclonal antibody.

Results: ALA decreased FRX mRNA levels dose dependently, but other substances tested did not (ALA 0; 100%, ALA 500 μM; 70 ±8 %, ALA 750 μM; 62 ±7%, ALA 1000 μM; 55 ±8 %). Western blot analysis similarly showed the decrease in FRX protein expression((ALA 0; 100%, ALA 500 μM; 69 %, ALA 750 μM; 62%, ALA 1000 μM; 42%) We also confirmed that serum ALA concentrations were higher in patients on MHD than control (84 versus 8.9 μg/L).

Conclusions: This is the first report that the expression of FRX in PMNL was significantly affected by heme precursor, ALA, which might be linked with erythropoiesis and mitochondrial dysfunction. ESA hyporesponsiveness could be associated with the dysregulated synthesis of heme and iron- sulfur clusters.

Funding: Private Foundation Support

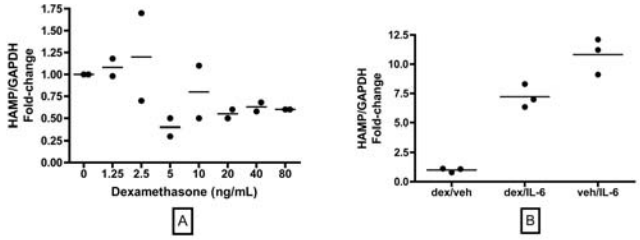
TH-PO845

Glucocorticoids May Inhibit *HAMP* (Hepcidin) mRNA in HEPG2 Cells Adam Rumjon, Iain C. Macdougall. *King's College Hospital, London, United Kingdom.*

Background: Hepcidin is the circulating hormone responsible for regulating the absorption and distribution of iron. Hepcidin levels are elevated in advanced renal failure, and this may impact on anaemia management. Previous studies have shown that hepcidin is suppressed by the sex steroids, oestrogen (*in vivo* and pre-clinical) and testosterone (clinical studies), but no data currently exist that examine the effect of glucocorticoids on hepcidin.

Methods: HepG2 cells, from a hepatoma cell line (ATCC), were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 5% penicillin and streptomycin. Cells were serum-starved overnight before the addition of increasing doses of dexamethasone (Sigma-Aldrich) from 1.25 ng/mL to 80 ng/mL, for a period of 18 hours. HepG2 cells were pre-treated with dexamethasone (10ng/mL) (or vehicle) for 4 hours before the addition of interleukin-6 (Life Technologies) at a concentration of 12.5 ng/mL, or vehicle, for 2 hours. Total cellular RNA was extracted (RNeasy - Qiagen) (1µg) and reversed transcribed (High Capacity RNA-to-cDNA kit-Applied Biosystems). Quantitative RT-PCR amplification reactions were performed using Taqman HAMP and GAPDH (housekeeping) primers.

Results: The dexamethasone-Hamp dose-response curve showed no suppression of HAMP at lower concentrations (<5 ng/mL), with maximal (40-50%) suppression demonstrated at concentrations ≥ 5 ng/mL (Figure A) up to 20 µg/mL (data not shown). In these preliminary experiments, IL-6 stimulated HepG2 cells that were not pre-treated with dexamethasone showed an 11-fold rise in HAMP compared to only a 7.5-fold increase in cells that were pre-treated with dexamethasone (p=0.028).



Conclusions: These preliminary data suggest that the glucocorticoid, dexamethasone suppresses HAMP expression *in vitro*, and it is possible that this is mediated via IL-6. Further confirmatory experiments will be required to test these hypotheses fully.

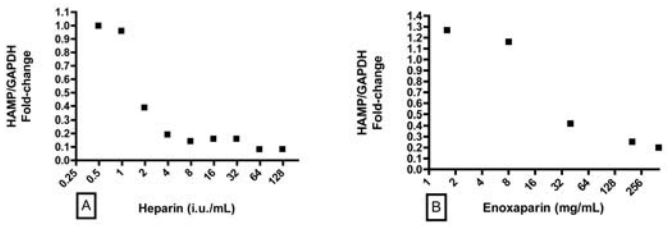
TH-PO846

Heparin May Reduce Hepcidin Levels in Dialysis Patients by Inhibiting *HAMP* mRNA Adam Rumjon, Iain C. Macdougall. *King's College Hospital, London, United Kingdom.*

Background: Hepcidin is the master regulator of iron homeostasis, and is key to the pathogenesis of anaemia in dialysis patients via its effect on regulating iron availability to the bone marrow. Understanding factors that impact on hepcidin levels are important in planning future strategies of anaemia management in CKD. A recent report suggested that there may be an effect of heparin on BMP-6-induced expression of hepcidin, and the aim of the present study was to further investigate this by examining the effect of varying concentrations of both unfractionated heparin and a low molecular weight heparin (enoxaparin) on *HAMP* mRNA.

Methods: HepG2 cells (ATCC), were cultured and grown to 70% confluence in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 5% penicillin and streptomycin. Cells were serum-starved overnight. Nine treatments of unfractionated heparin (LEO Pharma) (dose ranging from 0.5 to 125 i.u./mL) and six treatments of enoxaparin (Sanofi-Aventis) (0.32 to 400 mg/mL), were applied for a period of 18 hours. Total cellular RNA (1µg) was extracted using RNeasy kits (Qiagen) and reversed transcribed (High Capacity RNA-to-cDNA kit-Applied Biosystems). Quantitative RT-PCR amplification reactions were performed using Taqman HAMP (encoding hepcidin) and GAPDH (housekeeping) primers. All reactions were performed in triplicate.

Results: Both heparins inhibited hepcidin (*HAMP*) expression; maximum suppression of HAMP occurred with heparin from 4 i.u./mL (Figure A), and with enoxaparin from upwards of 32 mg/mL (Figure B).



Conclusions: This study provides further *in vitro* evidence that heparin has an inhibitory effect on hepcidin *HAMP* expression. Given that systemic anticoagulation is routinely used in haemodialysis patients to prevent extracorporeal line clotting, consideration should be given to the potential impact of regular heparinisation on hepcidin activity in this patient population.

TH-PO847

Impact of Iron Regimen on Iron Indices and Hepcidin during Roxadustat Anemia Correction in Incident Dialysis Patients Anatole Besarab, Lynda Szczech, Kin-Hung Peony Yu, Thomas B. Neff. *FibroGen, Inc, San Francisco, CA.*

Background: Roxadustat (FG4592) corrects anemia in incident ESA-naïve HD/PD patients assigned to no Fe, oral Fe, or IV Fe over a 12 wk period. Initial 6 wk ΔHb response was equal but maximal ΔHb and Hb at 12 weeks (EOT) was blunted with no Fe, while Hb response with oral iron was comparable to IV iron. This study evaluates the effect of each Fe regimen.

Methods: Post hoc study.

Results: Results are mean±SEM. At baseline (BL), iron parameters among iron regimens were similar reflecting iron depletion (TSAT/Ferritin (Ftn), but mean CHr 30.6pg; CRP 2 to 55 ng/ml; entry Hb 8.3 g/dL. CRP did not change; ΔHb was independent of BL CRP. Changes in iron parameters from BL to EoT are shown

Iron regimen (N)	ΔHb (g/dL)	Δ TSAT (%)	ΔCHr (pg)	ΔHepcidin (µM)	ΔFtn (µM)
No Iron (23)	1.98 ± 0.28[a]	-7.8 ± 1.43 [a]	-2.91 ± 0.63 [a]	-63.4 ± 13.3 [c]	-121.5 ± 12.2 [a]
Oral Iron (22)	2.68 ± 0.33 [c]	1.07 ±1 .87 [b,c]	-0.91 ± 0.13 [c]	-54.1 ± 18.4 [c]	- 51.9 ± 18.6
IV Iron (10)	3.25 ± 0.36 [c]	0.69 ± 2.89 [b,c]	-1.02 ± 0.14 [c]	-12.6 ± 31.6 [a,b]	-25.2 ± 33.5 [b]

[a] p <0. 01 from other 2 gps; [b] not different from zero; [C] no difference between pair TIBC increased in all 3 gps. Largest changes occurred in no Fe group. IV iron blocked hepcidin suppression and Ftn reduction. EOT TSAT (R^2 = 0.40), Ftn (R^2 = 0.41), and TIBC (R^2 = 0.31) correlated with EOT hepcidin in the no Fe group. EoT Ftn (R^2 = 0.73) and TIBC (R^2 = 0.40) correlated with EoT hepcidin in the po Fe group. No correlations in IV iron group. BL and EoT Ftn levels correlated (R^2 = 0.43 BL and 0.73 EOT) with hepcidin. Hepcidin correlated weakly with CRP [R^2=0.11, p=0.015] at BL but not at EoT.

Conclusions: FG4592 therapy is not impacted by inflammation and prevents iron-deficient erythropoiesis, removing parenteral iron-induced risks associated with IV iron. Even with IV iron, iron overload is avoided, likely attributable to HIF homeostasis. With FG4592, po Fe regimen was optimal and physiologic. The combination of FG4592 and po Fe offers short term and perhaps long term benefits for many intermediate iron markers and other benchmarks of anemia care.

Funding: Pharmaceutical Company Support - Fibrogen,Inc

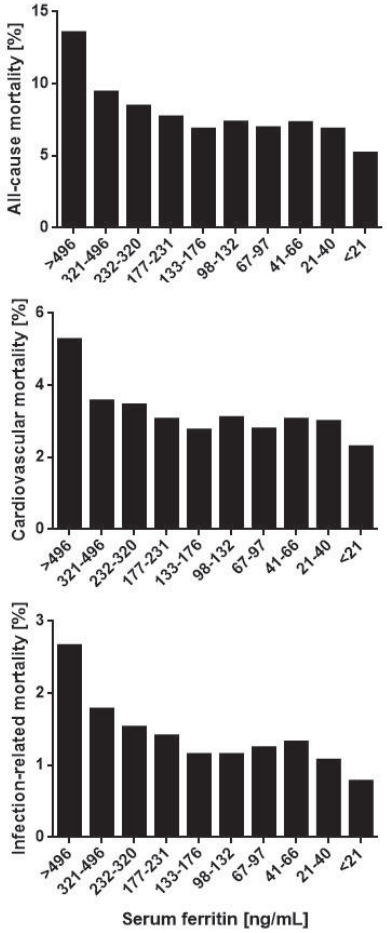
TH-PO848

A Higher Serum Ferritin Is Associated Not Only with All-Cause Mortality but also Infection-Related Mortality among Patients Receiving Hemodialysis in Japan Yukio Maruyama,[1] Keitaro Yokoyama,[1] Takashi Yokoo,[1] Takashi Shigematsu,[2] Kunitoshi Iseki,[2] Yoshiharu Tsubakihara.[2] *[1]Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan; [2]Committee of Renal Data Registry, Japanese Society for Dialysis Therapy, Tokyo, Japan.*

Background: The monitoring of serum ferritin is widely recommended in the management of anemia in patients receiving dialysis. However, target ferritin level varies by guidelines.

Methods: We collected the baseline data of 162,818 patients receiving HD thrice weekly (65 ± 13 years, males 61.4%, and median HD vintage of 61 months) extracted from a nationwide dialysis registry at the end of 2007 in Japan. Then we evaluated the patient survival and development of complication using the registry at the end of 2008.

Results: During one-year follow-up, 12,800 (7.9%) died of all causes including 5,216 (3.2%) cardiovascular death and 2,230 (1.4%) infection death. All-cause mortality, cardiovascular mortality, and infection-related mortality were higher in line with the increase of baseline serum ferritin.



Especially, a serum ferritin >500 ng/mL was associated with a markedly elevated mortality. In a multivariable logistic regression analysis, patients of the highest quartile of serum ferritin had higher all-cause, cardiovascular, and infection-related mortality compared with those of the lowest quartile (OR, 1.24; 95% CI, 1.13 to 1.36, and OR, 1.10; 95% CI, 0.96 to 1.26, and OR, 1.24; 95% CI, 1.02 to 1.52, respectively).

Conclusions: In this large observational cohort study, higher levels of serum ferritin were independently associated not only with all-cause mortality but also infection-related mortality among Japanese HD patients. Close monitoring of serum ferritin is thought to be useful for the management of renal anemia.

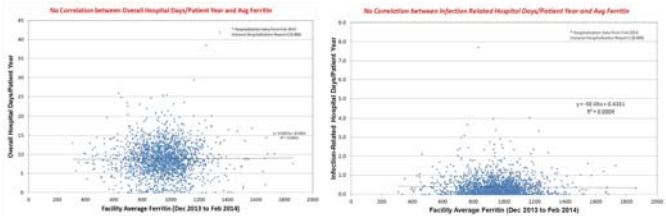
TH-PO849

Facility-Wide Mean Ferritin Levels of Hemodialysis Patients Are Not Associated with Either All-Cause Hospitalization Rates or Infection-Related Hospitalization Rates Norma J. Ofsthun, Julia I. Brennan, John W. Larkin, Len A. Usvyat, Jeffrey L. Hymes, Franklin W. Maddux. *Fresenius Medical Care North America (FMCNA).*

Background: In hemodialysis (HD) patients, higher ferritin levels are generally associated with higher levels of inflammatory markers, as well as, higher IV iron utilization. After observing that mean ferritin levels in HD patients had trended upwards with the availability of a Venofer® algorithm that includes maintenance IV iron administration, physicians in one of four geographic-based divisions of FMCNA requested an analysis to determine if hospitalization rates correlate with ferritin levels. Our aim was to determine whether there is a relationship between facility-based mean ferritin levels and all cause, or infection-related hospitalization rates.

Methods: A total of 1,984 FMCNA facilities met the inclusion criteria of ≥12 months of hospitalization data in the centralized data warehouse, and ≥25 patients with ferritin values between Dec 2013 and Feb 2014. Individual ferritin values of >10,000ng/mL were excluded. In total, 163,726 patients and over 250,000 ferritin results were analyzed. We computed each facility's mean ferritin for Dec 2013-Feb 2014, as well as, the number of all-cause and infection-related hospital days per patient year for Mar 2013-Feb 2014 in all permanent (non-transient) patients.

Results: Among FMCNA facilities, we found no relationship between all-cause hospital days and mean ferritin levels (r^2=0.0004), as well as, no association between infectious hospital days and mean ferritin levels (r^2=0.0001).



Conclusions: Among in-center HD patients, no relationship was observed on a clinic-wide basis between ferritin levels and all-cause hospitalization rates, or infection-related hospitalization rates.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

TH-PO850

Oxidative Stress Response to Intravenous Iron Administration to CKD Patients: Impact of Haptoglobin Genotype Zaher Armaly,[1] Amir Abd Elkadir,[1] Kamal Hassan,[3] Adel Rafik Jabbour,[1] Rawi Ramadan,[2] Bishara Shafik Bisharat.[1] *[1]Nephrology, Nazareth Hospital EMMS, Nazareth, Israel; [2]Nephrology, Rambam Health Campus, Haifa, Israel; [3]Nephrology, Western Galilee Hospital, Nahariya, Israel.*

Background: Anemia is a common disorder in CKD patients. It is attributed to decreased erythropoietin (EPO) production and iron deficiency. Therefore, besides EPO, therapy includes iron replenishment. However, the latter induces oxidative stress. Haptoglobin (Hp) protein is the main line of defense against the oxidative effects of Hemoglobin/Iron.There are 3 genotypes: 1-1, 2-1 and 2-2. Hp 2-2 protein is inferior to Hp 1-1 as antioxidant. So far, there is no evidence whether haptoglobin genotype affects iron-induced oxidative stress in CKD patients. The aim of the current study was to examine whether Hp genotype influences intravenous iron administration (IVIR)-induced oxidative stress in CKD patients, and its impact on the response of these patients to L-Carnitine therapy.

Methods: This study included 26 anemic (Hb=10.23±0.28) CKD patients (stages 2-4) that were given a weekly IVIR (Sodium ferric gluconate, [125 mg/100 ml] for 8 weeks, and during weeks 5-8 also received Carnitine (20mg/kg, IV) prior to IVIR. Weekly blood samples were drawn before and after each IVIR for Hp genotype, C-reactive protein (CRP), advanced oxidative protein products (AOPP), neutrophil gelatinase-associated lipocalin (NGAL), besides complete blood count and biochemical analyses.

Results: 8% of CKD patients were Hp 1-1, 19% Hp 2-1, and 73% Hp 2-2. IVIR for 4 weeks did not significantly increase hemoglobin levels, yet worsened the oxidative burden as was evident by elevated plasma levels of AOPP. The highest increase in AOPP was observed in Hp 2-2 patients. Simultaneous administration of Carnitine with IVIR abolished the IVIR-induced oxidative stress as was evident by preventing the elevations in AOPP and NGAL, preferentially in patients with Hp 2-2 genotype.

Conclusions: This study demonstrates that Hp 2-2 is a significant risk factor for IVIR-induced oxidative stress in CKD patients. Our finding, that co-administration of Carnitine with IVIR preferentially attenuates the adverse consequences of IVIR, suggests a role for Carnitine therapy in these patients.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

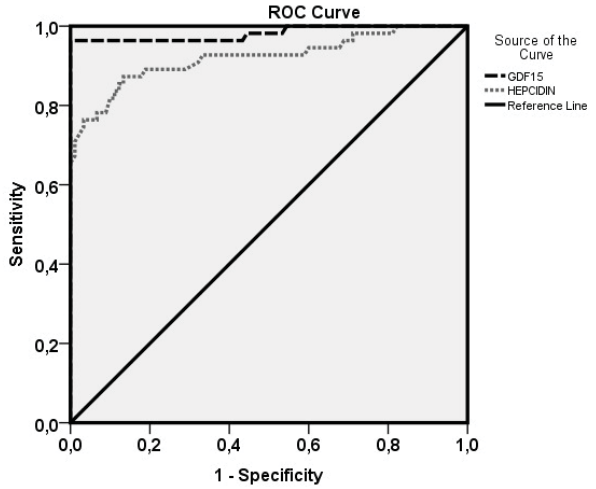
TH-PO851

Can Serum GDF-15 Be Associated with Functional Iron Deficiency in Hemodialysis Patients? Hakki Yilmaz,[1] Osman Inan,[2] Huseyin Tugrul Celik,[3] Ayse Mukadder Bilgic,[1] Nuket Bavbek,[1] Ali Akcay.[1] *[1]Internal Medicine, Section of Nephrology, Turgut Ozal Univ, School of Medicine, Ankara, Turkey; [2]Internal Medicine, Yenimahalle State Hospital, Ankara, Turkey; [3]Biochemistry, Turgut Ozal Univ, School of Medicine, Ankara, Turkey.*

Background: The incidence of functional iron deficiency(FID) is gradually increasing in hemodialysis(HD) patients. Recently, high levels of GDF-15 supressed the iron regulatory protein of hepcidin and expression of GDF-15 increased in patients with iron deficiency. The relationship between FID and GDF-15, hepcidin is not known exactly up to now. The aim of the present study was to evaluate the association between GDF-15, hepcidin and FID in chronic HD patients.

Methods: Serum GDF-15 and hepcidin concentrations were measured in 105 HD patients and 40 controls. FID is defined as serum ferritin>800ng/mL, TSAT<25%, Hb levels <11g/dL and reticulocyte haemoglobin content (CHr) <29pg.

Results: Serum GDF-15, hepcidin levels were increased significantly in HD patients with FID, compared with HD patients without anemia and the controls. GDF-15 correlated with ferritin, hepcidin and CRP in whole group. But GDF-15 was related to ferritin and CRP in HD patients with FID. GDF-15 is better diagnostic marker than hepcidin for detection of FID [AUC=0.982(0.013) versus AUC=0.921(0.027); P=0.0324].



Conclusions: GDF-15 was appeared to be promising tool for detection of FID. High levels ferritin and CRP correlated with GDF-15. Our results supported that GDF-15 may be a new mediator of FID via hepcidin, chronic inflammation or unknown pathways.

TH-PO852

Abstract Withdrawn

TH-PO853

Reduction of Oxidative Stress Marker with Enhancement of Iron Metabolism following Epoetin Beta Pegol Administration in Db/db Mice Mariko Noguchi-Sasaki, Yusuke Sasaki, Yukari Matsuo, Mitsue Kurasawa, Keigo Yorozu, Yasushi Shimonaka. *Product Research Dept, Chugai Pharmaceutical Co., Ltd., Kamakura, Kanagawa, Japan.*

Background: Erythropoietin (EPO) exhibits tissue protective effect by hypoxia improvement and EPO signaling activation. Epoetin beta pegol (C.E.R.A.), a novel long-acting erythropoiesis-stimulating agent (ESA) could promote utilization of iron for erythropoiesis through intensive suppression of serum hepcidin levels. Although iron is an essential element for various biological processes such as erythropoiesis, it has potential to induce oxidative stress. We hypothesize that the reduction of iron-induced oxidative stress by utilization of iron for erythropoiesis contribute the tissue protective effect of ESA, and investigated whether C.E.R.A. could ameliorate oxidative stress with enhancement of iron metabolism in db/db mice.

Methods: Eleven-week old male db/db mice were intravenously treated with 0.5 mg/head of Iron-Dextran (Fe0.5 group) or vehicle (Fe0 group). Five days after, C.E.R.A. (10 μg/kg) or vehicle were administered intravenously to both groups (Day0). Hemoglobin (Hb), serum hepcidin level and hepatic iron content were determined. Serum derivatives of reactive oxygen metabolites (d-ROMs) were analyzed as an oxidative stress marker by measuring the total amount of hydroperoxides via the Fenton's reaction.

Results: Serum d-ROMs and hepatic iron content significantly increased in Fe0.5 group compared to Fe0 group on Day0. On Day8, Hb level significantly increased after C.E.R.A. treatment in both Fe0 and Fe0.5 groups, whereas serum hepcidin level and hepatic iron content significantly decreased. Moreover, serum d-ROMs significantly decreased after C.E.R.A. treatment in Fe0.5 group on Day8.

Conclusions: C.E.R.A. treatment promoted iron utilization for erythropoiesis through mobilization of hepatic iron storage, consequently serum oxidative stress marker was decreased in db/db mice. Our results suggest a possibility that enhancement of iron metabolism by ESA, leading to the decrease in oxidative stress, contributes to tissue protective properties. ESA may have beneficial implications for improving prognosis by correcting oxidative stress-related disorders.

TH-PO854

Iron Sucrose (IS) and Iron CarboxyMaltose (ICM): Comparative Study in Hemodialysis Patients V. Menoyo, M. Touam, David Attaf, C. Muresan. *Echo Vannes, Vannes, France; Necker, Paris, France; Fresenius Medical Care, France; ECHO Vannes, France.*

Background: ICM, next-generation i.v. iron, is given as a bolus–push injection in haemodialysis (HD) while Iron Sucrose (IS) is given by perfusion of 100 mg. We aim to compare efficacy, safety and oxidative activity of both IS and ICM in HD.

Methods: 124 pts (73 y) are treated successively by IS 100 mg (01/2010 - 07/2011 = Period 1 (P1)) and by ICM 100 mg (08 /2011 - 12/2013 = Period 2 (P2)). P1 and P2 are compared according to : (1) biology (Hb, F, TSAT), (2) clinical status (Iron, ESA dose) and (3) oxidative stress (TSAT, generation of free iron). For the latter we assess TSAT and free iron in serum by Bleomycin-Detectable Iron (BDI) assay for different doses of i.v. iron (n=9 patients).

Results: Biology is reported in the following table:

	Hb g/dl	Ferrit. mg/l	TSAT %	ASE microg/w	Fer mg/month
P1	11,3 +/- 1	694 +/- 332	32,5 +- 15	98,8	694 +/- 332
P2	11,7 +-1,2	854 +/- 349	34,4 +- 12,5	61,8	854 +- 349
p	< 0,05	< 0,001	< 0,002	< 0,0001	< 0,07

FCM is tolerated, incidence of adverse events related to i.v. iron are similar in P1 and P2. Oxydative activities of IS (100 mg) and FCM (200 mg) are reported in the figure below:

	IS 100 mg		ICM 100 mg		ICM 200 mg TDI	
	TSAT %	BDI	TSAT %	BDI	TSAT %	BDI
Before i.v. iron	20±12	0.03±0.02	19±12	0.02±0.02	17±12	0.03 ±0.02
5 min	67±29	0.14±0.28	63±19	0.13±0.28	67±29	0.12 ±0.17
30 min	59±28	0.07±0.11	51±18	0.02±0.11	55±28	0.07 ±0.38
90 min	66±27	0.12±0.24	66±27	0.10±0.24	58±27	0.15 ±0.12
210 min	83±27	0.29±0.34	83±37	0.21±0.34	90±27	0.15 ±0.16
2-3 days after i.v. iron	20±16[a]	0.06±0.01[b]	18±06[a]	0.01±0.01[b]	19±16[a]	0.02 ±0.02[b]
Ref.	17–52 %	<0.10 µmol/l	17–52 %	<0.10 µmol/l	17–52 %	<0.10 µmol/l

Conclusions: ICM dose of 100 mg / session is well tolerated and provides comparable efficacy compared to FS. Morever ICM is less time consuming for the paramedical staff. The improvement of oxidative profile with ICM should be evaluated in appropriate clinical trials.

TH-PO855

Soluble Ferric Pyrophosphate (Triferic) Does Not Oversaturate Transferrin and Preserves Plasma Unsaturated Iron Binding Capacity Ajay Gupta,[1] Leanne Goldstein,[2] Vivian H. Lin,[1] Carrie D. Guss,[1] Raymond D. Pratt.[1] *[1]Rockwell Medical, Wixom, MI; [2]City of Hope, Duarte, CA.*

Background: Unsaturated iron binding capacity (UIBC) of transferrin in blood serves as a vital buffer for iron from dietary absorption or released from the RE system. UIBC minimizes labile plasma iron (LPI) and oxidative stress. The low molecular weight fraction of IV iron preparations contains free iron that can oversaturate transferrin, making UIBC undetectable.

Methods: Triferic™ is an investigational parenteral iron salt comprising covalent Fe-pyrophosphate-citrate complex that specifically donates iron to plasma transferrin. SFP was delivered continuously via hemodialysate (2 µM iron) to 47 CKD-HD patients at every hemodialysis session for 36 weeks (PRIME study).

Results: Changes in serum iron parameters observed during a single hemodialysis treatment using SFP dialysate are presented below.

Serum Iron Parameter Mean (SD) [Range]	Pre HD	Post HD	Post HD – Pre HD	Repeated Measures t-test P-value
Iron (µg/dL)	64.5 (25.2) [18.0-159.0]	212.8 (57.7) [54.0, 377.0]	148.2 (50.6) [4, 316.0]	<0.0001
TSAT (%)	27.8 (9.9) [10.1, 68.3]	74.8 (11.9) [35.5, 91.3]	47.1 (11.1) [1.6, 68.4]	<0.0001
TIBC (µg/dL)	232.1 (33.8) [127.0, 318.0]	281.5 (49.6) [147.0, 437.0]	49.4 (33.7) [-24.0, 185.0]	<0.0001
UIBC (µg/dL)	167.5 (33.6) [64.0-254.0]	68.8 (31.1) [26.0, 182.0]	-98.8 (30.9) [-170.0, -5.0]	<0.0001

There was no oversaturation of transferrin during 197 HD treatments with SFP. The minimum post HD UIBC was 26 µg/dL demonstrating significant residual iron binding capacity. The determinants of intradialytic decrease in UIBC were examined using a mixed effects linear regression model to evaluate change in average post HD UIBC. Significant model covariates included pre HD serum iron and TSAT, intradialytic iron and TSAT change and TIBC. Non-significant covariates were HD duration, average Qd, and IV iron during a previous hemodialysis visit.

Conclusions: Triferic delivered via dialysate (2 µM iron) does not oversaturate transferrin and preserves plasma UIBC throughout dialysis. The UIBC serves to buffer administered and dietary iron, thereby preventing oxidative stress and inflammation.

Funding: Pharmaceutical Company Support - Rockwell Medical

TH-PO856

Overcoming Functional Iron Deficiency in CKD-HD Patients: Triferic Bypasses RE Block and Reduces ESA Use Ajay Gupta, Carrie D. Guss, Vivian H. Lin, Raymond D. Pratt. *R&D, Rockwell Medical, Wixom, MI.*

Background: Uremic inflammation increases hepcidin levels leading to iron sequestration in the reticuloendothelial system (RES). As a consequence, functional iron deficiency (FID) and hyporesponsivness to ESAs develops.

Methods: Triferic™ (Soluble ferric pyrophosphate citrate) is a parenteral iron salt that donates iron directly to transferrin. A series of randomized, placebo-controlled clinical trials have now shown that Triferic delivered via hemodialysis can sustain iron balance by replacing obligatory iron losses, sparing ESA use [PRIME] and maintaining hemoglobin (Hgb) [CRUISE 1 and 2].

Results: The PRIME study randomized 104 iron-replete CKD-HD patients to Triferic or placebo for up to 36 weeks. ESA was titrated to maintain a target Hgb level and IV iron could be administered for serum ferritin <200 µg/L. At the end of treatment, Triferic reduced prescribed ESA by 35% (p=0.045) and IV iron by 51% (p=0.044) versus placebo while maintaining reticulocyte Hgb (CHr) and serum soluble transferrin receptor (sTfR) near baseline levels. The placebo group showed a significant decrease in CHr with increase in sTfRs (0.8±0.35 mg/L; p=0.03). Triferic did not increase serum hepcidin levels or increase markers of inflammation (CRP and IL-6) and oxidative stress (8-iso PGF2a, malondialdehyde (MDA) and isofurans). The CRUISE studies treated iron-replete CKD-HD patients with Triferic (N=299) or placebo (N=300) for up to 48 weeks. Once randomized, ESA dose change and IV or oral iron were prohibited. Triferic maintained Hgb at baseline in both studies, while placebo treated subjects showed significant declines. The primary endpoint, the mean change in hemoglobin from baseline to end of treatment between groups was 3.6 g/L in each study (p-value = 0.011). The safety profile of Triferic was similar to placebo in all studies, with no anaphylaxis and no increases in intradialytic hypotension, vascular access thrombosis, cardiovascular events or infections compared to placebo.

Conclusions: Triferic delivered via hemodialysate represents a new paradigm for iron therapy in CKD-HD patients addressing iron-restricted erythropoiesis and FID by circumventing iron sequestration syndrome.

Funding: Pharmaceutical Company Support - Rockwell Medical

TH-PO857

Changes in Reticulocyte Hemoglobin Equivalent Level and Efficacy of Continuous Erythropoietin Receptor Activator in Hemodialysis Patients Tadashi Kuji,[1,2] Tetsuya Fujikawa,[2,3] Midori Kakimoto-Shino,[2] Yoshiyuki Toya,[2] Satoshi Umemura.[2] *[1]Yokodai Central Clinic; [2]Medical Science and Cardiorenal Medicine, Yokohama City Univ Graduate School of Medicine; [3]Center for Health Service Sciences, Yokohama National Univ, Yokohama, Kanagawa, Japan.*

Background: Epoetin beta pegol or CERA (continuous erythropoietin receptor activator), a recently approved erythropoiesis-stimulating agents, has unique receptor-binding characteristics and has a long half-life. CERA was reported to cause transient decrease in reticulocyte hemoglobin equivalent (Ret-He) levels. However, the implication of the decrease in Ret-He on the erythropoiesis is unclear. This study assessed the relation between changes in Ret-He and efficacy of CERA treatment in hemodialysis patients.

Methods: A total of 47 patients were studied in treatment with monthly CERA. The target hemoglobin level was 10–11 g/dL. Maintenance CERA doses of 0, 50, 75, 100 and 150 µg were administered as appropriate. Iron supplementation was administered to maintain serum ferritin levels within the range of 50–200 ng/mL and TSAT within the range of 20%–40%.

Results: During a cycle of once-monthly CERA treatment, hemoglobin levels were higher at week 1 and 2 than those at time 0 ($P < 0.01$) and Ret-He levels were lower at week 1 and 2 than those at time 0 ($P < 0.01$). There were significant inverse correlations of changes in Ret-He levels within 2 and 3 weeks with administered CERA dose (r= -0.710, P <0.01; r=-0.515, P <0.01, respectively). Changes in hemoglobin levels were not significantly correlated with CERA doses. Significant inverse correlations were detected between changes in Ret-He within 2 weeks and changes in hemoglobin within 3 to 4 weeks (r= -0.498, P <0.01; r=-0.515, P <0.01, respectively).

Conclusions: CERA causes a significant increase in hemoglobin levels peaking 2 weeks and decrease in Ret-He level peaking one week of treatment. Greater decrease in Ret-He within 2 weeks is associated with greater increase in hemoglobin level. Decreased Ret-He levels during 2 weeks suggest relative shortage of available iron for highly-activated erythropoiesis by CERA, which may indicate the possibility of further improvement of CERA efficacy.

TH-PO858

Measurement of Annual Iron Loss By Blood Sampling and Residual Blood After Regular Hemodialysis Procedure in Japan Tatsuo Tsukamoto, Motoko Yanagita. *Nephrology, Graduate School of Medicine, Kyoto Univ, Kyoto, Japan.*

Background: Blood sampling and residual blood in the hemodialysis circuit mainly cause inevitable iron loss in hemodialysis patients. The standard of iron supplementation is different among guidelines, such as those of KDIGO and Japanese Society of Dialysis Therapy (JSDT). Since iron overload may lead not only to hemosiderosis and hemochromatosis but also worsening of systemic infection, the intravenous administering iron at the dialysis session, that is a common procedure in hemodialysis, must be strictly monitored. Although the annual iron loss by hemodialysis procedure had been reported as 1000mg~2000mg in 1990s, the advance of the technique might reduce this iatrogenic iron loss. For example, the rinse method with saline after hemodialysis is different between that in 1990s and the present in Japan. Thus, we measured iron in residual blood after hemodialysis, and calculated the annual iron loss by the JSDT standard hemodialysis.

Methods: 239 patients of Otowa Memorial Hospital were enrolled after informed consent. Men were consisted in 65.7%, and the mean age was 67±6.4 (m±SD). 43.2% was diabetic. The residual blood sample of each patient after a dialysis session was collected and diluted uniformly by 250ml saline after removal of the needle. The iron concentration was measured by the atomic absorption spectrometry methods.

Results: Since the mean concentration of iron in the residual blood diluted by saline was 670±433.5µg/dL (m±SD), the total iron loss was around 670x2.5x156=261.3mg. As the iron of the whole blood is predominantly distributed in hemoglobin (Hb), the 1mL of blood contains 34200µg/dL of iron when the Hb is 11g/dL. Thus, the iron loss by blood sampling was 205.2mg when the regular blood sampling volume is 50mL a month. Thus, the annual iron loss in hemodialysis patients would be 450~500mg.

Conclusions: 500mg of annual iron supplementation might be sufficient to maintain the iron status in hemodialysis patients in Japan, which is less than the recommendation dose by guidelines (1~2g/year). Further study is required to verify our measurement.

Funding: Government Support - Non-U.S.

TH-PO859

J-Shaped Effect of the Serum Ferritin Level on Adequate Hemoglobin Levels and a Good Prognosis in Hemodialysis Patients Chie Ogawa,[1] Fumiyoshi Kanda,[1] Kunimi Maeda,[3] Ken Tsuchiya,[2] Teiryo Maeda.[1] *[1]Maeda Inst of Renal Research, Kawasaki, Kanagawa, Japan; [2]Div of Nephrology, Dept of Medicine IV, Kidney Center, Tokyo Women's Medical Univ, Tokyo, Japan; [3]Div of Nephrology, Juntendo-Nerima Hospital.*

Background: The assessment of optimal iron storage for adequate anemia control and a good prognosis for hemodialysis (HD) patients is now on moving point and thought to be important. To clarify the appropriate iron status, a 10-year survey was performed to investigate the relationships among the serum ferritin (s-ft) level, transferrin saturation (TSAT), and mortality in HD patients.

Methods: 125 HD-outpatients treated with erythropoiesis-stimulating agents (ESA) were followed from May 2002 to April 2013. The ESA and low-dose iron supplement dosages were adjusted to maintain a hemoglobin (Hb) level of 10-11 g/dL, according to Japanese guidelines. Kaplan-Meier and log-rank tests were used to analyze the patient data. The Cox proportional hazards model was constructed to assess the relative risk of death, and the interaction among the Hb level, s-ft, and TSAT was analyzed using a multiple linear regression model.

Results: The patients were classified according to TSAT and s-ft cut-off values as follows: group 1 (s-ft < 30 ng/mL, n = 15); group 2 (s-ft, 30-80 ng/mL, n = 28); and group 3 (s-ft ≥ 80 ng/mL, n = 48). A TSAT < 20% was a predictor of a poor outcome. The survival rate in group 2 was significantly higher than in the other groups ($P = 0.013$), and a Cox hazard model analysis revealed a J-shaped effect of s-ft on the survival of the patients. The cause of death was analyzed, and the low TSAT and high s-ft groups had higher mortality rates from multiple causes. A multiple linear regression model showed that s-ft had a strong effect on Hb (log [s-ft], β-coefficient -0.45: 95% CI -0.65 to -0.26, $P < 0.001$).

Conclusions: Taken together, s-ft had a J-shaped effect on the outcome of patients with renal anemia and appeared to play a larger role than the TSAT based on an analysis of the 10-year survival ratio. The present study suggested that the s-ft level should be considered in detail for the ideal control of renal anemia and that this level might be lower than the previously established level.

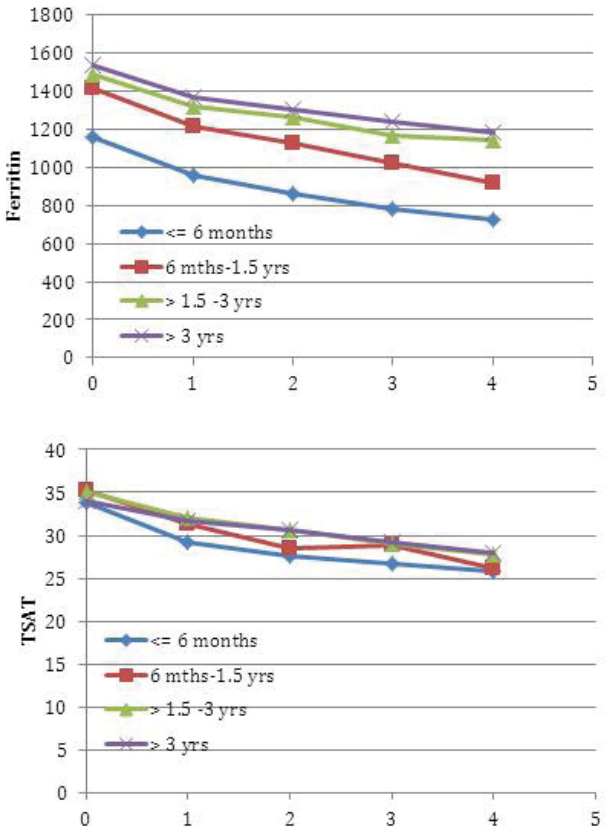
TH-PO860

Dynamics of Serum Ferritin and Transferrin Saturation (TSAT) in Hemodialysis Patients D. Miskulin,[2] S. Paine,[3] O. Myers,[1] Klemens B. Meyer,[2,3] A. Harford,[1,3] Philip Zager.[1,3] *[1]UNM, Albuquerque, NM; [2]Tufts, Boston, MA; [3]DCI, Nashville, TN.*

Background: In response to recent clinical trials and inclusion of ESAs in the 'bundle' providers are administering higher doses of iron. This has led to serum ferritin levels significantly in excess of KDIGO and the European Best Practices Guidelines. In order to design a clinical trial to assess the safety and efficacy of this practice it is important to assess serum ferritin and TSAT dynamics in response to increasing vintage and discontinuing iron therapy.

Methods: We studied patients (n=5876) dialyzed in facilities operated by DCI during 2012 and 2013. Baseline values for ferritin and TSAT were measured 13.8 ± 4.3 days after administration of ≥500 mg of iron in the preceding month. Ferritin and TSAT were then measured at regular intervals over periods of 1 to 4 months in which iron was not administered. To reduce confounding due to infection, we did not include ferritin or TSAT measurements within 14 days of an antibiotic order. Data were analyzed using mixed model regression analyses with patient random effects.

Results: Baseline least squares mean levels of ferritin (ng/ml) adjusted for age, sex and race were 1154, 1404, 1478, 1529 in patients who had been on dialysis ≤ 6, 6-18, 19-36 and >36 months, respectively ($p < 0.0001$). In contrast, there was no linear association between vintage and baseline TSAT values (p=0.27). Ferritin and TSAT decreased after iron was stopped with significant heterogeneity in the rates of decline by vintage p<0.001. Declines were faster in patients on dialysis ≤ 1.5 versus >1.5 yrs.



Conclusions: Baseline levels of ferritin but not TSAT increased with vintage, suggesting ongoing inflammation. The slow rates of decline in ferritin and TSAT after discontinuing iron suggests that a clinical trial of high versus low dose iron should be conducted among incident HD patients.

Funding: Clinical Revenue Support

TH-PO861

Assessing the Extent to which Research in Dialysis Is Consistent with Research Priorities Identified by Patients on Dialysis

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Background: There is a growing recognition that research focusing on priorities and outcomes considered relevant to patients is important. We sought to assess the extent to which recent research in patients on or nearing dialysis is consistent with the top-10 research priorities identified by dialysis patients, caregivers and clinicians.

Methods: We systematically searched the medical literature, national government and kidney research funders of Canada, U.S., UK, and Australia, and international randomized controlled trial(RCT) registries(2010-2013) to identify published original articles and funded studies(all designs-clinical studies) and registered RCTs, respectively, of adults on or nearing dialysis. All studies were grouped based on their correspondence with the top-10 dialysis research priorities(DRP) identified through a national Canadian survey and an in-person workshop of patients on or nearing dialysis, caregivers and clinicians.

Results: The search yielded 1123 published articles, 69 funded studies and 401 RCTs. The majority did not address the top-10 DRP; 195 articles(17%), 15 funded studies(22%) and 86 RCTs(21%), respectively, were relevant to these DRP.

Top-10 research priorities identified by patients on or nearing dialysis, their caregivers, and clinicians	Published medical literature; n=1123 (%)	Funding organizations; n=69 (%)	Clinical trial registries; n=401 (%)
1. How can communication between patients and care providers be improved?	1.5	8.7	0.5
2. How do the different dialysis modalities compare with one another?	4.4	1.5	0.8
3. What are the causes/effective treatments of itching in dialysis patients?	0.4	0	1.5
4. What is the best strategy to increase kidney transplantation?	1.9	0	0
5. What is the psychological/social impact of kidney failure?	0.8	0	1
6. What are the best ways to promote heart health?	5.6	4.4	9.2
7. What is the impact of dietary restrictions in dialysis patients?	0.5	0	0.5
8. What are the best ways to manage/prevent complications during/after hemodialysis?	0.9	2.9	4.5
9. What are the causes/effective treatments of depression in dialysis patients?	0.6	2.9	1.3
10. What is the best type of access for people on hemodialysis?	0.8	1.5	2.3
Not in the top-10 dialysis research priorities	82.6	78.3	78.5

In published articles not in the top-10 DRP, hemodialysis research outside the top-10 DRP(eg dialyzers;16.7%), other dialysis epidemiology(12.9%) and bone diseases(9.5%) were the most common research areas. Studies of symptom-related issues(eg itching), identified as high priority to dialysis patients, were lacking.

Conclusions: The majority of recently published or ongoing research in patients on or nearing dialysis is in areas outside the top research priorities identified by patients, caregivers and clinicians. Future dialysis studies should consider including the identified patient-centred research priorities in their design.

TH-PO862

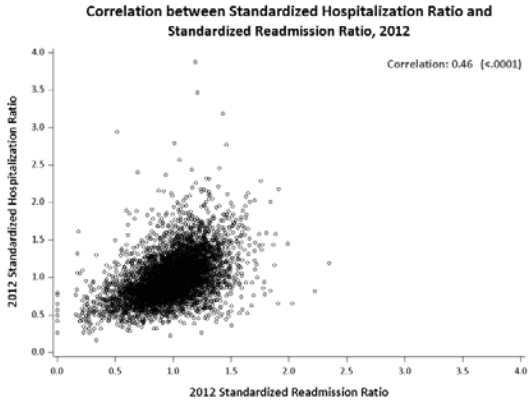
Measuring Dialysis Facility Performance on Hospital Utilization

John Kalbfleisch,[1] Zhi He,[1] Deanna Chyn,[1] John Wheeler,[1] Rajiv Saran,[1] Tempie H. Shearon,[1] Sarah Casino,[1] Claudia Dahlerus,[1] Jennifer Sardone,[1] Richard Hirth,[1] Joel S. Andress,[2] Yi Li.[1] *[1]Univ of Michigan; [2]Centers for Medicare & Medicaid Services.*

Background: Medicare uses the longstanding Standardized Hospitalization Ratio (SHR) and the more recent Standardized Readmission Ratio (SRR) to assess dialysis facilities' (DF) performance with respect to hospital usage. SHR is the number of hospital admissions divided by the expected number of admissions, adjusted for patient characteristics. The SRR is the number of hospital discharges that result in an unplanned readmission within 30 days divided by the expected number of readmissions, adjusted for similar variables plus discharging hospital.

Methods: We examined 2012 dialysis Medicare claims data for covariability in SHR and SRR among 5849 DFs and 534,609 patients. Using 95% intervals, we classified DF outcomes as being "as expected (E)," "better than expected (B)" or "worse than expected (W)."

Results: The correlation of SHR and SRR was 0.46 ($p<0.001$; see figure). 90.6% of DFs were classified E on both measures. 106 DFs classified E on SHR were classified W on SRR (see table). Although overall hospitalizations for these DFs are within national norms, they may have an opportunity to improve patient care after discharge through better care coordination. 205 DFs classified E on SRR were classified W on SHR; the rate of readmission for these DFs was within the national norm, but their higher SHR suggests an opportunity for improvement in preventing hospitalizations.



2012 Categorization of Facilities*, SHR v. SRR

	SHR Category			
SRR Category	Better than Expected	As Expected	Worse than Expected	Total
Better than Expected	11 (0.2%)	87 (1.6%)	1 (0.0%)	99
As Expected	49 (0.9%)	5077 (90.6%)	**205 (3.7%)**	5331
Worse than Expected	0 (0.0%)	**106 (1.9%)**	67 (1.2%)	173
Total	60	5270	273	5603

Note. Kappa statistic = 0.227 (SE = 0.024; 95% CI = 0.180–0.274).
*Restricted to facilities with an SHR and SRR.

Conclusions: Although there is an intrinsic correlation between SHR and SRR, the two measures are sufficiently distinct that they each carry important information about different aspects of quality of care as related to hospitalization. DFs can use SHR and SRR to motivate quality improvement and coordination in the post-discharge transition.

Funding: Other U.S. Government Support

TH-PO863

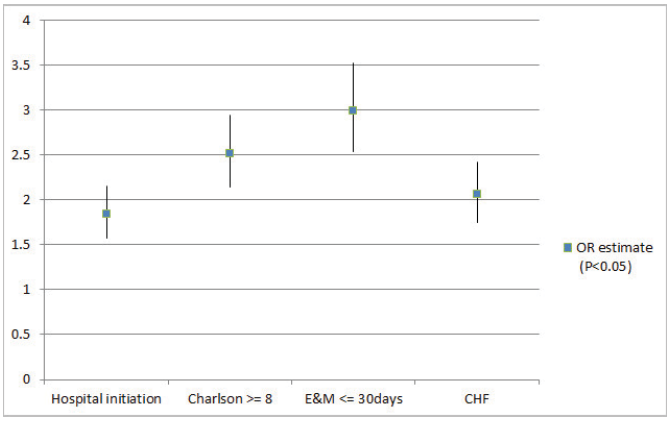
Predictors of 30-Day Readmissions among Incident Dialysis Patients: Registry Linkage to United States Renal Database System (USRDS) Data

LaTonya J. Hickson,[1] Bjoerg Thorsteinsdottir,[2] Megan Reinalda,[2] Priya Ramar,[2] Amy W. Williams,[1] Robert C. Albright,[1] Macaulay A. Onuigbo,[1] Andrew D. Rule,[1] Nilay D. Shah.[2] *[1]Nephrology, Mayo Clinic; [2]Health Care Policy &Research, Mayo Clinic.*

Background: Nearly 35% of Medicare's ESRD dollars are spent on inpatient care, much due to readmissions (ReADM). Identifying risk factors associated with Re-ADM is crucial in planning for outcome optimization.

Methods: AIM: To determine the cause, frequency, and predictors of 30-day Re-ADM over the first 2 years of dialysis in a systemwide registry. **METHOD:** Review of events in incident dialysis patients continuing therapy for ≥90 days from 2000-2010 with linkage to USRDS Medicare claims (n=2985).

Results: Mean age was 63±16, 59% males, 50% initiated dialysis in the hospital, 57% heart failure (HF), Charlson score≥8 (41%), and diabetes ESRD-cause (36%). Over 1.6±0.6 years, 81% patients were hospitalized with a rate of 1.8 PPY. Mean time to hospitalization after initiating dialysis was 6±6 months. Mean time to 30 day ReADM was 13.6+8.5 days. While, 924 (31%) patients had at least one Re-ADM, 467 had ≥2 Re-ADM. Primary diagnoses for Re-ADM were cardiovascular (21%), vascular (14%), infectious (13%), gastrointestinal (14%), and endocrine (11%). Aftercare E&M visits post ReADM were performed ≤30 days in 66%. Hospital dialysis start, Charlson≥8, HF, and an E&M visit were associated with an increased risk of ReADM (below)while gender, diabetes-ESRD, and time from first hospitalization were not.



In multivariable analysis, hospital dialysis start (OR 1.5, CI 1.3-1.8; p <0.001) and Charlson≥8 (OR 2.3, CI 1.9- 2.7; p <0.001) were the only independent predictors of readmission.

Conclusions: 30-day readmissions are common, especially within the first year of dialysis initiation. Hospital dialysis starters and those with multiple comorbidities appear to be at highest risk. Targeted interventions to reduce readmissions are needed.

TH-PO864

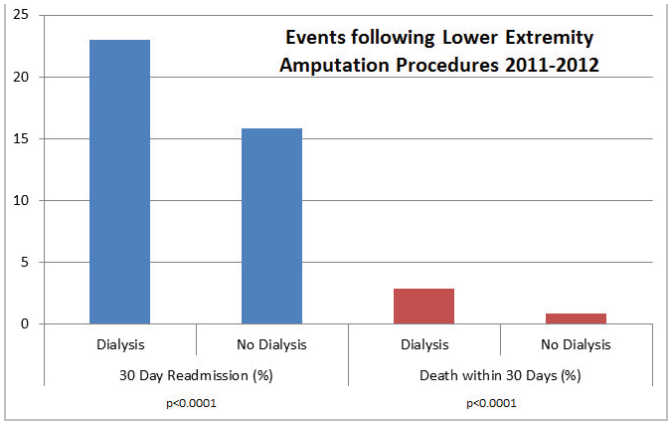
Increased Risk of 30-Day Readmissions following Lower Extremity Amputations among Dialysis Patients: American College of Surgeons National Surgical Quality Improvement Program Data (ACS-NSQIP)

LaTonya J. Hickson,[1] Andrew D. Rule,[1] Nilay D. Shah,[2] Bjoerg Thorsteinsdottir,[2] Raymond C. Shields,[3] Mark D. Fleming,[4] Daniel S. Ubl,[2] Amy W. Williams,[1] Elizabeth B. Habermann.[2] *[1]Nephrology, Mayo Clinic; [2]Health Care Policy and Research, Mayo Clinic; [3]Cardiology, Mayo Clinic; [4]Vascular Surgery, Mayo Clinic, Minnesota.*

Background: Dialysis patients have a heightened risk of lower extremity (LE) amputation. Identifying risk factors for readmissions (ReADM) and postoperative complications is crucial in care planning for optimizing outcomes following lower extremity (LE) amputation.

Methods: AIM: To determine the rate of 30-day ReADM and death following LE amputation based on dialysis-dependency. **METHODS**: Review of outcomes in all patients receiving thigh/femur and tibia/fibula amputation in the ACS-NSQIP database from 2011-2012. Patients with index length of stay >20 days were excluded.

Results: Of 5,346 patients (mean age 66±14, 37% females), 911 (17%) were dialysis dependent and 53% underwent thigh amputation. Comorbidities included: American Society of Anesthesiologists Physical Status Classification System (ASA) score ≥4 (39%), heart failure (HF) (6%), wound indication (12%), and history of revascularization or amputation (60%). Dialysis patients had more frequent ReADM and death events within 30 days of procedure.



In multivariable analysis, dialysis (OR 1.5, CI 1.22-1.76; p<.0001), ASA score ≥4 (OR 1.3, CI 1.09-1.48; p=0.002), HF (OR 1.5, CI 1.3-1.95; p=0.005), and female gender (OR 1.2, CI 1.02-1.37; p=0.03) were independent predictors of ReADM while age, race, prior revascularization, thigh amputation, and wound indication were not.

Conclusions: Independent of comorbidities, dialysis dependence is an important predictor of 30-day readmission and early death following LE amputation. Interventions aimed specifically at reducing perioperative morbidity in this high-risk population may be beneficial.

TH-PO865

Reductions in Hospital Admissions Associated with Interventions for Patients Predicted to Be at High-Risk for Hospitalization

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Background: Frequent hospital admissions in dialysis patients (Pts) are linked to increases in morbidities and mortalities. The aim of this pilot study was to investigate the potential impact of a high risk predictive model (PM) and clinical intervention program that was designed to reduce hospital admissions in dialysis Pts.

Methods: Six RRI dialysis facilities were utilized from March to October of 2013. Hospitalization rates were measured 90 days before and after initiating the program. Four of the dialysis facilities had high risk PM Pt identification and clinical interventions implemented, and two were controls. PM was performed to identify and target high risk Pts using clinical parameters and facility personnel assessments. Interdisciplinary facility personnel teams were deployed to design and implement clinical and non-clinical interventions.

Results: The high risk PM identified 44 Pts at four facilitates. Targeting of Pts and performing clinical interventions for 90 days was associated with a 44% reduction in hospital admissions and a decrease of 43% in hospital days, as compared to 90 days prior to any interventions. Control dialysis facilitates were monitored during the same time period; hospital admission rates were observed to have decreased by 17%, and hospital days were reduced by 15%. Accounting for concurrently matched controls, this high risk PM and intervention pilot study was associated with a 27% reduction in hospital admissions and a decrease of 28% in hospital days.

Conclusions: Predictive analytics can assist in identifying Pts at higher risk of hospitalizations. This process has the potential to aid renal care teams in understanding which Pts are at a priority to target for preventive interventions. Our project suggests that targeting of and interventions for Pts at higher risk of hospital admissions are associated with a significant reduction in hospitalizations.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

TH-PO866

Hemodialysis Patient Emergency Department Visits Frequently Result in Admission to Hospital with a High Proportion to Critical Care Areas

Eduard A. Iliescu, Frances Macleod, M. Khaled Shamseddin, Alexander R. Morton. *Kingston General Hospital, Kingston, ON, Canada.*

Background: Previous studies suggest that there is excessive use of the Emergency Department (ED) by Hemodialysis (HD) patients, however recent literature on the topic is lacking. The objective of this study is to measure the frequency of ED visits by HD patients and to examine their diagnoses and disposition.

Methods: This is a retrospective cohort study of prevalent HD patients in the Region of Southeastern Ontario. The study period was 6 months (January 1 to June 30, 2013). Patients were censored if they died, transferred out, recovered or were transplanted. Kingston General Hospital (KGH) is the only in-center regional dialysis program in the region. The primary variable was the frequency of visits to the KGH ED calculated as number of visits per patient-year. The other variables were diagnosis and disposition. The data were extracted electronically from the hospital ED database using unique patient KGH identifier.

Results: There were 429 patients, mean (SD) age 65 (15) years, 196 (46 %) female, 298 (69 %) satellite patients for a total observation time of 189 patient-years. During the observation time there were 306 ED visits for a frequency of 1.62 visits per patient-year. The visits occurred among 153 (36 %) of the HD patients (range 1 - 12 visits per patient). Of the 306 visits 115 (38 %) led to hospital admission, and of these 43 (37 %) were to critical care areas. The most frequent diagnoses were infection (22 %), cardiac (14 %), and bleeding unrelated to HD access (9 %).

Conclusions: The results of this study suggest the use of the regional center ED by HD patients is relatively low at 1.62 visits per patient-year, and that these visits are justified with a high proportion of them resulting in hospital admission often to critical care areas. The strength of this study is the regional respective excluding selection bias. The main weakness is that the study does not include visits to other types of urgent health care institutions or primary care physicians, however the objective of the study was to examine the use of the regional center ED by HD patients and not their use of health care in totality.

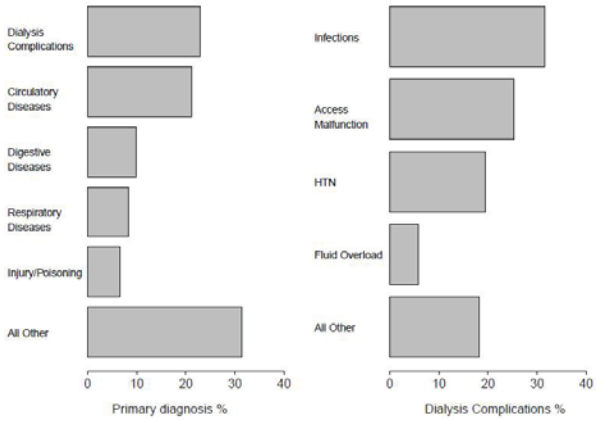
TH-PO867

Real Time Monitoring of Reasons for Inpatient Admissions among Maintenance Dialysis Patients Janet J. Singh, Fanna Liu, Jesse D. Schold, Susana Arrigain, Victoria Konig, Robert J. Heyka, Joseph V. Nally, Sankar D. Navaneethan. *Medicine, Quantitative Health Sciences and Nephrology, Cleveland Clinic.*

Background: Hospitalization rates in the dialysis population are associated with high morbidity and expenditures. We developed an inpatient dialysis registry that is being updated regularly and studied the rates and reasons for inpatient hospitalization in our health system.

Methods: We created an electronic health record (EHR) based dialysis registry that included adult patients on maintenance dialysis and met the following criteria: a) at least one overnight in-patient hospitalization, b) no previous transplantation, c) had no diagnosis for acute kidney injury within 8 weeks and d) billing details from the hospital records. Reasons for inpatient hospitalizations were categorized based on Clinical Classification System developed by the Agency for Healthcare Research and Quality.

Results: Our registry includes 6,247 maintenance dialysis patients with a total of 25,478 admissions (from January 2006 to May 2014). Mean age was 62.8 +/- 15.1 years, with 56% men. Median number of hospitalizations per patient was 2. Among the reasons of hospitalizations, dialysis related complications (23%) followed by cardiovascular diseases (21%) and gastrointestinal diseases (10%) were the leading causes. Among dialysis related hospitalizations. infection related admissions (32%) followed by dialysis access malfunction and hypertension related disorders were the common reasons for inpatient admissions.



Conclusions: In maintenance dialysis patients, dialysis related complications and cardiovascular disease accounts for half of inpatient admissions. Such real-time recognition of hospital admissions via EHR based registry may assist in developing programs to prevent rehospitalization in this high risk population.

Funding: Private Foundation Support

TH-PO868

Hospital Readmissions within 14 Days and 30 Days of Discharge: Differing Risks in Hemodialysis Patients Piangwarin Phaosawasdi,[1] Andrew I. Chin.[1,2] *[1]Dept of Medicine - Div of Nephrology, Univ of California, Davis School of Medicine, Sacramento, CA; [2]Dept of Medicine - Div of Nephrology, VA Health Systems, Mather Field, CA.*

Background: Patients on long-term hemodialysis (HD) have a high hospitalization and readmission rate. We postulate that factors associated with <14 day readmissions differ from those associated with <30 day readmissions.

Methods: Number of days from index hospitalization discharge to readmission were determined via CQI data over 2 years in adult HD patients. Factors analyzed before and after index hospitalization included: demographic data at the time of index hospitalization; HD vital signs before hospitalization and after discharge; HD access; serum albumin and hemoglobin before hospitalization; reason for admission; length of stay; and pre-existing co-morbidities.

Results: In a prevalent population of 351 HD patients, there were 1022 hospitalizations in 281 patients, 30 (11%) of whom were readmitted within 14 days (mean of 7.2 days from prior discharge) and 54 (20%) were readmitted within 30 days. Approximately 30% of patients were readmitted for a similar diagnosis as their index hospitalization, regardless of time to readmission. Using logistic regression analysis, significant factors associated with readmission included:

Factor	Odds for readmission	CI (95%)
14-day readmission		
Vintage	1.010	1.001 to 1.018
CHF co-morbidity	5.78	1.650 to 20.25
Serum albumin	0.33	0.12 to 0.93
Higher Systolic BP	0.96	0.95 to 0.98
Infection as admission Dx	0.034	0.002 to 0.535
30-day readmission		
CHF co-morbidity	2.28	1.021 to 5.099
Higher Systolic BP	0.98	0.97 to 0.99

We also looked at vital signs before and after hospitalization and noted that pre-HD systolic BP was significantly higher in those who did not get readmitted within 14 or 30 days. Pre-hospitalization weights were higher than post hospitalization weights, with no difference between <14 and <30 day groups.

Conclusions: Adult HD patients have a high rate of hospitalization and readmission. Factors associated with <14 day readmission versus <30 day readmission differ. Aggressive management of HD treatment parameters may decrease readmission rates; however, we found some factors that may not be easily modifiable.

Funding: Clinical Revenue Support

TH-PO869

The Clinical Outcomes in the Six Months after Initiation of Dialysis among Patients with Chronic Kidney Disease: A Nationwide Observational Study Hung-Bin Tsai,[1] Hsin-Ming Lu,[2] Likwang Chen.[2] *[1]Div of Hospital Medicine, Dept of Traumatology, National Taiwan Univ Hospital, Taipei City, Taiwan; [2]Inst of Population Health Sciences, National Health Research Insts, Miaoli County, Taiwan.*

Background: Prediction of survival and outcomes among chronic kidney disease (CKD) patients, especially those with geriatric multi-morbid condition is important for clinical shared decision-making.

Methods: We conducted a population-based cohort study based on the Taiwan National Health Insurance Research Database from 2000 to 2010. The CKD status was adopted at least one hospital stay or at least three outpatient visits with CKD diagnosis in one year immediately before the index day of dialysis initiation. The incident CKD dialysis patients were selected from those without any dialysis for at least two years before this index dialysis. The primary outcome is short-term mortality within 6 months of dialysis vintage. We applied the Cox proportional hazard and logistic regression model stratified by gender, age, or selected morbid conditions then performed post-estimation simulations to depict outcomes among selected patient subgroups.

Results: 50,276 incident CKD dialysis patients (49.48% male) were underlying 54% over 65 years, 6.8% malignancy including 10 reported metastasis, 5.21% severe heart failure, 1.65% severe stroke, 0.88% advanced liver cirrhosis, 0.48% organ transplant, 0.4% using mechanical ventilation at least 4 months, and 0.35% severe obstructive airway disease. The top six characteristics contributing to 1-month, 3-month, and 6-month mortality were those with prolonged mechanical ventilation (25.13%, 56.78%, 77.39%), cancer metastasis (40%, 40%, 70%), lymphoma (29.51%, 50%, 63.11%), advanced liver cirrhosis (27.5%, 51.36%, 60.91%), esophagus cancer (24%, 48%, 60%) and aged over 85 (25.67%, 56.78%, 58.88%) respectively.

Conclusions: Incident CKD dialysis patients with late stages of malignancy, advanced liver cirrhosis, oldest old had poor prognosis, and ventilator dependence had particularly worse outcome. Palliative dialysis or renal palliative care may be optional for both healthcare authority and providers to offer CKD patients with comprehensive holistic care.

Funding: Government Support - Non-U.S.

TH-PO870

Reducing Hemodialysis Therapy Non-Adherence: A Social Worker Initiated Program Stephanie Johnstone, Nien-Chen Li, Franklin W. Maddux, Amy R. Weissman-Hunt, Jessica Demaline, Eduardo K. Lacson. *Fresenius Medical Care, North America, Waltham, MA.*

Background: Missed hemodialysis (HD) treatments associate with poor outcomes. As a quality improvement (QI) project, a social worker (SW) initiated intensive program was implemented to improve treatment adherence.

Methods: The cohort included 348 patients completing ≥6 of the 8 session intervention (1 session every 1-2 weeks) from 195 FMCNA centers between 2/1/13 and 10/30/13, with at least a 6-month baseline and 6-month follow-up period. The intervention included patient-empowerment education and cognitive/behavioral counseling designed to address causes of non-adherent behavior and aggravating factors from 5 surveys: CESD-10, Stressors Screen, Sleep Quality Screen, and KDQOL-36. Pre- and Post-intervention survey scores were compared using paired t-tests, as well as 6-month baseline versus follow-up rate of missed treatments.

Results: The patients' mean age was 54 years, with 47% males; 61% white, 35% black; 56% had DM, 17% CAD, and 36% CHF. The baseline versus follow-up missed treatment rate was 146 versus 127 per 100 patient-months (p=0.008). Significant improvement (all p<0.05) of pre- to post-intervention survey scores were: Declines in CESD-10 (10 versus 7.5), Financial/Insurance Stressor (5.0 versus 4.3), Family/Relationship Stressor (4.7

versus 3.7), Health Symptoms Stressor (5.0 versus 4.6), Loss/Grief Stressor (2.7 versus 2.3), Difficulty Falling Asleep (4.3 versus 3.9), Difficulty Staying Asleep (4.5 versus 3.8), Difficulty Awakening (2.5 versus 2.1), Interrupted Sleep (4.4 versus 3.7), and Restless Legs (3.5 versus 3.0); with increases in PCS (35.7 versus 37.7), MCS (46.3 versus 49.2), Burden (60 versus 65.8), Symptoms (69.6 versus 76.7), and perception of Kidney Disease Effects (54 versus 62.6).

Conclusions: Preliminary results indicated that the intensive SW-initiated program reduced missed treatments in the short term (6 months). Furthermore, indicators of quality of life and well-being that potentially contributed to the non-adherent behavior also improved, which may help sustain the favorable results over the long term. This QI study is ongoing and updated outcomes will subsequently be reported.

TH-PO871

Dramatic Cost Reduction in a Globally Integrated ESRD Care Model: Population Management at the Top of the Care Pyramid Robert C. Albright, Kathryn Zavaleta, Jeffrey Sigrist, John J. Dillon, Ziad El-Zoghby, James T. McCarthy, Bradley D. Wick, Robert Warda, Jerilyn Sue Wilson, LaTonya J. Hickson, Amy W. Williams. *Div of Neph & HTN, Mayo Clinic, Rochester, MN.*

Background: Improving the value of care for patients with ESRD, improving outcomes while decreasing costs, requires focus on global care processes. Data from integrated care systems for ESRD patients capturing all costs along the trajectory of disease and across care sites are lacking, yet are crucial determinates of effective care. In 2010 an institution-wide project Reengineering Dialysis(RED) (CKD through renal replacement and death) began, emphasizing standardization of best practices including:robust patient education, smooth care transitions, pharmacy and palliative care partnerships.

Methods: Three years of global cost data was analyzed for an incident ESRD population whose entire care was provided by an academic multispecialty practice. Data were provided from the institutional Decision Support System, capturing all patient costs for the population studied from 2011(baseline)-2013. Costs per day reflect total cost of care divided by total calendar days of chronic dialysis care stated as % 2011(baseline) cost and were adjusted for inflation.

Results:

	2011	2012	2013	2011 vs 2013 (%)	P value (Mood median)
Number of Patients	191	181	182		
Age median	66.4	65.4	64.3		
Males (%)	116 (60.7)	110 (60.7)	111 (61.7)		
Global costs/day (*inflation adjusted stated as % of 2011 costs)	100.0	83.23	78.44	-21.6	0.004
Inpatient costs/day (*)	100.0	71.74	78.75	-21.5	0.014
Outpatient costs/day (*)	100.0	105.91	88.45	-11.6	0.024
n hospitalized (%)	129 (67.5)	108 (59.7)	100 (54.9)	-22	0.04 (Fisher exact)

Conclusions: A dramatic decrease in global costs of care among a cohort of ESRD patients exclusively managed by a multispecialty academic medical center was realized by designing and implementing a patient-centered population management strategy. This study demonstrates the value of downstream effects from managing the continuum of care for CKD and may serve as the future model of population care management, particularly in complex chronic diseases as the reimbursement structures for healthcare in our society change.

Funding: Clinical Revenue Support

TH-PO872

Survival, Convection Volume and Treatment Time in Hemodiafiltration: A Substudy of CONTRAST Camiel L.M. de Roij van Zuijdewijn,[1] Menso Jan Nubé,[1,2] Peter J. Blankestijn,[3] Pieter M. Ter Wee,[1,2] Renee Levesque,[4] Michiel Bots,[5] Muriel P. Grooteman.[1,2] *[1]Nephrology, VU Univ Medical Center, Amsterdam, Netherlands; [2]Inst for Cardiovascular Research, VU Univ Medical Center, Amsterdam, Netherlands; [3]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [4]Nephrology, Centre Hospitalier de l'Univ de Montréal, Montréal, QC, Canada; [5]Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Netherlands.*

Background: In hemodialysis (HD) patients, survival is positively influenced by treatment time. In hemodiafiltration (HDF) patients, it was recently shown that a high convection volume (CV>21.95L) is associated with a lower mortality as compared to patients treated with HD. Treatment time, however, is one of the major determinants of CV. The present study was designed to investigate whether the survival benefit of high CV HDF is mainly due to the magnitude of the CV itself or to treatment time.

Methods: Patients from CONTRAST (NCT 00205556) were subdivided into 4 groups: HD and 3 tertiles of CV in HDF (<18.18L, 18.18-21.95L and >21.95L). Death was used as end point. Patients were censored if alive at the end of follow-up, at renal transplantation, at the occurrence of a non-fatal adverse event or moving to a non-participating center. Using Cox regression, the effect of CV on survival was investigated in 3 models: (1) crude analysis, (2) adjusted for confounders and (3) as in model 2 and adjusted for treatment time. Any change in HRs between models 2 and 3 results from treatment time as this variable is the only difference. 95% CIs of models 2 and 3 were compared to investigate if the survival benefit of high CV HDF depends on treatment time.

Results: 700 patients were analyzed. Baseline characteristics did not differ between high CV HDF patients (n=115) and HD patients (n=356). Crude analysis showed a HR of 0.61 (p=0.02) for death in high CV HDF compared to HD. HR of high CV HDF in the model adjusted for confounders was 0.62 (p<0.05) and in the model adjusted for confounders plus treatment time 0.62 (p<0.05). No difference was observed between the two models.

Conclusions: The survival benefit of high CV HDF over HD is independent of treatment time.

TH-PO873

Extended Treatment Time on Hemodialysis: Effect on Hydration and Nutritional Status Seema Singh,[1] Lina Johansson,[1] Albert J. Power,[2] Charles D. Pusey,[1] Neill D. Duncan,[1] Edwina A. Brown.[1] *[1]Imperial College Hospital NHS Trust, United Kingdom; [2]North Bristol NHS Trust, United Kingdom.*

Background: The association of nutritional status on mortality is established. However the effect on nutritional status by extending Treatment time (TT) on in-centre day HD is unknown.

Methods: Randomised cross-over study of 29 in-centre HD patients with extended TT of 6 hr or standard TT of 4 hr for a period of 24 wks with a 4wk washout period between the two arms (NCT01721421). Nutritional status was measured using hand grip strength (HGS), bioimpedance spectroscopy (BIA) and Malnutrition inflammation score (MIS).

Results: Results are summarised below.

	Pre 6 hr	Post 6 hr	p value	Pre 4 hr	Post 4 hr	p value
Extracellular water (Kg)	17.6 +/- 2.9	18.2 +/- 3.1	0.33	17.5 +/- 2.9	18.4 +/- 2.7	<0.01
Dry Weight (Kg)	77.3 +/- 11	76.7 +/- 12	0.39	76.8 +/- 14	77.4 +/- 14	0.26
Malnutrition Inflammation Score	5.6 +/- 2.1	4.7 +/- 1.2	0.06	4.9 +/- 1.5	5.1 +/- 1.7	0.56
Serum phosphate (g/dl)	1.67 +/- 0.5	1.27 +/0.5	<0.0001	1.51 +/- 0.5	1.94 +/- 0.6	<0.01
Serum albumin (g/dl)	35.2 +/- 4.9	36.5 +/- 4.9	0.17	36.2 +/- 3.8	36.7 +/- 4.6	0.57
Hand Grip Strength (Kg)	18.6 +/- 9.9	20.3 +/- 9.4	0.21	18.3 +/- 9.3	18.0 +/- 7.8	0.83

29 HD patients (27 male, mean age 65.2 ± 15.2 years, pre study TT 4.6 ±0.4hrs) were assessed.There was a significant increase in extracellular water (ECW) on the 4 hr arm which may have masked a decline in flesh weight. On the 6 hr arm, there was no change in hydration status, but there was a trend towards improved nutritional status with a lower MIS (p=0.06). There was also a significant reduction (p<0.0001) in serum phosphate levels without increased phosphate binder use, despite matching blood volume processed to that of the 4 hr arm.

Conclusions: This randomised crossover study shows increased ECW during 4 hr TT and some improvement in nutritional markers during 6 hr TT. Further studies are needed to more robustly investigate the presence of a nutritional effect.

TH-PO874

Extended Treatment Time on Hemodialysis Is Associated with Improved Blood Pressure Control and Reduced Time to Recovery Seema Singh,[1] Albert J. Power,[2] Charles D. Pusey,[1] Neill D. Duncan,[1] Edwina A. Brown.[1] *[1]Imperial College Hospital NHS Trust; [2]North Bristol NHS Trust.*

Background: Observational studies and registry data suggest improved clinical outcomes with extended treatment time (TT) on haemodialysis (HD). This has not been rigorously studied for in-centre day time HD. We report the effects of extended TT on blood pressure (BP), time to recovery (TTR) and dialysis related parameters.

Methods: Randomised cross-over study of 29 in-centre HD patients with extended TT of 6 hrs or standard TT of 4 hrs for a period of 24 wks with a 4wk washout period between the two arms (NCT01721421).

Results: Results are summarised below.

	Pre 6 hr	Post 6 hr	p value	Pre 4 hr	Post 4 hr	p value
TTR (min)	425 +/-284	134 +/- 200	<0.001	382 +/- 319	505 +/- 524	0.07
MAP (mmHg)	103 +/- 17	93 +/- 12	<0.05	99 +/- 12	104 +/-15	<0.05
UFR (ml/min/Kg)	8.2 +/- 2.4	6.1 +/- 1.8	<0.001	9.2 +/- 1.4	9.3 +/- 2.1	0.97
IDWG (Kg)	2.3 +/- 1.0	2.2 +/- 1.2	0.92	2.2 +/- 0.8	2.5 +/- 1.3	0.22
BP Drugs (n)	1.6 +/- 1.2	0.8 +/- 1.2	<0.001	1.5+/- 1.3	1.5 +/- 1.3	0.58

29 HD patients (27 male, mean age 65.2 ± 15.2 years, pre study TT 4.6 ±0.4hrs)were assessed.Pre dialysis mean arterial pressure (MAP) was significantly reduced on the 6 hr arm accompanied by a significant decrease in the number of BP drugs. Intra- dialytic weight gain (IDWG) was not significantly changed on either treatment arm, but ultra-filtration rate (UFR) was significantly lower on the extended TT arm. This could explain the significantly lower TTR during the 6 hr arm.

Conclusions: This randomised trial confirms the positive effects of extended TT on BP control achieved with lowered UFR and less reliance on antihypertensive agents. The improvement in TTR may serve to promote the acceptability of extended TT to in-centre HD patients.

TH-PO875

Mortality Risk for Incident Hemodialysis Patients in Beijing Area: A Retrospective Cohort Study Xinju Zhao, Mei Wang, Li Zuo. *Nephrology, Peking Univ People's Hospital, Beijing, China.*

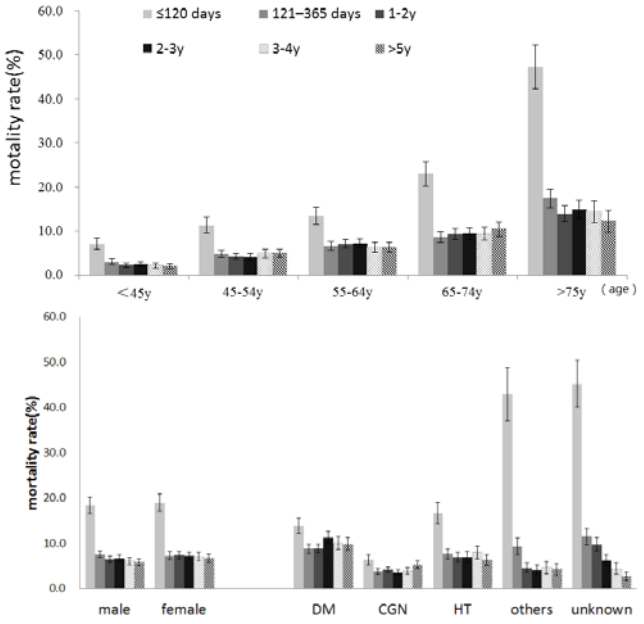
Background: Studies have shown that mortality is high for maintenance hemodialysis (MHD) patients, especially in their early dialysis stage. Here we demonstrate data from Beijing Hemodialysis Quality Control and Improvement Center (BJHDQCIC) to determine the mortality risk and pattern for incident MHD patients.

Methods: this study is based on reviewing all the incident MHD patients recorded in BJHDQCIC register system from Jan 1st2007 to Dec 31st 2012, with follow-up to the end of 2013. Their demographics, mortality, primary causes are investigated. Patients are divided into 6 groups according to their survival length, that is, whether or not died in Period (P) 1-6 (<120, 121–365 days; 1-2, 2-3, 3-4 and >5years) after initiation of dialysis. Stratified analysis by sex, age and primary disease are used.

Results: During a 6-year inclusion period, there are 11,955 patients. The patient characteristics are listed below.

follow-up time (months)	24.6±20.8
Age (mean)	57.7
18-44	22.0%
45–54	19.8%
55–64	21.8%
65–74	20.3%
≥75	16.2%
Male	56.4%
Primary causes (%)	
Diabetes(DM)	29.5
Chronic glomerulonephritis(CGN)	26.9
Hypertension(HT)	19.1
Unknown and others	24.5
Deaths	2561

The overall mortality rate is 8.2 per 100 patient-years. The rates are 7.9, 8.5 for male and female. Motality rates are 18.7, 7.5, 6.9, 6.9, 6.5 and 6.2 respectively in period 1-6. Higher mortality is observed in patients who are older, female and with other or unknown causes of ESRD. CGN group has the lowest rate. The early (P1) mortality constitutes 57.6% of the 1-year mortality and 25.2% of all death.



Conclusions: The overall mortality is relatively low in Beijing incident MHD patients. Generally, the mortality increases with age and varies among different etiology groups. Long-term survival is stable after the early dialysis period. Strategic improvement can be achieved by decreasing the early mortality rate.

Funding: Government Support - Non-U.S.

TH-PO876

Dialysis Modality and Mortality in Elderly Patients: Korean National Database and Meta-Analysis Seung Seok Han,[1] Jae Yoon Park,[1] Kyung Don Yoo,[1] Dong-Ryeol Ryu,[2] Hyunwook Kim,[3] Kwon Wook Joo,[1] Chun Soo Lim,[4] Yon Su Kim,[1] Dong Ki Kim.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Internal Medicine, School of Medicine, Ewha Womans Univ, Seoul, Korea; [3]Internal Medicine, Wonkwang Univ College of Medicine, Gyeonggi-do, Korea; [4]Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea.*

Background: Identifying the appropriate choice between hemodialysis (HD) and peritoneal dialysis (PD) is an unresolved issue in elderly patients with end-stage renal disease, who are at high risk for mortality but have a low chance of receiving kidney transplantation.

Methods: Data on 13065 elderly Korean patients (HD, 10675; PD, 2390) were obtained from the Korean Health Insurance dataset. Multiple statistical approaches, including the multivariate Cox model, propensity score-adjusted model, marginal structural model, and one-to-one matching model were used to compare mortality between PD and HD patients. We then used a meta-analysis to provide a pooled hazard ratio (HR) based on previous comparison studies as well as the present study.

Results: During the follow-up period of 1.8 years (max. 5 years), the PD group had a higher mortality rate than the HD group [HR, 1.20 (1.125–1.275); $P < 0.001$ by multivariate Cox model]. The discrepancy between the two modalities was greater in the presence of certain conditions, such as diabetes mellitus or longer dialysis duration. These trends were similar across several statistical models. In the meta-analysis, 15 studies involving over 631421 elderly patients were reviewed. Compared with HD, the pooled HR of PD was 1.10 (1.008–1.198). When the meta-analysis was stratified by confounding factors, the survival benefit from HD was shown to be particularly strong in subgroups that had diabetes mellitus, had long dialysis durations (> 1 year), or contained cohorts starting dialysis in the 1990s.

Conclusions: Korean national data and a meta-analysis suggest a higher risk of mortality in elderly patients on PD than HD. A more precisely tailored choice of dialysis modality is needed under certain conditions.

TH-PO877

Early Mortality on Dialysis. Data from Emilia-Romagna Dialysis Registry Elena Mancini, Antonio Santoro. *Nephrology Dialysis Hypertension, Teaching Hospital Policlinico S.Orsola Malpighi, Bologna, Italy.*

Background: The mortality rates of chronic dialysis patients are much higher than those of the general population. Many Dialysis Registries do not include patients who die in the first three months of replacement therapy, so the actual mortality rate is highly underestimated, particularly as concerns the first few months of therapy. Increasing age and comorbidity of the incident dialysis patients instead greatly worsen the risk of early death.

Methods: Emilia-Romagna is a region of northern Italy, with nearly 4,500,000 inhabitants. The Dialysis Registry was established in 1994; at Dec 31, 2012 the prevalence on dialysis was 734.3 pmp (2,955 patients on HD and 324 patients on DP). Any patient entering on a chronic dialysis program is recorded in the Registry from the start of the substitution therapy. We analyzed the mortality of incident dialysis patients over 3 years (2009-2011), focusing on crude EARLY MORTALITY, defined as death occurring during the first 12 months of dialysis (the early mortality of a certain year thus includes both the incident patients of that year and the incident patients of the previous year).

Results: Early mortality was found for as many as 524 out of 1,714 patients who died in the period 2009-2011 (HD+PD), corresponding to nearly one third (30.6%) of the overall mortality. Most of the patients dying early (63.6%) were over 75 years of age. More than half (52.6%) of the patients with early mortality actually died before the third month of dialysis treatment. Nearly half of the patients on HD who died early had a temporary vascular access, often secondary to a late nephrological referral. The Karnofsky Score shows that most of the patients presented a medium-high level of inability. Cardiovascular events were the main causes of death (46.3%), especially for those patients who died in the first three months (54.9%).

Conclusions: Early mortality is a dramatic phenomenon that can be captured only by those Dialysis Registries recording all the patients incident to dialysis, independent of the survival period. The possibility of an early death should be discussed with the families of the older and more comorbid patients before starting up a chronic dialysis program.

Funding: Clinical Revenue Support

TH-PO878

Excess Mortality Among HD Patients Compared with the General Population: Leading Causes of Death and Demographic Risk Factors Using Multiple Causes of Death Eliezer Golan,[1,2] Carmit Libruder,[3] Tamy Shohat.[2,3] *[1]Meir MC; [2]Sackler Faculty of Medicine Tel-Aviv Univ; [3]IsraelCDC.*

Background: Multiple cause of death analysis is particularly relevant for assessing patterns of mortality in patients with chronic diseases, such as dialysis patients. We compared cause-specific mortality rates among HD patients with the Israeli general population and measured the risk associated with HD treatment, adjusted for age, sex, time periods and population group (Jews/Arabs).

Methods: The study cohort included incident HD patients aged >20 from the Israeli End Stage Renal Disease Registry, treated between1990-2008. Multiple causes of death were available for the years 1998-1999, 2003 and 2007-2008.Mortality data for the general population were obtained from the Central Bureau of Statistics. Causes of death were coded

according to ICD-10.We used direct standardization to correct for age differences between patients and the general population. Cause-specific mortality rates per 100 person-years were calculated and a negative binominal regression was used to determine the effects of established risk factors on mortality in HD patients, compared with the general population.

Results: 2,646 HD patients aged ≥45 died during the follow-up period. The age adjusted mortality rates were 9.4, 6.1, 5.8, and 2.5 times higher in patients compared with the general population, for heart disease, infectious diseases, diabetes and CVA respectively. Female sex was significantly associated with increased mortality risk from heart disease, diabetes, CVA and infectious diseases (HR=1.28, 1.30, 1.36 and 1.55 respectively). Arabs had a significantly decreased mortality risk from heart disease, diabetes and infectious diseases (HR=0.59, 0.63 and 0.78 respectively). The differences in mortality rates between patients and the general population decreased with age.

Conclusions: As expected, mortality from heart disease, infectious diseases and diabetes is markedly higher among HD patients compared with the general population. Further research is needed to explore survival advantage among Arabs and survival disadvantage among females on HD, with particular attention to mortality by infectious diseases.

Funding: Government Support - Non-U.S.

TH-PO879

Improved Survival Rate of Korean Patients Initiating Dialysis in 2008

Dong-Ryeol Ryu,[1] Hyunwook Kim,[2] Hee Sung Ko,[1] Shina Lee,[1] Jung-Hwa Ryu,[1] Mina Yu,[1] Seung-Jung Kim,[1] Duk-Hee Kang,[1] Kyu Bok Choi.[1] *[1]Dept of Internal Medicine, School of Medicine, Ewha Womans Univ, Seoul, Korea; [2]Dept of Internal Medicine, Wonkwang Univ College of Medicine Sanbon Hospital, Gunpo, Korea.*

Background: The aim of this study is to investigate whether survival rate among Korean dialysis patients was significantly improved during the period between 2005 and 2008, and to compare the survival rate between HD patients and PD patients initiating dialysis in 2008 in Korea.

Methods: A total of 32,357 patients who began dialysis between January 1, 2005 and December 31, 2008 were eligible for analysis. Baseline demographics, comorbidities and mortality data were obtained from the database of the Health Insurance Review and Assessment Service.

Results: Kaplan-Meier curves according to the year of dialysis initiation showed that the survival rate was significantly different (log-rank test, $P = 0.005$), most notably among PD patients ($P = 0.000$), but not among HD patients ($P = 0.497$). In multivariate analysis, however, patients initiating either HD or PD in 2008 also had a significantly lower risk of mortality compared to those who began dialysis in 2005. Subgroup survival analysis among patients initiating dialysis in 2008 revealed that survival of PD patients was significantly higher than HD patients ($P = 0.001$), and the survival benefit of PD over HD remained in non-diabetic patients aged less than 65 years after adjustment of covariates.

Conclusions: Survival of Korean patients initiating dialysis from 2005 to 2008 has improved over time, particularly in PD patients. Younger non-diabetic patients may have a survival benefit of PD over HD.

TH-PO880

Development and Validation of a Modified Charlson Comorbidity Index to Predict Mortality in Incident Hemodialysis Patients: Result From a National Population-Based Database and a Nationwide Cohort in Korea

Jae Yoon Park,[1] Seung Seok Han,[1] Dong-Ryeol Ryu,[2] Hyunwook Kim,[3] Hajeong Lee,[1] Jung Pyo Lee,[4] Sejoong Kim,[5] Kwon Wook Joo,[1] Chun Soo Lim,[4] Yon Su Kim,[1] Dong Ki Kim.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Internal Medicine, School of Medicine, Ewha Womans Univ, Seoul, Korea; [3]Internal Medicine, Wonkwang Univ College of Medicine Sanbon Hospital, Gyeonggi-do, Korea; [4]Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea; [5]Internal Medicine, Seoul National Univ Bundang Hospital, Seoul, Korea.*

Background: Prediction of survival for patients with end-stage renal disease is difficult. The Charlson comorbidity index(CCI) has been used and validated to evaluate the influence of comorbidity on survival in patients with various kinds of diseases.The purpose of this study is to adjust the CCI in incident hemodialysis(HD) patients to develop a new scoring system;to validate in a separate cohort;and to compare its performance with the CCI.

Methods: We used data from a nationwide patient registry cohort to identify Korean citizens who received their first HD between 2005 and 2008(N=24,738). They were characterized by 15 comorbidities as used in CCI.The risk score was developed from coefficients of independent risk factors from Cox proportional hazard model.Findings were validated in a separate nationwide prospective cohort(N=1,309).

Results: Mortality correlated with the new scoring system(log-rank $P<0.001$).The appearance of survival curves across increasing comorbidity scores was similar for both indices,with anticipated worse survival for the higher scores($P<0.05$).The new scoring system showed higher ***c*** statistics than the CCI at 1,2,and 3 years follow-up($P<0.05$).The analyses using continuous net classification index revealed that the new scoring system improved the risk classification of mortality for 3 years since first HD started(25.8%, 29.8%, and 43.9%,$P<0.05$).The integrated discrimination improvement showed that the new scoring system improved the model performance at 2 and 3 years follow-up(0.009 and 0.016,$P<0.05$).

Conclusions: The new scoring system might predict mortality of incident HD patients more accurately than original CCI.

TH-PO881

Survival of Patients with Multiple Myeloma or Light Chain Amyloidosis on Chronic Dialysis: Data From the French REIN Registry from 2002 to 2011

Alexandre Decourt,[1] Bertrand Gondouin,[1] Jean Christophe Delaroziere,[2] Philippe Brunet,[1] Ariane Duval-Sabatier,[1] Stephane Burtey,[1] Noemie Jourde-Chiche.[1] *[1]Nephrology, Aix-Marseille Univ, Marseille, France; [2]Public Health, Aix-Marseille Univ, Marseille, France.*

Background: Multiple myeloma (MM) and light chain amyloidosis (LCA) with renal involvement can lead to end stage renal disease (ESRD). Few studies have focused on the prognosis of patients with MM or LCA on chronic dialysis. This study evaluated the survival of patients with MM or LCA on chronic dialysis in France.

Methods: Between 2002 and 2011, a total of 1459 patients with MM or LCA started chronic dialysis and were registered in the Renal Epidemiology and Information Network (REIN). We analysed survival censored for renal transplantation, recovery of renal function and loss to follow-up. Risk factors and causes of death were analyzed.

Results: A total of 860 (59%) patients with acute myeloma kidney, 265 (18%) with LCA and 334 (23%) light chain deposition disease (LCDD) were registered. Mean age was 70.5 years, and 56% were men. Within a median follow-up of 14.3 months, 977 (67%) patients died. Median survival on dialysis was 17.6 months. Causes of death were progression of hematologic malignancy in 348 (36%), infections in 119 (12%) and cardio-vascular in 102 (10%) patients. Risk factors of death were: age (OR = 1.05 [95%IC 1.03-1.073]), initiation of dialysis on a central venous catheter (OR = 1.84 [95%IC 1.05- 3.24]). Protective factors were: walking autonomy (OR = 0,44 [95%CI 0.29-0.66]), serum albumin (OR = 0.97 per g/L [95%CI 0.94-0.99]) and year of dialysis initiation (OR = 0.64 per year [95%CI 0.57-0.71]).

Conclusions: Survival of patients with MM and LCA on chronic dialysis is poor, but has improved over the past decade. Progression of hematologic malignancy is the main cause of death in this population.

TH-PO882

Enrollment of Hemodialysis (HD) Patients in an Integrated Pharmacy Management Program Is Associated with Reduced Mortality

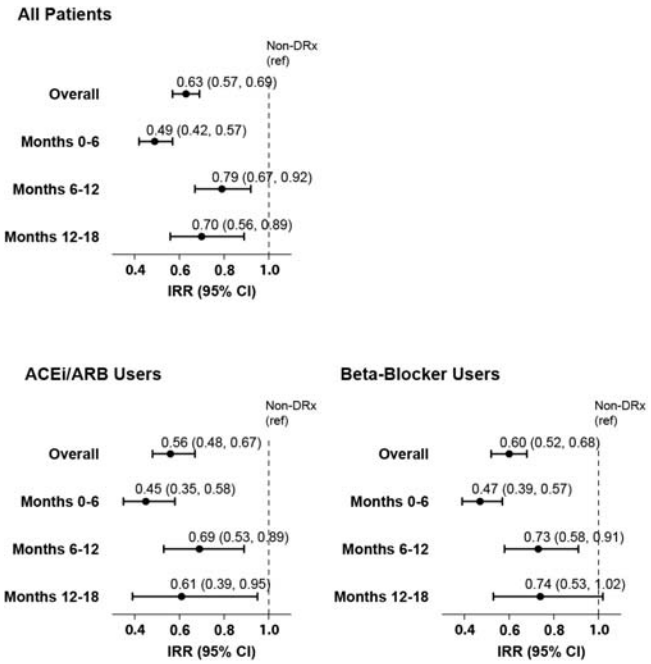
Steven M. Brunelli,[1] Steven M. Wilson,[2] May Hoang,[3] Erik Michael Bengtsson,[3] Tracy Furgiuele.[3] *[1]DaVita Clinical Research; [2]DaVita HealthCare Partners Inc; [3]DaVita Rx.*

Background: DaVita Rx is a specialty dialysis pharmacy service providing delivery of medications to patients' homes or dialysis facilities, prescription reviews, prior authorization assistance, and refill management. We assessed the impact of DaVita Rx enrollment on mortality of HD patients, overall and within subgroups of patients treated with different drugs.

Methods: Patients received in-center HD at DaVita from Jan 2011 to Sep 2012. DaVita Rx patients were considered from time of enrollment and were propensity score-matched (2:1) to eligible controls who had never enrolled. DaVita Rx patients were censored on disenrollment. We assessed mortality rates over all follow-up time and over the intervals 0-6, 6-12, and 12-18 months following index month. Comparisons were made between DaVita Rx and controls, overall and in subgroups defined by baseline use of ACEi/ARB and beta-blockers. Incidence rate ratios (IRR) were estimated using Poisson regression.

Results: A total of 4949 DaVita Rx patients were matched to 9898 controls. Mortality rate was lower for DaVita Rx patients versus controls over all study time (9.3 versus 14.9 deaths/100 pt-years; IRR 0.63; $p<0.001$). The protective association of DaVita Rx was more pronounced over months 0-6 than later time periods, but was potent and statistically significant for all time periods. The protective association of DaVita Rx was more pronounced among ACEi/ARB (IRR 0.56; $p<0.001$) and beta-blocker users (IRR 0.60; $p<0.001$) than for the overall cohort. Temporal patterns were similar to the overall cohort.

Conclusions: DaVita Rx enrollment was associated with 37% lower mortality over the 18-month study. The association between DaVita Rx and improved survival was more potent among subgroups of ACEi/ARB and beta-blocker users than for the cohort overall.



Funding: Pharmaceutical Company Support - DaVita Rx

TH-PO883

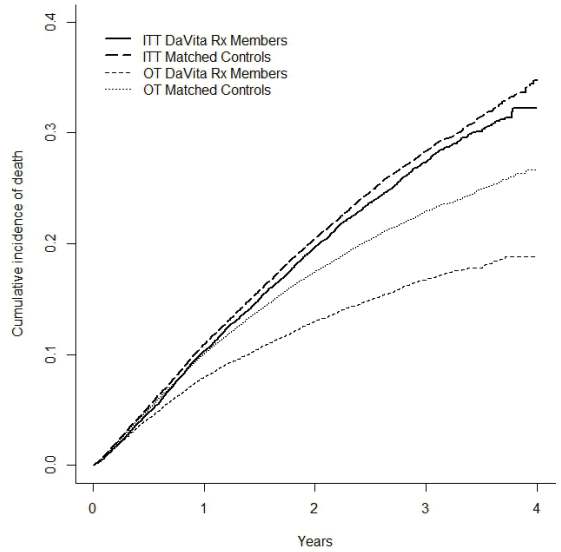
Lower Risks of Death and On-Treatment Hospitalization in DaVita Rx Members versus Matched Controls

Eric D. Weinhandl,[1] Wendy L. St. Peter.[1,2] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]College of Pharmacy, Univ of Minnesota, Minneapolis, MN.*

Background: Receiving integrated pharmacy services from DaVita Rx was associated with lower rates of death and hospitalization among poor hemodialysis patients, 2006-2008 (*AJKD*, 2012). We aimed to replicate these associations in a study of more contemporary patients, with broader inclusion criteria and stronger control of confounding.

Methods: We linked DaVita records with United States Renal Data System (USRDS) records. New DaVita Rx members had Medicare Parts A, B, and D coverage and first filled a Part D-covered medication at a DaVita Rx pharmacy between Jan 1, 2007, and Dec 31, 2010. For each member, we identified 3 matched controls at within-state DaVita facilities, according to an 81-factor propensity score of new DaVita Rx membership. We followed patients until Dec 31, 2010, with intention-to-treat (ITT) and on-treatment (OT) rules. We ascertained incidence of death and hospitalization from USRDS records.

Results: We identified 19,354 new DaVita Rx members and 58,045 matched controls. At 1 year, the ITT (OT) cumulative incidence of death was 10.3% (8.0%) versus 10.9% (10.1%) for members versus controls (figure). Overall, ITT and OT hazard ratios of death for members versus controls were 0.94 (95% CI, 0.90-0.98) and 0.82 (0.77-0.87), respectively. ITT and OT relative rates of hospital admissions were 0.98 (0.96-1.00) and 0.94 (0.92-0.96), respectively; for hospitalized days, corresponding relative rates were 0.96 (0.94-0.99) and 0.90 (0.87-0.92). In OT analysis, rates of hospitalized days due to heart failure and infection, particularly sepsis, were significantly lower among members ($P < 0.05$).



Conclusions: Receiving integrated pharmacy services was associated with significantly lower risk of death and, in on-treatment analysis, modestly lower risk of hospitalization.

Funding: Pharmaceutical Company Support - DaVita Clinical Research

TH-PO884

Increases in Medication Use after New Membership in DaVita Rx

Eric D. Weinhandl,[1] Wendy L. St. Peter.[1,2] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]College of Pharmacy, Univ of Minnesota, Minneapolis, MN.*

Background: Initiation of integrated pharmacy services, including medication management and refill management, may alter medication use. For medications related to cardiovascular disease and mineral and bone disorder, we aimed to compare treatment prevalence and adherence in DaVita Rx members and matched controls immediately after initiation of services.

Methods: We linked DaVita records with United States Renal Data System (USRDS) records. New DaVita Rx members had Medicare Parts A, B, and D coverage and first filled a Part D-covered medication at a DaVita Rx pharmacy between Jan 1, 2007, and Dec 31, 2010. For each member, we identified 1 matched control at a within-state DaVita facility, according to an 81-factor propensity score of new DaVita Rx membership. We followed patients until the earliest of death, kidney transplant or Dec 31, 2010, and retained pairs in which member and control were followed for >6 mo. We ascertained medication use during 6 mo before and after the start of follow-up and estimated treatment prevalence and the proportion of days covered (PDC) among users.

Results: We identified 13,114 pairs. Treatment prevalence and mean PDC were balanced at baseline. During follow-up, percentages of members with ≥1 fill of an ACE inhibitor/ARB, beta blocker (BB), and calcium channel blocker (CCB) were 3 to 4 percentage points greater than percentages of controls; for cinacalcet and phosphate binders, corresponding differences were +6 and +9 percentage points, respectively (table). Among users, mean PDC among members was 11 to 20 percentage points greater than among controls. All differences were significant ($P < 0.01$), before and after adjustment for small differences at baseline.

	Treatment Prevalence (%)		Mean PDC among Users	
	Member	Control	Member	Control
ACE inhibitor/ARB	56	52	73	60
BB	68	64	75	61
CCB	54	51	72	61
Cinacalcet	42	36	72	53
Phosphate binder	89	80	71	51

Conclusions: For medications related to cardiovascular disease and mineral and bone disorder, initiation of integrated pharmacy services was associated with increases in percentages of patients treated and proportions of days covered.

Funding: Pharmaceutical Company Support - DaVita Clinical Research

TH-PO885

Medication Adherence to Phosphate Binders: The CHEOBS Study

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Background: Noncompliance (NC) is not always intentional. The medical team rarely takes into account the non--intentional NC linked to the difficulty of taking drugs regularly and feelings of the patient, unless objective evidence is present. Better understanding of the triggers and determinants of NC would allow elaboration of educational tools designed to help out chronic patients with their treatment.

Methods: 340 hemodialysis patients in 9 centers in three areas in France were included on a voluntary basis. Among them, 10 patients responded to a qualitative interview focused on individual beliefs, attitudes and motivations towards phosphate binders' therapy. 26% of patients attended an educational program. Statistical methods consist of frequencies analysis and Exploratory Factor Analysis to determine combination of factors which significantly influence the compliance to phosphate binders. The semi--structured interviews were analyzed according to qualitative content analysis.

Results: 329 self--administered questionnaires (50 items) were analyzed, 297 were complete for analysis (mean age 61 years, 62% male, dialysis duration 4.5 years, number of medication 9 per day). The majority of patients considers treatment as important (80%). However, they mostly relativizes the treatment as vital (45%). Factor analysis helped to identify two kind of independent behaviors: those which indicate concerns for the treatment and those relative to the use of the treatment as a necessity. Age, level of education and gender influence these two factors. Older patients are more compliant. The higher the level of education the more frequently patients adapt the treatment. The swallowable tablets are preferred (75%). The shape and color has little influence on decision. 60% of the patients consider they received enough pre therapeutic information. The involvement into educational formation has a not high enough influence on adherence.

Conclusions: In conclusion, this large study provides clues to better understanding of non compliance to phosphate binders determinants. Based on these assumptions, educational program should be more efficient and fruitful to chronic dialyzed patients.

Funding: Pharmaceutical Company Support - Sanofi-Genzyme

TH-PO886

Sevelamer versus Calcium-Phosphate Binders on Dialysis Patients—A Meta-Analysis Xun Liu,[1,2] Caixia Wang,[1] Chenggang Shi,[1] Linsheng Lv,[3] Zhenda Zheng.[4] *[1]Dept of Nephrology, The Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [2]Div of Nephrology, Tufts Medical Center, Boston, MA; [3]Operating Room, The Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [4]Dept of Cardiology, The Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Compared with calcium-phosphate binders, the impact of sevelamer on cardiovascular calcification, all-cause mortality and other side effects of end-stage renal disease (ESRD) patients on dialysis is still uncertain.

Methods: We performed a meta-analysis. We searched the Medline, PUBMED, ASN-ONLINE, EMBASE, the Cochrane Library and 30 RCts with 7205 participants were included.

Results: In an analysis pooling, there was no difference in all-cause mortality or cardiovascular mortality, hospitalization, adverse events (AEs). But there showed a significant difference in change of coronary artery calcification scores (CACS) by -115.34 (mean difference, MD, 95% confidence interval, CI, -194.18, -36.49) and aortic calcification scores (ACS) by -451.91 (MD, 95% CI -742.88, -160.95) between sevelamer and calcium-based phosphate binder. Therapy of sevelamer, rather than calcium-based phosphate binders, decreased total cholesterol (MD, 0.55mg/dL, 95% CI -0.64, -0.45), LDL (MD, 0.72mg/dL, 95% CI -0.82, -0.61) and HDL (MD, 0.24 mg/dL, 95% CI -1.72, 1.24). Sevelamer also has a significant association with higher iPTH levels (MD, 0.23pg/ml, 95% CI 0.12 to 0.35), lower serum bicarbonate levels (MD, -1.76 mEq/L, 95% CI -2.18, -1.35), lower triglycerides levels (MD, -3.5 mg/dl, 95% CI -18.29, 11.28), higher alkaline phosphatase levels (MD, 38.34 U/L, 95% CI 32.37, 44.31), higher bone alkaline phosphatase levels (MD, 6.02 U/L, 95% CI 3.05, 9.00). The rate of hypercalcemia (defined as serum calcium >10.2-10.5 mg/dl and serum calcium >11.0mg/dl) was significantly smaller in sevelamer (Risk Ratio, RR = 0.44, 95%CI, 0.33, 0.58; RR = 0.24, 95%CI, 0.14, 0.41).

Conclusions: This meta-analysis suggests that sevelamer benefits dialysis patients in CACS, lipid, alkaline phosphatase, bone alkaline phosphatase, triglycerides and hypercalcemia.

Funding: Government Support - Non-U.S.

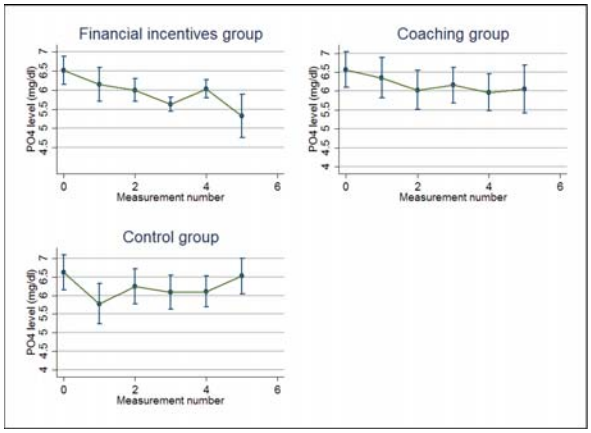
TH-PO887

A Pilot Randomized Controlled Trial of Financial Incentives or Coaching to Lower Serum Phosphorus in Dialysis Ofole Mgbako,[1] Adam S. Mussell,[1] Vishnu S. Potluri,[3] Simona Levsky,[1] Scarlett L. Bellamy,[1] Chirag R. Parikh,[2] Justine Shults,[1] Karen Glanz,[1] Harold I. Feldman,[1] Kevin Volpp,[1] Peter P. Reese.[1] *[1]Penn; [2]Yale; [3]Lankenau.*

Background: Among hemodialysis (HD) patients, hyperphosphatemia is common and associated with elevated mortality. Behavioral economics and behavior change theories may offer novel approaches for achieving PO4 control. The objective of this pilot RCT was to determine the feasibility of implementing these approaches in the HD setting.

Methods: In 3 urban HD units, we randomized 36 adults (1:1:1) with high PO4 (median >5.5 mg/dl over 3 months) to receive: 1) financial incentives for reducing or maintaining normal-range PO4, 2) structured coaching about medication/dietary adherence, or 3) usual care for 70 days. Serum PO4 was measured during routine unit operations. Each financial-incentives arm subject received the equivalent of $1.50/day if the PO4 was ≤5.5 mg/dl or >5.5 mg/dL, but had decreased by ≥0.5 mg/dl. In the coaching arm, the coach and subjects were instructed to be in contact at least 3X/week.

Results: Among 66 eligible patients, 36 (55%) enrolled. Median age was 53 years, 30 (83%) were black race and 28 (78%) were male. Mean final PO4 was 5.8 (+/-0.9) in the financial incentives arm, 5.9 (+/-1.7) in the coaching arm and 6.1 mg/dl (+/-1.4) in usual care. Median change in PO4 from initial to final measurement was -0.60 in the financial incentives arm, -0.80 in the coaching arm, and -0.45 mg/dl in usual care. All patients in the financial incentives and coaching arms expressed interest in receiving similar support in the future. The figure suggested that among subjects with more PO4 measurements, those receiving incentives had greater success lowering PO4.



Conclusions: Implementing novel behavioral health strategies to lower PO4 is highly feasible. A longer, larger trial is needed to determine if these strategies can achieve significant reductions in PO4.

TH-PO888

Short-Term Efficacy of Intensive Propaganda and Education for Hyperphosphatemia in Patients with Maintenance Hemodialysis Xun Liu,[1,3] Huijuan Ma,[1] Yitong Yao,[2] Huanling Guo,[4] Jianxiong Shi,[4] Ronglang Zhou.[5] *[1]Div of Nephrology, Dept of Internal Medicine, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen Univ, Guangzhou, Guangdong, China; [3]Div of Nephrology, Tufts Medical Center, Boston, MA; [4]Zhongshan School of Medicine, Sun Yat-sen Univ, Guangzhou, Guangdong, China; [5]School of Public Health, Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Hyperphosphatemia is an important determinant of mortality in chronic kidney disease patients undergoing hemodialysis. We aimed to assess the influence of intensive propaganda and education on serum phosphorus in patients with maintenance hemodialysis (MHD).

Methods: A total of 84 hemodialysis patients with hyperphosphatemia (serum phosphorus > 1.78 mmol/L) were enrolled, with 52 assigned to intensive propaganda and education group and 32 assigned to general propaganda and education group. Both of two groups received calcium carbonate ($CaCO_3$ 3.0g/day) for 12 weeks, with different follow-up intervals. Patients were guided to recognize the impact of phosphate on disease progression and be aware of the presence of phosphate in food and drinks and the benefit of phosphate binders. Blood phosphorus, blood calcium and blood intact parathyroid hormone were recorded.

Results: Five patients were excluded from intensive propaganda and education group and two from general propaganda and education group because of reasons such as death or renal transplantation. In all, 77 patients completed the analysis. In intensive propaganda and education group, the decreases of serum phosphorus in weeks 4 (P=0.001), 8 (P=0.006) and 12 (2.32 to 1.94 mmol/L, P<0.001) were significantly relative to that in week 0, whereas in the general group, it revealed no significant difference (2.21 to 2.07 mmol/L, P=0.182) after 12 weeks treatment. The decline of blood phosphorus in intensive propaganda and education group was significantly greater than that in general propaganda and education group (P=0.02).

Conclusions: Intensive propaganda and education could be more effective in the control of hyperphosphatemia in patients with MHD.

Funding: Government Support - Non-U.S.

TH-PO889

Effects of Lanthanum Carbonate to Serum Phosphate and Serum FGF-23 in Hemodialysis Patients with Hyperphosphatemia Xuemei Li, Jie Ma, Yang Yu. *Nephrology, Peking union Medical College Hospital, BeiJing, China.*

Background: High levels of circulating fibroblast growth factor 23 (FGF-23) are associated with chronic kidney disease (CKD) progression and high mortality. Klotho is a transmembrane protein that acts as a cofactor for fibroblast growth factor 23 (FGF23). Klotho also exists as a soluble circulating protein. The primary objective of this study was to assess the effects of 4 weeks of treatment with lanthanum carbonate on intact FGF23 (iFGF23) and Klotho levels in hemodialysis patients.The secondary objectives was to assess the effects of lanthanum carbonate on other factors including circulating levels of intact parathyroid hormone (iPTH), calcium, and phosphate.

Methods: A single center, open-labeled study from Peking Union Medical College Hospital was performed. Phosphate binders were discontinued during a two-week washout period.Patients with more than 1.78 mmol/L serum phosphorus after two-week washout period were eligible for the study.Lanthanum carbonate was administered to 12 MHD patients for 4 weeks.The dose was adjusted after first two weeks as necessary to achieve serum phosphorus control.

Results: All patients fulfilled the whole study.By the end of 4 weeks of lanthanum carbonate treatment, mean serum level of phosphorus was 1.77±0.10mmol/L, compared with the level before treatment 2.21±0.58mmol/L, *P*=0.005. Before treatment calcium-phosphate product was 5.36±0.21mmol^2/L^2, after 4 weeks treatment calcium-phosphate product was 4.39±0.28mmol^2/L^2, *P*=0.016;FGF-23, before:7554.9±1421.9pg/ml, after: 5605.8±1036.9pg/ml, *P*=0.027. Klotho levels, before: 597.2±53.2pg/ml, after:356.4±122.7pg/ml, *P*=0.001. While the level of serum calcium and serum intact parathyroid hormone kept steady. Of the 12 involved patients, 3 had adverse drug reaction, 2 had constipation, 1 had rash, the two patients all relieved after symptomatic treatment.

Conclusions: Lanthanum carbonatecan effectively control the serum phosphorus, reduce the levels of calcium-phosphorus product, serum FGF-23 and Klotho protein in the short term.

TH-PO890

Improvements in Relative Blood Volume Change during Hemodialysis Associates with Increases in Albumin, Declines in Systolic Blood Pressure and Lower Epogen® Utilization Cindy Allegretti,[1] Patricia McCarley,[1] Portia R. Scott,[1] Athena Palearas,[1] Patrice B. Taylor,[2] John W. Larkin,[1] Linda H. Ficociello,[1] Paul Balter,[1] Kevin Chan,[1] Len A. Usvyat,[1] Franklin W. Maddux,[1] Jeffrey L. Hymes.[1] *[1]Fresenius Medical Care North America (FMCNA); [2]FMC Renal Therapies Group.*

Background: Suboptimal volume control associates with increased morbidity and mortality in hemodialysis (HD) patients (Pts). FMCNA started utilizing Crit-Line® Blood Volume Monitors (CLM). We evaluated the temporal association of CLM implementation on hypertension, albumin, and Epogen (EPO) requirements in HD Pts.

Methods: From Jun 17, 2013 to Mar 31, 2014, BV was documented in 369 HD clinics. Pts with >36 treatments (tx) and >75% of tx with CLM were selected; average monthly slope of ending BV/tx/Pt was computed. Pts were stratified by ***declining BV*** (slope of BV negative and p-value significant), ***increasing BV*** (slope of BV positive and p-value significant), and remaining Pts were considered to have ***no change in BV***. Average monthly slope of albumin, post-dialysis weight, pre- and post-dialysis systolic blood pressure (pre-SBP/post-SBP), and EPO dose were computed; groups were compared using ANOVA.

Results: 1875 Pts were studied. Pts with declining ending BV had increases in albumin and pre-SBP, and decreases in post-dialysis weight, post-SBP, and EPO dose/tx. Conversely, Pts with increases in ending BV had decreases in albumin and post-dialysis weight, and increases in pre-SBP (more pronounced than Pts with a decline in BV), post-SBP, and EPO dose/tx. There were significant differences between Pts with declining, stable, and increasing BV in albumin change, post-dialysis weight, and post-SBP.

Figure 1. Comparison of groups of BV change

BV change	Number of patients	Monthly change in albumin (g/dL/month)	Monthly change in post-dialysis weight (kg/month)	Monthly change in Pre-SBP (mmHg/month)	Monthly change in Post-SBP (mmHg/month)	Monthly change in EPO dose (U/month)
Declined	180	0.031	-0.29	0.11	-1.33	-117
No significant change	1511	0.004	0.01	0.15	-0.21	-64
Increased	184	-0.016	-0.08	0.61	1.28	15
p-value		<.0001	0.0016	0.315	<.0001	0.561

Conclusions: Lower relative ending BV in HD Pts associates with increases in albumin and smaller increases in pre-SBP, as well as, declines in post-SBP, post-dialysis weight, and EPO dose requirements as compared to Pts with an increasing ending BV.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

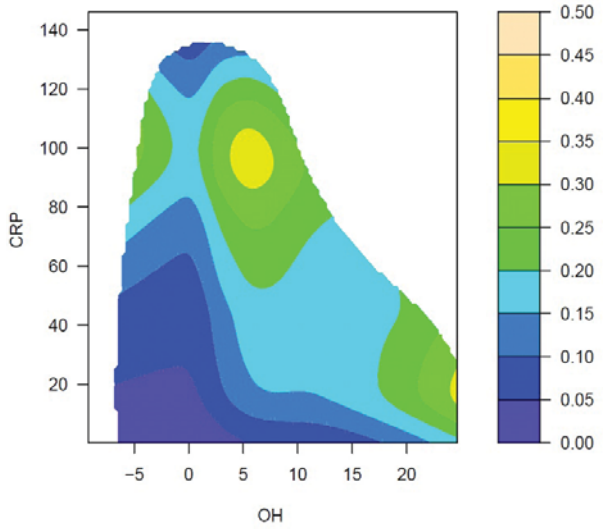
TH-PO891

Fluid Overload, Inflammation and Mortality: Results from a Large International Study Marijke J.E. Dekker,[1,2] Len A. Usvyat,[3,6] Constantijn Konings,[2] Frank van der Sande,[1] Karel M. Leunissen,[1] Peter Kotanko,[3] Yuedong Wang,[5] Daniele Marcelli,[4] Nathan W. Levin,[3] Bernard J. Canaud,[4] Jeroen Kooman.[1] *[1]Internal Medicine, Div of Nephrology, Univ Hospital, Maastricht, Netherlands; [2]Internal Medicine, Catharina Hospital, Eindhoven, Netherlands; [3]Renal Research Inst, New York; [4]Fresenius Medical Care, Bad Homburg, Germany; [5]Univ of California, Santa Barbara California; [6]Fresenius Medical Care North America, Waltham.*

Background: Fluid overload (FO) and systemic inflammation are associated with a higher mortality in hemodialysis (HD) patients. Previous studies showed a relation between FO and inflammation, the effect of the interaction between FO and inflammation on outcome however, has not yet been studied. The aim of this research is to evaluate the interaction between FO, inflammation and outcome.

Methods: We studied prevalent HD patients from the European Fresenius network (17 countries). Hydration status was assessed by Body Composition Monitor (BCM) measurements. We included patients with ≥ 1 BCM measurement and at least one C-reactive protein (CRP) measurement between 1/2011 and 12/2011 and recorded their survival between 1/2012 and 6/2012 (follow up). Probability of death was computed using a multivariate semi-parametic logistic regression model.

Results: We included 9,463 patients (mean age 63 years, 57.4% male, mean dialysis vintage 5.1 months). Of these patients, 476 (5.03%) died during the follow up period. FO and increased CRP levels were both associated with a higher mortality rate. However, especially the combination of OH and elevated CRP level was associated with an increased mortality.



Contour plot depicting the probability of death in 6 months as a function of hydration status (measured by BCM in liters) and CRP levels during the 12 months before.

Conclusions: HD patients with FO and high CRP levels have an increased mortality risk. However, especially the combined presence of both FO and inflammation is associated with a greatly increased risk of adverse outcome.

TH-PO892

6-Year Outcomes from a National Treatment Options Education Program for Chronic Kidney Disease Patients Eduardo K. Lacson, Shu-Fang Lin, Keith Lester, Teresa J. Close, Franklin W. Maddux. *Fresenius Medical Care, Waltham, MA.*

Background: We previously reported early outcomes from a national pre-dialysis Treatment Options (TOPs) program offered at no cost to patients with advanced CKD. This report provides a 6-year overview of outcomes since its initial launch in 2008.

Methods: Our CQI program tracked CKD patients initiating chronic dialysis therapy in all FMCNA facilities annually from 2008 to 2013. We tracked TOPs penetration rates and compared rates of home dialysis (PD and HD), vascular access for patients opting for HD, and dialysis survival 120 days later, between patients who attended TOPs and those who did not (non-TOPs).

Results: As incident patient admissions increased annually, the proportion attending TOPs increased each year from 8% (2008) to 18.5% (2013). The median age increased from ~65 to 66 years (Non-TOPs steady at ~64.5 years). The percentage of males increased from ~56.5% to 58% overall, similar in both TOPs and Non-TOPs patients. While a 7% gap in the proportion of black race attendees existed in 2008, it was abrogated to <1% difference between TOPs and Non-TOPs patients in 2013. Diabetes prevalence was similar in 2008, but only 40% of TOPs attendees had DM for 2013 (44% non-TOPs). With more TOPs attendees, patients opting for home dialysis decreased from 23% in 2008 to just over 16% in 2013, in contrast to a small but steady increase in home dialysis by non-TOPs attendees (+2% by 2013). Non-TOPs patients had ~0.5% decline to ~70% CVC for HD patients' 1st treatment, while TOPs attendees' CVC use declined from 54% (2008) to 47% (2013). Fistulas at 1st HD increased in both groups: 16% to 20% (Non-TOPs) and 33% to ~41% (TOPs). By 120 days, 9-10% more TOPs attendees proportionately remain in FMCNA care each year.

Conclusions: While a greater percentage of patients starting dialysis at FMCNA attended TOPs education, increased referrals are needed to cover more pre-dialysis CKD patients. Amid potential selection biases, trends for greater penetration of home therapies, favorable vascular access distribution in HD patients, and better >120 day high-risk period survival in TOPs attendees were maintained for 6 years.

TH-PO893

The Dialysate Purification and Introduction of High Flux Dialyzers in Cambodia Sovandy Chan,[1] Toru Hyodo,[1,2,3] Tomotaka Naramura,[3,4] Kenichi Kokubo,[3,5] Fumitaka Nakajima,[1,3,6] Haruki Wakai,[3,7] Nobuhisa Shibahara,[3,8] Sabo Ojano.[1] *[1]Cambodia-Japan Friendship Blood Purification Center, Sen Sok International Univ, Phnom Penh, Cambodia; [2]Kuratakai Eijin Clinic, Hiratsuka, Japan; [3]NGO Ubiquitous Blood Purification International, Yokohama, Japan; [4]Dept of Medical Engineering, Faculty of Health Sciences, Jubshin Gakuen Univ, Fukuoka, Japan; [5]School of Allied Sciences, Kitasato Univ, Sagamihara, Japan; [6]Moriguchi Keijinkai Hospital, Moriguchi, Japan; [7]Reiseikai Shinagawa Garden Clinic, Tokyo, Japan; [8]Arisawa General Hospital, Hirakata, Japan.*

Background: Dialysate purification systems have not been established in developing countries and so high flux dialyzers have not been introduced in such countries.

Methods: Blood Purification Center of Sen Sok International University (SSIU) Hospital was established at March 1st in 2010. The dialysis was performed by using low

flux dialyzers. At July in 2011, the endotoxin(ET) measurements and bacterial counting of the tap water, reverse osmosis water and dyalysate were checked in order to reveal whether high flux dialyzers could be used or not.

Results: The results of dialysate ET were 309, 246, 395 EU/mL and the bacterial counting were 800, 800, 900 cfu/mL. At July in 2013, the ET retentive filter (ETRF) was installed and the line after the ETRF was changed into the new one. Until December in 2013, no ET and bacteria were determined. We have started to use high flux dialyzers since December 14th in 2013. Mean B2-microglobrin(B2-MG) reduction rate was 56.9±1.4% (N=5) in the first use of dialyzers. The value of B2-MG in the 1st, 2nd, 3rd and 4th use of the single dialyzer (under reuse principle) were 37.9, 33.7, 31.9 and 30.1 mg/L (N=1).

Conclusions: The ET retentive filter is very useful to purify dialysate and make it possible to use high-flux dialyzers even under hard conditions in developing countries. But more efforts to clean RO water line should be needed.

Funding: Pharmaceutical Company Support - Nipro Japan Co. Ltd.

TH-PO894

Reduced Mortality Associated with Acetate-Free Dialysis: Findings From the French End-Stage Renal Disease Registry Lucile Mercadal,[1,2] Jeanna-Eve Franck,[2] Marie Metzger,[2] Wenlun Yuan,[2] Christian Jacquelinet,[3] Benedicte Stengel.[2] *[1]Nephrology Dpt, CHU Pitié-Salpêtrière, Paris; [2]INSERM U1018, CESP, Villejuif; [3]Biomedicine Agency, Saint-Denis.*

Background: Dialysis using hydrochloric acid (HCl) dialysate or acetate-free biofiltration improves cardiac output and per-dialysis tolerance when compared to standard hemodialysis (HD) using small amount of acetate. Whether acetate-free dialysis (AFD) is associated with reduced mortality is unknown.

Methods: Using the REIN registry, we classified all patients who started HD from 2008 to 2010 in 13 French regions into 3 exposure categories according to the type of dialysate used in their dialysis center: 100% HCl dialysate, both standard and AFD (mixed center) or standard dialysate only. Cox survival analysis was performed in 15,160 incident patients, adjusted for 15 baseline co-morbidities and biological data. We accounted for patient clustering within centers and used age as time-scale. Patient AFD exposure and hemodiafiltration (HDF) status were treated as time-dependent variables. Analysis was censored at Dec 31, 2011, or at kidney transplantation, lost to follow-up, dialysis weaning or transfer to peritoneal dialysis.

Results: Medians (interquartile range) of follow-up and age were 1.8 years (1.2-2.6) and 70.5 years (58.1-78.8) respectively. A total of 658 patients were dialyzed at least once in a 100% HCl center and 3,021 were dialyzed at least once in a mixed center and never dialyzed in a 100% HCl center. There was an interaction with age in the relation between AFD and mortality: patients older than 70 years had significant lower mortality risk associated with AFD, while no association was found in younger patients (table 1). Using HDF was associated with lower mortality risk at all ages.

	Person-years	Deaths	<70 years HR (95% CI)	≥70 years HR (95% CI)
AFD exposure status Dialyzed in a standard dialysis center	20,921	3,115	1	1
Dialyzed in a mixed dialysis center	3,489	544	1.03 (0.85-1.24)	0.83 (0.74-0.94)
Dialyzed in a 100% HCl center	775	132	1.24 (0.87-1.77)	0.79 (0.67-0.93)
HDF (ref: without HDF)	3,292	536	0.73 (0.59-0.90)	0.85 (0.76-0.96)

Conclusions: HD without acetate and HDF were associated with better survival in the REIN registry.

TH-PO895

Up-Regulation of Inflammatory Mediators and Markers of Metabolic Deraignment in End Stage Renal Disease as Studied by Biochip Array Analysis Vinod K. Bansal,[1] Daneyal Syed,[2] Debra Hoppensteadt,[2] Mushabar Syed,[3] Jawed Fareed.[2] *[1]Nephrology, Loyola Univ Medical Center; [2]Pathology, Loyola Univ Medical Center; [3]Cardiology, Loyola Univ Medical Center.*

Background: End stage renal disease represents a complex syndrome with multiple pathophysiological processes involving vascular, inflammatory, thrombotic, and metabolic deraignment. This study was designed to utilize biochip array technology to compare the inflammatory and metabolic syndrome biomarker profiles of a maintenance hemodialysis cohort (n=81) with healthy normal male and female volunteers (n=41).

Methods: The ESRD group represented patients who are under maintenance hemodialysis at the Loyola University Clinic (n=81) and a group of healthy normal individuals (n=50). High sensitivity inflammatory cytokine chips to profile IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-gamma, IL1-alpha, IL1-beta, MCP1, and EGF and metabolic array chip to analyze C-Peptide, ferritin, resistin, insulin, leptin, and PAI-1 were used employing Evidence Investigator (Randox, UK).

Results: In the inflammatory biochip array analysis, except for IL-2 and EGF, all the inflammatory biomarkers were found to be significantly higher than the normal group ($p<0.0001$). The ESRD group exhibited marked variations in the circulating levels of the inflammatory mediators and the extent of increase also varied. In the metabolic chip array analysis, all of the markers of metabolic deraignment were significantly increased ($p=<0.001$), except insulin, where a trend towards increased levels was noted.

Conclusions: These results clearly demonstrate the complexity of the pathophysiologic event in ESRD patients. A widespread increase in the inflammatory mediators, coupled with the higher levels of metabolic deraignment markers suggest that the ESRD patients not only have an ongoing inflammatory process but also sustain metabolic deraignment. Profiling of these mediators not only provides an understanding of the pathogenesis of this condition, but may be helpful in the risk stratification and clinical management of these patients.

TH-PO896

Imbalance of CD39/CD73 Cells and ROS in Maintenance Hemodialysis Eun Hye Seo,[1] Young-Il Jo.[1,2] *[1]BK21 plus, Konkuk Univ School of Medicine, Seoul, Republic of Korea; [2]Nephrology, Konkuk Univ Medical Center, Konkuk Univ School of Medicine, Seoul, Republic of Korea.*

Background: Recent evidences indicate that CD39 and CD73 expression by regulatory T (Treg) cells may contribute to renal protective effect in renal injury. CD39 is expressed on the surface of human Tregs that are important in constraining pathogenic Th17 cells. Our previous study showed that the frequency of Th17 cells had a decreasing tendency with decreased eGFR in chronic kidney disease. However, the role of CD39 and CD74 Tregs remain poorly understood. This research was designed to evaluate the frequency and role of Treg, CD39 and CD73 cells in ESRD patients on maintenance HD.

Methods: Fifteen ESRD patients on maintenance HD (age 61.8±3.0 yr, male 9, duration of HD 10.0±2.1 yr) and 15 healthy controls (age 45.0±3.1 yr, male 10) were recruited. Flow cytometry was used to analyze the proportion of CD39 and CD73 expressed by CD4+ T cells and intracellular reactive oxygen species (ROS) in Tregs in the peripheral blood of the subjects.

Results: ESRD patients on maintenance HD revealed an increased frequency of Treg (Control versus HD, 3.5±0.3 versus 11.0±3.1, p=0.001). An increase in the frequency of CD39+ Tregs increased (13.6±2.5 versus 37.2±6.0, p=0.01) and a decrease in the frequency of CD73+ Tregs decreased (10.0±2.2 versus 5.3±0.2, p=0.02) was observed ESRD patients on HD, in turn, the ratio of CD39+/CD73+ Tregs was significantly decreased in ESRD patients on HD [Figure1]. Intracellular ROS in Treg was significantly increased in ESRD patients on HD compared with healthy controls (8.8±2.2 versus 52.2±9.5, p=0.01). In addition, the expression of CD39+ Tregs was positively correlated with the expression of cellular ROS in Tregs (r=0.602, p=0.018).

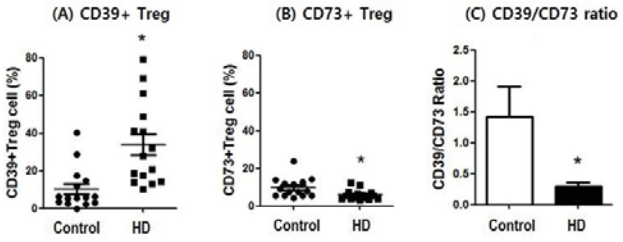


Figure. The expression of CD39+ and CD73 cells in healthy controls and ESRD patients receiving hemodialysis. (A) The frequency of CD39+ Treg , (B) The frequency of CD 73+ Treg, (C) The ratio of CD39/CD73 cells.

Conclusions: These results suggest that dysregulated balance of CD39+/CD73+ Tregs may play a role in the immune response of ESRD patients receiving maintenance hemodialysis.

Funding: Private Foundation Support

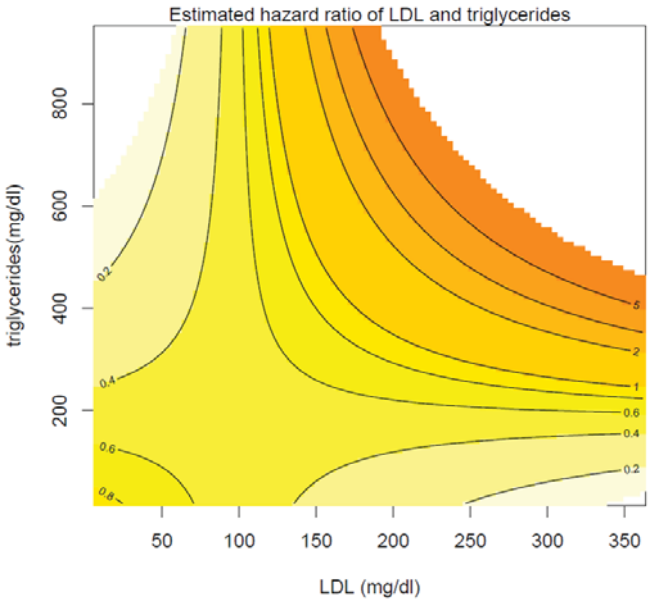
TH-PO897

Higher Low Density Lipoprotein Cholesterol Is Associated with Lower Infectious Events in a Large International Population of Hemodialysis Patients George A. Kaysen,[1,2] Yuedong Wang,[3] Len A. Usvyat,[2] Daniele Marcelli,[4] Bernard J. Canaud,[4] Peter Kotanko.[2,5] *[1]Medicine, UC Davis, Davis, CA; [2]Renal Research Inst, New York, NY; [3]UC Santa Barbara, Santa Barbara, CA; [4]FMC, Bad Homburg, Germany; [5]The MONDO Initiative.*

Background: Reduction in low density lipoprotein (LDL) is without effect on morality in hemodialysis (HD) patients. The second leading cause of death in HD after cardiovascular diseases is infectious. LDL absorbs and inactivates bacterial toxins. Human LDL can prevent endotoxin induced lethality in mice.

Methods: We explored the relationship between blood lipid levels and infectious outcomes in databases from Renal Research Institute (RRI) and Fresenius Medical Care (FMC) Europe (22,746 patients). All patients who had baseline measures of lipids and survived 12 months were selected. Time to all death. cardiovascular (CV) or infectious death or numbers of all cause, infectious or CV hospitalizations during the next 12 months were analyzed by Cox or Poisson models respectively.

Results: Risk of infectious mortality was significantly reduced by albumin and BMI, and increased by age, C reactive protein (CRP) and neutrophile lymphocyte ratio (NLR). LDL was protective against all cause and infectious mortality when TG was less than 200 mg/dL.



LDL was also protective against all cause and infectious hospitalizations when TG was less than 200 mg/dl. TG was less than 200 mg/dL in 75.3% of the population. LDL had no significant effect on CV death or hospitalizations regardless of the level of TG.

Conclusions: Higher LDL levels are associated with decreased all cause and infectious death and hospitalizations when TG are less than 200, a value present in over 75% of patients, possibly accounting for the observation that reducing LDL cholesterol has a limited effect on mortality outcomes despite potential adverse CV effects of LDL.

Funding: Pharmaceutical Company Support - Renal Research Institute

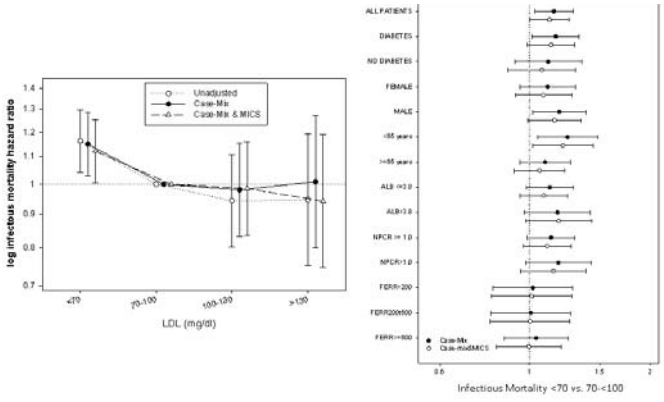
TH-PO898

Association of Low Density Lipoproteins with Infectious Mortality in Hemodialysis Patients Hamid Moradi,[1] Pouya Abhari,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Connie Rhee,[1] Csaba P. Kovesdy,[2] Rajnish Mehrotra,[3] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons, UC Irvine MC, Orange, CA; [2]Memphis VA MC, Memphis, TN; [3]Univ Washington, Seattle, WA.*

Background: Most studies on the association between plasma LDL and mortality have focused on the role of LDL in pathogenesis of cardiovascular (CV) disease and mortality. However, we have found paradoxical associations between plasma LDL level and all-cause mortality in hemodialysis patients, with low LDL levels being associated with worse outcomes. Numerous studies indicate that in an infectious or septic setting, LDL can sequester LPS and other endotoxins and potentially play a beneficial role. Since infection is the second leading cause of death in dialysis patients and based on the mentioned literature, we hypothesized that low plasma LDL levels may portend a worse infectious outcome in patients on maintenance hemodialysis (MHD).

Methods: We examined the association of plasma LDL level with infectious mortality in a 2 yr (2004-2006) cohort of 26412 MHD patients treated in clinics of a large dialysis organization using Cox models adjusted for demographics, case-mix and markers of malnutrition-inflammation complex (MICS).

Results: Patients were 58±15 yrs old and included 46% women, 18% Blacks, and 50% diabetics. Plasma LDL concentrations <70 mg/dL were associated with a significant increase in risk of infectious mortality when compared with levels of 70-100 mg/dL. Subgroup analysis revealed that this was most significant in men, diabetics and patients <65 yrs. Increasing levels beyond 100 mg/dL did not have an additional protective effect.



Conclusions: Reduced plasma LDL levels are associated with a significant increase in infectious mortality in MHD patients. Given that infections are the second leading cause of mortality in this patient population, therapies aimed at lowering LDL levels should be pursued with caution in light of these findings.

Funding: NIDDK Support

TH-PO899

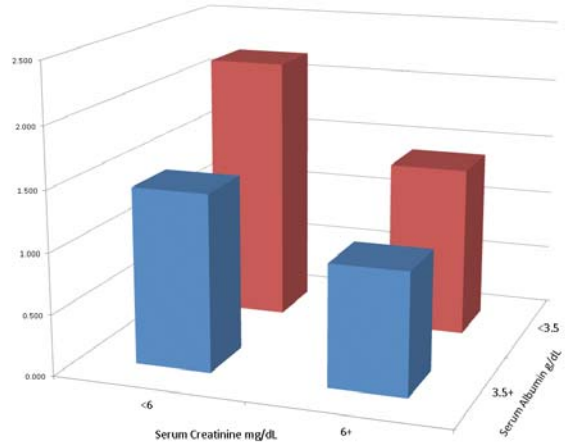
Concurrence of Serum Creatinine and Albumin with Mortality in Twice-Weekly Hemodialysis Patients Jialin Wang,[1,2] Elani Streja,[1] Connie Rhee,[1] Steven M. Brunelli,[1] Miklos Zsolt Molnar,[3] Rajnish Mehrotra,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Tianjin Union MC, Tianjin, China; [3]Univ of Toronto, Toronto, ON; [4]Univ of Washington, Seattle, WA.*

Background: Previous studies have found that both albumin (as measure of visceral proteins) and creatinine (as measure of skeletal muscle mass) are linearly associated with lower mortality in hemodialysis (HD) patients. But no data exist for <3 times/wk (usually twice-weekly) HD patients in whom larger residual kidney function may confound nutritional predictors. We hypothesize that the survival advantages of higher serum albumin and creatinine hold in these patients.

Methods: We identified 1,123 twice-weekly HD patients between 2007-2011 from a dialysis cohort of over 120,000 incident HD patients. Combination of serum creatinine (<6 versus≥6 mg/dl) and albumin dichotomies (<3.5 versus≥3.5 g/dl) yielded 2x2=4 groups. All-cause death risk was estimated using Cox models adjusting for demographics, comorbidities and laboratory measures.

Results: Patients were 70±14 yrs old and included 48% women, 13% Blacks, and 54% diabetics. In twice-weekly HD patients, compared to patients with combined higher creatinine and albumin levels, patients in the other 3 groups had higher mortality. Patients with combined lower creatinine and albumin had a 2.2-fold increased risk of mortality (HR:2.193, 95%CI:1.36-3.537).

Baseline Cr(mg/dl)	Baseline Alb(g/dl)	Mortality N	Total N	Mortality (%)	HR (95% CI)
<6	<3.5	90	287	31.36%	2.19 (1.36–3.54)
≥6	<3.5	14	55	25.45%	1.40 (0.70–2.80)
<6	≥3.5	137	588	23.30%	1.45 (0.93–2.28)
≥6	≥3.5	28	193	14.51%	Ref (1.0)



Conclusions: Lowest serum creatinine and albumin levels in twice-weekly HD patients are associated with highest mortality. Higher visceral protein and skeletal mass combined may confer greatest survival in twice-weekly HD patients, warranting controlled trials.

Funding: NIDDK Support

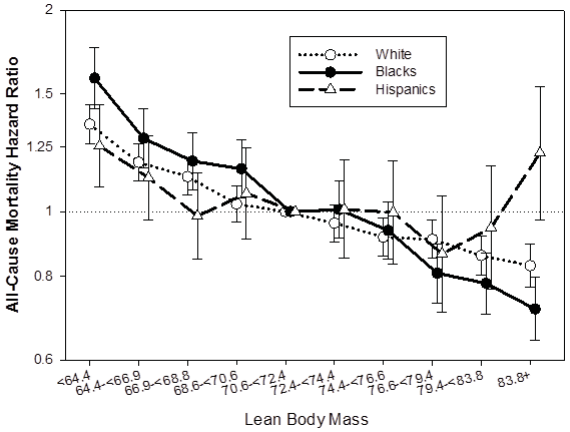
TH-PO900

Roles of Race and Ethnicity in the Risk of Mortality According to Estimated Lean Body Mass in Hemodialysis Patients Jialin Wang,[1,2] Elani Streja,[1] Connie Rhee,[1] Steven M. Brunelli,[1] Csaba P. Kovesdy,[3] Rajnish Mehrotra,[4] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Tianjin Union MC, Tianjin, China; [3]Memphis VA MC, Memphis, TN; [4]Harborview MC, Univ of Washington, Seattle, WA.*

Background: Lean body mass (LBM) is an important nutritional measure representing fat-free muscle mass and somatic protein compartments. Some observational studies have found that higher LBM is related to greater survival in hemodialysis (HD) patients, but there is little data on the role of LBM in the race/ethnicity-mortality relationship in HD patients. We hypothesized that there is no difference across race/ethnicity.

Methods: We identified 56,303 white, 35,382 black and 17,318 Hispanic HD patients between 2007-2011 in a large national dialysis cohort. We estimated LBM using the formula by Nouri/Kalantar-Zadeh: eLBM=0.34*Serum Creatinine (mg/dl) +5.58*gender+0.30*weight (kg) +0.67*height (inch)-0.23*URR-5.75. All-cause mortality risk was estimated using Cox models with adjustment for demographics, comorbidities and laboratory measures.

Results: Among whites, blacks and Hispanics treated with HD, the mean±SD age was 66±14, 58±15, and 58±15 years; 41%, 48% and 42% were women; 56%, 58% and 69% were diabetics, respectively. Compared to the reference eLBM group (70.6-<72.4), whites with the lowest eLBM (<64.4) had a 1.4-fold increased risk of mortality (HR:1.35, 95%CI:1.27-1.44) and blacks had a 1.6-fold risk of mortality (HR:1.58, 95%CI:1.43-1.76). Whites with the highest eLBM (≥83.8) had 17% lower death risk (HR:0.83, 95%CI:0.77-0.89) and blacks had 28% lower death risk (HR:0.72, 95%CI:0.63-0.80). Hispanics had a 1.3-fold increased death risk associated with both lowest and highest eLBM groups.



Conclusions: The relationship between mortality risk and eLBM is similar across race/ethnicity except among Hispanics with the highest eLBM. These discrepancies warrant further investigation.

Funding: NIDDK Support

TH-PO901

Interventions to Improve Restless Legs Syndrome Among Patients on Dialysis: A Systematic Review Kevin Quach, Mandark Gandhi, K. Scott Brimble, Michael Walsh. *McMaster Univ.*

Background: Restless legs syndrome (RLS) is common amongst patients receiving dialysis. It is unclear how to optimally treat RLS in dialysis patients. We conducted a systematic review to identify therapies to reduce the severity of RLS.

Methods: We systematically searched MEDLINE and EMBASE and hand searched narrative reviews for randomized controlled trials that assessed the use of pharmacological or non-pharmacological interventions to reduce the severity of RLS among patients receiving dialysis. The primary outcome was RLS symptoms.

Results: Of 621 abstracts identified, 11 studies that included 13 interventions met the inclusion and exclusion criteria. All trials reported interventions that significantly improved RLS severity. The interventions included dopamine agonists (n=6), gabapentin (n=2), intradialytic exercise (n=2) intravenous iron (n=1), and vitamins (n=2). In studies thats compared two interventions, ropinirole gabapentin, and intradialytic exercise were all more effective than L-dopa in indirect comparisons. However, all studies were at high risk of bias with the most common causes due to lack of allocation concealment, high losses to follow-up, and missing data. Further, follow-up for these studies was short-term ranging from 1 to 24 weeks.

Conclusions: Ropinirole, gabapentin, and intradialytic exercise appear promising to reduce RLS in dialysis patients.However, methodologically rigorous, appropriately powered, longer term RCTs are required to determine the optimal treatment strategy for RLS among dialysis patients.

TH-PO902

Pre-End-Stage Renal Disease Care and Mortality in Incident End-Stage Renal Disease Patients with Multiple Myeloma as Cause of Renal Failure Jason Cobb,[1] Laura Plantinga,[1] Janet R. Lynch,[2] Edwin D. Huff,[3] Sumit Mohan,[4] William M. McClellan.[1] *[1]Emory Univ, Atlanta, GA; [2]Mid-Atlantic Renal Coalition, Richmond, VA; [3]Centers for Medicare & Medicaid Services, Boston, MA; [4]Columbia Univ, New York, NY.*

Background: The relationship between mortality and pre-ESRD care in incident end-stage renal disease (ESRD) patients with multiple myeloma (MM) has not been examined. Most patients with MM are receiving medical care, and here, we hypothesized that pre-ESRD care would be more prevalent among these patients compared to other ESRD patients and that receipt of that care would be associated with lower mortality among MM-ESRD patients.

Methods: Among 439,206 incident U.S. hemodialysis patients with a primary cause of renal failure due to MM (6/1/05-5/31/09) identified using the U.S. Renal Data System, odds ratios for reported pre-ESRD care by ESRD due to MM (n=4561) versus other causes (n=434,645) were obtained using adjusted multivariable logistic regression models. The association of pre-ESRD care with subsequent mortality was examined using Cox proportional hazard models.

Results: MM-ESRD patients were less likely to have any pre-dialysis nephrology care (34.8% versus 58.5%, adjusted odds ratio [OR] =0.38, 95% CI 0.34-0.43) and less likely to have 6 months or more pre-dialysis care (26.2% versus 47.5%, OR=0.40, 95% CI 0.35-0.45) compared to patients with ESRD due to other causes. MM-ESRD patients more likely to have catheters on first dialysis (91.8% versus 75.6%, OR=4.16, 95% CI, 3.54-4.86). Receipt of any and 6 months or more pre-dialysis nephrologist care were significantly associated with lower 1-year mortality (fully adjusted HR for death 0.89, CI 0.82-0.97 and 0.88, CI 0.80-0.96 respectively). Catheter use was associated with 1.6-fold higher 1-year mortality (fully adjusted HR for death 1.55, CI 1.32-1.83).

Conclusions: MM-ESRD patients were less likely than their counterparts to have pre-dialysis nephrology care and were more likely to use catheters on first dialysis. However, pre-dialysis care is independently associated with reduced mortality in incident MM-ESRD patients. These results suggest that pre-ESRD care should be prioritized in MM patients approaching ESRD.

TH-PO903

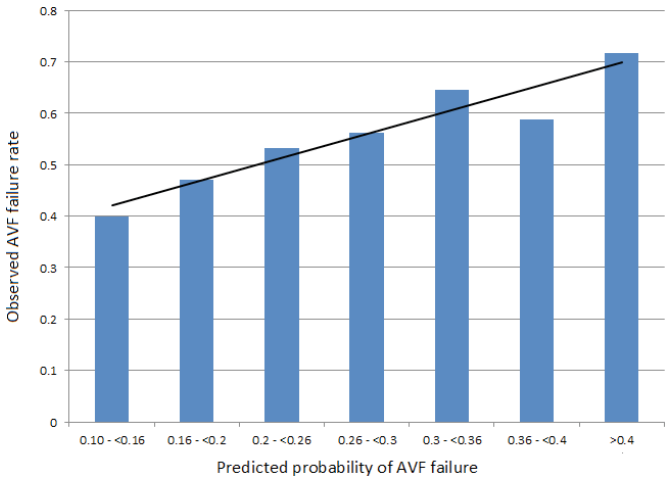
Prediction Model of AV Fistula Failure in Elderly Hemodialysis Patients Alexander S. Goldfarb-Rumyantzev,[3] Bhanu K. Patibandla,[1] Akshita Narra.[2] *[1]Dept of Medicine, St. Vincent Hospital, Worcester, MA; [2]Dept of Medicine, Univ of Connecticut, Farmington, CT; [3]Div of Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: AV fistula is the preferable access for hemodialysis and is associated with better clinical outcome. In some patients AVF established long time in advance of dialysis initiation is never used if the patient dies or transplanted prior to dialysis start. Furthemore, significant number of AVF established prior to dialysis fail, by some estimate close to 50% in specific populations. The goal of this study was to develop and validate a prediction model and risk-stratification tool to identify patients at greater risk for predialysis AVF failure.

Methods: We used United States Renal Data System (USRDS) data of ESRD patients 67 years of age and older, linked with Medicare claims. The AVF failure is defined by the fact that despite AVF being the initial access placed, dialysis was initiated using other than AVF vascular access. We used logistic regression to create our prediction model. Variables that demonstrated significant association with outcome were included in the prediction model. Prediction model was tested using calibration and ROC curve.

Results: The entire study cohort consisted of 20,360 subjects with mean age of 76.2±6.02 years, 58.5% males and 41.5% females. The dataset was divided into training subset (n=13,574) used for model development and testing subset (n=6,786) used for model validation. Prediction model and risk stratification algorithm were developed based on patient age, race, sex, cause of ESRD, history of diabetes, cerebro-vascular disease and CHF.

Conclusions: We developed prediction model and risk-stratification algorithm to identify ESRD patients 67 and older at risk for pre-dialysis AVF failure. Results of model validation might allow its practical use.



Funding: Clinical Revenue Support

TH-PO904

Citrasate versus Acetate Dialysate on Intradialytic Heparin Dose: A Randomized Crossover Study Kelvin Leung, Pietro Ravani, Robert R. Quinn, Nairne William Scott-Douglas, Jennifer M. MacRae. *Medicine, Univ of Calgary, Calgary, AB, Canada.*

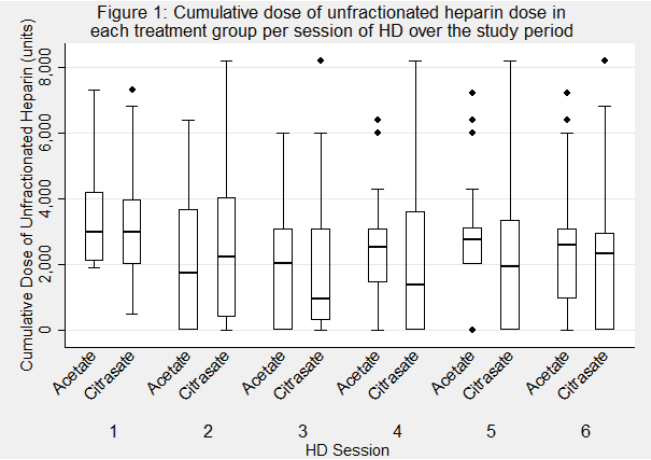
Background: Citrasate dialysate (CD) has an anticoagulant effect which may lead to a reduced cumulative heparin dose (CHD) as compared to standard acetate dialysate (AD).

Methods: We performed a randomized double-blind, crossover trial in maintenance HD patients to determine if CD is associated with a 30% lower CHD than AD (NCT01466959). Adults receiving HD three times per week who used heparin for anticoagulation were included. Heparin dose was adjusted with a heparin reduction protocol during a two-week, run-in phase. Patients who remained on heparin were then randomized to two weeks of CD or AD. Each patient underwent a one-week washout period before crossing over to the other arm. The primary outcome was CHD per HD session; secondary outcomes included changes in serum calcium, magnesium, and bicarbonate; QTc interval; and hemodynamic stability.

Results: Twenty-five patients entered the run-in phase, five achieved heparin free status, the remaining 20 were randomized; 19 completed the study. The mean CHD per session at baseline was 3646±1386 units and was reduced by 28% (2592±2234 units; p<0.01) at the

end of the run-in phase. At study end, CHD per session in the AD group was 2608±2120units compared to 2403±2427units in the CD group. This corresponded to a reduction of 1285units in CHD with AD and 1490units with CD (p=0.73, Figure 1). Results remained unchanged after adjustments. Ionized calcium was unchanged with AD, but decreased 0.10mmol/L with CD (p<0.01). There were no differences in serum magnesium or bicarbonate, QTc interval, number of intradialytic hypotensive events, or other hemodynamic parameters.

Conclusions: CD is a safe alternative to acetate dialysate, but the data does not support our study hypothesis of a reduction in the CHD.



Funding: Government Support - Non-U.S.

TH-PO905

Abstract Withdrawn

TH-PO906

Rotigotine in Patients with Restless Legs Syndrome and End-Stage Renal Disease Requiring Hemodialysis Virpi Rauta,[1] John W. Winkelman,[2] Yves Dauvilliers,[3] Markku Partinen,[4] Heike Benes,[5,6] Hanna Schröder,[7] Nadine Goldammer,[7] Elisabeth Dohin,[8] Erwin Schollmayer.[7] *[1]Helsinki Univ Central Hospital, Helsinki, Finland; [2]Massachusetts General Hospital, Boston, MA; [3]Hospital Gui de Chauliac, Montpellier, France; [4]Helsinki Sleep Clinic, Vitalmed Research Center, Helsinki, Finland; [5]Somni Bene Institut für Medizinische Forschung und Schlafmedizin, Schwerin, Germany; [6]Rostock Univ, Medical Center, Rostock, Germany; [7]UCB Pharma, Monheim, Germany; [8]UCB Pharma, Brussels, Belgium.*

Background: Restless legs syndrome (RLS) occurs in ~20% of end-stage renal disease (ESRD) patients and is linked to increased morbidity and mortality. RLS may be particularly bothersome when patients are immobile during dialysis. Periodic limb movements (PLMs) in sleep frequently occur in RLS. PLM Index (PLMI; PLMs/hs in bed) is an objective severity measure.

Methods: Double-blind polysomnographic (PSG) study (RenaLys:NCT01537042) of rotigotine (RTG) in patients with RLS (International RLS Rating Scale [IRLS]≥15; PLMI≥15) and ESRD requiring hemodialysis. Patients randomized to optimal dose (1-3mg/24h) RTG/placebo (PBO). PSG assessment at baseline (BL) and end of 2-week maintenance (EoM). Primary efficacy outcome: PLMI, assessed by ratio EoM/BL. Other outcomes (*p*-values exploratory): IRLS, Clinical Global Impression severity (CGI-1). Patients with evaluable BL and EoM data assessed for efficacy.

Results: Of 30 randomized patients (RTG:20; PBO:10), 25 (15;10) completed study with evaluable data. Mean±SD PLMI ratio at EoM: 0.7±0.4 for RTG; 1.3±0.7 for PBO (ANCOVA RTG/PBO ratio: 0.44 [95%CI: 0.22,0.88], *p*=0.0232). Mean±SD change in PLMI score: -23.7±38.7 for RTG (BL:81.8±37.5); 10.3±21.0 for PBO (BL:85.3±67.3). Mean±SD change in IRLS: -15.9±9.1 (BL:25.7±5.0) for RTG; -8.6±7.2 for PBO (BL:24.4±5.1); LS-mean treatment difference (RTG versus PBO): -6.08 [95%CI: -12.18,0.02], *p*=0.0508. 10/15 RTG and 2/10 PBO patients were CGI-1 responders (≥50% improvement). LS-mean treatment difference for CGI-1: -0.81 [95%CI: -1.94,0.33], *p*=0.1534. Hemodialysis did not impact unconjugated RTG concentrations. Adverse events were typical of dopaminergic stimulation.

Conclusions: RTG improved PLMs and RLS symptoms in ESRD patients requiring hemodialysis.

Funding: Pharmaceutical Company Support - UCB Pharma

TH-PO907

The Effects of Rotigotine Patch for Restless Legs Syndrome in Japanese HD Patients Hirotake Kasuga,[1] Ryo Takahashi,[1] Keiko Kimura,[1] Chieko Matsubara,[1] Kiyohito Kawashima,[1] Yasuhiko Ito.[2] *[1]Nephrology, Nagoya Kyoritus Hospital, Nagoya, Japan; [2]Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan.*

Background: Restless legs syndrome (RLS) is a common disease. The occurrence of this disease in HD patients is higher than general population. Recently, Rotigotine patch could be used for RLS in Japan. However the effect was not studied in detail in Japanese HD patients. The purpose of this study was to investigate morbidity and characteristics of RLS and the effects of Rotigotine patch in Japanese HD patients.

Methods: Two hundred nineteen HD outpatients in our hospital were screened based on the diagnosis criteria of International RLS Study Group (IRLSSG). We compared the patients' characteristics between RLS and non-RLS group. Nine patients with RLS were treated by Rotigotine patch for 3months. The effects of Rotigotine were evaluated by International Restless legs syndrome Rating Scale (IRLS) and Pittsburgh Sleep Quality Index (PSQI) before and 3 months after treatment.

Results: Twenty one (10.0%) HD patients were diagnosed as RLS by criteria of IRLSSG. Mean age of RLS patients was younger than that of non-RLS patients (59.1±9.2 years old versus 64.9±10.9 years old ; p=0.01), however there was no significant difference in sex, presence of diabetes and serum ferritin levels. Mean IRLS of 9 RLS patients before Rotigotine treatment was 23.9±7.2. Mean IRLS after treatment were 14.5±5.7 at 1 month (p<0.05, versus before treatment), and 18.9±7.5 at 3 months (p<0.05, versus before treatment). In 10 questions of IRLS, "daily affairs" (1.6±1.3 versus 0.9±1.1, p<0.05) and "mood disturbance" (2.1±0.9 versus 1.6±1.2, p<0.05) were significantly improved at 3 months. PSQI didn't improve significantly 3 months after treatment.

Conclusions: Morbidity of RLS in Japanese HD patients was similar to that of Western HD patients. Rotigotine patch was useful for RLS treatment in Japanese HD patients.

TH-PO908

Clinical Analysis of Restless Legs Syndrome in Long-Term Japanese Hemodialysis Patients Kazunori Yamada,[1] Toru Shibata,[2] Toshiaki Kimura,[2] Tsutomu Matsushima,[2] Remon Otake,[2] Akira Junicho,[2] Masatsune Hasegawa,[2] Mitsuhiro Kawano,[1] Toru Hasegawa.[2] *[1]Div of Rheumatology, Kanazawa Univ Hospital, Kanazawa, Japan; [2]Hasegawa Hospital, Toyama, Japan.*

Background: The reported prevalence of Restless legs syndrome (RLS) in end stage renal disease patients varies (6-70%) probably due to race and dialysis conditions including dialysis period. DOPPS annual report described that dialysis duration in Japan is longest in participating countries. RLS in long-term dialysis has not been well characterized. Our aim is to clarify RLS in long-term dialysis patients.

Methods: We enrolled 137 Japanese patients on HD (32 women and 105 men; mean age 64.5±12.3) in our hospital in Decenter 2013.Average HD period was 126.7±96.1 months. We analyzed the prevalence and severity of RLS. RLS was diagnosed using the criteria of the International Restless Legs Syndrome Study Group (IRLSSG), and severity of RLS was estimated using the International Restless Legs Syndrome Rating Scale. We retrospectively analyzed the impact and severity of RLS versus duration of HD, clinical data and complications. The impact of RLS on sleep disturbance was assessed with Epworth sleepiness Scale (ESS).

Results: The prevalence of RLS was 16.1% (22/137). The average IRLS was 7.4 (0-30). Six patients had moderate or severe RLS. The RLS group was significantly younger (59.7 years old versus 65.4 years, p=0.040) with higher serum calcium (9.5 mg/dL versus 9.3 mg/dL, p=0.041) than non-RLS group. No significant differences were seen in other factors including HD duration. Next, we divided RLS patients to two groups by IRLS: mild (n=16) and moderate-severe group (n=6). Patients in the latter showed significantly lower KT/V than those in mild group (1.31 versus 1.50, p=0.049), and tended to have a high prevalence of cardiovascular complications (66.7% versus 25.7%, p=0.140) and high ESS score (7.0 versus 4.5), p=0.145).

Conclusions: These results suggest young HD onset and serum level of calcium to be risk factors of RLS. Patients with moderate to severe RLS may be at increased risk for cardiovascular and sleep disorders.

TH-PO909

Assessing the Utility of Testing Aluminium Levels in Dialysis Patients Ashish K. Sharma,[1,2] Nigel David Toussaint,[1,2] Edward Robert Smith,[1] Stephen G. Holt.[1,2] *[1]Dept of Nephrology, The Royal Melbourne Hospital, Parkville, VIC, Australia; [2]Dept of Medicine (RMH), The Univ of Melbourne, Parkville, VIC, Australia.*

Background: Plasma aluminium (Al) is routinely tested in many dialysis patients. Al exposure may lead to acute toxicity and levels in excess of ~2.2µmol/L (60µg/L) should be avoided. Historically toxicity has been caused by excessive dialysate Al but modern reverse osmosis (RO) water should be Al-free. Nevertheless many units continue to perform routine annual Al levels on dialysis patients.

Methods: This single-center study retrospectively analysed plasma Al levels between Jan 2010 and Dec 2013 using our database (Nephworks 6), as well as assessing levels in the raw water feed and RO product with the aim of determining the utility of these measurements.

Results: 2058 plasma Al tests in 755 patients (61.9% male, mean age 64.7 years) were reviewed showing mean +/-SD of 0.41 +/-0.30µmol/L. 111 (5.4%) tests from 61 patients

had Al levels >0.74µmol/L and 45 (73.8%) of these patients were or had been prescribed Al hydroxide (AlOH) as a phosphate binder. Seven patients had Al concentrations >2.2µmol/L. In one of these patients, who was on home dialysis at the time, no source of Al was identified but the repeat Al level was normal. 166 patients taking AlOH (78.7% of all patients on AlOH) had levels ≤0.74µmol/L, the odds ratio of plasma Al >0.74µmol/L on AlOH was 9. The cost of plasma Al assay is AU$30.60, thus costs were AU$62,974.80 over the study period or over AU$1300/month. Despite RO feed water Al levels as high as 48µmol/L (1300µg/L), Al output from the RO was almost always undetectable (<0.1µmol/L). We detected dialysate Al levels >2.2µmo/L only 3 times since Jan 2010, and never in last 3 years.

Conclusions: Routine unselected testing of plasma Al appears unnecessary and expensive and more selective testing in dialysis patients should be considered.

TH-PO910

Relationship between Serotonin 1A Receptor Polymorphism and Emotional Distress and Health Related Quality of Life in Hemodialysis Patients

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Background: Emotional distress such as anxiety and depression may have significant impact on quality of life (QoL) in hemodialysis (HD) patients. Genetic polymorphism may be an important modulating factor in emotional distress and QoL in these patients. Serotonin 1A receptor polymorphism may be associated with vulnerability to emotional distress. Aim was to investigate the relationship between emotional distress and health-related QoL and genetic polymorphism in Korean hemodialysis patients.

Methods: Clinically stable HD patients were asked to participate in the study. Thirty-six-item Short-Form Health Survey (SF-36), Hospital Anxiety and Depression Scale (HADS) were used to assess health-related QoL and emotional distress, respectively. Sociodemographic factors such as age, sex, education and HD-related clinical factors (dialysis vintage and frequency, Kt/V), and laboratory parameters were assessed. Serotonin 1A receptor polymorphism was assessed using PCR. Regression analyses were conducted to find significant associations among these variables.

Results: Two hundred and sixty HD patients were enrolled. Mean age was 54.7 ± 11.9 years old and mean anxiety and depressive symptoms scores were 5.5 ± 3.8 and 7.4 ± 3.9, respectively. Age, depression symptom severities, and serotonin 1A receptor polymorphism were significantly associated with mental QoL but also with physical QoL in the final regression models. Anxiety symptom severity was only significantly associated with mental QoL. The comorbidity numbers, total HD duration were significantly associated with physical QoL in the final regression model.

Conclusions: After controlling multiple clinical variables, age, depressive symptoms, serotonin 1A receptor polymorphism were significantly associated with mental and physical QoL in HD patients. Chronic ongoing distress related to HD and genetic polymorphism may contribute to increase emotional distress and decreased QoL in HD patients.

Funding: Private Foundation Support

TH-PO911

Association of the Geriatric Nutritional Risk Index and the Glasgow Prognostic Score at the Initiation of Hemodialysis Therapy with All-Cause Mortality in the Nation-Wide Registry Cohort Study in Japan

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Background: The geriatric nutritional risk index (GNRI) is a screening tool for nutritional status in chronic dialysis patients. The Glasgow prognostic score (GPS) is known as an independent prognostic factor in cancer patients. Although the prognosis is still poor in incident dialysis patients, it remains unknown whether these composite scores are useful in predicting outcomes. So, we aimed this study to examine whether GNRI and GPS assessed at the introduction of hemodialysis (HD) are associated with overall mortality.

Methods: We studied 4,329 patients (male: 66%, age: 66.8±12.7 years) from the Japanese nation-wide registry data, who had started HD during 2006. We calculated GNRI and GPS using the data collected at the initiation of HD, and surveyed their outcomes at the end of 2007. GNRI was calculated by following formula, [14.89 × ALB (g/dL)] + [41.7 × (Dry weight / Ideal body weight (equivalent to 22 kg/m^2 of BMI))]. The patients were divided into the quartiles (Q) by GNRI; Q1: <81.1, Q2: 81.2-88.0, Q3: 88.1-94.8, Q4: >94.8. We also divided all patients into the 3 categories (C) of GPS as follows; C0: CRP ≤ 1.0 (mg/dL) and ALB ≥3.5, C1: CRP > 1.0 or ALB < 3.5, C2: CRP >1 and ALB < 3.5.

Results: During the almost 1-year follow-up, 421 patients (9.7%) had expired. Multivariate logistic regression analysis adjusted by cofounders identified that both GNRI and GPS were independent risk factors for overall mortality.

	OR	95% CI	p-value		OR	95% CI	p-value
GNRI Q1 (vs Q4)	3.166	2.257-4.440	<0.0001	GPS C1 (vs C0)	1.743	1.316-2.310	<0.0001
GNRI Q2	1.696	1.280-2.605	0.004	GPS C2	3.629	2.712-4.856	<0.0001
GNRI Q3	1.103	0.750-1.622	0.619				

Conclusions: A lower GNRI (< 88.0) and a higher GPS (≥ score 1) at the initiation of HD are both useful in predicting a short-term mortality in incident HD patients.

TH-PO912

Impact of Weight Evolution on Mortality and Nutritional State in Hemodialysis Patients

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Background: Nutritional status is a key factor for patient survival and malnutrition is a major risk factor for mortality and inflammation in hemodialysis (HD) patients (pts). Our aim was evaluate the impact of weight evolution in prevalent HD pts.

Methods: Observational prospective study in 149 prevalent Portuguese HD pts. We obtained baseline demographic data, blood biochemistry, comorbidities and prevalence of malnutrition by mini-nutritional assessment (MNA) and patient-generated subjective global assessment (PG-SGA). The follow up period time was 12 months. Risk of mortality was investigated adjusted to comorbidities, age and time on HD using log-rank test and cox proportional model.

Results: In our cohort 93 were male, mean age was 67 years, 39% had diabetes (DM) and average time on HD was 54 months. Comorbidity Charlson score was on average 4.4±2.3 and body index mass was 27±4.5. During the follow up, 6 pts died of CV events. According to MNA and PG-SGA, 59 and 71% of our pts were well nourished. 31% of the pts lost at least 5% of their weight. PG-SGA in pts with unintentional weight loss (WL) was 3.8±4 VS pts who maintained or increased weight 2.4±2.1(p=0.05). In a Kaplan Meier test, pts with WL had a lower survival (172 VS 331months; log rank 8.9; p=0.003). In a linear regression, lower serum albumin was associated with comorbidity Charlson score (Exp(B)-1.9;p=0.009; IC 95% -3.2 to -0.5). Hypoalbuminaemia was associated with higher mortality (binary regression: Exp(B)0.025; p=0.001; IC 95% 0.003 to 0.2). Both models were adjusted to time on HD. In a cox hazards model, unintentional WL was an independent predictor of mortality (HR=10.4; p=0.034; IC 95% 1.2-91.3), adjusted to age and DM. Basal MNA and PG-SGA were not predictor of death, but PG-SGA was an independent predictor of unintentional WL (Exp(B)0.86; p=0.023; IC 95% 0.8-0.98).

Conclusions: Unintentional weight loss in pts on HD was an indepent predictor of mortality. Basal nutrition screnning and close monitoring of weight evolution can be a tool of classification of risk and for prioriting pts care.

TH-PO913

Using a Simpler Creatinine Index as a Surrogate of Lean Body Mass Predicts Long-Term Survival in Chinese Hemodialysis Patients

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Background: Reduced lean body mass (LBM) is an indicator of malnutrition inflammation syndrome and is common in dialysis patients. Muscle mass and LBM inversely correlate with mortality in hemodialysis (HD) patients. Recently, a simpler creatinine index (CI) formula is developed. The aims of this study were to investigate the impact of LBM derived from the simple CI formula on HD patient outcomes.

Methods: We enrolled 1269 incident HD patients between Feb. 1981 and Feb. 2012 in three HD center of Taiwan and followed them until Feb. 2013. Clinical characteristics, HD-associated parameters, and serum chemistry profiles of each patient were collected at 1 month after initiating HD. LBM was estimated using creatinine index formula. Multiple linear regression analysis, Kaplan–Meier survival analysis, and Cox regression proportional hazard analysis were used to define independent variables and compare survival between groups.

Results: All patients were divided to three groups according to the tertile of estimated LBM between men and women. The patients in group 1 had the lowest LBM (n=423), group 2 had the intermediate LBM (n=423), and group 3 had the highest LBM (n=423) in men and women. Group 1 patients had shorter overall survival ($p < 0.01$). Each point increase in LBM reduced the hazard ratio for mortality by 22% after adjustment.

Conclusions: CI derived from the simple creatinine kinetic formula could be a surrogate of LBM to evaluate nutrition status and predict survival in HD patients. Besides, the simple formula could extend its use in clinical practice for HD patients.

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TH-PO914

Nutritional Status and RFLP Analysis in Patients Receiving Hemodialysis over 30 Years

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Background: Only 4% of 0.3 million patients receiving hemodialysis survive over 25 years after their initiation of hemodialysis even in Japan. To elucidate the clinical characteristics of long survival patients, we investigated their life-style and genetic factor. TT allele of methylene tetrahydrofolic acid reductase (MTHFR) C677T was reported as high risk factor for cardiovascular event and poor survival in CKD patients.

Methods: 78 of Japanese patients receiving hemodialysis over 30 years are enrolled. Their clinical profile, dietary intake and daily life activity was evaluated with diet history

questionnaires (DHQ), geriatric nutritional risk index (GNRI), and basal activity of daily life(BADL) scores. As their genetic factor, restriction fragment length polymorphism (RFLP) of MTHFR C677T were analyzed by PCR using their genomic DNA.

Results: Patients were 63.2±8.1 years old and included no diabetes. Male was 39.7%. Daily intake of energy, protein, and their achievement rate for Japanese guideline 2007 were 30.6±9.3 kcal/kg (97.7%),1.1±0.4 g/kg (101.9%). The frequency of TT allele was 26.9% and it was almost twice as average in Japanese population. BADL was 90, and their daily activities were highly maintained. In patients with TT allele, serum folic acid concentration were significantly lower, and serum homocysteine concentration was significantly higher than other groups, though daily intake of folic acid did not show significant difference.

Conclusions: Although the frequency of TT allele was higher than average population, our patients showed longer survival with high QOL and nutritional status based on our guideline. It is suggested that the proper life-style might overcome the genetic risk factors in patients receiving hemodialysis.

Funding: Private Foundation Support

TH-PO915

Efficacy of Oral Administration of LebeninS®, a Lactic Acid Bacteria Preparation, to HD Patients Yasuhisa Kurata,[1] Miho Hida,[1] Toru Hyodo.[2] *[1]Dept of Nephrology, Kuratakai Kurata Hospital, Hiratsuka, Japan; [2]Dept of Urology, Kuratakai Eijin Clinic, Hiratsuka, Japan.*

Background: The plasma levels of indoxyl sulfate (=indican, IS) are markedly increased in uremic patients and cannot be efficiently reduced by hemodialysis (HD) due to its albumin-binding property.Uremic toxicins as IS are produced in the intestine as bacterial putrefactive metabolites, and accumulate to a great degree in the feces of HD patients. We had demonstrated that oral administration of lactic acid bacteria in HD patients is effective in reducing the serum IS levels by inhibiting bacterial production by correcting the intestinal microflora[Nephron. 1996;74(2):349-55].

Methods: We administered LebeninS®, a lactic acid bacteria preparation, to 25 HD patients and assessed the mortality, complication, plasma IS and pentosidine levels, and the pulse wave velocity (PWV). The patients were divided into two groups: 11 of the patients were administrated 6g of LebeninS® daily for 12 months (LebeninS® group, Age: 79.0±0.68ys, HD history: 6.7±8.4ys, 4 diabetic, 5 female), and the other 14 patients(Age: 72.9±8.7 ys, HD history: 9.5±7.7ys, 6 diabetic, 7 female) were given nothing(control group). No statistical differences between these groups were observed in age, HD history, sex, diabetic, body mass index, and systolic and diastolic blood pressure.

Results: The frequency of complication during 12 months was 9.1% in LebeninS® group and 50% in the control group (P=0.0421). The plasma IS level (P=0.041) and PWV (P=0.006) significantly increased in the control group after 12 months. No adverse effects were observed.

	0 Month	12 months
PWL (Cont.) (cm/sec)	2173.6±415.8	2861.5±1042.3*
PWL (Lebenin)	2467.9±1003.8	2193.7±791.2
I S (Cont.) (mg/L)	190.0±70.1	257.0±102.7*
I S (Lebenin)	147.8±55.3	163.3±89.1
Pentosidine (Cont.) (ug/mL)	0.3±0.1	0.5±0.2*
Pentosidine (Lebenin)	0.3±0.1	0.4±0.2*

*: significant change.

Conclusions: These results demonstrate that oral administration of lactic acid bacteria preparations to HD patients is effective not only in reducing the plasma levels of uremic toxins such as IS, but also in clinical aspects including arteriosclerosis.

Funding: Pharmaceutical Company Support - Wakamoto Co Ltd.

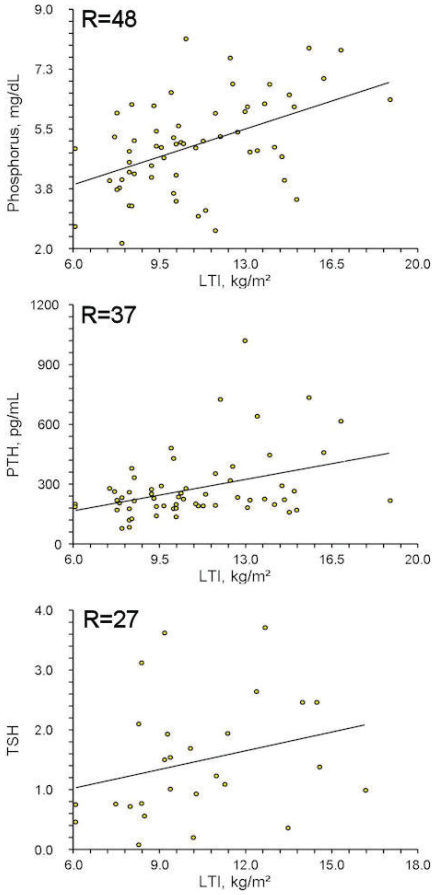
TH-PO916

Bioeletrical Impedance Analysis for Nutritional Status in Hemodialysis Patients: Two Sides of the Same Coin? Paolo Lentini,[1] Luca Zanoli,[2] Massimo de Cal,[1] Stefania Rastelli,[2] Anna Basso,[1] Andrea Contestabile,[1] Antonio Granata,[1] Roberto Dell'Aquila.[1] *[1]Nephrology, S. Bassiano Hospital, Bassano Del Grappa, Italy; [2]Internal Medicine, Univ of Catania, Catania, Italy; [3]Nephrology, S.Giovanni di Dio Hospital, Agrigento, Italy.*

Background: Malnutrition is a common feature associated with increased mortality in dialysis patients. Principal causes of malnutrition include dietary restrictions, metabolic acidosis, hormonal alterations, dialytic nutrient losses, catabolic properties of dialysis, and chronic inflammation. Inversely, excessive nutrition may yield to obesity and lead to high levels of phosphorus and hyperparatyrodism, with a high risk of vascular calcifications, fractures and CV events. Multifrequency bioimpedance spectroscopy, a tool for bioelectrical impedance analysis (BIA), is a practical, simple, repetitive, non-invasive, and inexpensive method to measure the body composition.**Aim** To determine the optimal nutritional status in HD patients.

Methods: HD patients underwent standardized evaluation of their fluid and nutritional status with a portable bioimpedance monitor (Body Composition Monitor-BCM Fresenius® Medical Care). All measurements were carried out after the short interdialytic interval. Lean Tissue Index (LTI), an index of the nutrition status, was calculated as the quotient of LTM/Height² (kg/m²).

Results: 60 HD patients were enrolled(age 64±16yrs,males 55%).High LTI was significantly associated with high phosphorus, PTH, TSH and Ca-P product (R=0.52),known markers of nutrition status,increased number of drugs for calcium-phosphorus metabolism (R=0.39) and mean blood pressure (R=0.31).



Conclusions: BIA is useful to monitoring nutritional status in HD and to select patients at higher risk for vascular calcification and cardiovascular events.Changes in body composition detected by BIA measurements may yield an overall picture of the patient's nutritional status.

TH-PO917

High Ferritin Level and Low Nutrition Status Predicts Higher Risk of Infection-Related Hospitalization in Incident Dialysis Patients: A Japanese Prospective Cohort Study Sawako Kato,[1] Shoichi Maruyama,[1] Bengt Lindholm,[2] Yukio Yuzawa,[3] Yoshinari Tsuruta,[4] Seiichi Matsuo.[1] *[1]Nagoya Univ Graduate School of Medicine, Nagoya, Japan; [2]Baxter Novum & Renal Medicine Karolinska Inst, Stockholm, Sweden; [3]Fujita Health Univ School of Medicine, Toyoake, Japan; [4]Meiyo Clinic, Toyohshi, Japan.*

Background: Iron management, evaluation of iron overload and its possible association with infections in dialysis patients (pts) are topics lacking consensus. This study aimed to clarify relations between ferritin and infection risk.

Methods: We enrolled 129 Japanese incident dialysis pts (84 males, 59 ± 11 years, 121 HD / 8 PD) and followed them prospectively for a median of 38 (range 1-74) months. We reviewed hospitalization due to infections and checked biomarkers and nutrition status by global subjective assessment (SGA: A, well nourished, B, mild/moderately malnourished, C, severely malnourished). The pts were divided into higher-ferritin group (H) and lower-ferritin group (L) by the median value.

Results: The median of serum ferritin levels was 82.0 (range 45.5-186.0) ng/ml. At dialysis initiation, 13.7% used a central venous catheter (CVC) and the ferritin levels of CVC users were significantly higher than in non-CVC pts (253 ± 280 versus 128 ± 138 ng/ml, P=0.02). In group H, the rate of hospital admissions due to infections was 0.23 (with 10.9 hospital days) per pts year, and in group L 0.09 (with 1.57 hospital days) per pts year. Moreover, the hospitalization days in pts with category A, B and C of SGA were 2.5, 10.1 and 27.7 days per pts year in group H and 0.2, 1.4 and 6.4 days in group L. The duration from dialysis initiation to first hospitalization due to infection was significantly shorter in group H (Log rank 4.44, P=0.035). High ferritin associated with significantly increased relative risk (Cox hazard model; crude RR 1.52 95% CI; 1.06-2.17), even after adjustments for age and CVC use (RR 1.50. 95% CI; 1.04-2.14).

Conclusions: Although ferritin levels and doses of iron administered to dialysis pts in general are lower than in Western counties, a higher ferritin level among these Japanese pts still associated with increased risk of hospitalization due to infection, especially in pts with poor nutritional status.

Funding: Government Support - Non-U.S.

TH-PO918

Associations of Appetite with Quality of Life and Depressive Symptoms in Hemodialysis Patients with and without Major Comorbidities
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Background: Reduced appetite, malnutrition, poor health-related quality of life (HRQOL), depression and comorbidities are problems that impair the well being of maintenance hemodialysis (MHD) patients. We explored associations of reduced appetite with HRQOL and depression symptoms and assessed if the associations is explained by nutritional status in patients with and without major comorbidities.

Methods: Cross-sectional analysis of data of 543 MHD patients (60.2% males, mean age of 48.1±13.7 yr) enrolled (Jan 2010-Jan 2011) in the PROHEMO cohort in Salvador, Brazil. 171 patients were diagnosed and 372 were not diagnosed with any of the following comorbidities: diabetes, heart diseases, cerebral vascular disease, peripheral vascular disease, pulmonary diseases and cancer. The patients were asked to rate the degree (absent, light, moderate, intense) of persistent reduction in appetite. The Malnutrition-inflammation score (MIS) was used to assess nutritional status (higher worse). SF-36 was used for HRQOL scores (higher better) and BDI for depression symptoms scores (higher worse). Linear regression was used for covariate adjustments and interaction.

Results: HRQOL scores decreased and both depression symptoms scores and MIS increased monotonically across the four categories of appetite rate (P for trend<0.01). In patients without major comorbidities, linear regression analysis with adjustments for sociodemographis, hemoglobin, vintage and vascular access showed higher BDI scores (difference=4.0, P=0.001) and lower scores for all HRQOL scales in patients referring reduced appetite with differences larger than 7 points for several components (P<0.05). Differences in HRQOL and depression symptoms were largely reduced after adjustments for MIS. Similar associations were seen in patients with major comorbidities (interaction coefficients appetite*comorbidity>0.15).

Conclusions: The results are consistent with a role of malnutrition in explaining the lower HRQOL and higher probability of depression in MHD patients with reduced appetite.

TH-PO919

Low Serum Neutrophil Gelatinase-Associated Lipocalin Level as a Malnutrition Marker in Hemodialysis Patients
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Background: Circulating concentration of neutrophil gelatinase-associated lipocalin (NGAL or LCN2) is elevated in acute or chronic kidney diseases and shows a positive correlation with poor renal outcome and mortality, but its clinical significance in maintenance hemodialysis (HD) patients remains elusive.

Methods: Serum NGAL levels were determined by ELISA in 79 HD patients. Their correlation to laboratory findings and morbidity (as development of severe infection or serum albumin reduction) was investigated using linear and logistic regression analyses.

Results: In a cross-sectional study, serum NGAL concentrations were determined independently by % creatinine generation rate (an indicator of muscle mass, standardized coefficient β=0.44), white blood cell count (β=0.37) and anion gap (which likely reflects the amount of daily protein intake, β=0.23, P<0.01 each). In a longitudinal study, serum albumin levels a year later were predicted by baseline NGAL (β=0.36) and age (β=-0.37, P<0.005 each). Within a year, patients with the lowest NGAL tertile showed significantly increased risk for marked decline in serum albumin levels [≥0.4 g/dl; adjusted odds ratio (OR) 4.6, 95% confidence interval (CI) 1.1-18.9, P< 0.05] and tendency of increased occurrence of severe infection requiring admission (OR 2.9, CI 0.5-16.0, not significant) compared to the middle and highest tertiles.

Conclusions: Low serum NGAL levels appear to be associated with current malnutrition and also its progressive worsening in maintenance HD patients.

TH-PO920

Serum Ferritin Level Is an Independent Risk Factor for All-Cause Mortality in Incident Hemodialysis Patients
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Background: Serum ferritin has been regarded as an indicator of inflammation as well as a marker of body iron storage in patients with end-stage renal disease (ESRD). In addition, high serum ferritin levels were significantly associated with poor clinical outcomes in prevalent dialysis patients. However, little is known about the prognostic impact of serum ferritin concentrations on the clinical outcome in incident hemodialysis (HD) patients.

Methods: A prospective cohort of 939 incident HD patients from 36 dialysis centers of the Clinical Research Center for ESRD in Korea was selected for this study. Serum ferritin levels were measured at the time of HD initiation. Patients were divided into tertiles according to log ferritin concentrations; <2.17, 2.17-2.50, and >2.50 ng/mL. Cox proportional hazard analysis was performed to determine the independent prognostic value of serum ferritin levels for all-cause mortality.

Results: The median ferritin concentrations were 217.8 (118.7-381.1) ng/mL. Pearson's correlation analysis showed that log ferritin levels were positively correlated with white blood cell counts (r=0.108, P=0.001) and log C-reactive protein concentrations (r=0.151, P<0.001), while negatively associated with serum albumin levels (r=-0.116, P<0.001). During a median follow-up duration of 20.7 months, 85 (9.1%) patients died. Kaplan-Meier analysis showed that all-cause mortality rates were significantly higher in the highest tertile ferritin group compared to the lowest tertile group (P<0.001). Multivariate Cox proportional hazard analysis demonstrated that log ferritin was independently associated with an increase in all-cause mortality risk after adjustment for confounding variables (per 1 ng/mL increase, hazard ratio=2.62, 95% confidence interval=1.45-4.73, P=0.001).

Conclusions: Serum ferritin concentration was a significant independent predictor of all-cause mortality in incident HD patients, suggesting that determining serum ferritin levels might be helpful to stratify mortality risk in these patients.

TH-PO921

Sex-Specific Difference According to Different Types of Cancer in End-Stage Renal Disease Dialysis Patients: Taiwan National Cohort Study
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Background: The distribution of site-specific cancer mortality and mortality between men and women in end-stage renal disease (ESRD) dialysis patients is unknown.

Methods: We enrolled, from the Taiwan National Health Insurance Research Database, a cohort of 40,833 ESRD patients who started maintenance dialysis between 1999 and 2004. They were followed from dialysis initiation until death, discontinuation of dialysis, or the end of 2008. We calculated the survival rate and mortality risk of ESRD patients with cancer.

Results: Of 40,833 dialysis patients, 2352 (5.76%) had new cancer diagnosed. Older age, diabetic mellitus, chronic lung disease, and chronic liver disease were associated with a significantly increased risk for cancers. Liver cancer (20.63%) was most frequent in men, followed by bladder (16.88%) and kidney (11.61%) cancers. In women, bladder cancer (25.57%) was most frequent, followed by kidney (16.31%) and breast (11.20%) cancers. Older age, male gender, DM, and cardiovascular diseases were independent predictors of all-cause mortality in patients with cancer. However, there were no statistical differences between women and men after site-specific cancer analyses. When comparing 5-year survival rates for various cancers, kidney cancer and bladder cancer were higher than others; in contrast, those for lung cancer, stomach cancer and liver cancer were lower.

Conclusions: The distribution of site-specific cancer mortality is different between men and women in ESRD dialysis patients. Higher mortality for men than women for cancer is due to difference in types of cancers. The survival rate for kidney cancer was the highest in ESRD dialysis patients while it was worst for lung cancer.

TH-PO922

Health Service Usage in Rural versus Urban End Stage Kidney Disease in Australia
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Background: ESKD is a significant burden upon health systems and health services are used differently by rural and urban patients. To compare health service usage in rural versus urban end stage kidney disease (ESKD) patients in Australia.

Methods: We identified all New South Wales (NSW) residents receiving renal replacement therapy 2000-2010 using the ANZDATA Registry. Patients were linked to the NSW Admitted Patient Data Collection (APDC- records all NSW hospitalizations). We separated people into rural (living out of highly accessible areas) and urban (living in highly accessible areas), using postcodes and the Accessibility remoteness index of Australia scores. Rates of hospitalization, inter-hospital transfers and lengths of stay (LOS) were compared excluding day-only and dialysis admissions.

Results: ANZDATA identified 11,036 people, 531 did not match within APDC or had missing data, leaving 10,505 patients (120,828 hospitalization records), with median follow-up of 4.41 years (IQR 2.24-7.82). Of these, 1,527 (15%) lived rurally and 8,978 (85%) urban. The rate of hospitalization/patient year was 1.77 for rural residents compared to 1.47 for urban residents. In univariate analysis, rurality increased likelihood of admission by 43% (IRR 1.43 95%CI 1.31-1.56; p<0.001). A total of 3,181 patients underwent an inter-hospital transfer, 774 rural and 2407 urban. In univariate analysis, rurality increased the likelihood of requirement for transfer by 163% (IRR 2.63 95%CI 2.49-2.77; p<0.001). There was no difference in LOS (4.0 days versus 4.0 days respectively; p=0.08), further confirmed in univariate analysis (IRR 1.00 95%CI 0.96-1.05; p=0.98).

Conclusions: Rural residents are hospitalized and transferred more than urban residents, but LOS is similar. Risk adjustment for differences in baseline characteristics will help explore the reasons underlying these differences.

Funding: Private Foundation Support

TH-PO923

Socioeconomic Deprivation and Patient Survival on Chronic Dialysis

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Background: Socioeconomic deprivation has been linked to worse end-stage kidney disease survival. The effect of socioeconomic deprivation on survival following initiation of chronic dialysis, including the impact of transplantation, was examined.

Methods: A retrospective, observational, cohort study was conducted to investigate the association of socioeconomic deprivation with patient survival on chronic dialysis, with censoring at the time of transplantation. All adult patients, with a valid postal address, commencing dialysis from 1990 to 2009 in a tertiary centre in Ireland were given a deprivation index (DI) score using the Pobal Haase-Pratschke Deprivation Index 2011. Cox proportional hazard and Kaplan Meier survival analysis were used to examine any association of socioeconomic deprivation with survival.

Results: The 1,794 patients included had a median follow-up of 3.8 years. The most deprived quartile were significantly younger than the most affluent, mean age 56.7 years versus 59 years, p=0.006, respectively. There was no association between DI score and survival in an unadjusted model (hazard ratio (HR) 1.00, 95% CI 0.99-1.01). However, survival in the most affluent quartile was superior to the most deprived in a multivariable adjusted model including age, gender and dialysis modality (HR 0.83, 95%CI 0.7-0.99, p=0.04). These results were largely unchanged by censoring at time of transplantation (least deprived versus most deprived, HR 0.85, 95% CI 0.7-1.03, p=0.09).

Conclusions: Whereas socioeconomic deprivation was not associated with poorer survival in dialysis patients in the Irish healthcare model, socioeconomically deprived patients tended to be younger and increasing affluence attenuated the mortality risk due to ageing in this setting. Further research should focus on identifying modifiable targets for intervention that account for this survival advantage in the more affluent.

TH-PO924

Fate Tracing Reveals Submesothelial Fibroblasts and Not Mesothelial Origin of Myofibroblasts in Peritoneal Fibrosis

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Background: Understanding the origin of myofibroblasts in peritoneum is of great interest because these cells are responsible for scar formation in peritoneal fibrosis after peritoneal dialysis. Recent studies suggest mesothelial cells are an important source of myofibroblasts through epithelial-mesenchymal transition; however, confirmatory studies *in vivo* are lacking.

Methods: We used tamoxifen-inducible Cre/Lox techniques to genetically label and fate map mesothelial cells and submesothelial fibroblasts in models of peritoneal fibrosis induced by sodium hypochlorite, peritoneal dialysis solution, or adenovirus expressing active TGF-β1.

Results: After pulse labeling induced by tamoxifen, the genetically red fluorescence protein labeled mesothelial cells did not generate transcripts of *collagen I (α1)* in normal peritoneum. Using red fluorescent protein as the fate marker, we found no evidence that mesothelial cells transmigrated into the thickened basal lamina and differentiated into α smooth muscle actin+ myofibroblasts *in vivo* although α smooth muscle actin could be induced in the primary culture of mesothelial cells *ex vivo* treated by recombinant TGF-β1. Cytokeratin+ mesothelial cells were found to express *collagen I (α1)* but not α smooth muscle actin after injury. No dilution of genetically labeled mesothelial cells was found, indicating the injured mesothelium was repaired by surviving mesothelial cells who had been genetically labeled. In contrast to no contribution of mesothelial cells to peritoneal myofibroblasts, genetically labeled submesothelial fibroblasts expanded and differentiated into myofibroblasts after peritoneal injury, accounting for a majority of myofibroblasts. No genetically labeled submesothelial cells expressed cytokeratin in peritoneal surface.

Conclusions: These data suggest that therapeutic strategies directly targeting myofibroblastic differentiation of submesothelial fibroblasts and promoting repair of mesothelium by surviving mesothelial cells may productively impact fibrotic peritoneal disease.

Funding: Government Support - Non-U.S.

TH-PO925

Vasopressin - 2 - Receptor Antagonist, Tolvaptan Provides Better Fluid Management and Improved Left Ventricular Hypertrophy in Peritoneal Dialysis Patients with Diabetes Mellitus

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Background: Last year we reported vasopressin-2-receptor antagonist, tolvaptan preserved residual renal function and ameliorated left ventricular hypertrophy. Here, we evaluated the effect of tolvaptan on peritoneal dialysis (PD) patients with DM for the relationship between urine volume (UV) and phosphate elimination, LVH, and inferior vena cava caliber (IVC).

Methods: Last year we reported tolvaptan preserved residual renal function and ameliorated left ventricular hypertrophy. Here, we evaluated the effect of tolvaptan on peritoneal dialysis (PD) patients with DM for the relationship between urine volume (UV) and phosphate elimination, LVH, and inferior vena cava caliber (IVC).

Results: At baseline, there was no difference in laboratory data between the groups. In Tolvaptan group renal Kt/V, and CCr were preserved, and LVMI and IVC at 12 months were lower and phosphate elimination in urine was higher than that of control group.

	baseline		at 6 months		at 12 months		p value		
	Control	Tolvaptan	Control	Tolvaptan	Control	Tolvaptan	Time	Treat	Time* Treat
Urine volume (mL/day)	1305 ± 389	1023 ± 297	573 ± 312	1096 ± 432	179 ± 288	883 ± 418	0.001	0.001	0.209
Phosphate elimination in urine (mg/day)	162 ± 71	177 ± 77	91 ± 54	171 ± 55	61 ± 52	149 ± 89	0.037	0.001	0.932
LVMI (g/m^2)	197 ± 42	229 ± 56	214 ± 74	175 ± 32	201 ± 49	161 ± 26	0.200	0.030	0.960
IVC (mm)	15.2 ± 3.0	16.6 ± 4.0	15.0 ± 4.1	12.7 ± 3.9	15.4 ± 2.4	12.3 ± 2.5	0.660	0.033	0.568

Significant correlation between LVMI and IVC was observed (r = 0.50, p = 0.0128). IVC was correlated with UV at 6 and 12 months (r=-0.403, p=0.037). ΔLVMI(LVMI at 12 month/ LVMI at baseline) and average UV was inversely correlated (r=-0.413, p=0.045).

Conclusions: Short term usage of tolvaptan was effective for residual renal function, phosphate elimination and IVC. Moreover, IVC indicates the predictor of short term volume control. While LVMI indicates the predictor of long term volume control.

TH-PO926

Cuboidal Change and Increased Pro-Fibrotic Reactions Accompanying Epithelial Mesenchymal Transition in Peritoneal Mesothelial Cells by Peritoneal Dialysis Solution with Enhanced Oxidative Stress in Rats

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Background: Functional change w/wo EMT of PMCs plays a pivotal role for progression of peritoneal fibrosis (PF) and sclerosis (PS) in patients on PD, and morphological change, i.e. increased surface area of PMCs, could reportedly predict extent of PS leading to encapsulating peritoneal sclerosis. High glucose and oxidative stress (OS) are supposed to be involved with the pathological mechanisms. Therefore, the present study aimed to elucidate the impact of PD solution, and solution with $FeCl_3$, on morphologic change and biological reactions of PMCs in vivo.

Methods: Male SD rats (8~10 weeks) were used for PD model: Sham group (n=4); PD dialysate only (neutral type, 2.5% glucose), group-A (n=5); PD dialysate with 0.5%$FeCl_3$ group, group-B (n=5). Rats were given the allocated test solution daily (20 mL) by intra-peritoneal injection for two weeks, and were subjected for peritoneal analysis. PMCs were collected by suspending of peritoneal tissue in trypsin solution. They were confirmed by flow-cytometry with HBME1 antibody staining. The pooled RNA of the respective groups was subjected for real-time PCR (housekeeping:CPH).

Results: Histologically, PMCs presented cuboidal shape (80%<) in group B, while they were flat in group A and sham. The fold changes of mRNA (versus sham) were shown in Table 1.

	Col-1	FN	TGF	MMP-2	SMA	Vimentin	E-cadherin	VEGF
PD (2.5%G)	x 4.57	x 8.72	x 1.87	x 66.14	x 0.82	x 2.42	x 1.33	x 1.42
$FeCl_3$					x 1.43	x 4.52	x 1.64	X 4.03

Conclusions: PD solution (glucose) increased pro-fibrotic reactions in PMCs, and oxidative stress enhanced EMT with accompanying angiogenetic reaction, indicating the primary pathological role of high glucose for PF and the critical role of oxidative stress for PS development. The cuboidal change of PMCs may be one of the histological step for enhanced PS in PD therapy.

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TH-PO927

Heme Oxygenase-1 Polymorphisms and Peritoneal Membrane Characteristics in Peritoneal Dialysis Patients

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Background: Baseline peritoneal membrane transport characteristics vary between individuals. Fast peritoneal small solute transport (PTR) is associated with oxidative stress and inflammation. Heme oxygenase 1 (HO-1) is an anti-oxidative and anti-inflammatory heme-degrading enzyme, expressed in the human peritoneum. Two functional promotor polymorphisms of the HO-1 gene are known: a $(GT)_n$ repeat polymorphism (short/long repeat: S/L) and a single nucleotide polymorphism (SNP) A(-413)T (S,A: higher activity). We studied the association between these polymorphisms and baseline PTR assessed by modified (3.86%) peritoneal equilibration test (PET) in peritoneal dialysis (PD) patients.

Methods: This is a retrospective study of 167 PDpatients from 3 Belgian hospitals (University Leuven n=123; Stuivenberg Antwerp n=30; Jessa Hasselt n=14) between 2002-2013. Baseline clinical and PET data ($D/P_{creatinine}$ and $D/D0_{glucose}$) were retrieved from patient files. Stored whole blood was used for DNA extraction and HO-1 genotyping.

Results: 56% of the patients were male. Mean age was 51±17 years. Distribution of the $(GT)_n$ repeat and A(-413)T SNP was: SS 10%; LL 48%; SL 42%; AA 30%; TT 18%; AT 52%. 35% of the patients had at least one S-allele AND one A-allele. This combination (Group A) is considered with higher HO-1 activity as compared to the remainder of the patients (Group B). Comparison of these groups revealed statistically significant differences in $D/P_{creatinine}$, $D/D0_{glucose}$, body mass index (BMI), serum albumin, total cholesterol and C-reactive protein (CRP) levels.

	Group A	Group B	p-value
D/P_{creat}	0,66 ± 0,14	0.69 ± 0.12	0,041
D/D_0 Glucose	0,33 ± 0,1	0,31 ± 0,13	0,015
BMI kg/m^2	23 ± 5	25 ± 5	0,004
Albumin g/L	39,4 ± 4,8	37,8 ± 4,4	0,024
cholesterol mg/dL	197,3 ± 50	177,1 ± 41,4	0,022
CRP g/L	5,0 ± 7,0	12,9 ± 36,2	0,011

Conclusions: Our data are supportive for a role of the HO-1 polymorphisms in the variability of PTR characteristics. Patients with higher HO-1 expression have a slower PTR and lower levels of inflammatory markers.

TH-PO928

LPA-LPA$_1$ Signaling Directs Peritoneal Mesothelial Cell Migration and Myofibroblast Differentiation in the Pathogenesis of Peritoneal Fibrosis Norihiko Sakai,[1] Takashi Wada,[1] Andrew M. Tager.[2] *[1]Div of Nephrology, Kanazawa Univ, Kanazawa, Ishikawa, Japan; [2]Pulmonary and Critical Care Unit, Massachusetts General Hospital, Boston, MA.*

Background: Peritoneal fibrosis is a serious complication of peritoneal dialysis, but the mechanisms by which it develops remain to be fully determined. We have previously found that the lipid mediator lysophosphatidic acid (LPA), acting through one of its receptors, LPA_1, stimulates peritoneal mesothelial cells (PMCs) to produce connective tissue growth factor through a myocardin-related transcription factor (MRTF)-serum response factor (SRF) pathway. In this study, we investigated additional effects of LPA-LPA_1 signaling on PMC biology, focusing on their migration and acquisition of a myofibroblast-like phenotype.

Methods: We isolated PMCs from wild-type(WT) and LPA_1-deficient (LPA_1KO) mice and determined their chemotaxis and expression of alpha smooth muscle actin (αSMA), a marker of myofibroblast differentiation, induced by LPA.

Results: WT-PMCs migrated toward LPA in a dose-dependent manner, whereas LPA did not stimulate LPA_1KO-PMC migration. The chemotactic activity of LPA on WT cells was completely inhibited by pertussis toxin, an inhibitor of the $G\alpha_i$ class of G proteins. In addition to migration, we found that LPA induced αSMA gene expression by PMCs, also in an LPA_1-dependent manner. To determine the molecular mechanisms through which LPA-LPA_1 signaling induces αSMA expression, we performed a series of experiments by inhibiting components of the MRTF-SRF pathway. We found that LPA-LPA_1 signaling mediates αSMA expression dependent on the MRTF-SRF pathway, through the $G\alpha_{12/13}$ class of G proteins. In a mouse model of peritoneal fibrosis induced by intraperitoneal injection of chlorhexidine gluconate (CG), we observed the presence of αSMA and E-cadherin co-expressing cells in WT mice throughout their mesothelium which was greatly expanded by extracellular matrix. In CG-challenged LPA_1KO mice, far fewer αSMA and E-cadherin co-expressing cells were present in the matrix of the peritoneum.

Conclusions: Our results suggest that LPA-LPA_1 signaling contributes to the pathogenesis of peritoneal fibrosis by driving PMC migration and myofibroblast differentiation.

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TH-PO929

Decorin Ameliorates Peritoneal Fibrosis in Peritoneal Dialysis Related Peritonitis Susan Yung, Na Jiang, Mel Chau, Qing Zhang, Daniel Tak Mao Chan. *Dept of Medicine, The Univ of Hong Kong.*

Background: Peritonitis is a leading cause of peritoneal membrane failure in patients on peritoneal dialysis (PD). Decorin is a dermatan sulfate proteoglycan that possesses anti-fibrotic properties. Its role in peritoneal fibrosis remains to be fully defined. This study examined the relationship between dialysate decorin level and that of pro-inflammatory and fibrotic mediators in patients with PD associated peritonitis, and investigated the role of decorin in fibrogenesis.

Methods: Serial PD fluid samples were collected from 43 patients with PD related peritonitis. Dialysate samples from 23 PD patients without peritonitis in the past 12 months were included as controls. Dialysate concentrations of decorin, TGF-β1, IL-1β, IL-6 and IL-8 were measured using commercially available ELISAs. Mesothelial cells were incubated with spent PD fluid in the presence or absence of exogenous decorin, and the expression of SNAIL and fibronectin assessed.

Results: Dialysate decorin level was significantly higher at the onset of peritonitis compared to non-peritonitis dialysate (6345.23±1169.38 versus 257.6±26.9 pg/ml, P<0.05). Decorin level peaked 3 days after the onset of peritonitis and remained significantly higher than non-peritonitis levels 3 months later (8729.14±1145.21 pg/ml, P<0.05). Dialysate decorin levels showed an inverse relationship with dialysate IL-1β, IL-6 and IL-8 levels (r= -0.34, -0.38 and -0.32 respectively, P<0.05 for all), but correlated with TGF-β1 levels (r= 0.31, P<0.05). Peritoneal dialysate obtained at the onset of peritonitis significantly induced SNAIL and fibronectin synthesis in mesothelial cells, and this induction was reduced by exogenous decorin. SNAIL, but not fibronectin, expression inversely correlated with dialysate decorin level (r= -0.46, P=0.0024).

Conclusions: Our data suggested a role of decorin in ameliorating the peritoneal fibrotic process induced by peritonitis, and that decorin may suppress epithelial-to-mesenchymal transition and fibrogenesis in peritoneal mesothelial cells.

Funding: Government Support - Non-U.S.

TH-PO930

The Peritoneal Endothelial Glycocalyx in Rats with Chronic Kidney Disease Exposed to Dialysis Solutions Carmen A. Vlahu,[1] Jan Aten,[2] Dirk Gijsbert Struijk,[1,3] Raymond T. Krediet.[1] *[1]Dept of Nephrology, Academic Medical Center, Amsterdam, Netherlands; [2]Dept of Pathology, Academic Medical Center, Amsterdam, Netherlands; [3]Dianet Foundation, Amsterdam, Utrecht, Netherlands.*

Background: Peritoneal dialysis (PD) leads to exposure of the peritoneal membrane to high concentrations of glucose and its degradation products, causing inflammation and angiogenesis, which may all alter the endothelial glycocalyx. Here, we investigated the changes in peritoneal endothelial glycocalyx induced by chronic kidney disease (CKD) and by long-term PD, using a conventional or a 'biocompatible' dialysis solution.

Methods: Forty-four Wistar rats were divided in 4groups: normal kidney function (NKF), CKD (induced by 70%nephrectomy), CKD exposed to Dianeal 4.25% (CKDD), or Physioneal 3.86% (CKDP). At 16 weeks, plasma levels of Syndecan-1 were measured by ELISA, and a peritoneal function test and Sidestream Darkfield imaging of the peritoneal vasculature were performed. The perfused boundary region (PBR), which reflects the erythrocyte permeable part of glycocalyx, and the valid microvascular density (VMD) were measured. The expression of syndecan-1, a heparan sulfate (HS) proteoglycan, and HS (10E4 epitope) was determined in peritoneal tissue specimens.

Results:

	NKF	CKD	CKDD	CKDP
Renal creat clearance (ml/min)	4.3±0.7	1.8±0.5*	2.3±0.5*	1.8±0.6*
D/P creatinine	0.4±0.06	0.5±0.03	0.6±0.08*	0.6±0.05*
PBR(μm)	2.2±0.2	2.1±0.4	2.1±0.2	2.1±0.1
Syndecan-1 (ng/ml)	9.4±6.1	19.6±10.0*	20.3±19.9*	12.2±6.7

*p<0.05 versus NKF.

Rats exposed to dialysis solutions had a higher VMD compared to NKF and CKD (p=0.04, p=0.007). A positive relationship was present between both PBR and VMD, and D/P creatinine (p=0.08, p=0.07). Syndecan-1 expression was decreased in rats exposed to dialysis solutions compared to NKF. HS as stained for the 10E4 epitope, was present mainly on the abluminal side of the endothelial cells.

Conclusions: Although the PBR was not different between groups, the decreased syndecan-1 expression in peritoneal blood vessels from the rats exposed to dialysis solutions suggests an alteration in the glycocalyx adjacent to the endothelial cell.

Funding: Pharmaceutical Company Support - Baxter NL

TH-PO931

CCL18 Production by Peritoneal Macrophages Is Down-Regulated by the Vitamin D Receptor Agonist Paricalcitol Rafael Selgas,[1] M. Auxiliadora Bajo,[1] Marta Ruiz-Ortega,[3] Guadalupe González- Mateo,[1,2] Teresa Bellon.[1] *[1]Nephrology, H. U La Paz. IdiPAZ. REDinREN.IRSIN, Madrid, Spain; [2]CBM, Madrid, Spain; [3]Univ Autonoma de Madrid. REDinREN, Madrid, Spain.*

Background: The use of peritoneal dialysis (PD) is limited by the membrane capacity to perform long-term diffusive and convective transports, whose worst functional consequence is ultrafiltration failure and peritoneal fibrosis. Continuous stress for PD fluids and peritonitis represent a risk for both.M2 polarized macrophages (MF) have been involved in fibrosis in numerous clinical settings and experimental models. CCL18, a product of M2 MF, has been related to fibrosis in different diseases. We identified a population of M2 MF secreting high levels of CCL18 in peritoneal effluents from PD patients suffering peritonitis. Anti-inflammatory properties have been attributed to Paricalcitol. **Aim** To determine the effects of Paricalcitol on fibrosis and CCL18 production of peritoneal MF.

Methods: a) Paricalcitol effects were explored in a mouse model of peritoneal membrane injured by PD fluids (3.86% glucose and PDGs). b) Purified CD66-CD14+ MFpopulations (N=9) isolated from PD effluents from patients with peritonitis were incubated with Zemplar® (equivalents of 10^{-9}M, 10^{-8}M, and 10^{-7}M paricalcitol) or vehicle. CCL18 production was evaluated in supernatants by ELISA at 24, 48 and 72 h. CCL18 levels were also measured in peritoneal effluents from patients with peritonitis.

Results: a) Paricalcitol attenuated fibrosis in the PD mouse model, significantly reducing IL-17 local levels. b) Peritoneal effluents from patients with peritonitis were highly enriched in CCL18 whose level was related to peritoneal MF counts. In vitro, CCL18 production was significantly reduced in peritoneal MF upon culture with Zemplar® (paricalcitol 10^{-8}M or 10^{-9}M). The results were confirmed by incubation with purified active drug paricalcitol and with Vit D3 in a few samples.

Conclusions: Paricalcitol attenuates peritoneal fibrosis in a PD mouse model, associated with reduced levels of IL-17, and down-regulates CCL18 production by M2 polarized macrophages in the human inflamed peritoneum. Both mechanisms might result in a synergistic protective action against fibrosis at the peritoneum.

Funding: Pharmaceutical Company Support - Abbvie

TH-PO932

Mesenchymal Stem Cells Inhibit Peritoneal Fibrosis Resulting From Peritoneal Dialysis via snail-1 Signaling Pathway Xiang-Mei Chen, Diangeng Li, Yan Mei, Kai Hou, Wang Nan. *Dept of Nephrology, Chinese PLA General Hospital, Chinese PLA Inst of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center of Kidney Diseases, Beijing, China.*

Background: Peritoneal dialysis (PD) is the main renal replacement therapy for end-stage renal disease (ESRD). However, PD associated chronic peritoneal fibrosis could cause ultrafiltration failure, which has been the most important reason for the dropout of PD patients and greatly limited the application and promotion of PD. Mesenchymal stem cells (MSCs) could inhibit inflammation and have the anti-fibrosis effect via paracrine secreting bioactive factor, thereby promoting injury repair. This study aimed to define whether MSCs could improve chronic peritoneal fibrosis resulting from peritoneal dialysis solution through inhibiting epithelial-mesenchymal transition (EMT) of peritoneal mesothelium cells (PMCs), and explore the specific mechanism for efficacy of MSCs.

Methods: In order to simulate chronic peritoneal fibrosis resulting from PD in clinical practice, we gave intraperitoneal injection of 4.25% peritoneal dialysis solution (100ml/kg per day) for 28 consecutive days to establish a chronic rat model of peritoneal fibrosis resulting from peritoneal dialysis solution. MSCs was used to make intervention in vivo via the tail vein injection on experimental model.

Results: The results showed that MSCs could improve peritoneal ultrafiltration function (29.65±20.33ml/kg, 28d), reduce peritoneal glucose transport function (16.88±1.74mmol/kg, 28d), peritoneal fibrosis degree (61.08±24.50μm, 28d) and blood vessel density (1.59±0.29/mm^2, 28d), inhibit the reduction of PMCs (the expression of E-Cadherin increasing), reduce the increase of peritoneal fibroblasts (the expression of α-SMA reducing), reduce the expression of TGF-β1. MSCs could increase the expression of E-Cadherin and reduce the expression of α-SMA by up-regulate the expression of snail-1 when co-culturing with PMCs stimulated by PD.

Conclusions: Our study suggest that MSCs could inhibit EMT of PMCs via snail signaling pathway in the model of PD associated chronic peritoneal fibrosis.

Funding: Government Support - Non-U.S.

TH-PO933

Nano-Kidney Possessing Oxidative Stress Suppression Character Yukio Nagasaki,[1] Tatsuya Yaguchi,[1] Takuma Matsumura,[1] Toru Yoshitomi,[1] Yutaka Ikeda,[1] Atsushi Ueda,[2] Aki Hirayama.[3] *[1]Dept of Materials Science, Master's School of Medical Sciences, and Satellite Laboratory, International Center for Materials Nanoarchitectonics (WPI-MANA), National Inst for Materials Science (NIMS), Univ of Tsukuba, Tsukuba, Ibaraki, Jap; [2]Tsukuba Univ Hospital Hitachi Medical Education and Research Center, Univ of Tsukuba, Hitachi, Ibaraki, Japan; [3]Center for Integrative Medicine, Tsukuba Univ of Technology, Tsukuba, Ibaraki, Japan.*

Background: The prevention of encapsulating peritoneal sclerosis (EPS) and the enhancement of dialysis efficiency are two important strategies that can improve the quality of life of patients undergoing peritoneal dialysis. We have thus far developed bionanoparticles that effectively scavenge reactive oxygen species (redox nanoparticles; RNPs). The objective of this study was to apply RNPs as a component of dialysate to reduce oxidative stress.

Methods: ROS scavenging nanoparticle was prepared by self-assembling of amhiphilic block copolymer possessing nitroxide radicals as a side chain of the hydrophobic segment. Porous silica nanoparticles were combined with RNPs to enhance the effective adsorption capacity of low-molecular weight compounds. Since the size of the siRNP thus prepared was ca. 40 nm, the dialysate was completely transparent even with siRNP in it.

Results: The silica-containing RNPs were confirmed to statistically decrease the level of creatinine and blood urea nitrogen in vivo. EPS model rats that underwent an intraperitoneal injection of chlorhexidine gluconate exhibited dysfunction of the peritoneal membrane. siRNP administration did not result in dysfunction of the peritoneal membrane. An LMW nitroxide compound, TEMPOL, also showed a weak peritoneal protective effect, although its efficiency was limited. No blood uptake of siRNPs was observed when they were administered into the peritoneal cavity. However, LMW-TEMPOL diffused into the blood stream, which might have decreased its effective concentration in the peritoneal cavity and led to adverse effects across the entire body.

Conclusions: Considering the above results, siRNPs are expected to be a new multi-functional nanomaterial for high performance peritoneal dialysis.

TH-PO934

Vascular Endothelial Growth Factor Receptor-3 Can Be a New Target to Improve Ultrafiltration Dysfunction in Methylglyoxal-Induced Peritoneal Injury Takeshi Terabayashi,[1] Yasuhiko Ito,[1] Masashi Mizuno,[1] Yasuhiro Suzuki,[1] Hiroshi Kinashi,[1] Fumiko Sakata,[1] Shoichi Maruyama,[1] Yoshifumi Takei,[2] Seiichi Matsuo.[1] *[1]Nephrology, Nagoya Univ, Nagoya, Aichi, Japan; [2]Biochemistry, Nagoya Univ, Nagoya, Aichi, Japan.*

Background: Ultrafiltration failure (UFF) is a major reason for discontinuation in peritoneal dialysis (PD). We recently reported that lymphangiogenesis developed in the peritoneal cavity associated with fibrosis via the TGF-b-vascular endothelial growth factor C (VEGF-C) pathway. The aim of this study is to investigate whether VEGF receptor-3 (VEGFR-3), the receptor for VEGF-C and -D, is a target to improve UFF by suppression of lymphangiogenesis.

Methods: Peritoneal injury model was induced by intraperitoneal injection of MGO in C57Bl6 mice. Lymphatic vessel proliferation in their parietal peritoneum and diaphragm was evaluated by immunohistochemistry (IHC) and quantitative PCR (qPCR). Subsequently, we performed inhibition studies of lymphangiogenesis using adenovirus (Ad) expressing soluble VEGFR-3 (sVEGFR-3). MGO models were treated with (1) Ad-LacZ or with (2) Ad-sVEGFR-3, and were assessed at day 22; (3) MGO models were treated with (3) AdLacZ+peritoneal lavage from day22 to day50 or with (4) AdsVEGFR-3+peritoneal lavage from day22 to day50, and were assessed at day 50. The mice were submitted to a peritoneal equilibration test using 4.25% glucose-based solution (G-PET) and 7.5% icodextrin solution (Ico-PET) at day22 or day50.

Results: Lymphangiogenesis was presented in MGO model especially in the diaphragm with upregulation of VEGF-D mRNA ($P<0.001$), LYVE-1 ($P<0.01$) and VEGF-R3 ($P<0.01$). Serum sVEGFR-3 was detected at day 50 after administration via tail vein injections. Ad-sVEGFR-3 successfully suppressed lymphangiogenesis in MGO models without significant impact on neoangiogenesis, inflammation and fibrosis. Drained volume in Ico-PET was improved by Ad-sVEGFR-3 at day 22 ($P<0.05$) and day 50 after inflammatory resolution ($P<0.01$)indicating that Ico-PET identifies changes of lymphangiogenesis. However, G-PET did not discriminate changes in lymphatic absorption.

Conclusions: These results indicate that VEGFR-3 is a new target to prevent UFF by suppressing lymphatic absorption.

Funding: Government Support - Non-U.S.

TH-PO935

Survival of Propensity Matched Incident Peritoneal and Hemodialysis Patients in a United States Healthcare System Victoria A. Kumar,[1] Margo A. Sidell,[2] Edward F. Vonesh.[3] *[1]Internal Medicine, Kaiser Permanente, Los Angeles, CA; [2]Research and Evaluation, Kaiser Permanente, Pasadena, CA; [3]Dept of Preventative Medicine, Northwestern Univ, Feinberg School of Medicine, Chicago, IL.*

Background: Favorable clinical conditions among patients who initiate peritoneal dialysis (PD) likely impact results of survival studies. In an effort to reduce case-mix bias, we compared survival among incident PD patients to propensity matched hemodialysis (HD) patients at our organization. All HD patients received pre-dialysis care and initiated dialysis with either a functioning arterio-venous fistula or graft.

Methods: We utilized databases at Southern California Kaiser Permanente to identify all adult patients who initiated PD or HD at our institution between 2001 and 2013. HD patients who used a central venous catheter (CVC) at any time period during the first 90 days of dialysis were excluded. Stratefied Cox proportional hazards models were used to compare survival in both intent-to-treat (ITT) and as-treated (AT) analyses.

Results: A total of 1,003 matched pairs were obtained from 11,301 incident patients (10,298 HD and 1,003 PD). Cumulative hazard ratios for death can be seen in the table below.

	As-treated			Intent-to-treat		
Year	Survival % PD	Survival % HD	Adjusted time dependent cumulative HR (95% CI)	Survival % PD	Survival % HD	Adjusted time dependent cumulative HR (95% CI)
1	96	91	2.38 (1.68-3.40)	96	91	2.10 (1.50-2.94)
2	91	87	1.38 (1.05-1.82)	90	87	1.26 (0.98-1.63)
3	86	82	1.26 (0.98-1.63)	84	82	1.09 (0.87-1.38)
4	78	78	0.95 (0.74-1.22)	76	78	0.84 (0.67-1.05)
5	73	74	0.93 (0.72-1.21)	70	73	0.84 (0.67-1.06)
6	68	68	0.99 (0.75-1.32)	64	67	0.87 (0.68-1.11)
7	64	59	1.19 (0.85-1.65)	57	59	0.95 (0.72-1.26)
8	53	50	1.10 (0.72-1.70)	49	50	0.93 (0.67-1.30)
9	38	48	0.72 (0.35-1.52)	42	47	0.83 (0.57-1.23)

Conclusions: The cumulative risk of death, as estimated by the cumulative hazard ratio, favored PD for nearly 3 years of follow-up in the AT analysis and nearly 2 years of follow-up in the ITT analysis with no differences thereafter. The higher adjusted rate of death observed for HD patients cannot be attributed to initial use of CVC's or lack of pre-dialysis care.

Funding: Pharmaceutical Company Support - Baxter Healthcare Incorporated

TH-PO936

Successful Use of Two Icodextrin Exchanges Alone Laura K. Troidle,[1] Fredric O. Finkelstein,[1,2] Peter Juergensen.[1] *[1]Metabolism Associates, New Haven, CT; [2]Medicine, Yale Univ School of Medicine, New Haven, CT.*

Background: Patients starting CPD therapy (Rx) often use several dextrose based solutions for dialysate. The use of such solutions exposes the patient to potential long-term peritoneal membrane damage. Some patients starting CPD Rx have significant residual renal function (RRF) that permits the achievement of a total Kt/V urea of >1.7 using low amounts of dialysate. Thus, CPD Rx using two long dwell icodextrin exchanges (2ICDE) has been effective in patients starting CPD therapy in meeting international standards of dialysis adequacy.

Methods: We previously reported our experience using 2ICDE and now expand our observations (Awuah et al, Adv Perit Dial 29:1-3, 2013). All patients starting CPD Rx with adequate RRF were considered to be candidates for 2ICDE. A total of 12 patients started CPD Rx with 2ICDE since January 2011.

Results: The patients were maintained on 2ICDE for an average of 9.3 months (range 1-26 months). The average Kt/V urea, including RRF, after starting 2ICDE was 1.97 (range 1.75-2.24). Additional manual or cycler therapy was added to the 2ICDE for patients unable to maintain adequate clearances with 2ICDE alone. Two patients were unable to continue beyond one week. One of these patients developed and ICD allergy, and, the other patient preferred to change to cycler therapy. Since there has been a concern that the use of 2ICDE may result in false glucose readings with certain glucometers, we obtained simultaneous glucometer and lab glucose determinations in seven patients. The glucometer reading was an average of 10.6% higher than the serum glucose lab measurement (range 8-18%) in 5 patients who had elevated serum glucose measurements. Two patients with normal serum glucose had an 18-33% higher reading with the glucometer.

Conclusions: We conclude that 2ICDE can provide adequate Kt/V urea for patients with RRF, thus, limiting glucose exposure as well as the amount of time needed to perform CPD Rx. Glucose determinations may be 10% higher with a glucometer in patients using 2ICDE. Patients with normal serum glucose measurements still had normal readings with a glucometer.

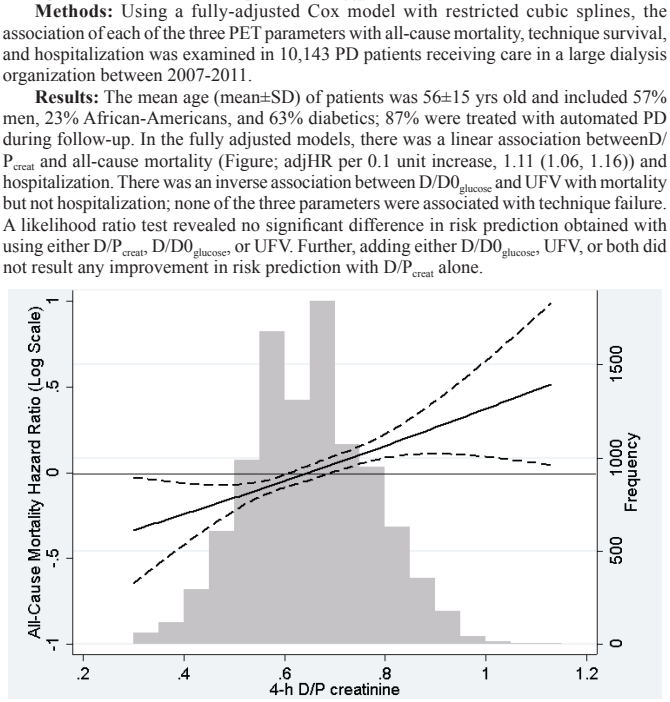
TH-PO937

Peritoneal Equilibration Test (PET) and Patient Outcomes in a Multi-Ethnic Contemporary Cohort Vanessa A. Ravel,[1] Elani Streja,[1] Sooraj Kuttykrishnan,[2] Miklos Zsolt Molnar,[3] Kamyar Kalantar-Zadeh,[1] Rajnish Mehrotra.[2] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Univ of Washington, Seattle, WA; [3]Univ of Toronto, Toronto, ON.*

Background: Even though a standard PET yields data on three different parameters (4-hr dialysate-plasma creatinine, D/P_{creat}, 4-hr to 0-hr dialysate glucose, $D/D0_{glucose}$, ultrafiltration volume, UFV), virtually all studies have focused on the prognostic value of D/P_{creat}. Since $D/D0_{glucose}$ and UFV may be superior to predicting total daily ultrafiltration, the likely mechanism that explains the association of PET results with patient outcome, we hypothesized that they are superior to D/P_{creat} for risk prediction.

Methods: Using a fully-adjusted Cox model with restricted cubic splines, the association of each of the three PET parameters with all-cause mortality, technique survival, and hospitalization was examined in 10,143 PD patients receiving care in a large dialysis organization between 2007-2011.

Results: The mean age (mean±SD) of patients was 56±15 yrs old and included 57% men, 23% African-Americans, and 63% diabetics; 87% were treated with automated PD during follow-up. In the fully adjusted models, there was a linear association betweenD/P_{creat} and all-cause mortality (Figure; adjHR per 0.1 unit increase, 1.11 (1.06, 1.16)) and hospitalization. There was an inverse association between $D/D0_{glucose}$ and UFV with mortality but not hospitalization; none of the three parameters were associated with technique failure. A likelihood ratio test revealed no significant difference in risk prediction obtained with using either D/P_{creat}, $D/D0_{glucose}$, or UFV. Further, adding either $D/D0_{glucose}$, UFV, or both did not result any improvement in risk prediction with D/P_{creat} alone.



Conclusions: Analysis of data from a large contemporary multi-ethnic cohort validates for the first time D/P_{creat} as the most robust predictor of outcomes in patients undergoing peritoneal dialysis.

Funding: NIDDK Support

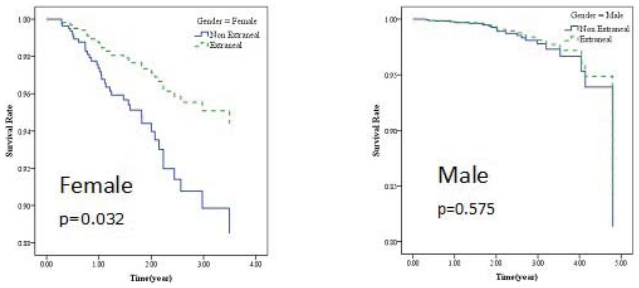
TH-PO938

Once-Daily-Use of Icodextrins Improved Survivals of Asian Peritoneal Dialysis Patients- A Propensity Score Matched, Nationwide Population Study Chiu-Ching Huang,[1,2] Wen-Yin Kuo,[3] I-Kuan Wang,[1,2] Wen-Chen Tsai.[3] *[1]Kidney Inst, China Medical Univ Hospital, Taichung, Taiwan; [2]College of Medicine, China Medical Univ, Taichung, Taiwan; [3]Dept of Health Services Administration, China Medical Univ, Taichung, Taiwan.*

Background: There are controversies whether icodextrin (ICO)-use can improve patient survivals in incident PD patients. This Asian study compared the risk of death between ICO-users (study group)and propensity score matched non-ICO-users (control group).

Methods: All incident PD patients in Taiwan National Health Insurance Research Database who had at least survived more than 90 days after PD from January 1, 2004 to June 30, 2009 were recruited. ICO were prescribed once daily for high risk patients, e.g. (1) diabetic with HbA1C > 7%, or (2) high transporters, or (3) used high-glucose containing dialysates. Patients were followed until death or transfer to HD or renal transplantation or Dec 31, 2009. Patient survivals were compared between ICO users and propensity score matched controls. The multivariate Cox regression models were used to calculate the impact of ICO-use on mortality and to plot survival curves.

Results: A total of 1627 incident PD patients were identified. Among them, 524 ICO-users were matched with 524 non-ICO-users for age, sex, co-morbidities, and monthly incomes. Ico-users had better patient survivals than control cohort (HR 0.6 for ICO versus non-ICO users, 95% CI: 0.37-0.99, p=0.045). Female ICO users had significently better patient survival than non-ICO users (HR 0.48, 95% CI: 0.25-0.94, p=0.032). but it is not significant in male patients (HR 0.79, 95%CI 0.35-1.79,p=0.575)



Conclusions: Comparing to a propensity score matched control cohort, once-daily-use of ICO in high risk Asian PD patients are beneficial to patient survivals, particularly in female population. Further randomized controlled studies are necessary to confirm our observations.

Funding: Government Support - Non-U.S.

TH-PO939

Association of Alternative Approaches to Normalizing Peritoneal Dialysis Clearance with Clinical Outcomes Suzanne Boyle, Yimei Li, Francis Perry Wilson, Joel D. Glickman, Harold I. Feldman. *Univ of Pennsylvania, Philadelphia, PA.*

Background: Kt/V is the standard for assessing PD adequacy. Calculation of V, the volume of total body water, ignores variability in fat:fat-free mass ratio potentially distorting Kt/V among those at extremes of BMI, leading to inappropriate PD prescriptions and promoting earlier mortality or technique failure (TF). We explored the relationship of mortality and TF to non-normalized Kt and to peritoneal Kt/V calculated with V based on actual body weight(standard practice), ideal body weight, and adjusted body weight.

Methods: The Dialysis Morbidity and Mortality Study Wave 2, a prospective study of incident dialysis patients enrolled from 1996-1997 at 799 randomly-selected U.S. dialysis facilities, was linked with USRDS data for ESRD treatment history and mortality through 2010. Patients were included in the analysis if data to calculate peritoneal Kt/V at study entry were available.The association of 4 metrics of peritoneal urea clearance with death and TF were assessed with Cox proportional hazard models.

Results: 534 individuals had sufficient data for analysis. Median age was 59. 54% were male; 72%, white; 91% on CAPD. 5-year mortality was 37% and 5-year TF, 60%.Varying normalization strategies led to profound differences in calculated clearance, particularly among the underweight and obese (Table).

Median and IQR of Achieved Peritoneal Clearance by BMI

BMI (kg/m²)	KT	Kt/V_{actual}	Kt/V_{ideal}	$Kt/V_{adjusted}$
Underweight < 18.5 (n=27)	47.9 (42.6-55.5)	1.75 (1.56-2.06)	1.61 (1.41-1.98)	1.66 (1.48-2.02)
Normal 18.5 – 25 (n=207)	58.6 (51.8-66.4)	1.73 (1.44-1.97)	1.74 (1.42-1.98)	1.72 (1.44-1.98)
Overweight 25 – 30 (n=172)	57.8 (51.9-64.7)	1.54 (1.27-1.77)	1.70 (1.41-2.03)	1.63(1.37-1.90)
Obese >30 (n = 128)	61.7 (56.4-67.8)	1.42 (1.20-1.61)	1.83 (1.57-2.16)	1.65 (1.42-1.92)

After multi-variable adjustment, we did not detect a relationship between any clearance metric and the outcomes of mortality or TF in either the whole cohort or within any BMI stratum.

Conclusions: Patients at extremes of BMI are at risk for false estimation of V. Although we did not detect an association between any clearance metric and clinical outcomes, large variation in clearance across BMI groups suggests the need for studies to optimize PD clearance targets among the underweight and obese.

Funding: NIDDK Support

TH-PO940

The Impact of Timing of Dialysis Initiation on Mortality in Patients with Peritoneal Dialysis Yong Kyun Kim, Ho Cheol Song, Euy Jin Choi, Chul Woo Yang. *Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

Background: The impact of timing of dialysis initiation on mortality is controversial in patients with peritoneal dialysis (PD). In this study, we analyzed the impact of timing of dialysis initiation on mortality in the incident PD population.

Methods: Incident patients with PD were selected from the Clinical Research Center (CRC) registry for end-stage renal disease (ESRD), a prospective cohort study on dialysis in Korea. Patients were categorized into three groups according to the estimated glomerular filtration rate (eGFR) at the initiation of PD using the Modification of Diet in Renal Diseaseequation.Group Awas defined as eGFR < 5 ml/min/1.73m^2, group Bas eGFR 5-10 ml/min/1.73m^2, and group Cas eGFR > 10 ml/min/1.73m^2. Cox regression analysis was used to calculate the adjusted hazard ratio (HR) of mortality with group B as the reference. The primary outcome was all-cause mortality.

Results: A total of 495 incident PD patients were included. The number of patients in group A was 109, group B was 279, and group C was 107. The median follow-up period was 23 months. Multivariate Cox regression analysis showed that group A had a significantly higher risk of all-cause mortality compared with group B (HR 4.13, 95% CI, 1.55-11.03, P = 0.005) after adjustment for age, gender, cause of ESRD, serum albumin level, diabetes mellitus, and cardiovascular diseases. There was no significant difference in mortality between group C and group B (HR 1.50, 95% CI, 0.59-3.80, P = 0.398) after adjustment for clinical variables.

Conclusions: An eGFR < 5 ml/min/1.73m^2 at the initiation of PD was a significant risk factor for death, while an eGFR >10 ml/min/1.73m^2 at the initiation of PD was not associated with improved survival compared with an eGFR of 5-10 ml/min/1.73m^2 at the initiation of PD.

TH-PO941

Lower Education Level Is a Risk Factor for Peritonitis and Technique Failure but Not a Risk for Overall Mortality in Peritoneal Dialysis
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Background: It is speculated thata lower education levelis a risk factor for higher peritoneal dialysis (PD)-associated peritonitis, potentially resulting in technique failure. We have analyzed the influence of lower education level on the development of peritonitis, technique failure and overall patient mortality.

Methods: This is a single-center, retrospective cohort study. Subjects who started PD at Seoul National University Hospital between January 2000 and December 2012 were enrolled. Subjects were divided into three groups: lower (academic year≤9 years, n=102), intermediate (9 <academic year≤ 12 years, n=229), and higher (academic year>12 years, n=324) education groups.Cox proportional hazards models were employed to analyze the influence of the education level on the outcomes.

Results: A total of 655 incident PD patients (60.9% male, age 48.4±14.1 years) were analyzed. Mean follow-up duration was 48.1±33.8 months. During follow-up, 250 subjects (38.2%) experienced more than one episode of peritonitis, 140 subjects (21.4%) underwent technique failure and 78 subjects (11.9%) died. After adjustment to the age, gender, diabetes, other comorbidities, visual disturbance, hemoglobin, C-reactive protein, albumin, glomerular filtration rate, lower education level was shown to be an independent risk factor for peritonitis (hazard ratio [HR] 1.66; 95% confidence interval [CI] 1.13-2.43) and technique failure (HR 1.71; 95% CI 1.01-2.90). However, it was not associated with increased overall mortality (HR 1.28; 95% CI 0.63-2.59).

Conclusions: Although lower education level was a significant risk factor for peritonitis and technique failure, it was not associated with increased mortality in PD patients. Subjects with lower education level should not be discouraged from choosing PD as their first-line renal replacement therapy. Comprehensive training in PD exchange procedures and multidisciplinary education is warranted for overcoming the lower education level in subjects undergoing PD therapy.

TH-PO942

Skin Autofluorescence as Mortality Predictor in Patients on Peritoneal Dialysis
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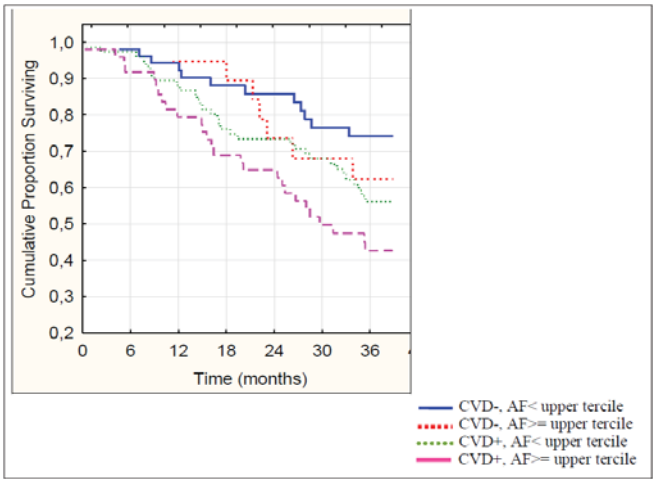
Background: Skin autofluorescence (SAF) is a known and accepted prognostic factor of mortality in hemodialysis patients. Traditional and non-traditional risk components are similar in peritoneal dialysis (PD), and as well cardiovascular disease (CVD) is the leading cause of death. Moreover peritoneal glucose absorption accelerates degenerative processes of the connective tissues, like in diabetes. In our study we examined the predictive value of SAF for mortality in PD population.

Methods: Data were taken from 198 prevalent adult Caucasian PD patients. Initially we evaluated the factors affecting SAF and CVD by multivariate linear regression. Registering the clinical and demographic data associations with mortality during the next 36 months were estimated using Kaplan-Meier method, analysis were stratified on presence of CVD and SAF level above or below the upper tercilis 3.61.

Results: SAF was influenced by CVD (p<0.01; CI 0.1-0.5) and white blood cell count (p<0.001; CI 0.031-0.117). According Spearman correlation it connected with peritoneal cumulative glucose exposure (p=0.02) and elapsed time in PD (p=0.008). CVD related with age (p<0.001; CI 1.24-1.65) and diabetes (p<0.001; CI 2.58-10.66). More death were observed in the high SAF group, than in the low SAF group (34/68 v. 44/130; p=0.04). Comparing the CVD(-) low SAF group survival (mean 33.9 month; SE 1.39) to CVD(+) low SAF (mean 30.5 month; SE 1.37; p=0.03) and to CVD(+) high SAF group (mean 27.1; month; SE 1.83; p=0.001) the difference was significant.



Conclusions: Among patients on PD the SAF value over 3.61 seems to be predictor of mortality. Relationship with glucose-exposure, CVD and diabetes suggests its suitability to characterize systemic cumulative glucose load.

TH-PO943

Endothelial Dysfunction Is Associated with Major Adverse Cardiovascular Events in Peritoneal Dialysis Patients
Seonghun Kim,[1] Chang-Yun Yoon,[2] Mi Jung Lee,[2] Hyung Jung Oh,[2] Jung Tak Park,[2] Seung Hyeok Han,[2] Tae-Hyun Yoo,[2] Shin-Wook Kang.[1,2] *[1]Brain Korea 21 PLUS, Severance Biomedical Science Inst, Yonsei Univ, Seoul, Korea; [2]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea.*

Background: Endothelial dysfunction is implicated in high cardiovascular risk in the general population. However, the impact of endothelial dysfunction on cardiovascular outcome has not been extensively investigated in peritoneal dialysis (PD) patients. In this prospective observational study, therefore, we elucidated the prognostive value of endothelial dysfunction in PD patients.

Methods: Endothelial function was determined by brachial artery endothelium-dependent vasodilation (flow-mediated dilation; FMD) in 143 non-diabetic PD patients and 32 sex- and age-matched controls. The primary outcomes were major adverse cardiac and cerebrovascular events (MACCEs). Cox proportional hazard analysis was performed to ascertain the independent prognostic value of brachial FMD for MACCEs.

Results: Brachial FMD was significantly lower in PD patients than that in controls [2.9 (1.3-4.7) versus 6.2 (5.4-8.3)%, P<0.001]. During a mean follow-up duration of 42 months, the primary outcomes were observed in 25 patients (17.5%). When patients were dichotomized by the median value of FMD (2.9%), the incidence rates of MACCEs were significantly higher in patients with lower FMD compared to the higher FMD group (7.2 versus 3.0 per 100 person-years, P=0.03). In multivariate Cox analysis, low FMD (≤2.9%) was a significant independent predictor of MACCEs (hazard ratio=2.73, 95% confidence interval=1.03-7.22, P=0.04). Furthermore, multivariate fractional polynomial analysis showed that the risk of MACCEs increased steadily with lower FMD values.

Conclusions: Impaired brachial FMD was a significant independent predictor of MACCEs in PD patients. Determining endothelial dysfunction by brachial FMD may be useful for stratifying cardiovascular risk in these patients.

TH-PO944

Cardiac Performance Is Improved in Incident Peritoneal Dialysis Patients with Slow Rates of Residual Renal Function Decline
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Background: Reduced residual renal function (RRF) is associated with left ventricular (LV) hypertrophy as well as all-cause and cardiovascular (CV) mortality in patients with end-stage renal disease. However, the serial changes in echocardiographic findings according to the decline rates of RRF have not been fully elucidated in incident peritoneal dialysis (PD) patients.

Methods: A total of 81 patients, who started PD between 2005 and 2012 at Yonsei University Health System and underwent baseline and follow-up echocardiography within the first year of PD, were recruited. Based on the 1-year median RRF decline slope (-1.6 mL/min/yr/1.73m^2), patients were divided into 'faster' and 'slower' RRF decline groups. Time-dependent serial changes in echocardiographic parameters were compared between the two groups by linear mixed model (LMM).

Results: Baseline RRF and echocardiographic parameters were comparable between the two groups. The decline rate of RRF was an independent factor for the changes in LV end diastolic volume index (LVEDVI) (r=17.95, P=0.019), left atrial volume index (LAVI) (r=8.54, P=0.006), and LV mass index (LVMI) (r=21.00, P=0.005). The LMM further confirmed that LVEDVI, LAVI, and LVMI were significantly more improved in the 'slower' RRF decline group than those in the 'faster' RRF decline group (P=0.047, P=0.048, and P=0.001, respectively). Compared to the 'faster' RRF decline group,

composite CV outcome (P=0.098), technique failure (P=0.006), and PD peritonitis (P=0.064) occurred less frequently in patients with 'slower' RRF decline. Multivariate Cox regression analysis revealed that 'faster' RRF decline was a significant risk factor for each clinical outcome [composite CV outcome, HR=4.82, 95% CI=1.15-20.22, P=0.03; technique failure, HR=4.44, 95% CI=1.15-17.18, P=0.03; PD peritonitis, HR=7.37, 95% CI=1.43-38.04, P=0.02].

Conclusions: Cardiac performance was significantly improved in incident PD patients with slow RRF decline, which might partly contribute to better clinical outcomes in this group.

TH-PO945

A Prognostic Model for Risk of Encapsulating Peritoneal Sclerosis Incorporating the Risk of Death During Peritoneal Dialysis: Results From PD-CRAFT

Mark Lambie,[1] Lucy Riley,[1] David W. Johnson,[2] Simon J. Davies.[1] *[1]Health Services Research Unit, Keele Univ; [2]Univ of Queensland.*

Background: Encapsulating peritoneal sclerosis(EPS) is an uncommon complication of peritoneal dialysis (PD), where the risk increases significantly with time on therapy. To aid decision making around switching from PD to haemodialysis to minimise the risk of EPS, we developed prognostic models for death and EPS.

Methods: We combined Australia and New Zealand Dialysis and Transplant (ANZDATA) and Scottish Renal Registry (SRR) datasets with complete data on EPS diagnosis and the denominator population. Patients aged ≥15 years were included in competing risks models with outcomes of EPS or death. Comorbidity was classified by primary renal diagnosis. Prognostic models were developed with hierarchical backwards stepwise selection, using calibration, discrimination, and goodness-of-fit to determine the best models after 3, 4 and 5 years of PD, predicting the 5 year risks in each case.

Results: Therewere 112 cases in 17,912 patients. EPS incidence differed markedly between ANZDATA and SRR (10 year incidence 0.04 in ANZDATA, 0.25 SRR, p<0.001). Models were therefore generated for ANZDATA, to be updated using SRR and Dutch registry data. Age and high comorbidity were associated with a decrease in EPS risk. Predicted risks for sample patients are shown in the table.

		Age 40, Low comorbidity, No Diabetes	Age 60, Low comorbidity, No Diabetes	Age 80, High Comorbidity With Diabetes
3 years	EPS	2.59%	1.27%	0.07%
	Death	18.94%	41.55%	84.88%
4 years	EPS	4.51%	1.68%	0.19%
	Death	23.12%	46.13%	84.17&
5 years	EPS	6.45%	2.55%	0.35%
	Death	28.75%	49.90%	91.31%

Conclusions: The predicted risks for EPS and death show marked differences between patients, supporting the use of a prognostic model when discussing whether to stop PD. Results for ANZDATA showed lower risks, likely due to ascertainment bias, so the updated model will predict higher EPS risks.

Funding: Government Support - Non-U.S.

TH-PO946

Long-Term Prevalence of Lipid Abnormalities in Children on Peritoneal Dialysis

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Background: Little data is available for dyslipidemia in pediatric peritoneal dialysis (PD) patients. The aim of the study is to determine long-term prevalence of lipid abnormalities in these patients and its relationship with age, PD duration and treatment modality.

Methods: Fifty-eight pediatric PD patients (33 boys; 34 CCPD) who underwent PD at least 24 months were retrospectively analyzed. All available data on lipid parameters [serum triglyceride(TG), total cholesterol(TC), LDL-C, HDL-C] were documented and mean values were calculated for the baseline, second and fifth year on PD. Dyslipidemia was defined as TC≥200mg/dl, LDL-C≥130mg/dl and non-HDL-C≥145mg/dl. Hypertriglyceridemia was defined as serum TG≥100mg/dl (0-9 yr) and TG≥130mg/dl (10-19 yr). The cases were divided into three groups according to the age at the initiation of PD; Group I; 0-2 yr (n=17), Group II; 2-9 yr (n=17) and Group III; 10-19 yr (n=27).

Results: Median age at the initiation of PD was 9.6(0.1-17.4) years and median duration of PD was 41(24-107) months. The prevalence of dyslipidemia and the prevalence of hypertriglyceridemia were 83%(n=43), 70%(n=58), 76%(n=21), and 70%(n=45), 66%(n=58), 58%(n=21) at the baseline, second and fifth year on PD,respectively. TG levels showed significant difference between the three groups at the baseline (p=0.015) and the second year (p<0.001). The mean TG levels were significantly higher in Group I as compared to Group II and Group III at the baseline (383±279, 214±91, and 176±77mg/dl,respectively) and at the second year on PD (326±139, 215±105 and 163±69mg/dl, respectively). Baseline TG level was an independent predictor of a high level of TG at the second year on PD (β=0.339,p=0.02). Any other lipid parameters at the baseline or at the second year and none of the fifth year lipid parameters did not differ between the three groups. Neither TG nor TC levels changed during the 5 years of PD. None of the lipid parameters differed between CAPD and CCPD patients.

Conclusions: These findings underline the high prevalence of dyslipidemia in pediatric PD patients. Children younger than 2 years are especially at higher risk of hypertriglyceridemia.

TH-PO947

First Year Mortality in Peritoneal and Hemodialysis Patients: An Instrumental Variable Analysis

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Background: Peritoneal dialysis (PD) has been proposed as a better initial modality compared with hemodialysis (HD). We postulate that PD-first patients (where PD is the initial dialysis modality) will have lower first-year mortality compared with HD-first patients.

Methods: A total of 175,323 adult patients from 4,303 dialysis facilities (with >10 patients) who initiated and remained on the same dialysis modality for 90 days between 01/2010 and 12/2012 were analyzed using national U.S. End Stage Renal Disease (ESRD) data. To reduce potential confounding-by-indication, we used a two-stage residual inclusion instrumental variable (IV) methodology, adjusting for relevant covariates and using percentage of patients utilizing PD in each facility as the instrumental variable.

Results: The PD utilization rate varied greatly across facilities (mean 7.8%; range 0-100%). Facilities in the upper quartile of the PD utilization had a mean rate of 28.4%, while 60.7% of facilities had 0% PD utilization. PD-first patients were younger, healthier, more likely to be Asian and White, with a higher rate of being insured or employed than HD-first patients. Facilities with higher PD utilization rate had more patients, HD stations, nurses, social workers, dietitians and more patients having received pre-ESRD nephrology care and were located in geographic areas with higher income and education level. The adjusted hazard ratio (HR) for 1-year mortality among PD-first patients was 0.65 [95% CI (0.60, 0.69)] in comparison with HD-first patients. Results from the IV analysis, which further account for unmeasured confounding, confirmed a lower hazard ratio of 0.68 [95% CI (0.61, 0.75)] for PD-first patients.

Conclusions: This national study shows low utilization of PD-first and association of both facility and geographic factors with this practice. PD-first patients had lower one year mortality compared with HD-first patients. Whether overall facility-level outcomes could be improved by increased utilization of PD should be studied prospectively.

TH-PO948

Development of Telemedical Service Using Smartphones for PD Patients

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Background: The 2011 Great East Japan Earthquake had a vast impact on dialysis patients. Peritoneal dialysis (PD) was shown to be a more favorable dialysis modality than hemodialysis in the affected areas (AJKD2012). During evacuation, medical records including PD prescriptions were left behind or lost, but smartphones were the item, which many PD patients carried. Taking this into account, we have developed a system using smartphones to manage medical records. This allows checking of the patients' PD status in real time as well as confirmation of their safety and locations using GPS at the time of disaster.

Methods: We have invented a smartphone application for management of PD patient with a robust security system to ensure confidentiality. Not only will this application be useful at the time of disaster, but also it is a helpful tool in daily clinical practice.

Results: The aim of this application is to provide telemedicine to PD patients using smartphones. Using this application, patients can compile vital signs, body weight, urine and PD output with a function that allows fluid balance to be calculated automatically. Photographs of exit sites and characteristics of PD fluid drained out can also be stored together with other vital information on the cloud server. Using this information, messages can be sent from doctors to patients with appropriate advice in this application. Daily PD manuals and those for the time of disaster are also accessible to the users if necessary. Furthermore, automatic summarization of the data facilitates identification of clinical problems. This system enables doctors to receive detailed medical information in real time from remote places, helping to bring about early detection of PD peritonitis and exit-site infections. Early signs of hypervolemia can also be picked up in a timely manner. Therefore, patients can be encouraged to seek medical advice before developing congestive cardiac failure.

Conclusions: Telemedicine using smartphones is useful for management of PD patients in both emergencies and daily clinical practice.

TH-PO949

Evaluation of Patient-Related Factors in Peritoneal Dialysis Selection

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Background: There are patients who initially intend to undergo peritoneal dialysis (PD) but instead eventually select hemodialysis (HD). These patients select HD because of a number of obstacles to PD. Identification of the factors that limit the selection of PD may be important to provide specific support or interventions that would better enable patients to select it.

Methods: The aim of this study was to identify patient-related factors that are obstacles to PD selection. One hundred twenty-three patients who selected HD as initial renal replacement therapy at our hospital were enrolled and completed a questionnaire survey. The rates of factors such as physical factors (abdominal appearance), social factors (absence of family support or caregiver), technical factors (inability for self-care), and mental factors (safety concerns) were statistically compared between the group that intended to select PD and the group that did not intend to select PD.

Results: The mean age of the studied population was 66.9 years, and 56.7% of the patients were male. Thirteen percent of the study population intended to select PD. The reasons listed by the group of patients who did not intend to select PD included safety concerns (42.9%), concerns regarding self-care (40.1%), and the absence of a caregiver (20.5%). The factors that influenced the group of patients who intended to select PD, as compared with the group that did not intend to select PD, included technical problems (56.2% versus 37.3%, respectively), safety concerns (62.5% versus 42.9%, respectively), and lack of time for predialysis education (12.5% versus 6.5%, respectively). The rates of these factors were higher in those patients that intended to select PD as compared to those that did not intend to select PD, but there were no statistically significant differences between the two groups.

Conclusions: Technical and mental factors, rather than social factors, were the major obstacles listed by the patients who intended to select PD. Therefore, careful patient education with a greater emphasis on eliminating their safety concerns and ensuring them that PD is safe would be useful for those patients who initially intend to select PD.

TH-PO950

Comparison of 30 Day Readmission Rates between Hemodialysis (HD) and Peritoneal Dialysis (PD) Patients Julio E. Pena, Heidi Mae G. Timbol, Priyanka Khatri, Shirin Shirani, Chirag R. Parikh, Fredric O. Finkelstein. *Yale New Haven Hospital, New Haven, CT.*

Background: Readmissions for hospitalized ESRD patients are common and have a high economic burden to the health care system. Over 30% of ESRD patients admitted to the hospital are readmitted within 30 days of discharge. The present study was designed to retrospectively compare the 30 day readmission rates of HD and PD patients.

Methods: Discharge summaries between 2009 and 2012 of all HD and PD patients from 4 dialysis centers in New Haven admitted to the hospital were examined. A comorbidity scoring system (modification of the Davies score) was used to assess comorbidities (1 point each for CAD, HTN, CHF, and DM). The cause of readmission was adjudicated based on detailed chart review as the same, related or unrelated to the index hospitalization.

Results: 433 HD patients and 110 PD patients were hospitalized during the study period. The total number of admissions in the HD group was 2071 and 538 in the PD group. The mean ages of admitted HD and PD patients were 68 ± 14 and 64 ± 15. The mean comorbidity scores of admitted patients were 2.28 ± 1.11 for HD and 2.2 ± 1.04 for PD patients (p= 0.49). 705 hospitalizations in the HD group (34.0%) and 137 (25.5%) in the PD group were readmissions within 30 days of discharge (p= 0.0002). The mean comorbidity scores of readmitted patients (HD 2.35 ± 1.07 and PD 2.52 ± 1.07) were higher than the patients not readmitted (HD 2.19 ± 1.14 and PD 1.85 ± 0.89). 32.5% of the patients in the HD group and 31.4% in the PD group were readmitted for the same cause as for the index hospitalization; 21.6% and 17.1% for a related cause; and 45.9% and 51.3 % for an unrelated cause. 61% of the readmitted patients in the HD group and 69% in the PD group were readmitted within 2 weeks of discharge.

Conclusions: The 30-day readmission rate of PD patients was significantly lower than HD (p= 0.0002) despite similar ages and comorbidities. Comorbidity was higher in patients readmitted than in those not readmitted. About half of the readmissions in both groups were for a cause unrelated to the index hospitalization. More than 60% of the patients were readmitted within 2 weeks of discharge in both groups.

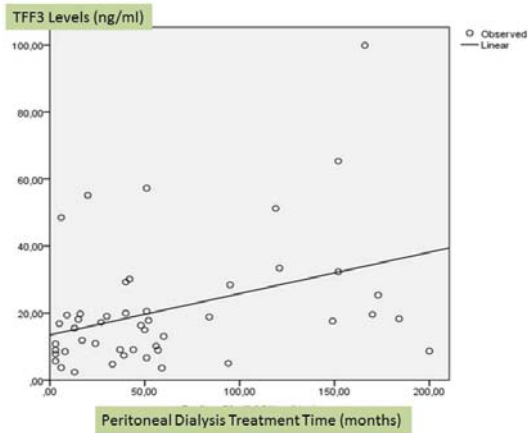
TH-PO951

Trefoil Factor 3 Levels in Peritoneal Effluents of Peritoneal Dialysis Patients Gökmen Aktas, Izzet Hakki Arikan, Derya Guler, Mehmet Koc, Serhan Tuglular, Cetin Ozener. *Nephrology, Marmara Univ Hospital, Istanbul, Turkey.*

Background: Peritoneal fluid of PD patients includes various substances. Trefoil Factor 3 (TFF3) is secreted from different kinds of epithelial cells. It increases survival of epithelial cells and facilitates the restoration of cells aganist any kind of injury. TFF3 levels in the effluent and serum of PD patients were measured and associated factors were investigated. Effluent CA-125 levels which is known as indicator of mesothelial cell mass were ascertained for the potential association.

Methods: Peritoneal fluid and serum samples of 48 patients were collected after a 12 hour dwell. ELISA kit (BioTek Instruments U.S.A.) was used for the detection of TFF3 levels. The patients' demographic and laboratory data as well as peritoneal membrane characteristics were recorded.

Results: Fourty-eight PD patients (24 men; mean age: 51,6 ± 13,9 years) who have a median treatment time of 43 months (range:3-200 months) were included. Median effluent TFF3 level was 17,07 ng/ml (range: 2,38-99,4 ng/ml). There was a positive correlation between effluent TFF3 levels and treatment time (r=0,349, p<0,015). Median serum TFF3 level was 1,56 ng/ml (range: 0,79-11,05ng/ml). There was no statistical association between effluent and serum TFF3 levels and effluent CA-125 levels. There was no statistical association with demographic features, labaratory results and treatment modalities except serum PTH levels (p<0,05).



Conclusions: High serum TFF3 levels were comparable with other studies including patients with chronic kidney disease. Effluent TFF3 levels increases with treatment time may be emanated from local production or peritoneal transport. Tissue localization of TFF3 in peritoneal microcirculation, determination of peritoneal clearance of TFF3 and indicating the association with pro-inflamatory cytokines could make TFF3 possible to be used as a biomarker in PD patients.

TH-PO952

Platelet-Derived Growth Factor Receptorβ (PDGFRβ) Expression in Human Peritoneum Stephan Segerer,[1] Harald Seeger,[1] Joerg Latus,[2] Mark Dominik Alscher,[2] Dagmar Biegger,[4] Rudolf P. Wuthrich,[1] Niko Braun.[2] *[1]Div of Nephrology, Univ Hospital, Zurich, Switzerland; [2]Dept of Internal Medicine, Div of General Internal Medicine and Nephrology, Robert-Bosch-Hospital, Stuttgart, Germany; [3]Dept of Diagnostic Medicine, Robert-Bosch-Hospital, Stuttgart, Germany; [4]Margarete Fischer-Bosch Inst of Clinical Pharmacology, Stuttgart, Germany.*

Background: Simple peritoneal fibrosis and encapsulating peritoneal sclerosis (EPS) are important lesions in the peritoneum of patients on peritoneal dialysis (PD). We previously described a population of podoplanin positive myofibroblasts in peritoneal biopsies from patients with encapsulating peritoneal sclerosis (EPS). PDGF receptor β (PDGFRβ) is a marker of pericytes and PDGFs might be involved in the fibrotic response of the peritoneum. This study aimed to describe PDGFRβ in the human peritoneum.

Methods: In this retrospective analysis we localized PDGFRβ in peritoneal biopsies from patients with EPS (n=6), on PD without signs of EPS (n=5), and compared them with normal peritoneum (n=4) and peritoneum from uremic patients (n=5). Consecutive sections were stained for smooth-muscle actin and podoplanin. Slides were scored semiquantitatively by two observers blinded to the diagnosis.

Results: PDGFRβ was expressed by cells of arterial walls in all biopsies. A prominent population of PDGFβR positive cells was present in the normal peritoneum. In EPS the majority of podoplanin positive cells were positive for PDGFRβ. In peritoneal biopsies from normal and uremic patients the expression of smooth muscle actin was mainly restricted to cells of arterial walls. Podoplanin expression was restricted to lymphatic vessels in normal peritoneum, in uremic patients, and patients on PD without EPS.

Conclusions: As podoplanin positive myofibroblasts express PDGFRβ, these cells might be related to pericytes (rather than other sources of fibroblasts). PDGFRβ might turn out to be a therapeutic target.

Funding: Pharmaceutical Company Support - Baxter, Private Foundation Support

TH-PO953

Does Peritoneal Dialysis Associated Peritonitis Risk Vary By Time on Treatment? Jeffrey Perl,[1] Sameer Parpia,[2] Sharon Nessim.[3] *[1]Nephrology, St. Michael's Hospital, Univ of Toronto, Toronto, ON, Canada; [2]Oncology, McMaster Univ, Hamilton, ON, Canada; [3]Nephrology, Jewish General Hospital, McGill Univ, Montreal, QC, Canada.*

Background: Retraining patients shortly after peritoneal dialysis (PD) initiation is recommended to reduce the risk of peritonitis due to breaches in technique. We sought to understand if peritonitis-risk varied by time on therapy and by organism.

Methods: Using the multicenter Baxter POET database (n=4247), time on PD was divided into 3-month intervals. Using prespecified organism categories, time-to-first peritonitis was analyzed by Kaplan-Meier analysis and using an adjusted multivariable model.

Results: Peritonitis-risk (all organisms) was highest in the first 3 months on PD (p=0.001). Organism-specific analyses revealed an increased risk of culture negative peritonitis in the first 3 months (p<0.001), but not for other organism categories.

Time (Months)	All Organisms	Coagulase Negative Staphylococcus (CNS)	S. *Aureus*	Gram Negative	Streptococcus	Culture-Negative
	OR (95%CI)					
Events	1363	409	118	328	172	384
0-3	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
>3-6	0.80 (0.68-0.95)	1.06 (0.75-1.50)	0.61 (0.31-1.20)	0.92 (0.65-1.28)	1.18 (0.72-1.93)	0.66 (0.49-0.88)
>6-9	0.69 (0.58-0.83)	1.23 (0.88-1.73)	0.94 (-.51-1.74	0.59 (0.39-0.87)	0.99 (0.58-1.69)	0.41 (0.28-0.58)
>9-12	0.81 (0.67-0.97)	1.37 (0.97-1.95)	0.90 (0.47-1.72)	0.62 (0.41-0.94)	1.00 (0.57-1.75)	0.57 (0.41-0.80)
>12-15	0.72 (0.59-0.89)	1.28 (0.88-1.86)	1.03 (0.54-1.96)	0.56 (0.36-088)	1.04 (0.58-1.84)	0.45 (0.31-0.66)
>15-18	0.71 (0.57-0.89)	1.46 (1.00-2.12)	0.86 (0.42-1.75)	0.75 (0.49-1.14)	1.00 (0.54-1.83)	0.44 (0.29-0.67)
>18-21	0.73 (0.58-0.93)	1.37 (0.91-2.05)	1.00 (0.49-2.04)	0.86 (0.56-1.31)	1.02 (0.54-1.92)	0.37 (0.23-0.6)
>21-24	0.70 (0.53- 0.91)	1.05 (0.66-1.69)	1.18 (0.57-2.41)	1.11 (0.74-1.69)	1.27 (0.69-2.37)	0.33 (0.19-0.57

Yeast not shown: 56 events, p=0.79, Adjustments: age, diabetes, ethnicity, gender, cause of ESRD, prior treatment modality and facility

Conclusions: Peritonitis risk is greatest in the first 3 months on PD, largely driven by an increased risk of culture negative peritonitis but not by CNS. Better understanding of this increased early peritonitis risk is warranted.

Funding: Pharmaceutical Company Support - CEC Grant: Baxter Healthcare

TH-PO954

The Peritoneal Dialysis Outcomes and Practice Patterns Study: Unifying Efforts to Improve Global Outcomes in Peritoneal Dialysis Jeffrey Perl,[2] Simon J. Davies,[3] Ronald L. Pisoni,[1] Keith McCullough,[1] David W. Johnson,[5] Hideki Kawanishi,[6] James A. Sloand,[4] Friedrich K. Port,[1] Bruce M. Robinson.[1] *[1]Arbor Research Collaborative For Health, Ann Arbor, MI; [2]Nephrology, St. Michael's Hospital, Toronto, ON, Canada; [3]Univ Hospital of North Staffordshire, Stoke-On-Trent, United Kingdom; [4]Baxter Healthcare, Deerfield, IL; [5]Univ of Queensland, Brisbane, Australia; [6]Tsuchiya General Hospital, Hiroshima, Japan.*

Background: Extending technique survival on peritoneal dialysis (PD) is a challenge to optimizing outcomes for patients on PD. The primary objective of The Peritoneal Dialysis Outcomes and Practice Patterns Study (PDOPPS) is to identify modifiable practices associated with improvements in PD technique and patient survival. In collaboration with the ISPD, PDOPPS also seeks to standardize PD-related data definitions and provide a forum for ongoing international collaborative clinical research in PD.

Methods: PDOPPS is an international prospective observational cohort study initially in Australia, Canada, Japan, the United Kingdom, and the United States. Countries are enrolling a random sample of incident and prevalent patients from national samples of 20 to 80 facilities that care for 15+ patients on PD. Enrolled patients will be followed over an initial 3-year study period. Demographic, comorbidity, and treatment-related variables, as well as patient reported data, will be collected over the study course. Outcomes data include PD technique failure (TF), cause-specific TF, death, and hospitalizations, with all-cause TF or death as the primary outcome.

Results: A high proportion of the targeted facility number has been recruited to date in each country. Several ancillary studies have already been funded, and local interest in expansion to new countries is high.

Conclusions: PDOPPS is the first large, coordinated study to follow a cohort of patients on PD longitudinally. With the breadth, detail, and standardization of data collected, PDOPPS will serve as an invaluable resource as a research platform for the PD community, as a means to understand variation in PD practices and outcomes, identify optimal practices, and to ultimately improve outcomes for patients on PD.

Funding: Pharmaceutical Company Support - The DOPPS program is supported by grants to Arbor Research from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, Ltd. Additional support for specific projects is provided in Canada by Amgen, BHC Medical, Janssen, Takeda, Kidney Foundation of Canada (for logistics support); in Germany by Hexal, DGfN, Shire, WiNe Institute; for PDOPPS in Japan by the Japanese Society for Peritoneal Dialysis; for PDOPPS by Fresenius Medical Care, Government Support - Non-U.S.

TH-PO955

Reliability of Markers of Sarcopenia in Peritoneal Dialysis Patients George Greenhall, Andrew Davenport. *Royal Free Hospital, London, United Kingdom.*

Background: Recently new definitions for muscle wasting (sarcopenia) have been proposed for patients with chronic kidney disease, including reduced percentage body fat (%BF) or skeletal muscle mass (%SMM), coupled with markers of inflammation (serum albumin, C-Reactive protein (CRP) and ESA resistance (ESAR, calculated as weekly ESA dose/gHb/kg).

Methods: To determine whether these proposed definitions would be appropriate for peritoneal dialysis (PD) patients, we examined the relationship between body composition and markers of inflammation. We correlated multifrequency bioelectrical impedance assessments (MFBIA) with laboratory data in 136 adult PD patients.

Results: Mean age was 54±15.7 years; 47% male; 28% diabetic; mean body mass index was 26.1±4.3kg/m^2. On multiple linear regression analysis, %BF was positively associated with age (β=0.159, p=0.02) and negatively associated with intracellular water (β=-0.649, p<0.001), dietary protein intake (as determined by total PD dialysate effluent and urinary urea losses, nPNA, β=-18.5, p<0.001), extracellular water over hydration (β=-3,62, p=0.001) and male sex (β=-4.06, p=0.026). Whereas %SMM was positively associated with male sex (β=0.61, p<0.001) and nPNA (β=9.7, p<0.001), and negatively associated with age (β=-0.43, p<0.001). Neither %BF or %SMM showed any association with serum albumin, CRP or ESAR.

Conclusions: In our cohort of PD patients, we found that body composition had no association with routine laboratory markers of inflammation or ESA resistance. However, %BF was negatively correlated with loss of cell mass, extracellular water overload and dietary protein intake; whereas %SMM was only associated with sex, age and protein intake. As such, definitions of sarcopenia in PD patients should use %BF rather than %SMM.

TH-PO956

Hepatocyte Growth Factor Is Associated with Uremia and Peritoneal Membrane Function Pedro M.S. Vieira, Ana Paula Bernardo, Maria Joao Carvalho, António Manuel Nunes Cabrita, Anabela Rodrigues. *Nephrology Dept, Hospital de Santo António, Porto, Portugal.*

Background: Biopsy studies have demonstrated peritoneal membrane(PM) submesothelial fibrosis before peritoneal dialysis(PD), implicating uremia as an injury factor. The antifibrotic Hepatocyte Growth Factor (HGF) can counteract epitelial mesenchymal transdiferentiation, and be a valuable marker of PM status. We aimed to investigate the relationship between uremia, HGF and PM function.

Methods: Cross-sectional study, 74 stable PD patients, mean age 50 years, 53% male, 60% PD-first. Uremia was assessed by residual renal function(RRF) and small solute adequacy parameters, average area under curve of RRF since PD initiation(avAUC_RRF) and by previous and total renal replacement therapy(RRT) vintages. Peritonitis were retrieved. A 4-hour modified 3,86% Peritoneal Equilibration Test(PET) was performed where effluent levels of HGF(eHGF) and transport of small solutes/fluid were determined.

Results: Median time on PD was 7(IQR 4-29) months. At the time of the PET 27% of the patients were anuric, the remaining had a mean RRF 6,1±3,7ml/min. We found significantly negative correlations between RRF/avAUC_RRF and eHGF(r=-0.244, P=0.036 and r=-0.283, P=0.015, respectively); conversely, previous and cumulative RRT vintages had significantly positive correlations with HGF (r=0.440, P=0.001 and r=0.416, P=0.001, respectively). On subset analysis, anuric versus non-anuric and non PD-first versus PD-first evidenced significant differences with higher eHGF on the first. Remarkably, concerning PM evaluation we found positive correlation between eHGF and D/P creat (r=0.29, P=0.012) and negative correlation with % corrected Free Water Transport (FWTc) (r=-0.39 P=0.001). In a multivariable linear regression model (r^2=0,43), previous RRT time, age, number of peritonitis, effluent VEGF and %FWTc remained independently associated with eHGF.

Conclusions: Uremia assessed by lower RRF and higher previous/cumulative RRT vintages is associated with higher eHGF. Moreover eHGF is associated with PM function changes possibly signaling an injured PM ongoing repairing process. Our study highlights the relevance of studying this effluent biomarker.

TH-PO957

Variations in KT/V Urea Measures in Peritoneal Dialysis (PD) Patients with Different Anthropometric Models of Volume Assessment Ranil S.P. Gajanayaka, Joni H. Hansson, Beth Holden, Neera K. Dahl, Fredric O. Finkelstein. *Yale New Haven Hospital, CT.*

Background: Kt/V urea is used to assess the efficacy of solute removal in dialysis patients. Current CMS guidelines target a minimum KT/V urea of 1.7 in PD patients. The denominator V represents total body water (TBW) and is generally estimated from different anthropometric formulas. Currently, the two largest dialysis organizations in the U.S.A. use different methods, Hume and Watson, to assess the TBW in PD patients. Furthermore, there is some controversy whether actual or ideal body weight should be used to calculate V. The present study was conducted to examine how the different ways of determining V impact Kt/V urea measurements.

Methods: We performed a retrospective analysis of data from all PD patients (age> 18) cared for by New Haven Home Dialysis. TBW was calculated from Watson and Hume formula for both actual and ideal body weight. Kt/V was then calculated and comparisons were made among BMI categories of<20, 20-39 >40.

Results: 28 males and 27 females were included. The mean age was 61.3 ± 16.3. The mean Kt/V difference between Hume and Watson with actual body weight was 0.4 ± 0.5 (0.1 – 2.4). This difference was 1.1± 1.4 (0.1-6.0) with ideal weight. The most striking differences occurred in patients with BMIs >40. The Kt/V values with different methods in each BMI categories are shown below.

	BMI (kg/m^2)		
Kt/V Formula	**<20**	**20-39**	**>40**
Actual weight-Hume	2.0 ± 0.5	2.3 ± 0.6	3.1 ± 1.2
Actual weight-Watson	2.2 ± 0.5	2.1 ± 0.4	2.5 ± 0.7
Ideal weight-Hume	1.5 ± 0.7	2.8 ± 1.3	4.6 ± 2.6
Ideal weight-Watson	2.0 ± 0.5	2.4 ± 0.4	3.3 ± 0.9

Conclusions: The present study shows significant variations of Kt/V urea measurements with the two most commonly used methods for assessing V. These differences are most marked in patients with BMIs >40. The differences in Kt/V using ideal or actual body weights to determine V is most dramatic in patients with high BMIs. These data suggest that it is important to develop standardization of Kt/V urea guidelines in terms of how V is determined if dialysis facilities are to be held accountable for achieving targeted Kt/V urea measures in PD patients.

TH-PO958

Effectiveness of Flushing and UV Light in the Disinfection of Peritoneal Dialysis Catheter Connections Glenn M. Chertow,[1] Julia Rasooly,[1] Tiffany L. Cummings,[2] Justin A. Lance,[2] Jim R. Kermode,[1] Niaz Banaei.[1] *[1]PuraCath Medical, Mountain View, CA; [2]Phoenix deVentures, Morgan Hill, CA.*

Background: Peritonitis remains the most common complica-tion associated with peritoneal dialysis (PD) and is associated with significant morbidity and technique failure. The aim of this in vitro study is to determine the individual and combined effects of flushing and the application of UV light on the growth of microorganisms within the fluid path of peritoneal dialysis connections.

Methods: The inside fluid path of the Y-set connector and transfer catheter were inoculated with 2.5µl of diluted inoculum containing Staphylococcus aureus. After connecting the transfer catheter to the Y-set, the fluid path was either flushed with 3mL of the aliquoted dialysate, exposed to UV light (UVC exposure = 254nm for 60 seconds), or a combination of both methods. Positive, negative and inoculant controls were also performed. Four samples were utilized for each test group with a single sample for each control. Following testing, dialysate from each group was collected, diluted, plated and stored overnight at the appropriate temperature. The log rate for reduction in bacterial growth for each test group was then determined.

Results: All test methods produced a significant reduction in growth of S. aureus versus the inoculant control. There was a significant reduction ($p = 0.0025$) in the viable count of S. aureus bacteria in the UV + flush group (2.12 $\log_{10}$ CFU/mL) compared to the UV (1.00 $\log_{10}$ CFU/mL) alone group.

Conclusions: This in vitro study suggests the application of UV light to the peritoneal dialysis fluid path in combination with a dialysate flush results in a significant reduction in bacterial growth compared to UV alone. Combining a UV light delivery system with the flush-before-fill method in Y-set systems may permit a reduction in the risk of peritonitis in PD patients.

Funding: Other U.S. Government Support, Pharmaceutical Company Support - PuraCath Medical

TH-PO959

Evaluation of Quick Start Use of Peritoneal Dialysis Catheter Ozair M. Ziauddin, Ankit Rawal, L. Tammy Ho, Stephen Haggerty. *NorthShore Univ HealthSystem, Evanston, IL.*

Background: PD is underutilized in the U.S. with est prevalence of 6.6% in adults initiating dialysis. Reasons cited include perceived high risk of infections, concern about inadequate dialysis, catheter issues and comfort level of physician. After placement of PD catheter (PDc), at least a 2 wk wait period is generally advised, depending on mode of placement. Hence, patients (pts) with ESRD requiring urgent initiation of dialysis will usually start HD with a central venous cath. As well pts who may opt for PD, often delay placement of PDc, leading to urgent HD start. Changing modalities after initiation is often difficult. Establishment of quick start PD (QS) may address these problems.

Methods: A pilot study was initiated to compare complications and dialysis adequacy associated with QS (use ≤7 days post catheter placement) to more traditional PD protocol (use >7 days post catheter placement, Trd). Between 2012-2014, 50 pts were initiated on PD via laparoscopic placement. 30 pts began using their catheter ≤ 7 days and 20 used their catheter > 7 days after placement. Outcomes, assessed at 1, 2, 4, 6, 12, 18, and 24 mos, included infection rate, catheter related complications, and dialysis adequacy.

Results: Mean age of pts was 63 versus 75 yrs (QS versus Trd respectively). QS pts used catheter on avg in 5.5 days, and Trd pts used catheter in 47.8 days. A total of 14,365 pts days were evaluated with QS and 10,012 pts days with Trd. No leaks were noted in either group. No significant difference in infections was noted in either group. Days to adverse event were 126 ±176 in QS and 234±204 in Trd (NS). There was 1 non-functioning catheter in QS. Episodes of pain were greater in QS (6 versus 1 pt in Trd ($p <0.22$)). Kt/V at the 1 mo interval was 2.61±0.6 in the QS group versus 1.74±0.3 in the Trd group ($p<0.01$). By 2 mo interval, Kt/V was not statistically different (2.6±0.5 and 2.3±0.8, respectively). Albumin levels were not statistically significant between the 2 groups.

Conclusions: Use of laparoscopically placed PDc does not appear to increase the frequency of mechanical and infectious complications. Achievement of adequate dialysis is not different. Quicker use of PDc may allow for direct initiation of PD eliminating the need for urgent HD.

TH-PO960

Initial Experiences with Urgent Peritoneal Dialysis Starts for New End Stage Renal Disease Patients in the United States Eduardo K. Lacson,[1] Leslie P. Wong,[2] Nien-Chen Li,[1] Joseph Kessler,[1] Stephanie Curd,[1] Keith Lester,[1] Melissa Herman,[1] Sheru Kansal,[2] Franklin W. Maddux.[1] *[1]Fresenius Medical Care, North America, Waltham, MA; [2]Cleveland Clinic, Cleveland, OH.*

Background: Peritoneal dialysis (PD) has routinely been used urgently for ESRD patients with imminent need for dialysis outside the U.S. We report the early combined experiences from 12 newly established American urgent start PD programs.

Methods: 28 of 29 referrals initiated urgent start PD from 12 FMCNA centers between 7/1/13 and 5/14/14. One patient had PD catheter malfunction due to adhesions and was unable to start therapy. We describe the characteristics, course, and outcomes of the 28 patients as of 5/15/14.

Results: The cohort's mean age was 50.5±14.5 years, 54% male, 50% white/46% black, and median BMI of 27.5 kg/m2. Eight patients (29%) did not have any insurance. PD catheters were inserted by laparoscopy in 82% and were problem free in 57%. Patients were followed on PD for a median of 84 days (range: 6-317). Of these, the median time for training was 10 days (range: 0-20), with one patient not getting a single full training day due to hospitalization and withdrew from dialysis therapy by day 6. Additionally, by the end of follow-up, 19 (68%) remained active on PD, 1 died, 1 recovered kidney function, and 6 switched to HD – 2 after peritonitis, 2 from catheter dysfunction (inability to drain/pain on drain), 1 for hernia and 1 by choice. 3 of the patients who switched had coexisting HD catheters in place while the 3 others had a new HD catheter placed. Among the latter, fistulas were subsequently placed within 11, 24, and 26 days of the switch.

Conclusions: Preliminary outcomes of urgent PD from 28 patients in 12 U.S. centers indicated that urgent start PD is a viable option – keeping 68% of patients active for a median of 127 days on PD as of 5/15/14. It allows for potential avoidance of HD catheters in a population that would most certainly have had to use them. Further evaluation with longer term follow-up and direct outcome comparisons to matched conventional PD/HD patients is planned.

TH-PO961

Educational Level and Survival in a Cohort of Incident Brazilian Peritoneal Dialysis Patients Kleyton Andrade Bastos,[1] Abdul Rashid Tony Qureshi,[2,3] Natalia Maria da Silva Fernandes,[4] Roberto Pecoits-Filho,[5] Antonio Alberto Lopes,[6] Jose C. Divino-Filho.[2] *[1]Dept of Medicine, Federal Univ of Sergipe, Aracaju, Brazil; [2]Div Renal Medicine, CLINTEC, Karolinska Instt, Stockholm, Sweden; [3]Baxter Novum, Karolinska Instt, Stockholm, Sweden; [4]Federal Univ, IMEPEN Foundation, Juiz de Fora, Brazil; [5]Center of Health and Biological Sciences, Catholic Univ of Paraná, Curitiba, Brazil; [6]Dept of Medicine, Federal Univ of Bahia, Salvador, Brazil.*

Background: There is evidence that limited educational level (EL) contributes to differences in health outcomes. The goal of this study was assessing the effect of EL on survival in a cohort of Brazilian peritoneal dialysis (PD) patients.

Methods: Prospective cohort study of incident PD patients enrolled in the Brazilian Peritoneal Dialysis Multicenter Study (BRAZPD) from December 2004 to October 2007. EL was categorized by school years: low EL, <4 (1309 patients); and high EL, ≥4 (643 patients). Survival analysis comparing low and high EL groups was performed using Kaplan-Meier survival curves and Cox proportional hazards model adjusting the results for age, gender, PD modality, first renal RRT, pre-dialysis care, family income, comorbidities, and calendar year.

Results: Median age was 59 years, 54% were women, 60% White, 41% diabetics, and 24% had cardiovascular disease. More than half were in continuous ambulatory PD (51%), had not received pre-dialysis care (58%), had <5 minimum wage (MW) monthly family income (80%), and <4 school years (67%). Low EL was associated with worse socioeconomic position indicators. High EL patients were predominantly men, White, older and more often on Automated PD. Cumulative patient survival was lower in the low EL group (Log Rank test $\chi2=4.29$, $p=0.03$) but technique survival did not vary significantly (Log Rank test $\chi2=0.90$, $p=0.34$) between the EL groups. In Cox-regression adjusted analyses, differences were not observed in the hazards of all-cause mortality (HR(hazard ratio)=0.97; 95% confidence interval [CI]=0.73-1.29; p=0.84) and technique survival (HR=1.06; CI=0.81-1.38; p=0.67).

Conclusions: EL is not independently associated with outcomes in this large cohort and should not be considered a barrier for PD indication.

TH-PO962

Paricalcitol, Vitamin D Receptor Activator Prevents Cardiovascular Fibrosis and Inflammation of 5/6 Nephrectomy Rat on Peritoneal Dialysis Min Jung Kim,[1] Il Young Kim,[1] Dong Won Lee,[1] Soo Bong Lee,[1] Byeong Yun Yang,[2] Sang Heon Song,[2] Eun Young Seong,[2] Ihm Soo Kwak.[2] *[1]Internal Medicine, Pusan National Univ Yangsan Hospital, Korea; [2]Internal Medicine, Pusan National Univ Hospital, Korea.*

Background: Vitamin D receptor expressions in kidney, myocardium and also peritoneum suggest the potential role of vitamin D receptor activators (VDRAs). We investigated whether the selective VDRA, paricalcitol (19-nor-1.25-dihydroxyvitamin D2) suppresses rennin-angiotensin-aldosteron system (RAAS), and thus reduces cardiovascular inflammation and fibrosis as well as peritoneal pathology of 5/6 nephrectomy rat model on peritoneal dialysis (PD).

Methods: Vitamin D receptor expressions in kidney, myocardium and also peritoneum suggest the potential role of vitamin D receptor activators (VDRAs). We investigated whether the selective VDRA, paricalcitol (19-nor-1.25-dihydroxyvitamin D2) suppresses rennin-angiotensin-aldosteron system (RAAS), and thus reduces cardiovascular inflammation and fibrosis as well as peritoneal pathology of 5/6 nephrectomy rat model on peritoneal dialysis (PD).

Results: After 4 weeks of PD, BUN and serum creatinine in group C and D were decreased ($p<0.05$ versus group B). D/P urea in group C and D were significantly increased ($p<0.05$). Paricalcitol treatment (group D) increased E-cadherin, and decreased αSMA and TGF-β significantly ($p<0.05$, versus group C). Peritoneal fibrosis scores and neovascularization in group D were decreased (p=NS versus group C). Peritoneal inflammation scores, thickness(μm) and αSMA staining in group D were decreased ($p<0.05$ versus group C). Moreover, myocardial fibrosis scores and aortic inflammation scores were decreased in group D ($p<0.05$ versus group C).

Conclusions: A selective VDRA, paricalcitol attenuates cardiovascular as well as peritoneal inflammation and fibrosis of 5/6 nephrectomy rat model on PD.

TH-PO963

Higher Serum 7-84 PTH Levels in Peritoneal Dialysis Than in Hemodialysis Patients. A Potential Contribution to the Higher Prevalence of Low Turnover Bone Remodeling in Peritoneal Dialysis?

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Background: Intact PTH (iPTH) assays do not reliably identify patients with low turnover bone disease (LTBD) and bone biopsy is not used in routine clinical care.

Methods: Cross-sectional study. Two dialysis units at tertiary care hospitals: 129 hemodialysis and 73 peritoneal dialysis (PD) patients. **Measurements:** iPTH (1-84PTH plus 7-84PTH), bio-PTH (1-84PTH), total serum calcium (tCa); ionized calcium (iCa) and carboxy-terminal telopeptides of collagen type I (βCTx). Calculation of PTH fragment ratios.

Results: iCa accounted for a higher percentage of tCa in PD than in hemodialysis (53% versus 39% $p < 0.001$) and 1-84PTH as a percentage of iPTH was lower in PD (44.0 ± 12.28 % versus 60.3 ± 10.82 %; $p < 0.001$). In the total group iCa was inversely correlated with serum 1-84PTH and 1-84PTH/7-84PTH ratio but not with iPTH. Serum levels ofβCTx correlated with 1-84PTH, 1-84PTH/ 7-84PTH ratio and iPTH. Use of the combined iPTH and 1-84PTH/7-84PTH ratio criteria proposed by Herberth et al (coexistence of 1-84PTH/7-84PTH ratio < 1 and iPTH < 420 pg/mL) resulted in a higher percentage of PD patients predicted to have LTBD (72.7% versus 16.3%, < 0.001). In a multivariate logistic regression analysis, dialysis modality was the main determinant of the percentage of calcium present as iCa. Similarly, the main determinant of LTBD (defined according to Herberth) was iCa.

Conclusions: PD is associated to a higher proportion of 7-84PTH fragments. This may be related to higher serum iCa and might contribute to the higher prevalence of LTBD in PD.

TH-PO964

Serum Response Factor Accelerates the High Glucose-Induced Peritoneal Membrane Fibrosis via Snail Signaling

Shiren Sun. *Dept of nephrology, xijing Hospital.*

Background: Epithelial-to-Mesenchymal Transition (EMT) induced by glucose in human peritoneal mesothelial cells (HPMCs) is a major cause of peritoneal membrane (PM) fibrosis and dysfunction.

Methods: To investigate serum response factor (SRF) impacts on EMT-derived fibrosis in PM, we isolated HPMCs from the effluents of patients with end-stage renal disease (ESRD) to analyze alterations during peritoneal dialysis (PD) and observe the response of PM to SRF in a rat model.

Results: Our results demonstrated the activation and translocation of SRF into the nuclei of HPMCs under extensive periods of PD. Accordingly, HPMCs lost their epithelial morphology with a decrease in E-cadherin expression and an increase in α-smooth muscle actin (α-SMA) expression, implying a transition in phenotype. PD with 4.25% glucose solution significantly induced SRF up-regulation and EMT in rat peritoneal mesothelial cells and increased peritoneal thickness, which could be protected by SRF inhibition. In immortal HPMCs, high glucose (HG, 60 mmol/L) stimulated SRF overexpression, nuclear translocation and a transformed fibroblastic phenotype. SRF-siRNA preserved HPMC morphology, SRF expression and localization, while transfection of SRF plasmid into HPMCs caused the opposite effects. Snail, a potent inducer of EMT, showed a similar response to HG with SRF in HPMCs. Evidence from electrophoretic mobility shift, chromatin immunoprecipitation and reporter assays further supported the hypothesis that SRF transcriptionally regulated Snail by directly binding to its promoter.

Conclusions: Our data suggested that activation of SRF/Snail pathway might contribute to the progressive PM fibrosis during PD.

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TH-PO965

Peritoneal Equilibration Testing in Practice: Errors and Efficacy

Eoin D. O Sullivan, William D. Plant. *Dept of Renal Medicine, Cork Univ Hospital, Cork, Ireland.*

Background: Peritoneal dialysis is the most common form of home renal replacement therapy in Ireland, representing 10% of all dialysis modalities. The functional integrity of the peritoneal membrane is central to the delivery of effective dialysis to patients. Peritoneal equilibrium testing(PET) is a semi quantitative measurement of peritoneal membrane transport capacity, and allows tailoring of dialysis prescriptions to patients, as well as having prognostic significance. Performing a PET is time consuming and prone to errors. An consistent and accurate PET is a requirement for good clinical care. Our aim was to examine PET accuracy, identify the most common errors, as well as establish risks for inaccurate testing.

Methods: All PET tests performed over a twenty year period were obtained (1993-2013) from an electronic database, and the patient demographic, clinical and technical variables were recorded.Technical variables included dialysate volumes as well as dialysate and serum glucose, creatinine and urea at specific time points.

Results: 215 patients have undergone peritoneal dialysis since 1993, receiving a mean of 5 PETs each. 1076 individual PETs were analysed. 54% of all PETs had an error rendering the results questionable. 71% of patients have had at least one invalid test at some stage during their care. The most common error was abnormally low dialysate glucose at initiation of the test (29%). This suggests incomplete emptying of the overnight dwell is the most common error in performing a PET. No correlation between any comorbidities, patient characteristics or membrane transport type and risk of PET errors were identified.

Conclusions: Peritoneal equilibrium testing is prone to errors, which can consequently lead to suboptimal dialysis prescriptions. The most common technical error was an incomplete drain of the overnight dwell at initiation of the test. Identification of the more common errors could help to improve the quality of PET testing, and allow more specific local protocols. This would save money by decreasing the need for repeat testing and improve patient care by ensuring dialysis prescriptions are based on accurate tests of membrane transport function.

TH-PO966

Hyperosmolality (Mannitol) Promotes Angiotensin II (AngII) AT1 Receptor (AT1R) Activation in Rat Peritoneal Tissue

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Background: Our laboratory has demonstrated that exposure of peritoneal mesothelial cells (PMC) in vivo and in vitro to peritoneal dialysis (PD) solutions resulted in increased peritoneal membrane thickness and increased cyclooxygenase-2 (COX-2) synthesis. The increases were mediated by activation of AT1R, particularly through NF-κB. The current study was designed to examine the role of one aspect of PD (ie high osmolality) on AT1R activation in rat peritoneal mesothelium.

Methods: PMC cultures were used to study the effects of high osmolality (mannitol) on AT1R trafficking, transcriptional activation of COX-2 (RT-PCR), and COX-2 protein synthesis (immunoblotting). HEK-293 cells expressing AT1R/GFP were used to examine the effects of mannitol on AT1R trafficking by laser scanning confocal microscopy. PMC surface AT1R were evaluated through radioligand binding assays. In order to examine the in vivo effects of long-term exposure of PMCs to high osmolality, male Sprague-Dawley rats were injected intraperitoneally twice daily with either 0.9% saline (controls) or 4.25% mannitol (high osmolality). At the end of 5 weeks, the peritoneal layer was examined for thickness and collagen deposition (Masson's Trichome stain).

Results: Compared to cells exposed to control isoosmotic conditions, cells exposed to high osmolality (240 mM mannitol) resulted in a 5-fold increase in AT1R internalization and a greater than 90% reduction in surface AT1R. PMC exposed to mannitol produced a 3-fold increase in mRNA levels and a 6-fold increase in protein expression for COX-2, compared to PMC under control conditions. These increases were blocked by the AT1R antagonist losartan or the NF-κB inhibitor RO-1069920. In vivo, experimental PD with hyperosmotic saline (240 mM mannitol) produced a significant thickening of sub-mesothelial collagen versus PD with isoosmotic saline (27.2 ± 4.8 versus 13.8 ± 4.6 μm, $P<0.05$, n=3-5).

Conclusions: These results indicate that the hyperosmotic nature of PD solutions may contribute to fibrosis associated with chronic PD by activating AT1R.

Funding: Veterans Affairs Support, Private Foundation Support

TH-PO967

Mupirocin versus Gentamicin in the Prevention of Exit-Site Infection and Peritonitis in Peritoneal Dialysis: A Meta-Analysis

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Background: Peritoneal dialysis (PD)-related infections remain the major causes of morbidity and mortality in PD patients. Exit-site infections (ESI) can be prevented by application of topical antibiotics. We conducted this systemic analysis to compare the efficacy of mupirocin versus gentamicin in the prevention of ESI and peritonitis in patients undergoing PD.

Methods: Recruited studies met the following criteria: they were randomized controlled trials (RCT) or cohort studies; subjects consisted of PD patients aged 18 and

older; mupirocin was administered to one arm and gentamicin was administered to the other arm. The primary extracted data were the difference in the episodes of ESI and peritonitis among mupirocin and gentamicin groups.

Results: Five studies (4 cohort studies and 1 RCT) and a total of 338 patients in mupirocin group versus 351 patients in gentamicin group were included in the analysis. There was no significant difference in the rate of ESI between mupirocin and gentamicin arms [mean difference (MD) 0.08/episode/patient-year, 95% confidence intervals (CI) -0.05 to 0.20; heterogeneity χ^2= 20.46, I^2= 80%, P= 0.004). There was also no significant difference in the rate of peritonitis [mean difference (MD) 0.10/episode/patient-year, 95% CI -0.04 to 0.24; heterogeneity χ2= 8.57, I^2= 53%, P= 0.07).

Conclusions: Gentamicin cream was not superior to mupirocin in the prevention of ESI and peritonitis.Both drugs can be recommended for prophylaxis of PD-related infections.

TH-PO968

Peritoneal Dialysis and Kidney Transplantation: No Fear for Encapsulating Peritoneal Sclerosis! Valerio Vizzardi, Massimo Sandrini, Silvio Sandrini, Elisabetta Devoti, Laura Bregoli, Giovanni Cancarini. *O.U. of Nephrology, Spedali Civili and Univ of Brescia, Brescia, Italy.*

Background: Objectives. Encapsulating peritoneal sclerosis (EPS) is the more dangerous complication of peritoneal dialysis (PD). Its prevalence has been reported as 0.3% to 3.3%; about 50% of cases of EPS are diagnosed after kidney transplantation (TX). PD discontinuation, use of calcineurin inhibitors (CNIs), corticosteroids (Sts) and mammalian target of rapamycin inhibitors (mTOR-Is) can affect the onset of post-transplantation EPS.

Methods: A retrospective study on PD patients undergoing TX in single Center, evaluation of immunosuppressive therapy and EPS prevalence.

Results: From July 1979 to December 2013, 920 patients started on PD. One hundred-seventy-two of them (18.7%) were transplanted. Median PD duration before transplantation: 27 months (IQR 17-47, range 1-148), median TX duration: 81 months (IQR 34-163, range 1-353). Therapy: CNIs= 49%; Sts= 13%; Sts + CNIs= 68 %; mTOR-Is associated with CNIs and/or Sts= 49%. During the time with functioning graft, two EPS cases occurred, 6 and 51 months after PD discontinuation. Three more episodes of EPS were diagnosed during dialysis in patients previously transplanted: one on hemodialysis (HD), two during PD. The median PD time at EPS diagnosis in the five patients previously transplanted was 79 months (IQR 54-95, range 36-100). Overall EPS prevalence on TX patients: 5/172 (2.9%). Two patients died for EPS (10 and 45 months after EPS diagnosis), one is on HD (78 months after) and two are on TX respectively (44 and 86 months after). At the end of 2013, 128/172 patients (74.5%) were on TX, 21 (12.2%) were on dialysis, 23 (13.3%) were died. During the same period other 21 EPS cases (2.8%) are occurred among the 748 PD patients never transplanted. No cases of EPS was manifested after direct transfer from PD to HD.

Conclusions: EPS prevalence among transplanted and not transplanted PD patients is similar. The reason could be both the low exposure to potential EPS risk factors (PD time <5 years, no peritonitis in 69% of cases) and the use of the mTOR-Is and/or corticosteroids. The transfer to hemodialysis or transplantation does not appear be a heavy risk factor for EPS.

TH-PO969

Title: Does Energy Expenditure Correlate with Standard Dosing of Dialysis in Peritoneal Dialysis Patients, or Are There Other Players in the Back Ground? Sally Salah El-Kateb,[1] Sivakumar Sridharan,[2] Ken Farrington,[2] Andrew Davenport.[1] *[1]UCL Centre for Nephrology (Royal Free Hospital), London, United Kingdom; [2]Lister Hospital, London, United Kingdom.*

Background: Guideline committees have proposed urea clearance targets (Kt/V) for peritoneal dialysis (PD) patients based on volume of distribution as surrogates for achieving adequate dialysis. However studies have failed to demonstrate any survival advantage from increasing weekly Kt/V urea clearance. We investigated whether differences in body composition determine total energy expenditure (TEE), and as such some patients may generate more waste products thus need greater clearance targets.

Methods: We assessed TEE in PD patients who had body composition measured by whole body Dual Energy x-ray absorptiometry (DEXA) and multi-frequency bioelectrical impedance assessments (MF-BIA) including extracellular water ECW) and total body water(TBW). TEE, resting EE (REE) and active EE (AEE) were calculated using validated equations.

Results: 148 PD patients (66.7% M), age: 60.7years (+/-1.4). DEXA showed a mean percentage of body fat (PBF%) 28.9%(+/- 1). MF-BIA determined mean ECW/TBW of 0.39 (+/-0.001). There were correlations between % body fat (PBF%) and both AEE (r=-0.33,p<0.001),and TEE (r=-0.33,p<0.001). ECW/TBW correlated with both AEE(r=-0.26,p=0.0056) and TEE(r=-0.26,p=0.0055). Protein intake (nPNA) was noted to be correlated with AEE (r=0.36,p=0.00091), TEE (r=0.36,p=0.00081), and REE (r=-0.31,p=0.004). On multiple regression analysis AEE correlated with PBF%[(β=-0.076, %CI(-.130 to-0.022),p=0.0005], In addition age was correlated with TEE [β=-6.27,95%CI(-0.8.7to-3.75),p=0.000019]. However there was no association between AEE, REE or TEE and weekly KT/V urea (total, peritoneal, urine) or litres of creatinine cleared per week (total, peritoneal or urine).

Conclusions: We showed that in PD patients TEE has no correlation with small solute clearance achieved. TEE is negatively associated with age, increasing PBF%, and volume overload (ECW/TBW) while it is positively associated with nPNA. As such, PD prescriptions should take into account TEE and body composition when deciding upon individual adequacy targets.

TH-PO970

Does Faster Peritoneal Protein Transport Lead to Extracellular Water Overload in Peritoneal Dialysis Patients? Cate Goodlad, Andrew Davenport. *Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: Faster transport status (4h D/P creatinine) has been associated with both extracellular water (ECW) expansion and adverse outcomes in peritoneal dialysis (PD) patients. Faster peritoneal transport is associated with increased peritoneal capillary surface area due to body size, hyperglycaemia or local peritoneal inflammation. Increased peritoneal protein clearance, through large pores, may be a marker of local peritoneal inflammation.

Methods: We studied the relationships between 4h D/P protein and ECW excess, using multifrequency bioimpedance assessments (MFBIA), in 103 PD patients with up to four years of annual prospectively collected PET results.

Results: The number of patients with data at start, 1, 2, 3 and 4 years was 103, 84, 82, 70 and 38 respectively. The 4hD/P protein was stable over time (K-W test, p=0.063). A trend to lower values was seen at 4 years (Dunn's test: start v 4 years, p < 0.05: median 0.0081 v 0.006), but this might represent a survivor effect. The 4hD/P creatinine was also stable in this group of PD patients (K-W analysis, p = 0.3357). Significant correlations between 4hD/P protein and 4hD/P creatinine were noted at all time points.

Conclusions: At the start of PD therapy, over-hydration (ECW excess) was seen with higher 4hD/P creatinine and 4h/P protein values, suggesting that initial exposure to PD fluids causes local inflammation. As 4hD/P protein was then stable over 3 years, continued exposure to dialysate did not lead to increasing peritoneal inflammation; 4hD/P protein correlated with 4hD/P creatinine at all time points. Over time we found no relationship between ECW over-hydration and faster large pore transport, suggesting perhaps that appropriate changes in PD prescriptions prevented ECW expansion.

TH-PO971

Limitation of Peritoneal Dialysis by Kidney and Liver Volume in Autosomal Dominant Polycystic Kidney Disease Satoshi Hamanoue,[1,2] Junichi Hoshino,[2] Tatsuya Suwabe,[2] Toshiharu Ueno,[2] Koki Mise,[2] Noriko Hayami,[2] Keiichi Sumida,[2] Kenmei Takaichi,[2] Yoshifumi Ubara.[2] *[1]Nephrology Center, Japanese Red Cross Kumamoto Hospital; [2]Nephrology Center, Toranomon Hospital.*

Background: In patients with autosomal dominant polycystic kidney disease (PKD), peritoneal dialysis (PD) can be limited due to excessive kidney volume(KV)and liver volume(LV). We evaluated how nephromegaly and hepatomegaly influenced the continuation of PD in patients with PKD.

Methods: Twenty-two PKD patients on PD at our hospital were evaluated retrospectively after being divided into two groups: **group 1** was 15 patients who started PD at our hospital and **group 2** was 7 patients who were referred from other hospitals at 47.1 ± 21.8 months after commencing PD for treatment of renomegaly by transcatheter arterial embolization(TAE).

Results: In group 1, KV was 2787±1945 mL and total volume (TV = KV + LV) was 4985 ± 1815 mL. In one patientwith a KV of 6816 mL (TV of 8912 mL) showing the largest TV, TV/BW ratio, and TV/BMI ratio among all 15 patients in group 1, PD was stopped due to dialysate leakage after infusion of peritoneal dialysate up to a maximum volume of 700 mL. The other 14 patients less than TV(7963 mL), TV/BW ratio (130.5), and TV/BMI ratio(353) did not experience any dialysate leakage with infusion volumes of 1200 ml to 1500 mL, although larger infusion volumes have not been employed because of abdominal fullness. In group 2, KV was 5822±1597 mL and TV was 7597 ± 1431 mL before TAE. Across both groups, 6 patients developed new abdominal hernias during 36 ± 5 months (6 - 55 months) after starting PD. At 12 months after TAE, KV showed a significant decrease from 5822 ± 1597 mL to 2925 ± 1326 mL (P<0.01), while the infusion volume was significantly increased from 1.53±0.37 L to 1.94±0.52 L (P=0.02).

Conclusions: Our data suggest that PD is limited by KV and LV in patients with PKD, and a TV/BMI ratio of 353 might represent the limit for dialysate leakage. TAE is a therapeutic option for these patients when residual renal function is minimal.

TH-PO972

Comparisons of Propensity-Based Matching to Marginal Structural Models in Mortality Comparisons of Peritoneal Dialysis and Haemodialysis John P. Ferguson,[2] Austin G. Stack.[1,2] *[1]Nephrology, Univ Hospital Limerick, Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Limerick, Ireland.*

Background: The optimal method for comparing non-randomised treatments between groups is debated with some favouring propensity-based matching while others advocate use of marginal structural models. We compared each approach in mortality comparisons of Peritoneal (PD) and Haemodialysis (HD).

Methods: From the U.S. Renal Data System, we matched PD and HD patients from 1,336,441 HD and 109,216 PD patients using 25 clinical characteristics and a propensity-based matching algorithm. Patients were followed from 90 days after dialysis initiation until death, transplantation, lost to follow-up, 31/12/2010. Interval Cox regression models evaluated the relative hazard of death (HR) by dialysis modality using an as-treated analysis. Marginal structural models compared HD to PD by calculating the probability of modality assignment to each modality during follow-up and using inverse probability weights to control for confounding using conditional logistic regression.

Results: The hazards of death for PD relative to HD decreased significantly from 1995-1998 to 2003-2006 with follow-up for 4 years. Time-dependent models gave similar results.

	Propensity Matched Model (HR)		
	1995-1998	**1999-2002**	**2003-2006**
Year1-Year4	1.14 (1.11-1.17)	1.01 (0.98-1.03)	0.89 (0.87-0.91)
Year 1	1.01 (0.97-1.06)	0.87 (0.83-0.90)	0.71 (0.68-0.74)
Year 2	1.32 (1.25-1.38)	1.19 (1.14-1.25)	1.08 (1.03-1.14)
Year 3	1.27 (1.20-1.34)	1.19 (1.13-1.25)	1.17 (1.11-1.23)
Year 4	1.33 (1.24-1.41)	1.22 (1.15-1.30)	1.09 (1.03-1.16)
	Marginal Structural Model (HR)		
	1995-1998	**1999-2002**	**2003-2006**
Year1-Year 4	1.09 (1.07-1.11)	0.96 (0.94-0.98)	0.86 (0.85-0.88)
Year 1	0.99 (0.95-1.03)	0.92 (0.87-0.96)	0.83 (0.78-0.88)
Year 2	1.21 (1.16-1.26)	1.05 (1.01-1.09)	0.93 (0.90-0.97)
Year 3	1.25 (1.20-1.31)	1.08 (1.04-1.12)	0.98 (0.94-1.02)
Year 4	1.27 (1.21-1.34)	1.08 (1.03-1.13)	1.01 (0.97-1.06)

Conclusions: The results from the marginal structural model were similar to those of the propensity-based method. Each method confirmed that PD survival has improved substantially from 1995 to 2006 and is significantly greater than HD over the first 4 years of treatment.

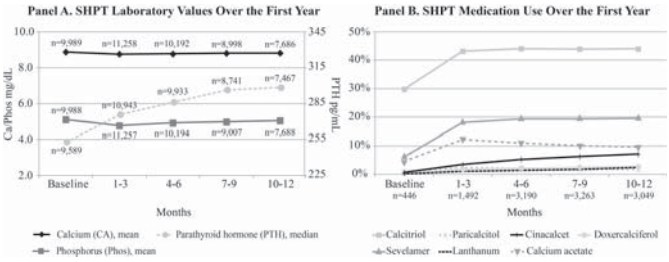
TH-PO973

Secondary Hyperparathyroidism Among Incident Peritoneal Dialysis Patients Scott Sibbel,[1] Paul Dluzniewski,[2] Thy P. Do,[2] Susan V. Yue,[2] Steven M. Brunelli,[1] Brian D. Bradbury.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]Amgen, Inc., Thousand Oaks, CA.*

Background: Data regarding secondary hyperparathyroidism (SHPT) among patients receiving peritoneal dialysis (PD) are sparse. We evaluated biochemical parameters and treatments for SHPT among a representative cohort of incident PD patients in the United States to better understand the burden of the disease and current treatment practices.

Methods: The cohort included all patients whose first renal replacement modality was PD at a large dialysis organization (LDO) between Jan 1, 2008, and Feb 28, 2014 (N=11,376). Demographic and clinical characteristics were evaluated during baseline (30 days following PD initiation). Laboratory indices for the entire cohort and medication use among the sub-sample enrolled in LDO pharmacy benefits program [n=4268] were evaluated over 1 year or until censoring for modality change, death, transfer, or transplant.

Results: Among the cohort, mean (±SD) age was 57±15.5 years, 43% were female, 57% were white, 21% were black, and 58% had diabetes. Laboratory values (total cohort) and medication utilization (subsample) are shown in the figure. Median parathyroid hormone (PTH) levels rose from 254 to 302 pg/mL over the first year. At baseline, mean (±SD) calcium and phosphorus levels were 8.9±0.9 mg/dL and 5.1±1.4 mg/dL, respectively, and remained fairly constant through follow-up. SHPT medication use among the cohort subsample was low at baseline, but increases in utilization over the 12-month follow-up reflected evidence of progressive disease: vitamin D use increased from 31% to 49%, while overall binder use increased from 11% to 31% and cinacalcet use increased from 1% to 7%.
Conclusions: SHPT was modest, yet progressed after PD initiation over the first year as evidenced by increases in PTH despite greater utilization of PTH-lowering therapies.



Funding: Pharmaceutical Company Support - Amgen, Inc.

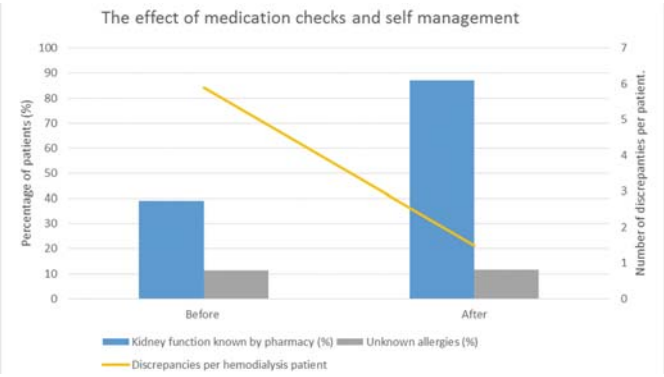
TH-PO974

Frequent Medication Checks and Structured Self Mangement Programme Improve Medication Safety in Hemodialysis Patients Marieke Kerskes,[1,2] Marijke J.E. Dekker,[1] Evelyn Cramer,[2] Johanna Bogers,[1] Maaike Hengst,[1] Constantijn Konings.[1] *[1]Nephrology, Catharina Hospital, Eindhoven, Netherlands; [2]Clinical Pharmacy, Catharina Hospital, Eindhoven, Netherlands.*

Background: In 2010 a prospective risk analysis showed that the medication registration process of hemodialysis patients on our ward was a high risk process. A prospective observational study (n = 143) showed that there were many discrepancies between public pharmacy drug records and drug records on our dialysis ward. In 60.8% of our patients the public pharmacy was even unaware of their chronic kidney disease.

Methods: In 2014 a second prospective observational study was performed (n = 77) to determine whether a series of interventions on the medication registration process, implemented on our ward in 2010, was effective in improving medication safety in our hemodialysis patients. The interventions consisted of a 3 monthly medication check, communication of this check with the public pharmacy and the use of a standardized and validated checklist on medication self management abilities of our patients.

Results: Awareness of chronic kidney failure of our hemodialysis patients in the public pharmacy (39.1% to 87.0%) and discrepancies in drug records between public pharmacy and our dialysis ward (5.9 to 1.5 per patient) were significantly improved.



Discrepancies consisted of prescribed dialysis medication unknown in public pharmacy (39.1%), dose discrepancies (17.3%) and medication prescribed by other physicians (30.9%), mainly ocular and dermal preparations. The nature of the discrepancies did not differ between the both studies.

Conclusions: Frequent medication checks and a standardized self management programme improve adequate medication registration of dialysis patients and may therefore be a usefull tool to improve medication safety in this group of patients.

Funding: Other NIH Support - Nierstichting Nederland

TH-PO975

Telehealth Delivery of Medication Therapy Management (MTM) in ESRD Patients Harold J. Manley,[1] Graham E. Abra,[2] Revkah Balingit,[2] Jessica L. Baugh,[1] Margaret Mcnamara,[1] Janet Beryl Mackay,[2] Jeraldyn Santiago,[2] Maria Lourdes L. Deguzman.[2] *[1]Reach MTM, Dialysis Clinic Inc, Albany, NY; [2]Satellite Health Plan, Satellite, San Jose, CA.*

Background: Medicare Part D plan sponsors offer MTM to targeted patients (pts) to ensure medications are appropriately used and therapeutic outcomes are optimized. Resolution of medication-related problems (MRP) via MTM programs improve clinical outcomes and reduce hospitalizations and costs. Unfortunately only 11-14% pts receive MTM despite meeting eligibility criteria.

Methods: An interdisciplinary (RN, RPh, MD) approach using telehealth (video and phone) technology to increase MTM services was provided to all enrolled ESRD pts. The principal RPh roles were to conduct comprehensive and targeted medication reviews (CMR, TMR respectively), create MTM intervention(s) for patient and provider(s), and generate patient and provider medication reports. The principal RN roles were to facilitate RPh-Patient video conference during dialysis sessions and to facilitate resolution of RPh recommendations to MDs. Weekly interdisciplinary calls were conducted to determine MTM interventions outcome(s).

Results: From June 2013-May 2014, 86 pts (age 58.7± 13.2 yrs; 12.2± 4.4 medications) received 224 MTM encounters (2.6± 1.5 per patient). 99 CMR (82.8% video; 17.2% telephonic) and 125 TMR were provided. CMR completion rate was 92.3%. 423 MRPs (mean 3.1 MRPs per pt). MRPs were related to dosage too high (19.4%) or too low (12.1%), compliance (16.8%), drug without indication (16.5%), indication without drug therapy (15.6%), adverse drug reaction (11.4%) and different drug needed (8.3%). MTM interventions were accepted 73.4%, rejected 5.9%, and noted 20.7% of time by the treating physician.

Conclusions: An interdisciplinary team approach increases the likelihood that pts receive MTM services. This improved CMR completion rate will hopefully lead to improved adherence, reduced patient risks, and lower drug and total cost of care.

TH-PO976

Home Visit Audits: Ensuring Patient Safety in Home Hemodialysis? Karlien Francois, Christopher T. Chan. *Div of Nephrology, Univ Health Network Toronto General Hospital, Toronto, ON, Canada.*

Background: Home visits (HV) and HV audits (HVA) are standard practice in home dialysis programs however with limited clinical evidence.

Methods: We performed a single-centre retrospective cohort study in patients starting home hemodialysis (HHD) at University Health Network (UHN). Data from the HVA, a questionnaire addressing the practice of HHD and patient well-being, surveyed at the start of HHD were collected to evaluate deficiencies noted as potential safety risk factors (SRF). All incident HHD patients starting dialysis at home between July 18, 2008 and June 30, 2013 and with baseline HVA available were included in the cohort and were followed until death, kidney transplantation, transfer to another center or study end, December 31, 2013. All medical and nursing HHD professionals involved in UHN's HHD program scored the SRF (0-5, 0 being unimportant) to grade degree of importance. Data are presented as mean±SD or median with IQR. Technique survival was assessed by Kaplan Meier analysis.

Results: HVA was surveyed in 56 patients. 35 items assessed by the HVA were considered a potential SRF. Overall scores for the degree of importance of SRF ranged from 13–31 with a median of 20 (IQR 18-26). We defined a SRF being an important SRF

when its overall score was ≥median. Overall, 18 SRF were at least once deficient. 7/20 important graded SRF were at least once deficient. Proportional to the total of questioned important SRF, 1.9% were answered deficiently and 18.2% unanswered. 11/15 unimportant graded SRF were at least once scored deficient with an overall deficiency rate of 4.0% and unanswered rate of 15.4%. 33/56 subjects presented ≥1 deficiencies in SRF with 17 patients presenting ≥1 deficiencies in the important graded SRF and 23 patients having ≥1 deficiencies in the unimportant graded SRF. Compared to the absence of SRF deficiencies, the identification of ≥1 SRF deficiencies, in the important or in the unimportant SRF group, did not affect technique survival (p= 0.98, 0.45 respectively).

Conclusions: The lack of association between safety concerns and technique survival warrants further refinement of risk assessment coupled with judicious resource use in HHD program.

TH-PO977

The Development of a Hemodialysis Safety Checklist Using a Structured Panel Process Samuel A. Silver,[1,3] Alison Thomas,[1] Andrea Rathe,[1] Pamela L. Robinson,[1] Ron Wald,[1,3] Chaim Bell,[2,3] Ziv Harel.[1,3] *[1]Nephrology, St. Michael's Hosp; [2]Internal Med, Mt. Sinai Hosp; [3]Univ of Toronto.*

Background: Research has demonstrated that 2% of deaths are a direct result of dialysis complications. The World Health Organization created a Surgical Safety Checklist to help reduce preventable adverse events in the operating room. A similar tool, to be completed by nurses and patients during a hemodialysis session, may also improve patient safety. Our objective was to develop a Hemodialysis Safety Checklist (Hemo Pause) to improve provider/patient communication and consistency of care, thereby reducing preventable adverse events.

Methods: A modified Delphi consensus technique was used to develop the Hemo Pause checklist. This involved an iterative process consisting of a literature review to identify safety measures (using PubMed and major society guidelines), individual rating of each measure by the panel, an in-person meeting wherein the panel refined the measures, and a final anonymous survey that assessed consensus among panelists. A 75% agreement threshold was required for consensus. The final version of the checklist was created using human factors engineering concepts, which address safety problems that arise due to the interaction between people, technology, and work environments.

Results: The literature review produced 31 patient safety measures. Examples included patient identity confirmation, medication administration, and patient falls. Individual review by panelists reduced the list to 25/31 measures, followed by a reduction to 19/25 measures at the in-person meeting. The final round of scoring yielded the following top 5 measures: 1)patient identification, 2)pre-dialysis weight, 3)physician orders transcribed, 4)dialysis solution administered as prescribed, and 5)pre-dialysis blood pressure. Revision using human factors principles yielded a final checklist of 17-items.

Conclusions: A novel 17-item Hemodialysis Safety Checklist (Hemo Pause) has been developed to standardize the hemodialysis procedure and improve provider/patient communication. Quality improvement efforts are underway to test the feasibility of using this checklist to reduce adverse events and strengthen the safety culture in the hemodialysis unit.

TH-PO978

Decreasing After Hours Hemodialysis in Hospitals: A Way to Improve Patient Safety and Reduce Hospital Costs Natasha Sharda, Omid Bakhtar, Sangeetha Murugapandian, Bijin Thajudeen. *Nephrology, Univ of Arizona, Tucson, AZ.*

Background: Inpatient Hemodialysis (HD) is ideally performed under a controlled setting with a team of medical professionals available. Staff is paid overtime when HD is initiated after business hours, resulting in extra costs to the hospital. In addition, safety issues may arise as ancillary staff is not readily available. This quality improvement project sets out to analyze and reduce the number of after-hours HD cases.

Methods: A Chart review for all after-hours HD cases between July 2011 and June 2012 was completed. Initial variables collected included: reason for HD, time of order / start time, reasons for delay if present and adverse events. After-hours HD was defined as HD started between 8PM and 6AM Monday to Saturdays and anytime on Sundays. A standard protocol was subsequently developed for initiating after-hours HD. This included anticipating dialysis treatments early and placing orders before 8AM, bedside presence of ordering physician, enforcing laboratory parameters/ clinical criteria, and postponement of HD to the next day in non-emergent cases.

Results: A total of 3360 cases were reviewed. Of these 2.26% (75) met criteria for after-hours HD despite 79% of orders being placed during working hours. Indications for HD in patients whom met criteria were as follows: 13.1% for acidosis, 13.1% for fluid status and 23.6% for hyperkalemia. Identifiable treatment delays are as shown.

Reason for delay in HD	N=38
Dialysis catheter placement	3
Major surgery	3
Chest tube placement	1
Transfer to ICU	4
Imaging	2
Other procedures	2
Unknown	23

Overall 9 adverse events were reported for patients within this group. Once the standard protocol was implemented a dramatic decrease in after-hours HD was noted. Within 3 month only 11/798(1.3%) cases occurred after-hours with no reported delays and no adverse events.

Conclusions: This quality improvement project demonstrates an effective way to reduce hospital costs, by promoting HD during business hours which can also help ensure patient safety. In addition, coordination between health care providers is imperative to prevent treatment delays.

TH-PO979

Impact of Hurricane Sandy on Hemodialysis (HD) Patients and Preparedness for Natural Disasters Naoka Murakami, Hira B. Siktel, James F. Winchester, Nikolas B. Harbord. *Medicine/ Nephrology, Mount Sinai Beth Israel, New York, NY.*

Background: Dialysis patients live in a complex sociomedical situation and are highly dependent on technologies to sustain their lives; such as transportation, electricity and water for the dialysis apparatus. Interruption of this infrastructure by a natural disaster can result in devastating outcomes. We measured the impact of Hurricane Sandy on HD patients to explore possible interventions to improve natural disaster-related outcomes.

Methods: The study included adults receiving outpatient maintenance HD during the landfall of Hurricane Sandy in NYC (October 2012) at 5 regional centers in Manhattan where electricity had been deprived. Demographic data and dialysis-specific preparation for natural disasters (from National Kidney Foundation recommendations) were assessed between November 2013 and April 2014.

Results: 357 patients completed the study; average age 60±14.8 years, 60% male, 30% African American, 29% Hispanic/Latino. 94 (19.9%) missed dialysis (2.12±1.54 sessions (mean±s.d.)) and 236 (66.1%) received dialysis away from their regular unit. 61 (17.1%) patients reported improvement in their dialysis-specific preparedness from before the storm. The improvement was significantly higher in the centers which distributed "emergency information package" after the storm (p<0.001, Fisher's exact test). The Poisson regression assessed the impact of variables on the frequency of missed HD sessions. Higher dialysis-specific preparedness scores were predictive of a significant reduction in the incidence rate of missed HD sessions (IRR=0.914, 95% CI 0.87-0.98, p=0.001). (Table 1).

Conclusions: HD patients are underprepared for natural disasters. The intervention by facilities to provide emergency instructions effectively improved disaster preparedness among dialysis patients, which may reduce disaster-related missed dialysis sessions in the future.

Table 1: Poisson regression analysis on the number of missed dialysis sessions

	IRR	95% CI	*p* value
Dialysis-specific preparedness	0.91	0.87-0.98	0.001
Racial Ethnicity (compared with African American)			
Caucasian	0.67	0.40-1.1	0.129
Hispanic/Latino	0.81	0.58-1.1	0.199
Other	0.34	0.20-0.57	0.000
HD in other units (compared with none received)			
ER	1.1	0.73-1.8	0.569
Other affiliated units	0.69	0.51-0.94	0.019
Evacuation			
Yes	1.6	1.1-2.3	0.018
Living situation affected			
Yes	2.3	1.6-3.2	0.000
Age	0.98	0.97-0.99	0.000

IRR, incidence rate ratio; CI, Confidence interval

TH-PO980

Disaster Preparedness in Dialysis Patients via Multidisciplinary Approach Anuradha Wadhwa, Stephanie Pesenko, Vinod K. Bansal, Karen A. Griffin. *Loyola Univ.*

Background: Emergencies and disasters can occur with or without notice, placing dialysis patients in life threatening situations: they rely on dialysis treatment to survive. Patient education on an individual level is the cornerstone of a successful disaster plan. Despite the availability of informational brochures, preparedness of dialysis patients is suboptimal. In this quality improvement study, we assessed disaster preparedness in our hemodialysis patients and evaluated multidisciplinary approach to disseminate this information.

Methods: The study consisted of a similar initial and follow up survey (yes/no answers). Patients were approached during dialysis to participate. On completion of the initial survey, patients were provided disaster preparedness related education by a multidisciplinary team of physicians, nurses, dieticians and social workers. Each member reviewed preparedness-relevant topics with the patients. Disaster preparedness was defined as subjective preparedness (survey question) and a positive response to at least three key questions-having a plan they have had discussed with a family member or dialysis unit, knowledge of backup dialysis facility and familiarity with emergency diet plan. A follow up survey was provided to the patients at the end of the month.

Results: Of the 132 eligible subjects, 124 completed all the surveys. Average age of the participants was 62 years, 52% were males and 58% were African Americans. While 60% of the patients thought they were prepared for an emergency (subjective), based on our defining criteria:80% of patients were *not* prepared for an emergency. About 50% of them did not have a plan or know about a backup facility. 35% were unaware of an emergency diet plan. 95% of the patients were interested in learning about emergency preparedness and 99% found the information provided during the project useful. Using similar criteria for preparedness, follow up survey showed 80% of the patients were better prepared.

Conclusions: Emergency preparedness in dialysis patients was lacking, but they were willing to learn. This study highlights that a multidisciplinary approach in an outpatient dialysis unit setting is feasible and effective in educating patients about disaster preparedness.

TH-PO981

Incidence of Angioedema with Lisinopril and Losartan in the Veteran Population Joseph A. Abdelmalek,[1,2] Jill Susan Simonian,[1] Margaret Mendes,[1] Rashid Kazerooni,[1] Nermeen Madkour,[1] Dena E. Rifkin.[1,2] *[1]Veterans Administration, San Diego, CA; [2]UCSD, San Diego, CA.*

Background: Angioedema is a well-recognized, potentially fatal adverse effect of angiotensin converting enzyme inhibitor (ACE-I) and angiotensin receptor blocker (ARB) use. We aim to characterize and compare rates of this adverse event in the veteran population for lisinopril and losartan, the first-line ACE-I and ARB, respectively.

Methods: The number of unique patients for lisinopril and losartan during fiscal year 2013 nationally was obtained using ProClarity, a VA outpatient prescription data repository. Combination products with hydrochlorothiazide for each respective product were also included in the analysis. The number of documented cases of angioedema for this analysis was retrieved from VAADERS (VA Adverse Drug Event Reporting System).

Results: We identified 1,626,863 unique patients prescribed lisinopril during the study period, with 1,592 cases of documented angioedema (incidence of 0.98 events per 1000 patients). There were 308,797 unique patients prescribed losartan, and 60 documented cases of angioedema (incidence of 0.19 events per 1000 patients). The incidence ratio of angioedema for lisinopril compared to losartan was 5.16. If, as described in the literature, there is minimal cross-reactivity between ACEI and ARB angioedema, prescribing losartan primarily instead of lisinopril would have resulted in approximately 1,267 fewer cases of angioedema in 2013. This translates into an estimated cost savings of approximately 2.3 million dollars per year nationally, based on 2013 VA expenses (assuming an ER visit and 24 hour inpatient observation per case).

Conclusions: Angioedema is associated with significant morbidity, and more commonly encountered with ACE-I use than ARBs. With the increasing number of indications for ARB use and the suggestion of a better safety profile, it may be beneficial to consider ARBs as first line therapy over ACE-Is in disease states where both drugs are considered equally efficacious.

TH-PO982

Objective Quantification of Fall Risk in Hemodialysis Patients Ken Wilund, Hyunwoo Hank Park, Peter J. Fitschen, Brandon Kistler, Jacob J. Sosnoff. *Dept of Kinesiology and Community Health, Univ of Illinois, Urbana, IL.*

Background: Maintenance hemodialysis (MHD) patients are at an increased risk of falling and are likely to suffer greater consequences such as hip fracture when they do fall. Yet, there is a significant lack of research on falls in MHD patients. Improving fall-risk prediction would be an important step in preventing falls in this high risk population.

Methods: 44 MHD patients from clinics throughout Illinois were recruited. All patients provided demographic information, a blood sample, fall history over the previous 12 months, and underwent a series of assessments including a multidimensional assessment of physical function, mobility, and postural control.

Results: Fourteen participants (31.8% of the sample) reported at least one fall in the previous 12 months, with a further 11 of these 14 fallers (78.6% of fallers) reporting two or more falls during the same period. Fallers were more likely to be diabetic but had normal albumin levels. Fallers had significantly lower physical function status (reduced endurance and muscle strength), balance, and mobility function than non-fallers.

Conclusions: The observations identify potentially modifiable targets for fall reduction interventions in MHD patients.

Funding: NIDDK Support

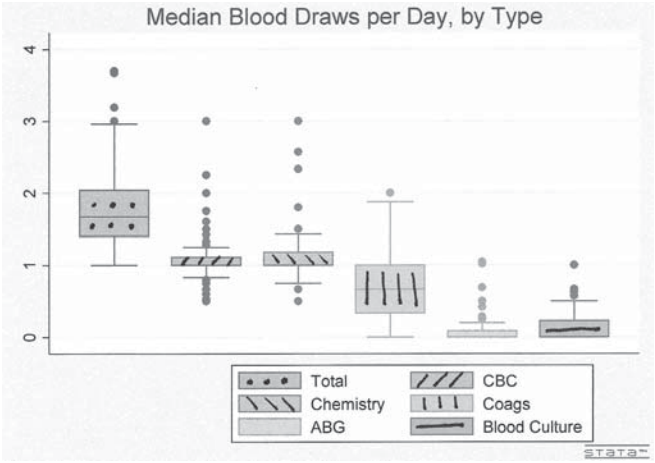
TH-PO983

Laboratory Venipuncture in a Tertiary Care Hospital Sushil Ratnaparkhe,[1] Sneha Shrivastava,[1] Rita L. McGill.[2] *[1]Internal Medicine, Allegheny General Hospital, Pittsburgh, PA; [2]Nephrology, Allegheny General Hospital, Pittsburgh, PA.*

Background: "Routine daily labs" have become a cultural expectation without an obvious evidence base. Potential harms include discomfort, iatrogenic anemia, and venous injury. In patients with CKD, venipuncture ought to be minimized. This study aims to describe laboratory testing behavior in the medicine population in a teaching hospital.

Methods: We analyzed 100 consecutive admissions on a hospital teaching service. For each subject, lab drawing behavior was recorded, including frequency, "stat" versus routine orders, and category of labs ordered. Hemoglobin was recorded at admission and discharge.

Results: All data were right-skewed by outliers with excess labwork, so data are presented as median and IQR, illustrated by box plots. Length of stay was 4(3,7) days. Patients underwent lab punctures 1.67(1.4,2.04)/day for lab testing, which was primarily driven by daily complete blood counts [1(1,1.12)/day] and chemistries [1(1,1.18)/day].



20(13,33)% of labs were ordered stat. Hemoglobin dropped by 0.7(0,1.7) grams over admissions. Few patients had CKD, precluding a subgroup analysis.

Conclusions: Medical inpatients in a teaching hospital are exposed to a high burden of daily lab testing due to an expectation of 'routine' daily blood counts and chemistries, exposing patients to significant blood loss, discomfort, and depletion of peripheral veins. This practice may explain the explosive increase in PICC use, and presents obvious hazards for patients who develop CKD. Given the prevalence of CKD in the general population, outreach toward Internal Medicine trainees and Patient Safety Committees is warranted. Evidence-based lab ordering parameters may improve vascular access prospects, and promote more cost-effective care.

TH-PO984

Usefulness of Doppler Ultrasound for Detection and Management of Complications following Renal Biopsy (RB) Maite Rivera, Nuria Rodriguez Mendiola, Victor Burguera, Viviana Raoch Michaels, Sandra Elias, Rodrigo Hernandez Loyola, Fernando Liano, Carlos Quereda. *Nephrology, Hospital Univ Ramón y Cajal.UAH Univ, Irycis, Madrid, Spain.*

Background: Asymptomatic vascular complications following renal biopsy can only be detected using doppler ultrasound, and it is of great help to decide surgery or embolization. We analyze the utility of doppler ultrasound in the detection and management of the RB complications.

Methods: 238 consecutive RB were analyzed. Data were collected prospectively from the database of our Diagnostic and Interventional Nephrology Unit. 64% were men. Mean age 55±15 years. All patients underwent a routine doppler ultrasound (color and pulsed) into the 24 h following RB. 113 (47.5%) were renal transplants (RT) and 125 (52.5%) native kidneys (NK). All RB were performed with an automatic biopsy-gun. Patients with RT and NK were similar in age, diabetes, number of samples and glomeruli obtained. Antiagregants and/or anticoagulants intake was similar in both groups (13.3% RT, 12% NK). There were more men in RT Group (71.7% versus 42.4%) and more HBP (85.8% versus 60.8%). Complications were divided into clinical (macroscopic hematuria, transfusion with or without shock and embolization) and ultrasound detected complications (hematoma, pseudoaneurysm (PSA) and arteriovenous (AVF)). All RB and ultrasounds were performed or supervised by a senior nephrologist.

Results: The rate of clinical complications was 4.2 % (2.6% in RT (2 hematuria, 1 transfusion+ shock+embolization) and of 5.6% in NK (6 hematuria, 1 transfusion without shock) and embolization was of 0.8% (2 RT). There were no nephrectomies or deaths. Ultrasound detected 38 hematomas<2 cm (4RT, 34 NK) and 7 hematomas>2 cm (7 NK). Doppler detected 30 asymptomatic AVF (19RT, 11NK) and 1 asymptomatic PSA (1RT) wich was later embolized.

Conclusions: Ultrasound-guided RB is an invasive procedure but safe. The post biopsy doppler ultrasound detects potentially serious vascular complications which clinically go unnoticed. We recommend the routine performance of Doppler- ultrasound following RB in order to know the real rate of complications and improve patient management by making it possible to individualize the postbiopsy care.

Funding: Government Support - Non-U.S.

TH-PO985

Safety and Hemoglobin Effect of the First 28-Day Dose Cycle of Sotatercept 0.7 mg/kg Compared with Lower Doses and Placebo for Correction of Anemia in Hemodialysis Subjects: Interim Analysis Mohamed A. El-Shahawy,[1] James Cotton,[2] Charles J. Kaupke,[3] Nelson P. Kopyt,[4] Suktae Choi,[5] William T. Smith.[5] *[1]Academic Medical Research Inst, Los Angeles, CA; [2]Tyler Nephrology Associates PC, Tyler, TX; [3]Nephrology Specialist Medical Group, Orange, CA; [4]Lehigh Valley Health Network, Allentown, PA; [5]Celgene Corporation, Warren, NJ.*

Background: This ongoing randomized, single-blind, placebo-controlled study of sotatercept, an ActRIIA-IgG1 fusion protein ligand trap for the correction of anemia in hemodialysis (HD) subjects, evaluated the PK, safety, tolerability, and hemoglobin (Hb) effect. We previously reported interim results of the 0.3 and 0.5 mg/kg dose groups.

Methods: Erythropoietin-stimulating agent (ESA)-responsive subjects were washed out of ESA effects until Hb was <10 g/dL and randomized to sotatercept or placebo (PBO). Reported are interim results for PK, safety, Hb, and home BP effects of PBO (n=8) versus sotatercept 0.3 mg/kg (n=9), 0.5 mg/kg (n=8), and 0.7 mg/kg (n=6) administered subcutaneously every 28 days for up to 8 dose cycles, without intrasubject dose escalation permitted. Treatment failures (Hb <9 g/dL) were rescued with ESA or transfusion. Enrollment in the 0.7 mg/kg group is ongoing.

Results: Cumulative safety shows that AEs occurred in 63%, 89%, 75%, and 100% and serious AEs in 25%, 44%, 13%, and 33% of PBO, 0.3, 0.5, and 0.7 mg/kg subjects, respectively. Events were mostly mild/moderate, unrelated to study drug, relatively similar between groups, and generally consistent with subjects' medical histories. No antidrug antibodies, injection site reactions, or hypersensitivity reactions were observed. One death occurred in the PBO group. In the first 28-day dose cycle, peak mean Hb responses were 0.0, 0.5, 0.8, and 1.0 g/dL and rescue was required in 38%, 12%, 13%, and 0% of PBO, 0.3, 0.5, and 0.7 mg/kg subjects, respectively. There were no dose-dependent changes in home BP.

Conclusions: Sotatercept 0.7 mg/kg appears to be tolerated with an acceptable safety profile in HD. There are dose-dependent peak Hb responses in the first dose cycle, with improved long-term efficacy and no increase in home BP.

Funding: Pharmaceutical Company Support - Celgene Corporation; Acceleron Pharma, Inc.

TH-PO986

Quality Improvement Protocol for Administration of Epoetin Alfa in Admitted Patients with End Stage Renal Disease Elliot M. Charen, Oladayo O. Bolarinwa, Nazia A. Siddiqi, Christina L. Bradshaw, Nijal R. Sheth, Hira B. Siktel, Nikolas B. Harbord, James F. Winchester. *Div of Nephrology and Hypertension, Mount Sinai Beth Israel, New York, NY.*

Background: Anemia is common in hemodialysis (HD) patients and is treated with intravenous iron and Erythropoietin Stimulating Agents (ESA) at outpatient hemodialysis centers. Admitted End Stage Renal Disease (ESRD) patients are at risk for worsening anemia in the setting of illness and frequent blood draws.

Methods: Chronic HD patients dialyzed during hospitalization and followed by the renal consult service were included. Data on admission hemoglobin and ESA dosing were collected retrospectively for the "study phase" (January 1-31, 2014). The hospital medication record was checked to see if ESA was ordered and administered by the day of first HD or within 72 hours of admission. If not given, the reason why was determined. In the "implementation phase" of the study (March 1-31, 2014), the ESA protocol for admitted HD patients was implemented with Kidney Disease Improving Global Outcomes (KDIGO) anemia guidelines as a reference.

Results: In the implementation phase (March) as compared to the study phase (January), a significantly greater percentage of patients had ESA ordered within 48-72 hours of admission or by first HD (74% versus 21%, p<0.005); had ESA dosed within 48-72 hours of admission or by first HD (53% versus 10%, p<0.005) and were administered ESA during their hospitalization (84% versus 34%, p<0.005). The most common reason for missed ESA dosing was that the patient was not going for dialysis that day.

Conclusions: Instituting a program for ESA ordering improves ESA dosing.

TH-PO987

Pediatric Left Renal Vein Entrapment Syndrome Diagnosed by ^{99m}Tc-Albumin-Conjugate Scintigraphy Keisuke Sugimoto,[1] Tomoki Miyazawa,[1] Takuji Enya,[1] Hitomi Nishi,[1] Shinsuke Fujita,[1] Hidehiko Yanagida,[2] Mitsuru Okada,[1] Tsukasa Takemura.[1] *[1]Pediatrics, Kidai Univ Faculty of Medicine, Osakasayama, Osaka, Japan; [2]Pediatrics, Sakai Hospital Kidai Univ Faculty of Medicine, Sakai, Osaka, Japan.*

Background: Left renal vein entrapment syndrome (LRVES) sometimes manifests as gross hematuria after intense exercise, and abdominal or flank pain with exacerbation. Ultrasonography is usually performed first in imaging to demonstrate LRVES, however it may make misdiagnosis of LRVES regarding the accuracy of this examination. Procedures for diagnosis of LRVES in children have been either invasive or limited in accuracy. To establish a noninvasive diagnostic protocol for children whose ultrasonographic, MRA, and/or 3-dimensional computer tomography (3D-CT) results suggest LRVES, we compared pressures between the IVC and LRV using a simplified Bernoulli equation in interpreting Doppler ultrasonography (DUS), and also performed ^{99m}Tc -HSA-D scintigraphy.

Methods: Thirteen patients provisionally diagnosed with LRVES by ultrasonography combined with other imaging such as magnetic resonance angiography and 3D-CT were examined. We examined ^{99m}Tc-HSA-D scintigraphy in childhood LRVES, demonstrating selective left renal nuclides excretion. We also measured peak velocity using pulse DUS, calculating pressure differences between inferior vena cava and left renal vein using a simplified Bernoulli equation.

Results: Four children showing repeated gross hematuria all showed pressure differences exceeding 3.0 mmHg. Selective left renal albumin excretion was demonstrated by ^{99m}Tc-HSA-D scintigraphy. SPECT also showed accumulation in a site consistent with the left renal pelvis. Among 9 children manifesting mainly orthostatic proteinuria, selective left renal albumin excretion examined by ^{99m}Tc-HSA-D scintigraphy was demonstrated only in those with proteinuria exceeding 1 g/g•Cr after standing in a lordotic position. Pressure differences in patients with orthostatic proteinuria were unrelated to proteinuria severity.

Conclusions: The newly method combining US and SPECT added to ^{99m}Tc-HSA-D scintigraphy is noninvasive and safe in children, may suffice for diagnosis of LRVES, especially with gross hematuria.

TH-PO988

Iatrogenic Hypoglycemia following Hyperkalemia Treatment in the Emergency Department: A Common and Preventable Complication Bairbre A. McNicholas, Katrina Carli, Arthur E. Anderson, Hien Pham, Bessie A. Young. *Div of Nephrology, Univ of Washington, Seattle, WA.*

Background: Hyperkalemia is a common presentation in the emergency department (ED), with fatal complications if untreated. Insulin and dextrose are frequently used to treat hyperkalemia as part of treatment plans. Protocolized treatment plans may leave patients at risk for iatrogenic hypoglycaemia if not carefully monitored.

Methods: Retrospective study of all patients who presented with K^+>6mEq/L to an ED from June 1 to December 31 2013. Data related to presentation, laboratory values and pharmacological management were collected using chart review. Two tailed student t-test and chi-squared statistics were used to determine significance for continuous and categorical variables.

Results: Over a 6 month period 125 patients were treated for 155 presentations of K^+>6mEq/L. End stage renal disease was present in 49(39%), acute/chronic kidney disease in 62(49%). 32(26%) were diabetics. EKG changes were present in 60(48%). Laboratory values and treatment is outlined in table 1. Pharmacological treatment was used in 99(63%) of cases and 62(40%) were part of a protocolized plan. Of those receiving insulin, 21(27%) received 10 units of IV insulin. 19(23%) who received IV insulin developed hypoglycemia. Mean time to a hypoglycemic event was 2.1±1.2 hours. There was no difference in incidence of hypoglycemia when treatment was protocolized or when treated with 5 versus 10units of insulin, There was a trend for ESRD patient who were treated with 10 units of insulin to develop hypoglycemia versus those treated with 5 units of insulin (4/28 versus 5/13 p=0.1)

Laboratory Values on Admission	
Potassium	6.7±0.7mEq/l
Glucose	186±227mg/dl
Creatinine	7.5±5.8mg/dl
Pharmacological treatment	
IV Calcium	78(50%)
Sodium bicarbonate	40(25%)
Beta-agonist	26(16%)
Resin	64(41%
Insulin	76(49%)
Dextrose	73(96%)

Conclusions: Hyperkalemia is a common problem in the ED and treatment with IV insulin is commonly associated with iatrogenic hypoglycemia. Careful monitoring of blood glucose and adjustment of dextrose/insulin dosage are critical particularly in patients with reduced renal function and should be part of protocolled treatment plans.

TH-PO989

Urinary Markers and Renal Function in Hepatitis C Experienced Patients on Pegylated Interferon/Ribavirin and Boceprevir Triple Therapy in a Third-Level Hospital in Mexico City Juan Carlos Garcia Ya?ez, Karen Andrade. *Nephrology, Inst Mexicano del Seguro Social Hospital de Especialidades La Raza, Distrito Federal, México Distrito Federal, Mexico.*

Background: Recently there is some evidence that the use of boceprevir is associated with decline in eGFR and the mechanism by which it could cause it has not been demonstrated. We aimed to identify alterations in serological and urinary markers of renal function during triple therapy treatment.

Methods: We conducted an observational, longitudinal, prospective study, in hepatitis C experienced patients, partial or null responders which started triple therapy with PEG-IFN/RBV and Boceprevir. Serum and urinary markers were taken at baseline and at weeks 4, 8, 12 and up to week 24 during treatment with triple therapy, eGFR was estimated using the MDRD-4 creatinine equation. Fractional excretion rates (FER) of calcium, phosphate and uric acid as well as urinary creatinine and protein in urine were tested.

Results: We included 21 patients (17 with urinary markers); 52% were men, median age was 51 years old (IQR 44-58), median weight was 67 kg (IQR 60-74). Median baseline eGFR was 87.4 ml/min (IQR 78.2-97.8) and at week 12, 87.7 ml/min (IQR 79-98) (p=0.825). Median baseline FER of calcium, phosphate, and uric acid were; 1% (IQR 0.7-1.5), 9.7% (IQR 8.9-11.9), and 5.5% (IQR 4.5-7.5) respectively. At week 12; 0.75% (IQR 0.3-1.39), 9.7% (IQR 7.1-11.7), and 6.1% (IQR 4.7-11), with p=0.064, p=0.245 and p=0.460 respectively when Wilcoxon rank test whas performed. At week 24; 0.85% (IQR 0.59-1.7), 9.8% (IQR 8-10.6%), and 6.5% (IQR 4.3-8.5), with p=0.062, p=0.534, and p=0.285.

Conclusions: no significant alterations of urinary markers could be identified. However, patients on triple therapy require complete and periodical renal function assessment.

TH-PO990

Low Dose Tinzaparin as Venous-Thromboembolism Prophylaxis in Advanced Kidney Disease Suzanne H. Forbes,[1] Sean Platton,[2] Peter Maccallum,[2] Neil Ashman.[1] *[1]Dept of Nephrology & Transplantation, Royal London Hospital, London, United Kingdom; [2]Dept of Haematology, Royal London Hospital, London, United Kingdom.*

Background: Many hospital policies advocate low molecular weight heparins (LMWH) for venous thromboembolism (VTE) prophylaxis. The dose, predictability and safety of LMWHs in advanced chronic kidney disease (CKD), a population at increased bleeding

risk, is uncertain. Most trials exclude these patients. We conducted a prospective study to ascertain prescribing practice in our unit and to assess safety, as guided by evidence of drug accumulation and clinical events.

Methods: We performed a spot audit of all renal inpatients on a given day to identify those prescribed LMWH (tinzaparin) VTE prophylaxis and prospectively followed them for up to 5 days, monitoring regular trough anti-Xa levels and one peak. Haemodialysis (HD) patients also receiving tinzaparin for HD anticoagulation had peak dialysis anti-Xa levels measured. We noted any bleeding or thrombotic events. We repeated this cycle 5 times.

Results: Overall we assessed 214 patients. Of these 64 received tinzaparin.

Modality (eGFR ml/min/1.72m^2)	n
CKD (<15)	7
CKD (>15)	5
Transplant (<15)	7
Transplant (>15)	4
HD	29
PD	2

We excluded acute kidney injuries and those with eGFRs >40ml/min/1.73m^2 leaving 54 patients followed prospectively for an average 4 days. Mean age was 64 and median eGFR 9ml/min/1.73m^2. Median dose of tinzaparin was 47.8 IU/kg. We measured an average of 4 troughs per patient; 16 were >0.05units(u)/mL, the highest 0.17u/mL (<0.2u/mL is expected with prophylaxis). The highest peak was 0.34u/mL (bioaccumulation is >0.4u/mL). Anti-Xa levels with iv tinzaparin on HD are well described, the peak at 30 minutes expected to be <1u/mL. In the HD patients also receiving prophylaxis all were <0.6u/mL. There was no correlation between anti-Xa level and weight, dose/kg or non-dialysis eGFR. In the LMWH group there was 1 fatal intracerebral bleed (TP eGFR 17ml/min/1.73m^2). In those not given prophylaxis there were 13 bleeding events. No patient had a thrombotic event.

Conclusions: This study shows low dose tinzaparin in patients with advanced CKD is not associated with accumulation of anti-Xa activity and has no impact on HD anti-Xa levels.

TH-PO991

Management of Cancer Patients with Simultaneous Chronic Kidney Disease or at Risk of Developing It. Three Years' Experience of an Ambulatory of Onco-Nephrology Laura Cosmai,[1] Wanda Liguigli,[2] Marina Foramitti,[1] Maurizio Gallieni,[3] Camillo Porta,[4] Fabio Malberti.[1] *[1]Nephrology, Istituti Ospitalieri, Cremona, Italy; [2]Medical Oncology, Istituti Ospitalieri, Cremona, Italy; [3]Nephrology, San Carlo Borromeo Hospital, Milan, Italy; [4]Medical Oncology, IRCCS San Matteo Univ Hospital Foundation, Pavia, Italy.*

Background: Onco-Nephrology is a novel subspeciality dealing with: renal toxicity from chemotherapy (CT), targeted agents (TA) or contrast medium (CM), active cancer treatment (aTx)-related electrolyte disturbances, alterations of the Ca-P metabolism and hypertension, management of pts nephrectomized for a malignancy. Here we report 3 years' experience of an ambulatory of Onco-Nephrology.

Methods: This ambulatory, run by a Nephrologist, takes place once a week within an Oncology outpatient ward, in order to allow closer interaction between specialists and easier access to pts' data. Until now, we have followed 349 cancer pts with CKD on aTx, and 92 untreated cancer pts with CKD; 127 pts were nephrectomized for a localized or metastatic renal cell carcinoma (RCC); beyond RCC, patients had also lung (48 cases), gastric (50), prostate (34), bladder (38), or other cancers (52).

Results: Among 47 pts nephrectomized for metastatic RCC under aTx, we had only 4 aTx interruptions, while among the 80 previously nephrectomized pts for a localized RCC (not on aTx), at a median follow-up of 12 months, we did not observe any CKD progression (versus an expected percentage of 63% at 3 years). Only one patient (out of 15) treated with cisplatin had to discontinue CT because of renal toxicity. Only 10 pts have developed an episode of acute kidney injury (AKI), but all were able to resume aTx; 6 pts with advanced CKD began dialysis while on aTx. Furthermore, no cases of AKI from CM were observed, thanks to the implementation of specific protocols. Finally, previously unreported renal toxicities were observed.

Conclusions: Our experience shows that an early Onco-Nephrological assessment may improve pts' outcome. Further development of Onco-Nephrology (e.g. dedicated ambulatories and specific trials) is warranted.

TH-PO992

PJP - *Pneumocystis* (*carinii*) *jiroveci*: A Reemerging Threat to the Immunosuppressed Patient John Niles, Andrew P. Murphy, Katherine M. Cosgrove, Karen A. Laliberte. *Renal, Massachusetts General Hospital, Boston, MA.*

Background: PJP is one of the most common opportunistic infections. Newer immunosuppressive therapies in autoimmune disease poses a potential threat of more or different opportunistic infections. PJP chemoprophylaxis with trimethoprim/sulfamethoxazole (T/S), atovaquone or dapsone is widely recommended but there is no consensus about the exact indications and durations for these therapies.

Methods: Two pts from outside clinics, with ANCA vasculitis, were seen. At the time of referral, neither had been given PJP prophylaxis and both subsequently developed PJP. Pt #1: 16yo newly diagnosed ANCA with RPGN presented after 2.5 months of tx with Rituxan (RTX), plasma exchange, cyclophosphamide, high dose prednisone plus 13 pulses. Prednisone was at 40mg/d at the visit. Prednisone tapered to 30mg /day was initiated with T/S prophylaxis. The next day she developed fever, hypoxia and LDH was 656. Pt #2: 79yo male with relapsing ANCA disease and CKD 4 treated with RTX x 4 doses and prednisone 40mg/day for 3 months because of nonspecific pulmonary findings. He was admitted from clinic with hypoxia and LDH of 886. Hospital: Both required high flow oxygen. Pt #1 was treated with T/S and continued steroids. Pt # 2 was given Atovaquone and steroids. Early on, both had pulmonary deterioration with attempts to wean steroids.

Results: Both pts were treated with immunosuppressive therapy without prophylaxis and had extended steroid courses for severe and persistent mainfestations of vasculitis. Examination of the medical records of pt #1 revealed an elevated LDH at week 7 of treatment, 3 weeks before admission. Pt #2 had a very high LDH at the time of consultation. With steroid taper both developed severe hypoxia and required hospitalization. During hospitalization, both had worsening pulmonary status during attempts to wean their steroids, despite institution of full anti-PJP antibiotics.

Conclusions: There has been a shift in treatment to biologic agents, with rapid steroid tapers. However, steroids are often used to help with persistent or severe disease. These two cases demonstrate the ongoing need for safe prophylaxis in patients requiring immunosuppressive therapy, including biologic agents.

TH-PO993

An Exploration of 30-Day Re-Admissions in Chronic Hemodialysis Patients David J.R. Steele, Merranda S. Logan. *Div of Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: The United States Renal Data System's 2013 report revealed that among hemodialysis patients prevalent in 2011, 36 percent of hospitalized patients were re-hospitalized within 30 days of discharge. In comparison only 17.4 percent of patients aged 66 and above without chronic kidney disease required re-hospitalization within 30 days of discharge. The aim of this quality improvement project was to determine common characteristics amongst chronic hemodialysis patients re-hospitalized within 30 days of hospital discharge and to identify system factors that may reduce preventable re-admissions.

Methods: All attending nephrologists participated in directed chart reviews of chronic hemodialysis patients re-admitted to our hospital within 30 days of hospital discharge over a 6 month period. Patients re-admitted to other hospitals were not included. Baseline data on factors related to re-hospitalization were collected. Reviewers were asked to rate the preventability of each re-hospitalization.

Results: Forty-eight re-hospitalizations were reviewed. Common themes associated with re-hospitalization included polypharmacy, greater than five medication changes during the index admission, ESRD diagnosis for less than 90 days and anemia. Common themes associated with preventable re-admissions included patient education, communication between inpatient and outpatient providers, medication side-effects and medication reconciliation.

Conclusions: Systematic review of re-hospitalized patients enabled us to identify potentially actionable opportunities for improvement in care delivery. As a result of this study, quality improvement projects directed at standardizing discharge communications, anemia management and discharge medication reconciliation were recommended.

TH-PO994

Evaluation of Antibiotic Dosing in Patients Receiving Sustained Low-Efficiency Dialysis Leigh A. Keough,[1] Joanna Hudson,[1,2] Amy G. Krauss,[1] Beth Segars.[1] *[1]Methodist Univ Hospital, Memphis, TN; [2]The Univ of Tennessee College of Pharmacy, Memphis, TN.*

Background: Sustained low efficiency dialysis (SLED) is a form of dialysis that combines aspects of conventional intermittent hemodialysis (IHD) with features of traditional continuous renal replacement therapy (CRRT). As a "hybrid" dialysis modality, SLED combines the use of conventional IHD machines with smaller filters and reduced blood and dialysate flow rates that typically are "in between" that of IHD and other types of CRRT. Antibiotic dosing recommendations for IHD and CRRT are not appropriate for SLED. Antibiotic dosing during SLED is variable and there is substantial concern for underdosing. In this study, we characterized the adequacy of dosing of select antibiotics during SLED treatment.

Methods: A retrospective chart review was performed on patients admitted to the Methodist Le Bonheur System from October 2010 through August 2013 who received SLED and at least one of the selected antibiotics: cefepime daptomycin, piperacillin/tazobactam, meropenem, and vancomycin. For all patients identified, the administered antibiotic dosing regimen was defined as "adequate" or "inadequate" based on recommendations available in the literature. Dosing regimens were evaluated each day the patient was receiving one of the selected antibiotics concurrently with SLED. Reasons for inadequate dosing were also evaluated.

Results: A total of 59 patients met inclusion criteria: 59% male, 53% African-American, mean age 57 + 13 yrs. SLED was required for 69% of patients with AKI and 31% with ESRD. SLED parameters were: average blood flow rate 220 + 24 ml/min, dialysate flow rate 265 + 39 ml/min, and duration 9.3 +1.7 hours. The median number of days on SLED of 7 + 5. The total percent of adequate antibiotic days was vancomycin 85%, cefepime 64%, daptomycin 57%, meropenem 37%, and piperacillin/tazobactam 22%. Underdosing accounted for 63% of inadequate antibiotic days.

Conclusions: A high percentage of the antibiotics being administered were inadequate based on current literature recommendations. There is a need to educate prescribers on appropriate dosing practices in this patient population as more data become available.

TH-PO995

Exposure to Ionizing Radiations in Patients on Hemodialysis

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Background: Patients on hemodialysis often carry a large burden of co-morbidities and are frequently exposed to ionizing radiations (IR) through various radiological examinations. We aimed to estimate exposure to IR in a cohort of hemodialysis patients.

Methods: Records of previous radiological examinations were obtained for patients receiving maintenance in-centre hemodialysis at a teaching hospital and affiliated centres in United Kingdom (covering five dialysis units). Exposure to IR per patient was assessed by determining cumulative effective dose (CED) for all radiological examinations.

Results: 126 patients, who consented for their records to be accessed, were included; median age: 69 (range: 22-87) years; males 82 (62%). The median duration on hemodialysis was 3.8 (range: 0.2 - 31.8) years which collectively represented 617 person-years of dialysis treatment. In this period, exposure to IR was recorded at 2,749.5 millisieverts (mSv) for the whole cohort; mean CED therefore was estimated to be 4.5 mSv per person per year. Investigation types and their relative contribution to total IR exposure are presented in figure 1. Cardiac investigations accounted for the largest contribution (35.6%) to this exposure followed by vascular (28.7%), gastrointestinal (15.8%), respiratory (7.2%), musculoskeletal (2.3%), urinary system (1.7%) and other (8.7%) examinations.

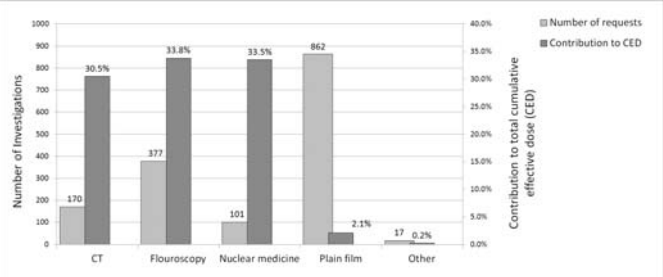

Fig 1: Numbers and types of investigations, and their relative contribution to total cumulative effective dose (CED) in 126 patients on hemodialysis. CT: computed tomography.

Conclusions: Exposure to IR due to radiological examinations among patients on hemodialysis is higher than that reported in general population. Certain radiological and nuclear medicine scans deliver a particularly high dose of radiation; their targeted use must be encouraged in order to reduce this burden.

TH-PO996

Maternal Diabetes Modulates Kidney Formation in Progeny: The Functional Interaction Between Catalase and Hedgehog Interacting Protein (Hhip)

Xin-Ping Zhao,[1] Shiao-Ying Chang,[1] Min-Chun Liao,[1] Isabelle Chenier,[1] Julie R. Ingelfinger,[2] John S.D. Chan,[1] Shao-Ling Zhang.[1] *[1]Research Center, CRCHUM, Montreal, QC, Canada; [2]Pediatric Nephrology, Mass. Gen. Hosp, Boston, MA.*

Background: Previously, we reported that impaired nephrogenesis induced by maternal diabetes is mediated, at least in part, via augmented Hhip gene expression. Overexpression of catalase (CAT) in renal proximal tubular cells (RPTCs) could prevent maternal diabetes-impaired kidney formation. The present study aims to investigate the functional interaction between Hhip and CAT gene expression in maternal diabetes modulated kidney formation in progeny and examine the potential underlying mechanisms *in vivo* and *in vitro.*

Methods: Young male offspring (newborn to 3 weeks of age) of non-diabetic and diabetic dams of Hoxb7-green fluorescence protein (GFP)-transgenic (Tg) and Hoxb7/Catalase (CAT)-Tg mice were studied. Renal morphology, immunohistochemistry, reactive oxygen species (ROS) generation and gene expression were assessed by standard methods. Mouse *endothelial cells (SVEC4-10)* and rat immortalized RPTCs (IRPTCs) were used as *in vitro* studies.

Results: As compared to the offspring from non-diabetic Hoxb7-GFP-Tg dams, renal dysmorphogenesis, nascent nephron apoptosis, increased ROS and heightened Hhip and TGFβ1 gene expression were observed within the kidneys of offspring of Hoxb7-GFP-Tg dams with severe maternal diabetes. These changes were ameliorated in male offspring of diabetic Hoxb7/Cat-GFP-Tg dams. In studies *in vitro*, high glucose stimulated Hhip gene expression, and subsequently targeted TGFβ1 signaling. CAT promoted Pax2, Shh andp27^{Kip1} expression to counter the stimulatory effects of high glucose on Hhip gene expression.

Conclusions: Our data suggest that the counterbalance of CAT and Hhip gene expression plays a key role in the impaired nephrogenesis that is induced by maternal diabetes, both *in vivo* and *in vitro.*

Funding: Government Support - Non-U.S.

TH-PO997

DNA Methyltransferase 1 Is Required for Rat Metanephric Development and Its Abundance Is Reduced by Maternal Nutrient Restriction

Midori Awazu, Mariko Hida. *Dept of Pediatrics, School of Medicine, Keio Univ, Tokyo, Japan.*

Background: We previously reported that ureteric bud branching and metanephric growth were inhibited by maternal nutrient restriction in the rat. Maternal nutrient restriction also reduced global DNA methylation of the embryonic kidney and the methylation of genes involved in the negative regulation of branching morphogenesis. Decreased DNA methylation may be due to the downregulation of DNA methyltransferases (DNMTs). DNMT1 is essential for the maintenance of DNA methylation, and DNMT 3A and 3B are required for *de novo* methylation. In the present study, the expression of DNMTs during normal kidney development was investigated and compared with that in kidneys of offspring from nutrient deprived mothers. The role of DNMT1 in ureteric bud branching and metanephric growth was further investigated in an organ culture system.

Methods: The kidneys of embryonic day 15 (E15) to 18 (E18) fetuses and neonatal day 1 (N1) to 15 (N15) rats from dams given food ad libitum (CON) and those subjected to 50% food restriction throughout pregnancy (NR) were examined. The expression of DNMTs was assessed by immunoblot analysis. Embryonic day 13 metanephroi from normal rats were subjected to organ culture in the presence or absence of a DNMT1 inhibitor 5-aza-2 '-deoxycytidine (Aza) 20 μM for 3 days. Ureteric buds were visualized by whole-mount staining with pancytokeratin.

Results: DNMT1 and DNMT3B were expressed up to N1 and N15, respectively, with expression becoming downregulated as development proceeds. On the other hand, DNMT3A was expressed only at E15. The expression of DNMT1 was dramatically and that of DNMT3A was slightly reduced in the NR kidney at E15. These differences were no more observed at E18. The ureteric tip number was significantly reduced by Aza (3.0±0.6 versus 8.2±1.4, n=6, P<0.005). The kidney surface area of Aza-treated metanephroi was also significantly reduced (1.39±0.10 versus 3.51±1.01, n=6, P<0.005).

Conclusions: DNMTs are strongly expressed in the E15 kidney, and DNMT1 is essential for the metanephric development. Reduced abundance of DNMT1 may play a role in the inhibited ureteric branching and metanephric growth by maternal nutrient restriction.

Funding: Government Support - Non-U.S.

TH-PO998

Novel Roles for Uroplakin 1b in Terminal Differentiation, Urothelial Homeostasis, and Structural Integrity in the Urinary Tract

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Background: Urothelium is uniquely specialized to maintain a barrier between tissue and urine. To do this, terminally differentiated cells elaborate a rigid apical plaque (urothelial plaque), composed of four major uroplakin (Upk) proteins. We hypothesize that disruption of a tetraspanin Upk, Upk1b, can lead to plaque destabilization and trigger widespread defects within the entire urinary tract.

Methods: Bladders and kidneys from *Upk1b*$^{+/+}$ (wild type) and *Upk1b*$^{RFP/RFP}$ (Upk1b disrupted) mice were analyzed by immunohistochemistry, brightfield and electron microscopy, western blot, and ultrasound.

Results: *Upk1b*$^{RFP/RFP}$ bladder urothelium was devoid of urothelial plaques and was consistently dysplastic compared to *Upk1b*$^{+/+}$. In addition to widespread proliferation, *Upk1b*$^{RFP/RFP}$ bladder urothelium exhibited increased expression of progenitor cell markers Krt5, Krt14, and Sonic hedgehog and loss of terminal differentiation markers Krt20 and uroplakins. In contrast to *Upk1b*$^{+/+}$, *Upk1b*$^{RFP/RFP}$ renal urothelium exhibited absent urothelial plaques, and renal ultrasound revealed progressive hydroureteronephrosis. Expression of uroplakins and additional urothelial proteins was significantly altered in the stratified *Upk1b*$^{RFP/RFP}$ renal urothelium as compared to the monolayered *Upk1b*$^{+/+}$. Interestingly, 12 of 85 (14%) *Upk1b*$^{RFP/RFP}$ and 0 of 138 *Upk1b*$^{+/+}$ and *Upk1b*$^{RFP/+}$ mice exhibited renal collecting system duplication, where parallel ureters exit both orthotopic and ectopic pelves (P < 0.0001, Fisher's exact test).

Conclusions: We implicate Upk1b and urothelial plaques in terminal differentiation, regulation of the urothelial progenitors and urinary tract development. Our data suggests that the acquisition of terminal differentiation by urothelial cells is important for the negative regulation of progenitors and the maintenance of urothelial homeostasis. Although, Upk1b is regarded as a structural protein in differentiated urothelial cells, our data indicates for the first time a role for Upk1b in metanephric development.

Funding: NIDDK Support

TH-PO999

The Prorenin and Soluble (Pro)renin Receptor May Be Associated with Prenatal Renal Development in Humans

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Background: The renin-angiotensin system (RAS) plays a key role in the development of the kidney. Although recent study demonstrated that (pro)renin receptor [(P)RR] was highly expressed in the developing kidney during embryonic development in the mouse, the mechanism by which (P)RR supports renal development in human are not fully understood. In this study, we examined plasma levels of prorenin and soluble (P)RR [s(P)RR] in cord blood and neonates, and the expression of (P)RR in kidney tissue.

Methods: Umbilical cord blood samples were collected from 56 preterm and 67 full-term neonates at birth. Blood samples were also obtained in 4 days and 4 weeks after birth. Prorenin and s(P)RR levels were measured using enzyme-linked immunosorbent assays. Additionally, we performed immunohistochemical (IHC) analysis of kidney tissues from neonates (n = 3, 33 to 37 weeks-gestation) and healthy children (n = 5, 3 to 7 years-old) in order to assess (P)RR expression in kidney.

Results: Mean plasma prorenin and s(P)RR levels in cord blood were significantly higher in preterm neonates than in full-term neonates ($P < 0.0001$ and $P = 0.0295$, respectively). Mean plasma prorenin levels in 4 days after birth were higher in preterm neonates than in full-term neonates ($P = 0.0001$), but s(P)RR levels were not ($P = 0.3809$). These differences were no longer evident for either plasma prorenin or s(P)RR levels between the two groups, 4 weeks after birth ($P = 0.2742$ and 0.5754, respectively). Importantly, the plasma prorenin levels in cord blood and in 4 days after birth were inversely correlated with gestational age ($P<0.001$). Furthermore, IHC study showed that renal expression levels of (P)RR in neonates was stronger than that in healthy children ($P < 0.0001$).

Conclusions: These results suggest that prorenin-s(P)RR signaling pathway is associated with prenatal renal development in humans.

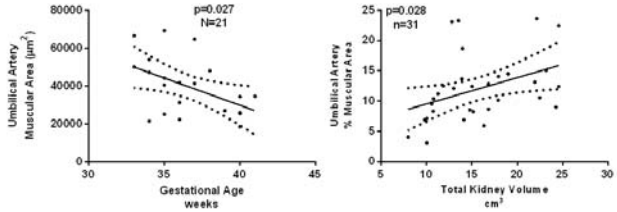
TH-PO1000

Umbilical Artery Histomorphometry Provides Insight into Angiogenesis and Nephrogenesis in Term and Preterm Infants Marissa J. Defreitas,[1] Deepan Mathur,[2] Teresa C. Cano,[1] Wacharee Seeherunvong,[1] Chryso P. Katsoufis,[1] Marta G. Galarza,[3] Salih Y. Yasin,[4] Maria Matilde Rodriguez,[2] Carolyn L. Abitbol.[1]
[1]Pediatric Nephrology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [2]Pediatric Pathology, Univ of Miami, Miami, FL; [3]Neonatology, Univ of Miami/ Holtz Children's Hospital, Miami, FL; [4]Obstetrics/ Perinatology, Univ of Miami/ Holtz Children's Hospital, Miami, FL.

Background: Preterm birth is associated with early and late renal insufficiency, a nephron deficit, hypertension and cardiovascular disease in later life. Since the umbilical arteries ultimately develop into the aorta, we hypothesized that the morphometry of these vessels could provide insight into the process of angiogenesis at birth.

Methods: A cohort of 155 infants were enrolled at birth into the Infant Kidney Study with assessment of renal mass as total kidney volume (TKV) by ultrasound. The umbilical cords of 31 infants were cut and sectioned for histomorphometric analysis including 9 term controls, 12 singleton and 10 preterm infants. The transverse section analyzed the amount of muscle and collagen within inner and outer layers of each artery by trichrome stain, averaging the measurements of the 2 vessels. The ratio between muscle and collagen was calculated.

Results: The density of the outer muscular-rich area was greater in preterm compared to term infants and correlated inversely with gestational age ($p<0.03$) in singleton infants. Moreover, per cent of inner muscular area correlated positively with renal mass ($p<0.03$) in all subjects.



Conclusions: Umbilical artery histomorphometry provides insight into early vascular morphology that is in synchrony with the development of the central vascular tree (aorta) and renal mass. This non-invasive technology merits further investigation with prospective follow-up of the infants identified at risk.

Funding: Private Foundation Support

TH-PO1001

Umbilical Artery Histomorphometry Provides Insight into the Fetal Development of the Central Vascular Tree (Aorta) in Preterm Twins Marissa J. Defreitas,[1] Teresa C. Cano,[1] Wacharee Seeherunvong,[1] Salih Y. Yasin,[4] Shahnaz Duara,[3] Deepan Mathur,[2] Maria Matilde Rodriguez,[2] Carolyn L. Abitbol.[1]
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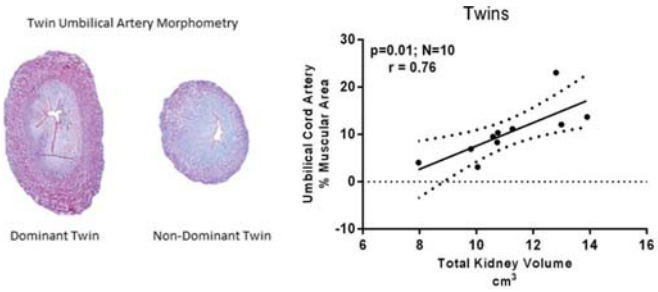
Background: Prematurity is a risk factor for hypertension, aortic stiffness, nephron deficit, and adult onset cardiovascular and renal disease. The effect of preterm birth on angiogenesis is largely unknown. Twin gestation offers a venue for studying angiogenesis in a competitive intrauterine environment. We hypothesized that the umbilical artery would provide insight into the central vascular tree (Aorta) in preterm twin infants.

Methods: A cohort of 155 newborn infants was enrolled into the Gerber Infant Kidney Study. Ultrasound was used to measure total kidney volume (TKV). Umbilical cord specimens were obtained in 9 term singleton, 12 preterm singleton, and 10 preterm twin infants. The umbilical cord was sectioned, stained with Trichrome, and then digitalized. Muscular and collagenous areas of the umbilical artery were measured in pixels using Image J 1.48q software and converted to μm^2.

Results: The dominant twins had an increased umbilical artery muscle wall area (40 ± 19 versus 24 ± 12 nm^2; $p<0.03$) and an increased TKV (15 ± 5 versus 10 ± 1 cm^3; $p<0.05$) when compared to the co-twins. There was also a significant positive correlation between umbilical artery muscular area and kidney mass ($r=0.76$; $p=0.01$).



Conclusions: Twin gestation imposes discordance in vascular muscular endowment with the dominant twin having an advantage in vascular wall muscle density and presumed elasticity as well as renal mass. Prospective longitudinal studies are needed to determine if this anatomical discordance predisposes to functional impairment in twins.

Funding: Private Foundation Support

TH-PO1002

Angiotensin Converting Enzyme Inhibitor Fetopathy: A Report of the Midwest Pediatric Nephrology Consortium (MWPNC) Shahid Nadeem,[1] Katherine Davis Westreich,[3] Ibrahim F. Shatat,[5] Marissa J. Defreitas,[2] Carolyn L. Abitbol,[2] Myra L. Chiang,[4] Donald J. Weaver,[6] Julia M. Steinke,[7] Larry A. Greenbaum.[1]
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Background: Renin angiotensin system (RAS) blockers are commonly used to treat hypertension and to reduce microalbuminuria or proteinuria. Fetopathy from in utero exposure to these agents was described more than 30 yrs ago; however, cases continue to occur. There are limited studies describing the full spectrum of this entity and no information on why fetal exposure occurs despite the presence of a black box warning.

Methods: This was a retrospective study performed through the MWPNC. Centers were asked to identify cases of ACE or ARB fetopathy. Case data included renal and extrarenal manifestations, timing of exposure, and the explanation for the fetal exposure.

Results: 13 cases were identified. RAS blocker exposure after the first trimester was associated with more severe renal manifestations. Chronic renal replacement therapy (RRT) was required in 6/7 patients (pts) with RAS blocker exposure after the first trimester and 0/6 pts with exposure restricted to the first trimester ($p=0.0047$). Pts had a variety of extrarenal manifestations, some not previously noted, including CNS anomalies (cystic encephalomalacia, cortical blindness, sensorineural hearing loss, arachnoid cysts) and pulmonary complications (pneumothorax, pneumomediastinum). RAS blocker exposure was usually secondary to absent or poor prenatal care or undiagnosed pregnancy.

Conclusions: ACE fetopathy continues to be a cause of considerable morbidity, with the need for chronic RRT more likely in affected pts exposed after the first trimester. A variety of serious extrarenal manifestations occur in these pts. Clinicians should emphasize the risk of fetopathy when prescribing RAS blockers to women of childbearing age.

TH-PO1003

Abstract Withdrawn

TH-PO1004

Serum Creatinine During Physiological Perinatal Dehydration May Estimate Individual Nephron Endowment Gianluigi Ardissino,[1] Francesca Tel,[1] Ilaria Possenti,[1] Mariangela Pavesi,[2] Bianca Castiglione,[1] Sara Testa,[1] Patrizia Salice,[3] Dario Consonni.[4]
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Background: It is well known that the nephron endowment of healthy subjects is highly variable and that individual nephron mass has potentially important implications both in health and disease. However nephron count is technically still impossible in living subjects. We hypothesised that serum creatinine levels during the physiological perinatal dehydration, might be helpful in identifying subjects with a reduced nephron mass.

Methods: The serum creatinine levels of normal Caucasian neonates were determined 48-96 hours after birth and their association with a family history of arterial hypertension (AH) was evaluated. Moreover, renal volume measured between the second and third month of life, in a subset of subjects, was correlated with the level of perinatal serum creatinine.

Results: Serum creatinine levels were determined in 182 normal newborns (90 males) at a mean of 61+8 hours after birth (range 46-82). Newborns with paternal AH had a higher mean serum creatinine level (0.7+0.3 versus 1.0+0.3; p<0.006). No differences in mean serum creatinine levels were found in relation with mother or grandparent's history of AH. Kidney volume negatively correlated (slope of -13.5 mL per unit of serum creatinine; 95% CI: -26.2 to -0.9; p=0.04) with serum creatinine levels in the subset of children who underwent kidney ultrasonography.

Conclusions: The determination of serum creatinine level during perinatal dehydration seems a promising tool for identifying normal subjects with a reduced nephron mass with potential important implications in understanding the individual outcome of renal diseases (including drug toxicity), expanding our knowledge on the pathophysiology of common renal and extrarenal diseases and providing clues for individualized preventive measures.

TH-PO1005

Enalapril Induces Dysregulation of Angiogenesis-Related Genes and Loss of Microvasculature in the Neonatal Rat Kidney Hyung Eun Yim, Kee Hwan Yoo, In Sun Bae, Young Sook Hong, Joo Won Lee. *Pediatrics, Korea Univ Guro Hospital, Seoul, Republic of Korea.*

Background: Pharmacological inhibition of the renin angiotensin system (RAS) during the neonatal period in rodents has shown vascular abnormalities that are followed by deterioration of kidney structure and function. Although angiotensin II appears to play an important role in kidney angiogenesis, mechanisms that govern the development of the kidney vasculature by the RAS are poorly understood. We aimed to investigate the effect of angiotensin II inhibition on the localization and expression of angiogenesis-related genes and microvasculature endothelial cells in the developing rat kidney.

Methods: Newborn rat pups were treated with enalapril (30 mg/kg/d) or vehicle for 7 days after birth. We investigated the intrarenal expression of angiopoietin-1, angiopoietin-2, the angiopoietin receptor Tie-2, and thrombospondin-1 with Western blotting and immunohistochemical staining at postnatal day 8. For determination of glomerular and peritubular capillary density, the endothelial cell markers of CD-31 and aminopeptidase P (JG-12) were also assessed.

Results: Light microscopic survey of kidney sections from enalapril-treated rats revealed fewer and immature glomeruli and disrupted tubules. In the enalapril-treated group, angiopoietin-2, Tie-2 and thrombospondin-1 protein expression were significantly increased, whereas angiopoietin-1 expression was decreased, compared with the control group ($P < 0.05$). Immunohistochemical staining of kidney sections for the endothelial cell markers of CD-31 and JG-12 showed a dense capillary network in cortex and medulla from control rats; however, enalapril-treated kidney showed a reduced endothelial immunostaining within both the glomeruli and the tubulointerstitium.

Conclusions: Our findings suggest that inhibition of the RAS during kidney development induces imbalanced angiopoietin signaling, upregulation of angiogenesis inhibitor, and loss of microvasculaturein the developing rat kidney. Angiotensin II-stimulated angiogenesis can play an important role in postnatal microvascular development and maturation in the developing rat kidney.

TH-PO1006

Next-Generation-Sequencing-based Molecular Diagnostics for Congenital and Inherited Kidney Disease Bert van der Zwaag, Albertien M. van Eerde, Martin Elferink, Patrick Van Zon, Marijn F. Stokman, Kirsten Y. Renkema, Nayia Nicolaou, J.k. Ploos van Amstel, Nine V. Knoers. *Medical Genetics, Univ Medical Center Utrecht, Utrecht, Netherlands.*

Background: Congenital and inherited kidney diseases constitute the leading cause of chronic kidney disease in children. Molecular diagnostic analysis of heterogeneous renal disorders has long been hampered by the size and numbers of genes involved, but has become feasible with the advent of Next-Generation-Sequencing (NGS).

Methods: We set up and implemented an NGS-based test in our ISO15189 certified laboratory to enrich and sequence 376 genes known to be causal in or associated with kidney and urinary tract disorders. To reach a genotyping accuracy of at least 99%, a minimal vertical coverage of 15 individual reads per base is required. To deliver a comprehensive analysis of the genes collected in a genepanel, a horizontal coverage of at least 98% of targeted bases is requested. When the coverage by NGS drops below requested coverage thresholds, additional "Sanger"-based sequencing is performed to fill in the gaps. In this fashion, a mutation detection rate of >95% is achieved for the genes analysed.

Results: Based on the enrichment design 23 disease related genepanels were formed, including renal cysts (50 genes), Bardet-Biedl syndrome (14 genes), Joubert syndrome (21 genes), nephronophthisis (15 genes), congenital anomalies of the kidney and urinary tract (40 genes), nephrotic syndrome (16 genes) and focal segmental glomerulosclerosis (8 genes).

Conclusions: To conclude, comprehensive genetic testing by NGS will boost the diagnostic yield from 3-5% per gene tested to potentially >40-50% molecular diagnoses within one clinical genepanel analyzed. Thereby delivering a swift answer to the patient and a leap in efficacy and cost reduction for healthcare providers.

TH-PO1007

ARegPKD - A Web-Based European Registry Study on ARPKD Kathrin Ebner,[1] Carsten Bergmann,[2] Anke Doyon,[3] Ali Duzova,[4] Heike Goebel,[1] Dieter Haffner,[5] Bernd Hoppe,[6] Thomas Illig,[5] Martin Konrad,[7] Mieczyslaw Litwin,[8] Djalila Mekahli,[9] Bruno Ranchin,[10] Sara Testa,[11] Lutz Thorsten Weber,[1] Dorota Wicher,[8] Franz S. Schaefer,[3] Max C. Liebau.[1] *[1]Univ Hospital of Cologne, Germany; [2]Bioscentia Center for Human Genetics Ingelheim, Germany; [3]Univ Children's Hospital Heidelberg, Germany; [4]Hacettepe Univ Faculty of Medicine Ankara, Turkey; [5]Hannover Medical School, Germany; [6]Univ Hospital Bonn, Germany; [7]Univ Hospital Muenster, Germany; [8]The Childrens Memorial Health Inst Warsaw, Poland; [9]Univ Hospitals Leuven, Belgium; [10]Univ de Lyon, France; [11]Fondazione IRCCS Milano, Italy.*

Background: Autosomal Recessive Polycystic Kidney Disease (ARPKD) is the rare but frequently severe pediatric form of polycystic kidney disease. Morbidity and mortality remain relatively high even in most-advanced medical centers and there is currently no causative treatment for ARPKD. There are also no clinical classifications, known clinical risk factors or detailed treatment guidelines. Experience remains sparse even in large pediatric centers.

Methods: The multinational ARegPKD registry study aims to provide an observational evidence base for clinical treatment concepts for ARPKD. We characterize patients in a pseudonymized way using a web-based retro- and prospective registry study approach. A detailed basic data questionnaire and yearly follow-ups visits will give insights into phenotypic variability and long-term clinical developments. An ARPKD-specific biobank and reference histology complete the approach.

Results: Here we present data of the first included international patients. Im May 2014 we are following 30 patients of 0 to 43 years of age, including patients on renal replacement therapy, patients after isolated kidney transplantation or after combined liver-and-kidney transplantation. Detailed retrospective data exist for up to seven years of follow-up. Almost 50 centers in 10 different countires have already registered for ARegPKD.

Conclusions: ARegPKD is a novel web-based registry study for ARPKD in Europe and aims to contribute to the understanding of this severe renal disorder of early childhood.

Funding: Private Foundation Support

TH-PO1008

Differential Urinary Proteomics in Primary Hyperoxaluria-1 Ellen Brooks,[1,2] Bernd Hoppe,[3] Dawn S. Milliner,[4] Gulsah Vural,[2] Eduardo C. Salido,[5] Craig B. Langman.[1,2] *[1]Feinberg Medical School, Northwestern Univ, Chicago, IL; [2]Ann & Robert H Lurie Children's Hosp, Chicago, IL; [3]Univ of Bonn Children's Hosp, Bonn, Germany; [4]Mayo Clinic, Rochester, MN; [5]Hospital Univ Canarias, La Laguna Tenerife, Spain.*

Background: Primary Hyperoxaluria-type 1 (PH1) is marked by hepatic oxalate (Ox) over production, high urinary (U) Ox, renal Ox crystal deposition, calculi, nephrocalcinosis, and progressive CKD. However, mechanisms mediating CKD remain relatively unknown.

Methods: 24 Hr. urine aliquots were analyzed for proteomic profiles using proprietary Luminex-based bead technology with families of proteins statistically tested.

Results: 47 PH1 patients were dichotomized into 2 subgroup sets by median (Md) UOx= 1.045(IQR=0.26-3.05)mmoL/L/1.73m^2 or Md eGFR=77.8(IQR=8.0-164.1)ml/min/1.73 m^2. No differences were seen for eGFR between UOx>Md or UOx<Md or for UOx level between eGFR>Md or eGFR<Md. See Table 1 for protein differences by subgroup.

Table 1. Differential PH1 Proteomic Responses

Variables	UOx>Md	UOx<Md	eGFR>Md	eGFR<Md
IGF-1; IL-15	↑			
KIM-1, NGAL		↑		↑
Apo C-III & D; B_2 Microglobulin, CD40, CD40L, IL-1rα, IGFBP-2, SOD-1, TNFR-2, VCAM-1, VEGF		↑		↑
Calgranulin, HGF, ICAM-1, IFN-γ, LOX-1, MMP-7, RANTES, TRAIL-3		↑		
OPN		↑	↑	
THP			↑	
α_1AT, α_1M, Fetuin A, IL-1β, IL-8, Microalbumin, TIMP-1				↑
TFF-3	↓		↓	

Conclusions: Patients with lower but not higher levels of UOx [UOx<Md] had increased tubular injury, inflammation, oxidative stress, lipid peroxidation, apoptosis, and fibrosis markers. We noted similarities with eGFR<Md, which also had a higher TIMP-1 level, another fibrotic inhibitor. Increased mineralization inhibitors were present in UOx<Md and eGFR>Md; but only eGFR<Md had higher Fetuin A. The data imply an overwhelming protective response to tissue injury in PH1 but only when UOx is lower, suggesting that the response is limited and insufficient as Ox rises. In those with UOx>Md, IL-15, and low TFF-3, factors to reduce apoptosis and augment tissue growth and repair were present uniquely. Our findings may lead to novel therapeutic approaches in PH1 based on the UOx phenotype.

Funding: NIDDK Support

TH-PO1009

Molecular Effects of Four Mutations Identified in the D3 Region of Thrombomodulin in Patients with Atypical Hemolytic Uremic Syndrome
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Background: Atypical hemolytic uremic syndrome (aHUS) has been associated with dysregulation of the alternative complement pathway. Mutations in CFH, CFI, CFB, thrombomodulin (THBD), C3, and MCP predispose to the development of aHUS. We analyzed the molecular effects of four THBD gene mutations including T500M, identified in Japanese patients, on the alternative pathway of complement activation.

Methods: We examined four missense mutations in the THBD gene (D486Y, P495S, T500M and P501L). These mutation residues were located in the third domain (D3) of THBD. The molecular structure of D3 was theoretically constructed on the basis of 5000-picosecond molecular dynamic simulation, because the homologous structure of D3 has not been established.

Results: We carried out structural analysis of the D486, P495, T500 and P501 residues on the molecular surface of our THBD D3 model. The D3 sequence has the glycosaminoglycan (GAG) attachment site motif All of 4 mutation residues were located at positions near the motif, and the mutation at T500 in particular appeared to affect the GAG binding function. In addition, mutations of two proline residues were considered to affect the thrombin-binding function by changing the molecular direction to the lipid bilayer of the membrane, because D3 was directly connected to the sixth EGF-like domain of the thrombin binding site.

Conclusions: We conclude that these mutations of the THBD gene including mutations T500M are causative mutations of aHUS.

TH-PO1010

Glomerular Basement Membrane Injuries in Thin Basement Membrane Disease
Yusuke Kajimoto,[1] Seiichiro Higo,[1] Go Kanzaki,[1] Shinya Nagasaka,[1] Kiyotaka Nagahama,[1] Yukinari Masuda,[1] Shuichi Tsuruoka,[2] Akira Shimizu.[1] *[1]Analytic Human Pathology, Nippon Medical School, Tokyo, Kanto, Japan; [2]Nephrology, Nippon Medical School, Tokyo, Kanto, Japan.*

Background: Thin basement membrane disease (TBMD) is diagnosed by diffuse thinning of glomerular basement membrane (GBM) in electron microscopy (EM), and is characterized clinically by benign familial hematuria. The presence of hematuria may indicate GBM injury in diffuse thinning of GBM. In the present study, we analyzed the clinico-pathological characteristics of TBMD, focusing on GBM injuries, using immunostaining for α2(IV) and α5(IV) chains of Type IV collagen, and a new technique, low-vacuum scanning EM (LV-SEM), which can exhibit the three dimensional surface of GBM.

Methods: Between 2006 and 2014, TBMD was identified in 23 cases (1.8%) of 1275 renal biopsies in our department. We investigated their clinical, laboratory and histopathological findings. In 13 cases, we also carried out double immunostaining of α5(IV) and α2(IV), as well as LV-SEM.

Results: The average age of the 23 cases is 38±19 years (5-64). 22 cases have hematuria and 16 cases have proteinuria.17 cases (73.9%) were indicated to have hematuria or proteinuria from under 20 years of age. In 4 adult cases, moderate to severe decline in eGFR developed. In TBMD cases, all cases we had examined showed morphological and qualitative abnormalities of the GBM, in addition to the diffuse thinning of GBM. Alterations of the expression of α2(IV) and α5(IV) of type IV collagen were noted, including reduced α5 expression with or without increased α2(IV) in the wavy GBM. In addition, in the LV-SEM observations, various alterations of the three dimensional surface of the GBM were evident, including thinning and flattering of GBM with multiple small holes. These GBM alterations were more prominent in the adult cases than those in the young cases.

Conclusions: GBM alterations were frequently noted in TBMD, and were morphologically and qualitatively characterized by irregular attenuation of α5(IV) with or without enhancement of α2(IV) in type IV collagen, and thinning and flattering of GBM with multiple small holes. All of these findings might be associated with the hematuria, proteinuria, and/or renal dysfunction in TBMD.

TH-PO1011

Metabolic Phenotyping in Steroid Resistant Nephrotic Syndrome
Claire Boulange,[1] Manuja Kaluarachchi,[1] Isabel Garcia-Perez,[1,2] Anthony Dona,[2] Samantha Louise Lodge,[2] Elaine Holmes,[1,2] John C. Lindon,[1,2] Sven Schnaidt,[3] Franz S. Schaefer.[4] *[1]Metabometrix Ltd, Metabometrix Ltd, London, United Kingdom; [2]Computational Systems Medicine, Imperial College London, London, United Kingdom; [3]Inst of Medical Biometry and Informatics, Univ of Heidelberg, Heidelberg, Germany; [4]Pediatric Nephrology Div, Heidelberg Uiversity Hospital, Heidelberg, Germany.*

Background: Metabolic phenotyping of biofluids and tissues characterises changes in small molecule metabolites due to genetic differences, environmental influences and disease or drug perturbations. NMR spectroscopy and mass spectrometry provide sensitive and reproducible detection of hundreds to thousands of metabolites. SRNS is an etiologically heterogeneous disorder that may be caused by immune-mediated podocyte dysfunction or genetic abnormalities of podocyte specific proteins. Here we investigated whether metabolic phenotyping of urine can be utilized to identify specific molecular signatures of individual SRNS entities.

Methods: We report preliminary findings in spot urine samples obtained from children with genetic (28 NPHS2, 5 WT1 nephropathy), immunosuppressant-sensitive (IS) in disease relapse (n=20) or remission (n=23), or multidrug resistant SRNS (MDR, n=36). Samples were analysed by 600 MHz ^{1}H NMR spectroscopy (detecting predominantly small molecules >1uM) and UPLC-MS (detecting water-soluble molecules > 0.05 nM). Multivariate statistical modelling was used to elucidate class differences and to determine the spectroscopic discriminators.

Results: Whereas no differences between genetic, IS, and MDR groups were detected by NMR, multivariate modelling of the UPLC-MS profiles discriminated genetic from IS-REL SRNS. The metabolite profiles of MDR resembled those observed in genetic SRNS. Notably, both NMR and UPLC-MS identified a signal discriminating NPHS and WT1 nephropathy, which was tentatively assigned as phenylacetyl-glutamine. Further biomarker validation is ongoing, as well as application of additional metabolic profiling technologies.

Conclusions: Urine metabolic profiling may prove useful to classify renal disorders such as different SRNS entities by their specific renal metabolite signatures and lead to the discovery of novel molecular disease biomarkers.

TH-PO1012

Do B Cells Have a Role in Minimal Change Disease?
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Background: Proteinuria in Minimal Change Disease (MCD) is currently thought to be due to a T-cell mediated circulating factor. Rituximab, which induces B cell depletion, has been recently reported to induce or prolong remission in MCD. The purpose of this study is to see the effect of stimulated B cell on T cell number and function in MCD.

Methods: Seventeen MCD patients (10 remission and 7 relapse) and 10 controls were selected. Peripheral blood mononuclear cells (PBMC) obtained by Ficoll gradient were cultured in RPMI 1640. For each patient, PBMC were stimulated in vitro with antihuman mouse IgM antibody at 10 microgram/ml. At 48 hours, B and T cell proliferation was assessed using flow cytometry. B and T cell count with and without stimulation as well as the fold changes in B and T cell proliferation for relapse, remission patients and control were compared. Supernatant from the PBMC culture were obtained at 24 and 48 hours. IL -10,IL-2, INFγ and TGFβ were measured by ELISA. Statistical analysis was performed using Kruskall Wallis and Wilcoxon paired tests. *P* value <0.05 was considered to be statistically significant.

Results: Analysis of non-stimulated versus stimulated PBMC showed a significant difference in B and T cell count within each group (relapse, remission and control). But when B and T cell count were compared among the 3 groups, there was no significant difference in B and T cell count both on non- stimulated and stimulated PBMC. There were no significant differences in PBMC culture cytokine (IL-10, IL-2, INFγ and TGFβ) production at 24 hrs and 48 hours among MCD patient (relapse and remission) and control.

Conclusions: 1) Non stimulated B cells do not induce T cells proliferation in MCD during relapse. (2) Stimulated B cells induce a significant T cell proliferation both in MCD patients and in controls. (3) In MCD, T-cell proliferation induced by B cell was no different than those seen in the control group. (4) In MCD, T-cell cytokine production by B cell activation was no different than those seen in the control group. These studies do not support role of B cell in MCD.

TH-PO1013

Calcineurin Inhibition Is Not Nephroprotective in Experimental Podocin Nephropathy
Helga Denc,[1] Tanja Wlodkowski,[1] Mansoureh Tabatabaeifar,[1] Ivana Simic,[1] Geraldine Mollet,[2] Corinne Antignac,[2] Franz S. Schaefer.[1] *[1]Pediatric Nephrology Div, Heidelberg Univ Hospital, Germany; [2]Inserm U983, Hopital Necker, France.*

Background: Anecdotal observations have suggested a non-immune mediated antiproteinuric effect of calcineurin inhibitors (CNI) in hereditary nephrotic syndrome, which has been attributed to preservation of synaptopodin abundance and stabilization of podocyte actin cytoskeleton. Mutations in NPHS2 (podocin) constitute the most common cause of hereditary nephrotic syndrome. In order to investigate the antiproteinuric and nephroprotective efficacy of CNI in podocin nephropathy, we administered tacrolimus in knock-in mice carrying the most common human NPHS2 mutation. Analogous to human disease, these mice develop heavy proteinuria, podocyte loss, focal segmental glomerulosclerosis and progressive renal failure.

Methods: In C57BL/6 mice with Nphs2$^{Flox/R140Q}$, Cre^{+} genotype hemizygosity for mutant podocin was induced by tamoxifen injection. Starting at the time of induction, the animals were administered tacrolimus at 0.33 (L) or 0.66 mg/kg/d (H) or vehicle (V) via subcutaneous osmotic mini-pumps. Weight, blood pressure and proteinuria were monitored weekly. Biochemical and histopathological changes were examined after 4 weeks of treatment.

Results: All animals developed massive proteinuria, without a difference between the groups (H, 139%; L, 118% of controls). Hypoalbuminemia at 4 weeks was also not attenuated (H, 23.4 L, 20.4 V, 21.8 g/dl; n.s.) whereas serum urea levels were increased (H, 162, L, 182, V, 92 mg/dl; $p<0.05$) in the tacrolimus-treated animals. Podocyte loss was unchanged (podocytes per glom: H, 75%, L, 82%, V, 62% of healthy animals), and the glomerular sclerosis index was higher in both CNI groups compared to controls (H, 2.2 L, 1.8 V, 1.3; $p<0.001$). Podocin abundance was totally lost in all treatment groups.

Conclusions: In an *in vivo* model of hereditary podocin nephropathy, CNI showed no beneficial effect on proteinuria and podocyte loss but aggravated glomerulosclerosis and renal failure. Our findings argue against a nephroprotective action of CNI in this hereditary podocytopathy.

TH-PO1014

Urinary Angiotensinogen as a Specific Biomarker of Intrarenal Renin-Angiotensin System Status Associated with Glomerular Injury in Pediatric IgA Nephropathy Patients Maki Urushihara,[1] Shuji Kondo,[1] Hiroyuki Kobori,[2] Toshiaki Tamaki,[3] Shoji Kagami.[1] *[1]Dept of Pediatrics, The Univ of Tokushima Graduate School, Tokushima, Japan; [2]Dept of Pharmacology, Faculty of Medicine, Kagawa Univ, Kida, Kagawa, Japan; [3]Dept of Pharmacology, The Univ of Tokushima Graduate School, Tokushima, Japan.*

Background: We recently demonstrated that the renal expression levels of angiotensinogen (AGT) are enhanced and urinary AGT levels are increased in pediatric patients with chronic glomerulonephritis including IgA nephropathy (IgAN). This study was performed to test the hypothesis that urinary AGT level provides a specific index of intrarenal renin-angiotensin system (RAS) status related to the RAS blockades treatment in pediatric IgAN patients.

Methods: Paired renal biopsies were done before and after 2 years of RAS blockades treatment in 24 participants with IgAN in childhood. We performed immunohistochemical analysis of AGT, angiotensin II (Ang II), and TGF-β, that is fibogenic molecule induced by Ang II, and measured plasma and urinary AGT levels and urinary TGF-β levels. Paired test were used to analyze changes from baseline to study end.

Results: Although there was no change in plasma AGT levels ($P=0.1264$), urinary AGT ($P<0.0001$) and TGF-β ($P=0.0158$) levels were significantly decreased after the treatment with RAS blockades, accompanied by the expression levels of AGT ($P<0.0001$), TGF-β ($P<0.0001$), and Ang II ($P<0.0001$), and the scores for mesangial hypercellularity ($P<0.0001$), mesangial matrix ($P=0.0205$) in renal tissues. Baseline urinary AGT levels positively correlated with diastolic blood pressure ($P=0.0126$), urinary protein levels ($P=0.0240$), scores for mesangial hypercellularity ($P=0.0150$), and the renal expression levels of AGT ($P=0.0031$), Ang II ($P=0.0023$), and TGF-β ($P=0.0043$). Multiple regression analysis showed that the expression level of Ang II and urinary protein level can account for urinary AGT level ($R^2=0.7688$, $P<0.0001$).

Conclusions: These data indicate that urinary AGT is a useful biomarker of intrarenal RAS activation and involved with glomerular injury during the course of treatment by RAS blockades in pediatric IgAN patients.

TH-PO1015

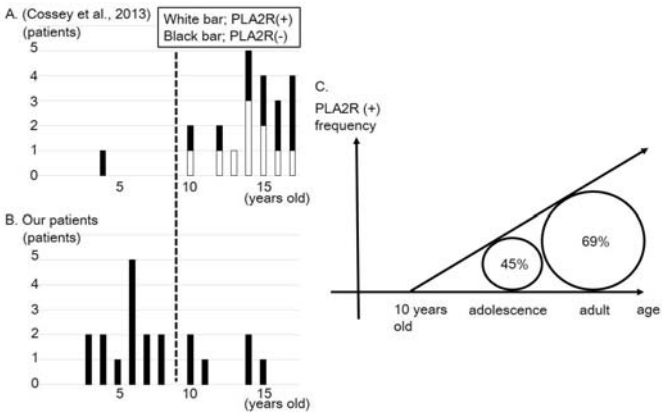
Antiphospholipase A2 Receptor (PLA2R) Expression in Glomeruli in Pediatric Idiopathic Membranous Glomerulopathy (iMN) Patients Shoichiro Kanda, Yuko Akioka, Tomoo Yabuuchi, Yuri Nawashiro, Naoto Kaneko, Norimasa Tada, Ohara Shinichiro, Takayuki Miyai, Noriko Sugawara, Kiyonobu Ishizuka, Motoshi Hattori. *Dept of Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan.*

Background: PLA2R is detected in sub-epithelial deposits along glomerular capillary loops of 69% of adult iMN patients (Hoxha et al., 2012) (Svobodova et al., 2013). On the other hand, this antigen is detected in 45% of pediatric iMN patients, the mean age of which is 14.0±3.0 years old (Cossey et al., 2013). The involvement of PLA2R in younger pediatric patients remains unclear.

Methods: During the time of observations, 20 pediatric iMN patients visited our hospital. We evaluated the expression of PLA2R using rabbit polyclonal anti-PLA2R antibodies (Sigma-Aldrich) at a dilution of 1:50. We also examined other candidate antigens, neutral endopeptidase, manganese superoxide dismutase and bovine serum albumin. We also examined the deposition of IgG subclasses along the glomerular capillary wall.

Results: The mean age, when the disease developed, was 8.0±3.7 years old. Male female ratio was 3:1. IgG1 deposition was occurred in all patients. IgG3 was detected in 13/20 (65%) patients and IgG4 was in 5/20 (25%). We confirmed PLA2R expression along the capillary wall in concurrence with IgG deposition in adult iMN samples. However this staining was not exhibited in all pediatric iMN patients. Other candidate antigens were not detected in all pediatric iMN patients either.

Conclusions: Considering our data and Cossey's data, no PLA2R-positive patient under 10 years old has been reported and the proportion of PLA2R-positive patients increases with age.



The etiology of pediatric iMN is different from that of adult patients and remains to be clarified.

TH-PO1016

The Long-Term Follow-Up of Children Presenting with Atypical Hemolytic Uremic Syndrome in Australia Between 1994 and 2001 Anne M. Durkan,[1] Elizabeth Elliott.[2] *[1]Nephrology, Children's Hospital Westmead, Sydney, NSW, Australia; [2]APSU, Australia.*

Background: There is no data available about the presentation and outcomes of atypical Hemolytic Uremic Syndrome (aHUS) in Australian children prior to the use of eculizumab.

Methods: The Australian Paediatric Surveillance Unit (APSU) prospectively collected data on all children presenting with HUS between Jul 1994 and Dec 2001. The clinicians of children with aHUS identified in this study were then sent questionnaires, at the time of patient transition to the adult services.

Results: In the initial APSU study 146 children were diagnosed with HUS of whom 14 had atypical HUS. Nine children had a diarrhoeal prodrome for a mean of 3 days and 2 children had bloody diarrhoea. Ten children required acute dialysis and two children died in the acute phase of the illness. Nine children received plasma exchange and a further two had infusions of FFP. Of the twelve children surviving to 1 year follow up data was available on 11. Nine children were on maintained on dialysis and 6 had recurrent bouts of aHUS. Long-term data was available on all of these 11 children at a mean of 14.3 years after initial presentation. Three further children had died. Eight children had been transplanted and 5 had developed recurrent aHUS. Five children received plasma exchange beyond the acute phase and 2 of these children reacted to the FFP causing the cessation of treatment in 1. Six primary transplants were lost at a mean of 5.3 years (5 due to aHUS and 1 due to BK virus). Five children have been retransplanted with recurrent disease in 3 and loss of the graft in 2. Only 2 children have maintained normal native renal function. Six children have a family history of aHUS, including 2 siblings in the study. Three children have mutations in complement factor H (CFH) and one had low CFH levels. Six children had no genetic testing or complement investigations.

Conclusions: Atypical HUS is a devastating disease with significant morbidity and mortality. The results presented here are worse than those from registries and this may reflect the prospective nature of the data. The outcomes are also affected by the level of knowledge and treatment options available at the time the children presented.

Funding: Pharmaceutical Company Support - A non-directed grant from Alexion

TH-PO1017

Adequate 25-Hydroxyvitamin D Levels Are Associated with Less Poteinuria and Attenuate Renal Failure Progression in Children with CKD Rukshana Shroff,[1] Helen Aitkenhead,[1] Augustina Jankauskiene,[2] Stefano Picca,[3] Aleksandra Zurowska,[4] Mieczyslaw Litwin,[5] Antonella Trivelli,[6] Ali Anarat,[7] Franz S. Schaefer,[8] Elke Wuehl.[8] *[1]Great Ormond Street Hospital, London, United Kingdom; [2]Vilnius, Lithuania; [3]Rome, Italy; [4]Gdansk, Poland; [5]Warsaw, Poland; [6]Genova, Italy; [7]Adana, Turkey, Turkey; [8]Univ of Heidelberg, Heidelberg, Germany.*

Background: Angiotensin converting enzyme inhibitors [ACEi] for renin-angiotensin-aldosterone system (RAAS) blockade are routinely used to slow CKD progression. Studies have shown that vitamin D may promote renoprotection by suppressing renin transcription, and possible antiproliferative and antifibrotic effects through cross-talk between RAAS and vitamin D-FGF23-klotho pathways. We hypothesise that vitamin D levels influence proteinuria and CKD progression in children with non-proteinuric renal disease.

Methods: This is a post-hoc analysis of the ESCAPE (Effect of Strict Blood Pressure Control and ACE Inhibition on Progression of CKD in Pediatric Patients) cohort. Serum 25-hydroxyvitamin D [25(OH)D], FGF23 and klotho were measured at baseline (before ACEi treatment) and after a median of 8 months on ACEi. Annualized GFR loss and renal survival analysis (composite end-point of loss of GFR>50% or start of renal replacement therapy) were measured.

Results: Of the 167 children (median 11.4 yrs, 96 males), 129 had renal hypodysplasia, 15 glomerulopathies and 23 hereditary or other renal diseases. Median eGFR was 51 ml/min/1.73m^2, median urinary protein/creatinine ratio 0.79 (IQR 0.2-2.1) and none

had nephrotic syndrome. Serum 25(OH)D showed an inverse association with urinary protein/creatinine ratio both at baseline (p=0.01) and follow-up (p=0.01). ACEi treatment significantly increased FGF23 and klotho (p<0.001 for both), without notable changes in calcium or phosphorus. The annualized loss of eGFR was lower in patients with higher baseline 25(OH)D (p=0.0003, r=0.28). 5-year renal survival was 75% in patients with baseline 25(OH)D >50nmol/L compared to 50% in patients with lower 25(OH)D (p<0.0001).

Conclusions: 25(OH)D is inversely associated with proteinuria and loss of eGFR in children with CKD. Prospective studies on the renoprotective effects of vitamin D are required.

TH-PO1018

The Canadian Childhood Nephrotic Syndrome (CHILDNEPH) Project

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Background: Evidence-based clinical practice guidelines are available for childhood nephrotic syndrome. However, dose and duration of steroid treatment for first presentation and relapses are variable among physicians and care centers. Reasons for variability and how these affect patient outcomes are unknown.

Methods: This mixed methods study will determine center-, physician-, and patient-level factors associated with cumulative steroid exposure and length of steroid treatment. The quantitative component is a national, 13-center prospective observational cohort study of 400 steroid-sensitive children over 2.5 years. Qualitative focus groups with nephrology team members (MDs, RNs, pharmacists) at each site will provide an understanding of the attitudes, beliefs and local factors driving treatment variation. Our analytic approach will integrate quantitative and qualitative results to explain care variability.

Results: To date, 3 centers have consented 27 patients (15M, 12F; median age 3.5 years, IQR 2.0-5.3). Median cumulative steroid dose (mg/m^2) for: first presentation=2,206 (range: 691-4,350); first relapse=998 (range: 348-2,171); second relapse=805 (range: 389–3,273). Median length of treatment (days) for first presentation=112, IQR 72-112. Four sites completed focus groups. Emerging themes explaining treatment variability are physician preferences based on prior training and experience, and the need to individualize therapy for patients.

Conclusions: This is the first national, Canadian population-based evaluation of children with nephrotic syndrome. Early results suggest wide variation in steroid prescriptions for first presentation and relapses. Physician factors play key roles in care variability.

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TH-PO1019

Renal Disease Progression and Complications in Autosomal Recessive Polycystic Kidney Disease (ARPKD): A Report from the Chronic Kidney Disease in Children (CKiD) Study

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Background: ARPKD is an inherited renal cystic disease with a high rate of ESRD, but prospectively collected data are limited. The goal of this study was to define the rates of GFR decline, hypertension (HTN), proteinuria and growth complications in CKiD subjects with ARPKD versus 2 control groups w/other congenital renal diseases.

Methods: We compared ARPKD subjects (n=22) w/controls from 2 groups: aplastic/hypoplastic/dysplastic(n=44) and obstructive uropathies(n=44). Subjects were matched 2:1 w/controls for baseline GFR, age at study entry and at diagnosis. GFR decline, HTN control (#of meds, LVH), proteinuria, height (Ht)%ile and growth hormone (GH) use were analyzed and differences between 2 study groups (ARPKD versus each control group) were examined by Wilcoxon rank sum test. GFR annualized change (ml/min/1.73m^2 and %change) and Ht Z-score were examined and matched differences analyzed with Wilcoxon signed rank tests.

Results: ARPKD subjects and controls were successfully matched on the selected factors. GFR annualized change was not different among the groups (-1.4 versus -1.0 versus -2.7 ml/min/1.73m^2; -6% versus -2% versus -7%). There were no significant differences in HTN or LVH rates, but ARPKD subjects had more ACEI use (82% versus 36% [p=0.0004] versus 27% [p=0.0001]) and a higher % on ≥3 BP meds (32% versus 0% in control groups, p<0.0001). ARPKD subjects had less proteinuria (prot:creat=0.1 versus 0.6 in control groups, p<0.005). There were no significant differences in median Ht%ile (14 versus 20 versus 28), % <3rd%ile (14% versus 19% versus 23%) and GH use (18% versus 9% versus 14%). Matched analyses showed no significant differences in GFR decline or Ht trajectory.

Conclusions: Rates of GFR decline, HTN, LVH and growth complications in ARPKD subjects did not differ from those of other congenital renal diseases. ARPKD subjects had lower rates of proteinuria, which may reflect high rates of ACEI use.

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TH-PO1020

Prevalence of Sleep Disorders in Pediatric Nephrology Patients

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Background: Sleep disorders are associated with hypertension, chronic kidney disease, metabolic syndrome and obesity. Our objective was to do a comprehensive study of prevalence of sleep disorders in children visiting Nephrology clinic using validated questionnaires.

Methods: Children attending pediatric nephrology (PN) and general pediatric clinic (GP) completed pediatric sleep questionnaire (PSQ) and pediatric daytime sleepiness scale (PDSS). Sleep related breathing disorder (SRBD) and periodic limb movements in sleep (PLMS) scores of >0.33 (calculated from PSQ) were considered to indicate high probability of these disorders.

Results:

	PN (N=60) Mean ± SD or n(%)	GP (N=94)Mean ± SD or n(%)	p-value
Age (yrs)	14.1 ± 3.5	12. 2 ± 3.4	0.001
Gender M/F (%)	35 (58%)	47 (50%)	0.31
BMI percentile	70.3 ± 27.8	66.4 ± 30.0	0.42
SRBD scale >0.33	13 (22%)	16 (17%)	0.47
PLMS Scale >0.33	22 (37%)	11 (12%)	0.0002
Weekday sleep time (WDST) minutes	531.9 ± 85.3	551.1 ± 82.3	0.33
Insufficient Sleep WDST≤ 8 hrs (%)	21 (35%)	23 (24%)	0.16
PDSS total	11.6 ± 5.1	11.4 ± 6.3	0.80
Excessively Sleepy (≥4 questions on PDSS as ≥3	6 (10%)	17 (18%)	0.17

A linear relationship was seen between SRBD score and SBP (r^2=0.275,β=0.177,p=.001) as well as DBP (r^2=0.265,β=0.194,p=.002) in PN group adjusting for age, gender and BMI SDS but not in GP group. WDST showed significant inverse correlation with SBP (r^2=0.275,β=-0.214,p=.001) and DBP(r^2=0.23,β=-0.01,p=.005) in PN group adjusting for age, gender and BMI SDS.

Conclusions: There was a high prevalence of sleep disorders (probable SRBD, probable PLMS, insufficient sleep and excessive sleepiness) in our patients. The prevalence of probable PLMS was significantly higher in PN group. In adults, CKD is associated with PLMS but CKD was rare in our patients. It is possible that renal disorders without CKD may predispose to PLMS too. SRBD score was directly and WDST was inversely correlated with BP parameters suggesting they may be risk factors for hypertension. These relationships need further exploration in children.

TH-PO1021

Are Pediatric Oncology Patients Clinically Hydrated prior to Chemotherapy? How Do We Know?

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Background: Chemotherapy-associated renal toxicity is ameliorated with hydration. The Children's Oncology Group (COG) protocols assess hydration by urine specific gravity (SG) before starting chemotherapy. Despite receiving aggressive volume expansion, patients often miss targets for SG, forcing oncologists to make decisions to begin chemotherapy without supporting laboratory evidence of hydration. We sought to assess markers of hydration prior to chemotherapy administration in pediatrics.

Methods: Chart reviews determined the amount of fluid administered prior to chemotherapy, urine output prior to chemotherapy administration, urine specific gravity before and after hydration therapy, admission weight and the next available weight post hydration and admission serum creatinine and the next available creatinine post hydration. Using Pearson correlation coefficients, we estimated a sample size of 47 pre-chemotherapy hydration instances to achieve moderate (r=0.4) correlation with p<0.025.

Results: Eight patients received 120 cycles of pre-chemotherapy hydration between May 2011 and September 2013, of which 93 cycles had sufficient data to analyze. Patient gender (3F:5M), age (16.0 +/- 3.3 years), diagnosis (7 osteosarcoma, 1 Ewing's sarcoma), fluid administered (45.6 +/- 17.5 ml/kg) and urine output during hydration (23.3 +/-14 ml/kg) were noted. Correlation between fluid balance and % body weight change (r=0.07, p=0.25), % change in eGFR (Schwartz) (r=-0.06, p=0.21), and % change in urine SG (r=-0.12, p=0.12) were not statistically significant.

Conclusions: Our study demonstrated that existing markers of hydration are not well correlated and that calculated relationships are weak, supporting the observation that existing measures of pre-chemotherapy hydration are unreliable. Existing protocols recommending the use of SG as a measure of pre-chemotherapy hydration should reflect the diagnostic uncertainty of these bedside assessments.

Funding: Clinical Revenue Support

TH-PO1022

Measuring Health Care Transition Readiness among Mexican Adolescents with Chronic Kidney Disease Guillermo Cantu,[1] Mara Medeiros,[2] Sarah Elizabeth Cohen,[3] Maria E. Ferris.[3] *[1]Medicine and Ehtics, Univ Panamericana, Mexico City, DF, Mexico; [2]Pediatric Nephrology, Hospital Infantil de México Federico Gómez, Mexico City, DF, Mexico; [3]UNC KIdney Center, Univ of North Carolina, Chapel Hill, NC.*

Background: There is a lack of valid health care transition readiness (HCT) scales in Spanish. We determined initial validation of the UNC TRxANSITION Scale™ among Mexican youth with chronic kidney disease (CKD) at the Hospital Infantil de Mexico Federico Gómez.

Methods: We performed translation and back translation of the 10-domain, provider-administered UNC TRxANSITION Scale™. This 33-question scale measures knowledge about diagnosis, treatment, diet, reproductive health, school/work, insurance, and the ability to self-manage or look for new providers (max score of 10). We invited 10-18 year-old patients. Univariate linear regression determined how sensitive the Scale was to increasing age. Dialysis patients were compared to others (excluding transplant patients) and transplant patients were compared to others (excluding dialysis patients).

Results: The 103 participants had a mean age of 14.8 (± 2.23) and 51 were females (49.5%). Of those with known cause of CKD, 38 had glomerular disease and 33 had non-glomerular disease. There were 9 dialysis patients (9%), 21 (20%) transplant patients and 73 (71%) had CKD. The cohort's overall total score ranged from 0.97 to 9.5 with a mean of 5.45 (± 2.10); 5.29 (±2.17) for males and 5.62 (±2.03) - no significant difference by sex-. The univariate linear regression yielded a beta coefficient of 0.197 ($p < .05$). Dialysis patients new more about their diet/food labels than those not on dialysis. ($F=6.913$, $p=.010$). Transplant patients had higher self-management ($F=16.063$, $p=.000$) and knew more about insurance ($F=12.135$, $p=.001$). ESRD patients had the highest level of self-management, while patients with non-glomerular disease had the lowest ($F=4.876$, $p=.003$). Patients with ESRD also had the highest knowledge of insurance, while patients with glomerular disease had the lowest ($F=5.383$, $p=.002$).

Conclusions: The Spanish version of the UNC TRxANSITION Scale™ for youth appears to be a useful tool to measure HCT readiness. Further validation is underway.

Funding: Private Foundation Support

TH-PO1023

Acute Peritoneal Dialysis in Premature Infants Weighing Less Than 1,000 g: A Single-Center Experience Mariko Sawada.[1] *[1]Pediatrics, Kurashiki Central Hospital, Kurashiki, Okayama, Japan; [2]Nephrology, Kurashiki Central Hospital, Kurashiki, Okayama, Japan.*

Background: Acute renal failure (ARF) is a common complication in premature infants. We performed peritoneal dialysis (PD) in neonates with ARF several decades ago, but there are no guidelines for PD in neonates and no appropriate devices, such as catheters, circuits, and peritoneal dialysates. Each center follows its own PD protocols for premature infants. Here we report our single-center experience with PD in premature infants.

Methods: We retrospectively analyzed the medical records of all patients treated with PD at the neonatal intensive care unit of Kurashiki Central Hospital between December 2001 and March 2014 and analyzed the patients' characteristics, cause of ARF, and 1-and 3-month outcomes.

Results: A total of 386 extremely low birth weight infants (ELBWIs) underwent neonatal intensive care during this period. Ten ELBWIs (2.95%; 5 with cardiogenic shock, 5 with septic shock) underwent PD during this period, 7 of whom were boys. The average gestational age was 25.4 ± 2.4 weeks, while the average age and body weight were 22 ± 15 days and 607 ± 147 g, respectively. All of these patients had multiple organ failure. The mean PD duration was 17.3 ± 20.2 days. We used nephrostomy catheters (5 Fr in 7, 6.5 Fr in 3) as PD catheters. The patients underwent 24 cycles of PD each day using bicarbonate of lactate dialysate. All of the patients were treated manually. PD-related peritonitis was noted in 3 patients. The overall 1- and 3-month mortality rates were 40.0% and 60.0%, respectively. After 2010, 3 of 4 patients survived and discontinued PD at the time of discharge.

Conclusions: Our results indicate that the mortality rate has decreased since 2010 and that peritonitis is much more common in premature neonates. PD might become a more effective and safer therapeutic method in neonates with ARF who weigh < 1,000 g if appropriate devices are used.

TH-PO1024

Novel Screening Algorithm for Identification of Renal Magnesium Wasting in Children with Renal Disease Kazuya Matsumura, Midori Awazu. *Dept of Pediatrics, School of Medicine, Keio Univ, Tokyo, Japan.*

Background: Fractional excretion of magnesium (FE_{Mg}) is reported to predict tubulointerstitial fibrosis in adults. No such studies have been performed in children. Also, FE_{Mg} can be affected by serum creatinine concentration (SCr). Therefore, we have developed a screening algorithm to detect Mg wasting using serum Mg (SMg), FE_{Mg}, and urine Mg-to-creatinine ratio (Mg/Cr), and investigated the prevalence of Mg wasting in children with renal disease especially in those with chronic renal insufficiency.

Methods: One hundred and nineteen subjects (59 males and 60 females, aged 2 months-48 years, median 15 years) with renal disease were studied. Estimated GFR (eGFR) was determined by new Schwartz formula (<2 years), quintic equation for Japanese children (2 to 19 years), or formulas for Japanese adults (≥19 years) by age and gender. Renal Mg wasting was defined as 1) FE_{Mg} above 2% if SMg is less than 1.8 mg/dL, and in subjects with normal SMg, 2) FE_{Mg} above 4% if eGFR is normal (≥90 ml/min/1.73 m^2), and 3) Mg/Cr above age-specific reference values if eGFR is reduced.

Results: A total of 43 patients (36%) were categorised as having Mg wasting. Sixteen of all the patients (13%) had hypomagnesemia (1.2-1.7 mg/dL) and all had high FE_{Mg} (9.5 ± 11.7%, mean±SD). In normomagnesemic patients with normal eGFR, 31% (21/67) had high FE_{Mg} (5.8 ± 2.0%). In normomagnesemic patients with low eGFR, 78% (28/36) had high FE_{Mg} but only 17% (6/36) had high Mg/Cr. Among 21 patients with normal SMg and eGFR categorised as having Mg wasting by FE_{Mg}, 14 (67%) had high Mg/Cr. In these subjects, ROC curve analysis (AUC = 0.934) determined that a cut-off point for FE_{Mg} 4.1% yielded 93% sensitivity and 82% specificity for high Mg/Cr. This cut-off value precisely agreed with that used in the present study. Underlying conditions associated with Mg wasting included low eGFR (15/45, 33%), CAKUT (12/34, 35%), hematuria (8/13, 62%), nephrolithiasis (7/12, 58%), Fanconi syndrome (8/11, 73%), ciliopathy (2/5, 40%), and calcineurin inhibitor (2/4, 50%).

Conclusions: We have developed a novel screening algorithm to detect Mg wasting. Mg wasting is not uncommon in children with renal disease including chronic renal insufficiency.

TH-PO1025

Racial Disparities in Children on Renal Replacement Therapy in Western Europe Lidwien Tjaden,[1,2] Marlies Noordzij,[1] Karlijn J. Van Stralen,[1] Franz S. Schaefer,[3] Jaap Willem Groothoff,[2] Kitty J. Jager.[1] *[1]Dept Medical Informatics, Academic Medical Center, Amsterdam, Netherlands; [2]Dept Pediatric Nephrology, Emma Children's Hospital, Amsterdam, Netherlands; [3]Dept Pediatric Nephrology, Univ Children's Hospital, Heidelberg, Germany.*

Background: Racial disparities have been reported in the quality of care among adult patients with end-stage renal disease (ESRD), but little research exists for the paediatric renal replacement therapy (RRT) population. We aimed to assess differences in patient and treatment characteristics between racial groups in children on RRT in Western Europe.

Methods: Using data from the ESPN/ERA-EDTA Registry, we included patients aged <19 years who started RRT in 2006-2011. To avoid confounding by economic factors, analyses were restricted to high-income countries. Racial groups were defined as white, black, Asian and other. Differences between groups were examined by ANOVA and Chi-square tests. We studied access to transplantation using Cox regression analysis whilst adjusting for gender, country of residence and age at the start of RRT.

Results: 769 patients from 6 countries (Belgium, Greece, the Netherlands, Portugal, Slovakia, UK) were included. 77.9% were white, 5.9% black, 8.6% Asian and 7.7% from other racial groups. Black patients were 40% (HR 0.60; 95%CI 0.41-0.89) and Asians 39% less likely (HR 0.61; 95%CI 0.45-0.83) to receive a kidney transplant compared with white patients. This difference could partly be explained by a higher proportion of living kidney transplants in white (34.9%) versus black (17.9%), Asian (21.6%), and other (17.1%) patients ($P<0.01$). In addition, among those who received a kidney transplant from a deceased donor we found longer waiting times in non-white patients (medians 17.4-18.3 months) compared with white patients (14.7 months, $P<0.01$).

Conclusions: This is the first European study examining racial disparities in quality of care in the pediatric RRT population. We found that white pediatric ESRD patients are more likely to receive optimal treatment compared to racial minority groups. Further research is required to identify and address the contribution of medical and sociocultural barriers to optimal treatment among these groups.

TH-PO1026

Medication Adherence in Adolescents with Chronic Kidney Disease or Kidney Transplant: Patient/Caregiver Report versus Provider Perception Cozumel S. Pruette,[1] Tammy M. Brady,[1] Susan R. Mendley,[2] Barbara A. Fivush,[1] Shamir Tuchman,[3] Angela M. Green,[1] Michelle N. Eakin,[1] Kristin Riekert.[1] *[1]Johns Hopkins Univ; [2]Univ of Maryland; [3]Children's National Medical Center.*

Background: Medication adherence in children with chronic kidney disease (CKD) is not well described and adherence in adolescent kidney transplant (KT) recipients is poor, with non-adherence (NA) ranging 5-71%. It is important to identify appropriate measures of adherence to identify patients at risk for NA. The aim of this study is to compare two measures of medication adherence.

Methods: 53 teens from 3 medical centers, age 11-19 with CKD or KT and prescribed antihypertensive medication, and their caregiver completed the Morisky Adherence Scale. Categories of adherence were based on the number of affirmative responses: low adherence= >2, medium adherence= 1 or 2, high adherence= 0. Provider-perceived adherence was determined post-clinic visit, with adherent defined as ≥75% and NA as <75%. Chi-squared analyses and kappa statistics were used to compare teen and caregiver-reported to provider-perceived adherence.

Results: Teen-mean age 14.8yrs; 47% female; 40% Caucasian, 47% Black; 75% CKD, 25% KT. Caregiver-87% female, 29% household income <$50,000/yr. Provider-n=15; 80% female. Providers perceived 72% of patients as adherent while 0%, 34% and 66% of teens reported themselves as high, medium and low adherent and parents also reported 0% high, 36% medium and 64% low adherence. There were no significant differences in teen/caregiver demographics between those perceived by the provider as adherent versus NA. Agreement between provider and teen (41.5% agreement; kappa -0.11±0.06) and provider and caregiver (38% agreement; kappa -0.15±0.06) was poor. Positive predictive value (PPV) of provider-perceived adherence was 42% and negative predictive value (NPV) was 87% when compared to teen-reported adherence, and PPV was 47% and NPV was 93%compared to caregiver report.

Conclusions: Providers overestimate medication adherence for many teens with CKD or KT and their ability to identify NA is worse than that expected to occur by chance alone. Use of a simple adherence measure, like the Morisky Scale, may help identify NA in this population.

Funding: NIDDK Support, Private Foundation Support

TH-PO1027

Nutritional and Metabolic Bone Disease Markers for a Global Sample of Children and Young Adult Hemodialysis Patients Maria E. Ferris,[1] Daniele Marcelli,[2] Xiaoqi Xu,[3] Cristina Marelli,[4] John W. Larkin,[5] Aileen Grassmann,[2] Roberto Pecoits-Filho,[6] Michael Etter,[3] Peter Kotanko,[7] Len A. Usvyat.[5] *[1]Univ of North Carolina, NC; [2]Fresenius Medical Care, Bad Homburg, Germany; [3]Fresenius Asia Pacific, Hong Kong, Hong Kong; [4]Fresenius Latin America, Buenos Aires, Argentina; [5]Fresenius Medical Care, Waltham; [6]PUCPR, Curitiba, Brazil; [7]Renal Research Inst, NY.*

Background: Nutritional and metabolic bone disease need to be characterized in hemodialysis (HD) patients (Pts) of the global Pediatric Investigation and Close Collaboration to examine Ongoing Life Outcomes in the MONitoring Dialysis Outcomes (PICCOLO MONDO) Consortium.

Methods: From 2000-2012, 3244 Pts from 0-30 years of age (13% pediatric, age 0-18; 87% young adults, age 19-30) were grouped into 4 regions: North America (NA), South America (SA), Europe, and Asia. The mean of clinical/laboratory parameters were computed in the first 365 days on HD. Analyses of continuous variables was conducted using multiple linear regression.

Results: Among pediatric Pts (mean age: 14.7±3.3 years) with known ESRD cause, glomerular disease (GD) was most common in Asia and NA, while genitourinary causes were most common in Europe. In young adults, GD was most common cause in Asia and Europe, while "other" was listed most commonly in NA. Using SA as a reference, albumin was highest in Asia in both age groups ($p<0.01$). In pediatric patients, lower albumin was observed in Pts with GD ($p=0.05$). In young adults, diabetics had lower albumin ($p<0.001$), while males had higher albumin ($p<0.001$). Using SA as a reference, in pediatric patients, phosphate levels were higher in NA ($p<0.001$) and male ($p=0.05$). For young adults, phosphate was higher in Europe and NA ($p<0.001$), in males ($p<0.001$) and lower in Pts with ESRD of genitourinary etiology ($p<0.05$). Furthermore, intact-PTH was higher in NA in both pediatric and young adult patients ($p<0.001$) with SA as reference. In young adults with GD and diabetes as cause of ESRD, intact-PTH was lower ($p<0.001$).

Conclusions: In this global cohort, GD was the most common cause of ESRD. Markers of nutrition and metabolic bone disease vary by region and age group warranting further investigation.

TH-PO1028

Clinical Manifestations and Metabolic Evaluation in Korean Children with Urolithiasis Heeyeon Cho, Sang Taek Lee. *Pediatrics, Samsung Medical Center, Seoul, Republic of Korea.*

Background: There is little data for the metabolic etiology and prognosis in pediatric patients with urolithiasis. The aim of this study was to assess the clinical findings, metabolic etiology, and treatment in Korean children with urolithiasis.

Methods: The medical records of 52 children (29 boys, 23 girls) diagnosed as having urolithiasis from January 2012 to December 2013 were retrospectively analyzed. Data of clinical symptoms, urinary tract abnormality, serum biochemistry, urine metabolic evaluation, and treatment were recorded.

Results: The mean age at diagnosis was 10.5 years (range 0.6 – 18 years). The most common presenting symptom was renal colic (40.0%, n=21). The other symptoms included gross hematuria (17.3%, n=9) and renal colic with gross hematuria (9.6%, n=5). Sixty seven percent of the stones were unilateral. Urinary tract abnormality was found in 1 patient with dupex kidneys. Metabolic abnormalities were found in 20 (38.4%) patients, including hypercalciuria in 19.2% (n=10), hyperuricosuria in 7.7% (n=4), hypomagnesuria in 5.8% (n=3), hyperoxaluria in 3.8% (n=2), and cystinuria in 1.9% (n=1). Stone analyses were performed in 13 patients, including calcium oxalate (n=7), carbonate apatite (n=1), calcium apatite (n=4), and cystine stone (n=1). Spontaneous passage of stone occurred in 5.7 % (n=3) of patients.Extracorporeal shock wave lithotripsy and surgical evacuation were performed in 15.3% (n=8) and 1.9% (n=1) of patients, respectively.

Conclusions: The most common metabolic abnormalities in children with urolithiasis were hypercalciuria and hyperuricosuria. There is the need for extensive evaluation to find underlying metabolic disturbances in children presented with renal colic and/or gross hematuria.

TH-PO1029

Risk and Prevention of Hepatitis B Virus and Hepatitis C Virus Infections in Pediatric Hemodialysis Patients Ihab Z. Elhakim,[1] Ahmed Hussein Hassan,[1] Ahmed Hosam el-Din Baranek,[1] Manal H. El-Sayed.[2] *[1]Pediatric Nephrology, Ain Shams Univ, Cairo, Egypt; [2]Pediatric Hematology/Hepatology, Ain Shams Univ, Cairo, Egypt.*

Background: Children on hemodialysis are at a high risk of HBV and/or HCV infection-related chronic liver disease. HBV immunization is protective in healthy children, yet remains unsettled in those on hemodialysis. This study determines the prevalence of HBV and HCV infections, the immune status and response to HBV vaccination among pediatric hemodialysis patients.

Methods: One year prospective study for all children on hemodialysis in a single center (n=50; median age: 14 years; M:F 28:22). All were tested for liver transaminases, iron profile, HBsAg and HBsAb titre by ELISA and HCV using Oraquick HCV rapid antibody test (anti-HCV and HCV-RNA for positive cases). Children with HBsAb titre <100 IU/L were randomly assigned to receive HBV recombinant intramuscular vaccine either at 0, 1 and 2 months (group 1) or 0, 1 and 6 months (group 2) schedule. HBsAb titer was measured one month following the last dose in both groups.

Results: Seven (14%) were HCV positive by Oraquick (6 confirmed anti-HCV and 5 HCV-RNA positive) and 6 (12%) were immune against HBV infection (HBsAb titre >100 IU/L). Both groups had a highly significant increase in post vaccination HBsAb titer (group 1: mean 12.5±5.6 IU/L rising to 909.5±240.6 IU/L and group 2: mean 16.7±16.08 rising to 808.7±334.2 IU/L; $p=0.0000$), with no significant difference between both schedules.

Conclusions: Hemodialysis children have a lower prevalence of HCV than previously reported and not adequately protected against HBV infection. HBV vaccination at 0, 1 and 2 months has a similar seroprotection rate as the 0, 1 and 6 months schedule.

TH-PO1030

Bacteria and Calcium Kidney Stones Andrew L. Schwaderer,[1] David S. Hains,[2] Evan Barr-Beare,[1] Vijay Saxena,[1] Evann E. Hilt,[3] Alan J. Wolfe.[3] *[1]Research, Nationwide Children's, Columbus, OH; [2]Nephrology, LeBonheur Children's, Memphis, TN; [3]Microbiology, Loyola Univ, Chicago, IL.*

Background: The role of bacteria in formation of struvite stones is established. If bacteria also have a role in calcium oxalate stone formation has not been extensively evaluated. We hypothesize that the interaction between bacteria, calcium oxalate crystals and host immune response is an important lithogenesis component.

Methods: Human studies: Following consent, upper tract urine, bladder urine and kidney stone fragments were obtained at the time of surgical stone removal. An enhanced quantitative urine culture protocol that included aerobic, CO_2 and anaerobic atmospheric conditions and sequencing for bacterial DNA were performed on urine and pulverized kidney stones. In vitro studies: Calcium oxalate monohydrate and dihydrate crystals were generated and incubated with FITC-labeled Escherichia coli. Bacterial adherence along with crystal size, number and structure were evaluated. Mouse studies: Kidney stones were induced in C57Bl/6 mice (n=6) and RT^2 PCR bacterial response arrays (Qiagen) to evaluate for altered regulation of 84 key bacterial response genes were performed and compared to control mice (n=6).

Results: Five patients with calcium kidney stones were recruited. We found viable bacteria in 2/5 kidney stones and detected bacterial DNA in all kidney stone samples. Bacteria identified included *E. coli, Gardnerella vaginalis, Pseudomonas* and *Phyllobacterium.* Time-lapse microscopy revealed that *E.coli* preferentially adhered to calcium oxalate monohydrate crystals by 8 hours. By 10 hours, bacteria also became adherent to the dihydrate phase. RT^2 PCR arrays revealed upregulation (> 4-fold, p value < 0.01) of 16 bacterial response genes; none were downregulated. Key upregulated genes included the NLR family, apoptosis inhibitory protein 1, and toll-like receptor 2.

Conclusions: Bacterial DNA is consistently identified in calcium oxalate kidney stones. Calcium oxalate crystals attract *E. coli* and elicit a marked antibacterial response. Murine studies to assess if isolates of bacteria from human kidney stones enhance lithogenesis are ongoing.

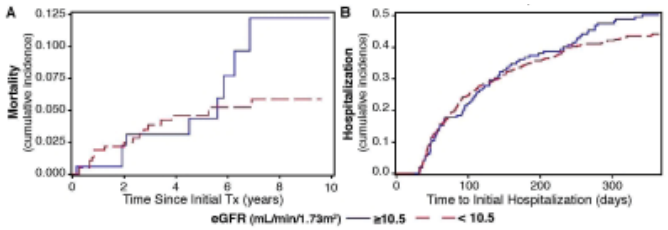
TH-PO1031

The Association of Timing of Dialysis Initiation with Hospitalizations and Mortality in Children Allison Dart,[1,8] Susan M. Samuel,[2,8] Manish M. Sood,[3,8] R. Todd Alexander,[4,8] Steven Arora,[5,8] Robin L. Erickson,[6,8] Braden J. Manns,[2,8] Michael Zappitelli.[7,8] *[1]Pediatrics and Child Health, Univ of Manitoba, Winnipeg, MB, Canada; [2]Univ of Calgary; [3]Univ of Ottawa; [4]Univ of Alberta; [5]McMaster Univ; [6]Univ of Saskatchewan; [7]Univ of Montreal; [8]CANN-NET.*

Background: Evidence guiding timing of dialysis initiation in children is limited. We assessed time to first hospitalization and mortality in children starting dialysis with an eGFR ≥ *versus* < 10.5 ml/min/1.73m^2.

Methods: Incident Canadian dialysis patients <22 yo, between 2001-2010 from 9 Canadian provinces were identified from the Canadian Organ Replacement Registry. Outcomes were time to first hospitalization and mortality *after* dialysis initiation, evaluated in competing risk Cox Proportional Hazards models (with transplant). The primary exposure was early *versus* late dialysis initiation (eGFR ≥ *versus* < 10.5 ml/min/1.73m^2). Models were adjusted for age at dialysis initiation, sex, ethnicity, ESRD etiology, initial modality (peritoneal *versus* hemodialysis), and income quintile. Sensitivity analyses were performed using eGFR thresholds of 6 and 15 ml/min/1.73m^2.

Results: 254/562 (54%) were hospitalized in the first year and 39 (6.7%) children died. There were no statistically significant mortality differences (Hazard Ratio (HR) 1.25; 95% CI 0.55-2.82) (Fig. A) or in first year hospitalizations (HR 0.98; 95% CI 0.77-1.26) between ≥ *versus* < 10.5 groups (Fig. B). Children 0-4 yrs old had a HR of death of 9.28 (95% CI 1.08-79.59) *versus* 5-14 yrs. Caucasians were less likely to be hospitalized (HR 0.78; 95% CI 0.62-0.97). Sensitivity analyses using eGFR thresholds of 6 and 15 ml/min/1.73m2 had similar results.



Conclusions: Early initiation of dialysis was not associated with clinical benefit in this study. A prospective randomized study is required to confirm these results.

Funding: Private Foundation Support

TH-PO1032

Impact of Histological Abnormalities on Implant Biopsy in Living Kidney Donors on Allograft Outcomes Silvi Shah,[1] Andres G. Chiesa-Vottero,[2] Saul Nurko,[1] Richard A. Fatica,[1] Brian R. Stephany,[1] Ziad S. Zaky,[1] Emilio D. Poggio.[1] *[1]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [2]Pathology, Cleveland Clinic, Cleveland, OH.*

Background: Renal transplantation is the best treatment for patients with end stage renal disease. Living kidney donation (LKD) is associated with better outcomes than deceased donation (DD). Donor histological changes found on implant biopsies is independently associated with allograft outcomes in DD. However, these associations are not well studied in LKD.

Methods: Data was retrospectively collected on 234 donor-recipient pairs transplanted between 2005 and 2011. Implant biopsies were routinely obtained in all donated kidneys. Acute tubular necrosis (ATN) in the implant biopsy was reported if at least two of the following histological changes were present: tubular dilation, tubular vacuolization or mitotic figures. Chronic histological changes recorded were arteriosclerosis, glomerulosclerosis, interstitial fibrosis and tubular atrophy (IFTA) and were reported positive if any involved greater than 5% of the biopsy specimen. Recipient estimated GFR (eGFR) was calculated using the CKD-EPI equation. The primary outcome analyzed was allograft function at 12 months post-transplant.

Results: ATN was present in 20.7% of implant biopsies. The prevalences of arteriosclerosis, glomerulosclerosis and IFTA were 30.3%, 16.3% and 9.2% respectively. Implant biopsies with versus without ATN were associated with lower recipient eGFR at 12 months [(56 ml/min/1.73m2 versus 50 ml/min/1.73 m2), P = 0.01]. Implant biopsies with IFTA were also associated with lower recipient eGFR at 12 months [(55 ml/min/1.73m2 versus 44 ml/min/1.73m2), p= 0.003)]. There was no significant association between arteriosclerosis and glomerulosclerosis on implant biopsy and recipient eGFR at 1 year.

Conclusions: ATN and IFTA on LKD implant biopsies predict lower allograft function at 1 year. However their long-term impact is not known and merits further study.

TH-PO1033

Cost Analysis of Incidental Findings during Living Kidney Donor Evaluation Anubha Mutneja,[1] Lauren Saling,[2] Motoyo Yano,[2] Anitha Vijayan.[1] *[1]Renal Div, Washinton Univ in St. louis, St. Louis, MO; [2]Mallinckrodt Inst of Radiology, Washington Univ in St. Louis, St. Louis, MO.*

Background: Living kidney donor (LKD) transplantation is an important alternative for the ESRD population and is preferred due to superior outcomes when compared to dialysis or deceased donor transplantation. LKD evaluation includes a computed tomography angiogram (CTA) for assessment of renal anatomy. Incidental findings noted on CTA often lead to additional testing and procedures. Their economic impact for transplant programs and donors remains uncertain.

Methods: We conducted a retrospective analysis of the incidental findings noted during CTA of 632 LKDs evaluated at our institution from 2008 to 2013. Follow-up evaluation of incidental findings was ascertained after thorough chart review. Cost analysis was performed using Medicare reimbursement data.

Results: There were 525 extra-renal incidental findings in 632 potential living donors. These findings were further categorized based on organ systems.

	Number of incidental findings N (%)	% requiring further testing	Follow-up cost (USD)
Pulmonary	82 (13)	39	14,277
Hepatic	159 (25)	9	7,732
Pancreatic-biliary	38 (6)	23	11,675
Adrenal	19 (3)	21	5,268
Bowel	76 (12)	7	4,087
Gynecological	63 (10)	27	10,435
Miscellaneous	88 (14)	24	9,611
Total	**525 (83)**	**20**	**63,035**

Appropriate clinical follow-up (further diagnostic imaging and lab tests, physician consultations and procedures) were required in 20% at an additional cost of USD 63,035. Additional cost per incidental finding requiring follow-up was USD 407±818. The median cost for follow-up was USD 168 (168-317).

Conclusions: This is the largest study evaluating the economic impact of the incidental findings noted during living kidney donor CT angiograms. Incidental findings during imaging lead to apprehension for the donor and contribute to additional costs. There is significant variability among transplant centers regarding payments for additional testing. Typically if a donor is rejected based on the incidental finding, then the responsibility for further evaluation is dependent on their insurance coverage. Appropriate counseling is warranted prior to evaluation of the living kidney donor.

TH-PO1034

Co-Morbidity Risk Score at Initiation of Renal Replacement Therapy in Living Kidney Donors Who Developed End Stage Renal Disease Amarpali Brar,[1] Rahul Jindal,[2] Moro O. Salifu.[1] *[1]Dept of Medicine, SUNY Downstate Medical Center, Brooklyn, NY; [2]Walter Reed National Military Medical Center, Uniformed Services Univ of Health Sciences, Bethesda, MD.*

Background: Although rare, there are living kidney donors who progress to End Stage Renal Disease (ESRD), but data is limited on co-morbidities at initiation of dialysis in this population.

Methods: We used USRDS (U.S. Renal Database System) retrospectively to identify living kidney donors who progressed to ESRD from 1995-2009. We identified co-morbidities and cause of ESRD in these patients .We also calculated a co-morbidity risk score (CRS) comprising of history of diabetes mellitus, hypertension, ischemic heart disease, cerebrovascular disease and age greater than 55 years, by assigning a value of 1 to each co-morbidity. Data on these variables was obtained from 2728 forms. Analyses were performed using SPSS. Chi square testing for categorical variables and Student's t test for continuous variables.

Results: A total of 70373 transplants were performed in U.S. in the study period .We identified 477 cases using code V59.4 for kidney donor using Medicare claims. Cause of ESRD was listed as diabetes in 104 (21.8%), glomerulonephritis in 154 (32.2%) and polycystic kidney disease in 19 (3.9%) patients. Sixty three percent were male. Race was listed as White in 326, Black in 78 and Asian in 15 patients. Majority were on hemodialysis (73.6%). Mean co-morbidity risk score was 1.138+- 0.91. Mean CRS in Blacks was 1.32+-0.76 versus 1.06+-0.94 in Whites (p=0.33). Mean CRS was significantly higher in diabetics 2.34+-0.76 versus non-diabetic with mean CRS 0.86+- 0.68 (p=0.03).

Conclusions: A small percentage (0.67%) of living kidney donors develop ESRD. We found a high prevalence of de novo diabetes and glomerulonephritis in presumably healthy kidney donors who developed ESRD. These findings may be due to variability in selection criteria of living kidney donors. Prospective studies with uniform selection criteria are needed to assess if long-term follow-up with serial CRS with emphasis on life style modification and early intervention could reduce this risk of ESRD in this population.

TH-PO1035

Malignancy following Live Kidney Donation Hassan N. Ibrahim,[1] Danielle M. Berglund,[2] Robert N. Foley,[3] Arthur J. Matas.[2] *[1]Medicine, Univ of Minnesota, Minneapolis, MN; [2]Surgery, Univ of Minnesota, Minneapolis, MN; [3]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN.*

Background: While living kidney donors are screened for cancer, the risks of cancer development following nephrectomy have not been adequately studied. We therefore sought to determine the incidence of cancer in donors compared to controls.

Methods: In 2003 we began efforts to ascertain the outcomes of all living kidney donors from our institution of which 85% have actively participated with follow-up > 2 years from donation. Information regarding cancer diagnoses was gathered from self-reports, medical records, and death certificates as available. Standardized incidence ratios (SIR) of the various cancer types were compared to the National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER) database.

Results: In our donor population (n=4188), we found non-skin cancer diagnosis in 371 individuals; prostate (n=65), breast (n=46), lung (n=34), colon (n=22), and kidney cancer (n=9). Compared to the SEER data, donors had a 40% lower incidence of breast cancer (SIR = 0.6, 95% CI 0.6, 0.6), 40% lower incidence of lung cancer (SIR = 0.6, 95% CI 0.6, 0.6), and 50% lower incidence of colon cancer (SIR = 0.5, 95% CI 0.5, 0.6). However we observed a 10% higher incidence for prostate cancer (SIR 1.1, 95% CI 1.1, 1.1) and a 60% higher incidence for cancer in the remnant kidney.

Cancer Type	Incidence per 100,000 years (95% CI)	SIR (95% CI)	*p*-value
Remnant Kidney(n=9)	13.4 (12.5-14.3)	1.6 (1.4-1.6)	<0.05
Lung(n=34)	50.7 (49-52.4)	0.6 (0.6-0.6)	<0.05
Colon(n=22)	32.9 (31.5-34.3)	0.5 (0.5-0.6)	<0.05
Prostate (n=65)	97.4 (95.1-99.8)	1.1 (1.1-1.1)	<0.05
Breast, female (n=46)	68.8 (66.8-70.7)	0.6 (0.6-0.6)	<0.05

Conclusions: Donors studied have a lower incidence of breast, lung, and colon but higher incidence of prostate and kidney cancer. These data need to be continued as a larger cohort as the event rate particularly for renal cell cancer is low.

Funding: Other NIH Support - NIAID

TH-PO1036

The Long Term Follow-Up of Renal Dynamics following Living Donor Nephrectomy Colin R. Lenihan, Bryan D. Myers, Jane C. Tan. *Divison of Nephrology, Dept of Medicine, Stanford Univ, CA.*

Background: Over five thousand living kidney donor nephrectomies are performed in the U.S. annually. While the physiological changes early post-nephrectomy are well described, less is known about late post-donation glomerular dynamics.

Methods: We enrolled 21 adult living kidney donors to undergo detailed long term clinical, physiological, morphometric and radiological evaluation before, early (median 0.8 years) and late (median 6.3 years) after kidney donation. Blood pressure was measured using Dynamap. Glomerular filtration rate and renal plasma flow were quantified by urinary clearances of iothalamate and para-aminohippuric acid, respectively. Renal cortical volumes were estimated using either CT or MR imaging.

Results: Parallel increases in single-kidney renal plasma flow, renocortical volume and glomerular filtration rate occurred early and were sustained through to late post-donation. Using the equation derived by Deen et al to model whole kidney ultrafiltration coefficient (K_f), assuming a fixed glomerular transcapillary pressure (ΔP) of 40 mmHg, we found that K_f increased early post-donation and remained elevated late post-donation (figure 1). When we modelled whole kidney K_f based on the observed magnitude of renocortical hypertrophy, we found that post-donation GFR was maintained without any increase in ΔP (figure 2).

Figure 1

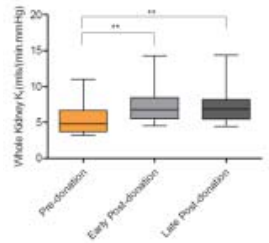
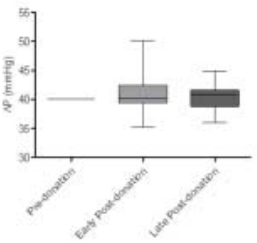


Figure 2



The use of hypertensive medication increased from 14% to 57% late post-donation. No subjects had albuminuria at any time.

Conclusions: We conclude that persistent hyperfiltration post-donation can be attributed exclusively to compensatory renocortical hypertrophy with ensuing glomerulomegaly. The resulting enhancement of glomerular filtration surface area explains K_f elevation which, along with hyperperfusion, accounts for the observed constant level of post-donation hyperfiltration in these donors.

Funding: NIDDK Support, Private Foundation Support

TH-PO1037

Validity of Current Equations to Estimate Glomerular Filtration Rate in Middle Eastern Kidney Donors Post Nephrectomy Osama M. El-Minshawy,[1] Eman Elbassuoni.[2] *[1]Medicine/Nephrology, El-Minia Univ School of Medicine, El-Minia, Egypt; [2]Physiology, El-Minia Univ Schoolf Medicine, El-Minia, Egypt.*

Background: Kidney donation is associated with little bad outcome in living donors, accurate determination of donor kidney function has important long term implications for donor health. **Aim:** is to investigate accuracy of eGFR equations to predict renal function in kidney donors after donation 10±4 month versus measurement of true GFR by diethylene triamine pentaacetic acid (^{99m}Tc- DTPA).

Methods: The study included160 living kidney donors 93 males (58%), GFR was estimated using MDRD, aMDRD, Walser, Nankivell, Cockcroft-Gault, Mayo clinic and CKD-EPI.

Results: All 7 eGFR correlated with ^{99m}Tc- DTPA clearance ($p<0.05$), but their r^2 was low ranging from 0.69 to 0.54. Their respective r^2 values were; MDRD 0.69, CKD-EPI 0.63, aMDRD 0.62, Cockcroft-Gault 0.58, Mayo Clinic 0.55, Walser 0.55, Nankivell 0.54

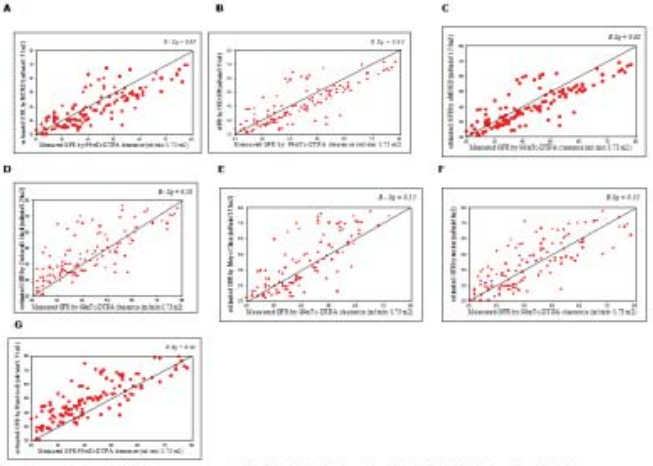


Figure 1: Correlation between measured GFR by ^{99m}Tc- DTPA and estimated GFR by 7 Equations in all Donors

Conclusions: After kidney donation all 7 equations are far from being ideal but eGFR MDRD was superior to other equations in precision and accuracy

	Mean ±SD	Range
Age (years)	37±7	21-50
Weight (Kg)	70±8	52-81
Height (meter)	1.7±0.1	1.51-1.96
BMI Kg/m^2	25±2	19-32
Serum creatinine (mg/dl)	1.1±0.2	0.8-1.6
BUN (mg/dl)	17±3	9-24

Table 1: Donor Data

	Within ± 10% error	Within± 30% error	Within 50% error
MDRD	36%	64%	78%
CKD-EPI	35%	63%	73%
aMDRD	30%	62%	71%
Cockroft-Gault	29%	61%	71%
Mayo Clinic	27%	61%	70%
Walser	25%	58%	68%
Nankivell	25%	58%	67%

Table 2: % of Prediction Error in Equations

GFR in ml/min/1.73m^2	Mean ± SD	Median	Range
True GFR	64±15	49	21-76
eGFR MDRD	60±11	47	23-78
eGFR CKD-EPI	62±13	47	22-74
eGFR aMDRD	61±11	48	20-67
eGFR Walser	66±14	52	20-78
eGFR Cockroft-Gault	68±12	54	24-79
eGFR Mayo Clinic	69±21	51	23-79
eGFR Nankivell	50±18	58	22-80

Table 3: True *^{99m}Tc- DTPA GFR &* eGFR by different equations

TH-PO1038

Abdominal Aortic Calcification in Older Living Kidney Donors: Effect on Graft Function and Histology David Wojciechowski,[1] Benjamin M. Yeh,[2] Meyeon Park,[1] Antonio C. Westphalen,[2] Zhen Jane Wang,[2] En-Haw Wu.[2] *[1]Medicine/Nephrology, UCSF; [2]Radiology, UCSF.*

Background: Many living kidney transplants are from older donors, who have a greater risk of vascular calcification. We assessed the prevalence of abdominal aortic calcification (AAC) in older donors and the effect of AAC on recipient graft function and histology.

Methods: We identified 292 consecutive living donor-recipient pairs with donor age ≥50 between 2003-2014 (mean age 56; range 50-78; F/M: 1.8). Donor AAC was determined by pre-nephrectomy unenhanced CT. Recipient 12, 24, and 36-month eGFR and spot urine protein: creatinine ratios (UPCR) were recorded and compared with student t-test in allografts from donors with versus without AAC. 180 recipients had 6-month protocol biopsies; presence of interstitial fibrosis (IF), tubular atrophy (TA), vascular intimal thickening (cv), and arteriolar hyaline thickening (ah) was recorded. Fisher's exact test was used to compare the percentage of allografts with any abnormal pathology from donors with versus without AAC. Multivariable regression analysis was used to assess the independent predictors (donor AAC, age, gender, recipient age, gender, and tacrolimus level at biopsy) of the 4 categories of abnormal pathology.

Results: AAC was present in 40.7% (119/292) of donors. Donors with AAC were older ($p<0.0001$) and more likely to be male ($p=0.004$). There was no significant difference in eGFR or spot UPCR in recipients of allografts from donors with versus without AAC up to 36-months. On protocol biopsy, there was a higher % of allografts with vc and ah from donors with versus without AAC ($p<0.0001$ for both). Multivariable regression found that presence of donor AAC independently predicts presence of vc (OR:6.3; CI:2.4-16.2) and ah (OR:8.6; CI:2.8-26.1) in allografts at 6 months. Tacrolimus level was not an independent predictor of ah. Donor age independently predicts allograft IF (OR: 1.1; CI: 1.02-1.18) and TA (OR: 1.1; CI: 1.05-1.22).

Conclusions: AAC was common and independently predicts cv and ah in allografts from older donors. The presence of donor AAC may improve prediction of graft histology and may identify recipients who would benefit from a less nephrotoxic immunosuppression regimen.

Funding: Clinical Revenue Support

TH-PO1039

Risk Factors of Impaired Renal Adaptation following Nephrectomy in Kidney Donors Hee Jin Kwon,[1] Do Hee Kim,[1] Kyungho Lee,[1] Seung Yeon Son,[2] Hye Ryoun Jang,[2] Jung Eun Lee,[2] Wooseong Huh,[2] Yoon-Goo Kim,[2] Dae Joong Kim,[2] Ha Young Oh.[2] *[1]Dept of Medicine, Samsung Medical Center, Sungkyunkwan Univ School of Medicine; [2]Nephrology Div, Samsung Medical Center, Sungkyunkwan Univ School of Medicine, Seoul, Korea.*

Background: Compensatory hyperfiltration that occurs in the remaining nephrons following donor nephrectomy was reported as a main mechanism of maintaining adequate renal function in kidney donor. Although postoperative renal function recovers smoothly by early renal adaptation in many donors, some donors show impaired recovery of renal function. The aim of this study was to identify factors predicting the degree of renal adaptation after donor nephrectomy and to identify donors with a high risk of developing chronic kidney disease (CKD).

Methods: A total of 265 living kidney donors receiving donor nephrectomy from January 2010 to October 2013 were retrospectively analyzed. Serum creatinine was serially followed up pre- and post-operative periods (1st, 2nd-3rd, and 4-14th postoperative day, 1st and 3rd-6th postoperative month). Renal function recovery was defined as the percentage of postoperative MDRD-based eGFR versus preoperative eGFR (%MDRD). Post-donation CKD was defined as eGFR measured at 3rd-6th months < 60 ml/min/1.73m^2.

Results: The mean age (M:F= 133:132) was 41.1±9.9 years. During follow-up, 148 donors (55.8%) developed CKD. Donor age (p=0.0115), BMI (p=0.0295), preoperative MDRD-eGFR (p<0.001), and change in eGFR at postoperative day (POD) 1 (p=0.0447) and 1 month after surgery (p=0.0003) compared to baseline were identified as independent predictors of developing CKD. Impaired renal recovery (defined as %MDRD < 66%) positively correlated with age (p=0.0001) and changes in eGFR from baseline to POD 2-3 (p=0.0024) and to 1 month after surgery (p=0.0001). Early renal adaptation (eGFR decline in immediate postoperative period within one month) determined renal function in postoperative 3rd-6th months. Old age, male, and lower residual kidney volume of CT angiography were associated with poor early renal adaptation.

Conclusions: The degree of early renal adaptation may help identify donors at high risk for poor recovery of renal function and development of postoperative CKD.

TH-PO1040

Impact of Obesity on Short-Term Outcomes After Kidney Donation Harini A. Chakkera,[1] Yu-Hui Chang,[1] William Knowler,[2] Hatem Amer,[3] Lilach O. Lerman,[3] Aleksandar Denic,[3] Andrew D. Rule.[3] *[1]Mayo Clinic Arizona; [2]NIDDK, Phoenix; [3]Mayo Clinic Rochester.*

Background: Coexistence of donor obesity and reduced number of functioning nephrons after nephrectomy is a risk for kidney disease. The body mass index (BMI) threshold to exclude an otherwise healthy potential donor is not uniform across transplant centers.

Methods: Cohort study of 1073 living kidney donors between 1999 -2008 at the Mayo Clinic in Rochester, MN. **Aims: 1.** Examine association of glomerular volume (estimated from the biopsy at time of donation) and measures of obesity. Cohort included a subgroup of 20 donors with the largest glomerular volume similar in age and gender to the 20 donors with the smallest glomerular volume –mean age 44 years and 70% female in both groups. Measures of obesity included BMI and pre-donation abdominal CT scan parameters (waist circumference, subcutaneous fat, visceral fat and peri-renal fat). **2**. Among 1073 donors, examine the association of strongest associative measure of obesity identified in Aim 1 and short-term kidney function in donor including GFR (iothalamate clearance), 24-hr urine albumin, and systolic BP in the 1st year after donation.

Results: BMI was most strongly associated with higher glomerular volume compared to all other measures of abdominal obesity: OR=5.03 (per 5 kg/m^2 difference), p=0.002. Thus, we assessed the association of BMI at donation with short-term outcomes in kidney function among 1073 donors (mean follow up 6.8 months), but found no significant association (Table).

BMI	Uncorrected Iothalamate clearance (% change, p value)	Systolic BP (% change, p value)	24 hour urine albumin >20 mg (OR, p value)
< 25 kg/m^2	Referent	Referent	Referent
25-30 kg/m^2	-4.1%, 0.25*	2.3%, 0.06*	1.53, 0.35*
30-35 kg/m^2	-4.5%, 0.26*	-0.05%,0.97*	1.31, 0.61*
≥ 35 kg/m^2	-4.1%, 0.45*	-0.06, 0.97 *	1.95, 0.29*

Models: age and gender adjusted * versus <25 kg/m^2.

Conclusions: Donor BMI is the obesity measure that most strongly associates with underlying glomerulomegaly, but does not clearly affect kidney function in the first year after donation. Long-term follow up is required to ascertain effect of BMI at donation on the renal function.

TH-PO1041

Impact of Donor Renal Reserve on 1-Year Post-Transplant Kidney Graft Function Maria Cora Giordani, Carlos Schreck, Silvia Rosana Groppa, Cesar Andrés Mombelli, Carlos Guido Musso, Nora Cristina Imperiali, Guillermo Javier Rosa Diez. *Nephrology, Hospital Italiano de Buenos Aires, Ciudad Autonoma de Buenos Aires, Argentina.*

Background: Renal reserve is defined as a 20% or larger increase in GFR (glomerular filtration rate) after an acute protein load. Presumably, a higher renal reserve would correlate with a higher capacity to adapt to a diminished nephron mass in kidney transplant receptors. We analyzed the renal reserve in living donors as a predictor of 1 year post-transplant kidney graft function.

Methods: Renal Reserve (> 30% versus < 30%) of living donors (2008-2011) was retrospectively correlated with 1-year post-transplant receptor Scr (serum creatinine). Other variables were used for univariate and multivariate analysis. From donors: age, hypertension, BMI (body mass index) and gender. Receptor variables included: age, gender, BMI, rejection, immunosuppression, missmatch number and DGF (delayed graft function). Statistics: T-test was used for continuous data with normal distribution. Wilcoxon analysis was used for those without normal distribution. Chi^2 test was used for categorical variables. Multivariate regression analysis was done using STATA software. p<0,05 was considered significant.

Results: n= 59 donor-receptor pairs. Data are expressed as mean ± SD. Univariate analysis

	SCr (mg/dl)	SCr (mg/dl)	p
Renal Reserve (>30% vs < 30%)	1.25 +/- 0.52	1.62 +/-0.76	0.02
Donor age (<50 vs > 50 years old)	1.05 +/- 0.37	1.65 +/- 0.66	<0.001
Receptor gender (female vs. male)	1.03+/- 0.17	1.39 +/- 0.23	<0.001
Receptor age (<33 vs >33 years old)	1.22 +/- 0.6	1.5 +/- 0.6	0.04
Rejection (no vs. yes)	1.14 +/- 0.5	1.8 +/- 0.54	<0.001
Receptor BMI (>24 vs < 24)	1.14 +/- 0.42	1.53 +/- 0.7	0.006
DGF (no vs yes)	1.3 +/- 1.1	2.29 +/- 0.87	<0.001

Multivariate analysis (odds ratio-p) for donor age (> 50 years old), receptor gender and rejection were: 8.99-0.042, 19.8-0.02 and 11.9-0.019.

Conclusions: Donor Renal Reserve >30 % correlates with a significantly lower 1 year-post transplant receptor Scr when analyzed separately (univariate analysis). In multivariate analysis, however, renal reserve does not affect 1 year-post transplant receptor Scr.

TH-PO1042

The Biomarker of Deceased Donor for the Prediction of Renal Graft Outcomes Tai Yeon Koo,[1] Jong Cheol Jeong,[1] Hee Jung Jeon,[1] Hyuk Yong Kwon,[2] Sik Lee,[3] Sung-Joo Kim,[6] Seok Ju Park,[4] Kook-Hwan Oh,[5] Curie Ahn,[1,5] Jaeseok Yang.[1] *[1]Transplantation Center, Seoul National Hospital, Seoul, Republic of Korea; [2]Dept of Internal Medicine, BHS-Han Seo Hospital, Busan, Republic of Korea; [3]Dept of Internal Medicine, Chonbuk National Univ Hospital, Jeonju, Republic of Korea; [4]Dept of Internal Medicine, Inje Univ Busan Paik Hospital, Busan, Republic of Korea; [5]Dept of Internal Medicine, Seoul National Univ, Seoul, Republic of Korea; [6]Dept of Surgery, Samsung Medical Center, Seoul, Republic of Korea.*

Background: Acute kidney injury(AKI) negatively impacts on renal allograft outcomes after kidney transplantation(KT). Neutrophil gelatinase-associated lipocalin(NGAL), kidney injury molecule-1(KIM-1), clusterin, and liver-type fatty acid binding protein(L-FABP) are biomarkers known to be associated with AKI. We performed a prospective, multicenter study to evaluate the predictive values of these biomarkers from deceased donor (DD) for renal allograft outcomes including graft recovery and acute rejection(AR).

Methods: We included 59 DDs and their 59 recipients from 4 transplantation centers in Korea (Seoul National University Hospital, Chonbuk National University Hospital, Samsung Medical Center and Inje University Busan Paik Hospital). The biomarkers were detected using donor urine samples before the operations. Graft recovery was classified into immediate graft function, slow graft function(SGF) and delayed graft function(DGF).

Results: The mean age of DDs and recipients were 43±16 and 45±13 years. The incidence of AR and graft loss were 18(31)% and 3(5%), respectively. SGF and DGF were observed in 20 and 14% of recipients. The median levels of NGAL, KIM-1, clusterin, and L-FABP were 6484μg/mgCr, 0.13ng/mgCr, 1.45ng/mgCr and 328ng/mg, respectively. KIM-1(p=0.006) and L-FABP(p=0.008) were significantly higher in patients with AR than those in patients without AR. Receiver operating characteristic curve analysis showed that KIM-1(area under the curve (AUC) 0.74) and L-FABP(AUC 0.73) predicted AR. However, no biomarkers predicted DGF or graft failure.

Conclusions: Biomarkers of AKI such as KIM-1 and L-FABP obtained from donor urine samples can predict adverse renal allograft outcomes after KT.

TH-PO1043

Knowledge, Attitudes, and Counseling Practices for CDC High Infectious Risk Donor Organ Offers: A National Survey of Nephrologists Lauren Marie Kucirka, Mary G. Bowring, Natasha Gupta, Eric Chow, Michael J. Choi, Bernard G. Jaar, Tariq Shafi, Dorry L. Segev. *JHU.*

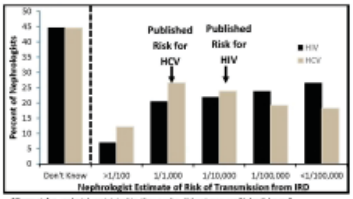
Background: Kidneys from donors classified by the CDC as increased infectious risk (IRDs) comprise >13% of transplants, yet have 1.5-fold higher odds of discard. In considering organ offers, studies show that patients most highly value the opinions of their nephrologists. The goals of this study were to 1) describe nephrologists' knowledge and attitudes towards IRDs, and 2) examine how knowledge influences counseling practices.

Methods: We surveyed 519 nephrologists caring for ESRD patients in Dec 2013. Modified Poisson regression was used to examine associations between nephrologist characteristics, IRD knowledge, and routinely recommending IRD decline (defined as recommending acceptance < 10% of the time).

Results: Of nephrologists who discuss organ offers with their patients, 62% had been asked about an IRD. However, 35.4% reported they are minimally/not familiar with IRDs, and 39% that they are not prepared to counsel a patient regarding an IRD offer. When asked what they would tell a patient about the risk of HIV and HCV transmission from an IRD, >40% answered "I don't know"; among those who said they knew, perceived risk estimates ranged from >1/100 (much higher than published risks) to <1/100,000 (much lower, Figure 1). Likelihood of recommending decline and being uncomfortable giving a recommendation increased with increasing patient age and dialysis vintage(Figure 2). Among those who reported being asked about IRDs, there was wide variation in counseling practices (25.7% never recommend IRD acceptance; 29.5% recommend >80% of the time.) Reporting minimal/no preparation for IRD patient counseling was associated with 1.78 times the rate of routinely recommending IRD decline in adjusted analysis (Table 1).

Conclusions: IRD education for nephrologists is critical to facilitate shared decision making.

Figure 1: Responses to Survey Question, "What would you tell a patient who asked about the probability of contracting HIV and HCV from a kidney donor classified as "IRD/CDC High Risk" who tested negative for HIV by ELISA?



*Percent for each risk restricted to those who did not answer "I don't know"

Figure 2: Responses to Survey Question: "For each hypothetical patient, please indicate whether you would recommend acceptance or decline of a CDC High Risk Donor Kidney"

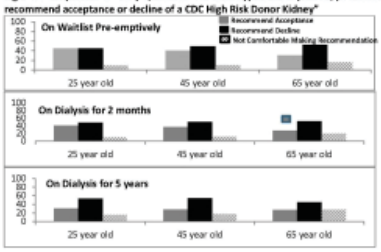


Table 1: Characteristics Associated with Routinely Recommending Decline Among Patients Receiving Kidney Offers from Infectious Risk Donors in a Multivariate Model

Characteristic	IRR (95% CI)	p-value
Gender		
Male	Referent	
Female	1.12 (0.76-1.65)	0.6
Years practicing since fellowship	1.01 (1.00-1.03)	0.1
Currently completing/have completed Transplant Nephrology Fellowship	0.52 (0.29-0.92)	0.03
Affiliated with University Hospital Performing Transplants	0.87 (0.61-1.25)	0.4
Not at all or minimally prepared to counsel a patient on acceptance or decline of an IRD-KT offer	1.78 (1.23-2.57)	0.002

Funding: NIDDK Support

TH-PO1044

"Expanding the Donor Pool": Successful Use of Cancer Survivors as Kidney Donors Alekhya Potluri,[1] Gregory Malat, PharmD,[1] Oleg Grapp,[1] Sharon M. West,[2] Karthik M. Ranganna,[1] Alden Michael Doyle.[1] *[1]Transplant Nephrology, Drexel Univ College of Medicine, Philadelphia, PA; [2]Gift of Life Donor Program, Philadelphia, PA.*

Background: There is a burgeoning disparity between supply and demand for transplantable kidneys, with over 100,000 patients on the waitlist this year. One way to address this shortage is to broaden the acceptance criteria for suitable donors. Herein, we report a long term follow up of a large series of patients transplanted with kidneys from donors that were felt to be marginal based on a history of documented treated cancer.

Methods: We retrospectively examined 125 kidney transplant recipients from our local Region 2 Organ Procurement Organization, who received an allograft from 72 different deceased donors who had been previously treated for cancer. We used descriptive data analysis to compute our results.

Results: Donors were 68% female, with median age of 56.5 years. Donors had a variety of cancers including breast, prostate, cervical, uterine, lymphoma, melanoma, bladder and thyroid. All donors were felt to be cancer-free at the time of their death. Median kidney donor risk index was 1.35. Median for number of turndowns by patients for cancer donor kidneys offered was 122.5. Of the 144 kidneys from 72 donors, only 125 have been transplanted. Recipients were 55% males with median age of 62 years. Unadjusted median patient and graft survival post-transplant were 47.29 and 42.37 months respectively. 36.6% had delayed graft function, 6.6% patients experienced rejection leading to allograft loss. 7.5% had pre transplant and 6.7% had post-transplant malignancies, all of which were unrelated to donor pathology. Of these, 3 patients died of complications of this malignancy. 24.2% patients have since died due to other causes.

Conclusions: We believe that these data represent successful transplantation with donors that would commonly be turned down based on their history of malignancy. Although not definitive, this experience adds to what has been previously reported and suggests that there may be an opportunity to further broaden our current acceptance criteria to best utilize the scarce resource of suitable transplant organs.

TH-PO1045

Kidney Donor Profile Index: The Older You Are, the Harder It Gets Ankit P. Shah,[1] Amay Parikh,[1] Michelle Gagnon,[2] Sheenu Chandwani.[2] *[1]Dept of Medicine / Div of Nephrology, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ; [2]Public Health, Rutgers-School of Public Health, New Brunswick, NJ.*

Background: The Kidney Donor Profile Index (KDPI) as part of the new Organ Procurement and Transplantation Network allocation scheme raises concerns given its unproven advantages. This study examines the association between KDPI, mortality and graft failure, and explores its potential negative impact for older recipients.

Methods: United Network for Organ Sharing data on adult transplants from January 2004 to December 2009 receiving deceased donor kidneys were analyzed. KDPI was calculated where a lower percentage predicts better graft survival. Estimated Post-Transplant Survival (EPTS) was calculated where a lower percentage predicts better recipient survival. Associations of ≤20 versus >20% KDPI (BTRK versus WRSK) and <50 versus ≥50 years of age (YNGR versus OLDR) with patient mortality and graft failure were examined using Cox proportional hazards models.

Results: A total of 51,434 recipients were included (WRSK 79.3%). Among OLDR patients, the unadjusted hazard ratio (HR) for mortality for WRSK versus BTRK was 1.5 ($p<0.001$). Compared to BTRK/YNGR, HRs were BTRK/OLDR 1.5 (1.3, 1.8), WRSK/YNGR 1.2 (1.02, 1.41), and WRSK/OLDR 1.9 (1.6, 2.3) for mortality ($p<0.05$) after adjusting for race, gender, expanded criteria donor status, EPTS, and treatment for rejection within 1 year post transplant. There was no significant interaction by age. Graft survival was significantly higher for BTRK irrespective of age (p=NS); however WRSK/YNGR had better graft survival than all OLDR ($p<0.05$). Applying the new allocation scheme, an additional 9,176 YNGR candidates would be eligible for BTRK kidneys, and an additional 3,372 OLDR candidates would be eligible for WRSK kidneys.

Conclusions: Allocating better functioning kidneys to the younger may limit transplants for older recipients. This new scheme widens the gap of potential recipients favoring either YNGR/BTRK or OLDR/WRSK pairings. Older patients are more likely to experience poor outcomes despite KDPI levels. Under the new scheme, transplant centers cognizant of outcome measures will be less likely to transplant this population.

TH-PO1046

The Impact of Zero Time Biopsy on Graft Outcomes after Deceased Donor Kidney Transplantation Ana C. Matos,[1] Niels O.S. Camara,[1,2] Lucio Roberto Requiao-Moura,[1] Marcelino Souza Durao,[1,3] Alvaro Pacheco-Silva.[1,3] *[1]Renal Transplantation Unit, Hospital Israelita Albert Einstein, Sao Paulo, Brazil; [2]Immunology, Univ of São Paulo, Sao Paulo, Brazil; [3]Renal Transplant Unit, Univ Federal de Sao Paulo, Sao Paulo, Brazil.*

Background: Zero-time biopsy (T0-Bx) provides important information about the organ quality; however its role as acute or chronic renal function predictor is not established.

Methods: We analyzed data from 136 T0-Bx in deceased transplant patients realized from 11/2008 to 5/2012. Patients had a follow up of at least 6 months. We analyzed presence of ATN, arteriolar hyalinosis (AH), intimal thickness (IT), interstitial fibrosis (IF) and glomerulosclerosis (GS). We also analyzed the impact of donor features with the following outcomes: delayed graft function (DGF), renal function measured by CrCl at hospital discharge, 6 and 12 months and chronic allograft dysfunction (CAD) defined as CrCl < 60ml/min.

Results: We studied 136 patients with a mean follow up of 805 days. The age of donors were 41 yr, 26% were extended criteria donors (ECD), 33% had hypertension and 50% had cerebral vascular accident (CVA) as the cause of death. ATN was present in 87% of these bxs, AH in 31%, IF in 21% and GS >10% in 7%. DGF occurred in 80% and CAD in 53% of our patients. No histological finding at zero-time biopsies was associated with DGF. CrCl at discharge and at 6 mo had no association with histological findings. Patients with AH had a lower CrCl at 12mo, compared to patients without it (49.8 ml/min x 64.5 ml/min, p=0.02). In a multivariate analysis, variables associated with CAD at one year were acute rejection OR:8,9 IC 95%: 2,2-35,9, p=0,002, HA OR: 3,9 IC 95%: 1,4-10,7, p=0,007, male gender OR:3,2 IC 95%: 1,2-8,2, p=0,018 and donor hypertension OR:2,9 IC 95%: 1,1-7,8, p=0,031. In multivariate analysis, risk factors for AH were donor age > 50 yr OR:2,4 IC 95%:1,1-5,4, p=0,03 and CVA as the cause of death OR:2,3 IC 95%: 1,0-5,1 p=0,04.

Conclusions: Vascular abnormalities were associated with a one year poorer renal function. T0-Bx can be useful to manage immunosuppression in order to improve long term graft function.

TH-PO1047

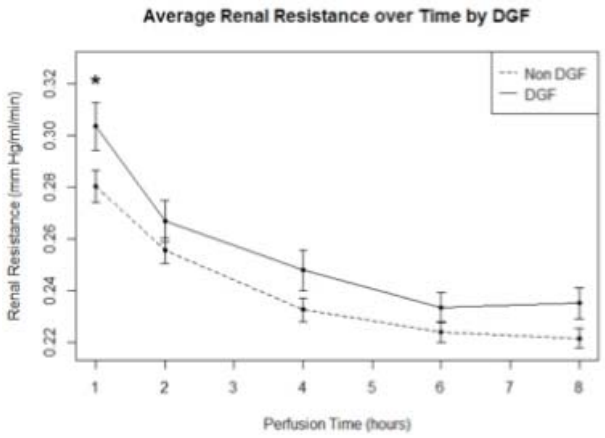
Association of Perfusion Parameters with Kidney Allograft Function after Deceased-Donor Kidney Transplantation Ronik S. Bhangoo,[1] Peter P. Reese,[2] Isaac E. Hall,[3] Mona D. Doshi,[4] Francis L. Weng,[5] Bernd Schroppel,[6] Chirag R. Parikh.[3] *[1]Univ of Arizona College of Medicine, Phoenix, AZ; [2]Univ of Pennsylvania, Philadelphia, PA; [3]Yale Univ, New Haven, CT; [4]Wayne State Univ, Detroit, MI; [5]Barnabas Health, Livingston, NJ; [6]Univ Hospital, Ulm, Germany.*

Background: Marginal quality kidneys are often placed on a pump to improve graft outcomes. We conducted a prospective multi-center observational study to determine the relationship between machine perfusion parameters and allograft outcomes after deceased-donor kidney transplantation.

Methods: Deceased donors were enrolled at four organ procurement organizations. Pump parameters (renal resistance and perfusate flow) were recorded at five time-points (1^{st}, 2^{nd}, 4^{th}, 6^{th}, and 8^{th} hour) or until the kidney was prepared for transplantation. Univariate and multivariate analyses (adjusted for the Kidney Donor Risk Index) were performed to determine the effect of perfusion parameters on delayed graft function (DGF, defined as dialysis in the first week) and one year graft failure.

Results: 748 kidneys from 405 donors were analyzed. 34% of kidneys met criteria for DGF (n=258) and 5% for one year graft failure (n=39). Renal resistance at one hour was significantly higher in the DGF group (0.28 versus 0.26 mmHg/ml/min, p=0.033) and decreased over time for both DGF and non-DGF recipients (Figure 1). Perfusate flow at one hour was significantly lower in the DGF group (100 versus 105 ml/min, p=0.016). In multivariable analysis, renal resistance at one hour demonstrated an independent association with DGF (relative risk 1.89 [1.01-3.55]). At no time point was renal resistance or perfusate flow significantly associated with one year graft failure.

Conclusions: Renal resistance at one hour is an independent risk factor for DGF in deceased-donor kidney transplantation, but is not associated with graft failure at one year.



Funding: NIDDK Support

TH-PO1048

Machine Perfusion following Static Cold Storage in Kidney Transplantation Decreases the Duration of Delayed Graft Function and Time to Hospital Discharge Alvaro Pacheco-Silva,[1,2] Marcelino Souza Durao,[2] Lucio Roberto Requiao-Moura,[2] Ana C. Matos.[2] *[1]Laboratory of Clinical and Experimental Immunology, /Nephrology, Escola Paulista de Medicina- UNIFESP, Sao Paulo, Brazil; [2]Transplantation, Hospital Israelita Albert Einstein, Sao Paulo, Brazil.*

Background: In Brazil the incidence of DGF is very high (60-70%) mainly due to an inadequate care of the donors and long cold ischemia time. This high incidence of DGF is associated to a longer hospitalization and poorer long term graft survival.

Methods: The objective of this work is to analyze the incidence of DGF, its duration and the time of hospitalization after transplantation in patients who received a kidney preserved in the machine perfusion (MP Group) after a long time of cold storage.

Results: We report the data from forty kidneys from DD preserved in the MP transplanted from 2/2013 to 12/2013 and compare their evolution to 136 kidney transplants preserved by Cold storage (Control Group), realized from 11/2008 to 5/2012 at our hospital. Results: The mean total ischemia time was 32,8±6,5 (22,7±5,1 CS plus 10±2,1 MP) for MP and 22,7±4,8 for Control Group (P<0,0001). Donor age (46,5 x43 Y), Creatinine (1,35 X 1,30) and death by stroke (57,1% X 50%) were not significantly different between MP and Control group patients. DGF incidence was 62,5% for MP compared to 76% in the control group (P= 0,14). However, DGF duration (days on dialysis) was 3,8 in the MP compared to 10 days in the control group (p=0,021). The hospital discharge was 13,8 days after transplantation for the MP and 19 days for the control group ((p=0,011) and the mean creatinine at discharge was 2,14 for MP and 2,6 for the control group.

Conclusions: In conclusion, in a group of patients with a high incidence of DGF the use *of MP after cold storage did not decrease DGF but contributed to a faster recovery of renal function and to a shorter time of hospitalization.*

Funding: Government Support - Non-U.S.

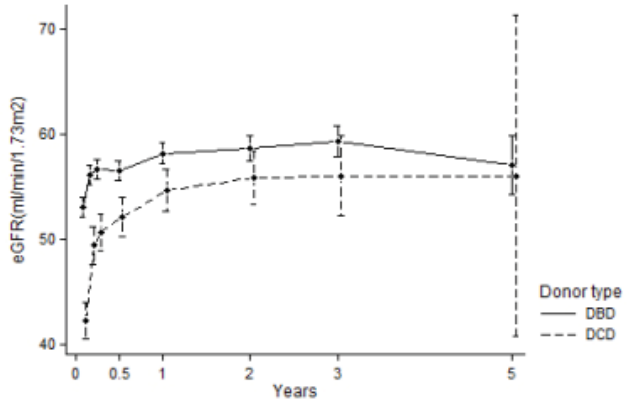
TH-PO1049

Outcomes af Donation after Cardiac Death Kidney Recipients in Australia Vinoi George David,[1] Kristine De Jesus,[2] Philip A. Clayton,[3] Richard Allen,[4] Stephen P. McDonald.[1,3] *[1]Renal Unit, Royal Adelaide Hospital, North Terrace, Adelaide, SA, Australia; [2]Univ of Sydney, Sydney, NSW, Australia; [3]ANZDATA Registry, Adelaide, SA, Australia; [4]Renal Unit, Royal Prince Alfred Hospital, Sydney, NSW, Australia.*

Background: A growing discrepancy between demand for organs and their availability from donors after brain deaths(DBDs) has led to an increase in the use of donors after circulatory death(DCDs) in Australia.We examined trends in use and outcomes of DCD kidney transplants.

Methods: Retrospective review of the ANZDATA registry cohort over 2007-12. Descriptive statics,linear models and Cox regression were used for the analysis.

Results: Of 2976 transplants over 2007-12, 542(18%)of were DCDs when compared to less than 2% of DCDs in the preceding 5 years. Compared to DBDs,DCDs were predominantly male(70 versus 55%, p<0.01),fewer diabetic donors (4.6%) and higher rates of cerebral hypoxia as cause of death.Donor creatinine at admission was similar to DBD,but terminal creatinine was significantly lower (median 74 versus 69 umol/l,ranksum p<0.05).Recipients of the DCDs had a longer waiting period (median 3.6 versus 3.1 years from first dialysis, p<0.01) for transplant, similar HLA and total ischemic time but had significant higher rates of delayed graft function (dialysis post transplant 53 versus 20%, p<0.01).Compared to DBD kidneys, mean eGFR of DCD recipients kidneys at 1,2,3,6,12 months(p<0.01) was significantly lower.The difference waned by 2 years (p=0.06).



There was no difference in rejection(21 versus 20%),patient and graft survival.In multivariate analysis,DCD remained a predictor for lower eGFR.Survival analyses of graft failure did not show a significant difference,p=0.22.

Conclusions: DCD kidneys were associated with higher rates of delayed graft function and lower eGFR in the first 2 years.There was no difference observed in graft survival,however this may become apparent with a longer duration of follow up.

TH-PO1050

HLA -DP Mismatch among the Zero HLA -A, -B, and -DR Mismatched Deceased Donor Kidney Transplant Population Is Associated with Higher Risk of Both Graft Rejection and Death Censored Graft Loss Nissreen S. Elfadawy,[1] Stuart M. Flechner,[1] Peter Lalli,[2] Jesse D. Schold,[1] Emilio D. Poggio,[1] Richard A. Fatica,[1] Saul Nurko,[1] Brian R. Stephany,[1] Medhat Askar.[1] *[1]The kidney Transplant program, Cleveland Clinic, Cleveland, OH; [2]Histocompatibility and Flow Cytometry Laboratory, Carolinas Healthcare System, Charlotte, NC.*

Background: Zero HLA mismatch (0MM) is defined by UNOS as the absence of HLA-serologic level mismatches at HLA-A, B, and DR antigens (Ag), and results in better graft outcomes after kidney transplantation. It is unclear whether mismatches at HLA- Cw, DQ or DP also have an independent impact on graft outcome.

Methods: In this retrospective study, we identified 265 recipients who had shared 0MM deceased donor (DD) kidney transplants at our center (1990-2012). The study population was classified according to HLA –CW, -DQ, -DP allele mismatch to investigate the isolated impact of mismatch on graft outcomes. Kaplan–Meier plots and log-rank tests were used to determine incidence of biopsy-proven acute graft rejection and graft loss in the different groups.

Results: The prevalence of HLA –CW, -DQ, and –DP mismatch in our 0MM cohort was 27%, 12%, and 77% respectively. HLA –DP mismatches were an independent risk factor for graft rejection (12 versus 3%, HR 4.0, 95% CI 1.1-26.1, p=0.03), death censored graft loss (26 versus 18%, HR 1.9, 95% CI 1.1-3.9, p=0.03), and shorter graft half-life (6 versus 8 years, p 0.01); after adjusting for other HLA-loci mismatches and confounding factors such as previous transplant history and degree of sensitization prior to transplantation. There was no association between groups stratified by HLA –CW, or -DQ mismatch with graft outcomes.

Conclusions: These results suggest that the majority of HLA -A, -B, -DR 0MM transplants are mismatched for HLA -DP. Our findings provide evidence to suggest that HLA –DP matching in renal transplant is clinically relevant and prospective typing and matching warrant further prospective investigation.

TH-PO1051

What Kinds of Graft/Body Size Mismatches Do Influence the Renal Outcomes of Recipients and Donors? Mikiko Yoshikawa, Kentaro Nakai, Shunsuke Goto, Hideki Fujii, Shinichi Nishi. *Div of Nephrology, Kidney and Dialysis Center, Kobe Univ Graduate School of Medicine, Kobe, Hyogo, Japan.*

Background: Several previous reports proved body size mismatches between graft and recipient or graft and donor affected the post-operative graft function. However, which parameters can precisely predict the deterioration of graft and donor remnant kidney function has not been clearly elucidated yet.

Methods: We performed a retrospective cohort study including 60recipient and donor pairs who received living kidney transplantation at our center from April 2003 to March 2013 and surveyed their post-operative clinical courses focusing on the mismatches between graft and recipient or graft and donor body sizes.

Results: Donor body weight (DBW), BSA (DBSA), and BMI (DBMI) were significantly correlated with graft weight. Among them, DBW showed the strongest correlation with graft weight (GW) (r= 0.714, p<0.001). DBW/RBW, DBSA/RBSA, DBMI/RBMI, and GW/ recipient body sizes revealed statistically significant correlation with recipients' ΔeGFR within post-operative 1year. Among them DBW/RBW showed the most significant negative correlation with recipients' ΔeGFR. (r= 0.469, p<0.001). Multiple regression analysis also proved that DBW/RBW was the most significant independent predictor of recipients' ΔeGFR. (β=–13.8, 95% confidence interval: –6.7 to –21.0, p<0.001). On the donor side, the lower GW/donor body size highly developed to CKD stage 3.

Especially, GW/DBMI < 8.0 (g/Kg/m^2) of donor was a high risk marker suggesting the progression to CKD stage 3 (P=0.001). Cox regression analysis also showed that GW/DBMI < 8.0 (g/Kg/m^2) was an independent risk marker to the progression of CKD stage 3 (HR=2.09, 95% confidence interval: 1.105 to 3.947, p=0.023). Recipient / donor body sizes or GW / donor sizes had no significant relationship with proteinuria.

Conclusions: DBW/RBW and GW/DBMI were the best predictors of graft and donor kidney function in our cases. Obesity and body weight control of recipient and donors before kidney transplantation is very important for prevention of recipient and donor size mismatches.

TH-PO1052

Association Between Preconception GFR and Pregnancy Outcomes in Female Kidney Transplant Recipients Serban Constantinescu,[1] Peter Axelrod,[1] Lisa Coscia,[2] Michael J. Moritz,[3] Vincent T. Armenti.[2] *[1]Temple Univ School of Medicine; [2]Gift of Life Inst, Philadelphia, PA; [3]Lehigh Valley Health Network.*

Background: Graft function may influence pregnancy outcomes in kidney recipients.

Methods: Data were collected by the National Transplantation Pregnancy Registry via questionnaires, phone interviews and hospital records. We analyzed 984 singleton pregnancies in 695 kidney recipients, divided in 4 groups according to preconception eGFR (MDRD). Analyses for linear trends were done by Chi square and least squares regression.

Results: With stepwise decrease in GFR, there was a significant increase in rejection and hypertension during pregnancy and graft loss within 2 yrs post-pregnancy. In parallel, there was a significant decrease in live births, and increased prematurity, low and very low birthweight and miscarriages.

	GFR ≥60	GFR≥45 to <60	GFR≤30 to <45	GFR <30	p value
Recipients	241	237	174	43	
MATERNAL FACTORS					
Pretransplant pregnancy	25.5%	30.6%	31%	42%	0.04
Age at transplant (yrs)	23.9±6.2	24.8±6.3	23.1±5.8	24.8±5.5	NS
Planned pregnancy	61.3%	67.1%	62%	60.5%	NS
Transplant to conception interval (yrs)	5.6±4.2	5.4±4.1	5.4±3.9	5.8±4.5	NS
Treated hypertension during pregnancy	41.9%	51.5%	59%	79.6%	<0.001
Preeclampsia	27.1%	24.7%	31.3%	50%	NS
Treated rejection during pregnancy	0.3%	1.8%	2.1%	6.1%	0.004
Graft loss within 2 yrs	2.5%	5.1%	6.1%	28.6%	<0.001
PREGNANCY OUTCOMES	359	331	245	49	
Miscarriage	10.3%	18.1%	15.5%	22.5%	0.006
Live births %(n)	87.5% (314)	78% (258)	82% (201)	71.4% (35)	0.003
Mean gestational age (wks)	36.8±2.9	36.2±3.3	35.6±3.4	34.2±3.1	<0.001
Prematurity (<37 wks)	38%	43%	57%	77%	<0.001
Mean birthweight (g)	2822±685	2654±742	2420±743	2022±724	<0.001
Low-birthweight (<2500 g)	25.8%	36.8%	47.8%	80%	<0.001
Very low-birthweight (<1500 g)	5.1%	7.4%	14.9%	23%	<0.001
Cesarean section	56.9%	52%	45.8%	60%	NS

Conclusions: Progressive decrease in preconception GFR is associated with increased maternal complications, graft loss, and adverse fetal outcomes. Graft function preconception should guide transplant professionals in counseling female kidney recipients contemplating pregnancy.

Funding: Pharmaceutical Company Support - Astellas Pharma US, Inc., Bristol-Myers Squibb Company, Pfizer Inc.

TH-PO1053

Living Donor Kidney Transplantation (LDKT) from HBsAg (+) Donor to HBsAg (-) Recipient with or without Anti-HBs Jin M. Kong, Hyuk Yong Kwon, Sung Hyun Son. *Internal Medicine, Han Seo Hospital, Pusan, Korea.*

Background: HBsAg positivity is currently regarded as a contraindication of kidney donation to HBsAg(-) recipients due to the risk of HBV transmission. Donor organ shortage is serious on this planet. The use of the HBsAg(+) donor organ may help to increase the opportunity of transplantation. We developed a protocol that enables LDKT from a HBsAg (+) donor to a HBsAg(-) recipient.

Methods: 1) Hepatitis B vaccine in recipients without protective titer (≥10 mIU/ml) of anti-HBs 2) Entecavir in donors with detectable HBV DNA in serum to reduce the viral load for 1-2 months before transplantation 3) HBIG in recipients on transplant day 4) Entecavir prophylaxis in recipients for 2-3 post-transplant months 5) Monitoring of HBV transmission in recipient.

Results: Seven LDKT from a HBsAg(+) donor to a HBsAg(-) recipient in 6 patients. In one case, anti-HBs of the recipient was negative, which was converted to positive (15mIU/ml) by HB vaccine before transplantation. All recipients had undetectable HBV DNA after LDKT and maintained HBsAg(-)/anti-HBs(+) status throughout the median follow up of 32(2-52) months.

Conclusions: Kidneys from HBsAg (+) living donors can be safely transplanted to HBsAg (-) recipients with or without anti-HBs.

TH-PO1054

Physical Activities Does Not Recover in 6 Months after Living Kidney Donor Transplantation Makoto Tsujita, Yoshihiko Watarai. *Transplant Surgery, Nagoya Daini Red Cross Hospital, Japan.*

Background: Since renal dysfunction and immunosuppressive drugs cause muscle degeneration, physical activities decrease in CKD, dialysis patients and transplant recipients. In addition, elderly kidney transplant recipients are increasing in Japan, so improving physical activities and increasing muscle strength and volumes are important to prevent fracture and fall accident.

Methods: Consecutive 41 patients older than 40 years were enrolled in this study from 2012. Immunosuppression basically contains steroid, cyclosporine or tacrolimus, and mycophenolate mofetil or everolimus. Parameters of physical activities such as hand grip, SMI (skeletal muscle index) using by DEXA(dual-energy x ray absorptiometry) and CPX(Cardio Pulmonary exercise test) were measured and compared at prior and 6 months after kidney transplantation. Recipients were encouraged to do daily exercise for 30 to 60 minutes everyday.

Results: There were not significant differences between male and female demography.

	Male (n = 22)	Female (n = 19)
Mean age (year)	55.2	53.7
Preemptive kidney transplantation (n)	11(50%)	10 (53%)
Mean duration of dialysis (month)	34.8	49.7
Diabetes mellitus (n)	7 (32%)	3 (16%)
Cyclosporine use (n)	10 (45%)	12 (63%)
Tacrolimus use (n)	12 (55%)	7 (37%)
Mycophenolate mofetil use (n)	16 (73%)	15 (79%)
Everolimus use (n)	6 (27%)	4 (21%)

Hand grip increased at 6 months, but any other tests did not improve after transplantation in male patients. BMI and SMI decreased at 6 months but any other tests was unchanged in female patients(Table2).

Table 2 Results of this study for male

	Male (n = 22)			Female (n = 19)		
	Baseline	6 months after transplantation	P value	Baseline	6 months after transplantation	P value
Body weight (kg)	63.6±12.8	63.2±11.8	0.72	50.0±8.1	49.9±8.7	0.85
BMI (kg/m^2)	22.4±3.4	21.9±3.3	0.33	20.4±3.2	19.6±3.3	< 0.05
Body fat mass (kg/m^2)	4.8±3.7	4.6±2.5	0.18	5.2±2.2	5.4±2.1	0.55
SMI (kg/m^2)	7.1±0.8	7.0±0.9	0.65	5.7±0.8	5.1±0.8	< 0.05
Hand Grip (kgw)	28.0±9.4	30.0±11.1	< 0.05	20.1±5.9	20.8±8.0	0.67
Peak VO2 (ml/kg/min)	17.8±4.5	18.4±4.9	0.33	16.1±4.4	16.5±9	0.43
BMI : body mass index						

Physical activities seemed rather worse after transplantion in female patients.

Conclusions: In both male and female recipients, physical activities do not seem to recover in 6 months after kidney transplantation. More interventions and more studies are needed to know the effect of daily exercise on physical activities.

TH-PO1055

HLA Gene Dosage Effect on Renal Allograft Survival Ji-Eun Lee,[1,2] Eugene Kwon,[1,2] Jeung-Min Park,[1,2] Hee-Yeon Jung,[1,2] Ji-Young Choi,[1,2] Jang-Hee Cho,[1,2] Sun-Hee Park,[1,2] Yong-Lim Kim,[1,2] Chan-Duck Kim.[1,2] *[1]Dept of Internal Medicine, Kyungpook National Univ School of Medicine, Daegu, Republic of Korea; [2]Clinical Research Center for End Stage Renal Disease, Republic of Korea.*

Background: Matching for the HLA-A, B and DR antigens has a beneficial long-term effect on renal allograft survival. However, gene dosage effect of mismatched HLA on transplant outcomes has not been known. We investigated influence of HLA gene dosage on allograft survival in kidney transplant recipients (KTRs).

Methods: We analyzed HLA typing of KTRs and kidney donors at Kyungpook National University Hospital from January 1982 to December 2012. KTRs were divided into two groups: recipients from homozygous HLA donor and those from heterozygous HLA donor. Death-censored graft survival of KTRs was compared according to allele state of kidney donors.

Results: 697 KTRs were enrolled in the study. According to the Kaplan–Meier analysis, mean survival of recipients from homozygous HLA-DR donor and homozygous HLA-B donor was 14.7 and 6.4 years compared to 16.9 and 20.1 years in those from heterozygous HLA-DR donor and heterozygous HLA-B donor respectively. In the multivariate Cox's proportional hazards model, homozygosity of HLA-DR gene and HLA-B gene was an independent risk factor for death-censored graft survival (hazard ratio [HR], 3.521 {95% confidence interval [CI], 1.227-10.104}; P=0.019 for HLA-DR gene, HR, 6.093 {95% CI, 1.305-28.454}; P=0.022 for HLA-B gene). Death-censored graft survival was not associated with HLA-A and HLA-A, B, DR allele states.

Conclusions: Allograft survival was influenced by gene dosage of HLA, especially DR and B. As well as HLA mismatch, allele state of donor was also considered to predict transplant outcomes.

Funding: Government Support - Non-U.S.

TH-PO1056

The Role of Hemodialysis Social Networks in Transplantation: Five Year Follow Up of an Urban Hemodialysis Cohort

Akshta Pai, Avrum Gillespie. *Nephrology, Hypertension and Kidney Transplantation, Temple Univ Hospital, Philadelphia, PA.*

Background: Racial and socioeconomic disparities are well known barriers to kidney transplantation (KT), especially among minority populations. Emerging evidence suggests social networks exist within hemodialysis clinics. The role of these social networks on KT is not yet fully understood.

Methods: A convenience sample from two urban hemodialysis clinics in Philadelphia resulted in a cohort of 116 English-speaking patients. The survey was conducted between July 2008 and December 2008 using the Dialysis Patient Transplant Questionnaire (DPTQ) to address barriers to KT. The survey was self administered by 29% of the cohort. Clinical and transplant data were extracted from the electronic medical record. Data was analyzed using independent t-tests, Fischer's exact test, and chi-square analysis.

Results: The mean age at enrollment was 58-years-old (22-87). Eighty seven percent self identified as non-Hispanic black, 9% as Hispanic, and 1% as White or other. Over a 5-year period, 12% of the cohort received a KT. The majority of the KT recipients (73%) were listed on the transplant list at the time of the interview (p=0.001). The rate of transplant was 11% among non-Hispanic blacks, and 40% among Hispanics (p=0.003). When asked about the likes and dislikes of hemodialysis, patients who received a KT liked the social aspect of hemodialysis (p=0.049) and did not like the physical benefits of hemodialysis treatment (p=0.031). There were no differences in the external social networks of patients who received a KT. There were also no racial/ethnic differences in liking the social aspects of hemodialysis.

Conclusions: In this cohort of urban patients with a low transplant rate, the hemodialysis clinic social network seems to provide a positive role toward transplantation. The dichotomy of transplant rates within the same hemodialysis clinic social network among Hispanics versus non-Hispanic blacks requires further study in order to help develop effective interventions.

TH-PO1057

The Effects of Organ Procurement Organization Network Centrality on Kidney Discard and Transplant Outcomes

Neel Butala,[1] Peter P. Reese,[2] Richard Formica,[3] Chirag R. Parikh.[3] *[1]Massachusetts General Hospital; [2]Perelman School of Medicine; [3]Yale School of Medicine.*

Background: Given growth in kidney transplant waiting lists and discard rates, donor kidney acceptance is an important problem. We hypothesize that kidneys may be more likely to be accepted when OPOs form more relationships that can lead to regular exchange of organs. We apply the novel tools of social network analysis to examine whether OPO network properties have an effect on discard rates and recipient outcomes.

Methods: We identified 106,160 deceased-donor kidneys recovered for transplant from 2000-2010 in SRTR. We constructed the transplant network by year with each OPO representing a node and each kidney-sharing relationship between OPOs representing a directed tie between nodes. Primary exposures were OPO out-degree centrality and in-degree centrality. Primary outcomes were discard, cold-ischemia time, delayed graft function, and 1-year graft loss. We constructed multivariable regression models, restricting analysis to the 50% of OPOs with highest discard and stratifying remaining OPOs into 2 groups by kidney volume. Models controlled for kidney donor risk index, waitlist time, and year/region dummies.

Results:

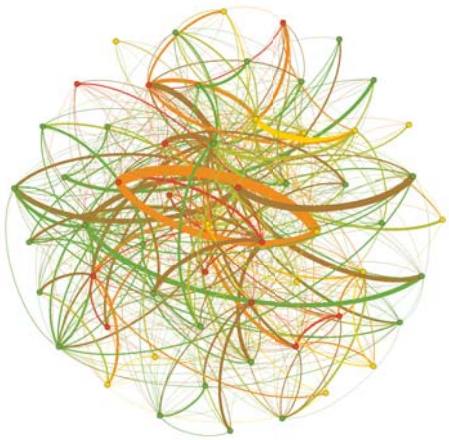


Figure 1 shows the 2010 kidney transplant network with edge size weighted by sharing volume. Among OPOs procuring high kidney volume, an increase in one additional OPO to which a kidney was given or from which a kidney was received by a procuring OPO in a year was associated with a 2% lower likelihood of discard for a given kidney (OR:0.979, CI:0.967,0.991 and OR:0.978, CI:0.963,0.994, respectively) with mixed associations with recipient outcomes.

Conclusions: Our study highlights the value of social network analysis in revealing how broader kidney sharing between OPOs may lead to greater organ acceptance. We conclude interventions to promote broader inter-OPO sharing could be developed to reduce discard.

TH-PO1058

UCF-101 Protects against Cold Storage Induced Renal Tubular Cell Apoptosis

Swati Jain, Daniel Keys, Charles L. Edelstein, Alkesh Jani. *Renal, Univ of Colorado, Aurora, CO.*

Background: Delayed graft function (DGF) independently predicts reduced 5 yr kidney transplant survival. Cold ischemia (CI) is a major risk factor for DGF and results in renal tubular cell (RTEC) apoptosis, but the mechanism by which this occurs is unknown. RTEC apoptosis of donor kidneys predicts the development of DGF. Our published data show that mouse kidneys subjected to CI have significantly increased RTEC apoptosis and cleaved caspase-3 (CC3). X-linked inhibitor of apoptosis (XIAP) is the most potent, naturally occurring inhibitor of CC3. UCF-101 is a chemical inhibitor of XIAP degradation. We hypothesized that (a) CI leads to reduced XIAP resulting in increased CC3 and RTEC apoptosis (b) Treatment of donor kidneys with UCF-101 will prevent activation of CC3 and RTEC apoptosis.

Methods: RTEC apoptosis, XIAP, and caspase-3 protein and activity were examined (a) *in vivo* in C57BL/6 mouse kidneys exposed to 24 hrs of CI with and without 100 uM UCF-101 (b) *in vitro* in LLCPK cells subjected to CI in UW solution with and without 50 uM UCF-101. Apoptosis *in vivo* was quantified by a nephropathologist in a blinded fashion. Annexin V and PI staining was used to evaluate apoptosis *in vitro* by flow cytometry.

Results: Kidneys exposed to CI *in vivo,* and RTEC exposed to CI *in vitro* had significantly decreased XIAP expression and increased CC3 protein, caspase-3 activity and apoptosis. UCF-101 treatment during CI (a) increased XIAP expression (b) decreased CC3 protein, caspase-3 activity and apoptosis.

	In vivo			***In vitro***		
	control	CI	CI+UCF-101	control	CI	CI+UCF-101
CC3	+	+++	+	ND	+++	+
XIAP	++	+	+++	+++	+	+++
caspase-3 activity (fold change)	1±0.1	10±0.01*	5±0.02	1±0.003	4±0.004*	2±0.005
Apoptotic cells/10hpf	0.1±0.1	10±2*	3.3±0.8	3.9±0.5%	40±0.3*%	13±0.4%

n=4;*p<0.01 vs. control and CI+UCF-101, ND=not detectable.

Conclusions: CI injury results in degradation of XIAP and subsequent RTEC apoptosis. Prevention of XIAP degradation by UCF-101 results in decreased CC3, caspase-3 activity and prevention of apoptosis during both *in vitro* and *in vivo* CI. UCF-101 may be an important therapy to prevent apoptosis during CI of donor organs and potentially improve DGF.

Funding: Other NIH Support - R03 DK96151-01 to Alkesh Jani

TH-PO1059

Acute Tubular Injury at Reperfusion and Transplant Outcomes

Yaa Oppong, John Farber, Maria P. Martinez Cantarin. *Thomas Jefferson Univ, Philadelphia, PA.*

Background: Conflicting data exist with respect to pathological features that predict allograft outcomes at the time of kidney reperfusion. Acute tubular injury is common at reperfusion, but its relationship with graft function is unclear. The present study aimed to evaluate in reperfusion biopsies the relationship between severity of acute tubular injury, as assessed by a new standardized scoring system, and the extent of chronic changes as defined by Banff criteria and graft outcomes.

Methods: Reperfusion biopsies from kidney transplants performed at our institution from January 2004 to December 2012 were retrospectively reviewed. Data from 476 biopsies were analyzed. Acute tubular injury (AI) was assessed by scoring apical blebbing, hydropic swelling, and cell sloughing. Chronic injury (CI) used the Banff grading system. Graft outcomes included graft rejection (GR), creatinine levels, and estimated glomerular filtration rate (eGFR) at 1 and 5 years. This study had the approval of the Institutional Review Board of Thomas Jefferson University Hospital.

Results: We reviewed 292 biopsies from deceased donors and 184 from live donors. A little over half of patients were male and white, with an average age of 51 years. In multivariable analysis, the AI score was not significantly associated with graft rejection, creatinine levels, or eGFR at 1 or 5 years post transplantation. On the other hand, an association was evident between the CI score and eGFR at 1 year. Patients with a moderate/severe CI score had significantly lower eGFR at 1 year (p=0.002) and a significantly higher creatinine at 1 year than those with mild scores (p=0.019). The association between the CI score and eGFR was not apparent at 5 years of follow up. No association was evident between the CI score and rejection in our cohort.

Conclusions: In summary, mild to moderate acute tubular damage at implantation does not affect transplant outcomes during the first year. Chronic changes graded by Banff are associated only with eGFR at 1 year and not at 5 years.

TH-PO1060

Effect of Early HHV-6 Viremia on Short- and Long-Term Kidney Transplant Outcomes Michael E. Seifert,[1,2] Ana Paula Rossi,[2] Timothy A. Horwedel,[2] Gregory A. Storch,[2] Daniel C. Brennan.[2] *[1]Southern Illinois Univ; [2]Washington Univ in St. Louis.*

Background: Reactivation of latent herpesviruses such as cytomegalovirus (CMV) is associated with poor kidney transplant outcomes. We previously reported the incidence of human herpesvirus (HHV)-7 viremia at 89%, as well as its resistance to antiviral therapy. The impact of HHV-6 reactivation is unclear. We sought to characterize the incidence of early HHV-6 viremia post-kidney transplant, examine interactions between HHV-6 and CMV, and determine the effects of early HHV-6 viremia on short- and long-term outcomes.

Methods: This was a prospective study of kidney transplant recipients (n=193) previously enrolled in a polyomavirus monitoring study with 10 years of follow up. We grouped subjects by donor/recipient CMV status to analyze interactions between HHV-6 activity and CMV exposure: CMV+ (donor or recipient positive) or CMV- (donor and recipient negative). Primary exposure was HHV-6 viremia through 16 weeks post-transplant determined by weekly PCR. Primary outcomes were leukopenia and anemia during year-1 post-transplant and a composite of acute rejection, graft loss, or death by 10 years post-transplant.

Results: HHV-6 viremia occurred in 10/193 (5.2%) during the first 16 weeks post-transplant, with 9/10 cases in the CMV+ group. Leukopenia occurred in 11% and and anemia in 6% of HHV-6 negative subjects but neither occurred in HHV-6 viremic subjects. In Kaplan-Meier analysis we observed a trend for more HHV-6 viremic subjects achieving the composite outcome than HHV-6 negative subjects (P=0.10). Using Cox regression, HHV-6 viremia was independently associated with the composite outcome after including age at transplant, CMV exposure and number of HLA-antigen mismatches in the final model [HR 2.422 (95% CI 1.002, 5.854)].

Conclusions: To our knowledge, this is the largest kidney transplant cohort assessed for HHV-6 reactivation. Early HHV-6 viremia occurs infrequently compared to other herpesviruses, but may occur more often in CMV-exposed subjects. HHV-6 viremia is not associated with leukopenia or anemia. Early HHV-6 viremia may be predictive of poor 10-year transplant outcomes, but the low incidence of HHV-6 viremia limits broad statistical inference.

Funding: NIDDK Support, Other NIH Support - NCATS KL2 Career Development Award via Washington University's CTSA

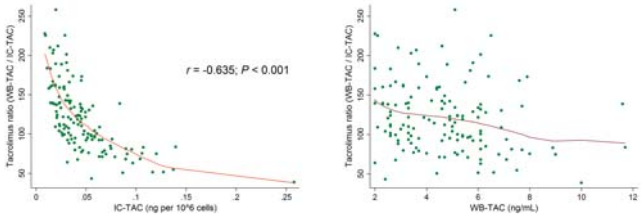
TH-PO1061

Monitoring Intracellular Tacrolimus Concentration in Kidney Transplant Recipients with Stable Graft Function Seung Seok Han,[1] Seung Hee Yang,[2] Ji In Park,[1] Hajeong Lee,[1] Jung Pyo Lee,[3] Dong Ki Kim,[1] Yon Su Kim.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [2]Kidney Research Inst, Seoul national Univ, Seoul, Korea; [3]Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea.*

Background: Monitoring the intracellular concentration of immunosuppressive agents can be promising to improve and individualize clinical practice in patients receiving transplantation. However, this issue remains unresolved in kidney transplant recipients.

Methods: Both whole blood and intracellular concentrations of tacrolimus (WB-TAC and IC-TAC, respectively) were simultaneously measured in 215 kidney recipients with stable graft function using LC-MS/MS. The tacrolimus ratio was defined as WB-TAC/IC-TAC. Furthermore, the genetic polymorphisms of P-glycoprotein 1, such as rs1128503, rs2032582, and rs1045642, were examined.

Results: The ranges of the WB-TAC and IC-TAC levels, and tacrolimus ratio were 2.0–11.7 ng/mL, 0.009–0.258 ng/mL, and 38.7–258.0, respectively. The correlation coefficient (r) between WB-TAC and IC-TAC was 0.759 (P < 0.001). The tacrolimus ratio was more strongly influenced by IC-TAC.



Genetic polymorphisms of P-glycoprotein 1 were not associated with IC-TAC or the tacrolimus ratio. Among baseline covariates, the duration of transplantation was the strongest predictor of IC-TAC and the tacrolimus ratio: patients who had a long period since transplantation had lower IC-TAC and higher tacrolimus ratio. Among the other covariates, patients with deceased donor, high proportion of lymphocyte, and high hematocrit level had lower IC-TAC and high tacrolimus ratio than their counterparts.

Conclusions: To our knowledge, this is the first study to identify the factors associated with the IC-TAC. These results will be helpful for monitoring and predicting IC-TAC or the tacrolimus ratio in kidney transplant recipients.

Funding: Government Support - Non-U.S.

TH-PO1062

Proton Pump Inhibitors Are Associated with Severe Hypomagnesemia in Renal Transplant Patients Mohamad Alhosaini, David J. Leehey, Kavitha Vellanki. *Medicine, Loyola Univ Medical Center, Maywood, IL.*

Background: Proton pump inhibitors (PPI) have replaced H2 receptor blockers (H2B) for GI prophylaxis at many transplant centers, despite lack of evidence for superior efficacy of PPI. Although PPI are generally safe medications, hypomagnesemia due to impaired GI absorption can occur. We hypothesized that PPI would result in a higher prevalence and severity of hypomagnesemia when compared to H2B.

Methods: Medical records of all adults receiving kidney alone transplants at our center between January 2010 and December 2012 were reviewed. Exclusion criteria were: delayed graft function, death within one year of the transplant date, and use PPI or H2B for less than one year. Outcomes were incidence of hypomagnesemia (mean serum Mg of < 1.8 mg/dL during the first year after transplant), incidence of severe hypomagnesemia (serum Mg < 1.3 mg/dL on any occasion) and use of Mg supplements.

Results: Of 187 patients receiving kidney transplants during the study period, 83 met the above eligibility criteria. Mean age was 52.2 ± 15.8; 59% were male and 59% were Caucasian. All were taking CNI. 43 patients were treated with PPI and 40 patients were treated with H2B. Overall, 65% had hypomagnesemia. Mean serum Mg was 1.70 ± 0.12 in the PPI group versus 1.79 ± 0.17 in the H2B group (p = 0.006). Hypomagnesemia occurred in 77% of patients taking PPI and in 60% of patients taking H2B (p = 0.10). However, severe hypomagnesemia was more common in patients taking PPI versus H2B (21% versus 5%, p =0.032). Use of magnesium supplements was more common in the PPI group than the H2B group (47% versus 23%, p = 0.022). Incidence of diabetes mellitus, serum albumin, and diuretic use were similar between the two groups. On logistic regression analysis, both PPI and diuretic use were independent risk factors for severe hypomagnesemia.

Conclusions: The use of PPI after renal transplantation causes more severe hypomagnesemia and leads to greater use of oral magnesium supplements compared to H2B. Given the lack of confirmed superior benefit of PPI versus H2B and the potential harmful effect of hypomagnesemia, we suggest restricting prophylactic PPI use in renal transplant patients to those who are at high risk for GI bleeding.

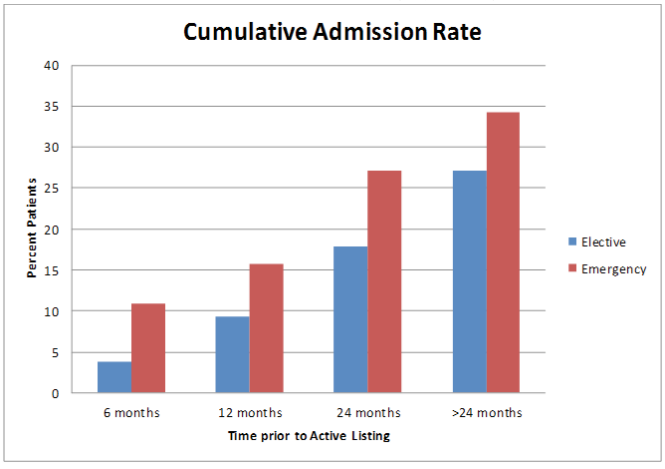
TH-PO1063

Novel Report of Fitness of Wait-Listed Dialysis Patients and Prevalence of Occult Cardiovascular Disease Arunraj Navaratnarajah, Menelaos Pipis, Bev M. Nicol, Ruhena Sergeant, Damien Ashby, David Taube, Neill D. Duncan. *Imperial College Renal and Transplant Centre, London, United Kingdom.*

Background: Our aim was to evaluate fitness of patients wait-listed at our centre based upon protocolised care.

Methods: An exhaustive examination of 4 haemodialysis centres and the peritoneal dialysis programme was made 21 Nov 2013. 184/676 dialysis patients were active on deceased donor wait-list. Last clinical review and hospitalisations were recorded. Our protocol is for 3 monthly general medical review, and coronary angiography in all symptomatic patients and asymptomatic high risk patients (aged >50 years and all diabetics). Biochemical surrogates a month prior to censure were used for nutrition (albumin), CKD-MBD (PTH), inflammation (CRP) and anaemia (Hb and erythropoietin requirement EPO/Wk).

Results: Median time from clinical review 40 days (1-227 days).



One third of emergency admissions were due to sepsis, 9.5% cardiac cause. 40% patients had clinical review <6 months of hospital admission. 7 patients (4%) had cardiac symptoms. 71% of listed patients had coronary angiography (99% of those >50 years and 95% diabetics). 20% of patients with angiography proceeded to revascularisation. Echocardiography performed in 66%, 16% systolic dysfunction, 9% valvular disease, and 25% others; diastolic dysfunction, regional wall abnormalities, pulmonary hypertension. Month prior to census, 5% had albumin <30g/L, 25% had PTH >70pg/ml, 16% had CRP >10mg/dL. 9% had Hb <10g/dL and 6 patients were EPO resistant with EPO/Wk >24k units.

Conclusions: Our study demonstrates acuity of illness in wait-listed patients, and the need for frequent clinical surveillance of patients. A minority of patients had cardiac

symptoms, there was adherence to protocol for high risk asymptomatic patients and 20% required revascularization. Biochemical surrogates also demonstrate a significant burden of chronic disease.

TH-PO1064

Impact of Cytomegalovirus Infection in Kidney Transplant Recipients. Experience at Our Centre Lourdes de la Vara Iniesta, Inmaculada Lorenzo Gonzalez, Francisco Llamas Fuentes, Carmen Gomez Roldan. *Univ Hospital of Albacete.*

Background: Cytomegalovirus (CMV) is one of the most important pathogens in kidney transplant patients. Early detection and prophylactic treatment reduce the impact of the disease. Objective: to analyse and describe our centre's experiences.

Methods: 235 transplant patients were observed. Prophylaxis was performed in the high-risk patients (D+/R- or treatment with antilymphocyte globulin), the remaining patients were monitored with Ag-pp65/PCR-CMV for 16 weeks or when there was suspicion of affectation. We considered infection (CMVI): Ag-pp65>10 cells/PCR>1000 copies, without associated clinical, and disease (CMVD) when associated symptomology.

Results: Of 235 patients, 151(64.30%) were male. 69 patients (29.36%) considered high-risk, the average prophylaxis period: 17.62±5.54 weeks (range:6.43-32.86). Of these, 16(23.18%) presented CMVI and 1(1.44%) developed CMVD (post-prophylaxis). The average onset period (AOP), since the transplant: 5.85±3.66 months. The remaining 166 patients (70.60%) were considered low-risk, 43(25.90%) presented CMVI, 2(1.20%) developed CMVD, AOP: 2.61±3.75 months. We did not find differences in the incidence of CMV when comparing both groups, or between patients that received a different period of prophylaxis, ≤ or >15 weeks. There were 7 relapses (5 in low-risk patients). Comparing patients treated <7, ≥7 and <15, y ≥15 weeks, we found 37.5%, 12.5% and 8.3% relapses respectively (p=0.20). On comparing affected patients with healthy, in the multivariate analysis, being male acts as a risk factor (p=0.001), glomerular filtration in the first year post-transplant was worse in affected patients, and no increase was observed in the incidence of neoplasms and acute rejection, or decrease patient and graft survival.

Conclusions: Early detection and prophylactic treatment in situations of risk have reduced the impact of the disease. The prophylaxis period >15 weeks does not improve the results and increase costs. With a longer treatment period, there are fewer relapses. CMV infection is associated to males and poorer kidney function during the first year post-transplant, without repercussion for survival of either the graft or the patient.

TH-PO1065

Comparison of Outcomes From Spousal Donor Graft Between ABO Compatible and Incompatible Kidney Transplantation Ji Hyun Yu,[2] Byung Ha Chung,[1,2] Bum-Soon Choi,[1,2] Cheol Whee Park,[1,2] Yong-Soo Kim,[1,2] Chul Woo Yang.[1,2] *[1]Transplantation Research Center; [2]Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

Background: In recent era of organ shortage, both spousal donor and ABO incompatible kidney transplantations (KTs) are good alternatives for living related ABO compatible donor KT. The aim of this study was to compare the outcomes of spousal donor in ABO compatible and ABO incompatible KTs.

Methods: Between Jan. 2009 and Dec. 2012, total 326 living donor KTs were performed at Seoul St. Mary's hospital. We compared the clinical characteristics and outcomes including survival rates for grafts and patients between two spousal donor groups.

Results: Percentage of spousal donor in ABO incompatible KT group (group1, 22 of 56 KTs, 39.3%) was higher than that of in ABO compatible KT group (group2, 62 of 270 KTs, 23%)(p=0.013). 3-year BPAR-free survival rate was higher in group 2 (65.3 versus 86.5%, p=0.043). But after exclusion of high titer (defined as baseline anti-A or anti-B immunoglobulin G isoagglutinin titer over 1:512) ABO incompatible KT recipients (n=5), 3-year BPAR-free survival rates showed no difference between two groups. 3-year grafts survival rate (85.2 versus 95%, p=0.065) and patients survival rate (95.5 versus 100%, p=0.093) were comparable between two groups. Serum creatinine level was lower at first 3 months in group 1 (1.25 ± 0.24 versus 1.45 ± 0.37 mg/dL, p=0.028), but after first 3 months, the difference of renal function between two groups has been disappeared. In ABO compatible KT recipients, there was no difference in 3-year BPAR-free, grafts and patients survival rates among living related donor (LRD), living unrelated donor (LUD) and group2. In ABO incompatible KT patients, there was no LUD. 3-year BPAR-free, grafts and patients survival rates also showed no difference between ABO incompatible LRD KT recipients and group1. Spousal donor KT itself and spousal type did not affect the grafts survival.

Conclusions: Outcomes of spousal donor graft in ABO incompatible KT is comparable to those of ABO compatible KT in respect of grafts and patients survival rates.

TH-PO1066

Prevention of Complications Associated with Benign Prostatic Hypertrophy After Renal Transplantation Michelle L. Lubetzky, Maria Ajaimy, Anjali Gupta, Layla Kamal, Graciela De Boccardo, Enver Akalin, Liise K. Kayler. *Transplantation, Montefiore Medical Center, Bronx, NY.*

Background: More than half of males over 50 have benign prostatic hypertrophy (BPH). As most patients with ESRD are anuric, BPH is under diagnosed in this population. BPH, if diagnosed late, may increase complications following kidney transplantation (KTX).

Methods: A single center retrospective cohort review of consecutive male patients over 50 years of age transplanted from Jan. 1, 2010 until Sept. 30, 2013 was performed to assess outcomes between patients with and without BPH. We also reviewed whether pre-transplant diagnosis of BPH and initiation of medical therapy had an effect on acute urinary retention (AUR), discharge with foley catheter, urinay tract infection (UTI), graft function, and patient survival.

Results: Of 147 patients, BPH was diagnosed in 34.2%, 15.1% prior to KTX and 19.1% after KTX. Mean follow-up was 673.9± 394.8 days and was not different between those with and without BPH (p=0.37). Compared to those without BPH, BPH was significantly associated with older age, higher pre-transplant PSA, discharge with Foley catheter, re-admission for AUR, and UTI after KTX. Patients who were placed on medical therapy at the time of transplant (Pre-KTx diagnosis of BPH) had significantly less UTIs, AUR, and hospital readmissions with AUR. There were no differences in graft function or graft/patient survival between the groups.

	No BPH (n=94)	BPH (n=53)	P Value
Mean Follow up (days)	653.9±402.7	709.5±381.5	0.37
Age	61.0±6.7	64.6±6.7	0.002
Race (AA)	23.4%	18.9%	0.52
Induction (Thymo)	80.1%	73.6%	0.31
Pre-KTX PSA	1.16±0.86	2.40±2.9	0.001
Most recent creatinine	1.8±1.4	1.7±1.0	0.95
Double J Ureteral Stent	42.6%	41.5%	0.90
Graft Loss	3.3%	0%	0.18
P[illegible]t surv[illegible]	98.9%	96.2%	0
Uri[illegible]tion after transp[illegible]	0%	2[illegible]	<[illegible]1
D[illegible]arge w[illegible] Foley	0%	13[illegible]	<[illegible]1
Re-admission for Urinary Retention	0%	28.3%	<0.01
UTI (within 1 year)	18.0%	45.2%	<0.01

	Pre-KTX BPH diagnosis (n=25)	Post-KTX BPH diagnosis (n=28)	P Value
Mean Follow up (days)	652.2±341.3	760.7±341.3	0.29
Urinary retention after transplant	4%	46.4%	<0.01
Discharge with Foley	4%	21.4%	0.06
Re-admission for Urinary Retention	8%	46.4%	<0.01
UTI (within 1 year)	24%	57.1%	0.01

Conclusions: Following KTX, urinary tract complications are common in patients with BPH. Pre-transplant assessment of BPH and initiation of medical therapy significantly diminished the incidence of complications. This data points to the need for earlier diagnosis of BPH in pre-transplant assessment of ESRD patients.

TH-PO1067

Sarcopenic Obesity in Male Renal Transplant Recipients Vasileios Devetzis, Uyen Huynh-Do, Spyridon Arampatzis. *Nephrology, Hypertension and Clinical Pharmacology, Bern Univ Hospital, Bern, Switzerland.*

Background: Although abnormal body compositions such as sarcopenic obesity (SO), which describes the condition in which high fat mass (obesity) co-exists with low muscle mass (sarcopenia), are clinical relevant phenotypes, data on their prevalence and impact on bone mineral status in renal transplant recipients (RTR) are currently lacking.

Methods: To investigate the prevalence of sarcopenia and obesity after renal transplantation and their impact on bone mineral density, we conducted during a 48 months period a cross sectional analysis in 78 male RTR with a stable renal function (eGFR>30 ml/min). Body composition and bone mineral density were evaluated by dual X-ray absorptiometry (DXA). Obesity was defined as percentage of whole body fat mass >27% and sarcopenia as appendicular skeletal muscle mass ≤7.26 kg/m2.

Results: The prevalence rates of sarcopenia and obesity in our cohort were 28% (22/78), and 51% (40/78), respectively. Sarcopenic obesity was present in 15% (12/78) of RTR. Those classified as SO had similar clinical (age, months after transplantation, BMI, glucocorticosteroid doses, rejection episodes) and biochemical (eGFR, serum intact parathyroid hormone and 25-hydroxyvitamin D levels) profiles when compared to non-sarcopenic RTR. Bone mineral density was significantly lower at any measured skeletal site in SO (mean ± SD lumbar spine: 1.030 ± 0.168 g/cm^2, femoral neck: 0.764 ± 0.125 g/cm^2 and proximal femur: 0.916 ± 162 g/cm^2) compared to non- sarcopenic RTR (lumbar spine: 0.930 ± 0.142 g/cm^2 p=0.03, femoral neck: 0.645 ± 0.137 g/cm^2, p=002 and proximal femur: 0.790 ± 0.154, p=0.008).

Conclusions: Obesity and sarcopenia are highly prevalent after renal transplantation and may potentiate each other, thus maximizing their deleterious effects on skeletal health.

TH-PO1068

Immunization Status of HIV Positive Patients on Kidney Transplant List: A Single Center Experience Purna Bindu Nandigam,[1] Talal A. Khan,[1] Ankit Parikh,[2] Amit A. Deshpande,[1] Akshay Sharma,[1] Karthik M. Ranganna,[1] Dong Heun Lee,[2] Alden Michael Doyle.[1] *[1]Div of Nephrology, Drexel Univ, Philadelphia, PA; [2]Div of Infectious Disease, Drexel Univ, Philadelphia, PA.*

Background: Immunizations are a key component of transplant care, as patients who are immunosuppressed are at heightened risk for infectious diseases. The best time to vaccinate is prior to transplantation as the response rates are higher before the

immunosuppressive medications have been added. Although the recommendations are clear, rates of immunization for transplant recipients have remained stubbornly below target. We sought to examine the rates of vaccination of an especially at-risk population, HIV+ patients who have been waitlisted for kidney transplantation in order to begin to develop a plan to boost pre-transplant immunity rates.

Methods: We retrospectively reviewed charts of 52 HIV+ ESKD patients who were waitlisted for kidney transplant. We recorded their immunization status including flu, pneumonia, Tdap and hepatitis B vaccination.

Results: The mean age of our cohort was 49 years old with 39/52 males, 40/52 Black, 8/52 White, 4/52 Hispanic. Patients had serologic evidence of exposure to EBV (41/52) and CMV (42/52). HIV was generally well-controlled, with 50/52 patients with CD4 counts over 200, 38/52 had undetectable viral loads. HCV co-infection was present in 4/52 patients. The vaccination rates of our patients are shown below:

Number of Patients	Flu vaccine administered	Pneumonia vaccine administered	Tdap vaccine administered	Hepatitis Bs Ab +
52	23/52(44.2%)	26/52(50%)	15/52(28.8%)	37/52(71.15%)

Conclusions: We found a relatively low rate of vaccination in HIV+ patients wait listed for kidney transplant. We surmise that we would find as low or lower rates of vaccination for HIV- populations who did not have an infectious disease provider. At our center we are developing educational and outreach programs to target wait listed patients so that patients that are called for organ offers have been immunized as completely as possible and have been educated about the central role that vaccines play in transplant care.

TH-PO1069

The Effects of Thyroid Function on Clinical Outcomes after Kidney Transplantation Jung Nam An,[1] Yun Kyu Oh,[1] Su-Kil Park,[2] Chun Soo Lim,[1] Jung Pyo Lee.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Asan Medical Center and Univ of Ulsan College of Medicine, Seoul, Republic of Korea.*

Background: The abnormalities in thyroid function, especially triiodothyronine (T3) levels, are observed in patients with chronic kidney disease and end stage renal disease. Few previous studies evaluated the correlation between thyroid function after kidney transplantation and various clinical outcomes, in particular, in Asian patients.

Methods: During the period of January 1997 to January 2012, the data about a total of 400 kidney transplantation recipients who had thyroid function test measured within 5 years after kidney transplantation was analyzed through the retrospective review of electronic medical records of two medical centers.

Results: Posttransplant thyroid-stimulating hormone (TSH) level was 1.9 ± 4.2 μIU/mL, free thyroxin (fT4) level was 1.3 ± 0.4 ng/dL, and T3 level was 112.4 ± 34.1 ng/mL. After follow up duration of 77.3 ± 53.0 months, 41 patients (10.3%) were diagnosed allograft failure. In cox-regression analysis model, posttransplant T3 level was negatively correlated with graft failure (hazard ratio [HR] 0.98; 95% confidence interval [CI] 0.97-0.99; $P = 0.001$), however, posttransplant TSH and fT4 level were not associated. Divided to four groups of T3 level, the risk for development of graft failure was the highest in the lowest group of T3 level (HR 6.90; 95% CI 1.45-32.85; $P = 0.015$). After adjusting other risk factors for graft failure, posttransplant T3 level was also significantly associated with kidney allograft failure. In Kaplan-Meier curves, in patients with lower T3 level had the higher risk for allograft failure (Log Rank $P = 0.037$).

Conclusions: Triiodothyronine level measured after kidney transplantation is an independent risk factor for allograft failure. Regular measurement and monitoring of thyroid function test should be the critical focus of post-transplant care.

TH-PO1070

Incidence of CMV (Cytomegalovirus) Viremia in High Risk Kidney Transplant Recipients Induced with Alemtuzumab. A Single Center Experience Pradeep Vaitla,[1] Ushma Patel,[2] Catherine G. Staffeld-Coit,[1] Ryan C. Mascarenhas,[1] Antonio G. Jimenez,[1] Stephanie Anders,[2] George E. Loss,[3] Ari J. Cohen,[3] Abdul Moiz,[1] Jorge C. Garces,[1] Humberto Bohorquez.[3] *[1]Nephrology, Ochsner Clinic Foundation; [2]Pharmacy, Ochsner Clinic Foundation; [3]Transplant Surgery, Ochsner Clinic Foundation.*

Background: It is still a matter of debate the risk of CMV infection in recipients receiving induction therapy with T-cell depleting versus other agents.

Methods: We performed a retrospective review of electronic medical records from November 2007 to December 2011. A total of 441 kidney transplants were performed during that period. Three hundred and eleven were induced with Alemtuzumab 30 mg IV single dose. Patients also received Methylprednisolone 500, 250 and 125 post-operative days 0, 1 and 2, they were maintained on Tacrolimus (target level 7-10 ng/ml) and Mycophenolate mofetil 500 mg oral twice daily. They received Valgancyclovir (VGC) prophylaxis. Our protocol dictates to use VGC for 180 days for high risk recipients (D+R-) and 90 days for everybody else.

Results: Fifty three patients were considered high risk (D+/R-) for CMV infection and 258 low risk. Among the patients induced with Alemtuzumab 18 patients developed CMV viremia (5.78%), 9 in the high risk (16.9%) and 9 in the low risk group (3.48%).

Conclusions: After a median follow-up of 12 months Alemtuzumab showed a better risk profile for the development of CMV infection compared with data from recent literature using induction therapy with ant-thymocyte globulin reporting rates as high as 24% in high risk groups compared with 16.9% in our cohort.Alemtuzumab seems to be safe in this particular population however long-term data and randomized controlled studies are needed.

TH-PO1071

BK Nephropathy in Kidney Transplant Recipients Induced with Alemtuzumab. A Single Centre Experience Pradeep Vaitla,[1] Ushma Patel,[2] Ryan C. Mascarenhas,[1] Antonio G. Jimenez,[1] Abdul Moiz,[1] George E. Loss,[3] Stephanie Anders,[2] Catherine G. Staffeld-Coit,[1] Ari J. Cohen,[3] Jorge C. Garces,[1] Humberto Bohorquez.[3] *[1]Nephrology, Ochsner Clinic Foundation; [2]Pharmacy, Ochsner Clinic Foundation; [3]Transplant Surgery, Ochsner Clinic Foundation.*

Background: BK nephropathy is increasingly identified as a cause of kidney injury and graft failure. Current literature suggests, higher dose of immunosuppression is associated with increased incidence of BK viruria, viremia and nephropathy. Most transplant centers across the country use antibody induction with rabbit antithymocyte globulin or Alemtuzumab to minimize maintenance immunosuppression. Our current protocol for immunosuppression includes single dose of Alemtuzumab 30 mg, Tacrolimus to achieve serum trough level 7-10 ng/ml, Mycophenolate mofetil (MMF) 500 mg oral twice daily, methylprednisolone 500 mg post opertive day(POD) 0, 250 mg POD 1 and 125 mg POD 2 with rapid steroid withdrawal.

Methods: We performed a retrospective chart review of 441 kidney transplants performed from November 2007 to December 2011 of which 311 recipients received Alemtuzumab for induction immunosuppression and we obtained demographic, laboratory and clinical data including biopsy proven acute rejection during one year of follow up. Our current protocol includes screening with PCR for BK virus in urine at 3,6,9,12,18 and 24 months post transplant. A BK viral load >10 million copies/ml is an indication for serum BK PCR every 2 weeks and a possible kidney biopsy.

Results: The incidence of BK viruria was 29% (90 out of 311 recipients), incidence of BK viremia was 9% (9 of 311) and incidence of BK nephropathy was 0.9% (3 of 311). For the entire cohort the incidence rate of biopsy proven rejection rate was 8% and graft survival rate was 99% at the end of 1 year. No allografts were lost due to BK nephropathy during the follow up period.

Conclusions: Induction therapy with Alemtuzumab in renal transplant recipients, combined with screening protocol with BK virus PCR is effective and has a low incidence of BK nephropathy compared with other protocols reported in the transplant literature.

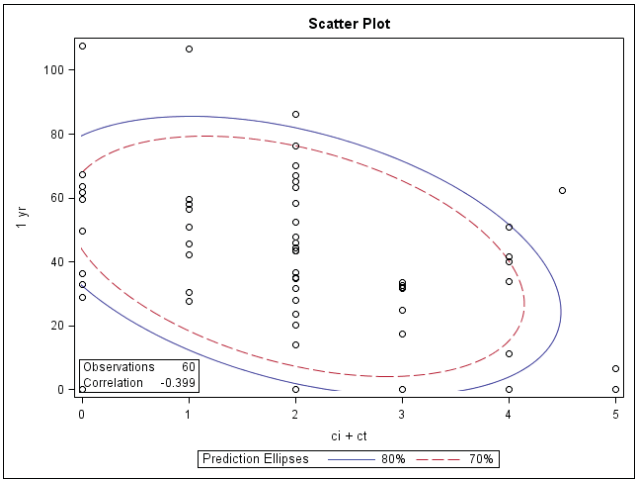
TH-PO1072

The Correlation between eGFR with Degree of Allograft Histological Changes in HIV + Kidney Transplants Recipients Amit A. Deshpande, Disha Narula, Alden Michael Doyle, Gregory Malat, PharmD. *Drexel Univ.*

Background: Hahnemann University Hospital Kidney Transplant Program has performed over 100 HIV (+) kidney transplants. Allograft biopsies were performed as needed and per protocol. These biopsies were analyzed as a tool for establishing an association between eGFR and degree of Interstitial Fibrosis and Tubular Atrophy.

Methods: 62 HIV+ kidney transplant recipients had allograft biopsies approximately 1 year post-transplant. This cohort was further stratified according to 1 yr eGFRs; eGFR> 40ml/min (group1) and eGFR<40ml/min (group2). Biopsies reported no IFTA, mild IFTA, and moderate/severe IFTA. Demographics were compared between the groups. Results analyzed correlation between eGFRs at 1 year and the degree of IFTA.

Results: Please See Scatter Plot for results.



Conclusions: Preliminary results indicate that moderate to severe interstitial fibrosis and tubular atrophy can be present even in groups with higher eGFRs in the HIV (+) kidney transplant recipients. This demonstrates the need for biopsies in this population to determine graft survival rather than relying on eGFRs.

TH-PO1073

Urinary Exosomal mRNA Analysis for Non-Invasive Diagnosis of Post Kidney Transplant Complications Taku Murakami,[1] Masato Mitsuhashi,[1] Hiroshi Harada.[2] *[1]Hitachi Chemical Research Center, Inc., Irvine, CA; [2]Sapporo City General Hospital, Sapporo, Hokkaido, Japan.*

Background: Monitoring of post-transplant kidney condition is important for the management of long term graft survival. Conventional urinary markers are not sensitive and specific, and kidney biopsy is invasive. Exosomes and microvesicles (EMV) are released into the urinary space from all the areas of the nephrons by encapsulating the functional cytoplasmic molecules of the cell of origin. To evaluate the diagnostic values of EMV, we analyzed EMV mRNA profiles of urine samples from kidney transplant patients.

Methods: Urine samples were collected from post-transplant patients (N=225). Post-transplant complications were diagnosed based on eGFR, urinary protein and kidney biopsy with Banff criteria. After urinary cells and casts were removed by low speed centrifugation, urine supernatants containing EMV were applied to EMV collection tubes (Hitachi Chemical Research Center, Inc. (HCR)), followed by mRNA isolation and cDNA synthesis using oligo(dT)-immobilized microplate (HCR) and real-time PCR. The obtained mRNA data were analyzed based on the diagnosis of post-transplant complications and Banff codes of kidney biopsy.

Results: Among 68 mRNAs we analyzed, several mRNAs including CCL2, DEFA3 and SLC12A3 were down regulated in both T-cell mediated rejection (TCMR) (N=5) and antibody-mediated rejection patients (ABMR) (N=12) comparing to those with stable recovery (N=165). Specifically to TCMR patients, several mRNAs such as AQP1, CCL4, GMFG, IL1B and CXCL10, were differentially expressed. Logistic regression analysis suggested that combinations of 3 to 4 genes including the above genes diagnose TCMR with 100% sensitivity and >95% specificity, and ABMR with >75% sensitivity and >85% specificity.

Conclusions: We analyzed urinary EMV mRNA from kidney transplant patients. We discovered differentially expressed genes in TCMR and ABMR patients comparing to those with stable recovery. We further developed preliminary diagnostic formulas to identify these complications using logistic regression analysis. Such formula will be a candidate of future diagnostics of TCMR and ABMR where only invasive kidney biopsy is available.

Funding: Pharmaceutical Company Support - Hitachi Chemical Research Center, Inc.

TH-PO1074

Use of Urine Biomarkers to Predict Favorable Kidney Allograft Histology Wisit Cheungpasitporn, John C. Lieske, Fernando G. Cosio, Hatem Amer. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Allografts with minimal fibrosis and without inflammation on protocol (surveillance) biopsies have a good prognosis. This study evaluated the ability of a panel of specific urinary proteins that reflect different pathophysiologic processes to identify allografts with favorable histology, in order to reduce the need for surveillance biopsies.

Methods: Urine samples of 391 KTx patients were biobanked beween 2005 and 2009 at the time of allograft biopsy. Patients with urine total protein (tP) to creatinine (Cr) ratio greater than 1 g/g were excluded (n=14). Urinary α1microglobulin (α1m), retinol binding protein (RBP), neutrophil gelatinase-associated lipocalin (NGAL), Immunoglobulin (Ig) G, IgM, albumin (A), and tP, were measured and normalized to Cr concentration. Acute tubulointerstitial, chronic tubulointerstitial, and acute and chronic glomerular damages were graded using combined tubular + interstitial (t+i), chronic tubular + chronic interstitial (ct+ci), and acute glomerular + chronic glomerular (g+cg) scores, respectively. Favorable allograft biopsies were defined as no inflammation (t+i scores=0), minimal fibrosis (both ct and ci scores<2) and no glomerular findings (no glomerulonephritis and g+cg=0).

Results: Correlations(r) between urine biomarkers and combined severity scores are summarized in the Table.

Combined scores	Log NGAL/ Cr	Log IgM/ Cr	Log α1M/ Cr	Log IgG/ Cr	Log RBP/ Cr	Log A/ Cr	Log tP/ Cr
t+i scores	0.12	0.27	0.21	0.17	0.24	0.23	0.22
ct+ci scores	0.01	0.26	0.12	0.18	0.21	0.28	0.28
g+cg scores	0.01	0.14	0.17	0.26	0.20	0.34	0.36

Favorable biopsy findings were present in 239 biopsies. The best-fit parsimonious model employed IgM, α1m and A with an AUC of 0.71. A score>1.03 predicted a favorable allograft biopsy with 27% sensitivity and 95% specificity, potentially eliminating the need for 64 of 368 biopsies (17%).

Conclusions: RBP and IgM are good indicators of acute and chronic tubulointerstitial injury, whereas albumin correlates better with glomerular injury. A pattern of urinary protein excretion predicts favorable renal allograft histology, and could potentially be used to eliminate the need for nearly 20% of allograft biopsies.

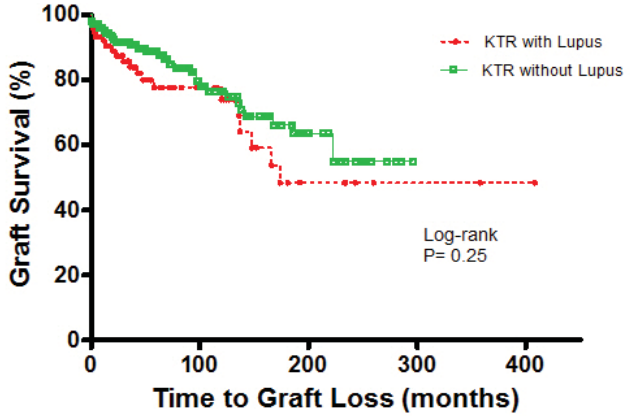
TH-PO1075

Kidney Graft Survival in Lupus Nephritis: A Mexican Cohort with 25 Years of Follow-Up Hugo Enrique Chavez,[1] Michael P. Wagner,[2] Olynka Vega,[1] Juan Carlos Ramirez-Sandoval,[1] Ricardo Correa-Rotter.[1] *[1]Nephrology and Mineral Metabolism, Inst Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico; [2]Univ of Minnesota, Minneapolis, MN.*

Background: Long-term graft survival of kidney transplant recipients (KTR) with end-stage kidney disease due to lupus nephritis (LN) is still controversial. Our aim was to evaluate the 25 years follow-up survival of KTR with LN compared with paired controls in a single center cohort in Mexico.

Methods: We performed a retrospective case-control study in which we analyzed KTR with diagnosis and biopsy proven LN (cases). To compare overall-survival, graft survival (serum creatinine>5mg/dl or dialysis), and time elapsed between transplant surgery and first immunological rejection, we paired these patients with non LN KTR (controls, 1:2) matched by age, sex, immunosuppressive treatment, HLA matches, transplant period, and immunosuppressive treatment.

Results: We identified 74 cases, 61 were female (82 %), mean age 32 years (± 10.2), 49 grafts were obtained from a living related donor (66%), of which 11 had 0-HLA matches. The 148 controls were paired as described. Graft loss occurred in 20 cases (28%) and 32 controls (22%). The mean graft survival time was 246 months (95% confidence interval [CI] 189-303) in cases and 214 months (95% CI 191-238) in controls. There were no differences between both groups for graft survival (log Rank test p=0.25) and overall-survival (p=0.31).



Similarly, there were no differences between the mean time elapsed from transplant surgery to first episode of immunological rejection (141 months [95% CI 89-194] versus 146 months [95% CI 120 -171], Log-Rank Test p=0.18). Only six cases had biopsy-proven lupus nephritis recurrence.

Conclusions: KTR with LN had a long-term overall survival, graft survival, and immunological risk similar to KTR matched by confounding factors.

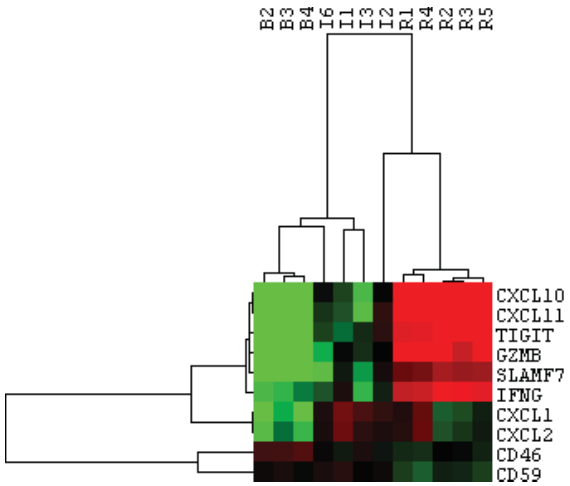
TH-PO1076

Gene Expression Patterns May Help in the Differential Diagnosis of Acute Rejection and Acute Pyelonephritis in Renal Allograft Biopsies Anjali A. Satoskar, Steve Oghumu, Uday S. Nori, Cherri N. Bott, Gyongyi Nadasdy, Sergey V. Brodsky, Ronald Pelletier, Tibor Nadasdy. *Ohio State Univ Wexner Medical Center, Columbus, OH.*

Background: Differentiating between acute rejection (AR) and acute pyelonephritis (APN) on renal allograft biopsies can be challenging, since both show mixed tubulointerstitial inflammation and urine culture results because of long-term prophylaxis with sulfa antibiotics. Our aim was to look for differences ivariable n gene expression between AR and APN using biopsy tissue.

Methods: Gene expression profiling was performed using Nanostring assay (total 513 genes) on a training set of 12 allograft biopsies (3 baselines, 4 unequivocal APN and 5 AR). Ten selected genes were validated using real-time PCR. A test set with 15 biopsies (2 baselines, 11 APN by histology and 2 AR), were studied for the same ten genes by PCR.

Results: Gene expression pattern in APN appears to be intermediate between normal and AR biopsies. AR shows profound upregulation of inflammation-related genes compared to APN and normal kidney. Differential expression of selected CXC-chemokine transcripts was seen. Significant upregulation of CXCL10 and CXCL11 but low CXCL1 and CXCL2 was seen in all AR biopsies (R in fig). Opposite pattern (low CXCL10 and CXCL11, comparable or lower than CXCL1 and CXCL2) was seen in the 4 unequivocal APN biopsies (I in fig) in the training set.



In the test set, 5/11 APN biopsies showed this profile, 5/11 APN biopsies showed AR pattern and 1/11 was indeterminate. In the test set 6/11 APN cases, had negative concomitant urine cultures and 4 of these improved after steroids.

Conclusions: Histologic features of APN in renal transplant biopsies may not be specific. Some cases may actually represent AR or a combination of APN and AR and steroid treatment may be helpful in selected cases. Chemokine profiling may help better design treatment.

TH-PO1077

Carotid Intima-Media Thickness of Pediatric Kidney Transplant Recipients

Kristen Sgambat, Sarah Clauss, Margaret Lasota, Asha Moudgil. *Children's National, Washington DC.*

Background: Increased carotid intima-media thickness (CIMT) is a known predictor of increased cardiovascular (CV) risk in adults, but there are limited data in pediatric kidney transplant (Tx) recipients. A prospective controlled study was conducted to investigate CIMT of lean and obese children in a predominantly African American (AA) pediatric kidney Tx cohort compared to healthy controls as well as change in CIMT over time after Tx.

Methods: Pediatric Tx recipients had the following parameters measured at baseline, 18, and 30 months post tx: CIMT, fasting glucose, HDL, triglycerides (TG), HbA1c%, waist circumference percentile (WC), and blood pressure (BP). CIMT was measured using B-Mode ultrasound imaging of right and left common carotid arteries and carotid bulbs. Tx children with metabolic syndrome (MS) were identified as those meeting ≥ 3 of the following criteria: HbA1c%>5.6 or fasting glucose>100, BP>90th percentile, central obesity (WC≥95th percentile), HDL <5th percentile, TG > 95th percentile. CIMT of Tx recipients were compared to 24 healthy pediatric controls using Wilcoxon ranksum analysis.

Results: Study group was comprised of 28 pediatric kidney Tx recipients (8 obese, 20 lean), mean age 13.1±0.75 years, 59.2% AA. Controls were 24 healthy children of similar age and race. CIMT of Tx was greater compared to healthy controls (0.47±0.002 versus 0.46±0.001, p=0.05). CIMT of AA Tx was greater than AA controls (0.49±0.002 versus 0.47±0.002, p=0.000). Mean CIMT of tx group improved over time from 0.47±0.002 mm at baseline to 0.46±0.002 mm at 18 months (p=0.01) and 0.44± 0.003 mm (p=0.000) at 30 months post-tx. Obese Tx had greater CIMT than lean Tx (0.48±0.004 versus 0.46±0.002, p=0.001). Tx with MS had greater CIMT than those without MS (0.49±0.004 versus 0.46±0.002 mm, p=0.000). AA Tx had greater CIMT compared to non-AA Tx (0.49±0.002 versus 0.43±0.001, p=0.000).

Conclusions: In conclusion, pediatric kidney Tx recipients carry increased CV risk, particularly those with MS, obesity, and AA race. This is the first study to demonstrate that AA have higher CIMT compared with non-AA pediatric Tx recipients. Further studies are needed to investigate strategies for decreasing CIMT after kidney Tx, particularly in the higher risk groups.

TH-PO1078

Impact of Tonsillectomy on Recurrent IgA Nephropathy after Kidney Transplant Recipients Based on Protocol Biopsy

Ken Sakai, Yasushi Ohashi, Toshiyuki Aoki, Reibin Tai, Nobuhiko Joki, Seiichirou Shishido, Atsushi Aikawa. *Nephrology, Toho Univ Faculty of Medicine, Tokyo, Japan.*

Background: Recurrent nephritis leads to graft-loss. Recently, tonsillectomy and additional steroid pulse therapy has been established as therapeutic option for primary IgA nephropathy (IgAN), but therapy for recurrence of IgAN is yet to be established.

Methods: Since 1984, kidney transplantation was performed 697 patients at our institution. Among those patients, 23 (24%) were diagnosed as recurrent IgAN in 96 recipients with IgAN confirmed as primary nephropathy. Recurrent IgAN was defined as newly appearance of urinary findings with histologic IgA deposition, but carried-in IgAN has been eliminated. Among 23 recurrent IgAN, 12 recipients (32.8 ± 9.4 yrs. at transplantation) who could follow up more than 5 years were enrolled and divided into 2 groups: with tonsillectomy (n=5) and without (n=7). After showing therapeutic option of tonsillectomy, we performed it in only the obtained agreement. Protocol biopsy was evaluated by Banff 2007 and Oxford classification. Usual immunosuppressive therapy has continued with ARB/ACE-I since diagnosis of recurrent IgAN.

Results: During the study period (117.4 ± 29.5 months), in no tonsillectomy group, the proteinuria at recurrence was 0.07 ± 0.19 g/gCr, and was significantly increased to 0.97 ± 1.09 at 60 months after recurrence (p=0.043). But in tonsillectomy group, proteinuria did not change (0.33 ± 0.52 to 0.40 ± 0.53, p= 0.87). eGFR decreased as follow: tonsillectomy group, 47.7 ± 14.6 ml/min/1.73m^2 to 34.8 ± 12.2 (p=0.11); no tonsillectomy group, 39.1 ± 5.8 to 30.4 ± 10.2 (p=0.03). Tonsillectomy group did not show any histopathological alteration. However, in no tonsillectomy group, mesangial matrix (mm) increased from 0.14 ± 0.38 to 2.14 ± 0.69 (p=0.002), mesangial hypercellularity (MS) increased from 0.20 ± 0.36 to 0.69 ± 0.37 (p=0.003), and segmental glomerulosclerosis (SS) increased from 0 to 0.57 ± 0.54 (p=0.03). The pathological rejection except borderline changes was absent in both.

Conclusions: While the natural history of recurrent IgAN remained stable with less amount of proteinuria in both groups (<1g/day), tonsillectomy protected from the histological damage.

TH-PO1079

Type 2 Diabetes Patients on Renal Replacement Therapy: Probability to Receive Renal Transplantation and Survival after Transplantation

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Background: Type 2 diabetes (T2DM) patients on renal replacement therapy (RRT) seldom receive a kidney transplant, which is partly due to age and comorbidities. Adjusting for case mix, we investigated whether T2DM patients have equal opportunity for renal transplantation compared to other patients on dialysis, and whether survival after transplantation is comparable.

Methods: Patients who entered RRT in Finland in 2000-2010 (n=5314) were identified from the Finnish Registry for Kidney Diseases. Of these, 20% had T2DM, 14% type 1 diabetes (T1DM) and 66% other than diabetes as cause of ESRD. Univariate and multivariate survival analysis techniques were employed to assess probability of kidney transplantation after start of dialysis and survival after transplantation.

Results: T2DM patients had a relative probability of renal transplantation of 0.16 (95% CI 0.13-0.21, P<0.001) compared to T1DM patients, this increased to 0.48 (95% CI 0.33-0.71, P<0.001) after adjustment for case mix (age, gender, laboratory values and comorbidities). When T2DM patients were compared to other than diabetes patients, the corresponding relative probabilities were 0.22 (95% CI 0.18-0.28, P<0.001) and 0.53 (95% CI 0.36-0.77, P<0.001). The risk of death within five years from transplantation was 12% (95% CI 5-19%) for T2DM, 10% (7-13%) for T1DM and 6% (5-8%) for other patients. After adjustment for age and gender, relative risk of death was 1.42 (95% CI 0.81-2.50) for T1DM patients and 0.62 (0.37-1.03) for other patients compared to T2DM patients.

Conclusions: T2DM patients had a considerably lower probability of receiving a kidney transplant, which could not be fully explained by differences in the patient characteristics studied. Survival after transplantation is comparably good in T2DM patients. This should encourage procedures to make kidney transplantation better available in this patient group.

TH-PO1080

Fludrocortisone Use in Tacrolimus Induced Hyperkalemia: A Pilot Study

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Background: Tacrolimus (FK-506) induced hyperkalemia is a serious concern among renal transplant patients. FK-506 induced hypoaldosteronism and mineralocorticoid receptor downregulation are implicated mechanisms. The utility of fludrocortisone, a mineralocorticoid, in the treatment (tx) of FK-506 induced hyperkalemia is unclear.

Methods: We collected data on our single center retrospective cohort by chart review. We defined hyperkalemia as K≥5meq/dl. K level on day 0 (pre tx) and day 90 (post tx) of fludrocortisone were compared. Exclusion criteria: ACEi, ARB, or ß blocker use; kayexalate use; fasting glucose≥ 300mg/dl; serum TCO_2 ≤15meq/dl; and biopsy-proven rejection or acute kidney injury. Paired T-test or Wilcoxon rank sum test was used to compare quantitative variables pre and post tx. Analyses were performed using SAS, v.9.3.

Results: The mean age of the study population (N=25) was 55±10.5 years, predominantly men (80%) and hispanic (47.6%) and white (33.3%) race. Serum K, creatinine (Cr), TCo_2, sodium (Na), weight, and blood pressure (BP) changed significantly pre and post tx with fludrocortisone (table1). The decrease in serum K remained significant after adjustment for Cr, TCo_2, Na, weight, and BP (p<0.001).
Paired Analysis

		PRE TX	POST TX	
	N	**Mean ± SD**	**Mean ± SD**	**P- Value**
Weight Kg	15	71.4 ± 12.3	74.4 ± 12.1	0.001*
SBP mmHg	15	129.7 ± 21.0	140.5 ± 18.3	0.045
DBP mmHg	14	72.9 ± 17.7	84.9 ± 27.5	0.048**
Na meq/L	21	138.9 ± 5.1	141.5 ± 6.1	0.008**
K meq/L	21	5.9 ± 0.5	4.8 ± 0.5	< 0.001
TCO2 meq/L	21	20.7 ± 2.7	24.0 ± 2.8	< 0.001
Cr mg/dl	21	2.2 ± 2.3	1.29 ± 0.24	< 0.001**
Glucose mg/dl	20	126.2 ± 75.6	118.6 ± 57.2	0.709**
Mg mmol/dl	17	0.9 ± 0.1	0.8 ± 0.1	0.062**
FK trough	20	11.3 ± 4.5	9.5 ± 3.1	0.131

*p-value for Paired T test,
**p-value for Wilcoxon rank sum test

Conclusions: Fludrocortisone significantly reduced FK-506 induced hyperkalemia, independent of renal function and metabolic acidosis in our study. A modest but significant rise is serum Na, weight, and BP was also observed. Larger studies are needed to confirm these findings.

TH-PO1081

Mechano-Growth Factor and VEGF Expression Are Increased in Glomeruli of Chronic Allograft Rejection Kidneys, and via GLUT1 May Promote Glomerulosclerosis

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Background: Our previous work in human Chronic Allograft Rejection (CAR) identified increased GLUT1 glucose transporter expression in CAR glomeruli and renal tubules. Excess GLUT1 increases cellular glucose uptake, metabolism, and in mesangial cells (MC) leads to excessive extracellular matrix (ECM) production. Here we investigated potential regulators of GLUT1 in CAR glomeruli to better understand how the increase in GLUT1 develops.

Methods: All studies were approved by the IRB for the University of Florida. Deidentified, archived, formalin-fixed, paraffin embedded kidney samples of normal controls (n = 3 – 6) and CAR (n = 3 – 6) kidneys were cut in 5µm sections for examination and semiquantitation of Mechano-Growth Factor (MGF), PKCß1, TGFß1, and VEGF immunolabeling, scored 0 – 4+ and normalized to open tuft area. Immunohistochemistry for MGF in individual glomerular cell types and renal cortical tubules was noted as positive or negative. The Mean ± SD (and ± SEM) for each measurement was determined for statistical comparisons with P < .05 significant.

Results: PAS staining of NL and CAR kidneys revealed a 2.5-fold increase of matrix in CAR, P < .02. The increased MGF correlates with 6.8-fold higher glomerular GLUT1 we previously identified in CAR. In contrast, active-PKCß1 and TGFß1 did not increase in CAR. VEGF, a stimulus to MC GLUT1, was increased 3.3-fold in CAR glomeruli, P < .001. Glomerular cell types expressing MGF included podocytes, parietal epithelial cells, and MC, in both NL and CAR. MGF was also identified in cortical collecting ducts, and to a lesser extent proximal tubules, in NL and CAR.

Conclusions: 1. This is to our knowledge, the first report of MGF in human kidneys. 2. Glomerular MGF and VEGF, both GLUT1 inducers, were increased in CAR, with potential to facilitate glomerulosclerosis. 3. Neither active-PKCß1 nor TGFß1 contributed to CAR. 4. Future investigation should identify signaling pathways from MGF, VEGF and GLUT1 to excess ECM production in CAR.

Funding: Clinical Revenue Support

TH-PO1082

Post Kidney Transplant Re-Admissions between 30 and 365 Days

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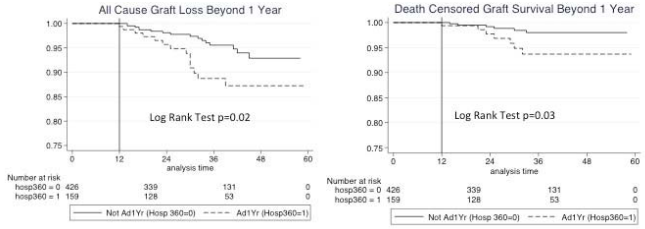
Background: In 2010 we established an ongoing quality assessment and practice improvement project (QAPI) monitoring early post kidney transplant readmissions for which there is scarcity of data regarding predictors, causes and effects. Previously we reported on admissions within 30 days (Ad30) from transplant. Here we focus on admissions from 30 to 365 days (Ad1yr).

Methods: We developed a working group that reviews all readmissions of solitary kidney transplant recipients to ascertain causes of readmissions within one year following kidney transplant surgery. Causes for readmission were grouped into 7 categories. We assessed the predictive ability of nine easily obtained and objective baseline characteristics on Ad1yr (Age, Gender, Race, Live Donor, Pre-emptive transplant, Re-transplant, BMI, diabetes, vitamin D levels).

Results: From 2009 through 2012, 607 patients received solitary kidney transplants at our institution. Mean age 52 (±14) years, 58% males, 90 % Caucasian, 83% living donor, 41% preemptive, 80% first transplants, 25% diabetic, with a mean BMI 28.5 (±5.9). 141 (23%) of patients were readmitted within 30 days and 166 (27%) experienced an admission between 30-365 days. Of the pre transplant characteristics only Age OR (p) 1.2 (0.01) per decade, BMI 1.04 (0.008) per unit, and diabetes 2.0 (0.001) predicted Ad1yr. Causes of Ad1yr were N (%): Surgical 65 (23) Allograft 50 (17), Cardiovascular 29(10), Fluids/Lytes 5 (2) GI 22(8), Infection 77(27), and Other 39 (14). Ad1yr portended worse graft survival HR (p) 2.3 (0.02) censored for death 3.1 (0.04) see Figure 1.



Conclusions: Re-admissions beyond 30 days and within one-year of transplant can be predicted, have varied causes and identify patients who could benefit from closer follow-up.

Funding: Clinical Revenue Support

TH-PO1083

Early Hospital Re-Admission and Outcomes After Kidney Transplantation

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Background: Early hospital re-admission (EHR) after kidney transplantation has been identified as a risk factor for subsequent hospitalizations, and increases graft failure and patient mortality. However, the associations between EHR, late hospital readmissions (LHRs), and graft outcomes remain unclear in the Canadian healthcare context.

Methods: This single-centre cohort study included 1,531 kidney transplant recipients from 1 Jul 2004 and 31 Dec 2012, with at least 1 year of follow-up. EHR was defined as hospitalization within 30 or 90 days post-discharge from the transplant admission. Recipient, donor, and transplant characteristics were measured at baseline. The primary outcomes were kidney function (using the CKD-EPI formula) at 1-year post-transplant, total graft failure (including death), and LHR (i.e., hospitalizations between 31 and 365 days [for 30-day EHR) or 91 to 365 days [for 90-day EHR) post-transplant). Multivariable linear regression models were used to assess kidney function while Cox proportional hazards models were used to examine EHR, LHR, and graft outcomes.

Results: EHRs at 30 days and 90 days were significantly associated with lower kidney function at 1 year post-transplant (β = -5.33 [95% CI: -8.23, -2.44], and β = -6.14 [95% CI: -8.69, -3.58], respectively). EHRs at 30 days and 90 days were also significantly associated with a greater risk of LHR (hazard ratio or HR 3.76 [95% CI: 2.66, 5.32], and HR 3.81 [95% CI: 2.64, 5.51], respectively). EHR was significantly associated with an increased risk of total graft failure (HR 1.57 [95% CI: 1.08, 2.30] at 30 days, and HR 1.91 [95% CI: 1.35, 2.70] at 90 days) and death-censored graft failure (HR 2.22 [95% CI: 1.31, 3.78] at 30 days, and HR 2.56 [95% CI: 1.55, 4.22] at 90 days). LHR was not associated with graft failure.

Conclusions: EHR is associated with adverse clinical outcomes for kidney transplant recipients. This reaffirms the need to develop and implement strategies to reduce EHR after kidney transplantation.

TH-PO1084

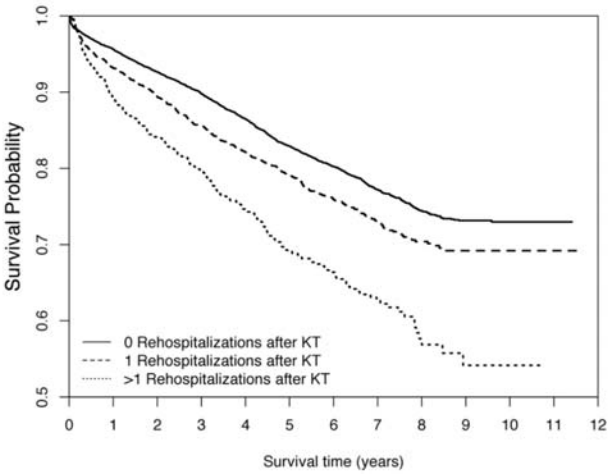
Distinct Predictors of Single versus Multiple Early Hospital Readmissions after Kidney Transplant

Meera Nair,[1] Wei Wang,[2] Alexander Stephen Hill,[2] Adam S. Mussell,[1] Roy D. Bloom,[1] Harold I. Feldman,[1] Peter P. Reese.[1] *[1]Medicine, Univ of Pennsylvania School of Medicine, Philadelphia, PA; [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: Early hospital readmission (EHR) within 30 days of discharge after kidney transplant (KT) is a common and costly event with diverse causes. Many KT recipients are readmitted more than once within 30 days. Risk factors for 1 EHR after KT may be distinct from multiple EHRs.

Methods: We linked datasets from the Organ Procurement and Transplantation Network and Medicare and performed a retrospective cohort study of 10,483 adults on dialysis who received KT from 6/12/2000 – 6/13/2010. KT recipients were categorized as having 0, 1, or >1 EHRs. We fit a multinomial logistic regression model using 0 EHRs as the reference category.

Results: Median age was 50 years; 34% were black and 63% male. 2048 subjects (19.5%) experienced 1 EHR after KT while 624 subjects (6%) experienced >1 EHR (max=4). Compared to 0 EHRs, the following variables were associated with having either 1 or >1 EHRs: older age (>70 years), diabetes, more years on dialysis, greater number of hospitalizations in 12 months prior to KT, an expanded criteria kidney donor, and delayed graft function. Black race (OR 1.14, p=0.02) and long wait times (1.4, p=0.04) were independently associated with having 1 EHR, but not >1. Lower rates of survival at 5 years were associated with a greater number of EHRs after KT (0.83, 0.79, 0.69 for 0, 1, and >1 EHRs, respectively, p<0.0001).



Conclusions: Our results suggest that distinct patient attributes predict single versus multiple EHRs after KT. Recipients with multiple EHRs after KT have the highest risk of death. A single EHR after KT may be less indicative of medical complexity/complications than >1, and these differences warrant further investigation.

Funding: NIDDK Support

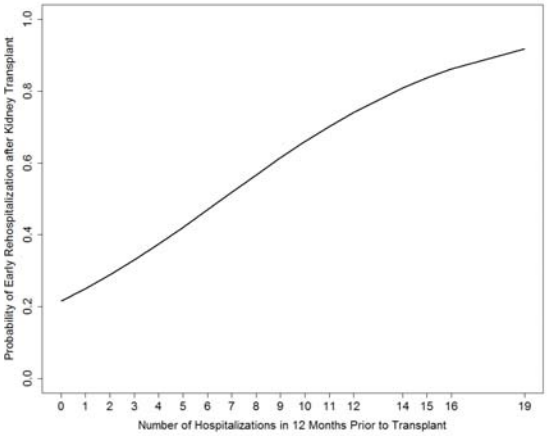
TH-PO1085

Pre-Transplant Hospitalizations Predict Early Hospital Readmission after Kidney Transplantation Meera Nair,[1] Alexander Stephen Hill,[2] Wei Wang,[2] Adam S. Mussell,[1] Roy D. Bloom,[1] Harold I. Feldman,[1] Peter P. Reese.[1] *[1]Renal Electrolyte & Hypertension Div, Univ of Pennsylvania School of Medicine, Philadelphia, PA; [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA.*

Background: While many patients awaiting kidney transplant (KT) have high rates of health care utilization, KT results in rapid changes in health status. The association of previous hospitalizations with early hospital readmission (EHR) within 30 days from discharge after KT is unknown.

Methods: We linked datasets from the Organ Procurement and Transplantation Network and Medicare and performed a retrospective cohort study of 10,483 adults on dialysis who received KT from 6/12/2000 – 6/13/2010. Our primary exposure was number of hospitalizations to an acute care facility in the 12 months prior to KT.

Results: Median age was 50 years; 34% were black and 62% male. 2672 KT recipients (25.4%) experienced EHR. Median time to EHR was 9 days (mean 11.1, SD 7.9). 5575 recipients (53.2%) had >1 hospitalization in the 12 months prior to KT (max=19). In multivariable adjusted analysis, number of prior hospitalizations was an independent predictor of EHR (OR=1.2 per hospitalization, p<0.001). Older recipients (age>70, OR=1.5, p=0.0003), those with prior KT (OR=1.2, p=0.01), greater years on dialysis (1.04 per year, p<0.0001), diabetes (OR=1.2, p=0.0001), and delayed graft function (OR=1.6, p<0.001) were also at increased risk of EHR. Adjusting for prior hospitalizations, EHR remained a predictor of mortality at 1 and 5 years post KT (OR=1.5, p < 0.0001 and OR=1.3, p<0.0001, respectively).



Conclusions: While KT improves health status and survival among patients with advanced chronic kidney disease, prior health care utilization is strongly associated with EHR after KT. Knowledge of prior hospitalizations can help clinicians risk stratify patients at the time of discharge from KT.

Funding: NIDDK Support

TH-PO1086

Early Hospital Readmission After Kidney Transplantation: Incidence, Causes, and Risk Factors Olusegun Famure, Esther D. Kim, Johnny Huang, Roman Zyla, Joseph Kim. *Kidney Transplant Program, Univ Health Network, Toronto, ON, Canada.*

Background: In the immediate post-transplant period, kidney transplant recipients are at an increased risk for a number of potentially serious medical or surgical complications that can result in early hospital readmission (EHR). Understanding the risk factors and causes of EHRs is essential to identifying strategies for reducing this burden of this problem for both patients and hospitals.

Methods: This single-centre cohort study included 1,531 kidney transplant recipients from 1 Jul 2004 and 31 Dec 2012, with at least 1 year of follow-up. EHR was defined as hospitalization within 30 or 90 days post-discharge from the transplant admission. Recipient, donor, and transplant characteristics were measured at baseline. Associations between these baseline characteristics and EHR within 30 days and 90 days post-transplant were determined using multivariable Cox proportional hazards model.

Results: The rates of EHR were 20.3% at 30 days, and 27.7% at 90 days post-transplant. The median times to the first EHR were 8 days and 13 days within 30 and 90 days post-transplant, respectively. Infectious complications were the most common reasons for EHR within 30 and 90 days post-transplant. Factors associated with EHR within 30 and 90 days in multivariable models were recipient history of peripheral vascular disease (hazard ratio or HR 2.16 [95% CI: 1.19, 3.91]), chronic lung disease (HR 1.98 [95% CI: 1.12, 3.5]), time on dialysis (HR 1.09 [95% CI: 1.01, 1.17]), and length of transplant hospitalization (HR 1.02 [95% CI: 1.00, 1.05]). Additional factors associated with EHR at 90 days after adjusting for baseline covariates were recipient history of cancer (HR 0.45 [95% CI: 0.21, 0.95]), and donor history of hypertension (HR 1.47 [95% CI: 1.01, 2.15]).

Conclusions: Early hospital re-admission is common after transplantation in Canadian kidney transplant recipients. The 30-day re-admission rate of 20.3% compares favourably to the 31% rate in the U.S. but strategies to reduce the burden of early hospital re-admissions is needed for all patients.

TH-PO1087

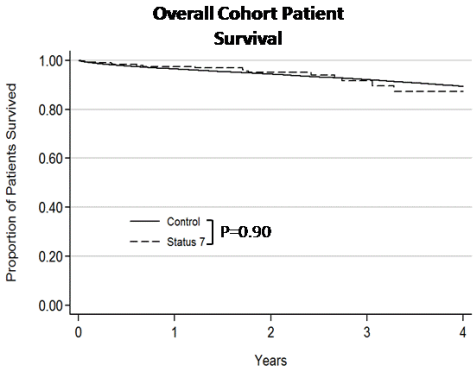
Pre-Transplant Weight Loss and Survival After Kidney Transplant Edmund Huang, Anake Yoosabai, Nattawat Klomjit, Suphamai Bunnapradist. *Nephrology, UCLA School of Medicine, Los Angeles, CA.*

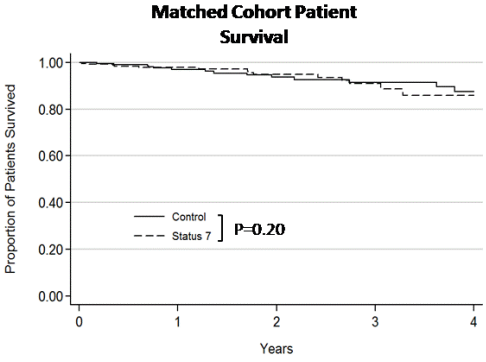
Background: We studied patient/graft survival of kidney transplant (txp) recipients who were once temporarily listed inactive (status 7) due to obesity and explored the impact of pre-txp weight loss on txp outcomes.

Methods: Adult kidney recipients from OPTN/UNOS registered with a BMI≥30 kg/m^2 from 2006-10 and designated status 7 due to a "weight inappropriate for txp" were included (n=328). Patient/graft survival were compared to a contemporaneous control of all other adult recipients (n=74,006). To assess if pre-txp weight loss associated with differences in outcomes, patient/graft survival were compared within status 7 stratified into quartiles of % BMI change while waitlisted. Status 7 recipients (n=257) were then 1:1 matched to controls on age, gender, diabetes, donor type, and list BMI for status 7 and txp BMI for controls.

Results: Median list and txp BMI for status 7 exceeded controls (38 versus 27 kg/m^2; 36 versus 27; p<0.001). Actuarial 4-year patient (status 7=87%;control=89%;p=0.90) and graft survival (status 7=83%;control=82%,p=0.75) did not differ. After multivariate analysis, status 7 was not associated with death (HR: 0.96, 0.59-1.57) or graft loss (0.95; 0.66-1.38). No difference in patient/graft survival was seen when status 7 was stratified into quartiles of % BMI change while waitlisted. In the matched cohort, median waitlist BMI change was greater for status 7 than controls (-1.6 versus 0.6 kg/m^2;p=0.001), but no difference in patient (status 7, 86%; control, 87%, p=0.20) and a slight increase in graft survival was seen (status 7, 82%; control, 79%,p=0.03).

Conclusions: Obese status 7 recipients had comparable survival to the overall kidney population. Pre-txp weight loss in obese kidney candidates had limited benefit on post-txp outcomes with no mortality benefit seen with greater weight loss and only a modest increase in graft survival when compared to matched controls.





TH-PO1088

Referral of Dialysis Patients for Transplant Based on Hypothetical Patient Scenarios Kevin C. Roe,[1] Ming Wang,[2] Surju Patel,[1] Ankita Tandon,[1] Nasrollah Ghahramani.[1] *[1]Medicine, Penn State College of Medicine, Hershey, PA; [2]Public Health Sciences, Penn State College of Medicine, Hershey, PA.*

Background: Compared to dialysis, kidney transplant (KT) is associated with expected improved quality of life and increase in expected life years. We analyzed the referral decisions of nephrologists to evaluate factors that influence KT in end-stage renal disease (ESRD) patients on dialysis.

Methods: In an online survey, 250 nephrologists were asked whether they would recommend kidney transplant for hypothetical patients who were already on dialysis. The 15 scenarios varied in age, race, sex, living situation (alone or with spouse), employment, and rural location.

Results: 214 nephrologists provided complete responses. In multivariate analysis, age <50 (OR:1.70; p<0.005) and living with spouse (p<0.01) were associated with higher likelihood of being recommended for KT; unemployment (OR:0.55;p=0.01) and rural location (OR:0.52;p=0.001) were associated with lower likelihood. Based on logistic regression, the following nephrologist-related variables were associated with higher likelihood of choosing KT for their patient: academic affiliation (OR:2.23;p=0.04), attending 4 or more national nephrology meetings (OR: 4.62; p<0.01), and a coordinator for workup of KT candidates (OR:3.21;p=0.05). Rural practice (p= 0.04), years from fellowship (p= 0.001), and fewer years practicing in the current location (p=0.02) were associated with lower likelihood of recommending KT.

Conclusions: Identifying factors that influence referral practices and educating physicians may improve KT distribution by acknowledging the ways in which location, patient living situations, patient age and physician education affect nephrologists' and ultimately patients' decisions to pursue transplant.

Funding: Other NIH Support - K23DK084300

TH-PO1089

The Influence of Socioeconomic Deprivation on Allograft and Patient Survival following Kidney Transplantation Frank Ward,[1] Patrick O'kelly,[1] Fionnuala Donohue,[2] Coilín O. Oh Aiseadha,[2] Trutz Haase,[3] Jonathan Pratschke,[3] Declan G. de Freitas,[1] Howard Johnson,[2] Peter J. Conlon,[1] Conall M. O'Seaghdha.[1] *[1]Dept of Tranplantation and Renal Medicine, Beaumont Hospital, Dublin, Ireland; [2]Health Intelligence Unit, Health & Wellbeing Directorate, Health Service Executive, Dublin, Ireland; [3]Social and Economic Consultants, Health Service Executive, Dublin, Ireland.*

Background: Whether socioeconomic deprivation confers worse allograft or patient outcomes after kidney transplantation is not known. We examined its influence on allograft and patient survival following kidney transplantation in Ireland.

Methods: We identified all adult deceased donor first kidney transplant recipients from 1990 to 2009, inclusively. Those with a valid Irish postal address on record were assigned a deprivation score based on the Pobal Hasse-Pratschke deprivation index (2011). Cox proportional regression analysis and Kaplan Meier survival analysis by quartile were used to investigate any association of socioeconomic deprivation with allograft and patient survival.

Results: We identified 1,977 eligible kidney transplant recipients, with 33 subsequently excluded due an invalid/missing address. The median follow-up time was 8.2 years (interquartile range 4.4 – 13.3 years). Patient survival was not associated with deprivation score (HR 1.0, 95% CI 0.93-1.08, p=0.88). The deprivation score was not associated with reduced uncensored or death-censored allograft survival (HR 0.997, 95% CI 0.989-1.003, p=0.33 and HR 0.995, 95% CI 0.986-1.004, respectively), with no difference amongst quartiles in either uncensored or death-censored allograft survival at 5- and 10-years. The most deprived quartile had a longer duration of dialysis therapy before transplantation compared to the least deprived (22months versus 18months, p=0.02).

Conclusions: In a healthcare model affording free or subsidised medication and healthcare coverage for eligible patients, there was no disparity in allograft or patient outcomes following kidney transplantation based on socioeconomic status. This gives further impetus to calls in other jurisdictions for universal healthcare and medication coverage for kidney transplant recipients.

TH-PO1090

Reduction in Perioperative Antibiotic Exposure After Kidney Transplantation Leads to a Decrease in Urinary Tract Infections with Extended Spectrum Betalactamase-Producing Bacteria Anja Susanne Muehlfeld,[1] Felix Wenzel,[1] Jürgen Floege,[1] Simone Scheithauer.[2] *[1]Div of Nephrology, Uniklinik RWTH Aachen, Aachen, Germany; [2]Zentralinstitut für Krankenhaushygiene und Infektiologie, Universitätsmedizin Göttingen, Göttingen, Germany.*

Background: Because of the high incidence of urinary tract infections (UTI) after kidney transplantation, especially with multiresistent strains, we changed our perioperative protocol in November 2008 from a prophylactic antibiotic treatment with cefuroxime (mean 11.2±6.0 days) during the time of percutaneous urostomy (group 1) to preoperative single shot antibiotic administration and internal urinary stenting with a double J catheter (group 2).

Methods: We performed a retrospective cohort study in 183 consecutive patients who received a renal transplant at our center between 2006 and 2011. The incidence of UTI, the spectrum of pathogens as well as the antimicrobial sensitivities during the first year after kidney transplantation were recorded.

Results: The abandonment of prolonged antibiotic prophylaxis reduced the time of antibiotic treatment from 35±38 to 25±47 d/patient within the first year after kidney transplantation (p<0.001). Despite this, there was no increase in the incidence of UTI (2.1±2.0 UTI/patient in group 1 versus 1.9±2.7 UTI/patient in group 2) while the incidence of UTI with ESBL (extended spectrum betalactamase) producing bacteria fell from 48% of all UTI to 23% during the two time periods (p<0.001). This was in contrast to the general trend at our hospital where UTI with ESBL producing bacteria increased in the same time from 4 to 14 %. In addition, the bacterial spectrum changed with respect to more UTI with E. coli (33% in group 1 versus 43% in group 2; p<0.05) and fewer UTI with pseudomonas aeruginosa (19% versus 8%; p<0.05).

Conclusions: A reduction in perioperative antibiotic exposure did not increase the rate of UTI within the first year after kidney transplantation and rather reduced the incidence of UTI with ESBL producing bacteria. In addition, the spectrum of isolated bacteria changed to more cefuroxime sensitive E. coli and fewer cefuroxime resistant strains (pseudomonas).

TH-PO1091

Secular Trends in Cardiovascular Disease in Kidney Transplant Recipients: 1994 to 2009 Ngan Lam,[1] Kyla Lynn Naylor,[1] Eric Mcarthur,[2] Joseph Kim,[3] Greg A. Knoll,[4] Salimah Z. Shariff,[2] Amit X. Garg.[1,2] *[1]Western Univ; [2]Inst for Clinical Evaluative Sciences; [3]Univ of Toronto; [4]Univ of Ottawa.*

Background: Cardiovascular death remains the number one cause of mortality in kidney transplant recipients. Cardiovascular events alone are associated with significant morbidity.

Methods: We conducted a retrospective cohort study using Ontario's linked healthcare databases to follow all first-time kidney-only transplant recipients between 1994 to 2009. Our primary outcome was a composite of death or first major cardiovascular event defined as one of myocardial infarction, coronary angioplasty, coronary bypass surgery, or stroke within three years of the transplant date.

Results: There were 4954 first-time kidney-only transplant recipients during the study period, of which 63% were male. The median age steadily increased from 43 years (interquartile range [IQR] 33-54) in 1994 to 53 years (IQR 42-62) in 2009 as did the proportion of recipients aged 65 years old older (3.8% in 1994 to 20.4% in 2009). There was also an increase in the proportion of recipients with coronary artery disease (23.7% in 1994 to 37.7% in 2009) and diabetes (19.2% in 1994 to 29.9% in 2009). A total of 444 recipients (9.0%, 95% confidence interval 0.082 to 0.098; 3.15 events per 100 person-years) died or experienced a major cardiovascular event within three years of transplantation with no significant change over time (P=0.59).

Conclusions: Despite transplant centres accepting recipients who are older with more co-morbidities, the three-year incidence of death or major cardiovascular event has remained stable from 1994 to 2009. These results are reassuring for transplant programs.

TH-PO1092

Early Post-Operative Acute Myocardial Infarction in Kidney Transplant Recipients: Incidence, Risk Factors, and Outcomes Maya Deeb,[1,2] Christopher Brian Overgaard,[2] Yanhong Li,[1] Olusegun Famure,[1] Joseph Kim.[1,2] *[1]Multi-Organ Transplant Program, Toronto General Hospital, Toronto, ON, Canada; [2]Univ of Toronto, Toronto, ON, Canada.*

Background: The epidemiology of acute myocardial infarction early after kidney transplantation has not been well studied. We examined the incidence, risk factors, and outcomes of early post-operative acute myocardial infarction (EAMI) in a cohort of Canadian kidney transplant recipients.

Methods: A total of 1,464 patients who underwent kidney transplantation at our center from 1 Jan 2000 to 30 Sep 2012 (followed until 30 Sep 2013) were included. A nested case-control design was used to study EAMI risk factors with a conditional logistic regression model. EAMI cases (within 3 months post-transplant) were adjudicated by a single cardiologist using the consensus definition set by the American Heart Association. Each case was matched to 5 controls on follow-up time, transplant year, and donor type. To assess the association of EAMI with graft loss and/or death, a Cox proportional hazards model was fitted to the total study cohort.

Results: A total of 49 (3.3%) kidney transplant patients had an EAMI episode. Most cases occurred within 3 days post-transplant (61.2%). Recipient factors that predicted EAMI included older age (OR 1.06 [95% CI: 1.03, 1.09]), female sex (OR 0.36, [95% CI: 0.16, 0.79],) pre-transplant diabetes (OR 3.91 [95% CI: 2.05, 7.45]), history of cardiac disease (OR 8.70 [95% CI: 4.04, 18.73]), and smoking (OR 1.97 [95% CI: 1.02, 3.80]). History of cardiac disease (OR 5.11 [95% CI: 2.23, 11.73]) and longer duration of dialysis (OR 1.21 [95% CI: 1.07, 1.37]) independently predicted EAMI after multivariable adjustment. EAMI was associated with significantly elevated relative hazards for total graft failure (HR 2.12 [95% CI: 1.24, 3.64]) and death with graft function (HR 2.42 [95% CI: 2.42 [95% CI: 1.27, 4.62]), but not death-censored graft failure (HR 1.58 [95% CI: 0.61, 4.08]).

Conclusions: Patients with a cardiovascular history and longer duration of dialysis were at elevated risk of EAMI. While the incidence of EAMI in kidney transplant recipients is low, it is associated with a significant risk of graft failure (including death).

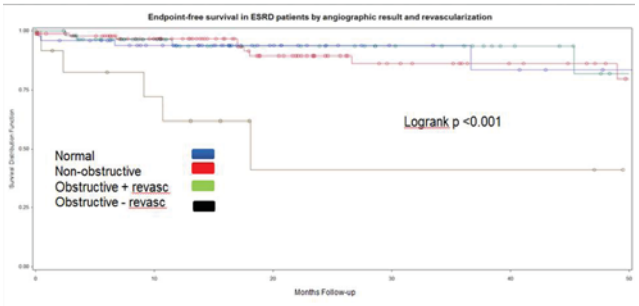
TH-PO1093

Cardiovascular Assessment in Pre-Renal Transplantation Candidates Eric M. Lindley,[1] Amanda K. Hall,[2] Jo Abraham.[2] *[1]Cardiovascular Medicine, Univ of Utah, Salt Lake City, UT; [2]Nephrology, Univ of Utah, Salt Lake City, UT.*

Background: There is lack of uniformity in the cardiovascular (CV) assessment of prospective kidney transplant recipients. In 2012, the American Heart Association (AHA) and the American College of Cardiology (ACC) issued a scientific statement, endorsed by the National Kidney Foundation, suggesting noninvasive stress testing for candidates who had 3 or more CV risk factors (age >60, diabetes, hypertension, dyslipidemia, >1 yr on dialysis, smoking, left ventricular hypertrophy, or known CV disease). However, many transplant programs take a more aggressive approach.

Methods: We conducted a historically prospective study evaluating the CV assessment, outcomes in a single center.

Results: Overall, 685 patients were evaluated. 472 candidates underwent some form of stress testing. 229 patients underwent coronary angiogram and 72 (31%) of those patients had obstructive coronary artery disease (CAD). Of the 72 patients, 10 were not revascularized. Patients who had normal coronaries, non-obstructive CAD, or CAD with intervention had significantly higher event free survival compared to patients with obstructive CAD without intervention.



The sensitivity of stress testing to detect obstructive CAD was (0.26). There were 338 patients transplanted in the follow-up period. In transplanted patients who had an angiogram (n=77), there were no clinic events (death, myocardial infarction (MI), stroke, urgent revascularization, graft failure) in the first 30 days post-transplant. Of transplanted patients who did not have an angiogram (n=289), there were 8 clinical events (6 MI's), in the first 30 days (p=0.15).

Conclusions: Our results indicate that stress testing in an ESRD population being evaluated for renal transplantation has a very poor sensitivity, and that none of the patients who had angiograms had a clinical event in the first 30 days post-operatively.

TH-PO1094

Cardiovascular Work Up for Kidney Transplantation Shuaib Ahmed Quraishi, Shankar Kumar, Hwee Jeon, Juan Carlos Kaski, Rajan Sharma, Debasish Banerjee. *Renal Dept, St. George's Healthcare NHS Trust, United Kingdom.*

Background: Cardiovascular (CV) event rates in CKD patients are high, during waiting, peri-operative and post-operative transplant periods. The aim of this study was to explore the value of pre-operative cardiac investigations to predict such events.

Methods: Data was collected retrospectively in 201 patients. The cardiac investigations were performed using a pre-specified protocol. The low risk patients had exercise treadmill testing (ETT) and echocardiogram. High risk patients (>60 y, diabetes, CAD) underwent dobutamine stress echocardiography (DSE). Patients underwent coronary angiogram (CA) if the ETT or DSE were positive or had angina. The patients were followed up from the time of the first cardiac testing to first event including ACS, stroke, CHF, PVD or death.

Results: The clinical characteristics were: age 54±12 years, BMI 28±5 kg/m^2, women 41%, diabetes 31%, hypertensive 92%, smokers 18% and dyslipidaemia 58%. During the follow up there were total of 45 events. 42 low risk patients performed ETT and 7 had positive test. 6 out of 7 with positive ETT had a dobutamine stress echocardiography (DSE) and 4/6 were negative. 2 patients with positive DSE had normal angiogram. The remaining 1 patient with abnormal ETT also had a normal angiogram. There was no significant difference in event rates between patients with abnormal ETT 0/7 and with normal ETT 3/32 (chi sq 5.17 p=1.00). 73 high risk patients underwent DSE. 18 patients with positive DSE had angiogram of which 7 were normal. The patients with abnormal DSE had higher rates of events, 7/19 compared to patients with normal DSE 7/54 (chi sq 5.17, p=0.039). 53 patients underwent CA and 31 were abnormal. 16 patients underwent coronary interventions. Patients with abnormal CA were more likely to be diabetics (24/31 versus 9/20; p=0.034). There was no statistically significant difference in event rates between patients with abnormal CA 6/22 and normal CA 16/31 (chi sq 3.1, p=0.096).

Conclusions: This study shows the value of DSE as a predictor of CV events, ETT in low risk patients did not predict CV events, while CA in selected patients resulted in interventions but did not predict events.

TH-PO1095

Exploring Variation in the Practice Patterns of Assessing Patient Suitability for Renal Transplantation in the United Kingdom Rishi Pruthi,[1] Paul J. Roderick.[2] *[1]UK Renal Registry, United Kingdom; [2]Univ of Southampton, on Behalf of ATTOM Study Group, United Kingdom.*

Background: The Access to Transplant and Transplant Outcome Measures (ATTOM) study is the largest UK transplant study to date exploring equity in access to renal transplantation. In conjunction with ATTOM this national survey aimed to investigate whether centre variation existed in the assessment of patients for deceased-donor renal transplantation in the UK.

Methods: Thematic analysis of 43 semi-structured qualitative interviews with key stakeholders conducted across a purposive sample of 9 renal centres in the UK informed the development of an online survey distributed to the Clinical Directors of all renal centres in the UK. This survey measured differences in centre assessment processes and their evaluation of patient's age, body mass index(BMI), cardiovascular disease(CVD) and malignancy.

Results: All 71 renal centres(100%) in the UK responded, including all 23 transplanting centres. 26% of centres did not discuss transplantation as a treatment option with all their patients aged <75 years and 28% did not have a formal assessment protocol. Only 3 centres in the UK had a cut-off age limit(<75 years) although 83% of centres excluded patients with a high BMI, median 35(range 30-40). There was considerable variation in the investigation of CVD and exclusion criteria based on cardiovascular status of the patients. Cardiac investigations were risk-stratified in 90% of centres. In high-risk patients a thallium stress test was the first line investigation in 32% of centres, while only 3 centres opted for coronary angiography. 59% of centres did not routinely screen for malignancies. Surgical involvement in assessing suitability varied with 10% of centres listing patients for transplantation without any formal surgical review.

Conclusions: There was marked variation in the assessment of patients for renal transplantation across the UK. Future work will investigate if unit factors influence listing independently of patient factors in a prospective cohort in ATTOM. Further research and high quality evidence-based guidelines are necessary to inform a uniform assessment process and to ensure equity of access to the renal transplant waiting list.

TH-PO1096

Long-Term Evaluation of Coronary Calcifications in Kidney Transplanted Patients: A Follow Up of 5 Years Carlo M. Alfieri,[1] Laura Forzenigo,[2] Maria Meneghini,[1] Biljana Danilovic,[2] Anna Regalia,[1] Donata Cresseri,[1] Piergiorgio Messa.[1] *[1]Nephrology, Dialysis and Kidney Transplantation, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Milan, Italy; [2]Radiology, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Milan, Italy.*

Background: Coronary artery calcifications(CAC) have been related to the increased CV mortality in CKD patients. Few data are available on the long term behavior of CAC in kidney transplantation (KTx). Using coronary CT we evaluated: 1) the prevalence,the clinical and biochemical factors related with CAC; 2) the factors implicated with CAC progression in the long run.

Methods: We evaluated 87 pts (M=51) transplanted in our unit between 2007 and 2008. Clinical parameters, blood and urinary samples were collected for 5years. For the statistical analysis the mean values of these parameters were considered. At baseline

(T0) and 5yrs after KTx (T5), coronary TC for the evaluation of CAC (Agatson score) was performed. Both at T0 and at T5, the patients were categorized in 4groups according to the Agatson score: 1)0-10;2)10-100;3)100-400;4)>400. The progression of CAC was determined using the formula proposed by Sevrukov(A-progr),or as passage in a higher CAC score group(B-progr).

Results: At T0 and T5,43% and 33% of pts were in the 1st group,15% and 17% in the 2nd,24% and 23% in the 3rd, 13% and 26% in 4th, resp. CAC at T5 were higher than T0. Both at T0 and T5,CAC correlated directly with age. The CAC-score values at T5 were highly dependent on the T0 levels.The 22%of pts, who had a significant increase in CAC score (A-progr), had lower mean levels of PTH and ALP. The 27% of pts, who worsened their category of CAC(B-progr), had only moderately lower levels of PTH. In a logistic model, PTH was the only independent factor inversely related with CAC progression (A-progr). During the 5 year of KTx one patient died for cerebral hemorrhage and 4pts restarted dialysis for chronic allograft dysfunction.

Conclusions: The prevalence of CAC in CKD patient is quite high, and is related to the patient age. CAC worsening on the long term was observed in almost ¼ patients characterized by higher T0 CAC score. CAC progression resulted mostly associated with lower PTH levels and, at a lesser extent, lower ALP levels.

TH-PO1097

Effects of Kidney Transplantation on Left Ventricular Remodeling and Risk Factors for Post-Transplant Left Ventricular Hypertrophy

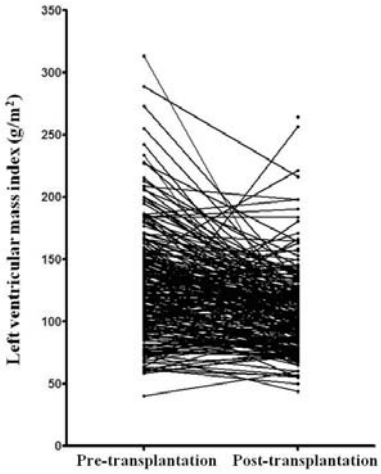
Jung Nam An,[1] Yun Kyu Oh,[1] Su-Kil Park,[2] Yon Su Kim,[3] Chun Soo Lim,[1] Jung Pyo Lee.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Asan Medical Center and Univ of Ulsan College of Medicine; [3]Dept of Internal Medicine, Seoul National Univ Hospital.*

Background: Cardiovascular disease is a leading cause of mortality in patients with end-stage renal disease, even undergoing transplantation. Left ventricular hypertrophy (LVH) is the most common feature and an independent risk factor for cardiac complications in kidney transplant recipients. The aim of this study was to identify cardiac alteration after kidney transplantation (KT) and analyze predictors of the post-transplant LVH.

Methods: Among 2957 kidney transplant recipients in a multicenter cohort from 1997 to 2012, a total of 221 patients who conducted echocardiography before and one year after transplantation were enrolled in this study. LVH was defined as left ventricular mass index (LVMI) > 95 g/m^2 for women and > 115 g/m^2 for men, respectively.

Results: KT significantly reduced mean LVMI from 130.5 ± 46.9 g/m^2 to 108.0 ± 34.4 g/m^2 ($p < 0.001$).

Figure 1.



After KT, prevalence of LVH decreased (66.1% versus 49.3%, $p < 0.001$), and ejection fraction improved (59.5 ± 7.6% versus 62.3 ± 6.2%, $p < 0.001$). Diastolic dysfunction and valvular regurgitations also decreased. After adjusting other risk factors including age, gender, underlying renal diseases, and immunosuppressants, higher pre-transplant LVMI (OR 1.02, 95% CI 1.01-1.02, $p < 0.001$) and uncontrolled blood pressure after transplantation (OR 1.02, 95% CI 1.00-1.05, $p = 0.025$) were independently associated with persistent LVH after KT.

Conclusions: Cardiac morphology and function were significantly improved by kidney transplantation. Optimal management of hypertension is crucial in regression and prevention of persistent LVH in kidney transplant recipients.

TH-PO1098

The Prognostic Significance of Preoperative Diastolic Dysfunction of the Left Ventricle and Left Atrial Enlargement on Clinical Outcomes in Kidney Transplantation

Jung Nam An,[1] Hyosang Kim,[2] Jaeseok Yang,[3] Curie Ahn,[3] Su-Kil Park,[2] Yon Su Kim,[4] Jung Pyo Lee.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Asan Medical Center and Univ of Ulsan College of Medicine; [3]Transplantation Center, Seoul National Univ Hospital; [4]Dept of Internal Medicine, Seoul National Univ College of Medicine.*

Background: Echocardiography is commonly performed as a screening test to evaluate cardiac function before kidney transplantation (KT). Identification of high-risk patients for cardiovascular (CV) disease is important to offer appropriate management before KT.

Methods: We reviewed 2,957 adult recipients who underwent pretransplant echocardiography from 1997 to 2012 in order to evaluate the prognostic significance of preoperative markers of LVDD and LAE. The LVDD was defined referring to the recommendations of the European Study Group on Diastolic Heart Failure and was divided by 4 grades: 0 (normal); 1 (relaxation abnormality); 2 (pseudonormalization); and 3 (restrictive pattern). The patients with grade 0 and grade 1 and patients with grade 2 and grade 3 were combined and analyzed, respectively.

Results: During the observation period (mean 54.1 months), the recipients with LVDD grade 2-3 showed higher occurrence of major adverse cardiac events (MACE) (P=0.001) and graft failure (P=0.005). All-cause mortality was not different between the groups depending on the LVDD grades. The recipients with LAE tend to be associated only with the occurrence of the MACE (P=0.002). In a multivariate analysis, increased age (P=0.001), previous history of CV event (P<0.001) and LVDD of grade 2-3 (HR 4.076; 95% CI 1.667-9.966; P=0.002) were associated with MACE. The recipients with LAE also showed significantly higher occurrence of MACE in a multivariate analysis (HR 3.172; 95% CI 1.069-9.410; P=0.037). Graft failure and all-cause mortality were not showed significant differences in both recipients with LVDD and LAE in a multivariate analysis.

Conclusions: The echocardiographic findings of LVDD and LAE before kidney transplantation may increase the risk of developing cardiovascular event.

TH-PO1099

Cardiac and Vascular Changes in Patients with Chronic Kidney Disease Before and After Kidney Transplantation: A Prospective Study

Azhar Ali,[1] Andrea Dell'Aquila,[1] Louise E. Ross,[1] Nabeel Sheikh,[1] David Gaze,[1] Juan Carlos Kaski,[1] Debasish Banerjee.[1,2] *[1]St. George's Univ of London, United Kingdom; [2]St. George's Healthcare NHS Trust, United Kingdom.*

Background: Chronic kidney disease (CKD) is associated with increased risk of cardiovascular (CV) morbidity and mortality with event rates being almost fifty times higher than those in the general population. Recent studies suggest that CV event rates improve following kidney transplantation, however the exact mechanism of the changes is not known. We investigated functional and structural changes taking place in the CV system following kidney transplantation in patients with CKD.

Methods: 10 non-diabetic (3 predialysis, 7 dialysis) patients were enrolled in the present study. Cardiac structure and function using the echocardiogram, endothelial function using brachial artery flow mediated dilatation techniques and circulating biomarkers were assessed within 7 days before and 6 to 12 months after renal transplantation. Blood biomarkers of inflammation, oxidative stress and cardiac function in the study included: hsCRP, ICAM-1, SOD, H_2O_2 and NT-proBNP using standard ELISA techniques. Left ventricular mass (LVM) was calculated using the Penn formula and further indexed to height and corrected for body surface area to give the left ventricular mass index (LVMI).

Results: The clinical characteristics of the patients at baseline were; age (51±13 years), women (20%), BMI (24.9±5.3 kg/m^2), hypertensives (80%), dyslipidaemics (30%), current smokers (0%), history of MI (10%), history of TIA/stroke (10%), systolic BP (138±22 mmHg), diastolic BP (78±12 mmHg), total cholesterol (4.9±1.4 mmol/L), haemoglobin (111±190 g/L), corrected calcium (2.32±0.14 mmol/L) and phosphate (1.66±0.48 mmol/L). During follow up (244±25 days), LVMI improved from 140±37 g/m^2 to 115±30 g/m^2 ($p<0.05$). FMD increased from 2.45±1.67% to 4.67±1.23% ($p<0.05$). Furthermore, hsCRP levels decreased from 1.4±14.1 mg/L to 0.7±2.3 mg/L ($p<0.05$).

Conclusions: This study demonstrates an improvement of hsCRP, FMD and LVM in non diabetic patients 6-12 months post kidney transplantation. We propose that a reduction in low grade inflammation may result in improved endothelial function and LVM.

TH-PO1100

Aspirin Use and Cardiovascular Disease and Kidney Outcomes in Stable Kidney Transplant Recipients: The FAVORIT Trial

Taimur Dad,[1] Alin A. John,[1] Hocine Tighiouart,[1] Myra A. Carpenter,[2] Lawrence G. Hunsicker,[3] John W. Kusek,[4] Marc A. Pfeffer,[5] Andrew S. Levey,[1] Daniel E. Weiner.[1] *[1]Tufts Medical Center; [2]North Carolina; [3]Iowa; [4]NIDDK; [5]Brigham and Women's Hospital.*

Background: Cardiovascular disease (CVD) is the leading cause of death in kidney transplant recipients. The role of aspirin for primary or secondary prevention of CVD and reduction of other adverse outcomes is uncertain.

Methods: This *post hoc* analysis of FAVORIT, an RCT evaluating the role of homocysteine-lowering vitamin therapy to reduce CVD in 4,110 stable kidney transplant recipients, used a propensity score with a 0.1 standard deviation caliper to match aspirin users to aspirin non-users. Outcomes were primary cardiovascular (CV) events, all-cause death, kidney failure, and a composite of kidney failure and death. Stratified Cox models,

adjusting for patient characteristics, including age, sex, race, diabetes, self-reported CVD history, hypertension, smoking status, cholesterol, eGFR, urine ACR, and transplant donor type and vintage, evaluated the effect of aspirin use on study outcomes.

Results: There were 1125 aspirin users matched to 1125 non-users, with no standardized difference above 5%. Mean age was 52 years with 37% women and 16% African Americans; 44% had history of diabetes, 93% history of hypertension, and 20% history of CVD. Mean eGFR was 49 ml/min/1.73m^2, median ACR 22 mg/g and mean graft vintage 5.2 years. Over 4 years of follow-up, there were 331 CV events, 271 deaths, 158 kidney failure events and 387 composite events. In adjusted models, there was no difference in CV events [HR=1.17 (0.85,1.60)], all-cause death [HR=0.84 (0.59,1.19)], kidney failure [HR=0.81 (0.35,1.90)] or composite events [HR=0.95 (0.70,1.28)] by aspirin use. When examining the subset without baseline CVD in whom aspirin was presumptively taken for primary prevention, results were similar: HR=0.99 (0.64,1.51), HR=0.86 (0.52,1.41), HR=0.71 (0.23,2.16) and HR=0.89 (0.59,1.23) for CV, mortality, kidney failure and composite outcomes, respectively.

Conclusions: We found no association between aspirin use and adverse events in stable kidney transplant recipients with a low prevalence of CVD overall and in persons without CVD at baseline.

Funding: NIDDK Support

TH-PO1101

Does African American Race Impact Statin Efficacy in Renal Transplant Outcomes? Mukoso N. Ozieh,[1] David J. Taber,[1,2] Prabhakar Baliga,[1] Titte Srinivas,[1] Leonard Egede.[1,2] *[1]Nephrology, MUSC, Charleston, SC; [2]Internal Medicine, Ralph H. Johnson VAMC, Charleston, SC.*

Background: In the general population, compared to Caucasians, African Americans (AA) have smaller LDL particle size and are less likely to receive statin therapy. Yet, AAs equally benefit from this therapy. However no studies have assessed if race impacts the efficacy of statins in renal transplant (RTX).

Methods: This was a retrospective analysis of solitary adult RTX at our center between June 2005 and May 2013. Cox proportional regression modeling was used to examine the impact of statin therapy on graft loss, death and acute rejection and determine if significant interactions exist between statin therapy and race. Models were adjusted for demographics, socioeconomic status, cardiovascular history, medication use and transplant characteristics. SPSS version 21.0 was used for statistical analysis.

Results: 1,176 RTX were included (624 AA versus 552 non-AA). AAs and non-AAs were equally likely to receive statin therapy (45% versus 45%, p=0.922). RTX patients on statin therapy were older, had more CV disease burden, received more marginal kidneys and were more likely to be on ACEIs/ARBs, antiplatelet therapy or beta-blockers. The mean LDL and TG in AA was 94 mg/dL and 133 mg/dL compared to 90 mg/dL and 163 mg/dL in non-AA. AAs did not experience a significant reduction in LDL with statin therapy (p=0.133), while non-AAs did (p=0.006). Overall, after adjusting for covariates, patients who received statins were 37% less likely to have graft loss (HR 0.63, 95% CI 0.43-0.94) and 29% less likely to die (HR 0.71, 95% CI 0.42-1.18). Acute rejection was not influenced by statin use (HR 0.88 95% CI 0.58-1.32). There was a statistically significant interaction between race and statin therapy for death, but not for graft loss or rejection. After stratifying the analysis by race, statins reduced the risk of death in AAs (HR 0.43, 95% CI 0.20-0.94), but not in non-AAs (HR 1.09, 95% CI 0.49-2.44).

Conclusions: Statin therapy significantly reduces the risk of graft loss in RTX, with AAs experiencing a significant mortality benefit from this therapy. These results highlight the importance of optimizing statin therapy in RTX, especially in AAs.

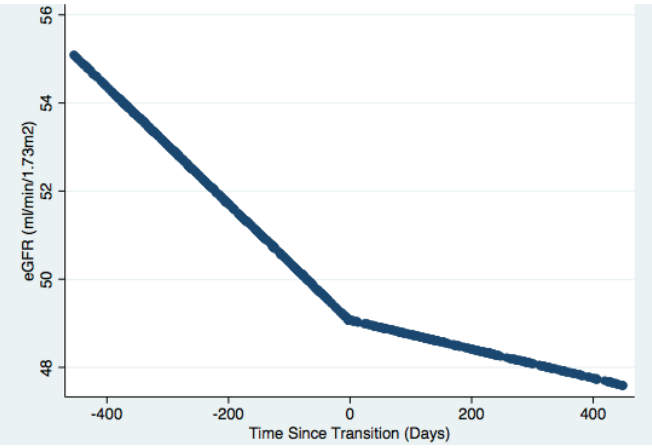
TH-PO1102

Effect of Transition to Adult Transplant Care on Kidney Function for Pediatric Kidney Transplant Recipients Hilda E. Fernandez,[1] Sandra Amaral,[2] Pamela A. Shaw,[1] Alden Michael Doyle,[3] Roy D. Bloom,[1] Melanie A. Mariano,[2] Alekhya Potluri,[3] H. Jorge Baluarte,[2] Susan L. Furth.[2] *[1]UPenn; [2]CHOP; [3]Hahnemann Univ.*

Background: Transition of care from pediatric (peds) to adult healthcare settings has been hypothesized as a risk factor for non-adherence and graft loss, however prior studies have been inconsistent in their findings and have focused on the late outcome of graft loss. We explored the effect of transition of care on changes in eGFR slope w/in a cohort of peds renal transplant (txplt) pts in a single center who transitioned from peds to adult txplt care.

Methods: Retrospective chart review study of peds kidney txplt pts (1999-2011) in a single U.S. peds txplt center. We examined change in eGFR (modified Schwartz formula) for all pts who transitioned txplt care from peds to adult healthcare through 2012. Date of transition was defined as last peds txplt clinic visit. Analysis was performed to assess change in eGFR pre versus post transition via a mixed-effects model.

Results: 48 patients had at least 2 time points pre/post transition to analyze. Median age at txplt was 16.5y (8.5-19.5). Most subjects were male (69%), white (65%), had CAKUT (42%), and HLA MM>3 (83%). 50% had LRKT. Median age at transition was 20y (17.4-23.6) and median eGFR at time of transition was 49 ml/min/1.73m2 (9-104). 6 pts had allograft loss w/in 365 days post-transition (24-347). Compared to pre-transition eGFR (prior to day 0 in Figure) there was a decrease in the decline of eGFR following transition (p<0.05).



After excluding pts w/ allograft loss w/in 1 yr after transition, this interaction was non-significant.

Conclusions: Transition of care was not independently a/w acceleration in eGFR decline, rather declines in eGFR preceded changes in healthcare settings. Future analysis will investigate effects of race, age at transition, acute allograft rejection, *dn*DSA as risk factors for decline in eGFR over time.

Funding: Other NIH Support - Kidney Disease Epidemiology T32 - Minority Supplement

TH-PO1103

Outcomes of Renal Transplantation in HIV Associated Nephropathy: Experience at a Tertiary Care Hospital in Maryland Sana Waheed, Mohamed G. Atta. *Dept of Medicine, Johns Hopkins Univ, Baltimore, MD.*

Background: Recent studies have shown that renal transplantation in both safe and effective in HIV infected patients. However, none of these studies have specifically looked at outcomes in patients with HIVAN. The focus of our study was to evaluate outcomes of renal transplantation in biopsy proven HIVAN patients.

Methods: Transplant and pathology records of all adult HIV infected patients undergoing kidney transplantation at Johns Hopkins Hospital between September 2006 and January 2014 were reviewed. Clinical characteristics and demographics of renal transplant recepients with biopsy proven HIVAN were compared to HIV infected transplant recepients without HIVAN. Graft survival and rejection rates over a 3 year period were calculated using the Kaplan-Meier method.

Results: Of the 16 HIV patients who underwent a renal transplant, 11 had HIVAN as the cause of their ESRD. 64 % of HIVAN patients developed delayed graft function (DGF) and 54% required post-operative dialysis within one week of transplant, compared to none in the non-HIVAN group. Antithymocyte globulin was the induction agent used in all but one of our patients and there were no serious infectious complications. The median follow up was 3.3 years. The 1 and 3-year graft survival rates in HIVAN patients were 100% and 81% respectively compared to 100% and 80% in non-HIVAN patients. The 1 and 3 year rejection rates were 18% and 27% in the HIVAN group, which were similar to rates of 20% and 40% in the non-HIVAN group.

Characteristics and Outcomes of patients with HIVAN compared to patients without HIVAN

Characteristics	HIVAN	No HIVAN	p-value
Patients	11	5	
Demographics			
Age [Mean(SD)]	47.6 (15.4)	46.1 (8.1)	0.26
Male [Number (%)]	9 (82)	4 (80)	0.70
African American [Number (%)]	10 (91)	4 (80)	0.54
Hepatitis C infection [Number (%)]	4 (36.4)	1(20)	0.5
Apolipoprotein homozygosity[Number (%)]	100 (3/3)	100 (1/1)	0.25
Transplant Related Variables			
Deceased Donor [Number (%)]	9 (82)	5 (100)	0.46
CD 4 count [Mean (SD) cells/mm^3]	606 (243.6)	648(301.4)	0.80
Delayed Graft Function [Number (%)]	7 (63.6)	0 (0)	0.03
Postoperative Dialysis [Number (%)]	6 (54.5)	0 (0)	0.06
CMV viremia [Number (%)]	1 (20)	0 (0)	0.33
BK viremia [Number (%)]	0 (0)	0 (0)	NA
Immunosuppression			
Induction with anti-thymocyte globulin [Number (%)]	10 (91)	5(100)	0.69
Induction with daclizumab [Number (%)]	1 (9)	0 (0)	
Long Term Follow up			
Follow up Duration [Mean (SD) years]	3.33 (1.9)	3.34 (2.8)	
One year rejection rate (%) [95% CI]	18 (4-53)	20 (1-70)	
Three year rejection rate (%) [95% CI]	27 (39-93)	40 (17-93)	
One year graft Survival (%)	100%	100%	
Three year graft Survival (%)	81%	80%	
Creatinine at 24 months post-transplant [Mean (SD) mg/dL]	2.01 (1.41)	2.03 (1.36)	0.98
Long Term Dialysis [Number (%)]	2(18)	1(20)	0.70
Graft Failure [Number (%)]	2(18)	1(20)	0.70
Malignancy [Number (%)]	1 (9.1)	1 (20)	0.54

Conclusions: Despite higher rates of DGF and need for post-operative dialysis, renal graft survival and rejection rates in HIVAN patients are similar to patients with HIV and an alternate cause of renal failure. However, overall rejection rates remain higher than the general population which can partially be explained by interaction of antiretroviral therapy with immunosuppressants.

TH-PO1104

Hyperuricemia and Mortality Post-Liver Transplantation Sana Waheed,[1] Christine Adefuin Murakami,[1] Joseph Craig Longenecker,[2] Mohamed G. Atta.[1] *[1]Dept of Medicine, Johns Hopkins Univ, Baltimore, MD; [2]Dept of Community Medicine, Kuwait Univ Faculty of Medicine.*

Background: The impact of hyperuricemia on renal function and mortality after orthotopic liver transplant (OLT) is poorly understood. This non-concurrent cohort study assessed the prevalence of hyperuricemia in the pre- and post OLT period, and its association with post-OLT mortality.

Methods: Medical records of 304 adult patients undergoing OLT at Johns Hopkins Hospital from 1996 to 2009 were reviewed. Clinical data, including a median of 90 post-OLT uric acid (UA) values per patient, were recorded. Mortality was assessed using NDI and UNOS. Post OLT hyperuricemia was defined as a mean UA level >6.5 mg/dl, averaged over all values during the first quarter after OLT. The primary outcomes were 1) change in UA level from the quarter before to the quarter after OLT; and 2) total mortality during follow-up after OLT. Post OLT survival according to UA level was assessed using the Kaplan Meier method. Multivariate Cox proportional hazards models were constructed to adjust for age, gender, and time-dependent eGFR category.

Results: The prevalence of hyperuricemia increased from 3 months pre-OLT (26.6%) to 3 months post-OLT (53.5%; p<0.0001). Overall, UA levels increased by +1.1 mg/dl from pre- to post-OLT period. The increase was higher in those with eGFR<60 mL/min/1.73m^2 (+1.5 mg/dl) compared to eGFR≥60 (+0.3 mg/dl; p=0.048). In both the pre- and post-OLT period, a GFR<60 mL/min/1.73m^2 was significantly associated with higher mean serum UA (p= <0.0001). Older patients were more likely to have a higher UA level (p=0.02). The overall mortality rate was 48.9 deaths/1000 person-years. Post-OLT hyperuricemia was independently associated with mortality, but only among those with GFR <60 mL/min/1.73 m^2 (HR=3.7; [C.I. 1.1-12.0] p=0.03), and not those with GFR>60 mL/min/1.73 m^2 (HR=1.0, p=0.95; p-value=0.046).

Conclusions: The pre- to post-OLT increase in UA levels is greater in patients with CKD compared to no CKD. Hyperuricemia in the post-OLT period is an independent predictor of mortality in patients with CKD. Further studies are needed to assess the effect of treatment of hyperuricemia on kidney function and mortality.

TH-PO1105

Increased Cancer Incidence among Dialysis and Transplant Patients Is Not Associated with Increased Dialysis Duration Vathsala Anantharaman,[2] Gek Hsiang Lim.[1] *[1]National Registry of Diseases Office, Singapore; [2]National Univ Centre for Organ Transplantation, Singapore.*

Background: There is an increased risk of cancer in End stage renal disease (ESRD). Skin cancer, Kaposi's, lymphoma and cervical cancer are more frequent after kidney transplant (KTX) whereas genitourinary (GU) cancers are more common after dialysis (DIAL). Although GU cancer is more common after KTX, there is controversy over whether increased DIAL duration increases this risk. This Registry analysis evaluated cancer incidence in KTX and DIAL patients and examined its relationship to demographics and DIAL duration.

Methods: 9565 DIAL patients and 937 KTX performed between 1998 and 2012 were studied. Incident cancers were ascertained through cross referencing with the Singapore Cancer Registry. Standardised Incidence Ratios (SIRs) of cancer were computed using age-, sex- and calendar-year specific population cancer incidence rates.

Results: 537 and 73 cancers were detected among DIAL and KTX respectively; compared to the general population, SIR for cancer for KTX was 3.9 (95%CI:3.1-4.9) versus 1.8 (95%CI:1.7-2) for DIAL patients. GU cancer was the most common for both KTX and DIAL patients (mean interval of 3 years after KTX or DIAL). Cancer risk was highest during the 1st year of either DIAL or KTX and decreased with time.

	DIALYSIS			KTX		
	No of Cancers	Crude SIR	95%CI	No of Cancers	Crude SIR	95%CI
	537	1.8	1.7-2.0	73	3.9	3.1-4.9
Years after DIAL/ KTX						
<1	90	5.5	4.4-6.9	12	29.8	16.5-53.8
1-2	105	2.8	2.3-3.4	9	8.1	4.2-15.7
3-5	188	1.6	1.4-1.8	24	4.5	3.0-6.7
6-10	128	1.4	1.2-1.7	21	2.7	1.7-4.2
>10	26	1.1	0.7-1.6	7	1.7	0.8-3.6
Total Therapy Time (Years)						
0-9	476	1.8	1.6-2.0	12	7.7	4.4-13.6
10-19	25	1.1	0.7-1.6	46	4.2	3.1-5.6
>19	0	-	-	11	2.3	1.3-4.2

SIR for cancers after KTX, in relation to DIAL duration pre-KTX, were 5.0(<1.5 years), 5.5(1.5-2.5 years), 3.5(2.5-4.5 years), 3.7(>4.5 years).

Conclusions: Cancer risk is higher among KTX than DIAL and higher in both groups than the general population. DIAL duration was not associated with increased cancer risk in either group. These results advocate for increased cancer surveillance, especially GU cancer, early after renal replacement therapy initiation.

TH-PO1106

Recommendation of Pre-Emptive Transplant Based on Hypothetical Case Scenarios Surju Patel,[1] Ming Wang,[2] Kevin C. Roe,[1] Ankita Tandon,[3] Nasrollah Ghahramani.[1] *[1]Dept of Nephrology, Penn State College of Medicine, Hershey, PA; [2]Div of Biostatistics and Bioinformatics, Penn State College of Medicine, Hershey, PA; [3]Dept of Internal Medicine, Penn State College of Medicine, Hershey, PA.*

Background: Pre-emptive renal transplant (PRT) is recognized as an underutilized option for patients with end-stage renal disease (ESRD). Reasons for the infrequent use of PRT remain unclear. We assessed Nephrologists' decisions regarding patient referral for PRT to identify factors influencing choices.

Methods: In an online survey, 250 Nephrologists were asked whether they would recommend pre-emptive kidney transplant (PKT) for hypothetical patients with stage 4 CKD not yet on dialysis. The 6 scenarios varied in age, sex, living situation (alone or with spouse), distance from transplant center (TC), rural location.

Results: 213 Nephrologists provided complete responses. In multivariate analysis, distance from TC< 10 miles (OR:3.26;p< 0.001) was associated with higher likelihood, and rural location (OR: 0.31;p < 0.001) with lower likelihood of being referred for PKT. Based on logistic regression, academic affiliation was associated with higher likelihood of recommending PKT (OR=5.12; p=0.045). Practice as non-transplant Nephrologist (p<0.01), increased years from fellowship (p<0.001), and fewer years practicing in the current location (p<0.001) were associated with lower likelihood of recommending PKT.

Conclusions: PRT avoids dialysis associated morbidity and reflects early access to kidney transplant. The underutilized nature of PRT has been attributed to lack of education of Nephrologists and patients, and notably the late referral for transplant evaluation. We identify close proximity to the transplant center and academic affiliation as increasing likelihood of PRT. Lack of transplant specific training, increasing years from fellowship or practicing in an area of a shorter period of time all decrease the likelihood of PKT.

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TH-PO1107

Survey of Protocol Kidney Transplant Biopsy Practices Adam Safdi,[1] Daniel R. Salomon,[2] John J. Friedewald.[1] *[1]Northwestern Medicine, Chicago, IL; [2]The Scripps Research Inst, La Jolla, CA.*

Background: Protocol kidney transplant biopsies (PBx) done at fixed intervals post transplant are used to monitor the graft. Our center performs PBx on all kidney recipients (KTR) but this practice varies. We developed a survey to explore this practice and opinions on PBx.

Methods: An 11-question survey on SurveyMonkey.com was distributed to the Centerspan transplant email listserv. We asked respondents who care for kidney recipients to best represent the practices of their home institution.

Results: Survey data were collected from 50 respondents from 33 transplant centers in the U.S.A. and 3 international centers. 34% routinely performed PBx on all KTR, 38% do not perform any PBx, and 28% only for certain patients, with the most common reasons being delayed graft function or ABO incompatibility. For those who do not perform PBx, when asked why not, 34.3% said they do not believe there is an indication for PBx, 31.4% said they believe in Pbx only for selected patients, and 22.9% said the logistics were difficult to arrange for PBx at their centers (answers not mutually exclusive). Respondents indicated an array of time intervals for PBx. When asked who performs the majority of PBx, 59% reported nephrologists, 24% interventional radiologists, and 16% transplant surgeons. Regardless of who performs the biopsies, the nephrologist responds to the biopsy results 78% of the time, unless it is a new (< 3 months) graft, then it is either the surgeon alone or a team response. When there is rejection on the PBx without graft dysfunction (subclinical acute rejection), 91% favor treating, but only 47% will repeat a biopsy after treatment. 51% treat Banff borderline cellular rejection, 20% do not, and 29% say it depends on the clinical situation. When asked if they would use a biomarker to replace PBx, 69% of respondents said yes, 10% said no, and 21% weren't sure.

Conclusions: The practice and attitudes towards PBx still vary widely throughout the kidney transplant community. More comprehensive data regarding the effectiveness of a PBx protocol are likely still needed. Clinicians are open to the use of biomarkers to replace PBx.

TH-PO1108

Infection and Immunosuppression following Renal Transplant Failure Claire T. Kassakian, George P. Bayliss. *Nephrology, Alpert Medical School at Brown Univ, Providence, RI.*

Background: Renal transplant patients whose grafts have failed are frequently kept on an immunosuppressive regimen. Continuation of such regimens introduces an increased risk of infections, malignancy, and side effects from long-term corticosteroid utilization. Infection remains one of the most common causes of mortality in this population. Small published retrospective studies have previously illustrated that patients who continue on even low dose immunosuppressive regimens are at an increased risk of infection. The objective of this study is to evaluate the risk and type of infection in renal transplant patients whose allografts have failed, comparing those who continue on an immunosuppressive regimen to those who do not.

Methods: Retrospective cohort study of renal transplant recipients from Lifespan who received transplants between 1999 to the present described as either on hemodialysis or deceased. Patients were excluded if no allograft failure was present prior to death or if inadequate data was present in the electronic medical record. Electronic chart review. The primary outcome is the incidence of infections requiring hospitalization.

Results: There were 189 patients with failed renal transplants in the cohort, 110 of whom had adequate data for review in the electronic medical record. There were a total of 112 infections. The incidence of developing any infection in the immunosuppressed group was 71%, and 64% for the non-immunosuppressed group (p-value 0.45). The odds ratio (OR) of developing an infection was 1.4. The three most common types of infections in the immunosuppressed group were pneumonia, UTI, and dialysis catheter infections.

Conclusions: In this study population, there is a greater incidence, albeit non-statistically significant, of infections in renal allograft failure patients who are continued on an immunosuppressive regimen. As prior literature has established, there is an increased risk of line infections in patients who are continued on immunosuppressive regimens. This study also demonstrates that these patients also have a higher incidence of other infections as well.

TH-PO1109

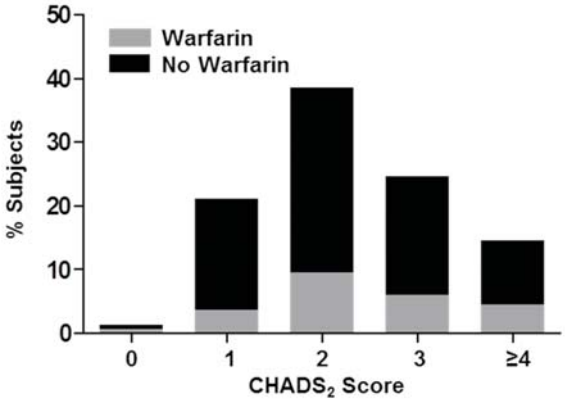
Correlates and Outcomes of Warfarin Initiation in Kidney Transplant Recipients Newly Diagnosed with Atrial Fibrillation Colin R. Lenihan, Maria E. Montez-Rath, Jenny I. Shen, John D. Scandling, Tara I. Chang, Wolfgang C. Winkelmayer. *Divison of Nephrology, Dept of Medicine, Stanford Univ, CA.*

Background: In the kidney transplant population with atrial fibrillation (AF), evidence regarding the effectiveness and safety of warfarin treatment is lacking.

Methods: We used fee-for-service Medicare claims to identify kidney transplant recipients with newly diagnosed AF from the U.S. Renal Data System. Warfarin use within 30 days of AF diagnosis was ascertained from Medicare Part D prescription claims (2007-2010) or using a validated algorithm (1997-2010). The study endpoints were 1) the composite of death, stroke or gastrointestinal (GI) bleed, 2) death and 3) death-censored graft failure. Warfarin user and non-users groups were balanced using inverse probability of treatment weighting and hazard ratios estimated using Cox regression.

Results: Among 434 subjects with available prescription data, 25.6% initiated warfarin treatment within 30 days of AF diagnosis. Age was the only independent correlate of warfarin use (odds ratio=1.02 per year; 95% CI: 1.00-1.04). Figure 1 shows warfarin use stratified by CHADS2 score.

Figure 1



In the larger cohort of 6170 patients with AF, warfarin use [22.3%] versus non-use [77.7%] was associated with a 13% reduction in the composite of death, stroke or GI bleed (table 1).

Table1	Hazard Ratio (95% CI) Warfarin use versus non-use
Composite of Death, Stroke or GI Bleeding	0.87 (0.78-0.98)
Death	0.89 (0.79-1.01)
Death-censored graft failure	0.87 (0.72-1.05)

Conclusions: Our data suggest a benefit for warfarin treatment in kidney transplant recipients with newly diagnosed AF. Given this evidence, warfarin appears to be underutilized in this population.

Funding: NIDDK Support, Private Foundation Support

TH-PO1110

Simultaneous Liver and Kidney Transplantation in the Nephrologist Led Era: Characteristics and Outcomes Scott Reule, Donal J. Sexton, Hassan N. Ibrahim. *Medicine, Univ of Minnesota, Minneapolis, MN.*

Background: Formal metrics to decide between liver transplant alone (LTA) versus simultaneous liver-kidney transplantation (SLK) have not been developed. On January 1st, 2009, the University of Minnesota adopted an approach whereby a nephrologist is given full autonomy to decide whether LTA versus SLK should be offered to patients with end stage liver disease and impaired kidney function. Whether this policy has impacted patient outcomes such as survival and rehospitalization, is unknown.

Methods: Chi-square analysis was performed for categorical variables and logistic regression was performed for comparisons of type of transplant performed, adjusted for age, race, sex and era. Clinical outcome comparisons were performed using Cox proportional hazards models, with hazards ratios adjusted for age, sex and race (AHRs).

Results: Outcomes of LTA (n =384) and SLK (n = 63) were compared before and after policy implementation. Overall percentage of SLK decreased over the time (17.1% in 2003 to 8.1% in 2012). The strongest associations with transplantation in a recent era included Hepatitis C positivity for those receiving LTA (20.4% versus 40.9%; AOR 3.34) and a lower likelihood of hypertension for those receiving SLK (97.4% versus 62.5%; AOR 0.05). Survival of recipients of SLK was comparable ($p = 0.07$) whereas those receiving LTA appeared to have improved survival in the most recent era ($p < 0.05$). No era differences in hospitalization post transplantation were observed in those receiving LTA, whereas those receiving SLK were less likely to be hospitalized in the first 30 days post transplantation in the most recent era ($p = 0.03$). No significant differences in dialysis post transplantation by era were observed (SLK; $p = 0.86$, LTA $p = 0.8$).

Conclusions: These data suggest that delegating the responsibility of allocating LTA versus SLK in patients with kidney disease to nephrology may result in more efficient use of kidneys with no appreciable adverse consequences to recipients.

TH-PO1111

Risk Factors for Asymptomatic Bacteriuria after Renal Transplantation and Its Impact on Renal Allograft Function Ramandeep Singh,[1] Suzanne E. Geerlings,[2] Ineke Ten Berge,[1] Frederike J. Bemelman.[1] *[1]Renal Transplant Unit, Academic Medical Center, Amsterdam, Netherlands; [2]Div of Infectious Diseases, Academic Medical Center, Amsterdam, Netherlands.*

Background: Asymptomatic bacteriuria (ASB) is common after renal transplantation. In 2007 we implemented the administration of trimethoprim-sulfamethoxazole (TMP-SMX) as *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis. The aims of this study are to describe the risk factors for the development of ASB, as well as the influence of TMP-SMX its incidence. We also evaluated the impact of ASB on renal allograft function 1 year after transplantation.

Methods: Retrospective cohort study with adult renal allograft recipients transplanted between 2004 and 2009, with follow-up of 1 year after transplantation. Bacteriuria events occurring after discharge of the renal transplant ward were analysed. ASB was defined as positive urine culture without symptoms of the urinary tract, fever/malaise. Bacteriuria with these symptoms was considered as urinary tract infection (UTI).

Results: In total 431 recipients were analysed; 48 (11.1%) had only ASB within 1 year after transplantation, 85 (19.7%) had a UTI and 298 (69.1%) did not develop any bacteriuria at all. Multivariable analysis showed that in comparison to the group who did not develop any bacteriuria, the risk factors for ASB were age, OR=1.05 (95%CI=1.05-1.08,p=0.003); female gender, OR=2.15 (95%CI=1.05-4.39,p=0.037) and indwelling urological catheters, OR=18.76 (95% CI= 6.20-40.06,p<0.001). TMP-SMX did not reduce the incidence of ASB, (OR=1.62, 95%CI=0.81-3.27,p=0.17). With TMP-SMX prophylaxis, the rate of TMP-SMX resistance was higher than without prophylaxis (74% versus 38%,p=0.02). Recipients who experienced ASB had comparable median eGFR (MRDR) of 50.5 ml/min/1.73m^2 versus 52.0 ml/min/1.73m^2 in the group without bacteriuria, p=0.251. The median 24 hour urine protein excretion was also comparable; 200 mg in the group with ASB versus 160 mg in the group without bacteriuria, p=0.269.

Conclusions: Age, female gender and indwelling urological catheter are associated with ASB. TMP-SMX as PJP prophylaxis does not reduce the incidence of ASB, but increases TMP-SMX resistance. ASB does not impair renal allograft function.

TH-PO1112

Time-Dependent Kidney Function Is a Risk Factor for Cardiovascular Disease in Kidney Transplant Recipients Johnny Huang,[1] Monica Abdelmasih,[1] Olusegun Famure,[1] Yanhong Li,[1] Joseph Kim.[1,2] *[1]Multi-Organ Transplant Program, Toronto General Hospital, Toronto, ON, Canada; [2]Div of Nephrology, Toronto General Hospital, Toronto, ON, Canada.*

Background: The relation between kidney function and cardiovascular disease (CVD) in kidney transplant recipients is complex due to time-varying confounding by factors like blood pressure (BP). BP may simultaneously confound and mediate the effect of kidney function on the future risk of CVD. We used novel statistical methods to examine the role of kidney function as a risk factor for CVD while accounting for the time-varying role of BP.

Methods: A cohort of 1221 kidney transplant recipients from 1 Jan 2000 to 31 Dec 2011 at a single center was studied. Kidney function was based on the CKD-EPI estimated glomerular filtration rate (eGFR) formula. The primary outcome was major adverse cardiovascular event (MACE), which included non-fatal MI, target lesion revascularization, or death. The secondary outcome was MACE excluding death. We used time-fixed, time-varying, and marginal structural Cox proportional hazards models to assess the association of time-dependent eGFR and MACE while accounting for time-varying BP. Other baseline recipient, donor, and transplant characteristics were also included in multivariable models.

Results: During 5696.7 person-years of follow-up (median 3.9 years), 172 MACE were observed (including 65 deaths). A time-fixed Cox model showed that every 10 mL/min decrease in eGFR was associated with an increased risk of MACE (hazard ratio or HR 1.15 [95%CI: 1.04, 1.27]) and MACE excluding death (HR 1.15 [95%CI: 1.01, 1.31]). Somewhat stronger associations were observed using a time-varying Cox model (HR 1.25 [95%CI: 1.16, 1.36] for MACE and HR 1.31 [95%CI: 1.17, 1.46] for MACE excluding death). Furthermore, the marginal structural Cox model confirmed that eGFR is a significant independent risk factor for MACE and MACE excluding death (HR 1.26 [95%CI: 1.13, 1.41] and HR: 1.32 [95%CI: 1.14, 1.52], respectively) after accounting for time-varying BP.

Conclusions: Time-dependent eGFR is an independent risk factor for MACE (and MACE excluding death) in kidney transplant recipients after accounting for time-varying BP and other factors.

TH-PO1113

Renal Transplant Recipient Perspectives on Physical Activity and Exercise
Laetitia H. Lloyd-Davies, Amy L. Clarke, Stephanie Amy Brown, Katherine Leigh Hull, James O. Burton, Alice C. Smith. *Leicester Kidney Exercise Team, Univ of Leicester, United Kingdom.*

Background: Transplantation offers those with end stage renal failure improved quality of life and increased functional capacity, but many renal transplant recipients (RTR) are undesirably sedentary despite the increasing appreciation of the risks involved with such behaviour. Patient-centred strategies to increase physical activity are required. As a first stage in intervention development, this study aimed to explore RTR attitudes and perspectives towards exercise using a mixed-methods approach.

Methods: 220 RTRs completed Exercise Self-Efficacy (ESE) and Stage of Change (SoC) questionnaires (time with graft >3 months, 53% male, median age 52, range 19-83 years). A purposive sample of 18 RTRs were recruited to 6 semi-structured interviews and 2 focus groups to explore perspectives in greater depth. Audio files were transcribed verbatim and qualitative analysis was completed using a framework approach.

Results: From the SoC, only 19% of respondents were in Pre-Contemplation stage (not interested in exercise). ESE correlated with intention to increase activity (rho=0.448, p<0.001). In the qualitative phase, patients anticipated a number of health and wellbeing benefits of exercise but described many physical barriers including fatigue, breathlessness and muscular pain at the site of surgery, and psychological fears of injury and damaging the graft. Messages around exercise from health professionals were primarily cautionary and mainly given soon after the time of surgery, leaving patients wanting specific advice about how to exercise safely. Positive effects of peer support and education were seen in the focus groups, and showed the potential of group interactions to contextualise these concerns and increase self-efficacy.

Conclusions: This study indicates that many transplant recipients want and need more help to build confidence around exercise and increase habitual activity levels. It supports the inclusion of peer education and vicarious learning in a structured intervention with different levels of support and instruction tailored to the needs of the individual.

Funding: Private Foundation Support

TH-PO1114

The Effect of Renin-Angiotensin System Blockade on Long-Term Outcomes in Renal Transplant Recipients
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Background: Blockade of the renin-angiotensin system (RAS) with angiotensin converting enzyme inhibitors or angiotensin II receptor blocker reduces cardiovascular mortality in the general population and among patients with diabetic chronic kidney disease (CKD). Similarly, RAS blockade decreases proteinuria in CKD. It remains controversial, however, whether this translates to improved patient or graft survival among transplant recipients.

Methods: We analyzed 2967 renal transplant recipients at the University of Wisconsin-Madison between 1990 and 2010 who survived at least 6 months after transplantation to assess the association of RAS blockade use with patient and graft survival.

Results: 429 (14.5%) patients used RAS blockade at the start of follow-up. More than half (n=1680; 56.6%) of patients used RAS blockade at some point during follow-up, though only 227 (7.7%) patients used RAS blockade during the entire follow-up. Median follow-up time was 5.6 years. 392 deaths, 396 death-censored graft failures, and 765 total graft failures occurred during follow-up.

Outcome	Unadjusted HR (95% CI)	Adjusted† HR (95% CI)
Mortality	0.93(0.76-1.13)	0.65(0.53-0.81)
Death-censored graft failure	1.26(1.03-1.54)	0.61(0.48-0.76)
Total graft failure	1.09(0.94-1.26)	0.62(0.53-0.72)

HR; hazard ratio, CI; confidence interval
†Adjusted for year of transplantation, duration of dialysis before transplantation, number of prior transplants, donor type, recipient age, gender, race, body mass index, sensitization, delayed graft function, biopsy-confirmed acute rejection, glomerular filtration rate at discharge, diabetes, preexisting cardiovascular disease, blood pressure, albuminuria, LDL cholesterol, and hematocrit

Conclusions: Our findings suggest that RAS blockade use is associated with improved patient and graft survival after renal transplantation. Widespread use of RAS blockade may improve patient and graft survival in renal transplant recipients.

TH-PO1115

Survival, Morbidity, Sensitisation and Retransplantation Outcomes in a Cohort of Dialysis Patients After Renal Graft Loss and Transplant Nephrectomy by Indication
Andrew K. Coutinho, Richard W. Corbett, Sarah Hildebrand, David Taube, Neill D. Duncan. *West London Transplant and Renal Centre, Imperial College Healthcare NHS Trust, London, United Kingdom.*

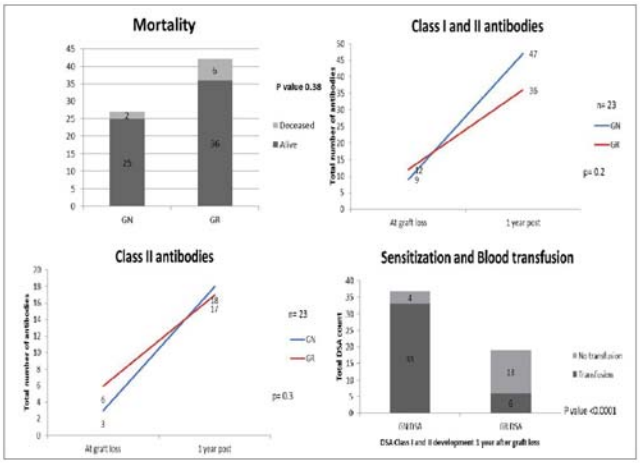
Background: A quarter of prevalant dialysis patients have Dialysis After Graft Loss with unique factors modifying outcomes. The indication for graft nephrectomy in patients with DAGL at our centre is evidence of systemic inflammation.

Methods: 121 patients had DAGL between Jan 2007-Aug 2013, we excluded those with previous, multiple or immunologically complex grafts. We made a retrospective observational study in the 69 patients with DAGL after exclusions. 27 patients had graft nephrectomy(GN), 42 had graft retained(GR).The median age at transplantation was GN-38.5 yrs versus GR-53 yrs.

Results: Total deaths in the GN (2/27) and GR (6/42) groups was not significant (p=0.38). Hospitalisation rate over a year for GN group was lower.

	GN group	*GR group*	*p-Value*
All hospital admissions	97/100 patient yrs	168/100 patient yrs	0.03
Non cardiac and non infective admissions	58/100 patient yrs	110/100 patient yrs	0.01
Outpatient antibiotic use	30.4/100 patient yrs	13.3/100 patient yrs	0.6

No difference was seen in surrogate markers of inflammation measured monthly - CRP, albumin and haemoglobin (p=0.2, 0.14 and 0.45 respectively) and in retransplant waitlisting between the groups (13/27 GN group versus 12/42 GR group, p=0.09).More DSA's were seen after graft loss in both groups of waitlisted patients but this was statistically significant with relation to blood transfusions only.



Using the UK NHSBT Relative Chance of Kidney Transplantation Calculator, 4/13 waitlisted GN patients had a 50% or greater chance of a retransplant versus 2/12 waitlisted GR patients at 5 years (p=0.7).

Conclusions: Hospitalisation was higher for GR patients. Graft retention did'nt adversely affect rates of survival, infection, markers of inflammation or chances of retransplantation.

TH-PO1116

Current Practices in Screening and Management of Prostate Cancer in Renal Transplant Recipients: A Survey of U.S. Transplant Centers
Ross Beckman,[1,2] Dorothy Skierkowski,[3] Reginald Y. Gohh,[1,4] George P. Bayliss.[1,4] *[1]Alpert Medical School, Brown Univ, Providence, RI; [2]Surgery, Johns Hopkins, Baltimore, MD; [3]Biostatistics, Rhode Island Hospital, Providence, RI; [4]Medicine, Rhode Island Hospital, Providence, RI.*

Background: Immunosuppression following renal transplantation is thought to put patients at increased risk of malignancy. But prostate cancer is often slow-growing and relatively low-risk, and its response to immunosuppression has not been widely studied. Optimal management of potential transplant recipients found to have prostate cancer prior to transplantation also has not been established.

Methods: After receiving institutional review board approval, a 16-question survey was sent to 225 transplant center medical and surgical directors at 135 centers asking about each center's practices in screening for and managing prostate cancer found during pre-transplant evaluations and post-transplant follow-up.

Results: We received 37 responses from 33 unique transplant centers representing all UNOS regions. 91% of respondents said they screened for prostate cancer pre-transplant, but only 50% said their institution screened patients in the general population; 34% said they did not know if they screened for prostate cancer in the general population. There was no significant difference between pre-transplant handling of low grade prostate cancer and whether respondents thought low-risk prostate cancers should always undergo definitive surgical treatment before transplantation. Those respondents who felt that the risk of dying from prostate cancer on immunosuppression was no greater than in the general population were significantly more likely to consider the risk of death from delaying transplant to be higher than of cancer post transplant. Those who said they were more aggressive in treatment of low-grade prostate cancers post transplant were significantly more likely to base their decision on experience rather than evidence.

Conclusions: We conclude there is no consensus among U.S. renal transplant centers on the management of prostate cancer in the transplant population, likely due to a lack of evidence to guide such practices.

Funding: Clinical Revenue Support

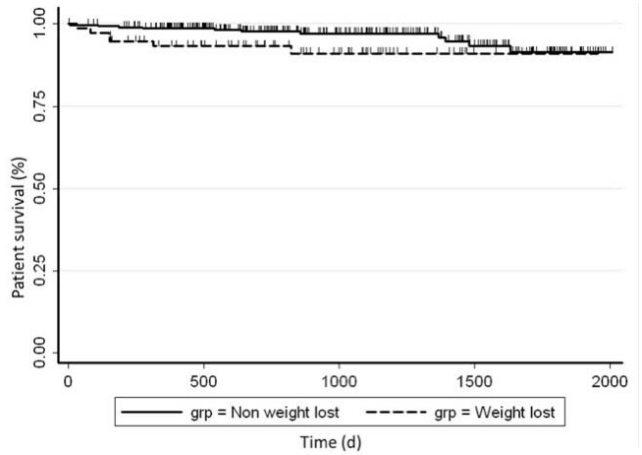
TH-PO1117

Impact of Weight Loss Before Renal Transplantation in Obese Patients: Risk of Early Mortality Anne-Elisabeth Heng,[23] Fernando C. Fervenza,[4] Mikel Prieto.[1] *[1]Div of Transplant Surgery, The William J von Liebig Center for Transplantation and Clinical ReGeneration, Mayo Clinic, Rochester, MN; [2]Nephrology Dept, Pole R.E.U.N.N.I.H.R., CHU G Montpied, Clermont-Ferrand, Puy de Dome, France; [3]Faculté de Médecine, Univ d'Auvergne, Clermont-Ferrand, Puy de Dome, France; [4]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: The obese patient's survival is significantly better after transplantation than dialysis. Some doctors encourage patients to lose weight but it could be harmful than beneficial.

Methods: In a retrospective study from the Mayo Clinic Rochester database (893 renal transplanted patients from 2007 to 2010) we compared data from non obese patients with those from patients with an history of obesity (obese patients). In this group, datas from patients with a significant weight loss (decrease in weight observed during waiting before transplant > 10% of initial weight) (WL) were compared to those from obese patient who have not lost weight (NWL).

Results: 42% of the transplanted patients were obese. In comparison with non obese, obese patients are older, have more comorbidities and surgical complications, warm ischemia time tends to be longer but patient and graft survivals are the same. A significant weight loss is observed in 78 of 380 patients. No differences in demographic characteristics were observed between WL and NWL group exept a greater proportion of African-American in the WL. No difference in surgical or medical complications was seen between the WL and NWL groups. However, the patients survival adjusted to age and comorbidities was less good especially the first year post transplantation.



Conclusions: Weight loss before transplantation in obese patients do not decrease surgical complications and may be harmful.

Funding: Pharmaceutical Company Support - novartis

TH-PO1118

Induction Regimen and Status of Epstein-Barr Viral Infection in Pediatric Renal Transplants Dongmei Huang,[1] Fu L. Luan,[2] Neal B. Blatt.[1] *[1]Univ of Michigan, Ann Arbor, MI; [2]Saint Barnabas Medical Center, Livingston, NJ.*

Background: Post-transplant lymphoproliferative disorder (PTLD) is more likely to occur in children due to their frequent EBV naïve status. Induction regimens incorporating Thymoglobulin show increased the risk of PTLD compared to regimens using anti-interleukin-2 receptor (aIL2R) antibodies, however, the rates of Epstein-Barr Virus (EBV) viremia following these induction regimens is unknown. We hypothesized that Thymoglobulin induction would be associated with increased EBV viremia.

Methods: EBV viral loads were monitored for 2 years post-transplant by PCR in all pediatric kidney transplant patients from 1998-2011 at C.S. Mott Children's Hospital. Positive EBV viremia was defined as any detectable copies and high titer EBV as ≥2000 copies/mL. Multivariate logistic regression analysis was used to test the relationships between EBV viral load, time to EBV viral load, and induction regimen.

Results: Similar numbers of patients received thymoglobulin or aIL2R induction and they had similar rates of PTLD (see Table). 73 patients were EBV naïve at transplant with 81% (N=59) having an EBV(+) donor. More aIL2R patients were EBV naïve at transplant. In multivariate analysis, the odds of having a high titer EBV was higher with aIL2R induction [OR 10.7 (2.8-41.47), p 0.001], AA race [(OR 12.8 (2.0-82.8), p 0.007], and having a EBV D(+)/R(-) status at transplant [7.2 (2.4-21.6), p<0.001]. Patients who received aIL2R had significantly shorter time to high titer EBV than those who received Thymoglobulin [HR 2.74 (1.1-6.9), p=0.032].

Table 1:

	Total	Thymoglobulin	aIL2R	p-value
Induction (N, %)	159	71 (45%)	88 (55%)	
PTLD	14 (9%)	7 (10%)	7 (8%)	0.67
EBV naive	73 (46%)	26 (37%)	47 (53%)	0.035
EBV PCR(+)	84 (53%)	32 (45%)	52 (59%)	0.003
EBV ≥2000	39 (46%)	6 (8%)	33 (38%)	<0.001
Age (mean±SD)	11.3 ± 5.4	11.7 ± 5.5	11.0 ± 5.3	0.29

Conclusions: Contrary to our hypothesis, aIL2R induction was associated with increased risk of developing high titer EBV, but did not result with increased PTLD. Although our study is retrospective and limited to a single center, our findings challenge the hypothesis that EBV viremia post-transplant is associated the development of PTLD.

TH-PO1119

Reduced Rejection Rates and Improved Graft Survival with a Dedicated Young Adult Service Jeroen Bastiaan van der Net, Paul N. Harden. *Oxford Kidney Unit, Oxford Univ Hospitals NHS Trust, Oxford, United Kingdom.*

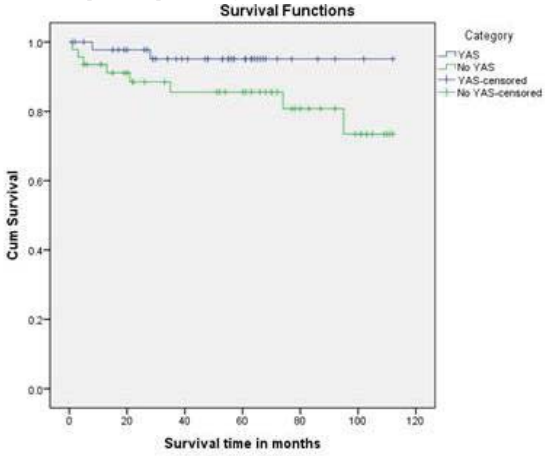
Background: Non-adherence results in the poorest 5yr allograft survival rates in 18-29 year old kidney transplant recipients (KTRs) compared with all other age categories. We explored the impact of a restructured dedicated young adult service(YAS) for 16-30 year old KTRs comparing rejection rates and graft survival with standard clinic care between 2005 to 2014 in a single adult center.

Methods: Eligible patients were identified using the electronic patient record; all patients records were carefully reviewed to document demographics, immediate and late outcomes of transplantation.

Results: Since January 2005, 93 KTRs aged 17-30 were transplanted (60m,33f; 79(85%) white); 47 (51%) were involved in the YAS. Main causes of ESKD were reflux nephropathy (17.2%), IgA nephropathy (10.8%) and dysplastic kidneys (8.6%). 53 (57%) received a kidney from a living donor. Mean age at transplantation was 24.2 (17-29) years, HLA mismatch 2.15 (0-5), 19/87 (21.8%) pre-emptive, 19/93 (20.4%) previously transplanted, 83/93 (89%) primary function. YAS patients were more frequently male and less likely to have graft loss 2/47 (4%) versus 8/46 (17%), p-value 0.041, or late rejection 1/47 (2%) versus 6/46 (13%), p-value 0.046.

	YAS (n=47)	No YAS (n=46)	p value
Age at transplantation (years)	23.5(0.48)	24.9(0.55)	0.08
Living Donor recipient (%)	30 (64%)	23(50%)	0.18
HLA mismatch	2.15 (0.19)	2.14 (0.17)	0.98
Male sex (%)	40 (85%)	20 (44%)	<0.001
Pre-emptive (%)	11(23%)	8(20%)	0.70
Median Serum Creatinine at 12 months post-transplant (umol/L)	125 (107-148)	118 (100-151)	0.57

Survival analysis of graft loss shows diverging Kaplan Meier curves for YAS patients versus non-YAS patients (p-value log rank 0.095).



Conclusions: The YAS resulted in a significant reduction in late rejection and graft loss, and is a useful tool to improve patient outcomes and reduce healthcare costs.

TH-PO1120

Immunosuppression and Pregnancy Outcomes in Female Kidney Transplant Recipients Serban Constantinescu,[1] Peter Axelrod,[1] Lisa Coscia,[2] Michael J. Moritz,[3] Vincent T. Armenti.[2] *[1]Temple Univ School of Medicine; [2]National Transplantation Pregnancy Registry, Gift of Life Inst, Philadelphia, PA; [3]Lehigh Valley Health Network.*

Background: Immunosuppressive regimen may influence pregnancy course and outcomes in kidney recipients.

Methods: Data were collected by the National Transplantation Pregnancy Registry via questionnaires, phone interviews and hospital records. All pregnancies with mycophenolate (MMF) exposure were excluded.

Results: We analyzed 1514 pregnancies in 895 kidney recipients on 3 immunosuppressive regimens: azathioprine (AZA) cyclosporine (CsA) and tacrolimus (TAC).

	AZA	CsA	TAC	p value
Conception yrs	1967-2013	1982-2013	1993-2013	
Recipients/Pregnancies	240/441	466/778	189/295	
MATERNAL FACTORS				
Planned pregnancy	59%	58%	73%#	<0.001
Mean SCr preconception (mg/dL)	1.1±0.4	1.4±0.4#	1.1±0.3	<0.001
Mean SCr during pregnancy (mg/dL)	1.2±0.5	1.4±0.7#	1.2±0.6	0.003
Mean SCr postpartum (mg/dL)	1.2±0.6	1.6±0.9#	1.3±0.5	<0.001
Treated hypertension during pregnancy	26.1%#	61.5%#	51%#	<0.001
Treated rejection during pregnancy	0.5%	0.9%	1.4%	NS
Graft loss within 2 yrs	4.2%	7.2%	4.8%	NS
PREGNANCY OUTCOMES*	456	808	303	
Miscarriage	10.8%	13.5%	19.1%#	0.004
Live births %(n)	81.6% (372)	77.8% (629)	77.6% (235)	NS
Mean gestational age (wks)	36.5±3.3#	35.8±3.4	35.4±3.7	<0.001
Prematurity (<37 wks)	46.2%	52.2%	53.2%	NS
Mean birthweight (g)	2745±710#	2502±750	2530±828	<0.001
Low-birthweight (<2500 g)	34.7%	44.8%	41.6%	<0.01
Birth Defects	2.2%	4.2%	6.8%	0.02

*includes multiple births, # p<0.01 compared to each other group

TAC group had significantly more planned pregnancies and miscarriages. CsA group had significantly higher HTN and serum creatinine (SCr). The incidence of rejection and graft loss was comparable among groups. While live births were similar among groups, AZA newborn had significantly higher gestational age and birthweight.

Conclusions: Pregnancy outcomes in kidney recipients on AZA, CsA or TAC, without MMF exposure resulted in similar rates of live births, and low incidences of rejection and graft loss within 2 yrs of pregnancy. For all groups birth defect rate was comparable to the general population.

TH-PO1121

Pregnancy and Maternal Outcomes in Kidney Transplant Recipients with the Diagnosis of Alport Syndrome Lisa Coscia,[1] Michael J. Moritz,[2] Serban Constantinescu,[3] Vincent T. Armenti.[1] *[1]National Transplantation Pregnancy Registry, Gift of Life Inst, Philadelphia, PA; [2]Lehigh Valley Health Network, Allentown, PA; [3]Temple Univ School of Medicine, Philadelphia, PA.*

Background: The purpose of this study was to analyze pregnancy and maternal outcomes in female kidney transplant recipients with the diagnosis of Alport syndrome.

Methods: Data were collected by the National Transplantation Pregnancy Registry (NTPR) via questionnaires, phone interviews and medical records.

Results: Out of 960 kidney recipients reporting 1,687 pregnancies to the NTPR, there are 15 female recipients with Alport syndrome. The kidney donors were: living related 6, living unrelated 2, deceased 6, and unknown 1. There were 22 pregnancies with 24 outcomes (includes twins); 22 live births and 2 miscarriages. The mean age at transplant was 24.8±5.5 yrs. and mean transplant to conception interval was 4.8±2.96 yrs. Immunosuppression during pregnancy was tacrolimus based in 9, cyclosporine based in 8 and azathioprine in 5. Comorbid conditions during pregnancy included: hypertension 50%, insulin use 14%, preeclampsia 20% and rejection 0. Two recipients had rejection postpartum; 1 treated with OKT3, lost graft 6 mos. postpartum and was re-transplanted, and 1 effectively treated with methylprednisolone. Graft function at last maternal follow-up (11.9±8.4 yrs): 10 adequate, 1 reduced, 3 had died, and 1 lost to follow-up. Mean gestational age was 35.8±2.1 wks and mean birthweight was 2665±655 g. There were 16 male and 6 female newborn. Two infants had birth defects: 1 pyloric stenosis and megaureter and 1 undescended testicle. There were 5 children (4 male, 1 female) diagnosed with Alport syndrome, one of whom required a transplant; another female child was identified as a carrier of the disease. At last child follow-up (mean age 11.2±6.9 yrs; range 0.09-21.9 yrs), all were reported developing well.

Conclusions: Kidney transplant recipients with the diagnosis of Alport syndrome are able to have successful pregnancies. As with all pregnancies post-transplant they are considered high-risk and should be managed by a multi-disciplinary team. Genetic counseling is particularly important in the pre-pregnancy planning for these recipients.

Funding: Pharmaceutical Company Support - Astellas Pharma US, Inc., Bristol-Myers Squibb Company, Pfizer Inc.

TH-PO1122

Growth Post Pediatric Renal Transplantation after Steroid Withdrawal or Avoidance: A Meta-Analysis Anne K. Tsampalieros,[1,2] Amber O. Molnar,[2,3] Dean Fergusson,[3] Greg A. Knoll.[2,3] *[1]Nephrology, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; [2]Epidemiology, Ottawa Hospital Research Inst, Ottawa, OH, Canada; [3]Medicine, Ottawa Hospital, Ottawa, ON, Canada.*

Background: A variety of steroid withdrawal and avoidance protocols have been developed for after kidney transplantation. The objective of this meta-analysis was to identify and evaluate the literature published on the use of steroid withdrawal and/or avoidance after pediatric kidney transplantation that focused specifically on analyzing growth. As a secondary objective we evaluated its safety using risk of acute rejection and graft function.

Methods: A systematic search using MEDLINE and EMBASE was performed from 1990 to February 2014. This review included all pediatric randomized controlled trials (RCT's) which compared either early (within 7 days post transplant), intermediate (>7 days to < 1 year), late (>1 year) steroid withdrawal or complete avoidance to participants who were maintained on daily or alternate day steroids after kidney transplantation. The primary outcome assessed was growth post transplant (measured as change in height standard deviation score [SDS]) and secondary outcomes included acute rejection, graft function, graft and patient survival.

Results: We screened 855 citations of which 184 were selected for full text review. Five studies met eligibility criteria (n=522).

Study	Total Sample Size	Intervention	Follow up
Meriqc et al, 2013	24	Early Withdrawal	1 year
Sarwal et al, 2012	130	Complete Avoidance	1 year
Benfield et al, 2010	132	Intermediate Withdrawal	1 year
Grenda et al, 2010	196	Early Withdrawal	6 months
Weber et al, 2010	42	Late Withdrawal	1 year

Using pooled data, we found the experimental group (those who underwent steroid withdrawal or avoidance) had improved growth, with a mean height SDS difference of 0.16 95% C.I. of 0.09-0.23, p=0.01 compared to controls (those who continued on steroids). The risk of a rejection episode did not differ significantly between the two groups (p=0.63) nor did the final reported eGFR (p=0.4).

Conclusions: Based on the summarized findings of these trials, steroid withdrawal and/or avoidance post pediatric kidney transplantation allows for better growth.

TH-PO1123

Kinetic Estimation of GFR Improves Prediction of Dialysis or Recovery After Kidney Transplantation Timothy J. Pianta,[1] Zoltan H. Endre,[1] John W. Pickering,[2] Nicholas Buckley,[1] Philip Peake.[1] *[1]Prince of Wales Clinical School, Univ of New South Wales, Australia; [2]Medicine, Univ of Otago, New Zealand.*

Background: Prediction of delayed graft function (DGF) after kidney transplantation is suboptimal. Kinetic estimates of GFR under non-steady-state conditions, KeGFR, have been advocated in assessment of acute kidney injury (AKI) and renal recovery.

Methods: We investigated KeGFR in the prediction of DGF. KeGFR derived from sCr ($KeGFR_{sCr}$) was calculated at 4h, 8h and 12h in 81 patients using initial serum creatinine (sCr) concentrations, the estimated creatinine production rate, volume of distribution, and the difference between consecutive sCr values. The utility of sCr for prediction of DGF was compared with $KeGFR_{sCr}$, plasma cystatin C (pCysC), and $KeGFR_{pCysC}$ similarly derived from pCysC concentrations.

Results: At 4h the area under the receiver operator characteristic curve (AUC) for prediction of DGF was 0.60 (95% CI: 0.45 – 0.76) for sCr. Prediction of DGF was improved by using $KeGFR_{sCr}$ [AUC: 0.76 (0.62 – 0.90), p = 0.04]. After adjustment for pre-operative variables, integrated discrimination improvement analysis showed that sCr provided no benefit for predicting DGF at 4h and 8h, and only modest benefit at 12h. In contrast, the $KeGFR_{sCr}$ improved the prediction of DGF at each time point. pCysC and $KeGFR_{pCysC}$ similarly outperformed sCr. In this cohort, a $KeGFR_{sCr}$ between 12 and 16 mL/min/1.73 m^2 predicted DGF at 4h, 8h or 12h with 90% sensitivity.

Conclusions: Calculation of $KeGFR_{sCr}$ facilitates early prediction of DGF within 4h of renal transplantation and may allow trials of early intervention aimed at ameliorating ischemia reperfusion injury.

Funding: Government Support - Non-U.S.

TH-PO1124

The Role of Plasma Exchange in Focal Segmental Glomerulosclerosis Recurrence Post Renal Transplant: A Systematic Review Abdullah Kashgary,[1,2] William F. Clark.[2] *[1]Dept of Medicine, King Abdulaziz Univ Hospital, King Abdulaziz Univ, Jeddah, Saudi Arabia; [2]Dept of Nephrology, Western Univ, London, Canada.*

Background: The role of plasmapheresis in recurrent focal segmental glomerulosclerosis post renal transplant has not been well established.To assess the effectiveness of plasmapheresis in the treatment of recurrent FSGS post renal transplant and to evaluate its long-term effect on graft function.

Methods: We performed a systematic literature review of English-language articles. We included all study designs of recurrent FSGS post renal transplant treated with plasmapheresis from 1950-2012.Key measures included patient characteristics,time to recurrence,histological features at diagnosis and recurrence,proteinuria at diagnosis,treatment,use of Rituximab therapy,and graft outcomes.Our primary outcome was FSGS remission; the secondary outcome was end-stage renal disease (ESRD) at end of follow up.

Results: Out of 77 articles included,420 patients met the inclusion criteria.Median follow-up time was 19 months. Three quarters of patients responded to plasmapheresis (25% of these had partial remission);the remaining 24% were non-responders. Responders were younger than non-responders (23 versus 26 years, respectively),had lower pre-treatment proteinuria(7.0 mg/day versus 9.3 mg/day),were less likely to progress to ESRD(12% versus 63%),and had a longer time from diagnosis to ESRD(3.0 versus 2.3 years).Histological evidence for FSGS at the time of the recurrence diagnosis was similar in both groups.Of 41 patients treated with Rituximab for FSGS recurrence,70% experienced remission.Rituximab responders were younger than non-responders(17 versus 41, respectively),but had higher levels of pre-treatment proteinuria(9.6 g/day versus 4.7 g/day).Responders were less likely to develop ESRD(0 versus 40%) and time to ESRD was longer(5.0 versus 3.5 years).

Conclusions: Our study suggests that plasma exchange may be effective in treating post renal transplant recurrence of FSGS. The majority of patients experienced remission and

preserved their renal function with this treatment.The level of proteinuria at diagnosis,older age and shorter time from diagnosis to ESRD could predict the non-responsiveness to plasmapheresis.

TH-PO1125

Urinary Potassium Excretion and Risk of Graft Failure and Mortality in Renal Transplant Recipients Michele F. Eisenga,[1] Michel M. Joosten,[1] Sabita Soedamah-Muthu,[2] Else Van den Berg,[1] Petronella Deetman,[1] Gerjan Navis,[1] Rijk O.B. Gans,[1] Carlo A. Gaillard,[1] Stephan J.L. Bakker.[1] *[1]Internal Medicine, UMCG; [2]Human Nutrition, Wageningen Univ, Netherlands.*

Background: Low potassium intake has consistently been shown to induce kidney injury in experimental animal models. This is thought to be mediated through stimulation of ammoniagenesis. We hypothesized that urinary potassium excretion (UKV) as gold standard for dietary potassium intake is inversely associated with graft failure in renal transplant recipients (RTR).

Methods: Dietary intake was assessed by 24 hour UKV and food-frequency questionnaires (FFQ). Graft failure was defined as return to dialysis or retransplantation. Cox regression analyses were used to investigate prospective associations with outcome.

Results: We included 705 stable RTR (age 53±13 yrs; 57% males at 8.1±7.6 yrs after Tx). UKV was 73±24 mmol/d and urinary ammonia excretion was 21±12 mmol/d. During follow-up for 3.1 (2.7–3.9) yrs, 45 RTR developed graft failure and 83 RTR died. UKV was positively correlated with potassium intake according to FFQ (r=0.44, p<0.001). RTR in the lowest tertile of UKV were at increased risk of graft failure (HR 2.70 [95%CI 1.13-6.44]) and mortality (2.36 [1.35-4.12]) compared to the highest tertile (figure 1). In multivariable analyses with UKV as a continuous variable, UKV was inversely associated with both graft failure (0.76 [0.64-0.91]; p=0.002) and mortality (0.84 [0.75-0.94]; p=0.002), independent of potential confounders, including eGFR, plasma potassium, SBP, and urinary ammonia.

Conclusions: Low potassium intake measured by UKV is associated with higher risk of graft failure and mortality. This association seems not mediated through stimulation of ammoniagenesis. Because many RTR are advised to limit potassium intake prior to transplantation, specific attention for adequate potassium intake after transplantation may be warrented.

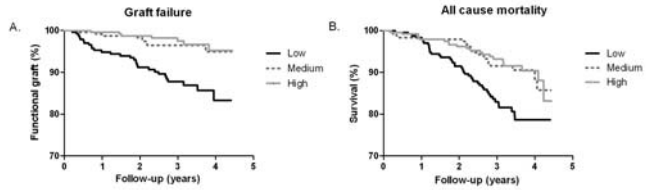


Figure 1 Survival curves for graft failure (A) and mortality (B) according to tertiles of UKV

Funding: Government Support - Non-U.S.

TH-PO1126

Proximal Tubular Dysfunction Is Common after the First Year of Kidney Transplantation Gaël Ensergueix,[1] Julien Allard,[1] Franck Saint-Marcoux,[2] Benoît Marin,[3] Sandra Bodeau,[2] Jean-Claude Aldigier,[1] Pierre Marquet,[2] Marie Essig.[1] *[1]Nephrology, Dialysis, Transplantation, CHU Limoges, France; [2]Pharmacology, Toxicology, CHU Limoges, France; [3]Epidemiology, Biostatistics, Methodology, CHU Limoges, France.*

Background: After the initial period of kidney transplantation, the prevalence of proximal tubular dysfunction (PTD) and its consequences are unknown. The aim of this study was to evaluate PTD prevalence in kidney transplant recipients, after the first year, and to analyze its risk factors.

Methods: We conducted an observational and cross-sectional study between April 2012 and April 2013 at Limoges university hospital (France). The inclusion criteria were: kidney transplantation for at least one year, and eGFR [MDRD] > 15 mL/min/1,73m². PTD was assessed by the association of urine cystatin C (UCC)/urine creatinine > 20 µg/mmol and UCC > 100 µg/L.

Results: 356 patients were included, among them 335 could have a full analysis (64.8% males and 35.2% females, with a mean age of 57.8 years (20.0 to 84.4)). 92.9% of patients were treated with calcineurin inhibitors, (Cyclosporine (CsA) : 51.6%, Tacrolimus (Tac) : 41.3%), 81.8% with anti-metabolites (Azathioprine : 3.3%, Mycophenolic Acid : 7.5%, Mycophenolate Mofetyl (MMF) : 71%) and 7.5% with m-TOR inhibitors. PTD prevalence was 22.7 %. The tubular proteinuria and the fractional excretion of uric acid were significantly associated with PTD (p < 0.0001). The prevalence of PTD increased with donor age (OR = 1.4, 95% CI [1.1 ; 1.7]) but was independent of cold ischemia time (p = 0.9980). An eGFR [MDRD] above 30 mL/min/1,73m² was protective (OR : 0.36, 95% CI [0.144 ; 0.92]). The immunosuppressive regimens " MMF + m-TOR inhibitors " and " MMF + Tac " were more frequently associated with PTD than " MMF + CsA " (OR = 6.4, 95% CI [1.8 ; 23.0] and OR = 3.0, 95% CI [1.3 ; 6.8], respectively).

Conclusions: In renal transplant patients, after the first year post-transplantation, about one quarter of the recipients are affected by PTD, which seems partly related to the immunosuppressive regimen. Since proximal tubular cells lesions could progressively lead to tubular atrophy and interstitial fibrosis, PTD early detection and treatment may help to increase graft survival.

TH-PO1127

Magnetic Resonance Elastography: A Novel Non-Invasive Modality for Chronic Renal Allograft Injury Detection Darren A. Yuen, General Leung, Nishigandha Burute, Stephen G. Szeto, Derek Sun, Serge Jothy, Jeffrey S. Zaltzman, G.V. Ramesh Prasad, Warren Foltz, Craig A. Simmons, Anish Kirpalani. *Univ of Toronto, Toronto, ON, Canada.*

Background: Chronic allograft injury (CAI) is a common cause of renal transplant loss, and is driven by multiple factors, including apoptosis and fibrosis. Biopsy is the gold standard for diagnosis and prognosis of CAI, but is limited by risk and sample bias. While preliminary reports suggest that injured organs stiffen due to fibrosis, apoptosis-mediated parenchymal loss and diminished perfusion may conversely lead to reduced stiffness. We report our preliminary results using MR elastography (MRE), a gadolinium-free imaging technique that can detect renal stiffness, in a cohort of renal transplant patients.

Methods: Patients with grafts > 1 yr were eligible for recruitment. All subjects underwent MRE to measure kidney stiffness. 12 regions of interest (ROI) were placed per kidney on a coronal section cut through the hilum, with two ROIs each in the upper, middle and lower pole cortex and medulla. Clinical data (age, gender, donor type, transplant vintage, plasma creatinine, eGFR, urinary albumin excretion) was also collected. All patients underwent graft biopsy, and interstitial fibrosis was measured on trichrome-stained sections.

Results: 4 patients were studied. Mean age was 42 ± 17 yrs and 75% were male. 2 of the 4 patients received a living donor kidney, and transplant vintage was 59 ± 7 mos. Plasma creatinine was 147 ± 25 µM (eGFR 46 ± 8 mL/min/1.73 m²), and urinary albumin:creatinine ratio was 7.8 ± 13 mg/mmol. Mean cortical and medullary stiffness values were 5.0 ± 2.0 kPa and 4.3 ± 1.4 Pa respectively. Stiffness was reduced in patients with eGFR < 45 mL/min (n = 2) versus those with eGFR > 45 mL/min (n = 2) (cortex: 3.5 ± 0.3 kPa versus 6.6 ± 1.0 kPa and medulla: 3.2 ± 0.2 versus 5.4 ± 0.7 Pa).

Conclusions: MRE-derived stiffness measurement in the transplant kidney is feasible. In this pilot study, bulk measures of graft stiffness appear to decline with more severe injury. Further studies are required to confirm this finding, and to delineate the underlying mechanisms. Development of MRE may enable non-invasive quantitation of injury in patients with CAI.

Funding: Pharmaceutical Company Support - Astellas Pharma Canada

TH-PO1128

The Clinical Impact of BK Virus Surveillance on Outcomes in Kidney Transplant Recipients Se-Hee Yoon,[1] Jang-Hee Cho,[2] Hee-Yeon Jung,[2] Won Min Hwang,[1] Sung-Ro Yun,[1] Ji-Young Choi,[2] Sun-Hee Park,[2] Yong-Lim Kim,[2] Chan-Duck Kim.[2] *[1]Internal Medicine, Konyang Univ Hospital, Deajeon, Republic of Korea; [2]Internal Medicine, Dept of Internal Medicine, Kyungpook National Univ School of Medicine, Daegu, Korea & Clinical Research Center for End Stage Renal Disease, Republic of Korea.*

Background: The objective of this study was to investigate the clinical impact of BK virus surveillance to prevent BK virus associated graft injury in kidney transplantation.

Methods: We evaluated the prevalence of BK viremia using plasma quantitative polymerase chain reaction (PCR) and BK virus associated nephropathy (BKVAN) and the clinical impact of BK viremia on graft outcomes in the kidney transplant recipients between January 2008 and June 2013.

Results: In this study, 213 kidney transplant recipients were included. The prevalence of BK viremia and high BK viremia (>1X10⁴ copies/mL) were 66.7% (142/213) and 17.4% (37/213) respectively. Nine cases (4.2%) were finally diagnosed as BKVAN by allograft biopsy. Although low BK viremia (<1X10⁴ copies/mL) group had comparable eGFR after transplantation, high BK viremia group showed significantly lower eGFR at 18 months after transplantation when compared with no viremia group. In receiver operating characteristic curve analysis, area under the curve of peak BK viremia for the diagnosis of BKVAN was 0.980. We found 92,850 copies/mL was significant cut-off level to predict BKVAN with 89% sensitivity and 94.6% specificity. The risk factors for viral loads ≥ 10000 copies/ml were CMV infection, steroid pulse therapy and acute rejection.

Conclusions: Early detection of BK viremia after renal transplantation using a frequent screening standardized surveillance protocol and treatment of those with significant viremia with systematic reduction in immunosuppressant are effective strategies that identify subclinical BKVAN, preserve renal function, and prevent graft loss from BKVAN.

TH-PO1129

Low Rate of BK Viremia and Nephropathy in Large Single Center Series of HIV+ Kidney Transplant Recipients Shabnum Haleem, Talal A. Khan, Gregory Malat, PharmD, Karthik M. Ranganna, Alden Michael Doyle. *Drexel Univ College of Medicine, Philadelphia.*

Background: BK virus is a polyoma virus that is benign in immunocompetent hosts but can lead to allograft graft dysfunction and loss for kidney transplant recipients .The reported estimated prevalence of BK-induced nephritis is 1 to 10 percent, with a mean of approximately 5 percent. There are multiple risk factors associated with BK virus nephropathy(BKVN) including male gender, glucocorticoids maintenance immunosuppression, diabetes mellitus, treatment of acute rejection, delayed graft function and ureteral trauma. The dominant risk factor is intensity of immunosuppression and the primary treatment modality is reduction in immunosuppression. Although the risk of BK viremia and nephropathy has not been well characterized in HIV+ kidney transplant recipients, it stands to reason that it might be greater than seen in HIV- populations because of the immune dysregulation imposed by the HIV virus on the host's immune system.

Methods: We retrospectively examined 59 consecutive HIV+ patients who received kidney transplant at our center between 2004-2014.At our center Urine BK virus PCR was checked sporadically .After 2009 serum BK virus PCR was checked as a protocol. We recorded their induction and maintenance immunosuppression, Urine and serum BK virus PCR, any evidence of rejections and any allograft biopsy proven diagnosis of BK nephropathy.

Results: Six out of 59 cases of HIV positive kidney transplant recipients had BK viuria. Only 1 out of 6 had BK viremia. None of the patients had evidence of BKVN from allograft biopsies. Most of the patients had induction with basiliximab and they were on standard triple maintenance immunosuppression with calcineurin inhibitors, mycophenolic acid and prednisone. All patients were maintained on stable ART therapy for HIV control. Thirty seven patients had episodes of acute rejections.

Conclusions: Our results suggest that despite the compound immunosuppression imposed by the combination of HIV serostatus and maintenance immunosuppression, HIV+ kidney transplant recipients were found to have a low risk for BK virus nephropathy. Why there is a lower risk for BK viremia and BKVN is not clear but deserves further inquiry.

TH-PO1130

Cerebral Lymphoma in Renal Transplantation in Australia and New Zealand Jobert Anjelo,[1] Vinoi George David,[1] Blair S. Grace,[2] Stephen P. McDonald,[2] Randall James Faull.[1] *[1]Nephrology, Royal Adelaide Hospital, Adelaide, South Australia, Australia; [2]ANZDATA Registry, Royal Adelaide Hospital, Adelaide, South Australia, Australia.*

Background: Post-transplant lymphoma is a major complication of organ transplantation, but cerebral lymphoma (CL) is rarely reported in literature.

Methods: Retrospective review based on ANZDATA Registry of patients with CL after renal transplantation in Australia and New Zealand from 1989-2012 cohort.

Results: Among 21390 people who received 24832 grafts, there have been 34 reports of cerebral lymphoma post transplantation, 327 reports of lymphoma in other sites, and 103 reports of PTLD CL was not reported in grafts prior to 1980, but was reported among 0.12, 0.32 and 0.15% respectively of recipients transplanted in the 1980s, 1990s and 2000s.

	Cerebral Lymphoma	Lymphoma[not Cerebral 0r PTLD]	PTLD	No lymphoma
Number of Recipients	34	302	102	20952
Age at first transplant [Median, years]	45[30-53]	43[30-53]	41[23-53]	44[31-54]
Age at diagnosis [Median,years)]	51[47-61]	52[42-63]	48 [35-61]	
Time from first transplant	2570	3183	2185	
EBV IgG neg recipient [OR=odds ratio]	7/20[35%] OR 2.7 [1.1- 6.7] P <0.04	36/90[40%] OR 3.3 [2.2- 5.1], P<0.001	30/57(53%) OR 5.5[3.3-9.3] P<0.001	
Use of CD3 agent [OR]	OR 2.5 [1.2-5.2] p=0.04	1.4 [1.1-1.9] P<0.001	1.3 [0.8-21], P=0.3	
Number of deaths	21	199	55	
Survival post diagnosis [median (IQR),days]	603[26-3486]	1264[76-5410]	1605[45-.]	

Risk factors: previous exposure to CD3 agents and seronegative recipient EBV IgG (collected from 1990) were associated with increased incidence. While median survival of people with CL was almost 20 months after diagnosis, this was poorer than other groups and more than 25% had died within 1 month.

Conclusions: Cerebral lymphoma is a rare presentation of post-transplant lymphoma in renal transplantation. Like lymphomas at other sites, it is associated with EBV negative serostatus and previous use of ATG / ALG / OKT3. It carries poor prognosis once diagnosed.

TH-PO1131

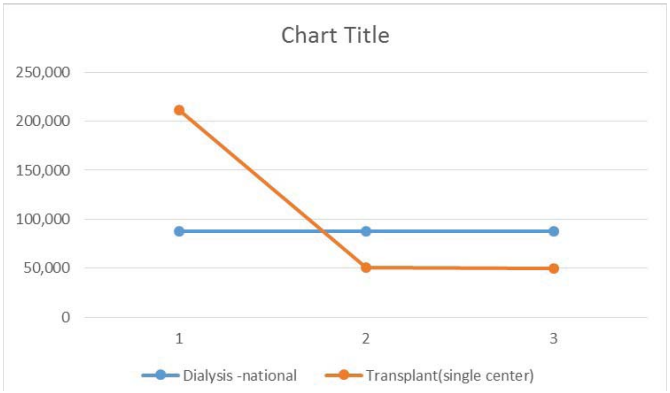
Comparative Cost Analysis of Renal Transplantation and Dialysis Therapy Neenu Sukumaran, Parvathy Madhavan, Sapna M. Jairath, Aditya Kadiyala, Umashankar Chandrashekaran, Celenia Reynoso, Madhu C. Bhaskaran, Ernesto P. Molmenti. *Renal Transplant Center, North Shore Univ Hospital, Manhasset, NY.*

Background: Multiple studies have demonstrated the cost effectiveness of renal transplantation in comparison to long term dialysis therapy in ESRD patients. However many changes in the cost structure for dialysis and renal transplant have occurred in recent years including, new cost saving measures and incentives for institutions. Also the criteria for transplant recipient selection is more aggressively regulated. We are determining the break-even point in the costs of these treatment modalities at a single center under the current payment system and evaluating the cost efficiency against the national financial average.

Methods: We conducted analysis of average annual costs of dialysis per patient and compared it to the average annual cost of renal transplant per patient using data collected from a single center. The values calculated were weighted against the national averages of dialysis and transplant costs. Several variables including reimbursements per visit, comorbidities, complications, length of inpatient stay and variation in reimbursements depending on these were recorded. Evaluating the above, we determined the time frame at which the cost of renal transplantation matched the cost of dialysis (break-even point).

Results: The results are as stated in the table and graph below:

	Single Center	National Costs
Average cost per transplant recipient in 2011	$211,313	$262,900



Conclusions: On analysis of the average costs of renal transplant versus dialysis in our center, the data obtained shows values comparable to the national average. Costs at this center are reduced through reduction in post-surgical hospital stay and the use of generic medication.

FR-PO001

Volume Replacement Improves Vasoconstriction but Not Glomerular Filtration Rate (GFR) Reduction Induced by Furosemide in Rats
Magali Araujo, Christopher S. Wilcox, William J. Welch. *Medicine, Georgetown Univ, Washington, DC.*

Background: Although loop diuretics reduce GFR by unknown mechanisms, we tested the hypothesis that a rise in proximal tubule hydrostatic pressure (P_{PT}) reduces GFR despite inhibition of tubuloglomerular feedback (TGF) and evaluated the effect of volume replacement (VR).

Methods: GFR and renal blood flow (RBF) were measured by inulin/PAH clearance in SD rats before and after i.v infusion of furosemide (F) (0.1, 0.3 and 1.0 mg/kg.h^{-1}) versus vehicle and TGF by changes in PT stop flow pressure (SFP) during loop perfusion of artificial tubular fluid (ATF) at 40 nl min with F (10^{-10} M to 10^{-4} M) versus vehicle.

Results: There was a dose-dependent increase in diuresis with F of 100%, 250% and 400% respectively. Both GFR and RBF decreased significantly 40-60 minutes following the higher i.v.doses of F by 40% and 46% respectivelly, $p<0.01$) without changes in MAP or heart rate. Volume replacement simultaneously with F (0.3mg/kg/h^{-1}) prevented a significant fall in RBF (6.1±0.8 versus 5.4±0.4 ml/min/g kidney, NS) but not in GFR (1.3±0.1 versus 0.9±0.1 ml/min/g kidney, $p<0.02$). P_{PT} (mmHg) increased 20 minutes after high dose of F (13.5±0.2 versus 22.4±0.4 mmHg, $p<0.01$) but returned to baseline within 60 minutes, yet the GFR and RBF remained reduced. Direct intratubular perfusion of F blunted TGF at 10^{-5} and 10^{-4} M.

Conclusions: Although P_{PT} increased sufficiently after F to reduce GFR, it cannot account for the persistent falls in GFR and RBF. A reduction in extracellular volume after F could account for the fall in RBF but not in GFR. Therefore, the reduction in GFR after F is largely independent of increases in P_{PT} or decreases in extracellular volume and occurs despite inhibition of TGF.

FR-PO002

The Intrinsic Circadian Clock in Renal Tubular Cells Does Not Play a Major Role in the Maintenance of Water and Salt Balance and Blood Pressure
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Background: Recent study demonstrated that the renal circadian timing system plays an important role in maintaining extracellular fluid homeostasis and blood pressure. However, the role of the intrinsic circadian timing system located in specific renal cell types is largely unknown.

Methods: To study role of the intrinsic circadian timing system in renal tubules, we generated inducible nephron specific knockout mouse model for Bmal1, an essential element of the molecular clock. Bmal1$^{lox/lox}$ mice were crossed with mice expressing Cre recombinase in renal tubular cells upon doxycycline administration (hereafter Bmal1$^{lox/lox}$/Pax8:LC1Cre mice).

Results: Bmal1 was inactivated in the renal tubules as well as partially in the liver in Bmal1$^{lox/lox}$/Pax8:LC1Cre mice. Bmal1$^{lox/lox}$/Pax8:LC1Cre mice showed no difference in body weight but a significant reduction in kidney weight/body weight ratio by more than 20% comparing to control mice. Histological analysis revealed the presence of mesangial sclerosis in Bmal1$^{lox/lox}$/Pax8:LC1Cre mice. There was no difference in water, sodium and potassium excretory rhythms between Bmal1$^{lox/lox}$/Pax8:LC1Cre and control mice. The blood pressure and blood pressure dipping pattern were not different between control and Bmal1$^{lox/lox}$/Pax8:LC1Cre mice. These findings correlated with the fact that all major water, sodium and potassium transporters exhibited similar expression levels between control and Bmal1$^{lox/lox}$/Pax8:LC1Cre mice. However, deep sequencing has revealed important differences in genes related to several cellular pathways, including glucose homeostasis (GYS2, GSK3b, PYGL, PPARd) and oxidative stress (fibrinogen alpha beta and gamma chains, Arg2, Cdkn1a, Loxl4, Angptl4).

Conclusions: Our data suggest that the intrinsic circadian timing system in renal tubules does not have a significant impact on salt/water transport and blood pressure homeostasis. Further studies are required to define the role of the clock system in renal metabolism and oxidative stress protective mechanisms.

FR-PO003

$[Ca^{2+}]_i$ Oscillations and Il-6 Release Induced by a-Haemolysin from *Escherichia coli* Require P2 Receptor Activation in Renal Epithelia
Mette G. Christensen, Steen K. Fagerberg, Randi G. Bjaelde, Jens G. Leipziger, Helle A. Praetorius. *Dept of Biomedicine, Aarhus Univ, Aarhus, Denmark.*

Background: Urinary tractinfections are commonly caused by a-haemolysin (HlyA)-producing *E. coli*. HlyA forms pores in cell membranes that renders the attacked cells permeable to ions and water. In erythrocytes, the effect of HlyA is strongly amplified by P2X receptor activation and our recent data support that ATP pass through the HlyA pore itself as an early event after pore insertion into the membrane. We therefore hypothesise that HlyA-induced $[Ca^{2+}]_i$ oscillations in renal epithelia is mediated by ATP release and following P2 receptor activation.

Methods: Here we confirm that HlyA, added at a concentration that cause 50% lysis of human erythrocytes, initiate marked $[Ca^{2+}]_i$ oscillatory activity in renal epithelia. This was quantified by live cell fluorescence microscopy in both MDCK cells and freshly isolated medullary thick ascending limb (mTAL) from mice.

Results: HlyA clearly triggered ATP release from MDCK cells and accordingly the HlyA-induced $[Ca^{2+}]_i$ oscillations were completely prevented by non-selective P2 receptor antagonists and by ATP-scavenging in MDCK cells. The HlyA-induced oscillations were reversible and did not cause permanent cell damage. To confirm these results, we tested the effect of HlyA on ATP biosensor cells. In native 132-1N1 astrocytoma cells that do not express any P2 receptors, HlyA barely caused any changes in $[Ca^{2+}]_i$. Transfection of the cells with a hP2Y_2 receptor resulted in an extensive increase in the HlyA-induced $[Ca^{2+}]_i$ oscillatory activity, which were sensitive to P2 receptor inhibition. Moreover, the HlyA-induced $[Ca^{2+}]_i$ oscillations were markedly reduced in mTAL isolated from P2Y_2 receptor deficient mice compared to wild type. Interestingly, the HlyA-induced interleukin 6 (Il-6) release was absent in the P2Y_2 receptor knockout (P2$Y_2^{-/-}$) mice, whereas it was readily detectable in the wild type (P2$Y_2^{+/+}$) mice.

Conclusions: These results suggest that HlyA triggers ATP release from renal epithelia, which via P2 receptor activation is responsible for the HlyA-induced $[Ca^{2+}]_i$ oscillations and following Il-6 release.

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FR-PO004

Claudin-2 Null Mice Reveal Increased Renal Transcellular Na Transport and Kidney Oxygen Consumption
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Background: 60% of filtered Na is reabsorbed in renal proximal tubules (PT), 40% of which is transported paracellularly. Claudins are a family of tight junction proteins that regulate paracellular permeability. Cation-selective claudin-2 (cldn2) is expressed in PT.

Methods: To evaluate the role of cldn2 in renal salt handling, we placed wild-type (WT) and cldn2 knockout (KO) mice on a Na deficient diet, and urine was collected every 8 hours. To test if there is any functional increase in transcellular Na transporters, we performed diuretic challenge test.

Results: The cldn2 KO mice were able to conserve Na to the same extent as WT.Quantitative RT-PCR did not reveal compensatory upregulation of other claudin isoforms. Immunoblotting for a panel of transcellular Na transporters showed a 23% decrease in the abundance of Na-H antiporter 3, only borderline increases in phosphorylated NaCl cotransporter and the l subunit of the epithelial Na channel, and no change in total or phosphorylated Na-K-2Cl cotransporter (NKCC2). The natriuretic response to the NKCC2 inhibitor, furosemide (25 mg/kg ip), was 25% higher in KO compared to WT ($P<0.05$), while the responses to hydrochlorothiazide and benzamil were not significantly different. Thus, loss of PT Na transport was compensated distally, primarily by a functional increase in transcellular Na transport in the thick ascending limb. We hypothesized that the increased transcellular transport would lead to increased renal oxygen consumption. Thus, we determined the ratio between whole kidney Na reabsorption (T_{Na}) and oxygen consumption (Q_{O2} = RBF X A-V_{O2}) in WT and KO mice. Cldn2 null mice exhibited lower T_{Na}/Q_{O2}. Renal O_2 extraction was also higher in KO mice.

Conclusions: These data indicate that the efficiency of oxygen utilization is decreased in cldn2 null mice. In conclusion, our findings suggest that in cldn2 KO mice, loss of PT paracellular Na transport is compensated by increased Na reabsorption by distal transcellular transporters maximally conserving Na at the expense of increasing kidney oxygen consumption.

FR-PO005

Protein Carbonylation of the Na/K-ATPase a1 Subunit Regulates Na/K-ATPase Signaling and Sodium Transport in Renal Proximal Tubular Cells
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Background: We have shown that cardiotonic steroids signaling through the Na/K-ATPase regulate sodium reabsorption in renal proximal tubule (RPT). Here we report that oxidative modification of the a1 subunit is critical in modulation of Na/K-ATPase signaling and sodium transport.

Results: In pig RPT LLC-PK1 cells, ouabain (100nM) and glucose oxidase (GO, 1 and 3 mU/ml) induced ROS production, stimulated activation of c-Src and accumulation of Na/K-ATPase a1 and NHE3 in early endosome (EE) fractions, and inhibited active transepithelial $^{22}Na^+$ flux. Pretreatment with the antioxidant, N-Acetyl-L-Cysteine (NAC) can either partially or completely prevent these effects in a dose-dependent manner. Induction of heme oxygenase-1, an enzyme with potent antioxidant capacity, by CoPP attenuates ouabain-induced signaling and protein carbonylation. Furthermore, disruption of the Na/K-ATPase signaling (a1 subunit knock-down, caveolin-1 knock-out, or Src knock-out) abolished ouabain-induced protein carbonylation. LC-MS/MS analyses identified that ouabain/GO stimulated direct carbonylation of Pro^{222} and Thr^{224} in pig a1. These two amino acid residues are located in the a1 actuator (A) domain, highly conserved and exposed, facing the nucleotide binding (N) domain. To test the role of Pro^{222} carbonylation, two stable a1 mutant cell lines were generated. Mutation of Pro^{222} appears to abolish ouabain-induced protein carbonylation and activation of c-Src and ERK1/2. However, a "scramble" control mutation of Ala^{414} appears to have no effect on ouabain-induced protein carbonylation and activation of c-Src and ERK1/2.

Conclusions: Protein carbonylation of the Na/K-ATPase a1 subunit regulates Na/K-ATPase signaling and related sodium handling in RPTs. The data indicate that direct carbonylation of Pro^{222} might dictate the Na/K-ATPase signaling and that the Na/K-ATPase might be a functional receptor of ROS.

Funding: Other NIH Support - NIH HL-109015

FR-PO006

NKCC2 Phosphorylation Attenuates Its Clathrin-Mediated Internalization Anna Daigeler,[1] Christin Dathe,[1] Bridget S. Wilson,[2] Sebastian Bachmann,[1] Kerim Mutig.[1] *[1]Vegetative Anatomie, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Cancer Research Facility, Univ of New Mexico School of Medicine, Albuquerque, NM.*

Background: The Na-K-2Cl cotransporter (NKCC2) of the thick ascending limb of Henle's loop (TAL) plays an important role in renal salt handling. Activity of the transporter depends on its surface expression and phosphorylation at conserved N-terminal threonine and serine residues. We have recently shown that phosphorylation of NKCC2 interacts with its apical trafficking. In this study we focused on the endocytic retrieval of NKCC2. We hypothesized that the activating phosphorylation attenuates its clathrin-dependent internalization and thereby facilitates its function.

Methods: Cellular distribution of NKCC2, phospho-NKCC2, and clathrin was evaluated in cultured TAL cells and rat kidney using immunofluorescence and immunogold labelling. Protein-protein interactions were studied by GST pull-down assay. Vasopressin (AVP)-deficient Brattleboro rats were treated with a vasopressin V2 receptor agonist dDAVP (1 ng/g of body weight; 30 min) to study the interaction between NKCC2 phosphorylation and internalization *in vivo*.

Results: Fine structural analysis of the apical TAL membrane using immunogold labeling showed significant co-localization of NKCC2- and clathrin-signals (~20%), whereas phospho-NKCC2 was barely co-localized with clathrin. GST pull-down assays performed with rat kidney lysates using N- or C-terminal tails of rat NKCC2 as baits revealed association of clathrin with both cytoplasmic NKCC2 moieties. Analysis of interactions between clathrin and NKCC2 mutants mimicking (de) phosphory-lation of the transporter at functionally relevant N-terminal residues (T96, T101, T114, and S126) suggested that phosphorylation abrogates N-terminal binding of NKCC2 with clathrin. Along the same line, stimulation of the vasopressin (AVP) /V2 receptor axis in AVP-deficient Brattleboro rats substantially increased NKCC2 phosphorylation and surface expression and simultaneously inhibited its interaction with clathrin.

Conclusions: Our data suggest that N-terminal NKCC2 phosphorylation may facilitate its function by reducing its clathrin-mediated internalization.

FR-PO007

Changes in Distal Sodium Transporters in the Development and Maintenance of Obesity-Related Hypertension Matthew R.P. Davies,[1,2,3] Marina Katerelos,[1] Kurt Gleich,[1] Scott Andrew Fraser,[1] Peter F. Mount,[1,2,3] David A. Power.[1,2,3] *[1]Nephrology, Austin Health, Australia; [2]Medicine, Univ of Melbourne, Australia; [3]Inst of Breathing and Sleep, Australia.*

Background: Enhanced tubular reabsorption of Na^+ is critical in the pathogenesis of obesity-related hypertension (ORH). Initially Na^+ is actively retained; later Na^+ retention is maintained despite hypertension. The Na^+ transporters responsible have not been identified. The distal transporters NKCC2 (target for frusemide) and NCC (target for thiazides) can be regulated by expression, membrane trafficking and phosphorylation; key activating sites are S126- and T96/101-NKCC2, and T58-NCC. ENaC (target for amiloride) is primarily regulated through membrane trafficking. We studied the status of distal Na^+ transporters at different time points in the development of ORH.

Methods: C57BL/6 mice were fed control or high fat diet (HFD) for 2.5 or 14 weeks. Protein expression and phosphorylation were determined by Western blotting. Surface expression was determined by IF. Serum, BP and diuretic studies were performed at 14 weeks.

Results: After 2.5 weeks of HFD, expression of NCC was increased 2-fold ($p<0.001$) with a similar increase in pT58-NCC. No differences were found in NKCC2 or ENaC at this time. At 14 weeks HFD mice were obese, hypertensive, hyperinsulinaemic and hyperleptinaemic. At this time the changes in NCC had returned to baseline but phos (S126)-NKCC2 was increased 2.5-fold ($p<0.001$) while phos (T96/T101)-NKCC2 remained unchanged in whole kidney preparations. Natriuretic response to frusemide *in vivo* was increased 1.6-fold ($p<0.05$), confirming increased NKCC2 activity. Response to amiloride was also increased ($p<0.05$), but no increase in subunit expression or surface localisation was found to explain enhanced ENaC activity.

Conclusions: In this HFD model increased expression of NCC was the key early change identified that could help explain the establishment of Na^+ retention in the pathogenesis of ORH. In contrast, increased phos (S126)-NKCC2 was identified as a mechanism that would maintain Na^+ overload in the presence of hypertension. ENaC activity was also increased in established obesity, but the mechanism causing this remains elusive.

FR-PO008

The Natriuretic Effect of the Glucagon-Like Peptide 1 Receptor Agonist, Exendin-4, Is Independent of eNOS and Phosphorylation of NKCC2 and NCC, and Absent in STZ-Diabetic Mice Yiling Fu,[1,2] Maria Gerasimova,[2] Takahiro Masuda,[1] Yasaman Alam,[1] Falk Bernhard Batz,[1] Volker Vallon.[1,2] *[1]Div. Nephrology, Univ of California San Diego, La Jolla, CA; [2]VA San Diego Healthcare System, San Diego, CA.*

Background: Exendin-4 (EX-4) activates the glucagon-like peptide 1 receptor (GLP-1R) and is used as an antidiabetic drug, but also induces natriuresis, as reported in normoglycemia as well as in type 2 diabetic db/db mice. The natriuretic effect has been proposed to involve endothelial nitric oxide synthase (eNOS) in renal vascular and phosphorylation/inhibition of Na-H-exchanger 3 in proximal tubules, but the renal localization of the GLP-1R and the involvement of the distal nephron remains ill defined.

Methods: i) In eNOS ko/wild type (WT) mice and ii) in streptozotocin (STZ)-diabetic mice, EX-4 (10 mg/kg) or vehicle was applied i.p. together with an oral NaCl load (30 ml/g bw of 0.85% saline) and the urine was quantitatively collected over 3 hours in metabolic cages. iii) One hour after EX-4 or vehicle application to WT mice, kidneys were harvested and renal membrane expression of phosphorylated Na-2Cl-K cotransporter NKCC2 (pS126) and Na-Cl cotransporter NCC (pS71) (phosphorylation of both associated with transporter activation) determined by Western blot (normalized to b-actin).

Results: i) Absence of eNOS did not affect the natriuresis during vehicle application (23±1 versus 22±1 nmol/min/g in wild type) or the EX-4-induced increase in natriuresis (14±2 versus 13±2 nmol/min/g) (not significant different (NS); n=8/group). The EX-4-induced natriuresis ii) was absent in STZ-diabetic mice (EX-4 versus vehicle: 17±2 versus 17±2 nmol/min/g, NS; n=8), and iii) in WT mice occurred in the absence of significant changes in the renal membrane expression of pS126-NKCC2 or pS71-NCC (94±5 and 101±8% of vehicle, respectively; NS; n=5/group).

Conclusions: The results argue against a role of eNOS or the phosphorylation of NKCC2 and NCC in the EX-4-induced natriuresis in mice. The mechanism for the blunted natriuretic effect in STZ-diabetic mice remains unclear.

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FR-PO009

A Role for aENaC in Connecting Tubules in PPARg Agonist-Induced Fluid Retention Yiling Fu,[1,2] Maria Gerasimova,[2] Falk Bernhard Batz,[1] Alexander Kuczkowski,[1] Yasaman Alam,[1] Edith Hummler,[3] Volker Vallon.[1,2] *[1]Div of Nephrology, Univ of California, San Diego, La Jolla, CA; [2]VA San Diego Healthcare System, San Diego, CA; [3]Univ of Lausanne, Switzerland.*

Background: Thiazolidinediones (TZDs, such as rosiglitazone (RGZ)) are peroxisome proliferator–activated receptors (PPARg) agonists used to treat type 2 diabetes. TZDs can induce fluid retention, and studies with amiloride suggested a possible role for the epithelial sodium channel ENaC. ENaC in the connecting tubule (CNT), but not in the collecting duct (CD), appears critical for normal NaCl and K balance. Since RGZ-induced fluid retention was maintained in mice with aENaC knockdown in the CD, here we aimed to test the role of ENaC in the CNT.

Methods: Mice with conditional inactivation of aENaC both in CD and CNT were generated using aquaporin2 (AQP2)-Cre (aENaC lox/lox AQP2-Cre; aENaC CD/CNT KO) and compared with littermate controls (aENaC lox/lox; WT). Mice were treated with RGZ (320 mg/kg diet) for 10 days in regular cages. Body weight (BW) was monitored, and body water volume determined by bioelectrical impedance analysis (BIA) before and after RGZ.

Results: On regular NaCl diet, aENaC CD/CNT KO had normal BW, body water volume, hematocrit, and plasma Na and K, whereas plasma aldosterone was modestly increased compared with WT (686±85 versus 503±42 pg/ml; $P<0.05$). The RGZ-induced increase in BW observed in WT was attenuated in aENaC CD/CNT KO (6.1±0.4 versus 3.4±0.3%, n=12-20; $P<0.05$). In WT, RGZ increased total body water (TBW) by 8±3% and extracellular fluid (ECF) by 10±1% (each $P<0.01$) and tended to increase intracellular fluid by 8±4% ($P=0.052$). No significant changes were observed in TBW (1±2%), ECF (4±2%) and ICF (0±2%) in aENaC CD/CNT KO in response to RGZ. Consistent with effective knockdown, we confirmed the impaired NaCl and K homeostasis in aENaC CD/CNT KO when challenged with a low NaCl diet.

Conclusions: Knockdown of aENaC in CNT/CD attenuated RGZ-induced fluid retention. Together with previous studies showing fully maintained RGZ-induced fluid retention in mice with knockdown of aENaC in the CD, the current results indicate a role for ENaC in CNT in this response.

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FR-PO010

Regulation of NCC by WNK4 and by High Dietary K Intake Yih-Sheng Yang, Jian Xie, Chou-Long Huang. *Internal Medicine, UT Southwestern Medical Center, Dallas, TX.*

Background: The role of WNK4 and SPAK cascade in the activation of NCC in DCT remains unclear. High K intake decreases the activity of NCC, which is expected to increase Na delivery and reabsorption via ENaC and thus K secretion by K channels in CNT/CCD. Role of WNK4, other WNKs and SPAK in the regulation of NCC by high K diet is unknown.

Methods: WT or WNK4-KO mice were pair-fed a control K (CK; 1% K) or a high K (HK; 5.25%) diet for 48 hr (day -2 to 0), and administered at day 0 (at 10 AM) either vehicle, furosemide (5mg/Kg BW), hydrochlorothiazide (HCTZ; 50 mg/Kg BW) or amiloride (5mg/Kg BW). Urinary Na and K excretion rate (mmole/hr) was measured for 24 hr pre-diuretic (baseline) and for two periods (0-4 hr and 4-24 hr) after diuretic.

Results: NCC activity ([HCTZ-induced Na excretion at 0-4 hr] minus [vehicle-induced Na excretion]) was reduced in WNK4-KO mice (versus WT) by ~67% (19±6 versus 56±3; $p<0.01$). NKCC2 and ENaC activity was respectively increased in WNK4-KO mice (versus WT), likely reflecting compensatory upregulation. Consistently, baseline Na excretion rate was not significantly different between WT and WNK4-KO. High K diet decreased NCC activity by ~35% (HK versus CK: 37±4 versus 56±3; $p< 0.01$) in WT mice. The inhibition of NCC by high K intake appeared blunted in WNK4-KO mice; significance of this finding yet remains uncertain because the low baseline activity of NCC in WNK4-KO mice may prevent detectable inhibition by high K diet. Thus, we are further generating mice that carrying homozygous Wnk4-null allele and a constitutive-active SPAK kinase

allele. These mice may have NCC constitutively phosphorylated and activated by SPAK kinase independently of WNKs and thus allow us to examine the role of phosphorylation by WNKs-SPAK cascade and/or role of de-phosphorylation by phosphatases in the regulating NCC by high K intake.

Conclusions: Our results support the notion that, under a normal Na and K diet, WNK4 is a positive regulator of NCC. Decrease of NCC activity in WNK4-KO mice is accompanied by compensatory upregulation of NKCC2 and ENaC activity. High K intake inhibits NCC activity. Future studies will elucidate role of WNK4, other WNKs and SPAK cascade in regulating NCC by high K diet.

Funding: NIDDK Support

FR-PO011

Role of SPAK/OSR1 in WNK1 Regulation of NKCC2 and NCC

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Background: Mice with intron deletion of Wnk1 gene mimicking human PHA2 disease exhibit increased phosphorylation and activation of NCC, phenocoping human disease. Compared to WNK4, functional evidence supporting WNK1 activation of SLC12 transporters, NKCC2 and NCC, via OSR1/SPAK yet remains relatively scarce. WNK1-null mice die in utero from angiogenesis defects. Here, we investigate the role of OSR1/SPAK in WNK1 regulation of NKCC2 and NCC using genetic mouse models.

Methods: We generated mice carrying a constitutive-active (CA) SPAK kinase allele and crossed with Wnk1-null mice. Baseline BP and urinary Na excretion of WT and mutant mice and their response to furosemide (5mg/kg BW), hydrochlorothiazide (50 mg/kg BW) or vehicle were studied. Diuretic-induced urinary Na excretion rate (mmole/hr) was measured for 24 hr pre-diuretic and for two periods (0-4 hr and 4-24 hr) afterward. The activity of NKCC2 and NCC was assessed using urinary Na excretion (mmole/hr) induced by respective diuretic under pair-feeding (excretion at 0-4 hr post-diuretic minus vehicle-induced excretion).

Results: Wnk1-null mice carrying CA-SPAK allele ("WS" mice) were born alive and with no gross defects except a smaller body weight, supporting that WNK1 regulates angiogenesis via OSR1/SPAK cascade. Body weight (g at 3 mo: 17±1 versus 25±1.3; $p<0.05$) and SBP was lower for mutant WS versus WT mice (mmHg: 83±12 versus 112±12; $p<0.05$). Steady-state daily urine output (1.7±0.1 versus 0.69±0.1 ml) and Na excretion (170±7 versus 119±15 mmole) were significantly higher in WS mutant versus WT with free access to normal rodent diet and water, indicating Na wasting in mutant WS mice. Mice were fed a normal Na diet for a week and switched to a Na-deficient (ND) diet. While urinary Na excretion fell during Na-deficient diet for both, it was significantly higher for WS than WT (day4 ND diet, mmole/day: 2.3±0.6 versus 1.3±0.2, $p<0.05$). NCC activity was not different between WS mutant and WT mice. NKCC2 activity was decreased in WS mutant versus WT (14.9±2.0 versus 23.3±1.6; $p<0.01$).

Conclusions: SPAK/OSR1 acts downstream of WNK1 to regulate NCC. Reduced NKCC2 activity in WS mutant suggests presence of a SPAK-independent mechanism for activation of NKCC2.

Funding: NIDDK Support

FR-PO012

Disruption of STE20/SPS1-Related Proline/Alanine-Rich Kinase (SPAK) Binding Lowers Blood Pressure and Recapitulates Gitelman Syndrome in Mice

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Background: The salt-wasting hypotensive phenotype of Gitelman syndrome is caused by low Na^+-Cl^- Cotransporter (NCC) activity. In the kidney WNK4 phosphorylates SPAK (p-SPAK) at threonine residue 243. This activated p-SPAK increases NCC phosphorylation (p-NCC) and thus NCC activity. Previously we demonstrated SPAK T243A mutant mice recapitulate Gitelman syndrome, thus confirming the integral role of SPAK activity in blood pressure control. For signalling to occur WNK4 and NCC bind to SPAK at leucine residue 502 in the conserved C-terminal.

Methods: To test this *in vivo* we generated homozygous SPAK L502A mice which we hypothesised would exhibit a Gitelman syndrome phenotype. To investigate renal salt-wasting, wildtype and SPAK L502A mice (n=14) were fed a 3% w/w sodium diet for 10 days and switched to a 0.03% w/w sodium diet with urine electrolytes measured at 0hr, 3hr, 6hr, 12hr, 24hr and 96hr post-switch and normalised to urine creatinine. Two weeks post-switch blood pressure was measured by catheterisation of the right carotid artery under anaesthesia (isoflurane). After sacrifice by exsanguination, the kidneys were harvested for western blot analysis. All data are mean±SEM.

Results: Na^+ wasting at 6hr post-switch was observed in SPAK L502A versus wildtype mice [77±17 versus 41±7 arbitrary units; $P<0.05$]. Mean arterial blood pressure on 0.03%w/w sodium diet was also strikingly lower in the SPAK L502A [62±1mmHg versus 78±1mmHg; $P<0.001$] and the inhibition of SPAK binding resulted in a 3-fold decrease in levels of p-NCC in SPAK L502A from whole kidney blots.

Conclusions: In conclusion, SPAK binding is crucial for blood pressure maintenance and pharmacological inhibition of this binding is an attractive antihypertensive strategy.

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FR-PO013

Acute Potassium Intake Does Not Reduce Renal Na-Cl Cotransporter Phosphorylation during Chronic Angiotensin II Hypertension

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Background: Potassium (K) rich diets have the beneficial effect of lowering blood pressure, but molecular mechanisms are not clear. We aimed to investigate whether K could exert a beneficial effect in experimental Angiotensin II (AngII) dependent hypertension. Two findings stimulated the study: 1) During Ang II hypertension, DCT Na-Cl cotransporter phosphorylation (NCCp) and NCC mediated Na transport increase 2-4 fold; 2) a single K rich meal provokes a 50% decrease in NCCp, which drives Na reabsorption downstream to CCD ENaC which powers K secretion. Motivated by these findings, we aimed to test the hypothesis that the elevation in NCCp observed during AngII dependent hypertension would be reduced by a K rich meal.

Methods: Three groups (n=5-6) of male Sprague Dawley rats were compared: 1) Control rats fed 0%K meal over 3 hr, 2) AngII infused rats (400 ng AngII/kg/min x 14 d) fed 0%K meal, and 3) AngII infused rats fed a 2%K meal. Renal Na transporter abundance and -p were assayed by immunoblot in cortical homogenates.

Results: The following changes in abundance were measured in AngII infused versus controls after 0%K meal: NCC (2.2 fold), NCCpS71 (3.4), NCCpT53 (3.4), NCCpS89 (5.2), SPAK (2.5), SPAKpS373 (2.3), and cleaved ENaC-gamma (3.3). The 3 hr 2%K meal did not change abundance or –p of these in the AngII infused rats. Further, AngII infused rats exhibited kaliuresis and K depletion compared to controls: overnight urine K (UKV,mM) was 4.3 ± 0.1 in AngII infused versus 2.3 ± 0.3 mM in controls, plasma [K, mM] was 3.3 ± 0.1 in AngII infused versus 3.9 ± 0.1 in controls. After a K rich meal, plasma [K] climbed to 4.6 ± 0.1 mM in AngII infused versus to 5.5 mM in controls. Additionally, UKV was not elevated in the AngII infused fed a 2%K meal suggesting K conservation status.

Conclusions: The results indicate that a K rich meal does not reduce NCCp in AngII infused rats, perhaps due to the accompanying K deficiency. That is, AngII infused rats use the K meal to replenish K stores rather than excrete it. The rise in NCCp during AngII hypertension may be, at least in part, a compensatory response to conserve K.

Funding: NIDDK Support, Private Foundation Support

FR-PO014

The Effect of WNK4 on the NaCl Cotransporter Is Modulated by Intracellular Chloride

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Background: The phenotype of the Familial Hyperkalemic Hypertension (FHHt) is mostly a consequence of increased activity of the renal Na^+:Cl^- cotransporter (NCC), due to altered regulation by WNK1 or WNK4. However, the effect exerted by WNK4 on NCC has been a matter of debate since inhibition and activation by this kinase have been reported in both, *in vitro* and *in vivo* systems. It was recently shown that WNK1 is a chloride-sensitive kinase, activated by a low Cl^- concentration (Piala et al. Science Sig, 2014). We thus hypothesized that contradictory results in WNK4 activity could be due to modulation of WNK4 activity by intracellular chloride concentration $[Cl^-]_i$.

Methods: We assessed the functional expression of NCC using *Xenopus* oocytes by measuring the thiazide-sensitive $^{22}Na^+$ uptake and the N-terminal phosphorylation by Western blot, two days after microinjection with NCC cRNA alone or together with mouse or human wild type or mutant WNK4 or SLC26A9 transporter cRNA. Site directed mutagenesis was used in human WNK4 for L322F, L324F, and/or D321A substitutions. For uptake assay or protein extraction oocytes were exposed to control conditions or to low chloride hypotonic stress (hypotonic medium with no chloride) that is known to reduce the $[Cl^-]_i$.

Results: In control conditions NCC activity was either decreased or unaffected by WNK4. The reduction of $[Cl^-]_i$ either by low chloride hypotonic stress or co-injection of oocytes with the SLC26A9 cotransporter, turned WNK4 into an NCC activator, increasing NCC-dependent Na^+ transport in a kinase-dependent manner by 3 fold. L322F substitution in WNK4 (homologous to that forming a chloride-binding pocket in WNK1), converted WNK4 into a constitutively NCC activating kinase because reduction of $[Cl^-]_i$. was not longer required for WNK4 to increase the activity (3 fold) and phosphorylaiton of NCC.

Conclusions: Our study shows that WNK4 inhibitory and activating effect on NCC coexist and are modulated by intracellular chloride concentration.

Funding: Government Support - Non-U.S.

FR-PO015

Choride-Sensing by WNK Kinases Mediates Effects of Dietary Potassium on Systemic Ion Balance

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Background: Low dietary K^+ stimulates the sodium chloride cotransporter (NCC). We investigated how plasma K^+ signals NCC.

Methods: Mouse diets: Normal salt (0.49% NaCl), high salt (HS, 6% NaCl), normal K^+ (NK, 1%), and low K^+ (LK, 0%) Drug treatments: Amiloride (50 mg/L) Cells: HEK 293 cells transfected with indicated constructs. Intracellular Cl measurements: MQAE dye.

Results: HS/LK diet increased NCC and pNCC compared with HS/NK. Plasma aldosterone remained unchanged, while plasma angiotensin II decreased. Effects on

NCC were preserved in type I angiotensin II receptor null mice. These data suggest the changes in NCC were not mediated by the RAAS. To determine if plasma K^+ signals NCC independent of diet, we treated animals with amiloride (Amil). Amil caused hyperkalemia and volume contraction and decreased NCC and pNCC, suggesting K^+ was determining NCC status. LK diet, which normalized plasma K^+, prevented effects of Amil on NCC. In cells, low K^+ medium increased pNCC and pSPAK/pOSR1, signaling via WNK kinases, as shown through siRNA studies. Treatment with Ba^{2+}, which depolarizes cells, reduced pNCC. Transfection with EAST syndrome mutant KCNJ10 channels, depolarized cells compared with wild (WT) type KCNJ10; mutant KCNJ10 also reduced pNCC, compared with WT. Low K^+ conditions decreased $[Cl^-]_i$, and the KCNJ10 mutants increased it. To test if decreased $[Cl^-]_i$was required for low K^+ to increase pNCC, we showed that effects of low K^+ on pNCC were inhibited by culture in high Cl^- medium or treatment with DIDS. As chloride exit from DCT cells is mediated partly by CLCNKB, which is mutated in type III Bartter syndrome, we compared effects of mutant and WT CLCNKB channels. Mutant channels decreased effects of low K^+ on NCC compared with WT channels. Disrupting the Cl^--binding motif of WNK1 (which mediates its inhibition by Cl^-(Sci Sig 2014)) also prevented effects of low K^+ on pNCC.

Conclusions: Results suggest plasma K^+ stimulates NCC directly, independent of signaling hormones; this effect likely requires WNK-SPAK/OSR1. Cl^- sensing by the DCT may mediate the effects of dietary K^+ on systemic ion balance.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO016

The European Eel NCCb Gene Encodes a Non-Thiazide Sensitive NaCl Cotransporter Eduardo R. Argaiz,[1] Erika Moreno,[1] Christopher Cutler,[2] Norma Hilda Vázquez,[1] Maria Chavez-Canales,[1] Gerardo Gamba.[1] *[1]Molecular Physiology Unit, INCMNSZ-IIB-UNAM, Mexico City; [2]Georgia Southern Univ, Statesboro, GA.*

Background: Thiazides are among the most frequently prescribed drugs in the world for their antihypertensive effect through inhibition of the renal Na^+:Cl^- cotransporter, NCC, which in mammals is encode by one gene (SLC12A3). In contrast, two NCC genes are expressed in the European eel (Anguila anguila): NCCa and NCCb. Flounder and mammalian NCC exhibit different affinity for thiazides and we have previously reported a single amino acid residue (C575 in rat NCC) responsible for this difference (Castañeda-Bueno et al AJP Renal 2011), but nothing is known regarding residues conferring specificity for thiazide diuretics in NCC.

Methods: To study the functional properties of NCCb, a full length cDNA encoding NCCb from eel intestine was constructed by RT-PCR, inserted into pgh19 vector and fully sequenced. Functional expression was assessed by $^{22}Na^+$ uptake using *Xenopus laevis* oocytes microinjected with NCCb cRNA. Rat and flounder NCC cRNA were used as controls.

Results: NCCb encodes a 1043 amino acid residues exhibting a 55, 55, and 43 degree of identity with flounder, rat and human NCC, respectively. It is expressed along the eel gastrointestinal tract and absent in the kidney. NCCb cRNA injection into oocytes induced the appearance of a Na^+ uptake pathway that is Cl^- dependent, K^+ independent, but is not sensitive to a variety of thiazide type diuretics (up to 1 mM concentration), to furosemide, DIDS or DIOA. A small 30% inhbition was observed with acetazolamide (100 mM). No $^{86}Rb^+$ uptake was induced by NCCb. The Cl^- transport kinetics revealed a Km for Cl^- about 13 mM (previously observed for flounder NCC was 15 mM and rat NCC 2 mM). Interestingly, in contrast to flounder and mammalian NCC, eel NCCb is not activated by intracellular chloride depletion or co-injection with WNK informs.

Conclusions: NCCb encodes for an electroneutral Na^+:Cl^- cotransporter that is not sensitive to thiazide-type diuretics, with different regulatory properties when compared with mammalian NCC. Structure function studies of NCCb could lead to define the amino acid residues responsible for thiazide specificity in the NaCl cotransporter.

Funding: Government Support - Non-U.S.

FR-PO017

Dissecting WNK Complex Formation and Signaling with the CRISPR/Cas9 System Ankita Roy,[1] Bridget F. Donnelly,[1] Joshua H. Goodman,[1] Arohan R. Subramanya.[1,2,3] *[1]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA; [2]Cell Biology, School of Medicine, Univ of Pittsburgh, Pittsburgh, PA; [3]VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: The cation chloride cotransporter NCC mediates salt reabsorption in the distal convoluted tubule, where it is activated by the kinases SPAK and OSR1. With-No-Lysine (WNK) kinases are the only known upstream SPAK/OSR1 activators, although the contributions of individual WNKs to downstream kinase activation are unclear. WNKs can associate with each other, although the composition of native WNK complexes in cells remains unknown.

Methods: To clarify the role of the individual WNKs in activating downstream targets, we are employing RNA-guided CRISPR/Cas9 nucleases to delete WNK kinases in mammalian cells. The WNK KO cell lines were used for biochemical assessment of protein abundance, Co-IP interaction, and downstream kinase activity toward SPAK/OSR1 and NCC.

Results: We present our experience generating a genetically and biochemically validated WNK1 KO cell line with this method. The knockout cells were generated by using a single guide RNA targeting exon 1 of the WNK1 gene, which generated indels that completely abrogated WNK1 protein expression. WNK1 KO cells exhibited a 43% reduction in endogenous WNK4 protein, indicating that WNK1 is required for WNK4 stability. Consistent with its critical role in SPAK/OSR1 activation, WNK1 KO cells exhibited a 90% reduction in SPAK/OSR1 regulatory domain phosphorylation. Surprisingly, the reduced SPAK/OSR1 activity did not downregulate NCC abundance and phosphorylation, which were increased by 12.5% in WNK1 KO cells. This unexpected increase in NCC activation correlated with enhanced WNK3 interaction.

Conclusions: In summary, we successfully used the CRISPR-Cas9 system to create a WNK1 knockout cell line that will be useful for studying cation chloride cotransporter regulation. Our data are consistent with a model in which the WNKs form interdependent complexes and can compensate for each other. They also support the stimulatory effect of WNK3 on NCC, and suggest that NCC phosphorylation at its activation sites may be subject to regulators other than SPAK and OSR1.

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FR-PO018

14-3-3 Gamma Modulates NCC through a Nedd4-2-Dependent Mechanism Xiuyan Feng,[1,3] Dingying Gu,[4] Courtney Marie Caroti,[1,3] Abdel A. Alli,[2] Robert S. Hoover,[1,3] Hui Cai.[1,2,3] *[1]Renal Div, Emory Univ School of Medicine; [2]Dept of Physiology, Emory Univ School of Medicine; [3]Renal Section, Atlanta VAMC; [4]Renal Div, Second Affiliated Hospital, Wenzhou Medical Univ; [5]Dept of Anesthesiology, Vanderbilt Univ Medical Center.*

Background: To investigate whether 14-3-3 gamma modulates the sodium chloride cotransporter (NCC) through a Nedd4-2-dependent mechanism.

Methods: Cell culture, transfection, RT-PCR, western blot analysis, immunostaining and immunoprecipitation were used in this study.

Results: We transfected HEK 293 cells with GFP-NCC and a series of doses of 14-3-3 gamma. Western blot analysis showed that over-expression of 14-3-3 gamma significantly inhibited NCC total protein expression in a dose-dependent manner (1 ± 0.032, 0.757 ± 0.085, 0.288 ± 0.080, $p<0.01$) whereas it did not change the NCC mRNA level. Surface biotinylation results showed that 14-3-3 gamma decreased the NCC surface expression in a dose-dependent manner. We also found that 14-3-3 gamma binds to NCC by immunoprecipitation. In addition, immunostaining and confocal microscope showed that both 14-3-3 gamma and NCC co-localized in the distal convoluted tubule (DCT). We further found that 14-3-3 gamma increased ubiquitinated-NCC in a dose-dependent manner. Furthermore, when we knocked down the 14-3-3 gamma expression by 60% using siRNA technique in mDCT cells, we found that total endogenous NCC expression increased by 2.02 times while the levels of both total-Nedd4-2 and phospho-Nedd4-2 (S448) decreased compared to the control group.

Conclusions: These results suggest that 14-3-3 gamma modulates NCC through a Nedd4-2-dependent mechanism.

Funding: Veterans Affairs Support

FR-PO019

Aberrant Activation of WNK-SPAK/OSR1 Signaling in Drosophila Pacemaker Neurons Disrupts Circadian Rhythm Aylin R. Rodan, Jeffrey N. Schellinger. *UT Southwestern, Dallas, TX.*

Background: Circadian fluctuations in intracellular Cl^- in mammalian pacemaker neurons have been proposed to play a role in circadian rhythmicity, and could be controlled by the relative amount of Cl^- ingress and egress by the sodium-potassium-2-chloride cotransporter (NKCC) and potassium-chloride (KCC) cotransporters, respectively. Here, we tested roles for fly NKCC and KCC, as well as the chloride-sensitive WNK-SPAK/OSR1 kinase cascade, in *Drosophila* pacemaker neurons.

Methods: Circadian rhythm period was determined by measuring 24-hour locomotor activity of male flies. Pacemaker cell expression and knockdown of genes of interest was performed using the GAL4-UAS system.

Results: Flies carrying a homozygous null mutation in the NKCC Ncc69 (*Ncc69^{r2}*) have increased period length (25.2±0.3 versus 24.0±0.05 hours in controls, $p<0.0001$). Expression of wild-type Ncc69 in the pacemaker neurons rescued the long-period phenotype (23.8±0.08 hours). Pacemaker neuron number and morphology were normal in the *Ncc69^{r2}* mutants. The long period of *Ncc69^{r2}* mutants was suppressed by knockdown of fly *kcc* in the pacemaker neurons (23.9±0.3 versus 26.4±0.2 hours in *Ncc69^{r2}*, $p<0.0001$), suggesting that the *Ncc69^{r2}* long-period phenotype may be due to low intracellular Cl^-. Since Cl^- inhibits WNK autophosphorylation and activation, we tested whether the long-period phenotype is due to aberrant activation of WNK-SPAK/OSR1 signaling. *wnk* knockdown in the pacemaker neurons suppressed the long-period phenotype of *Ncc69^{r2}* (24.7±0.3 versus 26.7±0.1 hours in *Ncc69^{r2}*, $p<0.0001$). Similarly, knockdown of *fray* (the fly SPAK/OSR1 homolog) also suppressed the *Ncc69^{r2}* long period (24.3±0.1 versus 26.3±0.1 hours in *Ncc69^{r2}*, $p<0.0001$). Conversely, expression of constitutively active Fray^{T206E} in the pacemaker neurons resulted in a long period (25.6±0.3 versus 24.3±0.09 hours in controls, $p<0.0001$) that was dependent on the kinase activity of Fray. This phenotype was suppressed by the *Ncc69^{r2}* mutation (23.8±0.1 hours), suggesting a feedback loop.

Conclusions: Coordinated activity of NKCC and KCC are required to maintain appropriate WNK-SPAK/OSR1 signaling in the regulation of circadian rhythm by the pacemaker neurons, suggesting precise regulation of intracellular Cl^-.

Funding: NIDDK Support

FR-PO020

Inwardly-Rectifying Potassium Channels in Drosophila Renal Tubule Function Aylin R. Rodan, Yipin Wu, Michel G. Baum, Chou-Long Huang. *UT Southwestern, Dallas, TX.*

Background: Flies eat a K^+-rich diet and secrete a KCl-rich fluid from the main segment of the renal tubule. We have previously shown that ~1/3 of transepithelial K^+ flux through the cation-conducting principal cell is via the basolateral NKCC, Ncc69. Ncc69 function is dependent on the Na/K-ATPase, but is not required for the kaliuretic effect of cAMP. Here, we examined the role of inwardly-rectifying K^+ (Kir) channels.

Methods: Transepithelial potential difference (TE_{PD}) was measured in isolated perfused tubules, and transepithelial K^+ flux was measured in isolated tubules using the Ramsay assay. Principal cell knockdown of the three fly Kir channels, Ir, Irk2 and Irk3, was performed using the GAL4-UAS system. Knockdown efficiency was determined by qRT-PCR.

Results: 2 mM peritubular barium, a Kir inhibitor, reversibly decreased TE_{PD} from 30.5±5 to 9.5±2 mV ($p<0.05$), returning to 30.5±4 mV after washout. K^+ flux was also decreased in 1 mM barium-treated tubules, but unchanged with vehicle treatment (Table). Knocking down *Irk3*, alone or in combination with *Ir* or *Irk2*, did not alter K^+ flux, nor did knocking down *Ir* or *Irk2* individually, despite similar efficiencies of transcript knockdown. Double knockdown of *Ir* and *Irk2* significantly decreased K^+ flux, without a further decrease in *Ir,Irk2,Irk3* triple knockdown tubules (Table). K^+ flux was increased by 1 mM cAMP and decreased by 100 mM ouabain in both control and *Ir,Irk2* double knockdown tubules (Table).

Genotype/condition	K^+ flux pmol/min/tubule	p value
Control, pretreatment	48±10	NS
Control, vehicle	56±19	
Control, pretreatment	43±9	<0.0001
Control, barium	9±7	
Control	109±10	<0.01
Ir,Irk2 knockdown (KD)	60±12	
Ir,Irk2,Irk3 KD	77±12	NS (compared to *Ir,Irk2)*
Control, vehicle	56±8	<0.01
Control, ouabain	25±6	
Ir,Irk2 KD, vehicle	43±9	<0.05
Ir,Irk2 KD, ouabain	15±3	
Control, vehicle	35±6	<0.05
Control, cAMP	64±10	
Ir,Irk2 KD, vehicle	11±3	<0.05
Ir,Irk2 KD, cAMP	37±9	

Conclusions: The principal cell Kir channels Ir and Irk2, acting basolaterally, have redundant roles in fly renal tubule transepithelial K^+ transport, act independently of the Na/K-ATPase, and are not required for tubule kaliuretic response to cAMP.

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FR-PO021

Collecting Duct (CD) Prorenin Receptor (PRR) Contributes to Renal Sodium Reabsorption *via* Regulation of ENaC Expression Renfang Song, Graeme James Preston, Ihor V. Yosypiv. *Pediatrics, Tulane Univ, New Orleans, LA.*

Background: We demonstrated that targeted inactivation of the *PRR* in the ureteric bud (UB) epithelia in mice using *Hoxb7*^{Cre} (*PRR*$^{UB-/-}$) is essential for the concentration of the urine (PloS One, 2013). Here, we hypothesized that CD PRRcontributes to renal sodium reabsorption *via* regulation of epithelial sodium channel (ENaC) expression.

Methods: *PRR*$^{UB-/-}$ and control mice (n=4 mice per genotype) were housed in metabolic cages, fed a standard chow and allowed free access to water. Plasma and urine creatinine, sodium (Na) and potassium (K) was measured on P30 by HPLC and flame photometry, respectively. ENaC a subunit mRNA and protein levels in the cortex and/or medulla were determined by qRT-PCR, Western blotting and immunohistochemistry (StressMarq). The intensity of aENaC immunostaining was quantitated by Slidebook 4.1 software.

Results: Although plasma Na levels did not differ (132±0.9 versus 135±2.1, p=0.12), urinary Na excretion was reduced in mutant compared with control mice (mM/L: 47±4 versus 82±3, $p<0.01$; mM/24h: 143±4 versus 199±13, $p<0.01$). qRT-PCR and Western blotting demonstrated that aENaC mRNA ($p<0.05$) and protein ($p<0.05$) levels were increased in the cortex of mutants compared with controls.aENaC immunostaining was increased in the CD of mutant compared with control kidneys (1.99±0.13 versus 1.41±0.04 pixels, $p<0.01$). Plasma K levels did not differ (9.0±0.17 versus 8.9±0.18, p=0.56). Urinary K excretion was reduced in mutant compared with control mice (mM/L: 89±5 versus 165±14, $p<0.01$; mM/24h: 279±9 versus 412±37, $p<0.01$), suggesting that reduced urinary Na excretion in *PRR*$^{UB-/-}$ mice is not mediated by aldosterone.

Conclusions: We conclude that CD PRR regulates renal ENaC expression and contributes to sodium reabsorption in the CD.

FR-PO022

AMP-Activated Protein Kinase Regulates the Epithelial Na^+ Channel and Nedd4-2 Phosphorylation in Kidney Collecting Duct Cells Suet-Wan Choy,[1] Hui Li,[1] Edwin Kamau,[1] Dietbert Neumann,[2] Kenneth R. Hallows.[1] *[1]Renal-Electrolyte Div, Univ of Pittsburgh, Pittsburgh, PA; [2]Molecular Genetics, Maastricht Univ, Maastricht, Netherlands.*

Background: Kidney collecting duct epithelial Na^+ channels (ENaC) play a key role in total body volume and blood pressure regulation. The metabolic sensor AMP-activated protein kinase (AMPK) inhibits ENaC by enhancing ENaC binding to the ubiquitin ligase Nedd4-2 through unclear mechanisms. We hypothesized that AMPK phosphorylation of Nedd4-2 is required for the regulation of ENaC by AMPK and is a key determinant of Nedd4-2 protein stability in cells and *in vivo*.

Methods: We used mass spectrometry to define AMPK phosphorylation sites in Nedd4-2 *in vitro*. We used mouse polarized kidney cortical collecting duct ($mpkCCD_{c14}$) cells for Western blot analysis and short-circuit current (I_{sc}) measurements of ENaC following AMPK activity modulation. We monitored Nedd4-2 phosphorylation at the AMPK target site using a phospho-specific (pS328) antibody and examined the role of Nedd4-2 phosphorylation by AMPK regulation of ENaC and Nedd4-2 stability.

Results: Mass spectrometry studies revealed that AMPK phosphorylates Nedd4-2 *in vitro* at S444 (Xenopus numbering, S328 in mouse). AMPK activation decreased stability of the b and cleaved g subunits of ENaC in cycloheximide chase assays performed in $mpkCCD_{c14}$ cells. Preliminary studies suggest that acute AMPK activation increases pS328 in $mpkCCD_{c14}$ cells as detected by an antibody directed against pS328. However, inducible shRNA-mediated AMPK-a1 knockdown in $mpkCCD_{c14}$ cells did not inhibit phosphorylation at S328. We propose that the lack of inhibition by AMPK knockdown may be due to a chronic compensatory increase in phosphorylation at that site by other participating kinases.

Conclusions: AMPK phosphorylates Nedd4-2 at S444, which enhances Nedd4-2 protein stability. AMPK also induces cellular ENaC turnover/degradation. Additional studies in stably transfected $mpkCCD_{c14}$ cells that overexpress wild-type or mutant Nedd4-2 will provide insight into the effect of these mutations on ENaC stability. Further studies to assess the physiological relevance of AMPK phosphorylation of Nedd4-2 *in vivo* are also underway.

Funding: NIDDK Support, Pharmaceutical Company Support - Roche

FR-PO023

NBCe2 Mediates Thiazide-Sensitive Sodium Reabsorption in the Distal Nephron Donghai Wen,[1] Ryan J. Cornelius,[1] Thomas Boettger,[2] Steven C. Sansom.[1] *[1]Cellular and Integrative Physiology, Univ of Nebraska Medical Center, Omaha, NE; [2]Cardiac Development and Remodeling, Max Planck Inst for Heart and Lung Research, Bad Nauheim, Germany.*

Background: A previous study reported that the Na^+-dependent Cl^-/HCO_3^- exchanger NDCBE mediates thiazide-sensitive Na^+ reabsorption in the cortical collecting duct (CCD). The electrogenic Na^+- HCO_3^--co-transporter (NBCe2) is also located in the apical membrane of principle cells in the CNT and collecting duct, where it functions to reabsorb Na^+ and HCO_3^- under acidic conditions. In the present study, we determined whether NBCe2 also mediates thiazide-sensitive Na^+ reabsorption in the distal nephron.

Methods: Wild type (WT) and NBCe2 knockout (KO) mice were fed a Na^+ deficient diet plus acid water for 7-10 days. Hydrochlorothiazide (HCTZ), amiloride (Amil), inhibitor of the epithelial Na^+ channel (ENaC), or polyethylene glycol (Veh) was administered intraperitoneally and the mice placed in metabolic cages for 12 hours. Urine was collected and the rates of Na^+ and K^+ excretion (UNaV; mmol/day and UKV; mmol/day, respectively) were determined.

Results: In Veh, UNaV was not significantly different in KO, compared with WT (WT: 3.9 ± 0.5, KO: 9.8 ± 3.0), while UKV was much higher in KO (WT: 91.8 ± 19.4; KO: 185.3 ± 27.9). With HCTZ treatment, both UNaV (WT: 106.1±8.4; KO: 38.6 ± 7.) and UKV (WT: 439.9 ± 24.0; KO: 156.4 ± 27.1) were much higher in WT, compared with KO. However, with Amil treatment, UNaV was higher in KO, compared with WT (WT: 99.4 ± 13.9; KO: 206.6 ± 31.4), while there was no difference in UKV (WT: 27.4 ± 6.2; KO: 27.1 ± 12.7). The HCTZ-sensitive urinary Na^+ reabsorption (WT: 102.1 ± 8.4; KO: 28.9 ± 7.9) and K^+ excretion (WT: 318.7 ± 30.9; KO: -29 ± 38.9) were significantly higher in WT compared to KO, while the Amil-sensitive urinary Na^+ reabsorption (WT: 114.6 ± 21.9; KO: 196.8 ± 37.1) and K^+ excretion (WT: 64.3 ± 20.4; KO: 154.3 ± 15.5) were higher in the KO.

Conclusions: Our results reveal that NBCe2 mediates thiazide-sensitive Na^+ reabsorption in the distal nephron, and deficiency of NBCe2 causes up-regulation of ENaC-mediated Na^+ reabsorption.

Funding: NIDDK Support

FR-PO024

Epigenetic Control of Epithelial Sodium Channel a-Subunit (a-ENaC) Transcription by Lysine-Specific Demethylase 1 (LSD1) Zhiyuan Yu, Qun Kong, Bruce C. Kone. *Internal Medicine, The Univ of Texas Medical School at Houston, Houston, TX.*

Background: ENaC in the distal nephron constitutes the rate-limiting step for renal sodium reabsorption. aENaC transcription in collecting duct is under complex genetic and epigenetic control in part coordinated by DNA-binding protein Af9, which binds to the aENaC promoter. LSD1 is a histone H3K4 and H3K9 demethylase that also interacts with histone deacetylases (HDAC) and corepressor of repressor element 1 silencing transcription (CoREST). We explored the role of LSD1-mediated histone demethylation and HDAC interaction in regulating aENaC transcription in mIMCD3 cells.

Methods: Transient overexpression and siRNA knockdown were used to test the effects of LSD1 and HDAC2 on aENaC mRNA and the activity of an aENaC promoter-luciferase construct stably expressed in mIMCD3 cells. ChIP and re-Chip assays were used to determine LSD1 occupancy and histone methylation states along the aENaC promoter. Co-immunoprecipitation (co-IP) assays were used to assay interaction of LSD1 with HDAC1, HDAC2, and CoREST.

Results: LSD1 overexpression resulted in 30% higher aENaC promoter activities versus control. Conversely, LSD1 depletion lowered aENaC mRNA levels and promoter activity by 30%. Mutation of the Af9 site in the aENaC promoter abrogated the LSD1 effect, but co-IP assays failed to detect LSD1-Af9 interactions. ChIP/qPCR assays revealed that LSD1 is basally enriched at multiple subregions of the aENaC promoter and mediates H3K4me2 and H3K9me2 demethylation. Co-IP and ChIP/re-ChIP assays showed that LSD1 complexes with HDAC2 and CoREST in mIMCD3 cells and the three proteins co-occupy the aENaC promoter. Like LSD1, overexpression of HDAC2 increased aENaC promoter activity. Combined overexpression of LSD1 and HDAC2 synergistically enhanced aENaC promoter activity.

Conclusions: LSD1, through H3K4me2 and H3K9me2 demethylation and functional interactions with Af9 and HDAC2, serves as an epigenetic activator of aENaC transcription in mIMCD3 cells. Our data also provide the first example of dual erasure of these histone marks associated with a single gene. The balance of histone lysine methylation and demethylation at the aENaC promoter governs aENaC transcription.

Funding: NIDDK Support

FR-PO025

Distinct Subunit Requirements for N-Glycans During Epithelial Na^+ Channel Processing and Expression Ossama B. Kashlan,[1] Carol L. Kinlough,[1] Mike M. Myerburg,[1] Brandon M. Blobner,[1] Teresa Buck,[2] Paul A. Poland,[1] Jeffrey L. Brodsky,[2] Rebecca P. Hughey,[1] Thomas R. Kleyman.[1] *[1]Medicine, Univ of Pittsburgh, Pittsburgh, PA; [2]Biological Sciences, Univ of Pittsburgh, Pittsburgh, PA.*

Background: The three epithelial Na^+ channel (ENaC) subunits undergo N-linked glycosylation in the ER where they assemble into a trimeric abg complex. There are six, thirteen and five consensus sites (Asn-x-Ser/Thr) for N-glycosylation within the extracellular domains of the mouse a, b and g subunits, respectively. Previous studies suggest that most of these sites are used. N-glycans on newly synthesized proteins facilitate their interaction with ER folding chaperones, and are required for recognition by quality control mannosidase-like proteins. Most N-glycans on folding-competent glycoproteins that transit to the Golgi complex are remodeled to a complex type. These complex N-glycans on mature proteins have varied functions including (i) stabilization of the folded state, (ii) protection of the protein core from protease digestion, and (iii) interaction with other proteins, such as galectins.

Methods: Using site-directed mutagenesis, we removed all of the consensus N-glycosylation sites from each ENaC subunit. We then biochemically and functionally characterized channels where one or more subunits lacked N-glycosylation sites.

Results: Although prior work indicated that a subunit N-glycans are dispensable for expression of functional channels, we found that a lack of N-glycans on the b or g subunit dramatically reduced channel activity, surface and whole cell ENaC protein expression, as well as a and g subunit proteolytic processing. Furthermore, the inhibitory response to external Na^+ was largely lost, suggesting a defect in channel folding. In addition, we observed that the b subunit co-precipitated with GST-tagged galectins-1, -4, -8 and -9, suggesting that ENaC surface expression may be modulated by crosslinking of the b subunit with galectins.

Conclusions: In summary, our results suggest that N-glycans on both the b and g subunits are required for proper processing and expression of ENaCs.

Funding: NIDDK Support

FR-PO026

The Role of Renal Collecting Duct Cathepsin D Overexpression in Regulating ENaC Activity Franziska Theilig, Alina Khramova, Suresh K. Ramakrishnan. *Medicine, Anatomy, Fribourg, Switzerland.*

Background: The epithelial sodium channel (ENaC) plays an important role in the maintenance of the sodium balance, extracellular volume and blood pressure. Proteolytic cleavage of ENaC is one of the key regulatory steps in the channel activation process. It has been reported that proteolytic cleavage of ENaC was executed by endogenous and /or exogenous serine proteases acting directly or in an indirect fashion. In many disease states, such as nephrotic syndrome, a dysregulation of the ENaC was identified.

Results: Performing microarray studies, we have detected significantly increased Cathepsin D expression levels in rats with puromycin aminonucleoside (PAN) induced nephrotic syndrome (NS) and with glomerulonephritis (GN) compared to controls (CatD mRNA (control=61,4; PAN=230,7; GN=122,7). Western blot analysis of renal membrane preparations and urine showed a 3 to 10-fold increase of CatD expression level and excretion, respectively, in rats with PAN-induced NS and GN compared to control. Next, to elucidate the effects of CatD overexpression an inducible CatD overexpressing collecting duct cell line was produced. In this cell line analysis of ENaC subunits demonstrated the occurrence of an increased cleavage of the aENaC, whereas b and gENaC remained unaffected. In agreement, epithelial voltage measurements showed a significantly higher conductance in CatD overexpressing clones (90+/-5 $\mu A/cm^2$ versus 11+/- 3 $\mu A/cm^2$) compared to normal $mpkCCD_{cl14}$ cells.

Conclusions: In conclusion, CatD plays a putative role in maintaining sodium balance by cleaving the a-subunit of ENaC and thereby proteolytically activating the channel.

Funding: Government Support - Non-U.S.

FR-PO027

Epithelial Sodium Channel Activity in the Distal Nephron Is Modified by the Aldosterone Induced Protein, Ankyrin G Christine Anne Klemens, Michael Butterworth. *Cell Biology, Univ of Pittburgh, Pittsburgh, PA.*

Background: The heterotrimeric epithelial sodium channel (ENaC) is the limiting entry step for sodium (Na^+) reabsorption in the aldosterone sensitive distal kidney nephron. Aldosterone instigates a number of changes to augment Na^+ reabsorption, including increasing ENaC subunit translation and modifying key regulators that alter ENaC function and/or membrane density. We identified ankyrin G (AnkG), as a novel aldosterone induced protein, capable of regulating ENaC activity. AnkG is a scaffold protein that traffics transmembrane proteins to the plasma membrane and links them to the cytoskeleton. The bENaC subunit contains a putative AnkG binding motif 6 amino acids downstream to the Nedd4-2 PY motif. We propose that AnkG regulates ENaC mediated Na^+ transport by modulating the Nedd4-2--ENaC interaction, to stabilize ENaC at the apical membrane and increase residency time.

Methods: ENaC activity was determined by short-circuit current measurements in Ussing chambers in either a mouse kidney collecting duct epithelial cell line (mCCD-cl1) or Fisher rat thyroid (FRT) cells.

Results: AnkG co-immunoprecipitates with bENaC. Deletion of the purported AnkG binding motif reduced ENaC current by 25%±4% compared to WT in a heterologous expression system. AnkG over expression in mCCD-cl1 cells increased ENaC current by 67%± 11% (n=12), whereas knockdown reduced ENaC activity by 69%±2% (n=15). Membrane permeabilization experiments confirmed the reduction in Na^+ transport was due to reduced ENaC function, and not Na^+/K^+ATPase activity.

Conclusions: These findings confirm AnkG as an aldosterone induced protein and novel regulator of ENaC that therefore alters Na^+ reabsorption in the distal nephron. Future studies will investigate the interplay between AnkG and Nedd4-2 in regulating ENaC surface expression.

Funding: Private Foundation Support

FR-PO028

A Common Golgi Export Signal Patch in Basolateral Kir Channels of the Distal Nephron Xiangming Li, Paul A. Welling. *Physiology, Univ of Maryland SOM, Baltimore, MD.*

Background: Polarized distribution of inward rectifying K^+ (Kir) channels the distal nephron is essential for K^+ excretion. Here we explore a new polarized trafficking mechanism, focusing on a Golgi trafficking step, which couples Apical/Basolateral membrane (BLM) cargo selection and clathrin-coated vesicle (CCV) formation to Golgi export. Our previous work on the prototypical Kir channel, Kir2.1, identified a novel signal patch embedded within its tertiary structure that forms a recognition site for interaction with the AP1A adaptor, marking channels for inclusion into CCVs at the trans-Golgi network (*Ma et al Cell, 2011*). Here we test if the Golgi export patch is conserved in the basolateral Kir channels for polarized trafficking to the BLM.

Methods: Common residues in the Golgi export patch of Kir2.1 were identified by sequence analysis and atomic resolution modeling. A common "SY" motif at the focal point of the putative signal was found. External HA-tagged Kir channels bearing deletions on "SY" motif (DSY) were compared to WT channels.Channel cell surface expression was quantified by surface antibody binding and analytical luminometry. Intracellular localization was evaluated by fluorescence microscopy.

Results: Kir4.1, mutations in which underlie EAST/SeSAME syndrome, Kir 5.1, and Kir2.3 contribute to the basolateral K^+ conductance in the distal nephron. Kir4.1 forms heteromers with Kir5.1; oligomerization of the subunits is thought to "mask" an ER retention signal in Kir5.1. We found Kir4.1, Kir2.3 and Kir4.1/Kir5.1 channels bearing DSY mutations fail to traffic to the cell surface, and accumulate in the Golgi, consistent with disruption of a common Golgi export signal. Coexpressing WT Kirs with the DSY mutants, does not rescue the surface expression of Kir4.1DSY or Kir5.1DSY, and these heteromultimeric channels largely accumulate in the Golgi, further supporting a dominant role of "SY" motif in Golgi export. WT Kir4.1/Kir5.1DSY channels, accumulate in the ER as well as the Golgi, suggesting the ER retention signal can be unmasked when channels are unable to exit the Golgi.

Conclusions: Basolateral Kir channels in the distal nephron rely on a common Golgi export signal patch to gain access to the cell surface.

FR-PO029

Are Potassium Homeostasis and Adaptation Similar in Male and Females Rats? Luciana C. Veiras, An Tran, Julie Jiyang Han, Donna Lee, Alicia A. McDonough. *Cell and Neurobiology, Keck School of Medicine of USC, Los Angeles, CA.*

Background: Plasma potassium (K) must be maintained within a narrow range because it is a key determinant of membrane potential. Dietary potassium (K) intake reduces phosphorylation (-P) and abundance of renal Na-Cl cotransporter (NCC) in male rodents. This response redistributes sodium (Na) downstream for reabsorption by epithelial sodium channels (ENaC) which powers K secretion via K channels. Previous studies have not addressed whether there are sex differences in K homeostasis. This study aimed to compare both K homeostasis and adaptation in female versus male Sprague Dawley rats.

Methods: Sprague Dawley rats (n=6/group) were studied in metabolic cages and fed defined gelled diets. Electrolytes were determined by flame photometry and renal transporter abundance by immunoblot of cortex homogenates.

Results: At baseline (after overnight fast), UV, UNaV, UKV and food intake were similar in both sexes. However, plasma Na, K and osmolality were lower in females versus males ([Na]: 115±1 versus 118±1 mM, [K]: 3.20±0.06 versus 3.90±0.06 mM, Osm: 296±3 versus 306±2 mOsm; $p< 0.05$; n=6 for all). The lower plasma K in females may be due, in part, to significantly higher abundance of renal ENaC subunits (alpha: 1.4 fold and cleaved gamma: 1.5 fold higher versus males). Additionally, in females, higher NCC (1.8 fold) and NCC-P (4.3 fold) abundance were observed versus males. Previously, we reported that in males fed a 2%K meal, plasma K increased to 5.5± mM. After females consumed a 2%K containing meal, urinary K increased 7 fold and plasma K increased from 3.20±0.06 to 3.9±0.1 mM, $p< 0.05$; n=6; in 0%K fed females K remained at 3.2±0.1. Even though plasma K remained below 4 mM in females, the 2% K meal decreased renal NCC and NCC-P to 0.8 and 0.5 fold versus the 0% K fed females (no changes in ROMK or ENaC subunits).

Conclusions: We conclude that baseline plasma K set point is significantly lower in female than male SD rats, perhaps secondary to higher ENaC activity, and that the renal responses to an acute K meal are indistinguishable in males and females. NIH DK 083785.

Funding: NIDDK Support

FR-PO030

Role of the H,K-ATPase Type 2 in the Regulation of Blood Pressure during K^+-Depletion

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Background: Alteration of blood pressure (BP) level is one of the side effects associated to K^+-depletion. However, the mechanisms linking K^+ balance to BP remain unsettled and debated. Adaptation to K^+-depletion requires ability to retain K^+. In kidney and colon, this is handled by the stimulation of H,K-ATPase type 2 (HKA2) and its absence leads to severe K^+-depletion under low-K^+ diet condition. Our aim is to investigate whether HKA2 may contribute to determination of BP level under low-K^+ diet.

Methods: Wild-type (WT) and HKA2-null mice (HKA2 KO) were fed a normal or a low-K^+ diet for up to 13 days and their blood pressure and metabolic parameters were measured daily. At day 4 of a normal or low-K^+ diet, plasma volume (Blue Evans dye), renal expression of ion transporter (PCR and WB), sensitivity to thizaide and to vasopressin were measured in both WT and HKA2 mice.

Results: Wild-type mice (WT) displayed a decrease of BP after 10 days of low-K^+ diet (from 113 to 103 mm Hg). In HKA2-null mice, the decrease of BP (109 to 100 mm Hg) occurred much earlier (day 2-3). At early stage of K^+-depletion, plasma volume remained constant in WT but decreased by 20% in HKA2-null mice, indicating that decrease of BP is linked to a the development of hypovolemia. The origin of this hypovolemic status is the transient renal "leak" of Na^+ and water observed in HKA2-null mice the first four days of the low-K^+ diet. The mechanisms of this salt and water loss could be related to a differential regulation of renal Na^+ transporter expression (mainly NCC) and a blunted response to vasopressin.

Conclusions: The presence of HKA2 is therefore required to limit the decrease of plasma K^+ value and to protect against development of hypotension.

Funding: Government Support - Non-U.S.

FR-PO031

Aldosterone Induces a BK-a/b4-Mediated Kaliuresis in Mice on a Low Na, High K Diet

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Background: BK-a/b4 channels mediate K secretion in mice on a low Na, high K, alkaline diet (LNaHK) in association with a 5-fold increase in urinary flow. The LNaHK diet yields a very high plasma [aldosterone] (P[aldo]), which increases K secretion via enhanced Na reabsorption and apical BK-a/b4 expression in the distal nephron. However, the relation between the high P[aldo] and distal flow rate is not yet understood.

Methods: To study this, we used C57BL/6 wild type mice (WT) or BK-b4 knockout mice (KO) that underwent sham or bilateral adrenalectomy (ADX). ADX mice were given a low or high dose aldo replacement by osmotic pump before being fed LNaHK.

Results: The low dose aldo (ADX-LA; 25 mg/kg/day) yielded a P[aldo] of 300-600 pg/ml and the high dose aldo (ADX-HA; 500 mg/kg/day) yielded a P[aldo] of 3,000 to 6,000 pg/ml. The K excretion rate (UKV) and transtubular K gradient (TTKG) were decreased in WT ADX-LA compared with WT sham, but were reversed to WT sham levels in WT ADX-HA. KO sham exhibited a decreased UKV and TTKG compared to WT sham; however, the UKV and TTKG of KO ADX-LA were not different from WT ADX-LA. Immunofluorescent staining showed decreased apical and total cellular BK-a expression in WT ADX-LA compared to WT sham; whereas BK-a expression of WT ADX-HA was similar to WT sham. Urinary flow was decreased in WT ADX-LA, compared to WT sham, and returned to WT sham levels in WT ADX-HA. To determine the effect of cortical distal K secretion on flow, mice were treated with amiloride to inhibit the ENaC-mediated Na reabsorption for K secretion exchange. When given amiloride (versus vehicle), WT control diet, WT LNaHK and KO LNaHK had increased isotonic luminal [Na], and decreased isotonic [K]. Amiloride caused an increased distal flow and isotonic luminal [K] + [Na] in WT control, a decreased distal flow and decreased isotonic luminal [K] + [Na] in WT LNaHK, and did not affect distal flow and luminal [K] + [Na] in KO LNaHK.

Conclusions: These results show that mice on LNaHK have increased urinary flow due to an aldo-induced increase in BK-a/b4-mediated K secretion. The increased ratio of K secreted per Na reabsorbed in mice on LNaHK creates an osmotic kaliuresis.

FR-PO032

Cholesterol (chol)-Rich Lipid Rafts (LRs): Mechanosensitive Structures to Transduce Fluid Flow in the Collecting Duct (CD)

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Background: Essential hypertension (eHTN) is associated with hypercholesterolemia, but how chol contributes to eHTN is unknown. Chol increases plasma membrane (PM) viscosity, partly, by incorporating into LRs. Dietary chol induces ENaC-dependent surge in blood pressure while salt-sensitive rats have elevated PM viscosity and suppressed renal prostaglandin E2 (PGE2) synthesis to augment Na absorption. Flow rate in CDs effects PGE2 release, and led us to hypothesize that chol, and its presence in LRs, regulates flow-mediated cyclooxygenase-2 (COX-2) and PGE2 expression in the CD.

Methods: PGE2 and COX-2 are measured in CD cells and dissected CDs after manipulating chol.

Results: Cortical CDs (CCDs) were microperfused at 0, 1, and 5 nL/min and then incubated in media. Secreted PGE2 was similar between no and low flow (151±28 versus 121±48 pg/mL/mm) CCDs; but, PGE2 was greatest from high flow (578±146 pg/mL/mm; $p<0.05$) CCDs. Next, mice were fed 0% or 1% chol diet, injected with saline to produce high urine flows, and CCDs microdissected for PGE2 secretion. PGE2 secreted from control was 271±73 versus 117±25 pg/mL/mm ($p<0.05$) from chol-fed mice. To evaluate total COX activity, PGE2 was measured after incubating CCDs in arachidonic acid. The induced PGE2 from control was 668±58 versus 313±36 pg/mL/mm ($p<0.05$) in chol-fed mice. Next, chol was extracted (with methyl b-cyclodextrin) from a CD cell-line which stimulated PGE2 release and COX-2 expression, similar to cells exposed to fluid shear stress (FSS), while chol integration suppressed PGE2 and COX-2. We tested whether chol, within PM LRs, constrained activation of mechanosensitive signaling proteins. To test this, extraction of chol mimicked FSS induced-PGE2 and COX-2 expression through p38 activation. Next, p38 was localized to LRs containing mechanosensitive caveolin-1, but chol extraction translocated p38 from LRs to heavier fractions.

Conclusions: LR chol regulates COX-2 expression, in part, by constraining p38 activity. We speculate that FSS raises LR fluidity to release p38, similar to chol extraction, which stimulates PGE2 and COX-2 expression.

Funding: Veterans Affairs Support

FR-PO033

Fluid Shear Stress (FSS) Induces the Translocation of BK Channels Out of Lipid Rafts in the Cortical Collecting Duct (CCD)

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Background: The Ca^{2+}- and stretch-activated BK channel, present in principal (PC) and intercalated (IC) cells of the CCD, mediates flow-induced K secretion (FIKS), enhanced by dietary K loading. BK channel activity is tonically inhibited under low flow conditions by MAPKs (Li *et al*, *PNAS*, 2006) and PKA (Liu *et al*, *AJP*, 2009). Cumulative evidence suggests that FIKS requires release of BK channel inhibition directly or indirectly by these kinases. Studies by others have shown in non-renal cells that the activity of BK channels, localized to sphingolipid-cholesterol-rich lipid rafts (LRs), is regulated by the composition and/or integrity of LRs. We hypothesized that BK channels in the CCD localize to LRs which provide a structural foundation for signaling complexes that regulate channel activity.

Methods: Monolayers of MDCK C7 and C11 cells (PC and IC models, respectively) were stably transfected with c-myc-tagged BKa, grown on glass slides and subjected to 0, 0.1 (low) or 0.4 (high) dynes/cm2 of FSS x 30 min. LRs were isolated from MDCK cells by sucrose density fractionation and analyzed by Western blotting (WB) for c-myc (BKa), ERK1/2, and Cav-1.

Results: MDCK C11 cells express more apical BKa than C7 cells, but in both lines, the channel colocalizes with Cav-1. Immunodetectable BKa, ERK1/2 and Cav-1 were identified in the same sucrose buoyant fraction, corresponding to the migration pattern of LRs, in cells exposed to no or low FSS, but after high FSS, BKa was detected in a denser fraction consistent with a translocation of the channel out of the LRs.

Conclusions: BKa in the CCD localizes to LRs under no and low FSS conditions, where it is tonically inhibited. High FSS stimulates translocation of BKa to non-raft plasma membrane domains where we speculate it can be activated to mediate FIKS.

Funding: NIDDK Support

FR-PO034

Hypokalemia and Diabetes Insipidus in a Kidney Specific Kir4.1 Knock Out Mouse

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Background: We recently developed a kidney specific Kir4.1 KO mouse using the cadherin 16 promoter to investigate SeSAME/EAST syndrome (Gray D et al, JASN 24 (2013), 539A). On control diet, a severe urinary concentrating defect associated with hypernatremia, grossly enlarged kidneys but normokalemia was seen. On low K, low Mg diet x 1 wk, KO mice showed marked hypokalemia (K 1.5 versus 3.0 mM in WT), metabolic alkalosis and the concentrating defect but hypomagnesemia was not seen, suggesting intact DCT function.

Methods: Immunoblots were done on whole kidney lysates. Snap frozen kidneys were homogenized in lysis buffer with protease inhibitors. Samples were spun at 1000g x 15 min, the supernatant spun at 18,000g x 6 hrs at 4C, pellets resolubilized in lysis buffer, samples then BCA quantified, treated with DTT, loaded onto SDS-PAGE gels, transferred and blotted.

Results: Immunoblots on whole kidney lysates showed a ~40% reduction in Kir4.1 protein expression in KO versus WT. Immunofluorescence using antibodies to Kir4.1 along with nephron specific markers showed bright staining for Kir4.1 in DCT > CNT and CCD for WT. In the KO, DCT staining was mildly reduced but signal in the CNT and CCD was minimal or absent, consistent with the preserved Mg transport (in DCT) but compromised K transport (in CNT and CCD) seen in the functional studies described above. To determine the etiology of the urinary concentrating defect, immunoblots were done using aquaporin 2 and aquaporin 3 antibodies. Neither showed decreased expression in the KO. In addition, no significant reduction in surface expression was seen in KO versus WT kidney sections stained with these 2 antibodies. However, the Na/K/2Cl cotransporter was decreased in KO immunoblots, suggesting that a disrupted medullary gradient may underlie the diabetes insipidus seen in these mice.

Conclusions: Basolateral K recycling by Kir4.1 in the CNT and CCD is essential for K adaptation when dietary K is restricted. A disrupted medullary gradient may underlie the concentration defect seen with the KO.

FR-PO035

Differential Regulation of K Channels in the Aldosterone Sensitive Distal Nephron by WNK1 Isoforms Tennille N. Webb,[1] Rolando Carrisoza-Gaytan,[2] Anna Rued,[1] Arohan R. Subramanya,[1] Lisa M. Satlin,[2] Thomas R. Kleyman,[1] Marcelo D. Carattino.[1] *[1]Renal-Electrolyte Div, Univ of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Renal K^+ secretion is accomplished in the aldosterone sensitive distal nephron by the ROMK and the Ca^{2+}- and stretch-activated BK channel. Familial hyperkalemic hypertension (FHHt) is an inherited form of low-renin hypertension with decreased K^+ secretion and increased Na^+ reabsorption, which has been linked to genes encoding WNK1 and WNK4. The WNK1 gene produces two isoforms by alternative promoter usage, a long WNK1 (L-WNK1) that is ubiquitously expressed and a kidney specific (KS-WNK1) isoform that lacks the kinase domain.

Methods: We examined the role of WNK1 isoforms on BK channel whole-cell and surface expression in HEK293 cells.

Results: Our studies showed that the overexpression of L-WNK1 promotes a significant increase in BK channel whole-cell and surface expression. Noticeably, the overexpression of a kinase dead L-WNK1 mutant (K233M) did not alter whole-cell BK expression, suggesting that catalytic activity is required for L-WNK1-mediated regulation of BK expression. When we coexpressed BK channel with L-WNK1, KS-WNK1 or a combination of both in HEK293 cells, we observed that KS-WNK1 antagonized the effect of L-WNK1 on BK whole-cell expression. In immunoperfused CCDs, L-WNK1 labeling, identified in intercalated but not principal cells, was enhanced in rabbits fed a high K^+ (HK) diet for one week.

Conclusions: Taken together, our studies and previous studies suggest that L-WNK1 exerts different effects on renal K secretory channels, inhibiting ROMK and activating BK. A HK diet-induced increase in L-WNK1 expression may contribute to enhanced BK channel-mediated K^+ secretion. An increased expression of BK in individuals with FHHt due to WNK1 mutations may explain why hyperkalemia is corrected with a thiazide diuretic in this setting.

Funding: NIDDK Support

FR-PO036

Mechanisms of Intracellular pH Regulation of Native Renal ClC-K2 Chloride Channel Laurent Pinelli, Stéphane Lourdel, Jacques Teulon, Marc Paulais. *INSERM/UPMC/CNRS U1138 ERL8228, Paris, France.*

Background: Basolateral ClC-Ka/b Cl^- channels, two members of the ClC transporters family, are distributed along the distal nephron. In the thick ascending limb, where ClC-Kb plays a key role in NaCl absorption, loss-of activity ClC-Kb mutations result in type III Bartter syndrome. Still, the physiologically relevant regulations of ClC-Kb properties that might influence chloride transport have to be described.

Methods: Using the inside-out configuration of the patch-clamp technique, the sensitivities of ClC-K2 channels to membrane voltage and intracellular pH (pH_i), two hallmarks of most ClC channels, were investigated in the basolateral membrane of intercalated cells of microdissected mouse connecting tubule, where ClC-K2 is thought to underlie the major chloride channel (*Nissant et al, Am. J. Physiol. 290, F1421-F1429, 2006*).

Results: Channel activity (*NPo*) was highly dependent upon membrane voltage, *NPo* increasing from 24 ± 0.6 % at -80 mV to 100 % at +80 mV at pH_i 7.4 (n = 19). Varying pH_i had a dramatic effect on this voltage-dependence, intracellular alkalinization flattening the channel *NPo/V* relationship through an increase in the maximal number of active channels per patch (from 12 ± 1.3 at pH_i7.0, n = 8, to 49 ± 8.6 at pH_i 7.8, n = 6). At either positive (+80 mV) or negative (-80 mV) clamp membrane voltages, rising pH_i from 7 to 7.8 induced dramatic increases in patch activity, *NPo* rising from ~ 20 % at pH_i 7.0 to 100% at pH_i 7.8 (n = 5). The apparent maximal number of active channels (*N*) was clearly the most sensitive parameter at both +80 and -80 mV, *N* rising from ~25% at pH_i 7.0 to 100% at pH_i 7.8, while single channel activity (*Po*) did not change with pH_i at +80 mV, although increasing with pH_i at -80 mV.

Conclusions: The present study demonstrates that pH_i regulates renal ClC-K2 channel by reducing its voltage-dependence and increasing the number of active channels upon intracellular alkalinization and suggests that it may have a significant impact on the physiology of HCO_3^- transporting intercalated cells.

Funding: Government Support - Non-U.S.

FR-PO037

2Cl/H Exchange Function of CLC-5 Is Required for the H-ATPase-Mediated Maximal Endosome Acidification Nobuhiko Sato,[1] Hideomi Yamada,[1] Osamu Yamazaki,[1] Shoko Horita,[1] Masashi Suzuki,[1] Motonobu Nakamura,[1] Yoshitsugu Kaku,[2] Daisuke Yamamoto,[3] Akira Ashida,[3] Takashi Sekine,[4] George Seki.[1] *[1]Internal Medicine, Tokyo Univ; [2]Fukuoka Children's Hospital; [3]Osaka Medical College; [4]Toho Univ.*

Background: In a typical Dent's disease patient with low-molecular-weight proteinuria and hypercalciuria we have identified a novel "gating glutamate" mutation E211Q that may alter the functional mode of CLC-5, but its disease-causing mechanism remains to be clarified.

Methods: In Xenopus oocytes, CLC-5 currents and surface pH were measured by two-electrodes voltage-clamp method and pH-sensitive microelectrodes, respectively. In HEK293 cells, cell pH recovery from acid-load was monitored with BCECF in hypotonic Na-free solution to estimate the vacuolar H-ATPase activity, and the pH sensitive ratiometric GFP mutant VAMP2-pHluorin was used to measure endosome pH.

Results: Analyses of CLC-5 currents and cell surface pH responses to depolarizing pulses in Xenopus oocytes confirmed that E211Q as well as the artificial mutant E211A functioned as a pure Cl channel with linear I-V curve. In HEK293 cells, the bafilomycin-sensitive basal H-ATPase activity was very low (0.03 +/- 0.001 pH/min). Although the H-ATPase activity was stimulated by E211Q (0.22 + 0.02 pH/min) and E211A (0.28 +/- 0.04 pH/min), these effects were less than that by WT CLC-5 (0.60 +/- 0.08 pH/min). While the basal endosome pH (6.89 +/- 0.04) was significantly lowered by E211Q (6.67 +/- 0.04) and E211A (6.67 +/- 0.05), these effects were again less than that by WT (6.25 +/- 0.05). Bafilomycin abolished the effects of CLC-5 constructs on endosome pH. Analyses with confocal microscopy and Western blotting confirmed that WT, E211Q, and E211A showed the similar intracellular distribution, without changing total or cell surface expression of H-ATPase B2 subunit.

Conclusions: Consistent with the proposed model for CLC-7, our data indicate that 2Cl/H exchange is more efficient than simple Cl conductance in the functional activation of H-ATPase. Loss of 2Cl/H exchange function of CLC-5 by E211Q mutation may cause Dent's disease by impairing maximal endosome acidification.

Funding: Government Support - Non-U.S.

FR-PO038

Chloride Channel Activity of ApoL1 John C. Edwards. *Internal Medicine, Saint Louis Univ, St. Louis, MO.*

Background: Variants in the protein ApoL1 are known to contribute to accelerated progression of chronic kidney disease in people of African descent. ApoL1 is thought to function as a chloride channel in trypansomes, but the properties of the ApoL1 channel have not been fully described. Whether it functions as a channel in kidney cells is unknown. A more complete characterization of the channel activity of ApoL1 and the disease-associated variants may shed light on the mechanism by which variant ApoL1 exacerbates kidney disease.

Methods: ApoL1 was expressed as a fusion protein in bacteria, with GST replacing the N-terminal signal sequence, and with a C-terminal V5/6His tag. A thrombin cleavage site separates the GST domain from ApoL1 sequence. Protein was purified from crude bacterial lysate in the presence of detergent by binding to glutathione agarose. ApoL1 with C-terminal tag was released by thrombin digestion. Protein was further purified over mono Q column. A control preparation was generated from bacteria expressing GST alone with no fusion protein, subjected to the exact same purification. Purified protein was reconstituted into lipid membranes by detergent dialysis. Alternatively, detergent was removed by dialysis and detergent-free protein was incubated with pre-formed vesicles. Channel activity was detected as potential-driven chloride efflux detected by a chloride-selective electrode. Analysis of variance was used to determine significance of any differences.

Results: For reconstituted samples, initial rates of efflux were 0.804%/sec for purified ApoL1, and 0.308%/sec for the control ($P<0.01$). Activity from direct insertion was assayed at pH 5.0 and at pH 7.5. At pH 5.0, initial efflux rates were 0.112 %/sec for ApoL1 and 0.076 %/sec for control ($P<0.05$), while at pH 7.5, rates were 0.86 %/sec for ApolL1 and 0.72 %/sec for the control (n.s).

Conclusions: Purified recombinant ApoL1 supports chloride selective, voltage driven efflux when reconstituted into phospholipid vesicles, or when allowed to directly insert into pre-formed vesicles. The direct insertion activity shows strong pH sensitivity, with activity at low but not neutral pH. The activity of the kidney disease associated variants has not yet been characterized.

Funding: Other NIH Support - NHLBI

FR-PO039

Paracellular Properties of Isolated Perfused Inner Medullary Collecting Ducts and Ascending Thin Limbs of Loop of Henle at Different States of Diuresis Nina Himmerkus, Svenja Sonntag, Annalisa Krause, Markus Bleich. *Inst of Physiology, Christian-Albrechts-Univ, Kiel, Germany.*

Background: The ability of the mammalian kidney to concentrate urine strongly depends on the counter current flow and on the properties of the medullary epithelia namely the descending and ascending parts of the loop of Henle together with the vasa recta and the collecting ducts. We investigated the paracellular properties of inner medullary collecting duct (IMCD) and inner medullary ascending thin limbs (IMaTL) under different states of diuresis.

Methods: 4-6 week old rats were kept for three days either under water restriction (0.18 ml/gBW/d) or water load (0.53 ml/gBW/d). Urinary output and urine osmolality were monitored. Paracellular properties were assessed in isolated perfused IMCDs or IMaTLs by measurement of transepithelial resistance R_{te} and of diffusion potentials. These potentials were generated by perfusion of luminal 245 mM NaCl versus basolateral 50 mM NaCl, both 600 mosm/kg.

Results: Water restriction led to a daily urinary production of 0.05±0.003 ml/gBW/d and a urine osmolality of 2090±121 mosm/kg. Water load increased the urinary flow to 0.29±0.012 ml/gBW/d at 351±8 mosm/kg. In IMCDs R_{te} (27±3.5 Wcm^2 versus 24±3.5 Wcm^2) as well as the permeability ratio P_{Na}/P_{Cl} (0.98±0.02 versus 0.99±0.01) were not different between the two experimental groups. In contrast IMaTL showed a more cation selective paracellular pathway (P_{Na}/P_{Cl} 1.31±0.18) after water-load in comparision to P_{Na}/P_{Cl} 0.99±0.04 under water restriction. R_{te} was not significantly different (9.9±4.8 Wcm^2 versus 3.6±1.0 Wcm^2, respectively).

Conclusions: IMaTLs but not IMCDs change their paracellular properties under different states of diuresis. Under water load with nearly plasma isotonic urine the paracellular pathway of IMaTL became more cation selective.

Funding: Government Support - Non-U.S.

FR-PO040

The Effects of Sodium Glucose Transporter Type 2 (SGLT2) Inhibitions on the Renal Medullary Circulation Yusuke Ohsaki,[2] Takefumi Mori,[1] Kento Akao,[1] Yoshimi Nakamichi,[2] Chika Takahashi,[2] Sadayoshi Ito.[1] *[1]Nephrology, Endocrinology and Vascular Medicine, Graduate School of Medicine, Tohoku Univ, Sendai, Miyagi, Japan; [2]Div of Integrative Renal Replacement Therapy, Graduate School of Medicine, Tohoku Univ, Sendai, Miyagi, Japan.*

Background: Renal medulla is generally hypoxia and reduction of medullary circulation by body fluid loss could result in ischemia. Sodium glucose transporter type 2 (SGLT2) inhibitor reduces sodium and glucose reabsorption in the proximal tubule and thereby control blood glucose level in diabetes but induces osmotic diuresis. Therefore, the present study designed to evaluate the effect of SGLT2 inhibitor tofogliflozin on renal medullary blood flow (MBF) and renal madullary oxygen pressure (pO_2).

Methods: Catheter was inserted in femoral artery and vein in male Sprague-Dawley rats, to monitor blood pressure, heart rate and to infuse drugs, respectively. Renal MBF and pO_2 were measured with laser-Doppler flowmetry or oxygen microelectrode, respectively. Tofogliflozin (1 mg/kg bw/h) or furosemide (0.5mg/kg bw/h) was intravenously administered for 90 min. Urine was collected in 30 min interval.

Results: Tofogliflozin and furosemide administration significantly increased urine volume from 0.39±0.09 to 1.42±0.24, from 0.28±0.03 to 1.38±0.31 g/30 min respectively) but not different between groups. Blood pressure and heart rate did not change either by tofogliflozin or furosemide treatment. Furosemide significantly reduced MBF and increased pO_2 (92.6±1.6% and 119±7.1%, average of 76-90 min data compared to baseline period, respectively), while tofogliflozin did not show significant difference in MBF and pO_2 compared to baseline period (107.3±3.3% and 104.2±4.4%, respectively).

Conclusions: Tofogliflozin did not alter renal medullary circulation and local pO_2 despite of significant increase in urine volume, while furosemide decreased renal MBF but increased renal medullary pO_2 by inhibition of oxygen dependent Na transport. These results indicate that SGLT2 inhibition could play a beneficial role in the body fluid control without altering medullary circulation and oxygen.

Funding: Pharmaceutical Company Support - Chugai Pharmaceutical

FR-PO041

Interplay between Disulfide Bonding and N-Glycosylation Defines SLC4 Na^+-Coupled Transporter Extracellular Topography Quansheng Zhu, Liyo Kao, Rustam Azimov, Natalia Abuladze, Debra Newman, Ira Kurtz. *Medicine, Univ of California, Los Angeles, Los Angeles, CA.*

Background: The extracellular loop 3 (EL-3) of SLC4 Na^+-coupled transporters contains 4 highly conserved cysteines and multiple N-glycosylation consensus sites. In the electrogenic Na^+-HCO_3^- cotransporter NBCe1-A, EL-3 is the largest extracellular loop and is predicted to consist of 82 amino acids. The role of these conserved cysteines in NBCe1-A-EL-3 topological folding and NBCe1-A ion transport function has been unclear.

Methods: In the present study, we designed various cysteine and N-glycosylation mutant combinations in NBCe1-A-EL-3 and analyzed their roles in EL-3 topological folding. These constructs were expressed in the HEK293 cells and subjected for protein chemistry analysis, chemical labeling, cross-linking studies and functional measurement.

Results: Our results demonstrate that the 4 highly conserved cysteines form 2 intramolecular disulfide bonds, Cys583-585 and Cys617-642 respectively that constrain EL-3 in a folded conformation. The formation of the second disulfide bond is spontaneous and unaffected by the N-glycosylation state of EL-3 or the first disulfide bond, whereas formation of the first disulfide bond relies on the presence of the second disulfide bond and is affected by N-glycosylation. Importantly, EL-3 from each monomer is adjacently located at NBCe1-A dimeric interface. When the two disulfide bonds are missing, EL-3 adopts an extended conformation highly accessible to protease digestion.

Conclusions: The unique adjacent location of two symmetrically-folded EL-3 loops from each monomer resembles a domain-like structure that is potentially important for NBCe1-A function *in vivo*. Moreover, the formation of this unique structure is critically dependent on the finely tuned interplay between disulfide bonding and N-glycosylation in the membrane processed NBCe1-A dimer.

Funding: NIDDK Support, Private Foundation Support

FR-PO042

Identification of a Unique Inhibitory Region in the N-terminal Segment of the Pancreatic Na+:HCO3- Cotransporter NBCe1B Electrogenicity Hong Chao Li,[1,3,4] Jingyu Xu,[3] Sunil Yeruva,[3] Brigitte Riederer,[3] Manoocher Soleimani,[1,2] Ursula E. Seidler.[3] *[1]Dept of Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Research Services, Veterans Affairs Medical Center, Cincinnati, OH; [3]Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Lower Saxony, Germany; [4]Pharmacology, Physiology and Toxicology, Joan C. Edwards School of Medicine, Marshall Univ, Huntington, WV.*

Background: The sodium bicarbonate cotransporter NBCe1 plays a crucial role in the reabsorption and secretion of HCO3- in the kidney and pancreatic ducts, respectively. The two major isotypes of NBCe1 include NBCe1A (kidney) and NBCe1B (pancreatic), which exhibit differential tissue distribution and electrogenicity. It is hypothesized that an inhibitory mechanism in the unique N-terminal region of NBCe1B regulates its activity. Inhibitory motif (s) in the unique N-terminal region of NBCe1B regulate its function and electrogenicity.

Methods: Various pNBCe1 mutant constructs were generated via mutagenesis and expressed in either *Xenopus laevis* frog oocytes or HEK 293 cells. The activities of cells expressing the wild-type NBCe1 protein and its mutants were analyzed and compared in the presence of HCO3-.

Results: Deletions of a. a. 31 to 41 and substituted mutations: V35A, P36A, Y39A in subdomain 31 to 41 of NBCe1B resulted in the disinhibition and increased NBCe1B activity in the oocyte expression system. The intracellular pHi recovery rates following intracellular acidosis were significantly higher in HEK293 cells transfected with NBCe1B del 31-41 and Y39A mutants than those in cells transfected with the wild type NBCe1B.

Conclusions: Our results suggest that there are multiple specific subdomains and individual amino acid residues located in NBCe1B unique N-terminal end that are responsible for its inhibition and reduced electrogenicity. In particular, the amino acid residue Y39 of the unique N-terminal domain of NBCe1B plays an inhibitory role in the activity and electrogenicity of NBCe1B. Whether the inhibitory effect of this amino acid or motif is mediated through changes in the conformational structure of the cotransporter or is via binding with other molecules remains to be determined.

FR-PO043

Effect of Aging on Basal and Acidosis-Stimulated Renal Ammonia Metabolism Hyun-Wook Lee,[1] Mary E. Handlogten,[1] Gunars Osis,[1] Jill W. Verlander,[1] I. David Weiner.[1,2] *[1]Div of Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL; [2]Nephrology and Hypertension Section, NF/SGVHS, Gainesville, FL.*

Background: Aging is associated with functional changes in the kidney. Because renal ammonia metabolism is a primary method through which the kidneys maintain acid-base homeostasis, we examined age-related changes in basal and acidosis-stimulated ammonia metabolism.

Methods: We studied young (6~10 months) and aged (18~28 months) C57Bl/6 mice. Mice were acid-loaded by feeding chow mixed with HCl, 0.4 M for 7d; daily 24 hr urines were collected, and then tissue was obtained. Mice with combined collecting duct-deletion of both Rhbg and Rhcg (CD-Rhbg-Rhcg-KO), generated using Cre-loxP technique, were used to examine the role of Rhbg and Rhcg in aging.

Results: Under basal conditions, urinary ammonia excretion, urine pH, and urine volume were similar in young and old mice. In aged mice, cortical Rhbg expression was decreased; outer medullary Rhbg and cortical and outer medullary PEPCK, PDG, glutamine synthetase (GS) and Rhcg expression were unchanged. Acid-loading increased ammonia excretion in both young and old mice and the increase did not differ significantly between groups; urine pH was lower in young mice than in aged mice on days 2 and 5 of acid-loading. After acid-loading, aged mice exhibited greater changes in renal cortical Rhcg, outer medullary Rhbg, and cortical PEPCK expression than did young mice. To determine the functional importance of Rhbg and Rhcg in aged mice, we examined effects of CD-Rhbg-Rhcg-KO on response to acid loading. CD-Rhbg/Rhcg-KO in aged mice decreased acidosis-stimulated ammonia excretion, compared to Cre-negative littermates, by ~35%; this was despite greater changes in GS expression.

Conclusions: We conclude: 1) ammonia excretion in response to an acid load is maintained in aged mice through mechanisms involving age-related changes in acidosis-stimulated PEPCK, Rhbg and Rhcg expression; (2) Acidosis-induced changes in Rhbg and Rhcg are greater in aged than in young mice, and are essential for the response to metabolic acidosis in aged mice; and (3) GS changes in aged, acid-loaded Cre-neg mice are submaximal.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO044

Effect of Low Protein Diet on Renal Ammonia Metabolism Hyun-Wook Lee,[1] Mary E. Handlogten,[1] Gunars Osis,[1] Jill W. Verlander,[1] I. David Weiner.[1,2] *[1]Div of Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL; [2]Nephrology and Hypertension Section, NF/SGVHS, Gainesville, FL.*

Background: Both low protein diets and systemic acid-base status can affect chronic kidney disease progression. Because ammonia metabolism is a major determinant of renal acid-base homeostasis, we examined the effect of dietary protein restriction on ammonia metabolism in normal mice and in mice with deletion of Rhbg and Rhcg.

Methods: Wild-type C57Bl/6 (wt) mice were fed either control (20%) or low (6%) protein diets for 13 days with daily 24 hr urine collection, then tissue was obtained for immunoblot analysis and immunohistochemistry (IHC). To determine the functional necessity for either Rhbg or Rhcg, we studied mice with global Rhcg deletion (Rhcg KO) or with collecting duct-specific Rhbg deletion (CD-Rhbg-KO).

Results: In wt mice, low protein diet decreased urinary ammonia excretion by ~80%. PEPCK and cortical PDG expression decreased, consistent with decreased ammonia production. In addition, cortical glutamine synthetase (GS) increased, consistent with increased glutamine regeneration via GS. Rhcg expression was unchanged. Rhbg expression in inner stripe of outer medulla (ISOM) increased significantly and in the cortex was unchanged. Quantitative IHC showed increased Rhbg expression in ISOM intercalated cells. Rhcg KO did not alter the ammonia excretion response to low protein diet, but resulted in greater changes in PEPCK, cortical PDG, and cortical GS expression than occurred in mice with intact Rhcg expression. CD-Rhbg-KO did not alter ammonia excretion in response to low protein diet.

Conclusions: (1) Low protein diet decreases ammonia excretion by decreasing ammoniagenesis and by increasing GS-mediated reaction of ammonia with glutamate to reform glutamine. (2) It also increases Rhbg expression; because Rhbg deletion did not alter ammonia excretion, Rhbg may have roles unrelated to ammonia excretion. (3) Changes in PEPCK, PDG and glutamine synthetase expression in Rhcg KO mice suggest they compensate for the absence of Rhcg in low protein conditions, indicating Rhcg is involved in response to dietary protein changes.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO045

Hypercalcemia in Mice with High CaP Diet Co-Operatively Stimulated Renal Alpha and Beta Intercalated Cells (IC-A and -B) via Basolateral Ca-Sensing Receptor in IC-B Yukiko Yasuoka,[1] Yuichi Sato,[2] Hiroshi Nonoguchi,[3] Katsumasa Kawahara.[1] *[1]Physiol., Kitasato U. Sch. of Med., Sagamihara, Japan; [2]Mol. Diagnostics, Kitasato U. Sch. of Allied Health Sci, Sagamihara, Japan; [3]Internal Med., Kitasato U. Medical Center, Kitamoto, Japan.*

Background: It is believed that hypercalciuria stimulates the urinary acid excretion to prevent urolithiasis through activation of luminal Ca-sensing receptor (CaSR) in IC-A. Basolateral CaSR in IC-B (Yasuoka Y, ASN 2011) may co-operatively stimulate alkali excretion to maintain the plasma pH in normal.

Methods: C57Bl/6J mice (10 weeks, male) were fed with a control diet (1% Ca by $CaCO_3$ (CaC)) or high calcium diet (control diet + CaC or Ca-phosphate (CaP)) for 7 and 28 days. Blood and urine samples were analyzed. By using a quantitative in situ hybridization technique, we examined changes in the anion exchanger (AE1), carbonic anhydrase (CAXII), H^+-ATPase, Pendrin and CaSR mRNAs expression in collecting ducts.

Results: No unexpected changes were observed in blood and urine samples of mice with CaP diet of 7 d and in mice with CaC diet of 7 and 28 d. In mice with CaP diet (28 d), plasma [Ca^{2+}] slightly, but significantly increased (hypercalcemia) and urinary Ca excretion markedly and significantly increased from 96.0 to 139.2 (μg/d). Further, urinary pH significantly decreased from 6.5 to 6.2. Interestingly, the expression levels of H^+-ATPase, AE1 and CAXII mRNAs in IC-A and of Pendrin and CaSR mRNAs in IC-B increased co-operatively. The cell heights of IC-A and IC-B increased similarly by 29.3% and 38.1% ($P < 0.05$). On the other hand, in mice with CaC diet (28 d),Ca excretion significantly and more largely increased from 90.1 to 341.0 (μg/d). However, urinary pH oppositely increased from 6.7 to 7.6 ($P < 0.05$). The cell height of IC-B, not IC-A, increased by 23.1% and its expression levels of Pendrin and CaSR mRNAs increased significantly.

Conclusions: There was no evidence to indicate that hypercalciuria stimulated urinary acidification through the direct activation of IC-A. Co-operative activation of IC-A and IC-B during chronic high CaP diet may prevent urolithiasis and maintain plasma acid-base balance by increasing urinary acid and alkali excretion.

Funding: Government Support - Non-U.S.

FR-PO046

Direction-Dependent Block of an Electroneutral Na-HCO_3 Cotransporter: Insights from Simultaneous Intracellular and Surface pH Measurements in the Presence of $DIDS^=$ Raif Musa-Aziz,[1,2] Mark Parker,[2] Fraser John Moss,[2] Walter F. Boron.[2] *[1]Physiol and Bioph, Univ of Sao Paulo, Sao Paulo, Brazil; [2]Physiol and Bioph, Case Western Reserve Univ, Cleveland, OH; [3]Physiol and Bioph, SUNY at Buffalo, Buffalo, NY.*

Background: Na^+-coupled HCO_3^- transporters (NCBTs) play important roles in cellular and whole-body acid-base homeostasis. Most are blocked by the divalent anion $DIDS^=$. Previous studies of NBCe1, an electrogenic NCBT, demonstrate that the initial, rapid phase of NCBT inhibition by $DIDS^=$ is reversible and mainly results from ionic interaction of $DIDS^=$ with conserved lysine residues at the outer end of transmembrane segment 5. Preliminary voltage-clamping studies have shown that reversibly bound $DIDS^=$ blocks NBCe1-mediated HCO_3 influx better than efflux, an observation consistent with the idea that exiting HCO_3 knocks $DIDS^=$ off from its extracellular interaction site. In the present study, on an electroneutral NCBT, we further investigate the phenomenon of direction-dependent block by monitoring the effect of reversibly-bound $DIDS^=$ on HCO_3 influx and efflux without imposing an electrical field that could in itself influence DIDS binding.

Methods: We expressed a hyperactive human-NBCn2 (lacking its N-terminal autoinhibitory-domain) in *Xenopus* oocytes and exposed the cells to a solution containing 5%CO_2/33mM HCO_3. We used microelectrodes to simultaneously monitor membrane potential (V_m), intracellular pH (pH_i), and the maximum transient change in surface pH (DpH_S).

Results: Following the familiar fall in pH_i caused by the rapid influx of CO_2, we observed a slower pH_i recovery and a pH_S that was consistently more acidic than the bulk extracellular pH, indicating HCO_3 uptake. We observed no substantial changes in pH_i or pH_S in parallel experiments performed on H_2O-injected oocytes. The addition of 200mM DIDS to the bath affected a complete yet reversible block of NBCn2-mediated HCO_3 influx. When we reversed the direction of transport by replacing extracellular Na^+ with Li^+, pH_i declined and pH_S transiently increased—indicating HCO_3 efflux—regardless of the presence of $DIDS^=$.

Conclusions: This work shows that $DIDS^=$ affects a directional block of NBCn2, reinforcing the idea that exiting HCO_3 displaces reversibly-bound $DIDS^=$.

Funding: NIDDK Support, Other NIH Support - NIH grants NS18400 and DK81567

FR-PO047

A Novel Assay for Quantitating Carbonic Anhydrase Activity and Assessing Red Blood Cell Hemolysis R. Ryan Geyer,[1,2] Pan Zhao,[2] Mark Parker,[3] Walter F. Boron.[2] *[1]Biochemistry, Univ of Sao Paulo, Sao Paulo, Brazil; [2]Physiology and Biophysics, Case Western Reserve Univ School of Medicine, Cleveland, OH; [3]Physiology and Biophysics, State Univ of New York at Buffalo, Buffalo, NY.*

The primary role of the red blood cell (RBC) is to transport O_2 to and CO_2 away from tissue and cells. The RBC membrane is an extremely resilient structure, capable of withstanding extreme mechanical stress and harsh conditions. However, with certain genetic diseases, bacterial infection, or age, RBC fragility can increase appreciably, and hemolysis can have a variety of untoward consequences. We hypothesized that we could exploit the release of carbonic anhydrase II (CAII) from RBCs to quantitate the degree of hemolysis. Here we report a novel method that combines stopped-flow fluorescence spectroscopy, out-of-equilibrium (OOE) CO_2/HCO_3^- solutions, and the pH indicator dye pyranine to monitor the acceleration of pH changes by carbonic anhydrases, including CAII released during hemolysis. One OOE component was buffered with 16mM HEPES (pH 7.05 at 10°C) and the other, with 44 mM HCO_3^- (pH 8.41, ~1%CO_2). Mixing of the two components yields the following initial OOE values: [CO_2]=0.5%, [HCO_3^-]=22 mM, and pH@7.23 at 10°C. Over time, the reaction $HCO_3^- + H^+ \rightarrow CO_2 + H_2O$ produces an exponential pH increase (rate constant, *k*). Adding purified bovine CAII substantially increases *k* in a [CA]-dependent fashion. As a proof of concept, we combined varying amounts of freshly prepared, intact murine RBCs (0.3% hematocrit) with RBC lysate, keeping the total [hemoglobin] constant at 5 mM to simulate different degrees of hemolysis (0-100%). In the OOE assay, *k* increases linearly with simulated % hemolysis. We found that although the initial hemolysis before loading into the SF machine is <1 %, the estimated initial hemolysis in the OOE SF assay is ~5%, presumably due to mechanical forces of rapid mixing. Our results show that the OOE stopped-flow fluorescence approach can measure CAII activity with high resolution in a simplified system composed of only CAII, as well as in a more complex system that mimics hemolysis.

Funding: Other U.S. Government Support

FR-PO048

Effect of Varying Electrode Tip Diameter on Extracellular Surface-pH (pH_S) Changes Caused by CO_2 Fluxes across an Oocyte Plasma Membrane Rossana Occhipinti,[2] Jessica Kabutomori,[1] Walter F. Boron,[2] Raif Musa-Aziz.[1] *[1]Physiol and Bioph, Univ of Sao Paulo, Sao Paulo, Brazil; [2]Physiol and Bioph, Case Western Reserve Univ, Cleveland, OH.*

Background: The use of a blunt electrode to monitor pH_S of an oocyte provides insights into CO_2 fluxes across the membrane. Exposing an oocyte to CO_2/HCO_3 leads to a rapid influx of CO_2, which causes a fall in intracellular pH (pH_i). The CO_2 influx also creates a CO_2 deficit at the extracellular surface, which is replenished by diffusion of CO_2 from the bulk and the reaction $HCO_3+H^+ \rightarrow CO_2+H_2O$. This reaction causes (surface pH) pH_S to rise to a peak and then decay, following a single exponential (t_{pHS}) as the CO_2 influx wanes. The opposite occur as we remove extracellular CO_2/HCO_3.

Methods: We record the pH_S signal with a microelectrode (20-mm tip diameter), controlled by an ultra-fine micromanipulator that positions the pH_S electrode on the oocyte surface, near the equator, until we observe a slight dimple of 40mm in the membrane. The maximal height of the pH_S transient (DpH_S) reflects the maximal CO_2 influx or efflux. A reaction-diffusion mathematical model developed by our collaborators and us suggests that the special volume between the pH_S electrode and oocyte surface may accentuate the effect of the extracellular reaction $HCO_3+H^+ \rightarrow CO_2+H_2O$, thereby amplifying the DpH_S sensed by the electrode. Here, we investigate this special volume on H_2O-injected oocytes by systematically varying the inner diameter (ID) of the pH_S electrode tip from 5 to 20 (our standard), to 40, to 80mm—the maximal feasible diameter.

Results: We observe that the maximal rate of pH_i change and the magnitude of the CO_2-induced pH_i decrease are identical for all IDs, and that reducing the ID to 5mm has little effect on DpH_S or t_{pHS}. On the other hand, increasing ID from 20 to 40 to 80mm

raises DpH_S and t_{pHS}, both for CO_2 addition and removal. The time to peak for pH_S—time difference between the initiation of the CO_2-induced upswing in pH_S and the peak of the pH_S spike—also increases as ID rises from 20 to 80mm.

Conclusions: These data confirm that increasing the ID beyond 20mm accentuates reaction ($HCO_3+H^+ \rightarrow CO_2+H_2O$) within, and reduces CO_2/HCO_3 diffusion to/from, the special environment beneath the pH_S electrode.

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FR-PO049

Vacuolar H^+-ATPase (V-ATPase) Regulation by Aurora Kinase A (AURKA) in Kidney Carcinoma Cells Mohammad M. Al-Bataineh,[1] Rodrigo Alzamora,[3] Allison L. Marciszyn,[1] Hui Li,[1] Fan Gong,[1] Kenneth R. Hallows,[1,2] Nuria M. Pastor-Soler.[1,2] *[1]Medicine, Univ of Pittsburgh, Pittsburgh, PA; [2]Cell Biology, Univ of Pittsburgh, PA; [3]Physiology and Biophysics, Univ of Chile, Chile.*

Background: The V-ATPase mediates ATP-driven transport of H^+ across membranes against a gradient and is involved in metastasis. V-ATPase A subunit phosphorylation at Ser-175 is important for PKA-mediated V-ATPase activity in intercalated cells (ICs). Although Ser-175 is a target for PKA, this residue is also located within a larger phosphorylation consensus sequence for Aurora kinases, which are important in the phosphorylation of proteins that contribute to the pathogenesis metastatic carcinomas. We thus hypothesized that AURKA overexpressed by aggressive carcinomas regulates the V-ATPase in human kidney carcinoma cells (Caki-2) via Ser-175.

Methods: We used Caki-2 cells transfected with either wild-type V-ATPase A subunit (WT-A) or a phosphorylation-deficient mutant A subunit at Ser-175 (S175A-A). We performed co-immunoprecipitation and immunoblot studies and phosphorylation assays *in vitro* and in intact Caki-2 cells. Extracellular pH measurements, *in vitro* wound-healing assays, immunoflorescence labeling and confocal microscopy were also performed on Caki-2 cells.

Results: AURKA is abnormally expressed in Caki-2 cells, where it directly binds the V-ATPase A subunit in an AURKA phosphorylation-dependent manner. An AURKA activator increased V-ATPase expression and activity at the plasma membrane of cultured Caki-2 cells. AURKA phosphorylates the V-ATPase A subunit at Ser-175 *in vitro* and in Caki-2 cells. Furthermore, immunolabeling revealed that an AURKA activator induced marked membrane accumulation of WT-A subunit in cell projections of transfected Caki-2 cells compared to untreated cells. Moreover, the AURKA activator did not induce plasma membrane accumulation of the phosphorylation-deficient mutant S175A-A. Finally, AURKA activation enhanced the rate of wound healing in Caki-2 cells compared to untreated cells.

Conclusions: AURKA mediated V-ATPase regulation via Ser-175 phosphorylation may have a role in the metastatic potential of kidney carcinomas and may represent a potential therapeutic target.

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FR-PO050

The Auto-Recessive Mutation R298S of the Sodium Bicarbonate Cotransporter NBCe1-A Leads to a Trafficking Defect That Underlies Proximal Renal Tubule Acidosis and Myriad Ocular Pathologies Harry S. Gill, Kun-Young R. Choi, Anastas Popratiloff. *Medicine, The George Washington Univ & Medical Faculty Associates, Washington, DC.*

Background: Proximal renal tubule acidosis (pRTA) is a devastating disease in which afflicted individuals fail to reabsorb bicarbonate in the proximal segment of the nephron and thus are unable to maintain blood pH. The electrogenic NBCe1-A is an integral membrane co-transporter that normally reabsorbs Na^+ and $HCO3^-$ across the basolateral membrane. A naturally occurring mutation, R298S, in the cytoplasmic, N-terminal domain (Nt) of NBCe1-A leads to pRTA. We hypothesize that the R298S genetic mutation results in a fragile monomer-dimer equilibrium of NBCe1-A, leading to intracellular aggregation and the inability to incorporate into the plasma membrane.

Methods: To investigate the basis of the R298S, we expressed NBCe1-A and NBCe1-A-R298S in cultured human proximal tubular (HK-2) cells to monitor intracellular trafficking and localization by confocal microscopy. The molecular basis of the disease was investigated by in-depth biophysical studies of the Nt and Nt-R298S (including determining Nt crystal structure at 2.4-Å resolution, homodimer dissociation constant analyses, molecular mass measurements by multiangle-light scattering, and hydrodynamic radii temperature dependence).

Results: Confocal studies show that, unlike NBCe1-A, NBCe1-A-R298S is largely retained in the endoplasmic reticulum and post-Golgi vesicles of proximal tubule cells. The Nt crystal structure reveals a dimer with putative conduits in the interior responsible for substrate transport. But biophysical experiments reveal a significantly higher K_D of the NBCe1-A-R298S dimer compared to wildtype, with a propensity to fall out of solution that is dependent on pH, temperature, and concentration.

Conclusions: The R298S mutation is predominantly a trafficking defect, where cellular localization at the plasma membrane is dependent on growth temperature of the cell line and expression levels. Patients afflicted with this mutation can be characterized with a failure to recover bicarbonate in the proximal tubule due to low expression levels of the cotransporter at the plasma membrane.

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FR-PO051

Silencing Prolyl hydroxylases 2 Protects against Cobalt Induced Hypoxia Injury in Human Renal Epithelial Cells by Autophagy Inactivation Hui Zhang, Bingying Zhang, Sheng Wu, Yi Fang. *Nephrology, Zhangshan Hospital, Fudan Univ, Shanghai, China.*

Background: Prolyl hydroxylase domain protein 2 (PHD2) is a cellular oxygen sensor that regulates hypoxia-inducible factor, a key transcription factor involved in cell survival. Here we studied whether and how PHD2 silencing in cultured human renal proximal tubular epithelial cell (HK2) enhances their renoprotective effects under hypoxia.

Methods: We studied the role of HIF/PHD pathway in $CoCl_2$-induced cell apoptosis/autophagy by employing small-interfering RNA (siRNA). Dynamic profiles of apoptosis/autophagy markers of HK-2 cells within 48h after exposing to $CoCl_2$ (200mM) were recorded.

Results: Our results showed that silencing PHD2 had significantly increased the expression of HIF-1a ($P < 0.01$) but had little effect on HIF-2a. Meanwhile, both knockdowning PHD2 or 3-Methyladenine (an autophagy inhibitor) treatment rescued cell death through autophagy inactivation ($P<0.01$). Of interest, coadministration of HIF-1a siRNA with PHD2 siRNA abrogated renoprotetive effect conveyed by PHD2 siRNA alone, suggesting that activation of endogenous HIF-1a-dependent pathways mediated the autophagy inactivation effects of PHD2 silencing.

Conclusions: Apoptosis and autophagy are crucial mechanisms regulating cell death and homeostasis under hypoxia condition. Direct inhibition of PHD2 promoted renal epithelia cell survival against $CoCl_2$-induced cell apoptosis/autophagy. Activation of the HIF-1a signaling pathway is required to reduced apoptosis and autophagy via up-regulating the expression of Bcl-xl protein.

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FR-PO052

Evaluation of Bardoxolone Methyl as a Modulator of Human Kidney Cell Injury Amandla Roque Atilano,[1] Lauren Aleksunes,[3] Melanie S. Joy.[1,2] *[1]Univ of Colorado, Skaggs School of Pharmacy and Pharmaceutical Sciences, Aurora, CO; [2]Div of Renal Disease and Hypertension, Univ of Colorado, School of Medicine, Aurora, CO; [3]Rutgers Univ, School of Pharmacy, Piscataway, NJ.*

Background: Cisplatin is prescribed for the treatment of solid tumors and exhibits toxicity to kidney tubules. Nuclear factor, erythroid 2-like 2 (NRF2) is a transcription factor in the antioxidant pathway. This investigation sought to study short-term treatment with BARD (NRF2 activator) as a nephro-protectant against cisplatin injury.

Methods: Human proximal tubule epithelial cells (hPTCs) were exposed to cisplatin (0-100mM) or control (DMSO) to measure cell viability by MTT assay. Studies conducted to determine changes in expression of *NFE2L2,* selected detoxifying enzymes (*GCLC*, *NQO1),* and transporters *(SLC22A2, ABCC2)* administered cisplatin (5, 25, 80uM) alone, BARD (100 nM) alone and given pre- or post-exposure (3h) to cisplatin. Cells were harvested, mRNA isolated, RT-PCR and immunofluorescence performed.

Results: Cisplatin 25-100uM resulted in reductions in cell viability *versus* control. Pre- and post-treatment with BARD yielded statistically significant increases in cell viability: 40% and 27% with pre-treatment at 24h and 48h, respectively, and 25% and 16% with post-treatment at 24h and 48h, respectively *versus* control. An increase in *NFE2L2* expression (2.7-fold) and dose and time dependent increases in *NQO1* (5.2-fold) and *GCLC* (4.7-fold) were demonstrated after exposure to cisplatin alone. Treatment with BARD alone resulted in increased *GCLC* (1.9 and 4.4-fold) and *NQO1* (9.3 and 41-fold) expression at 12h and 24h, respectively. Treatment with BARD pre- or post-cisplatin resulted in increased *GCLC* (1.8 and 68-fold*), NQO1* (9.9 and 13-fold), and *SLC22A2* (1.6-fold) and decreased *ABCC2* (0.36 and 0.41-fold) expression. Immunofluorescence demonstrated decreased NRF2 expression over time.

Conclusions: Human PTCs exposed to cisplatin demonstrated enhanced cell viability and increased mRNA expression of detoxifying enzymes (*NQO1* and *GCLC*) and *NFE2L2* with BARD administration. Bardoxolone methyl, given intermittently with planned exposures to cisplatin is a promising approach to mitigate acute kidney injury.

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FR-PO053

Cell Specific Gene Expression of Collecting Duct in Response to Cystitis Using RNA-Tagging Technique Neal A. Paragas, Tian Shen, Jonathan M. Barasch. *Medicine, Columbia Univ, New York, NY.*

Background: Many components of the mammalian immune system are likely involved in the protection of the urinary tract from bacterial invaders. As bacteria ascend the urinary tract they may encounter more than 50 different epithelial and mesenchymal cell types, each of which might contribute to innate defense. As a result, understanding host transcriptional responses at a cellular and temporal level requires a new technology. To determine gene expression in a single cell type at a single time point during a urinary infection (UTI) we generated a Lox-Stop-Lox Rosa26 uracil phosphoribosyltransferase (R26-UPRT) knock-in mouse that can convert 4-thiouracil (4TU) to 4-thiouridine. 4-thiouridine is incorporated into newly transcribed RNA where UPRT is expressed and the thio-RNAs can be biotinylated and isolated with streptavidin beads. We crossed the R26-UPRT mouse with a HoxB7-Cre mouse to specifically label and pull down RNAs from the HoxB7 expressing compartment of the nephron in mice with a UTIs.

Methods: (1) Developed Rosa26-UPRT knockin mouse. (2) Developed bioluminescent uropathogenic bacteria. (3) Pulled down labeled RNAs during the acute phase of a UTI.

Results: 14 week old Rosa26-UPRT/HoxB7-Cre mice with C57BL/6 background where challenged with a transurethral bladder injection of a bioluminescent strain of uropathogenic E. coli (UPEC-Lux, CFT073). Cystitis was confirmed 24 hrs after UPEC-Lux challenge by optical imaging. UPRT mouse was i.p. injected with a 4-thiouracil (4TU) which is converted to 4-thiouridine. TU-tagged RNAs where biotintylated and pulled down and analyzed by qPCR. qPCR of cell type specific markers of the collecting duct confirmed labeling and enhancement of the HoxB7 cell lineage (Pendrin >20-fold increased). Markers of epithelial bacterial activation also showed enrichment (NGAL, IL1a1, Cxcl1 all > 2-fold).

Conclusions: Using the R26-UPRT mouse we showed that specific cells of the distal nephron responded to a distant acute UTI. These data imply that a yet unknown mechanism activates the antimicrobial response of the kidney before bacteria ascend. Furthermore, we were able to show cell specific and time dependent labeling of RNAs for gene expression analysis.

Funding: NIDDK Support

FR-PO054

In Vivo versus in Culture Dynamic Mobility of Renal Epithelial Cell Tight Junction Proteins Kurt Amsler,[1] Josephine Axis,[1] Danielle Janosevic,[1] Robert L. Bacallao.[2] *[1]Biomedical Sciences, NYIT College of Osteopathic Medicine, Old Westbury, NY; [2]Medicine, Indiana Univ School of Medicine, Indianapolis, IN.*

Background: Transepithelial reabsorption of water and solutes across the renal epithelium is mediated by transcellular pathways, through cells, and paracellular pathways, between cells through the tight junction (TJ). Both in vivo and in vitro studies have demonstrated that the permeability of the TJ can be modulated under both physiologic and pathophysiologic conditions. The TJ is composed of multiple membrane proteins, e.g., claudins and occludin, and multiple associated cytoplasmic proteins, e.g., ZO1, -2, -3, and cingulin, organized into an extended multiprotein complex. Recent studies on cultured renal and other epithelial cell types have indicated that multiple TJ proteins can move into and out of the TJ structure.

Methods: We examined the dynamic behavior of GFP-tagged occludin protein (GFP-occludin) transfected into MDCK cells by Fluorescence Recovery After Photobleaching.

Results: Transfected GFP-occludin was localized to the TJ structure/region. Following photobleaching of a portion of the TJ joining a cell couplet, GFP-occludin from outside the photobleached area moved into the photobleached area. The halftime for fluorescence recovery was 50 +/- 22 seconds (n=29). A small number of experiments yielded a substantially longer recovery halftime (189 seconds; n=2). Treatment with hydrogen peroxide at concentrations that increased paracellular permeability 1.5X-3X increased recovery halftime (64 +/- 30 seconds; n=42; p=0.022). The fraction of GFP-occludin that did not move during the experiment timeframe was 53% +/- 16% (n=31). Treatment with hydrogen peroxide did not alter this parameter (54% +/- 14%; n=42; p=0.402). In rats in vivo, transfected GFP-ZO-1 protein was localized to the TJ. GFP-ZO-1 was immobile following photobleaching, i.e., there was no recovery of GFP-ZO-1 into the photobleached area over the timeframe of the experiment.

Conclusions: These results indicate that, in vitro, TJ proteins are dynamic and mobility can be affected by hydrogen peroxide treatment. In vivo, however, at least some TJ proteins are not mobile under basal conditions.

Funding: NIDDK Support

FR-PO055

Identification of a Receptor for Extracellular Renalase Gary V. Desir,[1,2] Ling Wang,[1,3] Heino Velazquez,[1,2] John J. Chang,[1,2] Robert L. Safirstein.[1,2] *[1]Medicine, Yale School Med, New Haven, CT; [2]Medicine, VACHS; [3]Medicine, Renji Hsopital, Shanghai.*

Background: An increased risk for developing essential hypertension, stroke and diabetes is associated with single nucleotide gene polymorphisms in the gene for renalase, a newly described secretory protein. Gene deletion causes hypertension, and aggravates acute ischemic kidney (AKI) and cardiac injury. As a monoamine oxidase, renalase binds and degrades catecholamines; it also acts as an oxidase/anomerase to convert a-NAD (P) H to ß-NAD+, with hydrogen peroxide as reaction byproduct. Independent of its intrinsic enzymatic activities, extracellular renalase activates MAPK signaling and prevents acute kidney injury (AKI) in wild type (WT) mice. Therefore, we sought to identity the receptor for extracellular renalase.

Methods: RP-220 is a previously identified, 20 amino acids long renalase peptide that is devoid of any intrinsic enzymatic activity, but it is equally effective as full-length recombinant renalase at protecting against toxic and ischemic injury. We used biotin transfer studies with RP-220 in the human proximal tubular cell line HK-2, and protein identification by mass spectrometry.

Results: PMCA4b was identified as a renalase binding protein. This previously characterized plasma membrane ATPase is involved in cell signaling and cardiac hypertrophy. Co-immunoprecipitation and co-immunolocalization confirmed protein-protein interaction between endogenous renalase and PMCA4b. Down-regulation of endogenous PMCA4b expression by siRNA transfection, or inhibition of its enzymatic activity by the specific peptide inhibitor caloxin1b each abrogated RP-220 dependent MAPK signaling and cytoprotection. In control studies, these maneuvers had no effect on epidermal growth factor mediated signaling, confirming specificity of the interaction between PMCA4b and renalase.

Conclusions: We conclude that PMCA4b functions as a renalase receptor, and a key mediator of renalase dependent MAPK signaling.

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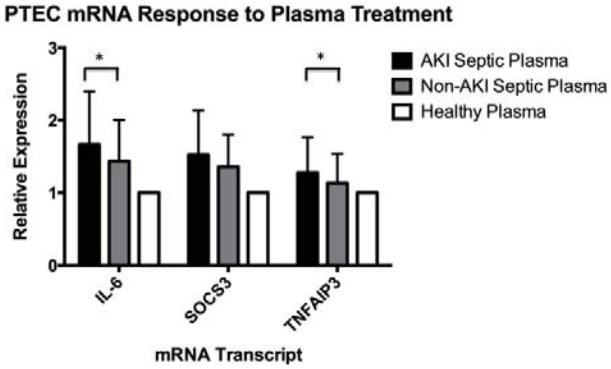
FR-PO056

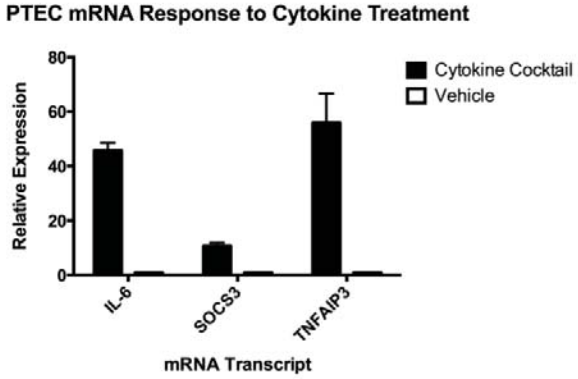
Human Septic Plasma Induces Inflammatory Gene Expression Response in Proximal Tubular Epithelial Cells Jennie Lin, Michael G. Shashaty, Nuala J. Meyer, Muredach Reilly. *Perelman School of Medicine, Univ of Pennsylvania, Philadelphia, PA.*

Background: The pathophysiology of sepsis-associated acute kidney injury (AKI) is not well understood, necessitating novel human translational approaches to gain mechanistic insight.

Methods: We collected plasma from patients with severe sepsis and followed them for subsequent development of AKI, defined by Acute Kidney Injury Network (AKIN) criteria. We stimulated immortalized HKC-8 human proximal tubular epithelial cells (PTECs) for 12 hours with 5% plasma from 12 patients who developed AKI and 12 who did not. Controls included PTECs treated for 12 hours with 5% plasma from healthy subjects, cytokine cocktail (50 ng/mL IL-6, 50 ng/mL TNF-a, 20 ng/mL IFN-g), and serum-free cell culture medium. Real-time quantitative polymerase chain reaction targeting five genes previously associated with either inflammatory pathways or AKI was performed on cDNA reverse transcribed from PTEC RNA from each experimental condition.

Results: Across six experiments performed in triplicate, plasma from septic AKI patients, compared to plasma from septic non-AKI patients, induced increased expression of IL-6 (up to 1.73-fold, $p < 0.001$) and TNFAIP3 (up to 1.39-fold, $p = 0.037$), with a similar trend for SOCS3. PTECs treated with supraphysiologic doses of cytokines similarly had increased IL-6, SOCS3, and TNFAIP3 expression compared to cells treated with serum-free medium (all $p<0.001$), though the increases were more marked than those seen in PTECs exposed to septic AKI versus septic non-AKI plasma. NGAL and IL-18 were not differentially expressed after stimulation with either septic plasma or cytokine cocktail.





Conclusions: PTEC stimulation with septic human AKI plasma induces a differential transcriptional response for inflammation-associated genes such as IL-6 and TNFAIP3, supporting use of this model to study inflammation's contribution to sepsis-associated AKI.

Funding: NIDDK Support, Other NIH Support - NHLBI

FR-PO057

Molecular Mechanism of Hsp70 Inhibitable, Nucleophosmin-Mediated Apoptotic Renal Cell Death Steven C. Borkan, Ramon G. Bonegio, Andrea Havasi, Zhiyong Wang. *Dept of Medicine, Boston Univ Medical Center, Boston, MA.*

Background: During stress, nucleophosmin (NPM), a nuclear DNA binding protein, is released from nucleoli, exits the nucleus and accumulates in the cytosol, presumably acting as a mitochondrial Bax chaperone. We hypothesize that stress-induced cell death is preventable by: (1) reducing NPM nuclear translocation into the cytosol and/or (2) limiting NPM-Bax complex formation required for Bax-mediated mitochondrial injury. To test these hypotheses, the effects of over-expressing wild type Hsp70, an anti-apoptotic protein, or Hsp70 mutants with translocation defects on NPM-mediated cell death were investigated.

Methods: To selectively over-express target proteins, Hsp70-knockout cells were infected with lentivirus containing empty vector (EV), wild-type Hsp70 (WT) or Hsp70

mutants with defective nuclear (985A) or nucleolar (M45) localization. These cells were subjected to ATP depletion, an insult that activates Bax and releases NPM into the cytosol. Cell survival, Hsp70 localization, cytosolic NPM, NPM-Hsp70 and NPM-Bax complex formation, mitochondrial Bax accumulation as well as mitochondrial membrane injury (AIF leakage) were compared in each group.

Results: ATP depletion caused marked nuclear accumulation of only WT and M45 Hsp70. However, WT and both Hsp70 mutants significantly increased cell survival after stress. Only 40% of EV-exposed cells survived stress. In contrast, 90% of WT, 86% of M45 and 60% of 985A expressing cells survived ($P < 0.05$ for each group *versus* EV). WT and both Hsp70 mutants enhanced Hsp70-NPM interaction, reduced NPM-Bax complex formation, mitochondrial Bax accumulation and outer membrane injury. However, only Hsp70 with nuclear access (wild type and M45) decreased the cytosolic accumulation of NPM after stress.

Conclusions: Both cytosolic NPM translocation and NPM-Bax complex formation mediate stress-induced renal cell death. Nuclear Hsp70 inhibits death by preventing nuclear NPM release and cytosolic Hsp70 inhibits NPM-Bax complex formation. Interference with NPM-mediated mitochondrial injury represents a novel mechanism by which Hsp70 protects renal epithelial cells and identifies a new target for preventing stress-induced acute kidney injury.

Funding: NIDDK Support

FR-PO058

Protective Effects of Cilastatin on Gentamicin-Induced Apoptosis, Oxidative Stress and Renal Injury in Rats Juan Carlos Jado,[1] Blanca Humanes,[1] Jose Manuel Lara Martinez,[2] Emilia Cercenado,[3] Alberto Tejedor Jorge,[1] Alberto Lázaro Fernández.[1] *[1]Medicine and Surgery Unit, Inst I.S. Gregorio Marañón, Madrid, Spain; [2]Pathology, Hospital G.U. Gregorio Marañón, Madrid, Spain; [3]Clinical Microbiology, Hospital G.U. Gregorio Marañón, Madrid, Spain.*

Background: Gentamicin (G) is an antibiotic that unfortunately causes nephrotoxicity in 10-20% of therapeutic courses, side effect that seriously limits its use. We have found that cilastatin, a renal dehydropeptidase I inhibitor has protective effects from cisplatin-induced renal damage. Here, we have investigated the potential use of cilastatin as protector on G-induced renal injury in vivo.

Methods: Male Wistar rats were divided into 4 groups: control rats, cilastatin-control rats, G-injected rats (80 mg/kg, daily ip), cilastatin-treated G-injected rats (150 mg/kg, daily, ip). Nephrotoxicity was assessed 9 days after the first dose of G treatment, by measuring serum creatinine, BUN, proteinuria and renal morphology. Renal apoptosis was measured by determination of TUNEL-positive cells and apoptotic mediator's levels. Renal oxidative stress (OS) was assessed by determining lipid peroxidation (4 hidroxy-2-nonenal -4HNE-) and antioxidant capacity. G uptake into kidneys was measured by TDX specific assay.

Results: G-treated rats showed significant elevations in BUN, creatinine and proteinuria, with severe morphological changes such as vacuolization and hyaline cast in the tubular lumen. The treatment with cilastatin resulted in amelioration of renal functions as shown by reduction of these parameters and improved histological damage. Cilastatin also reduced renal caspase 3-activation, bax, bax/Bcl-2 ratio, Fas ligand and TUNEL-positive apoptotic cells, previously increased by G. Moreover, G increased the levels of OS, increasing 4-HNE and decreasing total antioxidant capacity and specifically Cu/Zn SOD and catalase levels. Cilastatin reversed these changes significantly and also attenuated G uptake by kidney tissue.

Conclusions: This study provides evidence that cilastatin reduces in vivo G nephrotoxicity by ameliorating apoptosis and OS. The mechanism of the beneficial effect could be attributed, at least in part, to a decrease in drug accumulation by the cells.

Funding: Government Support - Non-U.S.

FR-PO059

Synchronized Tubular Cell Death Is Mediated by Ferroptosis in Acute Kidney Injury Andreas Linkermann,[1] Nina Himmerkus,[2] Shrikant R. Mulay,[3] Matthias Hackl,[4] Agnes Prokai,[4] Christin Dewitz,[1] Markus Bleich,[2] Ulrich Kunzendorf,[1] Hans J. Anders,[3] Joel M. Weinberg,[5] Stefan Krautwald.[1] *[1]Nephrology and Hypertention, Christian-Albrechts-Univ, Kiel, Germany; [2]Inst for Physiology, Christian-Albrechts-Univ, Kiel, Germany; [3]Nephrology Unit, Medizinische Klinik und Poliklinik IV, Munich, Germany; [4]Dept II of Internal Medicine and Centre for Molecular Medicine, Univ of Cologne, Cologne, Germany; [5]Div of Nephrology, Dept of Internal Medicine, Univ of Michigan, Ann Arbor, MN.*

Background: Necroptosis (Linkermann and Green, NEJM 2014) depends on receptor-interacting protein kinase 3 (RIPK3), and we recently found RIPK3-ko mice to be protected from ischemia-reperfusion injury (IRI) (Linkermann et al., PNAS 2013). However, these studies could not exclude a direct effect of RIPK3 on organ perfusion which was suspected given the high RIPK3-expression in renal endothelial cell lines.

Methods: To directly investigate the effect of necroptosis in kidney tubules, we generated conditional inducible tubule-specific caspase-8 and FADD-deficient mice, both of which show no major abnormalities upon transgene induction and thus could not explain synchronized tubular cell death, but rather suggested a vascular effect which was quantified by intravital microscopy.

Results: In contrast to necroptosis-deficient primary isolated RIPK3-ko and MLKL-ko tubules prevention of ferroptosis in primary tubules increased tubular survival in diverse disease settings. Different IRI settings and four *in vivo* models of acute kidney injury demonstrate the pathophysiological importance and the immunogenicity of ferroptosis. We developed a novel thrird generation ferrostatin which is stable *in vivo,* and demonstrate strong renoprotective effects employing this compound *in vivo* and *ex vivo*.

Conclusions: Ferroptosis appears to be the central cell death mechanism relevant in vivo in tubules whereas RIPK3 mediates a perfusion-effect, but does not directly affect the tubules themselves in AKI. Therefore, the beneficial effects of prevention of necroptosis (Linkermann and Green, NEJM 2014) may be largely mediated by perfusion benefits, but the parenchymal cell death occurs through ferroptosis.

Funding: Pharmaceutical Company Support - Pfizer, Novartis, Fresenius

FR-PO060

Osteopontin and Monocyte Chemotactic Protein-1 Are Targets of Meprin A During Acute Kidney Injury Christian Herzog, Sudhir V. Shah, Gur P. Kaushal. *Internal Medicine, UAMS, Little Rock, AR.*

Background: Meprin A, an oligomeric metalloproteinase composed of a- and b-subunits is anchored to the brush border membrane (BBM) by its b-subunit. During AKI meprin A is redistributed towards the basolateral side. We recently demonstrated that following AKI, meprin A is shed by ADAM-10 by cleaving at the b-subunit (JBC: 289, 2014). Shed meprin may be deleterious in AKI due to its high degradative potential. We have previously reported that meprin A is able to activate proIL-1b to IL-1b. In the present study we examined whether other proinflammatory chemokines like osteopontin (OPN) and monocyte chemotactic protein-1 (MCP-1) are proteolytic targets of meprin A.

Methods: Recombinant OPN was digested with activated promeprin a and b purified from stably transfected HEK cells in a time dependent manner. Likewise OPN was digested with meprin A purified from rat kidney. OPN proteolysis was visualized by westerns blot. Similarly the chemokine MCP-1 was digested with meprin a and b as well as with meprin A and the resulting digests visualized on western blots. Cleaved fragments of MCP-1 were analyzed by Edman degradation and LC/MS/MS to determine their peptide sequence. Urinary excretion of MCP-1 was monitored *in vivo* by ELISA in a mouse model of cisplatin AKI.

Results: OPN was rapidly (within 1 min) and completely degraded by meprins without forming a stable intermediary fragment. Meprin b cleaved OPN much faster than meprin a and meprin A. The cleavage of OPN may play an important role in AKI since OPN deficiency causes apoptosis in IR. MCP-1 was cleaved by meprins into stable fragments. As determined by LC/MS/MS meprin b cleaved MCP-1 after ^{74}Ser. Both meprin a and meprin A truncated MCP-1 into a 10 kDA fragment (N-terminally of ^{7}Ala and C-terminally most likely after ^{73}Arg). The P1 cleavage sites for MCP-1 differed from the sites obtained for proIL-1b.

Conclusions: The findings that meprins can degrade the proinflammatory OPN completely and cleave MCP-1 into fragments together with the already established fact of meprins ability to activate proIL-1b highlight the importance of meprins in the modulation of the inflammatory processes following AKI. Meprins may become targets for therapeutic intervention.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO061

Blocking JAM-C Promotes the Recovery of Cisplatin-Induced Acute Kidney Injury Sun Chul Kim, Hyojeong Chang, Myung-Gyu Kim, Sang-Kyung Jo, Won-Yong Cho. *Korea Univ Anam Hospital.*

Background: For resolution of inflammation, infiltrated leukocytes should be removed. Recent studies demonstrating 1) presence of neutrophil transendothelial migration in a reverse direction (rTEM) following ischemia/reperfusion injury (IRI), 2) endothelial junctional adhesion molecule-C being a negative regulator of rTEM, 3) identification of neutrophils that have migrated in a reverse direction by their high expression of ICAM-1 led us to test the effect of JAM-C blocking antibody on the resolution of kidney injury and inflammation in a mouse model of cisplatin-induced acute kidney injury (AKI).

Methods: Male C57BL/6 mice (19-23g) were given a single intraperitoneal (i.p.) injection of cisplatin (18mg/kg). Monoclonal anti-mouse JAM-C blocking antibody (clone H33; Millipore; Billerica, MA, U.S.A.) or control IgG was administered i.p. at 1.5mg/kg and the injection was delayed until day 4 and 5 following cisplatin injection to restrict the effect of antibodies on recovery phase. Biochemical, histological analyses as well as flow cytometry to detect reverse migrated ICAM-1$^+$ neutrophils were performed at 4, 5 and 6 days after cisplatin injection.

Results: After cisplatin injection, serum creatinine and histologic injury peaked on day 4. Treatment with a JAM-C blocking antibody on day 4 and 5 promoted the functional and histologic recovery of cisplatin induced AKI on day 5 and 6. Facilitating recovery by JAM-C blocking Ab was associated with significantly increased circulating ICAM-1$^+$ Tomm Horsfall protein (THP)$^+$ neutrophils as well as significantly decreased renal neutrophil infiltration and IL-6 levels, indicating that facilitating rTEM of neutrophils from kidney to peripheral circulation partially mediate resolution of inflammation and recovery.

Conclusions: We suggest that rTEM is involved in resolution of neutrophilic inflammation in cisplatin induced AKI and JAM-C is an important regulator of this process. Therefore, blockade of JAM-C could be considered for the new strategies to facilitate recovery in cisplatin induced AKI.

FR-PO062

NQO1 Regulate Autophagy in Cisplatin Induced Renal Injury Youngjung Kim, Sora Park, Hyun Tae Kim, Tae Won Kim, Si Yun Ryu, Ju Young Jung. *Veterinary Medicine & Inst of Veterinary Science, Chungnam National Univ, Daejeon, Korea.*

Background: Autophagy is a catabolic process that degrades damaged proteins and organelles in mammalian cells and activated under various pathologic condition including acute renal failure. It is generally accepted that ROS induce autophagy. NADPH:quinone oxidoreductase 1 (NQO1) is known as antioxidant enzyme, regulate the generation of ROS.

Methods: To invest regulation of autophagy by the NQO1, we used the NQO1 deletion and pretreated b-Lapachone known as NQO1 activator in cisplatin induced acute nephropathy *in vivo* and *in vitro*. We measured the serum blood urea nitrogen (BUN), creatinine (Cre) and performed the immunoblot and immunohistoemistry.

Results: NQO1 deletion (NQO1 -/-) mice was increased BUN and Cre under the cisplatin treatment, compared with wild type mice. NQO1 deletion resulted in increased autophagy with LC3B, LAMP2 and ATG7 levels and intense immunoreactivity of these proteins founded in the proximal tubule of outer medulla. However, treatment of b-Lapachone decreased autophagy and related proteins in cisplatin nephropathy. We also show the cisplatin induces autophagy in human kidney adenocarcinoma cells (ACHN). Levels of LC3B, LAMP2 and ATG7 were increased in treatment of Si NQO1 and decreased in b-Lapachone treatment.

Conclusions: These finding suggested NQO1 may be regulate renal autophagy in acute nephropathy induced by cisplatin treatment.

Funding: Government Support - Non-U.S.

FR-PO063

Cisplatinum-Induced Nephrotoxicity Activates ATM-Associated DNA Damage Response (DDR) and Offers Novel Avenues for Renal Protection Samriti Dogra,[1] Sriram Bandi,[3] Preeti Viswanathan,[2] Yogeshwar Sharma,[3] Sanjeev Gupta.[3] *[1]Pediatric Nephrology, Montefiore Medical Center, Bronx, NY; [2]Pediatric Gastroenterology, Montefiore Medical Center, Bronx, NY; [3]Medicine and Pathology, Albert Einstein College of Medicine, Bronx, NY.*

Background: Better insights into mechanisms of drug-induced nephrotoxicity will assist discovery of therapeutic agents to prevent or to promote tissue repair in kidneys. We first studied DDR typical of toxic chemotherapy, e.g. cisplatinum, in mouse renal tubular epithelial cells and next examined the possibility of renal protection within the context of DDR.

Methods: The studies were performed in MCT proximal tubular epithelial cells from the mouse. After exposing cells to IC50 concentrations of cisplatinum, we performed MTT assays for cell viability, qRT-PCR arrays for DNA damage associated changes, including ATM signaling pathway members, Comet assays for double-stranded DNA breaks, and cytostaining for gH2AX expression. To determine potential protection through ATM signaling, we examined ATM promoter expression with tdT reporter in MCT cells, with or without cisplatinum and growth factors. For in vivo effects, we treated mice with 30 mg/kg cisplatinum i.p. and sacrificed mice 3 d later for analysis of kidney tissue, including Comet assays and gH2AX staining.

Results: These studies showed typical evidences of DDR in cisplatinum-treated MCT cells. Specifically, we observed gH2AX expression, Comet formation and altered expression of ATM-related genes at mRNA level. Moreover, treatment of MCT cells with cisplatinum induced ATM promoter activity. Also, gH2AX expression in proximal tubular epithelial cells of cisplatin-treated kidney tissue indicated DDR in vivo. In a miniscreen of cytotrophic growth factors, we found G-CSF, VEGF, and FGFs could decrease cisplatinum-induced ATM promoter activity and improve cell viability.

Conclusions: Cisplatinum-induced DDR related to ATM signaling was observed in MCT cells as well as kidneys of intact mice. The ability to interfere with DDR through regulation of ATM promoter activity indicated potential protective role of growth factors in cisplatin-induced nephrotoxicity. These findings will be of clinical significance.

Funding: NIDDK Support

FR-PO064

Role of Sirt3 in Cisplatin-Induced Nephrotoxicity Won Kim, Dal Kim, Aesin Lee, Yujin Jung, Tung Nguyen-Thanh, Sik Lee, Sung Kwang Park, Kyung Pyo Kang. *Internal Medicine, Chonbuk National Univ Medical School, Jeonju, Republic of Korea.*

Background: Cisplatin based chemotherapy is commonly used in therapeutic strategies for solid tumor. However, limitation of this agent is adverse effect on normal tissue such as kidney, ear, and peripheral nerves. Mechanisms of cisplatin nephrotoxicity are proposed as oxidative stress, inflammation, cellular apoptosis and death, and cell cycle regulation. Sirt3 is one of sirtuins family, which is NAD^+-dependent enzymes with homology to the *Saccharomyces cervisiae* gene silent information regulator 2 (Sir2). Sirt3 is located in mitochondria and involved in mitochondrial energy metabolism and function. However, the role of sirt3 in cisplatin-induced is not yet clarified. In this study, we investigated the role of sirt3 in cisplatin-induced nephrotoxicity *in vivo*.

Methods: We used Sirt3 knockout mice (*Sirt3*$^{(-/-)}$) and their wild type mice. Cisplatin nephrotoxicity was induced by intraperitoneal injection of cisplatin (20 mg/kg). After 3 days after cisplatin injection, blood and kidney tissues were harvested. Renal function and histology were evaluated. Tubular apoptosis, cell adhesion molecule expression, cytokines and inflammatory cells were evaluated by immunohistochemistry, Western blot analysis and ELISA.

Results: After induction of cisplatin nephrotoxicity, renal function was significantly aggravated in *Sirt3*$^{(-/-)}$ mice group. Tubular injury and inflammatory cell infiltration was significantly increased in *Sirt3*$^{(-/-)}$ mice group compared to wild type mice. TUNEL positive tubular cells and renal MCP-1 expression were increased in *Sirt3*$^{(-/-)}$ mice group compared to wild type mice.

Conclusions: In summary, absence of Sirt3 is more aggravated in renal injury by increase of renal inflammation and tubular apoptosis. Sirt3 may have important role in cisplatin-induced nephrotoxicity. We need to further studies for their molecular mechanisms.

Funding: Government Support - Non-U.S.

FR-PO065

Identification of CDK4/6 Inhibitors That Ameliorate Cisplatin Nephrotoxicity Through Inhibition of OCT2 Function and Suppression of Cell Cycle Progression Navjotsingh P. Pabla, Alice A. Gibson, Alex Sparreboom. *Pharmaceutical Sciences Dept, St. Jude Children's Research Hospital, Memphis, TN.*

The organic cation transporter 2 (OCT2) mediates renal uptake of cisplatin and is a critical regulator of cisplatin nephrotoxicity. Pharmacological compounds that inhibit OCT2 have the potential to significantly mitigate cisplatin induced renal injury. In a chemical screen for OCT2 inhibitors, we identified two CDK4/6 inhibitors (palbociclib and LEE011) that potently inhibit OCT2 function. Mechanistically, palbociclib and LEE011 inhibit OCT2 in a non-competitive manner. Importantly, in mouse models of cisplatin nephrotoxicity, a single dose of palbociclib (150 mg/kg) prior to cisplatin administration significantly reduced cisplatin nephrotoxicity markers BUN and serum creatinine (vehicle versus palbocilib, P>0.05). Cisplatin nephrotoxicity was reduced by palbociclib in both wild-type and OCT2-null mice, indicating that the protective effects are mediated through OCT2 and an OCT2-independent mechanism, presumably cell cycle inhibition. We also found that CDK4/6 pathway is activated *in vivo* during cisplatin nephrotoxicity, and this is significantly inhibited by palbociclib. These results suggest that the CDK4/6 pathway is a therapeutic target for acute kidney injury and support the future clinical exploration of CDK4/6 inhibitors as modulators of cisplatin toxicity.

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FR-PO066

Functional Analysis of iPSC-Derived Myocytes from a Patient with Carnitine Palmitoyltransferase II Deficiency Tetsuhiko Yasuno. *Internal Medicine, Fukuoka Univ, Fukuoka, Japan.*

Background: Carnitine palmitoyltransferase II (CPT II) deficiency is an inherited disorder involving b-oxidation of long-chain fatty acids (FAO), which leads to rhabdomyolysis and subsequent acute renal failure. The detailed mechanisms of disease pathogenesis remain unknown; however, the availability of relevant human cell types for investigation, such as skeletal muscle cells, is limited, and the development of novel disease models is required.

Methods: We generated human induced pluripotent stem cells (hiPSCs) from skin fibroblasts of a Japanese patient with CPT II deficiency. Mature myocytes were differentiated from the patient-derived hiPSCs by introducing myogenic differentiation 1 (*MYOD1*), the master transcriptional regulator of myocyte differentiation. Using an *in vitro* acylcarnitine profiling assay, we investigated the effects of a hypolipidemic drug, bezafibrate, and heat stress on mitochondrial FAO in CPT II-deficient myocytes and controls.

Results: CPT II-deficient myocytes accumulated more palmitoylcarnitine (C16) than did control myocytes. Heat stress, induced by incubation at 38°C, leads to a robust increase of C16 in CPT II-deficient myocytes, but not in controls. Bezafibrate reduced the amount of C16 in control and CPT II-deficient myocytes.

Conclusions: In this study, we induced differentiation of CPT II-deficient hiPSCs into mature myocytes in a highly efficient and reproducible manner and recapitulated some aspects of the disease phenotypes of CPT II deficiency in the myocyte disease models. This approach addresses the challenges of modeling the abnormality of FAO in CPT II deficiency using iPSC technology and has the potential to revolutionize translational research in this field.

FR-PO067

Effects of Vesicules (Vs) Derived from Renal Pluripotent Stem Cells (rPSCs) on the LPS Toxicity in Immortality Human Mesangial Cells (iHMCs) and Renal Epithelial LLCP-K_1 Cells Luciana Aparecida Reis,[1] Gerson D. Keppeke,[2] Rita De C.S.G. Coimbra,[3] Nestor Schor.[1] *[1]Nephrology Div, UNIFESP/EPM, Sao Paulo, Brazil; [2]Rheumatology Div, UNIFESP/EPM, Sao Paulo, Brazil; [3]Eletronic Microscopy Div, UNIFESP/EPM, Sao Paulo, Brazil.*

Background: In this study we investigate the effect of Vs derived from rPSCs on the iHMCs or LLCP-K_1 treated with LPS.

Methods: rPSCs and characterized by immunofluorescence and FACS and Vs by TEM and Western blot. In order to enhance the production of Vs, the rPSCs were treated with LPS (100µg/ml), gentamicin (G; 2mM) or cisplatin (Cis; 6mM) (preconditioned - PC) or not (nPC) for 24 h. iHMCs or LLCP-K_1were treated with LPS (100µg/ml) or PBS (vehicle) for 72 h. iHMCs and LLCP-K_1 were treated with LPS+Vs nPC (35µg/ml) or PC (35µg/ml) for 24 h with LPS, G or Cis. Cellular viability were evaluated for Tripan blue and acridine orange; caspase 3, IL2, IL6 and IL10 by Western blot and NO by Griess.

Results: rPSCs were positive by Wnt1, CD24, PAX2 and ZO1 and Vs to CD9, CD81 and CD63. LPS decreased cell viability *versus* PBS 24h (150±7.9 *versus*408±18.8; 82.3±7.6 *versus* 97.8±9.3 %; p<0.05) or 72h (67.5±7.4 *versus* 385±12.6; 74.3±6.7 *versus* 98.1±13.4%; p<0.05). nPC Vs increase in cell viability 24h (312±7.4 *versus* 150±7.9; 89.4 *versus* 74.3±6.7%; p<0.05) or 72h (266±14.6 *versus* 76.6±5.7; 88.4±4.9 *versus* 76.6±5.7%; p<0.05) in iHMCs. LPS 72h increased caspase 3, IL2, IL6 and decreased IL10 *versus* CTL. When iHMCs received nPC-Vs decreased IL2, IL6 and caspase 3 and increased IL10. NO (nmoles/mg) increased in LPS *versus* CTL 72 h in iHMCs (82.8±9.5 *versus* 171.2±27.8; p<0.05) and LLCP-K1 (24.5±1.8 *versus* 129.8±16.4; p<0.05) and decrease in nPC-Vs (145.4±4.6 *versus* 171.2±27.8; p<0.05); (129.8±16.4 *versus*72.6±6.6; p<0.05) and PC-Vs with LPS, G or Cis for 24h (109.5±11.2; 115.8±5.8; 103.2±9.3; p<0.05); (76.3±5.9; 41.7±5.1; 33.8±1.3; p<0.05); respectively.

Conclusions: Results suggested that the Vs derived from rPSC have the ability to protect the toxicity in iHMCs and LLCP-K_1 induced by LPS. The protective effects were mediated by Vs-derived from rPSCs and intensified when the Vs-rPSC were previously stimulated by toxins, especially with different insult. In this case the stimulation by Cis presented higher protective effects.

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FR-PO068

Mesenchymal Stem Cell Homing to Kidneys Is Suppressed by Disrupting Interleukin 1 Alpha, Tumor Necrosis Factor Alpha, or Cyclooxygenase-2 Signaling

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Background: Optimal homing of iv-infused mesenchymal stem cells (MSC) may improve regenerative medicine therapies. Pulsed focused ultrasound (pFUS) enhances renal MSC homing sufficient to rescue cisplatin AKI 3 days after injection. pFUS generates a "molecular zip-code" of increased chemoattractants (cytokines, chemokines, cell adhesion molecules) in healthy murine kidneys. The molecular zip-code includes early elevations of TNFa, IL1a, and cyclooxygenase-2 (COX2) expression. We examined if inhibiting these molecules with clinically-relevant drugs abrogates the molecular zip-code and suppresses renal MSC homing.

Methods: C3H mice received inhibitors or vehicle pretreatment then unilateral kidney pFUS (8MPa). Kidneys were harvested 10min-72hr after pFUS. Pretreatment with drugs included: ibuprofen (30mg/kg, po, 15min before pFUS); etanercept (100mg, ip; 72 and 24hr before); or anakinra (IL-1a inhibitor, 200mg, ip; 48, 24, and 1hr before). In a second set, animals were given MSCs (10^6 human MSC iv) 3 hrs after pFUS. Kidneys were harvested 24hr later and MSC were detected by immunofluorescence.

Results: Renal pFUS elevated kidney TNFa and IL-1a early (10min) and upregulated COX2 (1–4hr). The subsequent (2-24hr) molecular zip-code included many chemoattractants necessary for MSC homing, and returned to baseline by 72hr. Kidneys from mice pretreated with ibuprofen, etanercept, or anakinra did not generate the molecular zip-code after pFUS. There was a mean decrease of ~64%, ~63%, and ~50% in MSC homing post pFUS for mice pretreated with ibuprofen, etanercept and anakinra, respectively. MSC also failed to home to pFUS-treated kidneys in COX2-knockout mice, demonstrating a specific, essential role for COX2.

Conclusions: pFUS increases MSC homing via signaling mechanisms that are TNFa, IL-1a, and COX2 dependent. Drug/MSC interactions remain unexplored and uncontrolled for in clinical trials. These data suggest common drug-host interactions could undermine cell-based therapies in regenerative medicine.

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FR-PO069

The Bone Marrow-Derived Mesenquimal Stem Cells (MSC) or Conditioned Medium (MSC-CM) Repair the Acute Kidney Injury (AKI) Induced By Acyclovir (ACY)

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Background: The ACY is an antiviral drug used to treat herpes simplex type 1 and 2 and varicella zoster and may induce nephrotoxicity with AKI.Several groups have reported the contribution MSC in repair AKI processes. The aim of this study was to investigate the role of MSC or its conditioned medium BMSC-CM in this model of AKI.

Methods: After standart characterization the MSC were used at 4th passages for all experiments. The female Wistar rats received ACY (80mg/Kg/BW) or water (CTL) daily, for 5 days i.p. (N=10) and 72 hours after the 5th day of treatment, they received i.v. MSC ($1X10^6$cells) or MSC-CM (500ml) injection (1 doses). Then, blood were collected for urea (U) and creatinine (Cr). As well as histological evaluation.

Results: The AKI model was confirmed by increase in in creatinine (1.7±0.1versus0.7±0.01mg/dl) and urea (174.5±0.2versus56.0±0.1mg/dl; p<0.05),respectively after ACY, compared CTL.The ACY+MSC, after 72 h,presented lower creatinine (0.9±0.2 versus1.3±0.1mg/dl) and urea (89.2±0.2versus143.5±0.2 mg/dl; p<0.05) compared with ACY. Also the ACY+MSC-CM presented lower values for creatinine (1.0±0.1versus1.7±0.1mg/dl, p<0.05) compared with ACY. The MSC or CM decreased in pro-inflammatory cytokines and increased in anti-inflammatory cytokine.

Serum cytokines (pg/mL)	CTL	Acyclovir 5 days	Acyclovir+ PBS 72 hours	Acyclovir +MSC 72 hours	Acyclovir+Cell culture medium conditioned 72 hours
IL-1α	9.0±8	50.1±9	48.2±9	29.4±07*	29.3±4#
IL-1β	40.2±6	220.0±4	240.0±1	130.0±8*	165.0±6#
IL-10	12.2±3	25.6±7	34.2±9	120.0±5*	84.8±5#
TNF α	7.0±8	42.9±2	41.0±3	11.8±4*	24.5±5#
IFNγ	30.3±4	110±9	98.0±5	35.2±3*	64.8±12#
IL-6	60.1±10	417.0±9	378.0±7	227.0±8*	246.0±12#

Values are expressed as the mean±SEM *P<0.05 CTL vc Acyclovir+MSC 72 hours (n = 5), vs acyclovir+cellculture medium conditioned 72 hours # (n = 5), (One-Way ANOVA tukey pos-test).

Conclusions: These results strongly suggest that MSC and MSC-CM minimize AKI induced by ACY and thus, could be a new strategy to treat this nephrotoxicity.

FR-PO070

Inhalation of Hydrogen Gas Is Beneficial for Preventing Contrast-Induced Acute Kidney Injury in Rats

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Background: Oxidative stress plays a key role in the pathogenesis of contrast-induced acute kidney injury (CIAKI). Administration of contrast media has been shown to increase the concentration of reactive oxygen species, resulting in the renal vascular constriction and tubular cell apoptosis. The present study aimed at investigating the effect of novel anti-oxidant, hydrogen (H2) gas, on the severity of CIAKI in a rat model.

Methods: CIAKI was induced in rats by intravenous injection of contrast medium, Ioversol, in addition to reagents inhibiting prostaglandin and nitric oxide synthesis. During the injection of these reagents, the rats inhaled H2 gas or control gas.

Results: One day after the injection, serum levels of urea nitrogen were significantly lower in H2 gas-inhaled CIAKI rats (17.6 ± 2.3 mg/dl) than those in control gas-inhaled CIAKI rats (36.0 ± 7.3 mg/dl), although they both were elevated as compared to untreated rats (14.9 ± 0.9 mg/dl). Consistently, creatinine clearance in H2 gas-treated CIAKI rats was higher than control gas-treated counterparts. Moreover, renal histological analysis revealed that the formation of proteinaceous casts and tubular necrosis was improved by H2 gas inhalation. Mechanistic analyses showed that inhalation of H2 gas significantly reduced renal tubular cell apoptosis, expression of cleaved caspase 3, and expression of an oxidative stress marker, 8-hydroxy-deoxyguanosine, in injured kidneys.

Conclusions: These results suggest that H2 gas inhalation is effective in ameliorating the severity of CIAKI in rats by reducing renal cell apoptosis and oxidative stress.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO071

The Role of Nrf2 Expression in Radiocontrast Induced Nephropathy

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Background: Radiocontrast induced nephropathy (CIN) is the third common cause of acute renal failure. Although the number of patients taken the exams using radiocontrast is increasing, little has been progressed in the treatment for CIN. The pathophysiology of CIN was known as tubular injury by oxidative stress. Nuclear factor-erythroid-2-related factor 2 (Nrf2) has been known as a key player to coordinate intracellular antioxidative process. We investigated the role of Nrf2 in CIN.

Methods: CIN were established in vivo and vitro model by administration of iohexol in C57BL/6J mice and rat tubular cells (NRK-52E) according to previous study. To determine the role of Nrf2 in CIN, Nrf2 expression was reduced in vivo study using Nrf2 KO mice (B6.129X1-Nfe2l2tm1Ywk/J) or by siRNA treatment in vitro study. Tubular cell injury was examined after iohexol treatment.

Results: Increased expression of Nrf2 was noted after iohexol treatment both in mice kidneys and rat tubular cells. Serum creatinine at 24hrs after iohexol injection was significantly increased in Nrf2 KO mice compared to controls (control versus KO: 1.41±0.28 versus 2.11±0.44 mg/dL, p<0.05). Histologic examination showed tubular vacuolization and structural disruption induced by iohexol were aggravated in Nrf2 KO mice, and significant increase of apoptosis and F4/80 (+) inflammatory cells infiltration were also demonstrated in Nrf2 KO mice compared to controls. In vitro study, Nrf2 inhibition with siRNA decreased cell viability measured by MTT assay (vehicle versus siRNA: 0.480±0.05 versus 0.346±0.05, p<0.05) and increased caspase 3 expression. Increase of reactive oxygen species (ROS) after iohexol treatment was augmented with Nrf2 inhibition both in vivo and vitro study measured by CM-DCFDA. Decreased expression of anti-oxidant factor such as HO-1 was accompanied after Nrf2 inhibition, which led to increase of cytochrome c and NF-kB expression examined by western blot.

Conclusions: Nrf2 was suggested to be implicated in the pathogenesis of CIN through modulating anti-oxidant, anti-apoptotic, and anti-inflammatory process. Targeted therapy for proper function of Nrf2 to mitigate CIN should be studied further.

FR-PO072

Heme Oxigenase 1 Counteracts Contrast-Induced Acute Kidney Injury in Diabetic Rats Cassiane Dezoti Fonseca, Mirian Watanabe, Maria de Fatima Vattimo. *Experimental Laboratory of Models Animals, School of Nursing- USP, Sao Paulo, Brazil.*

Background: Contrast-induced acute kidney injury (CI-AKI) is a toxic nephropathy that results from intrarenal vasoconstriction and direct tubular toxicity with generation oxygen species (ROS). The decreased tissue oxygen in medula in preexisting renal dysfunction, as in Diabetes Mellitus (DM), can be a risk factor for CI-AKI. This study evaluated the effect of HO 1 in the restoration of oxidant injury of CI-AKI in diabetic rats.

Methods: Adult male Wistar rats (250-300g) were used. Physiological parameters; renal function (creatinine clearance, crCl); oxidative injury (urinary peroxides, UP, tiobarbituric acid reactive substances-TBARS and thiols in renal tissue) and kidney histological analysis were evaluated. Rats were submitted to left uninephrectomy (Nx) on the 1° day. The DM was induced by a single dose of intravenous streptozotocin (65mg/kg i.v.) on the 20° day. The iodine contrast (IC) meglumine ioxithalamate (6ml/kg) and hemin (HO 1 inducer;10mg/kg; i.p.;60 minutes before IC) were infused on the 85° day. Groups: Control (Nx+Citrate); Nx+DM; Nx+DM+IC; Nx+DM+IC+H.

Results: Diabetic groups showed polyphagia, polydipsia, polyuria, increase in the blood glucose and reduction in body weight ($p<0.05$). IC reduced the crCl and thiols in renal tissue with a proeminent increase in UP and TBARS. These paramethers were significantly changed by hemin. Kidney histology showed tubular cells vacuolization and edema with moderate injury in IC animals.

Groups/n	cr/Cl/100g ml/min	Urinary Peroxides nmol/g creatinine	TBARS nmol/g creatinine	Thiols nmol/mg total protein
Control/6	0.5±0.8	9±2	0.2±0.7	12±4
Nx+DM/6	0.3±0.5α	46±21α	2.2±0.6α	7±2
Nx+DM+IC/6	0.2±0.3αβ	45±10α	4.2±1.0αβ	5±1α
Nx+DM+IC+H/6	0.4±0.7αβδ	26±13αβδ	1.6±0.2αδ	14±3βδ

Renal function, oxidative index.αp<0.05vsControl;β vs Nx+DM;δ vsNx+DM+CI.

Conclusions: The data confirm DM as risk factor for CI-AKI and highlight the HO 1 renoprotective effect in oxidative damage in this model of nephropathy.

FR-PO073

Lp25 Membrane Protein from Pathogenic *Leptospires* Is Associated with Rhabdomyolisis and Oliguric Acute Kidney Injury in Leptospirosis Antonio C. Seguro,[1] Daniele Canale,[1] Patricia A. Abreu,[2] Denize Monaris,[2] Tatiana B. Gotti,[2] Larissa R. Matos,[2] Thales de Brito,[1] Antonio J. Magaldi.[1] *[1]LIM-12 Nephrology, Hospital das Clínicas FMUSP, São Paulo, Brazil; [2]Lab. de Bacteriologia, Inst Butantan, São Paulo, Brazil.*

Background: Acute kidney injury (AKI) in leptospirosis is frequently nonoliguric with hypo or normokalemia. Higher serum potassium levels were observed in nonsurvivor patients and may have been caused by more severe AKI, metabolic derangement, or rhabdomyolysis. An association between the creatine phosphokinase (CPK) level and the maximum serum creatinine level was observed in these patients and could suggest that rhabdomyolysis may contribute to severe AKI and hyperkalemia (Clin Infect Dis 1999;29:1561). LipL 32 and Lp25 are membrane proteins from pathogenic *Leptospires*. The purpose of this study was to evaluate the effect of these proteins on renal function in guinea pigs.

Methods: Three groups of animals were studied: 1- Sham (phosphate-buffered solution), n=7; 2- LipL32, n=7 and 3- Lp25, n=8. All animals received daily 400µl injections intraperitoneally of each substance (160µg of LipL32 or Lp25/dose) for 4 days, after which they were placed in metabolic cages for 24 hour urine collection. We measured urinary volume (Ur V mL/24h), creatinine clearance (ml/min/100gBW), serum potassium (mEq/L), CPK (U/L), uric acid (mg/dL), phosphate (mg/dL). Data are mean±SEM.

Results:

	Cr Cl	Ur V	K	CPK	Uric acid	P
sham	1.10±1.18	27.7±4.8	4.8±0.3	499±48	1.08±0.19	6.9±0.3
LipL32	1.08±0.11	19.4±6.1	5.7±0.6	656±152	1.07±0.31	6.1±0.3
Lp25	$0.48±0.05^{b,d}$	$11.1±1.8^{a}$	$6.8±0.5^{a}$	$2852±495^{b,e}$	$4.15±0.48^{c,e}$	$9.6±0.6^{c,e}$

$^{a}p<0.05$ $^{b}P<0.01$ $^{c}p<0.001$ versus sham $^{d}p<0.05$ $^{e}p<0.001$ versus LpL32.

Conclusions: These data demonstrated that Lp25 is a membrane protein from pathogenic Leptospires responsible for the development of oliguric AKI in leptospirosis associated with hyperkalemia induced by rhabdomyolysis (elevated CPK, uric acid and serum phosphate). Therapeutic maneuvers to inhibit this protein or even to attenuate rhabdomyolisis- induced AKI may protect animals and even patients from severe forms of this disease and decrease mortality. (CNPq,FAPESP).

FR-PO074

Curcumin Prevents Maleate-Induced Nephropathy, Oxidative Stress, Apoptosis and Decrease of Mitochondrial Oxygen Consumption and in the Activity of Respiratory Complex I Edilia Tapia,[1] L. Gabriela Sanchez-Lozada,[1] Wylly Ramses Garcia,[2] Fernando E. García-Arroyo,[1] Horacio Osorio,[1] Magdalena Cristobal,[1] Abraham Said Arellano-Buendia,[1] Maria L. Loredo,[3] Eduardo Molina-Jijon,[4] Jacqueline Hernandez-Damian,[2] Mario Negrette-Guzmán,[2] Jose L. Reyes,[4] Cecilia Zazueta,[5] Magdalena Madero,[1] Jose Pedraza-Chaverri.[2] *[1]Laboratory Renal Physiopathology. Nephrology, INCIC, Mexico City, DF, Mexico; [2]Biology, Facultad de Quimica, UNAM, Mexico City, D.F., Mexico; [3]School of Medicine, U Panamericana, Mexico City, D.F., Mexico; [4]Physiology CINVESTAV, IPN, Mexico City, D.F., Mexico; [5]Cardiovasc Med, INCIC, Mexico City, D.F., Mexico.*

Background: The potential protective effect of the dietary antioxidant curcumin against the renal injury induced by maleate was evaluated.

Methods: Maleate was injected IP (400 mg/Kg) by a single injection in rats; other group received curcumin (120 mg/kg for 6 days) in addition to maleate. RBF, GFR, urine sodium, glucose, NAG, NGAL, renal structural changes, and the expression of claudin-2 and KIM-1, tubular proteinuria were evaluated. Oxidative stress was determined by measuring the oxidation of lipids and proteins and diminution in renal Nrf2 levels. Studies were also conducted in mitochondria isolated from kidneys of all groups and in renal epithelial LLC-PK1 cells.

Results: Maleate-induced renal injury included increase in RVR, proteinuria, glucose, sodium, NGAL and NAG, upregulation of KIM-1, decrease in RBF and claudin-2 expression besides of necrosis and apoptosis of tubular cells on 24 h. Maleate treatment reduced oxygen consumption in ADP-stimulated mitochondria and diminished respiratory control index when using malate/glutamate as substrate. The activities of both complex I and aconitase were also diminished. In *in vitro* studies, maleate-induced cell death and reactive oxygen species (ROS) production in LLC-PK1 cells in culture. All the above-described alterations were prevented by curcumin.

Conclusions: Curcumin is able to attenuate maleate-induced nephropathy. The *in vivo* protection was associated to the prevention of oxidative stress and preservation of mitochondrial oxygen consumption and activity of respiratory complex I and the *in vitro* protection was associated to the prevention of ROS production.

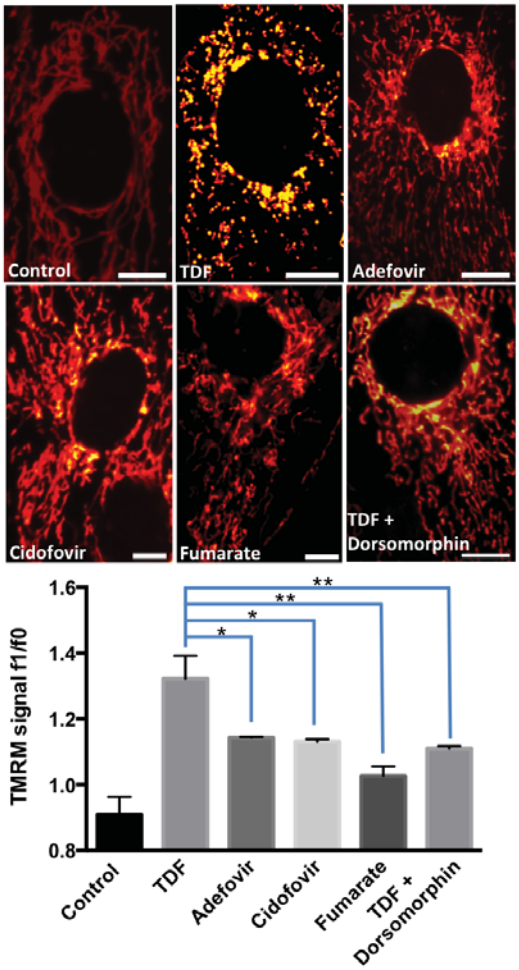
FR-PO075

Tenofovir Induces Mitochondrial Dysfunction in the Kidney via AMP Activated Protein Kinase Holly R. Courtneidge,[1] Eilidh Craigie,[2] Claire M. Peppiatt-Wildman,[3] Robert J. Unwin,[1] Andrew Michael Hall.[2] *[1]Centre for Nephrology, UCL, London, United Kingdom; [2]Swiss National Centre of Competence in Research, Univ of Zurich, Zurich, Switzerland; [3]Urinary System Physiology Unit, The Univs of Kent & Greenwich at Medway, Medway, United Kingdom.*

Background: Renal proximal tubule (PT) toxicity is recognized in patients taking the NtRTI Tenofovir disoproxil fumarate (TDF). *In vitro* tests suggest TDF is not toxic to mitochondria, but abnormalities in mitochondrial morphology are a feature of TDF toxicity. We used live multiphoton imaging to establish the mechanism of TDF toxicity, revealing TDF as a direct activator of AMPK.

Methods: To study mitochondrial membrane potential (DY_m) and reactive oxygen species (ROS) production, we used TMRM (tetramethylrhodamine-methyl-ester) and MitoSOX, respectively, with multiphoton microscopy.

Results:



TDF 200 µM caused a rapid increase in DY_m in Llcpk-1 cells. After 30 minutes, mean TMRM signal in TDF cells was greater (≤P<0.05) than: control, fumarate, NtRTIs cidofovir and adefovir. DY_m increase was followed by mitochondrial fragmentation (<30 min) and cell death in TDF cells. Fumarate, cidofovir and adefovir did not change mitochondrial morphology. Effects were blocked by treatment of AMPK inhibitor dorsomorphin prior to TDF insult (fig1). Similar changes were seen in PT cells in live kidney slices. TDF increased ROS production in Llcpk-1 cells; after 30 minutes MitoSOX signal was higher (P<0.05) in TDF cells than in control cells.

Conclusions: Live imaging in cultured cells *and* intact tissue, show TDF directly activates AMPK in PT cells. DY_m and ROS production are increased, leading to mitochondrial fragmentation and cell death. Effects of TDF are blocked by an AMPK inhibitor, and are unique to the drug rather than a class effect. Our findings implicate AMPK in TDF nephrotoxicity, and suggest inhibition of this pathway could be an effective preventative strategy.

FR-PO076

SRT1720 Prevents Renal Mitochondrial Biogenesis Suppression in Cholestatic Mice Zhi Zhong,[1] Qinglong Liu,[1] Hasibur Rehman,[1] Yasodha Krishnasamy,[1] Gyda C. Beeson,[1] Craig Cano Beeson,[1] Rick G. Schnellmann.[1,2] *[1]Drug Discovery & Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC; [2]Ralph H. Johnson VA Medical Center, Charleston, SC.*

Background: Cholestasis causes liver cirrhosis and frequently leads to acute kidney injury (AKI) by unclear mechanisms. This study tested the hypothesis that suppressed mitochondrial biogenesis (MB) during cholestasis contributes to AKI.

Methods: Mice were subjected to sham operation or bile duct ligation (BDL) and received vehicle or SRT1720 (2 mg/kg, ip, daily), a MB stimulator, for 2 wks.

Results: Blood bile acids increased ~150-fold after BDL and hepatic focal necrosis and overt liver fibrosis were observed. Serum creatinine increased >2-fold. Moderate histological changes were observed in the kidney, including loss of brush border and dilation of proximal tubules, atrophy and vacuolization of tubular cells in the cortex and medulla, and decreased cortical thickness. Renal ATP synthase-b (AS-b) and NADH dehydrogenase-3 (ND3), mitochondrial oxidative phosphorylation (OXPHOS) enzymes that are encoded by nuclear and mitochondrial DNAs, respectively, decreased markedly after BDL, suggesting suppression of MB. Expression of PGC-1a, the master regulator of MB, decreased substantially after BDL, revealing disruption of MB signaling in vivo. Incubation of primary cultures of renal proximal tubular cells (RPTC) with 0-200 mM chenodeoxycholic acid for 24 h reduced maximal oxygen consumption in a concentration-dependent manner, demonstrating direct inhibition of mitochondrial function in RPTC by toxic bile acids. SRT1720 treatment of cholestatic mice increased PGC-1a deacetylation and expression and enhanced AS-b and ND3 expression in the kidney. SRT1720 treatment also prevented decreases in renal cortical thickness, attenuated other renal pathological changes, and reduced serum creatinine.

Conclusions: MB suppression most likely plays an important role in AKI occurrence in mice with cholestatic liver fibrosis. MB is a novel therapeutic target to prevent/treat hepatic failure-induced AKI.

Funding: NIDDK Support

FR-PO077

The Specific Sphingosine Kinase-2 (SK2) Inhibitor ABC294640 Decreases Renal Injury after Orthotopic Liver Transplantation in Rats Zhi Zhong,[1] Qinglong Liu,[1] Yasodha Krishnasamy,[1] Hasibur Rehman,[1] John J. Lemasters,[1] Charles D. Smith,[1,2] Rick G. Schnellmann.[1,3] *[1]Drug Discovery & Biomedical Sciences, Medical Univ of South Carolina, Charleston, SC; [2]Apogee Biotechnology Corporation, Hummelstown, PA; [3]Ralph H. Johnson VA Medical Center, Charleston, SC.*

Background: Acute kidney injury (AKI) occurs frequently in perioperative liver transplant (LT) recipients, which increases hospital stay, acute rejection, infection and mortality. This study investigated whether ABC294640 attenuates renal injury after LT.

Methods: Livers were explanted from Lewis rats and stored in the UW storage solution with/without ABC294640 (100 µM) for 18 h before implantation in Lewis recipients. Liver, kidney and blood were collected 18 h after transplantation.

Results: Focal necrosis, apoptosis and inflammatory responses occurred in liver grafts and serum ALT and total bilirubin increased markedly after LT. ABC294640 decreased liver injury and inflammation and improved liver graft function. Seven-day survival after LT was reduced to 25% and ABC294640 increased survival to ~85%. After LT, serum creatinine increased >2 fold and NGAL expression in the kidney increased ~17 fold. Mild to moderate histological changes were observed in the kidney, including loss of brush border, vacuolization of tubular cells in the cortex, cast formation and necrosis in some proximal tubular cells. TLR4 expression, NF-kB p65 phosphorylation, myeloperoxidase and ED-1 increased in the kidney after LT, indicating inflammatory responses. ABC294640 treatment attenuated pathological changes and inhibited inflammatory responses in the kidney and decreased serum creatinine after LT.

Conclusions: AKI occurs after transplantation of liver grafts exposed to extended cold storage, which may also contribute to increased mortality after LT. ABC294640 attenuates hepatic graft failure-induced AKI.

Funding: NIDDK Support

FR-PO078

Cardiac Mitochondria Injury Induced by Renal Ischemia: Possible Role of Heart-Kidney Crosstalk in AKI Maki Sumida,[1] Kent Doi,[2] Emi Ogasawara,[1] Tetsushi Yamashita,[1] Yoshifumi Hamasaki,[1,3] Naoki Yahagi,[2] Masaomi Nangaku,[1] Eisei Noiri.[1,4] *[1]Nephrology and Endocrinology, Univ of Tokyo; [2]Emergency and Critical Care Medicine, Univ of Tokyo; [3]22nd Century Medical and Research Center, Univ of Tokyo; [4]Science and Technology Research Partnership for Sustainable Development, Japan Science and Technology Agency/ Japan International Cooperation Agency, Tokyo, Japan.*

Background: Experimental evidences have clarified distant organ dysfunctions induced by acute kidney injury (AKI). Although crosstalk between kidney and heart, which has recently been recognized as cardiorenal syndrome, appears to play an important role in the clinical settings, the mechanisms by which AKI causes cardiac injury remains poorly understood.

Methods: Renal ischemia reperfusion (IR) injury was induced by bilateral renal artery clamping for 30 minutes in eight-week old male C57BL/6 mice. Mitochondrial morphology in the heart at 24 hours after renal IR was evaluated by electron microscopy. Mitochondria dynamics regulatory proteins including Drp1, Mfn1/2, OPA1 were analyzed by western blot. Apoptosis in the heart was examined by cytochrome c release, activation of caspase-3, and TUNEL staining. A Drp-1 inhibitor mdivi-1 at the dose of 50mg/kg was administered to clarify the role of Drp-1 in mitochondrial fragmentation in the heart in renal IR model.

Results: Renal IR significantly reduced mitochondrial area in the heart compared with sham-operation (IR 0.45±0.26mm^2 versus Sham 0.85±0.35mm^2, n=5/group, p<0.01). Among the mitochondria regulatory proteins, only Drp1 was increased in the mitochondrial fraction of heart tissue (p<0.05). Apoptosis in cardiac cells were confirmed by increased cytochrome c in the cytosol fraction, positive staining of TUNEL and immunohistochemistry of activated casepse-3. Treatment of mdivi-1 suppressed decrease of mitochondrial area (IR 0.51±0.27mm^2 versus IR+midiv-1 0.88±0.44mm^2, n=8-10/group, p<0.01) and subsequent apoptosis in the heart, although renal dysfunction was partially improved by mdivi-1.

Conclusions: The present study demonstrated that renal IR injury induced mitochondria fission and apoptosis in the heart. This remote organ injury induced by AKI was mediated by mitochondrial fission protein Drp1.

FR-PO079

Charting the Course of Renal Cryo-Injury and Repair Brian B. Ratliff, Wasan Abdulmahdi, Joseph A. Zullo, Lauren M. Nesi, Kei Matsumoto, Michael S. Goligorsky. *New York Medical College, NY.*

Background: Advancement in therapeutic strategies targeting kidney injury and repair has been hampered due to the lack of a relevant and consistent injury model that permits examination of renal repair processes. We sought to characterize a novel renal cryo-injury that allows investigation into endogenous repair mechanisms and the potential to maximize these processes intrinsically and exogenously.

Methods: We examined kidney repair in mice after induction of a cryo-injury, in which the transient superficial application of a liquid nitrogen cooled cryo-probe to the exposed kidney induces a localized injury. The resulting cryo-injury (and its subsequent repair) was examined immunohistochemically and by Laser-Doppler flowmetry.

Results: Within hours of cryo-injury induction, substantial glomerular, tubular and vascular injury was observed (H&E staining), while blood flow in the injured area was reduced over 60%. The injured area demonstrated a peak in tubular and perivascular cell proliferation (Ki-67 staining) at 4 days post-injury, while apoptosis (TUNEL staining) peaked at 7 days. Infiltration of macrophages into the injured area was first observed at day 4 (F4/80 and CD11 staining), and peaked at 7 days post-injury. Vascularization of the injured area (CD31 staining) was lowest 7 days after injury, while fibrosis peaked at this same time point. After 4 weeks, the area of damage was reduced by 75% (H&E staining), while blood flow in the injury's penumbral area was restored to normal levels despite continued flow impediment in areas where damage was most severe. The exogenous application of LIF (Leukemia Inhibitory Factor), an inducer of epithelial repair, enhanced tubular and perivascular cell proliferation as well as vessel repair.

Conclusions: Kidney cryo-injury provides a facile model for the study of endogenous repair processes and dynamics. Furthermore, the cryo-injury model also allows examination of the therapeutic potential of various factors that can be introduced exogenously to the injury, such as LIF which exhibited therapeutic relevant effects when applied.

Funding: NIDDK Support, Private Foundation Support

FR-PO080

Recovery of Electrogenic Transport and Intracellular pH in Isolated Perfused Thick Ascending Limb After Acute Metabolic Ischemia Nina Himmerkus,[1] Andreas Linkermann,[2] Markus Bleich,[1] Paul S. Steels.[3] *[1]Inst of Physiology, Christian-Albrechts-Univ, Kiel, Germany; [2]Clinic for Nephrology and Hypertension, Christian-Albrechts-Univ, Kiel, Germany; [3]Biomed Inst, Univ Hasselt, Hasselt, Belgium.*

Background: We investigated the recovery of electrogenic transport and intracellular (pH_i) under different extracellular pH (pH_e) conditions after metabolic ischemia (MI) on the thick ascending limb of the loop of Henle (TAL) from mouse kidneys.

Methods: Freshly isolated TAL were microperfused and transepithelial voltage (V_{te}) and resistance (R_{te}) monitored parallel to pH_i using BCECF- microfluorimetry. NaCl transport was estimated by calculating the equivalent short circuit current (I'_{sc}). After an initial control period at pH_e 7.4 MI was induced by 10 mM 2-deoxy-glucose and 2.5 mM Na-cyanide for 5min and the subsequent washout and recovery period was investigated according to 3 different pH_e protocols: (a) MI at pH_e 6.8, 10 min recovery at pH_e 7.4, (b) MI at pH_e 6.8, 10 min recovery at pH_e 6.8 followed by 5 min at pH_e 7.4, (c) MI as well as 10 min recovery at pH_e 7.4. Cell height at several positions along the tubule was measured before, during and after ischemia.

Results: We observed a fast I'_{sc} recovery to 30 ± 6 % of pre-ischemic I'_{sc} after protocol (a) and to 35 ± 10 % after protocol (b). Protocol (c) led to a recovery of 72 ± 10 %. The initial fast drop in pH_i was comparable between groups, however, a pH_e of 7.4 during MI allowed a partial recovery already in the ischemic phase and a fast and robust recovery (103 ± 3 %) in the wash-out phase in comparison to protocol (a) (96 ± 2 %). During the pH_e 6.8 recovery phase of protocol (b) pH_i dropped again markedly and only showed full recovery in the additional pH_e 7.4 wash-out phase (98 ± 3 %). In all cases cell height as a measure of cell swelling increased during MI and recovered afterwards. The time-point of regulatory cell volume decrease coincided with the beginning of I'_{sc} recovery.

Conclusions: In comparison to acidic pH_e during an acute pathophysiological state of MI, physiological pH_e of 7.4 during this period counteracted the drop in pH_i and ameliorated the transport function of TAL immediately after ischemia/reperfusion injury.

Funding: Government Support - Non-U.S.

FR-PO081

Megalin Recycling in Proximal Tubule: Compartments and Sorting Mechanisms Involved Andres E. Perez Bay, Ryan Schreiner, Enrique Rodriguez-Boulan. *Dept of Ophthalmology, Margaret Dyson Vision Research Inst, Weill Cornell Medical College, New York, NY.*

Protein reabsorption from the glomerular ultrafiltrate is an important function of the kidney proximal tubule (PT), mediated by the apical co-receptors Megalin/Cubilin. Mutations in these receptors that impair protein absorption cause proteinuria in several human syndromes. Furthermore, abnormally high absorption of immunoglobulin light chains in multiple myeloma patients by Megalin/Cubilin causes acute kidney failure. Megalin endocytosis requires endocytic signals, clathrin and the clathrin adaptors ARH, DAB2 and AP-2. In contrast, the recycling branch of Megalin is poorly understood: whereas a tyrosine apical sorting signal has been identified, the sorting adaptor (s) and the sorting compartments involved remain unknown. Here we studied the recycling pathway of Megalin comparatively with that of the well-characterized fast-recycling basolateral receptor, transferrin receptor (TfR), using the prototypical kidney cell line MDCK and a human PT cell line (RPTEC-hTERT) that polarizes efficiently and preserves the phenotype of kidney PT. Apically internalized Megalin recycles successively through WGA-positive apical sorting endosomes, TfR-positive common recycling endosomes (CRE) and Rab11a-positive apical recycling endosomes (ARE) with half-times of 7, 12 and 17 minutes, respectively. Apically internalized Megalin mixes with basolaterally internalized TfR at CRE, suggesting that this is the compartment where sorting of these recycling receptors occurs. From CRE, Megalin recycles back to the apical domain via ARE and TfR translocates directly to the basolateral domain, mediated by clathrin and the clathrin adaptor AP-1B. AP-1B knock-down in MDCK cells promotes TfR translocation from CRE to ARE and the apical surface via a novel transcytotic route that utilizes non-centrosomal microtubules, the kinesin KIF16B, the small GTPase rab11a (Perez-Bay et al, EMBO J 2013), a N-glycan apical signal in TfR and the sorting lectin galectin-4 (Perez-Bay et al., in revision). Current experiments are focused on characterizing the role of clathrin and clathrin adaptors in apical recycling of Megalin.

Funding: Other NIH Support - NIH grants GM34107 and EY08538

FR-PO082

Unique Transcriptional Programs in Subtypes of Acute Kidney Injury: Implications for Reclassification Katherine Xu,[1] Paul Rosenstiel,[1] Paolo Guarnieri,[1] Neal A. Paragas,[1] Christian Hinze,[2] Kai M. Schmidt-Ott,[2] Jonathan M. Barasch.[1] *[1]Columbia Univ; [2]Max Delbrueck Center for Molecular Medicine.*

Background: The current diagnosis of AKI is contingent on the rise of a single analyte, serum creatinine (sCr), but this test is confounded by delay (>24hrs), insensitivity (sometimes >50% of the kidney must be damaged), and non-specificity (causes include volume depletion [VD], ischemia, nephrotoxins, and obstruction).

Methods: To study AKI subtypes, we used laser capture to isolate glomeruli, cortex, outer and inner stripes of the outer medulla from kidneys of mice subjected to 10min of bilateral ischemia reperfusion injury (IR) or mice subjected to 72h of VD by water deprivation. Transcriptional profiles, differentially expressed genes (DEGs), and altered pathways were detected in each region of the kidney by RNA-Seq. To identify candidate secreted proteins we filtered genes with annotation databases (Secreted ProteinDB, Max Plank Unified Proteome) and then validated using urine samples collected in Emergency Departments.

Results: The two groups demonstrated similar changes in sCr, but VD mice demonstrated higher sNa and hemoconcentration. IR induced a greater transcriptional effect than VD (10x more DEGs). Overlapping transcriptional changes were only a small fraction of IR (2.6%) and VD (27%) DEGs. The outer stripe showed the strongest contribution in IR while the inner stripe was most activated in VD. In addition, functional enrichment analyses demonstrated little overlap in pathways. Identification of secreted proteins revealed 109 unique to IR and 10 to VD. In fact, some of these proteins may serve as biomarkers, because they distinguished prolonged (typical of IR) from rapidly reversible (typical of VD) AKI in patients.

Conclusions: We found that despite similar elevations of sCr, IR and VD affected (i) different regions of the kidney, (ii) different sets of genes and secreted proteins, and (iii) different pathways. Hence the diagnosis of AKI based solely on sCr erroneously combines conditions with region specific transcriptional profiles that have little overlap. Novel biomarkers of VD and IR may reclassify sCr-based diagnoses and provide appropriate patient management in urgent settings.

Funding: NIDDK Support

FR-PO083

Delayed Administration of a Glycogen Synthase Kinase 3b (GSK 3b) Inhibitor Improves Function and Ameliorates Renal Fibrosis following Acute Kidney Injury (AKI) Chunming Jiang,[1,2] Evelyn Tolbert, Weiwei Xu,[1] Hui Bao,[1] Rujun Gong,[1] Lance D. Dworkin.[1] *[1]Nephrology, Medicine, Brown Univ, Rhode Island Hospital, Providence, RI; [2]Nephrology, Drum Tower Hospital, Nanjing Univ, Nanjing, Jiangsu, China.*

Background: Specific inhibition of GSK 3b prior to kidney insult attenuates AKI and accelerates recovery. However, most patients with AKI present after injury is established and whether delayed inhibition of GSK 3b is similarly protective is unknown. The long term effects of GSK 3b inhibition on renal fibrosis and development chronic kidney disease (CKD) after AKI are also unclear. This study examined the effects of delayed administration of TDZD-8, a specific inhibitor of GSK 3b, on the acute and chronic phases of disease in mice with folic acid (FA) induced kidney injury.

Methods: C57BL/6 mice were randomly divided into four groups; sham, FA, TDZD-PRE (30 minutes prior to FA), and TDZD-POST (36 hours after FA). Kidney tissue was examined on days 0, 3, and 28 after FA.

Results: Kidney function assessed by serum creatinine was significantly improved in TDZD-PRE and TDZD-POST at day3 and 28 as compared to FA. Mice in both treated groups showed less tubular injury and apoptosis, more dedifferentiation and proliferation by staining for KIM-1 and NGAL, TUNEL, Pax-2 and Ki-67. Three days after injury, expression of TGF-β and CTGF was significantly decreased to 24.0% and 28.6%, 28.6% and 57.1% in TDZD-PRE and TDZD-POST, respectively. This was associated with a significant reduction in chronic renal fibrosis at 28 days assessed morphologically by Masson's Trichrome, collagen I and fibronectin staining. Quantitatively compared to FA, total kidney collagen was reduced by 42.5% (TDZD-PRE) and 54.7% (TDZD-POST).

Conclusions: More clinically relevant, delayed administration of a small molecule GSK 3b inhibitor improved kidney function and attenuated chronic persistent renal fibrosis effectively in mice with AKI. Reduction in renal fibrosis probably resulted from amelioration of acute injury and reduced expression of pro-fibrotic growth factors TGF-β and CTGF. Longer term studies are needed to determine if therapy also reduces the risk of progression to ESRD.

Funding: Clinical Revenue Support

FR-PO084

Soluble RAGE Prevents Unilateral Ureteral Obstruction-Induced Renal Fibrosis Tae-Hyun Yoo,[1] Seonghun Kim,[2] Eunyoung Lee,[1] Hye-Young Kang,[2] Ji Sun Paeng,[2] Bo Young Nam,[2] Shin-Wook Kang.[1,2] *[1]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [2]Brain Korea 21 PLUS, Severance Biomedical Science Inst, Yonsei Univ, Seoul, Korea.*

Background: Tubulointerstitial fibrosis is a typical pathological finding in chronic kidney disease and is also associated with the progression of renal dysfunction. Recently, high mobility group protein box1 (HMGB1) and receptor for advanced glycation end products (RAGE) have been demonstrated to be implicated in the pathogenesis of epithelial-mesenchymal transition in renal tubular cells (RTCs). Although soluble RAGE (sRAGE) has been proposed as a potential therapeutic agent by competitively inhibiting to bind RAGE, however, little is known about the efficacy of sRAGE on renal fibrosis in unilateral ureteral obstruction (UUO) model.

Methods: In vivo, Sprague-Dawley rats were treated 1 hour before and every 48 hours after UUO operation either with diluent (n=6) or sRAGE (n=6) intraperitoneally. At the time of sacrifice, blood and renal tissues were collected. In vitro, NRK-52E cells were cultured in DMEM media with or without HMGB1 (10 mg/mL). To examine the effect of sRAGE on HMGB1-induced RTCs injury, NRK-52E cells were also incubated with sRAGE (1 mg/uL).

Results: The protein expression of RAGE was significantly higher in the kidney of UUO rats compared to controls. The renal protein expressions of JNK, ERK, and fibrosis-related molecules such as fibronectin and collagen I were also significantly higher in UUO rats compared to controls. These changes in UUO rats were significantly ameliorated by sRAGE treatment. Moreover, histological tubulointerstitial injury was significantly improved after sRAGE injection in UUO rats. In vitro, the expressions of fibronectin and collagen I protein were significantly higher in RTCs stimulated by HMGB1 and these increases were significantly attenuated by sRAGE. NF-kB and MAPK pathways were also activated in HMGB1-stimulated RTCs, which were significantly abrogated by RAGE inhibition.

Conclusions: These findings suggest that RAGE plays a role in the pathogenesis of renal fibrosis and its inhibition by sRAGE may be a potential therapeutic strategy for renal fibrosis.

FR-PO085

Loss of Alpha (E)-Catenin Disrupts Actin Organization in the Aging Kidney LaNita A. Nichols, Elizabeth A. Borgmann, Alan R. Parrish. *Medical Pharmacology and Physiology, Univ of Missouri School of Medicine, Columbia, MO.*

Background: As the kidney ages, morphological and functional changes lead to renal dysfunction. We have previously shown the aging kidney has a marked loss of the cadherin/a-catenin complex in the proximal tubular epithelium. Cadherin-mediated cell adhesion influences cytoskeletal organization while the actin cytoskeleton is necessary for cadherin-mediated adhesion. a-catenin is a key regulator of the actin cytoskeleton, inhibiting Arp2/3 formation of branched actin while increasing formation of linear actin. Therefore, we hypothesize the loss of a-catenin leads to disruption of the cytoskeleton of the aging kidney leading to renal dysfunction.

Methods: Stable shRNA knock-down of a (E)-catenin was generated in NRK-52E cells. Single cell clones were selected for a non-targeted (NT3) control and a targeted knock-down (C2).

Results: qPCR and Western blots confirmed not only reduced a-catenin levels, but N-cadherin as well. Phalloidin staining of C2 showed overall reduced F-actin staining with increased branching and disorganization as compared to NT3 control; disorganization of the actin cytoskeleton was also seen in the tubular epithelium in the aged rat kidney. Gene expression from migrating C2 and NT3 cells was quantified using RT^2 Profiler PCR for Cytoskeleton Regulators (SA Biosciences). Was, a member of the WASP family, increased 2.87 fold in C2, and is one of only two up-regulated genes. Myosin Light Chain Kinase 2 (Mylk2), required for cellular contraction, is the most reduced at 0.19 fold. Fscn2 (Fascin homolog2), an actin bundling protein is also reduced. Interestingly, Arpc2 and Arpc3 gene expression was not significantly changed. Gene expression of young (4m) versus aged (24m) Fischer 344 rats for Was, Mylk2, and Fscn2 was confirmed by qPCR. Was increased 5.42±1.55 at 24m while Mylk2 and Fscn2 decreased 0.56±0.20 and 0.47±0.09, respectively.

Conclusions: These data demonstrate that the loss of a (E)-catenin affects the regulation of the cytoskeleton in the aging kidney, which has implications in renal function, as well as in injury.

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FR-PO086

No Evidence for an Improved Safety Profile of Oral Sodium Phosphate By Using Intestinal Phosphate Binders Stef Robijn, Geert Dams, Benjamin Arthur Vervaet, Patrick C. D'Haese, Anja Verhulst. *Laboratory of Pathophysiology, Univ of Antwerp, Antwerp, Belgium.*

Background: Prior to colonoscopy, bowel cleansing is performed for which OSP is frequently used. An increasing number of patients developing acute kidney injury (AKI) referred to as APN and characterized by severe nephrocalcinosis (NC) is reported after the use of OSP. US-FDA issued a safety alert stating that OSP should only be available by prescription and suggested that investigation should focus on preventing intestinal P absorption to improve the safety profile of OSP. In the current study it was evaluated whether the acute effects of OSP could be prevented by the use of intestinal P-binders without affecting bowel cleansing efficiency. Hereto a rat bowel cleansing model using OSP and resulting in APN was optimized.

Methods: OSP was administered by gavage to male Wistar rats in 2 doses of 1.2 g phosphate with a time interval of 12 hours. Serum P, calcium (Ca), creatinine, iPTH and iFGF-23 concentrations were measured at several time points after gavage. The degree of NC was assessed by measuring total Ca in renal tissue. P-binders were administrated to rats by gavage (1000mg lanthanum carbonate or aluminum hydroxide) immediately preceding OSP gavages.

Results: OSP administration resulted in significantly increased serum P levels (21.8±5.1mg/dl 6h after the 2nd dose versus 8.4±5.1mg/dl at baseline) and concomitantly decreased serum Ca levels (8.7±0.8 versus 11.3±0.5 mg/dl). Serum iPTH and iFGF-23 levels increased significantly 12h after the 1st and 3h after the 2nd gavage, respectively. Renal Ca content of rats receiving OSP increased significantly (0.815 versus 0.068mg/g ww in controls). Serum creatinine significantly increased (factor 1.5) in OSP treated animals. Finally severe diarrhea inherent to bowel cleansing was observed in OSP treated animals. P-binder treatment did not affect any of these parameters.

Conclusions: In conclusion, a clinically relevant rat model to study APN as a result of OSP was developed: animals showed an increase in serum P-levels similar to that reported in humans and developed APN (NC and increased serum creatinine levels). No evidence was found for an improved safety profile of OSP by using intestinal P-binders.

Funding: Government Support - Non-U.S.

FR-PO087

Estrogen Is Protective against the Development of Acute Renal Damage Induced By Aristolochic Acid Li Zhou, Ping Fu. *Nephrology/Medicine, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

Background: Aristolochic acid (AA) is a Traditional Chinese Medicine with anti-inflammatory properties and has been shown to be a key mediator of Aristolochic Acid Nephropathy (AAN). AAN is characterized by progressive renal fibrosis and renal failure, with unclear mechanisms and therapy. Apoptosis and transformation of renal tubular epithelium cell (TEC), together with matrix accumulation are main mechanisms in AAN. In patients with acute AAN, acute kidney injury (AKI) in male is more severe than in female. Our premier data also show that with the same dose, AKI in male mice is more severe than in female mice.

Methods: The present study further tested the hypothesis that estrogen is protective against the development of acute renal damage in mice AAN model in vivo. AA at a dose of 10mg/ (kg•day) for 3 days i.p. was used to inducing acute AAN in C57BL/6 male mice. Estrogen is injected i.p. one day before AA injection and last everyday. Histology, serum creatinine, Western blot for cleaved caspase-3 and TUNEL staining for apoptosis cells have been examined.

Results: AA at a dose of 10mg/ (kg•day) for 3 days i.p. was capable of inducing acute AAN in C57BL/6 male mice. In contrast, estrogen was protected against the development of acute AAN. This was determined by the findings that estrogen prevented AA-induced acute renal injury such as histology, an increase in serum creatinine, and reduced the severity of tubular apoptosis as evident by inhibiting cleaved caspase-3 and TUNEL+ cells.

Conclusions: Estrogen is protective against the development of acute renal damage induced by aristolochic acid. Fund support: The National Basic Research Program of China (973 Program): 2012CB517700.

Funding: Government Support - Non-U.S.

FR-PO088

Kainate Receptors in Renal Injury Jason A. Funk,[1] Stacy Steele,[1] P. Darwin Bell.[1,2] *[1]Div of Nephrology, Medical Univ of South Carolina, Charleston, SC; [2]Ralph H. Johnson Veterans Affairs Medical Center, Charleston, SC.*

Domoic acid (DA) is a glutamate analog, structurally similar to kainic acid (KA), and a well-characterized neurotoxin. Because the kidney is the primary site of systemic DA elimination, and because it expresses ionotropic glutamate receptors (GluR), we chose to evaluate the renal effects of DA exposure in four-month-old Sv129/Black Swiss mice. There was evidence of renal histopathology, including proximal tubule necrosis and apoptosis, at doses ≥1.0 mg/kg. More sensitive markers, such as urinary kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL), were elevated at doses ≥0.1 mg/kg, and the early response genes *c-fos* and *junb* were increased in the kidney at doses ≥0.005 mg/kg. Pretreatment with the kainate receptor antagonist CNQX attenuated the toxic response, and administration of KA (5 mg/kg, i.p.) produced a similar toxic response. When 12-month-old mice were administered DA for 24 to 72h, renal *kim-1* mRNA and urinary KIM-1 were elevated compared to their younger counterparts, demonstrating an age effect on susceptibility to DA toxicity. HK-2 cells, a human proximal tubule cell line, robustly

expressed the kainate receptor (KR) subtypes *grik2* and *grik5*, similar to what was previously observed in the mouse kidney. DA induced rapid accumulation of intracellular calcium in HK-2 cells loaded with Fluo-4, and *c-fos*, and *kim-1* mRNA were elevated with 1-10uM DA or KA. Taken together, the data suggest that DA, and potentially other KR agonists such as KA, is a potent nephrotoxicant that elicits direct effects on renal epithelial cells. Furthermore, renal toxicity may be potentiated in aged and other susceptible populations, and thus stricter precautions may be necessary to limit DA exposure in these individuals.

FR-PO089

Specific Macrophage Subtypes Participate in Rhabdomyolysis-Induced Kidney Injury Julie Belliere,[1,2] Audrey Casemayou,[1] Alexia Zakaroff-Girard,[1] Frederic Martins,[1] Jason S. Iacovoni,[1] Benedicte Buffin-Meyer,[1] Bernard R. Pipy,[1] Dominique Chauveau,[1,2] Joost Schanstra,[1] Jean-Loup Bascands.[1] *[1]U1048, Inserm, Toulouse, France; [2]Nephrology, Hospital, Toulouse, France.*

Background: Acute kidney injury (AKI) is a life threatening complication of rhabdomyolysis. While macrophage infiltration has been reported in a rat glycerol-induced AKI model and in a kidney biopsy from a patient with rhabdomyolysis, the role or phenotype of the macrophages remains to be determined.

Methods: In a mouse model of glycerol-induced rhabdomyolysis, macrophages were obtained from digested kidney and characterized using multi-color flow cytometry phenotyping and a new method coupling cell-sorting with microfluidic-based single-cell qPCR. Liposomal clodronate (LC) was used to deplete macrophages in a systemic manner.

Results: Diverse renal macrophage phenotypes were observed depending upon the stage of the disease. Two days after rhabdomyolysis, F4/80^{low}CD11b^{high}Ly6b^{high}CD 206^{low} kidney macrophages were dominant. By day 8, we observed a shift whereby the F4/80^{high}CD11b^{+}Ly6b^{low}CD206^{high} cells became the predominant macrophage cell-type. Single-cell analyses revealed that the subpopulations were heterogeneous and that individual cells simultaneously expressed both M1 and M2 markers. Liposomal clodronate-mediated macrophage depletion improved mice survival, kidney structure and function. This indicated that the early infiltration of F4/80^{low}CD11b^{high}Ly6b^{high}CD206^{low} macrophages plays an important role in disease progression. Transcriptionally regulated targets (such as fibronectin, collagen III and chemo-attractants) that could participate in the progression of the disease, were identified via single-cell analysis and verified to be expressed in kidney in a macrophage-dependent manner *in situ.*

Conclusions: These data suggest that early depletion of a macrophage subtype limits rhabdomyolysis-induced AKI.

Funding: Government Support - Non-U.S.

FR-PO090

Novel Near Infra-Red Fluorescent Probe for Measurement of Kidney DNase I Activity *In Vivo*, *In Situ* and *In Vitro* Alexei G. Basnakian,[1,2] Tariq Fahmi,[1] Eugene Apostolov,[1] Dae Song Jang,[1] Todd Fite,[1] Alena Savenka,[1] Sudhir V. Shah.[1,2] *[1]Pharmacology and Toxicology, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Nephrology, Central Arkansas Veterans Healthcare System, Little Rock, AR.*

Background: Kidney injury results in activation of apoptotic deoxyribonuclease I (DNase I), responsible for the fragmentation of DNA before and after cell death. This DNA fragmentation occurs in all kinds of cell death including necrosis, and is the most reliable marker of irreversible cell death.However, methods are not available to measure DNase I in its natural environment *in vivo*, *in situ* and *in live* cultured cells.

Methods: We have developed a novel cell-permeable near infra-red fluorescent (NIRF) DNA probe which can be used for *in vivo, in situ* and *in vitro* measurement of enzymatic DNA cleavage by DNase I.

Results: Intravital use of this probe in mice demonstrated that most of DNase activity is located in the kidneys as opposed to other organs, mainly in cytoplasm of tubular epithelium, not in glomeruli. Patterns of the images were, therefore, very similar to the TUNEL staining indicating that the TUNEL-type DNA fragmentation is likely catalyzed by DNase I. Comparison of DNase I knockout and wild-type mice showed that DNase activity in the kidney, pancreas and salivary gland is mainly presented by DNase I, while liver and spleen have mainly other endonucleases.

Conclusions: Kidney ischemia-reperfusion injury was associated with significant increase of the intravital DNase I activity in the kidney. Overall, the probe is a new useful tool for studying DNase activity in live kidney cells, frozen/thawed kidney sections, and the kidney during injury.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO091

Non–Invasive GFR Assessment in Conscious Rats with Puromycin Induced Nephropathy Lena William-Olsson, Ulrika Lagerkvist, Charlotte Ericson, Majlis Hermansson, Anette E. Ericsson, Anna Granqvist. *Bioscience, AstraZeneca R&D Molndal, Molndal, Sweden.*

Background: Current methods for serial assessment of renal function in conscious animals are cumbersome and time consuming with considerable stress for the animals during repeated restrainment and blood sampling.We tested a novel device for transcutaneous measurement of GFR (1) in an acute kidney disease model.

Methods: Rats were given a single intravenous dose of 75, 100 or 125 mg/kg puromycin aminonucleoside (PAN) (n=8/group) to induce renal injury. Six days after PAN administration the rats received an intravenous dose of the exogenous fluorescent marker FITC sinistrin (4 mg/100g body weight). A miniature (Non-Invasive Clearance (NIC), Mannheim Pharma and Diagnostics, Mannheim, Germany) device that excites FITC sinistrin and detects the emitted light through the skin was mounted on the back of the freely moving rats. Digitalized records of the elimination kinetics of FITC sinistrin was analysed using a NIC computer software (1).

Results: There was a dose-dependent decline in GFR (0.73, 0.52 and 0.34 ml/min/100g body weight) whereas the urine albumin/creatinine ratio increased with the dose of puromycin (3.0, 3.9 and 6.3 mg/mg) compared with normal rats (1.2 ml/min/100g body weight and 0.07 mg/mg respectively).

Conclusions: Transcutaneous GFR measurements resulted in highly reproducible GFR values in line with previously published data using the invasive Cr-EDTA method (2). This new technique is well in accordance with the 3R principle for refinement, replacement and reduction in experimental animal usage and allows serial measurements longitudinally. References 1. Schock-Kusch, D. et al. (2011) Transcutaneous assessment of renal function in conscious rats with a device for measuring FITC-sinistrin disappearance curves. Kidney international, 79, 1254-125. 2. Björnson Granqvist, A. et al (2006) Podocyte proteoglycan synthesis is involved in the development of nephrotic syndrome. Am J Physiol Renal Physiol. Oct; 291 (4):F722-30.

FR-PO092

Visualization the Calcium Dynamics of Ischemia-Reperfusion Injury in Rat Proximal Tubules by a Genetically Encoded Indicator Protein Rozsa Csohany,[1] Kornélia Szebényi,[2] András Füredi,[2] Orsolya Kolacsek,[2] Agnes Prokai,[1] Katalin Kis-Petik,[3] Attila J. Szabo,[4] Tamás I. Orbán,[2] Ágota Apáti,[2] Balazs Sarkadi.[2,5] *[1]1st Dept of Pediatrics, Semmelweis Univ, Budapest, Hungary; [2]Inst of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary; [3]Dept of Biophysics and Radiation Biology, Semmelweis Univ, Budapest, Hungary; [4]MTA-SE, Pediatrics and Nephrology Research Group, Budapest, Hungary; [5]MTA-SE Molecular Biophysics Research Group, and National Blood Center, Budapest, Hungary.*

Background: Cytoplasmic calcium changes in the kidney have a crucial role in determining pathological and pharmacological responses in major renal diseases. The epithelial cells of the proximal tubule (PT) play a key role in renal physiology. Because of their high energy demand these cells are especially sensitive for ischemia-reperfusion injury. We have established a transgenic rat strain stably expressing the GCaMP2 calcium indicator protein in the kidney PT.

Methods: The transposon-based method allowed the generation of homozygous transgenic rats, containing one transgene copy per haploid genome with a defined insertion pattern, and showing no genetic or phenotypic alterations. We performed *in vitro* confocal and *in vivo* two-photon microscopy to examine ischemia-reperfusion injury-induced alterations in PT epithelial cell calcium levels.

Results: The pathologically important renal ischemia caused a rapid and transient increase in PT cellular calcium level, while reperfusion induced again a rapid and significant secondary calcium load, significantly decreased by specific blockers of the angiotensin receptor and the Na-Ca exchanger.

Conclusions: Applying this new animal model with the combination of new imaging approach opens new possibilities for physiological and pharmacological investigations; particularly we provide valuable information about the changes in PT cellular calcium levels in ischemia-reperfusion disease model. Supported by: OTKA K-108688, TÁMOP 4.2.1.B-09/1/KMR-2010-0001, SE-MTA Lendulet LP2001-008/2014, KMR_12-1-2012-0112 and KTIA_AIK_12-1-2012-0025.

Funding: Government Support - Non-U.S.

FR-PO093

Long-Term Risk of Chronic Kidney Disease and Mortality in Children after Acute Kidney Injury: A Systematic Review Jason Henry Greenberg,[1,3] Steven G. Coca,[2,3,4] Chirag R. Parikh.[2,3,4] *[1]Dept of Pediatrics, Section of Nephrology, Yale Univ School of Medicine, New Haven, CT; [2]Dept of Internal Medicine, Section of Nephrology, Yale Univ School of Medicine, New Haven, CT; [3]Program of Applied Translational Research, Yale Univ School of Medicine, New Haven, CT; [4]Dept of Internal Medicine, Section of Nephrology, VA Medical Center, West Haven, CT.*

Background: Acute kidney injury (AKI) is associated with significant short-term morbidity and mortality in children. However, the risk for long-term outcomes after AKI is largely unknown.

Methods: We performed a systematic review and meta-analysis to determine the cumulative risk of proteinuria, hypertension, decline in glomerular filtration rate (GFR), and mortality after an episode of AKI. After screening 1934 published articles from 1985-2013, we included 10 cohort studies that reported long-term outcomes after AKI in children.

Results: A total of 346 patients were included in these studies with a mean follow-up of 6.5 years (range 2-16) after AKI.The studies were of variable quality and had differing definitions of AKI with five studies only including patients who required dialysis during the AKI episode.There was a substantial discrepancy in the outcomes of the primary studies due to study size and methodological differences. In addition, there was no non-AKI comparator group in any of the published studies. The cumulative rates for proteinuria, hypertension, abnormal GFR (< 90 ml/min/1.73m^2), GFR<60 ml/min/1.73m^2, end stage renal disease, and mortality per 100 patient-years were 2.9 (95% CI 1.2-4.6), 1.4 (0.2-2.7), 6.2 (3.1-9.3), 0.8 (0 -1.6), 0.9 (0.4-1.4), and 3.5 (1.7-5.3) respectively. The mortality rate after AKI appears to be higher when compared with population mortality rates in children and the long term follow-up of other hospitalized cohorts.

Conclusions: AKI appears to be associated with a higher risk of long-term renal outcomes in children. Future prospective studies with appropriate non-AKI comparator groups will be required to confirm these results.

Funding: NIDDK Support

FR-PO094

Off-Pump or On-Pump Coronary Artery Bypass Sugery for Better Kidney Risk Profile - A Systematic Review and Meta-Analysis

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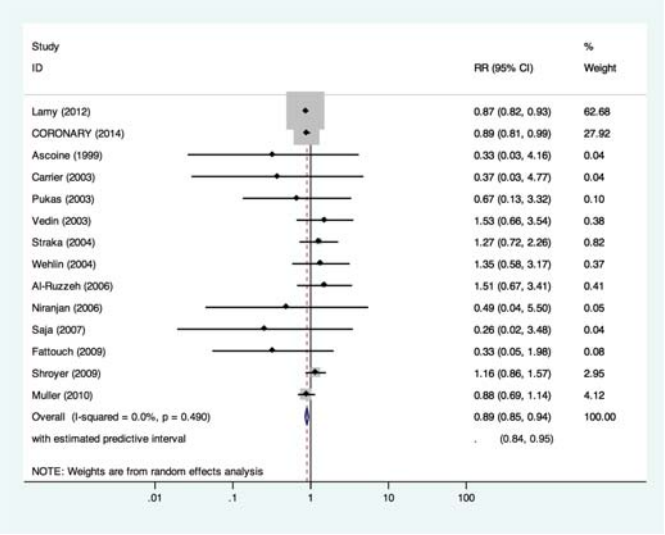
Background: Coronary artery bypass and grafting (CABG) surgery is a prevalent technique for coronary disease. Acute kidney injury (AKI) is a complication post CABG and leads to worse outcomes. Cardiopulmonary bypass (CPB) may protect the mocardium from injury, but may also exposes the patients in risk for AKI. The roles of CPB on renal outcomes are not determined. The aim of current study is to determine the role of off-pump technique on incident AKI from available literature.

Methods: The primary end-point was defined with serum creatinine-based definition for AKI. A systematic review and meta-analysis was carried out with random effect models, for presumed heterogeneity among studies. Only RCTs addressing the issue were included. We excluded studies done in pediatric patients (Age<18 years old). Studies with no events in both arms will be excluded. The heterogeneity among studies was assessed using tau-square, which will be judged high, moderate, or low, if >50%, 25%-50%, or <25%. A P-value less than 0.05 will be judged statistically significant.

Results: A total of 14 RCTs were left in final analyses, which was composed of 15,982 patients. AKI developed in 2051 (12.8%) of all patients. The tau-square was 0%. The summarized risk ratio of off-pump versus on-pump technique for incident acute kidney injury was 0.89 (95%CI=0.85-0.94). We detected no publication bias based on Egger's test (p = 0.235).



Conclusions: We demonstrated that Off-pump technique provide better kidney risk profiles than on-pump technique. The conclusion is robust based on low heterogeneiety detected.

FR-PO095

Relation of Radiographic Contrast to In-Hospital Acute Kidney Injury

Harlan Sparrow,[1] Nelda P. Wray,[1,2,3] Carol M. Ashton,[1,2,3] David Putney,[1] Linda W. Moore,[1,2] Wadi N. Suki,[1,3,4] A. Osama Gaber.[1,2,3] *[1]Houston Methodist Hospital; [2]Houston Methodist Research Inst; [3]Weill Cornell Medical College; [4]Baylor College of Medicine.*

Background: Administration of radiographic contrast (CON) is believed to increase Acute Kidney Injury (AKI) in hospitalized patients. However, most studies have neglected to include a control group when assessing CON-related AKI (CR-AKI).

Methods: In-hospital AKI was defined as an increase in serum creatinine (SCr) by >=0.3 mg/dL or >=50% or a decrease in estimated Glomerular Filtration Rate by >=25% over any 72h interval during the hospitalization. CRAKI was similarly defined based on comparing SCr baseline values to SCr changes within 72h post CON. The first hospitalization of patients admitted in 2012 to a tertiary academic medical center were included. Patients <18 years, those with preexisting AKI, stage 5 CKD, dialysis on admission, a maximum serum creatinine (SCr) <=0.4 mg/dL or AKI prior to CON were excluded as were, patients lacking SCr values for the ascertainment of AKI or CRAKI. Doses administered within 60min or less of each other were considered a single-dose.

Results: Demographic and hospital data of 13,406 unique patients included in the study were as follows: AKI 17%, 31% had one or more ICU days, 52% were female, and 64% were Caucasian. Patients with AKI were older (66 versus 61 yrs p<0.001) and more likely black (19% p=0.008). Approximately 30% of patients received CON of some type.

CON Exposure	% Total Population	% CR-AKI	% AKI any time post-CON
No CON	70		17
Oral Iodinated	0.01	4	6
IV Gadolinium	5	6	10
IV Iodinated Multi-Dose Same Day	0.01	7	14
IV Iodinated – Single Dose	21	7	15
IV Iodinated Multi-Dose Different Day	4	16	29

The great majority of AKI occurred more than 72 hours after CON administration. Further, the overall incidence of AKI after CON was lower than the AKI rate in patients who did not receive CON except for patients receiving multi-dose different day CON. All p values<0.001.

Conclusions: This retrospective analysis of nearly 14,000 patients provides no evidence that the use of radiographic CON in a single procedure increases the incidence of AKI compared to non-CON patients. However, multi-dose CON is associated with more AKI.

FR-PO096

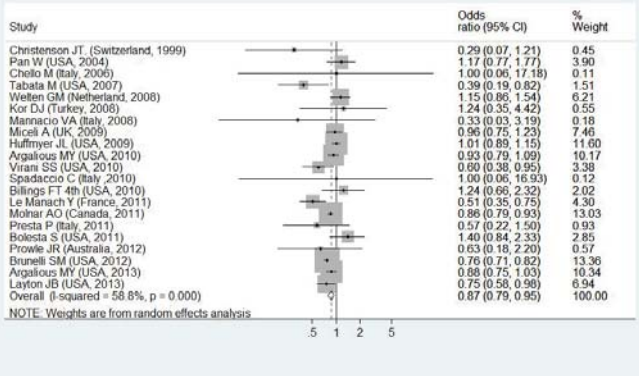
The Effect of Preoperative Statin Therapy on Postoperative Acute Kidney Injury in Patients Undergoing Major Surgery: Systemic Review and Meta-Analysis

Szu Yu Pan,[1] Taomin Huang,[1] Vincent Wu.[2] *[1]National Taiwan Univ Hospital, Yun-Lin branch; [2]National Taiwan Univ Hospital.*

Background: The effect of preoperative statin therapy on postoperative acute kidney injury (AKI) is contraversial. We performed a systemic review and meta-analysis to exam the association.

Methods: MEDLINE and EMBASE, from inception to April 2013, and the reference lists of relevant articles were searched. Trials comparing preoperative statin therapy with no preoperative statin in patients undergoing major surgery were included. Outcome measures of interest were the risk of cumulative postoperative AKI and postoperative AKI requiring renal replacement therapy (RRT). No language restrictions were applied. Authorship, country, year of publication, study population, study exposure, study outcome, crude and adjusted effect size, and confidence interval (CI) were extracted. Fixed or random effect meta-analysis was performed to derive summary effect estimates.

Results: In 5 randomized studies and 19 observational studies, comprising a total of 983,173 patients undergoing major surgery, 112,840 patients (11.4%) received preoperative statin therapy. Preoperative statin therapy was associated with a significant risk reduction for cumulative postoperative AKI (weighted summary odds ratio 0.87, 95% CI 0.79-0.95). (Figure 1) The effect of risk reduction was also significant when considering postoperative AKI requiring RRT (odds ratio 0.80, 95% CI 0.72-0.90). Various sources of heterogeneity existed among included studies. The types of surgery varied in each study. The specific type, dosage, and duration of statin therapy were not available in most studies. The definition of AKI was not uniform. The definition of AKI requiring RRT was based on clinical grounds.



Conclusions: In patients undergoing major surgery, preoperative statin therapy may be associated with a reduced risk for postoperative AKI.

FR-PO097

Iatrogenic Hospital Acute Kidney Injury: Results of the Implementation of a Prevention Protocol Anna Saurina,[1] Monica Pou,[1] Miquel Fulquet,[1] Ramon Roca-Tey,[2] Vicents Esteve,[1] Veronica Duarte,[1] Manel Ramírez de Arellano.[1] *[1]Nephrology, Consorci Sanitari de Terrassa, Terrassa, Barcelona, Spain; [2]Nephrology, Hospital de Mollet, Mollet del Vallés, Barcelona, Spain.*

Background: Analysis of Iatrogenic Hospital Acute Kidney Injury (HAKI) in a Hospital 162000 referred people. Elaboration and implementation of Prevention Protocol for HAKI.

Methods: Prospective single centre study in two phases. Definition HAKI: Acute increase of plasmatic creatinine up to 2 mg/dL in hospitalized patients with previous normal creatinine value, or its increase of more than 50% from baseline in patients with CKD-I-II-III. Patients with CKD IV or V, paediatric, ICU or palliative patients were excluded. First: identification and analysis of the HAKI during a 1st period of 9 months. Inclusion criteria for HAKI were detected automatically by the laboratory tests. Once detected, the clinic histories were revised by 2 physicians, collected a database and analysed the results. A Prevention Preventive Protocol of HAKI were developed, with participation of medical staff and nursing, laboratory and pharmacy. Second: new study of HAKI after the implementation of the Protocol for a 2nd period of 9 months.

Results: 394 HAKI episodes detected (167/227).Average age:75, 61.4% males, 36.3% diabetic and 75.4% with previous history of hypertension. Hemodynamic instability in 65.5% and infection in 36.3%. A 29.3% had a previous episode of HAKI. Prerrenal aetiology in 93% and 3.3% required dialysis. No differences between phases in terms of age, sex, grade of CKD, aetiology of HAKI and dialysis. After the implementation of the Protocol: reduction in the time of detection of HAKI: 4.95 versus 3.92 days (p=ns), Length of Stay 18.18 versus 13.74 days (p=0.087) and significantly decrease in its duration (8.99 versus 5.83, p=0.0000001). We detected a better monitoring on the collection of diuresis (74.4 versus 83.3% %, p = 0.012) and a decrease of iatrogenic HAKI (52.1% versus 37%, p= 0.0002) mainly due to a better correction of the hemodynamic instability (68.9 versus80.2%, p=ns).

Conclusions: The elaboration of a multidisciplinary prevention protocol for HAKI and its wide application, has allowed a significant reduction of the iatrogenic HAKI and all its consequences.

FR-PO098

Marked Improvent in Outcome of Acute Kidney Injury over the Past 20 Years Þórir E. Long,[1] Martin I. Sigurdsson,[2,3] Gisli H. Sigurdsson,[1,2] Olafur S. Indridason.[4] *[1]Faculty of Medicine, Univ of Iceland, Reykjavik, Iceland; [2]Dept of Anesthesia, Perioperative and Pain Medicine, Brigam and Women´s Hospital / Harward Medical School, Boston, MA; [3]Dept of Anesthesia and Intensive Care; [4]Div of Nephrology, Lanspitali - The National Univ of Iceland, Reykjavik, Iceland.*

Background: Acute kidney injury (AKI) is a serious condition associated with poor prognosis but it is unclear whether advances in medical management have resulted in improved outcome. The purpose of this study was to examine time trends in outcome of AKI with regard to kidney function and patient survival.

Methods: This was retrospective study that included all adult patients with AKI at Landspitali – The National University Hospital of Iceland from June 1st 1993 to May 31st 2013. AKI was diagnosed based on serum creatinine (SCr) values found in the electronic database of the clinical laboratory. The highest SCr value for each person was identified and a baseline value within the 6 preceding months used to categorize patients into stage 1, 2 and 3 of AKI based on the RIFLE criteria. Renal recovery was defined as the average of the last three available SCr measurements <1.5 x baseline SCr. Survival status was verified at Statistics Iceland. The 20 years time interval was divided into 4 equal periods and the periods compared with regard to outcomes of interest using Chi-squared, Kaplan-Meier and Cox proportional hazards analyses.

Results: In the 20 years period we identified 14,841 patients with AKI. Of those 7,524 (51%), 3,684 (25%) and 3,633 (24%) had stage 1, 2 and 3 AKI, respectively. The mean age was 69.1±16.9 years and 51% were female. The 90 day mortality was 40.3% in the first 5 year period, 39.7% in the second, 31.7% in the third and 25.9% in the last five year period (p<0.0001). Cox analysis showed a hazard ratio of 0.75 (95% CI:0.74-0.76) per 5 years. Improvements were seen for all stages of AKI. A total of 69%, 67%, 72% and 72% of patients achieved renal recovery in time periods one, two, three and four, respectively (p<0.0001).

Conclusions: Over the past two decades there have been significant improvements in survival and renal outcome for patients with AKI, probably as a result of improved medical care.

Funding: Government Support - Non-U.S.

FR-PO099

Acute Kidney Injury and Its Impact on Clinical Outcomes in Octogenarians following Cardiac Valve Replacement Surgery Michael A. Mao, Charat Thongprayoon, Qi Qian. *Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: The goal of this study is to investigate the occurrence, severity and outcomes of acute kidney injury (AKI) in octogenarians following cardiac valve replacement surgery.

Methods: Consecutive patients, age ≥80 years, undergoing cardiac valve replacement surgery at Mayo Clinic Rochester in 2002-2003 were enrolled. Dialysis patients were excluded. AKI was diagnosed based on AKIN criteria (abrupt s.Cr increase ≥0.3 mg/dL). AKI severity was determined using the RIFLE criteria.

Results: 209 consecutive patients undergoing valve replacement surgery with (58.4%) and without CABG were studied. Mean follow-up duration was 3.94±0.28 years (range: seven days, 12 years). 180 (85.7%) of the 209 patients underwent aortic valve replacement (AVR), 6.19% MVR, 0.9% TVR and 8.1% multivalve replacement. 34 (16.3%) had baseline CKD (s.Cr 1.7±0.04 mg/dL). Post-surgery, 98 of the 209 (47%) developed AKI. 79.8% (n=75) of the AKI was in stage R (risk), 9.3% in stage I (injury) and 14.4% in stage F (failure). 79.4% CKD patients developed AKI (ACKI). Of ACKI, 63% were in R and 25.9% in F. 14 of the 98 AKI patients required dialysis. 20% of them were ACKI versus 3.4% AKI without CKD, p<0.001. Length of hospital stay (LOS) was longer for AKI/ACKI patients, 14.4 versus 9.9 days in non-AKI, p=0.002. At hospital discharge, patients with AKI/ACKI had a higher sCr 1.42±0.49 mg/dL, compared to the non-AKI patients, 1.09±0.25 mg/dL, p<0.001. Patient survival at 30 days and 1 year for AKI/ACKI versus non-AKI were 89.8 versus 99.1% (p=0.003) and 76.5 versus 89.3% (p=0.03), respectively. Long-term survival also was reduced for AKI/ACKI patients by Kaplan-Meier analysis, p<0.001. Elevated Charlson Comorbidity Index was a risk factor for AKI, OR 1.19, 95% CI 1.02-1.40, p=0.03. Other adjusted AKI risk factors include CKD and surgical duration (ORs >1.0, CI >1-49, p<0.05).

Conclusions: Almost half of the octogenarians developed AKI following cardiac valve replacement surgery. AKI is associated with a worsening residual kidney function, longer LOS and reduced short-term and long-term patient survival. Comorbidity, preoperative CKD and surgery duration are independent risk factors for post-surgery AKI.

FR-PO100

Diabetes Mellitus Type 1 and Type 2 and Risk of Acute Kidney Injury following Coronary Artery Bypass Grafting Daniel P. Olsson,[1] Ulrik Sartipy,[3,4] Martin Holzmann.[1,2] *[1]Dept of Medicine, Karolinska Instt, Stockholm, Sweden; [2]Dept of Emergency Medicine, Karolinska Univ Hospital, Stockholm, Sweden; [3]Dept of Cardiothoracic Surgery and Anesthesiology, Karolinska Univ Hospital, Stockholm, Sweden; [4]Dept of Molecular Medicine and Surgery, Karolinska Instt, Stockholm, Sweden.*

Background: The objective was to investigate the risk for acute kidney injury (AKI) in patients with diabetes mellitus type 1 (DM1) and type 2 (DM2) undergoing coronary artery bypass grafting (CABG).

Methods: We included all patients (n=34 784) who underwent primary isolated CABG in Sweden during 2003 to 2013 from the Swedish national heart surgery register SWEDEHEART. Type of diabetes was retrieved from the Swedish National Diabetes Register. Acute kidney injury was defined according to the Acute Kidney Injury Network (AKIN) classification as an increase in postoperative compared to preoperative serum creatinine values: Stage 1, absolute increase by 0.3 mg/dL (26 mmol/L) or a relative increase by 50% to 100%; stage 2, 100% to 200% increase; and stage 3, ≥ 200% increase. The relative risk of AKI according to DM1 and DM2 compared to no diabetes diagnosis was calculated using logistic regression. Multivariable adjusted odds ratios with 95% confidence intervals (CI) were reported.

Results: In total 1.7% (n=605) of the study population had DM1 and 21% (n=7275) had DM2. Among patients with DM1 26%, 4.6% and 1.0% developed AKI stage 1, 2 and 3 respectively. Among patients with DM2 15%, 2.2% and 0.5% developed AKI stage 1, 2 and 3 respectively. In patients with DM1 the odds ratio for AKI stage 1 or higher was 4.38 (95% CI: 3.58-5.35) compared to patients with no diabetes diagnosis. In patients with DM2 the odds ratio for AKI stage 1 or higher was 1.32 (95% CI: 1.22-1.42) compared to patients with no diabetes diagnosis. The analysis was adjusted for age, sex, preoperative estimated glomerular filtration rate, left ventricular ejection fraction, chronic obstructive pulmonary disease, peripheral vascular disease and body mass index.

Conclusions: We found a strong and significant association between diabetes and AKI after CABG. The risk for AKI was markedly higher in patients with DM1.

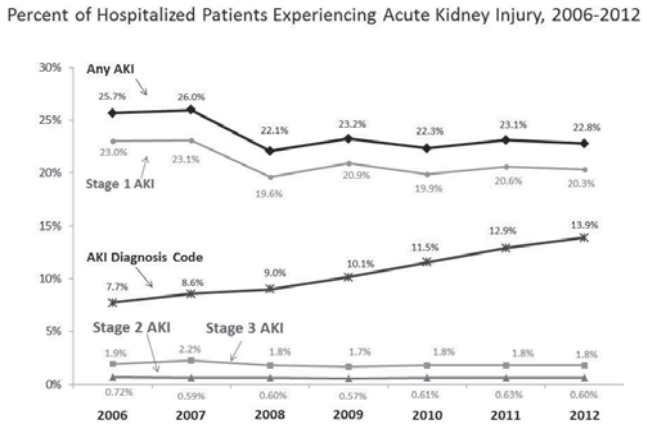
FR-PO101

Trends in Acute Kidney Injury Hospitalizations among U.S. Veterans: A Comparison between Clinical and Administrative Data Michael Heung,[1] Diane Steffick,[1] Chi-Yuan Hsu,[2] Neil R. Powe,[2] Nilka Rios Burrows,[3] Meda E. Pavkov,[3] Vahakn B. Shahinian,[1] Kara Zivin.[1] *[1]Univ of Michigan, Ann Arbor; [2]Univ of California San Francisco; [3]Centers for Disease Control & Prevention, Atlanta, GA.*

Background: Recent studies have suggested rising incidence of severe acute kidney injury (AKI). Most epidemiologic studies have relied on administrative data, while consensus AKI definitions emphasize use of clinical data. We examined recent trends in AKI hospitalizations using both administrative and clinical data.

Methods: Using national Veterans Administration data (5% sample:2006-2009; 100%:2010-2012), we identified patients with 1+ hospitalization. Patients with outpatient dialysis were excluded. The primary outcome was the proportion of hospitalized patients with an episode of AKI each year. We used a modified Poisson model to examine the effect of year on the likelihood of having AKI, adjusting for age, gender, ethnicity, diabetes and hypertension. Analyses were conducted separately for creatinine-defined (sCR) AKI and diagnosis code AKI.

Results: Over 360,000 patients were hospitalized in the VA annually. 23.6% of hospitalized patients met sCR criteria for AKI, decreasing from 25.7% in 2006 to 22.8% in 2012 (p<0.001). In the adjusted model, each increase in year was associated with a modest decreased risk for AKI (RR 0.99, 95% CI 0.99-1.00). Over the same period, the proportion of patients with a diagnosis code for AKI rose from 7.7% to 13.9% (p<0.001). After adjustment, each increase in year conferred a 10% increased risk for diagnosed AKI compared (RR 1.10, 95% CI 1.10-1.11).



Conclusions: Between 2006 and 2012, in the U.S. veteran population, diagnosed AKI rose markedly while the fraction of hospitalized patients with sCR-defined AKI decreased slightly. These findings highlight an important difference between clinical and administrative data for epidemiologic studies of AKI.

Funding: Other U.S. Government Support

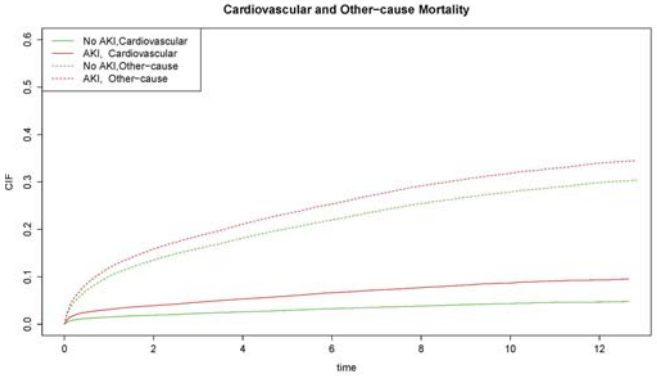
FR-PO102

Effect of Acute Kidney Disease on Competing Risks for Survival After Major Surgery Azra Bihorac,[1] Tezcan Ozrazgat-Baslanti,[1] Paul Thottakkara,[1] Wan Xu,[1] Shuo Zhang,[1] Matthew P. Huber,[1] Charles E. Hobson.[2] *[1]Anesthesiology, Univ of Florida, Gainesville, FL; [2]Surgery, Malcom Randall VA Medical Center, Gainesville, FL.*

Background: Acute kidney injury (AKI) affects up to 40% of surgical patients and is associated with increase in all-cause mortality. We determined the effect of acute and chronic kidney disease, age and comorbidity on cardiovascular–specific mortality in patients undergoing major surgery.

Methods: We performed survival analyses that accounted for competing risks in a single-center cohort of 51,457 adult surgical patients. We defined AKI using KDIGO (Kidney Disease: Improving Global Outcomes) criteria. We determined the presence of chronic kidney disease (CKD) prior to surgery and adjusted all analyses for age, comorbid conditions and type of surgery using multivariable subdistributional hazards model.

Results: Ten-year cumulative cardiovascular-specific mortality rates were significantly higher for AKI (10.2%), CKD with no AKI (10.3%), and ESRD (10.2%) patients compared to patients with no kidney disease (2.7%) whereas no significant difference was observed for cumulative cancer-specific mortality. Adjusted HR of cardiovascular-specific mortality for patients with AKI, CKD and ESRD were 2.1 (95% CI, 1.9-2.3), 1.8 (95% CI, 1.5- 2.2), and 3.2 (95% CI, 2.7-3.8), respectively, compared with those that had none. Hazards of cardiovascular-specific mortality were highest for patients with older age (HR 1.04, 95% CI 1.04-1.05), male gender (HR 1.2, 95% CI 1.1-1.3), Charlson comorbidity index score of at least 3 (HR 2.1, 95% CI 1.9-2.4), emergent surgery (HR 1.5, 95% CI 1.4-1.7), cardiothoracic surgery (HR 3.1, 95% CI 2.8-3.4) and hemoglobin of 8 to 10 (HR 1.3, 95% CI 1.1-1.5) compared to those with level ≥12.



Conclusions: Patients with acute kidney injury after major surgery are at high risk for cardiovascular-specific mortality within 10 years of diagnosis.

Funding: Other NIH Support - NIGMS

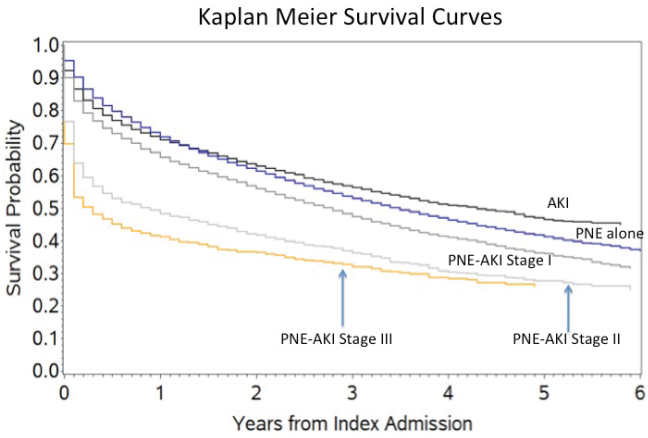
FR-PO103

Impact of Acute Kidney Injury on Patients Admitted to the Hospital with Pneumonia Ping Li,[1] Richard Amdur,[1] Carlos E. Palant,[1] Charles Faselis,[1] Paul L. Kimmel,[2] Lakhmir S. Chawla.[1] *[1]Medical and Research Service, DC VAMC, Washington, DC; [2]NIDDK, NIH, Bethesda, MD.*

Background: Acute Kidney Injury (AKI) is associated with major adverse kidney events-MAKE (death, dialysis, and durable loss of renal function[CKD]). CKD is associated with an increased risk of major adverse cardio-vascular events-MACE (MI, CVA, and CHF). We sought to determine incidence and impact of AKI on major adverse reno-cardiovascular events or MARCE (a combination of MACE and MAKE).

Methods: Patients in VA database with discharge diagnosis of ICD-9 code 584.xx (AKI), or 486.xx (PNE) were selected for analysis. Three groups were created, based on the index admission diagnosis and serum creatinine (SC) values: 1) AKI; 2) Pneumonia (PNE); 3) PNE with AKI. Patients with mean baseline estimated glomerular filtration rate (eGFR) < 45 ml/min per 1.73 m^2 were excluded.

Results: 55,088 subjects were analyzed. Mean age: 68.1 ± 12.0 yrs. Median follow-up:1.3 years following index admission (IQR = 0.4 to 2.9). At 5 years, death occurred most often for patients in the PNE+AKI group (64%), and least often (53%) for those with AKI admission. When AKI complicated an admission for PNE, mortality was increased OR 1.38 (1.34-1.42, p < 0.0001) in a Cox hazards model adjusted for age, baseline eGFR, gender, race, DM, and HTN. The severity of AKI was also linked to outcomes in a stepwise fashion. Patients with PNE and AKI-Stage III had worst outcomes as compared to patients with PNE-AKI-II, PNE-AKI-I, and AKI alone (mortality: 72.3%, 69.8%, 60.9%, and 49.4% [p < 0.0001], respectively



Conclusions: Hospital admission for AKI is associated with long-term outcomes comparable to hospital admission for PNE. When AKI accompanies PNE, outcomes are worse than either diagnosis alone for death, MACE, MAKE, and MARCE. The impact of AKI on PNE follows a stepwise worsening corresponding to the severity of the kidney injury.

FR-PO104

Acute Kidney Injury and Acute Myocardial Infarction: Partial Recovery of Kidney Function Is Associated with Improved Inpatient Mortality David G. Warnock,[1] T. Clark Powell,[1] John P. Donnelly,[1] Peter A. McCullough,[2] Henry E. Wang.[1] *[1]School of Medicine, Univ of Alabama at Birmingham, Birmingham, AL; [2]Baylor Heart and Vascular Inst, Dallas, TX.*

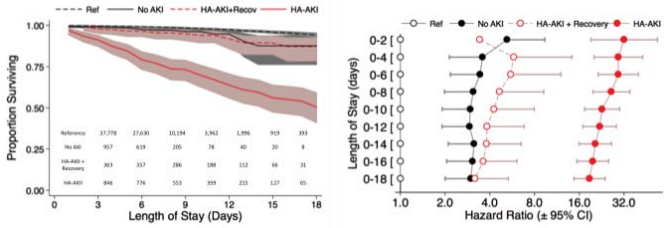
Background: Hospital-Acquired Acute Kidney Injury (HA-AKI; increase in serum creatinine (sCr) ≥0.3 mg/d), and acute ST-segment elevation myocardial infarction (STEMI) are associated with increased mortality (Newsome et al Arch Int Med 2008). We assessed mortality among patients with HA-AKI, STEMI, and partial recovery of sCr.

Methods: Hospital discharge, sCr and mortality data over 4 years (2010-2013) at UAB Hospital were analyzed for adults with >2 sCr values and no prior ESRD history, and length of stay (LOS) >1 and <22 days. STEMI events were coded with ICD9 410.*. Regression models were adjusted for demographics and baseline eGFR, with HA-AKI, STEMI and AKI_recovery (decreased sCr ≥0.3 mg/dL from peak sCr) as the effect variables.

Results: For 69,826 qualifying admissions; median LOS 4 days; mean age 56 years; 52% male, 36% black; baseline eGFRcreat, 77 ml/min/1.73 m^2. The time-averaged hazard ratio (HR; ±95% CI for inpatient mortality) was reduced from 3.43 for HA-AKI+STEMI to 1.0 with partial recovery of sCr, equal to cases with No AKI – STEMI (HR 1.00).

	No AKI		HA_AKI			
	–STEMI	+STEMI	–STEMI–Rec	–STEMI+Rec	+STEMI–Rec	+STEMI+Rec
Cases	37,379	980	6,544	6,326	414	420
Deaths (5)	358 (1%)	36 (3.7)	711 (11%)	388 (6.1%)	122 (30%)	67 (16%)
HR (±95% CI)	1.00 (Reference)	3.66 (2.60–5.17)	5.18 (4.55–5.91)	1.60 (1.38–1.87)	3.43 (2.34–5.04)	1.00 (0.65–1.54)

More striking were effects seen with shorter LOS.



Conclusions: HA-AKI and STEMI independently associate with inpatient mortality. Partial recovery of sCr is associated with decreased mortality among those with HA-AKI, and HA-AKI with STEMI. This finding could improve risk prediction for future intervention studies.

Funding: Other NIH Support - T32-HS013852, R01-NR012726, P30 DK079337 and ASN Kidney Research Clinical Scholars Grant

FR-PO105

Long Term Evolution from Acute Kidney Injury: A Prospective Study in Critical Ill Patients Carmen Bernis,[1] Laura Salanova,[1] Marta Chicot,[2] Diego Anibal Rodriguez-Serrano,[2] Ana Diaz,[3] Enrique Cereijo,[2] Jose-Antonio Sanchez-Tomero.[1] *[1]Nephrology, H.U Princesa CIFRA -CM, Madrid, Spain; [2]ICU, HU Princesa, Madrid, Spain; [3]Biochemistry, HU Princesa, Madrid, Spain.*

Background: In the past years there has been a great interest regarding the impact of an AKI episode on development of incident CKD and the value of markers of tubular injury as NGAL to predict long term outcomes. We study these questions in a group of ICU survivors.

Methods: A Prospective study of 415 patients admitted to a general ICU was initiated 2011. Patients (APACHE, SAPS, RRT, urinary NGAL, AKI defined by AKIN) were follow up until their discharge and a group of 108 survivors during 24 months. AKI developed in 99/415 patients and 29 died. No AKI patients were 316 and 25 died during hospitalization. We were able to follow up 54 AKI patients discharged alive (all of them in the geographical area of our hospital) and a control group of 54 no AKI survivors (similar charasteristics, age, sex).

Results: In 34/54 AKI patients renal function at 24 months was clearly worse (cr increment from discharge 1.1±1.8) versus only in a small increment in 14 /54 controls (cr increment 0,29± 0,15) p<0,001. CRD stage 5 needing dialysis was required in 7 AKI patients during follow up and in none controls. The no AKI group has a homogenous cr evolution with stable or small increase being the medium cr increment 0, 21± 0, 48. In the AKI group there were two patterns, (although the discharge creatinine was similar) one subgroup (34) get clearly worse and other subgroup (20) get better with a 24 months creatinine decrement of 0,61 ± 0,07. NGAL was higher in AKI group (772 +200 versus 84+ 42 controls p<0, 0001) but NGAL in AKI with good evolution was 809± 226 versus 525± 301 in AKI with bad evolution (pns). Mortality was 12/54 in AKI group versus 9/54 in controls (p.n.s).

Conclusions: In our prospective control trail of critical ill patients, AKI patients are clearly more likely to suffer accelerated loss of kidney function than controls. It is remarkable that AKI patients have two evolution patterns: 61% to worse and 29% to improvement. In our experience NGAL levels during AKI hospitalization cannot predict in the future progressive chronic kidney disease.

Funding: Government Support - Non-U.S.

FR-PO106

Chronic Kidney Disease and Mortality in Long-Term Survivors of an Episode of Acute Renal Injury Carlos Enrique Arias, Eva Rodriguez, Adriana Sierra, Sheila Bermejo, Alejandra Prada, Carla Burballa, Ali Berrada, Julio Pascual. *Nephrology, Hospital del Mar, Barcelona, Spain.*

Background: Several population studies have shown an association between acute kidney injury (AKI) and subsequent development of chronic kidney disease (CKD). However, there are few studies in our population that analyze this association. OBJECTIVES: The aim of this study is to evaluate the impact of AKI episode in the subsequent development of CKD, mortality and morbidity in the midterm follow-up (12 months) and long term follow-up (24 months).

Methods: Retrospective study of patients diagnosed with AKI (ICD- 9) as ADQI criteria definition, with full renal recovery at discharge. We included demographic, clinical and laboratory data at 12 months and 4 years follow-up.

Results: 498 patients were included, 61.8 % men, mean age 66.2 ± 19 years. At discharge, 386 patients (78.6 %) recovered renal function. Follow-up at 12 months showed that 122 (22.1 %) patients developed CKD and after 4 years follow-up, CKD was diagnosed in 106 patients (40.8%). Multivariate analysis showed that history of hypertension (OR: 1.62, 95% CI 1.2-2.6, p < 0.05) and serum creatinine > 2.6 mg / dl during the AKI episode (OR: 1.7, 95% CI 1.2-3.7, p < 0.05) are risk factors for CKD at 12 months, while at 4 years, the risk of CKD is associated with age (p < 0.05), hypertension (p < 0.05) and peripheral vascular disease (p < 0.05). Mortality was higher in patients who developed CKD at 12 months n = 38) (OR: 2.68 95% CI 1.26 -5.7, p < 0.05) and 4 years (n = 106) (OR: 4.13 95% CI 1.13 to 4.90, p < 0.05). Multivariate analysis showed that associated factors with mortality at 12 months were age and creat > 2.6 mg / dl; while 4 years mortality was significantly associated with male sex, age, ischemic heart disease, peripheral vascular disease and variables related to the severity of the episode of AKI (creatinine > 2.6 mg / dl, dialysis treatment and Failure and Loss RIFLE-staging).

Conclusions: Our data showed, that development of CKD after an AKI episode, is related, in the medium term, with severity of the AKI while in the long term is related with classical cardiovascular disease risk factors.

FR-PO107

Relationship between Recurrences of Acute Kidney Injury and Development of Chronic Kidney Disease, Cardiovascular Events and Mortality Carlos Enrique Arias, Eva Rodriguez, Sheila Bermejo, Adriana Sierra, Alejandra Prada, Carla Burballa, Ali Berrada, Julio Pascual. *Nephrology, Hospital del Mar, Barcelona, Spain.*

Background: Observational studies have shown that acute kidney injury (AKI) can be considered as a risk factor for later development of chronic kidney disease (CKD). However, there are few studies that evaluate the effect of AKI recurrences and the development of CKD, mortality or cardiovascular events. OBJECTIVE The aim of this study is to evaluate if the presence of various episodes of AKI, are related to the subsequent development of CKD and mortality and morbidity in these patients.

Methods: A retrospective study in 498 patients was conducted with a diagnosis of AKI (ICD-9) with full recovery of renal function. We reviewed medical records and we recorded the presence or abscense or new episodes of AKI, cardiovascular events and mortality. We also recorded clinical and laboratory variables.

Results: From an initial sample of 498 patients we had follow-up data at 4 years of 241, of these 147 (61 %) had ≥ 1 episode of AKI during follow-up Multivariate analysis showed that risk factors for new episodes of AKI were: previous type 2 diabetes mellitus (OR: 2.89 95% CI 1.33 to 1.67 p < 0.05), previous ischemic heart disease (OR: 2.62 95% CI 1.07 to 6.45 p < 0.05) and age > 67 years (OR: 1.02 95% CI 1.04 to 1.01 p < 0.05). Patients with ≥ 1 AKI episodes showed a higher risk of 4 years follow-up CKD (OR 2.43, 95% CI 1.16 to 5 p < 0.05), increased frequency of cardiovascular events (OR 4.25, 95% CI 2.05 to 8.81 p < 0.05) and higher mortality from all-cause (OR 3.12 95% CI 1.31 to 7.6 p < 0.05 comparing with 1 AKI episode survivors patients.

Conclusions: Previous hypertension, type 2 diabetes mellitus and ischemic heart disease are risk factors for AKI recurrence after an episode of AKI, also the fact for recurrence confers an increases the risk of developing CKD, cardiovascular events and higher mortality from all causes at 4 years.

FR-PO108

Acute Kidney Injury following Elective Total Joint Arthroplasty Rowan G. Walker,[1,2] Lara A. Kimmel,[1,2] Jyotsna Dinesh Janardan,[1] Susan Liew.[1,2] *[1]Alfred Hospital, Victoria, Australia; [2]Monash Univ, Victoria, Australia.*

Background: Total joint arthroplasty (TJA) is a frequently performed procedure shown to improve function and quality of life in patients with osteoarthritis. Incidence of acute kidney injury following TJA is generally low, with most studies reporting an incidence <2%, in the elective population, increasing to 9% when emergency orthopedic cases are included.

Methods: Retrospective medical record review of consecutive primary, elective TJA procedures was undertaken at a large tertiary hospital in Melbourne, Australia. Demographic, peri- operative and post-operative data were recorded. Multiple logistic regression was used to determine factors (clinical, biochemical and drug related) associated with AKI (RIFLE criteria).

Results: Of 429 patients undergoing TJA between January 2011 and June 2013; [254 total knee replacements (TKR) and 175 total hip replacements], 67 (15.6%) developed AKI, including 51 TKR. Increasing body mass index (AOR 1.12 95%CI: 1.06, 1.19), older age (AOR 1.06 95% CI 1.01, 1.11) and lower pre operative glomerular filtration rate (AOR 0.98 95%CI 0.96, 0.99) were associated with the development of AKI. Of drug related factors, taking angiotensin converting enzyme inhibitors (AOR 2.73 95%CI 1.18, 6.31) and angiotensin-II receptor blockers (AOR 2.49 95% CI 1.14, 5.43) were factors associated with worsening RIFLE criteria (after accounting for known confounders) whereas NSAIDs appeared protective (AOR 0.22 95%CI: 0.08, 0.64). AKI resolved by discharge in most cases although only 50% of the total population had renal function tests after discharge, rendering long term analysis difficult.

Conclusions: Although the incidence of AKI (>15%) in our TJA population is higher than previously reported, most associated factors were similar to other studies. The apparent protective nature of the NSIADs was surprising. Given AKI is associated with increased length of stay and long term complications, prospective research is needed to further understand the number and interrelationship of factors that might assist with prediction of AKI risk. Opportunities to maximise the pre-operative medical management and mitigate risk should be considered in this elective orthopedic population.

FR-PO109

Baseline Characteristics Associated with AKI after Hospitalization for Serious Infection T. Clark Powell,[5] Orlando M. Gutierrez,[2] Russell Griffin,[3] John P. Donnelly,[5] Monika M. Safford,[4] Henry E. Wang.[5] *[1]Div of Nephrology, Univ of Alabama School of Medicine; [2]Dept of Epidemiology, Univ of Alabama School of Medicine; [3]Div of Preventive Medicine, Univ of Alabama School of Medicine; [4]Dept of Emergency Medicine, Univ of Alabama School of Medicine, Birmingham, AL.*

Background: While ample data describe AKI, only limited data describe the risk of future AKI in healthy individuals. We sought to identify baseline characteristics of community dwelling adults associated with long-term risk of AKI after hospitalization for serious infection.

Methods: We used 8 years of data on 30,239 adults ≥45 years old from the REasons for Geographic and Racial Differences in Stroke (REGARDS) cohort. Using medical records, we identified serious infection hospitalizations. We defined AKI as a serum creatinine (sCr) increase ≥0.3 mg/dL from first inpatient measurement. We excluded individuals with prior renal transplant or ESRD, as well as hospitalizations with <2 sCr measurements (n=878). Examined baseline characteristics included sociodemographics, health behaviors, chronic medical conditions, and biomarkers. We identified associations with hospitalizations with AKI events using Generalized Estimating Equations.

Results: Over median follow-up of 4.5 years (IQR 2.4-6.3) we identified 2,074 serious infection hospitalizations among 1,547 individuals. Infection types included: lung 45.2%, kidney 16.2%, abdominal 18.0%, skin 10.8%, other 9.8%. AKI occurred in 312 hospitalizations (15.0%) (sCr rise 0.3-0.69 mg/dL - 8.7%; 0.7-1.19 - 2.7%, ≥1.20 or acute dialysis - 3.7%). Baseline characteristics independently associated with AKI after serious infection included male sex (adjusted OR 1.51; 95% CI: 1.11-2.05), history of diabetes (1.54; 1.13-2.08), Cystatin-C [CysC] ≥1.11 mg/dL (1.95; 1.37-2.78) and Albumin-to-Creatinine Ratio [ACR] ≥30 mg/g (1.69; 1.23-2.32). eGFR<60 was not associated with future AKI.

Conclusions: Male sex, history of diabetes, and elevated baseline CysC and ACR are independently associated with future AKI after serious infection. These findings highlight baseline characteristics that may be useful for risk-stratification and awareness of increased future AKI risk.

FR-PO110

Severity of Anemia Is Associated with AKI Recovery During ICU Stay Etienne Macedo,[1] Josee Bouchard,[2] Glenn M. Chertow,[3] Jonathan Himmelfarb,[4] T. Alp Ikizler,[5] Ravindra L. Mehta.[6] *[1]Univ of Sao Paulo; [2]Hospital du Sacre-Coeur de Montreal; [3]Stanford Univ; [4]Univ Washington; [5]Vanderbilt Univ; [6]Univ of California San Diego.*

Background: Anemia is a risk factor for development of AKI but has not been evaluated for renal recovery following AKI. We hypothesized that the severity of anemia would contribute for non-recovery from AKI in the ICU setting.

Methods: We included ICU survivors whose sCr increased consecutively for>3 days before recovery or dialysis. We stratified patients in quartiles according to the lowest Hb during ICU stay before dialysis. Recovery was defined as complete when ICU discharge sCr was within 20% of the reference sCr, and partial for sCr values >20% and not on dialysis. Non-recovery was defined as dialysis-dependence at ICU discharge. The likelihood of recovery was assessed in a logistic regression in reference to the lowest Hb quartile.

Results: Of the 212 ICU survivors, 40 (18%) were dialysis dependent at ICU discharge; and 64 (30%) had complete recovery. Median (IQR) Hb were (10.7G/dL (9.7-12.5) at ICU admission and the lowest during ICU stay were 8.6 G/dL (7.9-9.5). 25% of patients had Hb<8G/dL during ICU stay. Patients in the highest Hb quartile were less likely to be dialysis dependent and more likely to have complete recovery than those in the lowest quartile.

Hb quartiles during ICU stay	<7.9	8-8.5	8.6-9.5	>9.5
Complete recovery - ALL (212)	17 (32)	16 (28)	11 (19)	20 (42)
CKD (68)	3 (21)	5 (22)	3 (20)	8 (47)
Partial recovery - ALL	24 (45)	27 (48)	35 (62)	22 (46)
CKD	5 (35)	10 (45)	10 (66)	7 (41)
Dialysis dependency - ALL	12 (22)	13 (23)	10 (18)	5 (11)
CKD	6 (42)	7 (31)	2 (13)	2 (12)
OR for any recovery - ALL	1	3.30* (1.7-6.1)	4.6* (2.3-9.1)	8.4* (3.3-21)
CKD	1	2.14 (0.8-5.2)	6.5* (1.4-2.8)	7.5* (1.7-32)

*P<0.05

CKD patients in the highest Hb quartile had significantly higher recovery rates (88% versus 57%) and lower dialysis dependency those on the lowest quartile (43% versus 12%;p=0.024). The highest Hb quartile was associated with an 8.4 times higher likelihood of recovery.

Conclusions: Anemia is frequent in AKI patients during ICU and is associated with higher risk of RRT dependence at ICU discharge. Future studies should evaluate if prevention or attenuation of anemia could improve AKI recovery.

Funding: NIDDK Support

FR-PO111

Medication Usage and Survival After Acute Kidney Injury Sandeep Brar,[1] Neesh I. Pannu.[2] *[1]General Internal Medicine, Univ of Alberta, Edmonton, AB, Canada; [2]Nephrology, Univ of Alberta, Edmonton, AB, Canada.*

Background: The incidence of acute kidney injury (AKI) is continuing to rise, and there are currently no effective therapies for the treatment of AKI. Recent studies suggest that nephrology follow-up reduces the risk of mortality in AKI survivors and this may be due to the receipt of beneficial medications.

Methods: Retrospective cohort study of adults greater than 65 years of age, residing in Alberta, who were admitted to hospital between 2002 and 2008 and developed AKI during the index hospitalization, with progression to chronic kidney disease (CKD) within 120 days post discharge. Angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II–receptor blocker (ARB), beta-blocker and statin use was assessed within 120 days post discharge.All participants had a minimum of two years of follow up.

Results: n= 14,921 mean age 78.8 years. Within 120 days of discharge, 59.4% of the participants were on an ACEi or ARB, 36.7% were on a beta-blocker and 28.5% were on a statin. The adjusted hazard ratio of mortality for participants on an ACEi or ARB was 0.931 (95% confidence interval [95% CI], 0.868, 0.999), 0.931 for participants on a beta-blocker (95% confidence interval [95% CI], 0.868, 0.999) and 0.74 for participants on a statin (95% CI, 0.69, 0.80). Participants on an ACEi or ARB had a higher risk for all cause re-hospitalization (adjusted HR, 1.16; 95% CI, 1.1, 1.22). Participants on a beta-blocker also had a higher risk for all cause (adjusted HR, 1.07; 95% CI, 1.02, 1.13) and renal cause re-hospitalization (adjusted HR, 1.25; 95% CI, 1, 1.56). There was no significant difference in the rate of ESRD with any of the medications.

Conclusions: In AKI survivors, ACEi, ARB, beta-blockers and statins are associated with a decreased risk of mortality. However, ACEi or ARB and beta-blockers are associated with a higher incidence of re-hospitalizations.

Funding: Government Support - Non-U.S.

FR-PO112

Effect of Angiotensin-Converting Enzyme Inhibitor or Angiotensin-Receptor Blocker Use on the Progression of Acute Kidney Injury in Heart Failure Patients Ke Wang, Mayank Sardana, Payel Jhoom Roy, Youssef Rahban, Bailey Y. Chang, Ashish Upadhyay. *Nephrology, Boston Medical Center, Boston, MA.*

Background: Angiotensin-converting-enzyme inhibitors (ACEI) and angiotensin-receptor blockers (ARB) are widely used in patients with heart failure. ACEI/ARB lower intra-glomerular pressure and may impair renal auto-regulation during hemodynamic stress. We examined if early discontinuation of ACEI/ARB during in-hospital acute kidney injury (AKI) affected AKI progression in patients with heart failure.

Methods: We reviewed charts of patients admitted to Boston Medical Center's heart failure service from 2/2007 to 6/2013. Patients who developed in-hospital AKI while taking ACEI/ARB were included. Exact timing of ACEI/ARB discontinuation after onset of AKI was noted. We used regression analyses to assess the impact of the timing of ACEI/ARB discontinuation on doubling of serum creatinine (Cr), absolute change in Cr, length of hospital stay (LOS), and time to peak Cr. Results were adjusted for demographic characteristics and co-morbidities.

Results: 202 patients met the inclusion criteria. Group 1 included 27 patients with ACEI/ARB discontinuation within 24 hours after AKI, group 2 included 36 patients with ACEI/ARB discontinuation more than 24 hours after AKI, and group 3 included 139 patients whose ACE-I/ARB was continued during AKI. When comparing group 1 to groups 2 + 3, there was no difference in doubling of Cr (25.9% versus 22.8%, p=0.39), absolute change in Cr (0.99 mg/dL versus 0.80 mg/dL, p=0.07), LOS (10.5 days versus 8.7 days, p=0.11) and time to peak Cr (3.1 days versus 2.4 days, p=0.33). When comparing group 1 to group 2, group 1 had lower rates of doubling of Cr (25.9% versus 58.3%, p=0.01) and less absolute change in Cr (0.97 mg/dL versus 1.37 mg/dL, p=0.048). There was no statistically significant difference in LOS (10.1 days versus 12.2 days, p=0.27) and time to peak Cr (3.1 days versus 4.0 days, p=0.42).

Conclusions: Our results suggest that early discontinuation of ACEI/ARB after AKI may not affect AKI progression in heart failure patients. Among patients who had ACEI/ARB discontinuation, however, there was a trend towards better kidney outcomes in the group with early discontinuation.

FR-PO113

Acute Kidney Injury Complications in Critically Ill Patients: Implications on Mortality Rate and on the Efficacy of Renal Replacement Therapy Alexandre Braga Liborio,[1] Evandro Faria,[2] Eder Pinheiro Arantes,[1] Renata Almeida Leitao.[3] *[1]Clinical Medicine, Univ Federal of Ceara, Fortaleza, Ceara, Brazil; [2]Pronefron, Fresenius Medical Care, Fortaleza, Ceara, Brazil; [3]UNIFOR.*

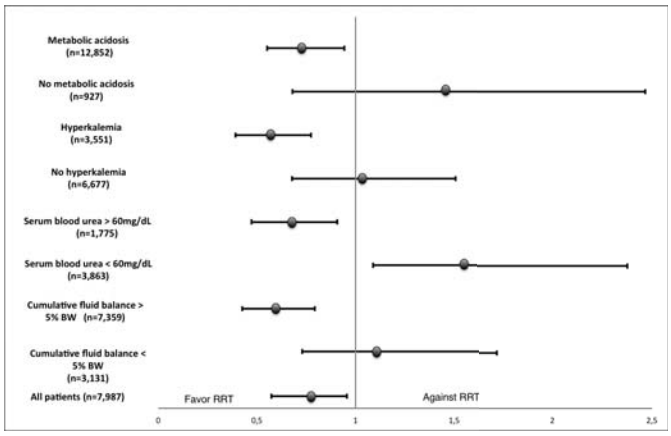
Background: it is unknown the contribution of AKI complications to AKI-related mortality and if renal replacement therapy (RRT) has any beneficial effect in critically ill patients mortality.

Methods: Retrospective study using data from MIMIC-II project comprising 23,529 patients.

Results: 10,245patients developed AKI.After adjustment for confounders (model 1), OR for hospital mortality are demonstrated.

	Model 1	Model 1+ hyperkalemia	Model 1+ hyperkalemia+ metabolic acidosis	Model 1+ hyperkalemia+ metabolic acidosis+ cumulative fluid balance
No-AKI	ref.	ref.	ref.	ref.
AKI stage 1	1.976 (1.737-2.247)	1.892 (1.661-2.154)	1.702 (1.492-1.942)	1.651 (1.446-1.885)
AKI stage 2	2.209 (1.858-2.626)	2.023 (1.696-2.413)	1.708 (1.428-2.044)	1.493 (1.243-1.794)
AKI stage 3	3.090 (2.529-3.775)	2.745 (2.235-3.371)	2.096 (1.637-2.589)	1.583 (1.268-1.996)

After including each AKI complication (hyperkalemia, metabolic acidosis and cumulative fluid balance), the excess of mortality rate was explained by 33% in AKI stage 1, 59 % in stage 2 and 72% in stage 3. The main burden of AKI-excess mortality was explained by metabolic acidosis and cumulative fluid balance. Long-term mortality was not explained by any of AKI complications.Subsequently,we used two different approaches to explore the association between RRT, AKI complications and hospital mortality: multivariate analysis and propensity-score matching. In both approaches, RRT was associated with better survival only in AKI-related complications subgroups



Conclusions: Majority of AKI excess risk of mortality can be explained by its complications, mainly in severe AKI.Also, for the first time we demonstrated RRT was associated with a better outcome only in those patients with AKI-related complications.

FR-PO114

Discordant Trends in Sepsis and Its Associated Acute Kidney Injury, 2004-2008 Jean-Sebastien Rachoin,[1] Andrew Moore,[2] Lawrence S. Weisberg.[1] *[1]Medicine, Cooper Univ Hospital, Cooper Medical School of Rowan Univ, Camden, NJ; [2]Medicine, Cambridge Health Alliance, Cambridge, MA.*

Background: Concerted efforts recently have been made to reduce the high mortality rate associated with sepsis in critically-ill patients. The impact of such efforts on the development of acute kidney injury (AKI) is unknown. We hypothesized that the incidence of AKI requiring renal replacement therapy (AKI / RRT) among patients with sepsis has not changed in recent years.

Methods: We performed a retrospective observational study of all patients in the U.S. and Canada admitted to a, ICU from 2000 through 2008 and entered in the Project Impact® database. We analyzed 28,238 records from21 hospitals deemed to have a sufficiently accurate time series without major gaps in the data. We constructed a cross sectional auto-regressive integrated moving average model (ARIMA) to smooth out seasonal variations in the data and examine trends more closely.

Results: There were 2,304 cases of sepsis in 2004, which increased to 2,496 in 2008. The number of cases of AKI / RRT was 218 in 2004 and 219 in 2008. After analyzing monthly variations and adjusting for confounding variables with our ARIMA model, we found that the temporally adjusted trend in sepsis cases showed a significant increase over time (p=0.0121), whereas that for AKI / RRT did not (p=0.4296.

Conclusions: Cases of sepsis increased significantly over time in our hospital ICU series from 2004 through 2008, without a corresponding significant increase in AKI / RRT. The factors associated with this discordance, including demographics, quality of care and goals of care, remain to be investigated.

FR-PO115

Acute Kidney Injury and Long-Term Outcomes after Major Abdominal Surgery: A Retrospective Cohort Analysis Joana Gameiro,[1] Catarina Bekerman,[1] Maria Ornelas,[1] Marta Pereira,[1] Sofia C.A. Jorge,[1] Rosario Rosa,[2] António Gomes da Costa,[1] Jose António Lopes.[1] *[1]Dept of Medicine, Centro Hospitalar Lisboa Norte, EPE, Lisboa, Portugal; [2]Dept of Surgery, Centro Hospitalar Lisboa Norte, EPE, Lisboa, Portugal.*

Background: Data regarding the influence of acute kidney injury (AKI) on long-term outcomes of patients submitted to major abdominal surgery are still lacking. We analyzed the impact of AKI on long-term renal outcome and mortality in a cohort of patients undergoing major abdominal surgery.

Methods: Patients who underwent major abdominal surgery in Centro Hospitalar Lisboa Norte, EPE, (Lisbon, Portugal) between January 2010 and February 2011 and were discharged alive from the hospital were retrospectively studied. AKI was defined by an increase in absolute serum creatinine (SCr) ≥0.3 mg/dl or by a percentual increase in SCr≥50%, and/or by a decrease in urine output to <0.5 ml/kg/hour for more than 6 hours, in the first 48 hours after surgery. Renal outcome was defined as the requirement for long-term dialysis and/or a 25% decrease in estimated glomerular filtration rate after hospital discharge. Cumulative survival curves were determined by the Kaplan-Meier method, and log-rank test was employed to analyze statistically significant differences between curves. Multivariate analysis was used to determine predictors of renal outcome and mortality. A two-tailed P value<0.05 was considered significant.

Results: Out of the 390 patients analyzed, 72 (18.5%) had postoperative AKI. Median follow-up was 38 months. Patients with AKI had worse renal outcome (47.2 versus 21.4%, P<.0001; unadjusted OR 3.2, 95%CI 1.9-5.4, P<.0001). In multivariate analysis, AKI still emerged as a risk factor for renal outcome (adjusted OR 2.2, 95%CI 1.2-4, P=0.013). At 1, 2 and 3 years of follow-up, the cumulative probability of survival of patients with AKI was 83.3, 68.4 and 56.8%, respectively, compared with 96.2, 93.4 and 90.9% in patients without AKI (log-rank, P<.0001). In multivariate analysis AKI was associated with increased mortality (adjusted HR 1.7, 95% CI 1.1-2.6; P = 0.016).

Conclusions: Postoperative AKI was associated with poor long-term renal outcome and mortality in patients undergoing major abdominal surgery.

FR-PO116

Acute Kidney Injury (AKI) Requiring Dialysis: Long Term Follow Up Survival and Effect on Kidney Function Paula A. Duran, Luis A. Concepcion. *Medicine Div Nephrology, Scott&White Hospital Texas A&M Health Science Center, Temple, TX.*

Background: Data on long term follow up after AKI requiring dialysis is scarce. Objective: Describe and Identify factors associated with survival, recovery of kidney function at discharge and long term follow up of renal function in AKI patients requiring hemodialysis (HD).

Methods: All AKI patients requiring HD (conventional and daily shift CVVHD (2000-2013) included. Data:mean (SD).

Results: 499patients 60.9%male33.8%diabetic75%w/dipstick proteinuria on admission.345discharged alive (mortality 30.6%).68died after discharge50% in the first 162 d,mortality at the end of study 41.7%.F/U (1-86 m).At discharge 273 recovered kidney function (51.8%).Of those who died in the hospital 88.1%did no recover kidney function (dialysis dependent).Baseline creatinine 1.5mg/dl (0.9) eGFR60.8ml/min (30.7),Peak creatinine6.0 (2.9) BUN85.4mg/dl (39.0).Discharge creatinine3.6 (4.0) eGFR 30.4 (28.0) 6months creatinine 1.8 (1.4) eGFR60.7 (44.7),at all f/u times creatinine was higher eGFR was lower than the baseline values (p<0.05).Non survivors were older 62.4y (15.2) versus 58.9 (16.9) had a lower peak creatinine 4.9 (2.3) mg/dl versus 6.5 (3) had lower eGFr at discharge 24.9 (16.4) versus 32.7 (31.5) ml/min.Mean survival time 39.9m.The survival of the patients who recover kidney function at discharge was longer than ones who did not recover (59.4 versus 13.2m p<0.05).Cox regression the factors significant for survival were peak creatinine and status at discharge (recovery versus nonrecovery) eGFR at baseline and days on HD. During follow up (up to 54 months) the percentage of patients with eGFR<60decreased from discharge88.5%to60.3% at 6months then increased to 75% at 36 months and longer.The percent with eGFR<30 decreased from67.1% at discharge to11% at 24 months to increase at a later date (30-36%).The percentage of patients with eGFR<15decreased from discharge (31.3%) to 10%at 18m of follow up to increase to 22% at later dates.

Conclusions: AKI requiring dialysis has a significant effect on GFR with almost 80% of the surviving having CKD stage 3 or less (GFR<60). Progression was observed on the long term follow up. Factors affecting the survival included peak creatinine and recovery of kidney function at discharge.

Funding: Clinical Revenue Support

FR-PO117

Acute Kidney Injury in Critically Ill Patients with Severe Acute Pancreatitis: Clinical Characteristics and Outcomes Jiaojiao Zhou, Yi Tang, Fang Liu, Yuliang Zhao, Ping Fu. *Div of Nephrology, Dept of Medicine, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

Background: Severe acute pancreatitis (SAP) is believed to be a frequent cause of acute kidney injury (AKI) in critical illness, but there is little information on SAP-induced AKI. We therefore studied the incidence of AKI defined by the Acute Kidney Injury Network (AKIN) criteria and the risk factors associated with outcomes in patients with AKI following SAP.

Methods: A multicenter retrospective study of critically ill patients with AKI due to SAP was performed from August 2009 to June 2013. Univariate and multivariate analysis were performed to investigate the risk factors on outcomes in the study population.

Results: In a total of 414 patients with SAP, 287 (69.3%) had AKI during intensive care units (ICU) stay by AKIN criteria and 16.7% was classified as AKI stage 1, 18.4% as AKI stage 2, and 34.3% as AKI stage 3. SAP patients with AKI was associated with a significantly higher ICU mortality (20.5% versus 44.9%, p<0.001) when compared without AKI. AKI was associated with older age and greater proportion of heart failure, chronic kidney disease, abdominal compartment syndrome (ACS), and operation than without AKI. In those patients, independent risk factors for ICU mortality included ACS (OR10.58; 95% CI: 5.98–18.72), renal replacement treatment (RRT) (OR 3.31; 95% CI: 1.77–6.19), sepsis (OR 2.46; 95% CI:1.10–4.01), Computed Tomography Severity Index (CTSI) (OR 3.01; 95% CI: 1.78–5.32), APACHE II score (OR 1.82; 95% CI: 1.23–2.72), AKI stage 3 (OR 1.38; 95% CI:1.19–1.77), duration of ICU stay (OR 1.04; 95% CI: 1.01–1.07), and multi-organ failure.

Conclusions: For the first time we have investigated the epidemiology of AKI due to SAP with the AKIN criteria in critically ill patients. We found that independent factors including ACS, RRT, sepsis, CTSI, APACHE II score, AKI stage 3, duration of ICU stay, and multi-organ failure were significantly related with ICU mortality.More effect should be taken to strengthen the management of AKI following SAP and improve the outcomes.

FR-PO118

Early Acute Kidney Injury in Critically Ill Combat Casualties Ian J. Stewart,[1] Jonathan Sosnov,[1] David Zonies,[2] Benjamin D. Morrow,[1] James D. Oliver,[3] Kevin Chung.[4] *[1]San Antonio Military Medical Center; [2]Oregon Health & Science Univ; [3]Walter Reed National Military Medical Center; [4]U. S. Army Inst of Surgical Research.*

Background: While acute kidney injury (AKI) has been studied in a variety of populations, there is a paucity of data in members of the U.S. Military injured in the wars in Iraq and Afghanistan. We sought to evaluate risk factors for the development of early AKI in this population and to determine factors associated with mortality.

Methods: We queried a military research database for subjects with combat injury that required ICU level care and survived to be evacuated from theater between 1 Feb 2002 and 1 Feb 2011. Data on creatinine, age, Injury Severity Score (ISS), Glasgow Coma Scale (GCS), race, in hospital mortality and presence of burn injury were extracted. Patients with at least one creatinine level within 2 calendar days of the date of injury were included for analysis. AKI was determined by KDIGO criteria. Univariate and stepwise multivariate logistic regression was performed to determine variables associated with AKI and mortality.

Results: A total of 1642 subjects were included for analysis. They had an average age of 25.7±6.0, median ISS of 18 (IQR 12, 27), median GCS of 15 (IQR 12, 15). The majority (98.4%) were male and 7.8% were African American. Burn injury was present in 13.9%. The in hospital mortality rate was 2.4%. AKI occurred in 13.9% of patients (12.1%, 1.2%, and 0.7% for KDIGO stages 1, 2 and 3, respectively). On univariate logistic regression, age (OR 1.02, 95% CI 1.00-1.05), ISS (OR 1.04, 95% CI 1.03-1.05) and GCS (OR 0.96, 95% CI 0.93-0.98) were associated with AKI. On multivariate analysis, only age (OR 1.03, 95% CI 1.00-1.05) and ISS (OR 1.04, 95% CI 1.03-1.05) were significantly associated with AKI. In the multivariate analyses for mortality, ISS (OR 1.07, 95% CI 1.04-1.09), GCS (OR 0.82, 95% CI 0.77-0.88) and AKI (OR 3.22, 95% CI 1.59-6.52) were significant. The c statistic of the model was 0.89.

Conclusions: Early AKI is common among combat trauma patients admitted to the ICU, occurring in 13.9%. Furthermore, the development of AKI is independently associated with a more than 3 fold rise in the odds of death in this population.

Funding: Other U.S. Government Support

FR-PO119

Renal Outcomes Post Orthoptic Heart Transplant in the Era of Left Ventricular Assist Device Siddiq Anwar, Pooja Koolwal, Mansumeet Singh, Jerrica Shuster, Timothy A. Horwedel, Justin Vader, Daniel C. Brennan. *Div of Medicine, Washington Univ School of Medicine, St. Louis, MO.*

Background: Hemodynamic support with a continuous-flow left ventricular assist device (LVAD) prior to orthotopic heart transplant (OHT) is increasingly common and introduces surgical complexity, but the incidence and extent of acute kidney injury (AKI) following OHT in these patients is not well-described.

Methods: Retrospective analysis of 190 consecutive OHT recipients from 2007-13 at a single center.

Results: Results are shown in Table 1.Of 190 OHT recipients, 86 (45%) had an LVAD pre-OHT and they were more likely to be male, white, obese, and to have a past history of hypertension. There was no statistical difference in the frequency or duration of renal replacement therapy (RRT) between VAD and non LVAD OHT patients. In the LVAD group there is no difference in LVAD duration (mean days ±SD) support pre- OHT between in those who did (374 ± 301.7) or did not die (355.1 ± 205.3) by 1 year (p=0.80) and also in those who needed RRT post OHT surgery (395.8 ± 427.2) and those who did not (365.4 ± 245.4) (p=0.70). In the LVAD group patients became RRT independent even up to 3 months after OHT. Creatinine clearance in the two groups was similar up to 60 months post OHT. Any need for RRT was associated with death by 1 year (23% RRT patients dead at 1 year versus 8% non-RRT dead at 1 year. p=0.02), but this was not different between VADs and non-VADs

Variables	No LAVD pre OHT=104	LVAD pre OHT=86	P-Value
Demographic			
Mean age ± Std. Deviation — in years	50.32 (13.34)	53.56 ± 11.99	0.08
Male sex (%)	63 (61%)	70 (81%)	0.002
Weight ± Std. Deviation- in KG	79.92 ± 19.37	87.85 ± 19.09	0.007
BMI ± Std. Deviation	26.91 ± 5.65	29 ± 5.55	0.014
Black (%)	10 (10%)	17 (20%)	0.012
Clinical			
Cause for endstage heart disease. (%)			0.021
Ischemic Cardiomyopathy	30 (29%)	33 (38%)	
Non-Ischemic Cardiomyopathy	21 (20%)	30 (35%)	
Hypertrophic Cardiomyopathy	5 (5%)	4 (5%)	
Viral	11 (11%)	6 (7%)	
Valvular	13 (13%)	4 (5%)	
Other	24 (23%)	9 (10%)	
Diabetes Mellitus	17 (16%)	21 (24%)	0.17
Smoking history (%)	46 (44%)	39 (45%)	0.88
Hypertension	55 (53%)	60 (70%)	0.018
LVAD duration pre OHT in days (Min, Max)	N/A	297(27.0,1879.0)	
Cold Ischemia in minutes± Std. Deviation	156.36 ± 50.91	178.2 ± 58.52	0.008
Days on ionotropes post OHT± Std. Deviation	9.98 ± 14.45	10.48 ± 10.41	0.79
Days on ventilator post OHT± Std. Deviation	4.46(4.86)	2.25(3.53)	0.08
Patients who developed Sepsis post OHT(%)	34(33%)	35(42%)	0.22
Baseline serum creatinine in mg/dl± Std. Deviation	1.25 ± 0.62	1.12 ± 0.54	0.1
Baseline creatinine clearence in ml/min	82.38 ± 41.50	93.74 ± 57.88	0.12
RRT pretransplant(no of patients)		1 3	0.28
RRT posttransplant			
Number of patients(%)	18 (17.3%)	19 (22%)	0.42
Mean ± Std. Deviation- in days	2.05 ± 6.57	5.01 ± 13.84	0.038
Number of days of RRT, Median(Min, Max)	7(2.0, 46.0)	17(1.0,92.0)	
Creatinine Clearence post OHT ± Std. Deviation in mls/min			
3 months± Std. Deviation	78.98 ± 28.93	75.1 ± 27.89	0.4
6 months± Std. Deviation	72.15 ± 26.72	72.12 ± 26.24	0.99
12 months± Std. Deviation	66.53 ± 26.74	66.22 ± 26.09	0.94
24 months± Std. Deviation	63.54 ± 22.13	64.14 ± 22.36	0.89
36 months± Std. Deviation	67.07 ± 23.65	63.73 ± 21.13	0.53
48 months± Std. Deviation	61.25 ± 27.04	69.67 ± 23.99	0.25
60 Months± Std. Deviation	68.00 ± 38.51	58.24 ± 19.53	0.44
Combined Heart Kidney Transplants		3 1	
End stage renal diease post OHT		1 2	
Death at one year	13 (13%)	8 (9%)	0.48
Overall mortality	14 (23%)	11 (27%)	0.66

Figure Legend: LVAD- Left venticular assist device, RRT: renal replacement therapy, BMI: body mass Index, OHT: Orthoptic Heart Transplant, N/A: Not applicable

Conclusions: Despite greater baseline risk factors for AKI, greater surgical complexity, and a trend to more frequent short term post-operative renal replacement therapy, OHT patients bridged with LVAD experience long term renal outcomes similar to OHT patients who were not bridged with LVAD.

FR-PO120

Long-Term Clinical and Renal Outcomes in Critically Ill Patients with Acute Kidney Injury Paul Hamilton Purvis,[1] Robert I. Docking,[2] Patrick B. Mark.[3] *[1]Univ of Glasgow, Glasgow, United Kingdom; [2]Glasgow Royal Infirmary, Glasgow, United Kingdom; [3]Inst of Cardiovascular and Medical Sciences, Univ of Glasgow, Glasgow, United Kingdom.*

Background: Critical illness is frequently complicated by acute kidney injury (AKI). The long-term outcomes of this patient group are poorly understood, with conflicting evidence regarding benefits of continuous versus intermittent renal replacement therapy (RRT). We studied short and long-term patient and renal outcomes in patients started on RRT in the intensive care unit (ICU).

Methods: Patient records were interrogated for all patients undergoing RRT in ICU at Glasgow Royal Infirmary between August 2010 and May 2013. Demographic, laboratory and clinical outcome data were retrieved, including creatinine and estimated glomerular filtration rate (eGFR) predating ICU admission (median 73 days pre-admission). Patients with pre-existing end-stage renal disease and ICU readmissions were excluded. Data were analysed for predictors of long-term renal outcome in hospital survivors, and comparison made between modality of RRT.

Results: 209 patients were studied. Modality of RRT was intermittent haemodialysis (IHD) in 88% and continuous veno-veno haemofiltration (CVVH) in 12%. Inpatient hospital mortality was 54%. Compared to hospital survivors, patients who died had higher APACHE score (26 versus 24, p=0.029) and non-significantly higher preadmission creatinine (1.23 versus 0.98mg/dL (p=0.09); other variables did not differ significantly. Baseline characteristics (age, sex, severity of acute illness, comorbidities) did not differ between modality of RRT. Those receiving CVVH did not differ in outcomes of renal function, survival or comorbidity. Hospital survivors had a non-significant decrease in renal function (pre/post hospital eGFR, 82.4/77.7 mL/min/1.73m^2). The only significant predictor of long-term eGFR or change in eGFR was baseline eGFR in a linear regression model.

Conclusions: Patients requiring ICU admission with AKI have a high mortality. We have shown a modest reduction in renal function following ICU admission, which is predicted by baseline eGFR; this may impact on long-term renal outcomes. Neither RRT modality showed superiority regarding long-term renal function.

FR-PO121

Acute Kidney Injury in Hospitalized Stroke Patients: Incidence, Risk Factors and Its Influence on the Outcomes Yang Luo. *Dept of Nephrology, Beijing Tiantan Hospital, Capital Medical Univ, Beijing, China.*

Background: Acute kidney injury (AKI) is a common and serious problem in hospitalized patients, however, there is a paucity of data describing clinical epidemiological characteristics of AKI among stroke patient. We therefore evaluate the incidence and risk factors of AKI and its impact on the outcomes in a group of hospitalized stroke patients.

Methods: We retrospectively analysed 647 consecutive patients with computer tomography confirmed stroke in neurological ICU between 2012 and 2013. Data were retrieved from electronic database of our hospital. AKI was identified and staged according to the 2012 KDIGO criteria. The incidence, risk factors of AKI and its influence of clinical outcomes of stroke were analysed. Stroke severity was assessed by the National Institutes of Health Stroke Scale (NIHSS) score.

Results: The mean age was 58.1±13.6 years old and 54.1% were males. AKI occurred in 135 (20.9%) patients with 84 (62.2%), 26 (19.3%), and 25 (18.5%) of AKI stage 1, 2, and 3, respectively. Patients with AKI appeared to be older, male, higher NIHSS score, and lower baseline eGFR than non-AKI patients (P<0.05 for all). Moreover, the rate of atrial fibrillation, hypertension, diabetes mellitus, sepsis, iodinated contrast media, ACEI/ARBs, antibiotics and diuretics administration were significantly higher in AKI patients (P<0.05 for all). A multi-logistic regression analysis showed that the independent risk factors of AKI in these patients were NIHSS score, baseline eGFR, hypertension, and sepsis [ORs (95%CI) were 1.027 (1.003-1.051), 0.985 (0.977-0.993), 1.592 (1.002-2.528), 3.385 (1.976-5.799), respectively; P<0.05 for all]. Patients with AKI had a significantly higher rate of all-cause mortality (36.3% versus 3.1%, P<0.01), and length of stay in hospital (15±6 days versus 6±2 days, P<0.01).

Conclusions: AKI is not rare among the stroke patients. Prevention and management of AKI is one of important measures among the stroke patients who are exposed to many risk factors of AKI.

FR-PO122

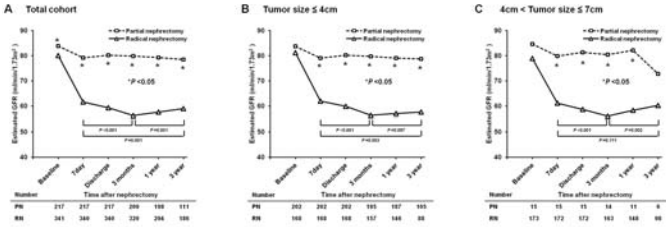
Impact of Partial Nephrectomy on Kidney Function in Patients with Renal Cell Carcinoma Chang Seong Kim, Eun Hui Bae, Seong Kwon Ma, Soo Wan Kim. *Dept of Internal Medicine, Chonnam National Univ Hospital, Gwangju, Korea.*

Background: The development of chronic kidney disease (CKD) is higher in patients with a small sized renal cell carcinoma (RCC) after radical nephrectomy (RN) than partial nephrectomy (PN). However, the renal outcomes between RN and PN are not well-

understood in a moderate sized RCC patients. This study aimed to compare the changes in kidney function and the association of tumor size and renal outcomes between patients with RCC who underwent RN and those who underwent PN.

Methods: A retrospective cohort study was conducted for 557 patients with an RCC ≤7 cm in diameter and normal contralateral kidney function who underwent PN or RN. PN was performed for 218 (39%) patients. Renal outcomes included the incidence of acute kidney injury (AKI), new-onset CKD and a ≥25% decline in eGFR 1 year after surgery.

Results: Serial changes in eGFR were compared during the 3 years of follow-up. Postoperative eGFR was significantly lower in patients undergoing RN than in those undergoing PN. The incidence of AKI and new-onset CKD was significantly higher in patients after RN (70.1% versus 24.3%, respectively; P < 0.001) than after PN (55.7% versus 6.2%, respectively; P < 0.001). According to the multivariable logistic regression analysis, RN was an independent risk factor for a ≥25% decline in kidney function after 1 year regardless of the tumor size, even after adjusting for various covariates.



Conclusions: Compared to PN, RN for even a moderate sized RCC leads to an increased incidence of AKI and new-onset CKD, and is a significant risk factor for kidney function decline. Therefore, PN should be considered as the choice of surgical treatment for RCCs that are ≤7 cm in diameter in order to preserve renal function postoperatively.

FR-PO123

The Effect of Renal Consults on Mortality in Patients with Acute Kidney Injury Iram Aqeel, Matthew J. Triano, Yevgeniy Gitelman, Yuliya Borovskiy, Michael G. Shashaty, Barry D. Fuchs, Francis Perry Wilson. *Univ of Pennsylvania.*

Background: Identifying which patients with acute kidney injury benefit most from renal consultation would increase the efficiency of care delivery and potentially improve outcomes.

Methods: We performed direct chart review of patients enrolled in a randomized trial of an electronic alert system for acute kidney injury (clinicaltrials.gov #NCT01862419). Renal consults were identified and abstracted by trained reviewers. We developed a propensity score for renal consultation based on demographics, time-varying laboratory parameters, and other clinical factors. Each patient who received a renal consult was propensity score matched with one patient who did not receive a renal consult in a greedy, nearest-neighbor fashion. Our primary outcome was inpatient mortality. Factors associated with consult benefit were evaluated by including interaction terms in a logistic regression model.

Results: Of 817 patients reviewed, 115 (14.1%) received a renal consult and were matched to 115 patients who did not receive a consult. The groups were similar with regard to age, sex, race, and all covariates used in propensity matching including serum creatinine, bicarbonate, potassium, blood urea nitrogen, lactic acid, and medical versus surgical status (all p>0.05). In the matched cohort, renal consult was not associated with a mortality benefit with inpatient mortality 17% among those who received a consult and 23% among those who did not (p=0.25). Renal consult was associated with a mortality benefit among those on the general floor as compared to the ICU (p=0.04), and among older patients (p=0.02). There was no detectable benefit to consultation among those with a shorter duration of AKI or among medical versus surgical patients. After exclusion of patients who went on to receive dialysis, consultation was associated with a reduced risk of inpatient mortality with OR 0.29 (0.11 – 0.78, p=0.01).

Conclusions: Renal consult for acute kidney injury may be associated with a survival benefit, particularly among those who are not yet receiving ICU care and those of older age. Randomized trials of renal consultation in AKI may be warranted to further elucidate this relationship.

Funding: NIDDK Support

FR-PO124

Outcome of Dialysis-Dependant Patients with Cast Nephropathy Treated or Not By Bortezomib: A Retrospective Monocentric Study Including 59 Consecutive Patients Levy Benedicte, Chadia H. Beaini, Denis Viglietti, Fabien Metivier, Denis Glotz, Marie-Noelle Peraldi. *Hopital Saint-Louis, Univ Paris 7, Paris, France.*

Background: Dialysis-dependant renal failure (DDRF) is associated with a poor outcome in multiple myeloma (MM), although some studies have shown improved overall survival in patients treated with novel agents such as bortezomib (borte).

Methods: This retrospective monocentric study included 59 consecutive patients with MM and DDRF for more than two weeks hospitalized in our unit between january 2004 and december 2010. Only patients with cast nephropathy were included. The aim of the study was to analyze the effects of borte on both patient and renal survivals. Statistical analysis were performed using the Mann-Whitney U test, Cox proportional hazard regression analysis and the Kaplan-Meier method for survival analysis.

Results: Median follow-up was 20.7 months (2-39). Median age of the patients was 60.7 years. In 22 cases (37%), DDRF occurred at diagnosis of MM. Thirty-nine patients were treated by Borte (66%). Borte treatment was associated with overall patients'survival improvment (P=0.03) with a median survival of 22 months in the Borte group *versus* 10 months in the other group. Multivariate analysis showed that only age <60 years (P=0.027), Borte treatment (P=0.033) and the occurrence of DDRF at diagnosis of myeloma (P=0.016) were factors associated with patient survival. In 16 patients (27%), dialysis could be withdrawn after 3.5 +/- 1.2 months. The use of either polysulfone or PMMA membranes did not influence the renal outcome. The single parameter significantly associated with dialysis withdrawal was the decrease of serum free light chain (P=0.001).

Conclusions: In DDRF patients with cast nephropathy included in this study, borte treatment was associated with improved survival, but not with the weaning of dialysis. The decrease of serum free light chain levels was the single parameter associated with dialysis withdrawal.

FR-PO125

Acute Kidney Injury in Crohn's Disease: Incidence, Risk Factors and Outcome Sarah Margaret Moran,[1] Michael Clarkson,[1] Carthage Patrick Moran.[2] *[1]Renal Medicine, Cork Univ Hospital, Ireland; [2]Gastroenterology, Univ College Cork, Ireland.*

Background: The association between interstitial nephritis and glomerulonephritides in Crohns disease (CD) is well described. However, the incidence of AKI and CKD is not known. Our aim was to assess the incidence and risk factors for AKI in patients with CD.

Methods: We retrospectively examined laboratory records of all patients with CD from a tertiary referral centre over a 7 year period. Patients who had more than 3 serum creatinine measurements were included. Episodes of AKI and CKD were classified using the KDIGO guidelines.

Results: 416 patients with Crohns disease were identified. 55.4% (232) were female. Mean age was 45.1 years (range 17-88 years, SD 14.2 years). Mean baseline creatinine was 70.8umol/L (range 32-227; SD 21.68). Mean baseline eGFR was 103.3 mls/min (range 19-232, SD 27.5). 3.8% (16) of patients had CKD with an eGFR less than 60mls/min. 3.3% (14) CKD stage 3 and 0.5% (2) had CKD stage 4 (none had CKD 5). Overall 20.2% (85) of patients with CD experienced an episode of AKI during the time period of evaluation. 23.5% (20) of affected patients had more than one episode of AKI: 17.6% (15) had 2 episodes, 2.3% (2) had 3 episodes, 1.1% (1) had 4, 5 and 6 episodes respectively. 77.3% (85) had AKIN stage I, 11.8% (13) had AKIN stage II and 10.9% (12) had AKIN stage III. No patients required renal replacement therapy. Peak creatinine values ranged from 72-955umol/L (mean of 178; SD of 146). 12.7% (14) of patients did not recover their renal function to baseline level. Episodes of AKI correlated with lower baseline GFR, higher CKD stage and increasing age (p<0.001). Increasing age correlated with higher AKI stage and baseline CKD. There was a 3.3% annualized risk of AKI in patients with CD. Recurrent episodes of AKI were associated with increasing severity of AKI (p=0.039), higher baseline creatinine and CKD (p<0.001).

Conclusions: We report the first study of the incidence and outcome of AKI in patients with Crohns disease. AKI is a common event in the natural history of this illness with an annual incidence of 3.3%. This data suggests the need for targeted strategies to prevent AKI and progressive CKD in this population.

Funding: Clinical Revenue Support

FR-PO126

Superimposed Community-Acquired Acute Kidney Injury in Patients with Chronic Kidney Disease: A Prospective Observational Study Patrick Saudan,[1] Belen Ponte,[1] Cyrielle Alves,[2] Fabien Stucker,[3] Pierre-Yves F. Martin.[1] *[1]Nephrology Unit, Dept of Medical Specialties, Geneva Univ Hospitals, Geneva, Switzerland; [2]Dept of General Internal Medicine, Geneva Univ Hospitals, Geneva, Switzerland; [3]Nephrology Unit, Hôpital de la Providence, Neuchatel, Switzerland.*

Background: Patients with CKD are prone to superimposed AKI. We aimed to better define characteristics, prognosis of community-acquired (CA-AKI) and use of potentially nephrotoxic drugs in CKD patients.

Methods: Prospective observational study within the Emergency Department of a University Hospital, screening for any patient > 16 years admitted with an eGFR < 60 ml/mn. Patients with CKD (previously known for a eGFR < 60 ml/mn) were included in this analysis and superimposed AKI was defined as a decline in eGFR compared to previous values according to KDIGO AKI criteria.

Results: From May 1st up to June 21st 2013, there were 8464 admissions and 664 patients (8%) had a eGFR < 60 ml/mn of whom 439 (66%) were known to have previous CKD. Mean age was 79 (12) yrs and 22% were diabetics. Use of ACEIs/ARBs, diuretics, NSAIDs and antibiotics was 47, 40, 3 and 3 % respectively. AKI was superimposed in 102 (23%) CKD patients. Etiology of superimposed AKI was prerenal (73%), renal (17%) and postrenal (9%).Stage I/II/III AKI were 8, 5 and 8% respectively. Multiple logistic analysis showed that occurrence of AKI was associated with male gender (OR 2.24; 95%CI:139-3.64, p=0.001) and diuretic use (OR 1.61; 95%CI:1-2.60, p=0.05).10 % of these patients went to ICU, and 4 % needed RRT. Length of stay (median+IQR) was increased in CKD patients with superimposed CA-AKI (11; 4-24 versus 8;8-17 days, p=0.01) Three month survivals were 93 % in patients with CKD and 90 % in CKD patients with superimposed CA-AKI.

Conclusions: These preliminary results show that community-acquired superimposed AKI in elderly CKD patients is more frequently encountered in male patients and those treated with diuretics may be at higher risk of CA-AKI. Some cases of CA-AKI may be avoided in CKD patients with clear education as to management of diuretics in case of disease-induced hypovolemia.

FR-PO127

Contrast Induced Nephropathy: Assessing the True Incidence of AKI Related to CT Scans with and without Contrast Juliya Hemmett,[1] Lee Er,[2] Chris Cheung,[1] Sean West,[3] Helen Chiu,[2] Ognjenka Djurdjev,[2] Adeera Levin.[1] *[1]Nephrology, St. Paul's Hospital and Univ of British Columbia, Vancouver, BC, Canada; [2]BC Provincial Renal Agency, St. Paul's Hospital, Vancouver, BC, Canada; [3]Vancouver Coastal Health Authority, Vancouver, BC, Canada.*

Background: Contrast-induced nephropathy (CIN) is the acute decline in renal function 48-72 hours after contrast media administration. Incidence rates vary in the literature. Since 100,000 CT scans are performed annually within one health authority (HA) in Canada, there is interest in implementing a standardized CIN prevention protocol (CIN-PP), to decrease risk of CIN.

Methods: We evaluated the efficacy of a pilot CIN-PP by measuring the incidence of CIN within the HA at baseline and after PP implementation. During two time periods, pre and 3 mo post CIN-PP implementation, all hospitalized patients who had 2 serum creatinine (sCr) values within a 7 day period pre and post CT scan, with and without contrast were included in the sample. Data included: patient (pt) demographics, type of CT scan, and sCr values. CT scans were excluded if they involved an extremity or if a pt received more than one scan within a 7 day period. Of 4919 scans done, there were 325 CT scans from the pre-protocol phase in Dec. 2012, and 518 CT scans from the post-protocol phase in Oct. 2013 meeting inclusion criteria. The primary outcome was the incidence of CIN, defined as a sCr increase of >26.5 mmol/L within 7 days post-CT scan.

Results: The mean age of the population was 70y, mean eGFR at baseline =70. Baseline and post-protocol implementation CIN incidence was similar (10.9 versus 10.0%;p=0.64). We evaluated the proportion of pts who received IV contrast in both time periods who had 2 sCr values; more pts post CIN-PP had 2 sCr values (73.6 versus 79.8%;p=0.14). The incidence of CIN did not vary between those who did and did not receive contrast in either time period.

Conclusions: The application of robust research methodology to the CIN-PP quality improvement initiative raises questions as to the value proposition of CIN-PP. Further understanding of factors contributing to AKI in those receiving CT scans, irrespective of contrast may guide future targeted interventions.

FR-PO128

Mortality and Hospital Re-Admission Rates for Patients with Stage III AKI Compared to all General Medical Admissions Pritpal Singh Virdee, Ben Talbot, Janet Campbell, Debasish Banerjee. *Renal Unit, St. Georges NHS Trust, London, United Kingdom.*

Background: Increased morbidity associated with acute kidney injury may precipitate further medical intervention or consultation following initial discharge. This has cost and resource implications which may be preventable. We compared mortality and re-admission rates for patients with Acute Kidney Injury stage III against all other patients admitted under General Medicine in a large tertiary referral hospital based in London.

Methods: A retrospective audit was performed to identify patients admitted to St Georges Hospital NHS Trust between July 2013 and March 2014. The hospital database was interrogated using information submitted via Electronic Patient Records (EPR) and iClip to create two patient groups with 30 day mortality and re-admission rates for each. Patients who were admitted under a treating general medical team following blood tests were identified. Patients admitted with AKI stage III were identified via AKI stage calculation performed by iSOFT pathology software and these patients formed the second group. All patients receiving acute or chronic dialysis were excluded from both groups prior to analysis.

Results: The average age amongst general medical patients and patients with AKI III were 64.51+/- 21.10 (1SD) and 67.17+/- 17.29 (1SD) respectively. 458 patients were admitted with AKI stage III not requiring dialysis. Amongst this group there were 141 deaths within 30 days (30.79%) and 30 re-admissions to hospital (6.55%). In the General medicine group there were 5458 admissions, 253 deaths (4.64%) and 418 readmissions (7.66%). There was statistical significance in mortality (P <0.0001) but not re-admission rates (P = 0.4418) between the groups.

Conclusions: 30 day mortality is significantly increased when patients are admitted with AKI stage III not requiring dialysis with a relative risk of death of 6.64 but risk of re-admission is not found to be increased. One factor may be increased mortality in patients with AKI stage III resulting in a smaller discharged patient cohort. Further investigation is required to determine what prompts re-admission to hospital amongst these patients and what the long term outcome is.

FR-PO129

Acute Renal Replacement in Critical Care: Short and Long-Term Prognosis Fernando Saldanha Thome,[1,2] Antonio Balbinotto,[1] Cássia M. Morsch,[1] Verônica Verleine Hörbe Antunes,[1] Aline Castello Branco Mancuso,[1] Pâmela Dalla Vecchia.[1] *[1]Nephrology, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; [2]Internal Medicine, FAMED-UFRGS, Porto Alegre, Brazil.*

Background: Our objective is to identify short and long term prognostic factors for acute kidney injury (AKI) with renal replacement therapy (RRT) in critical care patients.

Methods: A cohort of critical care patients needing RRT for stage 3 AKI was prospectively followed from 2006 to 2013. Chronic kidney disease stage 5 and renal transplants were excluded. RRT was intermittent hemodialysis-IHD (Fresenius™) for hemodinamically stable or continuous RRT-CRRT (Prisma™ or Diapact™) for unstable patients. Independent variables were: demographics, baseline creatinine, type of AKI, co-morbidities, APACHE II score, variables related to the treatments. Outcomes were mortality and dependence on dialysis during and after hospitalization. Statistics: univariate and mutivariate Poisson regression with robust variance analysis, SPSS 18.

Results: We followed 1828 patients, age 58.4±16.8years, 57%male, 86%white, 23%having baseline creatinine ≥ 1.5 mg/dl, 69%with clinical AKI, 77%septic, 93%with mechanical ventilation, 88%with vasopressors,APACHE II score 27.2±8.8. IHD (5.1±5.7 sessions) was used in 46% and CRRT (5.5±5.7 days) in 86%patients. Citrate was the anticoagulant in 71% of CRRTs. Time in the ICU was 14.7±16.7 days. Case-fatality rates were 65% in ICU and 72% in the hospital; 30% of patients became independent of dialysis in the hospital. Hospital survivors (n=489) were reached in a median of 24.1 months after discharge, seventy of them (14.3%) on chronic dialysis and 69%still alive. Factors associated with hospital mortality were sepsis, APACHE II score, use of mechanical ventilation or vasopressors, severe hepatic disease and age. Factors associated with mortality after discharge were baseline creatinine≥1.5 mg/dl, diabetes and cancer. The main factor related to dependence on chronic dialysis was baseline creatinine.

Conclusions: The high morbidity and mortality of critical care patients needing RRT because of AKI stage 3 remains beyond hospital discharge, but prognostic factors determining outcomes in the hospital or after discharge are different.

FR-PO130

The Impact of Serum Creatinine Adjustment for Fluid Balance on Diagnosis of Acute Kidney Injury in Critically Ill Patients Wisit Cheungpasitporn, Charat Thongprayoon, Kianoush Banaei-Kashani. *Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Fluid accumulation may delay recognition of acute kidney injury (AKI) in ICU patients commonly developing volume overload. This study aims to evaluate the impact of fluid balance on the diagnosis of AKI and associated outcomes in critically ill patients.

Methods: This is a single-center retrospective observational study conducted at a tertiary referral hospital. All adult ICU patients between January and March 2010 with at least one serum creatinine (SCr) measured in ICU were included. Patients with end stage renal disease or on dialysis before the ICU admission were excluded. AKI was defined as an absolute increase in SCr in ICU of ≥ 0.3 mg/dl or relative change of ≥ 50% from baseline. AKI was identified before and after serum creatinine adjustment for fluid balance. We used the following formula for the correction factor [admission body weight (kg) * 0.6 + cumulative fluid balance (L)]/ [admission body weight * 0.6].

Results: A total of 563 patients were enrolled in the final analysis. AKI was detected in 126 (22.4%) before adjustment and 148 (26.3%) after adjustment for fluid balance (P < 0.001). Among 124 patients who had AKI regardless of SCr adjustment SCr adjustment did not change the time of AKI diagnosis in most patients. Risk of 90 mortality in patients with AKI both before and after SCr adjustment for fluid balance showed significant increase compared to patients without AKI (RR = 2.33; 95% CI 1.59-3.42). In patients who met AKI criteria after but not before SCr adjustment for fluid balance, risk for 90-day mortality was not higher than patients without AKI both before and after adjustment (RR = 1.38; 95% CI 0.54-3.49), although there was a non-significant trend toward increased mortality in the former group.

Conclusions: Fluid management in ICU influences SCr and therefore diagnosis of AKI using SCr-based definition. SCr adjustment for fluid balance could detect earlier and more AKI cases. We would suggest using fluid balance adjustment for screening purposes as a highly sensitive definition. On the other hand when specificity is important AKI definition without fluid balance adjustment could be used.

FR-PO131

Renal Dysfunction and Fluid Overload in *Clostridium difficile* Infections Ravindra L. Mehta, Romana Turanovicova, Sareen Kaila Sandhu, Yu-Ting Christi Kao, Saima Aslam. *Univ of California, San Diego.*

Background: Clostridium difficile infection (CDI) is a devastating complication in hospitalized patients that increases morbidity, mortality and resource utilization. We sought to elucidate the role of fluid overload as a contributing factor to the link between renal disease and poor outcomes in CDI.

Methods: We retrospectively collected data from the electronic medical record of patients diagnosed with their first episode of CDI at an academic medical center during 2012. Data abstracted: demographics, clinical characteristics, daily fluid balance, body weight, laboratory values including creatinine, severity of CDI, treatment outcome, recurrence, readmission, and mortality in 90 days.

Results: Among 359 patients with CDI, 22.8% had AKI in the 2 weeks prior to CDI diagnosis and 8.4% were on hemodialysis; these were independent predictors of severe CDI in a multivariate model (p=<0.05). Dialyzed patients had a lower treatment success rate versus non-dialyzed patients (50% versus 69.5%, p=0.049); ≥5% fluid overload in the 48 hours following CDI diagnosis was as an independent predictor of treatment failure in this group. Additionally, cumulative fluid balance ≥ 10% in the first week prior to CDI diagnosis was associated with significantly longer length of stay (mean 29.1 versus 16.2 days, p=0.0007). Once CDI was diagnosed, fluid overload ≥ 5% in the following 48 hours was associated with diarrhea duration ≥ 5 days, accounting for severe diarrhea (≥10 stools/day) as well as severe CDI as confounders (OR 2.35, p=0.027). 13% of patients developed AKI in the 2 weeks following CDI diagnosis; this was associated with increased mortality independent of fluid overload (36% versus 8.9%, p=0.001). Patients that had severe CDI at the time of diagnosis were more likely to develop AKI in the following 2 weeks (p=0.001), independent of diarrhea severity.

Conclusions: Patients with renal disease have a higher rate of severe CDI, and development of AKI following CDI diagnosis is associated with increased 90-day mortality.

Fluid accumulation in dialyzed patients plays a role in the poor treatment outcomes of these patients. Fluid overload in general is also associated with prolonged diarrhea in CDI patients.

Funding: NIDDK Support

FR-PO132

Effect of Fluid Overload on Survival Rate in Critically Ill Patients with Acute Kidney Injury Receiving Continuous Renal Replacement Therapy

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Background: Extensive fluid overload (FO) leads to acute physiological changes such as metabolic imbalances of water and electrolytes, pulmonary edema, and respiratory failure. Thus we investigated whether FO is associated with mortality in critically ill patients with acute kidney injury (AKI) receiving continuous renal replacement therapy (CRRT).

Methods: We retrospectively reviewed 140 patients with AKI treated with CRRT between April 2005 and August 2011. We calculated fluid balance for each day using the sum of daily fluid intake (L) from which we subtracted total output (L) before initiation of CRRT. Patients were divided into three groups according to the tertile of FO: mild (FO1), moderate (FO2), and severe (FO3).

Results: Of the 140 enrolled patients, the mean follow-up period was 37.0 ± 77.8 days and the median duration of renal replacement therapy was 8 (1-594) days. Mean cumulative fluid balance was 9.0 ± 9.3 L (FO1, 1.1 ± 3.4L; FO2 6.8 ± 1.7L; FO3, 19.2 ± 8.5L). The FO1 group showed a significantly lower incidence of mechanical ventilation (63.8% versus 88.2%, *P*=0.001) and a lower use of inotropics (68.1% versus 91.4%, *P*=0.001) compared to the FO2 and FO3 groups. Survival rate was significantly higher in the FO1 group than in the FO2 and FO3 groups (P=0.003). In multivariate Cox analysis, patient survival was significantly higher in the FO1 group than in the FO2 and FO3 groups (hazard ratio 1.79, 95% confidence interval 1.15–2.80, P=0.011).

Conclusions: In critically ill patients with AKI, fluid overload was independently associated with mortality.

FR-PO133

Extracelular Hypervolemia and Prognosis in Acute Kidney Injury. Bioimpedance Analysis Aplication to Prevent Volemic Hyperexpansion

Francisco Javier Lavilla, Nuria Garcia-Fernandez, Paloma L. Martin Moreno, Jose Maria Mora Gutierrez, Maria Jose Molina Higueras, Diana Lopez Espinosa, Pelayo Moiron Fdez-Felechosa, Pedro Errasti. *Nephrology, Clínica Univ de Navarra, Pamplona, Navarra, Spain.*

Background: The aim of this study is to evaluate corporal hypervolemia by bioelectrical impedance analysis (BIA) as a marker of poor prognosis in acute kidney injury (AKI). Hypervolemia in AKI have consequences (cardiac failure, pulmonary edema, ICU and renal replacement theraphy). Another aim is evaluate application of cardiothoracic bioimpedance to prevent volemic hyperexpansion.

Methods: In a cohort of 122 patients (62 years-old SD 0.33, 72% male) with a diagnosis of AKI, a corporal bioimpedance analysis was made. Volemic bioelectricals parameters extracellular/intracellular water ratio (EC/IC), acute clinical index (individual severity index –ISI-), analytical parameters (C-reactive protein –CRP-, prealbumin –PRALB-) and mortality were evaluated. In a preliminary study we use hemodynamic bioimpedance analysis to evaluate Corporal Thoracic Fluid (CFT) and CFT index (CFTI). SPSS version 20.0 software was used.

Results: Exitus 10.4%. Extracelular hypervolemia was associated with poor prognosis in AKI. EC/IC showed a statistical significance correlationship with ISI (r = 0.286, p=0.001) andCRP (r = 0.323, p=0.001); as well as an inverse correlationship with PRALB (r = -0.405, p=0.010). EC/IC was associated with mortality risk (p= 0.008, OR 2.218 CI 95% 1.23-3.98) showing an AUC cut-off 0.776 (p=0.001;CI 95% 0.667-0.885). In a preliminary study with oliguric AKI we evaluated CFT and CFTI in 15 patients, showing higher CFT (> 37 l/kOhm) and CFTI (> 21 l/kOhm/m2) in 8/15 (53%). Patients with higher CFT and CFTI the intravenous hydration was stopped. No cardiorespiratory complications were found after adjustment of fluidotherapy during AKI.

Conclusions: Hypervolemia was associated with poor prognosis in AKI. Inflammatory status and hypoproteinemia were associated with hypervolemia. In oliguric AKI it may be possible to prevent complications of hyperexpansion by bioelectrical impedance (corporal and cardiothoracic). Bioimpedance is an useful tool in the evaluation and management of patient with AKI.

FR-PO134

Prognosis in Acute Kidney Injury. Unicentric Prospective Observational Study. Clinical Parameters Associated with Mortality and Renal Replacement Therapy

Francisco Javier Lavilla,[1] Siru Hu,[2] Nuria Garcia-Fernandez,[1] Paloma L. Martin Moreno,[1] Diana Lopez Espinosa,[1] Jose Maria Mora Gutierrez,[1] Maria Jose Molina Higueras,[1] Pelayo Moiron Fdez-Felechosa,[1] Pedro Errasti.[1] *[1]Nephrology, Clínica Univ de Navarra, Pamplona, Navarra, Spain; [2]School of Medicine, Univ de Navarra, Pamplona, Navarra, Spain.*

Background: The aim of this prospective study is to evaluate clinical parameters associated with mortality and renal replacement therapy (RRT) in acute kidney injury (AKI).

Methods: In a cohort of 2559 patients (62 years-old SD 0.31, 71.4% male) with a diagnosis of AKI (creatinine increase > 20%) between march1996 and march2014 in a tertiary hospital center, we evaluated acute clinical index (individual severity –ISI-) ISI formula=0.032* (age decade)-0.086* (male)-0.109* (nephrotoxic) +0.109* (oliguria) +0.116* (hypotension) +0.122* (jaundice) +0.150* (coma)-0.154* (consciousness) + 0.182* (assisted respiration) +0.210.), mortality and renal replacement therapy requirement (RRT). In 674 patients we analyzed analytical parameters during AKI (peak creatinine –C-, peak C-reactive protein –CRP-, peak brain natriuretic factor -pro BNP-, minimal albumin –ALB-, basal prealbumin –PRALB-, minimal haemoglobin –Hb-) to correlate its relationship. An univariant analysis with logistic regression was made to analyzed ISI with mortality and RRT. Multivariant analysis was performed to study analitical parameters and its relationship with oliguria. SPSS version 20.0 software was used for statistical analysis.

Results: The mortality rate was 17.7% with RRT required in 26.1% of the patients. Multivariant analysis with clinical parameters included in ISI showed a relationship between mortality and coma (p=.000, OR=8.186, CI 95% 5.993-11.18), as well as oliguria (p=.000, OR=6.592, CI 95% 5.140-8.453); while RRT was associated with oliguria (p=.000, OR=17.816, CI 95% 14.27-22.32). Oliguria showed an association in Multivariant analysis (including analytical parameters) only with PRALB (p=.004, OR=.911, CI 95% 0.855-.970).

Conclusions: Protein status was associated with oliguria risk. Oliguria was associated with RRT and mortality in AKI.

FR-PO135

Association between Phase Angle and Mortality Risk in Acute Renal Failure Patients

Francisco Javier Lavilla,[1] Jorge M. Nunez-Cordoba,[2] Maria Jose Molina Higueras,[1] Nuria Garcia-Fernandez,[1] Paloma L. Martin Moreno,[1] Jose Maria Mora Gutierrez,[1] Diana Lopez Espinosa,[1] Pelayo Moiron Fdez-Felechosa,[1] Pedro Errasti.[1] *[1]Nefrologia, Clinica Univ Navarra, Pamplona, Navarra, Spain; [2]Preventive Medicine and Public Health, Clinica Univ Navarra, Pamplona, Navarra, Spain.*

Background: The phase angle (PA) is defined as the relation between the 2 vector components measured by bioelectrical impedance analysis (BIA): resistance and reactance. A low PA has been suggested to be an adverse prognostic marker of survival, although no study has evaluated their prognostic influence on Acute Renal Failure (ARF) patients. The individual severity index (ISI) estimates the probability of death. We evaluated the association between PA and ISI, and between PA and death in AKI patients.

Methods: Clinical factors, PA, and death were prospectively registered in 69 AKI patients. ISI formula=0.032* (age decade)-0.086* (male)-0.109* (nephrotoxic) +0.109* (oliguria) +0.116* (hypotension) +0.122* (jaundice) +0.150* (coma)-0.154* (consciousness) + 0.182* (assisted respiration) +0.210. Each variable is evaluated when the nephrologist sees the patient the first time, and takes a value of 1 (presence) or 0 (absence), except for the age.

Results: Patients´ characteristics: Mean age, 65.5±15.2 years; men (72.5%); mean ISI, 0.25±0.11; and mean PA, 3.95±1.47. Deaths: 10 (14.49%). The association between PA and ISI index is showed in figure 1, and the relationship between PA and predicted mortality is shown in figure 2.

Figure 1

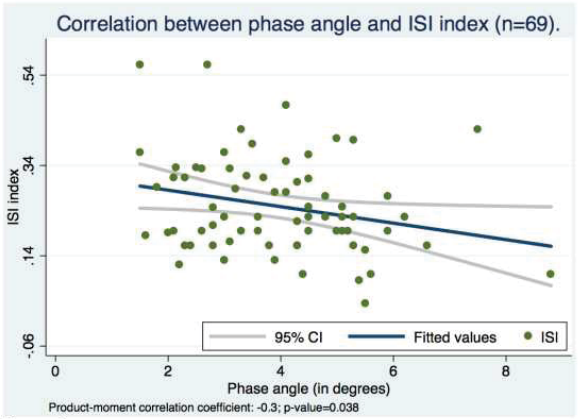
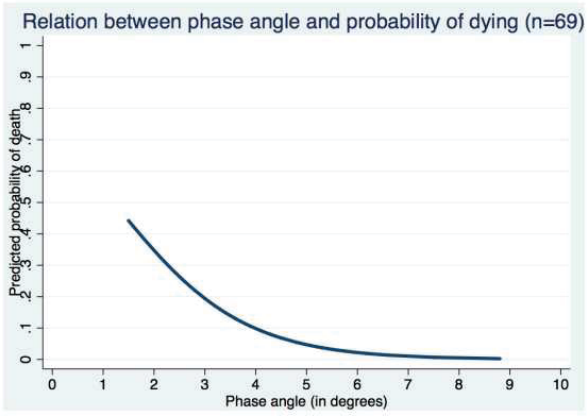


Figure 2



Conclusions: These findings suggest a statistically significant, moderate, and negative association between PA and ISI index. The PA appears to be a prognosis index of mortality in AKI patients.

FR-PO136

Obstetric Nephrology - Acute Kidney Injury in the Third Trimester of the Pregnancy and in the Puerperium Aline Gibson Notaro,[1] Luisa Queiroga Ferreira,[1] Ana Paula Gueiros,[1,2] Rafaella Barros da Paixão,[1] Raquel Leal de Alcantara Belfort,[1] Rodrigo Amblard Wanderley,[1] Ruben Andrade Filho,[1] Denise Maria do Nascimento Costa.[1,2] *[1]Nephrology Dept, Inst de Medicina Integral Prof. Fernando Figueira - IMIP, Recife, Pernambuco, Brazil; [2]Nephrology Div, UFPE, Recife, PE, Brazil.*

Background: Acute kidney injury in pregnancy (P-AKI) remains a cause of significant fetomaternal mortality. P-AKI occurs mainly during the late third trimester and around delivery. Hypertensive complications of pregnancy (HCP) are currently the leading cause of P-AKI. The aim of this study was to evaluate the factors responsible for P-AKI and its relation with outcomes.

Methods: Ninety-four women in the third trimester of pregnancy or in the immediate puerperium were included in this retrospective study. Demographic data, urine output (UO), HCP [preeclampsia (PE) /eclampsia (E) or hemolysis, elevated liver enzymes, and thrombocytopenia syndrome (HELLP)], sepsis and maternal mortality were noted and need for dialysis (HD) was considered. Biochemical tests were evaluated: creatinine on admission (Cr A), on arrival of nephrology (Cr N), 24-hour urinary protein (UP), hemoglobin (Hb), platelets (PT) and lactate dehydrogenase (LDH).

Results: The mean age of the patients was 26.2 ± 6.7 years and 72 (76.6%) were in immediate puerperium. The mean gestacional age was 30.4 ± 3.98 weeks. Twelve (12.8%) patients had CKD. The main causes of AKI were: Ischemic acute tubular necrosis (29.8%), PE + HEELP (25.5%), Sepsis (17%), PE (14.9%). Anuria or oliguria was observed in 29.7% cases and 35.1% required dialysis. Fourteen (15%) patients died, complete recovery was observed in 68 (72.3%), 4 patients (3 with CKD) remained on dialysis and 8 CKD patients returned to the basal creatinine or were not dependent on dialysis. Factors associated with HD were: Hb (p=0.028), Cr N (p<0.001), LDH (p<0.001) and UO (p<0.001). Maternal mortality was associated with HD (p<0.001), UO (p<0.001), sepsis (p<0.001) and LDH (p=0.01). Regarding fetal outcomes, 43.8% were preterm.

Conclusions: P-AKI is associated with a high risk for fetomaternal mortality and morbidity. HCP, notably HEELP syndrome, are the leading cause of P-AKI. LDH and UO are markers of worse outcome in pregnancy.y.

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FR-PO137

Outcomes in Acute Kidney Injury-Dialysis Dependent Population Rabeeh I. El-Refadi, Tadesse Beyene, Jerry Yee, Lenar T. Yessayan. *Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Background: Acute kidney injury (AKI) is an increasingly common complication of hospitalization affecting approximately 20% of all hospitalized patients and up to 67% of patients in the ICU. Approximately 4% of critically ill patients require renal replacement therapy (RRT). As a result of AKI, patients will have longer hospital stays and may require dialysis as outpatients. However, outcomes of renal recovery of dialysis-dependent AKI remain poorly described. **Aim:** The aim of this study is to examine the renal recovery rates of patients discharged on hemodialysis.

Methods: We prospectively enrolled 40 patients who developed dialysis-dependent AKI at a single center from January 1, 2013 to December 31, 2013. AKI causes, comorbid conditions and potential contributors to AKI as well as indications for RRT were recorded by time of initiation of dialysis. Dialysis dependence and mortality were assessed at 30, 60 and 90 days. For patients who eventually transferred to outside dialysis facilities, outcome data were collected by contacting dialysis units and patients or their surrogates.

Results: 40 patients initiated on RRT in intensive care units and were subsequently discharged on dialysis. Their mean age was 59 years. 9 (23%) had sepsis as a cause of AKI. 23 (58%) had underlying CKD; 28 (70%) had hypertension; 19 (48 %) had diabetes; and 12 (30%) had underlying liver disease. A total of 4 patients were lost to follow-up. The 90-day mortality rate was 4/36 (11%). Data at successive 30-day time intervals for renal recovery, death, loss to follow-up and cumulative probability of dialysis dependency are shown in the Classical Life TIME Table.

Days	0	30	60	90
No. at risk, n	40	30	23	14
Died, n	0	2	0	2
Lost to follow-up, n	0	1	2	1
Renal recovery, n (%)	0	7 (19%)	5 (18%)	6 (30%)
Probability of dialysis dependency	100%	82%	69%	51%

Conclusions: Prospective studies examining rates of recovery from dialysis-dependent AKI are lacking. Nearly one-fifth of hospitalized AKI patients who require RRT recover by 30 days and one-third by 60 days. Of those remaining dialysis-dependent after 60 days, nearly one-third recover.

FR-PO138

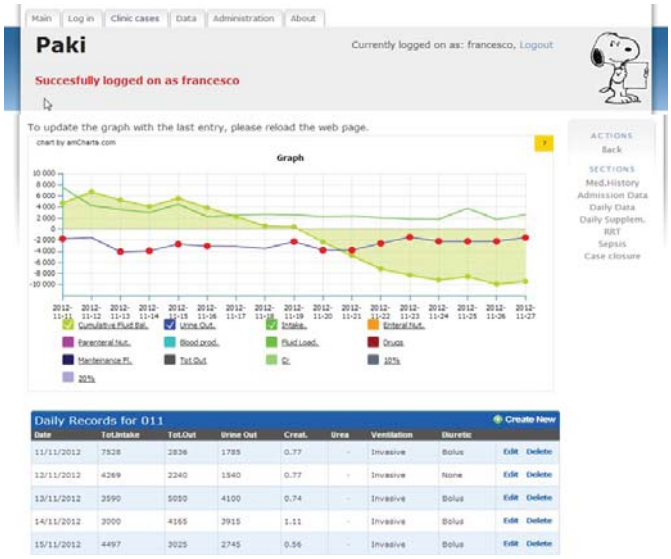
Development of a New Pediatric Acute Kidney Injury Registry Francesco Garzotto, Monica Zanella, Anna Lorenzin, Claudio Ronco. *Nephrology and IRRIV, St. Bortolo Hospital, Vicenza, Italy.*

Background: Advancements and improvements in care for critically ill pediatric population has led to fast changes in pediatric acute kidney injury AKI epidemiology. Prevalent causes leading to pediatric AKI are actually from single center's data. There is also a lack of information concerning AKI patients who did not require CRRT. The aim of the study is to: describe pediatric AKI patients, identify risk factor for AKI progression, evaluate the influence of fluid overload and type of fluids administrated (colloids, versus crystalloids versus nutrition) on clinical outcomes in AKI (w and w/o RRT) patients, describe current practices of RRT.

Methods: We developed a web-based password protected registry, dedicated to pediatric patients to support an observational and prospective international multicenter study in pediatric intensive care units pICU. Inclusion criteria: all patients admitted to pICU for almost 48h. We daily collect the amount of fluids (intake and output), diuretics, drugs, vasopressors and inotropes as well as scores and type of ventilation. Sepsis has also a dedicated section. RRT page includes dose calculation, circuit type and life, anticoagulants, type and location of catheter. Treatments done with the new carpediem machine also find their location in this section.

Results: After usability test, the registry has been designed on an open source based framework. Automated data verification system, pRIFLE calculation and alert, fluid management graph result as valid supports for fast data analysis helping in daily clinical activities.

Conclusions: The PAKI registry could represent a step forward in the description of the AKI epidemiology in pediatric patients and contribute to the resolution of the controversies regarding CRRT like initiation, dose, technique, anticoagulation.



Funding: Private Foundation Support

FR-PO139

Acute Kidney Injury among 234 In-Hospital Elderly Patients: A Single Center Retrospective Cohort Ederson Vidal Moura, Cintia Germana Mergulhão da Costa, Hugo Pinheiro, Têg Marcos Veiga, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Geraldo José de Amorim, Lucila Maria Valente. *Nephrology, UFPE, Recife, PE, Brazil.*

Background: Acute kidney injury (AKI) occurs in 2-7% of all hospital admissions and at a higher rate in elderly patients (EP). However data about epidemiological profile and outcomes are poorly known in this population.

Methods: We analyzed nephrologist referral (NR) medical records from January 2011 to December 2013. AKI was evaluated at the first day of NR based on AKIN Scr criteria and CKD was defined based on prior baseline Scr >1.3 mg/dL. EP were defined as patients older than 60 years-old.

Results: Among 778 medical records analysed, 319 of them were EP, with median age of 70y and 59% were male patients. AKI was found in 154 EP (48%), acute on CKD (A-CKD) was found in 80 patients (25%), ESRD in 66 patients (21%) and in the remainder (6%) renal injury could not be classified (missing data). Sepsis was found in 37% of in-hospital EP and it was the major cause of hospital entrance (20%) and mortality (65%). Ischemic etiology of AKI, including ATN and sepsis, was the most frequent mechanism of renal injury, followed by nephrotoxic and hypovolemic etiologies. Mean Charlson Comorbity Index (CCI) was 6.59 (95% IC: 6.18 - 7.01) in AKI patients and 5.98 (95% IC: 5.49-6.46) in A-CKD patients, without significant difference. AKI patients had higher rates of mechanical ventilatory assistance and need for vasoactive agents than A-CKD patients (P=0.001). Our overall mortality rate was 31% and univariated analysis showed higher mortality in AKI group when compared to A-CKD group (P= 0.001). Interestingly,

there was no difference in mortality rate relative to dialysis requirement in AKI patients (P=0.12) and A-CKD patients (P=1.0). NR occured in earlier stages of AKIN criteria in A-CKD group compared to AKI group (P=0.0001), but there was no diference in dialysis requirement between AKI and A-CKD groups (P= 0.32).

Conclusions: Among EP, AKI was associated with worse outcomes related to A-CKD, probably due higher rates of renal dysfunction at the NR and the high rates of cardiovascular and respiratory failure in the AKI group.

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FR-PO140

Blood Oxygen Level Dependent (BOLD) Imaging to Assess Disease Severity in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Background: MRI is the gold standard imaging technique to measure kidney volumes (KV) in ADPKD patients. However, KV does not provide information on non-cystic tissue. BOLD-MRI is sensitive to the blood concentration of paramagnetic deoxyhemoglobin, which acts as an MR contrast agent. Hypoxic regions present high R_2* (BOLD index), while fluid filled spaces (urine or cysts) have low R_2* values. We evaluated the use of BOLD-MRI for fast quantification of cystic and non-cystic tissue in a mouse model *Pkd1*R3277C (RC) of human ADPKD.

Methods: Fourteen *Pkd1*$^{RC/RC}$ mice; 9 and 12 mo old, and 4 wild type (WT) controls were scanned *in vivo* using a ultra-high field (16.4T) MRI system. R_2* was calculated for the entire kidney, and its distribution in all WT was averaged and fitted to a Gaussian distribution curve. Values within 95% C.I. were considered renal parenchyma. A threshold was then set to the lower limit, and the %cystic and non-cystic tissue calculated in the *Pkd1*$^{RC/RC}$. All mice were euthanized after imaging; blood was collected for BUN and kidneys harvested for histology. Cystic index (CIx) was determined from histological sections.

Results: Fig 1A shows BOLD maps and T2 images in 2 representative *Pkd1*$^{RC/RC}$ and a WT mouse. Fig 1B shows the histograms generated from BOLD maps in all mice. Average WT R_2* was 138 1/s (95% C.I.: 58-218 1/s), lines indicating the limits. CIx correlated well with BOLD-defined % of cystic-tissue (R^2=0.89) and BUN inversely with % non-cystic tissue (R^2=0.73).

Conclusions: The percentage of cystic and non-cystic tissue derived from BOLD-MRI correlate well with histological and functional parameters in *Pkd1*$^{RC/RC}$ mice. BOLD-MRI may be a marker of disease severity and useful for following disease progression in ADPKD

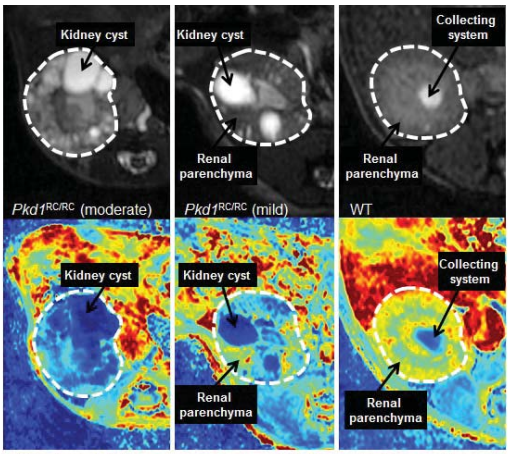

Figure 1A. T2 weighted MRI images (top row) and BOLD maps (bottom row) in 2 representative (moderate and mild disease) *Pkd1*$^{RC/RC}$ and a WT mouse.

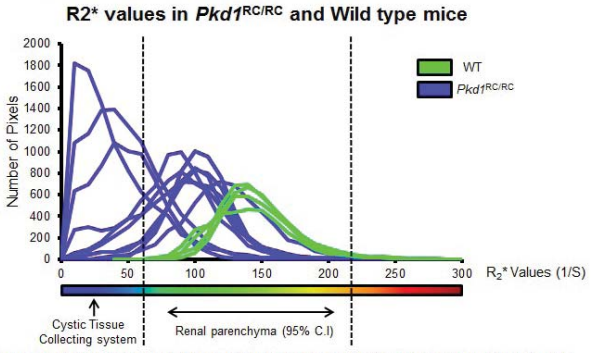

Figure 1B. Histograms generated from BOLD maps in *Pkd1*$^{RC/RC}$ (blue) and WT (green) mice.

Funding: NIDDK Support

FR-PO141

Gender Is a Disease Modifier in an Adult-Onset *Pkd1*-Mouse Model of Autosomal Dominant Polycystic Kidney

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Background: The NIH has recently called for greater study of the effects of gender in preclinical research. In this study, we have examined the relationship between gender and disease severity in a *Pkd1* mouse model in which conditional inactivation of Pkd1 in 40-days-old (P40) mice results in delayed-onset kidney cystogenesis starting 60 days post induction.

Methods: We injected tamoxifen in 135 P40 mice with floxed *Pkd1* alleles to delete *Pkd1* in 72 Cre-recombinase + mice (Mut: 39 males and 33 females) and 63 control mice (Cre-: 32 males and 31 females). We collected samples between P87 and P210 (Median: 160) and measured kidney/body weight (KBW; n=135), liver/body weight (LBW; n=49) and gene expression by microarray (n= 80).

Results: In mice <P100, the difference in KBW between mutant and control is not significant (n=6, p=0.6); in contrast, at P200, it is (n=6, p<0.02), consistent with the observation that kidneys look macroscopically normal ~60 days post-induction and get progressively larger and cystic with time. Analysis of covariance in cre+ mice fitting KBW to age and using gender as a categorical factor confirmed that age correlates with increased KBW (p<0.001), and showed that gender is a modifier of the rate of cyst growth (p<0.001; 2.5% to 97.5% confidence interval: 0.0072 to 0.0181), which corresponds roughly to an increase of 66% to 167% in mutant male KBW (compared to mutant females). In contrast, the liver disease shows the opposite trend: males have significantly better liver disease (p<0.05; 2.5% to 97.5% confidence interval: -0.0273 to -0.003), a LBW reduction of approximately 4% to 37%.

Conclusions: These data are consistent with reports of human studies and recapitulate an important and underappreciated aspect of ADPKD, suggesting that the model can be used to study the underlying mechanisms. The data also indicate that gender effects should be taken into account in ADPKD mouse studies. Furthermore, the magnitude of the effect is comparable to treatments tried in *Pkd1* mouse models, suggesting that understanding this phenomenon could provide novel therapeutic targets. Gene expression studies are being performed towards this goal.

Funding: NIDDK Support

FR-PO142

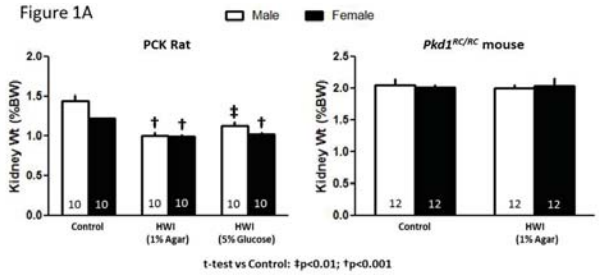
Effects of Hydration in Rodent Models of Polycystic Kidney Disease: Differences Between Rats and Mice

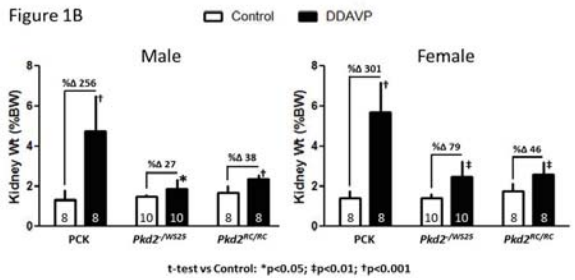
Katharina Hopp, Xiaofang Wang, Hong Ye, Maria V. Irazabal, Peter C. Harris, Vicente E. Torres. *Mayo Clinic.*

Background: Evidence that vasopressin and V2 receptor signaling promotes the progression of polycystic kidney disease (PKD) has raised the question of whether suppressing vasopressin release through enhanced hydration can delay disease progression. Enhanced hydration by adding 5% glucose to the drinking water is protective in a rat model orthologous to ARPKD. We wanted to explore the effect of enhanced hydration in a murine model orthologous to ADPKD and exclude an effect of glucose unrelated to enhanced hydration.

Methods: PCK rats were randomly assigned to control normal water intake (NWI) or to one of two high water intake groups achieved by adding 5% glucose to the drinking water (HWI-glucose) or by feeding a hydrated agar diet (HWI-agar). *Pkd1*$^{RC/RC}$ mice were assigned to NWI and HWI-agar groups. The cystogenic effect of 1-deamino-8-D-arginine vasopressin (DDAVP) was assessed in PCK rats and in *Pkd1*$^{RC/RC}$ and *Pkd2*$^{WS25/-}$ mice. Urine vasopressin, renal cAMP levels and phosphodiesterase activities were measured and histomorphometry used to assess disease severity.

Results: HWI-agar, like HWI-glucose, reduced renal cAMP levels and PKD severity in PCK rats, but not in *Pkd1*$^{RC/RC}$ mice (Fig1A). Compared to rat kidneys, murine kidneys had higher phosphodiesterase activity and lower cAMP levels and were less sensitive to the cystogenic effect of DDAVP (Fig1B).





Conclusions: The effect of enhanced hydration on PKD differs in rats and mice. A more powerful suppression of V2 receptor mediated signaling than that achievable by enhanced hydration alone may be necessary to affect the development of PKD in murine models.

Funding: NIDDK Support

FR-PO143

Blockage of Receptor for Advanced Glycation End Products Inhibits Cyst Growth in Polycystic Kidney Disease Jong Hoon Park, Eun Young Park, Eunji Lee, Eun Sun Chang. *Dept of Life Systems, Sookmyung Women's Univ, Seoul, Republic of Korea.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder, which is characterized by abnormal epithelial cell growth, fluid-filled cystformation, and interstitial fibrosis. Many studies have attempted to identify a new therapeutic target or striking biomarker for ADPKD,no drug has worked well in clinical trials. In this study, we focused on the receptor for advanced glycation end products (RAGE) gene as a novel target for ADPKD.

Methods: *In vitro* tests including viral infection, cell proliferation assay were performed with WT9-12 cell line, renal epithelial cells isolated from human patients with ADPKD. In addition, to examine the effect of RAGE on cyst enlargement *in vivo*, an adenovirus containing anti-RAGE shRNA was injected intravenously into PC2R mice, a severe ADPKD mouse model that we generated.

Results: We observed that RAGE gene knockdown resulted in loss of kidney weight and volume. Additionally, the cystic area that originated from different nephron segments decreased in size because of down-regulation of the RAGE gene. Blood urea nitrogen and creatinine values tended to be lower after inhibiting RAGE.

Conclusions: In conclusion, suppresion of RAGE using an adenoviral delivery system decreased cyst growth via inhibition of cell proliferation signals and improved renal function in PKD mouse. We propose that RAGE may play a role in cystogenesis and could be a new therapeutic target for PKD.

FR-PO144

Correlation of Expression of Ras-GTPase Isoforms in Rodent and Human Polycystic Kidney Disease Ayesha Irtiza-Ali,[1] Richard N. Sandford,[2] Patricia D. Wilson,[3] Bruce M. Hendry.[1] *[1]Renal Medicine, King's College London, United Kingdom; [2]Medical Genetics, Cambridge Univ, United Kingdom; [3]Nephrology, Univ College London, United Kingdom.*

Background: Small Ras GTPases act as central mediators for numerous effector cascades and are crucial in the control of cell proliferation, differentiation and apoptosis. Ras-GTPase signalling has been implicated in the pathogenesis of polycystic kidney disease (PKD), however the role of the 3 isoforms, Kirsten (Ki)-, Neural (N)- and Harvey (Ha)-Ras, in PKD are unknown. Our previous work has shown upregulation of Ki- and N-Ras isoforms across the proliferative and fibrotic phases of disease progression in an orthologous ADPKD mouse model. Here we extend our studies to examine Ras isoform expression in human polycystic kidneys, and a mouse model of recessive polycystic kidney disease.

Methods: Immunohistochemistry (IHC), qPCR and immunoblotting were used to characterize Ras isoform expression in normal and human polycystic kidney tissue, and in *pcy* mice.

Results: Similar to our findings in the *PKD1*$^{nl/nl}$ hypomorphic mice model, 2-3 fold rises in N- and Ki- Ras mRNA expression occur in pcy mice compared to wt between weeks 10 to 20, crossing both proliferative and fibrotic stages of disease ($p=<0.05$). Interestingly, in this model Ha-Ras mRNA is also elevated 2-fold during the early phase of cystic development ($p=<0.05$), declining after week 10. In human disease, Ras expression strongly localises to the epithelia of early and later stage cysts, as well as tubules in both the medulla and cortex of pre-dialysis stage polycystic kidneys (E-PKD), detected to a weaker degree in end-stage polycystic kidneys (ES-PKD), on IHC. Phospho-ERK similarly localises to these cells in human E- and ES-PKD. Furthermore, Ki-, N- and Ha- Ras isoform mRNA is detected in all stages of human PKD tissues.

Conclusions: These results show a close correlation in the cellular localisation of Ras and p-ERK expression between human ADPKD and PKD mouse models. In addition, a clear pattern of Ki- and N- Ras upregulation associated with different phases of disease progression occurs in 2 distinct models of polycystic kidney disease. These data further support a role for Ras-isoforms in PKD pathogenesis.

Funding: Private Foundation Support

FR-PO145

2'-O-Methoxyethyl Gapmer Antisense Oligonucleotides Are Efficacious for Selective Gene Targeting in Polycystic Kidney Disease Ayesha Irtiza-Ali,[1] Richard N. Sandford,[2] Dorien J.M. Peters,[3] Adam E. Mullick,[4] Bruce M. Hendry.[1] *[1]Renal Medicine, King's College London; [2]Medical Genetics, Univ of Cambridge; [3]Leiden Univ Medical Center; [4]Isis Pharmaceuticals.*

Background: Generation 2'-O-methoxyethyl (2'MOE) gapmer antisense oligonucleotides (ASOs) concentrate in the kidney as they are freely filtered through the glomerulus and efficiently re-absorbed by proximal tubular epithelial cells. Their use in PKD, where cysts can arise from all nephron segments, has not previously been extensively examined. Here, we demonstrate in several detailed studies the efficacy of 2'MOE gapmer ASOs in PKD.

Methods: 5 ASOs targeting different Kirsten-Ras sequences were investigated in normal mice, and 2 active ASOs selected for study in two murine PKD models, an ADPKD orthologous mouse, PKD1$^{nl/nl}$, and the Pcy mouse. Doses between 50-150mg/kg/week given subcutaneously for durations of 1, 2, 4 and 17 weeks were compared. Renal ASO activity and localization was analysed with qPCR and immunohistochemistry, and extra-renal effects assessed.

Results: Distribution of ASOs in normal kidney after 1 week at a dose of 60mg/kg was predominantly cortical, with uptake in the proximal and distal tubules, and collecting ducts. A striking shift in distribution occurred in the *PKD1*$^{nl/nl}$ and *Pcy* kidneys with uniform uptake across the medulla and cortex, localizing to the tubular and cystic epithelia, as well as the peri-cystic vascular endothelium, that was consistent irrespective of the dose or duration of ASO administered. Specific Ki-Ras knockdown of 60-85% was achieved in both *Pcy* and *PKD1*$^{nl/nl}$ kidneys at similar doses given for varying durations between 2 to 17 weeks. No significant differences in body weight gain, liver function tests, and spleen weights between vehicle and ASO treated groups, and no increase in renal expression of inflammatory markers MCP-1, IL1b, NFkB, and TNFa, were found.

Conclusions: 2'MOE gapmer ASOs reliably localize to target cells in cystic kidneys in two different PKD models, and achieve effective gene knockdown without significant adverse effects. We propose this as a strategy for selective gene silencing in the investigation and potential modulation of polycystic kidney disease.

Funding: Private Foundation Support

FR-PO146

Kidney-Selective AMPK Activator NT1021 Inhibits Cyst-Like Tubule Expansion in Pkd1 Mutant Kidneys Brenda S. Magenheimer,[1] Gail Reif,[1] Archana Raman,[1] James P. Calvet,[1] Ken W. Batchelor,[2] Darren P. Wallace.[1] *[1]Kidney Inst, Univ of Kansas Medical Center, Kansas City, KS; [2]NovaTarg Therapeutics, Research Triangle Center Park, NC.*

Background: In autosomal dominant polycystic kidney disease (ADPKD), aberrant cell proliferation and transepithelial fluid secretion are responsible for cyst enlargement. Inappropriate activation of mTOR promotes cyst epithelial cell proliferation, indicating that mTOR is potential therapeutic target for slowing cyst growth. Stimulation of AMP-activated protein kinase (AMPK), an energy sensor, inhibits mTOR mediated cell proliferation and CFTR-dependent Cl^- secretion. NT1021, a novel kidney selective biguanide that is selectively transported by organic cation transporter (OCT)-2 over OCT-1, is 38 times more potent than metformin, a clinically approved AMPK activator. Previously, NT1021 decreased levels of phosphorylated S6 kinase, a downstream mediator of mTOR signaling, cell proliferation and Cl^- secretion by human ADPKD cells. Here, we determined the effect of NT1021 on cAMP-dependent cyst expansion in Pkd1 mutant kidneys.

Methods: Embryonic kidneys (E15.5) were harvested from wildtype and Pkd1 mutant (Pkd1$^{+/-}$ and Pkd1$^{-/-}$) mice and placed on permeable cell culture inserts. The kidneys were bathed in media containing 100 mM cAMP ± NT1021 (concentrations ranging from 1 to 100 mM) or 1 mM metformin. Images of kidneys were captured using a digital camera connected to a dissecting microscope and cystic area was monitored for 5 days. For each experiment, cyst area of the treated kidney was compared to that of the contralateral control-treated kidney.

Results: cAMP stimulated cyst-like tubule expansion in Pkd1$^{+/-}$ and Pkd1$^{-/-}$ kidneys to a great extent than Pkd1$^{+/+}$ kidneys. Cystic area was decreased 1.7%, 23.7% and 96.7% in Pkd1$^{+/-}$ kidneys treated with 1, 10 and 100 mM NT1021, respectively, demonstrating a concentration dependent inhibition of cyst enlargement. In Pkd1$^{-/-}$ kidneys, cystic area was decreased 95% by 100 mM NT1021, compared to 50% with 1 mM metformin. The effect of NT1021 was reversible.

Conclusions: We conclude that NT1021, a kidney-selective AMPK activator, inhibits cyst-like tubule expansion in Pkd1 mutant kidneys.

Funding: NIDDK Support

FR-PO147

Role of NKCC1/CFTR-Dependent Fluid Secretion in Postnatal Cyst Formation in Mouse Pkd1 Kidneys Brenda S. Magenheimer,[1] Xia Zhou,[1] Gunda I. Georg,[2] Joseph S. Tash,[1] Xiaogang Li,[1] James P. Calvet.[1] *[1]Univ of Kansas Medical Center, Kansas City, KS; [2]Univ of Minnesota, Minneapolis, MN.*

Background: Cyst initiation in autosomal dominant polycystic kidney disease is thought to involve focal dilation of the renal tubule followed by expansion of the dilated area until a cyst pinches off from the tubule and becomes an isolated, self-contained structure. As the cyst continues to enlarge, it fills by secretion of fluid into the cyst lumen. Cysts would not enlarge to their enormous size if it were not for this fluid secretion. We previously showed using genetic models that polycystin-1 deficient kidneys in metanephric organ culture developed cyst-like expansions that depended on NKCC1/CFTR-mediated fluid secretion. The current study investigated the requirement for NKCC1/CFTR-mediated fluid secretion in postnatal cyst development in the mouse.

Methods: H2-gamendazole (H2-GMZ) treatment was carried out on Pkd1 floxed, Pkhd1-Cre mice using daily IP injections of 10 mg/kg H2-GMZ from postnatal day 8 to 24. H2-GMZ inhibits NKCC1 and CFTR and targets a number of other pathways in cystic kidneys. Pkd1 floxed, Hoxb7-Cre mice were crossed with Nkcc1 +/- mice and with Cftr +/- mice to test the effect of homozygous Nkcc1 or Cftr deletion on postnatal cyst growth.

Results: H2-GMZ targets Hsp90 and causes decreases in NKCC1 and CFTR, both being Hsp90 client proteins. H2-GMZ also acts as an open channel inhibitor of CFTR, and thus through a combination of activities it should effectively inhibit cyst-filling fluid secretion. Pkd1 floxed, Pkhd1-Cre mice treated with H2-GMZ had significantly ($P<0.001$) decreased kidney weight/body weight ratios and blood urea nitrogen, although the kidneys remained quite cystic. Interestingly, knockout of either Nkcc1 or Cftr had a minimal effect on cystic disease in Pkd1 floxed, Hoxb7-Cre mice examined at postnatal days 7-10.

Conclusions: Rapid postnatal cyst formation in Pkd1 conditional mouse models does not appear to depend significantly on NKCC1 and CFTR, possibly because at this stage rapid cystic dilation depends on glomerular filtration more than secretion. The effects of H2-GMZ on ameliorating PKD may be due to targeting pathways other than NKCC1 and CFTR.

Funding: NIDDK Support, Private Foundation Support

FR-PO148

Effect of Sodium-Glucose Cotransport (SGLT) Inhibition on Cystic Disease Progression in PCK Rats with Autosomal Recessive Polycystic Kidney Disease (ARPKD) Sarika Kapoor,[1] Daniel Rodriguez,[1] Meliana Riwanto,[1] Ilka Edenhofer,[1] Stephan Segerer,[2] Rudolf P. Wuthrich.[2] *[1]Univ of Zurich, Switzerland; [2]Univ Hospital Zurich, Switzerland.*

Background: The SGLT inhibitors Dapagliflozin (DAPA; SGLT-2 selective) and Phlorizin (PHLO; SGLT non-selective) induce profound renal glycosuria. The role of SGLTs and the therapeutic effect of these drugs in ARPKD have not been studied.

Methods: We examined the effect of DAPA (10 mg/kg/day po) or PHLO (400 mg/kg/day sc) in PCK rats, an orthologous animal model of ARPKD. Drugs or vehicle (CON) were administered to 6 week old male PCK rats (n=8 per group) for a total of 6 weeks. Blood and urine were collected at baseline and after 3 and 6 weeks of treatment to assess parameters of renal function. Rats were sacrificed after 6 weeks, and kidneys were excised for analysis of cyst growth.

Results: DAPA significantly increased urine output (DAPA 57.3±19.2, CON 19.3±2.3 ml/day at week 6) and resulted in higher osmolar excretion (DAPA 62.5±15.8, CON 23.9±2.8 mosm/day) and higher glucose excretion (DAPA 23.4±12.0, CON 0.3±0.3 mmol/day). After 3 weeks of treatment, DAPA-treated PCK rats displayed higher clearances for creatinine (DAPA 3.06±0.40, CON 2.56±0.54 ml/min) and BUN (DAPA 1.71±0.34, CON 1.23±0.31 ml/min) whereas after 6 weeks there was no difference between DAPA and CON. Furthermore, DAPA-treated PCK rats displayed a 3.5-fold increase in albumin excretion after 6 weeks of treatment. Surprisingly, there was a 23% higher total kidney weight after 6 weeks of treatment with DAPA. *In vivo* ultrasound imaging and histological analysis also showed an increase in the cyst growth, although there was no change in the level of renal cAMP content between both groups. Likewise, PHLO-treated rats displayed enhanced medullary cyst growth by ultrasound imaging.

Conclusions: Inhibition of glucose reabsorption with SGLT inhibitors caused significant glycuresis, hyperfiltration and albuminuria in PCK rats. Unexpectedly, the cyst growth was enhanced, suggesting that the factors which regulate cyst growth act independently from the factors which control GFR. The mechanisms which link glycosuria and hyperfiltration to distal cyst growth remain to be elucidated.

FR-PO149

Ischemia-Reperfusion Injury Causes Renal Cystogenesis with Long-Term Enhanced Expression of Polycystin-1 and Polycystin-2 Marie Trudel, Almira Kurbegovic. *Institut de Recherches Cliniques de Montréal, Montreal, QC, Canada.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by multiple cysts in both kidneys leading to renal insufficiency. ADPKD is associated with mutations mainly in *PKD1* (encoding polycystin-1/PC1) and less frequently in *PKD2* gene (encoding polycystin-2/PC2). We generated several Pkd1 mouse models that support gene dosage increase in PKD pathophysiology. Dosage-increased models with mild overexpression of full-length native Pkd1 gene ($Pkd1_{TAG}$) or of extracellular domain ($Pkd1_{extra}$) mimicking natural human *PKD1* mutations develop slow progressive PKDand renal failure at >1 year of age. Since ischemia-reperfusion injury (IRI) was reported to promote earlier cyst formation in Pkd1 dosage-reduced mouse models, we questioned whether cystogenesis could be accelerated in Pkd1 dosage increase mouse models via a similar cellular and molecular mechanism.

Methods: Mice from $Pkd1_{TAG}$ and $Pkd1_{extra}$ transgenic lines were subjected to unilateral renal IRI including control littermates at 3-months of age (90 ±3.0days) and sacrificed at different time points following reperfusion.

Results: From 23 to 120 days post-IRI, all mice showed hypoplasia of the IRI kidney and a compensatory hypertrophy of the non-IRI kidney independently of the genotype. Interestingly, all transgenic (n=22) and non-transgenic (n=36) mice develop 3 months after IRI moderate to severe renal cysts with epithelial hyperplasia, immune interstitial infiltrates and fibrosis. We then questioned the molecular and signaling mechanism leading to cystogenesis in these mice. Noticeably, stimulation of Hif1a expression was detected over time. Our data also showed significantly increased expression of Pc1, Pc2 and Tsc2 (~3-to 5-fold) in the IRI kidneys of non-transgenic and transgenic mice relative to non-IRI kidneys and to mice without exposure to IRI. Such overexpression in IRI non-transgenic kidneys is consistent with cystogenesis and with dosage increase PKD pathogenesis.

Conclusions: Our results provide evidence for a combinatorial role of increase Pc1 and Pc2 in renal injury and repair and insight into IRI progressive cellular pathophysiologic mechanism.

Funding: Government Support - Non-U.S.

FR-PO150

Autocrine Macrophage IL-10 Signaling Is Required for Macrophage-Promoted Polycystic Kidney Disease Cyst Cell Proliferation Jacqueline D. Peda, Sally M. Salah, Darren P. Wallace, Timothy A. Fields, Katherine Swenson-Fields. *The Kidney Inst, Univ of Kansas Medical Center, Kansas City, KS.*

Background: We have previously shown that ADPKD cyst cells secrete factor (s) that promote M2-like macrophage differentiation and that this macrophage phenotype is pathogenic, promoting cyst cell proliferation and cyst growth. We have also shown that these M2-like macrophages secrete IL-10. Here we examined the role of IL-10 in expression of the pathogenic macrophage phenotype.

Methods: Murine macrophage-like cells [RAW 264.7 (RAW macrophages)] were cultured with primary ADPKD cyst cells in the presence/absence of IL-10 neutralizing antibody and effects on PKD cell proliferation measured by direct cell counting. Similarly, primary, mouse bone marrow-derived macrophages (BMDM) from both wild type and IL-10 knockout mice (IL10KO) were co-cultured with primary ADPKD cells. Signaling was assessed using phospho-specific Western blot analysis.

Results: Neutralization of macrophage-derived IL-10 blocked the ability of RAW macrophages to stimulate ADPKD cell proliferation. Also, primary BMDM from wild type but not IL10KO mice stimulated ADPKD cell proliferation. Moreover, the pro-proliferative macrophage phenotype could be rescued by addition of exogenous, mouse-derived IL-10 to IL10KO BMDM during co-culture, while IL-10 alone had no effect on the PKD cells.

Conclusions: Macrophages in ADPKD kidneys can be programmed by PKD cyst cells to take on a pathogenic, pro-proliferative phenotype. While the signal that initiates this differentiation process is unknown, these data suggest that an autocrine IL-10 signaling loop in macrophages is required for expression of the pathogenic phenotype.

Funding: Private Foundation Support

FR-PO151

Role for the Primary Cilium in Inflammatory Responses and Cystogenesis During Kidney Maturation and Injury Cheng 'Jack' Song,[1] Bradley K. Yoder,[1] Michal Mrug.[2] *[1]Cell, Developmental, and Integrative Biology, Univ of Alabama at Birmingham, Birmingham, AL; [2]General Clinical Research Center, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Studies utilizing conditional IFT mutations suggest that the rate of cyst formation is dependent upon the timing of cilia loss. Induction of cilia loss in juvenile mice (before postnatal day 12) results in rapid cyst development while induction of cilia loss after P12 (critical switch point) or in adult mice causes a much slower progressing form of cystic disease. Interestingly, rapid cyst formation can be initiated in the adult-induced IFT mutants by ischemic reperfusion (IR) injury suggesting a possible role for inflammation in cyst development. This was recently confirmed by liposome clodronate (LC) mediated depletion of phagocytic macrophages that reduced cystic disease severity and improved renal function. The mechanism connecting an injury response, cilia dysfunction, and cyst formation remains poorly defined and it is not understood why cystogenic rates are markedly different in juvenile versus adult-induced cilia mutants. Here we investigate potential connections between primary cilia associated cystogenesis and an altered inflammatory response.

Methods: We used Immunofluorescence staining and flow cytometry to characterize different macrophage populations. qRT-PCR will be used to access the cytokine expression in these different macrophage populations.

Results: Our preliminary data show there is a similar kidney macrophage populations that accumulates in juvenile-induced as well as in adult-induced cilia mutants following IR injury. Importantly, this population of macrophages rapidly dissipates after the critical switch point and is not present in the adult-induced non-injured kidney.

Conclusions: We are testing the hypothesis that these are pathogenic macrophages capable of promoting cystogenesis. This would help explain the large differences in the rate of cyst progression observed in cilia mutant mice. This work will shed new light on mechanisms contributing to the rate of cyst formation associated with cilia dysfunction and provide possible targets for therapeutic intervention.

Funding: NIDDK Support

FR-PO152

Vasopressin V2 Receptor Independent Anti-Inflammatory Effect of Tolvaptan in Kidney Cells Osamu Takase, Masaomi Nangaku, Keiichi Hishikawa. *Dept of Advanced Nephrology and ReGenerative Medicine, Div of Nephrology and Endocrinology, Graduate School of Medicine, Univ of Tokyo, Tokyo, Japan.*

Background: Tolvaptan is a highly selective vasopressin V2 receptor antagonist, and is useful for the treatment of congestive heart failure. Lots of studies demonstrated renoprotective effects of long-term tolvaptan therapy, and tolvaptan is also expected to be a candidate for treatment of autosomal dominant polycystic kidney disease. However, the renoprotective effect of tolvaptan and its underlying mechanisms remain unknown. In this study, we investigated the vasopressin V2 receptor independent renoprotective effect of tolvaptan, especially on NF-kappaB activation, *in vitro.*

Methods: We studied the effect of tolvaptan related to inflammation and apoptosis in human renal tubular injury stimulated by LPS. NF-kappaB activity, NF-kappaB-associated inflammatory factors, and apoptosis were examined by EMSA, western blot, ELISA, and

Hoechst staining. Also we knocked down the NF-kappaB or IkBa activity using siRNA treatment in order to explore the effect on the NF-kappaB as a nuclear transcription factor, which plays a central role of inflammation.

Results: Tolvaptan inhibited the NF-kappaB activity and the expression of NF-kappaB-associated inflammatory factors in LPS-stimulated cultured human renal tubular cells. Pre-treatment with siRNA for IkBa significantly diminished inhibitory effect of tolvaptan on NF-kappaB activity. Interestingly, tolvaptan increased the expression of clusterin which stabilizes IkBa. These results showed that tolvaptan inhibited NF-kappaB activity by stabilizing IkBa or clusterin, independent of vasopressin V2 receptor. Tolvaptan also augmented LPS-induced apoptosis by NF-kappaB-AP-1 related extrinsic pathway, but not intrinsic pathway.

Conclusions: Our results for the first time demonstrated the direct inhibitory effect of tolvaptan on LPS-induced NF-kappaB activity independent of vasopressin V2 receptor in human renal tubular cells. Our results suggest the new anti-inflammatory renoprotective roles of tolvaptan beyond diuresis.

FR-PO153

Regulation of Fluid Secretion by Ouabain in Autosomal Dominant Polycystic Kidney Disease Gustavo Blanco,[1,4] Kyle Jansson,[1,4] Brenda S. Magenheimer,[3,4] James P. Calvet,[3,4] Darren P. Wallace.[2,4] *[1]Molecular and Integrative Physiology, Univ of Kansas Medical Center, Kansas City, KS; [2]Medicine, Univ of Kansas Medical Center, Kansas City, KS; [3]Biochemistry and Molecular Biology, Univ of Kansas Medical Center, Kansas City, KS; [4]The Kidney Inst, Univ of Kansas Medical Center, Kansas City, KS.*

Background: Enlargement of renal cysts in autosomal dominant polycystic kidney disease (ADPKD) requires the transepithelial secretion of fluid into the cyst lumen. We had previously shown that physiological levels of the hormone ouabain enhance cAMP-dependent fluid secretion in epithelial cells derived from renal cysts of patients with ADPKD (ADPKD cells) and cyst expansions in metanephric organs from ADPKD mice. In contrast, ouabain only slightly affects normal human kidney cells and metanephroi. Here, we have investigated the mechanisms by which ouabain stimulates ADPKD fluid secretion and cystogenesis.

Methods: The effects of physiologic amounts of ouabain (3nM) on ADPKD ion/fluid movement was studied in mouse metanephric organ cultures and in ADPKD cell cultures, using different transport assays.

Results: Ouabain, synergistically with cAMP, induced cystic dilations in embryonic kidneys from $Pkd1^{m1Bei}$ mice, but had no effect on $Pkd1^{m1Bei}$ mouse metanephroi that also lacked the cystic fibrosis transmembrane conductance regulator (CFTR). Similarly, in ADPKD cells, ouabain stimulation of cAMP-induced fluid secretion and microcyst growth required the activity of CFTR. Ouabain enhanced forskolin-dependent and CFTR mediated Cl^- efflux in ADPKD monolayers by increasing plasma membrane levels of CFTR and expression of the CFTR activator PDZK1. In addition, ouabain decreased ADPKD cell basolateral Na^+ transport by partially reducing Na,K-ATPase plasma membrane amounts and activity.

Conclusions: Ouabain enhances fluid secretion in ADPKD by both increasing the apical efflux of Cl^- via activation of CFTR, and by decreasing basolateral fluid reabsorption through down-regulation of the Na,K-ATPase. These mechanisms further highlight the importance of ouabain as a non-genetic factor capable of stimulating ADPKD cystogenesis.

Funding: NIDDK Support

FR-PO154

ADAM17 Regulates Cellular Bioenergetics in Polycystic Kidney Disease (PKD) Monika Beck Gooz,[1] Eduardo Maldonado,[1] Yujing Dang,[1] Hanna E. Abboud,[2] John J. Lemasters,[1] P. Darwin Bell.[1,3] *[1]Medical Univ of South Carolina, Charleston, SC; [2]Univ of Texas Health Science Center, San Antonio, TX; [3]R.H. Johnson VA Medical Center, Charleston, SC.*

Background: Recently, we found that the disintegrin metalloenzyme ADAM17 promotes proliferation of collecting duct kidney epithelial cells originating from the *Ift88*^{orpk} mice model of PKD through extracellular signal-regulated kinase (ERK) activation and increased glycolysis. In this study we investigated the effect of increased ADAM17 activation on mitochondrial bioenergetics in PKD cells.

Methods: We used immortalized control and PKD collecting duct cells. We assessed ERK activation and ADAM17 expression by Western blotting and ADAM17 activity by a fluorogenic substrate. We measured ROS production by the ROS substrate CM-H_2DCFDA, determined mitochondrial respiration by a Seahorse XF Analyzer, and measured nicotinamide adenine dinucleotide (NADH) autofluorescence by confocal multiphoton microscopy. We assessed mitochondrial membrane potential (DY) by tetramethyl rhodamine methyl ester fluorescence and expression of the mitochondrial uncoupling protein-2 (UCP2) by Western blotting.

Results: Expression and enzyme activity of ADAM17, and the level of phosphorylated ERK were higher (2-fold, 2-fold and 4-fold, respectively) in PKD cells compared to the less proliferative control cells. PKD cells also had higher basal respiration and ROS production than control cells: 440±31 versus 278±39 pmol O_2/min/10,000 cells and 0.41±0.04 versus 0.26±0.01 arbitrary units, respectively. NADH levels were 36±15% higher in PKD cells compared to control cells. Expression of UCP2 was 1.7-fold higher and DY was 50% lower in PKD cells compared to control cells. Chemical inhibition of ADAM17 (10 µM TMI-005, Pfizer) inhibited ERK activation, decreased ROS production, decreased both basal respiration (365±9 pmoles/min) and NADH levels and increased DY in PKD cells to the level of control cells.

Conclusions: Our data suggest that upregulation of the pro-proliferative ADAM17 activates an ADAM17/ERK/mitochondrial ROS loop, causing increased mitochondrial metabolism and aerobic glycolysis (incomplete Warburg effect) possibly due to UCP2 activation to promote cell proliferation in PKD.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO155

Exosome Based Biomarkers for Polycystic Kidney Disease Christopher James Ward,[1] Marie C. Hogan.[2] *[1]Div of Nephrology and Hypertension, KUMC, Kansas City, KS; [2]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a common cause of ESRD. Affected individuals inherit a defective copy of either the *PKD1* or *PKD2* gene, encoding the proteins polycystin–1 (PC1) or polycystin–2 (PC2) respectively. PC1 and PC2 are secreted on urinary exosome–like vesicles (ELVs) (100nm diameter vesicles), where PC1 is present in a cleaved form and may be complexed with PC2. Label free quantitative proteomic studies of urine ELVs in an initial discovery cohort (13 PKD1 and 18 Normals), revealed that of 2008 ELV proteins, nine (0.32%) showed a statistically significant difference between PKD1 and normals at a $p<0.025$.PC1 was reduced to 54% of the normal level ($p=0.011$) and PC2 reduced to 53% ($p=0.0004$).TMEM2, a protein with homology to fibrocystin, the product of the polycystic hepatic and kidney disease (*PKHD1*) gene, is increased 2.1 fold ($p=0.022$). The PC1/TMEM2 ratio correlated inversely with height adjusted total kidney volume (HtTKV) in the discovery cohort and the ratio of PC1/TMEM2 or PC2/TMEM2 could be used to distinguish PKD1 from normals in a confirmation cohort.

Methods: We have developed a novel high throughput western assay to measure the ratio of PC1 to TMEM2 or PDCD6IP (ALIX), a protein that is not altered in level. The assay uses 100ml of raw urine thus avoiding the use of an ultracentrifuge.

Results: We are able to detect PC1, TMEM2 and PDCD6IP in normal urine and measure the ratios of PC1/TMEM2 and PC1/PDCD6IP in normal and ADPKD urines.

Conclusions: Our high throughput western blotting technique offers the possibility of monitoring the PC1/TMEM2 ratio in ADPKD patients. We believe this ratio may correlate with disease status and might be useful in monitoring the disease state in individuals undergoing treatment for the disease.

Funding: NIDDK Support

FR-PO156

Polycystin-1 Regulates the Bioactivities of Exosomes Derived From Kidney Epithelial Cells Gang Yao, Hansong Xu, Yanan Chen, Maria Rasmussen, Maoqing Wu, Xuefeng Su, Jing Zhou. *Renal Div, Dept of Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: Polycystin-1 (PC1) is an integral membrane protein essential for tubule morphogenesis, and its disruption causes autosomal dominant polycystic kidney disease (ADPKD). Exosomes are small membrane vesicles with a diameter of 40-100 nm that originate as internal vesicles of multivesicular bodies (MVBs). They are secreted by various cell types and are involved in multiple biological processes. Exosomes are abundant in human urine and PC1 is abundant in these human urinary exosomes. However, the function of the PC1-containing exosome is unknown.

Methods: We isolated exosomes from the wild-type and *Pkd1*-knockout mouse kidney epithelial cells using a well established differential centrifugation protocol. By utilizing immunoelectron microscopy, biochemistry, cell biology, and live cell imaging, we investigated the functions of PC1-containing and PC1-free exosomes in several biological assays known to contribute to cytogenesis in human ADPKD patients and mouse ADPKD models.

Results: We show by immunoelectron microscopy that PC1 colocalized with exosomal marker CD63 and an FCH Bin–Amphiphysin–Rvs (F-BAR) protein important for vesicle trafficking in exosomes derived from cultured kidney epithelial cells. Depletion of the F-BAR protein significantly reduced the abundance of exosomal PC1. Comparative analysis of the effects of exosomes isolated from wild-type and *Pkd1*-knockout mouse kidney epithelial cells revealed that the PC1-containing exosomes were bioactive, promoted cell migration and cell signaling, yet inhibited cell proliferation. PC1-null exosomes were defective in these activities. We established a cilium-knockout kidney epithelial cell line and investigated the roles of primary cilium in the secretion of PC1-containing exosomes and bioactivities mediated by exosomes.

Conclusions: Our data demonstrates, for the first time, that PC1-containing exosomes are bioactive and regulate multiple cell behaviors in kidney epithelial cells. The abundance of PC1-containing exosomes is regulated by an F-Bar protein. This work also represents the first functional study of exosomes derived from cilium-deficient cells.

Funding: NIDDK Support

FR-PO157

The Role of Polycystin 1 GPS Cleavage in Vascular Development Patricia Outeda, Kathleen Mcavoy, Feng Qian, Terry J. Watnick. *Dept of Medicine, Div of Nephrology, Univ of Maryland, School of Medicine, Baltimore, MD.*

Background: Polycystin-1 (PC1) plays an important role during development. $Pkd1^{-/-}$ embryos die at mid-gestation with a variety of phenotypes including cystic kidneys and profound edema due to aberrant lymphatic vessel development. It was previously shown

that PC1 undergoes incomplete cleavage at the GPS domain (GPCR proteolytic site). Mice with disrupted PC1 GPS cleavage (*Pkd1*$^{v/v}$) appear normal at birth but develop polycystic kidneys during the postnatal period.

Results: To examine the role of GPS cleavage in the vasculature during development we harvested *Pkd1*$^{-/-}$, *Pkd1*$^{v/v}$ and controls (WT) embryos at E14.5. While severe polyhydramnios and edema were observed in *Pkd1*$^{-/-}$ embryos, no obvious phenotype was observed in *Pkd1*$^{v/v}$ embryos. We performed whole mount staining of skin with Lyve1 or Nrp2 (lymphatic endothelial cell (LEC) markers) and we found that *Pkd1*$^{-/-}$ embryos exhibited a dilated and disorganized lymphatic network with decreased branching compared to controls. In contrast, there was no difference in the lymphatic network between *Pkd1*$^{v/v}$ embryos and controls. Next, we focused on the placental vasculature that was previously shown to be abnormal in *Pkd1*$^{-/-}$ embryos. Paraffin sections were stained with Isolectin B4 (embryonic endothelial cell marker), and Laminin. Although *Pkd1*$^{-/-}$ embryos exhibited a decrease in fetal vessels with reduced branching, the placental vasculature appeared normal in the *Pkd1*$^{v/v}$ animals. We cultured MEF cells from *Pkd1*$^{-/-}$, *Pkd1*$^{v/v}$ and control embryos. We found that *Pkd1*$^{-/-}$ cells have an intrinsic defect in directed migration but *Pkd1*$^{v/v}$ cells behave normally.

Conclusions: Our results suggests that uncleaved PC1 is fully sufficient for proper vascular development.

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FR-PO158

Abstract Withdrawn

FR-PO159

RNA-Guided Editing of Mouse *Pkhd1* in Kidney Epithelial Cells Using CRISPR-Cas9 system Maoqing Wu, Benjamin S. Freedman, Xuefeng Su, Jing Zhou. *Harvard Center for Polycystic Kidney Disease Research and Renal Div, Dept of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Progress on research of autosomal recessive polycystic kidney disease (ARPKD) is hindered by the scarcity of human tissue samples, and the lack of a satisfactory mouse model that mimics the phenotypes in human ARPKD. The establishment of cell models by small hairpin RNA has been challenged by the large size of the *PKDH1* gene, the complexity of the splicing patterns.Mouse *Pkhd1* contains 67 exons and likely produces multiple isoforms. The clustered regularly interspaced short palindromic repeats (CRISPR) /Cas9 system is a newly developed technique and has become a powerful tool to mediate genome editing with high precision, which allows the one-step generation of animals and cell lines carrying mutations in multiple genes as well as distinct mutations in a single gene. It is particularly useful to edit genes encoding multiple alternatively spliced isoforms that may cause functional redundancy.

Methods: We searched for the CRISPR/Cas9 binding site conforming to the sequence G (N) $_{19}$NGG in the most commonly used exon (present in most of splicing variants) of *Pkhd1* as previously reported. We designed the guide (g) RNA and cloned it into pX330, a gRNA vector. The gRNA plasmids were then co-transfected with pCas9-GFP into mIMCD-3 cells. pCas9-GFP positive cells were selected by flow cytometry.

Results: Twelve lines derived from single GFP-positive cells were screened by PCR of the mutated region. Mutations in *Pkhd1* were identified in all lines by direct sequencing of PCR products. One homozygous line with deletion of the most commonly used exon was identified. Results on the characterization of these cell lines will be presented.

Conclusions: We have successfully established a *Pkhd1* deletion mutant cell line using CRISPR/Cas9 system. This is the first report of using the CRISPR/Cas9 system for functional studies of a PKD gene. We believe genomic editing is a powerful alternative to small hairpin RNA-mediated knockdown of complex genes such as *PKHD1*.

Funding: Private Foundation Support

FR-PO160

Genome Editing-Mediated Deletion of *Pkd2* in mIMCD3 Cells Disrupts Epithelial Morphogenesis Alexis Hofherr, Michael Kottgen. *Renal Div - Dept of Medicine, Univ Medical Center Freiburg, Freiburg im Breisgau, Baden-Württemberg, Germany.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent inherited cause for end-stage renal disease in the United States. It is defined by the age-dependent massive enlargement of both kidneys, which is characterized by dilated or cystic renal tubular segments. The two causative genes *PKD1* and *PKD2* encode together for a sensory receptor-channel complex that is required for renal tubular morphogenesis. Yet, the molecular function of the Polycystin-1/TRPP2 signaling module has remained elusive. To investigate the polycystin complex on a biochemical level, the establishment of suitable orthologous *in vitro* model systems is essential. Loss of function cell lines from knockout mice or RNAi-mediated depletion of PKD-genes have provided valuable insights, but are compromised by technical challenges including lack of cellular differentiation *in vitro*, poor scalability, incomplete knockdown, and putative RNAi off-target effects.

Methods: Here we present the site-specific genome engineering-mediated deletion of *Pkd2* in mouse inner medullary collecting duct (mIMCD3) cells using Transcription Activator-Like Effector Nucleases (TALENs). Wildtype mIMCD3 cells retain core differentiation characteristics of the collecting duct, e.g. apico-basolateral polarization, epithelial transport, and tight junctions.

Results: In contrast to the distinctive *in vivo* phenotype of loss of *Pkd2*, the epithelial morphology of these *Pkd2*$^{-/-}$ cells is not impaired in 2D cell culture. This is in line with previously described *Pkd2* depletion experiments. However, in a complex extracellular matrix environment *mIMCD3 PKD2*$^{-/-}$ cells show a marked defect of proper epithelial organization, with break down of the epithelial monolayer and cytoskeletal aberrations. In 3D culture these cells present with a prominent and easy to score phenotype, which may serve as a readout for impaired tubular morphogenesis *in vivo*.

Conclusions: In this proof of concept study we establish that *PKD2* is required for epithelial morphogenesis *in vitro*; and that the directed manipulation of PKD genes provides novel opportunities to model polycystin protein function.

Funding: Government Support - Non-U.S.

FR-PO161

An E3 Ligase Complex Is Critical for Polycystin-2 Ciliary Trafficking in *Drosophila* Weizhe Li, Stacey Cook, Terry J. Watnick. *Univ of Maryland Baltimore, Baltimore, MD.*

Background: Ciliary dysfunction can cause a variety of severe human diseases including Autosomal Dominant Polycystic Kidney Disease (ADPKD). Ciliary localization of Polycystin-1 and Polycystin-2, the protein products of ADPKD genes, is critical for their function, but how these proteins traffic to cilia remains largely unknown.

Methods: We have been using Drosophila as a model to address this question. The homologue of polycystin-2 in Drosophila (dPKD2) is localized at the tip of sperm tails, which can be regarded as specialized cilia. We used a collection of EMS mutant flies to screen for proteins that might be required for dPKD2 to reach the tip of the sperm tail.

Results: We identified one line, Z2-5905, that showed absent sperm staining for dPKD2, while the overall expression level in the testis was similar to wild type. We found that Z2-5905 harbors a truncating mutation in a gene, that we named zuba, which contains a UBA (ubiquitin associated) domain. We used homologous recombination to generate a knock-out allele and showed that it had the same phenotype as Z2-5905. We were able to rescue dPKD2 sperm localization in both zuba alleles using a genomic rescue construct. We developed an antibody against Zuba and found that the protein was almost exclusively expressed in the testis and mature sperm. In addition, we found that Zuba isolated from mature sperm is monoubiquitinated. We also identified an E3-ligase that we speculated might interact with Zuba. We used shRNA to knock down this E3 ligase and found that male flies show the same phenotype as Z2-5905, including male sterility and dPKD2 mislocalization at the tip of sperm tails. In addition, knock down of this E3 ligase resulted in the specific absence of the monoubiquitinated form of Zuba in mature sperm while keeping the unmodified form in testis intact. We are currently testing whether these proteins physically interact.

Conclusions: In conclusion, we found an E3 ligase complex that is required for polycystin-2 ciliary localization in flies. The E3 ligase and Zuba show 34% and 28% identity with their corresponding mammalian homologues. Future experiments will explore the role of vertebrate homologues in ciliary trafficking.

Funding: Other U.S. Government Support

FR-PO162

Cilia Trafficking Defective Mutation in PC2 Results in Complete Loss of Function *In Vivo* Xin Tian, Yiqiang Cai, Seung H. Lee, Stefan Somlo. *Internal Medicine/Section of Nephrology, Yale Univ School of Medicine, New Haven, CT.*

Background: Polycystin-2 (PC2) is expressed in cilia of kidney tubule epithelia and it has been demonstrated in LLC-PK1 cells that an N-terminal motif, R_6VxP is required for cilia localization of PC2.

Methods: To elucidate the function R_6VxP motif in vivo, we developed a BAC (Bacterial Artificial Chromosome) transgenic mouse model, *Pkd2*^{GAxA} -BAC expressing a substitution mutant R_6VxP motif, G_6AxA, that abrogates cilia trafficking *in vitro. Three founder lines (#17, 45 and 71) with germ line transmission had Pkd2*^{GAxA} -BAC transgene copy numbers of 2, 4 and 5, respectively.

Results: *Mice expressing Pkd2*^{GAxA} -BAC are viable, fertile and do not develop polycystic disease up to 12 months. We generated *Pkd2*^{GAxA} -BAC transgenic mice on the *Pkd2*$^{-/-}$ background to determine whether PC2 deficient in cilia trafficking can rescue any part of the embryonically lethal null phenotype. We documented expression of the transgenic PC2 and found that *Pkd2*^{GAxA} -BAC;*Pkd2*$^{-/-}$ mice are *embryonically lethal* by E15.5, comparable to *Pkd2*$^{-/-}$mice. *The left-right axis defect in Pkd2*$^{-/-}$ *was not affected by the Pkd2*^{GAxA} -BAC transgene. We also crossed the *Pkd2*^{GAxA} -BAC transgene with *Pkd2*^{flox};*Pkhd1-Cre* mouse line, in which *Pkd2* is inactivated exclusively in collecting duct. Histological and functional examination of kidneys from *Pkd2*$^{flox/flox}$;*Pkhd1-Cre;Pkd2*[/italic]^{GAxA} -BAC mice at P24 showed no significant change in kidney weight, cystic index and serum BUN when compared with *Pkd2*$^{flox/flox}$;*Pkhd1-Cre* mice. There was no significant difference in cyst lining cell proliferation (BrdU incorporation) and apoptotic activity (TUNEL) between two groups. Cilia in cyst lining epithelium appeared normal by immunofluorescence microscopy, and immunostaining with anti-PC2 antibody indicated that the PC2 cytosolic expression pattern was well maintained, but cilia expression was absent.

Conclusions: These results indicate that mutation in R_6VxP motif can prevent PC2 trafficking to cilia and result in complete loss of PC2 function *in vivo.*

Funding: NIDDK Support

FR-PO163

The Net Effect of Actin Binding Protein Filamin-A on Regulating Polycystin-2 Qian Wang,[1] Horacio Cantiello,[2] Xing-Zhen Chen.[1] *[1]Physiology, Univ of Alberta, Edmonton, AB, Canada; [2]Cátedra de Biofísica, Facultad de Odontología, Univ de Buenos Aires, Buenos Aires, Argentina.*

Background: Polycystin-2 (PC2), encoded by the PKD2 gene, is a Ca-permeable cation channel mutated in ~15% of autosomal dominant polycystic kidney disease (ADPKD). PC2 is mainly localized on the endoplasmic reticulum (ER) membrane, and is also present on the plasma membrane and primary cilium.

Methods: Lipid bilayer is used to study PC2 channel function in vitro by reconstituting purified PC2 protein; Live cell Ca imaging is to measure ER-localized PC2 function in vivo.

Results: Previously we reported that cytoskeletal actin-binding protein filamin-A (FLNA) reduces PC2 channel activity in a lipid bilayer system through direct binding with both the N- and C-termini of PC2. On the other hand, our data submitted for publication showed that FLNA stabilizes PC2 protein expression by anchoring it to the actin cytoskeleton. The apparent contradiction was solved by the observations that FLNA did not exhibit an inhibitory effect on PC2 channel activity in the absence of Ca in lipid bilayer system, and that Ca chelation by EGTA dramatically weakened the PC2-FLNA interaction. Thus, the inhibition of PC2 channel function by FLNA may be Ca-dependent in living cells. Next, we employed live cell Ca imaging technique to study the net effect of FLNA on PC2 (expression and function) in vivo. We found that PC2 silencing by siRNA increases ER Ca content, which is consistent with previous publication proposing that PC2 is an ER Ca leak channel. With FLNA silencing, a decreased ER Ca content was observed, indicating a high PC2 channel activity.

Conclusions: Thus, we think that the net effect of FLNA is to inhibit PC2 channel activity.

Funding: Government Support - Non-U.S.

FR-PO164

The Effect of Polycystin-2 Disruption on the Osmotic Response of Renal Epithelial Cells Brian J. Siroky,[1] Nancy Kleene,[2] Raven Gail Comer,[1] Steven Kleene,[2] Bradley P. Dixon.[1] *[1]Div of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Dept of Molecular and Cellular Physiology, Univ of Cincinnati College of Medicine, Cincinnati, OH.*

Background: Renal epithelial cells exposed to hyperosmolality in the inner medulla withstand these conditions by activating the osmotic stress response. Primary cilia detect mechanical and chemical stimuli including osmolality. We have previously shown that renal epithelial cells with loss of cilia formation due to *Kif3a* deletion have an attenuated osmotic response, and that TRPM3 is involved in cilia-dependent osmosensation. It is unknown whether the osmotic response is compromised in cells that harbor functional, rather than structural, defects in cilia. We characterized the osmotic response of renal epithelial cells deficient in polycystin-2 (PC2), a protein expressed in primary cilia that is defective in some patients with autosomal dominant polycystic kidney disease (ADPKD).

Methods: Murine inner medullary collecting duct (mIMCD3) cells were transduced with an shRNA construct targeting *Pkd2*, or a non-target control sequence. PC2 expression was reduced by 90% by the *Pkd2* shRNA construct as determined by immunoblotting, and cilia structure was normal in both cell lines as observed by immunofluorescence. Both cell lines were exposed to hyperosmolal culture conditions with NaCl (500 mOsm/kg), or maintained under isoosmolal conditions (300 mOsm/kg) in the presence of pregnenolone, an agonist for TRPM3, or DMSO vehicle. Western blotting for aldose reductase (AR), an osmotic response protein, was performed on whole cell lysates.

Results: Induction of AR expression by hyperosmolality was markedly reduced in PC2 knockdown cells compared to non-target controls. Pregnenolone attenuated the AR induction in non-target control cells only.

Conclusions: We demonstrate a novel finding of diminished osmotic response in mIMCD3 cells with reduced PC2 expression, but intact cilia structure, suggesting that impaired osmoregulation may play a role in ADPKD pathogenesis. Further, the impaired osmotic response in PC2-deficient cells may result from altered TRPM3 activity.

Funding: NIDDK Support

FR-PO165

Osteoblast-Specific Deletion of *Pkd2* Leads to Low-Turnover Osteopenia and Reduced Bone Marrow Adiposity Zhousheng Xiao, Leigh Darryl Quarles. *Univ of Tennessee Health Science Center, Memphis, TN.*

Background: Polycystin-1 (Pkd1) and polycystin-2 (Pkd2) are implicated in skeletal development. Selective deletion of Pkd1 the osteoblasts lineage in mice inhibits osteoblast differentiation and stimulates adipogenesis through the reciprocal regulation of Runx2 and PPARg. The osteoblast specific functions of Pkd2 have not been defined.

Methods: We conditionally inactivated Pkd2 in postnatal mature osteoblasts by crossing Osteocalcin (Oc)-Cre;$Pkd2^{/+}$ mice with floxed Pkd2 ($Pkd2^{flox/flox}$) mice to generate conditional heterozygous (Oc-Cre;$Pkd2^{flox/+}$) and homozygous (Oc-Cre;$Pkd2^{flox/null}$) Pkd2-deficient mice.

Results: Pkd2 expression was reduced by ~75% in bone of Oc-Cre;$Pkd2^{flox/null}$ mice. Oc-Cre;$Pkd2^{flox/null}$, and Oc-Cre;$Pkd2^{flox/+}$ demonstrated no cyst formation in the kidney. Compared to $Pkd2^{flox/+}$ control mice, Pkd2-deficiency resulted in a reduction in bone mineral density, trabecular bone volume, cortical thickness and mineral apposition rate that were associated with significant alterations in biomechanical properties, including a reduction in maximum force and stiffness. Similar to Pkd1 deficiency, loss of Pkd2 in osteoblasts resulted in diminished Runx2 expression in bone and impaired osteoblast differentiation of osteoblast cultures ex vivo. Expression of osteoblast-related genes, including, Osteocalcin, Osteopontin, Bone Sialoprotein, Phex, Dmp1, and Sost, were reduced proportionate to the reduction of Pkd2 gene dose in bone of Oc-Cre;$Pkd2^{flox/+}$ and Oc-Cre;$Pkd2^{flox/null}$ mice. Significant reductions in both serum concentrations and bone mRNA expression of FGF23, RankL and TRAP were also observed in Oc-Cre;$Pkd2^{flox/null}$ mice compared to controls. In contrast to Pkd1 deficient mice, loss of Pkd2 in bone resulted in diminished PPARg expression and reduced bone marrow fat in vivo and reduced adipogenesis in osteoblast culture ex vivo.

Conclusions: The selective loss-of-Pkd2 in osteoblasts inhibits both osteoblast differentiation and adipogenesis. Thus, Pkd1 and Pkd2 have concordant effects on osteoblast differentiation, but opposite effects on skeletal adipogenesis through signaling mechanisms that remain to be elucidated.

Funding: NIDDK Support

FR-PO166

Phenotype Rescue in the *cpk* Mouse Model By a Conditional, Inducible Transgene Targeted to the ROSA26 Locus Chaozhe Yang, Amber K. O'connor, Jacob A. Watts, Lisa M. Guay-Woodford. *Children's National Medical Center.*

Background: Mouse models have been instrumental in the characterization of polycystic kidney disease (PKD). Unfortunately, gene-targeting of *Pkhd1*, the mouse ortholog of the autosomal recessive PKD gene, results in minimal or no kidney disease. However, the *cpk* mouse renal lesion phenocopies human ARPKD. We previously identified *Cys1* as the disease-causing gene in this model. Interestingly, initial efforts to rescue the renal phenotype via standard, random-insertion, transgenic approaches were unsuccessful, despite *Cys1 re*-expression.

Methods: A *Cys1-GFP* fusion gene was cloned into a ROSA26 targeting construct to create a knock-in line (ROSA26-$Cys1^{GFP}$). $B6^{cpk/+}$ mice were then crossed with ROSA26-$Cys1^{GFP}$ mice to generate the ROSA26-$Cys1^{GFP}$;$Cys1^{cpk/+}$ line. In this model, gene activation requires the deletion of a stop-lox cassette using a Cre recombinase. Therefore, we mated this line to Ksp-Cre mice to express *Cys1-GFP* in the renal collecting ducts, the major nephron segment involved in ARPKD.

Results: At 6-weeks of age, kidneys harvested from putative rescue mice (ROSA26-$Cys1^{GFP}$;$Cys1^{cpk/cpk}$;Ksp-Cre) and control mice (ROSA26-$Cys1^{GFP}$;$Cys1^{+/+}$;Ksp-Cre and ROSA26-$Cys1^{GFP}$;$Cys1^{cpk/cpk}$) were comparatively evaluated by H&E staining, immunoblotting, and immunofluorescent analysis. In rescued kidneys, fluorescence was detected in collecting duct structures, confirming nephron-specific, Ksp-driven expression of the *Cys1-GFP* transgene. The renal phenotype in the rescue mice was comparable to normal kidneys, save for a few scattered cysts that were of proximal tubular origin.

Conclusions: Using a conditional, inducible *Cys1-GFP* transgene targeted to the ROSA26 locus, we have rescued the *cpk* phenotype by re-expressing *Cys1* within the collecting ducts. Our data suggest that a "set point" of *Cys1* expression is required for renal tubular homeostasis and significant alterations in gene expression are not tolerated, as has been observed for other cystoproteins.

FR-PO167

Tandem Affinity Purification (TAP) Identifies Arl3 as an Interacting Partner of Cystin, the Ciliary Protein Disrupted in the *cpk* Mouse Model of Polycystic Kidney Disease Jacob A. Watts,[1,2] Landon Shay Wilson,[3] Lisa M. Guay-Woodford.[1] *[1]Center for Translational Science, Children's National Med Center, Washington, DC; [2]Dept of Genetics, Univ of Alabama at Birmingham, Birmingham, AL; [3]Targeted Metabolomics and Proteomics Laboratory, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Autosomal recessive polycystic kidney disease (ARPKD) is a major cause of pediatric morbidity and mortality caused by mutations in *PKHD1*. Animal models are the mainstay for studies of human diseases, however, gene-targeting of *Pkhd1* results in mutants with little or no kidney disease. In contrast, disruption of the *Cys1* gene in the *cpk* mouse model closely phenocopies human ARPKD. We speculate that this phenotypic similarity suggests the protein products of *Pkhd1* and *Cys1*, FPC and cystin, respectively, belong to a common molecular pathway. The current study was designed to identify cystin-binding partners and the pathways that regulate the intracellular trafficking and nuclear function of cystin.

Methods: To identify cystin interacting partners we employed TAP using mIMCD-3 cells expressing a cystin-TAP construct. The SF-TAP vector contains a 4.6 kDa tag comprised two Strep and one FLAG domain on the N-terminus of a mutant form of cystin that lacks the myristoylation site, is unable to stably associate with the membrane and has enhanced trafficking to the nucleus.

Results: Mass spectrometry identified interacting partners, including the vesicular transport proteins Importin a1, a2, b1, b2, Rab5c, 7a, and 11a, as well as IFT74, and the GTP-binding protein, Arl3. Of note, Arl3-/- mice exhibit an ARPKD-like phenotype.

Conclusions: Based on our data, we propose that Arl3 may direct the localization of cystin via-IFT-mediated processes to the distal cilium. We have previously shown that cystin cilia localization is mediated in part by a myristoyl-electrostatic switch and that when released from the ciliary membrane, cystin traffics to the nucleus where it regulates c-myc expression. We speculate that loss of either cystin or Arl3 causes c-myc upregulation, a cardinal feature of proliferating cystic epithelia. Current knockdown studies are directed to address this proposed mechanism.

Funding: NIDDK Support

FR-PO168

Accelerated Epithelial Cell Cycle Causes Cyst Growth in the *Kif3a* Non-orthologous Mouse Model of Polycystic Kidney Disease Dongmei Lu,[1] Alysha Rauhauser,[1] Binghua Li,[1] Kayla McEnery,[1] Chongyu Ren,[1] Moumita Chaki,[1] Komal Vadnagara,[1] Anton M. Jetten,[2] Peter Igarashi,[1] Massimo Attanasio.[1,3] *[1]Internal Medicine, Univ of Texas Southwestern Medical Center, Dallas, TX; [2]Cell Biology Section, Div of Intramural Research, National Inst of Environmental Health Sciences, National Insts of Health, Research Triangle Park, NC; [3]Eugene McDermott Center, Univ of Texas Southwestern Medical Center, Dallas, TX.*

Background: The progressive growth of tubules is the hallmark of several genetic forms of polycystic kidney disease (PKD) that inexorably leads to nephron loss, but the molecular causes that sustain uncontrolled growth are largely unknown. Unlike autosomal dominant polycycstic kidney disease (ADPKD), kidneys in nephronophthisis (NPHP) are smaller in volume, suggesting that differences in proliferation rates may underlie the different phenotype.

Methods: Using the *Kif3a* knockout as non-orthologous mouse model of polycystic kidney disease and the *Glis2$^{mut/mut}$ mouse model of NPHP, we have found that genetic inactivation of Glis2* in *Kif3a* null mice results in decreased tubular proliferation and apoptosis, delayed cyst progression and preserved renal function. [/italic]

Results: *In vitro* studies on immortalized tubular cells indicate that *Kif3a* null cells fail to activate the G1/S checkpoint and have accelerated G1-to-S transition that leads to accumulation of spontaneous DNA damage and cell death. Inactivation of *Glis2* in *Kif3a* null tubular cells restores the G1/S checkpoint, reduces cell proliferation rates and DNA damage by activation of checkpoint kinase 1.

Conclusions: Our data indicate that accelerated cell cycle paired with compromised DNA damage-induced cell cycle arrest are primary causes of cyst growt in the *Kif3a* non-orthologous mouse model of polycystic kidney disease. These results further substantiate the emerging link between DNA damage and cystic kidney disease and shed light on the biological bases of the different kidney phenotype of PKD and NPHP.

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FR-PO169

Deficiency of Tuba, a cdc42 GEF, Disrupts Normal Zebrafish Ciliogenesis and Kidney Development Jeong-In Baek, Soo Young Choi, Xiaofeng Zuo, Joshua H. Lipschutz. *Renal Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia, PA.*

Background: Dysfunction of renal primary cilia leads to polycystic kidney disease (PKD). The exocyst, a highly-conserved eight-protein membrane trafficking complex, is essential for ciliogenesis, and is regulated by Rho and Rab family GTPases, such as cdc42. We previously showed that cdc42 deficiency disrupts renal ciliogenesis and causes PKD in zebrafish and mice, and that Tuba, a cdc42-specific GEF, is necessary for ciliogenesis in cultured renal tubular MDCK cells.

Methods: To determine how tuba affects ciliogenesis and kidney function *in vivo*, tuba knock-down was performed in zebrafish by microinjection of a translation blocking morpholino (MO). The phenotypic and histological defects caused by tuba deficiency were analyzed using whole-mount *in situ* hybridization, H and E staining and immunofluorescence.

Results: Tuba was expressed in several tissues containing primary cilia, including the brain, neuromast cells, and renal tubules. We found that tuba morphants phenocopied cdc42 morphants, with ciliary mutant phenotypes including: a curly tail, hydrocephalus, and abdominal fluid accumulation. These defects were rescued by human tuba mRNA, which was resistant to the morpholino due to a difference in primary base pair structure, indicating that the phenotype was due to tuba deficiency, and not off-target effects. In the tuba morphant kidney, pronephric cilia were short and disordered, and the glomeruli were also disorganized. Because tuba is a known GEF of cdc42, and tuba and cdc42 morphants shared the same phenotype, we next performed a genetic synergy experiment in which we knocked down both tuba and cdc42. Co-injection of tuba and cdc42 morpholinos at low concentrations, which individually resulted in no phenotype, caused a severe phenotype, suggesting that tuba and cdc42 act in the same pathway.

Conclusions: Our study showed that tuba deficiency causes an abnormal renal ciliary phenotype in zebrafish, demonstrating that tuba plays a critical role in ciliogenesis and kidney development in zebrafish. We are currently generating kidney-specific tuba knockout mice to confirm these results in metanephrogenesis.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO170

Greb1, a Gene Responsible for the Development of Renal Cysts Originating in the Distal Tubules in DBA/2 Mice Osamu Ichii,[1] Saori Otsuka,[1] Akira Yabuki,[2] Taro Horino,[3] Teppei Nakamura,[1,4] Yasuhiro Kon.[1] *[1]Laboratory of Anatomy, Dept of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido Univ, Sapporo, Japan; [2]Laboratory of Veterinary Clinical Pathology, Joint Faculty of Veterinary Medicine, Kagoshima Univ, Kagoshima, Japan; [3]Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi Univ, Nankoku, Japan; [4]Section of Biological Safety Research, Chitose Laboratory, Japan Food Research Laboratories, Chitose, Japan.*

Background: In cystic kidney disease, the localization of cysts depends on expression of the gene responsible. In this study, we found the development of renal cysts in DBA/2 mice, clarified their pathological features, and identified a responsible gene.

Methods: The histopathology of renal cysts was analyzed in C57BL/6 and DBA/2 of 10–32 weeks of age. In these strains, diabetes and acute kidney injures were induced by streptozotocin and folic acid, respectively. Genetic analysis of cyst formation was performed using F1, F2, and N2 progenies from C57BL/6 and DBA/2.

Results: With aging, DBA/2 but not C57BL/6 developed renal cysts characterized by columnar change, deciduation, and increased intercellular spaces of tubular epithelial cells. These epithelial cells expressed uromodulin and calbindin D-28K, markers for distal tubules. Some epithelial cells also expressed IL-1F6, a marker of injury of distal tubules, and showed BrdU incorporation. In diabetes and acute kidney injuries, DBA/2 showed severe distal tubular damage compared with C57BL/6. Genetic analysis revealed that cyst formation in DBA/2 is a monogenic autosomal recessive trait. Cyst formation was found to be significantly associated ($P < 0.01$) with the *D12Mit169–282* locus on chromosome 12, and we named this locus DBA/2-type renal cyst (*drecy*). Of the 26 genes encoded on *drecy*, mRNA expression of gene regulated by estrogen in breast cancer protein (*Greb1*) was significantly higher in the kidneys of DBA/2 compared to C57BL/6 (12-fold, $P < 0.0004$). The Greb1 protein primarily localized to the distal tubular epithelium in mouse kidneys.

Conclusions: *Greb1* is a strong candidate gene for *drecy* and shows promise as a novel molecular target for development of cystic kidney disease.

Funding: Government Support - Non-U.S.

FR-PO171

Loss of *Zeb2* Causes Glomerulocystic Kidney Disease in Mice Hila Milo Rasouly,[1] Stefanie Chan,[1] Anna Pisarek-Horowitz,[1] Yuriko Nishizaki,[2] Yujiro Higashi,[2] Weining Lu.[1] *[1]Renal Section, Boston Univ Medical Center, Boston, MA; [2]Inst for Developmental Research, Aichi Human Service Center, Kasugai, Aichi, Japan.*

Background: ZEB2 is a zinc finger E-box binding homeobox transcription factor. Mutations in *ZEB2* cause the Mowat-Wilson syndrome, an autosomal dominant disorder characterized by multiple congenital anomalies including kidney malformations. However, the role of ZEB2 in kidney development is unknown.

Methods: ZEB2 expression was analyzed in developing embryonic kidneys by immunostaining of a ZEB2-EGFP reporter knock-in mouse. Kidney specific *Zeb2* conditional knockout mice were generated by crossing the *Zeb2* floxed allele (*Zeb2$^{flox/flox}$*) with the *Pax2*-cre and *Six2*-cre alleles. The conditional knockout mice were analyzed from embryonic stage E14.5 to postnatal 8 weeks old. The mouse kidneys were analyzed by H&E staining for histological changes and immunostaining for apoptosis. Gene expression analysis was performed by TaqMan assays. Kidney function was assessed by measuring urine protein levels, serum creatinine and blood urea nitrogen.

Results: ZEB2 is highly expressed at E13.5 and E16.5 in the developing mouse kidney. Both *Zeb2$^{flox/flox}$*;*Pax2*-cre and *Zeb2$^{flox/flox}$*;*Six2*-cre conditional knockout mice developed kidney glomerular cysts starting at E16.5 dpc. *Zeb2$^{flox/flox}$*;*Pax2*-cre mice died at birth and *Zeb2$^{flox/flox}$*;*Six2*-cre mice developed abnormal kidney function at 5 weeks old. The cysts originate from the glomeruli with dilated Bowman's capsule and collapse of glomerular tufts. Reduced apoptosis was detected in the S-shaped and C-shaped bodies of *Zeb2* knockout embryonic kidneys compared to the wild type controls. Gene expression analysis revealed that both *Zeb2* conditional knockout mice had higher levels of *Pkd1* mRNA in the developing kidney than their wild-type littermates. Interestingly, overexpression of *Pkd1* has been reported to cause glomerular cysts in mice.

Conclusions: Loss of *Zeb2* causes glomerulocystic kidney disease in mice with increased expression of the *Pkd1* transcript.

Funding: Private Foundation Support

FR-PO172

Elongated Ciliary Length of Proximal Tubules in a PKD Model AQP11 Knockout Mouse Yuichi Inoue,[1] Eisei Sohara,[1] Katsuki Kobayashi,[2] Tatemitsu Rai,[1] Kenichi Ishibashi,[3] Shigeo Horie,[4] Xuefeng Su,[5] Jing Zhou,[5] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan; [2]Div of Molecular Genetics, Chiba-East National Hospital, Chiba, Japan; [3]Dept of Medical Physiology, Meiji Pharmaceutical Univ, Kiyose, Tokyo, Japan; [4]Dept of Urology, Juntendo Univ, Tokyo, Tokyo, Japan; [5]Renal Div, Dept of Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: It is well known that PKD gene products localize to the primary cilia. In addition, abnormal ciliary length has been reported in many renal cystic diseases, although molecular mechanisms regulating ciliary length remain unknown. AQP11 is a membrane-channel protein at endoplasmic reticulum (ER) and reported to be permeable to the water. The disruption of AQP11 gene in mice resulted in renal cystogenesis. We recently reported that aberrant glycosylation and localization of polycystin-1 (PC-1) could be key mechanisms of cystogenesis in AQP11 (-/-) mice. However, the involvement of primary cilia in the mechanism of cystogenesis in AQP11 (-/-) mice remains unknown. In this study, we examined whether AQP11 regulates length of primary cilia.

Methods: We performed immunofluorescence with anti-a-acetylated tubulin antibody in wild-type, AQP11 (-/-), and Tg^{AQP11}AQP11 (-/-) mice kidneys.

Results: Interestingly, elongated primary cilia of proximal tubules in AQP11 (-/-) mice were observed, compared to wild-type mice. In addition, in Tg^{AQP11}AQP11 (-/-) mice, AQP11 transgene expression normalized the ciliary length in AQP11 (-/-) mice, indicating that AQP11 may play a role in controlling ciliary length. Since we recently reported that Pkd1 (+/-) background resulted in increased severity of PKD in AQP11 (-/-) mice, we further examined whether Pkd1 affects elongated ciliary length in AQP11 (-/-) mice. However, the Pkd1 (+/-) background did not change the ciliary length in AQP11 (-/-) mice, indicating that elongation of cilia in AQP11 (-/-) mice might not be solely dependent on PC-1.

Conclusions: Our data demonstrated that AQP11 may regulate ciliary length. To the best of our knowledge, this case is the first in which ER protein regulates the ciliary length of kidney tubules.

FR-PO173

Metabolic Reprogramming Results in the Production of an Oncometabolite, 2-Hydroxyglutarate (2HG), in Polycystic Kidney Disease Robert H. Weiss,[1] Vicki Hwang,[1] Chaozhe Yang,[2] Hiromi Inoue Wettersten,[1] Lisa M. Guay-Woodford.[2] *[1]Internal Medicine/Nephrology, UC Davis, Davis, CA; [2]Center for Translational Science, Children's National Health System, Washington, DC.*

Background: Polycystic Kidney Disease (PKD) and cancer share many of the same characteristics including defects in cell growth control, altered metabolism, etc. Recently, the oncometabolite, 2-Hydroxyglutarate (2-HG), has been found to be increased in certain cancers due to mutated Isocitrate Dehydrogenase (IDH) 1 and/or 2 enzymes. Similarly, 2-HG was elevated in human cells proliferating in hypoxia in the absence of IDH mutations. 2-HG has been known to increase HIF1-alpha levels resulting in unregulated cell growth.

Methods: Human ARPKD tissues and normal tissue (adjacent to kidney cancer) were obtained under approved IRBs. Non-targeted metabolomics was performed in wt and *cpk* kidneys as well as human tissues using gas chromatography-mass spectrometry (GC-MS) based methods. Wild type IDH2 expression was analyzed in mouse cells and tissues from the *cpk* and *jck* mouse PKD models by western blot and immunohistochemistry.

Results: Using non-targeted metabolomics in the cpk mouse model of ARPKD, we identified 2-HG as being significantly increased in *cpk* over wt kidneys ($p<0.038$); this data was confirmed in two human ARPKD tissues ($p<0.001$). Correspondingly, a significant increase was found in citrate ($p<0.001$), and significant decreases were found in downstream metabolites of the TCA cycle, fumarate ($p<0.001$) and malate ($p<0.001$). An increase in IDH2 expression was found by IHC in the cyst epithelial cells in both *jck* and *cpk* kidney tissues.

Conclusions: Our results in two PKD mouse models and in a small number of human tissues suggest increased presence of 2-HG in cystic tissue which correlates with increased expression of wild type IDH2, possibly as a result of local hypoxia. This dysregulated expression of metabolites suggests a shift in the TCA cycle and metabolism, further supporting the parallels between oncogenesis and PKD. Such connections will allow us to tap into the vast resources of cancer basic research and cancer clinical trials to bring new treatment paradigms to PKD.

Funding: NIDDK Support, Other NIH Support - NCI, Veterans Affairs Support

FR-PO174

p21-Activating Kinase 4 (PAK4) Inhibition as a Novel Treatment for Autosomal Dominant Polycystic Kidney Disease Robert H. Weiss,[1] Arzu Ulu,[1] Hiromi Inoue Wettersten,[1] William T. Senapedis,[2] Erkan Baloglu,[2] Yosef Landesman.[2] *[1]Internal Medicine/Nephrology, UC Davis, Davis, CA; [2]Karyopharm Therapeutics, Natick, MA.*

Background: Due to its mechanistic similarities with cancer, the repurposing of cancer drugs is a largely untapped area of polycystic kidney disease (PKD) research. p21-activated kinase 4 (PAK4) is a mediator of filopodia formation and stabilizes β-Catenin which lies in a pathway which is integral to both nephrogenesis and cancer. In light of abundant evidence that defective ciliary signaling leads to renal tubular epithelial (RTE) cell proliferation, and because PAK4 is upregulated in most cancer cell lines, we asked whether specific inhibition of PAK4 inhibits activation of β-Catenin and thereby attenuates RTE cell proliferation.

Methods: Immortalized human autosomal dominant PKD (ADPKD) cells (WT9-12; WT9-7), human primary normal RTE cells (NHK) were incubated with a specific PAK4 inhibitor, KPT-8752, and cell viability assay and immunoblotting were performed.

Results: The PAK4 inhibitor, KPT-8752, decreases cell viability in both WT9-12 and WT9-7 as well as several renal cell carcinoma (RCC) cell lines (786-0 and ACHN). NHK cells were less sensitive to KPT-8752 when compared to ADPKD or RCC cell lines, which suggests the likelihood of decreased adverse effects when given in vivo. The cytotoxic effect of KPT-8752 was time- and dose-dependent in both ADPKD and RCC cell lines. KPT-8752 decreases protein levels of the activated form of PAK4 (phospho-PAK4) as well as phospho-β-Catenin at 48 and 72 hours.

Conclusions: A novel PAK4 inhibitor, KPT-8752, decreases cell viability in ADPKD cell lines. The mechanism of PAK4 inhibition appears to be driven by the changes in the β-Catenin/Wnt pathway which plays key roles in ciliary signaling and is deranged in the ciliopathies including PKD. Thus, once confirmed in vivo, PAK4 inhibition could be a new targeted therapy for human ADPKD.

Funding: NIDDK Support, Other NIH Support - NCI, Veterans Affairs Support, Pharmaceutical Company Support - Karyopharm

FR-PO175

Restoring Multidrug-Resistance Associated Protein 3 Suppresses Cell Proliferation in Polycystic Kidney Jong Hoon Park, Eun Sun Chang, Eun Young Park, Yu Mi Woo. *Biological Science, Sookmyung Women's Univ, Seoul, Korea.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by abnormal proliferation of renal tubular epithelial cells resulting in loss of renal function. Despite identification of the genes responsible for ADPKD, few effective drugs are currently available for the disease. Only few drugs such as tolvaptan or octreotide have shown powerful therapeutic potential in clinical trials. Thus, finding additional effective drug targets is necessary. The functions of multidrug-resistance associated protein 3 **(**MRP3) have been reported only in the field of drug-resistance, and the renal functions of MRP3 are mostly unknown.

Methods: Basal expression of MRP3 was measured in the kidneys of human patients with ADPKD and mouse PKD models. *Abcc3* RNAi or *ABCC3* clone was transfected respectively into MDCK cells to regulate MRP3 expression. To examine the effects of MRP3 on cell proliferation, we performed XTT (WST-1) assay and observed PCNA-positive cells by immunocytochemistry-florescence. *In vitro* cyst formation assay was performed with ABCC3 overexpression. Content of intracellular cyclic AMP was measured in WT9-12 cells and the activation of signaling molecules involved in B-Raf/MEK/ERK pathway were detected using Western blotting analysis. $p < 0.05$ was considered significant.

Results: MRP3 was significantly downregulated in polycystic kidneys compared with normal kidney tissues, particularly in the cystic epithelial lining cells. The RNAi-mediated downregulation of MRP3 induced cell proliferation through activating B-Raf/MEK/ERK signaling pathway. In contrast, restorating MRP3 suppressed cell proliferation and in vitro cystogenesis in MDCK cells. The level of cyclic AMP was reduced by MRP3 restoration and resulted in inhibition of PKA and Rap-1 which known as upstream of B-Raf in WT9-12 cells.

Conclusions: Significant decreases of MRP3 were observed in polycystic kidney tissues. In this context, MRP3 restoring could be one of therapeutic approaches through targeting cAMP/PKA-dependent signaling pathway.

FR-PO176

Cyclic AMP Regulates Glycogen Synthase Kinase-3b by a Feed Forward Mechanism in Polycystic Kidney Disease Vijayakumar R. Kakade, Shixin Tao, Reena Rao. *The Kidney Inst, Dept of Internal Medicine, Univ of Kansas Medical Center, Kansas City, KS.*

Background: Glycogen synthase kinase 3b (GSK3b), a protein kinase is crucial for vasopressin mediated renal cAMP generation. Cyclic AMP levels are elevated in polycystic kidney disease (PKD) and stimulate cyst expansion. Earlier we found that GSK3b protein levels are increased and associated with cyst lining epithelia in human and rodent models of PKD and its inhibition can reduce cyst expansion in PKD by reducing cAMP. The increase in GSK3b protein levels in PKD was an unexpected finding, as GSK3b is known to be regulated at the level of its activity rather than expression. Hence the current studies examined if cAMP mediated cell signaling can regulate GSK3b expression in a feed forward loop.

Methods: M1-cortical collecting duct cells and PKD-1 knockout or heterozygous cells were used.

Results: In M1 cells, forskolin treatment increased GSK3b protein and mRNA levels, and kinase activity. To examine if GSK3b is a cAMP responsive gene, we examined its promoter region and found five conserved potential cAMP-response element (CRE) binding sites. Forskolin treatment increased activated CRE-binding protein (pCREB) and GSK3b, while H-89, a Protein kinase-A (PKA) inhibitor reduced pCREB and GSK3b. This suggested that CREB could be involved in cAMP induced GSK3b expression. Hence we made GSK3b-promoter luciferase constructs to examine the effect of cAMP on CREB dependent GSK3b transcriptional activity. Luciferase activity was increased by forskolin or 8-Br-cAMP treatment and reduced by CREB inhibitor treatment. CREB inhibitor also reduced forskolin induced GSK3b protein levels. In PKD1 knockout cells, GSK3b expression as well as pCREB levels were significantly higher than control cells and CREB inhibitor treatment significantly reduced GSK3b expression.

Conclusions: Thus a unique feed-forward relation exists between cAMP and GSK3b, whereby increased cAMP levels can activate PKA and CREB mediated GSK3b expression, which in turn is essential for cAMP generation. Use of a GSK3 inhibitor can break this destructive cycle and may be therapeutically useful to reduce cyst expansion and preserve renal function in PKD.

Funding: NIDDK Support

FR-PO177

MitoQ Modulates Hypoxia-Inducible Factor-1a 1n ADPKD Cells
Wei Wang, Berenice Y. Gitomer, Kristen L. Jablonski, Robert W. Schrier, Michel Chonchol. *Univ of Colorado Anschutz Medical Campus.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disease that leads to end-stage kidney disease. So far there is no effective treatment other than dialysis or transplant. The pathophysiology of the disease is still unclear. One hypothesis is that cyst expansion results in pericystic hypoxia which leads to up-regulation of HIF-1a. Expression of HIF-1a is increased in the cyst epithelium in human and animal models of ADPKD. HIF-1a has many target genes including those involved in angiogenesis, cell proliferation and apoptosis which are involved in cyst growth and thus disease progression. Therefore agents that have an inhibitory effect on HIF-1a may have a beneficial effect in slowing progression of ADPKD. MitoQ, a mitochondrial-targeted antioxidant, was previously shown to ablate the hypoxic induction of reactive oxygen species and destabilize HIF-1a protein in various cancer cells. We hypothesized that HIF-1a is induced in ADPKD cells and that mitoQ may have an inhibitory effect on oxidative stress.

Methods: An immortalized human epithelial cell line derived from a single cyst from an ADPKD patient and normal renal tubular epithelial cells were used. A proteome profiler human cell stress array from R&D systems was used. Both cells were treated with 1μM mitoQ for 24hr.

Results: HIF-1a expression in ADPKD cells was increased by 60% as compared to normal tubular cells. MitoQ decreased the expression of HIF-1a in ADPKD cells by 51% as compared to untreated ADPKD cells.

Conclusions: The induction of HIF-a in PKD cells *in vitro* indicates that polycystin deficiency itself may have direct influence on HIF-1a regulation. The inhibitory effect of mitoQ on HIF-1a may imply a beneficial effect on ADPKD disease progression. *In vivo* studies are needed to confirm this observation.

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FR-PO178

Statin Effect on Human ADPKD Tubular Epithelial Cell Proliferation
Wei Wang, Melissa A. Cadnapaphornchai, Michel Chonchol, Godela M. Brosnahan, Robert W. Schrier, Berenice Y. Gitomer. *Univ of Colorado Anschutz Medical Campus.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic disease with no treatment. In our recently published study, pravastatin was shown to slow progression of structural kidney disease in children and young adults with ADPKD. However, the underlying mechanisms of statin's action are unclear. Statins have been shown to inhibit proliferation of cancer cells by inducing apoptosis. Cyst growth in ADPKD in some ways resembles characteristics of benign tumors. We thus hypothesized that statin may have an inhibitory effect on ADPKD cell proliferation through regulation of apoptosis.

Methods: An immortalized human epithelial cell line derived from a single cyst from an ADPKD patient and normal renal tubular epithelial cells were used. Both simvastatin (1 μM and 10μM) and pravastatin (1 μM and 10μM) were used. Cell viability was examined with trypan blue and cell proliferation by BrdU incorporation. Cell apoptosis markers were examined using proteome profiler human apoptosis arrays from R&D systems.

Results: Both simvastatin and pravastatin have a dose dependent inhibition of proliferation in ADPKD cells. Expression of the cell cycle regulator p21 increased more than two fold in simvastatin treated ADPKD cells. Similar results were observed by Western blot where pravastatin significantly increased the expression of p21.

Conclusions: P21 is a cyclin kinase inhibitor which has pleiotropic effects on the cell cycle. It is up-regulated by polycustin-1 and its expression is decreased in humans and animals with ADPKD. Thus the clinical benefit of pravastatin in ADPKD patients may well be related to its inhibitory effect on tubular cell proliferation through the regulation of p21. P21 in ADPKD merits further investigation as a target for future therapies.

Funding: NIDDK Support

FR-PO179

High Rates of Cellular Extrusion, Primary Cilia Defects, and Increased Apoptotic Sensitivity in Renal Epithelia with Inactivated Exocyst Sec10
Noemi Polgar, Amanda J. Lee, Vanessa H. Lui, Ben Fogelgren. *Univ of Hawaii.*

Background: The establishment and maintenance of epithelial barrier function are fundamental aspects of epithelial morphogenesis, and polarized exocytosis plays a key role in these processes. The eight-protein exocyst complex is responsible for the targeted trafficking of certain exocytic vesicles to specific subcellular locales. In Madin-Darby canine kidney epithelial (MDCK) cells grown on transwells, the exocyst has been reported to contribute to trafficking to primary cilia. Also, knockdown of exocyst subunit Sec10 (Sec10-KD) in MDCK cells prevented normal cyst formation when grown in 3D matrix.

Methods: We utilized the well-characterized MDCK cell line grown in 3D gel matrices to perform a detailed analysis of the consequences of loss of Sec10 and the exocyst complex on epithelial morphogenesis and primary cilia assembly. We also utilized our newly generated kidney-specific Sec10 knockout mouse strain to analyze primary cilia and epithelial characteristics *in vivo*.

Results: When grown in 3D collagen gels, late-stage Sec10-KD cysts (day 12) displayed high rates of apoptotic cells extruded from the basal surface of the cysts' epithelial layer. The early formation of these collagen-grown Sec10-KD cysts were marked by normal lumenogenesis (<8 days), however we observed significant defects in primary cilia assembly in Sec10-KD cysts beginning at day 8. As cell extrusion can be triggered by signals from dying cells, we found Sec10-KD MDCK cells demonstrated an increased sensitivity to apoptotic signals, and further analysis revealed defects in planar cell polarity and re-establishment of apical-basal polarity. Renal epithelia in mice with targeted inactivation of the Sec10 gene also displayed primary cilia defects and signs of cellular extrusion not observed in control littermates.

Conclusions: Based on these results, silencing exocyst component Sec10 in renal epithelial cells leads to defective primary cilia assembly, with an increased sensitivity to apoptotic signals and defective planar cell polarity. These factors lead to high rates of apoptotic cell extrusion from the epithelial monolayer in Sec10-silenced MDCK cysts and mouse tissues.

Funding: NIDDK Support, Private Foundation Support

FR-PO180

Aquaporin-1 Retards Renal Cyst Development in Polycystic Kidney Disease by Inhibition of Wnt Signaling
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Background: Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic diseases for which there is no effective treatment at present. Water channel aquaporin-1 (AQP1) is expressed at epithelial cell plasma membranes in renal proximal tubules and thin descending limb of Henle, as well as in epithelial cells lining the cyst wall in a subset of renal cysts in ADPKD kidneys.Our aim was to study the role of AQP1 in ADPKD development.

Methods: Postulating that AQP1 may enhance renal cytogenesis because its water transporting role, we investigated the role of AQP1 in ADPKD using *Aqp1* / *Pkd1* double knockout mice, embryonic kidney organ cultures, and matrix-grown MDCK cells.

Results: Contrary to expectations, kidney size and cyst number were significantly greater in AQP1 null PKD mice than in AQP1-expressing PKD mice, with the difference due mainly to a greater number proximal tubule cysts. In embryonic kidney cultures, AQP1 deletion increased cyst development by up to ~40%. Biochemical analysis revealed decreased β-Catenin phosphorylation and increased β-Catenin expression, suggesting enhanced Wnt signaling in AQP1 null PKD mice. In matrix-grown MDCK cells, AQP1 transfection inhibited cyst development and promoted tubulogenesis, and decreased β-Catenin and cyclinD1 expression, suggesting down-regulation of Wnt signaling. Co-immunoprecipitation showed AQP1 interaction with β-Catenin, GSK3b and LRP6.

Conclusions: These results implicate AQP1 as a novel determinant in renal cyst development that may involve inhibition of Wnt signaling by an AQP1-macromolecular signaling complex.

Funding: Government Support - Non-U.S.

FR-PO181

Epithelial Morphogenesis Can Be Rescued by Modulation of Actomyosin Contractility in Fibrocystin-Deficient MDCK Cells
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Background: Fibrocystin (FPC), a type I transmembrane protein of largely unknown function, is encoded by the *Pkhd1* gene, mutations of which cause autosomal recessive polycystic kidney disease (ARPKD). Among other potential functions, FPC appears to affects adhesion signaling of cells involving the FAK/Src axis. Contributions of epithelial cell adhesion and contractility to the disease process of ARPKD remain to be defined. The aim of this study is (i) to establish the link between FPC function and the capacity of renal collecting duct epithelial cells to generate normal epithelia, and (ii) to determine consequences of altered cell adhesion / contractility.

Methods: We analyze FPC function in Madin-Darby canine renal collecting duct epithelial cells (MDCK). In this cell line, formation of 3D spheroids can be employed to study establishment of polarity, lumen formation and ciliogenesis. A micro-pattered surface is used to confine (initial) adhesion / spreading of cells and induce spheroid formation. In this setup, establishment of polarity and lumen depend on the capacity of cells to properly control cell adhesion and actomyosin contractility. The impact of altered cell behavior is being addressed using modulators of actomyosin contractility e.g. blebbistatin and adhesion signaling.

Results: Pkhd1 siRNA-treated MDCK cells and controls were employed to analyze formation of spheroids (within three days), using specific markers for polarity, cell junctions and cilia. Knockdown of FPC strongly affects polarity and lumen formation in MDCK spheroids suggesting critical involvement of FPC protein in cellular control of adhesion signals and establishment of polarity. Furthermore, transient inhibition of actomyosin contractility using blebbistatin during the first day of spheroid formation restores 70% of (correct) epithelial morphogenesis in FPC-deficient cells.

Conclusions: FPC knock down in MDCK cells disturbs adhesion signaling, cell-cell interaction, and control of cell tension resulting in impaired epithelial morphogenesis, which can be rescued by transient reduction of cell contractility.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO182

Disruption of TRPV4 Reduces Survival of Renal Epithelial Cells in a Hyperosmolal Environment Charles D. Varnell,[1] Brian J. Siroky,[1] Nancy Kleene,[2] Bradley P. Dixon.[1] *[1]Div of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [2]Dept of Molecular and Cellular Physiology, Univ of Cincinnati College of Medicine, Cincinnati, OH.*

Background: TRPV4 localizes to primary cilia of renal epithelial cells, and is vital to cilia-mediated mechanosensation. Renal epithelial cells in the inner medulla are exposed to hyperosmolality, and survive such conditions through the osmotic response. We previously demonstrated attenuation of the osmotic response of renal epithelial cells by pharmacological modulation of TRPV4. To explore these findings further, we characterized the osmotic response and survival of ciliated and non-ciliated renal epithelial cells with disrupted TRPV4 expression.

Methods: Both renal epithelial cells lacking cilia due to an *Ift88* mutation and cells in which *Ift88* was re-expressed, rescuing cilia expression, were transduced with an shRNA construct targeting TRPV4 and compared to cells without this construct. TRPV4 expression was assessed by immunoblotting. Cells were exposed to basal osmolality or hyperosmolality induced with NaCl. Cell quantitation was assessed by crystal violet assay, and expression of *Akr1b3* and *Bgt1*, important in osmolyte accumulation in response to osmotic stress, was measured by qPCR.

Results: Cells lacking cilia had reduced *Akr1b3* and *Bgt1* expression and decreased survival in hyperosmolality compared to cells with intact cilia. Ciliated cells with reduced TRPV4 expression had reduced survival in hyperosmolal conditions compared to those with normal TRPV4 expression, whereas in non-ciliated cells, reduced TRPV4 expression did not diminish survival. Disruption of TRPV4 did not significantly alter *Akr1b3* and *Bgt1* expression in either ciliated or non-ciliated cells under hyperosmolal conditions.

Conclusions: We demonstrate a novel finding of reduced survival of ciliated renal epithelial cells with disrupted TRPV4 expression in hyperosmolal conditions. As disruption of TRPV4 expression did not alter expression of genes that regulate osmolyte accumulation, the reduced survival observed may be independent of this process but mediated by other components of the osmotic stress response.

Funding: NIDDK Support

FR-PO183

Periostin Activation of Integrin-Linked Kinase Stimulates Akt/mTOR and GSK3b/β-Catenin Mediated Proliferation of Human ADPKD Cells Archana Raman, Gail Reif, Lindsay A. Astleford, Darren P. Wallace. *Kidney Inst, Univ of Kansas Medical Center, Kansas City, KS.*

Background: Periostin, a matricellular protein, is highly overexpressed in ADPKD, ARPKD and several rodent models of PKD. Periostin binding to a_v-integrins (a_vb_3, a_vb_5) stimulates the proliferation of ADPKD cyst epithelial cells, suggesting that it is an important autocrine factor that promotes cyst growth. Genetic knockout of periostin in *pcy* mice, a slowly progressive model of PKD, reduced the number of proliferating cells in the kidney, cyst growth and interstitial fibrosis, and significantly improved renal function and survival of the mice. We **hypothesize** that periostin promotes the proliferation of cystic cells through integrin-linked kinase (ILK) activation of Akt/ mTOR and/or GSK3b/ β-Catenin signaling pathways.

Methods: ADPKD and normal human kidney (NHK) cells were grown for 4 days and ILK activity was determined using an immunocomplex kinase assay. ADPKD cells were treated with human recombinant periostin and levels of phosphorylated Akt and S6 kinase (S6K), a downstream target of mTOR, were evaluated by immunoblot analysis. To determine if β-Catenin signaling was altered, levels of phosphorylated GSK3b at Ser-9, an inhibitory site, and nuclear accumulation and transcriptional activity of β-Catenin were measured. ADPKD cells were treated with periostin ± CPD22, an ILK inhibitor, and then ILK activity and phosphorylated Akt, S6K and GSK3b were determined.

Results: Basal ILK activity was significantly elevated in ADPKD cells compared to NHK cells. Incubation of ADPKD cells with periostin increased ILK activity 1.5-fold and increased phosphorylated levels of Akt and S6K. Periostin also increased phosphorylated GSK3b and both the level and transcriptional activity of β-Catenin. Treatment with CPD22 blocked periostin-mediated phosphorylation of Akt, S6K and GSK3b and cell proliferation.

Conclusions: These data demonstrate that ILK is a key intermediate in periostin activation of signaling pathways involved in the proliferation of ADPKD cells and suggests that ILK may be a potential therapeutic target to slow cyst growth.

Funding: NIDDK Support, Other NIH Support - PKD Foundation

FR-PO184

TNFa and RANKL Regulates the Expression and Nuclear Translocation of Id2 in Renal Epithelial Cells Xia Zhou, Lucy Fan, Xiaoyan Li, Xiaogang Li. *Dept of Internal Medicine and Kidney Inst, Univ of Kansas Medical Center.*

Background: TNFa presents in the cyst fluid and promotes cyst growth in autosomal dominant polycystic kidney disease (ADPKD). The inhibitor of DNA binding 2 (Id2) regulates cystic renal epithelial cell proliferation through Id2-p21-Cdk2 axis. However, the cross-talk between TNFa and Id2 in ADPKD is unknown. It has been reported that receptor activator of NF-KB ligand (RANKL), a member of the TNFa cytokine family, is able to induce nuclear translocation of Id2 in mammary epithelial cells.

Methods: To test our hypothesis that TNFa or RANKL regulates the expression and nuclear translocation of Id2 in renal epithelial cells, we treated *Pkd1* wild type and mutant renal epithelial cells with either TNFa or RANKL alone, or plus different inhibitors of TNFa or RANKL mediated signaling molecules.

Results: We found that RANKL treatment increased the expression level of TNFa mRNA in renal epithelial cells. TNFa or RANKL stimulation increased 1) the expression of Id2 protein but not mRNA in *Pkd1* wild type and mutant renal epithelial cells; 2) the activation of mTOR in renal epithelial cells, whereas inhibition of mTOR with rapamycin decreased TNFa or RANKL induced upregulation of Id2; 3) the nuclear translocation of Id2 in *Pkd1* wild type renal epithelial cells; 4) the activation of PI3K, MAPK and CDK2, whereas inhibition of PI3K, MAPK and CDK2 with Ly294002, SB202190 and roscovitine, respectively, blocked TNFa or RANKL induced Id2 nuclear translocation. These results suggested that TNFa or RANKL induces Id2 expression via mTOR signaling pathway and regulates Id2 nuclear translocation via PI3K-MAPK-CDK2 pathway. In addition, we found that treatment with TNFa or RANKL significantly decreased the mRNA and protein levels of p21 in renal epithelial cells, which is an inhibitor of CDKs and the target of Id2.

Conclusions: TNFa or RANKL regulates 1) the expression and nuclear translocation of Id2 via mTOR and PI3K-MAPK-CDK2 pathways, respectively, in the renal epithelial cells, and 2) the expression of p21 and renal epithelial cell proliferation via Id2, which elucidates a novel mechanism for TNFa signaling in promoting cyst growth in ADPKD.

Funding: NIDDK Support

FR-PO185

Label-Free Quantitative Analysis of the YAP/TAZ Interactome Priyanka Kohli, Thomas Benzing, Bernhard Schermer, Markus M. Rinschen. *Univ Hospital Cologne, Dept of Internal Medicine, Cologne, Germany.*

Background: The Hippo signaling pathway is a potent regulator of cell proliferation and tissue growth. It has a significant effect on tumor development and progression, in addition to regulating organ size. Altered Hippo signaling has been suggested as a mechanism for cyst growth in autosomal-dominant polycystic kidney disease (ADPKD) and nephronophthisis (NPHP). YAP and TAZ are the downstream mediators of Hippo signaling in mammals.

Methods: To identify the interactomes of YAP and TAZ we expressed YAP and TAZ in a Flp-in single-copy cellular integration system. We performed single-step affinity purification with single-shot quantitative mass spectrometry analysis.

Results: Our study provides both comprehensive and comprehensive interactomes for both YAP and TAZ and does not only confirm the majority of previously described interactors but, strikingly, revealed uncharacterized interaction partners. Some of these interaction partners modulate YAP/TAZ TEAD-dependent transcription: YAP/TAZ TEAD-dependent transcription was inhibited by the transcription factor C/EBP and activated by Rassf8, a known modulator of Wnt signaling. In addition, our data allowed insights into complex stoichiometry and uncovered discrepancies between the YAP and TAZ interactomes.

Conclusions: Further studies will now characterize these novel interactors and unravel their dynamic role in regulating proliferation and ciliary signaling in ADPKD.

Funding: Government Support - Non-U.S.

FR-PO186

Hedgehog Defects in a Cep290 Model of Nephronophthisis John Andrew Sayer,[1] Ann Marie Hynes,[1] Rachel H. Giles,[2] Shalabh Srivastava,[1] Lorraine Eley,[1] Henry Ajzenberg,[2] Colin Miles.[1] *[1]Inst of Genetic Medicine, Newcastle Univ, Newcastle upon Tyne, United Kingdom; [2]Dept of Nephrology and Hypertension, Univ Medical Center Utrecht, Utrecht, Netherlands.*

Background: Nephronophthisis (NPHP) is the major cause of paediatric renal failure, yet the disease remains poorly understood, partly due to the lack of appropriate animal models. Joubert Syndrome (JBTS) is an inherited ciliopathy giving rise to NPHP and mutations in *CEP290* (*NPHP6*) are a common genetic lesion.

Methods: We have generated a Cep290 gene trap mouse model of JBTS that displays the kidney, eye and brain abnormalities and use isolated collecting duct epithelial cells in 3D culture as a model for assessing the response to Hedgehog signalling agonists.

Results: Mutant mice present with cystic kidney disease as neonates. Newborn kidneys contain normal amounts of Lef1 and Tcf1 protein, indicating normal function of the Wnt signalling pathway, however, an increase in Gli3 repressor reveals abnormal Hedgehog (Hh) signalling evident in newborn kidneys. Collecting duct cells from mutant mice have abnormal primary cilia and are unable to form spheroid structures *in vitro*. Treatment of mutant cells with the Hh agonist purmorphamine restores normal spheroid formation.

Conclusions: These data implicate abnormal Hh signalling as the cause of NPHP and suggest that Hh agonists may be exploited therapeutically.

FR-PO187

Mapping Genetic Modifiers of Autosomal Recessive Polycystic Kidney Disease (ARPKD) Michael Flister,[1] Bradley T. Endres,[1] William E. Sweeney,[2] Howard J. Jacob,[1,2] Ellis D. Avner,[2] Carol Patricia Moreno Quinn.[3] *[1]Physiology, Medical College of Wisconsin, Milwaukee, WI; [2]Pediatrics, Medical College of Wisconsin, Milwaukee, WI; [3]MedImmune, Cambridge, United Kingdom.*

Background: Autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations in PKHD1 in humans and the orthologous PCK rat model. Although ARPKD results solely from PKHD1 mutations, the disease onset and severity are highly variable, indicating that other unknown genetic risk factor (s) modify ARPKD-associated phenotypes. Here we performed genome-wide analysis of the PCK rat and two genetically distinct Pkhd1 congenic rat strains on the Fawn-Hooded Hypertensive (FHH) and the Dahl S (SS) rat backgrounds (denoted FHH.Pkhd1 and SS.Pkhd1, respectively) to identify genetic modifiers of ARPKD severity.

Methods: The PCK, FHH.Pkhd1, and SS.Pkhd1 rat strains were phenotyped for renal and hepatic cystogenesis, followed by genome-wide expression and sequence analysis.

Results: The FHH.Pkhd1 and SS.Pkhd1 strains had lower renal cyst formation at 30 days-of-age (5±2% and 8±2% cystic, respectively; P<0.001) compared to the PCK kidneys (26±4% cystic), which coincided with significantly reduced kidney weights in the FHH. Pkhd1 and SS.Pkhd1. In contrast, hepatic cystogenesis did not differ between PCK, FHH. Pkhd1, and SS.Pkhd1. These data indicated that the PCK genome harbors kidney-specific genetic modifier (s) of ARPKD severity that are not present in the FHH and SS genomes. Using high density SNP array genotyping and microarray expression analysis, we identified 50 potential modifiers of ARPKD severity in the PCK rat. Of these candidates, a damaging nonsynonymous variant in Nphp4 stood out as the most likely candidate based on variant segregation, protein modeling, network analysis, and gene ontology. Nphp4 mutations cause type 4 autosomal recessive nephronopthisis, but had not been previously implicated in the molecular or cellular pathophysiology of ARPKD.

Conclusions: These data provide genetic evidence of disease modifier (s) in the PCK model of ARPKD and prioritized multiple candidates, including NPHP4, for further investigation in ARPKD pathogenesis.

Funding: NIDDK Support

FR-PO188

Smad3 Gene Deletion Ameliorates Cyst Formation and Interstitial Fibrosis in cpk Mouce, a Model of ARPKD Taketsugu Hama,[1] Koichi Nakanishi,[1] Hironobu Mukaiyama,[1] Hiroko Togawa,[1] Masashi Sato,[1] Yuko Shima,[1] Masayasu Miyajima,[2] Kandai Nozu,[3] Shizuko Nagao,[4] Hisahide Takahashi,[4] Kazumoto Iijima,[3] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayama Medical Univ, Wakayama, Japan; [2]Laboratory Animal Center, Wakayama Medical Univ, Wakayama, Japan; [3]Pediatrics, Kobe Univ, Kobe, Japan; [4]Education and Research Center of Animal Model for Human Disease, Fujita Health Univ, Aichi, Japan.*

Background: Cystic epithelia acquire mesenchymal features in response to cyst enlargement, showing proliferative activity and interstitial fibrosis in PKD. In the context of this phenotypic alteration, we previously demonstrated that Smad3 phosphorylated at both linker regions and COOH terminal regions (pSmad3L/C) upregulated c-Myc through JNK/CDK4 dependent pathway. Therefore, pSmad3L/C may be a disease specific target. The aim of this study is to examine the effect of Smad3 gene deletion in *cpk* mice.

Methods: Mice with Smad3 gene deletion (EMBO J 1999;18:1280) were crossed with *cpk* mice to generate double-mutant (DM) mice (*cpk;smad3*$^{-/-}$).

Results: The ratio of kidney to body weight is significantly lower in DM mice than *cpk* (N=10 each, 0.13 ± 0.04 versus 0.22 ± 0.03, P<0.01). Western blot analysis revealed that protein expressions of nuclear pJNK, pCDK4 and c-Myc significantly decreased by 78% (P<0.05), 66% (P<0.05) and 63% (P<0.01), respectively, in DM mice kidneys compared to *cpk*. Compared with *cpk*, Ki-67 positive cells in cyst lining epithelia were significantly fewer in DM mice (50.7 ± 20.8 versus 35.8 ± 13.4%, P<0.01). Matrix deposition by Masson's trichrome staining in the peritubular interstitum and mRNA levels of collagen type I were significantly decreased in DM mice kidneys compared to *cpk* (9.4 ± 4.4 versus 14.4 ± 6.4, P<0.01 [% blue area]; 2.6 ± 0.6 versus 4.3 ± 1.3, P<0.01 [collagen type I mRNA/GAPDH mRNA]).

Conclusions: Study results suggest that Smad3 gene deletion inhibits deregulated cellular proliferation in cystic epithelia by suppressing the pSmad3L/C-JNK-CDK4-c-Myc cascade, and also reduces the interstitial fibrosis by suppressing TGF-β/Smad3 pathway. In conclusion, the modulation of Smad3 may offer a therapeutic strategy in PKD.

Funding: Government Support - Non-U.S.

FR-PO189

Temporal Profile of the Renal Transcriptome of HIV-1 Transgenic Mice during Disease Progression Ying Fan,[1] Chengguo Wei,[2] Wenzhen Xiao,[2] Niansong Wang,[2] Peter Y. Chuang,[2] John C. He.[2] *[1]Dept of Nephrology, Shanghai Jiao Tong Univ Affiliated Sixth People's Hospital, Shanghai, China; [2]Dept of Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Profiling of temporal changes of gene expression in the same kidney over the course of renal disease progression is challenging because repeat renal biopsies are rarely indicated in clinical practice.

Methods: Here, we profiled the temporal change in renal transcriptome of HIV-1 transgenic mice (Tg26), an animal model for human HIV-associated nephropathy (HIVAN), and their littermates at three different time points (4, 8, and 12 weeks of age) representing early, middle, and late stages of renal disease by serial kidney biopsy. We analyzed both static levels of gene expression at three stages of disease and dynamic changes in gene expression between different stages.

Results: Analysis of static and dynamic changes in gene expression revealed that up-regulated genes at the early and middle stages are mostly involved in immune response and inflammation, whereas down-regulated genes mostly related to fatty acid and retinoid metabolisms. We validated the expression of a selected panel of genes that are up-regulated at the early stage (CCL2, CCL5, CXCL11, Ubd, Anxa1, and Spon1) by real-time PCR. Among these up-regulated genes, Spon1, which is a previously identified candidate gene for hypertension, was found to be up-regulated in kidney of human with diabetic nephropathy. Immunostaining of human biopsy samples demonstrated that protein expression of Spon1 was also markedly increased in kidneys of patients with both early and late HIVAN and diabetic nephropathy.

Conclusions: Our studies suggest that analysis of both static and dynamic changes of gene expression profiles in disease progression avails another layer of information that could be utilized to gain a more comprehensive understanding of disease progression and identify potential biomarkers and drug targets.

Funding: NIDDK Support

FR-PO190

Linking Plasma Changes in ApoL1 Variants Causal for Nephropathy to Chronic Kidney Disease Maarten Hoek,[1] Julia Kozlitina,[2] Haihong Zhou,[1] Patricia Brown,[1] Rory Rohm,[1] Gulesi Ayanoglu,[1] Xiaoyan Du,[1] Eric Rimmer,[1] Dermot Reilly,[1] Thomas P. Roddy,[1] Doris F. Cully,[1] Thomas F. Vogt,[1] Daniel Blom.[1] *[1]MRL, Merck & Co., Kenilworth, NJ; [2]McDermott Center for Human Growth and Development, UT Southwestern Medical Center, Dallas, TX.*

Background: Two common missense variants in ApoL1 (G1 and G2) have been definitively linked to CKD in African Americans. However, not all individuals with the renal-risk genotype (RRG) go on to develop CKD and little is known about how ApoL1 variants drive disease. Because HDL is cleared by the kidney, it has been suggested that differences in the level or quality of mutant ApoL1-HDL particles could be causal and might serve as useful risk stratification markers.

Methods: Levels of G0 (low-risk variant), G1 and G2 ApoL1 were measured in plasma from 3552 individuals in the Dallas Heart Study using an LC-MS method that enabled the quantitation of the different variants. Additionally, native ApoL1-HDL from donors with no or two ApoL1 risk alleles was characterized by size exclusion chromatography and further analysis of immunopurified ApoL1-HDL particles.

Results: Though an association between APOL1 variant status and renal function in non-diabetic individuals was replicated ($p=7.1 \times 10^{-6}$ for microalbuminuria, 2.7×10^{-5} for GFR<60 ml/min per 1.73 m^2), there was no association between circulating ApoL1 levels and microalbuminuria or GFR after adjusting for genotype. This was true even when analysis was restricted only to subgroups such as G0 homozygotes, single risk allele carriers or double risk allele carriers. ApoL1 in the circulation existed in a heterogeneous high MW HDL pool, but no consistent size differences were observed between ApoL1-HDL from G0 and RRG donors. There were no differences in the relative amounts of Hpr and IgM in ApoL1-HDL purified from G0 and RRG individuals, though a potential change in ApoA1/ApoL1 stoichiometry was noted.

Conclusions: These data demonstrate that plasma levels of ApoL1 (G0, G1 or G2) are not useful to stratify risk of renal disease in ApoL1 RRG individuals and suggest that mechanisms underlying ApoL1-nephropathy cannot be easily attributed to obvious differences in the amount or quality of G0 or G1/G2 ApoL1 in the circulation.

Funding: Pharmaceutical Company Support - Merck & Co.

FR-PO191

Impact of Genetically Determined Ancestry Informative Markers on GFR Estimates and Kidney Disease Risk Girish N. Nadkarni, Miriam S. Udler, Gillian Belbin, Vaneet Lotay, Christina M. Wyatt, Omri Gottesman, Eimear Kenny, Inga Peter, Erwin P. Bottinger. *Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Self-reported race, genetic ancestry, and Apolipoprotein L1 (APOL1) polymorphisms contribute to variation in kidney function and related disease risk, yet it remains unclear what their relative contributions are.

Methods: We estimated global proportion of African ancestry (using genome-wide genotype data) and *APOL1* risk status for 9,048 unrelated individuals (self-reported as 3,189 African Americans, 1,721 European Americans, and 4,138 Hispanic/Latino Americans). We extracted CKD-EPI estimated glomerular filtration rate (eGFR), clinical and demographic characteristics from the electronic medical record. We assessed relationships between continuous variables and racial groups or *APOL1* variants using multivariable linear regression.

Results: In both the admixed African Americans and Hispanic/Latino Americans, there was significant trend of increasing creatinine as percent African ancestry increased (per 10% increase in African ancestry, 0.01 mg/dl increase in creatinine in African Americans, P=1x10^{-7} and 0.009 mg/dl in Hispanic/Latino Americans, P=1x10^{-8}).Estimated GFR was likewise significantly associated with African genetic ancestry in both populations. *APOL1* risk haplotypes were not associated with normal-range creatinine and eGFR, but were significantly associated with CKD, eGFR<45 mL/min/1.73m2, and ESRD, with effects of *APOL1* increasing with worsening disease states and the contribution of genetic African ancestry decreasing in parallel. Using genetic ancestry to reclassify patients as "Black" based on >50% African ancestry resulted in higher eGFR for 14.7% of Hispanic/Latino Americans and lower eGFR in 4.1% of African Americans, impacting CKD staging in 4.3% of Hispanic/Latino Americans and 1% of African Americans.

Conclusions: In summary,proportion African ancestry was significantly associated with normal-range creatinine and eGFR, while *APOL1* risk haplotypes drove associations with CKD states. Recalculation of eGFR based on genetic ancestry impacted CKD staging and warrants further investigation including correlation with measured GFR particularly in Hispanic/Latinos.

Funding: Private Foundation Support

FR-PO192

APOL1 Risk Variants Are Strongly Associated with HIV-Associated Nephropathy in Black South Africans Alex Nganga Kasembeli,[1] Raquel Duarte,[1] Michele Ramsay,[1,2] Pulane Mosiane,[2] Caroline Dickens,[1] Therese Dix-Peek,[1] Sophie Limou,[3] Efe Sezgin,[3,4] George W. Nelson,[3] Jeffrey B. Kopp,[5] Cheryl Ann Winkler,[3] Saraladevi Naicker.[1] *[1]Univ of the Witwatersrand, Johannesburg, South Africa; [2]National Health Laboratory Service, Johannesburg, South Africa; [3]NCI, Frederick National Laboratory, Frederick, MD; [4]Johns Hopkins Bloomberg SPH, Baltimore, MD; [5]NIDDK, Bethesda, MD.*

Background: APOL1 variants are strongly associated with HIVAN and FSGS in African Americans; however, their impact and prevalence on chronic kidney disease (CKD) in a setting of HIV-1 subtype C infection was not previously investigated. This study aims to determine the role of *APOL1* variants on HIVAN and CKD in a South African black population.

Methods: Archived kidney biopsies (N=124) were selected according to their histological diagnosis and DNA was extracted. The control groups comprised HIV positive individuals without CKD (N=54) and population controls (N=54). Genotypes were determined for APOL1 G1 (rs73885319 and rs60910145), G2 (rs71785313) and global ancestry informative markers. Association of APOL1 alleles with HIVAN and CKD was tested by Fisher's exact test (FET) and by logistic regression adjusting for age, sex, and genetic ancestry.

Results: Genotypes were determined for 38 HIVAN, 41 HIV positive CKD, and 41 HIV negative CKD cases (N=120) and 54 HIV positive controls and 54 population controls. 79% of HIVAN cases and 2% of the population controls carried two APOL1 risk alleles. In a recessive model, individuals carrying any combination of two *APOL1* risk alleles had 89-fold higher odds (95% CI 17.7-911.7; $p=1.2x10^{-14}$) of developing HIVAN. Population allele frequencies were 7.3% for G1 and 11.1% for G2. APOL1 risk variants were not significantly associated with other forms of CKD, including FSGS.

Conclusions: This is the first African study to report the impact of APOL1 risk variants on HIVAN in patients infected with HIV-1 subtype C. HIV positive, anti-retroviral therapy naïve South African blacks with two APOL1 risk alleles are at very high risk for developing HIVAN. Further studies are required to determine the impact of APOL1 risk variants on kidney disease in other regions of sub-Saharan Africa.

FR-PO193

Copy Number Variation in APOL1 Gene in African American Individuals and Its Role in Kidney Disease Rupam Ruchi,[1] Giulio Genovese, Jessica Lee,[1] Victoria Charoonratana,[1] David J. Friedman,[1] Martin R. Pollak.[1,2] *[1]Nephrology, Beth Israel Deaconess Medical Center/Harvard Univ; [2]Broad Inst of MIT and Harvard.*

Background: APOL1 associated kidney disease is largely autosomal recessive. However, in some studies, G1 heterozygotes develop kidney disease more frequently or have earlier onset of disease than individuals with two normal alleles. We hypothesize that structural variation in the APOL1 gene determines at least some of the phenotype seen in heterozygous individuals.

Methods: Sequence coverage analysis of 1000 genome project (KGP) phase 3 samples and exome sequencing data from our lab were screened for copy number variation by assessing read depth in the APOL1 region. A PCR-based assay was designed to confirm a putative APOL1 duplication. The frequency of this duplication was compared among heterozygous African American (AA) cases (n=123) and controls (n=255) by an allelic discrimination assay. A 314 bp region on the last exon of APOL1 (including the site of G1 SNP) was amplified, cloned and sequenced for haplotype identification.

Results: Analysis of data from 8 samples (including 5 heterozygous for G1) sequenced in KGP showed increased coverage over ~ 100kb region, including APOL2, APOL1 and part of MYH9, suggesting the presence of APOL1 copy number > 2. The qualitative PCR assay confirmed the presence of a duplication, which we subsequently observed in additional samples from heterozygous AA individuals with kidney disease. Sanger sequencing revealed identical sequences at the junction, indicating that the duplication likely occurred once and was co-inherited by all the subjects with the duplication. Bioinformatic analysis combined with cloning and Sanger sequencing revealed that most carriers of extra APOL1 copies were G0G0G1, but that G0G1G1 genotypes also existed. Taqman-based copy number assay confirmed the presence of multiple copies in these individuals. Comparison of cases and controls showed the presence of the APOL1 duplication in 4.06% of cases and 0.78% controls (p=0.03).

Conclusions: Copy number variation exists in APOL1 gene, and may be a genetic determinant of kidney disease in AA individuals who are heterozygous for the risk alleles by traditional genotyping techniques.

Funding: Pharmaceutical Company Support - Genzyme

FR-PO194

Association between Apolipoprotein L1 Gene Variants with Prevalent Kidney and Cardiovascular Disease: Systolic Blood Pressure Intervention Trial (SPRINT) Carl D. Langefeld,[1] Jasmin Divers,[1] Nicholas M. Pajewski,[1] Amret T. Hawfield,[1] David Reboussin,[1] D. Bild,[2] George A. Kaysen,[3] Paul L. Kimmel,[4] Dominic S. Raj,[5] Ana C. Ricardo,[6] Jackson T. Wright,[7] John R. Sedor,[7] Michael V. Rocco,[1] Barry I. Freedman.[1] *[1]Wake Forest Sch Med; [2]Patient-Centered Outcomes Research Inst; [3]Univ of California Davis Healthcare Sys; [4]NIDDK; [5]George Washington Univ Sch Med; [6]Univ of Illinois Coll Med at Chicago; [7]Case Western Reserve Univ.*

Background: Apolipoprotein L1 gene (APOL1) G1 and G2 coding variants are strongly associated with chronic kidney disease (CKD) in African Americans (AAs). APOL1 association was tested with baseline estimated glomerular filtration rate (eGFR), urine albumin:creatinine ratio (UACR), and prevalent cardiovascular disease (CVD) in 2,571 AAs from the Systolic Blood Pressure Intervention Trial (SPRINT). SPRINT is assessing effects of systolic blood pressure reduction on renal and CVD outcomes.

Methods: Logistic regression models adjusting for potentially important confounders were computed to test for association between APOL1 risk variants and baseline clinical CVD (defined as myocardial infarction, surgical or percutaneous coronary artery or carotid artery revascularization) and with CKD (eGFR<60 ml/min/1.73m^2 and/or UACR >30 mg/g).

Results: AA SPRINT participants were 45.3% female with mean+SD (median) age 64.3+9.3 (63) years, mean arterial pressure 100.7+12.2 (100) mmHg, eGFR 76.3+22.9 (77.1) ml/min/1.73m^2, UACR 49.9+188.6 (9.2) mg/g, and 8.2% had clinical CVD. APOL1 (recessive inheritance) was positively associated with CKD (p=0.0095, odds ratio (OR) 1.37, 95% confidence interval (CI) 1.08-1.73) and log UACR ($p=7.7x10^{-6}$,estimated slope [b] 0.33) and negatively associated with eGFR (p=0.0029, b -3.58). APOL1 risk variants were not associated with prevalent CVD (p=0.86, OR 1.02, 95% CI 0.82-1.27).

Conclusions: SPRINT data reveals that APOL1 nephropathy risk variants are associated with kidney disease in AA subjects with a UACR <1 gm/gm, but are not associated with clinical CVD. [Support: NIH HHSN268200900040C (SPRINT); RO1 DK084149 and RO1 DK070941 (BIF)].

Funding: NIDDK Support, Other NIH Support - NIH HHSN268200900040C (SPRINT)

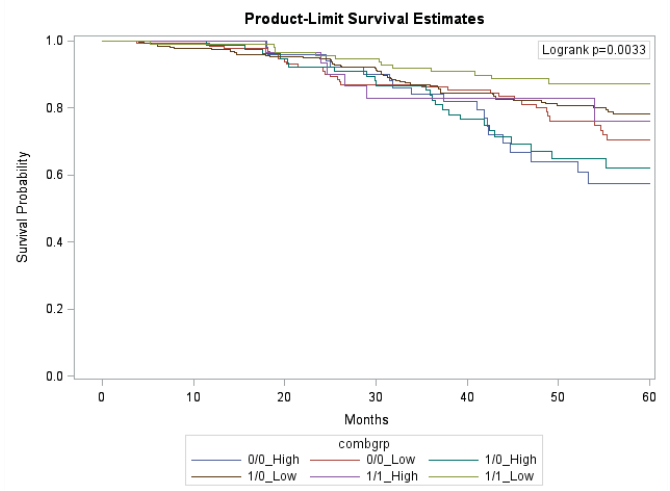
FR-PO195

Additive Effect of the *GSTM1 (0)* Null and *APOL1* High Risk Alleles in the African American Study of Kidney Disease (AASK) Trial Gabor Bodonyi-Kovacs,[1] Jennie Z. Ma,[2] Michael S. Lipkowitz,[3] Jeffrey B. Kopp,[4] Cheryl Ann Winkler,[5] Thu H. Le.[1] *[1]Renal Div., UVA, Charlottesville, VA; [2]Biostat. Sect., UVA, Charlottesville, VA; [3]Renal Div., Georgetown Univ, Washington, DC; [4]Kidney Diseases Branch, NIH, Bethesda, MD; [5]Center for Cancer Research, NIH, Frederick, MD.*

Background: Apolipoprotein L-1 (*APOL1*) G1 and G2 alleles are associated with chronic kidney disease (CKD) in African American (AA) patients. Glutathione-S-transferase Mu 1 (*GSTM1*) null allele, *GSTM1 (0),* is also associated with accelerated progression of CKD in hypertensive AA patients. We hypothesized that the *GSTM1 (0)* and *APOL1* high risk alleles interact to influence the clinical outcomes in the AASK Trial participants.

Methods: A total of 682 patients were classified into the combination of homozygous null for *GSTM1 (0/0),* heterozygous *(1/0),* and homozygous active *(1/1)* and *APOL1* high (*High*) and low (*Low*) risk genotypes. The survival differences among the six groups were assessed by log-rank test and in the Cox regression for time to the event of glomerular filtration rate (GFR, 50% or 25ml/min/1.73m^2 decline), dialysis, or death, or their composite events. Regression analyses were adjusted for age, gender, proteinuria, baseline GFR, MAP, CVD, drug class, and population admixture.

Results: The 6 groups differed significantly in the time to composite events of GFR, or dialysis or death (log-rank p=0.0033) (Fig.1).



Compared to *(1/1) _Low* group, the hazard ratios (HR) for the composite events were 2.6 (p=0.009) and 2.4 (p= 0.019) for the *(0/0) _*Low and the *(1/0) _High* groups, respectively. The HRs for the other groups did not reach statistical significance [*(1/1) _High* (HR 1.2, p = 0.73), *(1/0) _Low* (HR 1.78, p = 0.079), and *(0/0) _High* (HR 1.91, p = 0.097)].

Conclusions: *GSTM1 (0)* and *APOL1* high risk alleles have additive deleterious effects on CKD progression among hypertensive AAs.

Funding: NIDDK Support

FR-PO196

The Apolipoprotein L1 gene G3 Haplotype Is Not Associated with End-Stage Kidney Disease (ESKD) in African Americans Nicholette D. Palmer,[1] Maggie Ng,[1] Carl D. Langefeld,[2] Jasmin Divers,[2] Janice P. Lea,[4] Mark D. Okusa,[5] Robert P. Kimberly,[6] Donald W. Bowden,[1] Barry I. Freedman.[3] *[1]Biochemistry, Wake Forest; [2]Biostatistical Sciences, Wake Forest; [3]Nephrology, Wake Forest; [4]Renal Medicine, Emory; [5]Nephrology, Univ VA; [6]Rheumatology, Univ AL Birmingham.*

Background: Apolipoprotein L1 gene (APOL1) G1 and G2 nephropathy risk variants are strongly associated with progressive non-diabetic etiologies of kidney disease in populations with recent African ancestry. Selection for these variants likely occurred due to protection from human African trypanosomiasis (HAT). Resequencing of this region in ten genetically and geographically distinct African populations residing in HAT endemic regions identified 8 single nucleotide polymorphisms (SNPs) in strong linkage disequilibrium and comprising a novel G3 haplotype. The G3 haplotype could confer protection from HAT and/or risk for kidney disease (Ko WY et al, Am J Hum Genet 93:54-66, 2013).

Methods: To determine whether the APOL1 G3 haplotype was associated with nephropathy, G1, G2, and G3 SNPs and 70 ancestry informative markers spanning the genome were genotyped in 937 African Americans with non-diabetic ESKD, 965 with type 2 diabetes associated (T2D)-ESKD, and 1,029 non-nephropathy controls.

Results: Cases with non-diabetic ESKD and T2D-ESKD initiated renal replacement therapy at mean±SD 48.7±15.5 and 58.0±10.9 years, respectively. Controls were recruited at 50.0±11.9 years. Overall African ancestry was 80.0±11.6%, 79.9±11.4%, and 78.2±10.9% in non-diabetic ESKD cases, T2D-ESKD cases, and controls, respectively. In analyses adjusting for age, sex, APOL1 G1/G2 risk (recessive model), and global African ancestry, the APOL1 G3 haplotype was not significantly associated with ESKD (p=0.14 for non-diabetic ESKD; p=0.57 for type 2 diabetes-associated ESKD; p=0.38 for combined all-cause ESKD). Only one of eight G3 SNPs, rs136170, was significantly associated with non-diabetic ESKD and all-cause ESKD in the adjusted model ($P=3.9x10^{-4}$ and 0.017, respectively).

Conclusions: Variation in APOL1 G3 makes nominal, if any, contribution to ESKD susceptibility in African Americans. G1 and G2 variants explain the vast majority of nephropathy susceptibility.

Funding: NIDDK Support

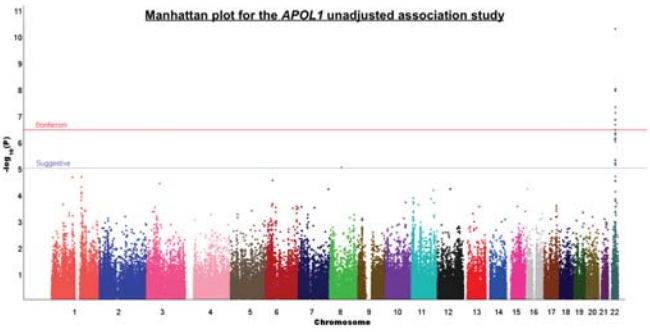
FR-PO197

Large-Scale Gene-Centric Exploration of FSGS Risk in African Americans Sophie Limou,[1] George W. Nelson,[1] Jeffrey B. Kopp,[2] Cheryl Ann Winkler.[1] *[1]Frederick National Laboratory, NCI, Leidos Biomedical Research, Inc., Frederick, MD; [2]NIDDK, NIH, Bethesda, MD.*

Background: Much of the increased burden of chronic kidney disease in people of African ancestry has been attributed to two *APOL1* alleles, termed G1 and G2. Approximately 10% of African Americans (AA) with focal segmental glomerulosclerosis (FSGS) or HIV-associated nephropathy (HIVAN) do not carry an *APOL1* renal risk allele, raising the possibility that other genetic variants may contribute to kidney disease.

Methods: We conducted a large-scale gene-centric association study on 181 AA with FSGS or HIVAN and 273 AA controls using the customized Illumina Human CardioMetaboloKidney Beadchips enriched in markers for kidney genes and 22q13.1 locus (198K). After quality control, 146,794 SNPs were tested for association with FSGS/HIVAN by logistic regressions adjusted for gender, HIV status, and the first two eigenvectors from the population stratification analysis.

Results: Thirteen chromosome 22 genetic variants were significantly associated with FSGS/HIVAN ($P<3.4x10^{-7}$).



After adjusting for the strong recessive *APOL1* G1/G2 effect ($P=8.4x10^{-17}$, OR=9.8), no SNP reached the study-wide significance level. However, two chromosome 1 variants met the suggestive significance threshold ($P<10^{-5}$, OR=2.7). Both are located in an estrogen-inducible gene whose encoded protein is localized in late endosome and lysosome, and plays a role in autophagy and cell survival under stress.

Conclusions: Our study confirmed the importance of *APOL1* association with FSGS/HIVAN in AA, and revealed a new candidate gene involved in autophagy as intracellular ApoL1 is thought to be. This new result warrants replication in a larger sample size and functional exploration in kidney cells.

Funding: NIDDK Support, Other NIH Support - Frederick National Laboratory for Cancer Research, NIH; Intramural Research Program of NIH, Frederick National Lab, Center for Cancer Research

FR-PO198

Common and Rare Codon-Altering Variants in the Functional Domains of APOL1 Sophie Limou,[1] George W. Nelson,[1] Jeffrey B. Kopp,[2] Cheryl Ann Winkler.[1] *[1]Frederick National Laboratory, NCI, Leidos Biomedical Research, Inc., Frederick, MD; [2]NIDDK, NIH, Bethesda, MD.*

Background: Much of the increased burden of chronic kidney disease in people of African ancestry has been attributed to two alleles, termed G1 and G2, in the *APOL1* gene, which encodes a component of the trypanosome lytic factor (TLF). Approximately 10% of African Americans (AA) with focal segmental glomerulosclerosis (FSGS) or HIV-associated nephropathy (HIVAN) do not carry an *APOL1* renal risk allele, raising the possibility that other *APOL1* variants may contribute to kidney disease. A recent report identified an additional *APOL1* haplotype, termed G3, which may be under selection by trypanosomes and might cause renal injury.

Methods: To determine the extent of variation in the terminal exon of *APOL1* encoding the functional domains, we resequenced 2,549 individuals: 1,112 individuals from 53 global populations from the Human Genome Diversity Project and International HapMap Project; 241 AA and 169 European Americans (EA) with FSGS; 54 AA with HIVAN; and 651 AA and 322 EA controls. To test for association of common and rare variants with FSGS and HIVAN, logistic regression, burden, and SKAT tests were performed. We computed the F_{ST} estimates to quantify the population divergence, and the EHH and iHS statistics to assess for signatures of recent selection. Plasma containing variant ApoL1 protein isoforms was tested for TLF-mediated lysis of *T.b. rhodesiense* and *gambiense*.

Results: None of the common or rare *APOL1* variants, including the G3 missense variants, were associated with FSGS or HIVAN beyond the strong association of G1 and G2 alleles ($P=3.31x10^{-58}$, OR=18). The selection analysis revealed signatures of recent selection only for G1, and to a lesser extent for G2, in West Africa. With the exception of plasma containing ApoL1 G1 or G2 isoforms, none of the human plasma containing other ApoL1 isoforms lysed trypanosomes.

Conclusions: Only *APOL1* G1 and G2 alleles are strongly associated with increased risk of FSGS and HIVAN, suggesting that sequencing *APOL1* would bring no additional benefit for prevention or clinical management of kidney disease compared to genotyping *APOL1* G1 and G2 risk alleles only.

Funding: NIDDK Support, Other NIH Support - Frederick National Laboratory for Cancer Research, NIH; Intramural Research Program of NIH, Frederick National Lab, Center for Cancer Research

FR-PO199

Fine Mapping of the HIVAN1 Susceptibility Locus on Chr. 3A1-A3 via Generation of Subcongenic Strains Natalia Papeta,[1] Ami Patel,[1] Travis Stanley Crevecoeur,[1] Vivette D. D'Agati,[2] Ali G. Gharavi.[1] *[1]Medicine, Columbia Univ, New York, NY; [2]Pathology, Columbia Univ, New York, NY.*

Background: HIV-1 transgenic mice on the FVB/NJ background (TgFVB) represent a validated model of HIV- associated nephropathy (HIVAN). A major susceptibility locus (*HIVAN1*), with CAST alleles associated with increased risk of disease, was previously identified on chromosome 3A1-A3 in a mapping study between TgFVB and CAST/EiJ (CAST) strains. Previously reported TgFVB-HIVAN1^{CAST}congenic strain, carrying a 50 Mb CAST interval (encompassing the *HIVAN1* locus), introgressed into the TgFVB genome, showed accelerated development of HIVAN compared to TgFVB strain.

Methods: We generated three subcongenic strains, which encompass proximal (SubII 4.2-38.55 Mb) or distal (SubIII 38.45-54.8Mb and SubIV 48.7-54.8Mb) regions of the reported FVB-HIVAN1^{CAST} locus (build 38.1). These strains were crossed with TgFVB strain and HIVAN pathology traits were analyzed to refine the *HIVAN1* locus.

Results: At 5-10 weeks of age renal injury parameters in Tg-SubII-FVB-HIVAN1^{CAST} mice were not different from those of TgFVB. In contrast, HIV transgenic SubIII and SubIV mice displayed significantly more severe kidney disease compared to Tg-SubII-FVB-HIVAN1^{CAST} mice (glomerulosclerosis 64.1+ 6.5% and 64.8 + 5.3% and versus 25.8 +3.5%, tubulointerstitial injury 38.5 +5.7% and 36.6+3.6% versus 14.2+ 1.9%, inflammation 28.1+5% and 28.4+3% versus 11.2+2.2%, P<0.01 for each comparison). The severity of disease correlated with the number of SubIV CAST alleles, indicating an additive effect. The identified *SubIV-HIVAN1* locus spans 7.3 Mb and encodes 31 genes, of which 4 have non-synonymous coding SNPs that differentiate CAST from FVB and 9 genes show expression levels, significantly different between these strains, according to our transcriptome data. High priority candidate genes include Pcdh18, Mgarp, Setd7, Exosc8 and Frem2.

Conclusions: These data further confirm that a gene on chr3A1-A3 increases susceptibility to HIVAN and reduce the *HIVAN1* locus to a maximal 7.5Mb interval with 5 high priority candidate genes based on sequence and transcriptome data.

Funding: NIDDK Support

FR-PO200

A Bioinformatics Approach to Understanding a Common Pathogenesis for Chronic Kidney Disease: Perspectives from Mendelian Genes and Proteome Analysis Chen-Han Wilfred Wu,[1] Jingtao Guo,[3] Robert H. Yenchek,[2] Jo Abraham,[2] Alfred K. Cheung,[2] Lynn B. Jorde.[1] *[1]Dept of Human Genetics, Univ of Utah, Salt Lake City, UT; [2]Div of Nephrology, Dept of Internal Medicine, Univ of Utah, Salt Lake City, UT; [3]Dept of Oncological Sciences, Univ of Utah, Salt Lake City, UT.*

Background: Chronic kidney disease (CKD) affects more than 13% of people in the United States, and is one of the leading causes of death. Genetics studies have provided molecular insights into CKD. However, genes identified so far showed locus heterogeneity across different functions. Yet the clinical manifestations of CKD are often shared across various etiologies. Is there a common pathogenesis that may explain these heterogeneous diseases? Here we employed a bioinformatics approach to identify one or a few common groups for CKD pathogenesis.

Methods: We first identified known causative genes associated with CKD curated by the Online Mendelian Inheritance in Man (OMIM). We explored their evidence of protein-protein interactions in the Database of Interacting Proteins (DIP). After parsing the genes and interactions, more candidate genes and interaction networks were discovered. A statistical hypothesis test was performed to see if these interactions are more abundant compared to the proteome. The extended candidate genes and networks are further analyzed for grouping for possible common pathogenesis.

Results: From OMIM, we identified 84 causative genes for CKD, and 16 of them have experimentally determined interactions in DIP. These resulted in 56 protein-protein interactions which is statistically more abundant than the interactions proteome-wide (p=0.0069). Through these interaction networks, 49 candidate genes were discovered, and 12 independent groups of possible pathogenesis pathways can be distinguished.

Conclusions: CKD causative genes have significantly more protein-protein interactions than that of the proteome. We identified 49 new candidate genes for CKD awaiting further verification. The networks form 12 independent groups. Although this model is still heterogeneous, further study may show additional reduction in heterogeneity towards shared common pathogenesis.

FR-PO201

GWAS-Derived Renal Genetic Risk Score and Renal Function in a Prospective Cohort of Type 2 Diabetic Patients – SURDIAGENE Study Philippe Zaoui,[1] Pauline Barbieux,[2] Samy Hadjadj.[2] *[1]Nephrology, CHU Michallon, Grenoble, France; [2]Endocrinology, CHU Poitiers, Poitiers, France.*

Background: Renal disease is a complex condition with ill-defined genetic components. Recent GWAS-identified loci seem associated with renal failure and/or function (see appendix). These genetic findings have not been yet tested for their clinical utility in prospective studies or in specified populations.

Methods: We used 16 SNPs identified by CKD consortium as associated with renal function or End-Stage Renal Failure (ESRF) to build a renal genetic risk score (RGRS): for a given SNP, homozygotes for a protective allele were given 0 point, heterozygotes 1 point and homozygotes for the risk allele 2 points. We tested the genetic risk score on 1420 type 2 diabetic patients of European origin from the real-life SURDIAGENE cohort, with 1136 patients of European ethnicity having the whole set of genetic determinations. Study endpoints were incidence of ESRF and/or sustained doubling of serum creatinine and trajectory of eGFR (CKD EPI) using a total of 20,898 determinations.

Results: The median (range) of RGRS was 17 (min=9, max=25). Follow-up duration was 70 months. RGRS was not different between CKD stage at baseline: CKD 1-2 versus CKD 3 versus CKD 4-5 (p=0.109). RSGS was not associated with occurence of renal events (72 events i.e. 1.1 % person-year). When analyzing eGFR trajectory with a mixed-linear model, RSGS was not associated with renal function decline (p=0.7115). In addition, none of the SNPs was individually associated with renal event or renal function decline.

Conclusions: The RGRS derived from 16 SNPs identified by the CKD consortium proved to add no major prognostic value for renal outcomes in type 2 diabetes patients. **Appendix:** gene (s) or putative gene (s) and corresponding rs NAT8_S143F rs13538; STC1 rs10109414; DAB2, C9 rs11959928; SLC7A9, CCDC123, ECAT8 rs12460876; GCKR, IFT172, FNDC4 rs1260326; UMOD, ACSM5, GP2, PDILT rs12917707; UBE2Q2, FBXO22 rs1394125; SHROOM3, CCDC158 rs17319721; ANXA9, FAM63A, PRUNE, BNIPL, LASS2, SETDB1 rs267734; TFDP2 rs347685; PIP5K1B, FAM122A rs4744712; DACH1 rs626277; SLC34A1, GRK6, RGS14, LMAN2, PRR7, F12, PFN3 rs6420094; ATXN2 rs653178; PRKAG2 rs7805747; VEGFA rs881858.

Funding: Government Support - Non-U.S.

FR-PO202

miRNome Expression Profiling in Plasma From Type 1 Diabetic Patients with Impaired Renal Function and an Increased Risk of Rapid Progression to End-Stage Renal Disease Eiichiro Satake, Marcus G. Pezzolesi, Kevin P. Mcdonnell, Adam Smiles, Andrzej S. Krolewski. *Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA.*

Background: MicroRNAs (miRNAs) are short endogenous, non-coding RNA molecules that have recently been shown to be expressed in a variety of human biofluids. These molecules are involved in gene regulation and play important roles in the pathogenesis of various renal diseases. The objective of this study was to determine the miRNA signature that is associated with impaired renal function in Type 1 diabetes (T1D) patients with nephropathy.

Methods: The expression levels of 1,811 miRNAs from the human miRNA genome (miRNome) were measured in baseline plasma samples from 78 proteinuric T1D patients who lost renal function at a rate of >3.3ml/min/year before reaching end-stage renal disease during 7-20 years of follow up.

Results: A total of 390 miRNAs were detectable in plasma samples from these study participants. To identify miRNAs associated with impaired function, miRNome expression profiles among individuals with normal (estimated glomerular filtration rates (eGFR) > 60 ml/min/1.73m^2) and impaired (eGFR= 30-59 ml/min/1.73m^2) renal function at baseline were compared. In total, 39 miRNAs (2.2%) with <0.5 or >2.0 fold changes were identified between individuals with normal and impaired renal function. Among 86 highly-expressed miRNAs, we identified 7 miRNAs with <0.5 or >2.0 fold change between the groups.

Conclusions: These data suggest that miRNAs are associated with eGFR difference in diabetic nephropathy. Furthermore, these differentially expressed miRNAs can be potential marker for renal function or therapeutic targets for diabetic nephropathy in T1D.

FR-PO203

Pro-Arrhythmic Genotypes and Sudden Cardiac Arrest Risk in Chronic Kidney Disease Patients Patrick H. Pun, Carol Haynes, Damian M. Craig, Megan Chryst-Ladd, John Paul Middleton, Laura P. Svetkey, Svati H. Shah. *Medicine, Duke Univ, Durham, NC.*

Background: CKD patients are at increased risk of sudden cardiac death (SCD); unfortunately, traditional risk factors have low utility in risk prediction in this population. The influence of genetic factors on SCD risk among CKD patients is unknown but could improve risk prediction. Genes encoding ion channels are intriguing candidates for SCD risk, since they underlie the majority of inherited arrhythmias, and subclinical mutations may promote arrhythmias when combined with CKD-related environmental stressors. We examined the SCD risk associated with common variants in the *KCNH2*, *SCN5A* and *NOS1AP* genes in CKD patients with coronary heart disease.

Methods: Using a biorepository of patients undergoing cardiac catheterization at our institution from 2001-2011, we genotyped candidate single nucleotide polymorphisms (SNPs). The primary outcome was a composite of SCD and clinically significant ventricular arrhythmias, as adjudicated from chart review. Analyses stratified by race and Cox proportional hazards models adjusted for covariates were used to compare the relationships among single SNPs (dominant model) and outcome in CKD patients (eGFR<60) and non-CKD patients (eGFR≥60).

Results: Of 9206 total subjects included, 26% had eGFR<60. There were a total of 832 composite events over a mean follow up of 5.5 yrs. All SNPs were in Hardy-Weinberg Equilibrium and met quality control metrics. SNPs in *SCN5A* and *KCNH2* were not associated with the composite outcome in the CKD population. There was a significant interaction between CKD and RS16847548 in *NOS1AP* (minor C allele frequency=0.2 in CKD population), with an association between genotype and outcome only in those with CKD (HR=0.65, 95%CI 0.50-0.83, p=0.0008). Stratification by race and adjustment for covariates did not alter the association (aHR=0.71, 95% CI 0.54-0.94, p=0.015).

Conclusions: A common SNP in the *NOS1AP* locus was associated with a ventricular arrhythmias and SCD in CKD patients. In contrast to prior studies, the SNP was associated with a diminished rather than augmented risk. This observation should encourage investigation into unique mechanisms of risk protection in the CKD population.

Funding: NIDDK Support

FR-PO204

Genomic Changes of Cardiovascular Disease in Patients with Chronic Kidney Disease Tzongshi Lu,[1,2] Li-Lun Ho,[3] Guerman Molostvov,[4] Lingsheng Dong,[2] Thomas F. Hiemstra,[5] Daniel Zehnder,[4,6] Li-Li Hsiao.[1,2] *[1]Renal Div, Brigham and Women's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Chemical Engineering, Massachusetts Insts of Technology, MA; [4]Warwick Medical School, Univ of Warwick, Coventry, United Kingdom; [5]School of Clinical Medicine, Univ of Cambridge, Cambridge, United Kingdom; [6]Univ Hospital Coventry and Warwickshire NHS Trust, Coventry, United Kingdom.*

Background: Over 20 million U.S. adults have chronic kidney disease (CKD). Cardiovascular disease (CVD) is the major cause of death among ESRD patients. The prevalence of acute myocardial infarction and congestive heart failure in patients with CKD is much higher than those without CKD. In this study, we explored the direct genomic effects of CKD on CVD.

Methods: Arteries were collected from patients with CVD with or without CKD in the absence of diabetes, hypertension or smoking history. RNA sequencing technology (RNAseq) was used to identify molecular markers. Gene selections are based on log 2 ratio using folds changes. Data analysis was using Kyoto Encyclopedia of Genes and Genomes (KEGG), and Database for Annotation, Visualization and Integrated Discovery (DAVID).

Results: When comparing arteries from CVD with CKD to those without CKD, 13 genes were up-regulated and nine genes were down-regulated. Pathway analysis among the up-regulated genes revealed that these genes are mainly involved in cardiomyopathy processes, such as NR1D1 (nuclear receptor subfamily 1, group D, member 1), a regulatory function gene in metabolic and inflammatory process; SGCG (gamma-sarcoglycan), a gene associated with hypertrophic/dilated cardiomyopathy or arrhythmogenic ventricular cardiomyopathy. Among the down-regulated genes, they were found to play roles in cell cycle regulation, apoptosis, oxidative stress and stress resistance. In addition, these genes also involve in HIF-1, which regulates the VEGF signaling pathway.

Conclusions: We have identified a group of genes of which expression is uniquely altered in the presence of CKD. This gene set may have pathophysiological significance and may serve to inform rational development of therapeutic strategies to prevent CVD adverse effects in CKD patients.

Funding: Private Foundation Support

FR-PO205

Uromodulin (UMOD) Gene Polymorphism Shows Different Associations with Chronic Kidney Disease in Euro-American versus African-American Individuals with Diabetes Mellitus

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Background: Single nucleotide polymorphisms (SNPs) in the *UMOD* gene encoding uromodulin (Tamm Horsfall protein) are associated with eGFR and BP in non-diabetic populations of European ancestry. Relationships between *UMOD* and HTN, CKD and subclinical cardiovascular disease are not as well characterized in T2D.

Methods: Associations between *UMOD* SNP rs4293393 and CKD, HTN and coronary artery calcified plaque (CAC) were assessed in 1117 European Americans (EAs) enriched for T2D from the Diabetes Heart Study (DHS), of whom 936 (83.6%) had T2D, and in 479 African Americans (AAs) with T2D from the AA-DHS. eGFR was calculated with the modified MDRD formula. Association analyses with eGFR and urine albumin creatinine ratio (UACR) were adjusted for age, sex, ACE inhibitor/angiotensin receptor blocker use, T2D and hypertension. Analyses with CAC were adjusted for age, sex, BMI, statin use, T2D, and smoking. Analyses with hypertension were adjusted for age, sex, and T2D.

Results: As shown in the Table below, rs4293393 was associated with higher eGFR (but not UACR) in EAs from the DHS; with a trend toward positive association with CAC. Conversely, rs4293393 was significantly associated with increased UACR in AAs with T2D, but not with eGFR or CAC.

Study Sample	Ancestry	N	Trait	P-value	Estimate (β)
Full DHS (T2D+non-T2D)	EA	1117	Hypertension	0.949	-0.008
Full DHS (T2D+non-T2D)	EA	1058	CAC	0.075	0.222
Full DHS (T2D+non-T2D)	EA	1117	eGFR	0.047	0.028
Full DHS (T2D+non-T2D)	EA	1092	UACR	0.725	0.028
T2D-affected DHS	EA	936	eGFR	0.056	0.030
T2D-affected AA-DHS	AA	479	UACR	0.043	0.27
T2D-affected AA-DHS	AA	479	eGFR	0.650	-0.05
T2D-affected AA-DHS	AA	479	CAC	0.290	0.37

Conclusions: We conclude that *UMOD* SNP rs4293393 associates with higher eGFR in EA populations enriched for T2D, but not in AAs with T2D. Rs4293393 was significantly associated with proteinuria in AAs. Differences in effects based on population ancestry require additional investigation.

FR-PO206

Overexpression of Uromodulin Induces Age-Dependent Renal Damage

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Background: Variants in the *UMOD* gene encoding uromodulin have been consistently associated with an increased risk for chronic kidney disease (CKD) in the general population. The *UMOD* risk variants, which are located in the promoter region in a LD, directly increase *UMOD* expression *in vitro* and *in vivo*. To gain insight into the role of uromodulin as a risk factor for CKD, we investigated renal function parameters and kidney pathology and structure in transgenic mice overexpressing *Umod*.

Methods: Renal function of transgenic and control mice (n= 6-8/group, age-, sex-matched) was monitored over time at 12, 16 and 20 months of age by sinistrin and creatinine clearance. We obtained urine by using individual metabolic cages and blood samples at time of sacrifice. Kidney samples were collected for mRNA and protein extraction, and for optical and electronic microscopy (EM).

Results: Transgenic uromodulin is specifically expressed in TAL segments. Increased uromodulin expression in transgenic mice (+80%) is comparable to the one observed in subjects homozygous for *UMOD* risk variants, relative to homozygous carriers of protective alleles. Baseline diuresis and urine parameters (creatinine, electrolytes, osmolarity) were within normal range and similar between strains up to 20 months of age. Renal function was also similar between groups. Increased microalbuminuria was observed in transgenic mice, starting at 16 months and present in about 50% transgenic mice by the age of 20 months. Renal damage in the kidneys of transgenic mice was evidenced by increased expression of lipocalin-2 (NGAL) and KIM-1, paralleled by segmental dilations and tubular casts, mainly affecting distal segments. There were no significant changes in the glomerular ultrastructure (EM) or in podocin and nephrin expression.

Conclusions: These results substantiate the causal link between *UMOD* variants, increased uromodulin expression and age-dependent renal damage. The preserved GFR/renal function in aged transgenic mice suggests that the effect of uromodulin as a risk factor for CKD may be dependent on additional conditions harming the kidney.

FR-PO207

Common *UMOD* Variants Influence Transcription, Urinary Excretion and Biochemical Properties of Uromodulin

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Background: Uromodulin is exclusively expressed in the kidney and is the most abundant protein in urine. Common variants in the promoter of the *UMOD gene* encoding uromodulin are associated with renal function and blood pressure. We recently demonstrated that such variants affect gene transcription but their impact on uromodulin excretion and biochemical properties remains unknown.

Methods: We analyzed urinary uromodulin (ELISA) in 14,404 Europeans from three genetic isolates and five urban cohorts. We measured plasma values in cases with low (2.0 ± 0.18 mg/g creat) and high urinary uromodulin (69.19 ± 2.84 mg/g creat) (N=53 pairs). Uromodulin qualitative analysis was carried out by immunoblotting and 2D-gel electrophoresis on urine samples from age- and sex-matched homozygous carriers of protective or risk *UMOD* variants (N=10 pairs).

Results: The lead *UMOD* SNPs rs12917707 and rs4293393, associated with increased risk for CKD and hypertension, are located in the gene promoter in a linkage disequilibrium block that extends to exon 6 in the coding sequence. In all cohorts, the common G allele of rs12917707 was associated with higher uromodulin levels in an additive fashion (geometric means 10.24, 14.05 and 17.67 mg/g creat for zero, one, or two copies respectively, $p<1x10^{-8}$). By contrast, plasma levels of uromodulin were not influenced by genotype. Similar plasma levels were also observed in subjects with extreme urinary values. The *UMOD* genotype was associated with a systematic difference in uromodulin immunoreactivity, independently of protein denaturation and glycosylation patterns. Individual differences were also observed within each genotype and were confirmed by 2D-gel electrophoresis.

Conclusions: These data indicate that common variants in the *UMOD* promoter region exert a strong influence on urinary uromodulin levels and are associated with significant modifications in uromodulin immunoreactivity. They provide insights into uromodulin biology and its association with renal function.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO208

Combination of Linkage Analysis and Whole Exome Sequencing for the Identification of Risk Variants in IgA Nephropathy

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Background: The pathogenesis IgA nephropathy (IgAN) is still not clear but familial clustering demonstrates a strong genetic involvement. Aim of our study was to find rare, high penetrant risk variants, combining family-based linkage analysis with full exome sequencing.

Methods: Genotyping and linkage analyses have been performed on 25 families of South Italian ancestry. Eight IgAN families that gave the strongest linkage signals were selected for exome sequencing. Variant calling and annotation were performed with standard procedures. High priority variants were chosen based on call quality, MAF, impact, co-segregation in affected family members and their presence within linked areas. Follow-up on these variants was performed using Sanger sequencing and TaqMan Assays on familial and sporadic IgAN patients and in-house population controls.

Results: We confirmed and refined our previously published identified regions on chromosome 4q26, 6q22-23 and17q21 linked with IgAN. Moreover, we detected additional regions on chromosome 1p and 3p, 8p. Our exome study identified a total of 150913 variants in at least one of the 24 samples. Sanger sequencing validated variants within different genes. Taqman assays showed that a missense variant segregated in affected individuals in five independent families and it was also detected in sporadic IgAN cases. Another coding variant showed a higher frequency than expected in both cases and controls, but a statistically significant difference was found.

Conclusions: Gene variants common to all affected IgAN patients have not been detected in our study confirming the already documented genetic heterogeneity of the disease. Sanger sequencing and taqman validation is still being performed on other selected variants and the most promising variants will be selected for functional studies.

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FR-PO209

Exome Array Analysis Identifies Rare Coding Variants Associated with IgA Nephropathy in Han Chinese

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Background: IgA nephropathy (IgAN) is one of the most common glomerulonephritis throughout the world and the major cause of ESRD in China. Our previous genome wide association study (GWAS) has identified several susceptibility loci, which are mainly

common variants in the non-coding regions. In order to identify rare and low-frequency non-synonymous variants associated with IgAN, we performed a comprehensive analysis of rare and common coding variants using an exome chip.

Methods: We performed the discovery analysis in 2378 cases and 15642 controls of Chinese Han population by using the Illumina HumanExome Beadchip. Genome-wide efficient mixed-model association (GEMMA) was used for SNP-based association study, and burden test and SKAT-O were used for the gene-based analysis. The replication analysis of top SNPs from the discovery analysis was done in an additional 4270 cases and 6017 controls by using the Sequenom iPLEX system and TaqMan assays.

Results: After sample and SNP quality control, 60676 SNPs, including 43977 non-polymorphic coding ones, were subjected association test. The validation analysis of top SNPs discovered two novel low-frequent risk variants for IgAN.

Conclusions: This study demonstrates that the genome-wide association analysis using exome array is a valuable approach in identifying low-frequency and rare coding variants that contribute to complex human traits. By identifying novel susceptibility variants, our study provides new insights into the biological variability in the development of IgAN.

FR-PO210

Genetic Association Study of Alpha-Defensin Gene DEFA1A3 Copy Number Polymorphism in Chinese Han Patients with IgA Nephropathy

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Background: Our recent GWAS study has confirmed a novel susceptibility locus, 8p23.1 (rs2738048) in Chinese Han patients with IgA nephropathy (IgAN). The association is within an LD block where several members of the DEFA family reside, including the DEFA1A3 gene which has extensive copy number variations. In this study, we aim to investigate the role of DEFA1A3 gene copy number polymorphism in Chinese Han IgAN patients.

Methods: 2400 Han Chinese subjects (1200 cases/1200 controls) were enrolled in the study. We combined two paralogue ratio tests (PRT) and four allelic ratio measurements to provide accurate measurements of DEFA1A3 gene number. Besides, we performed SNP genotyping of rs2738048 in all selected subjects. Then we did the association analysis using the trend test.

Results: The total copy numbers of the DEFA1A3 gene ranged from 2 to 16 copies and 6 copies was the most common in Chinese Han. DEFA1A3 copy number variants were significantly different between IgAN cases and controls. According to the association analysis, the total copy numbers of DEFA1A3, DEFA1, and DEFA3 were all strongly associated with IgAN susceptibility. SNP rs2738048 also showed significant association. But the significance of rs2738048 disappeared when conditioned on total DEFA1A3 or DEFA1,while it remained when conditioned on DEFA3. Lower copy numbers of total DEFA1A3, DEFA1 and DEFA3 were all more frequent in IgAN patients.

Conclusions: The case-control association study performed finds some evidence for an influence of variation in genes coding for alpha defensins in susceptibility to IgAN in Chinese Han. Previous significance found for SNP rs2738048 probably lies in the gene DEFA1A3. Lower copy numbers of DEFA1A3 are significantly associated with high risk of IgAN.

FR-PO211

Differential Expression of MicroRNA in Peripheral B Lymphocytesin Patients with IgA Nephropathy

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Background: IgA nephropathy (IgAN) is one of the most common causes of end stage renal disease (ESRD). The pathogenesis of IgAN is still unclear. Epigenetic alterations may play an important role in the pathogenesis of IgAN, but previous studies didn't focus on B lymphocytes that produce IgA1 molecules. In this study, our aim is to detecte the microRNAs expression profiles fromperipheral blood B lymphocytes, to find out significantly differential expressed microRNAs between IgAN patients andhealthy controls, and to explore the possible mechanism of microRNA in the pathogenesis of IgAN.

Methods: Five microliters peripheral blood was collected from 7 IgAN patients and 4 healthy controls. We selected B lymphocytes with magnetic Beads, and then isolated RNA from those lymphocytes. After hybridized on the miRCURY™ LNA Array (v.18.0), we compared the differential expression of microRNAs.

Results: Compared with healthy controls, there were 115 discriminating microRNAs with more than 2-fold change,85 of which were upregulated and 30 were downregulated in IgAN patients. Five significantly different microRNAs were picked out, four upregulated microRNAs, miR-3189-5p, let-7g-5p, miR-4258, miR-4695-3p and one downregulated microRNAs miR-99b-5p.

Conclusions: The expression profile of microRNAs from peripheral blood B lymphocytes in IgAN patients is different from healthy controls.

FR-PO212

Collagen Type IV Alpha Defect May Be Susceptibility Factor to IgAN

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Background: Increasing evidence have demonstrated that extracellular matrix components may play important roles in pathogenesis of glomerulonephritis. Familial IgA Nephropathy (IgAN), thin basement membrane nephropathy (TBMN) and Alport syndrome (AS) are primary glomerular disorders which all present with micro- or macroscopic hematuria, proteinuria and variable progression to ESRD. A previous genome-wide scan reported linkage of familial IgAN to chromosomes 2q36, which encompasses the collagen type IV gene COL4A3/A4 locus. Suggesting that collagen type IV alpha defects may be a susceptibility factor for IgAN.

Methods: We report five multigenerational kindreds referred for evaluation of familial IgAN. All kindreds were ascertained via an index case with renal failure, and a kidney biopsy demonstrating mesangial proliferation and dominant IgA deposition; each kindred featured multiple individuals with dominant transmission of hematuria/proteinuria with/without chronic renal failure, and absence of hearing or ocular defects. We performed mutational screening of all exons of the COL4A3, COL4A4 and COL4A5 genes in all affected members in these 5 [A1] pedigrees because we detected linkage peaks on 2q36 or rare coding mutations in one of the COL4A genes by exome sequencing the index case.

Results: Linkage analysis demonstrated linkage to the COL4A3/A4 or the COL4A5 loci in five families (LOD = 1.186, 2.1 and 2.2 at 2q35-37, and LOD = 3.6, 0.534 at Xq22-25). Mutational screening revealed a single segregating pathogenic mutation in five families (COL4A3 G291E, COL4A4 G852D, G1624V, and COL4A5 G174C, G1357V). These novel mutations occurred on conserved glycine residues, and segregated in all affected individuals without classic TBMN features. Additionally, these novel mutations were not present in at least 200 ethnically-matched controls.

Conclusions: The present case series suggest collagen type IV alpha may not be simply a coincidental finding and may represent a susceptiblilty factor for IgAN, motivating systematic investigation of rare coding mutations in COL4A genes in IgAN patients.

FR-PO213

Mannose-Binding Lectin Gene Polymorphism Is Closely Associated with Clinical Manifestation and Renal Prognosis in Lupus Nephritis

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Background: Lupus nephritis (LN) is one of the most serious SLE complications since it is the major predictor of poor prognosis.Complement system may play an important role in the pathogenesis of LN, such as mannose-binding lectin (MBL).

Methods: 140 LN patientswho received renal biopsy at our hospital from January 1, 2003 to December 31, 2012 were enrolled, and 150 healthy subjects were enrolled. Primary and secondary end-point was defined as entering ESRD or death, and Scr level increased >50% of baseline, respectively. ELISA was used to detect serum MBL level.Integrated capillary electrophoresis was used to detect gene polymorphism of MBL.

Results: The mean age of LN patients was 39.79±13.46 years old (129 females). During the following-up of 44.30±30.91 months, 40 patients entered end-point, and 7 patients entered ESRD. Compared with control group, LN patients with -550 GC gene type, -221 GG gene type, -435 GG gene type, +4 CC gene type and 54 codon GG gene type had lower serum MBL. LN patients had lower GG genotype frequencies at 54 codon compared with control group[124 (82.12%) versus 104 (74.29%),p=0.019]. Patients with SNP in -550 had higher systolic blood pressure, higher ds-DNA antibody level, higher serum creatinine, more proterinuria, higher SLEDAI score, and higher AI, but lower C3 and eGFR. Patients with SNP in +4 had higher ds-DNA antibody level, higher serum creatinine, more proterinuria, higher AI and CI, but also had lower eGFR. LN patients who progressed to end point events had less frequencies of GG, CC wild type at promoter -550 and +4 respectively compared with those LN patients without end point events. Kaplan-Meier analysis showed that patients with SNPs of -550, +4 were more likely to develope into ESRD. Cox regression analysis further showed that male (HR=5.654), SNP of +4 site (HR=5.142) and baseline serum creatinine (HR=1.012) were all independent risk factors of poor renal prognosis in LN patients.

Conclusions: Single nucleotide polymorphisms of MBL gene were related to clinical manifestation and renal prognosis of LN, and SNP of +4 site may predict progression of lupus nephritis.

Funding: Government Support - Non-U.S.

FR-PO214

Genetic Variants in ANCA-Associated Vasculitis: A Meta-Analysis

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Background: ANCA-associated vasculitis (AAV) is a systemic autoimmune disease of unknown etiology. Both genetic and environmental factors are believed to be involved in the pathogenesis of AAV. Evidence of a genetic component has been growing. This meta-analysis assessed the pooled effect of each genetic variant that is reproducibly associated with AAV.

Methods: Studies that assessed the association between genes and AAV were searched in PubMed, Embase, and Web of Science. All genetic variants that were significantly associated with AAV in an initial study and were subsequently independently reproduced

in at least one additional study were selected. All studies that assessed these reproduced genetic variants were subsequently included in the meta-analysis. Associations were calculated at the allele level, and the main measure of effect was a pooled odds ratio in a random-effects model.

Results: The literature search yielded 5020 articles. Genetic associations in AAV were investigated in 85 of these articles. We identified 16 replicated genetic variants, of which 13 remained significantly associated with AAV following meta-analysis. These variants were in or near the following genes: ARHGAP18, CD226, FCAR (CD89), COL11A2, CTLA-4, HLA, HSD17B8, LEPR, PTPN22, RING1, RXRB, and SERPINA1.

Conclusions: This meta-analysis identified 13 genetic variants associated with AAV. The contribution of the identified genetic variants in the pathogenesis of AAV should be the focus of future studies.

FR-PO215

Sequence Variants of PLA2R1 and HLA Loci in Caucasians and Asians with Idiopathic Membranous Nephropathy Gyungah Jun,[1] John Farrell,[1] Wei-Song Qin,[2] Zhihong Liu,[2] David J. Salant,[1] Laurence H. Beck.[1] *[1]Medicine, Boston Univ, Boston, MA; [2]Jinling Hospital, Nanjing, China.*

Background: Primary membranous nephropathy (MN) is associated with variants in *PLA2R1* and the MHC II region but it is unclear which sequence variants explain the association.

Methods: We conducted targeted resequencing of PLA2R1 and MHC II regions in 76 subjects from Caucasian (EA: 49 cases and 6 controls) and Asian (ASN: 12 cases and 2 controls) populations. Ninety-five percent of MN cases were anti-PLA_2R positive. All exonic variants and nominally significant ($p<0.05$) intronic variants were followed up by genotyping in independent (n=274) and the sequenced (n=73) samples. Two HLA genes (DRB1 and DQA1) from 109 subjects (total: EA=42 and ASN=66) including 8 cases that had been sequenced (total: EA=4, ASN=4) were typed in an independent HLA laboratory. With ethnic specific controls from the 1000 Genomes Project, a total of 581 EA (195 cases and 386 controls) and 442 ASN (83 cases and 359 controls) subjects were available for analysis. Association was conducted separately in each population and then meta-analyzed.

Results: No novel exonic variants in PLA2R1 were observed in MN cases. Minor alleles of six known variants in PLA2R1 were nominally significant with increased risk for MN in both EA and ASN (best variant, rs4664308, meta-analysis p value [meta-p]=$1x10^{-12}$, odds ratio [OR]=2.7, 95% confidence interval [CI]=1.7-2.9). Association of rs35771982 and rs3749117 in PLA2R1 was also confirmed (meta-analysis p~10^{-9}). Highly linked HLA subtypes, DRB1.03.01 and DQA1.05.01, were associated with similar effect sizes for MN (meta-p=$2x10^{-16}$, OR=5.6, CI=3.7-8.4). A novel independent HLA subtype, DRB1.11.04, was nominally significant in both populations (meta-p=0.006, OR=3.12, CI=1.39-6.98). Four additional DRB1 subtypes (07.01, 08.01, 15.01, and 16.02) were nominally significant only in the EA population.

Conclusions: Comprehensive resequencing of *PLA2R1* and MHC II suggests that MN risk is attributable to multiple HLA-D subtypes along with previously known *PLA2R1* risk variants. Further examination of additive effects of HLA subtypes and interactive effects between PLA2R1 variants and HLA subtypes is warranted.

Funding: NIDDK Support, Private Foundation Support

FR-PO216

Role of Mutations of Inverted Formin 2 in Pathogenesis of Familial Focal Segmental Glomerulosclerosis Xu Hao, Jingyuan Xie, Jun Ma, Weiming Wang, Hong Ren, Zhaohui Wang, Nan Chen. *Dept of Nephrology, Ruijin Hospital, Shanghai Jiaotong Univ, School of Medicine, Shanghai, China.*

Background: In our previous study, we identified two mutations of inverted formin 2 among seventy independent FSGS families by linkage analysis and Sanger sequencing methods. The aim of this study is to further investigate the effects of these two mutations on podocyte function.

Methods: Site mutagenesis was carried out to create S85W and S129_Q130insVRQLS mutant. Podocytes were transfected with plasmids. Quantitative PCR, western blot, immunofluorescence were performed to detect expression and localization of INF2, a-actinin4, Cdc42 and serum response factor (SRF). Immunoprecipitations were conducted to study the interaction of INF2 and Cdc-42. Adhesion assay, wound scratch assay and hoechst staining were performed to detect the effect of two mutations on adhesion ability and cell apoptosis.

Results: We found that protein and mRNA level of S85W decreased compared with wide type (WT) type podocyte. In addition, in WT podocyte, INF2 distribution was diffuse throughout the cell in WT podocytes; In S85W podocytes, INF2 had a perinuclear localization or aggregation in cytoplasm, whereas in S129_Q130ins VRQLS podocytes, the localization of INF2 was similar compared with WT pococytes. In addition, the expression of a-actinin4 was decreased compared with WT podocyte protein in levels of protein and mRNA. The phosphorylation of SRF decreased compared with WT podocyte and the localization of SRF in the podocyte changed in p.S85Wpodocyte, which indicated the activation of SRF decreased compared with WT podocyte. The result of Co-IP and inflorescence indicated that the interaction of INF2 and Cdc42 decreased in p.S85W podocyte. Adhesion assay and wound scratch assay revealed the decreased cell adhesion of both p.S85W and p.S129_Q130ins VRQLS. Hoechst staining demonstrated more apoptosis of p.S85W and p.S129_Q130ins VRQLS.

Conclusions: S85W could damage podocytes by affecting the cytoskeleton, and p.S129_Q130ins VRQLS has no effect on the cytoskeleton. Both p.S85W and p.S129_Q130ins VRQLS could decrease the adhesion ability and induce cell apoptosis.

FR-PO217

Reciprocal Regulation Between ANLN and AKT Influence Podocyte Motility in Podocytes Gentzon Hall,[1,2] Andrew F. Malone,[1,2] Paul J. Phelan,[1,2] Alison Homstad,[2] Guanghong Wu,[2] Thomas Lindsey,[2] Michelle P. Winn.[1,2] *[1]Nephrology, Duke Univ, Durham, NC; [2]Duke Molecular Physiology Inst, Duke Univ, Durham, NC.*

Background: Mutations of the F-actin binding protein anillin (ANLN) have been shown to cause FSGS. ANLN is a complex, multidomain protein that is known to play a role in cytokinesis and cell motility through interactions with phosphoinositol-3-kinase (PI-3K) signaling intermediates such as AKT. In prior studies, we demonstrated that ANLN modulates AKT activation in podocytes. In subsequent *in silico* analyses of the ANLN peptide sequence, we identified a candidate AKT consensus phosphorylation site within the ANLN F-actin binding domain (Ser659). Therefore, we hypothesize that ANLN and AKT modulate podocyte motility and cytoskeletal dynamics via reciprocal regulation.

Methods: Immunoblot and immunoprecipitation studies, siRNA-mediated gene knockdown studies and scratch wound healing assays were performed by standard methods in conditionally immortalized human podocytes (CIHP). Immunoprecipitation studies and scratch wound healing assays were performed in CIHPs stably overexpressing tGFP empty vector, tGFP-$ANLN_{WT}$, tGFP-$ANLN_{S659A}$, or tGFP-$ANLN_{S659E}$. Immunoblot and scratch wound healing studies were also performed in CIHPs with siRNA-mediated *ANLN* knockdown (KD).

Results: *ANLN* KD significantly reduced AKT and GSK-3b activation and basal motility in podocytes. ANLN phosphorylation and basal podocyte motility are significantly reduced in the presence of a highly selective pharmacologic antagonist of AKT. Finally, basal podocyte motility in phospho-null ANLN-overexpressing podocytes ($ANLN_{S659A}$) is significantly impaired relative to $ANLN_{WT}$ and $ANLN_{S659E}$-overexpressing cells.

Conclusions: ANLN is a critical modulator of podocyte cytoskeletal dynamics likely via the modulation of AKT activity. AKT is a modulator of ANLN activity via the direct phosphorylation of Ser659. Taken together, these novel findings elucidate the cellular effects of the dynamic interplay between ANLN and AKT and highlight a potential role for pharmacologic antagonism of AKT signaling in the treatment of FSGS.

Funding: NIDDK Support

FR-PO218

Genetic Loss of Function Mutations in Zebrafish and Human *CRB2*, a Regulator of Epithelial Polarity, Are Associated with Podocyte Foot Process Effacement and Focal Segmental Glomerulosclerosis Arindam Majumdar,[1] Lwaki Ebarasi,[1] Shazia Ashraf,[2] Agnieszka Bierzynska,[3] Hugh J. McCarthy,[3] Svjetlana Lovric,[2] Moin Saleem,[3] Friedhelm Hildebrandt.[2] *[1]Immunology, Genetics, and Pathology, Uppsala Univ, Uppsala, Sweden; [2]Div of Nephrology, Harvard Medical School, Boston Children's Hospital, Boston, MA; [3]Paediatric Renal Medicine, Univ of Britstol, Bristol Royal Hospital for Children, Bristol, United Kingdom.*

Background: Podocytes are epithelial cells, central to the glomerular filtration barrier, and a pathological target in glomerular diseases. In published work, we identified a requirement for zebrafish *crb2b*, an evolutionarily conserved regulator of epithelial apical basal polarity, in podocyte morphogenesis. The Crb proteins are transmembrane proteins which lie at the intersection of epithelial polarity, cell signaling, and membrane biogenesis.

Methods: We studied *crb2b* function in podocytes using a genetically heritable, loss of function mutation in zebrafish. Using homozygosity mapping in combination with exome sequencing and next generation sequencing, we then translated our findings into humans by determining whether the *CRB2* locus may be mutated in nephrotic syndrome patients.

Results: Homozygous *crb2b*$^{-/-}$ zebrafish podocytes show loss of foot process architecture and slit diaphragms and apical mis-localization of Nephrin protein. We identified recessive mutations in the *CRB2* in four different families with steroid resistant nephrotic syndrome (SRNS) and one family with congential nephrosis. All together, we present five new human mutations, including a putative null allele, which together constitute the first reported allelic series in human CRB2. By complementation rescue experiments of zebrafish *crb2b*$^{-/-}$ *embryos*, we show that three of these *CRB2* mutations resulted in loss of protein function. We show that Crb2 and Nephrin proteins molecularly interact.

Conclusions: Based on genetic analysis of CRB2 in zebrafish and humans we conclude that *CRB2* is required for proper podocyte morphological differentiation. Furthermore, our human genetic studies suggest that *CRB2* loss of function mutations may be causative to some forms of inherited nephrotic syndrome.

FR-PO219

The Genomic Landscape of Congenital Malformations of the Kidney and Urinary Tract Miguel Verbitsky,[1] David Fasel,[1] M. Sampson,[2] John M. Darlow,[3] Prem Puri,[3] Rik Westland,[1,4] Francesco Scolari,[5] Monica Bodria,[6,7] Landino Allegri,[7] Joanna Van Wijk,[4] Krzysztof Kiryluk,[1] Marijan Saraga,[8] Velibor Tasic,[9] Friedhelm Hildebrandt,[10] Cecile Jeanpierre,[11] Craig S. Wong,[12] David E. Barton,[3] Gian Marco Ghiggeri,[6] Ali G. Gharavi,[1] Simone Sanna-Cherchi.[1] *[1]Div of Nephrology, Columbia University, NY; [2]Dept of Pediatrics, University of Michigan, Ann Arbor, MI; [3]National Children's Research Centre, Dublin, Ireland; [4]Pediatric Nephrology, VUMC, Amsterdam, Netherlands; [5]Div of Nephrology, Hospital of Montichiari, Italy; [6]Pediatric Nephrology, G. Gaslini Hospital, Genoa, Italy; [7]Div of Nephrology, University of Parma, Italy; [8]Pediatric Nephrology, University of Split, Croatia; [9]Pediatric Nephrology, University of Skopje, Macedonia, The Former Yugoslav Republic of; [10]Div of Nephrology, Boston Children's Hospital, MA; [11]Lab des Maladies Rénales Héréditaires, Inserm U1163, France; [12]Dept of Pediatrics, University of New Mexico, Albuquerque.*

Background: Copy number variations (CNVs) predispose to multiple developmental phenotypes. We demonstrated that ~17% of cases affected by renal hypodysplasia (RHD) are caused by rare CNVs. We performed a CNV study across the clinical spectrum of congenital malformations of the kidney and urinary tract (CAKUT) from >2,000 affected individuals.

Methods: The study comprised 2,063 CAKUT cases and 23,362 controls, all genotyped with high-density SNP arrays. QC was performed using PLINK, CNV calls were determined using PennCNV.

Results: After QC, CNV analysis in 1,989 patients identified 2,851 large (>100 kb), high-quality, and rare (<1:1,000) CNVs. 337 cases carried a CNV with an overlap with a known genomic disorder, and 180 patients carried a novel large (>500 kb) CNV of potential pathogenic relevance. CNV burden was higher in patients with RHD compared to patients with obstructive uropathy or VUR. Besides the common 17q12 RCAD deletion, we identified multiple recurrent CNVs that can represent genomic disorder specific for urinary tract developmental phenotypes, such as the 1q21 deletion, 1p36 duplication, 16p11.2 deletion, 22q11 deletion.

Conclusions: This study describes the genomic landscape across the entire phenotype spectrum of CAKUT and identifies recurrent CNVs for urinary tract developmental phenotypes.

FR-PO220

Genome-Wide Joint Linkage and Association Analyses Confirm *SLC2A9* and *CNTN4 as C*andidate Genes for Serum Uric Acid Levels in Mexican Americans Geetha Chittoor,[1] Jack W. Kent,[2] Marcio Almeida,[2] Sobha Puppala,[2] Vidya S. Farook,[2] Shelley A. Cole,[2] Karin Haack,[2] Jean W. Maccluer,[2] Joanne E. Curran,[2] Melanie Carless,[2] Matthew Johnson,[2] Ravindranath Duggirala,[2] Michael C. Mahaney,[2] Anthony Comuzzie,[2] John Blangero,[2] V. Saroja Voruganti.[1] *[1]Nutrition, Univ of North Carolina at Chapel Hill, Kannapolis, NC; [2]Genetics, Texas Biomedical Research Inst, San Antonio, TX.*

Background: The variation in serum uric acid (SUA) levels is under significant genetic influence. Studies from our group and others have shown strong association of SUA levels with solute carrier family 2, member 9 (*SLC2A9*), a uric acid transporter gene. Our previous linkage study identified a novel QTL on chromosome 3p26 affecting SUA.

Methods: As a follow up, we examined genome-wide SNP data in an expanded cohort of 1281 Mexican Americans from the San Antonio Family Studies. We used a joint linkage/association (JLA) approach for each SNP, implemented in SOLAR, that tested each saturated model (including linkage and the fixed effect of the SNP) against a null model in which both effects were constrained to zero.

Results: JLA results showed a strong association of *SLC2A9* SNPs with SUA ($p<8x10^{-8}$), with the top three SNPs (rs10016075, rs6818672, rs12644047; MAF between 30 and 39%) showing association with lower SUA levels. Results also confirmed our previous linkage findings for SUA on 3p26 (LOD=4.9, $p=1x10^{-6}$) where we observe a strong association with SNPs in contactin 4 [*CNTN4*] (rs1153535, rs4685494, rs11720157; MAF between 17 and 44%; $p<3x10^{-6}$). Furthermore, association analysis of 2920 SNPS in *SLC2A9* in a subset of participants (n=447) confirmed our previous associations and identified novel SNPs (chr_pos: 4_9952938 and 4_9972304; MAF=9 and 32%, respectively) at $p<2.7x10^{-5}$ after accounting for multiple testing.

Conclusions: Our JLA results are especially interesting given the recent attention on the relative importance of common and rare genetic variants for risk of common complex diseases. These findings provide further confirmation of important roles for *SLC2A9* and *CNTN4* in the regulation of SUA levels in Mexican Americans.

Funding: Other NIH Support - R01DK092238

FR-PO221

CFH-Mutation Related Atypical Hemolytic Uremic Syndrome May Be Modulated by Coagulation Factor X Fengxiao Bu,[1] Nicolo Ghiringhelli Borsa,[1] William T.A. Tollefson,[2] Michael J. Schnieders,[2] Hela Azaiez,[1] Kai Wang,[3] Christie P. Thomas,[1] Carla M. Nester,[1] Richard J. Smith.[1] *[1]Molecular Otolaryngology and Renal Research Labs, Univ of Iowa; [2]Dept of Biochemistry, Univ of Iowa; [3]Dept of Biostatistics, Univ of Iowa.*

Background: Atypical hemolytic uremic syndrome (aHUS) is a complement-related rare renal disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. Mutations in *CFH* account for approximately 22% of aHUS cases. In familial cases, *CFH*-mutation penetrance ranges from 12.5% to 100% suggesting the involvement of other genetic factors/modifiers in the etiology of this disorder.

Methods: Five families carrying the same disease mutation c.3644G>A (p.Arg1215Gln) in *CFH* were included in this study. Using targeted genomic enrichment and massively parallel sequencing, all coding exons of genes in complement and coagulation cascades were screened for coding variants. Data were analyzed using a customized Galaxy pipeline. After filtering for quality and frequency, novel and rare variants were annotated based on computational algorithms and reported studies. For selected variants the predicted functional impact was confirmed *in vitro*.

Results: A known factor X deficiency variant (*F10* c.424G>A, p.Glu142Lys) was identified in a three-generation pedigree. No carriers of both the *F10* p.Glu142Lys and the *CFH* p.Arg1215Gln variant developed aHUS, although two persons carrying only the *CFH* p.Arg1215Gln variant developed disease in early childhood. Protein modeling shows that wild-type Glu142 hydrogen bonds with Cys129, while mutated Lys142 hydrogen bonds with Ser146, a shift that destabilizes an important intra-light-chain interaction between a two-stranded beta-sheet and a small alpha-helical secondary structure element. Consistent with this prediction, recombinant mutant factor X secretion was altered in HEK293 cells and its activity was reduced by 30%.

Conclusions: We have identified a variant in *F10*, p.Glu142Lys, that may modify *CFH*-related aHUS perhaps protecting *CFH*-mutation carriers from developing the phenotype. This finding may explain some instances of incomplete penetrance and offer new therapeutic targets to treat this life-threating disease.

Funding: Private Foundation Support

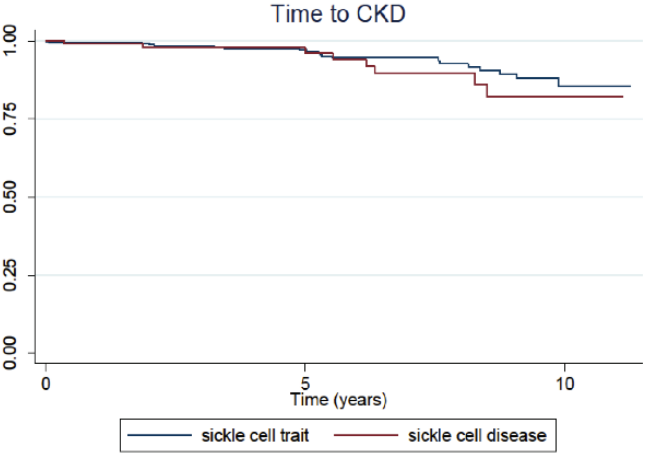
FR-PO222

Sickle Cell Trait a Possible Risk Factor for CKD Maulin Shah,[1] Iris C. De Castro,[2] Danielle Guffey,[1] Charles G. Minard,[1] Biruh Workeneh.[1] *[1]Baylor College of Medicine, Houston, TX; [2]Univ of Washington, Seattle, WA.*

Background: Patients heterozygous for sickle hemoglobin have a phenotypic spectrum including hyposthenuria, hematuria and papillary necrosis, but traditionally sickle cell trait (SCT) has been considered a generally benign state that does not result in progressive CKD and ESRD. However, given recent reports raised in the literature possibly implicated SCT in progressive CKD, we sought to determine whether this was the case in our region.

Methods: We performed database query at a large county hospital in Houston, TX. A total of 972 cases were identified, 790 with SCT and 134 that were confirmed by hemoglobin electrophoresis. We did not include a similarly matched control arm in our initial analysis, and hypothesized that patients with SCD would have significantly higher rates of CKD.

Results: There was no statistically significant difference in the risk for CKD between patients with SCT and SCD (P=0.40). The Kaplan-Meir plot (figure) shows the time to CKD by phenotype group.



After adjusting for age, HTN, diabetes and smoking status in a Cox Proportional Hazards model, the hazards ratio for CKD was 2.30 (95% CI: 0.96, 5.55; P=0.063) for those with sickle cell disease compared to SCT. When we included the interaction between HTN and diabetes (p=0.002 however, the hazards ratio was 2.61 (95% CI:1.11, 6.15; p=0.029).

Conclusions: The risk of CKD among those with trait appears to be more significant than originally thought in our population. We suspect that a prospectively designed study controlling for these other risk factors would more clearly determine whether patients with SCT have just as much risk for CKD as patients with CKD in our population. Further corroborative studies that also control for emerging genetic markers for CKD are required.

Funding: Private Foundation Support

FR-PO223

Effects of Renal Transplantation on Telomere Attrition Karin Luttropp,[1] Louise Nordfors,[2] Dagmara Mcguinness,[3] Abdul Rashid Tony Qureshi,[2] Peter F. Barany,[2] Paul G. Shiels,[3] Peter Stenvinkel.[2] *[1]Dept of Molecular Medicine and Surgery, Karolinska Instt, Stockholm, Sweden; [2]Dept of Clinical Science, Intervention and Technology, Karolinska Univ Hospital, Stockholm, Sweden; [3]Wolfson Wohl Translational Research Centre, Inst of Cancer Sciences, Univ of Glasgow, Glasgow, United Kingdom.*

Background: Telomere attrition is a feature of the process of aging and renal disease accelerates the attrition rate. It is unknown what effect a successful renal transplantation (RTx) has on telomere attrition. In this study, we investigated dynamic changes in telomere length following RTx its relation to age, CMV serology, homocysteine levels and biomarkers of oxidative stress and inflammation.

Methods: Telomere length was measured in whole blood DNA collected from 47 ESRD patients immediately prior to RTx and again 1 year after RTx. Telomere length determination was performed by qPCR using a Roche Light Cycler LC480.The relative T/S ratio (repeat copy number to single copy gene number) for each experimental sample was determined in relation to the control DNA sample. For comparison of data, Spearman's rank correlation (r) or linear regression was used, as appropriate.

Results: Baseline telomere length ($[T/S]_{base}$) was significantly correlated to telomere length at 1 year ($[T/S]_{1y}$) ($p < 0.0001$; adjusted $R^2 = 0.41$). Telomere length decreased one year after RTx (mean $[T/S]_{base} = 0.986$ versus mean $[T/S]_{1y} = 0.83$; $p<0.001$). $[T/S]_{base}$ was significantly associated to age ($p = 0.04$), IL-6 ($p = 0.01$), TNF ($p = 0.04$), PTX3 ($p = 0.01$) and homocysteine levels ($p = 0.0139$). An inverse association between changes in telomere attrition following RTx and baseline homocysteine levels ($p = 0.007$) was observed. No associations were found between $[T/S]_{base}$ and BMI, gender, fetuin-A, 8-OHdG, hsCRP, cardiovascular disease, folic acid, CMV serology or dialysis vintage.

Conclusions: Age and levels of IL-6, TNF and PTX3 were associated with shorter telomere length before RTx. One year after RTx, a significant decrease in telomere length was observed, which was inversely associated to levels of homocysteine before RTx.

Funding: Government Support - Non-U.S.

FR-PO224

Clinical Diagnostic Testing amongst Australians with Genetic Renal Diseases Using a Targeted Exomic Approach Andrew John Mallett,[1,2] Gladys Ho,[6] Hugh J. McCarthy,[3,6] Melissa H. Little,[4] Bruce Bennetts,[6] Stephen I. Alexander.[3,5,6] *[1]Dept of Renal Medicine, Royal Brisbane and Womens Hospital, Brisbane, Queensland, Australia; [2]CKD.QLD and School of Medicine, The Univ of Queensland, The Univ of Queensland, Brisbane, Queensland, Australia; [3]Dept of Paediatric Nephrology, The Children's Hospital at Westmead, Sydney, New South Wales, Australia; [4]Inst for Molecular Bioscience, The Univ of Queensland, Brisbane, Queensland, Australia; [5]Discipline of Paediatrics and Child Health, Univ of Sydney, Sydney, New South Wales, Australia; [6]Dept of Molecular Genetics, The Children's Hospital at Westmead, Sydney, New South Wales, Australia.*

Background: Massive parallel sequencing shows promise in enabling diagnostic interrogation of the protein-encoding exome that is enriched for mutations causing Mendelian disease. Genetic causes of kidney disease continue to rapidly expand representing a ripe target for such translational application.

Methods: Consecutive patients in an Australian adult and paediatric cohort with clinically identified likely genetic causes for kidney disease had DNA referred for disease-targeted exomic sequencing (AUSCam V3 Renal Panel, Illumina TruSight One). 458 genes were potentially analysed per patient according to phenotype with an average read depth of 200 (98% coverage).

Results: 22 patients with renal disease of likely genetic aetiology had DNA referred. Pathogenic mutations were detected in 8 patients with a further 3 detected requiring further family investigation. Causative genes included HNF1B, CEP290, ATP6V1B1, CFH, PKD1, PKD2, SALL1, ACTN4, DLX6, COL4A5 and LMX1B. Several variants of uncertain significance were detected amongst additional patients, including in COL4A4 and CR1.

Conclusions: An early national cohort of variable genetic renal disease has identified a number of clear mutations in diseases with significant genetic heterogeneity. Continued evolution and refinement of the local disease-targeted approach (AUSCam) continues and may result in a valuable tool for genetic diagnosis with implications for future treatment and management options.

Funding: Government Support - Non-U.S.

FR-PO225

Comprehensive Diagnosis of Hereditary Kidney Diseases By a Customized Diagnostic Panel of Targeted Exome Sequencing Takayasu Mori,[1] Kazuyoshi Hosomichi,[2] Eisei Sohara,[1] Tatemitsu Rai,[1] Sei Sasaki,[1] Ituro Inoue,[2] Shinichi Uchida.[1] *[1]Dept of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental Univ, Bunkyo, Tokyo, Japan; [2]Div of Human Genetics, National Inst of Genetics, Mishima, Shizuoka, Japan.*

Background: Gene identification of hereditary diseases by DNA sequencing is very important for precise diagnosis, following treatment, and genetic consulting. However, recent increases of causative genes of multiple diseases or even in a single hereditary disease make it difficult to perform DNA sequencing using conventional Sanger sequencing. Targeted multiple genome resequencing using next-generation sequencing (NGS) enables rapid and precise DNA diagnosis at a large scale, which may be helpful for the efficient diagnosis of hereditary kidney diseases.

Methods: With reference to Human Gene Mutation Database (HGMD^R), articles, opinion of experts, we created a customized capture RNA probe library (SureSelect Custom, Agilent Technologies) for about 70 hereditary renal diseases, including previously reported over 100 of disease-causing genes and their related genes. Using this newly developed DNA diagnosis panel, we performed genetic diagnosis for a total of 16 subjects including two healthy controls and 14 subjects who were clinically suspected hereditary renal diseases, such as Gitelman syndrome, nephrogenic diabetes insipidus, pseudohypoaldosteronism type II, Alport syndrome, etc. Captured genomic DNA libraries of target regions were analyzed with MiSeq (Illumina).

Results: The average coverage depth was 349.5 x with 96.3 % mapped rate and 99.7 % of the target bases covered by at least 10 reads. On-target rate was 65.1 %. Causative mutations were found in four subjects in the expected genes. These mutations were confirmed by Sanger sequence (accuracy of diagnosis by using this panel was 100%, 4 of 4). In other 10 subjects, several exonic non-synonymous mutations not reported as causative so far were also identified.

Conclusions: We succeeded in developing a customized DNA diagnostic panel for more than 70 hereditary kidney diseases using NGS, which provided us fast and accurate DNA diagnosis and may contribute to the improved managements of diseases.

Funding: Government Support - Non-U.S.

FR-PO226

Survey of the Performance of Protein Prediction Methods with Locus Specific Insights for Steroid Resistant Nephrotic Syndrome Denis Andrew Baird,[1] Agnieszka Bierzynska,[2] Ian N. Day,[1] Moin Saleem.[2] *[1]School of Social and Community Medicine, Univ of Bristol, Bristol, United Kingdom; [2]Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom.*

Background: Protein prediction tools are often used as a guide for determining pathogenic mutations from a background of many neutral variants. Mutational prediction is challenging and a wide range of tools using a variety of computational approaches exist. Although SRNS is genetically heterogeneous, single mutations in NPHS1 and NPHS2 account for the majority of Steroid Resistant Nephrotic Syndrome (SRNS) cases in childhood. This study compares the performance of 10 recent, commonly used web-based prediction tools on mutations within NPHS1 and NPHS2, as well as performing a leave one out validation to determine potentially important biological features contributing towards tool performance.

Methods: Two benchmark datasets were formed from pathogenic mutations in Human Gene Mutation Database (HGMD) and ClinVar and neutral mutations in dbsnp138, and submitted to 10 different protein prediction tools. Performance statistics (accuracy, specificity, sensitivity and Matthews Correlation Coefficient) and ROC curves were calculated. A leave one out analysis was carried out by removing mutations with specific biological features and comparing the change in performance statistics. The features investigated were mutations within a certain domain and allele frequency band (rare, intermediate, common), and mutations with certain physicochemical properties.

Results: All prediction tools performed well on NPHS1 (N=129), and most performed well on NPHS2 (N=82). Mutation Assessor 2 and PolyPhen2 performed best for NPHS1, and SIFT and PolyPhen2 performed best on NPHS2. The next step will involve annotating mutations within NPHS1 and NPHS2 to carry out the leave one out analysis.

Conclusions: Performance comparisons are usually performed on genome-wide benchmarks, with little attention given to performance of tools at a gene level. This study suggests that researchers interested specifically in NPHS1 and NPHS2 may wish to select Mutation Assessor 2, PolyPhen2 or SIFT.

FR-PO227

Understanding Cell Identity in the Kidney Using Novel Cell Type Specific Epigenome Mapping Pazit Beckerman, Yi-An Ko, Katalin Susztak. *Renal, Electrolyte and Hypertension Dept, Univ of Pennsylvania, Philadelphia, PA.*

Background: A single genome gives rise to over 200 cell types in the human body. The epigenome determines the unique gene expression program in each cell type through its effect on chromosomal architecture and transcription factor accessibility to DNA. Histone modifications serve both activating and silencing roles in transcription e.g H3K27ac is found at active promoters/enhancers, H3K36me3 at actively transcribed gene bodies and H3K27me3 marks repressed regions. Determining genome-wide distribution of histone marks in different cells can decipher their role in cell type specific transcriptional regulation and identify cell specific regulatory regions. Such maps are lacking in the kidney research. The aim of this study is to construct the first epigenome maps of the human kidney and of different kidney cell types.

Methods: Human kidney cortical tissue samples were processed to single cell using manual homogenizing. Human renal proximal tubule (HKC8) and podocyte cell lines were cultured. Chromatin was cross-linked, lysed and sonicated to generate fragments ~200bp. ChIP (chromatin immunoprecipitation) was preformed using antibodies for H3K4me3, H3K36me3, H3K4me1, H3K27me3 and H3K27ac. Libraries of ChIP-enriched DNA and control were prepared and sequenced. ChIP-Seq was validated using ChIP-PCR.

Results: Sequences obtained from human kidney tissue, proximal tubule and podocyte ChIP-seq were aligned to the human genome and histone modification maps were constructed. Using computational approaches, we integrated the chromatin datasets to build the first annotated epigenome maps of human kidney, proximal tubular cells and podocytes, defining cell specific enhancers, promoters and other regulatory regions.

Conclusions: We have constructed novel kidney-specific epigenomic regulatory maps. Their crucial significance lies in our ability to define kidney cell-specific critical genes and regulatory networks. Comparing epigenomic maps of different kidney cells will help us better understand cell identity in the kidney. We will be able to identify key transcription factors that take critical part in kidney cells regulation and in the pathogenesis of kidney disease.

Funding: NIDDK Support

FR-PO228

Broadening the Differential of Cystic Kidney Disease: Disease

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Introduction: Patients with bilateral renal cysts are often presumed to have polycystic kidney disease (PKD). In this case report we describe a patient who presented for a second opinion with a strong family history of chronic kidney disease (CKD).

Case Description: Our patient is a 41 year old male with a history of CKD (eGFR 24ml/min/m^2), dyslipidemia, and gout. His maternal grandfather passed away at age 55, with end stage kidney disease (ESKD). His mother passed away at age 65 after being treated with hemodialysis for 5 years. A renal biopsy was inconclusive in her case. His bother also has CKD and gout. A maternal aunt and two cousins have been diagnosed with presumed PKD. Recent workup includes a renal US, which confirmed normal sized kidneys (11.1cm on right, 11.5cm on left) and numerous cortical cysts bilaterally. Previous urinalyses have been negative for blood and albumin to creatinine ratio is 241. His blood pressure is controlled with an angiotensin receptor blocker and calcium channel blocker. He is on allopurinol for recurrent gout. Given the atypical appearance on ultrasound, strong family history of CKD and gout disproportionate to the level of renal impairment, there was a clinical suspicion for uromodulin associated kidney disease. DNA testing was done and a previously unreported mutation leading to a single amino acid substitution of an Asparagine to a Serine (N128S) was found in the uromodulin gene, confirming the diagnosis of uromodulin associated kidney disease.

Discussion: Uromodulin associated kidney disease is sometimes associated with corticomedullary cysts, and gout may be a prominent feature. This case could be differentiated from PKD because the cysts did not have characteristic features and the kidney size was preserved. Since genetic diagnosis of even rare kidney disorders is now readily available, this case serves as a reminder that multiple renal cysts are not necessarily diagnostic of PKD, and that recurrent gout and significant kidney dysfunction should raise suspicion for uromodulin-associated disease.

FR-PO229

An Unusually Severe Case of Polycystic Kidney Disease

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Introduction: Patients with polycystic kidney disease (PKD) can have extrarenal cysts. We present a severe case of PKD with extensive liver and renal involvement.

Case Description: The patient is a 43yo old woman with a known history of PKD, who was referred to renal clinic for evaluation of Stage IV chronic kidney disease. Her history was notable for a central nervous system aneurysm, as well as significant flank and back pain and severe shortness of breath. The patient reported she could only eat small amounts of food at one time, and was subsisting on 10-12 small meals a day. Subsequent abdominal CT scan revealed cystic disease involving a significant proportion of her abdominal space, including the majority of the liver parenchyma with shifting of the liver across the midline, and notable lung, gastric, and splenic compression. Remarkably, labs were all in the normal range, with the exception of creatinine of 2.3 mg/dL. Because of the extent of the cystic disease, surgical resection was not an option, and the patient was listed for a combined kidney/liver transplant. She underwent dual organ transplantation and her native liver and both kidneys were removed and sent to pathology. Her liver was noted to weigh 12,986 grams and measured 45x36x19 cm and contained cysts measuring up to 7 cm; her kidney was noted to be 22 x 14 x 8 cm and weighed 668 grams, containing cysts up to 3 cm in size.

Discussion: Although PKD is relatively common, this case is unusual because of the large extent of the extrarenal cysts, making a combined kidney/liver transplant clinically necessary.

FR-PO230

An Unusual Case of Familial Cystic Kidney Disease

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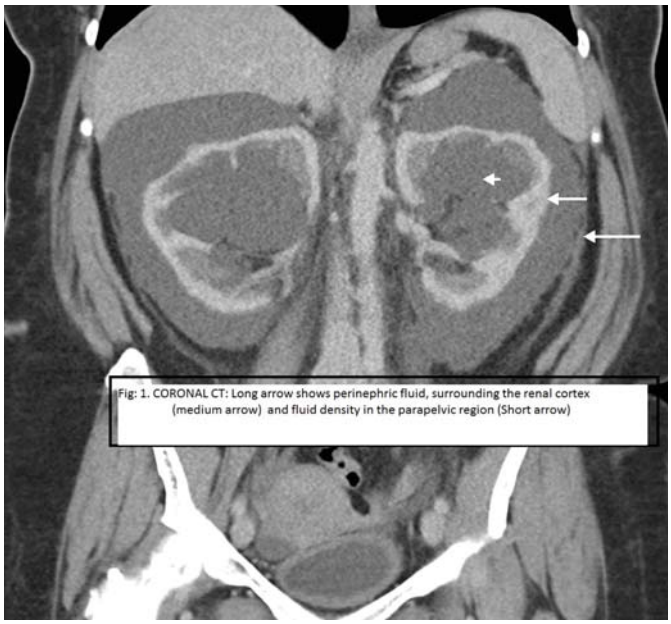
Introduction: We report an unusual case of bilateral renal lymphangiomatosis in a woman who was mistakenly believed to have autosomal dominant polycystic kidney disease (ADPKD). Given the familial and cystic nature of this disease it can be easily confused with ADPKD on ultrasound scanning (USS) unless there is a high degree of clinical suspicion or the typical imaging features are recognised.

Case Description: A 46-year-old woman with a prior diagnosis of ADPKD was referred to us with abdominal fullness. She was diagnosed with ADPKD by USS 10 years previously at a different renal unit and was lost to follow up. Her father at the age of 74 and her younger sister were diagnosed with ADPKD after her initial diagnosis. A repeat USS of the abdomen showed bilateral polycystic kidneys and a small amount of free fluid within the pelvis. A CT of the abdomen and pelvis was done to investigate pelvic free fluid. This showed bilateral perinephric fluid collections and a trace of free fluid. The appearances on CT were felt to be typical of renal lympangiomatosis rather than ADPKD.



Discussion: It is a rare disease characterised by cystic masses in the renal parenchyma, renal sinuses or perinephric spaces. It can be congenital or acquired and is caused by malformation of renal lymphatic drainage to retroperitoneal lymph ducts. It can run in families. The patients may be completely asymptomatic and diagnosed incidentally. Flank pain, abdominal distension, ascites, haematuria, fatigue, weight loss, hypertension and polycythemia have been described. The condition can be difficult to differentiate clinically, radiologically and surgically from ADPKD. Asymptomatic patients do not need treatment. When the pressure effects of enlarged kidneys give rise to significant symptoms treatments such as percutaneous drainage and repeated sclerotherapy have been reported to be safe, minimally invasive and effective techniques in comparison to surgery.

FR-PO231

Gitelman-Like Syndrome in a Patient with Primary Sjögren Syndrome

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Introduction: Sjögren syndrome is associated with different renal manifestations including tubulopathy, usually in the form of distal renal tubular acidosis. Here, we report a case Gitelman-like syndrome associated with primary Sjögren syndrome.

Case Description: Case Presentation: A 67 y o Caucasian female with a longstanding history of Sjögren syndrome on treatment with Cevimeline, presented with 6 weeks history of fatigue and generalized weakness. She denied any history of diarrhea or diuretic use. Physical examination revealed a BP of 117/60 mmHg and remarkable for dry eyes and oral mucosa. Laboratory studies showed hyponatremia, hypokalemic metabolic alkalosis and mild hypomagnesemia with high plasma renin activity and aldosterone level.

		Normal Value
Plasma Biochemistry		
Na^+	121	136 - 145 mmol/L
K^+	2.8	3.3 - 5.1 mmol/L
Cl^-	78	98 - 107 mmol/L
HCO_3^-	29	22 – 28 mmol/L
Arterial PH	7.51	7.35 - 7.45
Arterial PCO_2	39	35 - 45 mm Hg
Urea Nitrogen	19	6 - 20 mg/dL
Creatinine	0.84	0.40 - 0.90 mg/dL
Ca^{2+}	9.4	8.0 - 10.6 mg/dL
Mg^{2+}	1.6	1.5 - 2.8 mg/dL
Renin	3.2	0.2-1.6 ng/mL/hr
Aldosterone	40.9	≤ 16.0 ng/dL
24h Urine biochemistry		
Urine amount (mL)	2950	
Na^+	83	40 - 220 mmol/24h
K+	63	< 40 mmol/24h (in hypokalemic patients)
Cl^-	65	110-250 mmol/24h
Ca^{2+}	94	100 - 300 mg/24 h

Trans-Tubular potassium gradient (TTKG) was high at 13.6% indicating renal potassium wasting. 24h urine studies showed increased potassium excretion in the settings of hypokalemia along with mild hypocalciuria. These features are suggestive of Gitelman

syndrome. Her hyponatremia improved with isotonic volume expansion. She was started on Amiloride and potassium replacement. 1 month follow up revealed improvement in hypokalemia and metabolic alkalosis.

Discussion: While tubulointerstitial disease and distal tubular acidosis have been commonly associated with Sjögren syndrome, Gitelman syndrome has been exceptionally reported. Some reports suggested the presence of circulating autoantibodies to the sodium-chloride cotransporter (NCCT) in the distal convoluted tubules. Interestingly, the renal manifestations can be the presenting symptoms of the Sjögren syndrome and clinicians need to be aware of this possible association.

FR-PO232

Gitelman versus Bartter Syndrome Complicated by Diuretic Abuse
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Introduction: Bartter (BS) and Gitelman syndromes (GS) are autosomal recessive conditions that share common clinical features: hypokalemic metabolic alkalosis, hypomagnesemia, hyperreninemic hyperaldosteronism with normal or low blood pressure. BS usually has hypercalciuria as opposed to hypocalciuria in GS. We present a puzzling case with positive genetic mutation for BS and GS in addition to diuretic use.

Case Description: A 32 years old female had multiple admissions for refractory hypokalemic metabolic alkalosis. Pertinent positive data revealed K+ 1.9mEq/L, Ca++ 9.7mEq/L, Mg++ 1.7mEq/l, chloride 93mEq/L, bircabonate 34mEq/l, creatinine 1.01 mg/dl, phosphate 3.4, renin 130, and aldosterone 26.8. 24 hour collection revealed 6400ml urine with high potassium 158mmols/24hours (reference 25-125 mmol/24hours), hypercalciuria 378 mg/24 hour (reference 42-300 mg/24hour) and creatinine of 1.125g/24hour. Initial diuretic screen was negative on multiple occasions but was found positive 1 year later for hydrochlorathiazide and furosemide. Genetic analysis showed: (1) a duplication of CLCNKB gene with unknown duplication break points (2) a variant of unknown significance in SLC12A3. Diuretic abuse made the diagnosis and management difficult.

Discussion: CLCNKB gene mutation can be related to BS III causing an abnormal Na+-K+-2Cl- transporter in the thick ascending limb but its duplication has not been reported previously. SLC12A3 gene mutation is classically associated with GS that leads to an abnormal thiazide sensitive sodium-chloride symporter in the distal convoluted tubule. Interestingly, she had hypercalciuria intially but subsequent urine collection revealed hypocalciuria. Persistent profound hypokalemia under close inpatient supervision and genotype suggests GS, while a mutation in both CLCNKB and SLC12A3 may have contributed to mix picture. In CLCNKB mutations high, normal or even low urinary calcium levels have been reported.Diuretic abuse likely complicated the laboratory evaluation. This case demonstrates the importance of repeated evaluation of urine electrolytes and diuretic screens with understanding of genotype-phenotype variability.

FR-PO233

A Case of Compound Heterozygous Mutation of Gitelman Syndrome, Diagnosed During Preoperative Examination of Kidney Transplantation as a Donor
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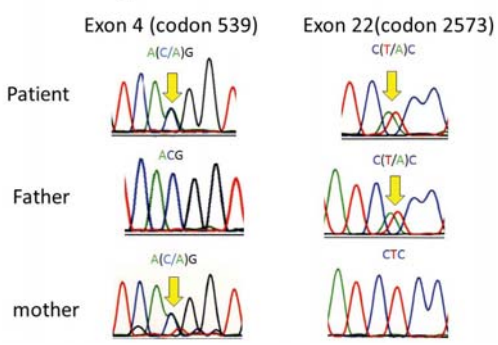
Introduction: Gitelman syndrome is an autosomal recessive disorder, which is caused by mutations in the solute carrier family 12, member 3 (SLC12A3) gene that encodes the thiazide-sensitive Na-Cl co-transporter (NCCT) in distal convoluted tubules (DCT).

Case Description: A 44-year old woman was admitted to our hospital for a preoperative assessment as a kidney transplant donor to her husband who was undergoing dialysis. During her preoperative examination, she presented with hypokalemia, hypomagnesemia, hypocalciuria, metabolic alkalosis and hyperreninemic hyperaldosteronism. The thiazide loading test did not affect chloride reabsorption. Hence, a diagnosis of Gitelman Syndrome was made. In this case, gene-sequencing analysis revealed compound heterozygous mutations of c.539C>A and c.2573T>A in SLC12A3 (ref. Figure). Family analysis of the patient confirmed an autosomal recessive inheritance. Her kidney tissue from the biopsy obtained during the transplant operation was stained with immunostaining anti-SLC12A3 antibody. The expression of SLC12A3 protein was detected in the apical membrane of DCT. This result suggests that the transporter mutation of this case does not cause the functional abnormality of the trafficking procedure to the cell membrane.

Figure: Genetic mutations of this case



Discussion: We found a rare case of Gitelman Syndrome with compound heterozygous mutations in SLC12A3.

FR-PO234

Two Cases of Successful Pregnancy in Patients with Gitelman's Syndrome
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Introduction: Gitelman's syndrome is characterized by hypokalemic metabolic alkalosis. Pregnancy in women with Gitelman's syndrome often results in severe hypomagnesemia and hypokalemia. We report two cases of successful pregnancies in patient's with Gitelman's syndrome, managed with aggressive oral and intravenous (IV) electrolyte repletion.

Case Description: The first case is a 25yo woman who presented at 6 weeks gestation with asymptomatic hypokalemia and hypomagnesemia. Patient had a history of 4 previous unsuccessful pregnancies, and was being managed as an outpatient with both oral and intermittent IV potassium and magnesium supplementation. Nephrology was consulted, and continuous IV electrolyte supplementation was initiated. The patient had labs monitored initially twice a week and then once a week, with continuously increased doses of electrolyte solutions to maintain a serum potassium of 2.5-3.3 mEq/L, and a serum magnesium of 1.7-2.5mg/dL. Her maximum repletion requirements were 120meq of IV potassium and 19g of IV magnesium per day. At 27 weeks gestation she was hospitalized and delivered a healthy infant. The second case is a 34yo woman on oral potassium and magnesium replacement. Her first pregnancy was lost in the first trimester. During her second pregnancy, one year later, she was maintained on an increased oral replacement from her prior pre pregnancy state. She had labs monitored at three-week intervals. Her electrolyte replacement was increased to a maximum daily dose of 200 meq oral potassium chloride and 768 mg oral magnesium chloride. This maintained serum potassium levels greater than 3.2meq/L and serum magnesium levels of 1.0-1.1mg/dl. She delivered a healthy infant at 32 weeks gestation.

Discussion: These cases are notable because of the high doses of electrolyte repletion required and the increased requirements over the course of the pregnancies. These cases also demonstrate that successful pregnancy outcomes are possible in Gitelman's syndrome with frequent laboratory monitoring and aggressive titration of oral and/or IV electrolyte replacement, even after previous miscarriages.

FR-PO235

Clinical and Molecular Genetic Correlations in Families with UMOD Variants
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Introduction: Mutations in *UMOD* underlie medullary cystic kidney disease type 2. The purpose of this investigation was to analyze the clinical and molecular genetic characteristics of families and individuals with *UMOD* mutations and sequence variants.

Case Description: Families with suspected autosomal dominant interstitial kidney disease were investigated including renal USS, biochemical data and genotyping of *UMOD*. Clinical characteristics including gout and progression of chronic kidney disease were retrospectively evaluated.

12 families were identified with *UMOD* mutations and sequence variants of uncertain significance. Individuals with *UMOD* mutations/variants suffered from chronic kidney disease with a variable age of onset of end stage kidney disease ranging from 36 to 68 years. Urinalyses revealed minimal protein and no blood. Ultrasounds of 22 individuals showed atrophic/cortical thinning in 6 cases and renal cysts in 4 cases. 9 families had a history of gout. One individual from a consanguineous family had a homozygous *UMOD* variant. The remaining sequence changes were all heterozygous. A deletion/insertion mutation affecting exon 4 was seen in 4 families. The T62P variant of uncertain significance was seen in 3 families.

Discussion: *UMOD* mutation results in progressive chronic kidney failure, gout occasional renal cysts and a bland urinary sediment. The age of onset of end stage kidney disease is highly variable. Mutations and variants may be throughout the *UMOD* gene, but are most commonly seen in exon 4.

FR-PO236

Paradoxical Response to Furosemide in Uromodulin-Associated Kidney Disease
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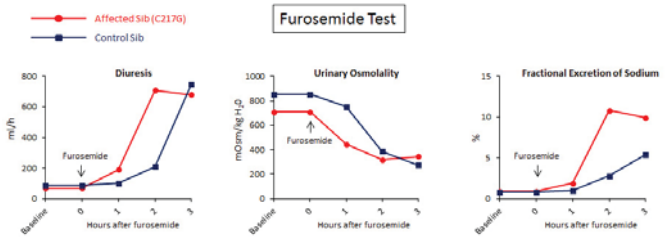
Introduction: Mutations in the *UMOD* gene encoding uromodulin (UMOD) cause autosomal dominant tubulointerstitial nephropathy. UMOD has been shown to regulate the Na^+-K^+-$2Cl^-$ cotransporter NKCC2 in thick ascending limb (TAL) cells. Whether these mutations induce functional alterations in the TAL in the early phase of the disease is unknown.

Case Description: The function of the TAL was tested by comparing the response to a single dose (80 mg) of furosemide and to a water deprivation test with desmopressin

perfusion in a 32-year-old female patient carrying the pathogenic *UMOD* mutation p.C217G and her unaffected 31-year-old sister. The affected sib had a lower GFR compared to her sister's (60 versus 99 ml/min/1.73m^2, respectively).

After furosemide, the affected sib presented intense headache with exaggerated decrease in blood pressure (D syst: 30 versus 20mmHg; D diast: 18 versus 5mmHg) and body weight (D2.6kg versus D0.1kg over 3h). The expected increase in diuresis with concomitant fall in urine osmolality (U_{osm}) was more important and detected earlier in the affected sib (Fig. 1.). Water deprivation was well tolerated and led to an increase in plasma osmolality (P_{osm}) and urine concentration in both subjects. In the healthy sib, desmopressin perfusion lowered P_{osm} by further increasing U_{osm}, whereas no changes in U_{osm} nor diuresis were observed in the affected sib, despite a continued increase in P_{osm} (from 298 to 305 mOsm/L).



Discussion: Despite a significantly lower GFR, the proband harbouring a pathogenic mutation of *UMOD* showed an exaggerated response to furosemide and a failure to maximally concentrate urine after water deprivation/desmopressin perfusion. These results confirm that mutations of *UMOD* cause specific transport defects in the TAL in the early phase of the disease.

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FR-PO237

Phenotypic Variability Associated with Combined Podocin Pathogenic Mutation and Variant R229Q Paul J. Phelan,[1,2] Delbert R. Wigfall,[3] Shashi K. Nagaraj,[3] John W. Foreman,[3] Andrew F. Malone,[1,2] Gentzon Hall,[1,2] David Howell,[4] Michelle P. Winn,[1,2] Rasheed A. Gbadegesin.[1,3] *[1]Duke Molecular Physiology Inst, Duke Univ, Durham, NC; [2]Dept of Medicine, Div of Nephrology, Duke Univ Medical Center, Durham, NC; [3]Dept of Pediatrics, Duke Univ Medical Center, Durham, NC; [4]Dept of Pathology, Duke Univ Medical Center, Durham, NC.*

Introduction: Mutations in podocin (NPHS2) are the most common cause of childhood onset autosomal recessive steroid-resistant nephrotic syndrome (SRNS). The disease is characterized by early onset proteinuria, resistance to immunosuppressive therapy and rapid progression to end stage renal disease. Compound heterozygous mutations involving the podocin variant R229Q and another pathogenic mutation have been associated with a milder phenotype. Disease onset is often considered only to occur in adolescence or early adulthood.

Case Description: We screened our entire cohort with childhood onset SRNS for mutations in *NPHS 1* (nephrin), *NPHS2* and *WT1* (Wilm's Tumor 1) genes, using Sanger sequencing.

We identified 2 families with 3 individuals presenting in childhood who are compound heterozygous for R229Q and one other pathogenic *NPHS2* mutation, either L327F or A291V. One child presented at age 4 years and the other two at age 13, one with steadily deteriorating renal function. Renal biopsy in the four year old child showed minimal change disease and focal segmental glomerulosclerosis in the other two children.

Discussion: These cases highlight the phenotypic variability associated with the NPHS2 R229Q + pathogenic mutation. Individuals may present with early aggressive disease suggesting that R229Q + pathogenic mutation should be screened not only in adults with familial FSGS but also in children.

FR-PO238

Renal Involvement in the Systemic Variant of Retinal Vasculopathy and Cerebral Leukodystrophy (RCVL) Associated with Novel TREX1 Gene Mutation Tiziana Stellato,[1] Andrea Stella.[2] *[1]Clinica Nefrologica, AO San Gerardo, Monza, Italy; [2]Dipartimento di Scienza della Salute, Univ degli studi di Milano Bicocca, Milano, Italy.*

Introduction: Retinal Vasculopathy and Cerebral Leukodystrophy (RCVL) is a rare autosomal dominant inherited disease linked with mutation TREX1 gene. It is characterized by retinal and cerebral progressive vascular lesions and in a systemic variant it compromises renal involvement. We describe a case of a 30 years old man who presented with malignant hypertension and renal dysfunction. Histological features at renal biopsy were consistent with thrombotic microangiopathy. A genetic screening identifield a novel T270fs heterozygous C-terminal frameshift mutations of TREX1 gene.

Case Description: In 2005 the patient presented to emergency department with severe hypertension (250/150 mmHg) and decreased visual acuity. Fundoscopy was consistent with severe hypertensive retinopathy. Brain CT scan did not show significant abnormalities. Laboratory tests showed serum creatinine 2.5 mg/dL, urinary protein excretion of 2.6 gr/24h, unremarkable autoimmunity tests. No mutation was found in CFH, CFI, MCP genes. Factor H level and enzymatic activity of ADAMS-13 were normal. In 2010 the patient developed a bilateral necrosis of hip's heads and few months later he developed left hemiparesis and dysarthria. Brain CT and MRI showed a right fronto-parietal lesion and multiple bilateral calcifications in the white matter. Later patient presented severe dysphagia necessitating a gastrostomy for enteral nutrition. Now renal function is stable (serum creatinine 2 mg/dl), levels of cholestasis indices (gammaGT 252 UI/ml, alkaline phosphatases 314 UI/ml) are increased, blood pressure is well controlled (120/80 mmHg) with ACE inhibitor.

Discussion: The systemic variant of RCVL should be suspected in patient presenting with features of malignant hypertension and renal microangiopathy. Genetic testing including TREX1 gene should be carried out whenever a progressive systemic disorder characterized by an occlusive vasculopathy arise in middle-age people.

FR-PO239

Identification of a Novel Mutation in SLC2A2 Leading to the Fanconi-Bickel Syndrome in an Adult with Associated Erythrocytosis Francesco Trepiccione,[1] Giovanna Capolongo,[1] Giovambattista Capasso,[1] Robert J. Unwin.[2] *[1]Dpt of Cardio-Thoracic and Respiratory Science, Second Univ of Naples, Naples, Italy; [2]UCL Centre for Nephrology, Univ College London, London, United Kingdom.*

Introduction: We described here the case of an adult male diagnosed with the Fanconi-Bickel syndrome associated with erythrocytosis.

Case Description: Clinical signs were first detected at two months of age with failure to thrive, hepatomegaly, glycosuria and fasting hypoglycaemia with ketonuria. No liver or renal dysfunction was reported in other family members (parents and two sisters). At five months of age he had a liver biopsy that was reported to show a 'glycogen storage disease'. He grew normally thereafter with no renal or other clinical problems. At age 25, he developed a JAK2 negative erythrocytosis with no elevation in erythropoietin concentration, which was treated by regular venesections. At this point he was referred to our clinical service and a clinical diagnosis of Fanconi-Bickel syndrome was made based on the history and evidence of a proximal tubulopathy.

Gene analysis of SLC2A2, which encodes the glucose transporter GLUT2, showed him to be a compound heterozygote for a nonsense mutation, p.R301*, and a splice site mutation, c.1068+1G>A. Each parent was heterozygous for one of these mutations and did not have any signs of disease. The nonsense mutation p.R301* has been described previously in homozygous patients with the Fanconi-Bickel syndrome, but not the splice site mutation, which is novel.

Discussion: Fanconi-Bickel syndrome has been described with erythrocytosis, although this may be a feature in other forms of the renal Fanconi syndrome, such as Dent's disease, and also in some forms of chronic liver disease, but usually with raised erythropoietin levels. Thus, we describe an adult patient with Fanconi-Bickel syndrome associated with non-erythropoietin-dependent polycythemia and a novel splice site compound heterozygote mutation in SLC2A2.

FR-PO240

Fanconi Syndrome Induced by Clofarabine: Evidence for Multiple Tubular Defects due to a Nucleoside Analogue Abdulmawla Albirini, Joe Ghata, Benjamin D. Cowley, Kai Lau. *Nephrology, Univ of Oklahoma, OKC, OK.*

Introduction: A 78-year-old 59-kg woman with newly diagnosed AML received 5 daily doses of clofarabine (Clofa) 40 mg/m^2/d. eGFR was 55 ml/min. In < 2 d, she developed several new lab findings [K <3.0 mM, Phos (P) <2.5 mg%, urate <1.5 mg% and glycosuria] despite no diuretics and frequent, robust replacement.

Case Description: Past medical history, ongoing issues and lab data were reviewed to examine potential etiology: low intake, GI, renal losses. Review of systems revealed anorexia but no vomiting/diarrhea. Serial exams showed mild volume depletion but no fever/hypotension. To test a renal tubular leak hypothesis, clearance and fractional excretion (FE) of solutes were repeatedly measured during hypophosphatemia, hypokalemia, hypouricemia and normoglycemia.

There was no glycosuria pre-Clofa but 3+ glycosuria on 3 separate days despite serum (S) glucose <150 mg%. Renal P wastage was repeatedly shown by high FEP (40, 55, and 56% in 3 spot urine) despite concurrent S-P <2.9 mg%. Off P therapy, 24 h urine had 1.35 g P and FEP of 67% despite S-P of 2.6 mg%. Renal K leakage was shown by FEK of 65, 67, and 66% in 3 spot urine despite concurrent S-K <2.8 mM. Renal urate wastage was supported by S-urate of 1.5 mg%, normal 24 h urine urate of 532 mg and FEurate of 80%. Renal tubular acidosis was suggested by urine anion gap of 50 mM and urine pH of 7 at S-HO3 of 21 mM. Proximal leakage of these solutes persisted >4 weeks. Measured creatinine clearance (56 ml/min) was stable.

Discussion: Our data showing marked tubular wastage of glucose, P, K, urate and HCO3 support the hypothesis of clofarabine-induced Fanconi syndrome. To our knowledge, this is the first documented case of tubular complications from a nucleoside analogue. We propose a pathophysiologic mechanism of proximal tubular injury similar to that caused by tenofovir, given their similarity in structure, proximal tubular handling, and ability to also impair GFR. Since Clofa is renally excreted but potentially nephrotoxic, we recommend initial and subsequent dosing based on GFR and checking serial creatinine during its infusion. Her persistent tubular defects beyond 4 weeks may reflect dose excess relative to intrinsically low GFR due to age and petite mass.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO241

Olanzapine-Induced SIADH: Correction of a Serum Na of 99 mEq/L Abdulmawla Albirini, Joe Ghata, Lukas Haragsim. *Nephrology, OUHSC, Oklahoma City, OK.*

Introduction: Hyponatraemia (serum (S) Na<136 mEq/L) is a dangerous medical comorbidity in psychiatric patients. Hyponatremia occurs as a rare adverse reaction to treatment with different psychotropic drugs. In these patients, it is important to rule out psychogenic polydipsia, characterised also by polyuria, as it is seen also in schizophrenia. In our patient, diagnosis of hyponatremia secondary to SIADH was established per consistent biochemical blood and urine test results/analyses.

Case Description: A 50 years old Caucasian obese male, smoker with schizophrenia (paranoia type) and hypertension who presented to ER with a chief complaint of weakness. Prior to labs, he was alert, following commands and answering questions appropriately. Labs and imaging were performed, which revealed a Na of 99 mg/dL, a S osm of 221 M, urine osm of 707 M and FENA of 0.2%. TSH was 0.81. CXR demonstrated no focal opacity. His baseline S Na was 137 mg/dL 3 months prior. Review of systems revealed no polydipsia/polyuria. Physical exam revealed no edema. He reported only Olanzapine use. The patient was soon intubated and transferred to the ICU after a protracted generalised tonic clonic seizure leading to an aspiration episode and subsequent intubation in the ER. CT Head, soon after, revealed cerebral edema.

Secondary causes of hyponatremia were sought, and none were found. Olanzapine was stopped. The patient was restricted from free water and given hypertonic saline (3%) x 5 days with an average serum Na increase of 6-8 mEq/L over 24 hour, until a serum Na of 124 on hospitalization day 5, whereupon the patient was free water restricted in addition to antibiotics in norma saline. Upon day 8, patient was extubated and transferred to the floor with a serum Na of 137 mg/dL. Prior to discharge, a repeat CT Head scan demonstrated no sequalae.

Discussion: SIADH is suspected in any patient with hyponatraemia, hypoosmolality, and a urine osmolality >100 mOsm/kg. Four cases of hyponatremia and concommitant severe SIADH have been attributed to Olanzapine, but never less than serum Na < 100. In our patient, the correction of hyponatremia, combined with the discontinuation of his Olanzapine, resulted in resolution of hyponatremia, without any further recurrence.

FR-PO242

Glomerular Nephropathy due to Lipids Deposition in a Woman with Niemann-Pick Type B Disease: First Reported Case in South America Hugo Pinheiro, Gisele Vajgel Fernandes, Luis H.B.C. Sette, Cintia Germana Mergulhão da Costa, Ederson Vidal Moura, Maria Alina G.M. Cavalcante, Geraldo José de Amorim, Nathalia K.N. Alecrim, André Luiz De Andrade Araújo. *UFPE.*

Introduction: Niemann-Pick (NP) type B disease is a rare pathology classified as a storage disease resulting from enzymatic deficiency of sphingomyelinase which leads to lysossomal accumulation of sphingomyelin. It affects many organs, including liver, spleen, lungs and bone marrow with progressive fibrosis due to inflammation around the deposit of lipids. Renal involvement is rare and nephropathy has been previously reported in association with NP once in an American girl.

Case Description: A 47y woman reported progressive edema, oliguria and episodic gross hematuria for the past six months. She was underwent splenectomy 25y later for thrombocytopenia. Liver biopsy showed structural changes suggestive of lipidosis. Intra-abdominal lymphadenopathy associated with anemia and lymphocytosis justified bone marrow biopsy where numerous filled macrophages were visualized by bluish inclusions (lipid accumulation). Tests showed proteinuria and hematuria and 24h urine protein 3.1g, SAlb 1.4mg/dL, SCr 2.7mg/dL; negative ANA, normal complement, no monoclonal spike on serum protein electrophoresis, serology for HBV, HCV and HIV were negative. Sphingomyelinaseactivity was 0.2nmol/h/mg protein (0.74-4.9) and quitotriosidase was 6nmol/h/mL (8.8-132). Kidney biopsy revealed 17 glomeruli with hypercellular proliferation of own cells, particularly endothelial cells (microvesicles in cytoplasm). Podocytes exhibit moderate diffuse degenerative changes. The capillary loops exhibit regular external contours, some with widening of subendothelial spaces and images in "double contour". Tubules had atrophy and mild interstitial fibrosis. Immunofluorescence staining was negative. Electron microscopy showed capillary loops occluded by cells with prominent vacuolization in cytoplasmic, podocytesdegeneration andretractionofpedicels.

Discussion: NP type B usually causes liver, spleen, bone marrow and pulmonary involvement and although proteinuria has been described in these patients, this is the second reported case of glomerular deposition of lipids.

FR-PO243

A Case of Multicentric Castleman's Disease with IgG4 Related Disease Like Multiorgan Lesions Including Tubulointerstitial Nephritis Takeshi Zoshima, Kazunori Yamada, Mitsuhiro Kawano. *Div of Rheumatology, Kanazawa Univ Hospital, Kanazawa, Japan.*

Introduction: Renal involvement in Multicentric Castleman's disease (MCD) is uncommon, but various types of renal disease have been reported. Some cases of MCD occur with infiltration of many IgG4-positive (IgG4+) plasma cells (PC) and elevated serum IgG4 levels. We report a case of MCD with IgG4 related disease (IgG4RD) like lesions, such as tubulointerstitial nephritis (TIN) with IgG4+ PCs, retroperitoneal fibrosis (RF), and periaortitis.

Case Description: A 64-year-old woman with a 2-month history of fever and night sweats was referred. Blood tests revealed severe inflammation (CRP 16.9 mg/dl, IL-6 113 pg/ml), anemia (Hb 8.5 g/dl), hyper IgG (IgG 4807 mg/dl, IgG4 235 mg/dl). PET-CT showed systemic lymphadenopathy, RF, hydronephrosis, and periaortitis. After admission, decreased renal function (Cr 0.99 mg/dl, eGFR 44 mL/min/1.73m^2, u-b2MG 18358 ng/ml, u-NAG 52.4 IU/l) was detected. Renal biopsy revealed TIN with clear margin, and IgG4+ PCs (54 /hpf, IgG4+/CD138+ PC ratio 32%). Supraclavicular lymph node (LN) biopsy showed hyperplasia of germinal centers and a few IgG4+ PCs, but no monoclonality. We diagnosed MCD, and prescribed prednisolone (PSL) 30 mg/day with little efficacy. Additional tocilizumab (TCZ) therapy improved her symptoms and laboratory data, and the lesions of LN, retroperitoneum and periaorta regressed transiently. But, the disease flared after PSL tapering, and severe anemia with cachexia developed. Although lymphoma was suspected, paraaortic LN biopsy revealed histological findings consistent with MCD but not those of lymphoma. Rituximab and cyclophosphamide therapy followed by restart of TCZ achieved a marked response.

Discussion: The present case showed IgG4RD-like lesions, but differed from typical IgG4RD in that marked inflammation and resistance to PSL were seen. Though a case of MCD with TIN and RF had been reported, the present case is the first one of MCD with IgG4RD-like TIN detected by renal biopsy, to our knowledge. Some MCD with IgG4+ PCs in LN, skin, and lung have been reported. The present case suggested that similar findings may also be detected in the kidney of MCD.

FR-PO244

Lack of Genotype-Phenotype Correlation in Two Brothers with Identical Mutations in Nephronophthisis 3 Gene Charu Gupta, Asha Moudgil. *Nephrology, Children's National Medical Center, Washington, DC.*

Introduction: Nephronophthisis (NPHP)-related ciliopathies are autosomal-recessive cystic kidney diseases. Phenotypic heterogeneity has been linked to more than 13 genes implicated in its pathogenesis. The cases presented here highlight the lack of genotype-phenotype correlation in two brothers with identical genetic mutations in the NPHP3 gene.

Case Description: Fifteen-year-old boy presented with fatigue and elevated serum creatinine (2.5 mg/dl, Schwartz eGFR 40 ml/min/1.73m^2). Lab evaluation revealed anemia (Hemoglobin 10.7 g/dl), metabolic acidosis (serum bicarbonate 18 mmol/l), glucosuria and mild proteinuria of tubular origin (UPC 0.41, b2 microglobulin 16,749 mcg/gm). His birth, past and family histories were unremarkable. There was no history of chronic medication intake, substance abuse or use of herbal supplements. His physical exam was normal with no extra-renal or skeletal abnormalities. Renal sonogram revealed bilateral echogenic and small kidneys (bilateral renal size two standard deviation below mean). Renal biopsy showed severe chronic tubulointerstitial fibrosis, tubular atrophy and dilatation with global glomerulosclerosis in 4 of 9 glomeruli, immunofluorescence was negative and focal foot process fusion was noted on electron microscopy. Whole exome sequencing showed that he was compound heterozygous for the novel c.958-2 A>G mutation (of paternal origin) and Q696P variant (of maternal origin) in the NPHP3 gene which encodes for nephrocystin 3. His 18 year old asymptomatic brother was noted to have serum creatinine 1.2 mg/dl (MDRD eGFR 79 ml/min/1.73m^2), his urinalysis showed no proteinuria or glucosuria and he had normal sized kidneys with mild increase in echogenicity. He was noted to have the same exact genetic mutations as his brother.

Discussion: NPHP is a common childhood ciliopathy which progresses to end stage renal disease. While phenotypic variability has been attributed to different genetic mutations, these cases highlight the lack of genotype-phenotype correlation with the same genetic mutations suggesting that other factors like genetic load, oligo-genetic inheritance, modifier-effect of other genes and other unknown modifiers may affect the phenotype.

FR-PO245

Acute Kidney Injury in Von Hippel-Lindau Disease Sunggeun Lee, Roger F. Carbajal Mendoza, Ashok P. Chaudhari, Donald I. Baumstein. *Nephrology, Metropolitan Medical Center, New York Medical College, New York, NY.*

Introduction: Von Hippel-Lindau disease (VHLD) is a autosomal dominant syndrome, usually complicated with renal cell carcinoma (RCC), Hemangioblastoma and Pheochromocytoma. For renal manifestation of NHLD, not only patients develops RCC and multiple renal cysts, also they seem to carry a higher risk for acute kidney injury (AKI). Here we describe a case of AKI in VHLD.

Case Description: 41 year-old male with medical history of VHLD and hypertension was consulted for AKI and hyperkalemia. He had right side nephrectomy for RCC one year ago, also had ventriculoperitoneal (VP) shunting for cervicomedullry hemangioblastoma with hydrocephalous. He did not have any complaints. The physical examination was normal. The laboratory tests showed BUN of 25mg/dL Cr of 2.0mg/dL (estimated GFR 37 L/min), K of 5.5mmol/L. FeNa of 0.5%.The renal sonogram revealed only left side kidney, size of 18.8cm,two hypoechoic masses, urinary retention. We concluded that AKI was from the hypovolemia with obstructive uropathy. Cr was improved to 1.3mg/dL and hyperkalemia was resolved with fluid and foley catheter insertion.

Discussion: There are several factors that VHLD patient are more prone to AKI. First of all, he was not able to function without assistance since the VP shunt procedure, which easily leads to poor oral intake and makes him prone to hypovolemia. He also has risk factors to have urinary retention, such as neurologic deficit and multiple pain medications. VHLD patients get frequent urinary tract infection, which also can contribute to AKI. The partial recovery from AKI might exacerbate the worsening of renal function, VHLD patients should be followed up closely to limit the risk factors for AKI. The bigger concern was that he had another new mass in his only remaining kidney. He will be on dialysis if he gets nephrectomy of his left kidney. The 69% of VHLD patients develop RCC by the age of 60. Most of them develop bilateral RCC eventually, partial nephrectomy is preferred

than radical nephrectomy. The nephron-sparing approaches, such as cryoablation and radiofrequency ablation also can be considered. The proper screening for RCC and nephron sparing surgery are the cornerstone of VHLD management.

FR-PO246

Sunitinib-Induced Persistent Hypomagnesemia with Hypocalcemia

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Introduction: Sunitinib is the FDA-approved treatment for renal cell carcinoma (RCC), known to cause hypomagnesemia and hypocalcemia. It acts by blocking vascular endothelial growth factor receptors (EGFR) to inhibit tumor growth. Because of abundance of EGFR and its ligands in renal tubular epithelial cells, renal toxicity mainly manifests as tubular dysfunction. Recently it was discovered that EGFR also play a role in renal magnesium regulation directly though transient receptor potential melastatin. Here we describe a case of persistent hypomagnesemia and hypocalcemia caused by Sumitinib.

Case Description: 58 year-old male with medical history of CKD stage III, renal cell carcinoma (RCC) with bone metastasis, hypertension, diabetes was admitted for severe hypocalcemia, complaining of jerky movements and constipation. Home medication includes calcium carbonate plus vitamin D,magnesium gluconate. The physical exam did not show chvostek's and trousseaus sign. The lab findings showed creatinine of 3.3mg/dl, BUN of 34mg/dl, calcium of 4.2mg/dl, albumin of 1.8g/dl, PTH of 207pg/ml, phosphate of 2.8mg/dl, Magnesium of 1.2mg/dl, vit D,25-OH of 14 ng/ml, vitD 1,25OH of 61 pg/ml. Electrocardiogram showed QTc prolongation (524 ms). Patient was treated with calcium gluconate IV, Calcium carbonate, rocaltrol, IV and oral magnesium sulfate. The main cause of hypocalcemia would be magnesium-wasting nephropathy in this case. 24-hour urinary magnesium excretion was 118mg, severe hypomagnesemia has been persistent since patient received Sunitinib 6 months ago. Sunitinib had to be switched to Temisirolimus because of the other complications, such as bleeding, pancytopenia and hand-foot syndrome. Despite discontinuation of Sunitinib, the hypomagnesiumia persisted and the patient continued to require IV magnesium. That further supports the permanent damage of the tubule by Sunitinib. Even though he also received radiation therapy for RCC later which also can damage the tubules, hypomagnesemia was started even before the radiation.

Discussion: It is important to aware hypomagnesemia as a possible adverse effect when using Sunitinib. The tubular damage can be permanent.

FR-PO247

Management of Hypernatremia in New-Onset Reversible Diabetes Insipidus

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Introduction: Hypernatremia is common in acutely ill patients with altered mental status. Diagnosis and treatment can be difficult especially if the cause is new-onset diabetes insipidus (DI).

Case Description: Case description: A 62 year-old man was admitted after fall with altered mental status, hypotension, and hypothermia. He had urosepsis, AKI (BUN/serum creatinine (SCr) of 86/1.9 mg/dL), and hypernatremia with serum sodium (SNa) of 156 mmol/L. In ED, a 4 L bolus of IV NS was given. Maintenance D5W was started, but SNa ranged from 147–159 mmol/L. Urine output (UOP) was up to 4.7 L/day. Serum and urine osmolality (Uosm) were 338 and 226 mOsm/kg, respectively (Figure1). Electrolyte-free water clearance (EFWC) was >61% of UOP. An initial diagnosis of partial nephrogenic DI from azotemia was made, but high SNa and UOP (4 L/day) persisted despite improved BUN/SCr of 18/1.2 mg/dL. DDAVP 2 mcg IV was started (hospital day 8). Urine osmolality increased to 534 mOsm/kg, indicating partial central DI and DDAVP 1 mcg SC twice a day was continued. D5W was restarted for a 2 day period when SNa increased again but as mental status improved PO fluids were substituted for D5W. On hospital day 27, SNa dropped to 133 mmol/L. DDAVP was stopped and SNa remained normal. He was discharged on hospital day 32 with SCr of 0.9 mg/dL and SNa of 139 mmol/L. A cause for the CDI was not found.

Discussion: Although water depletion is the most common cause of hypernatremia, some patients may have components of both nephrogenic and central DI that complicates treatment and delays the correction of the SNa. In this case both water and DDAVP were used to correct the hypernatremia. Over a hospitalization, the origin, and therefore treatment, of the DI may change. Careful monitoring of the UOP, Uosm, EFWC, and SNa, are important in the diagnosis and treatment of DI and hypernatremia.

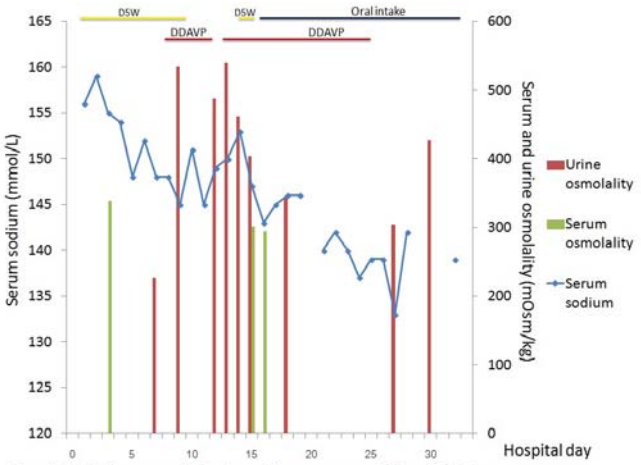

Figure1: *Clinical course and laboratory data over one month hospitalization.*

FR-PO248

Hypokalemia Induced Nephrogenic Diabetes Insipidus from Ifosfamide Use

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Introduction: Ifosfamide (IFO) is used as an anticancer drug for sarcomas and lymphomas. It causes nephrotoxicity in form of decline in GFR, tubular damage presenting as fanconi's syndrome, and albuminuria. Higher cumulative dose (>40-60 gm/m2), younger age of treatment, previous nephrectomy, previous use of cisplatin are risk factors for IFO induced nephritoxicity. Nephrogenic Diabetes Insipidus (NDI) is a rare toxicity of this drug, and only 4 cases have been reported in adults. We report a case of IFO induced Fanconi's syndrome and NDI in a patient with uterine sarcoma.

Case Description: 52 year old lady came with vomiting. Past medical history of uterine sarcoma and diabetes mellitus. She was treated with 29.5 gm IFO and 120 mg Adriamycin over a 4 week period, last dose being 2 weeks ago. Admission laboratory values revealed (meq/dl) hypernatremia (157), hypokalemia (2), hypophosphatemia (0.8) and metabolic acidosis (serum bicarbonate 19 meq/dl), along with glycosuria. She was diagnosed with Fanconi's syndrome, likely from IFO use. She had significant polyuria, daily urine output of upto 6-9 liters. Her serum osmolality (moms/dl) was high (314) and urine osmolality was low (196) suggestive of DI. She was given 2 doses of 15 mcg intranasal desmopressin with mild increment in her urine osmolality. She was diagnosed with hypokalemia driven partial NDI in the setting of IFO use. Potassium was repleted and Hydrochlorothiazide was initiated for continued improvement of her urine osmolality.

Discussion: Ifosfamide related fanconi's syndrome is rare in adult population. The mechanism is likely accumulation of IFO metabolite chloracetaldehyde which causes tubular toxicity. There have been 4 reported cases of NDI related to IFO use, all had coexistent fanconi's syndrome. Mechanism for this could be related to distal tubular damage done by IFO. More importantly, this process is likely driven by severe persistent hypokalemia, which causes downregulation of aquaporin2 channel expression. There was mild improvement of urine osmolality with desmopressin suggestive of partial down regulation of arginine vasopressin receptors. This is the second reported case of desmopressin responsive NDI with IFO use.

FR-PO249

Hypothermia Associated Hypokalemia: A Cautionary Note for an Increasingly Popular Treatment after Cardiac Resuscitation

Tanya Tocharoen Tang, Daniel Batlle, Robert M. Rosa. *Nephrology, Feinberg School of Medicine Northwestern Univ, Chicago, IL.*

Introduction: Induced hypothermia is a widely recommended intervention to improve neurological outcome in patients who have survived prolonged cardiac resuscitation. Hypothermia, however, can produce hypokalemia by a mechanism that has not been defined.

Case Description: We present a 60-year-old Caucasian male admitted after an out of hospital ventricular fibrillation cardiac arrest with prolonged CPR with intubation. Therapeutic hypothermia was implemented with intent to achieve a core temperature of 91.4 degrees over 24 hours. Serum potassium concentrations declined during induced hypothermia (Table). Ventricular ectopy recurred during hypothermia and K+ was replaced (a total of 400 mEq) without correction of hypokalemia. Upon rewarming, K+ rose to 6.8 with occurrence of sine waves and cardiac arrest (Table). Blood pH was in the range of 7.01-7.33 and bicarbonate was 13-24 throughout the entire episode. Patient had normal renal function throughout and urine output remained consistently between 100-200 cc/hr.

	---------Day 1--------		--------Day 2--------		----------Day 3----------		
Time	1 pm	9 pm	6 am	11 am	2am	6 am	9 am
°F	94.1	90.3	91.9	91.2	97.8	98.8	98.6
K^+	4.1	2.7	2.9	3.1	5.7	6.8	6.7
		------Ventricular Ectopy------ ------ 400 mEq KCl given----- INDUCED HYPOTHERMIA			-Cardiac Arrest, EKG sinus wave- REWARMING		

Discussion: This case illustrates the adverse effect of hypokalemia during induced hypothermia as well as the complication of hyperkalemia while rewarming. The mechanism of hypothermia-induced hypokalemia has been attributed to a shift of K^+ from extracellular space to the cellular space. Rather, we propose that extreme low body temperature is more likely to suppress the activity of skeletal muscle K channel (s) thereby decreasing the efflux of K^+ out of the cell. Physicians should be aware of this phenomenon and limit K replacement during rewarming.

FR-PO250

Adipsic Central Diabetes Insipidus Secondary to Gunshot Head Injury - A Case Report Chintan Shah, Roveena N. Goveas, Inder Patel, Wihib A. Gebregeorgis. *Nephrology, Wayne State Univ/DMC, Detroit, MI.*

Introduction: Trauma to the hypothalamus and posterior pituitary region is known to cause central diabetes insipidus (DI) leading to polyuria, hyperosmolality and in the alert patient, excessive thirst. Central DI with deficient thirst (Adipsic DI) as a complication of head trauma is extremely rare. We report a 39 year old man who was admitted for musculoskeletal shoulder pain and was incidentally found to have hypernatremia. Patient reported polyuria and nocturia but denied any history of excessive thirst. He estimated his habitual 24hr fluid intake to be 2-3 Liters. He was not on any medications. He has no history of psychiatric illness and has never been on lithium. Relevant past history included gunshot injury to the head at age 11. On examination, he was euvolemic with normal BP and PR. He was alert and oriented to time, place and person. Neurological examination was normal. Hypernatremia persisted throughout his hospitalization with a serum Na ranging 150-153mmol/L. Glucose level was normal. He denied feeling thirsty on several occasions when his serum Na was 150-153mmol/L despite being fully alert with free access to water. At a serum Na of 153mmol/L, his urine osmolality was 234mOsm/Kg but increased to 605mOsm/Kg after administration of DDAVP 5mcg SQ, consistent with central DI. Given the lack of thirst at serum Na of >150mmol/L despite intact sensorium, a diagnosis of adipsic DI was made. Head CT showed a bullet in the suprasellar/dorsum sella region with severe streak artifact related to the bullet limiting detailed evaluation of the sella turcica. There was no mass, pathologic enhancement, ischemia or hemorrhage. Treatment with DDAVP and scheduled water intake corrected his sodium to normal. In conclusion, we describe a rare case of gunshot injury to the pituitary/hypothalamic area resulting in combined deficits in ADH secretion and thirst sensation, a condition called adipsic DI. Adipsic DI has been previously reported with septo-optic dysplasia, tumors, aneurysms and sarcoidosis. On extensive literature review, we found two previous reports of head trauma causing adipsic DI. However bullet injury to the head as a cause of adipsic DI has not been reported.

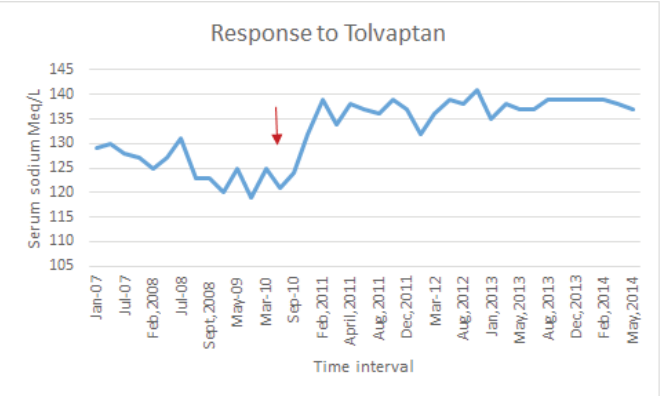
FR-PO251

Low Dose Tolvaptan for Chronic Outpatient Hyponatremia Sumedha Dhar, Daniel Batlle. *Nephrology and Hypertension, Feinberg School of Medicine, Northwestern Univ, Chicago, IL.*

Introduction: The use of vasopressin antagonists such as Tolvaptan, a selective V2 blocker, has greatly facilitated the therapy of hyponatremia in the hospital setting and obviated the need for fluid restriction that is often difficult to implement. However, long-term outpatient use has recently been limited to only a month because of concerns with potential liver injury.

Case Description: A 59 year old male patient with past medical history of C5-C6 injury during a motor vehicle injury resulting in quadriplegia, deep venous thrombosis, pulmonary embolism, neurogenic bladder with indwelling catheter and frequent urinary tract infections was referred to the nephrology clinic in 2010 for low plasma sodium. A diagnosis of syndrome of inappropriate antidiuretic hormone (SIADH) was made and attributed to central nervous system pathology. His lowest plasma sodium was 118 meq/L. Fluid restriction was not only difficult to follow but relatively contraindicated since the frequency of recurrent urinary tract infection could be worsened by reducing urine output during fluid restriction.

Tolvaptan 15 mg was started initially and then decreased to 7.5 mg for the last 3 years. The graph shows the fluctuation of plasma sodium before and after Tolvaptan over time. During the prolonged follow up, liver enzymes have always been in the normal range.



Discussion: This case illustrates that sustained correction of hyponatremia can be accomplished by low dose of Tolvaptan (half the lowest dose in the U.S. market) while fluid intake can be liberalized. The reported increases in liver enzymes have been observed in clinical trials for Adult Polycystic Kidney Disease (about 4%) for a different indication (to reduce cyst volume) where higher doses of Tolvaptan may be needed. Low dose Tolvaptan should be a logical option for treatment of hyponatremia in the outpatient setting.

FR-PO252

Hypokalemia, Rhabdomyolysis, and Nephrogenic Diabetes Insipidus from Excessive Soda Consumption Sharidan Parr, Jamie P. Dwyer. *Nephrology and Hypertension, Vanderbilt Univ Medical Center, Nashville, TN.*

Introduction: Excessive soda intake is a rare but important cause of severe hypokalemia. Under-recognized complications of hypokalemia include rhabdomyolysis and nephrogenic diabetes insipidus.

Case Description: A 37 year-old woman presented with a 4-day history of generalized weakness, myalgias, and paresthesias. She reported polyuria in the 2 months prior to admission. She denied vomiting or diarrhea. She consumes 24 cans (8.5L) of regular soda daily for several years. Past medical history includes lumbago, migraine, seizure disorder, hypokalemia, and remote history of cocaine use. She has no history of thyroid or autoimmune disease, and does not drink alcohol. Initial examination included BP 106/57 and HR 100. Neurologic examination revealed markedly diminished strength and sensation to light touch and pinprick in all extremities. The remainder of the physical exam was normal. Plasma laboratory values: sodium 142mEq/L, potassium 1.9mEq/L, chloride 107mEq/L, bicarbonate 31mmol/L, BUN <1 mg/dL, creatinine 0.5mg/dL, calcium 7.4mEq/dL, magnesium 1.6mEq/dL, phosphorus 3.8mg/dL, albumin 2.7g/dL, TSH 1.19µU/mL, and CPK 3029 Units/L. Urine laboratory values: specific gravity <1.005, sodium 73mEq/L, potassium 11mEq/L, chloride 72mEq/L, calcium-creatinine ratio 0.1, TTKG 3, osmolality 197mosm/kg. Urine output was >6L/day. Urine drug screen was positive for opiates. Plasma aldosterone and renin activity were <2.5ng/dL and <0.2ng/mL/hr, respectively. Her symptoms resolved with administration of potassium and magnesium, limitation of soda intake, and improved dietary solute intake.

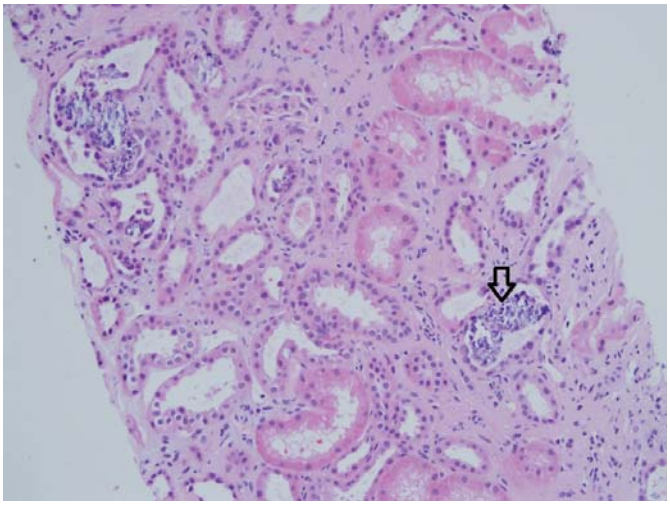
Discussion: In this case, while urinary potassium excretion was appropriately reduced, the obligatory potassium loss due to polyuria exceeded the dietary intake, causing severe hypokalemia. Elevated b-adrenergic activity from caffeine can cause intracellular shifts of potassium and further exacerbate hypokalemia. Skeletal muscle ischemia and rhabdomyolysis resulted from impaired vasodilation due to severe hypokalemia. Nephrogenic diabetes insipidus was likely due to both low medullary urea concentration from low protein intake, and hypokalemia-induced reduction of collecting tubule sensitivity to ADH.

FR-PO253

An Usual Case of Hypercalcemia: The Resurgence of the Human Adjuvant Disease Owolabi Ogunneye, Jo Abraham, Alfred K. Cheung. *Nephrology, Univ of Utah.*

Introduction: Oils have been injected by laymen into various parts of their bodies for cosmetic reasons. Various side-effects have emerged following the injections of these substances. Human adjuvant disease is associated with exposure to foreign substances.

Case Description: A 47 year old Hispanic male-to-female transgender presented to our Nephrology Clinic with several-year history of severely itchy bilateral thigh lichenification. Fifteen years prior, she had a transgender surgery that includes silicone-breast implantation and mineral-oil injection into the hips and buttocks. Five years preceding this presentation, she was hospitalized for AKI with serum creatinine of 4.0 mg/dl and calcium of 12.5 mg/dl. Renal biopsy at that time revealed calcium crystals in tubules.



One year prior to this visit, she had surgical excision of a granulomatous mass in the gluteal region. Her medical history was also notable for CKD stage IV secondary to nephrocalcinosis and hypertension. Chest x-ray was normal. Abdomen CT showed two 1-mm non-obstructing opaque calculi in the right kidney. She was maintained on

hydroxychloroquine 200 mg BID and ketoconazole 400 mg QD for the treatment of granulomatous hypercalcemia and calcium remained within normal level at 9.6 mg/dl.

Discussion: Exposure to adjuvants elicits both innate and adaptive immune responses. Extra-renal 1-alpha-hydroxylation of 25 (OH)-vitamin D by activated macrophages is likely responsible for the hypercalcemia in granulomatous disease. Ketoconazole that inhibits the 1-alpha-hydroxylation of 25 (OH)-vitamin, as well as hydroxychloroquine are effective in the treatment of hypercalcemia. While most patients who receive adjuvant injections for cosmetic reasons in general do not suffer from significant adverse events for a number of years, they should be followed carefully because granulomatous hypercalcemia may develop leading to chronic kidney disease.

FR-PO254

Persisting Hypocalcemia after Surgical Parathyroidectomy on Proton-Pump Inhibitor: The Differential Effectiveness of Calcium-Citrate versus Calcium-Carbonate

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Introduction: Successful surgical parathyroidectomy (PTX) elicits "hungry bone syndrome" with massive calcium-influx into the bones and potential for symptomatic hypocalcemia. The effectiveness of oral calcium may be contingent on patient's factors beyond compliance, such as PPI use and choice of calcium supplements.

Case Description: A 32 year-old Hispanic male with end-stage renal disease on peritoneal dialysis (PD) underwent successful surgical parathyroidectomy (initial parathyroid hormone level: 2,328 pg/mL; post-PTX: 287-69 pg/mL) but his post-operative course has been complicated by recurring hypocalcemia in the outpatient setting. At that point, the severe and recurrent hypocalcemia (corrected Ca: 4.8-5.6 mg/dL; nadir ionized Ca: 0.57-0.69 mM/L) was attributed solely non-adherence. During admissions, normocalcemia has been repeatedly restored on IV calcium (Ca)-gluconate infusion, but he has failed outpatient therapy, while on high-Ca PD dialysate fluid (1.5 mM/L), large doses of oral Ca-carbonate (3750 mg, x3/day) and calcitriol (2-4 mcg/day). During a subsequent admission, however, additional inquiry revealed that he was also taking a proton-pump inhibitors (PPI) for severe esophageal reflux and dysmotility, a therapy he could not abandon. Subsequently, oral Ca-supplement he was changed to calcium-citrate (3 gm x3/day), with prompt resolution of hypo-Ca (corrected Ca 8.1-8.3 mg/dL) and no further readmission necessary.

Discussion: Gastric acid contains hydrochloric acid, which reacts with calcium-carbonate to form calcium-chloride, easily bioavailable for absorption form the GI tract. Additionally, this chemical interaction results in net loss of acid and effectively converts the gastric mucosa and parietal cells into net bicarbonate secreting organ. In large epidemiologic studies, PPI have been associated with reduced bone mineral density. This current case emphasizes the disproportionate effectiveness of Ca-citrate under circumstances of achlorhydria.

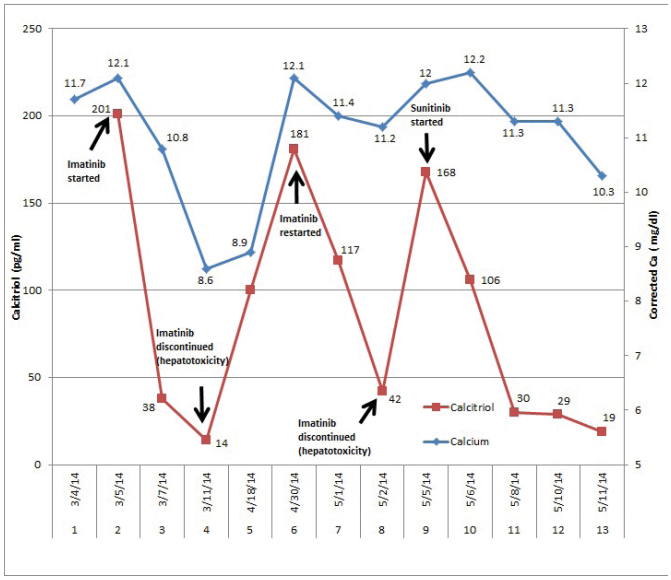
FR-PO255

A Dramatic Response to Imatinib in Calcitriol-Mediated Hypercalcemia

Surabhi B. Thakar, Milind Y. Junghare, Keith E. Eidman. *Nephrology, Univ of Minnesota.*

Introduction: Hypercalcemia is rarely reported in gastrointestinal stromal tumors (GIST) and is predominately parathyroid hormone-related protein (PTHrP) mediated. Calcitriol-mediated hypercalcemia is uncommon and described mostly in lymphomas and granulomatous disease. We report a case, only second in the literature, that demonstrates calcitriol-mediated hypercalcemia in GIST. This case is novel in showing a dramatic response to tumor suppression by tyrosine kinase inhibitors (TKIs) with rapid lowering of calcitriol and resolution of hypercalcemia.

Case Description: A 44 year-old man with recently diagnosed GIST developed refractory hypercalcemia. He had suppressed parathyroid hormone (PTH), undetectable PTHrP, no paraproteins but markedly elevated serum calcitriol. He remained hypercalcemic despite bisphosphonates and high dose steroids. Positron emission tomography (PET) showed marked tumor activity. On initiating imatinib, calcitriol declined rapidly with immediate resolution of hypercalcemia and PET activity. After developing ascites, imatinib was held and he had rebound hypercalcemia and elevated calcitriol. A rechallenge with imatinib again demonstrated an impressive response but with hepatotoxicity. Sunitinib therapy resulted in similar reduction in calcium and calcitriol.



Discussion: GIST are the most common mesenchymal tumors of the digestive tract, characterized by expression of receptor tyrosine kinase gene mutations. PTH and PTHrP are present in 90% of GIST and associate with tumor growth and differentiation. This case illustrates calcitriol-mediated hypercalcemia, independent of PTH and PTHrP. Plausible mechanisms are tumor production of humoral factors that increase 1-a hydroxylase activity or decrease metabolic inactivation of calcitriol. TKIs decreased calcitriol within 24 hours. In such cases, serum calcitriol could be used as a tumor marker indicating adequate and sustained response to therapy.

FR-PO256

Management of Severe Hyponatremia in Patients Requiring Continuous Renal Replacement Therapy

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Introduction: Severe hyponatremia, defined as a serum sodium of less than <125meq/L, is an electrolyte disorder that is frequently seen in the critical care setting. Continuous renal replacement therapy (CRRT) dialysate solutions contain a sodium concentration of 140 meq/L. This significant concentration gradient between the patient's serum and dialysate solution poses a risk for rapid sodium correction that can lead to an osmotic demyelination syndrome. Several case studies have demonstrated altering the dialysate solution in order to achieve a gradual correction, but this is not feasible in many hospital settings and other reviews propose complicated formulas for dialysis delivery. We propose a practical solution that involves using a lower CRRT dose, serial monitoring of sodium levels with titration of the CRRT dose.

Case Description: Case series of seven patients with severe hyponatremia and AKI stage III (severe) that required RRT. All the patients required vasopressors in the ICU. Nephrology was consulted due to the severe electrolyte/metabolic abnormalities and low-dose CRRT was started in the setting of severe hyponatremia. All the patients had serum sodium checked every hour and then every 2 hours once sodium of 125 mmol/L was reached.

Patient	Reason for admission to ICU	CRRT dose	Sodium before CRRT was started	Sodium 1 hour on CRRT	Sodium 6 hours on CRRT	Sodium 24 hours on CRRT
1	Septic shock	20 ml/kg/ hour	118	119	121	125
2	Septic shock	20 ml/kg/ hour	117	118	119	122
3	Liver failure and shock	15 ml/kg/ hour	120	120	122	124
4	Septic shock	20 ml/kg/ hour	115	116	118	120
5	Congestive heart failure/ pulmonary edema	20 ml/kg/ hour	116	116	120	126
6	Septic shock	20 ml/kg/ hour	115	116	118	123
7	Septic shock	20 ml/kg/ hour	117	117	119	125

Table 1. Patients, CRRT dose and changes in sodium over 24 hours.

With a CRRT dose of 15 – 20 ml/kg/hour we were able to correct the hyponatremia safely at a correction rate of 10 mmol/day, with close serum sodium monitoring.

Discussion: This study shows that severe hyponatremia in patients with kidney dysfunction can be managed safely and effectively with CRRT despite a set sodium concentration in the dialysate solution. We propose that instead of altering dialysate solution, lowering CRRT dose with close serum sodium monitoring is safe and practical in order to properly manage hyponatremia in the critically ill patient.

FR-PO257

Hyponatremia, It's All in the Head Zahra Deen,[1] Mahveen Sohail,[1] Muhammad Deen,[2] Sreedhar A. Mandayam.[1] *[1]Nephrology, Baylor College of Medicine, Houston, TX; [2]Medicine, Ziauddin Univ, Karachi, Pakistan.*

Case 1: 79 y/o female presented with 3 day h/o nausea, vomiting and decreased po intake. Serum Na was 111 mmol/L and nephrology was consulted. She had h/o hypothyroidism and was on thyroxine.

PE: Elderly lady with pallor, awake, lethargic and delayed deep tendon reflex relaxation but hemodynamically stable and euvolemic.

Labs: Urine osmolality 698 mOsm/kg >serum osmolality 236 mOsm/kg (suggesting elevated ADH) and urine sodium 51 mmol/L. Cortisol 0.8ug/dL (low) and TSH 0.15 uIU/mL (low).

As work up for hypoaldosteronism we checked pituitary hormone level and MRI of brain which showed low TSH, low FSH, low GH and pituitary macroadenoma. Hyponatremia, a consequence of secondary adrenal insufficiency due to panhypopituitarism normalized after starting on hydrocortisone. Case 2: 62 y/o Vietnamese female presented to ER with 5 day h/o epigastric pain, nausea and vomiting. PMH included HTN and recently diagnosed hypothyroidism. Serum Na was 120 mmol/L and nephrology was consulted.

PE: Middle age woman, awake, alert and oriented, hemodynamically stable and euvolemic.

Labs: urine osmolality 465 mOsm/kg > serum osmolality 236 mOsm/kg (high ADH), Urine sodium 56 mmol/L and a low uric acid 1.7 mg/dL. Low TSH 1.96 uIU/mL and cortisol 3.1ug/dL.

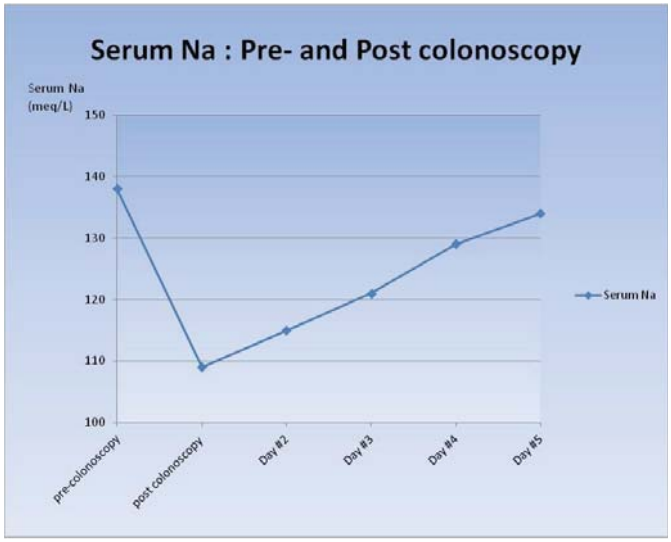
Work up for hypoaldosteronism showed low FSH, LH and IGF-1 and MRI showed intra and suprasellar cystic mass. As her hyponatremia resolved with hydrocortisone, we concluded that she had secondary adrenal insufficiency due to hypopituitarism. Discussion: Severe hyponatremia due to secondary adrenal insufficiency from panhypopituitarism is rare and usually overlooked. This is included in the category of "normovolemic hyponatremia". It present with nausea, vomiting, confusion, disorientation, somnolence or coma. On exam they have scanty pubic and axillary hair, pale and doughy skin and usually small testicles in male. In most patients with hyponatremic hypopituitarism, plasma ADH levels are inappropriately high, probably due to a failure of endogenous cortisol to suppress the hormone in a stressful situation. All patients show recovery soon after starting low-dose hydrocortisone due to normalization of ADH levels.

FR-PO258

Hyponatremia following the Administration of Bowel Prep Products for Colonoscopy Thet N. Zaw, Neil W. Lyman. *Dept of Nephrology, Saint Barnabas Medical Center, Livingston, NJ.*

Introduction: Polyethylene glycol (PEG) solution bowel prep agent induced hypernatremia is well recognized. Hyponatremia following the administration of PEG products is rarely seen. In our published literature search over the past 14 years, we encountered only 7 isolated case reports of PEG induced hyponatremia in patients over the age of 60.

Case Description: We report a case of an 84 year old Caucasian male who received PEG bowel prep for a screening colonoscopy. He had a normal sodium of 138 meq/L a month prior to the presentation. His daily medications include amlodipine and lisinopril for hypertension, synthroid for hypothyroidism, and flomax for benign prostatic hyperplasia. After the colonoscopy, the patient felt nauseous and had an episode of vomiting. He tried to hydrate himself at home by drinking 4 liters of water which was his usual habit. A day after the colonoscopy, the patient presented to the emergency department with gradually worsening lower abdominal pain. His basic metabolic panel showed a sodium level of 109 meq/L. Subsequently, he seized. The patient was treated with 3% saline and seizure terminated. He was then fluid restricted and his sodium slowly normalized over several days. Our workup confirmed the diagnosis of non-osmotic secretion of antidiuretic hormone (ADH).



Discussion: In this report, the advanced age (>75 years), nausea, and crampy abdominal pain which followed the bowel preparation may have led to inappropriate ADH secretion. The patient's vomiting and pure water ingestion were additional factors which ultimately resulted in his severe hyponatremia and seizures. We conclude that physicians should become thoroughly familiar with a patient's medical history and current medications before prescribing a bowel cleansing regimen. Furthermore, serum electrolytes should be checked if there are any changes in mental status after a colonoscopy procedure.

FR-PO259

A Heterozygous Female with Fabry Disease due to a Novel a-Galactosidase A Mutation (Pro210Ser) Exhibits a Unique Synaptopodin Distribution in Vacuolated Podocytes Mamiko Imada,[1] Naoki Takahashi,[1] Seiji Yokoi,[1] Kenji Kasuno,[1] Yoshinari Yokoyama,[1] Daisuke Mikami,[1] Hideki Kimura,[1] Masanori Hara,[2] Masayuki Iwano.[1] *[1]Div of Nephrology, Fukui Univ, Yoshida Gun, Japan; [2]Dept of Pediatrics, Yoshida Hospital, Tsubame, Japan.*

Introduction: Fabry disease (FD) is an X-linked ailment resulting from a deficiency in lysosomal enzyme a-galactosidase A (GLA) activity, which disrupts glycosphingolipid metabolism. As the clinical manifestation in heterozygous females is much more variable compared to homozygous males, it is difficult to diagnose FD in a heterozygous female with a novel mutation. In addition, there are no reports showing immunohistologically colocalization of deposited globotriaocylceramide (Gb-3) and podocyte markers in FD.

Case Description: A 42-year-old woman was referred to our hospital with moderate proteinuria (2.7 g/24 h), and a renal biopsy was performed. Light microscopic examination of the specimen revealed diffuse global enlargement of podocytes, which also showed foamy changes. Electron microscopy revealed abundant myeloid bodies in podocytes and focal mitochondrial abnormalities within the tubules. The patient exhibited none of the characteristic symptoms of FD except hypohidrosis and had no obvious family history. Genetic analysis revealed a novel missense mutation (Pro210Ser) in the GLA gene. She was ultimately diagnosed as FD by immunohistochemical staining indicating large amounts of accumulated Gb-3 in her podocytes, detection of urinary Gb-3 secretion using high-performance thin-layer chromatography, and structural modeling of the mutated GLA. Immunostaining the foamy podocytes using podocyte-associated antibodies (against podocalyxin, WT-1, vimentin and synaptopodin) revealed a unique distribution of cytoplasmic synaptopodin surrounded by Gb-3. Enzyme replacement therapy (agalsidase alfa) was started in January 2013 and her renal function has been stable for 16 months.

Discussion: To our knowledge, this is the first report of a patient with FD showing the augmented synaptopodin expression in foamy podocytes. The combined findings of vacuolated podocytes and cytoplasmic synaptopodin upregulation may be one of the helpful tools to suspect FD.

FR-PO260

A Novel Mechanism of Hyponatremia in HIV Disease Hector Madariaga, Apurv Khanna. *Div of Nephrology, SUNY Upstate Medical Univ Hospital, Syracuse, NY.*

Case Description: This is 22-year-old Black male prisoner who presents with exertional dyspnea, chest pain, relieved by resting, productive cough of clear sputum, weight loss. Pertinent physical examination demonstrated respiratory distress, tachycardia and decreased air entry at bases. A chest CT scan was obtained revealing ground glass opacities. His HIV 1 antibody was positive. In light of his pulmonary findings, treatment for Pneumocystis pneumonia with prednisone and Trimethoprim/sulfamethoxazole was started. His kidney function remained at baseline (0.5-0.8 mg/dL). Serum sodium on admission ranged from 129-135 mmol/L. Urine Na was 46 mEq/L. Serum bicarbonate ranged 20-21 mmol/L and measured osmolality, 258 mOsm/L. Serum potassium remained elevated ranging 4.8-5.8 mmol/L. Normal TSH and cortisol level. Given the suspicion for volume depletion, the patient received intravenous normal saline, but his urinary output increased. He had increased thirst and his fluid balance became negative since admission. His serum sodium decreased to 120 mmol/L and at this point we suspected a possible Glucocorticoid Resistance for which we initiated treatment with fludrocortisone. His serum sodium improved to 131-133 mol/L as well as his serum potassium. We monitored fludrocortisone dosage with plasma renin activity.

Discussion: Cortisol acts as a negative feedback mechanism on both corticotropin-releasing hormone and vasopressin. It has been reported that patients with hypopituitarism who have developed severe hyponatremia, ADH levels are high and get normalized after treatment with cortisone. Our Patient's cortisol level was normal, but he had an Adrenal Insufficiency picture, since he appeared to have sodium wasting in his urine, even though he was volume depleted. This is unique to HIV in which it has been reported that there is likely tubular dysfunction and impaired water handling that is independent of anti retroviral therapy and glomerular filtration rate. There is evidence in vitro and in vivo that there is direct invasion of tubular epithelial cells by HIV virus, even in the absence of viremia. More studies need to done on acquired Glucocorticoid Resistance and its physiopathology should be examined with more detailed.

FR-PO261

Adderall-Associated Severe Hyponatremia with Seizures

Robenson Jean Marie, Maria Saleem Khan, Ganesh B. Shidham. *Nephrology, OSUMC, Columbus, OH.*

Introduction: Adderall (Amphetamine stereoisomer Mixed Salts) is used in Attention deficit disorder (ADD) and works by increasing norepinephrine and dopamine neurotransmitters in brain. Antidiuretic Hormone (ADH) is regulated by osmotic and non-osmotic stimuli. In addition to Catecholamine, other biogenic amines (serotonin, dopamine), polypeptides and even cytokines are implicated in ADH release. Adderall increases thirst and also likely stimulates ADH secretion via non-osmotic pathways, predisposing to hyponatremia. We describe a young female with severe hyponatremia and seizures, on Adderall.

Case Description: 23-year-old female with ADD on Adderall, admitted with altered mental status. On admission day she was vomiting and three hours later experienced a witnessed seizure-like episode. She denied substance abuse or taking more than her usual dose of Adderall. Two months prior, in clinic, her sodium was 131 mEq/L. In the ED she was still lethargic and was intubated. Cardio-vascular examination was unremarkable. She was euvolemic. Sodium was 111 mEq/L and osmolality 229 mOsm/L. Urine sodium was 50 and urine osmolality 143. CT head showed brain edema. She was given 100 ml of 3% saline, switched later to normal saline. In about 5 hours her sodium overcorrected to 125 mEq/L. Saline was stopped and IV D5W was started. She woke up the following day and was extubated. EEG revealed no new seizure. Her sodium increased over 5 days to 135 mEq/L and she was discharged. During the hospital stay she was polyuric with 4-7 liters per day of dilute urine. Once awake she reported being always thirsty and drinking a lot of water at baseline.

Discussion: There has been no report of hyponatremia with Adderall. One can find blogs online where people on Adderall share experience with increased thirst. Our patient had all the risk factors for hyponatremia in the form of thirst and possible stimulation of ADH secretion by Adderall via non-osmotic stimuli. Her GI malaise, which triggered a surge of ADH, led to acute hyponatremia. SIADH is only reported with Ecstasy as amphetamines. Adderall is believed to be safe, however we think fluid intake and urine output should be monitored carefully in conditions of high ADH state like vomiting, pain, and volume depletion.

FR-PO262

Severe Symptomatic Chronic Hyponatremia in Guillain-Barré Syndrome Treated with Tolvaptan

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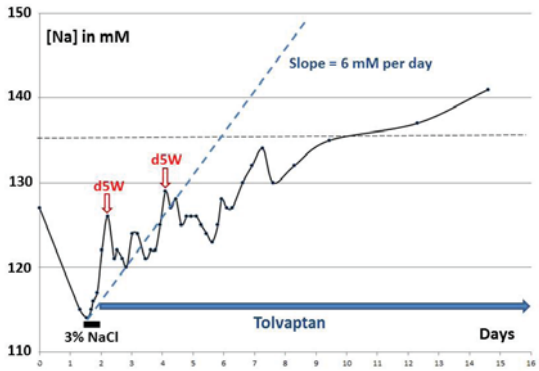
Introduction: Guillain-Barré Syndrome (GBS) is an autoimmune disease characterized by progressive ascending muscle weakness and autonomic dysfunction. Hyponatremia can occur in GBS and may be due to the syndrome of inappropriate antidiuresis (SIAD). Here, we report a case of a patient with symptomatic SIAD treated with Tolvaptan (TVP) —a selective V2 receptor blocker—rather than with hypertonic saline.

Case Description: A 65 year old man experienced muscular pain while mowing his lawn. He progressed to an unsteady gait and paresthesias. Physical examination showed a clinical euvolemia, diminished reflexes, muscle weakness, and limb ataxia suggestive of GBS, confirmed by LP and MRI. Upon admission, his [Na] was 127 mM, but 30 h later dropped to 114 mM. Initially, he was given 200-ml of IV 3% NaCl over 4 h, and then converted to oral TVP (15-mg) when [Na] was 114 mM and urine ([Na]+[K]) was 206 mM, indicating an inability to excrete free water. The course of serum [Na] is shown below, with the goal to increase [Na] by no more than 6 mM per 24 h, and to use free water to re-lower [Na] in case of overcorrection.



By day 4, his impaired short-term memory and impulsiveness had resolved. After 2 weeks on TVP, his [Na] was 141 mM and he was discharged home without fluid restriction on TVP 15-mg M-F, then restarted on Monday if serum [Na] < 136 mM and urine ([Na]+[K]) > serum [Na]. TVP was required for 11 weeks and [Na] was 138-141 mM, and remained in this range off TVP.

Discussion: This case of SIAD in the setting GBS illustrates that TVP can be used to safely treat inpatients with symptomatic chronic euvolemic hyponatremia. Growing data support the safety and efficacy of Vaptans in a dose-dependent fashion. In contrast to 3% NaCl, TVP can be easily continued after discharge without the need for fluid restriction as long as the patient is properly monitored.

Funding: Private Foundation Support

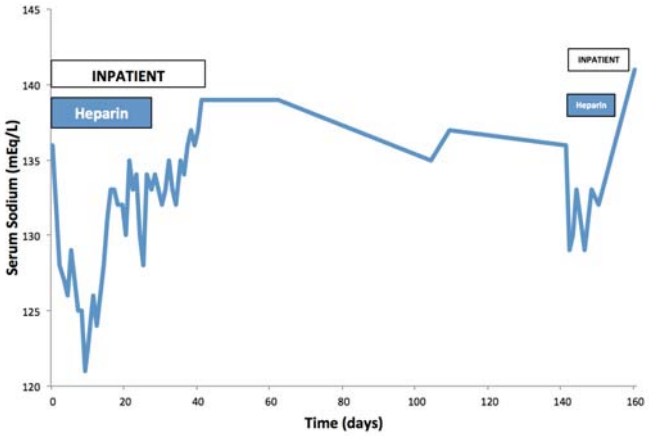
FR-PO263

Recurrent Hyponatremia Secondary to Subcutaneous Heparin Exposure

Yelena Rekhtman Drexler, Sumit Mohan. *Nephrology, Columbia Univ, New York.*

Introduction: Heparin-induced aldosterone suppression is well-described and thought to be secondary to reversible suppression of aldosterone synthase resulting in hyperkalemia commonly (8% of cases) and hyponatremia that is reported less frequently.

Case Description: We present the case of a 38-year-old man with no known medical history who was admitted with S. agalactiae-associated mitral valve endocarditis complicated by septic emboli to the brain and spleen, requiring a mitral valve replacement. Post-operatively, his serum sodium (sNa) decreased gradually to a nadir of 121 mEq/L by post-operative day (POD) #8 with stable renal function (sCr 0.9 mg/dL) and normal serum potassium (3.6–4.6 mEq/L). He developed orthostatic hypotension and had a urinary Na of 148 mEq/L for which free water restriction and sodium chloride tablets were started (then uptitrated to 12 g/d), resulting in a rise in serum sodium to 130 mEq/L but persistent orthostatic hypotension. On POD #20, he had an undetectable aldosterone level of <1.6 ng/dL (normal 4–31), plasma renin activity of 0.87 ng/mL/h (normal 0.25–5.82), and cortisol level of 27.8 ug/dL (normal 5–25), suggesting aldosterone suppression rather than insufficiency. Subcutaneous (SQ) heparin for DVT prophylaxis was stopped, with a resulting rise in sNa to 136 mEq/L and aldosterone to 4 ng/dL over 8 days with no further hyponatremia for the remainder of his hospitalization or subsequent outpatient followup. The patient was readmitted 4 months later for management of gangrene related to prior emboli and again developed hyponatremia (sNa 129 mEq/L) while on SQ heparin, which resolved with cessation of SQ heparin therapy.



Discussion: This case represents the first report of recurrent heparin-induced hyponatremia without concomitant hyperkalemia. Given the widespread use of SQ heparin, this should be considered as an often overlooked cause of hyponatremia with salt wasting.

FR-PO264

Severe Hypernatremia Causing Osmotic Demyelination Syndrome

Sandesh Joshi, Ali Javed, William DiFilippo. *Nephrology, Geisinger Medical Center, Danville, PA.*

Introduction: Rapid correction of hyponatremia is a known risk factor for osmotic demyelination syndrome, but severe hypernatremia can also cause this syndrome, which we should be aware of.

Case Description: A 54 years old women, with history of bipolar disorder on chronic Lithium therapy, chronic kidney disease, esophageal cancer treated with resection and gastrectomy, failure to thrive with protein calorie malnutrition on chronic tube feeds via J tube presented to outside hospital due to nausea, poor oral intake with diarrhea and confusion for few days. Blood work showed severe hypernatremia, sodium of 186 mmol/L prompting transfer to our center. On arrival, she was lethargic, looked very dry and delirious. Was tachycardic as well was hypotensive. A repeat sodium was 192 mmol/L with a fluid deficit of 10L. She was started on intravenous free water (5% Dextrose in water) with rate adjusted as necessary, with an aim to correct not more than 8 mmol/L of sodium in 24 hours. The rate of correction was challenging as she had high urine output. Given her long standing history of lithium use and low urine specific gravity, a diagnosis of nephrogenic diabetes insipidus was made and Hydrochlorothiazide was started. Rate of correction of sodium was appropriate and her sodium level remained stable at approximately 145 mmol/L after 10 days of admission. However, her mental status did not improve, and even after appropriate correction of hypernatremia, she remained very lethargic and slow to respond. An MRI of brain was done which was consistent with osmotic demyelination syndrome.

Discussion: Rapid correction of hyponatremia is a known risk factor for osmotic demyelination syndrome. However, this syndrome can also be found in correction of severe hypernatremia. Awareness of this phenomenon should always be considered in correction of sodium abnormalities.

FR-PO265

Factitious Hyponatremia: A Case Study Talal A. Khan, Purna Bindu Nandigam, Muhammad Awais Arif, Azka Arif, Bette Seamonds, Sandeep Aggarwal. *Drexel Univ College of Medicine, Philadelphia.*

Introduction: Hyponatremia is associated with significant morbidity and mortality. It is important to understand pathophysiology associated with hyponatremia.Clinicians should be aware of factitious Hyponatremia which can trigger un-necessary interventions. Here-in we report an interesting case of spurious hyponatremia secondary to severe triglyceridimia.

Case Description: 40-year-old white male with history of non-insulin dependent diabetes mellitus presented to the hospital with 2 days history of severe abdominal pain. On evaluation his vitals were stable. Initial serum chemistry showed sodium of 122 meq/L and serum triglycerides were 2629 mg/dL. Urine sodium and osmolality were 57mmol/L and 703 mOsm/Kg respectively.Serum glucose was 226mg/dL and total proteins were 6.6g/dL.We suspected falsely low serum sodium as his serum osmolality was 297 mosm/Kg. We sent an arterial sample that's analyzed by direct potentiometric assay, which revealed sodium of 137meq/L.

Upon confirmation it was concluded that this patient had spurious hyponatremia. He was diagnosed with acute pancreatitis which improved over the course of few days.He was started on subcutaneous insulin and oral omega-3 fish oil and as his serum triglycerides came down, his pseudo-hyponatremia improved, confirming our diagnosis.

Discussion: The mechanism underlying psuedohyponatremia is secondary to high serum triglycerides, which markedly reduce the fraction of serum water content. In normal subjects plasma water makes 93 percent of total volume while fats and proteins make rest of the 7%. However in cases of severe hyperlipidemia or hyperproteinemia, this percentage of plasma water falls below 80 percent. In such conditions plasma sodium concentration and plasma osmolality will be unchanged. But commonly used indirect potentiometric methods of serum sodium measurement will show falsely low serum sodium. To eliminate this erroneous laboratory abnormality, direct potentiometric assay (ion-specific) should be used. In our case we used the rapid blood gas analyzer that has direct potentiometric assay capability.Clinicians may find it useful to familiarize themselves with certain methodologoies used by the laboratory and their limitations.

FR-PO266

Manganese Toxicity as a Cause of Altered Mentation in a Chronic Dialysis Patient Karandeep Shergill, Richard E. Wing, Shkendie Velia. *Nephrology, Univ of Rochester, Rochester, NY.*

Case Description: A 60 years old woman with ESRD on hemodialysis for two years suffered a progressive decline in cognitive ability over several months prior to presentation. She presented acutely to the hospital with a 'few days' of confusion, lethargy, slurred speech and worsened tremor. Her past medical history also included hypertension, congestive heart failure, diabetes mellitus, and 'familial' essential tremor for six years. Medications included omeprazole, multivitamin, clonidine, melatonin, erythromycin, citalopram, gabapentin, and ondasetron. Social history was notable for use of well water. On presentation, the patient exhibited lethargy, slurred speech, and disorientation. Vitals: BP 104/56; pulse 56; R.R. 26; temp. 35.2 °C. Her physical exam was also notable for edema and a functional AVF. Her symptoms improved after holding sedatives; however, 1 week into her hospital stay she developed a profound agitation and delirium. EEG was suggestive of a moderate, diffuse encephalopathy. MRI brain showed symmetrical T1 hyper intensity within the bilateral globi pallidi and nigrostriatal tracts. Her Manganese (Mn) level was found to be elevated at 26.5 (Normal 4.2-16.5 mcg/L).On hospital day 23, a 10-day course of Calcium Disodium EDTA, 2 gram i.v. daily chelation therapy was administered. By the end of treatment, the patient's mentation had improved dramatically, coinciding with decreasing Mn levels.

Discussion: Manganese (Mn) circulates in the blood as Mn3+ bound tightly to transferrin or Mn2. High levels of Mn can be neurotoxic, with symptoms similar to those of Parkinson's disease, and include dis-coordination, loss of balance, and confusion. ESRD is significantly associated with an increased risk of Parkinson's disease but Mn toxicity is rarely diagnosed in patients with ESRD. In this case the Mn toxicity was thought to be due to a combination of hepatic cholestasis, Iron deficiency and the increased Mn content in the patient's drinking well water. In the right clinical setting, it may be prudent to check a Mn level on ESRD patients diagnosed with Parkinson Disease or other movement disorder.

FR-PO267

Succinylcholine-Induced Hyperkalemic Cardiovascular Collapse – Therapeutic Challenges Deepak Jasuja, Tarek M. El-Achkar. *Nephrology, Indiana Univ and Indianapolis VA Medical Center, Indianapolis, IN.*

Introduction: Succinylcholine is a depolarizing neuromuscular paralytic agent used for rapid sequence intubation for emergent airway management. It acts through the Acetylcholine receptors (AChRs) located in the junctional region. In certain pathological conditions including upper and lower motor neuron defects, immobilization, muscle atrophy, burns, severe infections, prolonged chemical denervation, muscle trauma, tumor or inflammation, patients are predisposed to a lethal hyperkalemic response. Succinylcholine induced hyperkalemia (SIH) can be initially missed, in predisposed patients who require rapid sequence intubation and develop cardiac arrest.

Case Description: We present the case of 70 kg 45 year old African American male patient with significant neurological history including multiple strokes, secondary right hemispheric encephalomalacia, thalamic dysfunction, seizure disorders and generalized debility. He was sent from a nursing facility with respiratory failure requiring emergent intubation; 120 mg succinylcholine was used as a paralytic prior to intubation. 5 to 10 minutes after intubation, he developed bradycardia followed by ventricular fibrillation and cardiac arrest. His potassium (K) during code was 8.3 meq/L; he was treated twice with calcium gluconate, Dextrose, insulin, and bicarbonate injections. He also received epinephrine pushes and defibrillation; repeat k after 5 minutes was 9.3; He was transferred to ICU and nephrology was urgently consulted for his hyperkalemia. His kidney function was normal. An emergent dialysis access was placed but a repeat k (measured 3 hrs after the code) came back as 3.1, and SIH was diagnosed.

Discussion: SIH typically lasts around 10-15 minutes but may last longer depending on the degree of upregulation in AChRs. Acute dialysis with low K bath to treat this self-resolving hyperkalemia could be detrimental. Cardiovascular instability, hyperkalemia with EKG changes that occurs within 2-5 minutes after intubation should alert providers to a potential diagnosis of SIH. It is imperative to follow up on potassium levels frequently to establish the diagnosis and avert additional complications.

FR-PO268

Normocalcemic Hyperparathyroidism and Calcium Phosphate Crystals during Pregnancy Ed Gould, Julia Lewis. *Div of Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

Introduction: The hormonal alterations seen both during pregnancy and lactation invoke a variety of clinically significant physiologic changes. Here we present the case of a woman who developed hematuria after beginning lactation secondary to calcium phosphate crystal precipitation with CaP crystals on renal biopsy.

Case Description: The patient was a 26 year old G1P0 female with no significant PMHx presenting with unremitting gross hematuria at the 36th week of pregnancy. Lactation began during her 34th week of pregnancy. Her hematuria was initially thought to be vaginal spotting and was induced during her 37th week. After delivery, she was referred to urology who did cystoscopy and ureteroscopy without identifying a lower GU source. At the time of Nephrology referral she was five months post-partum and continued to breast feed; her urine remained grossly bloody with every void. Further review found no culpable medications, no history of nephrolithiasis, no TB risk factors or trauma. Physical examwas unremarkable. She had normal renal imaging by sonography. Laboratory evaluation showed a Cr of 0.75, normal electrolytes including a calcium of 8.4, normal CBC, and no coagulopathy. Urinalysis with pH of 6.0, large blood, >200 RBCs, and +1 protein. No dysmorphic red cells or casts were identified and no crystals on urine microscopy. Limited serologic workup was negative. Serum phosphorous was 3.2, iPTH was measured at 131. After discussion, decision was made to proceed to renal biopsy. Biopsy showed intratubular calcium phosphate crystal deposition. A twenty-four hour urine collection revealed hyperphosphaturia and hypercalciuria. One year after delivery she stopped breast feeding. Within one month, her iPTH returned to normal and her hematuria resolved.

Discussion: Both nephrolithiasis and primary hyperparathyroidism are recognized clinical problems in pregnancy. In our patient, the temporal association of her symptoms with lactation – which can begin as early at the second trimester – and the development of normocalcemic hyperparathyroidism with calcium phosphate deposition without frank renal stones is interesting and has not been previously reported.

FR-PO269

Refractory Hypercalcemia in a Patient with Adult T Cell Leukemia/Lymphoma Laith Farah Al-Rabadi, Abdul Qadir, Abraham Cohen-Bucay, Amanda Rubenstein, Craig E. Gordon. *Nephrology, Boston Univ Medical Center.*

Introduction: Adult T cell leukemia/lymphoma (ATLL) is a rare malignancy of the peripheral T lymphocytes, caused by infection with human T cell lymphotropic virus -1 (HTLV-1). Hypercalcemia occurs commonly with ATLL. However, few cases of hypercalcemic crisis are reported.

Case Description: We present a 35 year old male patient presenting with abdominal pain, confusion and acute kidney injury. Serum calcium was 21.5 mg/dl, Creatinine 2.9 mg/dl, phosphate 5.5 mg/dl. He was resuscitated with 4 liters of isotonic saline and IV calcitonin was administered without immediate response. In light of a QT interval of 326 msec and the extreme hypercalcemia, urgent hemodialysis was performed using low-calcium dialysate (2[thinsp]mmol/L) to avoid large changes in calcium concentration leading to potential hemodynamic instability or cardiac arrhythmia. Dialysis was well tolerated with a decline in serum calcium to 13 mg/dl. However, the calcium level repeatedly rebounded to above 20 mg/dl mandating frequent sessions of dialysis. In the interim, denosumab, a human IgG2 monoclonal antibody that binds to human RANKL, was initiated with eventual improvement in serum calcium to normal values and dialysis was discontinued. Workup showed that PTH was suppressed at 8 pg/ml. AlP and LDH were significantly elevated at 432 U/L and 840 U/L, respectively. CT scan and MRI abdomen showed no lymphadenopathy. Skeletal survey showed no lytic lesions. MRI and PET scan showed increased bone activity. Ultimately, HTLV serology returned positive and a bone marrow biopsy showing abnormal T cell population. CHOP chemotherapy was initiated. Unfortunately, the patient ultimately succumbed to the illness within 6 months of diagnosis.

Discussion: Hemodialysis can be used in extreme hypercalcemia, with caution to avoid excessive lowering of serum calcium, while awaiting more definitive treatments. No guidelines are available about the indications for or timing of dialysis for extreme hypercalcemia, or the role and safety of calcium-free or low-calcium dialysate for extreme hypercalcemia. HTLV-associated ATLL is increasingly recognized as a cause of extreme hypercalcemia and should be investigated for in patients at risk.

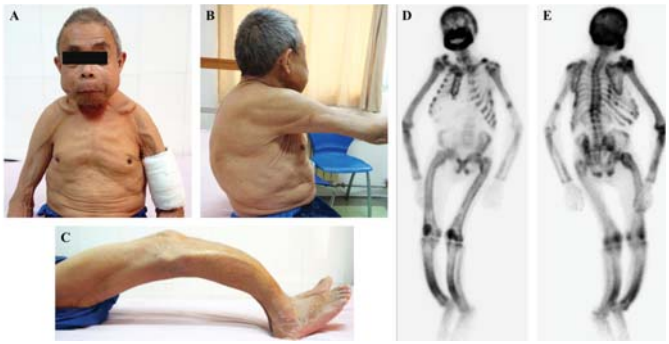
FR-PO270

Advanced Uremic Leontiasis Ossea Ameliorated by Total Parathyroidectomy
Ningning Wang, Jingjing Zhang. *Nephrology, First Affiliated Hospital of Nanjing Medical Univ, China.*

Introduction: Leontiasis ossea (LO) describes a lion-like face bone disease with overgrowth of maxilla and mandible. Uremic leontiasis ossea (ULO) is a rare systemic disease in patients suffered from chronic renal insufficiency and secondary hyperparathyroidism (SHPT). Here we report a 62-year-old male patient diagnosed of ULO.

Case Description: Global severe bone deformity of the patient was determined by technetium-99m (99mTc)-MDP bone scintigraphy. The lower limb and craniofacial bones was determined by radiographs and computed tomography respectively. Ultrasonography (US) and emission computed tomography (ECT) were used for preoperative localization to exclude ectopic and supernumerary parathyroid glands.

The main clinical features include advanced craniofacial disfigurement, dysarthria, pectus carinatum, kyphosis, left humerus fracture and severe bowing of lower extremities. The patient was shortened from 170cm to 150cm.Technetium-99m (99mTc)-MDP bone scintigraphy indicated: a) Increased activity in bones, especially in the axial skeleton, calvaria, mandible, costochondral junctions, long bones and a "tie" sternum;b) Increased bone-to-soft tissue ratio's; c) Higher radionuclide uptake in left humerus dues to prior fracture; d) Spinal kyphosis deformity owing to multiple thoracic and lumbar vertebra compression fracture; e) Severe bowing of legs.



The patient was under successful total parathyroidectomy (TPTX) with a remove of five parathyroid glands. Postoperative improvement tendency of craniofacial disfigurement, dysarthria, itching, osteodynia and blood biochemical indexes was observed within a 10-month follow-up.

	Serum Ca (mg/dl)	Serum P (mg/dl)	Serum ALP (U/L)	Serum iPTH (pg/ml)
Before PTX	11.12	5.13	1138.7	2183.2
After PTX	9.32	2.16	141	4.8

Discussion: ULO is a rare systemic disease in chronic kidney disease. Parathyroidectomy is critical to prevent the progression of clinical complications in these patients.

Funding: Government Support - Non-U.S.

FR-PO271

Neonatal Nephrolithiasis in an SLC7A9 Heterozygote with Cystinuria
Watfa S. Al-Dhaheri, Paul R. Goodyer. *Pediatric Nephrology, Montreal Children Hospital McGill Univ, Montreal, QC, Canada.*

Introduction: Cystinuria is a hereditary form of nephrolithiasis accounting for 1% of urinary tract stones. In Quebec, a newborn urine screening program identifies newborns with cystinuria. In previous publications we described a form of "transient neonatal cystinuria" in which babies with heterozygous null SLC7A9 mutations initially excrete cystine in the homozygous range, but urine cystine levels gradually fall below the stone-forming range as the renal proximal tubular undergoes functional maturation over the first 1-3 years of life.

Case Description: Here we present a girl identified by the newborn screening program whose urine cystine was confirmed to be 2370 mmol/g creat at 3 months of age. Her father's urine cystine 81 was within the normal range (<100 mmol/g creat) whereas her mother's urine cystine was 1069 mmol/g creat, suggesting that the baby was an SLC7A9 heterozygote with transient neonatal cystinuria. At the time of baseline renal ultrasonography (8 months), she was found to have two large non-obstructive stones in the left kidney (measuring 1.5 and 1.7 mm) and she was started on intensive potassium citrate therapy to alkalinize the urine and dissolve the stones. By 18 months, urine cystine had fallen to 1600 mmol/g creat and repeat ultrasonography showed that both stones had resolved. Molecular testing showed that the child had inherited a heterozygous mutant SLC7A9 allele bearing a deletion of Exon 12. No coding sequence mutations, deletions or rearrangements of the SLC3A1 gene were identified.

Discussion: To our knowledge this is the first known example of nephrolithiasis in transient neonatal cystinuria. Our case is important since it indicates the importance of baseline ultrasonographic screening in affected asymptomatic infants and demonstrates that the presence of one normal SLC7A9 gene does not protect against stone formation during the period of transient neonatal cystinuria.

FR-PO272

Association of Trimethylamine-N-Oxide (TMAO) with Nutrition, Inflammation, and Outcomes among Incident Dialysis Patients
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Background: Trimethylamine-N-oxide (TMAO) is a product of metabolism of phosphatidylcholine (lecithin) and carnitine by the intestinal microbiome that has been strongly associated with all-cause and cardiovascular (CV) mortality and CV hospitalizations in the general population.

Methods: We examined 235 participants in the Comprehensive Dialysis Study (CDS), measuring albumin, prealbumin, C reactive protein (CRP) and TMAO concentrations. TMAO was measured by liquid chromatography and online tandem mass spectrometry in CDS participants and in 3 pooled samples of serum from healthy controls. Data on creatinine, co-morbidities, hospitalization and death were obtained through the USRDS, and patients were followed for a median of 6 years (P25:3.9, P75:6.6). We used Cox proportional hazards modeling to examine the association of log TMAO with all-cause mortality and with time to CV death or CV hospitalization.

Results: TMAO was elevated compared to control (mean 48.4 ± 30.9 versus 1.41 ± 0.49 μM) and was directly correlated with albumin (Spearman rank correlation 0.24, [95% CI 0.12, 0.35]; p < 0.001), prealbumin (0.19 [0.07, 0.31]; p = 0.003), and creatinine (0.21[0.08, 0.33]; p = 0.002), and inversely with log CRP (-0.18 [-0.30, 0.06]; p = 0.005). For mortality, the multivariable HR for log TMAO was 0.84, 95% CI: 0.65-1.09; p =0.19. For the combined CV outcomes, the univariate HR for log TMAO was 0.95, 95% CI: 0.62-1.46; p=0.81.

Conclusions: TMAO levels were markedly elevated in patients new to HD and correlated directly with biochemical markers of nutrition and inversely with markers of inflammation. The hazard ratios for (log) TMAO predicting all-cause mortality or CV death or hospitalization were considerably smaller in our cohort than in the general population, suggesting that adverse vascular effects of TMAO may be counterbalanced by its association with better nutritional status in HD.

Funding: NIDDK Support

FR-PO273

Comparison of Angiotensin Receptor Blocker (ARB) versus ACE Inhibitor (ACEI) Administration on High Density Lipoprotein (HDL) Functionality in Patients on Maintenance Hemodialysis (MHD)
Ryohei Kaseda, T. Alp Ikizler, Nancy J. Brown, Valentina Kon. *Vanderbilt.*

Background: ACEI and ARB reduce cardiovascular events (CVE) in the general population. Although MHD are at high CVE risk, few studies have directly addressed their efficacy and recent findings suggest differences. Since CKD disrupts the normally protective HDL functions, we compared ACEI and ARB treatment on HDL functions in MHD.

Methods: After 3 week washout, we randomly assigned 40 MHD to placebo, ramipril or valsartan. HDL was isolated from blood at the start and 3-6 months later (pre/post). Cell cholesterol and efflux were assessed by gas chromatography and inflammatory markers (TNF-a, MCP-1, IL-1b) and cellular transporters (ABCA1/ABCG1) by RT-PCR in HDL-exposed human THP-1 cells and mouse macrophages.* P<0.05 **P<0.01.

Results:

	Placebo (pre/post)	ARB (pre/post)	ACEI (pre/post)
Efflux-THP-1	24.1±1.0/17.0±1.9**	24.2±1.0/22.4±2.2	23.3±1.6/22.2±1.2
Efflux-mouse cells	37.1±1.3/29.3±1.9**	35.6±5.6/42.7±3.6	34.5±0.9/33.2±2.0
ABCA1	0.84±0.06/0.95±0.05	0.94±0.09/0.95±0.06	1.13±0.07/1.41±0.09*
ABCG1	0.87±0.08/0.77±0.03	0.70±0.08/1.19±0.18*	0.70±0.04/0.85±0.04*
TNF-α	1.00±0.09/1.20±0.07	1.32±0.10/1.43±0.12	1.37±0.07/2.01±0.18*
MCP-1	0.97±0.13/1.15±0.10	1.25±0.17/1.28±0.13	1.44±0.11/1.89±0.13
IL-1β	1.63±0.07/1.97±0.13	1.93±0.10/1.97±0.12	1.73±0.11/2.78±0.23**

Pre/post in placebo found HDL becomes significantly less effective in facilitating efflux. ACEI or ARB abrogated this deterioration and increased cholesterol transporters. Neither ARB nor ACEI quelled HDL inflammatory response and ACEI potentiated TNF-a, IL-1b compared to placebo or ARB.

Conclusions: HDL cholesterol acceptor function in MHD deteriorates over time, but can be stabilized by ACEI and ARB, which stimulate transporters. By contrast, inflammatory effects of uremic HDL do not respond to the interventions and are amplified by ACEI. The findings reveal utility of antagonizing angiotensin in MDH and suggest a mechanism for superiority of ARB versus ACEI.

Funding: Other NIH Support - Heart, Lung, & Blood

FR-PO274

Lower C-reactive Protein and Better Hemodialysis Survival Are Associated with Regular Exercise Activity: Longitudinal Outcomes from the ACTIVE USRDS Special Study Nancy G. Kutner,[1] Rebecca H. Zhang,[1] Yijian Huang,[1] Jeanie Park.[2] *[1]USRDS Rehabilitation/Quality of Life Special Studies Center, Emory Univ, Atlanta, GA; [2]Medicine, Renal Div, Emory Univ, Atlanta, GA.*

Background: Inflammation markers are powerful predictors of hemodialysis (HD) patient mortality, and a sedentary lifestyle is a risk for the inflammatory syndrome, but the association of regular exercise activity with inflammation over time in an HD cohort has not been studied.

Methods: Longitudinal measures of physical activity and C-reactive protein (CRP) were obtained 2009-2013 for a multi-center cohort of 716 prevalent HD patients in a USRDS special study. Activity energy expenditure (kcal/week) was estimated from Minnesota Leisure Time Activity responses at baseline, 12-months, and 24-months; <500 kcal/week expenditure defined sedentary status (Dergance et al. 2003). Baseline CRP concentrations were right skewed. For inference testing CRP values were natural log transformed. The association of kcal/week with CRP at the three time points was examined in generalized estimating equations, and baseline activity status, CRP and mortality over a median follow-up of 718 days were investigated in a Cox proportional hazards model.

Results: After adjustment for demographic, clinical and HD treatment variables (including BMI, comorbid conditions, smoking, catheter use, HD hours/week, and vintage), sedentary patients on average were 0.21 higher than non-sedentary patients in CRP value on the log scale, (ß = 0.21, 95% confidence internal [CI] 0.04-0.39; P=0.02), and they had a more than 50% greater mortality risk (hazard ratio 1.58; 95% CI 1.03-2.42; P = 0.04). Among 360 patients who had baseline and 12-month activity and CRP data, sedentary patients' median CRP was 3.70 mg/L at baseline and 4.33 mg/L at 12-months, while active patients' values were 3.38 mg/L at baseline and 3.27 mg/L at 12-months.

Conclusions: Observed associations of HD patients' regular exercise activity with CRP and survival are consistent with the hypothesis that lower inflammation is one plausible biologic pathway by which greater physical activity may be associated with lower mortality risk.

Funding: NIDDK Support, Other NIH Support - NCATS grant UL1T2000454, Clinical and Translational Science Institute award program, National Center for Research Resources

FR-PO275

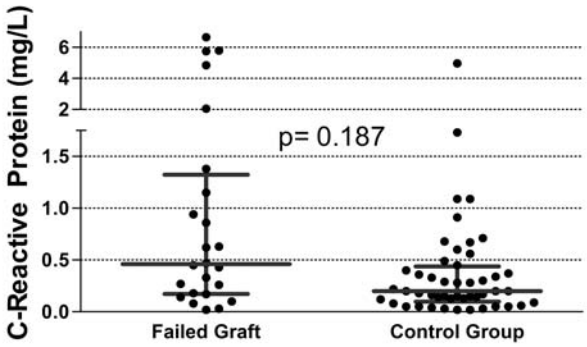
The Failed Kidney Allografts Are Not Associated with Increase in Ultrasensitive C-Reactive Protein Roxana Villca Gonzales, Raquel Aracely Vazquez, Josefina Alberú, Luis E. Morales-Buenrostro. *Nephrology, National Inst of Medical Sciences and Nutrition Salvador Zubiran, Mexico City, DF, Mexico.*

Background: A non-functional graft is associated with lower hemoglobin levels, erythropoietin resistance, and increased morbidity and mortality. Therefore, some experts advocate graft nephrectomy to improve their prognosis. **OBJECTIVE**: To establish if there is any difference in inflammatory status between patients on dialysis with non-functional graft versus non-transplanted patients.

Methods: Case-control study. Case were patient with retained non-functional graft, without surgical procedures or infections during previous 2 months. Control cases were defined as dialysis patients with similar characteristics but without previous transplant, paired 2:1 by age, gender, ESRD etiology, and time on dialysis. Ultrasensitive C-reactive protein was determinate by ELISA. Chi Square test, Student T test, and Mann-Whitney U test were used.

Results: A total of 24 cases and 48 controls were included. The relevant characteristics are showed in table. Levels of C-reactive protein in both study groups are depicted in Figure.

Variable	Failed Graft (N=24)	Controls (N=48)	p
Age	36.07±10.64	35.84±12.41	0.938
Male Gender n (%)	15 (62.5%)	30 (62.5%)	1.000
Hemoglobin (g/dL)	9.10±2.11	11.18±2.70	0.002
PTHi (pg/mL)	487.96±492.94	677.62±558.37	0.207
Albumin (mg/dL)	3.98±0.58	4.29±0.51	0.028
EPO dose (UI/w)	8333.33±4350.57	5666.66±5756.41	0.035
Ferritin (ng/mL)	323.10 (76.70 - 911.70)	248.00 (51.97 - 451.25)	0.291
Procalcitonin (ng/mL)	0.45 (0.19 - 0.63)	0.34 (0.16 - 0.68)	0.742



Conclusions: This study shows that having a non-functional graft NO necessarily results in an inflammatory status. Any case with suspected chronic inflammation should be studied comprehensively to exclude other causes before assigning it to the failed kidney allografts and propose nephrectomy.

Funding: Government Support - Non-U.S.

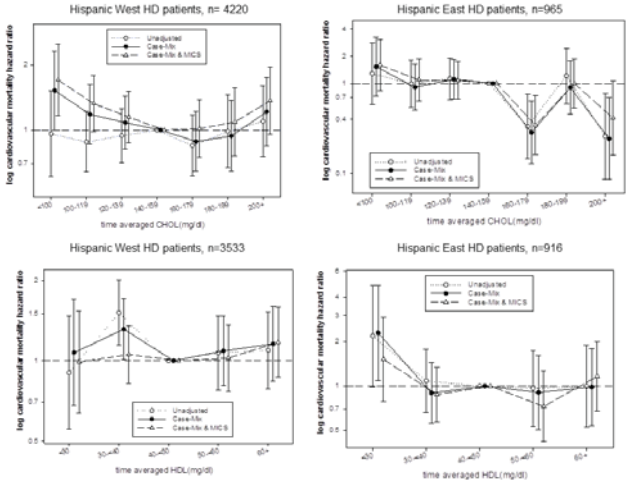
FR-PO276

Association of Lipid Profile with Cardiovascular Mortality in Dialysis Patients of Hispanic Origin in the East versus West Coast Is Significantly Different Pouya Abhari,[1] Hamid Moradi,[1] Madeleine V. Pahl,[1] Elani Streja,[1] Nosratola D. Vaziri,[1] Csaba P. Kovesdy,[2] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Memphis VA MC, Memphis, TN.*

Background: Previous studies have shown paradoxic associations between serum lipid levels and cardiovascular (CV) mortality in hemodialysis (HD) patients. Subgroup analyses have confirmed these findings in patients of Hispanic origin. However, significant racial-ethnic differences exist in patients of "Hispanic" background. For example, the Hispanic population of the West Coast consists mainly of those from Mexico and Central America, while on the East Coast there is a large Afro-Caribbean contingency. We hypothesized that major differences exist in association of dyslipidemia and cardiovascular survival in Hispanic HD patients on the West versus East Coast.

Methods: We examined the CV survival impact of serum lipids in a 2-year (6/2005-6/2007) cohort of 5,185 HD patients of Hispanic origin being treated in clinics of a large dialysis organization (in California, Texas, New York, New Jersey and Florida) using Cox models adjusted for demographics and case-mix and markers of malnutrition-inflammation complex (MICS) variables.

Results: In this cohort there were 4,220 West and 965 East Coast Hispanic patients. In fully adjusted models, significant differences were noted in the association of lipid markers and CV mortality between these 2 groups.



Conclusions: Significant differences exist in association of serum lipids with CV mortality in MHD patients of Hispanic background depending on whether they live on the West or East Coast of the United States. These geographical differences most likely reflect ethnic and racial differences which usually go unnoticed. Future studies should take into account these critical variations in a population of patients who will make up a majority of our society in the future.

Funding: NIDDK Support

FR-PO277

Protein Energy Wasting Is a Risk Factor for Infectious Complications in Both Hemodialysis and Peritoneal Dialysis Patients Raymond T. Krediet,[1] Anouk Van Diepen,[1] Tiny Hoekstra,[2] Joris I. Rotmans,[3] Marit M. Suttorp,[2] Dirk Gijsbert Struijk,[1] Friedo W. Dekker.[2] *[1]Nephrology, Academic Medical Centre, Amsterdam, Netherlands; [2]Clinical Epidemiology, Leiden Univ Medical Centre, Leiden, Netherlands; [3]Nephrology, Leiden Univ Medical Centre, Leiden, Netherlands.*

Background: Protein-energy wasting (PEW) has been linked to impaired immunity in both peritoneal dialysis (PD) and hemodialysis (HD) patients. The objective of our study was to investigate the association between protein-energy wasting and the risk for infections in both HD and PD patients.

Methods: In a prospective multi-center cohort study of incident dialysis patients (NECOSAD), the 7-point Subjective Global Assessment of nutritional status (SGA) was assessed every six months. Information about infections of all patients from 5 participating hospitals was retrospectively collected from the start of dialysis until censoring or 3 years of follow up. PEW was defined as SGA 1-5. Incidence rate ratios (IRR) were calculated with (Time-dependent) Poisson regression considering all infections in 3 years of follow-up. Models were adjusted for age, sex, ethnicity, primary kidney disease, smoking and comorbidity.

Results: This study included 400 patients, of whom 240 initially started on HD and 160 on PD. Thirty-two percent of HD patients and 18% of PD patients suffered from PEW

at dialysis start. In 3 years of follow up the incidence rate of infection was 0.46/HD year and 0.68/PD year. Both HD (Adjusted IRR: 1.42; 95%CI: 1.06, 1.92) and PD patients (1.37; 0.98, 1.92) suffering from PEW showed an increased risk for infection compared with patients with a normal nutritional status. Compared with HD patients with a normal nutritional status (reference group), 6-months adjusted IRRs for infection were 2.06 (95%CI: 1.60, 2.65) for PD patients with a normal nutritional status, 2.24 (1.65, 3.04) for HD patients with PEW, and 3.37 (2.27, 5.03) for PD patients with PEW.

Conclusions: Protein-energy wasting was associated with an increased risk for infection in both HD and PD patients. Routine screening of nutritional status is important in all dialysis patients.

FR-PO278

High Serum Hemojuvelin Levels in Hemodialysis Patients with Acute Infection Chia-Yu Wang, Kristin M. Corapi, Ishir Bhan, Jodie L. Babitt, Herbert Y. Lin. *Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Hemojuvelin (HJV), encoded by the *HFE2* gene, functions as a BMP co-receptor to activate hepcidin expression through the BMP/SMAD signaling pathway. Humans with *HFE2* mutations develop juvenile hereditary hemochromatosis. HJV can be cleaved and secreted as soluble hemojuvelin (sHJV), which can selectively bind to BMP ligands to inhibit hepcidin expression. Studies using cell culture and animal models suggest that HJV expression is regulated by inflammation and that sHJV levels increase in iron deficiency, conditions found in patients with chronic kidney disease and on hemodialysis (HD). In this study, we examined whether sHJV levels are altered in HD patients with acute infection.

Methods: After IRB approval, HD patients with (N=30) or without (N=28) acute infection were enrolled at the time of hospital admission and blood was collected at the start of the first inpatient HD session. sHJV levels were measured using a novel validated two-site enzyme linked immunosorbent assay (ELISA). Demographics, routine laboratory parameters, and dialysis details were compared using univariate analyses. Multivariable linear regression was used to adjust for possible confounders.

Results: Infected and non-infected HD patients were similar with respect to gender, race, dialysis vintage, and vascular access type. Infected HD patients were at 7.3 higher odds of having a detectable sHJV level (95% confidence interval 1.8 - 29.4, p=0.003). Patients with a detectable sHJV level were younger (median 57 versus 70 years, p=0.045), had lower iron levels (33 versus 50 mg/dL, p=0.001) and a smaller proportion had diabetes (29.4% versus 65.9%, p=0.01). Lower transferrin saturation and ferritin were noted, but not significantly different (p=0.08, and p=0.06 respectively). After adjusting for potential confounders, only age and iron level remained significant predictors of having a detectable sHJV level.

Conclusions: sHJV may be a new biomarker for acute infection in HD patients. Detailed mechanisms of how sHJV is regulated by inflammation and iron status need to be investigated.

Funding: NIDDK Support

FR-PO279

Serum Procalcitonin as a Predictive Test for Bacteremia in Acute Pyelonephritis Sihyung Park, Bongsoo Park, Kyubok Jin, Yang Wook Kim. *Dept of Internal Medicine, Inje Univ Haeundae Paik Hospital, Busan, Republic of Korea.*

Background: Serum procalcitonin is a specific biomarker for bacterial infection, which rises rapidly after bacterial infection. It is known that levels of procalcitonin are correlated with the severity of disease and the mortality of patients with pneumonia and sepsis. However, the effectiveness of procalcitonin in acute pyelonephritis is yet to be known. This study was aimed to evaluate the effectiveness of procalcitonin as a predictive test for bacteremia in acute pyelonephritis.

Methods: During the period of January 2012 to June 2013, a total of 140 patients admitted to Haeunade Paik Hospital of Inje University due to acute pyelonephritis and had a positive urine culture results finally. Serum procalcitonin, CRP, and white cell count at pre (D1) and post (D3-7) treatment were measured and blood and urine culture test were conducted in all patients. In order to access the ability to predict bacteremia of procalcitonin, the levels of procalcitonin between blood culture positive group and negative group were analyzed.

Results: Pre-treatment procalcitonin (D1) level was 0.77 ng/mL (95% CI: 0.42-1.60) in blood culture negative group, the level was 4.89 ng/mL (95% CI: 2.88-9.04) in blood culture positive group respectively. The level of procalcitonin in blood culture positive group increased to a statistically significant degree. Also, the CRP was increased significantly in blood culture positive group than that of the negative group (Table 1). Based on positive correlation between procalcitonin, CRP and bacteremia, receiver operating characteristic (ROC) curve analysis was performed. Area under the ROC curve (AUC) was 0.728 for procalcitonin while AUC was 0.614 for CRP. Thus procalcitonin level demonstrated more effectively to predict bacteremia, compared with CRP (Figure 1).

Conclusions: Serum procalcitonin is a useful predictive test for bacteremia in acute pyelonephritis. Through the early detection of bactermia, serum procalcitonin can help toestimate the prognosis and thecomplications such as multiple organ failure or sepsis.

FR-PO280

Immune Changes Associated with Home Hemodialysis Todd Fairhead,[1,2] Andreea Slatculescu.[1,2] [1]*Kidney Research Inst, Ottawa Hospital Research Inst, Ottawa, Canada;* [2]*Dept of Medicine, Univ of Ottawa, Ottawa, Canada.*

Background: Infection is a leading cause of death in patients with end-stage renal disease (ESRD). This is partly due to dysfunctional immunity, leading to poor immunization success, increased chronic or latent infections, and elevated levels of serum inflammatory markers. Frequent dialysis therapy improves patient outcomes and quality of life; but, it is currently unknown if this correlates with enhanced immunity.

Methods: We recruited 12 patients from the Ottawa Hospital currently on extended-home hemodialysis (EHHD) plus 18 healthy volunteers (HV) as controls for optimal immune function. We are now enrolling a matching-cohort of conventional in-center hemodialysis (CHD) patients. For this study, we used serum biomarkers of inflammation as predictors of overall immune status and we evaluated cellular immunity based on monocyte-derived dendritic cell (MDDC) maturation phenotype, dextran endocytosis, and T-cell activation capacity in an allogeneic mixed lymphocyte reaction using flow cytometric analysis. Intrinsic T-cell function was further measured by CFSE proliferation and cytokine production.

Results: Serum IL-6 and IL-10 levels were significantly higher in EHHD patients compared to HVs. However, our preliminary CHD cohort data showed that IL-6, CRP, and IL-10 levels were further elevated in CHD versus EHHD patients. MDDCs from EHHD patients exhibited a mature phenotype when stimulated with LPS and, compared to HVs. A significantly larger percentage of cells expressed co-stimulatory molecules (CD83 and CD40), adhesion molecules (CD11c), and endocytic receptors (CD206). We also observed robust T-cell proliferation and allogeneic activation in EHHD patients, although this did not differ statistically from HVs.

Conclusions: Serum pro- and anti- inflammatory markers are elevated in EHHD patients compared to HVs. This disrupted cytokine balance appears to increase the maturation status of MDDCs in individuals with renal failure, which possibly contributes to the chronic inflammation syndrome. Although EHHD therapy does not restore the healthy physiologic environment, it appears to be associated with decreased inflammation and cellular maturation/activation compared to CHD.

Funding: Private Foundation Support, Clinical Revenue Support

FR-PO281

Probiotic *Bifidobacterium animalis* subsp. *lactis* Bi-07 Restores Epithelial Tight Junction Proteins and Ameliorates Microinflammation in Experimental Uremia Meng Wei, Hongli Jiang. *Dialysis Dept of Nephrology Hospital, First Affiliated Hospital of Medicine School, Xi'an Jiaotong Univ, Xi'an, Shaanxi, China.*

Background: Uremia is characterized by intestinal bacterial translocation, which contributes to the development of microinflammation. Probiotic *Bifidobacterium animalis* subsp. *lactis* Bi-07 alleviates bacterial translocation and ameliorates microinflammation inexperimental uremia in previous study. This study investigated whether the probiotic *Bifidobacterium animalis* subsp. *lactis* Bi-07 enhance the intestinal barrier by restoring epithelial tight junction proteins.

Methods: Thirty Sprague–Dawley rats were divided into 3 groups of 10 rats each: the sham group, which underwent only laparotomy; the uremia group, which underwent 5/6 nephrectomy; and the uremia + probiotic group, which underwent 5/6 nephrectomy and daily intragastric administration of B. animalis subsp. lactis Bi-07 for 4 weeks. The animals were then euthanized and their ileum were removed. Ultrastructure of intestinal epithelial tight junction (TJ) complex was observed by scanning electron microscopes (SEM). The expression of the key constituents of TJ were evaluated by realtime polymerase chain reaction and western blot analysis.

Results: The uremic rats showed significant microinflammation. Administration of B. animalis subsp. lactis Bi-07, resulted in marked amelioration of microinflammation. Realtime polymerase chain reaction and western blot showed that constituent of TJ (ZO-1,occludin) in uremia + probiotic group were restored compared with uremia group. Ultrastructural observations by SEM reveal that probiotic B. animalis subsp. lactis Bi-07 treatment marked restored the epithelial intercellular space and microvilli, suggesting a benefit for TJ ultrastructural intergrity.

Conclusions: This study demonstrates the probiotic B. animalis subsp. lactis Bi-07 restores epithelial tight junction proteins, thereby, ameliorates microinflammation in uremia.

Funding: Government Support - Non-U.S.

FR-PO282

Targeting Microbiota Derived Uremic Retention Solutes with Antibiotics Ruben Poesen,[1] Bert Bammens,[1] Patrick Augustijns,[2] Pieter Evenepoel,[1] Bjorn Meijers.[1] [1]*Nephrology, Univ Hospitals Leuven, Belgium;* [2]*Drug Delivery and Disposition, Univ of Leuven, Belgium.*

Background: The gut microbiota contributes substantially to uremic retention solutes accumulating in CKD. p-Cresyl sulfate and indoxyl sulfate are representatives of this group of solutes and associate with adverse outcome in patients with renal dysfunction. Whether antimicrobial therapy has a direct and sustained effect on generation of these solutes in CKD patients has not been studied to date.

Methods: Serum levels and generation rates of p-cresyl sulfate and indoxyl sulfate were prospectively measured in CKD patients receiving antibiotics. To minimize the effect of systemic infection, we studied a homogenous population of peritoneal dialysis patients receiving flucloxacillin for exit site infection. We collected serum, urine and peritoneal

dialysate at baseline, as well as immediately and 6 weeks after treatment. Total removal rate, a surrogate of intestinal generation, was calculated using 24h urinary collection and dialysate. Differences were compared using the Wilcoxon signed rank test.

Results: In this ongoing trial, 11 peritoneal dialysis patients (mean age 55y, dialysis duration 11.8 months, weekly Kt/V 2.32) have already been included. Serum levels of p-cresyl sulfate significantly decreased during antimicrobial therapy (median decrease 46%, P 0.03), also accompanied by a trend of lower total removal rates (-60%, P 0.08). For indoxyl sulfate, we observed a trend of lower serum levels (-33%, P 0.12), and concomitant significant decreases in total removal rate (-21%, P 0.04) following antimicrobial therapy. However, there were no significant changes when comparing baseline serum levels and total removal rates of both solutes with those obtained 6 weeks after treatment.

Conclusions: Antibiotics decrease intestinal generation of microbiota derived uremic retention solutes in CKD patients. Therefore, antimicrobial therapy should be taken into account when interpreting solute levels in observational and intervention trials. Although single and short-term antibiotic exposure had no sustained solute-decreasing effect, probably due to recovery of gut microbiota, the long-term effects of frequent antimicrobial treatment remains unknown.

Funding: Government Support - Non-U.S.

FR-PO283

Relationship between Geriatric Nutritional Risk Index and Subpopulation Lymphocyte Counts in Hemodialysis and Peritoneal Dialysis Patients

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Background: No standard method for assessing the nutritional status in dialysis patients. In the present study, we undertook an evaluation to determine whether estimation of geriatric nutritional risk index (GNRI) and lymphocyte subset counts can be helpful in diagnosis of malnutrition in hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods: We examined the GNRI and lymphocyte subset counts of 50 HD patients (55.8 ± 12.7 years; 28 men and 22 women) and 16 CAPD patients (49.8 ± 14.5 years; 10 men and 6 women). The GNRI is calculated based on the serum albumin level and total lymphocyte count and uses the following equation: GNRI= [14.89 C albumin (g/dL)]+[41.7 C (weight/ideal body weight)]. Logistic regression analysis was performed for predicting malnutrition in dialysis patients.

Results: The average GNRI value was 100.1 ± 8.4 in HD patients and 99.2 ± 8.1, and GNRI values were normally distributed. lymphocyte subset counts were not different between HD patients and CAPD patients. Lymphocyte subset counts were lower in patients with higher GNRI (GNRI ≥ 100).

Table 4. Clinical characteristics of 66 dialysis patients according to CD19 count at start of the study

Variables	CD19 ≥ 100 (n=41)	CD19 < 100 (n=25)	P value
Age	55.1 ± 13.7	53.1 ± 12.7	0.565
Sex (Male/Female)	21/20	17/8	0.208
DM	15 (36.6%)	10 (40%)	0.799
HD/PD	32/9	18/7	0.768
Duration of dialysis (months)	50.6 ± 37.7	78.8 ± 59.6	0.022
Body Mass Index	21.4 ± 2.9	22.3 ± 3.5	0.511
GNRI	101.2 ± 8.0	97.9 ± 8.4	0.121
Systolic blood pressure (mmHg)	131.8 ± 30.6	145.4 ± 26.5	0.062
Diastolic blood pressure (mmHg)	78.2 ± 17.6	84.7 ± 10.2	0.065
Kt/V	1.74 ± 0.29	1.70 ± 0.22	0.504
Neutrophil Lymphocyte Ratio	0.42 ± 0.15	0.41 ± 0.15	0.931
Total Lymphocyte Count (/mm^3)	1525 ± 586	1537 ± 513	0.933
CD3 count (/mm^3)	1129 ± 406	796 ± 256	0.001
CD4 count (/mm^3)	701 ± 262	488 ± 169	0.001
CD8 count (/mm^3)	426 ± 177	302 ± 127	0.002
CD19 count (/mm^3)	176 ± 74	57 ± 26	0.001
CD4/CD8 ratio	1.7 ± 0.7	1.7 ± 0.7	0.905
Hemoglobin (g/dL)	11.1 ± 0.7	10.8 ± 0.9	0.145
Iron (ug/dL)	96.9 ± 35.9	83.7 ± 38.0	0.173
TIBC (ug/dL)	247.2 ± 38.2	249.7 ± 40.6	0.805
TSAT (%)	40.5 ± 17.5	33.5 ± 15.6	0.111
Ferritin (ng/mL)	366.4 ± 241.3	337.5 ± 295.4	0.672
BUN (mg/dL)	63.1 ± 17.1	66.1 ± 21.0	0.534
Cr (mg/dL)	9.9 ± 2.6	10.3 ± 2.2	0.472
Sodium (mEq/L)	137.8 ± 2.1	137.3 ± 4.0	0.487
Potassim (mEq/L)	5.1 ± 0.8	5.1 ± 0.8	0.987
Calcium (mg/dL)	8.9 ± 0.5	8.8 ± 0.5	0.656
Phosphorus (mg/dL)	5.1 ± 1.3	5.4 ± 1.5	0.282
Parathyroid hormone (pg/mL)	277.6 ± 251.4	171.6 ± 216.6	0.077
Albumin (g/dL)	3.9 ± 0.3	3.6 ± 0.4	0.022
Total cholesterol (mg/dL)	154.8 ± 37.0	176.1 ± 54.0	0.071
High density Lipid (mg/dL)	44.4 ± 19.0	50.7 ± 14.6	0.163
Low density Lipid (mg/dL)	67.8 ± 24.8	81.7 ± 33.1	0.064
Uric acid (mg/dL)	7.6 ± 1.6	7.4 ± 1.4	0.748

According to logistic regression for predicting malnutrition according to GNRI, age, female and CD 19 count predicted malnutrition in hemodialysis and peritoneal dialysis patients.

Conclusions: These results suggest that GNRI and lymphocyte subset counts (especially CD 19 count) may be a significant nutritional marker in HD and CAPD patients.

FR-PO284

Development of a Novel Index of Nutritional Status Based on Hemodialysis Patients' Mortality in Dialysis Outcomes and Practice Patterns Study (DOPPS)

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Background: For hemodialysis (HD) patients, their nutritional status strongly affects their quality of life and mortality. However, there is no single index that can be used for malnutrition diagnosis of HD patients. We developed a novel nutritional risk index for U.S. HD patients from the Dialysis Outcomes and Practice Patterns Study (DOPPS) database: the DOPPS nutritional risk index (DNRI).

Methods: We analyzed data from 5765 patients for DNRI development and those from 5610 patients for DNRI validation. To predict death within one year, DNRI was developed using logistic regression models.

Results: The final DNRI model included serum creatinine, albumin, and phosphorus levels, body mass index and its square, white blood cell count above 9000/ml, and age. DNRI showed higher accuracy in predicting death (area under curve [AUC] 0.714) than geriatric nutritional risk index (AUC 0.637).

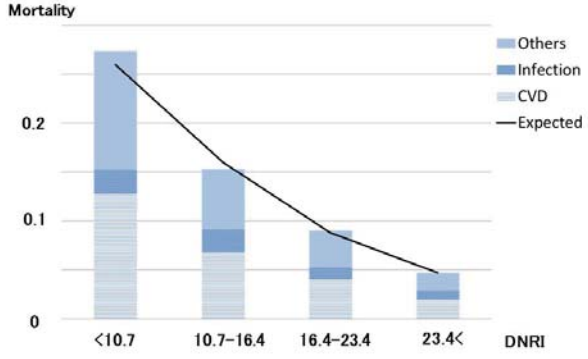

Fig.1 Probability of death expected on the basis of DNRI coincided with the observed incidence of deaths in US hemodialysis patients

Cox proportional hazard models for time-dependent DNRI showed that a 1 unit increase in DNRI decreased the risk of death (adjusted hazard ratio [aHR]: 0.83; 95% confidence interval [CI]: 0.80, 0.87), and that patients with a low DNRI (<12.9) showed a higher risk of death than patients with a high DNRI (aHR, 3.48; 95% CI, 2.27, 5.33).

Conclusions: DNRI may be a useful index to identify HD patients at higher mortality risk due to poor nutritional status.

FR-PO285

A Common microRNAs Pattern Expression Related to Malnutrition and Inflammation in Chronic Kidney Disease Mice Model

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Background: Malnutrition and inflammation are risk factors for ESRD; its relationship with the molecular regulators like miRNas is unknown. Our aim was to investigate the differential expression of miRNas in a Chronic kidney disease (CKD) mice model and its relationship with malnutrition and inflammation.

Methods: Three mice groups were performed: CKD group (Nx 5/6), I group (Inflammation induced with *E. coli LPS*) and C group (Control). We determined food intake in CKD group and was created a four group: FR (food restriction). Animals were sacrificed after eight weeks of treatment; plasma creatinine, albumin, and total proteins tests were measured, liver was extracted, and weighted. Total RNA was extracted from liver; miRNAs were obtained for miRNAs microarrays. Gene target prediction was made with "miRANDA" database.

Results: Food intake in CKD group was 59.2% less than control group (4.8±1.22 versus 8.1±1.47 g/day, p <0.005), in I group was 46.9% less than C (3.8±0.94 versus 8.1±1.47 g/day, p<0.005). Groups were confirmed with creatinine and albumin. Body and liver weight in CKD and FR groups were lower than control p<0.05. The mir-409, mir-29b, mir-206, and mir-30a showed common differential expression in CKD, I and FR groups; mir-212 expression is common in CKD and FR groups, mir-376 expression is common in I and FR groups; mir-376, mir-30b and mir-195, have common expression pattern in CKD and I groups.

Conclusions: A common microRNAs pattern expression was found in CKD, Inflammation and Food Restriction mice models, their predicted gene targets are cellular cycle, transcription and inflammation factors mRNAs. These results suggest that nutrition signaling is similar in CKD, inflammation and food restriction models.

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A Pharmaco-Metabolomic Study of Chronic Aristolochic Acid Nephropathy
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Background: Aristolochic acids (AA) belong to a family of carcinogenic, mutagenic, and nephrotoxic compounds that are found in the Aristolochiaceae plants which are commonly used in Chinese herbal medicine. AA are thought to be involved in pathogenesis of Balkan nephropathy, Chinese herbal nephropathy and urothelial cancer. CAAN is a rapidly progressive interstitial nephritis that can lead to ESRD and urothelial malignancy and is frequently associated with the long-term consumption of AA-containing herbs.

Methods: Serum metabolomics based on the ultra-performance liquid chromatography/ SYNAPT high-definition mass spectrometry was undertaken to explore the pattern of low molecular mass metabolites in the rat model of CAAN. Sixty-four rats were randomized into AA and control groups. The AA group was fed 20 mg/kgbody weight/day of AA for 12 weeks. Blood biochemistry, and Masson's trichrome staining and Western blot analysis of kidney tissues were performed. Data were analyzed using principal component analysis, orthogonal partial least squares-discriminant analysis, heatmap analysis and metabolic pathways analysis.

Results: As expected the CAAN rats exhibited progressive deterioration of renal function and renal histology, and up-regulation of TGF-β1, CTGF, PAI-1 and TIMP-1 during the observation period. This was coupled with a significant change in the global serum metabolites from CAAN compared to control rats at weeks 4, 8, and 12. These included bile acids, phospholipids, uremic toxins and eicosanoids. The changes in these metabolites correlated with progressive renal injury and upregulation of the TGF-β pathway in the CAAN rats.

Conclusions: This study provides longitudinal data on the metabolic profile across a wide range of biochemical pathways in response to AA-induced chronic renal injury. The observed changes highlight perturbations in the metabolism of bile acids, phospholipids, uremic toxins and fatty acids, in tandem with upregulation of inflammatory, oxidative stress and fibrosis pathways.

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Circulating Irisin Levels Are Associated with Sarcopenia and Carotid Atherosclerosis in Peritoneal Dialysis Patients
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Background: Sarcopenia is an important cardiovascular risk factor in patients with end-stage renal disease. Recent studies have indicated that skeletal muscles mediate their protective effect by secreting myokines. However, to date, the pathophysiologic role of irisin, a novel myokine, has not been evaluated in peritoneal dialysis (PD) patients. In this study, therefore, we investigated the association of irisin with sarcopenia and carotid atherosclerosis in these patients.

Methods: Serum irisin levels were assessed by enzyme-linked immunosorbent assay in 102 prevalent PD patients. To determine sarcopenia and carotid atherosclerosis, anthropometric indices including mid-arm muscle circumference (MAMC) and carotid intima-media thickness (cIMT) were measured. Linear and logistic regression analyses were performed to ascertain the independent association of irisin with sarcopenia and carotid atherosclerosis.

Results: The mean serum irisin levels were 184.2 ± 88.0 ng/mL. Thirty-seven patients (36.3%) had carotid atherosclerosis. In univariate linear regression analysis, serum irisin concentrations were positively correlated with MAMC, thigh circumference, and serum creatinine levels, whereas negatively correlated with residual renal function and cIMT. Multivariate analysis revealed that MAMC [b = 0.31, 95% confidence interval (CI) = 1.89 to 17.85, P = 0.02] and cIMT (b = -0.29, 95% CI = -92.53 to -17.11, P = 0.005) had independent association with serum irisin levels. In addition, serum irisin concentration was a significant independent risk factor for carotid atherosclerosis after adjustment for confouding variables (per 1 ng/mL increase, odds ratio = 0.987, 95% CI = 0.979 to 0.996, P = 0.004).

Conclusions: This study demonstrated for the first time that circulating irisin levels were significantly associated with sarcopenia and carotid atherosclerosis in PD patients, suggesting that irisin could mediate the detrimental effect of sarcopenia on cardiovascular disease.

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Angiotensin II Induces Lipid Accumulation in Hepatic Cells via the Disruption of Low Density Lipoprotein Receptor Pathway
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Background: Our previous studies demonstrated that activation of renin-angiotensin system (RAS) contributes to the progression of chronic kidney disease via the disruption of low density lipoprotein receptor (LDLr) pathway. This study aimed to investigate the effects of angiotensin II (Ang II) on lipid homeostasis in Human hepatoblastoma cell line (HepG2) cells and its underlying mechanisms.

Methods: HepG2 cells were cultured and divided into the control group (incubated with serum-free medium) and Ang II group (treated by 10^{-7} mol/L of Ang II for 24 hours). The effects of Ang II on lipid accumulation were examined by Oil red O staining and a quantitative assay of intracellular cholesterol. The expression of LDLr, sterol regulatory element–binding protein (SREBP) cleavage activating protein (SCAP) and SREBP-2 mRNA and protein were examined by Real-time polymerase chain reaction (PCR) and Western Blot. The colocalization of SCAP and Golgi in HepG2 cells was examined immunofluorescent staining using confocal microscopy.

Results: Ang II treatment increased intracellular lipid accumulation in HepG2 cells, which was correlated with increased mRNA and protein expression of LDLr, SCAP, and SREBP-2 in HepG2 induced by Ang II. Furthermore, results from confocal microscopy observation demonstrated that Ang II increased the translocation of SCAP/ SREBP-2 complex from endoplasmic reticulum to Golgi, thereby upregulating LDLr gene transcription.

Conclusions: Ang IIdisrupts LDLr feed-back regulation to increase cholesterol uptake and induce intracellular lipid accumulation. It suggests that local RAS activation in livers may contribute to the progression of non-alcoholic fatty liver disease via the disruption of LDLr pathway.

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Activation of the Renin-Angiotensin System Is Involved in Hyperlipidemia-Mediated Liver Injuries in Apolipoprotein Knockout Mice and HepG2 Cells
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Background: Our previous studies demonstrated thatdyslipidemia and activation of renin-angiotensin system (RAS) contribute to the progression of chronic kidney disease. This study aimed to investigate possible synergistic effects of intrahepatic RAS activation with hyperlipidemia in liver injuries of non-alcoholic fatty liver disease (NAFLD).

Methods: Apolipoprotein knockout mice were fed with normal chow diet (control) or high fat diet (HF group) for 8 weeks. Human hepatoblastoma cell line (HepG2) cells was treated without (control) or with 30 mg/ml cholesterol (lipid group) for 24 hours. The plasma lipid profile and RAS components were determined by clinical biochemistry assay and radiommunoassay, respectively. The gene and protein expressions of molecules involved in RAS components and biomarkers of epithelial mesenchymal transition (EMT) were examined by real-time PCR, immunochemical staining, and Western blot.

Results: The mice fed with high-fat diet showed significant hyperlipidemia with collagen deposition in livers compared to the controls. The plasma levels of renin, angiotensin I, and angiotensin II were no difference in two groups. However, the livers of HF group showed up-regulated RAS components, which were positively associated with increased plasma levels of triglyceride, total cholesterol, and LDL. These effects were further confirmed by *in vitro* studies. Lipid loading induced HepG2 cells underwent EMT, which was closely associated with the increased expressions of intracellular RAS components.

Conclusions: Local RAS activation was involved in hyperlipidemia-mediated liver injuries, suggesting that there are synergistic effects resulting from RAS activation with hyperlipidemia that accelerates the progression of NAFLD.

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Increased Expression of ADAM17 and Soluble TAM Receptor in Patients with Chronic Renal Failure
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Background: The receptor tyrosine kinases Mer and Axl (TAM receptors) are expressed on mononuclear cells and are important regulators of cytokine and toll-like receptor signaling. Mer is crucial for clearance of apoptotic bodies and resolution of vascular inflammation, and plays an anti-inflammatory role in lipopolysaccharide induced inflammation and autoimmunity. Preservation of membrane bound Mer is necessary for these functions. Proteolytic cleavage of membrane bound Mer by metalloproteinase (MMP) ADAM17 to its soluble form (sMer) causes its inactivation. Reactive oxygen species and toll like receptor signals, likely operative in inflammatory states such as chronic renal failure (CRF), are known inducers of ADAM17.

Methods: We measured levels of (sMer), soluble Axl (sAxl), ADAM17 and ADAM10 in the plasma and peripheral blood mononuclear cells of non-dialysis (CKD) and dialysis (HD) patients by real time PCR, ELISA and FACS.

Results: TAM receptors sAxl and sMer, were significantly increased by ELISA in the plasma of patients with CRF compared to controls. sMer was (Mean ± SD, ng/ml) 1.26±.65 in CKD, 1.16±.65 in HD and.54±.20 in controls. sAxl was 39 ± 14.3 in CKD, 60 ± 18.2 in HD and 27 ± 9.8 in controls, (p<.0001). Both sAxl and sMer were positively associated (r=.30, and r=.50 respectively, p<.0001) with levels of the TAM ligand Gas6 known to be elevated in patients with systemic inflammation and CRF. Only sAxl was inversely associated with eGFR, (-.32, P=.004). Given increased levels of soluble TAM receptor, expression of ADAM10 (cleaves Axl) and ADAM17 (cleaves Mer) was examined by PCR. We found HD monocytes expressed significantly more ADAM17 (6-fold) than controls. No difference was found for ADAM10.

Conclusions: The inflammation induced MMP ADAM17 cleaves membrane bound Mer, limiting Mer receptor ligation and subsequent regulation of inflammation in basic studies. In line with this, our data show increased expression of ADAM17 and high levels of sMer and sAxl in CRF. These findings suggest a new mechanism underlying chronic inflammation in patients with CRF.

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Insulin Secretory Defect in a Mouse Model of Chronic Kidney Disease
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Background: Disorders of glucose homeostasis are common in chronic kidney disease (CKD), but the presence of impaired insulin secretion are unclear. Recent data show that elevated levels of urea increase O-GlcNAc–modified insulin signaling molecules and cause insulin resistance. The aim of this study was 1- to examine whether insulin secretion is altered in a CKD mice; and 2- to ascertain whether urea has a direct negative impact on insulin secretion by increasing protein O-GlcNacylation in islets.

Methods: C57BL/6N mice underwent a 5/6 nephrectomy. At 3 weeks post-surgery,b-cell mass, insulin content and insulin secretion were measured. *In vivo,* insulin secretion was assessed by intraperitoneal glucose tolerance tests and hyperglycemic clamps. Isolated mouse islets were exposed to pathological concentrations of urea (20 mM) for 24 h and insulin secretion was measured. Total O-GlcNacylated proteins and O-GlcNAc transferase (OGT) levels were evaluated by Western blot.

Results: CKD mice were glucose intolerant compared to sham-operated controls (AUC glucose during the IPGTT: 315±31 versus 414±29 mmol/l*h, n=10, $p<0.05$). Hyperglycemic clamp revealed a decrease in both glucose- and arginine-induced insulin secretion in CKD mice. Thus, insulin levels during the steady-state phase of the clamp were 1.2±0.1 ng/ml versus 2.2±0.3 ng/ml in sham mice (n=7, $p<0.01$). Accordingly, isolated islets from CKD mice showed reduced insulin secretion in response to 16.7 mM glucose (-41±19 %, n=5-7, $p<0.05$) and 35 mM KCl (-55±12%, n=5, $p<0.05$). b-cell mass and islet insulin content were similar in both group. A similar reduction in insulin secretion was observed in normal mouse islets exposed to 20 mM urea for 24h (-46±17 % versus control islets exposed to 20 mM mannitol, n=4, $p<0.05$), without changes in insulin content. In addition, exposure of islets to urea led to an increase in total O-GlcNAcylated proteins and expression of OGT (+137±18 % versus control, n=3, $p<0.05$).

Conclusions: CKD is associated with an insulin secretory defect which likely lies at the level of exocytosis. This defect is recapitulated by prolonged exposure of islets to urea and is associated with an increase in protein O-glycosylation.

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FR-PO292

Urinary Nephrin Is an Early Biomarker for CKD in Maternal Caloric Restricted Rats
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Background: Inadequate nutrition during pregnancy is a major cause of low birth weight (LBW) and impaired fetal growth. LBW may lead to low nephron endowment and increased risk of CKD. Despite large numbers of infants with LBW, clinical guidelines for monitoring of infants at risk for CKD do not currently exist. Urinary biomarkers of renal injury would be helpful in identifying those individuals at greatest risk for CKD. We previously characterized a model of CKD, induced by nutritional deprivation, in which caloric- restricted offspring had low nephron number, and developed hypertension and albuminuria by adulthood. We hypothesized that urinary biomarkers of renal injury, cystatin C and/or nephrin, could be detected prior to the onset of disease, and help predict the development of CKD in maternal caloric-restricted rats with low nephron number.

Methods: Sprague Dawley rats were fed standard chow (control) or 50% caloric restricted diet (CR) starting at 11 days of gestation until weaning. All litters culled (8/lit). Both groups were fed standard chow postweaning. Urine was collected at 2 months from CR (n=6) and controls (n=11) and at 6 mos (control,n=8;CR,n=9). Spot urine albumin, cystatin C and nephrin were quantified by ELISA and normalized to urine creatinine.

Results: CR had similar albumin to creatinine ratios (ACR) compared to controls at 2 mos. At 6 mos, CR had a 3-fold increase in ACR compared to controls (290.2mg/mg ± 44.5 versus 94.3mg/mg ± 15.3, $p=0.002$). Urinary nephrin doubled in CR compared to controls at 2 mos (271.9mg/mg ± 38.6 versus 123.5mg/mg ± 25.4, $p=0.003$). At 6 mos, urinary nephrin increased by 3-fold in CR compared to controls (392.1mg/mg ± 63.5 versus 108.9mg/mg ± 16.4, $p=0.001$). Urinary cystatin c was not significantly different between groups.

Conclusions: Urine nephrin is significantly increased prior to the onset of albuminuria in caloric-restricted offspring, and may be an early biomarker for CKD. Cystatin C may not be a useful marker to detect increased risk of CKD. Longitudinal studies in humans will be necessary to determine if urinary nephrin is useful in identifying LBW infants at increased risk for kidney disease in adulthood.

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Sustained Beneficial Effects of a Soluble Guanylate Cyclase Activator on Diabetic Nephropathy
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Background: We hypothesized that a soluble guanylate cyclase (sGC) activator would result in a reduction in renal damage that would be maintained following discontinuation of treatment in the ZSF1 rat model of diabetic nephropathy.

Methods: Male ZSF1 rats implanted with telemetry devices were treated for 10 weeks with either BAY 58-2667 (sGC activator) in combination with enalapril, amlodipine in combination with enalapril, enalapril alone, or vehicle. Mean arterial pressure (MAP), heart rate (HR) and proteinuria were recorded. Following 10 weeks of treatment (Treatment phase), the compounds were discontinued and similar endpoints as above were measured for 5 weeks (Reversibility phase). Histopathological assessment of glomerulosclerosis and tubulointerstitial damage was performed at study termination.

Results: In the treatment phase, MAP reduction was greater in the amlodipidine + enalapril group, versus BAY 58-2667 + enalapril (-27.4 ± 3.9 mmHg versus -19.8 ± 3.0 mmHg, $p<0.05$). In contrast, reductions in proteinuria were similar between the 2 groups (-108.1 ± 63.4 mg/day versus -115.6 ± 41.3 mg/day). Upon discontinuation of treatment, MAP in both combination groups rebounded to a similar level. However, proteinuria appeared to rise to a greater extent in the amlodipine + enalapril group versus BAY 58-2667 + enalapril (224.6 ± 47.1 versus 190.8 ± 31.1 mg/day, $p<0.05$). Importantly, glomerulosclerosis and tubulointerstitial lesions were only significantly reduced in the BAY 58-2667 + enalapril group versus vehicle (-35.4% and -45% respectively, $p<0.05$); thereby indicating a persistent improvement in renal damage despite treatment discontinuation. Such an effect was not observed in the amlodipine + enalapril group, thus demonstrating its dissociation from MAP lowering.

Conclusions: An sGC activator resulted in disease modification in the ZSF1 rat model of diabetic nephropathy, as evident by beneficial effects maintained weeks after treatment discontinuation.

FR-PO294

A Receptor Fusion Protein (mIL-6-RFP-Fc) for the Inhibition of IL-6 in Preclinical Disease Models
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Background: IL-6 is a central regulator of inflammatory processes and has been shown to be markedly increased during glomerulonephritis, chronic inflammation, fibrosis, and acute kidney injury. Targeting of this cytokine during these states is highly desirable to develop potential therapeutic strategies. Fusion proteins derived from the extracellular parts of heteromeric cytokine receptors have turned out to be promising cytokine inhibitors.

Methods: We constructed an improved receptor fusion protein, mIL-6-RFP-Fc, consisting of the ligand binding domains of murine signal transducer gp130 and the murine IL-6Ra-subunit connected by a flexible polypeptide linker. The gene sequence was optimized for expression in mammalian cells. An engineered Fc fragment from murine IgG2a was added to increase half-life and to allow for purification through affinity chromatography. Further tagging with V5 and HA allowed for the reliable quantification of the fusion protein by ELISA during expression and purification.

Results: Codon optimization led to a 10-fold increase in protein yield from transfected HEK cells. mIL-6-RFP-Fc was a highly specific and highly potent inhibitor of murine, rat and human IL-6 *in vitro* as evidenced by downstream signaling and cell proliferation. Pharmacokinetics of mIL-6-RFP-Fc in mice was assessed following intravenous, intraperitoneal and subcutaneous application in mice showing availability for at least 8 hours after a single administration. Transient hydrodynamic expression in mice using a kidney and liver-specific promoter (PEPCK) resulted in organ expression and significant plasma levels (up to 0.5 µg/ml) of mIL-6-RFP-Fc. The inhibitory *in vivo* activity of mIL6-RFP-Fc was confirmed in murine models of IL-6-induced signaling responses.

Conclusions: mIL-6-RFP-Fc is ready for use in research and preclinical models. Encoded by a single gene, it is also well suited for organ-specific or inducible expression in transgenic mice.

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FR-PO295

Therapeutic Targeting of JAK/STAT/SOCS Pathway Reduces Diabetic Nephropathy in Mice
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Background: JAK/STAT pathway regulates a broad range of mediators implicated in cell proliferation, inflammation and fibrosis and is an important mechanism whereby hyperglycemia and dyslipemia contribute to the progression of diabetic complications. SOCS proteins negatively regulate JAK/STAT signaling and have emerged as a promising target for novel anti-inflammatory therapies. We investigate whether a cell-permeable peptide inhibitor of JAK/STAT pathway could decrease inflammation, fibrosis and renal injury in experimental diabetic nephropathy.

Methods: Type I diabetes was induced in apolipoprotein E KO mice by streptozotocin injection. Diabetic animals were treated with a peptide containing the SOCS1 kinase-inhibitory region (65 µg/day, i.p., 8 weeks, n=9) or vehicle (n=9). Kidney samples were analyzed for morphology (PAS), leukocyte infiltration (F4/80 and CD3) and collagen content (Picrosirius red). In vitro studies were performed in mesangial and tubular cells stimulated with cytokines (IFNg/IL-6) in the presence of inhibitory (SOCS1) and control mutant peptides. Cell migration was determined by wound-healing assay. STAT activation was evaluated by detection with phospho-specific antibodies. Expression of inflammatory and fibrotic genes was analyzed by real-time PCR.

Results: *In vivo*, SOCS1 peptide improved renal function and reduced the pathologic changes associated with diabetes, without impact on hyperglycemia and hyperlipidemia. Kidneys from SOCS1-treated mice exhibited a decreased infiltration of leukocytes and collagen content. SOCS1 peptide prevented STAT1/STAT3 activation and the expression of CCL2, CCL5,TNFa, TGFb and fibronectin. In cultured renal cells, SOCS1 peptide, but not control mutant peptide, inhibited cytokine-induced STAT1/STAT3 activation, target gene expression and cell migration.

Conclusions: SOCS1-based targeting JAK/STAT reduces renal inflammation and fibrosis and ameliorates renal dysfunction in experimental diabetic nephropathy. Mimicking SOCS1 functions may have a therapeutic potential to retard the progression of diabetic complications.

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Peptide-Based Modulation of NF-kB in a Mouse Model of Diabetes-Driven Nephropathy and Atherosclerosis Ainhoa Oguiza, Carlota Recio, Iolanda Lazaro Lopez, Benat Mallavia, Jesus Egido, Carmen Gomez Guerrero. *Renal and vascular pathology, IIS-Fundacion Jimenez Diaz, Autonoma Univ of Madrid, Madrid, Spain.*

Background: Hyperglycemia, dyslipidemia and inflammation participate in the progression of diabetes complications. Transcription factor NF-kB regulates the gene expression of many inflammatory factors involved in these pathologies. The NBD peptide selectively blocks the interaction of NEMO with IkB kinase complex and is proven to be effective in experimental models of acute inflammation but not in diabetes complications. Our aim is to inhibit NF-kB activation in an experimental model that combines hyperglycemia and hypercholesterolemia.

Methods: Type I diabetes was induced in apolipoprotein E deficient mice by streptozotocin injection. Diabetic animals were given i.p. injections of cell-permeable NBD peptide (40µg/day and 200µg/day) or vehicle for 8 weeks. Rhodamine-labeled NBD peptide was used for tissue distribution analysis. Tissues were analyzed for changes in morphology (PAS and ORO), cellularity (leukocytes and a-actin), collagen content (picrosirius red), gene expression (RT-PCR) and NF-kB activity (Southwestern). In vitro effects were studied in mesangial and tubular cells under hyperglycemic and inflammatory conditions.

Results: NBD peptide accumulated in kidneys and aorta of diabetic mice and suppressed NF-kB activation. NBD therapy improved renal function of diabetic mice and reduced glomerular and tubulointerstitial lesions without affecting metabolic parameters. Kidneys from treated mice exhibited less infiltrating cells and inflammatory molecules (MCP-1, RANTES and TNF-a), and reduced expression of pro-fibrotic genes (collagen, fibronectin and TGFb). In aorta, NBD peptide ameliorates atheroma plaques by reducing leukocyte content and inflammatory markers and increasing plaque stability. In cultured renal cells, NBD peptide disrupted the interaction of IkB kinase subunits, inhibiting NF-kB activation and pro-inflammatory gene expression.

Conclusions: Peptide-based modulation of NF-kB restrains the progression of renal and vascular damage in experimental diabetes and may be of potential interest in the treatment of diabetes complications.

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FR-PO297

A Novel Integrin Agonist Reduces Inflammation and Protects against Diabetic Nephropathy Mohd Hafeez Faridi,[1] Hatem A. Elshabrawy,[1] Johanna Guzman,[2] Alessia Fornoni,[2] Vineet Gupta.[1] *[1]Internal Medicine, Rush Univ Medical Center, Chicago, IL; [2]Medicine, Univ of Miami, Miami, FL.*

Background: Diabetic nephropathy (DN) is one of the major complications of diabetes mellitus leading to deterioration of kidney ultrastructure often culminating in end stage renal disease. Clinically, DN results in complex pathologic changes in the glomeruli, including basement membrane thickening, glomerular mesangial expansion, glomerulosclerosis and a significant loss of podocytes. Inflammation, often accompanied by leukocyte infiltration, has been shown to play a significant role in the development and progression of DN, suggesting that reducing leukocyte infiltration may be a promising therapeutic strategy. We recently identified a class of novel compounds (termed leukadherins) that exhibit a unique mechanism for reducing leukocytes infiltration into inflamed tissue. We report the therapeutic effect of leukadherins on DN progression in a murine type 2 diabetes model.

Methods: We used the BTBR ob/ob murine model of DN, in which the disease resembles human DN. At the onset of DN in these mice (4 wks of age), they were injected i.p. with our novel compound (Leukadherin 1 i.e. LA1) daily for eight weeks. Animals were monitored for blood glucose level, body weight, and renal function and for glomerular defects using histopathology at different time points.

Results: Daily administration of LA1 significantly reduced the number of infiltrating leukocytes in the glomeruli and preserved the kidney function in diabetic animals. We observed no effect of our interventions on hyperglycemia. Histopathological studies showed a significant reduction in glomerular mesangial sclerosis in LA1-treated animals.

Conclusions: Our results suggest that LA1 significantly preserves the kidney function in DN and provides a novel approach to delay the onset of DN.

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Urine Cell Quantitative RT-PCR for Analysis of Renal Inflammation in Diabetic Nephropathy Bairbre A. McNicholas,[1,2] Malgorzata Zurawska,[1] David Lappin,[2] Matthew D. Griffin.[1,2] *[1]ReGenerative Medicine Inst, School of Medicine, National Univ of Ireland, Galway, Ireland; [2]Nephrology Services, Galway Univ Hospitals, Ireland.*

Background: Urine cell analysis is a non-invasive approach to study and diagnose human kidney disease. We investigated technical feasibility and results of urine cell quantitative (q) RT-PCR for selected inflammatory mediators (IL-6, IL-6R, IL-17) in fresh urine samples from adults with type 2 diabetes (T2DM) and nephropathy of varying severity and from healthy adults.

Methods: Urine pellet mRNA from 160 T2DM outpatients with varying urine albumin creatinine ratio (ACR) and 20 healthy adults was extracted by modified Trizol® protocol and analysed for quantity and quality by NanoDrop and Bioanalyzer. Relative abundance of IL-6, IL-6R and IL-17 mRNA was quantified on a StepOne Plus® Real-Time PCR System by standard curve method normalized to b-actin and in relation to a calibrator sample. Samples with 260/280 ratio <1.7 and/or amplification threshold for b-actin >24 were deemed unsuitable. Transcript levels were correlated with HbA1c, BMI, MDRD-eGFR, ACR and systolic blood pressure (SBP).

Results: Only 72 (40%) cell pellets had adequate mRNA for qRT-PCR. Subjects with adequate compared to inadequate mRNA had similar age, ACR, BMI and HbA1c but lower eGFR (66±20 v 73±18 ml/min/SA, p=0.02). Among the 61 T2DM subjects with adequate urine cell mRNA, albuminuria grade was normal in 25, mild to moderate in 26 and severe in 10. Quantifiable mRNA for IL-6, IL-6R and IL-17 was present in 100%, 100% and 72% respectively of all adequate samples. Contrary to expectations, mean urine cell mRNA levels of IL-6, IL-6R and IL-17 were no different in T2DM compared to healthy adults. Furthermore, urine cell mRNA levels did not correlate with albuminuria grade, eGFR, BMI or SBP. Subjects with detectable urine cell IL-17 mRNA had higher albuminuria (11±18 v 41±85 mg/mmol, p=0.01).

Conclusions: 1. Even with optimized methodology, sample inadequacy significantly limits urine cell qRT-PCR for investigation of mild to moderate diabetic kidney disease. 2. For T2DM subjects with mild to moderate nephropathy and adequate urine cell RNA the results provided evidence against overt renal inflammation.

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Reduced Connexin 43 Expression Protects against the Progression of Experimental Glomerulonephritis in Mice Ahmed Abed,[1] Panagiotis Kavvadas,[1] Carlo M. Alfieri,[2] Jean-Jacques Boffa,[3] Christos Chatziantoniou,[1] Christos E. Chadjichristos.[1] *[1]UMR-S1155, INSERM, Paris, France; [2]Unit of Nephrology Dialysis and Kidney Transplantation, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico di Milano, Milano, Italy; [3]Nephrology Dpt, Tenon Hospital, Paris, France.*

Background: Glomerulonephritis (GN) includes a variety of renal pathologies which may progress to end-stage renal disease. We have recently observed an increased expression of the gap junctional protein connexin 43 (Cx43) in injured glomeruli of mice treated with nephrotoxic serum (NTS). The aim of our work was to study the role of Cx43 in this model of GN.

Methods: We compared the progression of the NTS-induced GN between two different groups of mice, one normally expressing Cx43 (WT) and a second in which Cx43 expression was genetically reduced by half (Cx43+/-) (n=10 mice per group). Mice were sacrificed at day 15. After sacrifice kidneys were assessed for morphometry, inflammation and interstitial fibrosis.

Results: Renal function was improved in Cx43+/- mice two weeks after NTS administration. Indeed, proteinuria (11.1±1.1g/mol for Cx43+/- versus 16±0.5g/mol for WT, p<0.05), blood urea nitrogen (35±5.3mmol/l for Cx43+/- versus 55±9.mmol/l for WT, p<0.05) and serum creatinine (25±4.4mmol/l for Cx43+/- versus 56±9mmol/l for WT, p<0.01) were significantly decreased. Consequently, renal structure was preserved as crescent-like formation (30±3.3% of crescentic glomeruli in Cx43+/- versus 45±4.5% in WT, p<0.05), tubular dilation (1.8±0.4 index for Cx43+/- versus 2.5±0.3 for WT, p<0.05), monocyte infiltration (cortex surface of F4-80 immunostaining 3±0.5% for Cx43+/- versus 7±1.5 for WT, p<0.05) and interstitial renal fibrosis (2±0.45% and 4.5±0.9% of cortex surface for Cx43+/- and WT respectively, p<0.001) were blunted in these mice. Colocalization experiments with nestin indicated that Cx43 was mainly induced in damaged podocytes. Interestingly, western blotting demonstrated that neprhin expression was preserved in Cx43+/- mice confirming the beneficial effect of Cx43 deletion for the integrity of glomerular podocytes.

Conclusions: Our study demonstrates the deleterious role of Cx43 in NTS-induced GN and suggests that targeting Cx43 may protect against the progression of the disease.

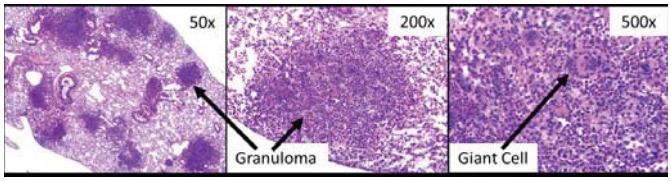
FR-PO300

Induction of Pulmonary Granulomatosis in Mice By Anti-MPO IgG: An Animal Model of ANCA Granulomatosis Peiqi Hu, Hong Xiao, Hua Su, Kemper Ramsey, Ronald J. Falk, J. Charles Jennette. *Pathology and Laboratory Medicine, Univ of North Carolina, Chapel Hill, NC.*

Background: Extravascular granulomatosis is a major pathological feature of some variants of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis. Understanding the pathogenic mechanism for granulomatosis has been hampered by the lack of animal models. Here we show a mouse model of lung granulomatosis induced by anti-MPO IgG that closely mimics the early acute pulmonary lesions of ANCA granulomatosis.

Methods: 9-10 weeks old B6, 129S6 wild type (WT), 129S6 rag2-/-, 129S6 Fc gamma RIIb-/- mice were injected i.v. at day 0 and day 1 with 75ug/g body weight of anti-MPO IgG. Mice were sacrificed at day 6 and kidney, lung and other tissue collected for pathologic assessment.

Results: Six days after two dose i.v. injections of anti-MPO IgG on consecutive days, lung granulomatous lesions and necrotizing glomerulonephritis (GN) developed in all mouse strains with the least severe disease in B6 mice and the most severe in 129S6 Fc gamma RIIb-/- mice lacking inhibitory Fc receptor. The nodular lung lesions contained neutrophils, monocyte and macrophages, including prominent multinucleated giant cells.



Conclusions: 1) The same batch of anti-MPO IgG that induces only renal limited GN when given as a single dose causes pulmonary granulomatosis when administered in two sequential doses. The first dose positions primed neutrophil and monocytes in the lung for activation by the second dose. 2) Induction of granulomatosis by anti-MPO IgG in Rag2-/- with no functioning T cells mice documents that the granulomatosis is not T cell-mediated. 3) Absence of the inhibitory Fc gamm R IIb results in more severe pulmonary granulomatosis.

Funding: NIDDK Support

FR-PO301

Altered Phenotypeof Anti-GBM Glomerulonephritis following Conditional and Kidney-Specific Knockdown of Stanniocalcin-1 Luping Huang, Luan D. Truong, Huiming Ju, David Sheikh-Hamad. *Medicine/Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Inflammation is the hallmark of anti-GBM GN. We have shown stanniocalcin-1 (STC1) inhibits macrophages, stabilizes endothelial barrier function, and diminishes trans-endothelial migration of leukocytes; consistently, transgenic (Tg) overexpression of STC1 protects from anti-GBM glomerulonephritis (GN). In the following experiments, we sought to determine the phenotype of anti-GBM GN following conditional and kidney-specific knockdown of STC1.

Methods: We generated STC1 shRNA Tg and scrambled shRNA Tg mice that express STC1 shRNA or scrambled shRNA, respectively, upon removal of floxed reporter (PGK-EGFP); delivery of pTie2-Cre to the kidney using ultrasound microbubbles, initiates expression of the shRNA, in kidney endothelial cells. STC1 shRNA expression provides global kidney knockdown of STC1 protein (70%) within 4 days, persisting through day 14. We observe no change in STC1 expression in similarly treated scrambled shRNA Tg mice. Sheep anti-mouse GBM antibody was administered at day 4, mice were killed at day 14 and the following were determined: blood pressure; urine output; proteinuria; serum creatinine; kidney morphology, fibrosis, T-cells and macrophages infiltration, C_3 and mouse IgG deposition, and cytokine array.

Results: The increase in serum creatinine and proteinuria, and decline in urine output, were similar in STC1 shRNA and scrambled shRNA Tg mice after anti-GBM GN; however, STC1 shRNA Tg mice displayed: higher blood pressure; greater tubulointerstitial expansion; marked tubular epithelial vacuolization and sloughing; massive cast formation and severe tubular dilatation; 7-fold higher glomerular necrosis/hyalinosis; 5-fold greater glomeruli with crescents. STC1 shRNA Tg kidneys had fewer T-cells and macrophages, less C_3 and mouse IgG deposition, and reduced cytokine expression.

Conclusions: Anti-GBM following kidney-specific knockdown of STC1 is characterized by: severe tubular and glomerular necrosis; massive cast formation. Surprisingly, inflammation is not a dominant feature, and we speculate that absent cytokine release from necrotic tubules may account for the paucity of inflammatory cells within the kidney.

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

FR-PO302

The Growth Factor Midkine Reflect the Disease Activity in Lupus Nephritis Tomohiro Masuda,[1] Kayaho Maeda,[1] Waichi Sato,[2] Tomoki Kosugi,[1] Yuka Sato,[1] Takuji Ishimoto,[1] Seiichi Matsuo,[1] Shoichi Maruyama.[1] *[1]Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan; [2]Nephrology, Fujita Health Univ School of Medicine, Toyoake, Japan.*

Background: Midkine (MK), a heparin-binding growth factor regulates cell growth, cell survival and migration in nephrogenesis and development. Its pathophysiological roles are diverse, ranging from the occurrence of acute kidney injury to progression of chronic kidney disease, often accompanied by hypertension and diabetes. In autoimmune diseases, however, molecular mechanism involving MK is unknown. We so far examined the association of MK in regulatory T cell population *in vitro.* In current study, we elucidated the role of MK in lupus nephritis (LN).

Methods: *In vivo* study: LN was induced in MK-deficient ($Mdk^{-/-}$) or wild-type mice ($Mdk^{+/+}$) with an intraperitoneal injection of pristane.Mice were sacrificed at 6 months later. Kidney, spleen and lipogranulomas were analyzed. Clinical study: Plasma and spot urine samples were collected from LN patients (N=42), who underwent renal biopsy in our affiliated hospitals, and healthy volunteer (N=33). MK values were measured by ELISA. MK expression in tissue samples from LN patients was assessed by immunostaining.

Results: *In vivo* study, glomerular injury in $Mdk^{+/+}$mice was severer than that of $Mdk^{-/-}$ mice. Particularly, mesangial and endothelial cells proliferations, component C3 deposition, $CD68^+$ macrophages, neutrophils and $CD4^+$ T cells infiltration were prominent in glomeruli of $Mdk^{+/+}$mice, consistent with the profiles of albuminuria and renal function. In addition, MK expression was increased in LN kidneys of $Mdk^{+/+}$ mice and positively correlated with pathological severity. In patients with LN, the close relationship between urine MK and LN disease activity was also observed. Interaction between immune complex (IC) and Fcg receptor (FcgR) is an important factor for LN progression. Interestingly, FcgR-dependent activation and cytokine production in ICs-regulated leukocytes was found in MK dose-dependent manner *in vitro.*

Conclusions: Lack of MK ameliorates glomerular injury of LN through the suppression of cytokine production in ICs-regulated leukocytes.

FR-PO303

Decay Accelerating Factor (DAF) Induction and Attenuation of Complement Activation in the Rat Glomerulus By Heme Oxygenase (HO)-1 Maria Detsika,[1] Pu Duann,[2] Elias A. Lianos.[2] *[1]Medicine, Univ of Athens, Greece; [2]Medicine, Rutgers Biomedical and Health Sciences, NJ.*

Background: Rat glomeruli express DAF exclusively in glomerular epithelial cells (GEC) thus attenuating complement (C)-mediated injury. HO-1 attenuates C-mediated injury via heme degradation products. An alternative mechanism may involve DAF upregulation. This was explored in normal rat glomeruli.

Methods: $hmox1^{+/-}$ and $hmox1^{-/-}$ rats were obtained by Zinc Finger Nuclease (ZFN)-mediated HO-1 gene disruption. Rats with GEC targeted HO-1 overexpression (GEC^{HO-1}) were generated by Sleeping Beauty Transposon mediated transgenesis using a nephrin promoter. Glomeruli from wild type (WT) or $hmox1^{+/-}$, $hmox1^{-/-}$ or GEC^{HO-1} rats were treated for 18h with Metalloporphyrins (MPs): Heme (Hemin) or Cobalt Protoporphyrin (CoPP) (HO inducers), Zinc (ZnPP) or Tin (SnPP) (HO inhibitors), and effect on glycosyl-phosphatidyl-inositol (gpi)-anchored DAF was assessed. Verification of gpi-DAF expression was confirmed by phosphatidylinositol specific phospholipase C (PI-PLC) treatment. Glomerular C3b deposition was triggered by incubation with 10% rat serum (spontaneous C-activation). DAF, HO-1 and C3b protein levels were assessed by western blot analysis.

Results: PI-PLC treatment resulted in ≥90% reduction in glomerular DAF. Constitutive gpi-DAF levels decreased by 2-fold and 4-fold in $hmox1^{+/-}$ and $hmox1^{-/-}$ glomeruli, respectively. All MPs dose-dependently induced gpi-DAF in WT glomeruli. In co-incubations with Hemin, ZnPP and SnPP had an additive effect on DAF induction. Hemin-mediated DAF induction in $hmox1^{+/-}$ and $hmox1^{-/-}$ glomeruli was attenuated in comparison to WT. In GEC^{HO-1}glomeruli, constitutive DAF expression was increased and Hemin-mediated DAF induction was augmented. C3b deposition was decreased by 40% at Hemin concentrations that induced gpi-DAF.

Conclusions: Glomerular gpi-DAF expression is HO-1 dependent. MPs induce gpi-DAF in a metal and HO-activity independent manner. In spontaneous C-activation Hemin-mediated gpi-DAF induction attenuates C3b deposition. MP-mediated DAF induction may point towards novel therapeutic strategies in C-dependent glomerulopathies.

Funding: Government Support - Non-U.S.

FR-PO304

Modulation of Heparan Sulfate in the Glomerular Endothelial Glycocalyx Decreases Leukocyte Influx during Experimental Glomerulonephritis Angelique Rops,[1] Markus A. Loeven,[1] Jasper J. Van Gemst,[1] Xander Van Wijk,[2] Henry Dijkman,[3] Toin Van Kuppevelt,[4] Jo H.M. Berden,[1] Ton J. Rabelink,[5] Jeffrey D. Esko,[2] Johan Van der Vlag.[1] *[1]Nephrology, Radboud Univ Medical Center, Nijmegen, Netherlands; [2]Cellular & Molecular Medicine, Univ of California, San Diego; [3]Pathology, Radboud Univ Medical Center, Nijmegen, Netherlands; [4]Biochemistry, Radboud Univ Medical Center, Nijmegen, Netherlands; [5]Nephrology, Leiden Univ Medical Center, Leiden, Netherlands.*

Background: The glomerular endothelial glycocalyx is postulated to be an important modulator of permeability and inflammation. The glycocalyx consists of complex polysaccharides, the main functional constituent of which, heparan sulfate (HS), is

synthesized and modified by multiple enzymes. The N-deacetylase-N-sulfotransferase (Ndst) enzymes initiate and dictate the modification process. Here we evaluated the effects of modulation of HS in the endothelial glycocalyx on albuminuria and glomerular leukocyte influx using mice deficient in endothelial and leukocyte Ndst1 ($TEKCre^{+}/Ndst1^{flox/flox}$).

Methods: Albuminuria, blood urea nitrogen, glomerular leukocyte influx, histology, electron microscopy, and glomerular HS expression were determined before and after anti-GBM nephritis induction. Leukocyte adhesion was evaluated i*n vitro* using mouse glomerular endothelial cells (mGEnC) with reduced Ndst1 expression and Ndst1-deficient granulocytes.

Results: In Ndst1-deficient mice, glomerular expression of a specific HS domain was significantly decreased, whereas the expression of other HS domains was normal. In the endothelial glycocalyx, this specific HS structure was not associated with albuminuria or with changes in renal function. However, glomerular leukocyte influx was significantly reduced during anti-GBM nephritis, which was associated with less glomerular injury and better renal function. *In vitro* decreased adhesion of wild-type and Ndst1-deficient granulocytes to Ndst1-silenced mGEnC was found, accompanied by a decreased binding of chemokines and L-selectin.

Conclusions: Modulation of heparan sulfate in the glomerular endothelial glycocalyx significantly reduces the inflammatory response in anti-GBM nephritis.

Funding: Government Support - Non-U.S.

FR-PO305

Identification of Activin A as a Novel Urinary Biomarker for Lupus Nephritis in MRL/lpr Mice Anastasie Kadiombo Tshilela, Akito Maeshima, Noriyuki Sakurai, Hidekazu Ikeuchi, Toru Sakairi, Yoriaki Kaneko, Keiju Hiromura, Yoshihisa Nojima. *Dept of Medicine and Clinical Science, Gunma Univ Graduate School of Medicine, Maebashi, Gunma, Japan.*

Background: Activin A, a member of TGF-beta superfamily, is known to regulate cell growth and differentiation in various tissues. It has been reported that activin A modulates ureteric bud branching in kidney development, inhibits tubular regeneration after renal ischemia, and acts as a potent inducer of renal fibrosis in a rat unilateral ureteral obstruction model. However, the precise role of activin A in the progression of kidney diseases is not totally understood. To address this issue, we used female lupus-prone MRL/lpr mice in this study.

Methods: The expression and localization of activin A in the kidneys of MRL/lpr mice at 12, 16, 19 weeks of age was analyzed by real-time PCR, western blot analysis, and immunostaining. Histological changes of the kidneys (PAS, HE, and EVG staining), urinary protein level, renal functions (BUN, creatinine), infiltration of inflammatory cells (CD3, CD19, and CD68) and urinary activin A concentration (ELISA) were examined. C57BL/6 mice were used as control.

Results: No expression of activin A was observed in the kidneys of normal C57BL/6 mice. In contrast, activin A was detectable in periglomerular or perivascular CD68-positive cells in the kidneys of MRL/lpr mice at 12 weeks and thereafter. Urinary activin A was not detected in normal C57BL/6 mice or in MRL/lpr mice at 14 weeks or less, but became detectable in MRL/lpr mice at 16 weeks and thereafter. Urinary activin A level was statistically correlated with the number of infiltrating CD68-positive cells, the number of crescentic glomeruli, EVG-positive fibrotic area, and urinary protein level, but not with renal functions.

Conclusions: These data suggest that urinary activin A is a new biomarker reflecting renal inflammation in MRL/lpr mice. Activin A produced by infiltrating CD68-positive cells might be involved in crescent formation and interstitial fibrosis in these mice.

Funding: Pharmaceutical Company Support - Astellas Pharma Inc.

FR-PO306

Complement Activation on Vascular Endothelial Cells Results in Polymorphonuclear Leukocyte Adhesion Magdalena Riedl,[1,2] Damien Gerard Noone,[1,3] Meraj Alam Khan,[1] Fred G. Pluthero,[1] Walter H. Kahr,[1] Nades Palaniyar,[1] Christoph Licht.[1,3] *[1]Research Inst, The Hospital for Sick Children, Toronto, ON, Canada; [2]Dept of Pediatrics I, Innsbruck Medical Univ, Innsbruck, Austria; [3]Div of Nephrology, The Hospital for Sick Children, Toronto, ON, Canada.*

Background: Atypical hemolytic uremic syndrome (aHUS) is a devastating disease caused by defective complement (C) regulation on vascular endothelial cells (EC) resulting in microvascular thromboembolism and organ failure. EC are protected from C attack via C regulators (e.g. CD46, CD55 and CD59).The clinical observation of infections often preceding aHUS events has suggested a key role for the inflammatory system. Combining these insights, we hypothesized that adhesion of polymorphonuclear leukocytes (PMN) will be enhanced following EC C challenge.

Methods: Calcein-labeled PMN were introduced at 1 dyne/cm^2 into a microfluidic system (Bioflux), seeded with blood outgrowth endothelial cells (BOEC). These EC were pretreated with a) GB24 (blocking CD46), b) GB24 and BRIC216 (blocking CD55) and BRIC229 (blocking CD59) or c) TNFa 20ng/ml as positive control (PC). 50% normal human serum (NHS), used as source for C, or heat-inactivated serum (HIS) as control were either introduced simultaneously or for 1h prior to PMN. PMN adhesion was quantified in static conditions using a fluorescent plate reader assay.

Results: In fluidic conditions significant PMN adhesion was observed 5min after PMN/50%NHS introduction, only when CD46, CD55 and CD59 were blocked. ($p<0.05$). This effect was abandoned with HIS. The same effect was seen after 1h incubation of serum, indicating an independent effect of direct C activation on PMN. C-induced EC activation upon blockade of all three C regulators was confirmed by immunofluorescence for C5b-9 and flowcytometry for C3c. In static conditions, significantly ($p<0.025$) increased PMN adhesion was observed upon blockade of CD59, CD59 in combination with CD46 or CD55, and triple block.

Conclusions: C challenge of EC results in significant increase of PMN adhesion in static and fluidic conditions. This finding supports a precipitating role of inflammatory events for aHUS pathogenesis and has the potential to guide ways towards novel treatment targets.

Funding: Government Support - Non-U.S.

FR-PO307

CXCL1, CXCL2 and CCL2 Binding to Mouse Glomerular Endothelial Cell Glycocalyx Is Differentially Mediated by Specific Heparan Sulfate Domains Jasper J. Van Gemst,[1] Angelique Rops,[1] Markus A. Loeven,[1] Toin Van Kuppevelt,[2] Jo H.M. Berden,[1] Ton J. Rabelink,[3] Johan Van der Vlag.[1] *[1]Nephrology, Radboud Univ Medical Center, Nijmegen, Netherlands; [2]Matrix Biochemistry, Radboud Univ Medical Center, Nijmegen, Netherlands; [3]Nephrology, Leiden Univ Medical Center, Leiden, Netherlands.*

Background: Heparan sulfate in endothelial glycocalyces is an important co-factor for binding of chemokines, cytokines, and is involved in leukocyte adhesion to activated endothelium. In glomerulonephritis, heparan sulfate (HS) in the glomerular endothelial glycocalyx could play a role in chemokine presentation and oligomerization, and in binding of selectins and integrins expressed by leukocytes.

Methods: We evaluated binding of three pro-inflammatory chemokines (CXCL1, CXCL2 and CCL2) to mouse glomerular endothelial cells (mGEnC-1) in ELISA in competition with GAG preparations and anti-HS single chain variable fragment (ScFv) antibodies.

Results: We showed a different affinity of CXCL1, CXCL2 and CCL2 to HS, chondroitin sulfate and dermatan sulfate in a competition ELISA with mouse glomerular endothelial cells (mGEnC-1) as a substrate. HS appeared to be the main contributor in the glomerular endothelial glycocalyx mediating endothelium-chemokine interactions. Blocking of specific HS domains with anti-HS ScFv antibodies revealed a domain-specific interaction of the tested chemokines to HS.

Conclusions: From these findings it can be concluded that CXCL1, CXCL2 and CCL2 binding to the glomerular endothelial glycocalyx is differentially mediated by specific HS domains. Our findings may lead to the development of specific GAG-based drugs specifically targeting chemokine-endothelium interactions.

FR-PO308

Concomitant Inhibition of Renin Angiotensin System and Toll-Like Receptor 2 Attenuates Renal Injury in Unilateral Ureteral Obstructed Mice Sarah Chung,[1] Jin Young Jeong,[1] Ye Jin Kim,[1] Dae Eun Choi,[1] Yoon-Kyung Chang,[2] Ki Ryang Na,[1] Kang Wook Lee.[1] *[1]Internal Medicine, ChungNam National Univ Hospital, Daejeon, Korea; [2]Internal Medicine, Saint Mary Hospital, Catholic Univ, Daejeon, Korea.*

Background: There has been controversy about the role of toll-like receptor (TLR) 2 in renal injury following ureteric obstruction. Although inhibition of the renin angiotensin system (RAS) reduces TLR2 expression in mice, the exact relationship between TLR2 and RAS is not known. The aim of this study was to determine whether the RAS modulates TLR2.

Methods: We used 8-week-old male wild type and TLR2-knock out (KO) mice on a C57Bl/6 background. Unilateral ureteral obstruction (UUO) was induced by complete ligation of the left ureter. Angiotensin (Ang) II (1,000 ng/kg/min) and the direct renin inhibitor aliskiren (25 mg/kg/day) were administrated to mice using an osmotic minipump. Molecular and histologic evaluations were performed.

Results: Ang II infusion increased mRNA expression of TLR2 in wild type mouse kidneys ($p < 0.05$). The expression of renin mRNA in TLR2-KO UUO kidneys was significantly higher than that in wild type UUO kidneys ($p < 0.05$). There were no differences in tissue injury score or mRNA expression of monocyte chemotactic protein-1 (MCP-1), osteopontin (OPN), or transforming growth factor-b (TGF-β) between TLR2-KO UUO and wild type UUO kidneys. However, aliskiren decreased the tissue injury score and mRNA expression of TLR2, MCP-1, OPN, and TGF-β in wild type UUO kidneys ($p < 0.05$). Aliskiren-treated TLR2-KO UUO kidneys showed less kidney injury than aliskiren-treated wild type UUO kidneys.

Conclusions: TLR2 deletion induced activation of the RAS in UUO kidneys. Moreover, inhibition of both RAS and TLR2 had an additive ameliorative effect on UUO injury of the kidney.

FR-PO309

The Axis Gremlin/VEGFR2 in Renal Damage Carolina Lavoz,[1] Matilde Alique Aguilar,[1] Raquel Rodrigues-Diez,[1] Janos Pato,[4] Gyorgy Keri,[5] Jesus Egido,[2] Sergio A. Mezzano,[3] Marta Ruiz-Ortega.[1] *[1]Cellular Biology in Renal Diseases Laboratory, IIS-Fundación Jiménez Díaz. Univ Autónoma, Madrid, Spain; [2]Nephrology, IIS-Fundación Jiménez Díaz. Univ Autónoma, Madrid, Spain; [3]Nephrology, School of Medicine, Univ Austral, Valdivia, Chile; [4]Vichem Chemie Ltd., Budapest, Hungary; [5]Pathobiochemistry, Semmelweis Univ, Budapest, Hungary.*

Background: Gremlin is a developmental gene up regulated in human chronic kidney diseases and proposed as a mediator of renal damage. Gremlin binds to bone morphogenetic proteins (BMPs) acting as an antagonist regulating, among other process, nephrogenesis and fibrosis. In cultured endothelial cells Gremlin binds to vascular endothelial growth factor receptor-2 (VEGFR2) to induce angiogenesis. Our aim was to investigate the direct effects of Gremlin in the kidney, evaluating the receptor subtype and mechanisms involved.

Methods: Gremlin was administered into C57BL/6 mice (renal parenchymal injection, 50 ng/g BW). Some mice were treated with the VEGFR2 inhibitor SU5416 (i.p; 0.1 mg/mice/day). In vitro studies were done in cultured tubular epithelial cells (HK2 cell line).

Results: Gremlin administration caused a rapid and sustained activation of VEGFR2 signalling in the kidney, mainly located in tubular epithelial cells. One of the earliest downstream mechanism activated was the nuclear factor-kB pathway (observed at 15 min). Gremlin via nuclear factor-kB pathway caused renal inflammation, characterized by up-regulation of pro-inflammatory factors (chemokines, adhesion molecules and cytokines) and infiltration of inflammatory cells, observed after 48 hours. VEGFR2 kinase inhibition diminished Gremlin-induced renal inflammation. In vitro, Gremlin activated VEGFR2 pathway independently of BMPs. VEGFR2 kinase inhibition or gene silencing inhibited Gremlin-mediated NF-kB activation and pro-inflammatory genes upregulation. In the model of renal damage by unilateral ureteral obstruction, Gremlin overexpression was associated to VEGFR2 pathway activation, and VEGFR2 kinase inhibition diminished renal inflammation.

Conclusions: These data demonstrate that Gremlin binds to and activates the VEGFR2 signalling pathway in the kidney linked to renal inflammation.

Funding: Government Support - Non-U.S.

FR-PO310

PEDF Protects Tubular Epithelial Cells and Ameliorates Renal Fibrosis via Inhibition of the Wnt/β-Catenin Pathway Xuemin He, Rui Cheng, Jian-Xing Ma. *Dept of Physiology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: Our previous studies revealed that pigment epithelium-derived factor (PEDF) confers reno-protective effects in diabetic nephropathy. The purpose of the present study was to evaluate the protective effect of PEDF on tubular epithelial cells in obstructive nephropathy and explore its molecular mechanism.

Methods: Two months-old PEDF$^{-/-}$ mice and wild-type (WT) mice received sham or unilateral ureteral obstruction (UUO) operation. Five and ten days after the surgery, renal fibrotic markers, inflammatory factors and the Wnt/β-Catenin pathway were evaluated by quantitative real-time PCR, Western blot analysis, immunohistochemistry and X-gal staining. In proximal tubular epithelial cell line (HKC-8), 4-HNE-induced cell death was determined by TUNEL and Trypan blue exclusion assay. Primary tubular epithelial cells (PTECs) were isolated from WT mice and PEDF$^{-/-}$ mice.

Results:: Renal levels of PEDF were significantly down-regulated at day 5 after UUO. Loss of PEDF led to aggravated UUO-induced renal tubular epithelial cell death compared with WT/UUO controls. Recombinant human PEDF (rhPEDF) or over-expression of PEDF using adenovirus significantly decreased 4-HNE-induced tubular epithelial cell death. Compared with WT/UUO controls, PEDF$^{-/-}$/UUO mice showed higher levels of p-LRP6, non-β-Catenin and β-Catenin activities in the kidney compared to WT/UUO mice. Further, PEDF$^{-/-}$/UUO kidneys exhibited more severe renal fibrosis and renal inflammation, including elevated levels of collagens, TGF-β1, TGF-β receptor type I and type II, fibronectin, ICAM-1, TNF-a, IL-6 and M-CSF, most of which were regulated by the Wnt/β-Catenin pathway. In PEDF$^{-/-}$ PTECs, Wnt3A conditioned medium (WCM) induced more prominent increases of p-LRP6, non-β-Catenin, fibronectin and TGF-β1levels relative to that in WT PTECs. In addition, rhPEDF suppressed WCM-induced Wnt/β-Catenin signaling and downstream genes in a dose-dependent manner in PTECs.

Conclusions: PEDF protects tubular epithelial cells against obstructive nephropathy. This effect may be ascribed to its anti-oxidant and anti-inflammatory activities through regulation of the Wnt/β-Catenin pathway.

Funding: Other NIH Support - NIH EY018659, EY019309, EY012231 and GM104934

FR-PO311

The Involvement of Histone Acetylation in the Progression of Nephrosclerosis Kumiko Muta,[1] Yoko Obata,[1] Takehiko Koji,[2] Tomoya Nishino,[1] Shigeru Kohno.[1] *[1]Second Dept of Internal Medicine, Nagasaki Univ School of Medicine, Nagasaki City, Nagasaki Prefecture, Japan; [2]Dept of Histology and Cell Biology, Nagasaki Univ School of Medicine, Nagasaki City, Nagasaki Prefecture.*

Background: Although histone acetylation, one of epigenetic modification, has been reported to be related to the progression of various diseases, its involvement in the progression of nephrosclerosis is unclear.

Methods: 6 week-old Dahl salt-sensitive rats were divided into 3 groups: (i) normal salt diet (NS) group, (ii) high salt diet (HS) group, (iii) HS group administered daily curcumin, a histone acetyltransferase (HAT) inhibitor, (HS+C group). At 6 week after salt load, the kidneys were dissected out. Morphologic changes were assessed by Masson's trichrome staining. The number of macrophages and fibroblasts, and histone acetylation—positive cells were assessed by immunohistochemistry. HAT and histone deacetylase (HDAC) activity were measured by Enzyme-linked immunosorbent assay. The genes affected to their expression by histone acetylation were examined by chromatin immunoprecipitation.

Results: Both HS and HS+C groups showed marked increase of systolic blood pressure since 2 week of salt load. At 6 week, serum creatinine was increased markedly in HS group, whereas it was suppressed in HS+C group. In HS group, interstitial fibrosis and glomerular sclerosis were observed, and the each number of macrophages and fibroblasts was increased more than those in NS group. HS+C group showed the inhibition of these inflammation and fibrosis. The level of histone acetylation was enhanced in HS group compared to NS group, whereas the curcumin administration suppressed histone acetylation. HAT/HDAC activity ratio was elevated in HS group and tended to be lower in HS+C group than that in HS group. In HS group, interleukin 6 (IL-6) gene and protein expression were increased by histone acetylation.

Conclusions: Our results suggested that increased IL-6 gene expression induced by the enhancement of histone acetylation might be involved in the progression of nephrosclerosis, independently of hypertension.

FR-PO312

Progressive Activation of Innate Immunity and Inflammasome Components in the 5/6 Renal Ablation Model Camilla Fanelli, Jessica K. Okuma, Fernanda F.F. Zambom, Simone C.A. Arias, Flavia G. Machado, Victor F. Avila, Viviane D. Faustino, Gizely C.S. Moreira, Orestes Foresto-Neto, Lisienny C.T. Rempel, Claudia R. Sena, Vivian L. Viana, Ricardo P. Mazzonetto, Denise M.A.C. Malheiros, Niels O.S. Camara, Clarice K. Fujihara, Roberto Zatz. *Univ of Sao Paulo, Brazil.*

Background: Innate immunity activation may play a key role in the development of chronic kidney disease (CKD). We showed previously that NFkB inhibition attenuates renal injury in the 5/6 nephrectomy model (NX). Here we examine whether activation of the inflammasome/IL-1b pathway occurs as renal injury associated with NX progresses.

Methods: Adult male Munich-Wistar rats underwent NX (N=20) or sham operation (S, N=10). Tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/24h), serum creatinine (S_{cr}, mg/dL), glomerulosclerosis index (GSI), % interstitial expansion (INT), macrophage infiltration (MF, cells/mm^2), % TLR4+ tubules (%TLR4+tub) and the gene expression of Casp1, Nrlp3, Tlr4, and Il1b were assessed 7, 30, 60 and 120 days after ablation.

Results:

	S	NX 7d	NX 30d	NX 60d	NX 120d
TCP	137±2	171±5^{a}	206±7^{ab}	211±9^{ab}	218±5^{ab}
ALB	5±1	55±16^{a}	88±15^{ab}	94±11^{ab}	175±14^{abcd}
S_{cr}	0.6±0.1	1.2±0.1^{a}	1.2±0.1^{a}	1.1±0.1^{a}	1.3±0.1^{a}
GSI	0±0	0±0	15±4	38±9^{ab}	108±20^{abcd}
INT	0.2±0.1	0.5±0.1	2.9±0.4^{ab}	4.7±0.8^{abc}	5.0±0.2^{abc}
МΦ	21±2	47±10	135±35^{ab}	153±29^{ab}	216±25^{abcd}
TLR4+tub	7±1	11±2^{a}	18±1^{ab}	20±1^{ab}	20±2^{ab}

Results are mean±SE: $^{a}p<0.05$ vs C, $^{b}p<0.05$ vs NX7, $^{c}p<0.05$ vs NX30, $^{d}p<0.05$ vs NX60.

Innate immunity activation followed closely the progression of renal injury and inflammation. At 7 days after NX, mRNA and protein expression of Tlr4 were statistically increased when compared to S group. By the end of the study, the expressions of Casp1, Nlrp3, Tlr4 and Il1b were 3-, 8-, 5- and 4-fold, respectively, those observed in S.

Conclusions: Activation of innate immunity/inflammasome tends to parallel, since early stages, the development of renal injury in NX, suggesting that these events may constitute early pathogenic factors and may offer novel targets for treatment of CKD.

Funding: Government Support - Non-U.S.

FR-PO313

Genetic Background Dictates the Incidence of Renal Fibrosis following Experimental Pyelonephritis Babitha Haridas,[1] Birong Li,[1] Hanna H. Cortado,[1] Robert Easterling,[1] David S. Hains,[2] John David Spencer,[1] Santiago Partida-Sanchez,[1] Brian Becknell.[1] *[1]Nationwide Children's Hospital, Columbus, OH; [2]Le Bonheur Children's Hospital, Columbus, OH.*

Background: Risk factors for post-pyelonephritis renal scarring are incompletely elucidated. We induced experimental pyelonephritis in inbred mice to test the hypothesis that renal fibrosis is genetically determined.

Methods: Six week old C3H/HeOuJ or C3H/HeN females were transurethrally inoculated with 10^8 CFU uropathogenic Escherichia coli (UPEC). Mice were sacrificed 1, 3, 7, 14, and 28 days post infection (dpi) to measure bacterial burden, gene expression, inflammation and fibrosis.

Results: UPEC challenged C3H/HeOuJ and C3H/HeN mice developed pyelonephritis with comparable frequency and bacterial burden 1 and 3 dpi. UPEC induced renal transcripts associated with response to bacterial lipopolysaccharide and renal injury in both strains, including Il6 and Lcn2 ($p < 0.05$). Beginning 14 dpi, UPEC burden was increased in C3H/HeOuJ compared to C3H/HeN kidneys ($p < 0.05$). UPEC clearance occurred in 57% of C3H/HeN kidneys by 14 dpi, whereas 100% of C3H/HeOuJ kidneys remained infected ($p < 0.001$). C3H/HeOuJ kidneys displayed cortical inflammation (25%) and scarring (17%) by

7 dpi, but these features were absent in C3H/HeN kidneys. Inflammation grade correlated with myofibroblast activation and interstitial fibrosis in C3H/HeOuJ kidneys (Spearman coefficients 0.85 and 0.69, respectively, $p = 0.01$). Flow cytometry identified increased absolute number of macrophages and neutrophils in C3H/HeOuJ kidneys 7 dpi compared to uninfected controls ($p < 0.005$).

Conclusions: C3H/HeOuJ kidneys demonstrate increased bacterial burden, prolonged infection, increased inflammation, and higher grades of fibrosis, compared to C3H/HeN following UPEC infection. C3H/HeOuJ serves as a preclinical model for the development of anti-inflammatory and antifibrotic therapies for pyelonephritis. Our study supports the hypothesis that host genetic determinants dictate susceptibility to inflammation driven renal fibrosis in pyelonephritis.

FR-PO314

HIP-PAP / RegIIIg Is a Urothelium Derived Antimicrobial Peptide Induced by Bacterial Infection in Humans and Mice Birong Li,[1] Martin S. Vonau,[3] Ashley R. Carpenter,[1] Kirk M. McHugh,[2] John David Spencer,[1] Brian Becknell.[1] *[1]Nephrology, Nationwide Children's Hospital, Columbus, OH; [2]Anatomy, Ohio State Univ School of Medicine, Columbus, OH; [3]College of Medicine, Ohio State Univ, Columbus, OH.*

Background: Antimicrobial peptides (AMPs) serve proposed roles in shielding the urinary tract from invading microbes. We hypothesized that global transcriptome profiling following experimental urinary tract infection (UTI) would identify key AMPs that serve this function *in vivo*.

Methods: We analyzed the bladder transcriptomes of C57BL/6 female mice transurethrally inoculated with 10^8 uropathogenic Escherichia coli (UPEC) 2 and 6 hours post-infection (hpi), using oligonucleotide microarrays. Human and mouse AMP mRNA was measured by QRT-PCR, and AMP protein was detected by immunoblotting, immunohistochemistry, and ELISA. AMP knockout or wild type female mice were inoculated with 10^7 UPEC and evaluated for bacterial burden 16, 24, 48, and 72 hpi.

Results: *RegIIIg* is the most upregulated AMP mRNA in UPEC infected bladders, with a 300-fold induction peaking 16 hpi ($p = 4 \times 10^{-5}$). RegIIIg protein localizes to the apical surface of infected bladder and renal urothelium. Bladder and urine RegIIIg protein levels increase with infection. In humans, the orthologous HIP/PAP protein localizes to urothelium and renal tubules. Kidney HIP/PAP protein levels increase in pyelonephritis. HIP/PAP is absent from sterile human urine and significantly increases in urine from patients with UPEC cystitis and pyelonephritis ($p = 0.009$). We next evaluated the functional consequences of *RegIIIg* deletion on bacterial clearance following experimental UTI. *RegIIIg* deficiency did not significantly affect UPEC bladder clearance 16, 24, 48 and 72 hpi.

Conclusions: Murine RegIIIg and human HIP/PAP are urothelial derived peptides enriched in infected urine. While *RegIIIg* is the most induced AMP mRNA in experimental cystitis, it is dispensable for UPEC clearance from the murine bladder. The function of RegIIIg and HIP/PAP during UTI and mechanism of their induction by UPEC require further study.

Funding: NIDDK Support

FR-PO315

Renal Effects of Dipeptidyl Peptidase-4 Inhibition in Rats with Doxorubicin Nephropathy Gheun-Ho Kim, Eun Young Choi, Sua Kim, Chor Ho Jo, Joon-Sung Park. *Internal Medicine, Hanyang Univ College of Medicine, Seoul, Republic of Korea.*

Background: Dipeptidyl peptidase-4 (DPP-4) inhibitors were known to have a protective effect on the diabetic kidney disease, possibly via reduction of oxidative stress and inflammation in the kidney. However, whether these potential mechanisms may exert in non-diabetic kidney diseases is not clear. We tested if DPP4 inhibitors may have beneficial effects on rat kidneys in doxorubicin nephropathy.

Methods: Two different animal experiments were carried out using sitagliptin (10 mg/kg/d) and linagliptin (3 mg/kg/d) for DPP-4 inhibition. In each experiment, male Sprague-Dawley rats were uninephrectomized and randomly divided into vehicle-treated controls and doxorubicin-treated (a single iv bolus 5 mg/kg BW) rats. A half of the doxorubicin-treated rats were then treated with a DPP-4 inhibitor given by daily oral gavage over 6 weeks. Systolic blood pressure, proteinuria, and serum creatinine were determined at 4 and 6 weeks, and then kidneys were harvested for histopathologic examination, immunoblot analysis, and quantitative PCR analysis.

Results: In doxorubicin-treated rats, remarkable hypertension and proteinuria were noted. Although systolic blood pressure was significantly reduced by linagliptin treatment (180 ± 5 versus 155 ± 4 mmHg, $P<0.05$), heavy proteinuria was not affected by DPP-4 inhibition. Increases in serum creatinine in doxorubicin-treated rats were insignificantly decreased by DPP-4 inhibition, but tubulointerstitial injury and interstitial fibrosis were significantly relieved by treatment with sitagliptin or linagliptin. The increase in renal protein expression of monocyte chemotactic protein 1 in doxorubicin-treated rats was significantly decreased by linagliptin treatment (205 ± 9 versus $114 \pm 24\%$, $P<0.05$). The increases in renal mRNA expression of gp91phox (175 ± 13 versus $104 \pm 13\%$, $P<0.05$) and RANTES (811 ± 39 versus $459 \pm 41\%$, $P<0.05$) were significantly reduced by sitagliptin treatment.

Conclusions: In rats with doxorubicin nephropathy, hypertension and renal injury may be attenuated by DPP-4 inhibition. Inflammatory responses associated with NADPH-oxidase 2 upregulation in doxorubicin nephropathy seem to be suppressed by treatment with DPP-4 inhibitors.

FR-PO316

Mild Acidic Milieu Alters Action Potential Threshold in Renal Neurons Wolfgang Freisinger,[1] Nadja Tzinis,[2] Tilmann Ditting,[2] Sonja Heinlein,[2] Jens Lutz,[1] Roland E. Schmieder,[2] Karl F. Hilgers,[2] Roland Veelken.[2] *[1]Med. Clinic, Nephrology, Universitätsmedizin Mainz, Mainz, Germany; [2]Med. Clinic 4, Nephrology and Hypertension, Univ Erlangen-Nürnberg, Erlangen, Germany.*

Background: Renal innervation has been shown to play an important role under pathologic conditions, e.g. renal inflammation. Recently, we found that afferent renal neurons exhibit predominantly a sustained firing upon current injection due to specific sodium channel expression. Additionally, we had hints that these particular neurons show inward currents mediated by acid sensitive channels, e.g. TRPV1 and ASIC. Therefore, we hypothesized that mild changes in pH, could alter the firing activity of renal afferent neurons.

Methods: Dorsal root ganglion (DRG) neurons (n=94) from Th_{11}-L_2, harvested from SD-rats were investigated. Retrograde labelling (DiI) identified renal afferent neurons. Current clamp was used to characterize neurons as "tonic", i.e. sustained action potential (AP) firing or "phasic", i.e. <5 APs upon electrical stimulation. Current injections (40pA-12000pA) were performed under physiologic (pH 7,4) and mild acidic (pH6,9) conditions. Acid channel blocker (Capsazepine, CPZ 10µmol/l and Amiloride, 10µmol/l) were added to investigate the respective channels.

Results: 73 renal (DiI-positive) and 21 non-renal neurons (DiI neg) were investigated. Non-renal neurons were exclusively "phasic" and unaffected by pH-change, also "phasic" renal neurons. In "tonic" renal neurons (47/73), exposure to pH 6,9 did alter the threshold of action potential activation (-17.4 ± 7.5 versus -13.5 ± 7.3*) and reduced firing frequency (22.3 ± 9.5 versus 15.9 ± 10.1/600ms, *$p<0.05$), other electrophysiological parameters (resistances, capacity) remained equal. Blockers did not reverse this effect.

Conclusions: We found that exposition to protons significantly decreased the firing rate of renal "tonic", more active neurons. On the other hand this was accompanied by a less negative threshold putatively facilitating activation of tonic neurons with reduced maximum firing rate. This effect seems to be independent of acid sensitive channels such as the ASIC and TRPV1.

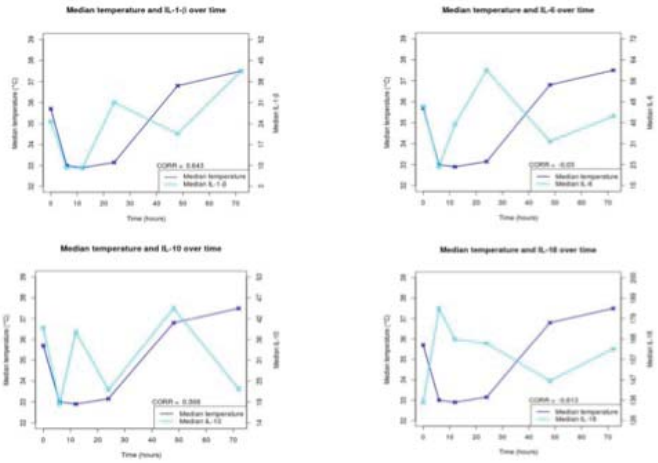
FR-PO317

Cytokines Response following Induced Hypothermia Silvia De Rosa,[1,2] Alessandra Brocca,[1] Massimo de Cal,[1] Stefano Marcante,[1] Silvia Pastori,[1] Massimo Antonelli,[2] Claudio Ronco.[1] *[1]Nephrology and Dialysis, International Renal Research Inst (IRRIV), San Bortolo Hospital, Vicenza, VI, Italy; [2]Intensive Care and Anaesthesiology, Univ Cattolica del Sacro Cuore, Rome, RM, Italy.*

Background: The impairment of cardiac contractility during endotoxemia causes overexpression of proinflammatory cytokines that can significantly alter metabolic response. In vitro studies suggest that proximal tubular cells are vulnerable to proinflammatory cytokines (i.e.IL6 and IL1b) mediated injury.Therapeutic hypothermia (TH) has been used to prevent damage connected with ischemia and hypoperfusion.The purpose of the study is to analyze the influence of hypothermia state (33°C) during TH on renal infiammatory response in patients after cardiac arrest with restoration of spontaneous circulation.

Methods: We performed a prospective observational study of 8 comatose patients resuscitated from out-of-hospital cardiac arrest and treated with TH.On Admission time and at 6,12,24,48,and72hrs after the start of TTTH,blood samples were collected.Plasma cytokines were measured by ELISA.

Results: At the beginning of TH and at 6,12,24,48,72 hrs,mean T were respectively 35 (±0.5),33 (±0.4),33 (±0.4),33.5 (±1.6),36 (±0.8),37 (±0.6).Correlations were calculated between temperature (T) and cytokines.



During the induction phase,we observed a decrease of IL6,IL1b and IL10 levels,and an increase of infiammatory during the maintenance phase.A similar initial effect is showed for IL18,but with an earlier proinfiammatory effect during induction phase.Furthermore,we assist an increase of IL10 and ILb during the rewarming phase.

Conclusions: In conclusion,data indicate that,during induction and rewarming phases there is a modification of infiammatory balance.It is important to define a real time window during which TH could provide a therapeutic effect on these mechanisms.Additional clinical studies are needed for an optimal maintenance period and strategy.

FR-PO318

NLRP3 Inflammasome Mediates Albumin-Induced Renal Tubular Injury through Impaired Mitochondrial Function Aihua Zhang, Songming Huang, Zhanjun Jia. *Nephrology, Nanjing Children's Hospital, Nanjing Medical Univ, China.*

Background: Proteinuria serves as a direct causative factor of renal tubular cell injury and is highly associated with the progression of chronic kidney disease (CKD) via uncertain mechanisms. The present study was undertaken to examine the role of NLRP3 inflammasome/mitochondria axis in albumin-induced renal tubular injury.

Methods: A total of 18 renal biopsy samples obtained from patients undergoing diagnostic evaluation at our department were selected. Nlrp3$^{-/-}$ and caspase1$^{-/-}$ mice on a C57BL/6J-129 background were used to examine the roles of NLRP3 and caspase-1 in albumin overload-induced renal tubular injury. Mouse proximal tubular cells (mPTCs), an immortalized cell line, were cultured. NLRP3, caspase-1, IL-1b, and IL-18 were examined by immuohistochemistry, immunoblotting, real time RT-PCR. Mitochondrial function including ROS production, mitochondrial membrane poteintial (MMP), and ATP content was evualuated.

Results: In patients with proteinuria, NLRP3 was significantly upregulated in tubular epithelial cells and was positively correlated with the severity of proteinuria. In agreement with these results, albumin remarkably activated NLRP3 inflammasome in both in vitro renal tubular cells and in vivo kidneys in parallel with significant epithelial cell phenotypic alteration and cell apoptosis. Genetic disruption of NLRP3 inflammasome remarkably attenuated albumin-induced cell apoptosis and phenotypic changes under both in vitro and in vivo conditions. In addition, albumin treatment resulted in a significant mitochondrial abnormality as evidenced by the impaired function and morphology, which was markedly reversed by invalidation of NLRP3/caspase-1 signaling pathway. Interestingly, protection of mitochondria function by MnTBAP or CsA robustly attenuated albumin-induced injury in mPTCs.

Conclusions: These findings demonstrated a pathogenic role of NLRP3 inflammasome/caspase-1/mitochondria axis in mediating albumin-induced renal tubular injury. The discovery of this novel axis provides some potential targets for the treatment of proteinuria-associated renal injury.

FR-PO319

IL13-Induced Hepatic Cholesterol Transport Defect in Rat Model of Minimal Change Nephrotic Syndrome (MCNS) Lauretta Danwei Low,[1] Chang Yien Chan,[1] Jinmiao Chen,[2] Henry He Yang,[1] Caroline G.L. Lee,[1] Hanry Yu,[1] Markus R. Wenk,[1] Hui Kim Yap.[1] *[1]Pediatrics, Biochemistry, Cancer Science Inst, Physiology, National Univ of Singapore, Singapore; [2]SIgN, A*STAR, Singapore.*

Background: Hypercholesterolemia in nephrotic syndrome is thought to be secondary to marked proteinuria. However, in our *IL13*-overexpression rat model of MCNS, hypercholesterolemia preceded development of proteinuria suggesting the role of a cytokine. This study aimed to investigate the mechanism of IL13-induced cholesterol dysregulation in our rat model of MCNS.

Methods: Plasmid containing rat *IL13* gene was transfected into Wistar rats. Liver RNA was used for microarray and qPCR. The direct effect of IL13 (100ng/ml) on rat primary hepatocyte cell culture was studied after 24-hour incubation using qPCR to evaluate gene expression, while cholesterol efflux via Abcg5 was measured using taurocholate as a cholesterol acceptor.

Results: *IL13*-transfected rats became nephrotic by Week 10 and both liver microarray analysis and qPCR showed 6-fold downregulation in *Abcg5*, with no change in *Lxra*. Moreover, compared to controls, we demonstrated downregulation in gene expressions of *Abcg5* (0.033±0.011 versus 0.091±0.022, p=0.021), *Abca1* (0.072±0.005 versus 0.094±0.007, p=0.025), and their transcriptional regulator, *Lxra* (0.21±0.01 versus 0.29±0.03, p=0.03), early on in *IL13*-transfected rats with hypercholesterolemia prior to onset of proteinuria. Similarly, IL13-stimulated rat primary hepatocyte cells showed downregulation in gene expressions of *Lxra* (0.010±0.002 versus 0.024±0.009, p=0.012) and *Abca1* (0.016±0.002 versus 0.027±0.004, p=0.012), as well as decreased cholesterol efflux (10.77±1.13% versus 13.31±1.24%, p=0.012) compared to unstimulated cells, consistent with the early events in the rat model.

Conclusions: In conclusion, our study demonstrated that IL13 was able to downregulate the Lxra-Abca1/Abcg5 pathway of hepatic cholesterol elimination, with resultant hypercholesterolemia in the *IL13*-transfected rats prior to development of proteinuria. Subsequent sustained severe hypercholesterolemia as seen in the nephrotic rats could result in normalization of *Lxra* expression due to the positive feedback stimulus possibly via oxysterols, which are natural agonists for Lxra.

Funding: Government Support - Non-U.S.

FR-PO320

HIV Enhances Kidney Cell Inflammasome Formation Both *In Vivo* and *In Vitro* Shabirul Haque, Partab Rai, Kartikeya Kashyap, Rivka Lederman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Inflammasome activates an inflammatory cascade by binding to pro-caspase-1 via ASC (apoptosis-associated speck-like protein containing CARD [caspase recruitment domain]) and cleaving caspase-1, the later, converts pro-interleukin (IL)-1ß to IL-1ß. Deregulated inflammasome activity has been linked to variety of inflammatory diseases. HIV-1 has been reported to stimulate NLRP3 expressing inflammasomes in immune cells. However, the effect of HIV on kidney cell inflammasome formation has not been studied. In the present study, we evaluated the effect of HIV on kidney cell inflammasome formation both *in vitro* and *in vivo*.

Methods: Renal cortices of four week old control (FVB/N, n=4) and HIV-transgenic (Tg26, n=4) mice were immunolabeled for molecular markers of inflammasomes including NLRP3, ASC1, pro-caspase-1, and pro-IL-1ß (n=4). cDNA and protein blots of renal tissues of control and Tg26 mice were probed for above mentioned markers. Serum levels of IL-1ß in control and Tg26 mice were measured. Human podocytes-transduced with either empty vector (EV) or HIV were treated with either caspase-1 inhibitor or buffer for 24 H, followed by Western blot analysis for ASC and IL-1ß. Since inflammasome formation is associated with pyroptosis (condensed nuclei with leaky cell wall), EV/HPS and HIV/HPs in the presence or absence were evaluated for induction of pyroptosis.

Results: Renal tissues of Tg26 mice displayed enhanced mRNA as well as protein expression of NLRP3, ASC, caspase-1 and IL-1ß. Renal cortices of Tg 26 mice also displayed enhanced kidney cell expression NLRP3, ASC, caspase-1 and IL-1ß. Serum of Tg26 mice displayed 4-fold higher levels of IL-1ß versus FVB/N mice. *In vitro* studies, protein blots of HIV/HPs displayed 2-fold increase in ASC and IL-1ß expressions. Whereas, caspase-1 inhibitor down regulated the effect of HIV on podocyte ASC and IL-1ß expressions**.** HIV/HPs displayed greater number of pyroptosed cells versus EV/HPs. Whereas, caspase-1 inhibitor-treated HIV/HPs displayed attenuated number of pyroptosed cells.

Conclusions: HIV enhances kidney cell inflammasome formation both in vivo and in vitro. HIV induces pyroptosis in podocytes.

Funding: NIDDK Support

FR-PO321

Mitigation of CD40 Dependent Renal Inflammation via CD40 Antisense Oligonucleotides Adam E. Mullick, Aaron Donner, Steve T. Yeh, Huimin Li, Mark Graham, Rosanne M. Crooke. *Antisense Drug Discovery, Isis Pharmaceuticals, Carlsbad, CA.*

Background: Experiments were conducted to test the hypothesis that renal CD40 is primed in kidney disease and that CD40 dependent renal inflammation would be mitigated by CD40 antisense oligonucleotide (ASO) treatment.

Methods: Activation of CD40 dependent renal inflammation was performed by IV administration of an activating CD40 mAb. ASO treatments consisted of SQ administrations of Gen 2.5 CD40 or control ASOs. Studies were conducted in two models of kidney disease. In the first, mice were given adriamycin and then 2 weeks later divided into groups with low or high adriamycin nephropathy (AN) based on proteinuria and urine NGAL. ASO treatments were started 30 days after initiation of AN. In a second model, ASO treatments were initiated in mice after unilateral ureter obstruction (UUO). In both studies, the CD40 mAb was administered after 10 days of ASO treatments and tissues harvested 24 hours afterward.

Results: In AN mice, CD40 mAb resulted in larger increases in kidney CD40, CCL5 and IL12 in mice with high AN versus low AN. E.g., CD40 mAb increased renal CCL5 by 38-fold in high AN mice but only 6-fold in low AN mice. CD40 ASO treatments strongly mitigated or prevented CD40 mAb-dependent inflammation in both subgroups of AN mice. In UUO mice, renal inflammation following the CD40 mAb was larger in the obstructed kidney versus the unobstructed kidney. E.g., following CD40 mAb renal CCL5 increased 120-fold in the obstructed kidney versus 10-fold in the unobstructed kidney. In both kidneys, CD40 ASO treatments reduced renal CD40, CCL5 and IL12 by up to 90% relative to control. This level of activity was comparable to that observed in vehicle treated UUO mice not given the CD40 mAb as well as CD40 knockout mice receiving UUO and the CD40 mAb.

Conclusions: These data demonstrate CD40 activation results in renal inflammation, with the extent of preexisting injury or inflammation priming the CD40 response. CD40 ASO treatments sharply attenuated or prevented CD40 dependent inflammatory responses and were equally efficacious in kidneys with either low or high preexisting injury. Such treatments could be beneficial in patients with aberrant or excessive renal CD40 activation.

Funding: Pharmaceutical Company Support - Isis Pharmaceuticals

FR-PO322

Inflammasome Activation in Renal Failure Is Exaggerated by High Fat Diet (HFD): Role of Lipopolysaccharides (LPS) Sam Righi, Siddhartha S. Ghosh, Daniel E. Carl, Richard Krieg, Todd W. Gehr. *Nephrology, VCU, Richmond, VA.*

Background: Inflammasome activation is shown to play a major role in various inflammatory disorders. Activation of inflammasome is generally mediated by two triggers (i) Activation of NFkB by LPS and (ii) membrane perturbation and K^+ efflux which can be mediated by iPLA2. We have seen increased iPLA2 and NFkB activation in renal failure (RF) and in animals on high fat diet (HFD). In this study we observe an increase in circulatory LPS in RF mice and mice on HFD therefore, we postulate that LPS activates inflammasome in RF which is aggravated by HFD. We use LPS binder Polymyxin (PM) to prove our hypothesis.

Methods: We used LDLR-/- mice since they are susceptible to inflammation following HFD. RF was induced in LDLR-/- mice by 5/8 nephrectomy. 24 Mice were divided into sham surgery (C) and C+HFD (HF), 5/8 nephrectomy with normal chow (Nx), Nx with 4 months of high fat diet (NxHF). Two NxHF groups received PM; 15 mg/l in drinking water; n=5.

Results: Creatinine, BUN,ACR of Nx and NxHF ($p<0.01$) were significantly higher than C and HF. HF was similar to C, suggesting no renal impairment in HF. Compared to Nx, creatinine, BUN and ACR of NxHF were significantly higher ($p<0.05$). Circulatory LPS levels of the groups were as follows: Nx>HF ($p<0.05$) and HF> C ($p<0.05$). LPS of NxHF was 2 fold higher than Nx ($p<0.05$) indicating HFD increased LPS. Inflammasome markers, pNFkB/NFkB, Caspase 1, IL-1b and ASC in the kidney were highest in NxHF

(p<0.05), followed by Nx>HF (p<0.05) HF>C (p<0.05). Since Nx+HF had highest levels of LPS and renal dysfunction we chose to treat this group with PM. PM reduced BUN and ACR 30% and 40% respectively (P<0.05) but not creatinine. PM also decreased the levels LPS by 55% (p<0.01). All the inflammasome markers were significantly reduced by PM.

Conclusions: This study show that in RF circulatory LPS is increased possibly due to increased permeability from the gut.This increase is further exaggerated by HFD. PM is a non-absorbable antibiotic with LPS binding properties hence its beneficial effect can only be imparted by modulating LPS permeability from the gut. By reducing circulatory LPS, PM significantly reduces inflammation and ameliorates RF.

Funding: Private Foundation Support

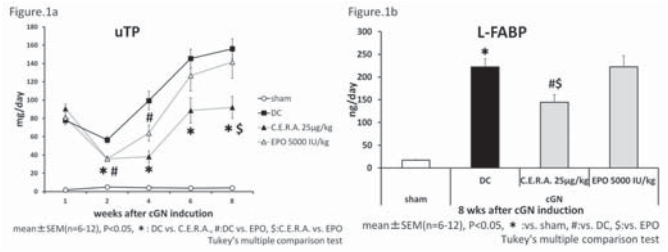
FR-PO323

Single Treatment of Epoetin Beta Pegol Halts Chronic Kidney Disease Progression in Chronic Glomerulonephritis Rats: Comparison with Epoetin Beta Ryohei Kawasaki, Ken Aizawa, Michinori Hirata, Yoshihito Tashiro, Kenji Yogo, Mika Yagoto, Koichi Endo. *Product Research Dept, Chugai Pharmaceutical Co., Ltd., Gotemba, Shizuoka, Japan.*

Background: Erythropoietin-stimulating agents (ESAs) show renoprotection in several acute kidney disease models, such as puromycin aminonucleoside and Thy1-induced nephritis models. However, the renoprotective effects of ESAs in progressive chronic kidney disease (CKD) models have not been fully studied. We reported previously at ASN 2012 that epoetin beta pegol (continuous erythropoietin receptor activator, C.E.R.A.), which has a longer half-life than any other ESAs, showed renoprotection in rats with chronic glomerulonephritis (cGN). The aim of the current study was to compare the renoprotective effect of C.E.R.A. with that of epoetin beta (EPO) in cGN.

Methods: The cGN model was established by injection of anti-Thy1.1-antibody (OX-7, 0.6 mg/kg, i.v.) to uninephrectomized rats (F344, 7 wks old, male). Vehicle (disease control: DC), C.E.R.A. (25 mg/kg), or EPO (5000 IU/kg) was intravenously administered 24 h after the operation. We then evaluated urinary total protein (uTP) at 1, 2, 4, 6 and 8 wks after induction of cGN, and liver-fatty acid-binding protein (L-FABP) at 2 wks and 8 wks.

Results: uTP excretion was higher than sham-operated rats at 1 wk after induction of cGN, and it gradually increased from 2 wks to 8 wks. C.E.R.A. and EPO showed almost the same level of renoprotection (decreased uTP and L-FABP compared with DC) at 2 wks after treatment. C.E.R.A. showed continuous suppression of uTP and L-FABP until 8 wks, whereas EPO did not.



Conclusions: Although both C.E.R.A. and EPO showed a renoprotective effect in the early phase of cGN, only C.E.R.A. suppressed CKD progression in the late phase. This is the first evidence that the long half-life of ESA might be one of the beneficial features in suppressing the progression of CKD.

FR-PO324

The Increases in Glomerular Permeability due to IL-1ß, TNF-a and IL-6 in Rats Are Inhibited By the Superoxide-Scavenger Tempol Bengt Rippe, Kristinn Sverrisson, Josefin Axelsson, Anna Rippe. *Dept of Nephrology, Lund Univ, Lund, Sweden.*

Background: This study was performed in order to investigate the actions of some common proinflammatory cytokines, interleukin-1 beta (IL-1ß), tumor necrosis factor alpha (TNF-a) and IL-6, on glomerular permeability in rats and to test whether these actions depend upon the release of reactive oxygen species (ROS), particularly superoxide.

Methods: In anaesthetized Wistar rats (~250 g) blood access was achieved and the left ureter was cannulated for urine collection. Rats were continuously infused i.v. with either IL-1ß (2 micro-g/kg/h), TNF-a (2 micro-g/kg/h) or IL-6 (4 micro-g/kg/h), with 1/4 of the dose given as an initial bolus, together with polydisperse fluorescein isothiocyanate (FITC)-Ficoll-70/400, Inulin and ^{51}Cr-EDTA for 1h. Plasma and urine samples were taken simultaneously at different time points and analyzed by high performance size exclusion chromatography (HPSEC) for determination of glomerular sieving coefficients (q) for Ficoll. The glomerular filtration rate (GFR) was assessed using ^{51}Cr-EDTA. In separate experiments the superoxide scavenger, tempol (15 mg/kg/h), was given before and during the cytokine infusions.

Results: IL-1ß and TNF-a caused rapid, partly reversible increases in glomerular permeability to large Ficoll molecules ($Ficoll_{50-80Å}$), peaking at 5-15 min, while IL-6 caused a more gradual increase in glomerular permeability, leveling off at 30 and 60 min. For TNF-a, q for $Ficoll_{70Å}$ increased from $2.7 \times 10^{-5} \pm 0.7 \times 10^{-5}$ to $2.1 \times 10^{-4} \pm 1.2 \times 10^{-5}$ at 15 min, while GFR and systemic arterial blood pressure remained largely unchanged. Co-infusion of tempol almost completely abrogated the permeability effects of the cytokines infused.

Conclusions: IL-1ß, TNF-a and IL-6, when infused systemically, can cause marked and partly reversible increases in glomerular permeability, which can be inhibited by the superoxide scavenger, tempol. Our data suggest that, like angiotensin II, the proinflammatory cytokines, IL-1ß, TNF-a and IL-6, all induce increases in glomerular permeability mediated via the release of ROS.

Funding: Government Support - Non-U.S.

FR-PO325

Inhibition of Dipeptidyl Peptidase-4 Suppresses Macrophage Infiltration via Glucagon-Like Peptide-1 Dependent Signaling in the Rat Thy-1 Nephritis Model Yoshiki Higashijima, Tetsuhiro Tanaka, Junna Yamaguchi, Shinji Tanaka, Masaomi Nangaku. *Div of Nephrology and Endocrinology, The Univ of Tokyo Graduate School of Medicine, Bunkyo-ku, Tokyo, Japan.*

Background: Dipeptidyl peptidase-4 (DPP-4) is an enzyme that inactivates incretin hormones such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). DPP4 inhibitors are now widely used for the treatment of type 2 diabetes. In contrast, widespread expression of the receptor for GLP-1 has called for alternative actions of incretins in extrapancreatic tissues. In the kidney, DPP4 inhibitors exhibited reno-protective effects against various models of diabetic kidney disease. However, little is known in the non-diabetic models. Thus, we examined the effect of DPP4 inhibitors in the rat Thy-1 nephritis model.

Methods: Rats were injected with OX-7 (1.2 mg/kg, iv) and treated with alogliptin (20 mg/kg/day, orally by gavage) or vehicle for 7 days.

Results: DPP4 inhibitors did not ameliorate glomerular injury and resulted in a non-significant trend toward a reduction in proteinuria. Instead, immunohistological analysis revealed that DPP4 inhibitors significantly reduced the number of CD68 positive inflammatory macrophages in the kidney. An additional DPP4 inhibitor, anagliptin, (300mg/kg/day, mixed with food) and a GLP-1 receptor agonist, exendin-4 (10 mg/kg, sc), similarly reduced CD68 positive macrophage infiltration to the kidney, raising a possibility that this is a class effect and that the anti-inflammatory effect was mediated by the GLP-1 receptor signaling. The reduction in macrophages was not associated with changes in the mRNA levels of chemokines such as MCP-1 and RNATES, but was further corroborated in ex-vivo studies. Boyden chamber assays using peritoneal macrophages revealed that exendin-4, but not the DPP4 inhibitor, dose-dependently reduced MCP-1-stimulated macrophage infiltration.

Conclusions: These data suggest that DPP4 inhibitors reduced macrophage infiltration directly via GLP-1 dependent signaling in the rat Thy-1 nephritis model, and offer a promising view that the control of inflammation by anti-diabetic drugs may be of use in models of non-diabetic kidney diseases.

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FR-PO326

Activated Macrophages Trigger Mesangial Cells to Produce Inflammasome Siddhartha S. Ghosh, Hiba Sheikh, Sam Righi, Daniel E. Carl, Todd W. Gehr. *Nephrology, VCU, Richmond, VA.*

Background: Renal injury is an inflammatory disorder and activation of inflammasome may play a role in renal failure (RF). Lipopolysaccharide (LPS) seen in the circulation of RF patients activates inflammasome which often require two triggers. We have observed an increase in circulatory LPS in mice with RF following 5/8th nephrectomy. We postulated that LPS activates inflammation/inflammasome in the kidney. However, it is not clear whether inflammasome activation detected in RF originates from the macrophages infiltrating the kidney and/or directly originates from the kidney cells. This study was undertaken to delineate the source of inflammasome activation.

Methods: 200 pg/ml LPS was used to simulate low levels LPS seen in the circulating plasma of RF animals. Mouse mesangial cells (MC) and macrophages (MO) were incubated separately with LPS for 4 hours (hrs). In separate experiment MO were incubated with LPS for 4 hrs washed and incubated with normal MO media. This MO media is conditioned media. MC were treated with 4 hrs LPS washed and MO conditioned media was added for 24 hrs. Western blot measurements of caspase 1, IL-1b and ASC were carried out to assess inflammasome activation. Ratios of phosphorylated NFkB and NFkB (pNFkB/NFkB) were also measured by immunoblot.

Results: The table shows low levels of LPS can activate MO but not MC however, conditioned media from activated MO can stimulate MC.

	MC treated with 200 pg/ml LPS	MO treated with 200 pg/ml LPS	MC treated with 200 pg/ml LPS washed and treated with MO Conditioned Media
Caspase 1	1.5 (NS)	1.7 fold; p<0.05	2.2 fold; p<0.05
IL-1β	1.7 (NS)	2.5 fold; P<0.05	3.5 fold; P<0.01
ASC	1.8 (NS)	2.8 fold; P<0.05	3.8 fold; P<0.01
pNFkB/NFkB	2.0 (p<0.05)	2.4 fold; P<0.05	3.5 fold; P<0.01

The values are fold increase from their respective controls. NS= non significant

Conclusions: NFkB activation in MC suggest low levels of LPS can cause inflammation but not inflmmasome activation. MO conditioned media activates inflammasome in the MC suggesting that substances released from MO produces second trigger to activate inflammasome in the MC. We conclude inflammasome activation seen in the kidneys of RF animals may be influenced by infiltrating MO.

Funding: Private Foundation Support

FR-PO327

Regulation of the Anti-Inflammatory Glucocorticoid Target Annexin A1 during the Course of Acute Anti-Thy-1.1 Nephritis Robert Labes,[1] Frank Stephan Hohberger,[1] Tanja Loof,[2] Harm Peters,[2] Sebastian Bachmann,[1] Alexander Paliege.[1,2] *[1]Anatomy, Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]Nephrology, Charité - Universitätsmedizin Berlin, Berlin, Germany.*

Background: Glucocorticoids are an essential component of immunosuppressive therapeutic strategies in the treatment of inflammatory renal disease. Annexin A1 (AnxA1) has been identified as a central mediator of anti-inflammatory glucocorticoid actions but its regulation during the course of nephritic disease is unknown.

Methods: We used the anti-Thy-1.1 rat model of human mesangioproliferative glomerulonephritis to study the expression of AnxA1 in a time- and cell-specific manner. Adult Wistar rats were injected with anti-Thy-1.1 antibody and examined after 24h, 5d, and 15d. Regulation of AnxA1 was studied by qPCR, immunohistochemistry and double-labelling immunofluorescence using markers for endothelial cells (rat endothelial cell antigen), fibroblasts (CD73), macrophages (CD68), and lymphocytes (CD4, CD8, CD20).

Results: Under control conditions, AnxA1 was expressed in the podocytes, Bowman's capsule, macula densa, thick ascending limb, medullary collecting duct, and in cortical fibroblasts. Rapid and sustained increases in renal AnxA1 mRNA abundance were recorded at d1 (+92±16%), d5 (+128±19%), and d15 (+78±33%) after the induction of anti-Thy-1.1 nephritis (compared to vehicle-treated controls; $p<.05$ for each time point). AnxA1 immunoreactive signal was enhanced selectively in cells of the cortical renal interstitium at d5 and d15 whereas in the epithelia, signal intensities were unchanged. Double staining immunofluorescence identified infiltrating macrophages as the major source for the interstitial signals (+150±40% on d5 and +378±43% on d15; $p<.05$). The number of AnxA1 immunoreactive cortical fibroblasts was increased as well in these stages. Lymphocytes and endothelial cells of the renal microvasculature were negative for AnxA1.

Conclusions: We have shown that AnxA1 gene expression and protein abundance are significantly increased during the course of anti-Thy-1.1 nephritis. Abundant expression of AnxA1 in macrophages and fibroblasts suggests an important role for these cells during the resolution of renal inflammation.

Funding: Government Support - Non-U.S.

FR-PO328

Activation of Innate Immunity Parallels the Progression of Renal Injury in Adriamycin Nephropathy Viviane D. Faustino, Simone C.A. Arias, Flavia P. Albuquerque, Camilla Fanelli, Victor F. Avila, Lisienny C.T. Rempel, Orestes Foresto-Neto, Gizely C.S. Moreira, Claudia R. Sena, Vivian L. Viana, Ricardo P. Mazzonetto, Denise M.A.C. Malheiros, Niels O.S. Camara, Roberto Zatz, Clarice K. Fujihara. *Univ of Sao Paulo, Brazil.*

Background: Excess filtered protein can promote the release of inflammatory mediators by tubular cells. The mechanisms underlying this effect are unclear. We investigated whether innate immunity/inflammasome assembly is activated in this setting.

Methods: Albuminuria (ALB) was induced in 15 male Munich-Wistar rats by adriamycin (AD), 5 mg/kg iv. Control rats (C, N=5) received saline only. ALB, mg/d, tail-cuff pressure (TCP, mmHg), serum creatinine (S_{Cr}, mg/dL), NGAL, mg/day, % Glomerulosclerosis (%GS), % cortical interstitium (%INT) and INT macrophages (MF, cells/mm^2) were assessed at Weeks 2 (AD_{2wk}), 4 (AD_{4wk}) and 20 (AD_{20wk}) after AD. Renal innate immunity was assessed by gene expression (IL-1ß, TLR4, NLRP3) and protein abundance (IL-1ß, TLR4).

Results:

	C	AD_{2wk}	AD_{4wk}	AD_{20wk}
ALB	7±2	336±34^{a}	356±49^{a}	505±34^{abc}
TCP	142±2	149±3	156±2^{a}	169±4^{abc}
S_{Cr}	0.7±0.1	0.7±0.1	0.7±0.1	1.6±0.2^{abc}
NGAL	28±3	126±23^{a}	97±14^{a}	94±35^{a}
%GS	0.1±0.1	0.7±0.6	1.1±0.5^{a}	47.2±10.2^{abc}
%INT	1±1	1±1	6±1^{ab}	15±2^{abc}
MΦ	9±2	76±34^{a}	142±19^{ab}	150±40^{ab}
IL-1β (ELISA)	0.4±0.1	0.6±0.2	1.6±0.3^{ab}	2.7±0.9^{ab}
TLR-4 (Western Blot)	0.2±0.1	0.2±0.1	0.3±0.1	1.5±0.3^{abc}

MeanSE, $^{a}p<0.05$ vs C, $^{b}p<0.05$ vs AN_{2wk}, $^{c}p<0.05$ vs. AN_{4wk}.
In addition, the mRNA levels of IL-1b, TLR4 and NLRP3 were significantly increased at Weeks 4 (68%, 106% and 177%, respectively) and 20 (127%, 215% and 74%).

Conclusions: AD was followed by massive albuminuria, early tubular injury (indicated by NGAL excretion), and MF infiltration; GS and INT expansion developed from Week 4 on. The expression and/or abundance of IL-1b and TLR4 were approximately parallel to the extent of renal injury. Innate immunity activation/inflammasome assembly may represent one important pathogenic link between tubular exposure to excess protein and renal injury. FAPESP/CNPq.

FR-PO329

Gadolinium–Based Magnetic Resonance Imaging Contrast–Induced Systemic Fibrosis Is Attenuated in Chemokine Receptor–Deficient Mice Brent Wagner. *Medical Service, South Texas Veterans Health Care System, San Antonio, TX.*

Background: Almost nothing is known about the pathobiology of nephrogenic systemic fibrosis. We have found that the monocyte chemoattractant protein–1 (MCP–1) /chemokine (C–C motif) ligand 2 (CCL2) is increased in the serum from contrast–treated animals. CCR2 was detected in the dermis of magnetic resonance imaging contrast–treated rats. Studies were conducted to demonstrate whether an association of gadolinium deposition correlated with tissue fibrosis and the fibrocyte receptor for MCP–1/CCL2, chemokine receptor 2 (CCR2).

Methods: C57 black mice, and CCR2–deficient mice (kindly provided by Seema Ahuja) were treated with magnetic resonance imaging contrast (2.5 mmol/kg), 20 doses over a 4–week period. Fixed, paraffin–embedded tissues were stained and examined by light microscopy. Frozen sections of tissue were homogenized in RIPA buffer, protein quantitated by immunoblot. Relative amounts of gadolinium were assessed by scanning electron microscopy equipped with energy–dispersive x–ray spectroscopy. Inductively–coupled plasma mass spectroscopy was employed to quantify gadolinium in a sample of contrast–treated mice (Jaqueline Ranger, Department of Analytical and Environmental Chemistry, Southwest Research Institute, San Antonio, TX).

Results: With respect to the control animals, there was an increase in dermal cellularity only in the wild–type contrast–treated mice. In parallel, there was an increase in skin fibronectin and a smooth muscle actin in the contrast–treated mice which was attenuated in the CCR2–deficient mice. Liver from contrast–treated animals invariably demonstrated a greater gadolinium signal (both from the Ma and La electron shell regions). Gadolinium was detected in skin, liver, and kidney from contrast–treated mice using inductively–coupled plasma mass spectroscopy.

Conclusions: That CCR2 is increased in contrast–treated animals, and that mice deficient in CCR2 have abrogated skin fibrosis supports the fibrocyte theory of nephrogenic systemic fibrosis. Gadolinium deposition in tissues may trigger the release of MCP–1/CCL2 that mediates the recruitment of CCR2–expressing fibrocytes to affected lesions.

Funding: Veterans Affairs Support

FR-PO330

Indole Derivatives Enhance Epo Production through AhR-XRE Pathway and Induces Cell Protectvie Gene NQO1 Yuki Oba,[1] Shun Watanabe,[1] Akihiro Matsuo,[1] Hisato Shima,[1] Yoichi Takeuchi,[1] Eikan Mishima,[1] Yasutoshi Akiyama,[1] Chitose Suzuki,[1] Takehiro Suzuki,[2] Ken-Ichiro Hayashi,[3] Sadayoshi Ito,[1] Takaaki Abe.[1] *[1]Tohoku Univ Graduate School of Medicine; [2]Brigham and Women's Hospital, Harvard Medical School; [3]Okayama Univ of Science.*

Background: In CKD patients, the accumulation of uremic toxins causes renal inflammation and the inflammatory cytokines (IL-1b, TNF-a) suppress erythropoietin (Epo). Recently, we have identified and reported that certain indole compounds potently enhance Epo production. 41 indole derivatives were further synthesized and shown to be in the same range as FG4592, a potent PHD nhibitor (ASN2013). The aim of this study is to identify the mechanisms underlying the effect of the compound on Epo-production and cell protection.

Methods: 1, An Epo-producing hepatoma cell line (Hep3B) was incubated under both 20% (normoxia) and 1% conditions. 2, a mouse erythropoietin promoter luciferase reporter vector and 4X XRE vector were used for transcriptional assay. 3. Signal transduction pathways were analyzed with a Cignal Transduction 45 Pathway Arrays reporter assay kit™ (Qiagen). 4. *siRNA* knockdown of AhR and NQO1 and QT-PCR was performed.

Results: Epo mRNA was markedly increased by 60 times that of control after the administration of Compound #5 in Hep3B cells under a normoxic condition, although FG4592-mediated upregulation was only two fold. Compound #5 also enhanced Epo protein by 3 fold under a normoxic condition. By signal pathway analysis, Compounds #5 was shown to be linked with the AhR-XRE pathway. The AhR inhibitor CH233191 blocked the Compound #5-induced Epo protein, but K7174 (a GATA inhibitor) and YC-1 (an HIF inhibitor) did not. The Epo mRNA increased by compound #5 was completely abrogated by AhR knock down. These data suggest the involvement of the AXR-EXR pathway in Epo production as well as known GATA and HIF pathways. We also found that NQO1, ananti-odxidant stress gene, was up-regulated by compound #5 and this increment was blocked by AhR knockdown.

Conclusions: This data suggests that indole compounds enhance Epo production by the AhR-XRE pathway as well as GATA and HIF, and the induction of NQO1 may be involved in its cell protective activity.

FR-PO331

Indoxyl Sulfate, a Potential Uremic Neurotoxin, Causes Accumulation of 8-Oxoguanine in Mitochondrial DNA in Differentiated SH-SY5Y Cells
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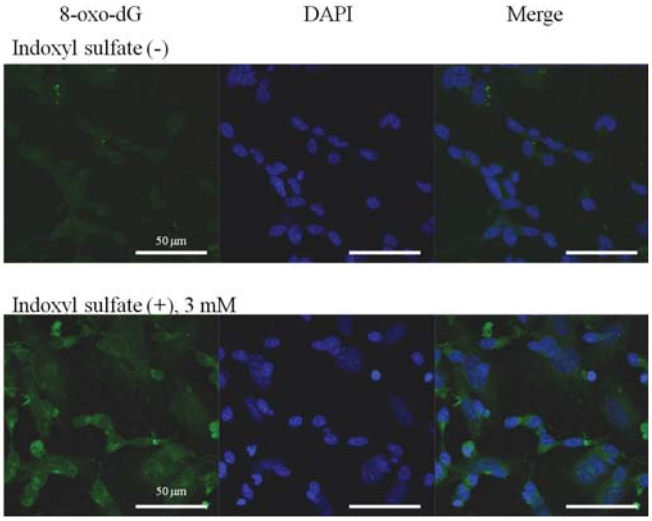
Background: Several reports suggested that indoxyl sulfate (IS) is elevated in the brain of uremia, hence it is implicated as a putative toxin in uremic encephalopathy. Recent experimental studies revealed that mitochondrial dysfunction and oxidative mitochondrial DNA damage are involved in many neurodegenerative disorders (i.e. Alzheimer's disease). We thus explored whether IS causes oxidative DNA damage in neuronal cells.

Methods: Human SH-SY5Y neuroblastoma cell line was treated with retinoic acid to induce neuronal differentiation, then the cells were exposed to 3 mM of IS. After assessment of cell viability, IS-induced production of reactive oxygen species (ROS) was visualized by dihydroethidium. To detect 8-oxo-2'-deoxyguanosine (8-oxo-dG), we performed immunofluorescence laser scanning confocal microscopy.

Results: Ninety six hours after exposure to IS, the number of live cells was significantly decreased (50.9±1.4 %; P <0.01, versus control cells).The level of intracellular ROS in IS-exposed cells was increased in a time dependent manner. In addition, we performed immunofluorescence microscopy for 8-oxo-dG 96 hours after exposure to IS. The nuclear levels of 8-oxo-dG in IS-exposed cells were not increased, but those in mitochondrial DNA were significantly increased after exposure to IS (Figure).



Conclusions: Exposure to IS increased accumulation of 8-oxo-dG in mitochondrial DNA in differentiated SH-SY5Y cells.

FR-PO332

A New Mosaic Mouse Model of Segmental Podocyte Ablation
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Background: Our recent study using chimeric mice demonstrated that injury of a fraction of podocytes can be spread to other initially intact podocytes, which may underlie the progressive expansion of glomerulosclerosis. Each chimeric mouse needs to be de novo generated by aggregating embryos, hindering a further study.

Methods: We aimed to establish a new mosaic mouse model in which a fraction of podocytes express hCD25 and can be injured by an hCD25-targeted immunotoxin, LMB2. For this purpose, lox2272 - loxP - stop - lox2272 - EGFP - polyA - loxP - hCD25 - IRES - tdTomato - polyA were inserted into the first intron of the ROSA26 locus. Upon mating with Nephrin-Cre mice, podocytes are designed to randomly and alternatively express either hCD25 + tdTomato, or EGFP.

Results: Analysis in frozen sections confirmed that glomeruli of this line showed mosaic patterns containing both EGFP-positive and -negative podocytes. Flow cytometry analysis of glomerular cells showed that 51.3±4.3% of podocin-positive podocytes were tdTomato-positive and 30.3±2.4% were EGFP-positive. RT-PCR analysis confirmed that hCD25 mRNA was expressed in tdTomato-positive cells, but not in EGFP-positive or double-negative cells. The mosaic mice showed no abnormal renal phenotype before LMB2 injection, with normal urinary albumin/creatinine ratio (ACR), 0.347±0.81 mg/mg. Nine mosaic mice were subjected to heminephrectomy and injected with LMB2. These mice showed moderate proteinuria with ACR 40.1±20 mg/mg, and focal segmental glomerular sclerosis two weeks after the injection. Of note, EGFP-positive, i.e. hCD25-negative, podocytes were also injured as evidenced by desmin staining and diminished nephrin staining.

Conclusions: While the subtotal nephrectomy model reproduces the spreading of damage from nephron to nephron in a late phase of renal failure, this new model reproduces the spreading of damage from podocyte to podocyte in all phases. This model will be useful for exploring the common therapeutic measure to block the progression of kidney diseases.

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FR-PO333

Podocyte Injury-Driven Endocapillary PAI-1 Promotes a Vicious Cycle of Podocyte Loss *via* beta-1 Integrin Endocytosis
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Background: We reported transgenic mice with podocyte-specific injury (NEP mice) showed up-regulation of the capillary plasminogen activator inhibitor-1 (PAI-1) in glomeruli, prior to thrombosis and proteinuria. PAI-1 is expressed from endothelial cells or platelets in damaged tissue. Both PAI-1 and uPA bind to uPAR, and make a complex with integrin on the cell membrane. Internalization of the complex induces cell detachment as a result of reducing integrin. The present study was aimed to show PAI-1 was involved in podocyte detachment through this novel mechanism.

Methods: Podocyte injury was induced in NEP mice with PAI-1 inhibitor (PI), anti-platelet antibody (PLab), heparin sodium (Hep) or with PBS as a control, by LMB2 injection. Histological and clinical parameters were analyzed on Day 12. We treated cultured podocytes either with uPA (control), PAI-1, PAI-1/uPA complex (P/U), or an antibody for blocking uPAR before P/U. After incubation, we analyzed the localization of b1 integrin and uPAR by immunofluorescence (IF) and immunolabeling electron microscopy (IEM), and western blot.

Results: The NEP mice with PI ameliorated podocyte number, thrombosis and proteinuria compared to the control. b1 integrin translocation to podocyte cytoplasm was inhibited. The NEP mice with PLab reduced PAI-1 expression and preserved podocyte number, whereas Hep loading showed no effect on proteinuria and podocyte number, despite a thrombi decrease. In vitro, attached cells in P/U were reduced compared to the other groups (p <0.01). IF showed cell membrane-localized b1 integrin translocation to cytoplasm in P/U. It was confirmed by b1 integrin western blotting of a biotinylated experiment and cytoplasmic fraction. IEM revealed co-localization of b1 integrin and uPAR in podocyte endocytotic vesicles in P/U.

Conclusions: PAI-1/uPA complex-mediated uPAR-dependent podocyte b1 integrin endocytosis is a novel mechanism of glomerular injury leading to progressive podocytopenia.

FR-PO334

Pharmacological Targeting of GSK3b Confers Protection against Podocytopathy and Proteinuria via Desensitizing Mitochondrial Permeability Transition
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Background: Mitochondria dysfunction, triggered by mitochondria permeability transition (MPT), has been centrally implicated in the pathogenesis of podocytopathy and involves a multitude of cell signaling mechanisms, among which, glycogen synthase kinase (GSK) 3b has emerged as the integration point and plays a crucial role. This study aimed to examine the role of GSK3b in podocyte MPT and mitochondria dysfunction.

Methods: The regulatory effect of GSK3b on MPT was examined in differentiated podocytes in culture and in a murine model of adriamycin induced podocytopathy by using 4-Benzyl-2-methyl-1,2,4-thiadiazolidine- 3,5-dione (TDZD-8), a highly selective small molecule inhibitor of GSK3b.

Results: TDZD-8 therapy prominently ameliorated proteinuria and glomerular sclerosis in mice with adriamycin nephropathy, associated with a correction of GSK3b overactivity in glomerulus and attenuated podocyte injuries, including foot process effacement and podocyte apoptosis. Consistently, in adriamycin injured podocytes, TDZD-8 treatment overrode GSK3b overactivity, improved cell viability and prevented apoptosis, concomitant with diminished oxidative stress, improved mitochondrial dysfunction and desensitized MPT. Mechanistically, a discrete pool of GSK3b was detected in podocyte mitochondria and colocalized with clyclophilin F, a key structural component of MPT pore, as revealed by dual color immunocytochemistry staining and confocal microscopy. Co-immunoprecipitation assay confirmed that GSK3b interacts with and phosphorylates clyclophilin F, which possesses multiple GSK3b phosphorylation consensus motifs, suggesting that GSK3b has a direct control of MPT. TDZD-8 treatment intercepted the GSK3b controlled phosphorylation and activation of cyclophilin F, desensitized MPT and alleviated mitochondria damages in podocytes upon adriamycin injury *in vivo* and *in vitro*.

Conclusions: Pharmacological targeting of GSK3b might represent a promising and feasible therapeutic strategy to protect podocytes against mitochondrial dysfunction induced by oxidative injuries.

Funding: NIDDK Support

FR-PO335

Overexpression of microRNAs May Be Key to Decreased Autophagy and PRR in the Pathogenesis of Focal Segmental Glomerulosclerosis

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Background: Focal segmental glomerulosclerosis (FSGS) is one of the most common glomerular causes of end-stage renal disease (ESRD). In this study, we aimed to explore the role of autophagy in the pathogenesis of podocyte injury, which is the key point in disease progression, and the roles of intrarenal microRNAs and the prorenin receptor (PRR) in the 5/6 nephrectomy and adriamycin nephropathy models of FSGS.

Methods: Using microarray technology, we assayed microRNA expression in kidney tissues from 5/6 nephrectomy rats, adriamycin nephropathy rats, and control rats. microRNAs were then validated using quantitative polymerase chain reaction (PCR). Beclin-1, microtubule-associated protein 1 light chain-3B (LC3B), Autophagy Protein 5 *(*ATG5), Autophagy Protein 7 (ATG7) and PRR expression were detected by western blotting, immunohistochemistry and ELISA.

Results: Beclin, LC3B, ATG5, ATG7 and also PRR were strongly expressed in podocytes of normal kidneys. In contrast, the expression of these autophagy markers were significantly decreased in the 5/6 nephrectomy group than the adriamycin nephropathy group ($p<0.05$), and both were lower than controls ($p<0,05$). These tended to correlate with the extent of global and segmental sclerosis ($rs<0.01$). miR212 (2.18 fold increase, $p<0.05$), miR132 (10.85 fold increase, $p<0.01$), miR146b (8.19 fold increase, $p<0.01$), miR34c (11.87 fold increase, $p<0.01$) and miR21 (5.44 fold increase, $p<0.01$) expression were significantly higher in the patients than in the controls.

Conclusions: These results suggest that increased level of microRNAs may be related to reduced expression of autophagy proteins and PRR, that may contribute to the development of FSGS. We speculate that the induction of autophagy and the overexpression of PRR may protect against the development of FSGS.

FR-PO336

Deficient Autophagy Results in Endoplasmic Reticulum and Mitochondrial Dysfunction and the Development of Focal and Segmental Glomerulosclerosis of the Kidney

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Background: FSGS is a heterogeneous fibrotic disease recognized as a component of other kidney diseases, whose etiology remains poorly understood. Autophagy is the process by which cells degrade damaged organelles, cell membranes and proteins. Deficient autophagy may contribute to the accumulation of cell damage and disturbance of cell homeostasis.

Methods: We explored autophagic deficiency in the kidney epithelium by mutating critical autophagy gene *ATG5* or *ATG7* during nephrogenesis only in kidney epithelium using Cre-Lox recombination.

Results: Mice with *ATG5* mutations develop foot process effacement, mild tubular cell dysfunction and albuminuria within 2 mths. By 4 mths they exhibit profound glomerular, tubular and interstitial disease, which bear remarkable similarity to the FSGS in humans, as well as severe albuminuria, hypoalbuminemia. They developing advanced renal failure sufficient to cause death by 6 mths. *ATG7* mutants develop a similar disease with slower progression. In addition to the characteristic features of FSGS, podocytes and tubular cells show vacuolization, enlarged damaged mitochondria, and changes to the endoplasmic reticulum. At 2 mths, ATG5 KO tubules and podocytes show ER and mitochondrial distress and produce high levels of ROS, the result of impaired autophagic organelle turnover in podocytes. FSGS biopsies also show mitochondrial morphometric abnormalities consistent with mitochondrial dysfunction in human disease.

Conclusions: We conclude deficient autophagy in the Nephron is sufficient to cause all the manifestations of FSGS.

FR-PO337

Vitamin D Attenuates Proteinuria by Inhibition of Heparanase Expression in the Podocyte

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Background: Heparan sulfate (HS) is a negatively charged polysaccharide that is abundantly expressed in the glomerular filtration barrier. HS expression is reduced in patients with proteinuria, which is associated with an increased expression of the HS-degrading enzyme heparanase. Heparanase is essential for the development of proteinuria in diabetic nephropathy. Several studies showed that proteinuria and podocyte loss could be reduced by treatment with activated vitamin D ($1,25\text{-}D_3$).

Methods: We evaluated the effects of vitamin D on heparanase expression in vivo and in vitro.

Results: In vivo, 1a-hydroxylase knockout (1a-OHase KO) mice, which are unable to hydrolyze 25-hydroxy vitamin D to $1,25\text{-}D_3$, developed proteinuria, which could be corrected by treatment with $1,25\text{-}D_3$. Heparanase expression and activity were increased in the 1a-OHase KO mice and normalized by $1,25\text{-}D_3$ treatment. In addition, adriamycin-exposed (AN) rats developed proteinuria, which could be ameliorated by $1,25\text{-}D_3$ treatment. Glomerular heparanase expression and activity were increased in AN, which was reduced by $1,25\text{-}D_3$ treatment. Furthermore, glomerular HS expression was reduced by induction of AN, and normalized by $1,25\text{-}D_3$ treatment. In vitro, adriamycin stimulation increased heparanase mRNA expression in the podocyte, which could be corrected by $1,25\text{-}D_3$ treatment. $1,25\text{-}D_3$ treatment alone also reduced heparanase mRNA expression. In line with these results, $1,25\text{-}D_3$ treatment dose-dependently reduced heparanase promoter activity. By chromatin immunoprecipitation we showed that the vitamin D receptor binds directly to the heparanase promoter. Finally, we showed that $1,25\text{-}D_3$ treatment reduced transendothelial albumin passage after adriamycin stimulation.

Conclusions: Our data suggest that the protective effect of vitamin D on the development of proteinuria in AN is mediated by reduction of the increased heparanase expression in the podocyte.

FR-PO338

Inhibitory Effects of Acthar® Gel in the Passive Heymann Nephritis Model

Paul Higgins, David Young. *Questcor Pharmaceuticals, Ellicott City, MD.*

Background: H.P. Acthar Gel, a purified preparation of ACTH derived from porcine pituitary gland, is an FDA approved therapy to induce a remission of proteinuria in the nephrotic syndrome without uremia of the idiopathic type or that due to lupus erythematosus. Membranous nephropathy (MN) is a common cause of idiopathic nephrotic syndrome, accounting for about one-third of the cases in adults. ACTH has been shown to be effective in alleviating proteinuria in patients with MN. However, the effects of Acthar have not been tested in an animal model of MN. We therefore tested the efficacy of Acthar in passive Heymann nephritis, an animal model which resembles MN in several clinical and histopathologic aspects.

Methods: Disease was induced in male Sprague-Dawley rats by i.v. injection of anti-Fx1A antibody (5 ml/kg). Ten days after disease induction, after proteinuria was well-established, Acthar was dosed at 40, 160, and 400 U/kg s.c. every other day for 4 weeks. Placebo gel (the vehicle for Acthar, not containing the active ingredient) was administered as a negative control. Urine was sampled weekly for determination of urinary protein and creatinine. After 4 weeks of drug administration, rats were sacrificed and kidneys harvested for semi-quantitative histologic analysis to assess inflammation, glomerular sclerosis, mesangial proliferation and tubular nephrosis.

Results: The results showed a progressive increase in urinary protein/creatinine ratio in animals treated with Placebo gel. Rats treated with all doses of Acthar exhibited significantly reduced protein/creatinine ratios compared to Placebo gel at week 3 (37-44% decrease, $p < 0.05$) and week 4 (58-67%, $p < 0.001$) of drug treatment. Histologically, reductions in kidney histology scores for inflammation (50-88% decrease, $p < 0.01$), glomerular sclerosis (46-65%, $p < 0.001$), mesangial proliferation (35-62%, $p < 0.0001$), tubular nephrosis (67-87%, $p < 0.01$) as well as total score (59-63%, $p < 0.01$) were all induced by Acthar treatment.

Conclusions: The present results demonstrate the in vivo efficacy of Acthar in the Heymann nephritis model and provide evidence to support the use of Acthar in the treatment of proteinuria in patients with idiopathic membranous nephropathy.

Funding: Pharmaceutical Company Support - Questcor Pharmaceuticals

FR-PO339

Fingolimod (FTY720), an Analog of Sphingosine Inhibited the Reduced Expression of the Slit Diaphragm Molecules and Ameliorated Proteinuria in PAN Nephropathy

Eriko Hasegawa,[1] Yoshiyasu Fukusumi,[1] Ayako Wakamatsu,[1] Natsumi Takashima,[1] Ichiei Narita,[2] Hiroshi Kawachi.[1] *[1]Cell Biology, Inst Nephrol, Niigata Univ Medicine, Niigata, Japan; [2]Medicine II, Niigata Univ Medicine, Niigata, Japan.*

Background: Fingolimod has a structural similarity to a sphingosine 1-phosphate (S1P), and acts as a functional antagonist. Fingolimod is used an immunosuppressant, because it can sequester lymphocytes to lymph tissues by binding to S1P receptor (S1PR) on lymphocytes. It is also reported that S1P-S1PR signaling regulates a variety of cell functions including survival, cytoskeletal rearrangement, and cell-to-cell barrier. In this study, we examined the effect of fingolimod in puromycin aminonucleoside (PAN) nephropathy, a mimic of MCNS.

Methods: First, the expressions of S1PRs and metabolic enzymes of S1P were analyzed in cultured mouse podocytes, and normal and PAN nephropathy rat glomeruli. Next, the effects of fingolimod on proteinuria and the expression of slit diaphragm molecules in PAN nephropathy were analyzed.

Results: mRNA expressions of sphingosine kinase (SK) 1,2, S1P phosphatase1, 2, S1P lyase, and S1PR1, 2, 3, 4, 5 were detected in normal rat glomeruli. In mouse cultured podocytes S1PR5 was not detected, although other S1P related molecules were detected. The treatment of fingolimod reduced the mRNA expressions of S1PR3, 4. mRNA expressions of S1PR3, 4, and SK1 were upregulated in cultured podocytes treated with PAN. In PAN nephropathy rat glomeruli, mRNA expressions of S1PR3, 4 and SK1 were clearly upregulated (S1PR3 488% to normal: S1PR4 208% SK1 162%). Daily administration of fingolimod (1mg/kg BW) reduced proteinuria in PAN nephropathy (day8, 103.4mg/day versus 5.3 $p<0.05$: day10, 152.3 versus 11.2 $p<0.05$), and prevented the reduced expressions of nephrin (IF score 1.78 versus 2.86 $p<0.05$) and podocin (1.62 versus2.62 $p<0.05$), but not ZO-1.

Conclusions: Increased S1P-S1PR signaling may contribute to the development of podocyte injury. Fingolimod ameliorated PAN nephropathy by suppressing the S1P-S1PR signaling in podocyte.

FR-PO340

Production of Congenic Mouse Strains with Mutated or Wild-Typed Tensin 2 Gene in Multiple Inbred Mouse Genetic Backgrounds to Elucidate the Mechanism for Renal Failure due to Tensin 2 Deficiency Hayato Sasaki,[1] Kiyoma Marusugi,[2] Junpei Kimura,[3] Hiroshi Kitamura,[4] Ken-Ichi Nagasaki,[5] Yasuhiro Kon,[3] Daisuke Torigoe,[1] Takashi Agui,[1] Nobuya Sasaki.[2] *[1]Laboratory of Laboratory Animal Science and Medicine, Graduate School of Veterinary Medicine, Hokkaido Univ, Sapporo, Japan; [2]Laboratory of Laboratory Animal Science and Medicine, Faculty of Veterinary Medicine, Kitasato Univ, Towada, Japan; [3]Laboratory of Anatomy, Graduate School of Veterinary Medicine, Hokkaido Univ, Sapporo, Japan; [4]Dept of Veterinary Physiology, School of Veterinary Medicine, Rakuno Gakuen Univ, Ebetsu, Japan; [5]Chitose Laboratory, Japan Food Research Laboratories, Chitose, Japan.*

Background: Tensin 2 (Tns2) is thought to be a component of the actin cytoskeletal structures linking actin filaments with focal adhesions and play a role as an intracellular signal transduction mediator through integrin in podocytes, and how it functions is unclear. *Tns2*-null mutation (*Tns2*^{nph}) leads to massive albuminuria following podocyte foot process effacement in ICR-derived glomerulonephritis (ICGN) mice, which is the origin of the mutation, and DBA/2J (D2) mice, but not in C57BL/6J (B6) mice and 129X1/SvJ (129) mice. Elucidating the reasons for these differences in diverse genetic backgrounds could help unraveling *Tns2* function in podocyte.

Methods: We produced the congenic mouse in which *Tns2*^{nph} is introgressed into FVB/NJ (FVB) background (FVB-*Tns2*^{nph}), and evaluated the progression of kidney disease. We also produced the congenic strain carrying wild-type *Tns2* on an ICGN background (ICGN-*Tns2* (+/+)).

Results: FVB-*Tns2*^{nph} mice developed albuminuria, renal fibrosis and renal anemia, like ICGN mice, whereas ICGN-*Tns2* (+/+) mice did not. In FVB-*Tns2*^{nph} mice, podocyte foot process effacement was observed with an electron microscope at as early as 4 weeks age.

Conclusions: Our mice reveal that FVB mice are susceptible to *Tns2* deficiency, and provide bona fide controls for ICGN mice that derived from ICR outbred mice. Now, we can provide the two resistant strains (B6 and 129) and the three susceptible strains (D2, FVB and ICGN-*Tns2* (+/+)) for comparative analyses to elucidate the mechanism for renal failure due to *Tns2* deficiency.

FR-PO341

Podocyte Nox5 Induces a RAS-Independent Systolic Blood Pressure Increase in Mice Chet E. Holterman, Chris R. Kennedy. *Ottawa Hospital Research Inst, Ottawa, ON, Canada.*

Background: Mice with podocyte Nox5 expression (Nox5^{pod+}) develop pathology reminiscent of diabetic nephropathy coupled with elevated systolic blood pressure (SBP). While the mechanisms underlying such effects are unresolved, the AngII/AT1R pathway is known to both activate and be regulated by Nox members, including Nox5. We therefore investigated whether activation of the renin-angiotensin system (RAS) is linked to Nox5-driven renal injury and SBP elevation.

Methods: We queried renal expression of RAS pathway family members in Nox5^{pod+} mice by qPCR. We employed a tetracycline-inducible mouse line to express Nox5 in podocytes (Nox5^{podInd}) and investigated potential interactions between AngII and Nox5. Nox5 expression was induced with doxycycline in Nox5^{podInd} mice and osmotic minipumps loaded with either AngII or Losartan were implanted. SBP was measured by tail cuff and albuminuria was determined via spot urine collection/albumin ELISA. Glomerular filtration rate (GFR) was measured via FITC-inulin clearance.

Results: Nox5^{pod+} transgenic mice displayed increased renal cortex expression of endogenous RAS pathway members, renin, AT1R, ACE2, as well as cyclooxygenase-2. Similar to Nox5^{pod+} animals, Nox5^{podInd} mice developed albuminuria and increased SBP following Nox5 induction. Exogenous AngII infusion in Nox5^{podInd} mice increased SBP and albuminuria to a greater extent than in non-transgenic mice ($P<0.05$). Interestingly, Losartan attenuated neither albuminuria nor SBP yet reduced GFR in Nox5^{podind} mice.

Conclusions: Our data reveal that podocyte-specific Nox5 expression renders mice more sensitive to the effects of exogenous AngII infusion including SBP elevation and albuminuria. In contrast, and despite AT1R blockade, Nox5-driven filtration barrier damage and SBP elevation remain unchallenged in mice without AngII infusion, thereby negating endogenous RAS involvement in such injury. However, activation of the endogenous RAS pathway may nevertheless compensate to maintain GFR in response to altered arteriolar tone brought about by oxidative stress in podocyte-specific Nox5 mice. These findings highlight the complex interplay between Nox5-driven oxidative stress and the renin angiotensin system.

FR-PO342

Podocyte Injury Promotes Glomerular Lipid Peroxidation and Foam Cell Infiltration Under Hypercholesterolemia Satoshi Hara,[1,2] Namiko Kobayashi,[1] Shun Manabe,[1] Tomo Suzuki,[1] Kazuo Sakamoto,[1] Yasutoshi Takashima,[1] Toshiharu Ueno,[1] Juri Hamada,[3] Taiji Matsusaka,[4] Michio Nagata.[1] *[1]Kidney and Vascular Pathology, Univ of Tsukuba, Tsukuba, Japan; [2]Rheumatology, Kanazawa Graduate School of Medicine, Kanazawa, Japan; [3]Tsukuba Advanced Research Alliance, Univ of Tsukuba, Tsukuba, Japan; [4]Internal Medicine, Tokai Univ School of Medicine, Isehara, Japan.*

Background: Glomerular macrophage (Mj)-derived foam cells (FCs) infiltration is frequently observed in various nephrotic diseases and it is associated with podocyte injury. We have reported that acute podocyte injury under hypercholesterolemia (NEP25/LDLRKO mice) accelerated glomerular lipid deposition. The present study analyzed the effect of acute podocyte injury on lipid peroxidation and chronic effect on glomerular Mj-derived FCs infiltration.

Methods: Matrix-assisted laser desorption/deionization imaging mass spectrometry (MALDI-IMS) was performed to examine oxidized LDL (oxLDL) in NEP25/LDLRKO mice as compared with NEP25 mice and LDLRKO mice. Then, uninephrectomized LDLRKO mice were induced of adriamycin (ADR) nephropathy. ADR + high fat diet (HFD) group was compared with ADR + normal diet and vehicle + HFD group in histology. Finally, using rat mesangial cell (MC) and glomerular endothelial cell (GEnC) line, the effect of oxLDL on cytokine signaling about Mj adhesion and migration was analyzed by quantitative RT-PCR.

Results: MALDI-IMS showed that lysophosphatidylcholine (LPC) 16:0 and 18:0 were accumulated only in the kidney of NEP25/LDLRKO mice. The amount of glomerular LPC16:0 and 18:0 was significantly high in NEP25/LDLRKO mice compared with control ($P < 0.05$). In ADR + HFD group, glomerular Mj and a LPC precursor oxidized phospholipid (oxPL) increased ($P < 0.05$) and Mj-derived FCs contained oxPL ($P < 0.05$) as compared with control. In vitro, LPC16:0 induced MCP-1, VCAM-1, ICAM-1 and Mj migration inhibitory factor (MIF) mRNA up-regulation in MC. LPC18:0 induced VCAM-1, P-selectin and MIF mRNA up-regulation in GEnC and MCP-1 mRNA up-regulation in MC.

Conclusions: Podocyte injury promotes LPC formation within the glomeruli, leading to glomerular Mj-derived FCs infiltration through chemokines and adhesion molecules expression in GEnC and MC.

FR-PO343

sPLA2 IB Induces Human Podocyte Apoptosis via the M-Type Phospholipase A2 Receptor Yangbin Pan,[1] Jian-Xin Wan,[2] Huiming Wang,[1] Yipeng Liu,[1] Qian Yang,[1] Wei Liang,[1] Guohua Ding.[1] *[1]Div of Nephrology, Renmin Hospital of Wuhan Univ, Wuhan, Hubei, China; [2]Div of Nephrology, First Affiliated Hospital of Fujian Medical Univ, Fuzhou, Fujian, China.*

Background: The M-type phospholipase A2 receptor (PLA2R) is expressed by human podocytes. Group IB secretory phospholipase A2 (sPLA2 IB), one of the ligands of PLA2R, has been found to be at a high level in patients with chronic renal failure than in normal controls. However, little is known about the role of sPLA2 IB in the pathogenesis of glomerular diseases. In the present study, we evaluated the effects of sPLA2-IB /PLA2R on human podocyte (HPC) injury and explored the underlying signaling pathway.

Methods: Western blotting, real-time polymerase chain reaction (PCR) analyses and immunofluorescence assays were performed to evaluate PLA2R, cPLA2a, ERK1/2 and p53 expressions. High-performance liquid chromatography (HPLC) was used to evaluate arachidonic acid (AA) content. Podocyte apoptosis was assessed by flow cytometry and Hoechst-33342 staining.

Results: PLA2R was expressed in the membrane of HPCs and medicated internalization of sPLA2 in HPC cytoplasm. sPLA2 IB was able to induce podocyte apoptosis in a PLA2R-dependent manner. Moreover, sPLA2 IB exposure promoted expression of PLA2R and phophorylation of cPLA2a and ERK1/2. HPLC analysis demonstrated that elevated levels of AA were found in the culture medium of sPLA2-exposed HPC in a dose-dependent manner. In addition, exogenous AA was able to provoke apoptosis and p53 pathway was involved in AA-induced apoptosis in HPC.

Conclusions: sPLA2 IB has a potential ability to combine PLA2R of human podocyte and induce podocyte apoptosis through activation of ERK1/2 and cPLA2a and release of AA.

FR-PO344

Heterozygous Actn4 Mutant Mice Are Susceptible to Glomerulopathy when Subjected to Hemodynamic Stress Mostafa Belghasem, Mei Cao, Joel M. Henderson. *Pathology & Laboratory Medicine, Boston Univ, Boston, MA.*

Background: a-Actinins are actin-binding and cross-linking proteins. Mutations in ACTN4, the gene encoding the actin binding protein a–actinin-4, can cause familial focal segmental glomerulosclerosis (FSGS) in humans. Previous studies showed that Actn4 knockout mice are prone to develop spontaneous glomerular disease. However, heterozygous Actn4 mutant mice carrying a human disease-associated mutation, K255E, do not. In this study heterozygous Actn4 mutant mice are challenged with systemic hypertension using the deoxycorticosterone acetate (DOCA)-salt uninephrectomy model, to determine if their kidneys are especially vulnerable to hemodynamic stress.

Methods: Heterozygous Actn4 mutant mice (n=4) and wild type (n=4) controls were subjected to uninephrectomy and DOCA-salt treatment for 6 weeks. During treatment, systemic blood pressure was measured by the tail cuff method, and kidney function was assessed with urine and serum biomarkers. At the end of the treatment period, kidney tissue was harvested for histological analysis.

Results: Both groups displayed a remarkable increase in systolic blood pressure in response to DOCA-salt uninephrectomy, with minimal difference between the groups. Survival was markedly reduced (25%) in heterozygous Actn4 mutant mice compared to controls (75%) prior to the end of the observation period (6 weeks). The heterozygous mouse that survived to the end of observation period showed severe glomerular injury, more extensive than that seen in controls. Glomerulosclerosis involved 27% and 10% of glomeruli in the heterozygous mouse and wild type controls, respectively. The glomerular lesions almost exclusively affected the juxtamedullary glomeruli in both groups.

Conclusions: Overt glomerular disease does not develop spontaneously in heterozygous Actn4 mutant mice. However, our findings suggest that additional modifying factors, such as hemodynamic stress, play a role in the development of glomerular disease in these mice. Furthermore, these mice display a remarkable decrease in survival in response to hemodynamic stress induced by DOCA-salt uninephrectomy.

Funding: NIDDK Support

FR-PO345

Podocyte-Preserving Strategies for Preventing Hypertrophy-Driven Progression Ryuzoh Nishizono, Masao Kikuchi, Su Qing Wang, Mahboob A. Chowdhury, Yan Yang, Larysa T. Wickman, Roger C. Wiggins. *Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: Uni-nephrectomy (to a lesser extent than 5/6 nephrectomy) can trigger accelerated podocytes detachment leading in susceptible individuals to ESKD over time. Model systems show that this process which is driven by compensatory hypertrophy is podocyte-dependent. We hypothesize that this mechanism common to all nephronopenic progression is also analogous to kidney transplantation ("reverse nephrectomy") where post-implantation hypertrophic events cause podocyte stress leading to accelerated podocyte detachment and progressive loss of renal function.

Methods: To investigate how to mitigate these events we used the AA-4EBP1 transgenic rat model where expression of the dominant AA-4EBP1 transgene is driven by the human podocin promoter. Homozygous AA-4EBP1 rats maintained on an ad lib diet developed proteinuria, podocyturia, progressive podocyte depletion, glomerulosclerosis, hypertension and progressed to ESKD by 12 weeks after nephrectomy. Three potentially mitigating strategies were tested: (i) reduced food intake by 40% or 20%, (ii) ACE inhibitor (enalapril) to inhibit angiotensin II, and (iii) rapamycin to slow growth.

Results: Each of these approaches was largely effective when started immediately after nephrectomy, although protection occurred by different mechanisms. Both food restriction and rapamycin prevented glomerular enlargement so podocytes did not become stressed and detach. In contrast ACE inhibitors did not prevent glomerular enlargement but prevented podocyte detachment thereby preventing proteinuria and progression. These strategies were also tested for their efficacy starting 3 weeks after nephrectomy when the urine protein:creatinine ratio was already elevated to 10 but prior to loss of podocytes from glomeruli to simulate treatment of already established glomerular disease. None of the strategies was completely effective if started late. However combinations were more effective than individual strategies alone.

Conclusions: These data provide a guide towards mitigating all forms of growth-dependent glomerular progression in the clinic.

Funding: NIDDK Support, Other NIH Support - Kidney Diseases group of the National Institutes of Health (grants DK RO1 46073)

FR-PO346

Podocyte Density Decreases with Age in Man Roger C. Wiggins, Su Qing Wang, Jeffrey B. Hodgin, Yan Yang, Larysa T. Wickman, Farsad Afshinnia, Mahboob A. Chowdhury, Ryuzoh Nishizono, Jocelyn E. Wiggins, Markus Bitzer. *Univ of Michigan, Ann Arbor, MI.*

Background: Human aging is associated with sclerosed glomeruli, decreasing eGFR and increasing ESKD prevalence. Podocytes are long-lived post-mitotic cells with limited capacity for renewal. Model systems show that podocyte depletion below a density of about 100 per 10^6 um^3 causes glomerosclerosis, and can occur because (i) podocyte number per glomerulus can decrease and/or (ii) glomerular volume can increase. We therefore tested the hypothesis that glomerular aging is associated with reduced podocyte density.

Methods: Podocyte density was estimated from archival tissue sections with recently developed technology (Venkatareddy et al. JASN 2014) using kidney donor biopsies (n=59 age 4-71) and normal nephrectomy samples (n=30, age 31-85).

Results: Podocyte nuclear density decreased linearly with age with a slope of -0.8% per year (R^2=0.43, P<0.001) corresponding to the approximate rate of decrease of eGFR with age, and reaching a podocyte density beyond which glomerulosclerosis is increasingly prevalent by 80 years of age. Decreased podocyte nuclear density with age was due to a combination of decreased podocyte number per tuft (-0.4% per year, R^2=0.15, P<0.01) and increased glomerular volume (+0.4% per year, R^2=0.19, P<0.01). As podocyte density decreased with age podocyte cell volume increased (measured by Glepp1 area density) (R^2=0.44, P<0.001) in parallel to podocyte nuclear volume (R^2=0.53, P<0.001) reflecting the well-established karyplasmic ratio relationship of cell size to nuclear size (R^2=0.64).

Conclusions: These observational data provide a logical explanation for kidney aging as a dynamic process whose variables can be understood and potentially modulated by pharmacologic and dietary factors. In successful glomerular aging increased podocyte cell size (estimated by two independent methods) compensates for decreasing podocyte density. Diabetes, hypertension and other glomerular disease can be seen as superimposed accelerators of age-associated reduction in podocyte density. Podocyte reserve is much reduced in older than in younger individuals.

Funding: Private Foundation Support

FR-PO347

MiR-21 in Renal Aging Christopher Lund O'Connor, Jinghui Luo, Harkamal Singh Jhajj, Jennifer Yi-Chun Lai, Markus Bitzer. *Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: Loss of podocytes is sufficient to cause progressive glomerulosclerosis (podocyte depletion hypothesis). Increased age, reduction in nephron number and hypertension are associated with development of glomerulosclerosis. We had previously shown that miR-21, a small regulatory RNA, antagonizes loss of podocytes. Therefore we investigated changes in glomerular pathology associated with increased age and after nephron mass reduction plus hypertension in miR-21-mutant mice.

Methods: Mature miR-21 expression was determined using qrt-PCR. Ubiquitously miR-21-deficient mice and wildtype littermates were aged to 24 months on ad-libitum diet (n=5 in each genotype) or underwent uninephrectomy + s.c. implantation of a DOCA tablet + saline drinking water (1% NaCl, 0.2% KCl) (UN/DOCA salt) at 3 months of age (n=5 in each genotype). Computer-assisted morphometric image analysis of immunohistochemistry for WT-1 for podocyte enumeration and PAS staining to measure mesangial matrix area.

Results: miR-21 expression was increased significantly in kidney cortex of 22 months compared to 3 months old wildtype mice (3.1-fold, n=5 per age-group, p<0.001) but not after UN/DOCA-salt (n=5). At 3 months of age miR-21-deficent mice did not exhibit differences in blood pressure, proteinuria, podocyte density or mesangial matrix area compared to wildtype littermates. Unchallenged 24 months old miR-21-deficient mice exhibit a significantly decreased podocytes density (p=0.0189) and higher glomerular volume per podocytes (p=0.045). Genotype had no significant effect on kidney weight, kidney-to-body weight ratio and mesangial matrix area. In UN/DOCA salt mice blood pressure increased after two weeks in all mice independent of genotype. miR-21-deficient UN/DOCA salt mice exhibited increased proteinuria (p=0.043), decreased podocyte density (p=0.004) but no difference in mesangial matrix area.

Conclusions: miR-21 antagonizes loss of podocytes associated with aging and after nephron mass reduction plus hypertension in mice consistent with our previous findings. Studies examining the underlying signaling events and molecular mechanisms and the effect of loss of miR-21 in specific renal cell-types are ongoing in the lab.

Funding: NIDDK Support, Other NIH Support - NIA

FR-PO348

Podocyte Depletion in Kidney Allografts Yan Yang, Jeffrey B. Hodgin, Su Qing Wang, Farsad Afshinnia, Larysa T. Wickman, Ryuzoh Nishizono, Mahboob A. Chowdhury, Milagros D. Samaniego-Picota, Roger C. Wiggins. *Univ of Michigan, Ann Arbor, MI.*

Background: Kidney allografts half-life is unexpectedly short in spite of improved immunosuppression. Podocytes are long-lived post-mitotic cells with limited capacity for renewal. Model systems show that podocyte depletion beyond a threshold density (about 100 per 10^6 um^3 glomerular volume) due to (i) decreased podocyte number per glomerular tuft and/or (ii) glomerular enlargement causes glomerulosclerosis. We therefore tested the hypothesis that allografts develop decreased podocyte density.

Methods: Podocyte density was estimated from archival histologic sections using recently developed technology (Venkatareddy et al, JASN 2014).

Results: Protocol kidney biopsy 3 months post-implantation shows 21% decrease in podocyte density from 214±73 to 173±45 per 10^6 um^3 (P<0.01) due to glomerular enlargement (compensatory kidney hypertrophy) without podocyte loss. By 12 months post-implantation podocyte density was not further decreased (157±53 per 10^6 um^3, P=0.37 versus 3 month biopsy). In contrast biopsies with glomerulopathy (excluding recurrent glomerular diseases) within 2 years post-implantation (mean 0.9±0.7 years) podocyte nuclear density had decreased to 92±32 per 10^6 um^3 (P<0.001) due primarily to reduced podocyte number per tuft from 463±85 to 305±55, P<0.001) and associated with >10-fold accelerated podocyte detachment rate estimated using a non-invasive urine pellet podocin mRNA assay (Wickman et al, JASN 2013). Grafts that survived longer term (mean 10.2±4.3 years post-implantation) biopsied for abnormal kidney function and with a pathologic diagnosis of transplant glomerulopathy had markedly reduced podocyte nuclear density at 70±33 per 10^6 um^3 (P<0.0001 versus control) due to a combination of glomerular enlargement and reduced podocyte number per tuft. Allograft eGFR correlates with podocyte density (R^2=0.42, P<0.001).

Conclusions: Podocyte depletion is a common phenomenon in allografts likely to be contributing to failure in both the short and long term.

Funding: Private Foundation Support

FR-PO349

Podocyte-Specific TRPC6 Overexpression-Induced FSGS in Mice Is Exacerbated by a Novel TRPC6 Mutation Zijin Sun,[1] Yaochun Zhang,[1] Kar Hui Ng,[1] Zakir Hossain,[2] Jun Li Ng,[1] Chang Yien Chan,[1] Isaac Liu,[1] Hui Kim Yap.[1] *[1]Dept of Paediatrics, National Univ of Singapore, Singapore; [2]Cancer Science Inst of Singapore, National Univ of Singapore, Singapore.*

Background: Mutations in the Transient Receptor Potential Cation Channel 6 *(TRPC6)* gene have been associated with familial focal segmental glomerulosclerosis (FSGS). We found one Chinese family with autosomal dominant FSGS carrying a novel *TRPC6* p.R68W missense mutation which increases *TRPC6* current amplitudes, suggesting gain of function. This study aimed to compare the phenotypes of transgenic mice with podocyte-specific overexpression (OE) of wild type (OE^{WT}) and p.R68W mutant (OE^{Mut}) *TRPC6* to ascertain the in-vivo function of p.R68W.

Methods: cDNAs of WT and p.R68W EGFP-*TRPC6* were subcloned independently downstream of the pNPHS2 podocin-specific promoter. Founders with the transgene were obtained by pronuclear microinjection. These were crossed with FVB/N WT mice to establish mouse lines with varying transgene copy numbers. Transgene expression was verified by reverse-transcription PCR and western blot analysis, while transgene copy number was estimated by qPCR. The phenotypes of OE^{WT} (n=5) and OE^{Mut} (n=6) mice with comparable transgene copy numbers were studied and compared with control mice (n=8) carrying only native *TRPC6*. Urine albumin:creatinine ratios were obtained monthly. The mice were euthanized at 6 months of age. Statistical analysis was performed using Mann-Whitney *U* test.

Results: At 5 months' of age, both OE^{WT} (36.5±5.7 ng/µg) and OE^{Mut} (48.3±19.6 ng/µg) mice had higher mean urine albumin:creatinine ratios compared to controls (25.3±7.8 ng/µg) ($P<0.01$). Moreover, OE^{Mut} had higher mean urine albumin:creatinine ratios compared to OE^{WT} mice ($P<0.05$). Histologically, both OE^{WT} and OE^{Mut} mice had FSGS and extensive podocyte foot process effacement. Immunogold studies with anti-EGFP antibody showed more extensive deposition on the podocyte foot processes of OE^{Mut} compared to OE^{WT} mice.

Conclusions: Podocyte-specific overexpression of both WT and p.R68W *TRPC6* in mice results in albuminuria and FSGS, with a more severe phenotype in p.R68W, suggesting an important role of p.R68W mutation in the pathogenesis of familial FSGS.

Funding: Government Support - Non-U.S.

FR-PO350

Podocyte Injury and Segmental Glomerulosclerosis in Heme Oxygenase (HO)-1 Knock Out Rats Harikleia Gakiopoulou,[1] Vasileios Atsaves,[2] Maria Detsika,[2] Elpida Poulaki,[1] Pu Duann,[3] Elias A. Lianos.[3] *[1]Pathology, Univ of Athens, Greece; [2]Medicine, Univ of Athens, Greece; [3]Medicine, Rutgers Biomedical and Health Sciences, Greece.*

Background: In the kidney,the inducible isoform of Heme Oxygenase (HO-1) protects against oxidative injury. It is unknown whether HO-1 is also important for preservation of renal structural/functional integrity. The aim of this study was to assess structural/functional consequences of HO-1 depletion in the rat kidney.

Methods: HO-1 depletion was achieved in Sprague-Dawley rats using Zinc Finger Nuclease technology to disrupt the HO-1 sequence 5'-GGT GGC CCA CGC ATA tac ccg cTA CCT GGG TGA CCT CTC AG-3' within Exon 3, and was documented in isolated glomeruli by western blot analysis. Urine albumin and creatinine excretion were measured at defined time points in sex and age-matched Heterozygotes (HO-1 depletion), homozygotes (HO-1 absence) and wild-type controls. Pathologic lesions were examined by routine histolology and immunohistochemistry techniques.

Results: Both heterozygotes and homozygotes developed albuminuria. Podocytes were hypertrophied with copious cytoplasm, enlarged nuclei, prominent nucleoli and presence of WT1 antigen in glomerular filtrate. There was also increased mesangial matrix and segmental sclerosis with synechial adhesions to Bowman's capsule or to the origin of the proximal convoluted tubule. Tubules displayed microcystic dilatation with proteinaceous casts and there was interstitial infiltration by inflammatory cells, more prominent in homozygotes.

Conclusions: HO-1 is important in preserving glomerular, particularly podocytic, and interstitial integrity. HO-1 depletion or absence results in FSGC.

FR-PO351

Dual Role of SDF-1 in Podocyte Injury and Regeneration Simone Romoli,[1] Santhosh Kumar Vr,[1] Shrikant R. Mulay,[1] Dirk Eulberg,[2] Hans J. Anders.[1] *[1]Nephrologisches Zentrum, Klinikum der Univ Muenchen, Germany; [2]NOXXON Pharma AG, Germany.*

Background: Stromal Derived Factor-SDF1 is a homeostatic chemokine, which promotes homing and activation status of stem cells. We speculated that SDF-1 regulates glomerular injury and regeneration.

Methods: Glomerular injury was induced by single intravenous injection of Adriamycin 13.4mpk (ADR) in male Balb/c mice that received either the SDF-1 inhibitor NOX-A12 (50mpk *s.c*) After 2 weeks all mice were sacrificed for analysis: ELISA, Histology. Human parietal epithelial cells (PECs) with renal progenitor cell properties were used for *in-vitro* cell culture studies, q-RT-PCR.

Results: ADR injection induced proteinuria with a peak at 7 days (injury phase), which subsequently declined without returning to baseline and was associated with FSGS lesions at the end of week 2. SDF-1 blockade significantly aggravated the injury phase at day 7. Despite this effect, at day 14 SDF-1 blockade had improved proteinuria and markers of glomerular disease versus control mice. SDF-1 inhibition only from day 7 to 14 displayed the same beneficial effect on the regeneration phase of ADR. To unravel these oppositional effects we focused on the Notch signaling pathway. Notch ligand and receptor mRNA expression profiling revealed their suppression during ADR, which was recovered by SDF-1 blockade. In vitro, we found SDF-1 to suppress PECs proliferation. The same results were obtained when podocyte progenitors where exposed to supernatants of necrotic podocytes, which implies that necrotic cells provide Notch ligands and SDF-1 to podocyte progenitors. SDF-1 also reduced the induced differentiation of PECs into podocytes by VRAD medium. In contrast, SDF-1 increased the viability of podocytes in vitro after Adriamycin stimulation.

Conclusions: These data unravel a bivalent role of podocyte-derived SDF-1 in glomerular injury. SDF-1 protects podocytes during injury phase, which may relate to its suppression of Notch and Notch-driven mitotic catastrophe of podocytes. However, SDF-1 also acts as a negative regulator of Notch-mediated podocyte regeneration; therefore, SDF-1 inhibition may be a suitable therapeutic strategy to enhance the regenerative capacity of the glomerulus after podocyte loss.

FR-PO352

Thymosin b4 Plays a Critical Role in the Progression of Glomerular Disease Elisa Vasilopoulou,[1] Maria K. Joannou,[1] Kathryn E. White,[2] Michael G. Robson,[3] Paul Winyard,[1] David A. Long.[1] *[1]Developmental Biology and Cancer, UCL Inst of Child Health, United Kingdom; [2]EM Research Services, Univ of Newcastle, United Kingdom; [3]MRC Centre for Transplantation, King's College London, United Kingdom.*

Background: Podocyte shape is essential to maintain the structure and function of the glomerular filtration barrier; hence molecules which alter this process may contribute to the progression of kidney disease. Based on this premise, we hypothesised that the actin-sequestering protein, thymosin-ß4 may have a critical role in the glomerular filtration barrier.

Methods: We assessed thymosin-ß4 expression in developing and adult kidneys by qRT-PCR, in-situ hybridisation and immunohistochemistry. To determine the role of thymosin-ß4 in healthy glomeruli we examined renal function and glomerular morphology in wild-type and thymosin-ß4 knockout (Tß4ko) mice. Glomerular disease was also induced in wild-type and Tß4ko mice by administration of nephrotoxic serum. In-vitro, thymosin-ß4 was downregulated by siRNA in differentiated immortalised mouse podocytes. Cell migration and podocyte process number and length were assessed.

Results: Thymosin-ß4 mRNA and protein were highly expressed in developing and adult mouse glomeruli, both in podocytes and endothelial cells. The kidneys of Tß4ko mice had normal renal function and glomerular morphology. Renal thymosin-ß4 expression was upregulated in mice following glomerular injury. Lack of thymosin-ß4 accelerated glomerular disease in mice administered nephrotoxic serum compared with wild-type littermates as demonstrated by significantly increased albuminuria, plasma creatinine, blood urea nitrogen and decreased creatinine clearance. Tß4ko mice also had increased glomerular histological damage, inflammation and fibrosis. In vitro, thymosin-ß4 downregulation increased podocyte migration and average process length.

Conclusions: Our results suggest that although thymosin-ß4 is not essential for glomerular function in healthy mice, it may be critical in glomerular disease. Modulation of thymosin-ß4 may be a novel treatment strategy for renal disease in the future.

FR-PO353

$P2X_7$ Promotes Podocyte Injury in the Experimental Model of Anti-Podocyte Nephritis Catherine Meyer-Schwesinger,[1] Marlies Sachs,[1] Björn Rissiek.[2] *[1]Dept of Internal Medicine, Nephrology, Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Inst of Immunology, Univ Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

Background: The $P2X_7$ receptor is a ligand-gated cation channel that is expressed by a variety of immune cells, including macrophages and lymphocytes. Activation of $P2X_7$ leads to an influx of Ca^{2+} ions and to an efflux of K^+ ions. Moreover, $P2X_7$ activation induces the assembly of the NALP3 inflammasome complex resulting in the release of IL-1b. Sustained activation of $P2X_7$ leads to cell death by pore formation. Increased expression of both $P2X_7$ mRNA and protein was reported in mesangial cells and macrophages infiltrating the glomeruli in animal models of antibody-mediated glomerulonephritis. Cultured podocytes were demonstrated to express mRNA of $P2X_7$ and to react to benzoyl ATP, a selective agonist of $P2X_7$. $P2X_7$ could therefore mediate injury in podocytes and could be a potential therapeutic target for podocyte disease.

Methods: $P2X_7$ expression was investigated by WB and by IP in cultured murine podocytes and functional assays were performed to search for effects of a $P2X_7$ activation in podocytes. Additionally, the experimental model of anti-podocyte nephritis was induced in $P2X_7$-deficient mice and $P2X_7$-specific nanobodies were used in wild-type mice to modulate $P2X_7$ function in the course of disease.

Results: $P2X_7$ expression was demonstrated by WB and IP in murine podocytes. Functional assays in cultured podocytes demonstrated that ATP-induced activation of $P2X_7$ increased the intracellular Ca^{2+} concentration and the phosphorylation of Erk1/2 of the MAP-kinase pathway. The course of anti-podocyte nephritis was attenuated in $P2X_7$ deficient mice and in mice treated with $P2X_7$-blocking nanobodies. Disease parameters were aggravated in mice treated with $P2X_7$-enhancing nanobodies. Moreover, histology and Western blot analyses indicated enhanced levels of $P2X_7$ in glomeruli during active APN.

Conclusions: Our results support a pro-inflammatory role for $P2X_7$ in podocyte injury and suggest that the $P2X_7$ receptor is a potential therapeutic target in podocyte damage.

FR-PO354

Studying the Mechanism of Apolipoprotein L1 Nephropathy through Transgenic Zebrafish Opeyemi A. Olabisi,[1,2] Khaldoun Al-Romaih,[2,5] Joel M. Henderson,[4] Ritu Tomar,[1] Iain A. Drummond,[1] Callum MacRae,[3] Martin R. Pollak.[2] *[1]Medicine, Massachusetts General Hospital, Boston, MA; [2]Medicine, Beth Israel Deaconess Medical Center, Boston, MA; [3]Medicine, Brigham and Women's Hospital, Boston, MA; [4]Pathology and Lab Medicine, Boston Univ School of Medicine, Boston, MA; [5]King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.*

Background: Risk variant APOL1 (G1/G2) are strongly associated with a spectrum of kidney disease in people of recent African descent. Mechanism of APOL1 nephropathy is unknown. Podocytes and/or endothelial cells are presumed mediators. Given the close homology between zebrafish (ZF) pronephros and human nephron, we study the effect of podocyte-or endothelium-specific expression of G0, G1, or G2 on the structure and function of ZF pronephros.

Methods: Podocyte-specific and endothelium-specific expression of APOL1 was driven by by podocin and Flk promoters, respectively, using Gal4-UAS system. Structural pronephric changes were studied with light and electron microscopy (EM). Proteinuria was assayed by measuring renal excretion of GFP -vitamin D binding protein. Puromycin aminonucleoside (PAN) was used as inducer of podocyte injury.

Results: Vivo-morpholino knock-down of APOL1-like sequence in ZF (~20% similarity to Hu-APOL1) showed no toxicity when splice blocking morpholino was used at concentrations of up to 0.5mM. Toxicity of translation blocking morpholino was seen, however it was similar to that caused by control-morpholino. Endothelial-specific transgenic expression of G1/G2 is associated with condensed appearing glomeruli on H&E and electron dense subendothelial deposits on EM. Podocyte specific expression of G1 is associated with segmental podocyte foot process effacement and irregularities relative to G0. Expression of G1/G2 alone in podocyte or endothelium is not associated edema or gross whole fish phenotype. Moreover, PAN produced equal pericardial edema in all transgenic fish as well as non-transgenic control.

Conclusions: Transgenic expression of risk variant APOL1 is associated with moderate structural changes in the ZF glomerulus, however, expression alone is insufficient to cause quantifiable functional dysfunction.

Funding: NIDDK Support

FR-PO355

BH3 Domain-Independent Apolipoprotein L1 Toxicity Rescued by Bcl2 Pro-Survival Proteins John F. Heneghan, Seth L. Alper, Martin R. Pollak. *Nephrology, Beth Israel Deaconess Med Ctr, Harvard Med School, Boston, MA.*

Background: The APOL1 gene encodes Apolipoprotein L1 (APOL1), an HDL3 component which lyses many pathogenic trypanosomes. However, "serum resistance-associated" protein (SRA) of *T. Brucei rhodesiense* neutralizes APOL1 to foster progression of trypanosomiasis. Expression of APOL1 variant G1 or G2 confers resistance against SRA but elevates kidney disease risk through mechanisms unknown, but previously suggested to include ionophore toxicity or BH3 domain-dependent autophagy.

Methods: We expressed APOL1 in *Xenopus laevis* oocytes from cRNA and noted rapid oocyte degeneration (toxicity). Partial replacement of extracellular Na^+ with K^+ substantially reduced APOL1-associated oocyte toxicity, allowing further investigation. Since APOL1, a BH3-only protein, might be modulated by Bcl2 family members, we expressed APOL1 in oocytes co-injected with cRNA encoding a Bcl2 family member, and documented morphology at 24, 48 and 72 hrs post-cRNA-injection. We measured influxes of $^{45}Ca^{2+}$ and $^{36}Cl^-$, and measured trans-membrane currents by two-electrode voltage clamp.

Results: Co-expression of a Bcl2 family member decreased APOL1-associated oocyte toxicity in the order Mcl1~BclW>BclXL~Bfl1A1>Bcl2. Deletion of 9 core codons from the APOL1 BH3 domain abolished toxicity (as previously reported in HEK-293 cells), but Ala substitution of the same 9 core BH3 codons abolished neither APOL1-associated oocyte toxicity nor its rescue by co-expressed Mcl1. Oocyte influxes of $^{36}Cl^-$ (blocked by DIDS) and of $^{45}Ca^{2+}$ (DIDS-insensitive, blocked by Gd^{3+}, inhibited by Na^+) paralleled in magnitude the severity of APOL1-associated oocyte toxicity, and were accompanied by increased Gd^{3+}-sensitive non-selective currents. Mcl1 coexpression with APOL1 reduced $^{45}Ca^{2+}$ flux and ion current, paralleling morphological rescue of APOL1-associated toxicity.

Conclusions: We propose the BH3-independent toxicity of APOL1 is rescued by Mcl1 and other Bcl2 prosurvival proteins either by an indirect mechanism, or directly via interaction (s) distinct from or only partially overlapping the BH3-binding groove of Bcl2 proteins.

FR-PO356

Cre-Recombinase Toxicity in Podocytes – A New Model for FSGS Kevin Schulte,[1] Madeleine Angela Frahsek,[1] Herdit M. Schüler,[2] Katja Berger,[1] Bart Smeets,[1] Jürgen Floege,[1] Marcus J. Moeller.[1] *[1]Div of Nephrology and Clinical Immunology, Univ Hospital of the RWTH Aachen Univ, Aachen, Germany; [2]Inst of Human Genetics, Univ Hospital of the RWTH Aachen Univ, Aachen, Germany.*

Background: The tetracycline inducible system has become a widely used tool to control gene expression in specific cell populations *in vivo*. Here, we show that inducible expression of Cre-recombinase in podocytes of newborn mice results in cellular toxicity and subsequent focal and segmental glomerulosclerosis (FSGS).

Methods: Transgenic Pod-rtTA/LC1 and PEC-rtTA/LC1 mice overexpress Cre-recombinase in a doxycycline inducible fashion specifically either in podocytes (Pod) or parietal epithelial cells (PEC). Transgenic mice were treated once with doxycyclin shortly after birth (p3) or after completion of nephrogenesis (p10). Kidneys were analysed after 1, 3, 6 and 13 weeks. Karyogram analyses were performed in primary cultures.

Results: In newborn Pod-rtTA/LC1 mice, overexpression of Cre-recombinase induced by administration of doxycyclin resulted in podocyte loss and progressive FSGS in 100% of transgenic Pod-rtTA/LC1 mice. At high doses of doxycycline, FSGS progressed rapidly resulting in premature lethality at the age of 6-8 weeks. Induction at a later time point (p10), or at any time in PEC-rtTA/LC1 or in mice lacking the LC1 transgene had no effect on renal function or histology. Numerous chromosomal aberrations were found in podocytes isolated from Pod-rtTA/LC1 mice treated with doxycyclin on p3, but not in controls.

Conclusions: The Pod-rtTA/LC1 transgenes mediate aberrant Cre-recombination exclusively in newborn mice – most likely because of the increased transcriptional activity of the podocin promoter in early developing podocytes. When induced at birth, the Pod-rtTA transgenic mouse represents a novel and highly reliable model for FSGS in mice.

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FR-PO357

Common Responses of Glomerular Epithelial Cells in Human Secondary FSGS Lesions Christoph Kuppe,[1] Hermann-Josef Groene,[2] Jürgen Floege,[1] Bart Smeets,[1] Marcus J. Moeller.[1] *[1]Dep. of Internal Medicine II, Div. of Nephrology and Clinical Immunology, RWTH Aachen, Aachen, Germany; [2]Dep. of Cellular and Molecular Pathology, German Cancer Research Center, DKFZ, Heidelberg, Germany.*

Background: Recently, it has been shown that parietal epithelial cells (PECs) are involved in the development of sclerotic lesions in focal and segmental glomerulosclerosis (FSGS). In FSGS, PECs become activated, proliferate, migrate onto the glomerular tuft and deposit extracellular matrix. Here, the role of PECs was investigated in secondary FSGS lesions in different primary glomerular diseases in human biopsies.

Methods: A total of 55 renal biopsies diagnosed as "secondary FSGS" were selected with an average of 13.4 glomeruli (11 systemic lupus erythematosus, 12 diabetic nephropathy, 7 membranous nephropathy, 7 membranoproliferative glomerulonephritis, 11 transplant glomerulonephritis, 2 amyloidosis, 2 Alport, 3 HIV-associated FSGS). One section each per biopsy was quadruple stained by immunofluorescence for: activated PECs (CD44 and CK19), PEC matrix (LKIV69) and nuclei (Hoechst); a second slide was stained for PECs (ANXA-3); podocytes (synaptopodin); PEC matrix (LKIV69) and Hoechst.

Results: Three major response patterns of PEC involvement in FSGS lesions were observed in all disease entities: 1. Early FSGS lesions (cellular adhesions between Bowman's capsule and tuft) were formed by podocytes and/or PECs. 2. PECs were always detected in more advanced FSGS lesions independent of the primary glomerular disease. 3. Proliferative (crescentic) lesions were also observed in all glomerular diseases and consisted of activated PECs. Several early FSGS lesions, which could not be identified on PAS sections yet, were detectable already by IF staining. Podocytes or PECs were absent in globally sclerotic lesions.

Conclusions: PECs are involved in the formation of secondary FSGS lesions independent of the primary glomerular disease.

FR-PO358

Parietal Epithelial Cell Reactions Follow Podocyte Loss in a Spectrum of Glomerular Lesions Astrid Weins,[1] Minna D. Balbas,[5] Marcel Tuecking,[2] Kirk N. Campbell,[3] Charles Sawyers,[4] Peter H. Mundel.[2] *[1]Pathology, BWH, Boston, MA; [2]Medicine, MGH, Boston, MA; [3]Medicine, MSSM, New York, NY; [4]MSKCC, New York, NY; [5]UCSF, San Francisco, CA.*

Background: Parietal epithelial cells (PECs) play an elementary part in the evolution of glomerular lesions in Adriamycin (ADR) nephropathy. We sought to address the question if PEC activation represents a ubiquitous response to podocyte loss independent of the cause of podocyte injury. We examined 2 mouse models of primary podocyte injury: 1) Mice deficient in the podocyte protein CD2AP, an established model of progressive proteinuria and focal segmental glomerulosclerosis (FSGS), and 2) Mice deficient in the scaffolding protein MAGI-2, characterized by slit diaphragm disruption, severe early podocyte loss and nephrotic proteinuria.

Methods: Kidney tissue from CD2AP and MAGI-2 knock out (KO) mice and age-matched controls was analyzed by light and electron microscopy. Glomeruli were studied by immunohistochemistry using antibodies against the podocyte marker synaptopodin (synpo), the PEC marker PAX2, and the proliferation marker KI67.

Results: Analysis of both models revealed a spectrum of distinct non-inflammatory glomerulopathies. The CD2AP KO showed FSGS and rare collapsing lesions by 5 weeks as described. As in the ADR model, KI67- and PAX2-positive, synpo-negative PECs populated the tuft in FSGS and in collapsing lesions. The MAGI-2 KO demonstrated a diffuse collapsing glomerulopathy (CG) at 5.5 weeks, including many glomeruli with extensive cellular crescents. Interestingly, proliferating cells within the crescents were diffusely PAX2 and KI67 positive, indicating they also represented activated PECs. In addition, podocyte loss preceded the formation of all light microscopic glomerular lesions, with the most severe loss seen in the MAGI-2 KO at 4-5 weeks.

Conclusions: Our findings suggest that PECs are activated in response to podocyte loss irrespective of the cause of primary injury, leading to distinct light microscopic patterns. We conclude that the severity or rate of podocyte loss governs the PEC response, resulting in the wide range of glomerular lesions currently included in the podocytopathy spectrum.

Funding: NIDDK Support

FR-PO359

The Glomerular Parietal Epithelial Cell Phenotype Depends on SM22 Alpha Levels Shokichi Naito,[1,2] Jeffrey W. Pippin,[1] Kouju Kamata,[2] Stuart J. Shankland.[1] *[1]Nephrology, Univ of Washington, Seattle, WA; [2]Nephrology, Kitasato Univ School of Medical Sciences, Sagamihara, Kanagawa, Japan.*

Background: Reduced podocyte number underlies proteinuria and glomerulosclerosis. Studies suggest glomerular parietal epithelial cells (PECs) are podocyte progenitors. Others show PEC epithelial-to-mesenchymal transition (EMT) associated with crescent formation, and a proliferative phenotype. We have shown that SM22a, an actin-binding protein considered a marker of smooth muscle differentiation, is upregulated in PECs in experimental glomerular models of podocyte injury. The purpose of this study was to investigate the role of SM22a in PECs transition and EMT.

Methods: Experimental glomerular disease was induced in SM22a +/+ and -/- mice by intraperitoneal injection of sheep anti-rabbit glomeruli antibody, and studies on days 7 and 14. Immunohistochemistry was used to determine PEC transition, defined as cells lining

Bowman's capsule co-expressing a podocyte (synaptopodin) and PEC (PAX2) protein, or NCAM (progenitor cells marker). Vimentin and a-smooth muscle actin were used as EMT markers. Podocyte number (WT-1) and proliferation (Ki-67) were also measured.

Results: The number of podocyte progenitors, defined as cells expressing both a PECs protein and a podocyte protein, and NCAM+ progenitor cells was significantly increased in diseased SM22a -/- mice lining Bowman's capsule (P<0.01 versus SM22a +/+) and in the tuft (P<0.01 versus SM22a +/+). Conversely, immunostaining for the EMT markers vimentin and a-smooth muscle actin were reduced in PECs in diseased SM22a -/- mice (P<0.05 versus SM22a +/+).

Conclusions: These data suggested that SM22a may inhibit PECs transition and augment EMT.

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FR-PO360

ARB, But Not Nonspecific Antihypertensive Drugs, Promotes Survival and Parietal Epithelial Cell to Podocyte Transition in 5/6 Nephrectomy Rats

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Background: We previously found that angiotensin receptor blocker (ARB) could regress existing sclerosis in short term, but not in long term follow-up in 5/6 nephrectomy (Nx) rats. ARB also prolonged the survival time in this model. We now further investigated whether ARB effects are blood pressure-dependent and effects on parietal epithelial cell (PECs)-podocyte transition.

Methods: Adult male Sprague Dawley rats underwent 5/6 Nx. Glomerular sclerosis index (SI, 0-4 scale) was assessed by renal biopsy (Bx) at 8 weeks. Rats were then divided into three groups with equal average systolic blood pressure (SBP), 24-h urinary protein (Uprot) and SI, and treated with high dose losartan (ARB, n=20), triple-therapy (hydralazine, reserpine, HCTZ, TRX, n=20) or no treatment (CONT, n=21) till 30 wks after 5/6 Nx.

Results: Both ARB and triple-therapy lowered SBP, but only ARB reduced proteinuria and increased survival rate when compared with control (median survival time ARB 27.1, TRX 25.0 and CONT 19.7 wks). Serum creatinine levels and glomerulosclerosis were similarly improved with ARB and TRX versus control (SI: ARB 2.37±0.19, TRX 2.28±0.11 versus CONT 2.88±0.15, p<0.05). ARB maintained density of WT1-positive podocytes, while TRX did not preserve podocytes (ARB 311.39±31.18, versus TRX 145.23±20.07 and CONT 139.28±18 N/mm^2, p<0.05). In parallel, there were more double CD44 and synaptopodin positive cells in the tuft area after ARB treatment than TRX and control (ARB 0.44±0.01, versus TRX 0.17±0.01 and CONT 0.13±0.01/glomeruli, p<0.05).

Conclusions: We conclude that both ARB and triple-therapy reduce glomerulosclerosis. However, ARB, but not triple-therapy, has survival benefit and podocyte protection. We further speculate that ARB has beneficial effects on permeability functions to enhance survival in the uremic environment.

Funding: NIDDK Support

FR-PO361

Soluble Urokinase Receptor and HIV-Associated Podocyte Dysfunction

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Background: Soluble urokinase receptor (suPAR) is a circulating factor that can become pathological when serum or urine levels increase, or if particular subtypes are present. In some patients, this leads to heightened podocyte avb3 integrin activation—causing foot process effacement, proteinuria, and initiation of early FSGS pathology. Because commercial ELISAs do not recognize all suPAR types, we developed cellular assays to decipher which suPAR-containing samples specifically lead to podocyte dysfunction. This comprehensive suPAR testing method was used to study suPAR's role in HIV-associated podocyte dysfunction, given its resemblance to FSGS.

Methods: suPAR was measured in urine and serum samples from 202 women (eGFR>30) enrolled in the Chicago Women's Interagency HIV Study. Selected HIV+ samples with elevated suPAR were then tested for podocyte avb3 integrin activation (detected by AP5 antibody). Differentiated human podocytes were treated with patient serum or urine, and IF staining was used to test for avb3 activation. High-throughput fluorescence microscopy (Opera LX) provided a novel, quantitative measurement of AP5/Paxillin intensity.

Results: Mean serum suPAR was 4872 pg/ml and 3775 pg/ml in HIV+ (n=172) and HIV- (n=30) patients respectively (p =0.005). Mean urinary suPAR/Cr was 4572 pg/mg and 2520 pg/mg for HIV+ and HIV- patients respectively (p <0.001). Significantly higher suPAR levels were seen in HIV+ patients even after adjusting for age, race, eGFR, and urine ACR. In the subset of serum and urine samples tested for avb3 integrin activation, significantly higher AP5/Paxillin intensities were noted in HIV+ patients, with activity localized to focal adhesions.

Conclusions: Serum and urine suPAR levels are significantly higher in HIV+ compared to HIV- individuals. Elevated suPAR levels coupled with podocyte integrin activation suggest functional similarity between FSGS and podocyte dysfunction in HIV. Since suPAR can partly be removed by plasmapheresis, there may be an avenue for improved therapies for early HIV-podocytopathy owing to this activated suPAR-avb3 integrin axis.

Funding: NIDDK Support, Pharmaceutical Company Support - Questcor

FR-PO362

HIV Genes-Induced Disparate Effects during the Initiation and Progression of HIV-Associated Nephropathy (HIVAN) Are Mediated through Vitamin D Receptor

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Background: Since angiotensinogen (Agt) is the substrate for renin, we hypothesized that mice with enhanced expression of Agt would display accelerated progression of HIVAN. We evaluated the effect of different copies of Agt in the initiation and progression of HIVAN in genetically engineered HIVAN mice (Tg26).

Methods: Control and Tg26 mice with 2 (Tg26/Agt-2) and 4 (Tg26/Agt-4) copies of Agt were evaluated for severity of renal lesions, arteriosclerosis and hypertension at 8 weeks and 16 weeks. Renal cortical sections were stained with sirus red and PAS. RNA was extracted from renal tissues and probed for AT1, AT2, PAI-1, VDR and molecules involved in Tert and epithelial mesenchymal transition (EMT) pathways.

Results: Tg26/Agt-4/8wks showed lower blood pressure (P<0.01) versus Tg26/Agt-2/8 wks. While Tg26/Agt-4/16wks displayed higher blood pressure versus Tg26/Agt-2/16wks. Tg26/Agt-4/8wks displayed attenuated expression of PAI-1 versus Tg26/Agt-2/8wks; however, Tg26/Agt-4/16wks showed 3-fold greater PAI-1 expression than to Tg26/Agt-2/16wks. Tg26/Agt-2/8wks displayed attenuated expression VDR and enhanced production of Ang II versus Tg26/Agt-4/8wks, however this pattern reversed at 16 wks. Tg26/Agt-4/8wks displayed attenuated expression of AT1 and AT2 and down regulation of Tert, TGF-β, Snail, and vimentin when compared to Tg26/Agt-2/8wks. However, all these markers were comparable between these groups at 16 wks of age. Tg26/Agt-2/8wks developed renal lesions which were more advanced than Tg26/Agt-4/8wks. Conversely, Tg26/Agt-4/16wks displayed more advanced renal lesions versus Tg26/Agt-2/16wks.

Conclusions: Tg26/Agt-4 displayed slowed progression of HIVAN at early time periods associated with enhanced renal tissue VDR expression and attenuated expression of AT1, TGB-b, PAI-1, Tert and EMT markers. However, Tg26/Agt-4 at 16 wks displayed accelerated growth possibly due to attenuated VDR expression, high blood pressure and upregulation of EMT and profibrotic molecules with ageing.

Funding: NIDDK Support

FR-PO363

HIV-Induced Kidney Cell Renin Generation Enhances Kidney Cell HIV Gene Expression

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Background: The activation of renin -angiotensin system has been demonstrated to play an important role for the development and the progression of HIV-associated nephropathy (HIVAN). However, the involved mechanism is far from clear. Recently, HIV has been demonstrated to stimulate renin generation by kidney cells *in vitro* studies. Moreover, we observed that renin directly enhances HIV replication in T cells (another **submisstion** to ASN). We hypothesized that HIV-induced kidney cell renin production might also be enhancing kidney cell HIV gene expression.

Methods: Immortalized differentiated human podocytes (IDHP) were trandudced with either empty vector (EV/IDHP) or HIV (NL4-3, HIV/IDHP). To increase endogenous renin production, EV/IDHPs and HIV/IDHPs were transfected with siRNA vitamin D receptor (siRNAVDR/HIV/IDHPs) or scrambled (Scr-siRNA/HIV/IDHP) siRNA. Protein blots were probed for renin and actin. To evaluate the effect of renin *in vivo*, mRNA expressions of HIV genes from renal tissues of HIVAN mice with high endogenous renin (genetically engineered Tg26 mice either with 2, 3 and 4 copies of angiotensinogen [Agt] or lacking VDR) were quantified by qPCR. To downregulate renal tissue renin expression, Tg26 mice were treated with either vehicle or a VDR agonist (VDA) for 2 weeks and then renal tissues were evaluated for HIV gene expression.

Results: HIV enhanced renin expression in human podocytes. Silencing of VDR in HIV/IDHPs further enhanced expression of Nef, Tat, and Vif. On the other hand, treatment of HIV/IDHPs with VDA downregulated HIV gene expression. Renal tissues of Tg26-Agt-4 displayed 2-4 fold increase in mRNA expression of gp120, Vpr, Tat, Nef and Vpu versus Tg26-Agt-2. Similarly, Tg26 mice lacking VDR displayed greater HIV gene expression when compared with wild type Tg26 mice. Moreover, VDA treatment of Tg26 downregulated renal tissue expression of HIV genes.

Conclusions: HIV-induced kidney cell renin generation enhances HIV gene transcription both *in vitro* and *in vivo*.

Funding: NIDDK Support

FR-PO364

Metformin Slows Down the Progression of HIV-Associated Nephropathy (HIVAN) through Down Regulation of Mammalian Target of Rapamycin (mTOR) Pathway

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Background: The mTOR pathway has been reported to play an important role in the development of renal lesions in patients with HIV infection. Since patients with HIV infection are now living almost a normal life style including the development of metabolic

syndrome. we hypothesized that use of metformin in this population would not only control insulin resistance but would also slow down the progression of kidney lesions related to HIV-associated nephropathy. To test our hypothesis, we studied the effect of meformin on the progression of renal lesions in a mouse model of HIVAN (doxycycline-inducible Vpr [podocyte specific] transgenic mice).

Methods: Vpr mice in groups of eight were fed either doxycycline with or without metformin for six weeks followed by evaluation for renal biomarkers (Blood urea nitrogen, urine protein:creatinine ratio, grading of severity of renal lesions and immunoblotting for phospho-mTOR and down stream molecular markers). In *in vitro* studies, mouse proximal tubular epithelial cells (MPTECs) were transduced with either empty vector (EV) or NL4-3 without *gag* and *pol* (HIV). EV/MPTECs or HIV/MPTECs were incubated in media containing either buffer or metfromin (0.5mM) for 48h. Protein blots of EV/MPTECs and HIV/MPTECs were probed for phospho-mTOR, phospho-p70S6 kinase, phospho-eEF2, p-eIF4B, and p-4EBP-1. The same blots were stripped and reprobed for actin.

Results: Vpr mice displayed sclerotic glomerular lesions, microcyst formation, proteinuria and activation of mTOR pathway; metformin not only attenuated proteinuria but also decreased severity of renal lesions. Moreover, metformin downregualed activation of the mTOR pathway. In vitro studies, protein blots of HIV/MPTEC displayed 2-fold increase in phospho-mTOR, 2.5-fold increase in phospho-p70S6K, and 2-fold increase both in p-eIF4B and p-4EBP-1when compared to EV/MPTECs. On the other hand, metformin inhibited HIV-induced mTOR phosphorylation and associated down stream signaling.

Conclusions: Metformin slows down the progression of HIVAN through down regulation of mTOR pathway.

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FR-PO365

Transmembrane Protein 14A Is Involved in the Development of Proteinuria

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Background: Proteinuria is an independent risk factor for cardiovascular mortality and progression of renal disease. To understand the underlying mechanism and find potential targets for treatment, identifying and investigating genes suspected of playing a role in the proteinuric pathway is required. Our group has previously performed a microarray experiment in spontaneously proteinuric Dahl SS rats. In this experiment, transmembrane protein 14A (TMEM14A) mRNA was found to be differentially expressed. Little is known of this gene and the protein it encodes. In this study, we have investigated whether blocking TMEM14A mRNA translation results in proteinuria.

Methods: Zebrafish (Danio rerio, ABTL strain) embryos were injected with a morpholino targeting zgc:163080, the zebrafish homologue of TMEM14A, during the one- to four-cell stage of embryonic development. Proteinuria was measured by intravenously injecting a mixture of dextran tracers at 4 days past fertilization (dpf). After fixing, embedding and sectioning, fluorescent microscopy was used to investigate tubular reabsorption of the dextran tracers that have passed the glomerular filtration barrier. Results were quantified by comparing the mean amount of 70kD droplets with an independent samples t-test.

Results: TMEM14A mRNA was significantly downregulated in Dahl SS rats. Morpholino injected zebrafish showed developmental defects and displayed a significantly ($p<0.05$) higher amount of 70kD droplets in the tubular epithelial cells compared to wildtype zebrafish embryos.

Conclusions: The higher amount of 70kD droplets in the tubular epithelial cells of the morpholino injected zebrafish indicates that their glomerular filtration barrier is more permeable to 70kD particles than in the control group. This indicates that blocking translation of zgc:163080 results in proteinuria. These results, taken together with our previous results from the microarray experiment in spontaneously proteinuric rats, strongly imply a role for TMEM14A in the development of proteinuria and warrant further research into its function.

FR-PO366

Intracellular Transcytosis and Exocytosis of Albumin in Human Glomerular Endothelial Cells

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Background: We have been reported the albumin endocytosis through caveolae into human glomerular endothelial cells (HRGEC) as a new pathway of albumin through HRGEC in addition to fenestrae, and its pathway may be recognized as the new mechanism of albuminuria. This study is the next step to show intracellular trafficking pathway and exocytosis of albumin in HRGEC after caveolar endocytosis.

Methods: HRGEC were incubated with Alexa Fluor 488 labeled bovine serum albumin (BSA) for 0.25, 0.5, 1, 2, 4, and 6 hours. Then immunofluorescence study was performed by using golgi zone as the Golgi apparatus marker, LANP2 as the lysosome marker, PDI as the endoplasmic reticulum (ER) marker, AE-8 as the microtubules marker, and anti actin antibody. HRGEC were also incubated with the immune gold labeled BSA for 15 and 30 minutes, and ultrastructural pathological study by electron microscope was performed.

Results: Alexa Fluor 488 labeled BSA were not co-localized with any marker of organelle, such as golgi body, lysosome and ER. Labeled BSA were also not co-localized with cytoskeletons, such as actin and microtubules. The intensity of colocalized BSA with any organelle and cytoskeletons were significantly lower than that with Caveolin-1, which was a main component of caveolae. In ultrastructual pathological study, gold labeled BSA were clearly observed to enter into HRGEC through caveolae. Caveolae were pinched off and detached from cell membrane. BSA particles coated with cavelae were transported through HRGEC without dropping on any organelle and cytoskeletons, and finally excreted to the other side of HRGEC.

Conclusions: We have shown transcytosis and exocytosis of albumin coated with caveolae through HRGEC was independent from the Golgi apparatus, lysosome, ER, actin and microtubules. Albumin was entered into HRGEC through caveolae form capillary lumen, caveolae coated pits include albumin were transported through HRGEC to the other side of cells and excreted to sub-endothelial space. This is the new caveolae dependent pathway of albumin through HRGEC and may become new pathogenesis of albuminuria independent from fenestrae.

FR-PO367

Ubiquitin-Specific Protease 2-69 Antagonizes Glomerulonephritis by Stabilizing Decorin

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Background: Decorin (DCN) is a well-known anti-sclerosis factor in kidney diseases because it inhibits mesangial cell (MC) proliferation and neutralizes the effect of TGF-β1. Our previous studies have shown that DCN is degraded by ubiquitin-proteasome pathway which can be reversed by deubiquitinases. However, no deubiquitinase related to DCN was reported. USP2-69, a recently found deubiquitinase, is implicated in the regulation of cell proliferation in cancer and highly expresses in kidney, but its deubiquitinated effect on DCN and role in glomerulonephritis (GN) have not yet been explored.

Methods: Firstly, we detected the interaction between USP2-69 and DCN. After overexpressed or silenced USP2-69 in rat MCs, ubiquitination of DCN, half-life of DCN and expression of collagen IV and TGF-β1 were examined. Then we examined USP2-69 and DCN in MCs treated with TGF-β1, TNF-a or PDGF-BB for 3h to 36h and in human GN such as MCD, IgA (type MsPGN) and LN (type IV). In addition, we transfected USP2-69-HA plasmid into the left kidney of rat anti-thy1.1 GN by using electroporation-mediated gene transfer via renal artery, and then the renal morphology was examined by H&E and PAS, expressions of PCNA, collagen IV, fibronectin and TGF-β1 were detected in both left and right kidneys.

Results: A novel physical interaction between USP2-69 and DCN was found. Overexpression of USP2-69 resulted in decreased ubiquitination and prolonged half-life of DCN and decreased collagen IV and TGF-β1, which was opposite after USP2-69 interference. Stimulation of TGF-β1, TNF-a or PDGF-BB led to a same decreased trend of USP2-69 and DCN. Both of USP2-69 and DCN expression increased in human IgA and LN compared to MCD. In anti-thy1.1 model transfected with USP2-69-HA plasmid, HA positive staining was detected in glomeruli, less cell proliferation and matrix was observed and decreased PCNA, collegen IV, fibronectin and TGF-β1 was examined in the left kidney compared to the right one.

Conclusions: This study showed that USP2-69 deubiquininated DCN specifically and antagonized GN in anti-thy1.1 model, indicating that USP2-69 is an effective approach targeting DCN for against glomerular sclerosis.

Funding: Government Support - Non-U.S.

FR-PO368

Nuclear Translocation of Dendrin in IgA Nephropathy

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Background: Dendrin is an 81-kD protein localized in the podocyte slit diaphragm. Recent studies have focused on the proteins pro-apoptotic signaling properties, and its accumulation in the podocyte nucleus in response to glomerular injury. With recent findings of an increased occurrence of dendrin-positive podocyte nuclei in IgA nephropathy (IgAN), we analyzed glomerular gene and protein expression of dendrin in renal biopsies from patients with IgAN and membranous nephropathy (MN).

Methods: Microarray was performed on the glomerular fraction of kidney biopsies from patients with IgAN (n=30), MN (n=5) and normal renal tissue (n=20). Podocytic dendrin expression was studied by iEM, on kidney biopsies from patients with IgAN with or without nephrosis (n=4 and n=5, respectively), MN (n=3) and normal renal tissue (n=5). The primary antibody was detected by gold-conjugated protein A and semi-quantification was performed.

Results: Gene expression analysis revealed a 2.01 fold significant up regulation of dendrin expression in IgAN compared to controls. In MN, gene expression of dendrin was not significantly changed. Dendrin was significantly increased in the podocytes nuclei in both IgAN, with and without nephrosis (0.9 ± 0.0 and 0.9 ± 0.2, respectively) compared to controls (0.4 ± 0.1). The increase of dendrin was also seen in the podocytes cytoplasm of IgAN with nephrosis (3.0 ± 0.6) compared to controls (1.5 ± 0.6). The amount of dendrin in MN was unchanged both in the podocytes nuclei (0.2 ± 0.1) and the cytoplasm (1.1 ± 0.3).

Conclusions: Our data demonstrates a two fold up regulation of dendrin gene expression in IgAN and a corresponding increase in podocytic protein expression. The increase in nuclear protein expression was not correlated to the degree of proteinuria. In MN, on the other hand both gene- and protein expression were unchanged. Our results supports the hypothesis that dendrin is translocated into the podocytic nuclei in IgAN. This may induce apoptosis and chronic glomerular injury, e.g. capsular adherences.

Funding: Government Support - Non-U.S.

FR-PO369

C4d Deposits Are Associated with Thrombotic Microangiopathy in IgA Nephropathy and Mark a Poor Renal Function Jamie S. Chua,[1] Malu Zandbergen,[1] Johan W. De Fijter,[2] Ron Wolterbeek,[3] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, LUMC, Leiden, Netherlands; [2]Nephrology, LUMC, Leiden, Netherlands; [3]Medical Statistics and Bio-Informatics, LUMC, Leiden, Netherlands.*

Background: Complement deposits such as C4d and MBL can be found in 25% of patients with IgA nephropathy (IgAN), the most prevalent primary chronic glomerular disease worldwide (Wyatt et al, NEJM 2013). We previously demonstrated that C4d marks thrombotic microangiopathy (TMA) in native biopsies with lupus nephritis. We hypothesized that C4d marks TMA in native biopsies with IgAN and that both C4d and TMA are clinically significant markers of poor renal function.

Methods: In this single center study, 132 native renal biopsies diagnosed with IgAN from 2003-2013 were included, of which 108 were previously diagnosed with IgAN and 24 with Henoch-Schönlein nephritis (HSN). Biopsies were re-evaluated according to the Oxford Classification for IgAN. Additional scored histologic parameters were: arterial intimal sclerosis, arteriolar hyalinosis and TMA (presence, localization and chronicity). C4d was scored in glomeruli (<25% or >25% of all glomeruli), in peritubular capillaries (Banff '07 criteria) and in arterioles (absent or present). Demographics and clinical data were collected retrospectively from medical records, and included blood pressure, history of hypertension and renal function.

Results: TMA was present in 27 biopsies (21%). TMA lesions were mainly chronic (67%) and mainly present in arterioles (85%). C4d staining was available for 126 biopsies and was associated with TMA ($p<0.001$). Glomerular and arteriolar C4d deposits were present in 15% and 13% of all biopsies respectively. C4d was absent in the peritubular capillaries of all biopsies. Biopsies with both TMA and C4d had significantly more E1 and higher T scores ($p<0.05$), whereas biopsies without TMA and without C4d had significantly less S1 scores ($p<0.05$). Patients with both C4d and TMA had significantly more frequently hypertension ($p<0.05$) and a significantly lower eGFR (30.6 ml/min/1.73m2) compared to TMA only (59.8), C4d only (48.2) or neither (74.9 $p<0.05$).

Conclusions: C4d is associated with TMA in IgAN and both C4d and TMA are clinically significant markers of a poor renal function.

FR-PO370

Gene Expression Analysis of the Renal Lesions in IgA Nephropathy. A Multicenter Study Claudia Curci,[1] Fabio Sallustio,[1] Grazia Serino,[1] Mirko Trpevski,[1] Francesco Pesce,[1] M. Rossini,[2] Mario Bonomini,[3] Vittorio Sirolli,[3] Paolo Felaco,[3] Giuseppe Lattanzio,[3] Gianluigi Zaza,[4] Antonio Lupo,[4] Isabella Squarzoni,[4] Patrizia Bernich,[4] Concetta Gangemi,[4] Francesco Paolo Schena.[1] *[1]C.A.R.S.O. Consortium; [2]Univ of Bari; [3]Univ of Chieti; [4]Univ of Verona.*

Background: The diagnosis of idiopathic IgA Neprhopathy (IgAN) may be based on two type of histomorphological classification. The "lumped system" (Schena classification, 2005) identify three grades (G) of severity. The "split system" (Oxford MEST classification, 2009) is based on 4 types of lesions: Mesangial hypercellularity (M 0-1), Endocapillary hypercellularity (E 0-1), Segmental glomerulosclerosis (S 0-1) and Tubular atrophy/interstitial fibrosis (T 0-2). Aim of our study was to elucidate the molecular changes that happen in the renal lesions at the time of biopsy.

Methods: We obtained total RNA from archival FFPE renal tissue samples of 34 IgAN, 24 non-IgAN patients (MCD 12, MGN 12) and 6 kidney living donors (KLD, as normal controls). Genome-wide expression profiles were generated by Illumina platform. Real Time PCR was used for validation of the identified transcripts.

Results: The principal component analysis showed that 152 transcripts partially separated IgAN renal biopsies from non-IgAN, while 107 transcripts clearly separated IgAN from KLD. We identified 28 genes exclusively modulated in IgAN biopsies. These genes belonged to apoptotic processes, renal cellular damage and immune system regulatory pathways. We identified genes were differentially modulatedthe different degrees of severity (G1-3). Finally, we differentiated the gene expression profiles of the 4 MEST lesions and identified specific gene clusters for each lesion. We have validated the candidate transcripts by qRT-PCR in anothere independent cohort of 35 IgAN biopsies.

Conclusions: Transcriptomics on FFPE renal biopsies offer the possibility to integrate genomics and histomorphologic renal lesions. Our study the involvement of specific gene pathways that are responsible for the development of MEST lesions in IgAN. This novel type of information has identify some gene classifiers that may be considered for target therapy.

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FR-PO371

Enhanced Glomerular Neutrophil Chemoattractants Expression in Experimental ANCA Associated Vasculitis Go Kanzaki, Shinya Nagasaka, Seiichiro Higo, Yusuke Kajimoto, Kiyotaka Nagahama, Yukinari Masuda, Akira Shimizu. *Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

Background: ANCA-neutrophil and neutrophil-endothelial cell interactions play an important role in the pathogenesis of ANCA-associated vasculitis (AAV). However, the mechanisms underlying the pathogenesis of crescent formation in AAV have not been completely elucidated. To unveil an involvement of these interactions in necrotizing crescentic glomerulonephritis (NCGN), we used an AAV rat model and investigated the effects of MPO, TNF-a, G-CSF, or subnephritogenic anti-GBM antibodies (Ab), as a pro-inflammatory stimulus.

Methods: NCGN was induced in WKY rat by immunization with human MPO (hMPO; 1600 μg/kg). Blood and urine samples were obtained at 8 weeks. NCGN was evaluated by renal histopathology. Furthermore, we examined whether the pro-inflammatory stimulus induced NETs in crescent formation in AAV rat model. At 8 weeks after NCGN induction, the expression of cytokines and adhesion molecules in isolated glomeruli were analysed by quantitative RT-PCR.

Results: Hematuria were noted from 4 weeks. The hMPO-immunized rats had serum anti-hMPO antibody titers of 1:10000 by ELISA. We demonstrated that the induced anti-hMPO antibodies cross-reacted with TNF-a or G-CSF primed rat neutrophils inducing IL-1b in vitro. Crescent formation with NETs in the AAV rat model is dramatically enhanced by the subnephritogenic anti-GBM Ab, whereas not significantly enhanced by the administration of TNF-a or G-CSF. AAV rats injected with the subnephritogenic anti-GBM Ab elevated serum TNF-a and IL-1b level with mild renal failure. TNF-a, CXCL-1, and CXCL-2, which are mainly involved in neutrophil-endothelial interactions, were induced or up-regulated in NCGN.

Conclusions: Although neutrophil stimulation by ANCA can trigger pro-inflammatory cytokines, neutrophil migration into glomerulus by the proinflammatory chemokines after exposure to ANCA might be necessary to develop NCGN with NETs in AAV.

FR-PO372

Structural Analysis of a New Epitope Causing Experimental Autoimmune Glomerulonephritis (EAG) in Rats Jitendra K. Gautam,[1] Palak D. Shah,[2] Kline Bolton.[1] *[1]Dept of Medicine, Div. of Nephrology/CIIR, Univ of Virginia (UVA) HS; [2]College of Arts and Science, UVA, Charlottesville, VA.*

Background: The a_3 (IV) NC1 domain causes Goodpasture's syndrome (GPS) in humans and similar disease in rats, EAG. We have shown that an immunodominant pure T cell epitope, p13, that induces EAG, is frequently associated with intra-and-intermolecular antibody (Ab) B cell epitope spreading along the a_3 (IV) NC1 and the a_4 (IV) NC1chains. Several other peptides can induce EAG, and EAG can be transferred by T cells. In our reported screen of 22 overlapping peptides, each 20 amino acids long, one peptide Col_131-150, induced severe EAG (JASN 24, 2013 FR-PO563). Cathepsin D cleavage sites are present within this region of a_3 (IV) NC1 and normal humans harbor autoreactive T cells reacting to the same region (JASN 19:396-404, 2008). This region has also been shown to be important for EAG in a humanized mouse model (JASN 24: 419-431, 2013).

Methods: In this study we used a structural biologic approach to visualize the 22 overlapping peptides in the 3D structure of a3 (IV) NC1 in reference to Cathepsin D cleavage sites. We used SWISS-MODEL (http://swissmodel.expasy.org/) to model human a3 (IV) NC1 based on related PDB 1T60 (JBC 279 (43):44723-30, 2004). Web Lab Viewer Pro was used for surface visualization.

Results: We assessed each peptide surface within the a3 (IV) NC1 structure for overall exposure and accessibility for Cathepsin D cleavage. Peptides varied in their surface accessibility of Cathepsin D sites. Peptide Col_131-150 partially overlapped with the well described surface exposed EB Ab epitope. The rest of the peptide was found to be buried inside rendering it inaccessible from the surface. The cryptic region also includes the reported Cathepsin D sites. Exposure of this cryptic site could play a role not only in the development of EAG and GPS, but also in augmentation of the disease phenotype.

Conclusions: A better understanding of the structural basis of this molecular epitope exposure and release, and its correlation with clinical and immunological data of GPS could enhance our understanding of disease pathogenesis. It may also help us design antigen specific therapeutic interventions.

Funding: NIDDK Support

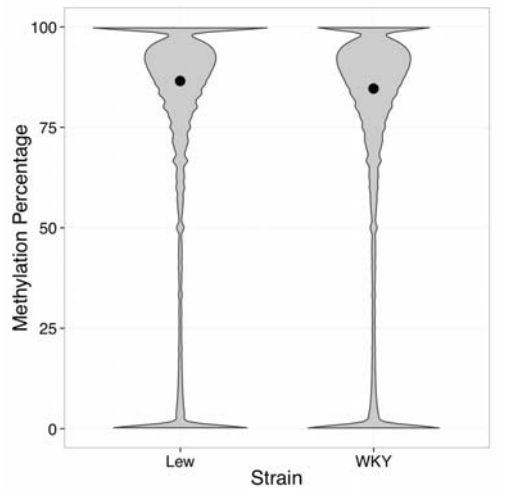
FR-PO373

The Role of DNA Methylation in a Rat Model of Crescentic Glomerulonephritis Thomas M. Oates,[1] Michael Mueller,[2] Charles D. Pusey,[2] H. Terence Cook,[2] Enrico Petretto.[1] *[1]MRC Clinical Sciences Centre, London, United Kingdom; [2]Imperial College London, London, United Kingdom.*

Background: The Wistar-Kyoto (WKY) rat is susceptible to crescentic glomerulonephritis (CRGN), whilst the Lewis (LEW) rat is resistant, making this a useful model to study underlying disease mechanisms. Previous work has implicated macrophages in CRGN and defined some of the genetic determinants underlying CRGN susceptibility. Recent data has linked epigenetic modifications to macrophage activation and autoimmune disease. Therefore, we hypothesized that variation in DNA methylation in macrophages could contribute to susceptibility to glomerulonephritis in the WKY rat.

Methods: Fragmented DNA libraries from WKY and LEW bone marrow-derived macrophages (BMDMs; 4 biological replicates/strain) were subject to whole genome shotgun bisulfite sequencing using an Illumina HiSeq 2000. Multiplexed PCR sequencing was used to verify whole genome methylation signatures at CpG dinucleotides. Bioinformatic analysis of bisulfite sequencing, histone and transcription factor binding site data was used to explore the interaction of methylation and gene regulatory sequences.

Results: Approximately 15 million CpG dinucleotides were analyzed. WKY BMDMs were generally less methylated than LEW ($P<2.2x10^{-16}$; Wilcoxon rank sum test).



More than 13,000 CpG dinucleotides were differentially methylated between the two strains and 70.5% of these were >5 kilobases from genes. As a result, we investigated the relationship to distant gene regulatory sequences. Our analyses suggested that differentially methylated CpGs were enriched at gene enhancer elements and at binding sites of transcription factors of functional importance in macrophage biology.

Conclusions: These data reveal an association between DNA methylation and susceptibility to CRGN and highlight macrophage genes and gene regulatory elements that could underlie this association.

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FR-PO374

Toll-Like Receptor 8 Contributes to Podocyte Injury in Murine Autoimmune Glomerulonephritis Junpei Kimura,[1] Osamu Ichii,[1] Teppei Nakamura,[1,2] Taro Horino,[3] Saori Otsuka,[1] Yasuhiro Kon.[1] *[1]Laboratory of Anatomy, Dept of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido Univ, Sapporo, Japan; [2]Chitose Laboratory, Japan Food Research Laboratories, Chitose, Japan; [3]Dept of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi Univ, Nankoku, Japan.*

Background: Glomerulonephritis (GN) is a major cause of chronic kidney disease. Recent studies have revealed that the Toll-like receptor (TLR) family plays a central role in local autoimmune response as well as pathogen sensing; however, the contribution of TLRs to GN progression remains unknown. In the present study, we investigated glomerular injury and expression of TLRs and their downstream factors using a murine model for autoimmune GN.

Methods: BXSB/MpJ-*Yaa* (BXSB-*Yaa*) and BXSB/MpJ mice were used as models of autoimmune GN and healthy controls, respectively. Glomerular histopathology and urinary albumin creatinine ratio (uACR) were evaluated. Glomerular expression levels of the TLR family, downstream factors, and glomerular functional factors were determined using isolated glomeruli. TLR8 protein and mRNA were localized using immunohistochemistry (IHC) and in situ hybridization (ISH), respectively. The serum and glomerular levels of miR-21, a candidate ligand of TLR8, were determined.

Results: BXSB-*Yaa* developed membranoproliferative GN and increased uACRs compared to controls. The glomerular expression levels of TLR-family genes (*Tlr1*, *2*, *7*, *8*, *9*, and *13*) and downstream factors (*Il1b*, *Il6*, and *Tnfa*) were significantly higher in BXSB-*Yaa* mice than in controls; *Tlr8* expression was particularly increased (107-fold), and both TLR8 protein and mRNA were localized to podocytes. The glomerular expression levels of podocyte functional markers (*Actn4*, *Cd2ap*, *Myh9*, *Nphs1*, *Nphs2*, *Podxl*, and *Synpo*) were significantly lower in BXSB-*Yaa* mice than in controls. The glomerular expression levels of *Tlr8* were significantly correlated with both those of podocyte functional markers and uACRs. The serum and glomerular mi-R21 expression levels were significantly higher in BXSB-*Yaa* than in controls.

Conclusions: These findings suggest that activation of the TLR8-mediated pathway contributes to podocyte injury in murine autoimmune GN.

Funding: Government Support - Non-U.S.

FR-PO375

Renal Iron Accumulation Precedes Albuminuria in the (NZBxNZW) F1 Model of Lupus Nephritis Erika I. Boesen. *Univ of Nebraska Medical Center.*

Background: Poorly-liganded iron damages tissue via several mechanisms. Lupus patients may be at risk of increased exposure to iron via hemolytic anemia or iron supplements to treat anemia of chronic disease. Several iron homeostasis proteins have been proposed as urinary biomarkers of lupus nephritis, but whether renal iron homeostasis is altered in lupus nephritis is unknown. Accordingly, we compared renal non-heme iron concentrations between the (NZBxNZW) F1 (NZBWF1) mouse model of lupus and age-matched NZW control mice.

Methods: Female mice fed normal chow were studied at 8 weeks, 20 weeks or 7-8 months (n=4-6 per group). Urine was collected for 24h by metabolic cage. Albuminuria was tested by dipstick, protein excretion by Bradford assay, lipocalin-2 excretion and plasma anti-dsDNA IgG by ELISA. Non-heme iron was measured in renal cortex by acid extraction and colorimetric assay.

Results: Renal cortical non-heme iron concentration was similar in NZBWF1 and NZW mice at 8 weeks (60±3 versus 56±53μM, P>0.05). At this age, 3 out of 4 NZBWF1 mice were negative for anti-dsDNA IgG. At 20 weeks, cortical iron was significantly higher in NZBWF1 mice than age-matched NZW mice (86±4 versus 69±53μM, P<0.05). Anti-dsDNA IgG were not yet significantly elevated in NZBWF1 versus age-matched NZW (19±5 versus 10±3kU/ml, P=0.2). Dipstick revealed only trace amounts of albumin in both groups, and 24h urinary protein excretion (1.8±0.3 versus 1.9±0.1mg/day, P>0.05) and lipocalin-2 excretion (69±5 versus 123+/-56ng/day, P>0.05) did not differ between NZBWF1 and NZW mice, suggesting that increased iron accumulation in NZBWF1 mice preceded overt renal injury. At the onset of albuminuria (≥2+ or 100mg/dL) at 7-8 months, cortical iron levels remained higher in NZBWF1 mice (109±11 versus 81±3μM in NZW, P<0.05), and anti-dsDNA IgG were markedly elevated in NZBWF1 versus NZW mice (332±60 versus 19±5kU/ml, P<0.01).

Conclusions: Our data show that renal iron accumulation is increased in the NZBWF1 model of lupus nephritis and precedes overt renal injury. This enhanced iron accumulation, which occurred without iron supplementation, may contribute to the development and progression of lupus nephritis. As a clinical perspective, our data suggest that lupus patients may be at increased risk of iron-mediated renal damage.

FR-PO376

Application of Proteomics to Glomerular Diseases Tadashi Yamamoto,[1] Hidehiko Fujinaka,[1] Shigeru Miyazaki.[2] *[1]Structural Pathology, Inst of Nephrology, Niigata Univ, Niigata, Japan; [2]Internal Medicine, Shinrakuen Hospital, Niigata, Japan.*

Background: Proteomics approaches are now applicabletoclinical nephrology. To understand systems biology/pathology of the kidney and also to discover biomarkers for kidney diseases in urine, an international collaboration project, Human Kidney and Urine Proteome Project (HKUPP) was established as an initiative in Human Proteome Organization (HUPO). In this presentation is introduced an application of proteomics to kidney tissue analysis and urine biomarker discovery.

Methods: By LC-mass spectrometry (MS) and immunohistochemistry (IHC), proteomes of normal human kidney tissues (cortex, medulla, glomerulus and other several compartments), CKD disease glomerulus (IgA normal volunteers are examined.Thousands of proteins with high confidence were identified by LC-MS, quantified by non-labeled quantitaion, normalized spectral index, of MS data and localized by IHC.

Results: Thousands of proteins with high confidence were identified by LC-MS, quantified by non-labeled quantitaion, normalized spectral index, of MS data and localized by IHC. Bioinformatics tools for network and pathway analysis of the glomerulus proteomes depicted interesting networks and pathways; networks of oxidative stress, immune response, fibrosis, and hypertension and pathways of GRAB2, snRNP, g-secretase, angiogenin and glutathione are demonstrated as enhanced ones in membranous nephropathy glomerulus. Proteins identified in healthy volunteer urine are annotated with their sources, cells or plasma, for construction of urinary proteome database.

Conclusions: Proteomics and bioinformatics analysis of kidney biopsy specimen provided new insights intomolecular mechanisms of glomerular diseases and may promote efficient personalized medicine in the near future. New biomarkers for earlier detection, pathologic diagnosis, or prognosis of CKD may be introduced by urine proteomic analysis.

Funding: Government Support - Non-U.S.

FR-PO377

Tubuloglomerular Crosstalk Is Affected by Type of Tubulointerstitial Injury Jae Won Yang,[1] Beom Jin Lim,[1] Ming-Zhi Zhang,[2] Raymond C. Harris,[2] Haichun Yang,[1] Agnes B. Fogo.[1] *[1]Pathology, Microbiology, and Immunology, Vanderbilt Univ, Nashville, TN; [2]Nephrology, Vanderbilt Univ, Nashville, TN.*

Background: Glomerular injury can cause subsequent tubulointerstitial ischemia and fibrosis. However, little is known about how tubulointerstitial injury causes glomerular injury. In this study, we evaluated the effect of different types of preexisting tubulointerstitial injury on glomerular function and sclerosis.

Methods: Nep25 transgenic mice express human CD25 receptor on podocytes, and develop glomerulosclerosis when immunotoxin (LMB2) is administered. To assess toxic tubulointerstitial injury, we mated DT/gGT transgenic mice with diphtheria toxin (DT) receptor in proximal tubular epithelial cells with Nep25 mice, to generate $Nep25^{+}/DT^{+}$ and $Nep25^{+}/DT^{-}$ mice and treated with DT. To assess crystal-induced tubulointerstitial injury, we mated Col I-luciferase mice, which have luciferase inserted in the collagen I promoter, with Nep25 mice to generate Nep25/Col I-luciferase mice, and treated with folic acid or vehicle. All mice underwent uninephrectomy (UNx) at day 42 to assess type and extent of tubulointerstitial fibrosis, and LMB2 injection 3 days later, and were sacrificed one week after LMB2.

Results: The kidneys from UNx showed diffuse interstitial fibrosis in $Nep25^{+}/DT^{+}$ but not $Nep25^{+}/DT^{-}$ mice. Although bioluminescence imaging showed higher score for collagen I in folic acid group, Masson staining of the UNx kidney showed only patchy and mild interstitial fibrosis along medullary rays. After UNx, but before LMB2 injection, all mice showed normal range albuminuria. After LMB2 injection, proteinuria increased 2.8 fold in $Nep25^{+}/DT^{+}$ mice versus 1.4 fold in $Nep25^{+}/DT^{-}$. Glomerulosclerosis at sacrifice was more widespread in $Nep25^{+}/DT^{+}$ versus $Nep25^{+}/DT^{-}$. In contrast, proteinuria and synaptopodin expression at sacrifice were similar in folic acid and vehicle groups.

Conclusions: We conclude that different types and severity of tubulointerstitial injury have varied effects on development of glomerular injury. Thus, crystal induced mild tubulointerstitial injury may not adversely impact glomerulosclerosis in the short term, in contrast to the deleterious effects of severe ischemic injury.

Funding: NIDDK Support

FR-PO378

Development of Atubular Glomeruli in Cisplatin-Induced Chronic Kidney Disease Robert L. Safirstein,[1,2] Heino Velazquez,[1,2] Richard Torres,[1,2] John J. Chang,[1,2] Gilbert W. Moeckel,[1] Gary V. Desir.[1] *[1]Nephrology Div, Yale Univ, New Haven, CT; [2]Nephrology, VACT HealthCare System, West Haven, CT.*

Background: Chronic Kidney Disease (CKD) develops in humans after repeated doses of cisplatin (CP). Here we report on a new model to investigate the mechanism of CP-induced CKD.

Methods: 8-week old C57Bl6 mice received a single or double doses (2 weeks apart) of CP (15 mg/kg) and were studied 2,4,6,9 and 16-25 weeks after the first dose. Glomerular filtration rate (GFR) was measured by inulin clearance. Renal tissue was prepared for quantitative 3-D multiphoton microscopy (MM) using a novel tissue clearing method that allowed for the collection of high resolution images at more than 1 mm depth.

Results: Cisplatin caused profound functional and morphologic changes. GFR dropped progressively to 50% of age-matched controls by 9 weeks without hypertension or loss of glomeruli. Kidney weight fell mainly by loss of cortical tissue. Extensive fibrosis developed, as measured by Sirius Red staining and confirmed by MM, first appearing 6 weeks after the first dose. Glomerulosclerosis failed to develop even as late as 25 weeks. After fibrosis, widespread peritubular vascular rarefaction appeared (by CD34 staining). An influx of macrophages, F4/80 staining, also was noted in the backdrop of an increase in both cell cycle activity (Ki67) and apoptosis (TUNEL). The earliest morphologic changes seen on MM involved the glomerulotubular (GT) junction and were characterized 1) by the loss of parietal cuboidal cells from Bowman's capsule and 2) by the loss of connection between the glomerulus and its proximal tubule resulting in an atubular glomerulus. Notably, these abnormalities developed before fibrosis and correlated well with the development of progressive renal insufficiency.

Conclusions: Using MM on a new murine model of CP-induced CKD, we identified the morphologic changes at the GT junction that occur earlier than fibrosis and that herald the development of CKD. Thus, this segment of the nephron may be integral to the onset and progression of CKD. Understanding the mechanisms that determine the development of the cellular changes at the GT junction is likely to yield new information on ways to prevent CKD.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO379

Proteinuria That Develops with Aging in the MWF Rats Results From Both Glomerular and Tubular Alterations Mark C. Wagner,[1,5] Silvia B. Campos-Bilderback,[1,5] Brittany N. Flores,[1] Sudhanshu Kumar,[1,5] Xianyin Lai,[2] Jered Myslinski,[1,5] Ruben M. Sandoval,[1,5] Sarah E. Wean,[1,5] Frank Witzmann,[3] Bruce A. Molitoris.[1,4,5] *[1]Medicine, Indiana Univ School of Medicine, Indianapolis, IN; [2]Biochemistry & Molecular Biology, Indiana Univ, Indianapolis, IN; [3]Celluar & Integrative Physiology, Indiana Univ, Indianapolis, IN; [4]Roudebush VAMC, Indianapolis, IN; [5]Indiana Center for Biological Microscopy, Indiana Univ School of Medicine, Indianapolis, IN.*

Background: While the clinical relevance of albuminuria is well documented, the role of glomerular and tubule contributions remains of considerable interest. Understanding protein handling in the PT cells requires a better understanding of their function and regulation under physiological and disease conditions.

Methods: Glomerular sieving coefficient (GSC) and PT uptake was measured in young (7-9wks) and old (32-46wks) MWF rats. Two-photon intravital microscopy was used to study handling of Texas Red RSA. Analysis of cortical protein changes identified proteins and pathways altered by age which may contribute to proteinuria.

Results: MWF female and male GSC increased from 0.0075 to 0.0117 and from 0.0052 to 0.0134, respectively. 24hr urine total protein increased from 49mg to 900mg in the females and from 126mg to 3000mg in the males. The increased urine protein does not directly correlate with the GSC changes. PT TRRSA uptake in males was reduced by 50%. To address cortical proteome changes in young and old MWF male rats, proteins were analyzed by LC-MS/MS. 1485 unique cortical proteins, splice variants or isoforms were identified, and compared. 255 proteins detected in both groups were differentially expressed ($p<0.05$). 32 proteins were detected only in the 32 wk group and 7 in the 7wk group. Prominent up-regulated proteins included ribosomal proteins Rps28 and Rps20, Filamin C, serum albumin, Vps29, and Lims. The largest down-regulations included proteasome proteins Rpn6 and Rpn11; mitochondrial proteins Dmgdh,Ckmt2, and Diablo homolog; ABC transporter G family member 2; GPI-anchor transamidase; and Rab-5c.

Conclusions: These data support a model in which the tubular epithelia play a greater role in albuminuria.

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FR-PO380

Apoptosis Is the Primary Cause of Renal Albumin Toxicity and Can Be Rescued By a Na,K-ATPase Triggered Cytosolic/Mitochondrial Calcium Signal Ievgeniia Burlaka,[1] Jacopo Maria Fontana,[1] Lena Scott,[1] Hjalmar Brismar,[1,2] Anita Aperia.[1] *[1]Karolinska Instt, Sweden; [2]Royal Inst of Technology, Sweden.*

Background: There is a great need for treatment that arrests progression of chronic proteinuric kidney disease (CKD). Increased levels of albumin in the primary urine lead to fibrosis and apoptosis of podocytes and proximal tubule cells. There have been many attempts to target fibrosis but few to target apoptosis, because of lack of appropriate therapeutic agents. The interrelationship between fibrosis and apoptosis is not well understood.

Results: Here we show with time-sequence imaging that excessive albumin uptake into primary rat proximal tubule cells (RPTC) promptly triggers the mitochondrial apoptotic pathway, while increased production of pro-fibrotic factors lag behind. Incubation of RPTC with delipidated, endotoxin-free albumin 2.5 -10 mg/ml results within an hour in reduction of the anti-apoptotic factor Bcl-xL, mitochondrial accumulation of the apoptotic factor Bax and depolarization of the mitochondrial membrane. We previously reported that the Na,K-ATPase ligand ouabain triggers an anti-apoptotic signal mediated via cytosolic calcium oscillations. Here we show that ouabain, in concentrations that do not inhibit Na,K-ATPase pumping function, attenuates albumin triggered mitochondrial membrane depolarization, lowers mitochondrial accumulation of apoptotic factor Bax, increases abundance of anti-apoptotic factor Bcl-xL and rescues from apoptosis. Using genetic Ca^{2+} indicators targeted to cytosol and mitochondria, we show that each cytosolic calcium wave triggered by ouabain is followed by a delayed mitochondrial calcium transient, which is similar in amplitude, but slower in recovery than the cytosolic wave.

Conclusions: We have shown that renal albumin toxicity is primarily due to mitochondrial dysfunction, that the Na,K-ATPase ligand ouabain rescues from mitochondrial dysfunction and that Na,K-ATPase may exert a feedback control on mitochondrial function and control of life and death.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO381

Deletion of the mTORC1 and -2 Induced Signalling Pathways Reduce Renal Proximal Tubular Endocytosis Franziska Theilig,[1] Florian Grahammer,[2] Mélanie Bousquenaud,[1] Alexey Larionov,[1] Suresh K. Ramakrishnan,[1] Tobias B. Huber.[2] *[1]Medicine, Anatomy, Fribourg, Switzerland; [2]Nephrology, Freiburg, Germany.*

Background: Receptor-mediated endocytosis is a pivotal function of the renal proximal tubule (PT) to reabsorb proteins from the ultrafiltrate to maintain body homeostasis. The signaling pathways involved in the regulation of endocytosis and their point of action remain uncertain. We aimed to elucidate the role of mTORC1 and -2-induced signaling pathways in clathrin-mediated endocytosis.

Methods: Therefore Raptor $^{fl/fl}$ and Rictor $^{fl/fl}$ Pax8rtTA*TetOCre as well as double mutants were analyzed.

Results: Renal function analysis of double mutants revealed a low molecular weight proteinuria, reduced FITC-inulin-GFR (4.3 versus 6.7 µl/min/g bw in double mutants versus controls, $p<0.01$) and increased plasma urea concentration (88 versus 37 mg/dl in double mutants versus controls, $p<0.001$). Single mutants did not differ from their respective controls. Raptor fl/fl and Rictor fl/fl Pax8rtTA*TetOCre and more pronounced the double mutants demonstrated a reduced proximal tubular HRP-uptake. Morphological analysis showed reduced BBM microvilli length and endocytic vesicle formation in single mutants and most pronounced in double mutants. Vacuoles formation and cystic dilation with loss of proximal tubular phenotype were observed in double mutants and to lesser extent in single mutants. Western blot and immunohistochemical analysis of endocytic parameters revealed in double mutants reduced clathrin vesicles, recycling endosome formation and ClC-5 expression whereas the early endosomes were increased. Megalin expression remained unaltered. Viral knockdown of S6K1 and rictor in opossum kidney cells demonstrated reduced albumin uptake and vesicle formation in ligand-induced endocytosis.

Conclusions: In conclusion, mTORC1 and -2 are important regulators of PT endocytic functions by altering vesicle formation and therefore intracellular endocytic transport mechanisms.

Funding: Government Support - Non-U.S.

FR-PO382

Graft Reconditioning with Mesenchymal Stromal Cells in Non Hearth Beating Donors Experimental Model Valeria Corradetti,[1] Eleonora Francesca Pattonieri,[1] Marilena Gregorini,[1] Chiara Rocca,[1] Samantha Milanesi,[1] Teresa Valsania,[1] Carolina Bianco,[2] Ilaria Benzoni,[2] Manuela Cannone,[1] Maria Antonietta Avanzini,[3] Melissa Mantelli,[3] Marcello Maestri,[2] Teresa Rampino,[1] Antonio Dal Canton.[1] *[1]Nephrology, IRCCS Policlinico San Matteo, Italy; [2]Chirurgia Generale, IRCCS Policlinico San Matteo, Italy; [3]Oncoematologia Pediatrica, IRCCS Policlinico San Matteo, Italy.*

Background: Grafts from non hearth beating donors (NHBD) have high risk for primary non and delayed-graft-function.Viability of NHBD kidneys is improved by perfusing them with hypothermic machine perfusion (HMP),but the outcome of NHBD grafts remains worse in spite of HMP.Mesenchymal stromal cells (MSC) are multipotent

cells and in a rat model of renal transplantation they have anti inflammatory effects.We evaluated whether perfusing the isolated kidney with MSC in HMP,NHBD graft is protected by ischemia/reperfusion injury.

Methods: Fisher rats (F) were used as kidney donors,Lewis rats (L) as MSC donors and Transgenic Sprague-Dawely rats expressing enhanced green fluorescence protein as MSC donors to track MSC.After 20' of warm ischemia,bilateral nephrectomy from F was performed and kidneys perfused with BelzerUW solution (A) or BelzerUW solution plus $3*10^6$ MSC (B) for 4 h, at 4°C.8 kidneys for each group were studied after 4 h perfusion. Proliferating cell nuclear antigen (PCNA) expression was evaluated by immunochemistry. Renal damage was graded according to semiquantitative score in 15 non-consecutive microscopic fields (0-4) (0:0%tubular casts/microscopic field;4:<75% tubular cast/microscopic field).Tubular mitotic index was measured as tubules with mitotic cells/total tubules number in 15 microscopic fields.Malondialdeide (MDA) was measured on perfusion liquid effluent.

Results: We found MSC in tubules,glomeruli,interstitium and no MSC capillary margination.PCNA positive cells were increased significantly in B compared with A (A 27.9±12.1;B 35.0 ±15.7,p<0.0001) Damage score was significantly lower in MSC perfused kidneys (A:3.4±1;B1.6±1.2,p<0.0001) Mitotic index was higher in B than in A (p<0.005). MDA levels were lower in B than in A (p<0.001).

Conclusions: Our results demonstrate that pre-transplant graft reconditioning with MSC protects from ischemia/reperfusion injury.

FR-PO383

Possible New Biomarkers for Transplanted Kidneys: The Indoleamine 2,3-Dioxygenase (IDO) and the Switch from Proteasome to Immuneproteasome (PS/iPS) Licia Peruzzi,[1] Andrea Ranghino,[2] Elisa Loiacono,[1] Alessandro Amore,[1] Alberto A.B. Boido,[2] Luca Vergano,[1] Giulio Mengozzi,[3] Paola Puccinelli,[3] Inna Lastauka,[4] Rosanna Coppo.[1] *[1]Nephrology Dialysis Transplantation, Città della Salute e della Scienza, Regina Margherita Hospital, Turin, Italy; [2]Nephrology Dialysis Transplantation, Città della Salute e della Scienza, Turin, Italy; [3]Laboratory Medicine, Città della Salute e della Scienza, Turin, Italy; [4]Belarusian Medical Academy of Postgraduate Education, Belarus.*

Background: There is a growing interest in biomarkers of immune system activation in transplanted subjects. Indoleamine 2,3 dioxygenase (IDO), an interferon-g induced enzyme degrading tryptophan (Trp) to kynurenine (Kyn) expressed in dendritic cells is triggered in acute rejection and downregulated by immunesuppressive drugs. Interferons regulate also the switch from proteasome (Ps) to immuneproteasome (iPs) favouring optimal antigen presentation.

Methods: We prospectively detected in 25 transplanted patients (T0, T15, T30, T60, T180 D) and 30 controls (HC) IDO activity (Kyn/Trp) (HPLC), mRNAs for IDO and 3 catalytic PS and iPS units (Taqman). T regulatory cells were investigated by forkhead box P3 (FoxP3), Th17-related factors (IL-17) and TGF-β1 mRNAs.

Results: At T0 IDO activity was significantly higher than in HC (Kyn 4.16±0.26 versus 2.05±0.07, p<0.0001; Trp 29.58±2.89 versus 54.02±1.63, p<0.0001; Kyn/Trp: 16.02±1.61 versus 3.83±0.15,p<0.0001) as well as IDO mRNA (p=0.004). TGF-β1 mRNA was also increased (2.13±0.2 versus 1.37±0.1,p=0.004) while IL-17 (1.49± 0.42 versus 0.81±0.10) as well as FoxP3 mRNA (1.16±0.23 versus 1.21±0.11) were similar to HC. A significant PS/iPS switch was detected at T0 (LMP2/b1,p=0.011 versus HC). A significant correlation was found between Kyn/Trp and TFG-b1 mRNA (p=0.005). Kyn was significantly correlated with LMP2/b1 (p=0.005). A significant reduction in IDO activity and IDO mRNA expression were found over the first month after transplantation (T15 and T1), with decrease in Kyn/Trp parallel to a decrease in PS/iPS switch (LMP2/b1mRNA). IDO activity progressively increased over T3-T6.

Conclusions: IDO and the proteasome/immunoproteasome switch represent possible biomarkers for monitorning the immune system activation after kidney transplantation.

Funding: Private Foundation Support

FR-PO384

Transcriptome Analyses of Zero Kidney Graft Biopsies Reveal Metallothioneins as Candidate Markers of Biological Age Johannes Leierer, Gert J. Mayer. *Dept of Internal Medicine IV, Nephrology and Hypertensin, Medical Univ Innsbruck, Innsbruck, Austria.*

Background: Structure and function of the kidney deteriorate with age and age-related diseases contribute to this process, leading to the high frequency of end-stage renal disease in the elderly. Despite some similarities the aging kidney and CKD differ but it is difficult to distinguish these two conditions. Thus biological markers for age might be useful tools to dissect the specific pathologies and gene expression analysis of healthy aged kidneys might be a reasonable approach to avoid confounding by disease.

Methods: Age-regulated gene expression changes in zero hour donor kidney biopsies were determined using microarray technology followed by ANOVA and SAM analysis. Expression changes of selected genes were confirmed by quantitative real-time PCR. *In situ* hybridization and immunohistochmistry was used to localize mRNA and protein expression in zero hour biopsies. Functional aspects were examined *in vitro* in RPTEC/TERT1 cells.

Results: Donors were classified into 3 age groups (<40, 40-59, >60 years). In the given Microarray data age-associated transcriptional changes were identified: 16 transcripts were found to be significantly upregulated in age group 3 as compared to age group 1. 8 of these transcripts encoded for metallothionein (MT) isoforms. *In situ* hybridization demonstrated localization of MT mRNA in renal proximal tubular cells.RPTEC/TERT1 cells overexpressing MT2a were less susceptible towards CdCl2 induced cytotoxicity.

Conclusions: Metallothionein (MT) expression might serve as a marker for biological age in zero hour biopsies. As MTs contribute to detoxification of heavy metals and homeostasis of essential metals, protect from ROS mediated oxidative stress and prevent apoptosis their upregulation with ageing might represent an intrinsic protective mechanism.

Funding: Government Support - Non-U.S.

FR-PO385

Decreased Kidney Oxygenation due to Mitochondrial Uncoupling in Renal Transplantation Diana A. Papazova,[1] Malou Friederich-Persson,[2] Jaap A. Joles,[1] Marianne C. Verhaar.[1] *[1]Nephrology & Hypertension, UMC Utrecht, Netherlands; [2]Medical Cell Biology, Uppsala Univ, Sweden.*

Background: Ischemia/reperfusion (IR) is a common cause for chronic allograft dysfunction. Lower renal oxygenation (pO_2) is observed after IR. We hypothesize that mitochondrial uncoupling via uncoupling proteins (UCP) increases oxygen consumption and leads to decreased pO_2. Furthermore, we examined whether mitochondrial-targeted antioxidant therapy can improve mitochondrial uncoupling and graft oxygenation after renal Tx.

Methods: Male Lewis rats were left untreated as 2K-controls, uninephrectomized as 1K-controls, or underwent syngenic renal Tx (Tx). Two weeks after Tx, we measured renal function glomerular filtration rate (GFR: inulin), renal plasma flow (RPF: PAH), renal blood flow (RBF: PAH clearance/1-HCT) and pO_2. Mitochondrial uncoupling was evaluated as decreased consumption after UCP-2 blockade with guanosine diphosphate. In a follow-up experiment, donors were treated with mitoTempo (200 ug/kg, TxT) or vehicle (0.9% NaCl, TxV) daily for 5 days prior to Tx. Recipients were followed for two weeks and renal function (GFR, RPF, RBF) and pO_2 and mitochondrial uncoupling were measured.

Results: Tx resulted in increased GFR (0.65±0.07 versus 0.47±0.05 ml/min/kidney/100g, P<0.001), RPF (2.6±0.2 versus 1.8±0.2 ml/min/kidney/100g, P<0.001), RBF (3.9±0.4 versus 3.0±0.3 ml/min/kidney/100g,p<0.01) in comparison to 2K-controls. Similar changes were observed in 1K-controls. However, Tx also decreased pO_2 (30.3±12.6 versus 58.3±15.8 μM, P<0.01) in comparison to 2K-controls, changes not observed in in 1K-controls (47.1±4.2 μM, NS). Tx resulted in mitochondrial uncoupling (-15.8±5.6 versus -1.9±2.1 pmol O_2/s,p<0.05), but not in 1K-controls (-2.3±1.4 pmol O_2/s,ns). MitoTempo pretreatment did not improve any of the measured in vivo parameters (GFR, RPF or pO2) in comparison to vehicle but resulted in less mitochondrial uncoupling (-7.20±1.02 versus -13.12±2.51 pmol O_2/s, P<0.01).

Conclusions: The present study demonstrates that mitochondrial uncoupling occurs early after Tx and is associated with decreased kidney pO_2 despite similiar oxygen delivery as compared to 1K-controls. This early hypoxia may contribute to progressive chronic damage in transplanted kidneys.

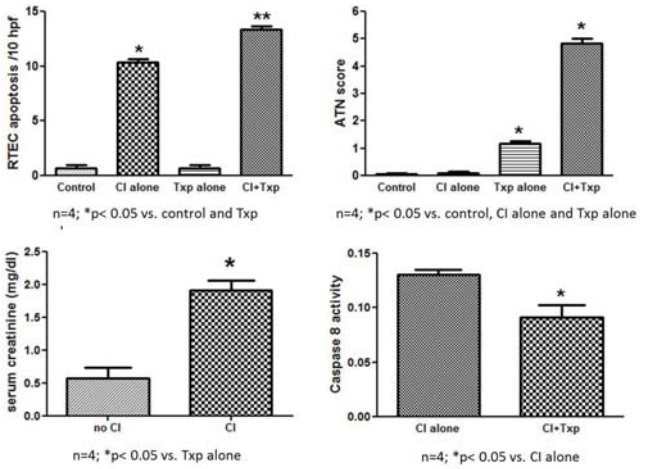
FR-PO386

The Changing Phenotype of Tubular Cell Death during Delayed Graft Function Swati Jain, Danica Ljubanovic, Charles L. Edelstein, Alkesh Jani. *Renal, Univ of Colorado, Aurora, CO.*

Background: Delayed graft function (DGF) independently predicts reduced 5 yr kidney transplant survival. Treatments of DGF are lacking. Cold ischemia (CI) is a significant risk factor for DGF but the mechanism by which CI leads to DGF is unknown. The effect of CI alone versus CI + warm reperfusion (WR) on renal tubular cell (RTEC) death are not well defined. The aim of this study was to determine the relative effects of CI versus CI+WR on donor kidneys in a kidney transplant model of DGF. We hypothesized that CI alone would produce a different injury phenotype to CI+WR.

Methods: Male C57BL6 mice aged 12 weeks were subjected to mouse kidney transplant. Donor kidneys subjected to 3 hours CI at 4°C in UW solution, were either processed immediately or subjected to syngeneic mouse kidney transplant. Renal function was assessed by serum creatinine (SCr). RTEC apoptosis and necrosis were assessed by a nephropathologist in a blinded fashion.

Results: CI alone significantly increased RTEC apoptosis but did not result in necrosis. In contrast, CI+WR after transplant caused RTEC apoptosis and necrosis. SCr was significantly increased in transplants subjected to CI versus no CI. Since caspase-8 is known to trigger apoptosis and also serves as a negative regulator of programmed necrosis, we examined caspase-8 protein expression and activity. Caspase-8 activity and protein expression were significantly decreased in CI+WR versus CI alone.



Conclusions: We have shown that CI alone results in RTEC apoptosis whereas CI+WR after mouse kidney transplant results in a different injury phenotype with the development of RTEC necrosis. A potential mechanism by which these differing phenotypes may occur is via decreased caspase-8 after CI+WR, thus removing negative regulation of programmed necrosis. These studies suggest that while inhibition of capsase-8 may prevent RTEC apoptosis, a consequence might be increased RTEC necrosis.

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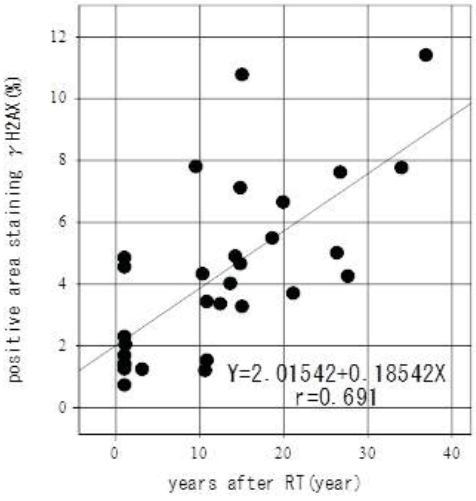
FR-PO387

DNA Double Strand Brakes Induced Intractable Glomerular Fibrosis in Renal Allografts Yuki Matsui, Hirokatsu Atsumi, Junko Imura, Keiji Fujimoto, Hiroki Adachi, Hiroshi Okuyama, Hideki Yamaya, Hitoshi Yokoyama. *Kanazawa Medical Univ, Kanazawa, Japan.*

Background: DNA injury in renal allografts has been reported in a few studies of cyclosporine nephrotoxicity, chronic renal allograft rejection, and renal reperfusion injury. However, the relationship between DNA injury and glomerular fibrosis was still unclear. In this study, we investigated DNA injury and glomerular fibrosis in renal allografts.

Methods: We evaluated 30 patients who underwent renal graft biopsy from 1.0 to 36.9 years after transplantation. We detected DNA double strand brakes by immunostaining using an anti-phospho-Histone H2A.X (Ser139; gH2AX) monoclonal antibody, and analyzed positive area by an image analyzing system. Subsequently, we identified glomerular fibrosis in renal allografts by collagen (COL) type III, IV, and VI accumulation.

Results: gH2AX staining area was positively correlated with years after renal transplantation (RT) (r=0.691 p<0.01). Similarly, type VI COL, which accumulated mainly in the subendothelial and mesangial area, was also positively correlated with years after RT (r=0.760, p<0.01). In multiple regression analysis, years after RT was selected as a significant factor for the accumulation of type VI COL in glomerular capillaries (b value=0.699, p<0.001), type III COL on vascular pole (b value=0.441, p=0.027), and gH2AX (b value=0.700, p<0.001). In addition, gH2AX staining area was also selected as a predictor of the accumulation of type VI COL in glomerular capillaries (b value=0.439, p=0.028). On the other hand, donor age at renal biopsy was a significant factor for type IV COL accumulation in glomeruli (b value=0.505, p=0.01).



Conclusions: These findings suggested that long-term RT induced DNA double strand brakes and accumulation of type VI COL in glomerular capillaries, which may progress to intractable glomerular fibrosis.

FR-PO388

CDKN2A Expression in Uremic Artery and Muscle - A Biological Marker of Ageing in Renal Disease Karin Luttropp,[1] Abdul Rashid Tony Qureshi,[2] Dagmara Mcguinness,[3] Peter F. Barany,[2] Louise Nordfors,[2] Peter Stenvinkel,[2] Paul G. Shiels.[3] *[1]Dept of Molecular Medicine and Surgery, Karolinska Instt, Stockholm, Sweden; [2]Dept of Clinical Science, Intervention and Technology, Karolinska Univ Hospital, Stockholm, Sweden; [3]Wolfson Wohl Translational Research Centre, Inst of Cancer Sciences, Univ of Glasgow, Glasgow, United Kingdom.*

Background: Using chronic kidney disease (CKD) patients as a clinical model of accelerated ageing, we investigated the expression of cyclin-dependent kinase inhibitor 2A (CDKN2A) in artery and muscle biopsies taken from CKD patients undergoing renal transplantation (RTx) and examined its applicability as a marker of ageing and cardiovascular disease (CVD).

Methods: 43 biopsies from epigastric artery and 81 biopsies from muscle were obtained at RTx and RNA was prepared using Trizol (see Table 1). CDKN2A gene expression was measured by qRT-PCR with reference to the HPRT gene on an ABI Prism® 7700 Sequence Detection System. TaqMan™ Primer/Probe sets were designed by Primer Express algorithm (Applied Biosystems, Austin, TX, U.S.A.). For comparison of data, Spearman's rank correlation (r) or linear regression was used, as appropriate.

	Artery	Muscle
Men, %	70	62
Age, median	42	46
DM, %	16	21
CVD, %	12	20
RQ CDKN2A, median	0.96	0.59

Results: CDKN2A expression was significantly higher in artery than in muscle (p = 0.01). In arteries, CDKN2A expression was significantly associated with CVD (p = 0.001), medial calcification (p = 0.04), and diabetes mellitus (DM) (p = 0.05). In muscle, CDKN2A expression was significantly associated to age (p = 0.003) and DM (p = 0.04). After correction for age, the association between arterial CDKN2A and CVD and DM remained significant whereas the relation to medial calcification was lost.

Conclusions: CDKN2A expression in muscle increases with age, demonstrating its validity as a biomarker of ageing. The age-independent association with CVD confirms previous genotype studies that have shown associations between this DNA locus and various forms of CVD. The finding that CDKN2A expression is higher in artery than in muscle indicates that vessels are prone to a more rapid ageing process than other organs/tissues in the uremic milieu.

Funding: Government Support - Non-U.S.

FR-PO389

Role of Complement in the Regulation of Anti-Senescence Protein Klotho (KL) in Kidney Transplantation Giuseppe Castellano,[1] Angelica Intini,[1] Alessandra Stasi,[1] C. Divella,[1] Margherita Gigante,[1] Paola Pontrelli,[1] Fabio Sallustio,[2] Anna Zito,[1] Marco Fiorentino,[1] Giovanni B. Pertosa,[1] Loreto Gesualdo,[1] G. Grandaliano.[3] *[1]Dept of Emergency and Organ Transplantation, Univ of Bari, Italy; [2]C.A.R.S.O. Consortium, Valenzano, Bari, Italy; [3]Dept of Medical and Surgical Sciences, Univ of Foggia, Italy.*

Background: The transmembrane KL protein is mainly produced at renal level and is released into blood as an endocrine anti-aging factor. Recent studies demonstrated a reduction of KL in acute kidney injury. Aim of the study was to investigate the modulation of KL in a swine model of Ischemia /Reperfusion (I/R) injury and in Delayed Graft Function (DGF) patients.

Methods: In an experimental model of I/R injury, 10 pigs underwent to 30 min of renal warm I followed by 24h of R. Five pigs (C1-Inh) were treated with recombinant C1-Inh. KL level was investigated by immunohistochemistry in renal biopsies from animal model and patients and by ELISA assay in patients serum. We evaluated KL *in vitro* in renal tubular cells (HK-2) stimulated with C5a by qPCR and Western Blot.

Results: We found a significant reduction in tissue KL in I/R pigs (Ctr) at 24h from R compared to basal condition (30.4%±1.2% versus T0:72.5%±2.1%,p<0.05). Complement inhibition preserved KL expression in vivo (T24C1-Inh:70.7%±1.9% versus T24Ctr,p<0.05). In accordance, Complement anaphylotoxin C5a led to a downregulation of KL gene (p<0,01) and protein (p<0,05) expression in vitro in HK-2 cells. Moreover, KL was not expressed in adult renal progenitor cells compared to primary tubular cells in vitro. In transplant patients, we found a significant reduction of tubular KL in DGF compared to pre-transplant (pre-Tx) biopsies (DGF:10.8%±0.6% versus pre-Tx:73.1%±2.5%,p<0.05). Moreover DGF patients showed low levels of serum KL at 2 years after transplantation compared to EGF patients with similar renal function (DGF:412pg/ml±106 versus EGF:900.25pg/ml±153.9,p=0.03).

Conclusions: Our study demonstrated that the Complement activation during renal I/R injury plays an important role in KL modulation. Considering the renoprotective functions of KL, its reduction in DGF patients might promote the senescence of kidney allograft.

FR-PO390

Fibroblast Growth Factor 23/Klotho Axis Is a Risk Factor for Kidney Transplant Loss Siren Sezer,[1] Ayse Zeynep Bal,[1] Mehtap Erkmen Uyar,[1] Handan Ozdemir,[2] Orhan Guliyev,[1] Saliha Yildirim,[3] Mehmet Haberal.[4] *[1]Nephrology, Baskent Univ Faculty of Medicine, Ankara, Turkey; [2]Pathology, Baskent Univ Faculty of Medicine, Ankara, Turkey; [3]Internal Medicine, Baskent Univ Faculty of Medicine, Ankara, Turkey; [4]General Surgery, Baskent Univ Faculty of Medicine, Ankara, Turkey.*

Background: Chronic allograft nephropathy (CAN) is a major cause of late kidney allograft loss. Increased circulating level of fibroblast growth factor 23 (FGF23) is an independent risk factor for cardiovascular disease. The aim of this study is to evaluate the relationship between FGF-23 and allograft loss and to identify risk factors for progression to ESRD.

Methods: We performed a study of 80 maintenance kidney recipients with stable allograft function who had received their transplant at least 36 months previously. All acute cellular and humoral rejections were excluded. According to renal biopsy patients divided four groups: Group CAD (n:38) was the patients diagnosed as chronic allograft dysfunction; group TED (n:18) was included the patients with tubular epithelium damage and group CON (n:24) was defined as control group. Samples for FGF-23 and Klotho were taken on the biopsy day and patients were followed at least 36 months after renal biopsy.

Results: FGF23 and parathyroid hormone (PTH) values were higher in CAD group, while the calcium levels and Klotho were significantly lower compared to other groups. A significant inverse correlation was found between FGF23 and MDRD, Klotho, serum calcium level and albumin level. FGF-23 levels were positively correlated with duration after transplantation, serum uric acid level, serum creatinine level, interstitial fibrosis score, glomerulosclerosis rate and proteinuria. Multivariable analysis showed that serum albumin, interstitial fibrosis score and percentage of glomerulosclerosis were associated with high FGF23 levels.

Conclusions: We conclude that elevated FGF23 and decreased Klotho were associated with chronic allograft dysfunction and an independent risk factor allograft loss in kidney transplant recipients. We suggest that FGF-23/Klotho axis could play a role as triggering factor and could be a prognostic marker in case of progressive renal injury.

FR-PO391

FGF23 Induced Vascular Endothelial Activation and Proliferation via the mTOR Pathway Eileen W. Tsai,[1] Yiping Jin,[2] Katherine Wesseling-Perry,[1] Isidro B. Salusky,[1] Robert B. Ettenger,[1] Elaine F. Reed.[2] *[1]Pediatrics, UCLA, Los Angeles, CA; [2]Pathology, UCLA, Los Angeles, CA.*

Background: Fibroblast growth factor 23 (FGF23) has been associated with allograft rejection, chronic kidney disease (CKD) progression, cardiovascular mortality and poor graft survival in renal transplantation. We recently discovered that FGF23 induces endothelial cell (EC) functional changes, including EC phosphorylation and proliferation, which we postulate contribute to transplant vasculopathy (TV). We, therefore, aimed to determine the intracellular signaling pathway mediating FGF23-induced EC signal transduction and proliferation.

Methods: Primary human aortic EC, passages 2-8, were treated with FGF23 100 ng/ml with and without mTOR inhibitors, rapamycin 30 nM (RAP) and everolimus 10 nM (RAD). Untreated EC were used as control. Phosphorylation of S6kinase389 and Akt Ser473 was measured by Western blot. EC proliferation was assessed by flow cytometry using CSFE labeling.

Results: Both RAP and RAD reduced FGF23 phosphorylation of S6KinaseThr369 and Akt Ser473 which are downstream targets of mTOR (Fig 1A). These phosphoproteins have been associated with EC proliferation. Moreover, RAP also directly reduced FGF23 stimulated EC proliferation (Fig 1B).

Conclusions: We show for the first time that mTOR inhibition reduced FGF23-induced EC signal transduction and proliferation, suggesting that FGF23 uses the mTOR pathway to exert functional changes on the endothelium. This could have important clinical and therapeutic implications, delaying chronic kidney disease progression and potentially TV.

Fig 1A Inhibition of FGF23 Induced EC Phosphorylation by Rapamycin & RAD

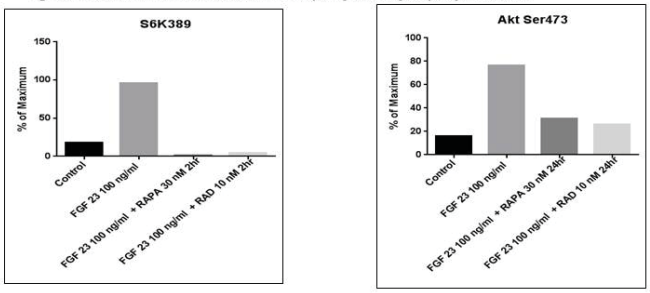
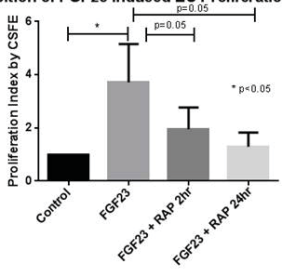


Fig 1B Inhibition of FGF23 Induced EC Proliferation by Rapamycin



FR-PO392

(Non-) Anticoagulant Heparinoids Alter Proteoglycan Mediated In- and Efflux of Interstitial Leukocytes in Experimental Renal Transplantation Ditmer Talsma,[1] Kirankumar Katta,[1] Saritha Adepu,[1] Miriam Boersema,[2] Saleh Yazdani,[1] Gerjan Navis,[1] Harry Van Goor,[2] Jan-Luuk Hillebrands,[2] Jacob van den Born.[1] *[1]Nephrology, UMCG, Groningen, Netherlands; [2]Pathology & Medical Biology, UMCG, Groningen, Netherlands; [3]Ronzoni, Milano, Italy.*

Background: Chronic renal transplant dysfunction (CTD) is characterized by loss of renal function and extensive tissue remodeling, including chronic inflammation and lymph vessel formation. Previously, we showed upregulation of matrix proteoglycans in CTD. We hypothesize that proteoglycans, via their glycosaminoglycan side chains act as docking platforms for L-selectin and chemokines, orchestrate in- and efflux of interstitial leukocytes, and could be target of intervention.

Methods: In a rat renal CTD model, collagens, chemokines and matrix proteoglycans were profiled by qRT-PCR in microdissected tubulo-interstitium. Heparinoid effectivity to dampen inflammation was tested in vitro and in the rat CTD model. Interstitial leukocytes were quantified by CD45 staining, whereas the in- and efflux of leukocytes was assessed by counting leukocyte numbers within vanWillebrand factor positive bloodvessels and podoplanin-positive renal lymphatics.

Results: qRT-PCR profiling revealed upregulation of Collagen I and IV, TGF-beta, chemokines CCL2, CCL5, and matrix proteoglycans perlecan and versican in CTD allografts compared to isografted and control kidneys (all $p<0.05$). In vitro (non-anticoagulant) heparin (oids) showed dose-dependent inhibition of both L-selectin and CCL2 binding to perlecan. In the rat CTD model daily treatment with non-anticoagulant heparinoid reduced tubulo-interstitial leukocyte numbers two-fold ($p<0.02$). Moreover, heparin (oid) treatment resulted in a decreased leukocyte adhesion to endothelium ($p<0,05$) but an increased leukocyte efflux into renal lymphatics ($p<0,001$).

Conclusions: We conclude that in renal CTD matrix proteoglycans via their glycosaminoglycan side chains stabilize chemokine gradients and interaction with L-selectin, thus promoting inflammation. Heparinoid treatment blocks these interactions and reduces interstitial leukocyte number both by reducing influx from blood vessels and by increasing leukocyte efflux into renal lymphatics.

FR-PO393

Nanoparticle Delivery of Donor Antigens for Transplant Tolerance Induction Jane Bryant, Xiaomin Zhang, Lei Zhang, Xun-Rong Luo. *Medicine, Northwestern Univ Feinberg School of Medicine.*

Background: Human islet cell transplantation is a promising treatment for type 1 diabetes; however, long-term donor-specific tolerance to islet allografts remains a clinically unmet goal. We have previously shown that recipient infusions of apoptotic donor splenocytes chemically treated with 1-ethyl-3- (3'-dimethylaminopropyl)-carbodiimide (donor ECDI-SP) can mediate long-term acceptance of full major histocompatibility complex (MHC)-mismatched murine islet allografts without the use of immunosuppression.

Methods: In this report, we investigated the use of poly (lactide-co-glycolide) (PLG) particles in lieu of donor ECDI-SP as a synthetic, cell-free carrier for delivery of donor antigens for the induction of transplant tolerance in full MHC-mismatched murine allogeneic islet transplantation.

Results: Infusions of donor antigen-coupled PLG particles (PLG-dAg) mediated tolerance in ~20% of recipient mice, and the distribution of cellular uptake of PLG-dAg within the spleen was similar to that of donor ECDI-SP. PLG-dAg mediated the contraction of indirectly activated T cells but did not modulate the direct pathway of allorecognition. Combination of PLG-dAg with a short course of low dose immunosuppressant rapamycin at

the time of transplant significantly improved the tolerance efficacy to ~60%. Furthermore, altering the timing of PLG-dAg administration to a schedule that is more feasible for clinical transplantation resulted in equal tolerance efficacy.

Conclusions: Thus, the combination therapy of PLG-dAg infusions with peritransplant rapamycin represents a clinically attractive, biomaterials-based and cell-free method for inducing long-term donor-specific tolerance for allogeneic cell transplantation, such as for allogeneic islet transplantation.

Funding: NIDDK Support, Private Foundation Support

FR-PO394

Interleukin-5 Therapy Prevents Chronic Allograft Rejection By Induction of T Regulatory Cells Bruce M. Hall,[1] Rachael Hall,[1] Giang Tran,[1] Catherine Robinson,[1] Chuanmin Wang,[2] Alexandra Sharland,[2] Suzanne Jean Hodgkinson.[1] *[1]Immune Tolerance Group, UNSW Australia, Sydney, NSW, Australia; [2]Collaborative Transplant Research Group, Univ of Sydney, Sydney, NSW, Australia.*

Background: Chronic rejection of allografts remains a major problem that may be controlled by promotion of alloantigen specific Treg cells. Naïve $CD4^+CD25^+Foxp3^+$ Treg activated by specific alloantigen (alloAg) in the presence of IL-4, not IL-2, express the specific receptor of IL-5 (IL-5Ra) and are more potent alloAg specific Treg than nTreg. IL-5 therapy (IL-5Rx) promotes expansion of Ag specific Treg to prevent autoimmunity.

Methods: We examined if IL-5Rx prevented chronic rejection of Lewis heterotopic cardiac allografts in F344 rats. rIL-5 was given ip daily at 5000 units for 10d, from 7d post-transplant. IL-5Rx was delayed to allow alloactivation to produce the IL-4 required to activate Ts2 cells. Grafts were monitored by palpation and scored as ++++ full function to 0 as fully rejected.

Results: Sham Rx rats developed rejection at 18d and by 28d all grafts were fully rejected (n=5). IL-5Rx prevented rejection, with all grafts ++++-+++ until cessation of IL-5 treatment at day 18 ($p<0.01$ compared to sham Rx). After 18d, there was rejection with a mean score of ++ until 50d (n=5) ($p<0.01$). Another group (n=5) had a transient rejection episode after changing on d18 to IL-5Rx 3x/wk, but by 30d the grafts recovered, so at 60d, 3 of 5 heart grafts were ++++, one +++, and one ++ ($p<0.05$ v short IL-5Rx; $p<0.01$ v sham Rx). Depleting $CD25^+$ cells or blocking IL-4 with mAb therapy abolished the effects of IL-5, all grafts rejected in <28d. IL-5Rx increased $CD4^+CD25^+$T cells at 6-8% versus 3-4% in controls. After IL-5Rx, $CD4^+CD25^+$T cells proliferated in MLC to Lewis, but not F344 alloAg, and this was enhanced by presence of IL-5.

Conclusions: In a model of chronic rejection, ongoing IL-5Rx reduced rejection and restored good graft function. IL-5Rx promoted growth of Ag specific $CD4^+CD25^+$Treg that were activated by host IL-4 and alloAg. In vitro, these cells had enhanced proliferation to donor Lewis that was further enhanced by IL-5. IL-5 therapy controlled chronic allograft rejection by the expansion of host Ag specific Treg.

Funding: Government Support - Non-U.S.

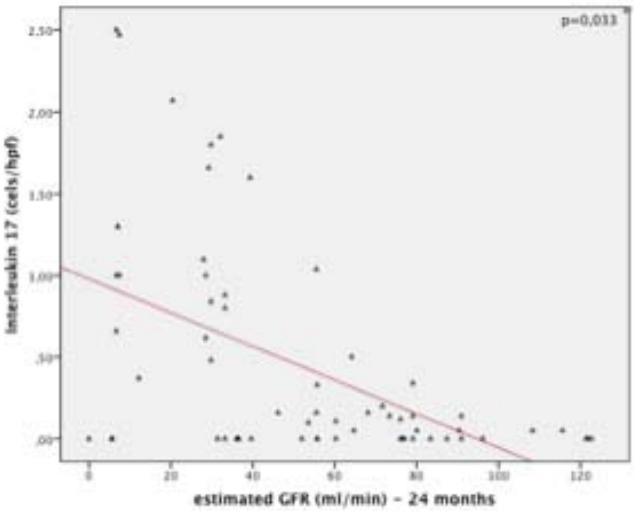
FR-PO395

Interleukin-17 Expression Is Associated with Worse Long-Term Kidney Graft Function Luciana Mello,[1,2] Lucio Roberto Requiao-Moura,[1,2] Denise M.A.C. Malheiros,[3] Alvaro Pacheco-Silva.[2] *[1]Kidney Transplant Unit, Hospital Israelita Albert Einstein, Sao Paulo, Brazil; [2]Nephrology Dept, Univ Federal de Sao Paulo, Sao Paulo, Brazil; [3]Pathology Dept, Univ de Sao Paulo, Sao Paulo, Brazil.*

Background: The identification of the family of interleukin-17 (IL-17) cytokines, defined a new cellular type called Th17 cells. Some studies point to the involvement of this new inflammatory pathway and others proinflammatory cytokines such as interleukin 6 and presence of B cells in acute rejection and graft loss. The aim of this study was to evaluate the presence of producing IL-17 cells in kidney transplant allograft tissue and correlate with long-term graft function.

Methods: Immunohistochemical staining for IL17, IL6, and CD20 was performed in 65 biopsy samples from patients who underwent kidney transplantation between 2007 and 2010 in Hospital IsraelitaAlbert Einstein, São Paulo, Brazil, and correlated with the presence of rejection and long-term graft function.

Results: We observed that the higher IL17+ cell count, the worse graft function found after one or two years was detected ($p<0,05$) regardless of the presence of rejection.



Neither interleukin-6 nor CD20 rich infiltrates were related to poor graft outcome. No significant difference was observed between Il17+ cell count in rejection and non-rejection groups. Instead, CD20 and IL-6 positive cellular infiltrates were more common in the rejection group ($p<0.001$) and ($p<0.005$), respectively.

Conclusions: The presence of IL-17 in renal transplant tissue is associated with a worse long-term kidney function regardless of whether associated with acute rejection. This finding makes room for a possible role of this interleukin in the immune component of chronic renal allograft dysfunction.

FR-PO396

Gene Expression Profiles in CD4+ T Cells Suggest an Interferon Alpha Signature in Chronic Antibody-Mediated Rejection (CAMR) of Kidney Transplantation Paola Pontrelli,[1] Annarita Oranger,[1] Matteo Accetturo,[1] F. Rascio,[2] Margherita Gigante,[1] Giuseppe Castellano,[1] Marco Fiorentino,[1] Anna Zito,[1] Gianluigi Zaza,[3] Giovanni Stallone,[2] Loreto Gesualdo,[1] G. Grandaliano.[2] *[1]Dept of Emergency and Organ Transplantation, Univ of Bari, Italy; [2]Dept of Medical and Surgical Sciences, Univ of Foggia, Italy; [3]Dept of Medicine, Univ of Verona, Italy.*

Background: CAMR is the main cause of graft loss, but its pathogenesis is still unclear. The aim of the present study was to investigate the molecular mechanisms underlying CAMR by the analysis of gene expression profiles of both total peripheral lymphomonocytes (PLM) and isolated CD4+ and CD8+ T cells.

Methods: We enrolled 14 patients with biopsy-proven CAMR and 12 stable transplant recipients with normal graft (control group). Gene expression profiles of PLM and CD4+ and CD8+T cells were assessed using Agilent microarrays. Results were evaluated by statistical (unpaired t tests, moderated t test, Benjamini-Hochberg correction, Storey q-value) and functional pathway analysis (Ingenuity Pathway Analysis) and then validated by q PCR in an independent set of patients. To analyze the genome-wide expression of CD4+ and CD8+T cells, we enrolled an independent cohort of 8 CAMR patients and 8 control subjects.

Results: Gene expression profiles of PLM identified a characteristic activation of the interferon (IFN)-alpha pathway in CAMR patients. Interestingly, the gene expression profiles of CD4+ and CD8+ T cells resulted completely distinct: only one gene (TNFSF10) was commonly expressed in CAMR patients versus control. CD4+T cells showed a specific IFNalpha feature ($p=5.06*10-11$), confirming the differential expression of genes such as IFIT1 (Fold change:+9.5) and IFIT3 (Fold change:+16.6). CD8+ T cell response resulted instead less unambiguous, as several pathways were involved (Tryacylglicerol biosynthesis $p=1.45*10-3$; TREM1 signaling $p=5.8*10-2$; Toll like receptor signaling $p=5.2*10-2$) except type I IFNs signaling.

Conclusions: Our data suggest a key role for IFN-alpha in modulating the immune response during CAMR, mainly influencing CD4+T cells response. This observation may open new perspectives for non-invasive diagnosis and to define new therapeutic targets.

Funding: Government Support - Non-U.S.

FR-PO397

The Onset of Post-Transplant Malignancies Is Related to a Change in Gene Expression Profiles in Peripheral Blood Mononuclear Cells Giovanni Stallone,[1] F. Rascio,[1] Paola Pontrelli,[2] Matteo Accetturo,[2] Annarita Oranger,[2] Margherita Gigante,[2] Giuseppe Castellano,[2] Marco Fiorentino,[2] Barbara Infante,[1] Loreto Gesualdo,[2] G. Grandaliano.[1] *[1]Dept of Medical and Surgical Sciences, Univ of Foggia, Italy; [2]Dept of Emergency and Organ Transplantation, Univ of Bari, Italy.*

Background: Malignancies are the third leading cause of death in renal transplant recipients and their incidence is increasing. Although the role of the immune system in malignancies development is known, mechanisms of tumor escape remain an unsolved problem.

Methods: We enrolled 8 patients with post-transplant neoplasia (PTN: 2 kidney graft, 3 native kidney, 2 prostate, 1 stomach), 8 transplanted patients without neoplasia (Tx-control) and 8 non-transplanted patients with cancer (neoplasm control: 4 kidney, 3 prostate,1 bladder). All groups were comparable for the main clinical characteristics/ demographics. Transplant patients were all receiving immunosuppressive therapy with calcineurin inhibitors, mycophenolate, steroids. The transcriptomic profiles of peripheral blood mononuclear cells were assessed by microarray. Results were analyzed statistically and functionally (Ingenuity Pathway Analysis).

Results: 4345 genes were differentially expressed between PTN and Tx-control. The most significantly represented pathway was linked to the mechanisms of cancer ($1.71*10^{-5}$, 87 genes). The first 6 network (score = 34) included regulation of cell cycle and gene expression, reparative mechanisms of RNA/DNA, the pathogenesis of immunological and metabolic disorders. The 29 differentially expressed genes between PTN and neoplasm control were included in two networks. The first (score = 56) consisted of 21 genes related to TGF-beta. Among the most activated pathway there was coagulation. In particular, the tissue factor was significantly increased in the PTN group (fold-change: +2.93).

Conclusions: Our data, using an "omic" approach, may allow us to identify the immunological mechanisms underlying the development of post-transplant malignancies and suggest new markers useful in the monitoring of transplanted patients.

Funding: Government Support - Non-U.S.

FR-PO398

The Histopathological Investigation of Kidney in Acute Graft-versus-Host Disease After Allogeneic Rat Bone Marrow Transplantation

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Background: Allogeneic hematopoietic and bone marrow cell transplantation are responsible for acute and chronic graft-versus-host disease (GVHD). Liver, gut, and skin are known to primary target sites of GVHD. However, the disorder of kidney in acute GVHD is not well-understood. We examined the clinical and histopathological characterizations of the kidney in acute GVHD in Dark Agouti (DA)-to-Lewis rat bone marrow transplantation (BMT) model.

Methods: Acute GVHD was induced in Lewis rats ($RT1^l$) by transplantation of DA rat ($RT1^a$) bone marrow cells (6.0×10^7 cells) after irradiation (10Gy) without immunosuppression. We examined the impact of acute GVHD on the kidney in allogeneic BMT rats and compared them with those in Lewis-to-Lewis syngeneic BMT and non-BMT control rats.

Results: In peripheral blood, almost white blood cells were constituted with DA phenotype. Between 21 and 40 days after BMT, severe acute GVHD was developed with decreased body weight (>20%), skin rush with alopecia, and diarrhea. Liver and kidney dysfunction was detected: asparate aminotransferase (AST: 271.8 ± 104.2 U/L), alanine aminotransferase (ALT: 82.8 ± 19.9 U/L), blood urea nitrogen (BUN: 33.9 ± 4.68 mg/dL) and urinry N-acetyl-b-D-glucosaminidase (NAG: 31.5 ± 15.5 U/L). In kidney, many numbers of CD3+ T-cells, CD8+ T-cells, and ED1+ macrophages infiltrated the interstitial inflammation around small arteries that expanded into peritubular interstitium with peritubular capillaritis. CD4+ T-cells also infiltrated the kidney. Acute glomerulitis and endarteritis in small arterioles were evident. Acute tubulitis with $CD3^+$ T-cells were also noted. No obvious IgG, IgM, and C3 deposition was detected in the kidney.

Conclusions: Kidney was one of the target organs of acute GVHD. The pathology of acute GVHD in the kidney was characterized by T cell-mediated injury for the microvascular endothelium and renal tubules, and these findings were very similar of acute T cell-mediated rejection in transplanted kidney grafts.

FR-PO399

Contractile Pericytes May Contribute to Calcineurin Inhibitor-Mediated Nephrotoxicity

Mark C. Kelly, Carol Crawford, Scott S.P. Wildman, Claire M. Peppiatt-Wildman. *Urinary Physiology, Medway School of Pharmacy, Kent, United Kingdom.*

Background: Cyclosporine A (CsA) and Tacrolimus (FK-506) are immunosuppressant calcinerium inhibtors (CNI's) prescribed to prevent allograft rejection post kidney transplant. Both agents are however nephrotoxic, which significantly impacts upon long-term kidney graft/patient survival. The precise mechanism of the CNI-induced toxicity is unknown, although impairment of endothelial cell function and reduced production of endogenous renal vasodilators has been noted with concomitant release of vasoconstrictors resulting in net vasoconstriction and exacerbated ischemia.Rapamycin is macrocyclic antibiotic that shows similar immunosuppression to CNI's, albeit through a different pathway, but more importantly, a reduced nephrotoxicity associated with its use, potentially offering an alternative immunosuppression regime for allograft transplantation without risk of acute rejection.

Methods: We have utilized a live kidney slice model, developed by our group [1], to investigate the mechanism (s) of CNI-induced nephrotoxicity in the renal medulla; a region of the kidney that is particularly sensitive to ischemia.

Results: Data presented here demonstrates both CsA (600 ng/ml) and FK506 (800 ng/ml) caused vasoconstriction (9.9%±0.88% and 9.2%±0.63%, n=7, respectively) of vasa recta via their specific action at contractile pericytes, whereas Rapamycin (44ng/ml) failed to evoke pericyte constriction. Application of CsA with Rapamycin produced a significantly enhanced contractile response compared to CsA alone (13.2% versus 9.9% respectively). The thiazide diuretic, hydrochlrothiazide, was shown to significantly reduce FK506-induced vaosconstriction (3.15% versus 11.35%, respectively) while the calcium channel antagonist, Diltiazem, significantly inhibited CsA-induced vasoconstriction (4.04% versus 8.98%, respectively). Medullary tubule diameter was unaffected by bath application of CsA, FK506 and Rapamycin.

Conclusions: Data suggesting that CNI-induced constriction of vasa recta by pericytes may contribute to the nephrotoxicity and exacerbated ischemia that can result in allograft dysfunction in renal transplant patients.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO400

Human Cytomegalovirus Infection Induces Viral Chemokine Receptor US28 Expression in Renal Allografts and Dedifferentiation of Infected Cells

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Background: Human cytomegalovirus (HCMV) infection is associated with decreased renal graft function and survival. HCMV expresses US28, a chemokine receptor that might alter cell phenotype by modifying intracellular signaling. We investigated US28 expression in renal biopsies and its effect on cell phenotype in vitro.

Methods: US28 immunohistochemistry was semi-quantitatively scored in recipient renal transplant biopsies (n=49) from HCMV-seropositive donors. Cultured primary vascular smooth muscle cells (VSMCs) and tubular epithelial cells (TECs) were infected with wild type HCMV and stimulated with PDGF-BB and TGF-β1. Expression of viral antigens and phenotypic markers was detected using qRT-PCR, and infection efficiency and dissemination using HCMV carrying a GFP-tag.

Results: US28 was expressed in all renal compartments, but was most prevalent (Kruskal-Wallis; $P<0.001$) in VSMCs (42% of cells positive) and TECs (30%). Cultured VSMCs were permissive to HCMV, underwent lytic infection and IEA, US28 and HCMVpol displayed distinct kinetics. Infection resulted in a phenotype transition characterized by loss of a-SMA and SM22a along with induction of Klf4 (27-, 13 and 2-fold). Infection of TECs reduced E-cadherin and induced vimentin. HCMV infection followed by PDGF-BB and TGF-β1 resulted in more extreme phenotypic marker changes.

Conclusions: HCMV induced US28 in renal allografts and dedifferentiation of cultured cells. Long-term renal graft survival remains poor after transplantation and US28 could link HCMV to transplant dysfunction. Phenotype transition can enable HCMV to initiate or instigate IF/TA and transplant vasculopathy; two major features of transplant dysfunction. The implication of US28 for transplant histopathology and recipient clinical outcome are now investigated. Further studies will elucidate the role of US28 and its potential for therapeutic intervention, so to prevent HCMV-induced transplant dysfunction.

Funding: Government Support - Non-U.S.

FR-PO401

Adherence to Immunosuppression in Kidney Transplant Patients Treated in a Federal Universal Healthcare System

Dustin J. Little,[1] Robert Nee,[1] Deepti S. Moon,[1] Matthew Ward,[2] Christina M. Yuan,[1] Kevin C. Abbott,[1] Rahul Jindal.[3] *[1]Nephrology, Walter Reed National Military Medical Center, Bethesda, MD; [2]Uniformed Services Univ, Bethesda, MD; [3]Organ Transplant Surgery, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Medication non-adherence following organ transplantation is common, and associated with increased risk of graft loss. Immunosuppression (IS) medication cost may be an important contributor to non-adherence, and leading nephrology organizations continue to advocate for changes to U.S. public policy to decrease patient out-of-pocket IS costs. To our knowledge, however, medication adherence has not been reported in U.S. patients who receive IS medications at no out-of-pocket cost. We therefore designed a study to evaluate medication adherence in organ transplant recipients treated in the military health system (MHS).

Methods: Study subjects reported medication adherence and depressive symptoms via the Medication Therapy Adherence Scale (ITAS-M) and Beck Depression Inventory (BDI), respectively. Subjects also completed the Immunosuppressant Therapy Barrier Scale (ITBS), which includes a question rating level of agreement with the following statement, "I skip doses of my immunosuppressant medication (s) when I am short of money."

Results: Nineteen of 21 (90%) subjects approached about the study provided consent and completed the study instruments. The median age and time from transplant to study participation were 52 years and 12 months, respectively. Twelve of 19 (63%) subjects were male. The mean ITAS-M was 11.5 ± 0.99, with 12, 5, and 1 subject reporting perfect (12/12 ITAS-M score), nearly perfect (11/12), and less than perfect (≤ 10/12) adherence, respectively. Fifteen, 1, and 3 patients reported minimal, mild, and moderate depressive symptoms, respectively. Thirteen patients strongly disagreed with the ITBS statement about skipping IS medications when short of money;2 subjects disagreed, 1 strongly agreed, and 2 did not answer the question.

Conclusions: Our preliminary results suggest that MHS transplant patients who receive free IS medications have low levels of medication non-adherence and depressive symptoms, and do not miss IS medications due to financial concerns.

Funding: Other U.S. Government Support

FR-PO402

Utilization of Smart Phones and Medication Adherence in Adolescents with Kidney Disorders Oleh M. Akchurin, Alexandra P. Hollman, Dionne Sears, Rebecca Hashim, Frederick J. Kaskel, Paula Marcus, Marcela Del Rio. *Montefiore Medical Center / Albert Einstein College of Medicine.*

Background: Medication non-adherence is widespread in adolescents with chronic conditions; however, little is known about the utilization of available smart phone-based technologies in community-based settings.

Methods: Study was approved by the IRB of Montefiore Medical Center and powered for 70 participants. Patients at pediatric nephrology clinic were surveyed about cell phone access and its utilization for medication adherence management. Medication adherence was assessed by self reports (objective assessment based on the drug blood levels is ongoing). Standardized adolescent medication barriers scale was used to identify obstacles for medication use in three domains (frustration, adaptation, and ingestion).

Results: The majority (77%) of teens continue to utilize such traditional techniques of improving medication adherence as pillboxes and incorporating medications into daily routines. While 93% of surveyed teens have a smart phone in their personal possession, only 29% are aware about medical mobile apps, although 50% reported that they utilized cell phones for some kind of reminders to take medications. Only 30% of patients use an electronic device to maintain their medication list / schedule. Boys were more likely to use cell phones to remember to take medications than girls (71% versus 17%) and the prevalence of 100% self-reported medication adherence was higher in teens who utilized cell phones for reminders (70% versus 14%). Patients who used cell phone reminders reported significantly fewer barriers for taking medications within the adaptation domain ($p<0.03$) and had a trend towards having fewer barriers on the frustration domain ($p=0.05$), while ingestion domain was not different ($p=0.2$).

Conclusions: A substantial number of teenagers with kidney disorders and transplants reported cell phone use for managing their medications; however, significant opportunity for utilizing smart phone-based technology to improve medication adherence remains. Utilization of cell phones was associated with beneficial self-reported outcomes in this study. Data collection and analysis on this project is currently ongoing.

FR-PO403

Peers4PATH: Feasibility of Medication Adherence Measures in Adolescent Transplant Recipients Sandra Amaral, Nina Foster, Hannah S. Dashefsky, Christopher S. Paterno, Susan L. Furth. *Pediatrics, Children's Hosp Phila, Phila, PA.*

Background: Adolescents with solid organ transplants face difficulties adhering to prescribed medication regimens. Although screening for nonadherence is tremendously important, nonadherence is challenging to identify and quantify, and there is no one "gold standard" for measurement.

Methods: Peers4PATH is an NIDDK-sponsored clinical trial to test the efficacy of peer mentoring to improve medication adherence in adolescent solid organ transplant recipients ages 14-23 years. Subjects are randomized to a peer mentor versus standard of care. Peer mentors interact with mentees over a one-year study intervention by phone, text, email or Facebook weekly and have in-person meetings at time 0, 6 months and 12 months. The primary outcome is medication adherence, captured in four different ways: pharmacy refill data, pill counts, self-report (by the modified Medication Adherence Measure-Medication Module) and coefficient of variation of immunosuppressive drug levels.

Results: To date, 46 subjects (of an anticipated 60) have completed their first visit and have baseline adherence data available. Pill counts were successfully obtained for only 30 subjects (65.2%) due to patients stockpiling medications and using bottles from prior time periods. Pharmacy refill records were successfully obtained for 32 subjects (69.6%) and were difficult to obtain for patients who use multiple pharmacies or mail orders and for those with frequent dose changes. 45 subjects (97.8%) completed the self-report measure and 45 (97.8%) had ≥ 2 drug levels to calculate coefficient of variation.

Conclusions: Though multiple measures of adherence are thought to provide a more complete picture of true adherence behavior, our initial findings suggest that the feasibility and interpretability of pharmacy refill data and pill counts is poor for adolescent solid organ transplant recipients. Although imperfect, self-report and immunosuppressive drug levels appear preliminarily more feasible to obtain for this population. With longer follow up, we will examine these four adherence measures as time-dependent covariates and consider the intra- and inter-individual variation between intervention and control groups.

Funding: NIDDK Support

FR-PO404

Generic Immunosuppression in Kidney Transplantation: A Systematic Review and Meta-Analysis Amber O. Molnar,[1,2] Anne K. Tsampalieros,[3,2] Dean Fergusson,[2] Alexandria Bennett,[2] Greg A. Knoll.[1,2] *[1]Nephrology, Univ of Ottawa, Ottawa, ON, Canada; [2]Epidemiology, Ottawa Hospital Research Inst, Ottawa, ON, Canada; [3]Nephrology, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada.*

Background: The use of generic immunosuppressants in the kidney transplant population has the potential for huge cost savings. However, there is growing concern that generic and trade name immunosuppressants are not bioequivalent and differ in terms of clinical efficacy.

Methods: We performed a systematic search of the literature using MEDLINE and EMBASE from 1980 to 2014. Studies comparing a trade name to a generic immunosuppressant in kidney transplant recipients were included. The main outcomes were bioequivalence and acute rejection. If appropriate, data was meta-analyzed.

Results: We screened 1,542 citations of which 159 were selected for full text review. 39 studies met eligibility criteria (14 trials, 14 non-randomized interventional studies and 11 observational studies). 3 studies compared Cellcept to a generic, 9 studies compared Prograf to a generic, and 27 studies compared Neoral to a generic. A total of 10 studies (Cellcept n=0; Prograf n=1; and Neoral n=9) reported and met the standard criteria for bioequivalence (90% confidence intervals [CI] for the relative means of the AUC and Cmax 0.80 to 1.25). In terms of clinical equivalence, the risk of acute rejection was similar for trade name versus generic immunosuppressants (Peto odds ratio [OR] 0.71, 95% CI 0.16-3.1 for Cellcept; Peto OR 1.06, 95% CI 0.50-2.27 for Prograf; Peto OR 0.93, 95% CI 0.61-1.41 for Neoral).

Conclusions: Few studies reported the criteria needed to determine bioequivalence. While acute rejection rates were similar, analyzed studies were primarily non-randomized, underpowered and had short follow up times.

FR-PO405

Post Hoc Subgroup Analysis of ZEUS: Outcome on Renal Function, Efficacy and Safety in Living-Donor Kidney Transplant Recipients after Conversion from a Calcineurin Inhibitor to an Everolimus Based Regimen Claudia Sommerer,[1] Klemens Budde,[1] Martin G. Zeier,[1] Rudolf P. Wuthrich,[3] Petra Reinke,[1] Ute Eisenberger,[1] Anja Susanne Muehlfeld,[1] Wolfgang Arns,[1] Rolf A. Stahl,[1] Katharina M. Heller,[1] Oliver Witzke,[1] Heiner H. Wolters,[1] Barbara M. Suwelack,[1] Ingeborg A. Hauser,[1] Martina Porstner,[2] Frank Lehner.[1] *[1]ZEUS, Study Group, Germany; [2]Novartis, Pharma, Germany; [3]ZEUS, Study Group, Switzerland.*

Background: To study renal function and patient outcome in living donation subgroup of kidney de novo transplant recipients after conversion to an everolimus (EVR) based regimen and withdrawal of calcineurin inhibitor (CNI) therapy.

Methods: Post hoc subgroup analysis from the prospective, open-label, controlled, multi-center study ZEUS. 300 renal transplant (Tx) patients were randomized at month 4.5 post Tx to either receive EVR plus enteric coated-mycophenolate sodium (EC-MPS) or cyclosporine (CsA) plus EC-MPS regimen, among them were 80 living donor recipients (EVR n=42; CsA n=38).

Results: In this subpopulation of living donation recipients, adjusted estimated GFR (Nankivell) at month 12 (the primary endpoint) was 74.3 (95%CI [70.7, 77.9]) mL/min/1.73m2 with EVR versus 63.8 (95%CI [60.0, 67.7]) mL/min/1.73m2) with CsA, a difference of 10.5mL/min/1.73m2 in favor of EVR ($p<0.001$). From randomization to month 12, adjusted estimated GFR increased by a mean of 9.8 (95%CI [6.2, 13.4]) mL/min/1.73m2 in the EVR subgroup, versus -0.7 (95%CI [-4.6, 3.1]) mL/min/1.73m2) ($p<0.001$) within the CsA group. Of six biopsy-proven acute rejection episodes in the EVR group, five were Banff grade I. Overall safety profile was similar between treatment groups. Discontinuation due to adverse events occurred in three EVR-treated patients (7.1%) and five CsA-treated patients (13.2%) between randomization and month 12.

Conclusions: EVR-based regimen with early elimination of CNI therapy in living donor kidney transplant recipients is associated with a significant renal benefit at 12 months post Tx without compromising safety and efficacy.

Funding: Pharmaceutical Company Support - Novartis Pharma Germany

FR-PO406

HERAKLES Trial after 36 Month: Follow-Up Results on Efficacy and Safety of Three Different Treatment Regimens in De Novo Renal Transplant Patients Claudia Sommerer,[1] Klemens Budde,[1] Wolfgang Arns,[1] Petra Reinke,[1] Thomas Rath,[1] Ingeborg A. Hauser,[1] Hans-Hellmut Neumayer,[1] Frank Lehner,[1] Peter Weithofer,[1] Johannes Jacobi,[1] Martina Porstner,[2] Daniel Baeumer,[2] Martin G. Zeier,[1] Oliver Witzke.[1] *[1]HERAKLES Study Group, Germany; [2]Novartis Pharma, Germany.*

Background: To compare safety and efficacy of 3 immunosuppressive regimen 3 years after renal Tx.

Methods: A prospective, open-label, randomized (rdz), controlled, multi-center study where all pts received induction with basiliximab, cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 months (Mo) post Tx 499pts were rdz 1:1:1 to either a) continue standard CsA (100-180ng/ml) +EC-MPS (n=166) (STD) or convert b) to calcineurin inhibitor (CNI)-free regimen with everolimus (EVR) (5-10ng/ml) and EC-MPS (n=171) or c) to a CNI-low regimen with EVR (3-8ng/ml) and low CsA (50-75ng/ml) (n=162). Steroids were continued. Mo36 FU visit was performed by 123 (89%) STD, 130 (95%) CNI-free, 123 (94%) CNI-low treated pts of the FU population.

Results: Since rdz to Mo36 BPAR was reported in 19/151 (13%) STD, 22/149 (15%) CNI-free and in 21/146 (14%) CNI-low pts (ITT). 3 deaths (2%) occurred in STD, 2 (1%) in CNI-free and 5 (3%) in the CNI-low group. 6 (4%) graft losses were observed in the STD, 5 (3%) in the CNI-free and 1 (1%) in the CNI-low group. Composite failure (BPAR,death,graft loss,loss to FU): 27 (19%) in STD, 28 (20%) CNI-free and 33 (23%) in CNI-low group; premature discontinuation due to AEs: 4/154 (3%) in STD, 4/150 (3%) in CNI-free and 1/147 (1%) in CNI-low group (safety set) from Mo12 to 36.

Safety Pop. n(%) *during FU (Mo12-36)	Standard	CNI-free	CNI-low
Infections	56 (36.4)	74 (49.0)	55 (37.7)
Severe infections	12 (7.8)	14 (9.3)	12 (8.2)
Infections leading to hospitalisation	40 (26.0)	47 (31.1)	41 (28.1)
CMV	4 (2.6)	6 (4.0)	6 (4.0)
BKV	1 (0.6)	2 (1.3)	2 (1.4)
Hospitalisation due to:			
Acute rejection	13 (8.4)	16 (10.6)	12 (8.2)
Cardiovascular event	7 (4.5)	4 (2.6)	2 (1.4)
GI event	7 (4.5)	6 (4.0)	7 (4.8)
Malignancy	2 (1.3)	8 (5.3)	3 (2.1)
Metabolic disorder	2 (1.3)	0	0

cGFR (Nankivell) was significantly improved at Mo36 by +7.0mL/min/1.73m2 in favor of the CNI-free group (ITT;p<0.01).

Conclusions: Mo36 results from HERAKLES show that an immuosuppression using EVR with low-dose or without CNI reflect an efficacious and safe therapeutic approach offering the opportunity for an individualized immunosuppression.

Funding: Pharmaceutical Company Support - Novartis Pharma Germany

FR-PO407

5 Year Data of the APOLLO Trial: Outcome on Renal Function of an Everolimus Based Therapy after Calcineurin Inhibitor Withdrawal in Maintenance Renal Transplant Recipients

Klemens Budde,[1] Thomas Rath,[1] Barbara M. Suwelack,[1] Petra Reinke,[1] Hermann G. Haller,[1] Oliver Witzke,[1] Martina Porstner,[2] Daniel Baeumer,[2] Wolfgang Arns,[1] Claudia Sommerer.[1] *[1]APOLLO Study Group, Germany; [2]Novartis Pharma, Germany.*

Background: To study renal function, safety and efficacy of an Everolimus (EVR) based regimen after Calcineurin-Inhibitor (CNI) withdrawal in maintenance kidney allograft recipients.

Methods: In an open-label, randomized,controlled,multi-center study 93 patients (pts) on stable immunosuppressive therapy consisting of CNI (CsA or Tac), Enteric-Coated Mycophenolate Sodium (EC-MPS) with or without steroids were randomized to either continue CNI/EC-MPS or convert to an EVR/EC-MPS based regimen. After completion of the 12-months (Mo) core study patients were included in an observational 4 year follow-up.

Results: 93 maintenance kidney Tx pts with a mean time of 6.4 years since most recent transplantation (Tx) were randomized to either EVR (n=46) or CNI (n=47) treatment. 78 pts completed 12Mo core study, 67 (72%) attended the final 60Mo visit. Mean trough levels were 81±34ng/ml in CsA, 5.2±1.9ng/ml in Tac and 6.1±2.4ng/ml in EVR treated pts. Mean time post-Tx at baseline was 82.6 months and 70.5 months in the EVR and CNI groups, respectively. At Mo60, adjusted mean eGFR (Nankivell) was 63.0 (95% CI 57.8, 68.2) mL/min/1.73m2 in the EVR versus 57.9 (95% CI 52.6, 63.1) mL/min/1.73m2 in the CNI group, a difference of 5.1 (95% CI -0.6, 10.8) mL/min/1.73m2 (p=0.076). Among pts who remained on randomized study drug until Mo60, mean eGFR (Nankivell) was 71.6 (95% CI 64.2, 79.0) mL/min/1.73m2 in EVR-treated pts (n=21) versus 60.6 (95% CI 55.1, 66.1) mL/min/1.73m2 in CNI-treated pts (n=29) (mean difference 11.0; 95% CI 3.6, 18.5 mL/min/1.73m2; p=0.005). No cases of BPAR occurred from randomization to Mo60 in either group. Graft loss occurred in 3EVR-treated pts and one CNI-treated patient. Until Mo60 one death had occured in the EVR group, three deaths in the CNI group. No unexpected safety concerns were observed in either group.

Conclusions: The late conversion to an EVR/EC-MPS treatment in maintenance renal Tx pts after CNI withdrawal is safe and led to a sustained better renal function in EVR treated pts 5 years post Tx.

Funding: Pharmaceutical Company Support - Novartis Pharma Germany

FR-PO408

Renal Function Outcomes Associated with Once-Daily MeltDose Tacrolimus versus Twice-Daily Tacrolimus, a Phase 3 Randomized Trial in De Novo Kidney Transplantation

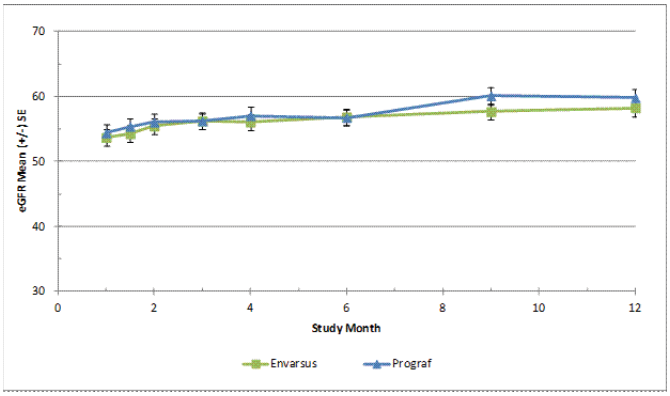
Lionel Rostaing,[1] Suphamai Bunnapradist,[2] Steven M. Steinberg.[3] *[1]Hôpital de Rangueil, Toulouse, France; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA; [3]California Inst of Renal Research, San Diego, CA.*

Background: Conversion of stable kidney transplant recipients (KTR) from twice-daily tacrolimus (tac) (Prograf) to a novel, once-daily, Meltdose® formulation of tac (Envarsus®; LCP-Tacro) showed greater bioavailability, noninferior efficacy and similar safety and with lower total daily dose (TDD) for Envarsus.

Methods: Here we report renal function results from a phase 3 double-blind, double-dummy, trial of de novo KTR randomized to Envarsus or Prograf. Renal function was assessed via the MDRD7 formula. Delayed graft function (DGF) was clinician-reported as an adverse event (AE) or a serious adverse event (SAE). eGFR at 12 months follow-up are reported; 2 year data are forthcoming.

Results: A total of N=543 (Envarsus, n=268; Prograf, n=275) KTR were included. Target trough levels were achieved earlier for Envarsus versus Prograf; within 1 day of first dose 66% of Envarsus patients versus 25% of Prograf achieved troughs of ≥6ng/mL, p<0.001. Total daily dose (TDD) and troughs were higher for Envarsus in the first 2 weeks; cumulative dose over the study was 14% lower for Envarsus. Incidence of DGF tended to be lower for Envarsus (7%) versus Prograf (11%). DGF reported as a SAE trended towards being lower for Envarsus (3%) versus Prograf (6%). Both Envarsus and Prograf were associated with good renal function over the first 12 months of the study period; 2 year data will be available by the time of the conference.

Conclusions: These results show that both tac formulations are associated with good renal function in the first year post-transplant. A higher initial Envarsus TDD was not associated with renal function decrement, either in terms of eGFR or DGF.



Funding: Pharmaceutical Company Support - Veloxis Pharmaceuticals

FR-PO409

Superior Renal Function in an Everolimus-Based Calcineurin Inhibitor Free Regimen Compared to Standard Cyclosporine/Mycophenolate and Low Cyclosporine/Everolimus: Follow-Up of the HERAKLES Study at Month 36

Wolfgang Arns,[1] Klemens Budde,[1] Claudia Sommerer,[1] Thomas Rath,[1] Volker Kliem,[1] Petra Reinke,[1] Johannes Jacobi,[1] Martina Porstner,[2] Daniel Baeumer,[2] Hans-Hellmut Neumayer,[1] Ingeborg A. Hauser,[1] Frank Lehner,[1] Oliver Witzke,[1] Martin G. Zeier.[1] *[1]HERAKLES Study Group, Germany; [2]Novartis Pharma, Germany.*

Background: To follow-up (FU) on renal function (RF) at month (Mo) 36 after kidney Tx in patients (pts) on immunosuppressive regimen with different calcineurin inhibitor (CNI) exposures.

Methods: 802 patients (pts) were included in a 1 year, prospective, open-label, randomized, multi-center study. After induction with basiliximab all pts received cyclosporine A (CsA), enteric-coated mycophenolate sodium (EC-MPS) and steroids. 3 months post Tx 499 pts were randomized (rdz) 1:1:1 to either a) continue standard (STD) CsA (100-180ng/ml) with EC-MPS (n=166), b) convert to CNI-free regimen with everolimus (EVR;5-10ng/ml) and EC-MPS (n=171) or c) convert to CNI-low regimen (CsA 50-75ng/ml +EVR 3-8ng/ml) (n=162). Mo36 FU visit was performed by 123 (89%) STD, 130 (95%) CNI-free and 123 (94%) CNI-low pts.

Results: Median trough levels were: CsA 98ng/ml in STD, 72ng/ml in CNI-low treated pts; EVR 6.0ng/ml in CNI-free and 5.4ng/ml in CNI-low pts. cGFR (Nankivell) was similar at rdz, had significantly improved at Mo12 by +5.6mL/min (95%CI:[+2.9;+8.3];p<0.001) and remained significantly improved by +7.0mL/min in favor of CNI-free group at Mo36 (p=0.009). 58% of CNI-free, 36% of CNI-low and 46% of STD treated pts had an improvement in RF at Mo36 (p=0.04 CNI-free versus STD). All groups had similar rejection rate since rdz (13%STD, 15%CNI-free, 14%CNI-low) and an overall comparable safety profile.

Conclusions: CNI-free as well as reduced CNI therapy in combination with EVR are both efficacious and safe regimen. CsA trough levels showed that in CNI-low group the CNI reduction was not fully met, hence lower effect on sparing RF should be expected compared to STD. However, CNI-free group showed better RF maintained for 3years post Tx. The results of this large trial confirm previous reports of improved RF after CsA withdrawal by an EVR-regimen in combination with EC-MPS.

Funding: Pharmaceutical Company Support - Novartis Pharma Germany

FR-PO410

Efficacy of Envarsus Once-Daily MeltDose Tablets versus Twice-Daily Prograf Capsules in Black De Novo and Stable Kidney Transplant Recipients: A Pooled Analysis of Two Phase 3 Trials

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Background: Black kidney transplant recipients (KTR) have shown increased risk for poor clinical outcomes post-transplant. Management of immunosuppression may be challenging in black KTR due to the high frequency of the CYP3A5 genetic polymorphism which increases clearance and lowers bioavailability of tacrolimus (tac). Envarsus® (LCP-Tacro), a once-daily MeltDose® formulation of tac, has shown non-inferiority, similar safety, improved bioavailability, and less peak and peak-trough fluctuations versus Prograf.

Methods: The efficacy of Envarsus in Black KTR was examined in a pooled analysis of data from two phase 3 randomized, controlled trials. Treatment failure (death, graft failure, centrally read biopsy-proven acute rejection [BPAR], or lost to follow-up) was compared between black KTR randomized to Envarsus versus Prograf. One trial included de novo KTR where initial dosing was: Envarsus: 0.17 mg/kg/day; Prograf: 0.1 mg/kg/day. In the other trial, stable black KTR were switched from Prograf to Envarsus at a conversion ratio of 0.85.

Results: N=93 black KTRs were enrolled in the two trials (Envarsus N=44; Prograf N=49; 26% were de novo KTR). Significantly more treatment failures occurred in black KTR in the Prograf group versus the Envarsus group at 12 months (18.4% versus 4.5%; difference [95% CI]: -13.82% [-27.22%, -0.31%]). Numerically, more black KTR in the Prograf group had BPAR versus the Envarsus group at 12 months (12% versus 2%; -9.97% [-22.12%, 1.57%]). In the de novo study, initial target tac troughs (6–11 ng/mL) were more rapidly achieved following first dose of Envarsus versus Prograf.

Conclusions: Envarsus appears to be efficacious in black KTR. This analysis shows that Envarsus may be associated with fewer treatment failures among black KTR. The increased bioavailability of Envarsus contributes to the rapid achievement of therapeutic tac trough levels in the de novo setting and the maintenance of target levels in the conversion setting.

Funding: Pharmaceutical Company Support - Veloxis Pharmaceuticals

FR-PO411

Mycophenolate Mofetil (MMF) Therapeutic Drug Monitoring and Dose Modification in Pediatric Kidney Transplant Patients Vimal Chadha,[1] Judith Sebestyen,[1] Chelsey Jensen,[2] Bradley A. Warady.[1] *[1]Children's Mercy Hospital; [2]Children's Hospitals and Clinics of Minnesota.*

Background: Exposure to MMF is measured by area under the time-concentration curve (AUC) of mycophenolic acid (MPA). A goal AUC of 30 – 60 mg*h/L, has been suggested. However, there is substantial inter-patient variability between standard fixed-dose MMF and exposure to MPA. Therapeutic drug monitoring (TDM) of MPA has the potential for optimization and individualization of MMF dosing and efficacy.

Methods: All patients received MMF at an initial fixed dose of 450 mg/m^2 twice daily within 2 weeks of kidney transplant. AUC was determined by obtaining plasma MPA levels at 0, 1, 2 and 4 hours post dose once the patients were receiving oral MMF. The dose was then adjusted to achieve desired AUC based on first order kinetics. The data from 47 patients who received a kidney transplant between January 2010 and December 2013 was analyzed.

Results: 42 patients with median age 10.9 yrs. (1.4 to 21.6 yrs.) had MMF kinetics at baseline. Their daily MMF dose was 866 ± 96 mg/m^2 which resulted in AUC of 40 ± 19 mg*h/L. There was poor correlation between MMF dose and AUC (r = 0.08). Less than half (48%) of the patients had an AUC in the desired range (30 – 60 mg*h/L). 36% of patients had AUC < 30 mg*h/L, and 16% had AUC > 60 mg*h/L. The patients with subtherapeutic AUC were younger (median 4.5 yrs) in comparison to those with AUC > 60 (median 17.1 yrs). To achieve the desired AUC, the daily MMF dose was increased to 1133 ± 153 mg/m^2, and decreased to 634 ± 63 mg/m^2, in the younger and older children, respectively. 22 patients had repeat MMF kinetics at median of 6 months post-transplant. While their current daily MMF dose 880 ± 284 mg/m^2 was statistically not different (p = 0.88) from their initial dose, their AUC increased from 42 ± 20 at baseline to 54 ± 20 mg*h/L at 6 months ($p < 0.05$).

Conclusions: Our data reveals that systemic exposure to MPA is unpredictable at a fixed-dose regimen in pediatric kidney transplant recipients. Age appears to be a significant influence on exposure and MPA exposure tends to increase in patient over time. Individualized MMF dosing based on TDM has the potential for improving the outcome of transplant patients.

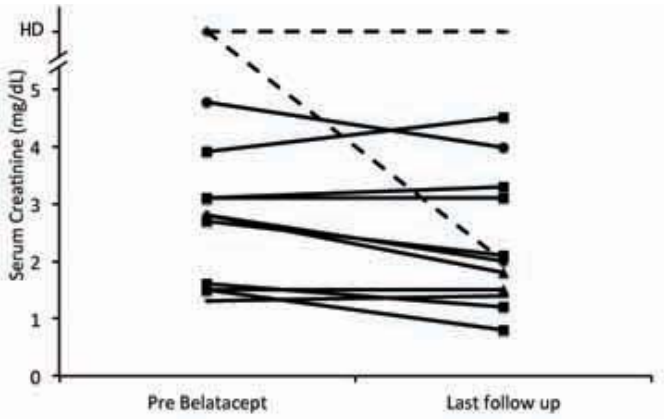
FR-PO412

Belatacept Conversion in Renal Transplant Recipients with Calcineurin Inhibitor Toxicity Russell J. Crew, Shefali Patel, Heather K. Morris, Lloyd Ratner, David J. Cohen, Sumit Mohan. *Columbia Univ Medical Center.*

Background: Although calcineurin inhibitors have improved kidney transplant and recipient survival, their use has significant nephro- and neuro-toxicities. Belatacept is a recently approved immunosuppressant for use at time of transplantation that does not share these properties. However, little is known about the safety and efficacy of conversion from a CNI based regimen to a Belatacept based regimen.

Methods: Conversion protocol: Patients received Belatacept 5mg/Kg on day 0, 14, 28, 42, 56 and then every 28 days with a 50% reduction of CNI after the second dose and cessation after the 5th dose of Belatacept.

Results: Since 2013, we have converted 13 patients from a CNI based regimen to Belatacept. for graft dysfunction (8), thrombotic microangiopathy (2), neurotoxicity (2) and bone marrow suppression (1). Patients were 54.3±15.7 years old, 54% male and were converted a median of 20 months (IQR: 12- 60) after transplant. After 8.8±7.7 months on Belatacept, mean+SD serum creatinine improved from 3.32 ±1.78 to 2.49±1.29 and includes renal recovery from dialysis dependence for one of two patients with severe TMA.



A single patient was hospitalized for pneumonia after conversion, recovered completely, but later had sudden cardiac death at home.

Conclusions: The short-term results of conversion to Belatacept show significant improvement in renal function and resolution of CNI related toxicity, but longer term follow up is needed to confirm its safety.

FR-PO413

Early Clinical Experience with Conversion from Calcineurin Inhibitors or Sirolimus to Belatacept in Renal Transplant Patients Marat Abdullin, Gregory Lee Braden, Michael J. Germain. *Baystate Medical Center, Springfield, MA.*

Background: Belatacept (Blt) demonstrated renal and metabolic benefits over a calcineurin inhibitors (CNI) based regimens in recent trials. No study has yet evaluated late conversion to Blt from CNI or mammalian target of rapamycin inhibitors (mTOR). We analyzed our experience in converting renal transplant patients from CNI or mTOR to Blt.

Methods: Patient switched to Blt from CNI or mTOR regimens with at least 4 months follow up were studied. Laboratory data including hemoglobin (Hb), serum creatinine (SCr), triglycerides (Tg), blood glucose (BG), urinary protein/creatinine ratio (PCR) before and after conversion were compared. We used three measurements before and after transition for SCr, Hb and BG, and two measurements before and after transition for Tg and PCR. Patients filled out a questionnaire to assess their experience with the conversion.

Results: 24 patients (14 male and 10 female) were switched from CNI (CNI group - 11 patients) or sirolimus (mTOR group - 13 patients) to Blt. Average age was 45.3 (17-74) years old. Average allograft age was 8 (1.5-20) years. Average follow up on Blt therapy was 13.2 (4-28) months. Indications for conversion were various side effects of CNI and mTOR, including proteinuria, pulmonary toxicity, diabetes, CNI renal toxicity, etc. SCr decreased in patients converted from CNI and remained unchanged in mTOR group. There was no change in Hb in either group. Tg and BG improved in both groups. PCR decreased in both groups.

	CNI	mTOR inhibitor	Combined
Creatinine, % change	- 9.7	- 0.9	- 4.9
Hemoglobin, % change	+ 3.1	+ 0.8	+ 1.9
Triglycerides, % change	- 23.7	-23.1	- 23.3
Fasting blood glucose, % change	- 21.1	- 7.1	- 13.3
Protein/creatinine ratio, % change	- 33.1	- 20.2	-25.7

No significant side effects with Blt were reported. No clinical rejections were noted. On a three point Lickert scale 10 out 14 patients reported feeling better after conversion.

Conclusions: Conversion to Blt from CNI and mTOR is safe, well-tolerated, associated with improved renal function in patients transitioned from CNI and stable renal function in patients transitioned from mTOR. It was associated with improved metabolic parameters and decreased proteinuria.

FR-PO414

Polyclonal and Monoclonal Antibodies for Induction Therapy in Kidney Transplant Recipients Penny E. Hill,[1] Nick Cross,[1] Nicholas Barnett,[2] Suetonia Palmer,[1] Jonathan C. Craig,[3] Angela C. Webster.[3] *[1]Christchurch Hospital; [2]Guy's and St. Thomas' NHS Foundation Trust; [3]Sydney School of Public Health.*

Background: Induction immunosuppression with antibody therapy is recommended for kidney transplant recipients but the relative benefits and harms of available agents remain uncertain.

Methods: Systematic review of randomised trials of all monoclonal or polyclonal antibodies (excluding interleukin-2 receptor antagonists) used as induction therapy in adult kidney transplant recipients. We appraised risk of bias and obtained estimates using random effects models.

Results: We identified 89 trials (8246 participants). Many study design details were incompletely reported. Compared to no induction, ATG prevented acute rejection (relative risk (RR) 0.63 95%CI 0.51-0.78) but increased CMV infection (RR1.56 95%CI 1.24-1.97). Estimates of treatment effects on mortality, graft survival, malignancy, PTLD, and NODAT were imprecise. In 6 trials (N=446) alemtuzumab, compared with ATG, had uncertain effects on acute rejection. Subgroup analysis of 4 trials with steroid withdrawal as a co-intervention

showed reduced acute rejection with alemtuzumab at 1 year (RR0.57 95%CI 0.35-0.93) compared to ATG but uncertain effects on mortality, graft loss, infection, malignancy and PTLD. Compared with triple therapy without induction antibody, alemtuzumab with steroid withdrawal increased CMV (RR2.28 95%CI 1.18-4.40) but estimates of treatment effects on mortality, graft loss, acute rejection, NODAT, PTLD and malignancy were imprecise.

Conclusions: Given a 45% acute rejection risk with no induction, 9 patients would need ATG to prevent 1 from having rejection. There would be 1 extra CMV case for every 10 patients treated with ATG. In steroid withdrawal trials 11 patients would require alemtuzumab to prevent 1 having rejection given a 21% rejection risk with ATG. Alemtuzumab and steroid withdrawal would cause 1 extra CMV case for 6 patients treated compared to no induction and triple maintenance without apparent benefits. ATG and alemtuzumab decrease acute rejection at a cost of increased CMV and patient centred outcomes of survival and reduced side effects do not appear to be improved.

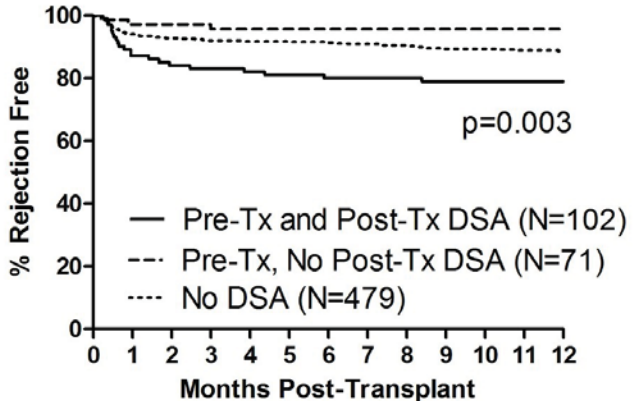
FR-PO415

Class, Timing and Persistence of Pre Transplant Donor Specific Antibodies (DSA) and Impact on Renal Allograft Outcomes Oluwafisayo O. Adebiyi, Jane Gralla, Alexander C. Wiseman, James E. Cooper. *Div of Renal Disease and Hypertension, Univ of Colorado, Aurora, CO.*

Background: Presence of low-level HLA donor specific antibodies pre-transplant (pre-tx DSA) often wanes prior to and following transplant. Specifically, few studies have evaluated the impact of DSA class, timing of pre-tx DSA detection and their persistence post-transplant on graft outcomes.

Methods: 652 consecutive recipients of a kidney or kidney/pancreas transplant with a negative pre-transplant FCXM between 09/2007 and 08/ 2012 at our center without desensitization therapy were analyzed. All patients underwent cell-based FCXM and SAB analysis on current and historic sera prior to transplantation. Patients were screened for DSA at post-transplant months 1, 6, 12, and annually thereafter. Graft outcomes were compared for patients with (DSA+) and without (DSA-) pre-transplant DSA.

Results: Of 652 patients with a negative FCXM and pre-tx DSA, 173 had DSA (DSA+, MFI $\geq$ 500) detected at any point prior to transplant by SAB analysis. Patients with pre-tx DSA that persisted post-transplant (N=102) had significantly higher acute rejection (AR) rates versus pre-tx DSA only versus no pre-tx DSA (p=0.003). With a median follow-up period of 2.3 years, presence, class type, timing of pre-tx DSA and persistence post-transplant was not associated with worse graft survival (p=0.71, p= 0.36, p=0.7 and p=0.44 respectively). Multivariate analysis of persistent pre-tx DSA impact on one year AR remains significant (p=0.05).



Conclusions: We present the first study to our knowledge evaluating the impact of timing, class type and post-transplant persistence of pre-DSA on renal allograft outcomes. Persistence of pre-tx DSA after transplant correlates with a modestly increased incidence of AR. However neither class type, timing of detection of pre-tx DSA, nor its persistence post-transplant impacts long term graft survival.

FR-PO416

Noninvasive Detection of Acute Renal Allograft Rejection by Measurement of Soluble Tim-3 in Urine Dajin Chen, Jianghua Chen. *Kidney Disease Center, The First Affiliated Hospital, College of Medicine, Zhejiang Univ, Hangzhou, Zhejiang Province, China.*

Background: The purpose of this study was to assess whether measurement of urinary soluble T cell immunoglobulin domain and mucin domain 3 (sTim-3) could be adopted as a new non-invasive diagnostic tool for acute rejection following renal transplantation.

Methods: 286 patients were enrolled between January 2001 and December 2009., including 116 with biopsy-proved acute rejection (AR), 80 patients with stable renal function and no abnormal histological findings (NO-AR), 23 patients with subclinical rejection (SCR) in protocol biopsy, 24 patients with biopsy-proven acute tubular necrosis (ATN) and 43 patients with biopsy-proven chronic allograft nephropathy (CAN). Urinary concentration of sTim-3 was determined by an enzyme-linked immunosorbent assay technique in 286 renal allograft recipients and 40 healthy controls.

Results: Patient with acute rejection (n=116) excreted urinary sTim-3 at a significantly higher level (4356±440.4ng/mmol creatine) than levels of patients with No-AR (n=80) and healthy controls (n=40) (P<0.001). Patients with acute tubular necrosis (n=24) excreted urinary sTim-3 at a significantly lower level (2060±217 ng/mmol creatine) than levels of patients with acute rejection. ROC curve was constructed to determine the discriminatory power of sTim-3 levels for diagnosis of acute rejection. The area under ROC curve was 0.874, which showed that sTim-3 was a suitable marker for the diagnosis of acute rejection. At a cut point of 1836 ng/mmol creatinine, the sensitivity was 89.4% and the specificity was 82.5% (P<0.001). Patients with steroid-resistant acute rejection (n=66) had significantly greater urinary sTim-3 concentration than patients with steroid-sensitive acute rejection (n=50) (5548±613.5ng/mmol creatinine versus 2653±391.7ng/mmol creatinine, P=0.0002).

Conclusions: This study demonstrates that the monitoring of urinary sTim-3 may be a useful non-invasive approach for the detection of acute rejection. Additionally, urinary sTim-3 levels were shown to predict the response to anti-rejection therapy.

Funding: Government Support - Non-U.S.

FR-PO417

Effect of Pre-Transplant Use of Rituximab on Post-Transplant Clinical Outcome in Highly Sensitized Patients Seon Deok Hwang, Byung Ha Chung, Bum Soon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

Background: Pre-transplant sensitization is important risk factor for the development of antibody-mediated rejection and can result in inferior long-term allograft survival in kidney transplantation (KT). The aim of this study is to investigate the effect of pre-transplant Rituximab infusion on the clinical outcome in highly sensitized patients.

Methods: Between Jan. 2002 and Aug. 2013, 52 patients showed the result of panel-reactive antibody higher than 50%, but showed negative crossmatch test before transplantation. Out of them, 32 patients who took pre-transplant Rituximab at a dose of 375 mg/m^2 belonged to Rituximab group and remained 20 patients were regarded as historic control group. We compared the development of antibody mediated rejection, the change of allograft function and allograft survival rate between 2 groups after KT.

Results: Between two groups, no difference was found in baseline characteristics such as age at KT, gender, pre-transplant dialysis duration, primary renal disease. The mean value of PRA and HLA mismatching number, the type of main immunosuppressive agent did not differ as well. Deceased donor turned out to be higher in non-Rituximab group (P=0.02). After KT, the development of antibody mediated rejection was significantly lower in Rituximab group (3/32 (9.4%)) compared to historic control group (8/20 (40%, P<0.009) and total rejection rate showed lower tendency in Rituximab group as well. (P=0.056). However, development of pooled infection and infection free survival rate did not differ between 2 groups. Allograft function assessed by MDRD eGFR at 14 days after KT was significantly higher in Rituximab group compared to historic control group, but at 1, 3, 6, and 12 months from KT, it did not differ between two groups. Allograft rejection free survival was significantly higher in Rituixmab group compared to historic control group.

Conclusions: In sensitized patients with high PRA but negative crossmatch, Rituximab was effective to prevent the development of antibody mediated rejection without increased risk for infection.

FR-PO418

Differential Clinical Outcomes in Kidney Transplant Recipients with De Novo C1q+ and C1q- Donor Specific Antibodies Maria Ajaimy,[1,2] Adriana Colovai,[1] Sumeyye Calp Inal,[2] Michal L. Melamed,[2] Joel Lindower,[1] Enver Akalin.[1,2] *[1]Einstein/Montefiore Transplant Center; [2]Renal Div, Albert/Einstein College of Medicine, Bronx, NY.*

Background: Development of de novo donor-specific anti-HLA antibodies (DSA) has been associated with increased risk of antibody mediated rejection (AMR) and poor graft outcome. However, it is not clear if C1q binding capacity of DSA plays role in clinical outcomes.

Methods: The study included 301 consecutive recipients who received renal transplant between May 2009 and December 2012. We excluded patients who were transplanted with pre-transplant DSA. HLA antibody testing and C1q binding capacity was performed using the Luminex Single Antigen Bead assay. Anti-HLA antibodies were monitored after transplantation at 3, 12 months and yearly thereafter, and at the time of clinically indicated biopsies.

Results: Thirty-five of the total 301 patients (11.6%) developed DSA during a median follow-up of 28.5 months (17, 50). 23% had class I, 46% had class II, and 31% showed both class I and II DSA. DSA directed to donor HLA-DQ were most frequently observed (49%). Of the 35 DSA+ patients, 12 were C1q+ (34%). C1q+ DSA patients had significantly higher mean fluorescence intensity (MFI) values compared to C1q- DSA patients (7282+3423 versus 3898+3428, respectively, p=0.002). There was a striking difference in clinical outcomes that C1q+ DSA patients had higher AMR and transplant glomerulopathy (TG) (42% and 50%) compared to C1q- DSA (13% and 13%) and DSA- patients (1% and 4%), respectively. The graft survival was lower in the C1q+DSA group (75%) compared to the C1q-DSA (87%) and DSA- (90%) groups. Patients with C1q+ DSA had higher creatinine levels compared to C1q-DSA and DSA- groups.

Conclusions: Our results suggest that de novo C1q+ DSA patients had more AMR and TG, and lower allograft survival compared to C1q-DSA patients.

	No DSA (N=266)	De novo DSA c1q + DSA (N=12)	De novo DSA c1q - DSA (N=23)	P-value
Median Age at TX	56	41	46	0.005
Female (%)	40	66	48	0.56
Live donor (%)	29	34	26	0.85
Previous tranplant (%)	9	0	8	0.53
ACR (%)	5	17	4	0.17
AMR (%)	1	42	13	<0.001
TG (%)	4	50	13	<0.001
Last mean creatinine (mg/dl)	1.3	2.2	1.5	<0.001
Graft survival (%)	90	75	87	0.21
Patient survival (%)	94	100	96	0.70

FR-PO419

Increased Risk of Antibody Mediated Rejection and Graft Loss in Sensitized Pregnant Kidney Transplant Recipients Maria Ajaimy, Michelle L. Lubetzky, Layla Kamal, Anjali Gupta, Graciela De Boccardo, Enver Akalin. *Einstein/ Montefiore Transplant Center, Bronx, NY.*

Background: Pregnancy and allograft outcomes in sensitized renal transplant recipients were not well documented. We aimed to examine the clinical outcomes of these immunologically high risk pregnancies.

Methods: This is a single-center retrospective cohort study of adult kidney transplant recipients who became pregnant from June 1, 2009 through December 31, 2012. Donor-specific anti-HLA antibodies (DSA) and panel reactive antibody (PRA) levels were studied by Luminex single antigen beads.

Results: There were 9 pregnant patients with a median age of 36 years (22, 38) and 33 % were African-American. Pregnancies occurred at a median of 3.1 years (1.1, 7.2) after transplantation. Patients' median serum creatinine levels and spot urine protein/creatinine ratio were 1.1 mg/dl (1.1, 2.1), and 0.55 g/day (0, 1.2) respectively. Of the total cohort, 6 patients were sensitized (PRA > 0%) and 3 had PRA of 0%. Of those sensitized patients, the median PRA level was 46% (25, 98) for class I and 0% (0, 86) for class II, and 2 had DSA. All patients were on triple drug immunosuppression with tacrolimus, mycophenolate mofetil and prednisone before pregnancy and mycophenolate mofetil was switched to azathioprine. The sensitized group had a higher incidence of adverse pregnancy outcomes; 1 stillbirth and 1 second trimester miscarriage. The remaining four high PRA patients and three 0% PRA patients delivered babies at a median of 34.5weeks (34, 36.5). During a median follow-up of 2.3 years (2, 4) after delivery, 3 high PRA patients (50%) developed AMR within one year after delivery that led to graft loss. In the low PRA group, no patients had rejection episodes and all maintained stable graft function (median creatinine level of 1.1 mg/dl (1.0, 1.2) and median proteinuria of 0.4 g/day (0.3, 0.5) without the development of new DSAs.

Conclusions: Our observational cohort suggests that there is an increased risk of AMR, graft loss and adverse pregnancy outcomes in sensitized kidney transplant recipients.These results should be assessed in a larger cohort for a better counselling of high immunologic risk patients who wish to become pregnant.

FR-PO420

C1q Binding and Humoral Rejection in Kidney Transplant Mohammad Sharif,[1] Navin Jaipaul,[2] Thanh Hoang,[1] Siegmund Teichman.[1] *[1]Loma Linda Univ Medical Center; [2]Loma Linda VA Healthcare System.*

Background: Donor specific antibodies (DSA) are associated with antibody-mediated rejection (AMR), detected by histology and C4d staining. However, the presence of DSA does not necessarily result in graft dysfunction. One way to distinguish when DSA are harmful is to determine when DSA can activate the classical complement pathway. C1q is the first step in the classical complement cascade activated by DSA. Two novel assays are available to detect this process, DSA by C1q by luminex (DSA-C1q) and C1 binding assay (which detects the presence of circulating immune complexes).

Methods: This is a single center retrospective cohort study of kidney transplanted patients above 18 years of age, who developed DSA post-transplant and subsequently underwent transplant kidney biopsy between 06/2012-07/2013. The aim was to compare the correlation of both assays with AMR and C4d staining on transplant kidney biopsy. All statistical analysis was performed using SPSS, version 17.0.

Results: Baseline characteristics were similar without significant differences between all groups. Results

		DSA-C1q		Total	C1q binding assay		Total
		Negative	**Positive**		**Negative**	**Positive**	
AMR	Positive	4	5	9	0	4	4
	Negative	3	1	4	8	0	8
Total		7	6	13	8	4	12
C4d staining	Positive	3	5	8	0	4	4
	Negative	4	1	5	8	0	8
Total		7	6	13	8	4	12

C1q binding was superior to DSA-C1q in correlation with biopsy-proven AMR: (r=1.0 versus 0.28; p <0.001 versus 0.35), sensitivity (100% versus 55.6%), specificity (100% versus 75%), positive predictive value (PPV) (100% versus 83.3%) and negative predictive value (NPV) (100% versus 42.9%) for C1q binding versus DSA-C1q, respectively. Also, C1q binding was superior to DSA- C1q in correlation with biopsy-positive C4d staining: (r= 1.0 versus 0.41; p<0.001 versus 0.16), sensitivity (100% versus 62.5%), specificity (100% versus 80%), PPV (100% versus 83.3%) and NPV (100% versus 57.1%) for C1q binding versus DSA-C1q, respectively.

Conclusions: This pilot study demonstrated that C1q binding correlated better than DSA-C1q in detecting DSA which activates the complement pathway to induce AMR. More studies are needed to confirm these results as it is a noninvasive and an inexpensive method for monitoring DSA in kidney transplant patients.

FR-PO421

Expression of IL-6 and Its Receptor IL-6Ra Is Increased in Kidney Allografts with Antibody Mediated Rejection (ABMR) Jolanta Kowalewska,[1] Hedyeh Shafi,[1] Cynthia C. Nast,[1] Arkadiusz Gertych,[1] Mark Haas,[1] Ashley Vo,[2] Mieko. Toyoda,[2] Stanley C. Jordan.[2] *[1]Pathology, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.*

Background: IL-6 is a multifactorial cytokine regulating immune response and hematopoiesis. It has 2 receptors: IL-6R (membrane-bound and soluble) and gp130. Membrane bound IL-6R is expressed on T and B lymphocytes, and other cells. IL-6 may be involved in transplant rejection; it is elevated in allograft recipients with glomerulitis, suggesting association with ABMR.

Methods: We studied renal allograft biopsies that met Banff criteria for cell mediated rejection (CMR) and ABMR. Controls included native kidneys with thin basement membrane disease (Nk) and allograft kidneys without rejection (Tx). Biopsies were retrospectively stained for IL-6 and IL-6Ra. Slides were digitized using a whole-slide ScanScope AT Turbo scanner (Aperio), then analyzed by Tissue IA software (Leica). Negative and positive cell number and staining intensity were assessed. IL-6 was expressed as number of positive cells/area (mm^2). IL6Ra positive glomerular and tubulointerstitial cells were counted manually and expressed as mean number /glomerulus or area (mm^2).

Results: 6 cases of Nk, 9 Tx, 12 CMR and 11 ABMR were analyzed. IL-6 was expressed by leukocytes and tubular epithelial cells. Biopsies with ABMR showed greater numbers of IL-6 positive cells compared to CMR, Tx and Nk (5001 versus 2669 versus 2890 versus 3023, respectively; p=0.02). IL-6Ra was expressed in infiltrating leukocytes, and the cytoplasm of intrinsic renal cells, including podocytes, distal nephron tubular epithelium and arterial smooth muscle cells. ABMR had the most IL-6Ra positive leukocytes in glomerular [1.1 (ABMR) versus 0.4 (CMR) versus 0.5 (Tx) versus 0.2 (Nk)] and tubulointerstitial (82.3 versus 16.8 versus 15.6 versus 13.5, respectively) compartments, although these differences were not statistically significant.

Conclusions: Allograft kidneys with ABMR have significantly increased IL-6 expression and have more leukocytes with IL-6R compared to CMR, Tx without rejection and Nk. These findings suggest a role of the IL6 /IL-6R pathway in tissue injury in ABMR.

FR-PO422

Anti-Angiotensin Receptor Type I Antibody: Role in Antibody Mediated Rejection and Response to Plasmapheresis Dhiren Kumar,[1] Gaurav Gupta,[1] Anne L. King,[1] Amit Sharma,[2] Adrian Cotterell,[2] Felecia McDougan,[2] Pam Kimball.[2] *[1]Nephrology, Virginia Commonwealth Univ, Richmond, VA; [2]Transplant Surgery, Virginia Commonwealth Univ, Richmond, VA.*

Background: In kidney transplant patients sensitization to Angiotensin type I receptor auto-antibody (AT1R-Ab) has been associated with antibody-mediated rejection (AbMR) and worse graft survival. It is unknown if AT1R-Ab sensitization independently causes AbMR in the absence of donor-specific HLA antibodies (HLA DSA). This study aims to examine the association of AT1R-Ab with the incidence of AbMR and response to therapy with plasmapheresis (PP).

Methods: Forty consecutive kidney transplant patients with biopsy proven AbMR between Jan '06 and Sep '13 were included. They were treated with 6 sessions of PP followed by CMVIg 150mg/kg. Flow cytometry and solid-phase assays were used to measure HLA DSA and At1R-Ab at the time of transplant, acute AbMR and post therapy. Based on prior reports a positive AT1R-Ab concentration was set at > 10U/ml.

Results: Most patients were African-American (26/40; 65%) and sensitized with a PRA>20% (21/40; 53%). At the time of rejection a substantial proportion of patients (14/40; 35%) had no HLA DSA, of these 3 (21%) had a positive AT1R-Ab. Thus, 3/40 (7.5%) episodes could be attributable to AT1R-Ab. Seventy percent (28/40) of the patients had a decline in AT1R-Ab levels after PP from a mean of 26U/ml (median 13, range 6.5-216) to 11U/ml (median 10, range 5-21) at the end of therapy. There was a trend towards greater graft loss among patients who had an AT1R-Ab>10U/ml (14/17; 82%) compared with patients with AT1R-Ab <10U/ml (13/23; 56%; p=0.08).

Conclusions: In this single-center study, we report that 7.5% of all cases of AbMR could be attributable to non-HLA AT1R-Ab. There was a trend towards greater graft loss among patients with a positive AT1R-Ab at the time of rejection although a clear association was not evident. It is possible that this effect was blunted by the efficacy of plasmapheresis in the removal of the AT1R-Ab. Further prospective studies are needed to determine the clinical course of kidney transplant patients with detectible AT1R-Ab and its impact on graft outcomes.

FR-PO423

T Regulators/Th17 Polarization in Cellular Rejection in Kidney Allograft
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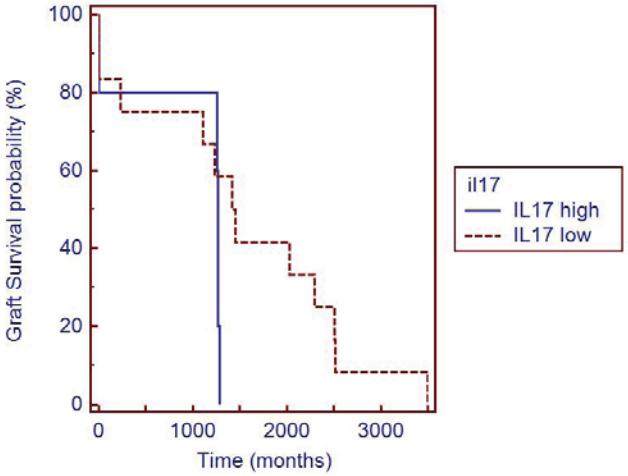
Background: Th17 in association with Th1, Th2 and Th follicular are the effectors cells in kidney rejection. Th17 have a pro-inflammatory activity by the production of IL17 cytokine. The T regulators (Tregs), marked by FOXP3, have several inflammatory suppressor effects. Nowadays, it is known that Th17 and Tregs have a common precursor. According the cytokine milieu, the Tregs could be converted in Th17 and vice-versa. The final immune response, the tissue lesion severity depends of the balance between Tregs/th17.

Methods: Reevaluation of all consecutive biopsies (n=45), between 2000-2001 unicenter, with a histological diagnosis of borderline alterations (BA), cellular rejection (CR) or vascular rejection (VR). Identification of Il17 and FOXP3 cells by indirect immunohistochemistry in paraffin preserved tissue. Numbers of cells/mm2 are determined. Cells are counted in all the biopsies. Areas are measured using digital methods. Biopsies are divided in two groups according the median: low IL17 and high IL17.

Results: Biopsies present a mean of 16.56±17.66 FOXP3/mm2 and a mean of 6.12±14.53 Th17/mm2. Th17 cells were not present in 16 biopsies. Biopsies with BA have less FOXP3 than CR (8.49±11.32 versus 25.33±19.66; p=0.0022) with a low and similar number of Th17 (2.39±4.14 versus 2.45±4.45; p=0.015). Biopsies with VR present lower FOXP3 (6.11±5.98 versus 25.33±19.66; p=0.0002) and higher Th17 (15.74±25.17 versus 2.45±4.45; p=0.015). We demonstrates 10 years graft survival according Th17 number at biopsy time.



Conclusions: It seems that FOXP3 was "substituted" by Th17 in Vascular rejection. That probably explains the severity of inflammation/ tissue destruction observed in vascular rejection. The number of Th17 in biopsy seems influence the 10 years graft survival after the rejection.

FR-PO424

Does Renal Allograft C1q Staining Predict Outcomes?
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Background: Antibody mediated rejection (AMR) results from activation of the complement cascade by donor specific antibody (DSA) leading to microcirculatory injury within the renal allograft. Studies have shown association between C1q binding of DSA and inferior graft outcomes. However, the relationship between renal allograft C1q staining and outcomes is unknown. We hypothesized that the presence of C1q staining on allograft biopsy (bx) portends worse graft outcomes.

Methods: We performed a retrospective chart review of kidney transplant recipients from 2007-2013 who underwent for-cause allograft bx and a had positive DSA defined as MFI>1000. The frozen bx tissue was retrieved and stained for C1q with immunofluorescence. The light microscopy of each bx was reviewed by a single pathologist blinded to clinical data. C1q binding of DSA closest to the time of bx was performed using Luminex platform. Graft outcomes were compared for C1q positive (C1q+) versus C1q negative (C1q-) bx.

Results: The study included 28 patients with average age of 50±14 years and mean follow-up of 26±20 months of which 11 had C1q+ bx. There were no differences in the incidences of acute cellular rejection (36% versus 29%, p=0.7), AMR (27% versus 29%, p=0.9), C4d staining (18% versus 29%, p=0.5), graft failure (36% versus 29%, p=0.7) or level of serum creatinine at 1-year (1.6±0.4 versus 1.6±0.9 mg/dl, p=1.0) and 2-year (1.6±0.4 versus 1.9±1.2 mg/dl, p=0.66) for C1q+ versus C1q- groups. Transplant glomerulopathy (p=0.03) and interstitial fibrosis (p=0.01) scores were significantly higher while scores on tubulitis (p=0.5), interstitial inflammation (p=0.15), glomerulitis (p=0.13) and peritubular capillaritis (p=0.4) were similar for C1q+ versus C1q- bx groups. C1q binding DSA was observed in 4 patients of which 2 had C1q+ bx.

Conclusions: Although presence of C1q in renal allograft bx did not translate to adverse short-term graft outcomes, the observation of increased transplant glomerulopathy and interstitial fibrosis in these patients may portend worse long-term graft outcome. Larger studies are needed to verify these findings.

FR-PO425

Immune Activation in Kidney Transplant Recipients After Sirolimus Conversion
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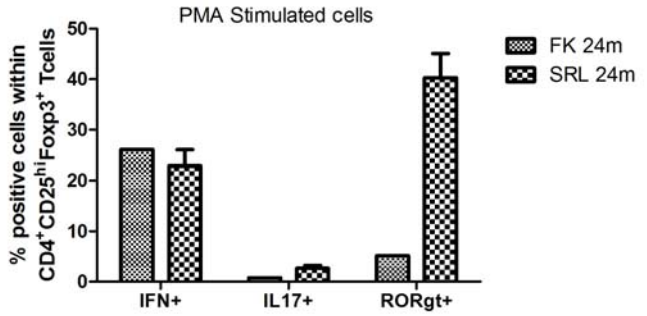
Background: Conversion from Tacrolimus (TAC) to Sirolimus (SRL) in kidney transplant recipients was aimed to improve calcineurin inhibitor nephrotoxicity and long term graft function. The results from multiple studies are not consistent. This study investigates the effects of SRL on immune cell functions after conversion.

Methods: The study included 30 renal transplant recipients from a randomized trial of SRL conversion (n=18) or TAC maintenance (n=12). The conversion started at 12 months post-transplant. PBMC were collected at 0, 6, 12 and 24 months post-randomization. T cell subpopulations were analyzed by flow cytometry. Cellular alloreactivity against irradiated donor cells (Direct), donor cell membrane sonicates (Indirect) and synthetic mismatched HLA peptides (Indirect) were tested by IFN-g ELISPOT assay.

Results: SRL conversion led to a significant increase in $CD4^+CD5^{hi}Foxp3^+$ T cells at 6, 12 and 24 months post conversion (1.58±0.09 (SRL group) VS 0.13±0.02 (TAC group) % of PBMC at 24 months (p<0.01). However, Intracellular cytokine and transcriptional factor staining showed that $CD4^+CD25^{hi}Foxp3^+$ T cells in SRL-converted group had more percentage of cells that co-expressed ROR-gt and IL-17 which are Th17 markers.



SRL conversion also showed an increase in indirect donor alloreactivity against both donor cell membrane sonicates and synthetic HLA peptides from 0 to 12 months post-randomization while the TAC maintenance showed a decrease alloreactivity resulted in a significant different over time between TAC and SRL group (p<0.01). No difference in direct alloreactivity was detected at 12 months.

Conclusions: Chronic immune alterations are induced after conversion from TAC to SRL. These findings have important implications for guiding the clinical use of mTOR-inhibitors.

Funding: NIDDK Support

FR-PO426

T Cell Receptor Deep Sequencing to Track Alloreactive T Cells: Evidence for Clonal Deletion in Tolerant Patients following Combined Kidney and Bone Marrow Transplantation
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Background: Alloreactive T cells recognize myriads of MHC/peptide specificities, thus far precluding the identification of those mediating graft rejection and graft-versus-host disease in HLA-mismatched transplantation. In addition, the need for new non-invasive tools to monitor rejection risk and/or operational tolerance in kidney transplant recipients is underscored by the morbidity associated with chronic immunosuppression.

Methods: We hypothesized that high-throughput deep sequencing of a transplant recipient's donor-reactive TCRb CDR3 regions expanded in one-way mixed lymphocyte reaction prior to transplantation could identify and allow tracking of donor-reactive T cells following transplantation. We tested this approach in four combined kidney and bone marrow transplantation patients in a trial aimed at achieving allograft tolerance (ITN036ST), and in two "conventional" kidney transplant recipients.

Results: We observed significant post-transplant reductions in donor-reactive CD4 and CD8 T cell clones only in the CKBMT patients who achieved allograft tolerance (3 of 4 CKBMT). In the "conventional" kidney transplant recipients, we found a significant increase in circulating anti-donor CD4 clones post-transplantation. Loss of donor-reactive TCR in the CKBMT patients identified tolerance more specifically than functional assays. In some instances, clonal reduction was specific for donor-reactive clones; in others, the reduction reflected repertoire turnover due to lymphocyte depletion during conditioning.

Conclusions: We have developed a method of tracking donor-reactive T cells and obtained evidence for clonal deletion as a mechanism of allograft tolerance in humans.

Funding: Other NIH Support - NIH grants ROI #AI084074, PO1 grant #P01 AI106697,

FR-PO427

Application of Flow Cytometry to Measure Anti-A/B Antibody in ABO Incompatible Kidney Transplantation Ji-Young Choi,[1,2] Ji-Eun Lee,[1,2] Jang-Hee Cho,[1,2] Hee-Yeon Jung,[1,2] Se-Hee Yoon,[3] Sun-Hee Park,[1,2] Yong-Lim Kim,[1,2] Chan-Duck Kim.[1,2] *[1]Dept of Internal Medicine, Kyungpook National Univ School of Medicine, Daegu, Korea; [2]Clinical Research Center for End Stage Renal Disease in Korea; [3]Dept of Internal Medicine, Konyang Univ, Daejeon, Korea.*

Background: Current methods of measuring anti-ABO antibody titer by hemagglutination are subjective and not reproducible. However, the flow cytometry (FCM)-based method was suggested to be reproducible and yield semi-quantitative results. The aim of this study is to compare anti-ABO antibody levels by FCM with the levels by column agglutination test (CAT) and to evaluate the clinical outcome according to the baseline mean fluorescence intensity (MFI) ratio obtained by FCM.

Methods: We reviewed 21 cases of ABO-incompatible kidney transplantation (ABO-i KT) performed from February 2012 to March 2014. In these patients, baseline IgG titers were measured using both FCM and CAT methods. We investigated the correlation between levels measured by FCM and levels measured by CAT using correlation coefficient. Patients were classified as the high MFI ratio group (≥200, n=7) or the low MFI ratio group (<200, n=14).

Results: The MFI ratio of FCM-based method was highly correlated with the titer of CAT (r=0.890, p=0.01). An equation between MFI ratio and CAT titer is as follows: Log (MFI ratio) = 0.879 x log (CAT titer) + 0.298. The number of pre-transplant plasmapheresis significantly increased with the increase of baseline MFI ratio (r=0.838, p = 0.01). The allograft function was immediately recovered and stable. The pre-transplant MFI ratios were reduced from 221.3 (baseline) to 8.0 (day of transplantation) and the highest MFI ratio at kidney transplantation was 32.6. There was no acute rejection in ether group during follow-up. The postoperative bleeding requiring reoperation immediately after KT occurred in 2 and 1 patients of the high MFI ratio and low MFI ratio groups, respectively.

Conclusions: Anti-ABO antibody levels measured by FCM-based method were highly correlated with the levels by CAT in ABO-i KT. The decreased level of anti-ABO antibody by FCM after plasmapheresis suggested its potential as effective method for assessment of anti-ABO antibody level.

FR-PO428

ABO Incompatible Kidney Transplantation Enabled by Non-Antigen Specific Immunoadsorption: A 5-Year Experience Luis Eduardo Becker,[1] Daniela Siebert,[1] Sebastian Markus Schaefer,[1] Caner Süsal,[2] Gerhard Opelz,[2] Albrecht Leo,[2] Ruediger Waldherr,[3] Stephan Macher-Goeppinger,[3] Martin G. Zeier,[1] Vedat Schwenger,[1] Christian Morath.[1] *[1]Nephrology, Univ of Heidelberg, Germany; [2]Immunology, Univ of Heidelberg, Germany; [3]Pathology, Univ of Heidelberg, Germany.*

Background: We recently proposed a protocol using non-antigen specific immunoadsorption (IA) and rituximab for the desensitization of ABO incompatible (ABOi) kidney transplant recipients. This protocol allows a high number of IA treatments at reasonable cost, and the depletion of HLA antibodies at the same time.

Methods: In the current analysis, we compared clinical outcomes from 34 ABOi kidney transplant recipients who were desensitized and transplanted by this protocol to 68 standard-risk living donor kidney transplant recipients matched for time posttransplantation. Patients with preexisting donor-specific HLA antibodies were excluded.

Results: Before desensitization, the 34 patients had a median isoagglutinin titer of 1:64 (1:2-1:1.024; Coombs technique). Patients required a median of 7 (5-12) preoperative IA treatments, and 24 patients had 1 (0-6) additional plasmapheresis treatment to reach the preoperative target isoagglutinin titer of ≤1:8. After a median postoperative follow-up of 22 (6-65) months overall allograft survival was with 94% in the ABOi group not significantly different from the 99% graft survival in standard-risk patients. Allograft function was also not significantly different between the groups after 1, 2 and 3 years, as was the incidence of cellular or antibody mediated rejection episodes (18% for both groups). ABOi patients had both a higher incidence of polyoma BK virus replication (>10*4 copies/mL plasma) and nephropathy (SV40 staining positive) compared to standard-risk patients (21% versus 6%; P=0.04 and 12% versus 0%; P=0.01, respectively). The incidence of CMV, fungal or bacterial infections was similar between the groups.

Conclusions: Outcomes of ABOi patients desensitized with a non-antigen specific IA device and rituximab do not differ from the outcomes in standard-risk patients. Special attention, however, must be given to the higher incidence of polyoma BK virus infection in the ABOi patients.

FR-PO429

The Role of Rituximab in ABO Incompatible Kidney Transplantation in Japan Kazuhide Saito,[1,2] Kota Takahashi.[1,2] *[1]Urology, Niigata Univ, Niigata, Japan; [2]Japan ABO Incompatible Kidney Transplantation Committee, Niigata, Japan.*

Background: Since the first success in January 1989, we have performed more than 2,400 ABO incompatible kidney transplantation (ABOIKT) to date. Especially after 2001, the concept of desensitization and rituximab has been introduced and patient and graft outcome dramatically improved. Purpose of this study is to confirm the benefit of rituximab for desensitization as a substitute for splenectomy and elucidate the factors affecting the patient and graft outcome in large Japanese ABOIKTx cohort.

Methods: Among 2,212 cases registered in Japan ABO incompatible kidney transplantation committee, we have selected 1,121 patients after 2001 era in whom the data for the use of rituximab and splenectomy status were available. We have categorized the patients into 4 groups according to the splenectomy (S) and Rituximab (R) status, S (-) /R (-): N=16, S (-) /R (+): N=738, S (+) /R (-): N=320 and S (+) /R (+): N=47. At first, we analyzed overall patient and graft survival, next, compared the outcome beween each group, finally analyzed the factors affecting patient and graft outcome by univariate and multivariate analysis.

Results: Overall patient survival rate at 1,3,5 and 10 years were 98.5, 97.3, 95.4 and 94.8%, and graft survival rate were 97.1, 94.5, 91.3, 59 and 84.8%, respectively. There was no statistically significant difference in patient and graft survival between 4 groups. The factors affecting the patient survival were postoperative antibody removal (PAR) (multivariate: p<0.004, HR 2.986, PI 1.419-6.283) and postoperative anticoagulation (PAC) (multivariate: p<0.020, HR 3.148, PI 1.199-8.261). The factors affecting the graft survival were PAR (multivariate: p<0.001, HR 4.238, PI 2.510-7.156) and PAC (univariate: p<0.021, HR 1.936, PI 1.107-3.385). There were no significant difference in patient and graft survival according to the preoperative anti-A/B IgG/IgM antibody titer.

Conclusions: For successful ABO-IKTx, desensitization with rituximab has become standard and the substitute of splenectomy for prevention of AMR and accommodation induction. ABOIKTx has become accepted as a therapeutic alternative treatment of choice for end-stage kidney disease.

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FR-PO430

Long Term Outcome of ABO-incompatible Living Donor Kidney Transplantation, Using the Desensitization Protocol and Timely Plasmapheresis after Transplantation Eunhye Shin, Hyosang Kim, Su-Kil Park. *Internal Medicine, Asan Medical Center, Seoul, Republic of Korea.*

Background: ABO-incompatible (ABOi) kidney transplantation (KT) has gradually increased in consequence of the development of desensitization protocol for overcoming the serious organ shortage. However, there are only few long term follow-up data in Korean patients receiving ABOi KT.

Methods: A retrospective review of medical records was conducted for individuals who underwent KT in Asan Medical Center from February 2009 to January 2012.

Results: A total of 666 patients were included; the mean age was 43.4±11.7 and the median follow-up period was 39 (interquartile range 31-48) months. 83 individuals who had ABOi living donor KT were compared with 583 individuals who had ABO-compatible (ABOc) KT. Kaplan-Meier survival analysis showed that ABOi KT group had slightly lower cumulative survival rate compared to ABOc KT group (p=0.030). However, graft survival, acute rejection episode, graft function, and postoperative complications were not significantly different between two groups. Additionally, ABO incompatibility was not a significant risk factor for acute rejection (HR, 1.47; 95% CI, 0.72-2.99; p=0.294) but, donor age, mismatch of HLA, and panel-reactive antibody (PRA) ≥20% were significant risk factors (HR, 1.04; p=0.001; HR, 1.20; p=0.046; HR, 3.17; p=0.002, respectively). In the ABOi KT group, initial high isoagglutinin titer (≥1:256) was related with titer-rebound after KT (HR, 1.05; 95% CI, 1.00-1.10; p=0.037), many preoperative plasmapheresis (HR, 3.39; 95% CI, 1.69-6.79; p=0.001), and low titer reduction rate (HR, 9.56; 95% CI, 1.14-80.5; p=0.038). However, patient survival, graft survival, and graft function were similar regardless of initial isoagglutinin titer or presence of titer rebound after KT.

Conclusions: Although ABOi KT group showed significantly lower survival rate, graft survival, graft function, acute rejection, and peri- or postoperative complications were comparable with ABOc KT group. Therefore, ABOi KT could be a safe option for patients whose only available donors are ABO incompatible.

FR-PO431

Do Pre-Existing Donor Specific Anti-Human Leukocyte Antigen (HLA) Antibodies Lead to Increased Risk of Cellular Rejection Post-Transplantation A. Regmi,[1] P. Singh,[1] S. Koenig,[3] O. Myers,[2] B. Masten,[3] K. Madden,[1] A. Harford.[1] *[1]Internal Medicine, Univ of New Mexico School of Medicine, Albuquerque, NM; [2]Biostatistics, Univ of New Mexico School of Medicine, Albuquerque, NM; [3]Pathology, Univ of New Mexico School of Medicine, Albuquerque, NM.*

Background: Some studies have shown that the presence of DSA can negatively impact renal allograft survival. In this study we examined the association between pre-existing DSA and outcomes post-transplant.

Methods: We retrospectively analyzed 213 consecutive renal transplants, at a single center between 1/2007 and 9/2013, for the presence of pre-transplant DSA and its strength expressed as mean fluorescence intensity (MFI).Univariate analysis and logistic regression analyses included the following variables: race, ethnicity, gender, age at transplant, ESRD cause, prior sensitizing events, donor type, cross match results, and induction agent. Outcomes analyzed were presence of cellular and antibody mediated rejection and eGFR at 1 year using MDRD.

Results: There were 39 episodes of rejection in 213 patients with an incidence of 18.3%. Of the patients with rejection, 18% had pre-existing DSA. Of those with pre-existing DSA and rejection 85.7% had cellular rejection and 14.3% had antibody mediated rejection**.** MFI levels for DSA were not different between those patients with rejection versus those without. The incidence of rejection was decreased in recipients of expanded criteria donor kidneys: 8% versus 19.7% in recipients of standard criteria donors and 19.4% in recipients of living donors. A higher incidence of rejection was observed among non-Hispanic whites at 23.8%

versus 16.3% in Hispanics and 11.6% in Native Americans. Patients with rejection had significantly lower eGFR (47.1ml/min) at 1 year versus those with no rejection (60.2ml/min), (P=0.004).

Conclusions: DSA and the strength of pre-existing antibody as expressed as MFI, were not associated with higher rates of rejection within the first year of transplant. There was a trend towards cellular rejection in those patients who had rejection and DSA. eGFR was lower in the group who experiencedrejection.

FR-PO432

De Novo Donor-Specific Antibodies in Pediatric Renal Transplant Recipients - Risk Factors and Treatment Effectiveness Mercy Rajesh, Samhar I. Al-Akash. *Pediatrics/Pediatric Nephrology, Driscoll Children's Hospital, Corpus Christi, TX.*

Background: Development of De Novo donor-specific antibodies (DSA) in renal transplantation (RTx) has been shown to be associated with decreased graft survival (GS). There is limited data on DSA in pediatric (Ped) RTx, and on the impact of treatment on graft function (GF) and GS. In this study we examine the risk factors for development of DSA and effect of therapy on GF and GS.

Methods: This is a retrospective study on Ped RTx recipients who received RTx at our center from 2006 to 2012 and had at least 12 months (mon) of follow up. 55 transplants were included. Our immunosuppressive regimen consisted of induction with a 5-day steroid taper and Daclizumab (ZEN) or rabbit-thymoglobulin (r-ATG) or Alemtuzumab (C1H), and maintenance with tacrolimus (Tac) and mycophenolate (Myc) or azathioprine (Aza). DSA were tested periodically after the first year and the time of biopsy-proven rejection. Single-antigen luminex beads were used to identify and measure DSA.

Results: 49 (89%) received deceased donor (DD) RTx. 18 pts (32.7%) developed DSA. [table 1] shows the differences between the DSA+ and DSA- pts. Risk factors for DSA were nonadherence (p 0.003, OR 9.1), and induction with Zen versus either r-ATG (p 0.01, OR 8.7; 1.5-49.2) or C1H (p 0.02, OR 5.1; 1.2-22.7). Treatment for DSA resulted in overall reduction of DSA mean florescence intensity (MFI) from 6998 to 2900 (P<0.001). DSA reduction of 80% or more was achieved in 65% of pts with MFI < 10000 and 38% in pts with MFI > 10000; p 0.0002). GF was significantly better in DSA- pts.

	DSA+	DSA-
Mean age at Tx	13.3 (3-22)	11.7 (1.5-19)
Primary vs. re-Tx (%)	94	89
Mean followup (mon)	49 (12-78)	40 (12-81)
Mean HLA mismatches	4.5	4.1
Induction % (Zen/r-ATG/C1H)	39/17/44	49/35/16
Mean eGFR ml/min/1.73m2		
- 3 mon	67	75
- 12 mon	69	71
- 24 mon	64	68
- 36 mon	60	68
- 48 mon	57	74
- 60 mon	47	72

GS was higher in DSA- pts, but did not reach statistical significance.

Conclusions: De Novo DSA is associated with worse GF in spite of treatment and reduction in DSA. Therapies and strategies directed at improving adherence and preventing development of De Novo DSA may be beneficial and should be explored.

Funding: Clinical Revenue Support

FR-PO433

Renal Allograft Outcomes with Alemtuzumab in Highly Sensitized Recipients. A Single Centre Experience Pradeep Vaitla,[1] Ushma Patel,[3] Ryan C. Mascarenhas,[1] Antonio G. Jimenez,[1] Abdul Moiz,[1] George E. Loss,[2] Stephanie Anders,[3] Jorge C. Garces,[1] Humberto Bohorquez.[2] *[1]Nephrology, Ochsner Clinic Foundation; [2]Transplant Surgery, Ochsner Clinic Foundation; [3]Pharmacy, Ochsner Clinic Foundation.*

Background: Pre-transplant sensitization is a well recognized risk factor for acute rejection and allograft failure. Higher dose of immunosuppressive agents is needed to minimize risk of allograft rejection.Several studies have been published with renal allograft outcomes using Alemtuzumab for antibody induction therapy but not much data are available for highly sensitized patients.We report our experience with Alemtuzumab (30 mg iv single dose) in pre-transplant sensitized patients.Our current protocol for immunosuppression includes Tacrolimus with a target trough level between 7-10 ng/ml, Mycophenolate mofetil (MMF) 500 mg oral twice daily,methylprednisolone 500 mg post opertive day (POD) 0, 250 mg POD 1 and 125 mg POD 2 with immediate steroid withdrawal.

Methods: We performed a retrospective chart review of 441 kidney transplants performed from November 2007 to December 2011 of which 311 recipients received Alemtuzumab for induction immunosuppression and we obtained demographic, laboratory and clinical data including biopsy proven acute rejection during 1 year of follow up. We stratified recipients based on Panel-reactive antibody (PRA) levels as 0%,1-50% and >50%.

Results: The overall incidence of biopsy proven acute rejection during one year follow up was 8% and graft failure of 3%. Recipients with PRA level 0% had a rejection rate of 4.2% and graft failure of 1.8%, recipients with PRA level 1-50% had a rejection rate of 14% and graft failure of 3%, recipients with PRA level >50% had a rejection rate of 19% and graft failure rate of 11%.

Conclusions: Alemtuzumab seems to be a cost-effective agent in high risk population with a 1 year rejection rate of 19% in highly sensitized recipients. It is well documented that increased pre transplant sensitization leads to reduced allograft survival. Alemtuzumab provides good rejection profile with a one year graft survival rate of 97% in recipients with PRA level 1-50% and 89% in recipients with PRA level >50%. More data are needed to address long-term follow-up with this agent.

FR-PO434

Treatment of Chronic Active Antibody-Mediated Rejection: What to Expect? Isabelle Houde, Stephanie Beland, Patrice Vallin, Real Noel, Isabelle Lapointe, Isabelle Côté, Eric Wagner, Olivier Desy, Julie Lesage, Sacha A. De Serres. *Renal Div, Dept of Medicine, CHU de Quebec Hôtel-Dieu, Laval Univ, Quebec City, QC, Canada.*

Background: Anti-rejection protocols for antibody-mediated rejection (AMR) reduce graft loss for acute AMR. However, little is known about the effect of treatment for chronic active AMR (CAAMR). Notably, it is unknown whether we should expect a return to previous baseline graft function following treatment.

Methods: This is a single center retrospective observational study of 50 patients treated for suspicious or confirmed CAABMR according to Banff criteria between June 2002 and January 2012. Patients were classified in 3 treatment groups: solumedrol only (S, n=18), solumedrol and IVIG (S+I, n=24) and solumedrol, IVIG and plasma exchange and/or rituximab (S+I±P±R, n=8). Therapeutic decision was made on clinical grounds. We studied a composite endpoint of death-censored graft failure or doubling of serum creatinine (Scr), using Chi-square, non-parametric paired and unpaired tests.

Results: Mean recipient age was 39±18yrs and 88% were recipients of a deceased donor. At biopsy, median time post transplant was 90mo and Scr was 2.2±1.0mg/dl. 30 patients (60%) reached the endpoint at a median time of 36mo. Among patients free of the endpoint, median Scr increased from 1.7mg/dl at biopsy to 1.8mg/dl at last follow-up (median 39mo), with a mean increase of 0.1±0.4mg/dl on an individual basis. Compared to patients treated with S, patients treated with S+I and S+I±P±R had similar Scr at biopsy (median 1.9 versus 1.8 versus 2.1 respectively; p=0.42). The endpoint was reached in 56%, 75% and 25% of S, S+I and S+I±P±R patients respectively (p=0.039). In patients free of the endpoint, mean Scr increased non-significantly in S and S+I groups and remained stable in the S+I±P±R (0.1±0.5, 0.2±0.6 and 0.0±0.2, p=0.16, 0.60 and 0.92 respectively, paired test).

Conclusions: The prognosis of CAAMR is dismal. A more aggressive therapy including S, I and a combination of P and/or R seems to be associated with better outcomes. Our data suggest that following successful treatment, Scr stabilizes rather than improves. These data need to be replicated in a larger cohort.

FR-PO435

Complement Inhibition in Antibody Mediated Rejection during Kidney Transplantation Michelle Elias, Erika Nnang Obada, Séverine Beaudreuil, Alyette Duquesne, Caroline Poitou, Laurentiu Iamandi, B. Charpentier, A. Durrbach, H. Francois. *Nephrology, Bicêtre Hospital, Univ Paris Sud, INSERM 1014, Le Kremlin-Bicêtre, France.*

Background: There is to date no gold standard treatment of antibody mediated rejection (AMR). Current therapeutic strategies rely on plasmapheresis combined with intraveinous globulins (IVIg). Eculizumab is a humanized monoclonal antibody that targets complement protein C5 and prevents the formation of the membrane attack complex. Therefore it could be efficient in treating complement mediated lesions during AMR. Few clinical cases describe the efficiency of Eculizumab in treating acute AMR.

Methods: We conducted a monocentric retrospective study of 15 patients (7 men and 8 women) that have been treated between October 2011 and June 2013 for severe AMR using eculizumab on top of plasmapheresis, IV Ig and a B-cell targeting agent. Patients were transplanted between January 2002 and November 2012. All AMR were documented by a graft biopsy according to the Banff 2011 criteria at diagnosis and we report patients and graft outcome after treatment. The biopsy was repeated whenever necessary.

Results: The mean follow up was 16,4 months. Complement inhibition was fullfilled in both treatment groups throughout follow up.8 patients out of 15 (53%) did not respond to the treatment and 6 lost their graft. 9 patients were diagnosed with AMR 27,2 months after their transplantation, out of which 7 (78 %) did not respond to treatment. The remaining 6 were diagnosed with AMR in the first year following transplantation and only one lost its graft (17%), p<0,05. Only one of the patients died with a functional graft and 9 patients experienced severe infections. In the responders group, the mean serum creatinine was 205 µmol/l 37,6 months after transplantation and 10,4 months after AMR diagnosis. No responding patients received eculizumab more than 3 months.

Conclusions: Eculizumab seems more efficient in early versus late AMR, which reflects a more prominent involvement of the complement cascade in early versus late AMR.

FR-PO436

Microcirculation Inflammation in Transplant Kidney Biopsies: Clinicopathological and Genomic Significance Anjali Gupta,[1] Pilib O Broin,[2] Yi Bao,[1] Aaron Golden,[2] Enver Akalin.[1] *[1]Einstein/Montefiore Transplant Program; [2]Computational Genomics, Albert Einstein College of Medicine.*

Background: We investigated the clinical and genomic significance of microcirculation inflammation (MI) score [peritubular capillarities (ptc) + glomerulitis (g)] in transplant kidney biopsies.

Methods: 345 clinically indicated kidney transplant biopsies were classified into 3 groups G1:MI=0 (n=199), G2:MI=1 or 2 (n=94), G3:MI>2 (n=52). Gene expression profiles were studied by Affymetrix HuGene 1.0 ST expression arrays in a subset of patients (n=93).

Results: G3 and G2 groups had higher median class I (23% and 17%) and class II (41% and 30%) calculated panel reactive antibody levels and Donor Specific Antibody (DSA) frequency (56% and 40%) compared to G1 group (0%, 2%, and 26%), respectively, p<0.01. Acute Antibody Mediated Rejection (AMR) were more frequent in G3 (10%) compared to G2 (3%) and G1 (0%), p<0.01. Chronic AMR and transplant glomerulopathy (TGP) were more frequent in G3 and G2 (40% and 41%) compared to G1 (13%), p<0.01. The Banff tubulitis, intimal arteritis, allograft glomerulopathy and ptc C4d staining scores were significantly higher in G3 (0.75, 0.22, 0.73 and 1.33) and G2 (0.43, 0.09, 0.61 and 0.59) compared to G1 (0.10, 0.01, 0.15 and 0.16), respectively, p<0.01. Gene expression profiles revealed increased intragraft Interferon-Gamma and Rejection Induced (GRIT), Quantitative Cytotoxic T-cell (QCAT), Natural Killer Cell (NKAT), T Regulatory (TREG) and Donor Specific Antibody Selective Transcripts (DSAST) in G3 compared to G2 and G1. QCAT and DSAST were the only increased pathogenesis based transcripts (PBT) studied in G2 samples compared to G1 samples. Endothelial cell associated transcript (ENDAT) expression was not different between the 3 groups.

PBT	G2 VS G1	G3 VS G1	G3 VS G2
GRIT	0.059	**0.01**	**0.01**
QCAT	**0.016**	**0.003**	**0.007**
TREG	0.08	**0.03**	**0.03**
BAT	0.09	**0.04**	0.10
NKAT	0.14	**0.037**	**0.02**
DSAST	**0.015**	**0.001**	**0.007**
ENDAT	0.40	0.17	0.10

Conclusions: Our results indicated that increased MI score is significantly associated with histological diagnosis of acute and chronic AMR/ TGP, positive C4d staining and increased intragraft gene transcripts related to AMR.

FR-PO437

C4d+ Endothelial Microparticles: A Highly Sensitive and Specific Noninvasive Biomarker of Acute Antibody-Mediated Rejection (AAMR) Behzad Najafian,[1] Aleksandra Kukla,[2] Nicolae Leca,[1] Niamh Kieran,[1] Kimberly A. Muczynski,[1] J. Ashley Jefferson,[1] Elizabeth A. Kendrick,[1] Christopher D. Blosser,[1] Paul Warner,[3] Krena A. Nelson.[3] *[1]Univ of Washington; [2]Univ of Minnesota; [3]Puget Sound Blood Center.*

Background: Antibody mediated rejection (AMR) is a major cause of kidney transplant (KTX) loss. Currently, AMR diagnosis depends on KTX biopsy (Bx). Injured cells release microparticles (MP). We hypothesized that since AAMR is associated with endothelial (endo) injury and endo C4d staining in Bx, AAMR is associated with increased C4d+ endo (C4d+/CD144+) MP, abbreviated EMP+.

Methods: Blood was collected prior or shortly after for cause KTX Bx from 62 patients, including 18 with AAMR and 44 with no AMR (NAMR) based on Bx and donor specific antibody (DSA). Platelet poor plasma was prepared, stained with anti-CD144 and C4d antibodies and studied for MP using flow cytometery. Results were compared with samples from 23 healthy subjects.

Results: Densities of total MP, CD144+, C4d+ and C4d+/CD144+ MP (EMP+) were greater in AAMR than NAMR and controls (P<0.00001), and greater in NAMR than controls (p=0.004). Importantly, there was no overlap between EMP+ density in AAMR and other groups. As such, a cut off of 3100 EMP+/uL had 100% sensitivity and specificity to diagnose AAMR with a perfect receiver operation characteristics curve (area under the curve =1.0) in this data set. Paired pre/post AAMR treatment samples from 10 patients with GFR improvement from 28±14 (pre) to 39±16 (post) ml/min/1.73 m^2 (p=0.006) showed reduced EMP+ density from 11,365±8,075 to 3,916± 3,031 /mL (p=0.02). EMP+ density inversely correlated with GFR in all KTX subjects (r=-0.26, p=0.045). EMP+ density was greater in Bx with Banff g2 or g3 compared to g0; ptc2 or ptc3 compared to ptc0; and cg2 or cg3 compared to cg0, confirming relationships between EMP+ and AMR Bx findings.

Conclusions: Our data identify EMP+ as a highly sensitive and specific biomarker of AAMR diagnosis with potential for treatment efficacy assessment and possible prognostic value. Further studies are needed to prepare for clinical use of EMP+ which may lead to early, non-invasive diagnosis of AMR with valuable implications for treatment and allograft outcomes.

Funding: Private Foundation Support

FR-PO438

Comparison of a Bortezomib Based and a Standard Regimen in Antibody Mediated Rejection following Kidney Transplant Aneesha A. Shetty,[1] Yvonne El Kassis,[1] Stuart M. Flechner,[1] Andres G. Chiesa-Vottero,[2] Richard A. Fatica.[1] *[1]Dept of Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH; [2]Dept of Anatomic Pathology, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Antibody mediated rejection (AMR) is a cause of graft loss after kidney transplant. Despite limited evidence, bortezomib has been used in AMR. This study compares graft outcomes in kidney recipients with AMR treated with a standard regimen of intravenous methylprednisone (IVMP), intravenous immunoglobulin (IVIG) and plasmapheresis (PLEX), and those treated with bortezomib plus the standard regimen.

Methods: We studied 28 renal transplant recipients treated for biopsy proven AMR by Banff criteria and donor specific anti-HLA antibodies (DSA). 12 patients received IVMP, a 2-week cycle of bortezomib 1.3g/m^2/dose and PLEX on days 1-4-8-11, and 4 doses of 0.5g/kg of IVIG. 16 patients in the control group got the standard regimen without bortezomib. 8 patients in this group also received Thymoglobulin. Primary outcome was death censored graft loss at 18 months. Secondary outcomes were time to graft loss, serum creatinine (SCr) and protein creatinine ratio (PCR) at 1 year, and treatment related adverse effects.

Results: Mean follow up was 43±15 and 32±17 months in the bortezomib and control group. De novo class II DSA were more prevalent than class I in both groups. Patients in the bortezomib group had a more favorable histology. There was no significant difference between the 2 groups in graft loss at 18 months, time to graft loss, SCr and PCR at 1 year, and treatment related adverse effects.

	Bortezomib	Control	p
Patients (n)	12	16	
Age (years)	62±11	57±15	0.82
Males (n)	10	9	0.12
African Americans (n)	5	7	0.65
Time to AMR (months)	18 (8,35)	22 (1,70)	0.83
SCr at AMR (mg/dL)	2.2 (1.9,2.9)	4.1 (2.4,4.9)	0.02
PCR at AMR	0.3 (0.2,4.4)	0.7 (0.2,2.5)	0.64
Graft loss at 18 months (%)	69±14	56±12	0.47
SCr at 1 year (mg/dL)	2.6 (1.4,5.1)	1.8 (1.4,2.4)	0.28
PCR at 1 year	1.6 (0.2,8.3)	0.1 (0.09,2.2)	0.03

Conclusions: The bortezomib based and the standard regimen resulted in similar graft outcomes. Larger prospective studies are needed to characterize the role of bortezomib in AMR in kidney transplant recipients.

FR-PO439

Off Label Thymoglobulin Induction for Belatacept Based Calcineurin Inhibitor Sparing Regimen in Kidney Transplantation Is Associated with Lesser Risk of Acute Allograft Rejection Ashish Kataria, Barbara Bresnahan, Ehab R. Saad, Brahm S. Vasudev. *Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Belatacept, a co-stimulatory blocker of antigen-presenting cells was approved by the U.S. Food and drug administration (FDA) in 2011 for use in preventing rejection in adult kidney transplant recipients in combination with Basiliximab induction, Mycophenolate mofetil (MMF), and corticosteroids. We describe a single center experience in using Belatacept with either Basiliximab or off label Thymoglobulin induction.

Methods: Since 2006, we followed 12 kidney transplants recipients (2 cadaveric and 10 live donors) which received Belatacept as their initial (de-novo) immunosuppression medication. The decision to use Belatacept was based on the discretion of the transplant nephrologist. 7 patients received Thymoglobulin for induction therapy while 5 patients received Basiliximab. All patients also received steroids and Mycophenolate mofetil.

Results: After a maximum follow up period of up to 8 years, we encountered 4 episodes of acute cellular rejection (all within first 3 months of transplant); 3 in the basiliximab induction group (60%) and 1 in thymoglobulin induction group (14%). One patient had Banff grade IIb vascular rejection in the basiliximab group. These rejection episodes were treated with intravenous steroid with or without Thymoglobulin. All patients responded and there was no allograft loss. There was no episode of antibody mediated rejection. We encountered a low incidence of opportunistic infections; BK nephropathy in 2 patients and Cytomegalovirus (CMV) viremia in 1. There was no post-transplant lymphoproliferative disorder (PTLD) observed. Allograft and patient survival is 100% and mean eGFR after the follow up period is 70 ml/min/1.73m^2.

Conclusions: Belatacept is a novel agent in the CNI free anti- rejection regimen. It's off label use with thymoglobulin may provide a lower rate and less severe acute rejection episodes without an increased incidence of opportunistic infections or malignancies. Randomized studies in this regard are the need of present time.

FR-PO440

Single Center Experience on Comparisons of Alemtuzumab and Thymoglobulin as Induction Immunosuppressant Sandesh Parajuli, Ali Olyaei, Joshua Wiegel, Eric D. Langewisch, Douglas J. Norman. *Oregon Health & Science Univ.*

Background: Induction therapy is the standard of care at a majority of kidney transplant centers. Different induction agents are used depending on patient risks and clinician preference. The benefits of induction agents come with the risk of increased rates of infections and malignancy. In today's practice, commonly used induction therapy in immunological high risk patients are Alemtuzumab (C1H) or Thymoglobulin (rATG).

Methods: This was a prospective study of 58 immunological high risk patients transplanted between 2011 and 2013 with a minimum of six months follow up. Twenty five patients received one dose of 30 mg C1H and 33 patients received rATG, 6mg/kg in four divided doses. There was one early allograft loss due to recurrence of disease. All patients received tacrolimus, mycophenolate mofetil and prednisone for maintenance. The primary end point was biopsy-confirmed acute rejection. Secondary endpoints were BK viremia and cytomegalovirus (CMV) infections at six months.

Results: There were total of 58 patients, 25 in C1H group and 33 in rATG group. Common causes of end stage renal disease in both groups were diabetes, hypertension and chronic glomerulonephritis. Average age at transplant was 50 in C1H and 47 in rATG group (P=NS). White race was predominant among donors and recipients in both groups. Average duration on dialysis was 32.2 for C1H and 31.3 months for rATg (P=NS). 72% and 88% of transplants were deceased donors in C1H and rATG respectively (P=NS). Rejection rates within 3 months were fewer in C1H v rATG groups, 20% v 44% (p<0.005). There were no differences in serum Cr at 3 months. The mean WBC count was lower in the C1H group v in the rATG group (5.1 v 6.4) (P<0.005) at three months. The incidence of BK and CMV were slightly higher in rATG group at six months but this was not statistically significant.

Conclusions: In our single center experience, biopsy proven acute rejection was significantly lower in the C1H group. Adverse event rates were similar in both groups. Other advantages of C1H induction include single-dose administration and lower drug cost [$11,500 saving per transplant].

FR-PO441

Induction Therapy with Alemtuzumab for Deceased and Living Renal Transplant Recipients. A Single Centre Experience Pradeep Vaitla,[1] Antonio G. Jimenez,[1] Ryan C. Mascarenhas,[1] Abdul Moiz,[1] George E. Loss,[2] Stephanie Anders,[3] Jorge C. Garces,[1] Humberto Bohorquez.[2] *[1]Nephrology, Ochsner Clinic Foundation, New Orleans, LA; [2]Transplant Surgery, Ochsner Clinic Foundation; [3]Pharmacy, Ochsner Clinic Foundation.*

Background: Antibody induction therapy has been used for induction in upto 80% kidney transplant recipients according to United Network for Organ Sharing. It has been established by multiple studies that induction therapy reduce the incidence and severity of acute rejection episodes, which may improve graft survival. Most transplant centers across the country use antibody induction with rabbit antithymocyte globulin or Alemtuzumab to minimize maintenance immunosuppression. We are presenting our experience with Alemtuzumab (30 mg iv single dose) in relation to graft survival and acute rejection rates. Our current protocol for immunosuppression includes Tacrolimus target trough level between 7-10 ng/ml, Mycophenolate mofetil (MMF) 500 mg oral twice daily, methylprednisolone 500 mg post opertive day (POD) 0, 250 mg POD 1 and 125 mg POD 2 with rapid steroid withdrawal.

Methods: We performed a retrospective chart review of of 441 kidney transplants performed from November 2007 to December 2011 of which 311 recipients received Alemtuzumab for induction immunosuppression and we obtained demographic, clinical data including biopsy proven acute rejection during one year follow up.

Results: The incidence of biopsy proven acute rejection during the one year follow up was 8% (25 of 311 patients). According to Banff classification 10 patients had type I and II rejection, 5 patients had type II alone, 4 patients had type I alone, 4 patients had type I,II and anti body mediated rejection (AMR), 2 patients had AMR alone. By the end of study period 0.3% (9) patients had graft failure requiring renal replacement therapy. Out of 311 patients induced with Alemtuzumab 0.12% (4) patients died during the study period.

Conclusions: In our experience with Alemtuzumab, using maintainance therapy of Tacrolimus, MMF and rapid steroid withdrawal (POD 2) is an effective agent with rejection rate of 8% and graft survival rate of 99% at one year post transplant.

FR-PO442

Induction with Alemtuzumab and Outcomes of Renal Allograft Transplants in African American Recipients Pradeep Vaitla,[1] Ushma Patel,[3] Antonio G. Jimenez,[1] Ryan C. Mascarenhas,[1] Abdul Moiz,[1] George E. Loss,[2] Stephanie Anders,[3] Ari J. Cohen,[2] Catherine G. Staffeld-Coit,[1] Jorge C. Garces,[1] Humberto Bohorquez.[2] *[1]Nephrology, Ochsner Clinic Foundation; [2]Transplant Surgery, Ochsner Clinic Foundation; [3]Pharmacy, Ochsner Clinic Foundation.*

Background: Induction with Almetuzumab is well documented in literature to reduce the incidence and severity of acute rejection episodes, which may improve graft survival. Not much data is available in African american population. We are presenting our experience with Alemtuzumab (30 mg iv single dose) in African american population. Our current protocol for immunosuppression includes Alemtuzumab (30 mg iv single dose),Tacrolimus target trough level between 7-10 ng/ml, Mycophenolate mofetil (MMF) 500 mg oral twice daily, methylprednisolone 500 mg post opertive day (POD) 0, 250 mg POD 1 and 125 mg POD 2 with rapid steroid withdrawal.

Methods: We performed a retrospective chart review of of 441 kidney transplants performed from November 2007 to December 2011 of which 311 recipients received Alemtuzumab for induction immunosuppression and we obtained demographic, clinical data including biopsy proven acute rejection during one year follow up.

Results: The incidence of biopsy proven acute rejection during the one year follow up was 8.8% (13 of 147) in African americans compared to 8% (25 of 311) in the study population. Incidence of graft failure by end of study period, in African american population was 0.27% (4 of 147) compared to 0.28% (9 of 311) in the study population.

Conclusions: In our experience Alemtuzumab is an effective agent with similar rejection rate and allograft survival in African American and non-African American population.

FR-PO443

A Prospective Study of Acute Clinical and Subclinical Rejection in a Rapid Steroid Withdrawal Protocol Rajil B. Mehta, Riyaj A. Kasekar, Puneet Sood, Chethan M. Puttarajappa, David Rothstein, Nirav A. Shah, Christine Wu, Sundaram Hariharan. *Medicine, Univ of Pittsburgh Medical Center, Pittsburgh, PA.*

Background: To estimate the incidence and risk factors for acute clinical rejection (ACR) and subclinical rejection (SCR) and evaluate response to therapy in a rapid steroid withdrawal protocol.

Methods: We prospectively followed 231 adults who underwent kidney transplantation between 1/1/2013 - 2/28/2014 at our center. Patients received induction with thymoglobulin (93%), simulect (6%) or campath (1%), CNI/MPA maintenance therapy and rapid steroid withdrawal by day 7. Protocol biopsy was performed at 3 months post transplant. Eight patients were excluded from the study due to early graft failure or death. The remaining 223 patients were divided into 4 groups. Grp I (n=27) had SCR on 3 month protocol biopsy and received therapy. Grp II (n=101) had no rejection. Grp III (n=25) had ACR on for cause biopsy and Grp IV (n=70) did not undergo biopsy for various reasons. Banff classification was followed for biopsy grading and patients were treated per our center protocol. Recipient and donor demographics, transplant and post transplant variables and serum creatinine (3 and 6 months) were compared using t-test, ANOVA or chi-square test as indicated.

Results: The incidence of SCR was 17.3% (27/153) and ACR was 11.2% (25/223). The distribution of variables is as shown. The incidence of DGF and CIT were higher in Grp I (SCR). Serum creatinine was higher at 3 and 6 mths in the ACR grp.

Groups (n)	I (27)	II (101)	III (25)	IV (70)	p value
Mean age (yrs)	52	50	48	55	0.23
Gender (M/F)	17/10	57/44	11/14	28/32	0.73
Race (W/AA/O)	23/4/0	78/20/3	19/6/0	61/8/1	0.0001
Tx type (LD/DD)	7/20	54/47	11/14	14/56	0.009
Donor age (yrs)	49.4	39.4	39.8	38.2	0.82
CIT (mins)	510	353	372	504	**0.04**
HLA DR mm	1.25	1.18	1.44	1.27	0.96
PRA	9.4	18.5	10.4	18.4	0.22
DGF (Y/N)	9/18	8/93	4/21	14/56	**0.008**
SCr at 3 mths	1.47	1.38	*2.17	1.3	**0.001**
SCr at 6 mths	1.51	1.39	*1.54	1.21	***0.0041**
Banff Gr (Bord/IA or greater)	5/22	NA	5/20	NA	NA

Conclusions: The incidence of SCR was 17.5% at 3 months in our study.CIT and DGF were higher in patients with SCR. Serum creatinine at 3 and 6 mths was higher in ACR grp.

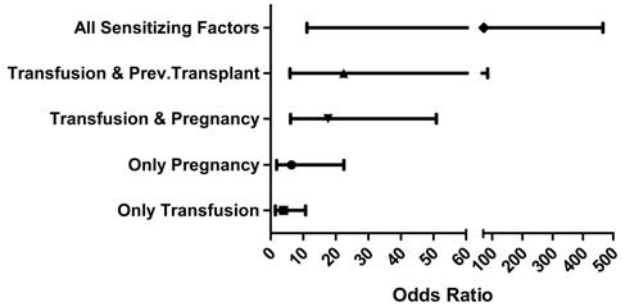
FR-PO444

Impact of Pre-Transplant Exposure to Allosensitizing Factors on the Generation of Anti-HLA Antibodies in Luminex Era Araminta Guichard, Lluvia A. Marino-Vazquez, Carlos Norman Velazquez Gutierrez, Norma O. Uribe-Uribe, Josefina Alberú, Luis E. Morales-Buenrostro. *Nephrology, Pathology & Transplantation, National Inst of Medical Sciences and Nutrition Salvador Zubiran, Mexico City, DF, Mexico.*

Background: Most studies of allosensitization were performed prior to the use of erythropoietin, new techniques of cell separation (leukoreduction) and prior to the use of Luminex to measure anti-HLA antibodies. So, the main causes of allosensitization demonstrated by these studies were transfusions, pregnancy, and transplantation of organs and tissues. OBJECTIVE: To establish the frequency of current exposure to sensitizing factors and their association with the presence of pre-transplant HLAabs measured by Luminex, a highly sensitive and specific technique.

Methods: Retrospective cohort study from January/2004 to December/2013. Variables of age, gender, %PRA and allosensitizing factors were included. Odds ratio were calculated for each sensitizing factor.

Results: In this period, 444 kidney transplants were performed and 430 (96.8%) were included. The mean age 34.7 ± 12.5 years, 56.7% males, 70.4% from living donor, and 94.4% first transplant. Of the 430 patients, 57.4% received transfusions which was associated with higher %PRA levels for both class I HLAabs ($p < 0.001$) and class II HLAabs ($p = 0.002$) compared with those negative. Only 5.6 % had a previous transplant and had higher levels of %PRA class I and II ($p < 0.001$). Of 186 women, 48.3% had one or more pregnancies, which was associated with higher %PRA levels of class I ($p < 0.001$), it showed trend for class II ($p = 0.052$). The risk to developing a %PRA greater than 20% if have been exposure to a sensitizing factor is depicted in Figure.



Conclusions: Despite use of erythropoietin and leukoreduction, the HLA sensitization is high. This study demonstrates that those recognized sensitizing factors continue to be valid in the Luminex era.

Funding: Government Support - Non-U.S.

FR-PO445

Predictors of the Response to Bortezomib in Antibody Mediated Rejection following Kidney Transplant Yvonne El Kassis, Aneesha A. Shetty, Richard A. Fatica. *Dept of Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Background: Bortezomib has been used in the treatment of antibody mediated rejection (AMR) in kidney transplant patients. However reported treatment outcomes have been variable and inconsistent, making selection of appropriate candidates for this therapy challenging. This retrospective study evaluates the pre treatment predictors of the response to a bortezomib based regimen in AMR, in an attempt to establish its best use.

Methods: We identified 24 adult renal transplant recipients treated with bortezomib for biopsy proven AMR and donor specific antibody (DSA) between 2008 and 2013. Patients also received adjuvant intravenous methylprednisone (IVMP), plasmapheresis (PLEX) and intravenous immunoglobulin (IVIG). Response to bortezomib was defined as graft survival at 1 year.

Results: Mean age was 41±13 years. There were 15 males and 9 African American. 12 patients (50%) got basiliximab and 9 (38%) got anti thymocyte globulin for induction at time of transplant. At the time of AMR, 16 patients (67%) were on maintenance immunosuppression with tacrolimus, mycophenolate mofetil and prednisone. Median serum creatinine (SCr) and Protein creatinine ratio (PCR) at AMR were 2.4 (1.8, 3.9) mg/dl and 0.3 (0.1, 2.4) respectively. Concomitant acute cellular rejection (ACR) was present in 13 patients (54%). At 1 year, 5 patients (21%) were on dialysis. Induction with anti thymocyte globulin was associated with a higher risk of graft failure at 1 year (p 0.03), likely a reflection of high-risk patients receiving thymoglobulin induction at our center. Patients with SCr >3mg/dL (p=0.03), higher PCR (p=0.03) and concomitant ACR (p=0.02) at the time of AMR were more likely to be on dialysis at 1 year. Time from transplant to AMR, DSA class and titer, and adjuvant treatment regimen were not statistically significant predictors of the response to bortezomib.

Conclusions: Kidney transplant patients treated with a bortezomib based regimen for AMR may have worse outcomes if they have concomitant ACR and if they show clinical evidence of advanced graft damage. This may be an important consideration for clinical decision-making in selecting appropriate patients for bortezomib therapy.

FR-PO446

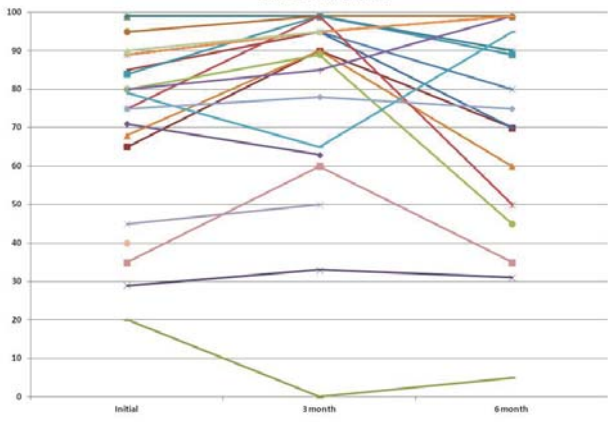
No Effect of Rituximab and IVIg on Sensitized Patients Waiting for Deceased Donor Renal Transplants Shefali Patel, Leigh-Anne Dale, Elena Rodica Vasilescu, Geoffrey K. Dube, Sumit Mohan, Lloyd Ratner, Russell J. Crew. *Columbia Univ Medical Center.*

Background: Approximately 30% of patients waiting for deceased donor renal transplant (DDRT) have anti-HLA antibodies against potential donors, leading to dramatically increased waiting times. Those sensitized patients who do receive a DDRT have higher rates of cellular (ACR) and antibody mediated rejection (AMR).

Methods: To improve access to transplantation, we attempted to desensitize 24 sensitized ESRD patients waiting for a DDRT with 2 doses of intravenous immunoglobulin (2 gm/kg in divided doses each time) separated by 4 weeks and a single dose of Rituximab 375 mg/m^2. We followed anti-HLA antibodies at 3, 6, and 12 months by cytotoxicity Panel of Reactive Antibodies (PRA) as well as by Luminex flow beads.

Results: We were unable to detect a change in PRA at any time point after desensitization.

Figure 1 - Change in PRA



However, 11/24 patients (46%) were transplanted at a mean 331 (range 26-692) days after desensitization. Transplanted patients had similar waitlist times (65.2 ± 41.6 versus 39.4 ± 30.8 months p=0.1) but lower initial PRA (63% versus 82%, p=0.038). Despite desensitization, 9/11 had donor specific antibodies (DSA) by Luminex and 6/11 had positive flow crossmatch. There was 1 allograft failure at 3 years, 1 AMR and 6 ACRs, and creatinine at 1 year=1.3 mg/dL. When compared to a contemporaneous sensitized control group matched by Luminex and flow crossmatch results, there were no differences in rejection rates, infections, or renal function.

Conclusions: We were unable to detect a decrease in PRA using IVIG and Rituximab in waitlisted patients. Nor did these patients experience a significantly different outcome compared to control sensitized patients.

FR-PO447

Clinical Outcome in Patients with Chronic Antibody-Mediated Rejection Treated with and without Rituximab and Intravenous Immunoglobulin Combination Therapy Tae Hyun Ban,[2] Byung Ha Chung,[1,2] Bum Soon Choi,[1,2] Cheol Whee Park,[1,2] Yong-Soo Kim,[1,2] Chul Woo Yang.[1,2] *[1]Transplant Research Center, Seoul St. Mary's Hospital, Seoul, Korea; [2]Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, Seoul, Korea.*

Background: We previously reported that rituximab (RTX) and intravenous immunoglobulin (IVIg) combination therapy (RIT) is effective in treating patients with chronic active antibody-mediated rejection (CAMR), and the proteinuria level can determine the response to RIT. However, the results were not compared to those of patients who did not receive RIT.

Methods: Fifty-nine patients with CAMR were divided into 2 groups: an RIT treated group (n=25) and a historic control (HC) group who had not received RIT (n=29). The RIT group was treated with RTX (375 mg/m^2) and IVIg (0.4 g/kg) for 4 days. We compared the decline in glomerular filtration rate/month (DeGFR), RIT-related complications, and allograft survival rate in both groups. We also compared the allograft survival rate between patients with high proteinuria (spot urine protein/creatinine [PC] ratio >3.5 g/g) and low proteinuria (PC ratio <3.5 g/g).

Results: DeGFR was significantly decreased in the RIT group compared with the HC group after 6 months ($P < 0.05$). No serious complications were associated with RIT, and only one case of herpes zoster infection developed. The overall allograft survival rate in the RIT group was significantly higher than in the HC group. In both groups, patients with low proteinuria survived better than patients with heavy proteinuria ($P < 0.05$). The allograft survival rate was greater in the high proteinuria RIT group than that in the HC group.

Conclusions: RIT treatment is recommended for delaying the progression of CAMR without serious complications, and is not limited by the presence of heavy proteinuria.

FR-PO448

Clinical Impact of HLA-Donor Specific Antibodies Measured By Luminex Technique in Kidney Transplant Recipients with Renal Allograft Dysfunction Ji Hyun Yu,[2] Byung Ha Chung,[1,2] Bum-Soon Choi,[1,2] Cheol Whee Park,[1,2] Yong-Soo Kim,[1,2] Chul Woo Yang.[1,2] *[1]Transplant Research Center; [2]Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

Background: In this study, we investigated the clinical impact of donor-specific anti-HLA-antibody (HLA-DSA) measured by the Luminex single-antigen assay in patients who took indicated biopsy due to allograft dysfunction.

Methods: Between Feb. 2010 and Feb. 2013, total 210 cases of indication biopsy were performed in Seoul St. Mary's hospital. In all cases, we examined the presence of HLA-DSA using Luminex technique when allograft biopsy was done. We investigated the clinical impact of the presence of HLA-DSA and the association to the pathologic finding of biopsy specimen in those cases.

Results: Anti-HLA antibody was detected in 86 cases (41%) and 39 cases (19%) were proved as HLA-DSA. HLA-DSA-DR was most common (26%) followed by HLA-DSA-B (11%) and HLA-DSA-A (8%). In comparison between HLA-DSA positive (+) group (n=39) and HLA-DSA negative (-) group (n=171), the rate of re-transplantation and the number of HLA-mismatched antigen were significantly higher (P<0.05 for each) in HLA-DSA (+) group. The number of patients who showed sensitization before KT was higher in HLA-DSA (+) group as well (P<0.05). In comparison of histologic finding, vasculitis (v), glomerulitis (g) and peritubular capillaritis (ptc) was more severe in HLA-DSA (+) group compared to HLA-DSA (-) group, hence microvascular inflammation score (g + ptc) was also higher in HLA-DSA (+) group (P<0.05). In allograft survival rate after biopsy, HLA-DSA (+) group showed significantly worse outcome compared to HLA-DSA (-) group (P<0.05). In addition, presence of HLA-DSA was independent risk factor for allograft failure in multivariate analysis.

Conclusions: HLA-DSA detected at indication biopsy is important prognostic factor for allograft outcome independent of the result of allograft biopsy and other factors.

FR-PO449

DSA Detected by ELISA-PRA Is Associated with High Risk for Development of ABMR in Stable Kidney Transplant Recipients Dong Ryeol Lee,[1] Mi Young Jeon.[2] *[1]Medicine, Maryknoll General Hospital, Busan, Republic of Korea; [2]Pathology, Marknoll General Hospital, Busan, Republic of Korea.*

Background: Donor HLA-specific antibodies (DSAs) have been implicated in poor graft outcome and decreased survival in solid organ transplantation. The purpose of this study was to evaluate the incidence of presence of DSA and the association of DSA and development of ABMR in stable kidney transplant recipients.

Methods: We screened 208 stable kidney transplant recipients by HLA-specific ELISA-PRA (panel reactive antibody) for DSA from January to December 2013. We compared the incidence of acute rejection between Group1 (DSA positive group, n=11) and Group2 (non-DSA positive, n=11). To evaluate response after ABMR treatment, ELISA-PRA levels were monitored at day 0, month 1 (M1) and month 3 (M3).

Results: 25 (12.0%) of 208 patients were positive ELISA-PRA. 22 of them were biopsied. At the time of biopsy, median total PRA was 7.5% (2.5-47.5). In Group 1, all eleven patients with positive DSAs (5 had DSA against class I, 5 had DSA against class II, one had DSA against class I and class II) were diagnosed with subclinical ABMR. ABMR was treated with 4 (4-7)) of plasmapheresis with low dose IVIG and 100mg of rituximab.

Total median PRA levels (n=11) decreased from 13.7% at M0 to 2.5% at M1 but increased 20% at M3. Class I PRA (n=6) decreased from 12.5% to 1.8% at M1 but increased 3.6% at M3. Class II PRA (n=6) decreased from 50% to 16.7% at M1 but increased 41.7% at M3. At 3month (M3) after treatment of ABMR, Three of 6 class 1 DSA positive became negative, however, none of 5 class II DSA positive became negative. In Group 2, 3 (27.3%) of 11 non-DSA positive patients had borderline change treated with steroid pulse therapy. Time to biopsy after transplantation was 6.5 (4month-14 year). The median creatinine at the time of biopsy was 1.1 (0.6-2.2) mg/dl, FK level 5.9 (4.5-8.1) ng/ml.

Conclusions: Our study demonstrated that ELISA-DSA positive has a significantly high risk for development of subclinical ABMR in stable renal transplant recipients.ELISA-PRA is a effective screening method with subsequent allograft biopsy may be useful to recognize patients are likely to develop ABMR. Long term effect of treatment for ABMR need to be determined.

FR-PO450

Intravenous Immunoglobulin and Rituximab Based Desensitization Is Ineffective for Highly Sensitized Patients Layal Abdel Rahman,[1] Pam Kimball,[2] Anne L. King,[1] Amit Sharma,[2] Adrian Cotterell,[2] Dhiren Kumar,[1] Muhammad Raza Qureshi,[1] Felecia McDougan,[2] Gaurav Gupta.[1] *[1]Nephrology, Virginia Commonwealth Univ, Richmond, VA; [2]Surgery, Virginia Commonwealth Univ, Richmond, VA.*

Background: ESRD patients with human leukocyte antigen (HLA) sensitization can have prolonged waiting times. This can result in increased morbidity and mortality. Studies focusing on non-African Americans (AA) have suggested that desensitization with rituximab/intravenous immunoglobulin (IVIG) might be efficacious.

Methods: 15 highly sensitized patients with a median calculated panel reactive antibody (cPRA) of 99% (range=69-100%) underwent desensitization. The desensitization protocol included 2 doses of IVIG (2 g/kg) on days 0 and 30 and 1 dose of rituximab (375mg/m²) on day 15. HLA antibody profiles of sera obtained before and after treatment were characterized, and flow cytometry crossmatch (FCXM) tests were performed for all kidney offers.

Results: 87% (13/15) patients were AA and 80% (12/15) patients had previous failed transplants. Twelve (out of 15; 80%) patients had no significant decline in cPRA over a follow-up of 9 months post-treatment. There was a trend towards a decline in Class I cPRA (median 64% to 48%; p=0.1) and the number of Class I unacceptable antigens (median 11 to 8; p=0.1) after treatment. Of the 3 patients who showed a clear decline in cPRA: 2 were Caucasian and 2 had a lower cPRA (mean=70%) with pregnancy as the sensitizing event. Eight (out of 15; 53%) patients got transplanted at a median of 11.8 months post-desensitization. Among these patients, retrospective FCXM testing using pre-treatment samples showed an unchanged FCXM suggesting that all patients would have been eligible for a transplant with their respective donors even before desensitization.

Conclusions: In this small single-center study we were unable to demonstrate successful use of Rituximab/IVIG for most AA sensitized patients. It is possible that this protocol might have some efficacy in non-AA patients with a cPRA<80% and with primarily Class I sensitization. The high rate of transplantation seen in our study is likely due to an increased threshold for accepting a borderline positive FCXM.

FR-PO451

Rescue of Mutant Laminin by Combined Treatment with a Chemical Chaperone and a Proteasome Inhibitor Yeawon Kim, Jeffrey H. Miner, Ying Maggie Chen. *Renal Div, Washington Univ, St. Louis, MO.*

Background: Laminin beta2 encoded by *LAMB2* is a component of the laminin-521 trimer, an important constituent of the mature glomerular basement membrane (GBM). Utilizing our established cell and knock/transgenic mouse model resembling human nephrotic syndrome (NS) caused by the C321R-LAMB2 mutation, we demonstrated that defective secretion of the mutant protein from podocytes to the GBM and concomitant podocyte endoplasmic reticulum (ER) stress are crucial contributors to the development of proteinuria (*Chen Y et al., JASN 24: 1223-1233, 2013*). Moreover, the C321R mutant is functional. Here we investigated a novel therapeutic approach.

Methods: We generated stably transfected HEK293T cells expressing fusion proteins in which the N-terminal fragments of wild-type (WT) or mutant beta2 were fused to Gaussia luciferase (Gluc). Secretion of the fusion proteins is directed by the beta2 signal peptide, and trafficking of the fusion proteins can be analyzed by luciferase assay.

Results: The steady-state level of C321R/Gluc was increased after proteasome inhibition by MG-132 for 24h compared to that of WT/Gluc. In addition, treatment with MG-132 induced a striking accumulation of polyubiquitinated C321R mutant protein, but much less of the WT protein, indicating that a much greater fraction of the mutant protein is indeed ubiquitinated and degraded by the proteasome. These results suggest that the ubiquitin-proteasome system has been activated by ER stress induced by the C321R mutation. Previously, we showed that a chemical chaperone tauroursodeoxycholic acid (TUDCA) enhanced secretion of the C321R mutant into the medium. In the current study, when combined with MG-132, TUDCA elicited an even more robust effect on secretion of the mutant protein: a 35.2 fold increase compared to vehicle control, and a 3.6 fold increase compared to TUDCA alone.

Conclusions: Currently there is no effective treatment for genetic forms of NS. Our results suggest that therapeutic strategies aimed at modulating both protein misfolding and degradation could lead to a significant leap forward in the treatment of certain loss-of-function types of NS.

Funding: NIDDK Support, Private Foundation Support

FR-PO452

AT1R Blockade during Adverse Milieus: Role of SMRT and Co-Repressor Complexes Kamesh R. Ayasolla,[1] Nirupama Chandel,[1] Rivka Lederman,[1] Guohua Ding,[1] Praveen N. Chander,[2] Ashwani Malhotra,[1] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Pathology, New York Medical College, Valhalla, NY.*

Background: Since AT1R blockade is associated with elevated renal tissue levels of renin, use of 1, 25$[OH]_2$D (VD) in combination with AT1R blockade has been demonstrated to provide better outcome in experimental animal models of diabetic nephropathy. We hypothesized that AT1RB would provide podocyte protection through silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and VD receptor (VDR) expressions in adverse milieus (AMs) such as high glucose and HIV.

Methods: Human podocytes (HPs) were treated with losartan (AT1RB) or VDR agonist (VDA) with or without AMs (48h) and evaluated for mRNA expressions of Cytochrome P450 Family 24 (CYP24) and VDR and protein expressions of co-repressor and co-activator complexes. Protein interactions in these complexes in response to AT1RB and VDA were evaluated by immunoprecipiatation (IP) studies followed by Western blot analysis.

Results: Both AT1RB and VDA enhanced podocyte VDR expression but they exhibited disparate effects on CYP24A1 and acetylated histone (AC-H) 3 expressions. Analysis of AT1RB- induced protein interaction complexes revealed presence of VDR, SMRT, and phospho-HDAC3; whereas, VDA-induced protein interaction complexes displayed presence of VDR and CBP/p300. AT1RB induced complexes attenuated expression of AC-H3 and thus indicating their deacetylase properties. Repressive nature of AT1RB complex was also manifested by its effect on podocyte CYP24A1 expression. However, net podocyte CYP24A1 expression depended on repression by co-repressor complex and presence of VD in the media. AT1RB-induced podocyte SMRT expression inhibited pro-apoptotic gene expression through down regulation of Wip1 and phosphorylation of Chk2. Since podocyte SMRT knockdown lacked AT1RB mediated protection against DNA damage, it appears SMRT is critical for DNA repairs during AT1RB.

Conclusions: AT1RB provides podocyte protection in AMs predominantly through SMRT expression and partly through unliganded VDR expression in VD deficient states, whereas, it contributes to liganded VDR expression in VD sufficient state.

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FR-PO453

Vegfa Expression by the FoxD1 Lineage Is Critical for Adequate Glomerular Development and Function Henrik Dimke,[1,2] Yoshiro Maezawa,[1] Rizaldy P. Scott,[1] Paul S. Thorner,[3] Yashpal S. Kanwar,[4] Susan E. Quaggin.[1,5] *[1]The Lunenfeld-Tanenbaum Research Inst, Mt. Sinai Hospital, Toronto, ON, Canada; [2]Dept of Cardiovascular and Renal Research, Univ of Southern Denmark, Odense, Denmark; [3]Dept of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada; [4]Depts of Pathology and Medicine, Northwestern Univ, Chicago, IL; [5]Feinberg Cardiovascular Research Inst and Div of Nephrology and Hypertension, Northwestern Univ, Chicago, IL.*

Background: Intraglomerular mesangial cells (GMC) lie in close contact with the fenestrated endothelial cells that line the capillary loops. It is well established that endothelial cells communicate with GMCs by secreting platelet-derived growth factor; however, cytokines generated by GMCs may also convey signals to endothelial cells, although such signaling pathways remain poorly defined. Here we explored whether mesangial Vegfa could play a role in cross-communication between GMCs and endothelial cells.

Methods: Transgenic mice carrying forkhead box D1 (FoxD1)-driven Cre recombinase were used to excise *Vegfa* from vascular mural cells and GMCs.

Results: Elimination of Vegfa from the FoxD1 lineage ($Vegfa^{FoxD1}$) resulted in the onset of proteinuria at postnatal day (P) 14. Kidneys from $Vegfa^{FoxD1}$ appeared smaller at P0, while mild to moderate expansion of the mesangium was noted at P7. Structurally, thickening of the glomerular basement membrane was visible by electron microscopy at P7, which was confirmed by silver stains. At P14, moderate to severe expansion of the mesangium was evident, with obvious thickening of glomerular basement membranes, obliteration of capillary lumina, loss of endothelial cells and accumulation of subendothelial deposits. Progressive worsening of the phenotype was evident at P21, at which time glomeruli appeared hypocellular with collapse of capillary tufts.

Conclusions: Production of *Vegfa* from cells within the FoxD1 lineage is necessary for proper development and maintenance of the glomerular filtration barrier. The present data suggest that Vegfa produced by GMCs may play a key role in cross-communication between the GMC and endothelial compartments.

Funding: Government Support - Non-U.S.

FR-PO454

Fine-Tuning of NFkB by Glycogen Synthase Kinase (GSK) 3b Dictates the Fate of Glomerular Podocytes Upon Injury Hui Bao,[1,2] Yan Ge,[1] Ai Peng,[2] Rujun Gong.[1] *[1]Nephrology, Brown Univ; [2]Nephrology, Shanghai Tenth People's Hospital, Tongji Univ, China.*

Background: NFkB acts as a double-edged sword in the pathogenesis of podocytopathy. In addition to directing the transcription of a multitude of mediators involved in podocyte injury, including proinflammatory cytokines as well as cytoskeletal remodeling molecules like lysosomal protease cathepsin L and co-stimulatory molecule B7-1, NFkB is also an

essential survival factor for cellular response to stress or injury for self-protection. The activity of NFkB is controlled by a myriad of signaling cascades and the effect of GSK3b on NFkB activation in podocytes was examined.

Methods: Podocyte injury was induced by liposaccharide (LPS) in cultured podocytes and in mice. The effect of GSK3b on NFkB activation was examined.

Results: In cultured podocytes, LPS elicited cytoskeletal disruption and hypermotility, associated with NFkB activation and induced expression of NFkB target molecules, including prosurvival Bcl-xL and podocytopathic mediators like MCP-1, cathepsin L and B7-1. Broad range inhibition of NFkB by PDTC or TPCK diminished the expression of all NFkB target genes, restored cytoskeleton integrity, but potentiated apoptosis. In contrast, inhibition of GSK3b by lithium or TDZD-8 selectively obliterated the expression of podocytopathic mediators, ameliorated podocyte injury, reinstated cytoskeleton integrity, but barely affected Bcl-xL expression or sensitized apoptosis. Mechanistically, GSK3b was sufficient and essential for phosphorylation of murine RelA/p65 specifically at serine 467 and thereby directed NFkB binding to MCP-1, cathepsin L and B7-1 genes, but not to Bcl-xL gene. *In vivo*, lithium or TDZD-8 treatment improved proteinuria and podocyte injury in LPS injured mice, concomitant with mitigated expression of MCP-1, cathepsin L and B7-1 but largely retained Bcl-xL expression. Contrarily, TPCK therapy conferred a similar but much blunted anti-proteinuric and podocyte protective effect, accompanied with marked podocyte apoptosis and suppression of glomerular expression all NFkB dependent molecules, including Bcl-xL.

Conclusions: The GSK3b dictated fine-tuning of NFkB might serve as a novel therapeutic target for podocytopathy.

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FR-PO455

Shank2 Impacts Endosome Abundance and Rates in Renal Epithelial Cells

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Background: Shank2 is a scaffolding protein that has been associated with endocytic trafficking of epithelial transport proteins in proximal tubule (PT) cells. Shank2 co-migrates with NaPiIIa during endosomal trafficking to the lysosomal compartment. The present studies utilized Shank2 knockout (KO) mice to determine the significance of Shank2 in the endocytic pathways.

Methods: Electron microscopy, intensity correlation coefficient (ICQ) analysis and Western blot analysis were used to examine endocytosis in Shank2 wild type and KO renal epithelial cells.

Results: Electron microscopy analysis of renal proximal tubule cells found Shank2 KO mice have significantly decreased amounts of endocytic vesicles in the proximal tubules. Like PT cells, renal podocytes actively endocytose proteins, including albumin. Western blotting and immunofluorescence studies show that Shank2 is expressed by podocytes *in vivo* and in culture. In cultured human urine-derived podocytes, intensity correlation coefficient analysis demonstrates that Shank2 colocalizes with endocytosed FITC-albumin, early endosome compartments (EEA1) and lysosomes (LAMP1). Lentiviral shRNA knockdown of Shank2 in cultured human podocytes diminished Shank2 levels by 64±16% but failed to demonstrate a significant decrease in albumin uptake (Shank2 knockdown cells had 49±8% less FITC-albumin compare to control shRNA-infected cells at 2 hrs of uptake at 37°C, n=3, p=0.06). To evaluate the impact on albumin uptake in the complete absence of Shank2, primary podocytes were isolated from Shank2 KO mice and cultured. Primary mouse podocytes express the podocyte markers podocin and WT-1. When compared to wild type podocytes, cells from Shank2 KO mice have significantly diminished rates of albumin endocytosis.

Conclusions: Measuring changes in both endosome abundance in PT cells and endosomal uptake of albumin in podocytes, the present studies are the first to directly demonstrate a functional role for Shank2 on the endosomal pathways in epithelial cells.

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FR-PO456

SIRT1 Maintains Actin Cytoskeleton by Deacetylation of Cortactin in Podocytes

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Background: Recent studies have highlighted the renoprotective effect of SIRT1. However, its molecular mechanism in podocytes is still unclear. We therefore investigated the function of SIRT1 in podocytes.

Methods: We established podocyte-specific *Sirt1* knockout ($SIRT1^{pod-/-}$) mice and induced glomerular injury by injection of anti-GBM antibody, and histological and functional analyses were performed. The expression of podocyte specific proteins was assessed by western blot analysis or immunofluorescent study. Immortalized murine podocytes and the SIRT1 inhibitors/activator were utilized to assess the effect of SIRT1 to podocytes. Furthermore, the association between SIRT1 and cortactin was investigated *in vivo* and *in vitro*.

Results: Seven days after glomerular disease induction, u-alb/cre, BUN and the ratio of glomerular injury in $SIRT1^{pod-/-}$ mice were significantly higher compared with wild-type mice. Consistently, significant decrease in podocyte-specific molecules was shown in $SIRT1^{pod-/-}$ mice. Electron microscopy revealed the exacerbation of foot process effacement and actin cytoskeleton derangement in $SIRT1^{pod-/-}$ mice. Similarly, actin cytoskeleton derangement in H_2O_2 (mediator of anti-GBM glomerulonephritis)-treated cultured podocytes became prominent by pretreating with SIRT1 inhibitors, while it was ameliorated by a SIRT1 activator. Furthermore, we assessed the link between SIRT1 and cortactin, which acts to support actin cytoskeleton. We revealed that SIRT1 deacetylated cortactin in the nucleus in podocyte and that the deacetylated cortactin was transported to the cytoplasm for maintenance of actin cytoskeleton. We confirmed this mechanism using cortactin siRNA or leptomycinB, nuclear export inhibitor.

Conclusions: SIRT1 regulates the functional state of cortactin by deacetylation, and thereby maintains actin cytoskeleton integrity in podocytes.

FR-PO457

Arp3 Mediated Actin Branching Controls Podocyte Cortical Tension and ECM Interaction via Modulation of the Actomyosin Machinery

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Background: Podocyte foot processes (FP) are essentially involved in the formation of the glomerular filtration barrier and provide at the same time tight adherence of the podocyte to the underlying GBM. We´ve previously demonstrated that N-WASP mediated actin branching is involved in the stabilization and maintenance of podocyte FPs. However, it remained so far unaddressed, whether and how actin branching might influence podocyte-GBM interaction.

Methods: We generated a set of complementary conditional mouse models allowing for deletion of N-Wasp and Arp3 at decisive developmental stages of podocytes. Furthermore, a novel primary podocyte culture system with proven genetic origin was employed to investigate cellular functions of either N-WASP or ARP3.

Results: Deletion of N-Wasp at different developmental stages demonstrated only minor alterations in podocyte FP morphology at first. Interestingly, also deletion of Arp3 as an ultimate effector of N-WASP resulted in comparable phenotypes. Analysis of Arp3 deficient primary podocytes showed a decreased spreading capacity, migrational speed and altered focal adhesion morphology. Employing traction force microscopy revealed that Arp3 deficient podocytes exert higher force levels compared to control cells and display an activated actomyosin machinery. Inhibition of the actomyosin machinery reversed the inefficient protrusion formation of Arp3 deficient cells in 3D culture conditions.

Conclusions: Our observations demonstrate that the mechanism of actin-branching is not essentially required for intial podocyte FP assembly, but rather for the stabilization of FPs under mechanical stress. Furthermore, we provide evidence for a novel link between ARP2/3 dependent actin networks, the actomyosin machinery and podocyte-GBM interactions. These findings further deepen our global understanding of actin dependent podocyte FP function and plasticity.

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FR-PO458

Rac1 Protects against Podocytes Injury Induced by DNA Damage

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Background: Podocytes are specialized epithelial cells that cover the outer surface of glomerular basement membrane and maintain the integrity of glomerular filtration barrier. Podocytes Injury in rodent models results in podocytes loss and proteinuria, and/or even focal segmental glomerulosclerosis. Rac1 is a member of small GTPase family, whose dysfunction is associated with podocytes injury and proteinuric kidney diseases. Mice with induced Rac1 expression in the podocytes develop foot process effacement and transient proteinuria.

Methods: In this study, we have generated transgenic zebrafish expressing either constitutive-active or dominant-negative Rac1 in the podocytes using Gal4-UAS system. No gross abnormality was seen either in the embryos or adults of these transgenic lines. To characterize the role of Rac1 in podocytes injury, we used an inducible podocytes injury model in which metronidazole (MTZ) is converted to a DNA damaging agent by the bacterial nitroreductase expressed specifically in podocytes of the transgenic zebrafish and DNA damage leads to podocyte apoptosis, which resembles adriamycin-induced podocyte injury in mice. Upon administration of MTZ, the transgenic zebrafish embryos developed varying degrees of peri-orbital edema and abdominal edema due to podocyte loss in a dosage-dependent manner.

Results: Inhibition of Rac1 activity by dominant-negative Rac1 significantly accelerates the onset of orbital edema while expression of constitutive-active Rac1 ameliorates the edema phenotype. This indicates that Rac1 plays a role in protecting podocytes against injury due to DNA damage. Consistently, administration of Rac1 inhibitor NSC23766 also aggravates podocytes injury in our model system. On the other hand, treatment with mineralocorticoid receptor inhibitor eplerenone does not affect the severity of MTZ-induced podocytes injury.

Conclusions: Taken together, our results unveil a distinct role of Rac1 in regulating podocytes apoptosis and suggest that modulating Rac1 pathway may be beneficial to podocytes under certain adverse or disease conditions.

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FR-PO459

Dynamin1 Regulates Actin Cytoskeleton in Podocytes in a Phosphorylation Dependent Manner Changkyu Gu,[1] Mario Schiffer,[2] Sanja Sever.[1] [1]*Nephrology, Massachusetts General Hospital, Charlestown, MA;* [2]*Nephrology, Hannover Medical School, Hannover, Germany.*

Background: Dynamin is an essential actin regulatory protein in podocytes, and loss of its function is closely connected to podocytes damage and proteinuria. Recently, our data has shown that dynamin directly regulates actin cytoskeleton via its oligomerization state. Therefore, it is important to maintain balance between dynamin assembly and disassembly. This dynamin oligomerization can be regulated through interaction with diverse cellular proteins, and it is reported that dynamin1 can differentially alter the affinity for its protein binding partners via phosphorylation by two different serine/threonine kinases, GSK3b and CDK5, in neurons. Based on these data, we hypothesize that phosphorylation-dependent dynamin1 oligomerization is an important molecular mechanism that regulates actin dynamics in podocytes.

Methods: Dynamin1 phosphorylation in podocytes was detected by western blot using phospho- dynamin1 antibodies in the presence of GSK3b or CDK5 inhibitor. Actin and paxillin in podocytes were stained to observe actin structures and focal adhesions. Cell migration and spreading assays were performed with podocytes expressing phosphor-dynamin1 mutants. For zebrafish proteinuria experiments, each phosphor-dynamin1 mutant was expressed in dynamin2^{KD} zebrafish.

Results: 1. Dynamin1 is phosphorylated by GSK3b and CDK5 in podocytes. 2. Expression of phosphor-dynamin1 mutants affects cell migration. 3. Expression of phosphor-dynamin1 mutants alters cortical actin cytoskeleton during cell spreading. 4. Expression of phosphor-dynamin1 mutants fails to rescue proteinuria in dynamin2^{KD} zebrafish.

Conclusions: The role of dynamin in actin cytoskeleton is essential to maintain the filtration barrier function of podocytes. Dynamin directly regulates actin structures via its oligomerization state. Our data suggest that dynamin1 phosphorylation is implicated in cortical actin dynamics of motile cells, possibly through regulating its oligomerization state via altering the affinity for its protein binding partners.

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FR-PO460

IQGAP1, a Downstream Effectors of Nephrin, Contributes to Actin Cytoskeleton Organization of Podocytes Yipeng Liu,[1] Wei Liang,[1] Yingjie Yang,[1] Qian Yang,[1] Xinghua Chen,[1] Huiming Wang,[1] Pravin C. Singhal,[2] Guohua Ding.[1] [1]*Div of Nephrology, Renmin Hospital of Wuhan Univ, Wuhan, Hubei, China;* [2]*Renal Molecular Research Laboratory, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: IQ domain GTPase-activating protein1 (IQGAP1) is a scaffolding protein involved in cytoskeleton regulation. Nephrin behaves as a cytoskeleton regulatory hub at slit diaphragm (SD) of podocytes. We hypothesized that IQGAP1 may be a downstream effectors of nephrin and contribute to actin cytoskeleton organization of podocytes.

Methods: Foot process (FP) fusion was observed by transmission electron microscope in puromycin aminonucleoside (PAN) –induced rats. Cytoskeletal reorganization was assessed in human podocytes. Cell migration and spreading assays were used to evaluate podocyte function. IQGAP1 plasmid and siRNA were introduced to investigate the role of IQGAP1 in actin cytoskeleton reorganization. Cytochalasin D-pretreated COS7 cells were used to evaluate the role of IQGAP1 phosphorylation in nephrin-related cytoskeletal regulation. The binding domain of IQGAP1 wtih nephrin was identified.

Results: The IQGAP1 expression and IQGAP1-nephrin colocalization in glomeruli were progressively suppressed and gradually recovered in line with the development of FP fusion and proteinuria in PAN-injected rats. In cultured human podocytes, PAN-induced disruption of F-actin and disorders of migration and spreading were aggravated by IQGAP1 siRNA, which was partially restored by IQGAP1 plasmid. Furthermore, the disorganized cytoskeleton treated by cytochalasin D in COS7 cells was recovered by cotransfection with IQGAP1 and nephrin plasmid, but not by single transfection of IQGAP1 nor by cotransfection of mutant IQGAP1[D1443 (S→A)] and nephrin. Co-immunoprecipitation assay in COS7 cells transfected with plasmids of truncated IQGAP1 and full length of nephrin demonstrated that the poly-proline binding domain (WW) and RasGAP domain in the carboxyl terminus (RGCT) of IQGAP1 were the target modules that interacted with nephrin.

Conclusions: Activated IQGAP1 is a downstream effectors of nephrin and contributes to actin cytoskeleton organization and functional regulation of podocytes.

FR-PO461

Podocyte-Specific Hyper-Activation of Rac1 Induces Glomerulosclerosis and Nephrotic Syndrome Richard Robins, Cindy Baldwin, Lamine Aoudjit, Indra R. Gupta, Tomoko Takano. *Nephrology, McGill Univ, Montreal, QC, Canada.*

Background: Rac1 is a member of the Rho-family of small GTPases that play critical roles in the regulation of actin cytoskeletal dynamics and cell motility of podocytes. Yu *et al*. have recently reported that podocyte specific activation of Rac1 results in transient proteinuria that rapidly recovers (Mol Cell Biol 2013). We hypothesize that podocyte-specific activation of Rac1 will result in sustained podocyte injury, foot process effacement and nephrotic syndrome in mice.

Methods: Transgenic mice carrying the tetracycline-inducible constitutively active mutant of Rac1 (L61, CA-Rac1, Flag-tagged) were bred with mice with the podocin-driven reverse tetracycline trans-activator to generate double transgenic mice (DTG). Mice aged 8-12 weeks were treated with doxycycline (Dox). Proteinuria was assessed by the albumin/creatinine ratio. Kidneys were evaluated histologically using light and immunofluorescence microscopy. Glomerular lysates were used for immunoblotting.

Results: After 5 days of Dox treatment, 8/12 DTG mice demonstrated heavy proteinuria which persisted for the duration of treatment (1 month), while 0/24 control mice developed proteinuria. In DTG treated with Dox for 1 month, FLAG staining was confirmed in podocytes by co-staining with WT1. 2/3 DTG mice displayed severe sclerosis, while 1 was mostly normal. Quantification of glomerulosclerosis with scores (normal=1 and global sclerosis=4) showed a significant increase of glomerulosclerosis in DTG mice (1.04 ± 0.02 for control versus 1.78 ± 0.3 for DTG; 45 glomeruli from 3 mice each, $p<0.05$). Podocyte number counted by WT1+ nuclei was significantly decreased in DTG (10.2 ± 0.21 for control versus 6.7 ± 0.4 for DTG; 30 glomeruli from 3 mice each, $p<0.001$). DTG mice showed synaptopodin and nephrin loss by immunofluorescence staining and immunobloting. In contrast, the podocyte injury marker, phospho-p38 was increased in glomerular lysates of DTG mice.

Conclusions: Podocyte-specific hyper-activation of Rac1 caused rapid and persistent proteinuria resulting in podocyte loss and glomerulosclerosis. Hyper-activation of Rac1 may contribute to the pathogenesis of primary or secondary nephrotic syndrome.

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FR-PO462

Loss of *Robo2* in Podocytes Alleviates Abnormal Kidney Phenotype in *Ilk* Knockout Mice Hila Milo Rasouly, Sudhir Kumar, Xueping Fan, Anna Pisarek-Horowitz, Stefanie Chan, Weining Lu. *Renal Section, Boston Univ Medical Center, Boston, MA.*

Background: ROBO2 is a transmembrane protein that plays an important role in kidney development. Recently we have shown that ROBO2 interacts with nephrin via adaptor protein NCK and loss of *Robo2* alleviates the abnormal podocyte phenotype in nephrin knockout mice. Integrin-linked kinase (ILK) is another important podocyte protein that interacts with NCK and nephrin. Podocyte-specific deletion of *Ilk* in mice results in proteinuria, renal failure and premature death. Therefore, we hypothesize that ROBO2 can also interact with ILK in podocytes via NCK and that loss of *Robo2* in podocytes would alleviate the abnormal kidney phenotype in *Ilk* knockout mice.

Methods: Protein complex formation was tested with yeast two-hybrid and co-immunoprecipitation assays. $Robo2^{flox/+};Ilk^{flox/+};Nphs2Cre^{+}$ podocyte-specific heterozygous knockout mice were crossed to generate $Ilk^{flox/flox};Nphs2Cre^{+}$ single and $Robo2^{flox/flox};Ilk^{flox/flox};Nphs2Cre^{+}$ double homozygotes which were followed up to one year. Urine protein levels were analyzed by Coomassie blue staining. Kidney histology was analyzed by H&E staining. Podocyte ultrastructure was analyzed by electron microscopy.

Results: Although there is no direct interaction between ROBO2 and ILK by yeast two-hybrid assay, co-immunoprecipitation analysis shows that ROBO2 and ILK form a protein complex through NCK and PINCH. At 6 weeks old, fewer *Robo2-Ilk* double homozygous mice developed proteinuria than did the *Ilk* single homozygous mice. Survival analysis of 86 mice up to one year also revealed that *Robo2-Ilk* double homozygous mice survived longer than *Ilk* single homozygous mice. Although no significant differences were observed at the histological level between the groups, electron microscopy showed that there was an improvement in podocyte ultrastructure of the *Robo2-Ilk* double homozygous mice in comparison to the *Ilk* single homozygous mice at 4 weeks of age.

Conclusions: ROBO2 and ILK form a protein complex through NCK and PINCH. Loss of *Robo2* reduces the prevalence of proteinuria and improves the survival of *Ilk* podocyte-specific knockout mice.

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FR-PO463

Induced Cathepsin L Activity due to Mitochondrial Dysfunction in CD2AP-Deficient Podocytes Julia van Tuijl, Hatem A. Elshabrawy, Mehmet M. Altintas, Jochen Reiser. *Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

Background: The podocytes in the kidney glomeruli play a key role in the kidney's filtration function. Their foot processes (FP) and interposed slit diaphragm form the final barrier to protein loss. The induction of cytosolic variant of the cysteine protease cathepsin L (cCatL) causes FP effacement by cleaving GTPase dynamin, synaptopodin and CD2AP. We have previously shown that acidification of cytosol during podocyte injury increases cCatL activity and glomerular disease-causing stimuli/conditions were associated with a downshift of the podocyte pH.

Methods: Conditionally immortalized podocytes were derived from wild type and CD2AP-/- mice and cultured at 33°C with interferon-g. To induce differentiation, podocytes were maintained at 37 °C for 14 days. Intracellular pH and cCatL activity were measured by using a fluorescent probe and a fluorescent substrate, respectively. Lactic acid, NADH and ATP were measured by using commercially available assay kits. Mitochondrial membrane potential was measured by FACS analysis. Mitochondrial shapes and sizes within fixed cells were analyzed by confocal microscopy.

Results: CD2AP KO podocytes had lower mitochondrial membrane potential when compared to WT podocytes. When mitochondrial function is impaired, ATP generation shifts from oxidative phosphorylation (OXPHOS) to glycolysis and generally a higher lactic acid production. We recently showed increased lactate production rates in several podocyte disease models. We also found that Complex IV, which plays a key role in the OXPHOS, was downregulated in CD2AP KO podocytes. Notably, inhibition of ATP synthase, another OXPHOS related protein, by Oligomycin increased the amounts of lactic acid in WT podocytes. We measured pHi for WT and CD2AP KO podocytes as 7.34±0.04 and 7.06±0.03, respectively. The enzyme activity versus pHi curves for both cell types indicated that the acidification of cytosol in CD2AP KO cells induced cCatL activity, which leads to podocyte damage.

Conclusions: Using an integrated approach, we have shown that the glycolytic metabolism and OXPHOS can be combined to generate a cellular profile, which explains the induced cCatL activity in diseased podocytes.

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FR-PO464

Involvement of Mitochondrial Hyperactivity in Puromycin-Induced Podocytes Injury Masayo Hino,[1] Shunya Uchida,[2] Ikuroh Ohsawa.[3] *[1]Ibaraki Prefectural Central Hospital, Ibaraki, Japan; [2]Teikyo Univ School of Medicine, Tokyo, Japan; [3]Tokyo Metropolitan Inst of Gerontology, Tokyo, Japan.*

Background: Puromycin aminonucleoside (PAN)-induced nephrosis is a model of human minimal change disease. We have previously reported that PAN induces apoptosis by increasing oxidative stress in murine podocyte cell line (MPC). Increased expression of B7-1 (CD80) by PAN was suppressed by mitochondrial respiratory inhibitors, but not by NADPH oxidase inhibitor, suggesting that mitochondrial oxidative stress play pivotal role in podoyte injury. In the present study, to explore mitochondrial involvement in podocyte injury and apoptosis, we examined gene expression level of respiratory complex IV and mitochondrial membrane potential in PAN-treated MPC.

Methods: 24 hours after the addition of PAN to MPC, transcription level of complex IV subunit II (cytochrome c oxidase subunit 2; CcO-II) in total RNA was examined by quantitative PCR (qPCR). To examine mitochondrial membrane potential, PAN-treated MPC was stained with MitoTracker Red (MTR).

Results: Analysis of qPCR showed that transcription of CcO-II was markedly up-regulated by PAN, which was suppressed by the addition of mitochondrial respiratory inhibitors, rotenone and antimycin A. Fluorescent intensities of MTR were also increased by PAN, indicating that PAN could activate mitochondrial membrane potential. After the addition of PAN, we observed that localization of mitochondria was changed from uniform distribution in the cytoplasm to periphery of the nucleus, and then apoptotic nuclear cleavage was induced.

Conclusions: These results showed that PAN increased mitochondrial activity transiently, which might facilitate the production of ATP and reactive oxidative species, probably leading to podocyte apoptosis. We propose here that mitochondrial hyperactivity plays a pivotal role in PAN-induced podocyte injury.

Funding: Government Support - Non-U.S.

FR-PO465

Nephrin Tyrosine Phosphorylation Is Required for Maintenance of Podocyte Foot Process Architecture and Response to Injury Nina Jones. *Molecular and Cellular Biology, Univ of Guelph, Guelph, ON, Canada.*

Background: Nephrin is a transmembrane component of the kidney slit diaphragm that plays a central role in organizing the unique morphology of actin-rich podocyte foot processes. The intracellular domain of nephrin contains several tyrosine residues that undergo phosphorylation, thereby initiating signaling events linking the slit diaphragm to the podocyte actin cytoskeleton. Human renal diseases characterized by podocyte foot process effacement and decreased filtration barrier function are associated with loss of this phosphorylation; however, it has not been clear whether phospho-nephrin signaling plays a causative or compensatory role in disease.

Methods: To explore the function of nephrin phosphorylation in vivo, we generated and analyzed knock-in mice bearing tyrosine to phenylalanine mutations at three key adaptor protein binding sites that disrupt overall nephrin tyrosine phosphorylation.

Results: Surprisingly, absence of nephrin phosphorylation does not affect the initial formation of podocyte foot processes; however, as the mice age, they develop proteinuria accompanied by structural changes in the filtration barrier, including dilated capillary loops, irregular thickening of the glomerular basement membrane and podocyte foot process effacement, all of which are influenced by genetic background. Furthermore, loss of nephrin phosphorylation enhanced the susceptibility to, and recovery from, experimental podocyte injury.

Conclusions: Our findings indicate that nephrin tyrosine phosphorylation is dispensable during development, but required for podocyte maintenance and response to stress. This is indicative of a model in which dynamic changes in phosphotyrosine-based signaling confer plasticity to the podocyte actin cytoskeleton.

FR-PO466

Integrin Dependent Nephrin Tyrosine Phosphorylation Rakesh Verma, Madhusudan Venkatareddy, Puneet Garg. *Div of Nephrology, Univ of Michigan, Ann Arbor, MI.*

Background: Events that result in Nephrin tyrosine phosphorylation have been an important gap in the field of Nephrin biology. Studies have shown that nephrin is tyrosine phosphorylated during development and following injury. Investigators have employed artificial means to phosphorylate Nephrin due to a lack of a physiological Nephrin ligand. A popular strategy involves "clustering" of transfected chimeric-Nephrin using an antibody that results in phophorylation of Nephrin cytoplamic domain. it is unlikely that this occurs *in vivo*. In fact, both *in vitro* and *in vivo* studies suggest that Nephrin phosphorylation is abrogated when ligated. Here we present data that suggests Nephrin tyrosine phosphorylation occurs following integrin activation at the basolateral aspect of the podocyte foot process. This effect is mediated via src kinase activation by integrins.

Methods: We used biochemical and cell biology techniques to demonstrate integrin dependent nephrin phosphorylation.

Results: Podocytes were plated on laminin-coated plates following suspension in serum free media. Cells were lysed at various time points and blotted for p-Nephrin and p-FAK. We observed an increase in Nephrin phosphorylation at the Y1193 and Y1217 residues within 15 minutes of plating. This observation was specific for laminin and not observed with collagen1 or plastic itself. To avoid bias from potential ligation of the N-ECD, we transfected Nephrin containing an amino terminal myristoylated sequence (membrane targeting) but lacking N-ECD. Following plating of transfected cells on laminin, Nephrin phosphorylation was observed even in the absence of N-ECD. In addition, use of a3b1 integrin- blocking antibody abrogated Nephrin phosphorylation confirming the specificity of this interaction.

Conclusions: To our knowledge, this is the first report explaining the mechanism involved in nephrin tyrosine phosphorylation that concurs with the observations made *in vivo*. Nephrin phosphorylation-dependent actin dynamics are important for foot process structural changes. Understanding mechanisms that result in nephrin phosphorylation are important to potentially abrogate or limit foot process spreading observed in various proteinuric kidney diseases.

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FR-PO467

Podocyte-Specific p53 Deletion Promotes Alport Syndrome Progression by Enhancement of Podocyte Proliferation and Migration Ryosuke Fukuda, Yukari Kai, Kohei Omachi, Mary Ann Suico, Tsuyoshi Shuto, Hirofumi Kai. *Molecular Medicine, Kumamoto Univ, Kumamoto, Japan.*

Background: p53 is thought to be as an apoptosis inducer of renal cells such as podocyte in diabetic nephropathy (DN) and several acute kidney injury (AKI) models. However the role of p53 in the development of renal disease that is caused by podocyte hyperplastic phenotype has not been investigated. Alport syndrome (AS) is a hereditary, progressive chronic kidney disease that is caused by mutation of *Col4a3,4,5* genes. AS is characterized with podocyte abnormal growth-induced glomerular crescent formation. Here we studied the role of p53 in podocyte hypercellularity using AS mouse model.

Methods: Podocyte-specific p53 deletion AS mouse (pod-p53+/+ or -/- AS) was established by crossing Podocin-CRE, p53-loxP and *Col4a5* G5X mutant mice. Renal function (U-Albumin, Proteinuria, BUN score) and histology (glomerular injury, fibrosis, PCNA staining, SEM, TEM) were compared between pod-p53+/+ AS and pod-p53-/- AS. Additionally, p53 fuction in podocyte differentiation, proliferation and migration was analysed by using primary podocyte and mouse podocyte cell line.

Results: Interestingly, pod-p53-/- AS mice showed aggravation of renal dysfunction compared to pod-p53+/+ AS mice. PCNA positive cells in glomerular region were significantly increased and foot process effacement were enhanced in pod-p53-/- AS. Furthermore, podocyte-speific p53 deletion in AS mouse induced numerous filopodia formation in podocytes. Consistent with *in vivo* data, we found that p53 regulates podocyte dedifferentiation, spreading from glomerular and migrative phenotype *in vitro*.

Conclusions: Present study showed that p53 functions as suppressor of podocyte abnormal growth and migration and loss of p53 expression in podocyte exacerbates AS mouse pathology. These results suggest that p53 is not just a malignant factor of renal dysfunction as in DN or several AKI but has the potential to maintain normal podocyte function and controls the development of podocyte hypercellularity disease such as AS.

FR-PO468

Blood Outgrowth Endothelial Cells as a Model to Study Atypical Hemolytic Uremic Syndrome Damien Gerard Noone,[1,2] Magdalena Riedl,[1,2] Lily Lu,[2] Yi (Emma) Quan,[3] Fred G. Pluthero,[2] Walter H. Kahr,[2,4,5] Christoph Licht.[1,2,5] *[1]Div of Nephrology, Hospital for Sick Children, Toronto, Canada; [2]Cell Biology Program, Hospital for Sick Children, Toronto, Canada; [3]Univ of Toronto, Toronto, ON, Canada; [4]Div of Haematology, Hospital for Sick Children, Toronto, Canada; [5]Dept of Paediatrics, Hospital for Sick Children, Toronto, Canada.*

Background: Atypical hemolytic uremic syndrome (aHUS) is due to defective regulation of the alternative complement pathway (CAP) which is constitutively active requiring a multi-layered defense system. It is unclear how this defense system functions as a whole to protect endothelial cells (EC) or why diminished function of one of these regulators can lead to aHUS. Mutations in membrane cofactor protein/CD46, a membrane-anchored CAP regulator leads to aHUS but with variable penetrance. We aimed to use blood outgrowth endothelial cells (BOEC), EC precursors that can be isolated from patients' blood, to study aHUS.

Methods: BOECs were cultured from subjects by a standard protocol. EC phenotype and presence of complement regulators was confirmed by immunofluorescence (IF), western blot, qPCR and flow cytometry. Cells were challenged with complement, with/without functional blockade of complement regulators. Adhesion of calcein-labelled platelets perfused across confluent BOEC in a microfluidic system was quantified by IF.

Results: BOECs were characterized by FACS (CD31/PECAM-1 and CD144/VE-cadherin positive; haemangioblast marker CD45 and immature endothelial progenitor cell marker CD14 negative). Surface expression of complement regulators was confirmed. A surrogate phenotype of aHUS modelled ex vivo (endothelial cell death and platelet adhesion) is not achieved with CD46 blockade alone. Complement deposition (C3) is enhanced by blocking complement regulators sequentially (four-fold increase when CD46, CD55 and CD59 are blocked;$p<0.001$) and results in cell death, apoptosis and an EC prothrombotic phenotype with platelet adhesion.

Conclusions: We have established an ex vivo method to model aHUS pathogenesis that can now be expanded to use patient-derived BOEC. Our data supports the hypothesis that an additional factor is required in order for a patient with a CD46 mutation to manifest disease.

FR-PO469

The Association of von Willebrand Factor and the Alternative Complement Pathway in Atypical Hemolytic Uremic Syndrome-Complement Amplifying or Protective? Damien Gerard Noone,[1,2] Magdalena Riedl,[2] Fred G. Pluthero,[2] Walter H. Kahr,[2,3,4] Mackenzie L. Bowman,[5] Paula James,[5] Christoph Licht.[1,2,4] *[1]Div of Nephrology, Hospital for Sick Children, Toronto, Canada; [2]Cell Biology Program, The Hospital for Sick Children, Toronto, Canada; [3]Div of Paediatric Haematology, Hospital for Sick Children, Toronto, Canada; [4]Dept of Paediatrics, Hospital for Sick Children, Toronto, Canada; [5]Clinical and Molecular Hemostasis Research Group, Queen's Univ, Kingston, Canada.*

Background: Atypical hemolytic uremic syndrome (aHUS) is due to defective regulation of the alternative complement pathway (CAP) and is associated with glomerular endothelial cell (EC) injury and platelet microthrombi. EC activated by complement release von Willebrand Factor (VWF). Recent evidence links CAP with VWF, and it has been suggested that this may amplify EC complement injury. We aimed to use blood outgrowth endothelial cells (BOEC), EC precursors that can be isolated from patients' blood, to further study this.

Methods: BOEC were cultured by standard protocol from healthy controls and patients with type 3 von Willebrand disease (VWD3 BOEC), which have no VWF secretion. EC phenotype was confirmed by immunofluorescence (IF), western blot (WB), qPCR and flow cytometry and was not different between cell lines. Cells were challenged with complement using 50% human serum, with/without functional blockade of complement regulators. EC apoptosis was quantified by IF staining for cleaved caspase 3.

Results: BOEC were characterized by FACS (CD31/PECAM-1 and CD144/VE-cadherin positive; haemangioblast marker CD45 and immature endothelial progenitor cell marker CD14 negative). VWF secretion from complement activated BOEC and a lack thereof in VWD3 BOEC was confirmed. Complement products including C5b-9 were found to associate with VWF multimers released from normal BOEC by IF and spinning disk confocal microscopy. Complement (C3) deposition however, is greater on VWD3 BOEC following complement challenge and results in an increase in cell death by apoptosis.

Conclusions: We have confirmed an association of VWF and CAP activation products, however, the finding of increased complement activation on BOEC devoid of VWF, argues for a protective effect of this association.

FR-PO470

Annexin A2 Protects the Aging Podocyte Biao Li,[1] Tuncer Onay,[2] Jessica Anne Quaggin-Smith,[1] Vera Eremina,[1] Chengjin Li,[1] Susan E. Quaggin.[1,2] *[1]Lunenfeld Tenenbaum Research Inst, Toronto, ON, Canada; [2]Nephrology, Feinberg Cardiovascular Research Inst, Chicago, IL.*

Background: Anxa2 (Annexin A2) is a calcium dependent phospholipid binding protein and one of 12 members of the Annexin family that is expressed extensively in a variety of tissues. Previously, we identified high expression of Anxa2 in lipid rafts of human podocytes and found that knockdown of Anxa2 results in dramatic changes in morphology and adhesion of podocytes in vitro. These data led us to hypothesize that Anxa2 plays a significant role in formation and maintenance of the actin cytoskeleton and foot processes in podocytes.

Methods: A floxed Anxa2 (Exon 4) mouse was generated and used to develop whole body and podocyte specific KO mice. Kidney function was evaluated by protein/creatinine ratio (P-C ratio) and histology. Northern blot was used to determine the expression of Anxa2 and other Annexin family members in glomeruli.

Results: While diffuse fibrin deposition was observed in and around glomerular capillaries of Anxa2 KO kidneys, histological analysis showed no other major differences between KO and control mice; P-C ratios were not different at 6 months of age. However, aged podocyte-specific Anxa2 KO mice (1.5 years of age) develop variable degrees of proteinuria, ranging from mild to severe (P-C ratio, 0.66-48.23ug/g). Histologic analysis of kidneys from KO mice demonstrated a spectrum of glomerular injury from focal changes to endstage sclerosis. qPCR analysis from isolated glomeruli showed increased expression of various Annexin family members at two months of age in KO mice. Northern blot analysis showed presence of new Anxa2 bands in KO mice, consistent with expression of alternative splice variants.

Conclusions: Deletion of Anxa2 from podocytes results in extensive glomerular fibrin deposition and mild changes in the podocyte cytoskeleton that appear to be well compensated by other Annexin family members in young mice. However, Anxa2 deficiency can lead to pronounced alteration of the podocyte cytoskeleton and severe proteinuria upon aging.

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FR-PO471

Micro-RNA-378 Is Upregulated in Proteinuric Kidney Diseases and Regulates Podocytic Nephronectin Expression Janina Müller-Deile,[1,3] Jan Dannenberg,[1] Jenny C. Nystrom,[2] Peidi Liu,[2] Johan M. Lorenzen,[1,4] Thomas Thum,[4] Hermann G. Haller,[1,3] Mario Schiffer.[1,3] *[1]Dept of Medicine/Nephrology, Hannover Medical School, Hannover, Germany; [2]Sahlgrenska Academy, Univ of Gothenburg, Gothenburg, Sweden; [3]Mount Desert Island Biological Laboratory, Salisbury Cove, ME; [4]Inst of Molecular and Translational Therapeutic Strategies, Hannover Medical School, Hannover, Germany.*

Background: Even though different genes and soluble factors have been previously described to cause primary glomerular disease, their clinical relevance remains elusive. Micro-RNAs (miRNAs) play an important role in gene regulation and therefore seem to be promising candidates involved in glomerular disease.

Methods: Baseline and stress-induced miRNA profiles of different human glomerular cells as well as miRNA profiles of urines from patients with different glomerular disease were used to screen for specific overlap of miRNAs. Regulation of miRNA target genes was investigated in cultured podocytes in after stimulation with TGF-beta, glucose, miRNA-mimics and -antagomirs. MiRNA-mimics and morpholinos for miRNA target genes were injected in zebrafish embryos and the resulting phenotypes and degree of proteinuria was analysed.

Results: We identified miRNA-378 specifically up-regulated in stressed human podocytes and in urines from patients with different primary glomerular disease. NPNT has been previously described as integrin interaction partner and is a confirmed target gene of miRNA-378. We detected NPNT expression in podocytes and confirmed a partial overlap with the actin cytoskeleton. NPNT expression was suppressed by miRNA-378 as well as by high glucose and TGF-beta in cultured human podocytes. Knockdown of NPNT by injection of a miRNA-378-mimic or a morpholino for NPNT in a developing zebrafish embryo resulted in a renal phenotype with edema and proteinuria.

Conclusions: Podocytic NPNT seems to play an important role for the maintenance of the in the glomerular filtration barrier. MiRNA-378 controlled NPNT expression seems to be a novel pathophysiological regulator of podocyte and basement membrane interactions. Furthermore, urinary miRNA-378 could be a novel non-invasive marker for active glomerular diseases in patients.

FR-PO472

Tight Control of Calcium-Calcineurin Signaling by miR-30 in Podocytes Junnan Wu, Chun-Xia Zheng, Xiao Wang, Lin Liu, Yu-Ting Ye, Jing He, Changming Zhang, Wanfen Zhang, Yuqiu Lu, Hong Xia, Wei-Song Qin, Shaolin Shi, Zhihong Liu. *National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, China.*

Background: Calcium-calcineurin signaling activation induces podocyte injury, but the mechanism underlying its regulation is largely unknown. Many components of the signaling are predicted to be miR-30 targets and they are upregulated in injured podocytes in which miR-30s are downregulated, we therefore hypothesize that miR-30s control calcium-calcineurin signaling by inhibiting the expression of its multiple components and miR-30 downregulation leads to the activation of the signaling.

Methods: Studies were performed with human podocyte cell line, podocyte-selective miR-30s sponge transgenic mice, puromycin aminonucleoside (PAN)-treated rats, miR-30a gene transferring *in vivo*, and FSGS patients' renal biopsies.

Results: We found that calcium-calcineurin signaling components, TRPC6, PPP3ca, PPP3cb, PPP3r1, and NFATc3, have abundant mRNA but not protein in normal podocytes, and that their protein but not mRNA levels are greatly elevated in the podocytes of FSGS patients. We confirmed by luciferase reporter assays that these genes are miR-30 targets and showed that. miR-30 overexpression reduced their expressions, as well as calcium influx, calcineurin activity and NFAT3c nuclear translocation in PAN-treated podocytes. *In vivo*, podocyte-selective miR-30s sponge transgenic mice displayed increased expressions of the five genes in the podocytes and the mice developed significantly higher levels of proteinuria than control mice when treated with PAN. The expressions of the five genes were also upregulated in the podocytes of PAN-treated rats, in which miR-30s were downregulated. As expected, the upregulation of these genes in the podocytes of PAN-treated rats was prevented by miR-30a gene transferring to the podocytes, accompanied by alleviation of proteinuria and podocyte injury.

Conclusions: miR-30s tightly control calcium-calcineurin signaling by inhibiting the expression of multiple components of the signaling, and miR-30 downregulation results in their upregulation and thus the signaling activation.

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FR-PO473

Optimization of TRPC5 Inhibitors for Podocyte Protective Therapy Sookyung Kim, Philip M. Castonguay, Dequan Tian, Frank Dubois, Anna Greka. *Glom-NExT, Center for Glomerular Kidney Disease and Novel Experimental Therapeutics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.*

Background: Podocytes are highly specialized cells in the kidney glomerulus, whose injury and loss leads to proteinuria and progressive kidney disease. We recently showed that inhibition of Transient Receptor Potential Canonical 5 (TRPC5) channels by ML204 prevents podocyte cytoskeletal collapse, and protects mice from filter barrier damage. Here, we tested two inhibitors of TRPC5 based on the ML204 chemical backbone, to test the hypothesis that these inhibitors may better protect the podocyte actin cytoskeleton compared to ML204.

Methods: We used patch clamp electrophysiology in HEK293 cells overexpressing TRPC5 or TRPC6 to test the potency of the two inhibitors compared to ML204. For cell culture experiments, podocytes were pretreated with inhibitor 20 minutes prior to exposure to Protamine Sulfate (PS). Phalloidin staining was performed for actin stress fiber quantification. The abundance of synaptopodin was also tested by Western Blot.

Results: We confirmed that the two compounds are specific TRPC5, and not TRPC6, inhibitors by patch clamp electrophysiology. Phalloidin staining and stress fiber quantification revealed one of the two inhibitors as superior to ML204 in protecting the podocyte actin cytoskeleton from PS-induced remodeling. The abundance of synaptopodin was also preserved in a dose-dependent manner after treatment with the inhibitor compared to control PS-treated cells.

Conclusions: Our studies have validated an optimized TRPC5 inhibitor *in vitro*. This work bolsters the notion that TRPC5 inhibition is a valuable podocyte protective strategy.

Funding: NIDDK Support

FR-PO474

Profoundly Altered TRPC6 Channel Gating Properties in Acquired FSGS Stuart E. Dryer, Hila Roshanravan, Eunyoung Kim. *Biology and Biochemistry, Univ of Houston, Houston, TX.*

Background: Gain-of-function mutations in TRPC6 channels give rise to familial FSGS[1,2]. An increase in TRPC6 protein was detected in acquired FSGS[3]. However, ion channels are regulated primarily by gating. Here we have used electrophysiology to examine the functional properties of podocyte TRPC6 channels in a rat model of acquired FSGS.

Methods: FSGS was induced in Sprague-Dawley rats by chronic treatment with puromycin aminonucleoside (PAN). PAN or saline were injected three times at 30 day intervals. Glomeruli were isolated 10 days after the third injection. Whole-cell recordings from podocytes were used to measure TRPC6 responses to chemical stimuli (OAG and ATP) and mechanical stimuli (hypoosmotic stretch). Podocytes are attached to the glomerular capillary during these recordings. Podocyte proteins were detected by immunoblot and FSGS was ascertained by PAS staining.

Results: Chronic PAN treatment resulted in segmental glomerulosclerosis and protein casts in tubules. Podocytes from PAN-treated rats showed ~10 fold larger TRPC6 currents in response to mechanical stimuli compared to controls. However, currents evoked by bath application of 50 μM ATP (P2Y agonist) or 100 μM OAG (canonical activator of TRPC6) were almost completely eliminated after PAN treatment. Podocytes from saline-treated rats exhibited modest and approximately equal sized cationic currents in response to mechanical stretch, OAG, or ATP. Podocin expression was markedly reduced, and TRPC6 was increased, in glomeruli from PAN-treated rats compared to controls.

Conclusions: Increased sensitivity to mechanical stimuli in acquired FSGS may over time lead to Ca^{2+} overload and further loss of podocytes. The change in gating properties seen here are similar to those seen after podocin knockdown[4]. Loss of TRPC6 responses to chemical stimuli suggest that Ang II signaling to podocytes is attenuated in acquired FSGS, suggesting that protective effects of RAS inhibition are exerted on other cell types. 1. Winn et al. (2005) Science 308:1801-4. Reiser et al. (2005) Nat Genet 37 (7):739-44. 3. Möller et al. (2007) J Am Soc Nephrol 18:29-36. 4. Anderson et al. (2013). Am J Physiol-Cell Physiol 305:C276-89.

Funding: Pharmaceutical Company Support - Pfizer

FR-PO475

A STAT3 Inhibitor Alters Morphology and Migration of Podocytes and Inhibits Expression of Synaptopodin Stuart E. Dryer, Mousa Abkhezr. *Biology and Biochemistry, Univ of Houston, Houston, TX.*

Background: Previous studies have suggested that the transcription factor signal transducer and activator of transcription-3 (STAT3) plays a complex role in the progression of HIV nephropathy, collapsing glomerulopathy, and crescentic glomerulonephritis[1,2] and in podocyte cell biology. The targets of STAT3 signaling are not well understood.

Methods: Immunoblot analysis of synaptopodin (Synpo) and STAT3 tyrosine phosphorylation, RT-PCR, confocal microscopy and migration assays in immortalized mouse podocytes.

Results: Application of 5 μM Stattic, a small molecule inhibitor of STAT3 activation, caused a drastic reduction in Synpo mRNA and protein in podocytes. This effect was complete within 30 minutes after the onset of Stattic treatment. Synpo protein levels declined at a similar rate after inhibition of protein synthesis using cyclohexamide. Effects of Stattic and cycloheximide were blocked by the cathepsin-L inhibitor E-64. These observations indicate that Synpo has a very high turnover rate due to ongoing proteolytic degradation (see also ref. 3). Stattic had no effect on the time course of Synpo disappearance after inhibition of protein synthesis or cathepsin-L. This suggests that Stattic, and by implication STAT3, act primarily on Synpo biosynthesis. We also observed that treating podocytes with agents that stimulate STAT3 tyrosine phosphorylation in pododcytes, i.e. Ang II and IL-6[4], caused corresponding increases in Synpo protein. Stattic application for 24 hr caused marked changes in podocyte morphology and also caused complete inhibition of their migration as assessed by wound assays.

Conclusions: Synpo protein has high turnover rate in podocytes owing to ongoing protelolytic degradation. Synpo biosynthesis is regulated in part by signaling to STAT3, and STAT3 may therefore play a role in regulating podocyte cytoskeleton and motility. It is possible that STAT3 effects on cytoskeleton are in some way related to cell cycle control. 1. Feng et al. (2009) J Am Soc Nephrol 20 (10):2138-46. 2. Dai et al. (2013) Kidney Int 84 (5):950-61. 3. Faul et al. (2008). Nat Med 14 (9):931-8. 4. Abkhezr and Dryer (2014) Mol Pharmacol in press.

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FR-PO476

Isolation and Characterization of Podocyte-Derived Microparticles in *In Vitro* Nephrotic Syndrome Environment Hoon Young Choi, Sung Chang Bae, Sung-Kyu Ha, Hyeong Cheon Park. *Dept of Internal Medicine, Gangnam Severance Hospital, Yonsei Univ School of Medicine, Seoul, Korea.*

Background: Microparticles (MPs) shed from cell membrane in various stimuli such as oxidative stress or hypoxia. The biological characteristics of MPs are dependent on activation stimuli or the microenvironment in which MPs are produced. Effacement of podocyte foot processes is the main pathologic features of nephrotic syndrome and FSGS. These changes could be induced by protamine sulfate experimentally. We investigated this study to perform the isolation and characteristics of podocyte-derived MPs in *in vitro* nephrotic syndrome environment.

Methods: Podocytes were cultured in serum deprived RPMI without or with protamine sulfate (300ug/mL) for 2~4 hours to mimic nephrotic syndrome condition. MPs were isolated from supernatants by differential ultrafiltration. The presence of MP was confirmed by flow cytometric analysis.

Results: Podocyte-derived MPs were mainly detected at a region below the forward scatter signal corresponding to 1μm beads, which were used as internal size standards in FACS analysis. Podocyte-derived MPs expressed nephrin, podocin, podocalyxin, which were also expressed on podocyte plasma membranes. FACS analysis showed that the expressions of podocyte specific markers and annexin V were more in MPs from podocyte treated with protamine sulfate (control versus protamine sulfate treatment: nephrin 8.6 ± 0.9 % versus 52.1 ± 7.8%, podocalyxin 15.6 ± 2.0 % versus 47.7 ± 8.9 %, podocin 9.2 ± 1.6 % versus 37.5 ± 11.7 %, $P < 0.05$).

Conclusions: This study showed that protamine sulfate mimicking *in vitro* nephrotic syndrome environment promotes more release of podocyte-derived microparticles expressing podocalyxin, podocin, and nephrin together with annexin V.

FR-PO477

Synaptic-Like-Microvesicle Regulated by SV2B in Podocyte Plays a Role in Intracellular Trafficking of Slit Diaphragm Molecules and Exocytosis of GBM Components Yoshiyasu Fukusumi, Natsumi Takashima, Ayako Wakamatsu, Eriko Hasegawa, Hiroshi Kawachi. *Dept of Cell Biology, Inst of Nephrology, Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan.*

Background: We have previously reported synaptic vesicle protein 2B (SV2B) was expressed on synaptic-like-microvesicle (SLMV) in podocyte, and SV2B knockout (KO) mice showed proteinuria. However, the function of SLMV in podocyte and the role of SV2B in SLMV are still uncertain.

Methods: The morphology and the expressions of the podocyte molecules, GBM components and synaptic vesicle molecules were analyzed in SV2B KO mice. The interactions of SV2B with podocyte molecules were analyzed. The expressions of SV2B and other synaptic vesicle molecules were analyzed with an embryonic rat kidney.

Results: SV2B KO mice showed foot process effacement and GBM thickness. The stainings of CD2AP, nephrin and NEPH1 were clearly reduced in the SV2B KO mice (IF score, KO versus WT: CD2AP, 2.6 versus 3.3, $p<0.01$; nephrin, 3.1 versus 3.4, $p<0.05$; NEPH1, 3.0 versus 3.3, $p<0.05$). However, those of other slit diaphragm (SD) molecules, podocin and ZO-1, and apical surface molecules podocalyxin and NHERF2 were not altered. The staining of lamimin was clearly reduced (3.1 versus 3.4, $p<0.05$), although other GBM components were not altered. Although mRNA expressions of SV2A, SV2C and other vesicle surface molecules, Rab3a, Rab4, and vGlut1 were not altered in the kidney cortex of SV2B KO mice, those of Munc18, synaptotagminand neurexin were clearly reduced (Munc18, 62%; synaptotagmin, 52%; neurexin, 43%). The precipitation assay showed SV2B interacted with CD2AP and nephrin, and that CD2AP interacted with neurexin. SV2B was exclusively expressed in glomeruli in embryonic rat kidney section, whereas SV2A was diffusely expressed. SV2B was first detected along the whole cell surface of podocyte in the early S-shaped body stage glomeruli, and with maturation the staining became to be concentrated to baso-lateral area.

Conclusions: SLMV plays a role in podocyte maturation and in intracellular trafficking of the SD molecules and exocytosis of GBM components. SV2B regulated the docking of SLMV to baso-lateral surface of podocyte.

Funding: Government Support - Non-U.S.

FR-PO478

Role of MAP Kinase-Activated Protein Kinase 2 (MK2)-Mediated Renal Stress Response in Adriamycin-Induced Nephropathy Shipra Agrawal,[1] Xiaojing Nie,[1] Melinda A. Chanley,[1] William E. Smoyer,[1,3] Ruma Pengal,[1] Rainer Benndorf.[1] *[1]Clinical and Translational Research, The Research Inst at Nationwide Children's Hospital, Columbus, OH; [2]Pediatrics, The Ohio State Univ, Columbus, OH; [3]Inst of Biochemistry, Hannover Medical School, Hannover, Germany.*

Background: The p38 MAPK signaling pathway plays a crucial role in glomerulopathies, whereactivation is associated with disease and inhibition is associated with disease amelioration. We have previously reported the specific role of the p38 MAPK→MK2→HSPB1 pathway in both *in vitro* models of podocyte injury and in an *in vivo* model of acute glomerulonephritis. Based on this, we hypothesized that MK2 also plays an important role in a model of chronic glomerulopathy (Adriamycin nephropathy) via its action on its downstream substrate HSPB1.

Methods: MK2 heterozygous knock-out (KO) mice (C57BL/6 background) were backcrossed with sv129 for 7 generations to obtain MK2-KO mice in an Adriamycin (Adr)-susceptible strain. At 11 weeks of age, male wild-type (WT) and MK2-KO mice were injected with 18 mg/kg Adr (N=12 per group) or PBS (N=6 per group) by tail vein injections. Serum BUN and proteinuria were measured for 21 days, when mice were sacrificed. Renal cortices were analyzed for the expression of MK2, and the stress proteins HSPB8, GRP78, HSPB1 and phospho-HSPB1.

Results: Both WT and MK2-KO mice, developed massive albuminuria and high serum BUN values after Adr. Renal cortical MK2 protein expression was completely abrogated in MK2-KO mice. HSPB1 was highly induced by Adr treatment in both WT and MK2-KO mice. While HSPB1 was highly phosphorylated in WT Adr-treated mice, its activation was greatly reduced in Adr-treated MK2-KO mice. Furthermore, HSPB8 expression was higher in Adr-treated mice compared to control mice in WT mice, but not MK2-KO mice. GRP78 expression remained unaltered.

Conclusions: Induction of Adr-nephropathy resulted in massive proteinuria and a strong renal cortical stress response via activation and increased expression of HSPB1 in a MK2-mediated manner. These studies suggest a crucial role of MK2 in regulating the renal stress response in a chronic model of glomerulopathy.

Funding: NIDDK Support, Private Foundation Support

FR-PO479

CLIC5A Signaling and Hypertension-Induced Glomerular Injury

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Background: Hypertension (HTN) is a major risk factor for glomerular disease progression. Glomerular capillary HTN triggers stretch-activated signals that lead to podocyte remodeling. We have shown that the podocyte-predominant protein CLIC5A stimulates PI[4,5]P2 accumulation, causing ezrin phosphorylation and ezrin coupling to actin. Rac1, a Rho GTPase activated by podocyte stretch, also stimulates ezrin phosphorylation and actin remodeling. We tested the hypothesis that HTN–induced podocyte Rac1 activation is CLIC5A-dependent.

Methods: GFP-CLIC5A or GFP were transiently expressed in COS-7 cells. Uninephrectomized CLIC5 deficient ($CLIC5^{-/-}$) and wild-type ($CLIC5^{+/+}$) mice were treated with deoxycorticosterone and 1% saline drinking water (DOCA/Salt) for 20 days. COS-7 cell and glomerular lysates were evaluated for activated Rac1-GTP, and for the Rac1 effector phospho-PAK1 (P-PAK1) and phospho-Ezrin (P-ERM).

Results: In COS-7 cells, GFP-CLIC5A, but not GFP, activated Rac1 and phosphorylation of PAK and Ezrin. GFP-CLIC5A-dependent ezrin phosphorylation was blocked by the Rac1 inhibitor NSC23766. In glomerular lysates, P-PAK1 and P-ERM levels were much lower in $CLIC5^{-/-}$ compared to $CLIC5^{+/+}$ mice. DOCA/Salt strongly stimulated podocyte-associated PAK phosphorylation in $CLIC5^{+/+}$, but not in $CLIC5^{-/-}$ mice. While the degree of DOCA/Salt-induced systemic HTN was similar in $CLIC5^{-/-}$ and $CLIC5^{+/+}$ mice, albuminuria and morphological injury were greater in $CLIC5^{-/-}$ mice.

	$CLIC5^{+/+}$	$CLIC5^{-/-}$
Systolic BP (mm Hg; baseline vs. day 20)	98±8 vs. 119±6	97±8 vs. 121±11
Urine Albumin:Creatinine Ratio (mg/g)	780±235	1,720±960*
Microaneurysms (% Glomeruli Affected)	31±17	57±18*
Foot Processes (#/µm GBM)	2.55±0.10	1.91±0.09**
Endothelial Fenestrae (#/µm GBM)	2.63±0.35	1.25±0.20**

(Mean±SEM, n=5/group, *p<0.05; **p<0.01).

Conclusions: Thus, CLIC5A-dependent Rac1 activation and downstream PAK phosphorylation are components of the podocyte response to HTN, at least in the DOCA/Salt model. The findings imply that protection from HTN-induced glomerular injury requires CLIC5/Rac1/PAK/Ezrin-dependent podocyte remodeling.

FR-PO480

Chloride Intracellular Channel 5 (CLIC5) Is Critical for Podocyte Adhesion and Regulation of Podocalyxin Stability

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Background: Podocytes are a common target cell of injury in the pathogenesis of many glomerular diseases that are the leading cause of end-stage renal and chronic kidney diseases. The cost of end-stage renal disease in 2009 was $25 billion, making this a major burden on our health care system. Mechanisms that promote podocyte attachment and repair are critical for podocyte survival and for the prevention of progression of glomerular disease. Chloride intracellular channel 5 (CLIC5) is a novel membrane protein that is enriched in podocytes. CLIC5 protein is decreased in the glomerulus of Minimal Change Disease compared to living donor patients. CLIC5-deficient podocytes have decreased podocalyxin (PODXL) protein expression and abnormal podocyte foot process ultrastructure. PODXL is an apical membrane protein necessary for the morphogenesis and maintenance of podocyte cytoarchitecture. We have determined the extent to which CLIC5 is critical to maintain podocyte adhesion and we measured CLIC5-mediated control of PODXL stability.

Methods: We utilized *Clic5*$^{+/+}$ and *Clic5*$^{-/-}$ differentiated podocytes to determine to what extent CLIC5 controls podocyte adhesion and migration capacity. We quantified the extent of podocyte loss into urine using qRT-PCR of podocin between *Clic5*$^{-/-}$ and *Clic5*$^{+/+}$ mice. We performed pulse-chase live-cell imaging of photoconvertable, fluorescently tagged PODXL to measure the stability of PODXL in differentiated *Clic5*$^{+/+}$ and *Clic5*$^{-/-}$ podocytes *in vitro*.

Results: *CLic5*$^{+/+}$mice shed 6-fold less urine podocytes compared with *Clic5*$^{-/-}$ mice at 10-12 months of age (N=5 *Clic5*$^{+/+}$ mice; N=7, *Clic5*$^{-/-}$ mice. P<0.01). *Clic5*$^{-/-}$ podocytes have a 3-fold decrease in adhesion and migrated 3-fold less compared to $CLIC5^{+/+}$ podocytes on fibronectin and laminin coated surfaces (P<0.05 and P<0.001, respectively). PODXL is degraded 4-fold faster in the absence of CLIC5 in podocytes (N=5 cells for each genotype, P<0.001).

Conclusions: CLIC5 is critical for podocytes to maintain their adherence to the glomerular matrix and to control PODXL stability.

Funding: NIDDK Support

FR-PO481

Protein O-GlcNAcylation Is Essential to Maintain Normal Podocyte Function

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Background: Posttranslational modification is essential for controlling cell function. It has been shown that the addition of O-linked b-N-acetylglucosamine (O-GlcNAcylation) to cytosolic and nuclear proteins serves as a nutrient/stress sensor for modulating cell functions, and is ubiquitous like phosphorylation. However, little is known about how O-GlcNAcylation affects normal podocyte function.

Methods: O-GlcNAc transferase (Ogt) is a critical enzyme for O-GlcNAcylation in mammals, and resides on the X chromosome. To examine the physiological role of O-GlcNAcylation in podocytes, we analyzed the renal phenotype of podocyte-specific Ogt-knockout mice. This mouse model was generated by crossbreeding Ogt-flox mouse with podocin-specific Cre mouse.

Results: We confirmed that O-GlcNAcylation was functionally diminished specifically in podocytes of podocyte-specific Ogt-knockout mice by immunofluorescent assay on O-GlcNAcylation. Podocyte-specific deletion of Ogt did not influence birth rate and showed a normal growth pattern until 16 weeks of age. Proteinuria began to develop at 4 weeks of age and increased with age until 16 weeks of age in podocyte-specific knockout mice. PAS and HE stains revealed little pathological alterations in the glomeruli and tubulointerstitial area of podocyte-specific Ogt-knockout mice at 16 weeks of age, compared to those of wild type mice. However, scanning and transmission electron microscopy analysis showed marked foot process effacement and enlarged cell body in podocytes of podocyte-specific Ogt-knockout mice. Also, an immunofluorescent assay on podocin revealed that the expression level of podocin significantly decreased and its expression pattern was visible as dots but not linear.

Conclusions: O-GlcNAcylation of intracellular proteins by Ogt is necessary to maintain normal foot process function in podocytes. Our results provide new insight into podocyte biology.

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Study in the Effect of IgA1 Glycosylation and Histone Acetylation on the Pathogenesis of IgA Nephropathy

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Background: IgA nephropathy (IgAN) is the most common glomerular disease worldwide. Its pathogenesis is unclear yet. The formation of immune complexes containing Gd-IgA1 is the key factor in the pathogenesis of IgAN. Histone acetylation modification is one of the histone modification, but its role in the pathogenesis of IgAN is unclear.

Methods: 59 IgAN patients and 30 health controls were respectively collected. The plasma level of IgA and Gd-IgA1, H3/H4 acetylation and H3K4/H3K9 methylation level in PBMC were measured by ELISA. HDACs, HAT, C1GALT1 and ST6GALNAC2 mRNA in PBMC were measured by qPCR and H3/H4 acetylation level at C1GALT1 and ST6GALNAC2 gene promoter detected by ChIP. The expression of HDAC1 and H3Ac in renal tissues was observed by immunofluorescence.

Results: We found IgA and Gd-IgA1 in IgAN patients plasma were significantly increased compared with the healthy controls (P<0.0001) and Gd-IgA1 in IgAN patients was positively correlated with the total amount of 24h urine protein (r=0.479, P<0.001). Transcription of C1GALT1 and ST6GALNAC2 mRNA of IgAN patients was 0.38±0.07 (P<0.01) and 2.31±0.48 folds (P<0.05). Increased H3 acetylation (P<0.05) and H4 acetylation (P<0.01) were observed in IgAN patients. The mRNA level of HDAC1, HDAC2, HDAC3, HDAC7, HDAC8, P300 and CREBBP from IgAN patients was respectively 2.20±0.18 (P<0.001), 0.44±0.08 (P<0.05), 0.98±0.18 (P>0.05), 1.02±0.21 (P>0.05), 1.47±0.25 (P<0.05), 2.81±0.39 (P<0.01) and 2.44±0.27 folds (P<0.01). H3 and H4 acetylation at C1GALT1 and ST6GALNAC2 promoter were elevated from IgAN patients. Immunofluorescence results indicated that the HDAC1 was increased obviously, but H3Ac was significantly reduced in the renal tissue of IgAN patients.

Conclusions: This study found that IgAN patients were in an abnormal histone acetylation state; IgA1 glycosylation and Histone acetylation take part in the pathogenesis of IgAN. The results enrich the pathogenesis of IgAN and provide new insights to the treatment of IgAN.

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Chinese Herbal Medicine for IgA Nephropathy, ShenPing Decoction, Blocks PDGF-Induced Signaling and Proliferation in Human Mesangial Cells

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Background: A prescribed Chinese herbal medicine, ShenPing Decoction (SP), has been effectively used in China to treat IgA nephropathy (IgAN) at its early stages for over 30 years. In IgAN, mesangial deposition of immune complexes, consisting of galactose-deficient IgA1 (Gd-IgA1) and auto-antibodies bound to Gd-IgA1, stimulate mesangial cell

(MC) proliferation, possibly *via* PDGF signaling. Locally produced IL-6 may be part of the pathogenic process. In this study, we investigated the effects of SP on PDGF signaling, using primary MCs.

Methods: MCs were incubated with PDGF for 24 h with or without SP. Cellular proliferation was measured with Syto60. RealTime RT-PCR was performed to determine changes in expression of IL-6. Signaling in MCs by PDGF with or without SP was evaluated by Western blotting with antibodies specific for multiple phosphorylated proteins, including ERK and p38.

Results: PDGF increased MC proliferation and SP inhibited this PDGF-induced activity in a dose-dependent manner. PDGF increased phosphorylation of multiple proteins, including ERK and of p38. SP inhibited this PDGF-enhanced phosphorylation in a dose-dependent manner, PDGF up-regulated expression of IL-6 by 5 fold; SP partially inhibited this PDGF-mediated upregulation of IL-6 gene expression in a dose-dependent manner.

Conclusions: PDGF increased MC proliferation, enhanced phosphorylation of multiple proteins, and up-regulated expression of IL-6. SP inhibited PDGF-induced MC proliferation and phosphorylation of multiple proteins, and partially inhibited expression of IL-6 mRNA. Future studies are needed to identify the active componentsof SP.

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Angiotensin II-Induced Podocyte Injury via the Nephrin-c-Abl-ship2-Akt Pathway Qian Yang, Yiqiong Ma, Yipeng Liu, Wei Liang, Xinghua Chen, Zhilong Ren, Huiming Wang, Guohua Ding. *Div of Nephrology, Renmin Hospital of Wuhan Univ, Wuhan, Hubei, China.*

Background: Previous studies have shown that nephrin plays an important role in podocyte injury induced by AngiotensinII. However, its specific mechanism remains unclear. C-Abl is a SH2/SH3 domain containing nonreceptor tyrosine kinase, which is involved in cell survival and cytoskeleton regulation. Phosphorylated nephrin is able to interact with molecules containing SH2/SH3 domain, which suggests that c-Abl may be a downstream molecular of nephrin signaling. In this study, we intend to study the role of c-Abl in nephrin signaling in process of podocyte injury.

Methods: Twenty-four male Wistar rats were randomly assigned to administer AngII (400 ng/kg/min) or saline with osmotic mini-pumps. In vitro, cultured murine podocytes were exposed to Ang II (10^{-6}M) for variable time. Western blotting was used for detecting expression and phosphorylation levels of nephrin, c-Abl, ship2 and Akt. The colocalization of nephrin and c-Abl was detected by laser confocal microscopy. The nephrin-c-Abl and c-Abl-ship2 interactions were assessed using co-immunoprecipitation. Cytoskeleton formation was evaluated by FITC-phalloidin staining and apoptosis was analyzed by flow cytometry.

Results: AngII-infused rats developed proteinuria and podocyte damage, which was accompanied with decreased expression of nephrin and fainter interaction between nephrin and c-Abl. *In vitro*, AngII exposure induced cytoskeleton reorganization and apoptosis in podocytes. In addition, Ang II-induced dephosphorylation of nephrin and Akt was in line with lower interaction of nephrin-c-Abl. Moreover, AngII exposure promoted phosphorylation of c-Abl and interaction of c-Abl-ship2. In contrast, c-Abl siRNA and STI571 (c-Abl inhibitor) significantly restored AngII-induced podocyte injury (cytoskeleton reorganization and apoptosis) and suppressed Ang II-induced interaction of c-Abl-ship2 and phosphorylation of ship2 without alteration of nephrin phosphorylation.

Conclusions: These findings indicate that c-Abl is a molecular chaperone of nephrin signaling and ship2-Akt pathway with released c-Abl contributes to Ang II-induced podocyte injury.

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FR-PO485

GLEPP1 Controls the Composition of the GBM in Mice Eva Koenigshausen,[1] Laura Lennartz,[1] Christian Weigel,[1] Thorsten Wiech,[2] Dontscho Kerjaschki,[3] Roger C. Wiggins,[4] Magdalena Woznowski,[1] Ivo Quack,[1] Lars C. Rump,[1] Lorenz Sellin.[1] *[1]Nephrology, Univ Hospital Duesseldorf, Germany; [2]Nephropathology, Univ Hospital Hamburg, Germany; [3]Pathology, Univ of Vienna, Austria; [4]Nephrology, Univ of Michigan.*

Background: Proteinuria is one symptom of glomerular kidney disease and evolves out of the altered glomerular filtration barrier. GLEPP1 is a receptor tyrosine phosphatase present in the podocyte foot processes. Mutations in the GLEPP1 gene were shown to be associated with MCD and FSGS. The precise function of GLEPP1 is not fully understood.

Methods: GLEPP1 -/- deficient and WT +/+ mice on a 129P3/J background were examined at the age of 4, 6 and 10 months of age. Mouse urine was analyzed. RNA was obtained from isolated glomeruli and qPCR was performed for collagen IV alpha1, 2, 3, 4, 5 as well as laminin alpha 1, 5 and beta 1, 2. Mouse kidneys were fixed. PAS staining was performed. For immunogold EM ultrathin sections of rat kidneys were labeled with a GLEPP1 antibody.

Results: GLEPP1 -/- mice display a significantly higher urinary ACR compared with age matched +/+ controls at the age of 6 and 10 months. At the age of 4 months there was no difference in proteinuria. Histologically, this correlated with focal thickening of the GBM in GLEPP1 -/- mice, resembling GBM humps. At the sights of these humps foot process effacement was observed. qPCR from glomeruli revealed significant upregulation of collagen IV alpha1 and 2 expression while alpha3 expression was significantly down regulated. qPCR also showed significant upregulation of laminin alpha1 and beta1 chains, while alpha5 and beta2 did not differ. Immunogold EM detected GLEPP1 expression mainly at the apical part of the foot processes but also in proximity to the glomerular slit.

Conclusions: GLEPP1 deficiency mediates the formation of GBM humps. With the occurence of the humps the type IV collagen alpha1 and 2 and laminin alpha1 and beta 1 is increased. There is evidence that GLEPP1 switches the GBM composition from a mature to an immature composition. This switch can only be seen in aging mice and results functionally in proteinuria. These findings stress the protective and beneficial role of GLEPP1 for podocytes.

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Albumin Modification Impacts Binding to the Transcytotic Receptor FcRn Mark C. Wagner,[1,2] Jered Myslinski,[1,2] Brittany N. Flores,[1,2] Bruce A. Molitoris.[1,2,3] *[1]Medicine, Indiana Univ School of Medicine, Indianapolis, IN; [2]Indiana Center for Biological Microscopy, Indianapolis, IN; [3]Roudebush VAMC, Indianapolis, IN.*

Background: Serum albumin is the most abundant carrier protein in blood and interacts with both small and large molecules. In addition, its long half-life in circulation enables more interaction with oxygen radicals, free sugars and other albumin reactive compounds present in blood. The focus of this study was to address specific *in vitro* albumin modifications and their impact on albumin-FcRn binding.

Methods: Since FcRn binds albumin strongly at acidic pH and releases it following transcytosis at physiological pH, binding at pH 6.0 and 7.4 was performed. Kd was measured using Microscale Thermophoresis (NanoTemper Technologies GmbH, Munich, Germany) which measures enzyme activities and biomolecule interactions under close-to-native conditions in immobilization-free and bioliquids of choice. The change in hydration shell of albumin is used to determine binding affinities with high accuracy and sensitivity.

Results: Purified soluble rat FcRn was incubated with normal rat albumin, human albumin and rat IgG and binding affinities agreed with previous studies. Glycated albumin results from the reaction of reducing sugars i.e. glucose, ribose which form a labile Schiff base that leads to a ketoamine adduct or Amadori product that overtime forms stable AGEs. Glucose, ribose and methylgloxal modified albumin (21day) all had reduced affinity to FcRn at pH 6.0 suggesting that these albumins would not be returned to the circulation via the transcytotic pathway. Interestingly, 7 day modified methylgloxal and glucose albumin retained pH sensitive binding suggesting that shorter exposure to reducing sugars does not alter albumin handling. Glycolaldehyde albumin had reduced binding at pH 6.0 but increased binding at pH 7.4. In CKD patients, urea leads to albumin carbamylation. Carbamylated RSA was found to have decreased FcRn binding at pH 6.0.

Conclusions: Understanding how modified albumins interact with this and other receptors i.e. Megalin/Cubilin, RAGE is critical to developing a complete picture of how the kidney handles this important protein.

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FR-PO487

Differential miRNA Expression in Parietal Epithelial Cells and Cellular Crescents of IgA Glomerulonephritis Jan U. Becker,[1] Bernd Hohenstein,[2] Catherine Meyer-Schwesinger,[3] Clemens L. Bockmeyer.[4] *[1]Inst of Pathology, Univ Hospital Cologne, Cologne, Germany; [2]Medical Clinic III, Nephrology, Univ Hospital Carl Gustav Carus, Dresden, Germany; [3]Internal Medicine, Nephrology, Univ Hospital Hamburg-Eppendorf, Hamburg, Germany; [4]Inst of Pathology, Hannover Medical School, Hannover, Germany.*

Background: miRNAs are known to regulate mRNA levels and mRNA translation, thus regulating cell functions including proliferation. Parietal epithelial cells (PECs) are key players in crescent formation. We hypothesize that altered miRNA expression contributes to the proliferation of PECs involved in crescent formation.

Methods: Each 5 human renal biopsies with non-crescentic IgA glomerulonephritis (IgA-GN), IgA-GN with fresh, cellular crescents (crescIgA-GN) and minimal tubulointerstitial nephritis as controls were chosen from our archive. PECs and crescents were microdissected from paraffin sections. miRNA expression was quantified in the microdissected fractions with RT-PCR low density arrays. Relative miRNA expression levels were compared with Tukey tests. Putative target genes and pathways of differentially expressed miRNAs were determined *in silico*.

Results: miR-708-5p and miR-21-3p were higher in crescents than in PECs of IgA-GN, crescIgA-GN and and controls. Putative targets of miR-708-5p include CD44, an activation marker of PECs, miR-708-5p is known to be involved in regulation of proliferation, negatively or positively depending on the cellular context. Putative targets of miR-21-3p include cyclin-dependent kinase 6 (Cdk6) and arginine/serine-rich coiled coil 2 (RSRC2), both involved in cell cycle control and expressed in PECs.

Conclusions: We found upregulation of miR-708-5p and miR-21-3p in crescents compared to PECs. While miR-708-5p rather appears to counteract proliferation, upregulation of miR-21-3p could contribute to PECs proliferation in crescent formation. *In vitro* studies with PECs will reveal the functional role of both miRNAs regarding proliferation and inflammatory activation.

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Transcriptome Analysis of Glomerular Disease Using PodNet, a Protein-Protein Interaction Network of the Podocyte Gregor Warsow,[1] Maja Lindenmeyer,[2] Nicole Endlich,[1] Matthias Kretzler,[3] Georg Fuellen,[4] Clemens D. Cohen,[2] Karlhans Endlich.[1] *[1]Anatomy and Cell Biology, Univ Medicine Greifswald, Greifswald, Germany; [2]Physiology, Univ of Zurich, Zurich, Switzerland; [3]Internal Medicine, Div. of Nephrology, Univ of Michigan, Ann Arbor, MI; [4]Biostatistics and Informatics in Medicine and Ageing Research, Univ Medicine Rostock, Rostock, Germany; [5]Genetics and Functional Genomics, Univ Medicine Greifswald, Greifswald, Germany.*

Background: Recently, we have built PodNet, a literature-based mouse network of protein-protein interactions (PPIs) in the podocyte (Warsow et al., *Kidney Int.* 2013). Mapping expression data of podocytes on PodNet, we gained valuable insight into podocyte differentiation in vivo and the loss of differentiation in culture. The aim of the present study was to analyze the glomerular transcriptome in various glomerular diseases using PodNet2, an updated and improved version of PodNet.

Methods: Recently published findings (2011-2013) were integrated into PodNet2. A mouse and a human version of PodNet2 were built and expanded to XPodNet2 by incorporation of PPIs from STRING database. The most differentially regulated interactions were identified with the Cytoscape plugin ExprEssence.

Results: After mapping glomerular transcriptomes of healthy and diseased kidney from the European Renal cDNA Bank on XPodNet2, the most differentially regulated interactions in FSGS, MCD and DN were identified. For example, small networks around collagen I and lipoprotein lipase were found to be up- and downregulated, respectively, in FSGS. Weighted gene correlation network analysis demonstrated that several modules with correlated gene expression changed in glomerular disease. As an example, FSGS modules with correlated gene expression contained frequently the gene ontology terms “cell cycle” or “mitosis”. Surprisingly, podocyte specific essential interactions (e.g. those of the slit diaphragm) exhibited a limited regulation in glomerular disease on a transcriptome level.

Conclusions: PodNet2 is a valuable tool for the comprehensive analysis of mouse and human expression data. Our analysis of human glomerular transcriptomes provides novel insight into gene expression in glomerular disease.

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FR-PO489

Inhibiting of Histone Deacetylase6 (HDAC6) Activity Alleviates Podocyte Injury in db/db Mice Yuxiong Lai, Wenjian Wang, Yuanhan Chen, Xiaofan Tan, Li Zhang, Yangyang Zuo, Lei Fu, Jianteng Xie, Jianxun Wu, Shuangxin Liu, Wei Shi. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Podocytes are a crucial cell type in kidney and play a vital part in the pathology of diabetic nephropathy. Histone deacetylase 6 (HDAC6) gets involved in neurodegenerative disorder and several kinds of cancer, but has rarely been studied on podocyte injury in diabetic nephropathy.

Methods: To assess the function of HDAC6 on podocyte injury, we firstly examined mRNA expression and HDAC6 activity in podocytes which were treated with advanced glycation end products (AGEs). Then a further study was carried out on a type 2 diabetic nephropathy animal model (db/db mice) and their age-matched wild-type (BKS) mice. At 12 weeks of age, half of the db/db mice were randomly chosen to receive tubacin, a selective inhibitor of HDAC6 (n = 4, i.p., 0.5 $mg \cdot kg^{-1}$, one time per 2 days for 8 weeks). In control group, 4 db/db and 4 BKS mice were given the same volume of DMSO without tubacin. Relevant physiological parameters were measured on schedule.

Results: When treated with AGEs, mRNA expression and activity of HDAC6 were up-regulated in podocytes. Tubacin markedly alleviates proteinuria in db/db mice, decreases mesangial expansion and prevents podocytes losses in gulermuli. Besides, tubacin treatment exhibites better renal protection effects bases on the urinary albumin excretion ($mg \cdot mg^{-1}$ creatinine), creatinine clearance analysis in db/db mice compared to the control.

Conclusions: These data indicate that HDAC6 plays an important role in podocyte injury. Tubacin, the inhibitor of HDAC6, demonstrates a markedly renopreotection effect on podocytes exposured to hyperglycemia. However the exact mechanism under this role needs further study.

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FR-PO490

Investigation of the Role of the Inflammasome Triggering PYHIN Interferon Gene Family in Systemic Lupus Erythematosus Model BXSB Allyson Catherine Egan,[1] Michael D. Jones.[2] *[1]Dept of Medicine, Hammersmith Hospital, Imperial College London; [2]Genomics Laboratory, MRC Clinical Sciences Centre, Imperial College London.*

Background: The telomeric region of chromosome 1 (C1q21-23) in murine models and a syntenic region in humans is associated with the development of Systemic Lupus Erythematosus (SLE). This interval encodes three candidate gene families – *PYHIN*, Slam and Fcgamma Receptor. The interferon inducible PYHIN gene family includes *AIM2*, a unique cytosolic DNA sensor which instigates the formation of the inflammasome yielding cell death via IL-1b and IL-18. This project sought to develop a novel subcongenic mouse model containing the *PYHIN locus, B10. Yaa.Bxs3.Ifi* to investigate the potential role of this gene family in SLE development.

Methods: (B10 x B10.*Yaa.Bxs3*) F1 male mice were backcrossed with B10 (non-autoimmune) females. Recombinants were genotyped at six loci using polymerase chain reaction and sequenced within the*Slam* locus. ANA slides were prepared using Hep2 cells. Renal H&E sections were graded according to mesangial hypercellularity and matrix increase. Flow cytometric analyses (FACs) of splenic B and T-cells were performed.

Results: Three novel subcongenics B10.*Yaa.Bxs3.Ifi*, B10.*Yaa.Bxs3.Ifi/Slam* and B10.*Yaa.Bxs3.Fcgr* were generated. Phenotypically, in B10.*Yaa.Bxs3.Ifi* there was significant difference in the levels of ANA titres, evidence of nephritis and elevated splenic weights and lengths in comparison to the non-autoimmune strain B10.*Yaa* at twelve months. These trends reflect the autoimmune parental strain B10.*Yaa.Bxs3*. FACS analysis of B10.*Yaa, Bxs3.Ifi* at six months demonstrated expanded B-cell $CD45^{-}$ populations similar to autoimmune B10.*Yaa. Bxs3*, which was not present in the non-autoimmune B10.*Yaa*.

Conclusions: The three novel subcongenic models uniquely facilitate the opportunity to dissection the candidate gene families *HIN200*, *Slam* and *Fcgramma's* contribution to the SLE phenotype. The *PYHIN* subcongenic, identifies that phenotypic features of SLE are present in the subcongenic at 12 months, supporting the role of this interferon inducible gene family as a candidate gene for the development of SLE.

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Development of an In Vitro Mesangial Cell Model for Progressive Glomerulosclerosis by Disruption of the ANAPC-10 Gene in Fvb Mice Yongxin Gao, Kathleen O. Heilig, Zaid Brifkani, Charles W. Heilig. *Medicine, Univ of Florida College of Medicine - Jacksonville, Jacksonville, FL.*

Background: We previously developed Fvb Os/+ mice with disruption of the ANAPC-10 gene. These mice exhibit rapidly progressive glomerulosclerosis (GS) with heavy albuminuria, early renal failure and death at approximately 12 weeks of age. Fvb Os/+ glomeruli have enhanced glucose uptake. Here we isolated primary culture mesangial cells (MC) from Fvb Os/+ mice and Fvb +/+ wild type control mice to investigate the mechanism of GS in Fvb Os/+.

Methods: Effects of the ANAPC-10 mutation (Anaphase Promoting Complex Subunit 10) in chromosome 8 were determined by analysis of cell growth curves, and analysis of Glucose Transporter-1 (GLUT1), Mechano-Growth Factor (MGF) and extracellular matrix (ECM) proteins in Western analyses, immunofluorescence microscopy (IF), and/or immunohistochemistry (IHC). RNA/DNA ratios were determined as a marker for cell size.

Results: Fvb Os/+ MC had a 16% lower RNA/DNA ratio suggesting reduced cell size. Fvb Os/+ MC also exhibited severely slowed growth in vitro, compared to Fvb +/+ control MC. Western analyses revealed Fvb Os/+ MC had increased MGF (3.5-fold) and GLUT1 (9-fold). These are both stimuli for ECM expression. Fibronectin (FN) was increased 4-fold, and type IV collagen (Col-IV) increased 9-fold in Fvb Os/+. All of these changes were $P < .005$ versus Fvb +/+ control. This was evident even after 8 passages of the MC's in culture. IF and IHC confirmed increased FN (an expanding lattice between cells) and Col-IV in the MC.

Conclusions: 1) ANAPC-10 gene disruption slowed the growth of Fvb Os/+ MC, an expected consequence of impairing mitosis. 2) Fvb Os/+ MC produced excessive FN and Col IV, providing an explanation for the rapidly progressive glomeruloscloerisis (GS) in Fvb Os/+ mice. 3) Increased MGF and GLUT1 in Fvb Os/+ MC provide a potential mechanism for the excessive ECM production these cells, likely via enhanced glucose uptake and metabolism which both of these proteins stimulate. 4) The Fvb Os/+ and Fvb +/+ MC provide a new in vitro model for investigation of mechanisms leading to progressive GS in vivo.

Funding: Pharmaceutical Company Support - Dialysis Clinics Inc.

FR-PO492

Mechano-Growth Factor Regulation of Mesangial Cell Growth and Extracellular Matrix Protein Expression: Implications for Diabetic Nephropathy Yongxin Gao,[1] Kathleen O. Heilig,[1] Raafat Farag Makary,[1] Zaid Brifkani,[1] Mohamed G. Atta,[2] Charles W. Heilig.[1] *[1]Medicine, Univ of Florida College of Medicine - Jacksonville, Jacksonville, FL; [2]Medicine, Johns Hopkins Univ, Baltimore, MD.*

Background: We previously identified Mechano-Growth Factor (MGF) as a glomerular mesangial and renal tubular protein expressed in normal mouse kidney, induced in glomeruli by type 1 and type 2 diabetes mellitus. MGF stimulated expression of GLUT1 and glucose uptake in mesangial cells (MC) in vitro. Here we investigated MGF effects on MC growth, fibronectin (FN) and type IV collagen (Col-IV) expression, +/- high glucose.

Methods: Primary culture mouse MC (C57BL6 background) were transduced with MGF-Sense (MGF-S cells) MGF-Antisense (MGF-AS cells), or Empty Vector (MGF-EV control cells) MoMuLV retroviral expression constructs, cloned, and studied in vitro for cell growth rate, RNA/DNA ratio (cell size), Western analyses of MGF, GLUT1, FN and Col-IV, and immunolabeling for selected proteins. MC were grown in standard 8mM glucose medium, or in 20mM high glucose medium where indicated.

Results: MGF-S MC overexpressed MGF > 10-fold, with an enhanced growth rate versus MGF-EV, and versus MGF-AS which had delayed growth. MGF-S had increased GLUT1 expression (4-fold), though MGF-AS did not have suppressed GLUT1. MGF-S have a 2 - 3-fold elevated ^{3}H2-deoxyglucose uptake rate which correlates with the increased GLUT1. MGF-S had RNA/DNA 27% higher than MGF-EV, indicating hypertrophy. In contrast, MGF-AS had 3.8% lower RNA/DNA, suggesting reduced cell size. MGF-S displayed increased extracellular matrix (ECM) protein expression: FN (1.6-fold) and Col-IV (1.7-fold), persistent after numerous cell passages. 20mM High glucose induced MGF (17-fold), GLUT1 (5.9-fold), FN (7.2-fold), and Col-IV (2.7-fold). MGF-AS was protected against 20mM high glucose induction of GLUT1 (by 31%), FN (by 11%) and Col-IV (by 56%).

Conclusions: 1.MGF, inducible by diabetes and an inducer of GLUT1, stimulated cell hypertrophy, proliferation, FN and Col-IV in vitro. 2. High glucose stimulated MGF, GLUT1, FN and Col-IV, and Antisense-MGF protected against these responses, indicating MGF involvement in mediating these effects of glucose. 3.Future studies will further delineate mechanisms by which MGF induces ECM and GS.

Funding: Pharmaceutical Company Support - Dialysis Clinics Inc.

FR-PO493

Effect on Cultured Mesangial Cells of Chinese Herbal Medication Prescribed for IgA Nephropathy Zhi Qiang Huang,[1] Xianwen Zhang,[1,2] Lin Wang,[2] Qi Bian,[1,3] Stacy D. Hall,[1] Bruce A. Julian,[1] Yiping Chen,[2] Jan Novak.[1] *[1]Microbiology, Univ of Alabama at Birmingham, Birmingham, AL; [2]Nephrology, Shanghai Univ of Chinese Traditional Medicine, Long Hua Hospital, Shanghai, China; [3]Nephrology/Medicine, ChangHai Hospital, Shanghai, China.*

Background: IgA nephropathy (IgAN) is an autoimmune disease induced by circulating immune complexes consisting of galactose-deficient IgA1 (Gd-IgA1) and autoantibodies against Gd-IgA1. IgAN is a mesangioproliferative disease in which activation of the PDGF pathway is thought to be involved in mesangial cell proliferation and subsequent glomerular injury. A prescribed Chinese herbal mixture, ShenAn decoction (SA) has been used to treat IgAN patients with renal insufficiency in China. Its mechanism of action is unknown. In this study, we investigated the effects of SA on mesangial-cell PDGF signal transduction and proliferation.

Methods: Cultured, quiescent human primary mesangial cells were treated with PDGF, with or without SA. Protein phosphorylation was analyzed with Western blotting of cell lysates with anti-phospho-tyrosine antibody (PY20), anti-phospho-ERK antibody, and anti-phospho-p38 antibody. Cellular proliferation was assessed 24 h after PDGF treatment with or without of SA, using Syto 60.

Results: PDGF increased mesangial-cell proliferation and SA blocked the stimulatory effects of PDGF in a dose-dependent manner. PDGF enhanced phosphorylation of multiple proteins, including ERK and p38. SA inhibited this PDGF-induced phosphorylation in a dose-dependent manner.

Conclusions: SA inhibited PDGF-induced mesangial-cell phosphorylation, possibly due to the presence of inhibitors of protein kinases. Identification of the active components of SA may provide explanations of the mechanisms of action for this treatment of IgAN.

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FR-PO494

Activation of Human Mesangial Cells by PDGF: Gene Expression and Signaling Study Qi Bian,[1] Stacy D. Hall,[1] Zhi Qiang Huang,[1] Leona Raskova Kafkova,[1,2] Xianwen Zhang,[1] Bruce A. Julian,[1] Jan Novak.[1] *[1]Univ of Alabama at Birmingham, Birmingham, AL; [2]Palacky Univ, Olomouc, Czech Republic.*

Background: Glomerular mesangial injury characterized by proliferation of mesangial cells (MCs) is an important feature in IgA nephropathy (IgAN). Platelet-derived growth factor (PDGF) is a potent stimulator of MC. Accumulating evidence indicates a critical involvement of PDGF signaling in MC in IgAN and other glomerular diseases. To better understand the effect of PDGF on MC, we studied signaling pathways and differential gene expression in PDGF-stimulated MC.

Methods: MC proliferation was measured by ^{3}H-thymidine or BrdU incorporation. Differential gene expression by PDGF after 24-h stimulation (proliferation peak) of quiescent primary human MCs was determined by high-density microarrays; genes were clustered by pathway mapping. Data were validated by RealTime RT-PCR (qPCR) and immunodetection. Signaling in MCs was evaluated by Western blotting with phospho-specific antibodies.

Results: In PDGF-treated MCs, 2,997 genes were expressed at higher levels, 2,197 genes at lower levels. Using cut off value $p<0.001$, 30 genes were significantly up-regulated and 7 genes significantly down-regulated by PDGF. By clustering analysis, 95 genes were induced and 10 genes repressed. These up-/down-regulated genes were involved in different biological processes, cellular proliferation, apoptosis, signaling, energy and lipid metabolism, ion-transport, immune regulation, and inflammation. qPCR confirmed 4.3- to 61.8-fold increased expression of PTX3, IL-8, IL-6, MCP-1, and TGF-β. Immunodetection confirmed increased protein expression of TIMP1, MMP9, and IL-8. Signaling studies revealed that PDGF increased phosphorylation of multiple proteins, including ERK1/2, p38, Akt1, and FLHRL1. MEK1/2 inhibitor reduced phosphorylation of ERK1/2 and Akt1, whereas PI3K inhibitor reduced phosphorylation of Akt1 but not of FLHRL1.

Conclusions: PDGF signaling through several pathways resulted in MC proliferation and differential gene expression. Targeting PDGF-specific pathways in MC may provide a new tool for treating mesangioproliferative diseases.

Funding: NIDDK Support

FR-PO495

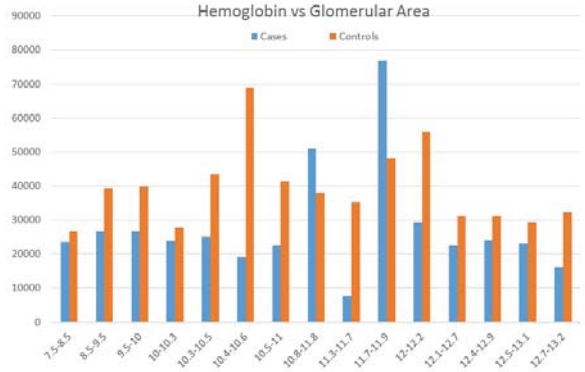
The Role of Anemia in Cardio-Renal Syndrome: Does It Impact Glomerular Size? Janice Berthe Desir,[1] Joel Neugarten,[2] Michal L. Melamed,[1] James M. Pullman,[2] Ladan Golestaneh.[2] *[1]Nephrology, Albert Einstein College of Medicine, Bronx, NY; [2]Nephrology, Montefiore Medical Center, Bronx, NY.*

Background: The presence of anemia among patients with congestive heart failure (CHF) is fairly common and found irrespective of kidney parenchymal disease. This impact on the renal histology of cardio-renal syndrome (CRS) is unclear. Patients with cyanotic heart disease are noted to have glomerulomegaly. The mechanism is purported to be polycythemia. Our recent case controlled study of anemic CHF patients showed small glomerular area. This suggests anemia may play a different role in glomerular size in patients with non-cyanotic heart disease.

Methods: We measured the total glomerular areas of 16 adult cases with end stage heart disease using the BioQuant Image Analysis program. Kidney biopsies were performed in the case patients during evaluation for heart transplant at Montefiore Medical Center between January 2007 and December 2013. Matching controls were selected from renal cell carcinoma nephrectomy samples.

Clinical Characteristcs	Cases	Controls
Gender (% male)	94 (15/16)	-
% DM	56 (9/16)	-
Mean BMI	29.8	31.8
Mean Age	59.7	60.4
Mean GFR at Biopsy	38.38	44.38

Results: 15/16 patients met the definition for anemia with hemoglobin (Hb) values less than 14 g/dL. The Hb values were 7.8-13.3 g/dL with a mean value of 11.2 g/dL among the cases and 8.2-14.8 g/dL with a mean value of 11.4 g/dL among the controls. The correlation coefficient between Hgb levels and glomerular area was 0.20 with p-value = 0.27. Hgb values were analyzed in both a continuous and dichomatous fashion and were found to have to no correlation with glomerular area.



Conclusions: This case controlled study demonstrates that patients with heart failure have smaller glomerular size that is not related to anemia. Future studies are needed to examine the histology of cardio renal syndrome.

FR-PO496

Podocytes Are a Target of Nicotine: Link to Proteinuria and Progression of Renal Disease? Leopoldo Raij,[2] Runxia Tian,[1] Bernice Acevedo,[1] Vasil Peev,[1] Sandra M. Merscher,[1] Alessia Fornoni,[1] Ming-Sheng Zhou.[1] *[1]Medicine, VAMC, Miami, FL; [2]Medicine, Univ of Miami, Miami, FL; [3]Research, SFVAFRE, Miami, FL.*

Background: Epidemiologically the link between cigarette smoking (CS), proteinuria and renal failure progression is overwhelming but the mechanisms remain obscure. We reported that Nicotine (NIC) increased superoxide anion (O_2^-) in human mesangial cells via non-neuronal nicotinic receptors (nAchRs) (AJP '07). Further, in human macrophages NIC increased O_2^-,and foam cell formation linked to increase B scavenger receptor CD36 and oxLDL uptake (AJP'13).Here we studied whether human podocytes (POD) are a target of NIC.

Methods: In immortalized human POD we determined by WB expression of 1) Synaptopodin (Synpo): key stabilizer of actin skeleton 2) Activated AMPK (pAMPK): suppresses NADPH, 3) CD36 and 4) *for the first time*: a2, a3, a4 and b3nAchRs. With lucigenin we determined O_2^- production in POD treated with NIC 100nmol/L, a plasma concentration attained with CS and E- CS. We preincubated POD with Hexametonium: blocker of nAchRs; DPI: inhibitor of NADPH; Catalase: inhibitor of H2O2 and AICAR: activator of AMPK and after 24hrs POD incubation with 50ug/ml oxLDL we measured POD cholesterol (Chol) ug/mg prot, and apoptosis (APOPT) flow.cytom. Stats.ANOVA-Bonferonni's- Scheffe's.

Results: NIC increased POD O_2^- production, max150% at 6 hrs; Synpo and active pAMPK were reduced 50% ($p< 0.05$). DPI and AICAR prevented reduction of Synpo, pAMPK and the increase in O_2^- ($p<0.05$). NIC upregulated POD CD36 40% ($p<0.05$) which was prevented by Hexametonium, DPI and Catalase. 24 hrs. oxLDL incubation increased POD Chol 28% whereas oxLDL+NIC increased Chol 54 % ($p< 0.05$) that was reduced to 17% in CD36 SiRNA pretreated POD ($p<0.05$) Only 2.8 and 2.6 % of POD incubated with NIC or oxLDL were APOPT whereas 9.4 % in oxLDL+ NIC were APOPT ($p< 0.05$ versus all); CD36-SiRNA reduced POD APOPT to baseline.

Conclusions: Concurrent $O2^-$ production, reduction in Synpo and up regulation of CD36 associated with oxLDL uptake and POD apoptosis, are sufficient to implicate nicotine as a risk factor for proteinuria and progression of renal failure linked to protracted CS and, likely, E-Cigarettes smoking.

Funding: Private Foundation Support

FR-PO497

Triptolide, an Extracted Phytomedicine Attenuates Glomerularsclerosis in Diabetic Nephropathy Rats via Regulation of Akt/AMPK/mTOR and TGF-β1/Smad Signaling Activities, Compared with Rapamycin Yigang Wan, Zhimin Mao. *Dept of Traditional Chinese Medicine, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing Univ Medical School, Nanjing, China.*

Background: Triptolide (TP), a natural extract from Tripterygium wilfordii has been applied extensively for treating glomerularsclerosis (GS) in patients with early diabetic nephropathy (DN) in China. Activation of mTOR plays a critical role in pathologic forms of hypertrophy and proliferation in kidneys under hyperglycemia other than classical TGF-β1/Smad pathway. Hyperglycemia increases mTOR activity by combined actions of Akt activation and AMPK inhibition. This study thus aimed to investigate effects and mechanisms in vivo of TP on GS, compared with rapamycin (mTOR inhibitor), through regulating Akt/AMPK/mTOR or TGF-β1/Smad signaling activities.

Methods: Rats were randomly divided into 4 groups, sham-operated group, TP-treated group, vehicle-given group and rapamycin-treated group, and sacrificed at week 8 after the induction of DN by 2 consecutive intraperitoneal injections of streptozotocin (STZ) with an interval of 1 week following unilateral nephrectomy. Daily oral administration of TP, rapamycin and saline were started after the second injection of STZ until the day of sacrifice. Proteinuria, UAlb, BG, biochemical indicators, renal pathological changes, as well as key protein expressions in Akt/AMPK/mTOR and TGF-β1/Smad pathways in kidneys were examined, respectively.

Results: Akt/AMPK/mTOR and TGF-β1/Smad pathways were concurrently activated in kidneys. TP, similar to rapamycin, markedly regulated protein expressions of p-Akt, p-mTOR, p-p70S6 kinase, p-Smad2/3 and TGF-β1 in kidneys, and ameliorated proteinuria, mesangial matrix expansion, a-SMA expression and collagen deposition in glomeruli, without lowering hyperglycemia. Additionally, retardation in glomerularsclerotic development was observed.

Conclusions: Activated Akt/AMPK/mTOR and TGF-β1/Smad pathways jointly contribute to glomerular injury. TP, as a natural regulator in vivo, effectively attenuates GS by potential molecular mechanisms involving reduction of mesangial matrix and suppression of Akt and mTOR activation, as well as regulation of TGF-β1/Smad signaling activity.

Funding: Government Support - Non-U.S.

FR-PO498

Lack of Protein Fatty-Acylation Can Cause Focal Segmental Glomerulosclerosis (FSGS) Giuseppina Federico,[1] Stefan Porubsky,[1,2] Francesca Rampoldi,[1] Christoph Kuppe,[3] Hermann-Josef Groene.[1] *[1]Cellular and Molecular Pathology, German Cancer Research Center, Heidelberg, Germany; [2]Inst of Pathology, Univ Medical Center Mannheim, Germany; [3]Dept of Nephrology and Clinical Immunology, RWTH Aachen, Germany.*

Background: Protein fatty-acylation is the attachment of fatty acids to proteins. Protein N-myristoylation represents one type of protein fatty-acylation and refers to the attachment of myristic acid to the N-terminal glycine. This modification is catalyzed by N-myristoyltransferases (Nmt1 and Nmt2). Our aim was to elucidate the role of N-myristoylation for the morphology and function of podocytes.

Methods: LoxP-sites were introduced into the *Nmt1* and *Nmt2* loci by recombination in embryonic stem cells and podocyte-specific deletion was achieved by expressing Cre under the control of podocine promoter (Pod^{Cre}). At day 1, 17, 35 and 70 after birth, morphological analysis and functional tests were performed.

Results: Mice with podocyte-specific deletion of both Nmt1 and Nmt2 ($Pod^{Cre}/Nmt1^{flox/flox}/Nmt2^{flox/flox}$) did not show proteinuria at day 1 after birth. However, starting at day 17, they developed a FSGS accompanied by an increasing proteinuria. At day 35, these changes progressed to a diffuse segmental and global glomerulosclerosis with an overt nephrotic syndrome leading to death before day 70 in most of the $Pod^{Cre}/Nmt1^{flox/flox}/Nmt2^{flox/flox}$ mice. Upon electron microscopy, podocytes lacking NMT activity showed a fusion of foot processes. In contrast, the glomerular scaring and proteinuria were significantly milder or absent in mice lacking only *Nmt1* or *Nmt2* respectively. We could show that cytoskeleton-associated proteins such as MARCKS (myristoylated alanine-rich C kinase substrate) need myristoylation activity for their function.

Conclusions: These results show that protein N-myristoylation is essential for a proper localization and function of proteins in podocytes. Investigations are currently done to determine which metabolic and immunologic diseases interfere with protein N-myristoylation in podocytes. We propose that metabolic alterations may potentially initiate the most frequent form of FSGS, which is associated with degenerative and immune complex diseases.

Funding: Government Support - Non-U.S.

FR-PO499

Anti-PDGFRb Antibody Protects Col4a3-/- Mice From the Development of Alport Nephropathy Naoki Nakagawa,[1] Ivan G. Gomez,[1] Shreeram Akilesh.[1] *[1]Medicine & Pathology, Univ of Washington, Seattle, WA; [2]Medicine & Pathology, Univ of Washington, Seattle, WA; [3]Medicine & Pathology, Univ of Washington, Seattle, WA; [4]Medicine & Pathology, Univ of Washington, Seattle, WA.*

Background: The platelet-derived growth factor receptor (PDGFR) has been implicated in a number of glomerular and interstitial fibrogenic diseases in the kidney. Inhibitors of the kinase activity of PDGFRa and PDGFRb have been shown to have activity in animal models of glomerular and interstitial disease. Recently we showed that the major fibrogenic cells in the kidney express PDGFRa and PDGFRb and that blocking these receptors with soluble receptor ligand trap or monoclonal antibodies had a profound impact on fibrogenic and inflammatory responses (Chen YT, et al. Kidney Int. 2011 80:1170-81; Lin et al Am J Pathol. 2011 178:911-23). In the following studies we tested the efficacy of monoclonal antibody 1B3 (Imclone), which specifically blocks PDGF-BB binding to PDGFRb and blocks downstream signaling pathways.

Methods: Col4a3-/- mice (n=12/group) on the 129SV background were given anti-PDGFRb antibody 1B3 or isotype control antibodies (40mg/kg ip twice/week) from week 3 to week 9. Mice were evaluated for kidney function and histology at 9 weeks.

Results: No toxic or deleterious effects were observed. Mice receiving 1B3 antibody had increased body weight at 9 weeks compared to control (indicative of improved health), had a 50% reduction in BUN levels, 50% reduction in albuminuria. Mice receiving active antibody had a 50% reduction in fibrosis, and marked preservation of tubule histology and function. Glomerular histology was preserved with many fewer glomeruli showing sclerosis.

Conclusions: Antibody mediated blockade of PDGFRb signaling is a promising strategy in the treatment of Alport Nephropathy and potentially other chronic kidney diseases.

FR-PO500

APOL1 Variants Induce Pyroptosis and Formation of Inflammasomes in Human Podocytes through the Activation of Caspase-1 Shabirul Haque,[1] Xiqian Lan,[1] Hongxiu Wen,[1] Ashwani Malhotra,[1] Karl Leon Skorecki,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Medicine, Rambam Health Care Campus, Haifa, Israel.*

Background: Expression of APOL1 variants (Vs) are associated with the occurrence of higher rates of FSGS in patients with Sub-Saharan African Ancestry. Since podocyte loss is a common pathway for the development of FSGS, we hypothesized that APOL1Vs may be contributing to podocyte loss through pyroptosis and the release inflammasomes, which promote the maturation of inflammatory cytokines such as interleukin (IL)-1 ß and IL-18. These interleukins participitate in multiple cellular activities, including interferon-g secretion, activation of lipid biosynthesis, and pyroptosis, a process of programmed cell death distinct from apoptosis.

Methods: To evaluate the role of APOL1Vs in the induction of podocyte injury, we overexpressed APOL1Vs (G1, G2 and G1+G2 combined) as well as APOL1WT (G0) in human podocytes (HPs). Vector/HPs, APOL1G0/HPs, APOL11 G1/HPs, APOL1G2/HPs and APOL1G1/G2/HPs were stained with H33342 and propidium iodide as a measure of pyroptosis (condensed nuclei with loss of cell wall integrity). RNA was extracted and cDNA was probed for NLRP3 expression. Protein blots were probed for podocyte caspase-1 and IL1-ß expression as markers of inflammasome formation. To test for a causal relationship, the effect of a caspase-1 inhibitor was evaluated on vector/APOL1-, APOL1G0- and and APOL1Vs-induced podocyte inflammasome formation and pyroptosis.

Results: APOL1G0/HPs and APOL1G1/HPs and G2/HPs displayed a greater ($P<0.01$) number of cells with evident pyroptosis when compared to Vector/HPs. However, strikingly, APOL1 G1/G2/HPs displayed the most significant ($P<0.01$) elevation of cells with evidence of pyroptosis. Protein blots of APOL1G0/HPs and APOL1G1/HPs and APOL1G2/HPs displayed enhanced expression of caspase-1 and IL-1ß. Since a caspase-1 inhibitor not only inhibited APOL1vs-induced IL-1ß beta production, but also attenuated induction of pyroptosis, it appears that APOL1Vs-induced pyroptosis is mediated through caspase-1 activation.

Conclusions: APOL1Vs induce pyroptosis and inflammasome formation through the activation of caspase-1.

Funding: NIDDK Support

FR-PO501

Enhancement of Wnt/β-Catenin Signaling Promotes Renal Fibrosis by Inducing Cell Cycle Arrest at the G2/M Phase in Tubular Epithelial Cells Minoru Satoh, Hiroyuki Kadoya, Seiji Itano, Atsushi Uchida, Chieko Ihoriya, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Recent studies have indicated that renal epithelial cell cycle arrest at the G2/M phase promote renal fibrosis. However, the mechanism of cell cycle arrest at the G2/M phase on the process of renal fibrosis is not fully elucidated. The forkhead transcription factors of the O subgroup (FOXO) functions at the G2/M checkpoint to induce a delay in G2 to M progression. FOXO transcriptional activity is enhanced by β-Catenin and concomitantly with oxidative stress, both of which are increased in the process of renal fibrosis. It is possible that increased levels of β-Catenin/FOXO complex may participate

in G2/M cell cycle arrest. We hypothesized that oxidative stress-dependent β-Catenin signaling could promote renal fibrosis by leading to cell cycle arrest at the G2/M phase in tubular epithelial cells.

Methods: In vitro experiment, we examined whether the profibrotic cytokine expression at the G2/M phase is increased by Wnt3a stimulation with oxidative stress in human proximal tubule epithelial cells. Next, we examined whether renal fibrosis could be reduced by antioxidant treatment in nephritic mice (ICGN).

Results: In vitro, cell cycle arrest in G2/M phase was increased by the Wnt stimulation under the presence of hydrogen peroxide. In addition, Wnt3a with hydrogen peroxide also increased growth arrest and DNA-damage-inducible protein 45a and cyclin G2 genes with increase of β-Catenin/FOXO complex. In the G2/M phase cells, transforming growth factor (TGF)-β and connective tissue growth factor (CTGF) expressions were increased. In vivo, ICGN mice were treated with or without tempol for 6 weeks. Tempol prominently decreased the tubular expressions of phospho-Histone H3, marker of the G2/M transition. Decreased expression of TGF-β1 and CTGF were observed in the tempol-treated ICGN micecompared with non-treated control ICGN mice.

Conclusions: Oxidative stress with Wnt stimulation induces G2/M cell cycle arrest and increases profibrotic cytokine production. Reduction of the oxidative stress could ameliorate the renal fibrosis.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO502

Kidney Fibrogenesis Is Determined by the Subcellular Localization of YB-1

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Background: Y-box binding protein-1 (YB-1) exerts pleiotropic functions in gene transcription and translation, e.g. of fibrosis-related genes. This led us to investigate the outcome of half-maximal YB-1 depletion in two models of renal fibrosis, namely unilateral ureteral obstruction (UUO) and cyclosporine A (CsA)-induced nephropathy. Heterozygous YB-1 knockout (Yb1+/d) mice exhibit half-normal YB-1 content on mRNA and protein level. Homozygous YB-1 KO mice are not viable and die before birth due to neuronal disorders.

Methods: Parameters of renal fibrosis were determined by immunohistochemistry (IHC), qRT-PCR and Western blot (WB) analyses. We performed ex vivo chromatin immunoprecipitation and mRNA precipitation in kidney tissues obtained from Yb1+/d and wildtype (wt) mice to dissect between nuclear and cytoplasmatic YB-1 functions. Nuclear shuttling of YB-1 was induced in vivo by a small chemical compound (HSc025).

Results: Renal damage and fibrosis development were markedly aggravated in CsA-induced nephropathy in Yb1+/d compared to wt mice. In contrast, Yb1+/d animals were protected from tubular injury and renal fibrosis following UUO (mean fibrosis score: wt: 1.54 ±0.20; Yb1+/d: 0.97±0.36), with significantly reduced ECM deposition (e.g. Col1A, fibronectin), alpha-SMA expression and impaired inflammatory responses (monocyte infiltration, chemokine expression). We could localize YB-1 in the renal cytoplasm in UUO and this contributed to enhanced Col1A mRNA stabilization and to the nucleus in the CsA model, where it repressed Col1A gene expression. In line with this, in human biopsy samples of calcineurin inhibitor-treated patients, YB-1 mainly localized to the nuclei of tubular cells. Forced nuclear shuttling of YB-1 in wt mice triggered by HSc025 attenuated renal fibrosis in the UUO model (mean fibrosis score: wt: 1.54±0.2; wt/HSc025: 0.85±0.26).

Conclusions: In conclusion, intracellular localization of YB-1 decides on aggravation or improvement of renal fibrosis, and forced nuclear YB-1 shuttling may be a novel anti-fibrotic approach.

FR-PO503

ASK1/p38 Signaling in Renal Tubular Epithelial Cells Promotes Renal Fibrosis in the Mouse Obstructed Kidney

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Background: The stress-activated kinases, p38 mitogen-activated protein kinase (MAPK) and c-Jun amino terminal kinase (JNK), promote renal fibrosis; however, the pathways by which these kinases are activated in kidney disease remain poorly defined. Apoptosis signal-regulating kinase 1 (ASK1/MAP3K5) is a member of the MAPKKK family which can induce activation of p38 and JNK. This study examined whether ASK1 induces p38/JNK activation and renal fibrosis.

Methods: Groups of 6 wild type (WT) and Ask1 deficient (Ask1-/-) mice underwent unilateral ureteric obstruction (UUO) and were killed on day 7. In separate studies, cultured renal fibroblasts were isolated from day 5 UUO kidneys, while cultured tubular cells were isolated from animals without surgery.

Results: Basal p38 and JNK activation in WT kidney was increased 3-5 fold on day 7 UUO in association with renal fibrosis. In contrast, there was no increase in p38 activation in Ask1-/- UUO, while JNK activation was only partially increased. Accumulation of a-SMA+ myofibroblasts and collagen deposition were significantly reduced in Ask1-/- UUO. However, cultured WT and Ask1-/- renal fibroblasts showed equivalent proliferation and matrix production, indicating that ASK1 acts indirectly on fibroblasts. Tubular epithelial cells are the main site of p38 activation in the obstructed kidney. Interestingly, angiotensin II but not IL-1 or LPS induced p38 activation and up-regulation of TGF-β1 and MCP-1 production was suppressed in Ask1-/- tubular epithelial cells, demonstrating that diverse stimuli can induce p38 signalling in tubular epithelial cells via different upstream MAP3K family members. In addition, macrophage accumulation was significantly inhibited in Ask1-/- UUO mice.

Conclusions: ASK1 is an important upstream activator of p38 and JNK signalling in the obstructed kidney, and ASK1 as a potential therapeutic target in renal fibrosis.

Funding: Government Support - Non-U.S.

FR-PO504

Tissue Transglutaminase (TG2), a Partner of Anti-Angiogenic Peptide Endostatin, Participates in the Renal Fibrosis of Aging in Mice

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Background: We have recently demonstrated (AJP: Heart, 2014) that accumulation of an anti-angiogenic fragment of collagen XVIII, endostatin (Endost), occurs in aging kidneys and may contribute to nephrosclerosis. TG2, a partner of endostatin, is a multifunctional protein richly expressed on the surface of vascular endothelium and in the extracellular matrix, where it cross-links fibronectin, collagen, vitronectin, osteopontin.

Methods: We used mice of different ages (from 2 mo to 24 mo on C57 background) to examine expression of TG2 in the kidneys by western blotting and immunofluorescence microscopy. Similar parameters were studied in collagen XVIII knockout (KO) mice of the same age groups and background.

Results: Endost was overexpressed in aged kidneys and suppressed ex vivo vascular sprouting. This effect was in part attributed to development of endothelial-mesenchymal transition. Immunodetection studies demonstrated upregulated expression of TG2 in aged vis-à-vis young mice, which paralleled increased nephrosclerosis. This was accompanied by enhanced TG2 activity, as judged by the extent of protein cross-linking detected using antibodies against N^{ε}-g-glutamyl-lysine residues in aging kidneys. We next examined the age-dependence of nephrosclerosis and TG2 expression and activity in Endost-deficient collagen XVIII KO mice. The kidneys from these mice showed nephrosclerosis of aging indistinguishable from wild-type mice, but the reduced levels of immunodetectable TG2 and N^{ε}-g-glutamyl-lysine cross-links. When ex vivo angiogenesis assays were performed in Matrigel supplemented with a recombinant TG2, vascular sprouting of aortic rings obtained from aSMA-GFP mice was reduced.

Conclusions: The data confirm progression of nephrosclerosis in aging mice. Data demonstrate that in vitro preconditioning of Matrigel with TG2 reduces vascular sprouting. The findings demonstrate that Endost deficiency reduces age-dependent increase in abundance and activity of TG2.

Funding: NIDDK Support, Private Foundation Support

FR-PO505

STAT3 Acetylation Is Associated with Fibrotic Responses in Renal Tubular Epithelial Cells and the Obstructed Kidney

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Background: Signal transducer and activator of transcription 3 (STAT3) is activated during the progression of renal fibrosis. Janus kinase 2 (JAK2) mediates the effects of angiotensin II (Ang II)-induced STAT3 phosphorylation, while p300 and SIRT1 are involved in regulating STAT3 acetylation. In this study, we explored the correlation between STAT3 acetylation and renal fibrosis.

Methods: Western blot was used to evaluate acteyl-STAT3 (Lys685), phospho-STAT3 (Tyr705), fibronectin, collagen IV, a-smooth muscle actin (a-SMA) and transforming growth factor-b1 (TGF-β1) in renal tubular epithelial cells and kidneys of a murine unilateral ureteral obstruction (UUO) model. Fluorescent immunocytochemistry was used to observe phospho-STAT3 (Tyr705). Transfection of a recombinant palsmid vector carrying p300 gene was used to up-regulate p300 expression. siRNA was used to knockdown p300 expression. Hematoxylin-eosin (HE) and Masson's trichrome staining were used to observe renal interstitial fibrosis.

Results: *In vitro* studies showed that Ang II increased phospho-JAK2 (Tyr1007/1008), phospho-STAT3 (Tyr705), fibronectin, collagen IV and a-SMA. Curcumin, an inhibitor of JAK2 and p300, blocked the effects of Ang II. Inhibition of acteyl-STAT3 (Lys685) by resveratrol, C646 or p300 siRNA decreased Ang II-induced up-regulation of phospho-STAT3 (Tyr705) and consequent fibrotic changes. AG490, a JAK2 inhibitor, repressed Ang II-induced phospho-STAT3 (Tyr705) and fibronectin expression. Both nicotinamide, an inhibitor of SIRTs, and p300 overexpression increased phospho-STAT3 (Tyr705) and the expression of relevant downstream proteins by up-regulation of acteyl-STAT3 (Lys685). *In vivo* studies showed that resveratrol inhibited acteyl-STAT3 (Lys685) and phospho-STAT3 (Tyr705) in obstructed kidneys, accompanied by attenuated expression of fibrotic factors and renal interstitial fibrosis in UUO mice.

Conclusions: These results suggested that STAT3 acetylation plays an important role in the activation of the STAT3 signaling pathway and consequent renal fibrosis.

Funding: Government Support - Non-U.S.

FR-PO506

Nesfatin-1 Ameliorates Unilateral Ureteral Obstruction Induced Renal Fibrosis in Rats Neslihan Tezcan,[1] Zarife Ozdemir,[2] Naziye Ozkan,[3] Dilek Ozbeyli,[2] Aysin Tulunay,[4] Sule Cetinel,[3] Berrak Yegen,[2] Mehmet Koc.[1] *[1]Depts of Nephrology; [2]Physiology; [3]Histology; [4]And Immunology, Marmara Univ, Medical Faculty, Istanbul, Turkey.*

Background: Unilateral ureteral obstruction (UUO) is a well-characterized fibrosis model exhibiting tubular injury and interstitial inflammation in the obstructed kidney. Nesfatin-1 (NS-1) is reported to have anti-inflammatory and antiapoptotic actions in several experimental models. However, the role of NS-1 in the development of fibrosis in a UUO model has not yet been elucidated.

Methods: In male Sprague-Dawley rats, UUO was performed by ligating left ureters. The rats were injected ip with either saline (SL) or NS-1 (10 μg/kg/day) for 7 or 14 days (n=8 in each group). On the 7th or 14th day, obstructed kidneys were removed for the isolation of leukocytes by flow-cytometry and for the assessment of histopathological changes and the determination of glutathione (GSH) levels and myeloperoxidase (MPO) activity.

Results: Renal MPO activity in the SL groups was significantly increased compared to sham-operated (SH) group ($p<0.05$). NS-1 treatment abolished renal MPO activity compared to corresponding SL groups ($p<0.05$). GSH levels, which were reduced in SL groups, were elevated by NS-1 treatment back to the levels observed in SH group. The percentages of lymphocytes and macrophages infiltrating the obstructed kidneys were increased in SL groups compared to SH group ($p<0.05$), while treatment with NS-1 did not prevent lymphocyte/macrophage infiltaration. Apoptosis scores were increased in SL groups compared to SH group ($p<0.05$) and NS-1 treatment for 7 days decreased the apoptosis score ($p<0.05$). The interstitial fibrosis score was increased in the SL groups compared to SH group ($p<0.05$), while treatment with NS-1 decreased the scores in both 7- and 14-day groups ($p<0.05$). a-SMA score was also significantly lower in the 7-day NS-1 treated group ($p<0.05$).

Conclusions: The present data demonstrate that UUO-induced renal fibrosis is ameliorated by NS-1, which appears to act by inhibiting the infiltration of neutrophils and preventing the oxidative stress. These data suggest that NS-1 may have a regulatory role in protecting against obstruction-induced tubular injury.

Funding: Government Support - Non-U.S.

FR-PO507

V-ATPase Promote Renal Tubulointerstitial Fibrosis By Activating (Pro) renin Receptor Qiongqiong Yang,[1,2] Yun Liu,[1,2] Xiaoyan Li,[1,2] Jinjin Fan,[1,2] Xueqing Cao,[1,2] Xueqing Yu.[1,2] *[1]Dept of Nephrology, The First Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Guangdong Provincial Key Laboratory of Nephrology, Guangzhou, Guangdong, China.*

Background: The (pro) renin receptor (P) RR activation plays an important role in the renal fibrosis. The fragment (P) RR, ATP6AP2, is associated with V-ATPase. The V-ATPase is a multi-subunit proton pump involved in diverse and fundamental cellular processes. This study is to investigate whetherV-ATPase be involved in the prorenin-induced renal tubulointerstitial fibrosis by activating (P) RR and its possible mechanism.

Methods: Prorenin, a-SMA, FN, (P) RR and V-ATPase subunits (B2, E and c) were tested on rat UUO models, and NRK-52E cells treated with prorenin in presence f Bafilomycin A1 (1nmol/L), losartan (10umol/L) or PD123319 (10umol/L). The V-ATPase activity was tested by ATPase in an ATP/NADH-coupled assay and proton transport by Na^+-independent intracellular pH recovery rate after acid load. The interaction of (P) RR and V-ATPase subunits were investigated by co-immunoprecipitates (IP), co-immunofluorescence (IF), and siRNA mediated (P) RR or B2 subunit knockdown.

Results: UUO was shown as significant upregulations of prorenin, (P) RR, V-ATPase subunits, a-SMA and FN in areas of tubulointerstitial injury, and the marked colocalization of the (P) RR and B2 subunit. Prorenin treatment resulted in upregulations of a-SMA,FN, (P) RR, and V-ATPase subunits and activity of NRK52E cells in a dose- and time-dependent manner. Furthermore, Bafilomycin A1 partially block the expression of (P) RR, FN and a-SMA, which was induced by prorenin. Co-IP and co-IF results showed that V-ATPase subunit B2 could bind to (P) RR, which was upregulated after prorenin stimulation. Si RNA (P) RR knockdown partially reduced prorenin induced FN and a-SMA expression, as well as the increase in V-ATPase activity and subunits expression. Meanwhile, siRNA B2 subunit knockdown also partially prevent FN, a-SMA and (P) RR expression induced by prorenin.

Conclusions: V-ATPase may promote renal tubulointerstitial fibrosis by activation of (P) RR and the V-ATPase B2 subunit might act as a key player in the process. (Supported by China National Natural Science Foundation 81370786).

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FR-PO508

Inhibitory Effect of the Chromatin Histone H3-Lysine 4 Methyltransferase (SET7/9) on the Tubulointerstitial Fibrosis Kensuke Sasaki, Ayumu Nakashima, Shigehiro Doi, Takao Masaki. *Nephrology, Hirohima Univ Hospital, Hiroshima, Japan.*

Background: Recent study showed that TGF-β1 increases H3K4 methyltransferase (SET domain containing lysine methyltransferase 7,SET7/9), playingan important role in production of exracellular matrix proteins (ECM). In this study, we investigated the signaling pathway of TGF-β1-induced SET7/9 expression and inhibitory effect of SET7/9 on renal fibrosis in unilateral ureteral obstruction (UUO) mice.

Methods: C57BL/6J mice underwent UUO and the gene expressions of histone modification enzymes were assessed by PCR array. In UUO mice, expressions of SET7/9, methylated H3K4, a-smooth muscle actin (aSMA), and collagen 1 were evaluated by western blotting (WB) and/or immunohistochemistry (IHC) with the administration of TGF-β1-neutralizing antibody, SET7/9 siRNA or Sinefungin (10 mg/kg/day). For *in vitro* experiment, rat renal tubular cells (NRK52E) and rat kidney fibroblasts cells (NRK49F) were used. TGF-β1-iduced expressions of SET7/9, methylated H3K4, aSMA were evaluated by WB with the pretreatment of Smad3 siRNA or Sinefungin (0.5-1.0 ng/mL). In addition, histologic analysis was performed to examine whether SET7/9 correlate with renal fibrosis on human kidney biopsy specimens.

Results: 1) PCR Array detected that SET7/9 gene expression was significantly increased at day 7 after UUO. 2) UUO-induced SET7/9 expression was inhibited by TGF-β1-neutralizing antibody. 3) TGF-β1-induced SET7/9 upregulation was inhibited by Smad3 siRNA treatment in NRK52E. 4) Administration of SET7/9 siRNA improved an increase of aSMA expression in UUO mice. 5) In human kidney biopsy specimens, SET7/9 expression was positively correlated with the degree of interstitial fibrosis ($r = 0.537$, $P < 0.05$). 6) Intraperitoneal injection of Sinefungin suppressed inductions of aSMA and collagen 1 in UUO mice. Furthermore, UUO-induced H3K4 mono-methylation was significantly suppressed by Sinefungin. 7) Sinefungin attenuated TGF-β1-induced aSMA as well as H3K4 mono-methylation in both NRK52E and NRK49F.

Conclusions: Sinefungin ameliorates renal fibrosis by inhibiting H3K4 mono-methylation.

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FR-PO509

Activation of eNOS/NO Signaling Attenuates Renal Interstitial Fibrosis in Mice by the Increase of β-Catenin Degradation Hiroyuki Kadoya, Minoru Satoh, Seiji Itano, Atsushi Uchida, Tamaki Sasaki, Naoki Kashihara. *Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.*

Background: Activation of the wnt/β-Catenin signaling pathway has been demonstrated to play an important role in the development of organ fibrosis. On the other hands, it is well known that systemic endothelial dysfunction is associated with chronic kidney disease. However, the role of the renal endothelium in the initiation and the progression of renal fibrosis remain largely elusive. We hypothesized that protein kinase G (PKG) activation by nitric oxide (NO) could phosphorylate β-Catenin and downregulate the β-Catenin signaling on the process of renal fibrosis.

Methods: WeusedCOS-7 cells and evaluated the effect of PKG activation on β-Catenin phosphorylation. Under the proteasome inhibition with MG132, cells were treated with 8Br-cGMP and mifepristone (Mfp) to activate PKG, and phosphorylated β-Catenin was evaluated by western blotting. We used wild type (WT) and endothelial NO synthase (eNOS)-deficient (eNOSKO) mice. GSK-3b inhibition by lithium (Li) can activate the canonical Wnt pathway by increasing β-Catenin. We administrated Li in drinking water to both WT and eNOSKO mice for 4 weeks. Some eNOSKO mice were treated with Bay41-2272 (10 mg/kg/day, i.p) as a soluble guanylate cyclase (sGC) stimulator to determine the role of the NO-sGC-PKG activation on fibrosis process.

Results: In vitro experiments, phosphorylation of β-Catenin was increased by activation of NO-PKG pathway. PKG facilitated ubiquitination of β-Catenin and promoted degradation of β-Catenin, resulting in reduced cytoplasmic β-Catenin levels. In vivo experiments, no renal fibrotic changes were observed in WT-Li compared to WT-vehi group. However, renal fibrosis was exacerbated in eNOSKO-Li compared with WT-Li group. Pro-fibrotic factors were also more prevalent in kidney from eNOSKO-Li compared with WT-Li mice. The protein expression of β-Catenin was remarkably increased in eNOSKO-Li mice. These changes were suppressed in eNOSKO-Li/Bay mice.

Conclusions: NO-PKG signaling pathway could counteract the β-Catenin accumulation by wnt activation. Activation of eNOS signaling would protect from renal interstitial fibrosis.

FR-PO510

Increased Synthesis of CD44s and Its Variants in Kidney Fibrosis of Lupus Nephritis Daniel Tak Mao Chan, Wan Wai Tse, Mel Chau, Susan Yung. *Dept of Medicine, The Univ of Hong Kong.*

Background: Lupus nephritis is characterized by immune-mediated renal injury leading to glomerular and tubulo-interstitial fibrosis. CD44 is the major cell surface receptor for hyaluronan (HA) which has been implicated in inflammatory and fibrotic processes. During pathological conditions, splice variants of CD44 may also be induced. We investigated renal expression of standard CD44 (CD44s) and its variants in patients and mice with lupus nephritis, and their role in renal fibrosis.

Methods: CD44s, CD44v3 and CD44v6 expression was determined in kidney biopsies from patients with diffuse proliferative lupus nephritis and normal kidney tissue using cytochemical staining. Expression of CD44s, CD44v3 and CD44v6 was examined in NZB/W mice during disease progression. Mesangial cells were isolated from the renal cortex of NZB/W mice to investigate the mechanisms of CD44 synthesis.

Results: There was intense staining for CD44s and its variants in the glomeruli and renal tubules in kidney biopsies from patients with lupus nephritis, compared with weak expression in controls. CD44v3, but not CD44s and CD44v6, was also noted in cells infiltrating the glomerulus, peri-glomerular area, and interstitium. Compared to mice before the development of nephritis, murine renal cortical CD44s gene expression was significantly higher during active nephritis (proteinuria >3g/l), and continued to increase during progressive glomerular and tubulo-interstitial fibrosis ($P<0.01$ and $P<0.001$ respectively). CD44s, CD44v3 and CD44v6 expression in NZB/W mice was associated with glomerular and tubulo-interstitial fibrosis. Glomerular CD44s co-localized with collagen deposition, while tubulo-interstitial CD44s expression was associated with inflammatory

cell infiltration, tubular atrophy and tubulo-interstitial fibrosis. CD44s, CD44v3 and CD44v6 were constitutive expressed in mesangial cells from NZB/W mice. Stimulation of mesangial cells with HA, IL-6, IL-1b, TNF-a, but not IFN-g, increased CD44s and CD44v3 but not CD44v6 synthesis.

Conclusions: Our data suggested a role of CD44s and its variants in renal fibrosis due to lupus nephritis, and the modulatory effect of pro-inflammatory cytokines on their expression.

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FR-PO511

GQ5 Hinders Renal Fibrosis in Obstructive Nephropathy by Selectively Inhibiting TGF-β1-Induced Smad3 Phosphorylation Jing Nie, Jun Ai, Fan Fan Hou. *Div of Nephrology, Nanfang Hospital, Guangzhou, Guangdong, China.*

Background: The TGF-β1, *via* its canonical, Smad-dependent signaling, plays a central role in the pathogenesis of renal fibrosis. This pathway has been recognized as a potential target for anti-fibrotic therapy. *Resina toxicodendri* is the dried resin secreted by *Toxicodendron vernicifluum* and has been used as an anti-inflammatory and anti-scarring agent in traditional Chinese medicine for centuries. In the previous study, we isolated and purified the major component of *Resina toxicodendri* GQ5, a small molecular phenolic compound.

Methods: We investigated the effect of GQ5 on renal interstitial fibrosis in unilateral ureteral obstruction (UUO) kidneys *in vivo* and in TGF-β1 stimulated renal proximal tubular cells (NRK52E) and renal fibroblast cells (NRK49F) *in vitro*. Western blot, immunohistochemical staining, and/or Real-time RCR were performed to detect the expression of α-smooth muscle actin (α-SMA), collagen I, fibronectin, p-Smad3, and p-Smad2. Immunoprecipitation was performed to detect the binding of Smad3 with the TGF-β type I receptor (TβRI). Immunofluorescence staining was performed to detect the nuclear translocation of Smads complex.

Results: In TGF-β1 stimulated NRK52E cells and NRK49F cells, GQ5 blocked the binding of Smad3 with the TbRI, inhibited subsequent phosphorylation of Smad3, reduced nuclear translocation of Smads complex, and suppressed the transcription of major fibrotic genes such as α-SMA, collagen I and fibronectin. Most importantly, intraperitoneal administration of GQ5 hindered the progression of renal fibrosis induced by UUO. Treatment with GQ5 selectively inhibited Smad3 phosphorylation in UUO kidneys, suppressed renal expression of α-SMA, collagen I and fibronectin, and resulted in impressive renal protection after obstructive injury. Late administration of GQ5 also effectively attenuated fibrotic lesions in obstructive nephropathy.

Conclusions: In conclusion, GQ5 hinders renal fibrosis byselectively inhibiting TGF-β1-induced Smad3 phosphorylation and might have therapeutic potential for intervention of renal fibrosis.

FR-PO512

Identification and Clinical Implication of Indole Derivatives Having Anti-Inflammatory and Antifibrotic Effects Hisato Shima, Eikan Mishima, Yasutoshi Akiyama, Takehiro Suzuki, Chitose Suzuki, Sadayoshi Ito, Takaaki Abe. *Tohoku Univ, Japan.*

Background: Renal fibrosis is a common cause of ESRD. Renal inflammation is one of the known factors in the progression of renal fibrosis. Recently, we have identified certain endogenous compounds that potently ameliorate TNF-a-induced Epo suppression. The aim of this study was to investigate the effect of these compounds on renal inflammation and fibrosis.

Methods: 1. Cell viability and fibroblastic formation was analyzed using the rat renal fibroblast cell line NRK49F.

2. To examine the antifibrotic effect, Ureteral Unilateral Obstruction (UUO) was induced in Male C57BL/6 mice. Tissue fibrosis, inflammation were detected by Elastica Masson staining, Sirius red staining and F4/80 staining, respectively.

3. RNA expressin was analyzed by QT-PCR.

4. Signal transduction pathways were analyzed by a Cignal Transduction 45 Pathway Arrays reporter assay kit™ (Qiagen), STAT3 a-screening and STAT3 reporter assay.

Results: 1. In NRK49F cells, compound #35 inhibited TGF-β_1-induced fibronectin and collagen type 1 mRNA upregulation without changing cellular viability.

2. In the UUO model, the fibrotic area and F4/80 positive area were increased in the kidney. After 4 day administration of compound #35, these areas were significantly reduced (Figure 1).

3. Compound #35 significantly suppressed UUO-induced fibronectin, collagen type 1, TGF-β_1, F4/80 and TNF-a mRNA (Figure 2).

Figure 1.

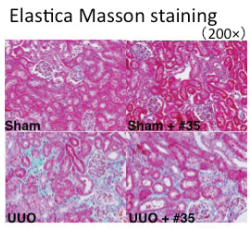

Figure 2.

4. By Signal Transduction analyses, we found that the STAT pathway was downregulated by 50% by compound #35. In addition, compound #35 inhibited STAT3 pathway in the same range as S3I-201, a representative STAT3 inhibitor.

Conclusions: These results demonstrate that compound #35 has anti-inflammatory and antifibrotic effects *in vitro* and *in vivo*. Therefore, compound #35 may be a novel tool for developing drugs that attenuate renal fibrosis and CKD.

FR-PO513

Smad Anchor for Receptor Activation (SARA) Mediates Anti-Fibrotic Activity of sKlotho Constance Runyan,[1] Tomoko Hayashida,[1] Youhua Liu,[2] Lili Zhou,[2] H. William Schnaper.[1] [1]*Pediatrics, Northwestern Univ, Chicago, IL;* [2]*Pathology, Univ of Pittsburgh, Pittsburgh, PA.*

Background: The secreted form of Klotho (sKlotho), which is downregulated in progressive kidney fibrosis, has anti-fibrotic properties that are not fully understood. Previously, we demonstrated that expression of the scaffold/adaptor protein, SARA, maintains human proximal tubular (HKC) epithelial cell phenotype, and may be protective against fibrosis. TGF-β-stimulated fibrogenesis in HKC, characterized by increased expression of type-I collagen and smooth-muscle a-actin, is prevented by overexpression of SARA. Here, we report that the antifibrotic action of sKlotho may result from its protection of SARA expression.

Methods: Kidney fibrosis in the unilateral ureteral obstruction (UUO) was assessed by pathology and qPCR. Overexpression or knockdown was performed in cultured HKC using plasmid expression constructs or lentiviral shRNA vectors.

Results: *In vivo*, kidneys from mice subjected to UUO showed >90% reduction in SARA expression. Ectopic expression of sKlotho, which greatly ameliorated renal fibrosis, was associated with partly restored SARA expression. Kidneys from Klotho hypomorph (kl/kl) mice showed a strong correlation between Klotho and SARA expression (r^2=0.892). *In vitro*, sKlotho overexpression or treatment with recombinant sKlotho increased HKC cell expression of SARA, with or without TGF-β in the culture. sKlotho inhibits TGF-β signaling at least in part by disrupting TGF-β interaction with its receptor. However, while TGF-β decreased SARA promoter activity, sKlotho did not affect the SARA promoter. Therefore it is unlikely that sKlotho increases SARA expression solely by interfering with TGF-β-stimulated SARA downregulation. While sKlotho protected against TGF-β-mediated EMT in control cells, it did not do so in SARA knockdown cells. Thus, SARA is a likely downstream antifibrotic effector of sKlotho.

Conclusions: Together, our data describe a previously unknown function of sKlotho in modulating SARA expression, and suggest that the antifibrotic action of sKlotho is mediated at least in part by SARA, both *in vitro* and *in vivo*.

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FR-PO514

SARA (Smad Anchor for Receptor Activation) Inhibits Fibrosis by Suppressing β-Catenin Activity Constance Runyan, H. William Schnaper. *Pediatrics, Northwestern Univ, Chicago, IL.*

Background: TGF-β/Smad and Wnt/β-Catenin signaling are critical fibrogenic mediators in progressive CKD. Progression appears to require cross-talk between these two pathways. Recent reports suggest that association of Smad3, β-Catenin and the transcriptional activator CBP may be critical for TGF-β-induced phenotypic changes associated with tubulointerstitial fibrosis, but dispensable for its anti-inflammatory effect. How β-Catenin is distributed to different cellular pools to regulate specific fibrogenic interactions is poorly understood. Our laboratory has studied the scaffold/adaptor protein, SARA (Smad Anchor for Receptor Activation). We have reported that high SARA expression protects renal tubular epithelial cells from TGF-β-stimulated expression of smooth muscle a-actin and collagen, whereas loss of SARA promotes fibrosis without requiring TGF-β. Here, we demonstrate that SARA also regulates fibrogenic signaling by β-Catenin.

Methods: Whole-cell and fraction lysates of HKC8 cells were examined by immunoprecipitation, western blot, or promoter/reporter assay.

Results: High levels of SARA expression inhibited β-Catenin nuclear accumulation and downstream transcriptional activity, either basally or induced by TGF-β or Wnt3a. As demonstrated by co-immunoprecipitation, SARA interacts directly with β-Catenin, and overexpression of SARA enhances that interaction. One proposed mechanism of TGF-β-mediated, β-Catenin-dependent fibrogenesis is TGF-β-induced dissociation of E-cadherin/β-Catenin/a-catenin-containing adherens junctions. We detected high levels of both SARA and β-Catenin in Triton-X-100 insoluble cell lysates, which include the adherens junction membrane compartment. SARA knockdown reduced β-Catenin association with this fraction and enhanced nuclear association between Smad3 and β-Catenin. Therefore, levels of SARA may control levels of cellular β-Catenin, its availability and localization, and the localization of Smad3 to either allow or inhibit Smad3/β-Catenin complex formation.

Conclusions: Together our data suggest that by acting as a regulator of both Smad2 versus Smad3 and β-Catenin signaling, SARA has multiple inhibitory effects on TGF-β- or Wnt-induced fibrogenesis.

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FR-PO515

Inhibition of G9a Attenuated Renal Fibrosis and Retained Klotho Expression in UUO Mice Taisuke Irifuku, Shigehiro Doi, Kensuke Sasaki, Ayumu Nakashima, Takao Masaki. *Dept of Nephrology, Hiroshima Univ Hospital, Hiroshima, Japan.*

Background: Histone H3K9 modification reportedly plays an important role in the development of the epithelial mesenchymal transition and transcriptional activity of the klotho gene in cancer cells. In this study, we investigated the inhibitory effects of H3K9 methyltransferase (G9a) on renal fibrosis and klotho expression.

Methods: C57BL/6J mice underwent unilateral ureteral obstruction (UUO) and expression of G9a, methylated H3K9, a-smooth muscle actin (aSMA), collagen 1 and klotho was evaluated by Western blotting (WB) and/or immunohistochemistry with administration of TGF-β1-neutralizing antibody, G9a siRNA or BIX01294 (200mg). For in vitro experiments, rat renal tubular cells (NRK52E), rat kidney fibroblasts (NRK49F) and human renal tubular cells (HK-2) were used and TGF-β1-induced expression of G9a, methylated H3K9, aSMA and klotho was assessed by WB with pretreatment with Smad3 siRNA or BIX01294. In addition, histologic analysis was performed to examine whether G9a levels correlated with renal fibrosis or klotho expression in human kidney biopsy specimens.

Results: 1) UUO-induced G9a expression was inhibited by TGF-β1-neutralizing antibody. 2) TGF-β1-induced G9a upregulation was inhibited by Smad3 siRNA in NRK52E. 3) Administration of G9a siRNA increased aSMA and reduced klotho expression in UUO mice. 4) Intraperitoneal injection of BIX01294 not only suppressed induction of aSMA and collagen 1, but also retained klotho expression in UUO mice. Moreover, UUO-induced H3K9 mono-methylation (me1) was significantly suppressed by BIX01294. 5) BIX01294 suppressed TGF-β1-induced aSMA as well as H3K9me1 in NRK49F. 6) Pretreatment with BIX01294 reduced klotho expression and H3K9me1 by TGF-β1-stimulation of HK-2. 7) In human kidney biopsy specimens (n = 36), G9a expression was positively correlated with interstitial fibrosis ($r = 0.69$, $P < 0.05$), whereas it was inversely correlated with klotho expression ($r = -0.44$, $P < 0.05$).

Conclusions: TGF-β1-induced G9a plays an important role in the progression of renal fibrosis and reduced klotho expression through H3K9me1.

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FR-PO516

Molecular Mechanism of Hypoxia-Inducible Factor (HIF) Action in Fibrogenesis Bethany Baumann, Tomoko Hayashida, H. William Schnaper. *Pediatrics, Northwestern Univ, Chicago, IL.*

Background: Although hypoxia has been identified as a progression factor for CKD, the pro-fibrotic actions of HIF are not well understood. We have found that TGF-β increases HIF expression in normoxia, and that HIFs contribute significantly to normoxic TGF-β induction of type-I collagen, suggesting a key role for HIFs in fibrotic kidney disease regardless of oxygen tension, such as in glomerulosclerosis.

Methods: The mechanism of collagen1 transcriptional regulation by HIF-a was investigated in renal epithelial cells and primary human mesangial cells. HIF-1a and HIF-2a were manipulated by siRNA or overexpression of non-degradable (ND) HIF-a isoforms and deletion mutants. We evaluated collagen1 protein, mRNA, and COL1A2 proximal promoter reporter construct (COL1A2-luc) activity. Site-directed mutagenesis identified key sites in this promoter. HIF effects on Smad3 activity were evaluated with Smad3 responsive reporter (SBE-luc) and Smad3-Gal4 transactivation assays.

Results: HIF-a siRNA reduced TGF-β-induction of collagen1 protein, mRNA and COL1A2-luc activity. ND HIF-a expression increased COL1A2-luc activity. Site-directed mutagenesis of COL1A2-luc identified a functional hypoxia response element (HRE); binding of HIF-1a to this HRE was confirmed by DAPA assay. ND HIF-2a was much more robust in activating COL1A2-luc, and enhanced its transcription even when the HRE was mutated. ND HIF-2a, but not ND HIF-1a, significantly increased Smad3 activity in SBE-luc and Smad3-Gal4 transactivation assays. No physical interaction was detected between Smad3 and HIF-2a, and it was determined that both the transactivational and DNA-binding domains of ND HIF-2a are required for its effects on Smad3 and collagen1.

Conclusions: HIF-1a and particularly HIF-2a are likely key non-canonical mediators of the response to TGF-β, and of collagen1 deposition in CKD, regardless of oxygen tension. HIF-2a may act by inducing an additional, non-HIF, translation factor. These findings are consistent with our previous report that HIF-2 targets are upregulated in glomeruli of mice with glomerulosclerosis (AJP-Renal 305: F1312, 2013) and suggest that HIF regulation is a promising target for treating renal fibrosis.

Funding: NIDDK Support

FR-PO517

Low-Dose Paclitaxel Ameliorates Renal Fibrosis by Suppressing Transforming Growth Factor-β1-Induced Plasminogen Activator Inhibitor-1 Signaling Cascade Eun Sook Jung,[1] Jeonghwan Lee,[2] Nam Ju Heo,[1] Sejoong Kim,[1] Kwon Wook Joo,[1] Jin Suk Han.[1] *[1]Internal Medicine, College of Medicine, Seoul National Univ, Seoul, Korea; [2]Internal Medicine, Hallym Univ Hangang Sacred Heart Hospital, Seoul, Korea.*

Background: Plasminogen activator inhibitor-1 (PAI-1), a major downstream target of transforming growth factor-b1 (TGF-β1), plays an important role in the pathogenesis of renal fibrosis. SMAD and non-SMAD pathways including mitogen-activated protein kinase and p53 are required for PAI-1 induction by TGF-β1. Previous reports have indicated that stabilizing microtubules with paclitaxel can modulate TGF-β/SMAD signaling. We investigated the effects of low-dose paclitaxel on renal fibrosis and attempted to elucidate the potential mechanism involved.

Methods: Male Spraue-Dawley rats were divided into 4 groups (n=6 per group): sham, sham/paclitaxel, UUO (unilateral ureteral obstruction) and UUO/paclitaxel. The rats received intraperitoneal injection of paclitaxel at a dose of 0.3 mg/kg twice a week and were sacrificed at day 7 after UUO or sham operation. Histological changes were examined using periodic acid-Schiff and Masson's trichrome staining. TGF-β1-induced PAI-1 signaling cascade, status of various extracellular matrix proteins and epithelial-mesenchymal transition markers in renal cortex were evaluated by western blotting.

Results: UUO injury induced tubular damage, excessive interstitial collagen deposition and increased protein expression of collagen I, fibronectin and a-smooth muscle actin. Administration of low-dose paclitaxel markedly suppressed all these fibrotic responses and enhanced E-cadherin expression levels. Low-dose paclitaxel was also effective in suppressing UUO-induced expression of PAI-1 and its E-box-binding protein, upstream stimulatory factor-2. Furthermore, the levels of TGF-β1 and the phosphorylation of SMAD2/3, p53 and ERK1/2 were substantially increased by UUO, and they were all significantly attenuated by low-dose paclitaxel.

Conclusions: Our data demonstrate that low-dose paclitaxel inhibits TGF-β1-induced PAI-1 signaling cascade and renal fibrosis. Therefore, low-dose paclitaxel may have potential as a therapeutic target for chronic kidney disease.

Funding: Private Foundation Support

FR-PO518

Targeting N-Ras Reduces TGFβ1 Expression and Collagen Deposition in a Murine Model of Tubulointerstitial Fibrosis Subash Somalanka,[1] Stephen Sampson,[2] Tracy White,[2] Mysore K. Phanish,[1] Claire C. Sharpe,[3] Mark E. Dockrell.[1] *[1]South West Thames Inst for Renal Research; [2]Dept of Histopathology, Epsom & St. Helier Univ Hospital NHS Trust; [3]King's College London, United Kingdom.*

Background: Tubulointerstitial fibrosis (TIF) is a hallmark of progressive chronic kidney disease. Our group has published evidence that N-Ras is an important regulator of renal fibrosis in a human cell model. Antisense Oligonucleotides (ASO) are effective in targeting Ras GTPases in renal disease, as shown previously by Wang et al in the unilateral ureteric obstruction model. In a parallel abstract, we have demonstrated that induction of connective tissue growth factor (CTGF) and autoinduction of TGFβ1 are dependent on N-Ras in a murine renal cell culture. Hence, we extended that work to a mouse model of chronic folic acid induced nephropathy (CFAIN).

Methods: CFAIN was attained in CD1 male outbred mice by an injection of folic acid (FA, 125mg/kg) or vehicle (V) on d 1 and 21. Mice were culled at d 84. RNA extracted from kidneys was probed for Ras isoforms, CTGF, TGFβ1, fibronectin (FN), Collagen (Col) 1a2 and a-SMA. Paraffin sections were stained with Picro-Sirius Red (PSR). TIF was blindly scored by a histopathologist. 6 ASO were screened in healthy mice to assess their efficacy and toxicity with renal N-Ras knockdown assessed by qPCR and toxicity monitored by transaminases and BUN assay. Selected ASO3 and 4 were injected over 7 weeks after d 35 in CFAIN. PSR stained kidney tissue was analysed by NIS-Elements BR 3.1 software.

Results: Cortical TIF (10 – 15 %) was observed in the CD-1 mice after d 84 with increased mRNA expression of CTGF, FN, Col1a2 and TGFβ1. Renal impairment at day 84 was assessed by BUN ($p=0.056$) and Cystatin C ELISA ($p=0.02$) for V versus FA groups. ASO knockdown of N-Ras mRNA was significant ($p<0.001$). No hepatotoxicity and nephrotoxicity was seen with ASO. ASO3 inhibited Col1a2 ($p=0.024$), TGFβ1 ($p=0.006$) and collagen deposition ($p<0.01$). CTGF and FN were not affected by N-Ras ASO. No significant improvement in renal function was noted in N-Ras treated mice.

Conclusions: ASO is safe and effective in mice. Selective N-Ras knockdown with ASO3 has inhibited TGFβ1 and Col1a2 expression but interestingly did not reduce CTGF.

FR-PO519

Construction and Verification of a Chimeric Small Molecule which Induces the Degradation of Smad3 via Ubiquitin Proteasome System Xin Wang, Jinjin Fan, Xiaoyan Li, Xueqing Yu. *Nephrology, The First Affiliated Hospital, Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Smad3 is a key signal protein in renal fibrosis. Protein-targeting chimeric molecule (Protac) is a chimeric small molecule targeting proteins for degradation via ubiquitin proteasome system. Typically it's composed of 3 components: an E3 ligase recognition motif, known as hydroxylated pentapeptide of hypoxia-inducible factor-1a that would recruit the activity of Von–Hippel–Lindau (VHL) ubiquitin ligase (E3), linker, and ligand (protein recognition). We aimed to design a new Protac to prevent renal fibrosis by targeting Smad3 protein.

Methods: Computer aided drug design technic was utilized to find the specific ligand targeting Smad3. Surface Plasmon Resonance (SPR) was applied to choose the best fit one from screening results. The synthesized Protac structure validity was analyzed by two stage mass spectrometry. Protac' specificity to VHL (E3 ligase) was proved by 2 kinds of cell lines: 786-0 (does not express VHL) and ACHN (express VHL) *in vitro* assay. The ubiquitination and degradation of Smad3 was detected by Western blot (WB).

Results: (1).13 small molecular compounds (SMC) were got from the Enamine library by using GLIDE molecular docking program based on the active site of Smad3 (depression area where Phe247 and His248 located). (2).SPR results showed that 8# SMC (EN300-72284) had the best combination with Smad3 (K_D=4.547×10^{-5} M). (3). Protac were synthesized by Shanghai science peptide biological technology co.,ltd and Mass Spectrometry showed the synthesized Protac had the right total and seperate molecular weights of peptides. (4).WB showed Smad3 can be degraded by Protac plus whole cell lysate of ACHN but not 786-0. Only the degradation not the ubiquitination of Smad3 can be inhibited by proteasome inhibitor MG132.

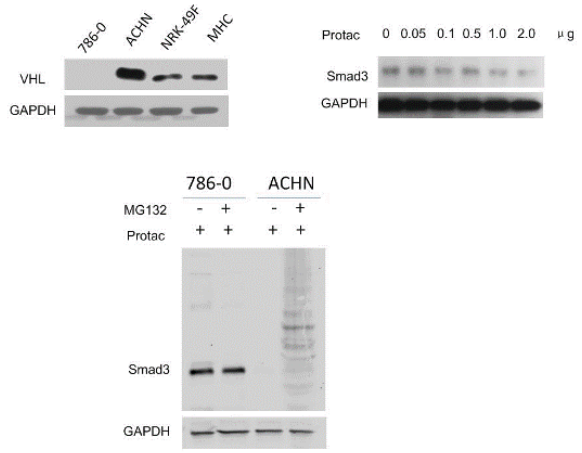

Figure 1: The ubiquitination and degradation of Smad3 (Western Blot)

Conclusions: The new Protac can target Smad3 for ubiquitination and degradation.

Funding: Government Support - Non-U.S.

FR-PO520

Anti-microRNA21 Therapy on Top of ACE-Inhibition Enhances Nephroprotection in a COL4A3-Knockout Mouse-Model for Alport Syndrome Diana Rubel,[1] Deidre Mackenna,[2] Oliver Gross.[1] *[1]Clinic fpr Nephrology and Rheumatology, Univ Medicine Goettingen, Goettingen, Germany; [2]Regulus Therapeutics, San Diego, CA.*

Background: COL4A3 -/- Alport mice serve as animal model for progressive renal fibrosis. Testing the hypothesis of micro-RNA21 (miR21) contributing to the progression of tubulointerstitial damage we investigated the nephroprotective effects of anti-miR21 therapy in combination with ACE-inhibition in these mice.

Methods: 64 COL4A3 -/- mice were treated in 4 groups: (miR21) with anti-miR21 compound 25 mg/kg, or (PLAC) vehicle s.c. twice a week, (ACE+PLAC) with Ramipril 10 mg/kg/day p.o. plus vehicle, and (miR21+ACE) with anti-miR21 plus Ramipril. Ramipril monotherapy started preemptively in week 4, anti-miR21 monotherapy started late in week 6 in animals already showing renal damage (proteinuria >1g/l, mild renal fibrosis). In the miR21+ACE-group, Ramipril was started in week 4 and anti-miR21 was applied in 6 week old animals and lasted until death from renal failure. Six animals were sacrificed after 7.5 and 9.5 weeks, respectively, and kidneys were further investigated using histological, immunohistological and Western blot techniques. Survival until end stage renal failure was determined in the remaining animals.

Results: The combination of anti-miR21 therapy on top of ACE-inhibition MiR21+ACE significantly prolonged median lifespan by more than 30% compared to ACE-monotherapy. The effect of anti-miR21 monotherapy was restricted to tubular accumulation of extracellular matrix and renal scarring in our rather rapid progressive animal model (SvJ/129 background). Dual therapy with miR21 on top of ACE showed the best effect in immunohistochemistry.

Conclusions: In COL4A3 -/- Alport mice, the anti-miR21 therapy in combination with ACE-inhibition reduces fibrosis and prolongs survival compared to ACE-monotherapy. Despite the late onset of miR21 therapy, this nephroprotective effect is significant on top of early-on ACE-inhibition and presents a promising new therapy for patients with Alport syndrome.

Funding: Private Foundation Support

FR-PO521

Inhibitory Effect of Vascular Endothelial Growth Factor-C on Renal Interstitial Inflammation and Fibrosis through Lymphangiogenesis in Mouse Unilateral Ureteral Obstruction Shoko Hasegawa,[1] Toshiaki Nakano,[1] Akihiro Tsuchimoto,[1] Kumiko Torisu,[1] Kazuhiko Tsuruya,[2] Takanari Kitazono.[1] *[1]Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: The interaction between lymphangiogenesis and renal injury has been shown in chronic kidney disease (CKD); however, the precise role of lymphangiogenesis in renal injury remains unknown. The lymphatic network is responsible for the removal of interstitial protein and fluid. In the present study, we investigated the interaction between lymphangiogenesis and renal interstitial inflammation and fibrosis using unilateral ureteral obstruction (UUO) model mice.

Methods: Seven-week-old male C57BL6 mice were divided into the 3 groups; the control mice (Con, n=5), UUO mice (UUO, n=5), and UUO mice administrated vascular endothelial growth factor-C (VEGF-C), an inducer of lymphangiogenesis (UUO+VEGF-C, n=5). VEGF-C was administered at a dose of 100 μg/kg/day using osmotic pumps for 14 days. We investigated lymphangiogenesis (anti-LYVE-1 immunostaining), interstitial fibrosis (Sirius red staining), expression levels of fibrosis-related gene [Collagen I mRNA and a-smooth muscle actin (a-SMA) protein], and macrophage infiltration (anti-F4/80 immunostaining) in each group.

Results: In UUO mice, along with progression of interstitial fibrosis, the density of lymph vessels, the levels of VEGF-receptor 3 (VEGF-R3) mRNA, Collagen I mRNA, and a-SMA protein, and macrophage infiltration increased with time in the obstructed kidney. In UUO+VEGF-C mice, the density of lymph vessels (1.21-fold, p=0.3) and the level of VEGF-R3 protein (2.18-fold, p<0.05) increased, whereas the levels of Collagen I mRNA (0.45-fold, p<0.05) and aSMA protein (0.68-fold, p<0.05), and interstitial fibrosis (0.53-fold, p<0.01) decreased compared with UUO mice.

Conclusions: In UUO mice, administration of VEGF-C suppressed renal interstitial inflammation and fibrosis through lymphangiogenesis, suggesting that lymphatic vessels might have an inhibitory effect on renal interstitial inflammation and fibrosis in the progression of CKD.

FR-PO522

A Novel Dipeptidyl Peptidase IV Inhibitor DA-1229 Ameliorates Tubulointerstitial Fibrosis in Cyclosporine A-Induced Nephrotoxicity in Mice Dae R. Cha,[1] Sung Jin Kim,[1] Jung Eun Kim,[1] Jungyeon Ghee,[1] Hye Kyung Song,[1] Hye Sook Min,[1] Mi Jin Lee,[1] Jin Joo Cha,[1] Young Sun Kang,[1] Ji Eun Lee,[2] Hyunwook Kim,[2] Mihwa Lee,[1] Jee-Young Han.[3] *[1]Nephrology, Korea Univ, Ansan, Korea; [2]Nephrology, Wonkwang Univ, Sanbon, Korea; [3]Pathology, Inha Univ, Incheon, Korea.*

Background: Dipeptidyl peptidase IV (DPPIV) is a peptidase that are involved in glucose metabolism, inflammation, cell migration, and cell differentiation. Although DPPIV is highly expressed in proximal renal tubular cells, the role of DPPIV inhibition in renal disease is not fully understood. The aim is to investigate the effects of DA-1229, a newly developed DPP IV inhibitor, on cyclosporine A-induced nephropathy model.

Methods: Male ICR mice at 6 wks were treated with a low-salt diet (0.01% Na), and mineral oil (vehicle) containing CyA at 30mg/kg/day for 4weeks. Experimental groups were divided into four groups; vehicle (n=10), CyA (n=10), DA-1229 (300mg/kg/day) (n=10) and CyA+DA-1229 (n=10).

Results: DPP-4 activities in plasma and kidney did not significantly different between control group and CyA group, but DPP-4 activity in plasma and kidney showed markedly suppressed in DA-1229 treatment group. Serum creatinine level was significantly increased in CyA group, and DA-1229 treatment restored serum creatinine level to control group. There were no significant difference in food intake, water intake, urine volume and systolic blood pressure among groups. Urinary protein and albumin excretion were significantly increased in CyA group at 4weeks, and those were markedly decreased after DA1229 treatment. DA-1229 treatment also significantly suppressed urinary levels of 8-isoprostane, an oxidative stress marker in kidney. Additionally, DA-1229 treatment improved pro-fibrotic molecule synthesis including CTGF, type I collagen and a-SMA. In addition, DA-1229 also markedly suppressed renal expression of inflammatory cytokines including IL-1b, MCP-1 and TLR4. Furthermore, DA1229 treatment decreased macrophage infiltration determined by F4/80 staining.

Conclusions: These results suggest that a novel DPP IV inhibitor, DA-1229 provide renal protective effects in animal model of CyA-induced nephrotoxicity through anti-inflammatory and anti-fibrotic mechanisms.

FR-PO523

Glucose-Regulated Protein 78 Modulates Renal Interstitial Fibrosis in the Angiotensin II/DOCA Salt Model of Chronic Kidney Disease

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Background: Endoplasmic reticulum (ER) stress is a pathophysiological condition associated with proteinuria and renal interstitial fibrosis during the development of chronic kidney disease (CKD). The ER stress regulator, Glucose-regulated protein (GRP) 78, is induced in kidneys from patients with nephrotic syndrome and glomerular nephritis as well as in animal models of CKD. Therefore, we hypothesized that modulation of GRP78 will affect CKD progression.

Methods: CKD was induced in male C57BL/6 mice by uninephrectomy and subcutaneous implantation of a slow release deoxycorticosterone acetate (DOCA) pellet and Angiotensin (Ang) II osmotic infusion pump. Mice were given 1% sodium chloride in their drinking water. To study the impact of GRP78 modulation on CKD, GRP78 heterozygous mice (~50% GRP78) were used. During the course of the study, blood pressure and 24 h total urinary protein were measured. On day 21, mice were sacrificed and renal tissue was collected and stained with PAS for intertubular casts and with a-smooth muscle actin for renal interstitial fibrosis. RNA was extracted for NanoString gene expression analysis.

Results: GRP78$^{+/-}$ animals that underwent the CKD model experienced significant increases in systolic and diastolic blood pressure, proteinuria, albuminuria and protein cast formation comparable to WT mice exposed to the model. However, NanoString analysis showed a decrease in fibrotic gene expression, Collagen Type 3, FN1, LOXL2 and a-smooth muscle actin in GRP78$^{+/-}$ mice compared to WT mice. These results were confirmed with a-smooth muscle actin staining, demonstrating renal interstitial fibrosis in WT and its inhibition in GRP78$^{+/-}$ mice.

Conclusions: The Ang II/DOCA salt model of CKD developed in the C57BL/6 mouse resembles the pathological features found in patients with late stage CKD, including proteinuria, hypertension and renal interstitial fibrosis. We have shown that GRP78 modulates renal interstitial fibrosis, an important factor in CKD progression. These findings could lead to the development of novel therapeutics to halt the progression of CKD.

Funding: Government Support - Non-U.S.

FR-PO524

Multigenerational Adaptation of the Renal Fibrogenic Response in Mice

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Background: Ancestral liver damage is reported to promote adaptation of the hepatic wound-healing response. (Nat Med, 18;1369-77, 2012) Therefore, we investigated whether renal fibrosis leads to heritable adaptation of the renal fibrogenic response in male mice.

Methods: Adult male 129/J mice were divided into 2 groups and underwent unilateral ureter obstruction (UUO) and sham-operation, respectively (F_0 generation), and after 2 weeks both groups was bred with females. The male offsprings from each group underwent relevant manipulation again (F_1 generation), and were bred with females. The male offsprings (n=5) from each F_1 groups underwent UUO (F_2 generation). By using the kidneys with Day 5 and 7 UUO from F_2-generation male mice with or without a family history of renal fibrosis, levels of mRNAs encoding profibrogenic molecules and area of interstitial fibrosis were measured with qRT-PCR and Sirius Red staining, respectively.

Results: In the kidneys with Day 7 UUO, levels of mRNAs encoding fibronectin EIIIA (FN), but not either transforming growth factor-b1 (TGF-β1) or a1 procollagen type I (COLI), were significantly reduced in mice with a family history of renal fibrosis (UU) compared to mice without it (NN) (UU, versus NN; 10.7 ± 1.4, versus 37.0 ± 5.3 ($p<0.05$; FN), 8.4 ± 2.3, versus 7.6 ± 2.9 (n.s.; TGF-β1), 65.7 ± 25.7, versus 52.3 ± 20.5 (n.s.; COLI)). This tendency was also observed in the kidneys with Day 5 UUO. Additionally, ancestral renal fibrosis significantly narrowed fibrotic area (%) in the kidneys with Day 5 UUO, but not Day 7 UUO (UU, versus NN; 7.3 ± 3.7, versus 10.1 ± 4.5 ($p<0.05$; Day 5), and 12.7 ± 5.4, versus 13.7 ± 4.4 (n.s.; Day 7)).

Conclusions: We found that a family history of renal fibrosis corresponds with transmission of a suppressive adaptation of the renal FN expression to the male F_2 generations. We are planning to test whether epigenetic remodeling is involved in this anti-fibrogenic adaptation of renal FN expression.

Funding: Government Support - Non-U.S.

FR-PO525

KIM-1 Regulates Fibronectin Production by Renal Epithelial Cells

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Background: Kidney Injury Molecule-1 (KIM-1) is upregulated in proximal tubular cells (PTC) following kidney injury. Conditional expression of KIM-1 in PTCs in mice leads to the development of renal and extra-renal complications of chronic kidney disease (CKD) similar to humans. Here, we report that KIM-1 mediates the production of fibronectin, an extracellular matrix component involved in the repair and fibrosis of the kidney.

Methods: Full-length KIM-1 or phosphotyrosine mutant (Y350) were overexpressed in LLC-PK1 and HEK cells and fibronectin levels were evaluated using RT-PCR, Western and a luminex assay. KIM-1-mediated regulation of fibronectin was investigated by treating the above cell lines with specific inhibitors for src, PI3K, JNK, MAPK, p38 MAPK and TGFb. KIM-1 expression was reduced in kidney cancer cell line (ACHN) using lentiviral shRNA. To investigate the association between KIM-1 and fibronectin *in vivo*, different animal models of renal injury were employed. Mice were subjected to either 20 and 30 min of ischemia followed by 1 or 7 days of reperfusion (I/R), unilateral urethral obstruction (UUO) or aristolochic acid (AA, 5 mg/kg) treatment.

Results: KIM-1-expressing renal epithelial cells produced increased levels of fibronectin both at mRNA and protein levels as compared to controls. The increase in fibronectin production was abrogated in Y350F mutant cells indicating that the involvement of KIM-1 phosphotyrosine-mediated signaling in fibronectin production. Blockade of PI3K-AKT, and p38 MAPK, using specific inhibitors, decreased KIM-1-mediated fibronectin production. Fibronectin levels were significantly reduced when endogenous KIM-1 expression was silenced in RCC cell lines. In mouse models, KIM-1-expressing tubular epithelial cells co-localized with high fibronectin expression in I/R, UUO and AA models of renal injury.

Conclusions: We demonstrate that KIM-1 regulates fibronectin expression and production using gain-of-function and loss-of-function approaches. Thus, chronic expression of KIM-1 may lead to deposition of excess fibronectin which contributes to fibrosis. Blocking KIM-1 signaling may be therapeutically beneficial to halt or reduce fibrosis and CKD progression.

FR-PO526

Increased Expression of miRNA in IgA Nephropathy Fibrotic Lesions

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Background: Progressive IgA nephropathy (IgAN) leads to glomerular and interstitial fibrosis. Fibrosis grade is a key determinant of disease prognosis. MicroRNAs (miR), are small non coding RNA, acting as post-transcriptional gene regulators. miRNAs are implicated in various fibro-proliferative disorders. Recently, miR-21 has been suggested to be involved in the renal fibrosis process.The aim of this study was to assess the implication of miR-21 in kidney fibrosis occurring in IgAN.

Methods: A cohort of 56 patients with IgAN (biopsy proved) was selected. For each patient, the renal biopsy was scored according to Oxford classification, including mesangial proliferation (M score), endocapillary hypercellularity (E score), segmental glomerulosclerosis (S score) and interstitial fibrosis (T score). After extraction of total RNAs, the level of miR-21 was measured in renal biopsies by Q-PCR. Furthermore miR-21 expression was studied by *in situ* hybrydization (ISH).

Results: Renal expression of miR-21 was significantly higher in renal tissue of patients with IgAN and severe lesions of interstitial fibrosis (miR-21 relative quatification: for T0 score: 1.64±1.67; for T1 score: 5.38±3.8; for T2 score: 8.88±9.37; p=0.001 for T0 versus T2, or for T0 versus T1). miR-21 expression was independent of the M and E scores and was also associated with renal survival (OR for renal replacement therapy in patient with miR-21 tissue expression above the median value: 7,3, IC 95% [1,72;31,18]). Finally ISH analysis revealed that miR-21 is highly expressed in the fibrotic interstitial area.

Conclusions: Our data suggest that miR-21 is involved in renal fibrosis occurring during IgA nephropathy. In this context, miR-21 may be an interesting non invasive biomarker of fibrosis and/or an innovative therapeutic target in IgAN.

FR-PO527

Urinary and Serological Markers of Collagen Degradation Are Elevated in Three Different Rat Models of Kidney Fibrosis

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Background: In this study we explore the use of specific matrix metalloproteinase (MMP)-generated collagen degradation fragments as urinary and serological markers of fibrosis in three rat models of chronic kidney disease associated with fibrosis.

Methods: The concentration of two Protein Fingerprint markers of collagen type I and type III degradation was measured in serum (sC1M and sC3M) and urine (uC1M and uC3M) of rats after 5/6 nephrectomy (moderate disease, n=6; severe disease, n=6), chronic anti-Thy 1.1 mesangioproliferative glomerulonephritis (n=6), adenine nephropathy (n=5) and compared to healthy controls (n=6).

Results: All three models developed significant renal fibrosis shown by histology and immunohistochemistry and reduced renal function. uC1M and uC3M levels were significantly elevated in diseased animals compared to controls in all three models, and correlated with disease severity in the 5/6 nephrectomy rats. sC1M and sC3M were also significantly elevated in rats with 5/6 nephrectomy and adenine nephropathy, whereas no changes were observed in anti-Thy1.1 glomerulonephritis. The urinary markers and sC1M correlated with serum creatinine, proteinuria, and collagen type I and type III staining in kidney ($p<0.001$).

Table 1: Mean uC1M, uC3M, sC1M, sC3M concentration and collagen I and collagen III % area of staining in the different CKD models.

	Healthy	Moderate 5/6 Nx	Severe 5/6 Nx	Anti-Thy 1.1	Adenine nephropathy
uC1M/Cre (ng/µg creatinine)	0.2#	0.9	5.7***	5.7*	5.0***
uC3M/Cre (ng/µg creatinine)	0.1#	1.8	5.8***	4.3*	17.6**
sC1M (ng/ml)	58.6#	58.3	90.1**	77.3	168.2***
sC3M (ng/ml)	20.2#	45.2	74.9**	16.8	35.0*
Col I (%)	3.0	7.2	18	13.8	20.8
Col III (%)	2.4	7.6	17.3	8.8	16.4

**** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05, as compared to #

Conclusions: The measurement of markers of MMP driven collagen turnover in biological fluids, in particular in the urine, may represent a non-invasive tool to determine the presence of kidney fibrosis.

FR-PO528

Nephrogenic Systemic Fibrosis: Detection of Gadolinium in and the Histologic Effect of Gadolinium–Based Magnetic Resonance Imaging Contrast in Rodents with Uninephrectomy

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Background: Nephrogenic systemic fibrosis (NSF) can develop after intravenous exposure to gadolinium-based magnetic resonance imaging (MRI) contrast in those with renal impairment. It is characterized by diffuse skin thickening as well as internal organ fibrosis. Different formulations of contrast, each with different affinities for gadolinium ion (expressed as the thermodynamic stability, Ktherm), may carry variable risks for triggering the disease. The heart is one organ purportedly effected in NSF, therefore the effects of low– and high–Ktherm contrast agents,—gadodiamide and gadoteridol, respectively—were compared in a model of renal insufficiency.

Methods: Rats with 5/6 nephrectomy were treated with either gadodiamide or gadoteridol (2.5 mmol/kg IP) for 20 doses over a 4-week period. Immunoblot quantitated fibronectin in homogenized tissue. Gadolinium was quantified by scanning electron microscopy equipped with energy–dispersive spectroscopy (EDS).

Results: Gadodiamide induced dermal hypercellularity, fibronectin accumulation, and the recruitment of fibrocytes to a greater degree than gadoteridol. There was no evidence of fibrosis by histology or immunoblot in any of the groups. Although gadolinium was detectable in skin, liver, kidney, and spleen, cardiac tissue did not register a significant increase in gadolinium from control by EDS (see figures 1 and 2).

Conclusions: These data demonstrate that there is organ–specific accumulation of gadolinium in a rodent model of nephrogenic systemic fibrosis.

Funding: Veterans Affairs Support

FR-PO529

Fear and Loathing in the Lanthanides: A Comparison of Gadodiamide and Gadoteridol in a Rat Model of Nephrogenic Systemic Fibrosis

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Background: It has been presupposed that the thermodynamic stability (K_{therm}) of gadolinium–based magnetic resonance imaging chelates relate to the risk of precipitating nephrogenic systemic fibrosis (NSF). This study compared low–K_{therm} gadodiamide to a high–K_{therm} gadoteridol in cultured fibroblasts and rats with uninephrectomies.

Methods: Cultured fibroblasts were serum–deprived and treated with therapeutic levels of magnetic resonance contrast. Rats with uninephrectomy were treated with magnetic resonance contrast (2.5 mmol/kg IP) for a total of 20 doses over a 4–week period. Gadolinium content was assessed using scanning electron microscopy equipped with energy–dispersive x–ray spectroscopy in paraffin–embedded tissues.

Results: Cultured human fibroblasts demonstrated dose–dependent fibronectin generation, transforming growth factor b production, and expression of the activated myofibroblast stress fiber protein a smooth muscle actin. There were negligible differences with respect to toxicity or proliferation between the two contrast agents *in vitro*. In a rodent model of chronic kidney disease (animals were status post uninephrectomy), gadodiamide treatment led to greater skin fibrosis and dermal cellularity with respect to gadoteridol. Either contrast agent induced renal proximal tubule vacuolization as well as increased fibronectin accumulation. Despite relatively large detectable gadolinium signals in spleen, skin, muscle, and liver from the gadodiamide–treated group, contrast–induced fibrosis appears to be limited to skin and kidney; skeletal muscle, liver, and spleen did not demonstrate pathology.

Conclusions: These findings 1) support the hypothesis that low thermodynamic stability chelates have a greater propensity to elicit nephrogenic systemic fibrosis, and 2) demonstrate that certain tissues are resistant to these effects. An understanding of the latter could help in finding a cure or guide in testing rational prophylactic therapies.

Funding: Veterans Affairs Support

FR-PO530

mTOR Component Deptor Contributes to TGFbeta-Induced Collagen I (alpha-2) Expression in Proximal Tubular Epithelial Cells (PTEC)

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Background: In progressive kidney disease, TGFbeta (TGFb)-induced collagen I (alpha-2) (collagen) expression in PTEC contributes to renal fibrosis. Along with canonical Smad 3, we have recently shown involvement of both mTOR complexes (C1 and C2) in non-canonical TGFb signaling. How TGFb maintains mTOR activity to increase collagen expression is not known. Deptor is a component of both mTORC1 and C2, and inhibits their activity.

Methods: Cultured PTECs were used. Immunoblotting, quantitative RT-PCR, chromatin immunoprecipitation assay and reporter transfection assay were used.

Results: TGFb decreased levels of deptor, resulting in increased collagen expression due to elevated Akt, mTORC1 and mTORC2 activity. Expression of deptor inhibited both mTORC1 and mTORC2 activity, resulting in reduction of expression of collagen protein, mRNA and its transcription. Conversely, shRNAs against deptor increased collagen transcription, mRNA and protein expression concomitant with enhanced mTORC1 and mTORC2 activity. As a mechanism, we identified Hif1alpha (Hif1a) responsive element in the collagen gene, which bound Hif1a in PTEC. Expression of deptor inhibited TGFb-induced Hif1a expression and its recruitment to the collagen gene. In contrast, shDeptor markedly increased Hif1a binding to the collagen gene similar to TGFb treatment. Furthermore, shDeptor-induced increase in collagen transcription and protein expression was inhibited by siRNA against Hif1a. Moreover, TGFb- and shDeptor-mediated increase in Hif1a and collagen expression was significantly inhibited by shRNA against raptor, an exclusive component of mTORC1, but not by downregulation of rictor, the required constituent of mTORC2 for its activity.

Conclusions: These results for the first time demonstrate a role for deptor in collagen expression, which contributes to renal fibrosis. We uncover a specific role of mTORC1 for collagen transcription via Hif1a in the presence of bystander activation of mTORC2.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO531

Mycophenolate Mofetil Is Superior to Cyclophosphamide or Azathioprine Induction Therapy for Lupus Nephritis in Mexican Mestizo Population

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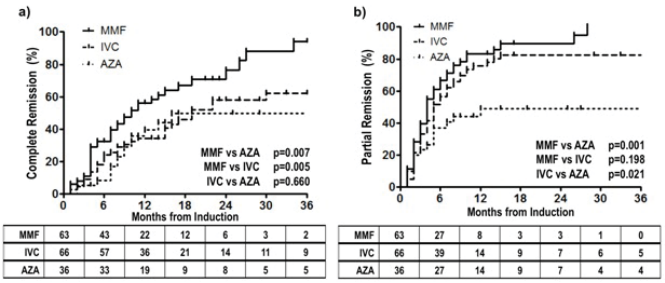
Background: Studies comparing induction to remission strategies in lupus nephritis (LN) suggest that hispanic ethnicity and Latin America region patients may have a better response to mycophenolate mofetil (MMF) over intravenous cyclophosphamide (IVC); but these studies are frequently subanalysis with a great regional and hispanic population variability. Here, we report a comparison of MMF, IVC and azathioprine (AZA) as induction treatment regimens for active LN in a mexican one-center lupus nephritis retrospective cohort.

Methods: A total of 165 patients with ISN/RPS clases III through V LN, distributed in MMF (dosage >2g/d per 6 months), IVC (0.7g/m^{2}BSA) and AZA (dosage >1.5mg/kg/d per 6 months) groups with a median 31 +-18 months follow-up were analyzed. The primary end-point was complete remission as defined in LUNAR trial. Secondary end-points included partial remission, doubling of serum creatinine, end-stage renal disease development.

Results: Complete remission achievement was higher with MMF at 36 months (94.1%) compared to IVC (62.1%) and AZA (49.7%). Partial remission achievement was higher with MMF (100%) and IVC (85.5%) groups compared to AZA (67%). There were no differences between treatment groups in serum creatinine doubling or end-stage renal disease development. Presentation proteinuria (HR 0.91), IVC (HR 0.49) and AZA (HR 0.31) induction treatment, and absense of vascular damage (HR 2.04) were independent predictors of complete remission.

Figure 1. Complete remission (a) and Partial remission (b) at 36 months.



MMF	63	43	22	12	6	3	2
IVC	66	57	36	21	14	11	9
AZA	36	33	19	9	8	5	5

MMF	63	27	8	3	3	1	0
IVC	66	39	14	9	7	6	5
AZA	36	27	14	9	7	4	4

Conclusions: MMF induction therapy may be superior to IVC and AZA in LN patients of mexican-mestizo race.

FR-PO532

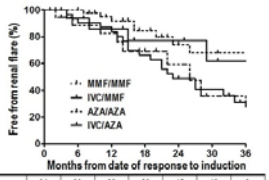
Mycophenolate Mofetil Is Superior to Azathioprine as Maintenance Therapy in Hispanic Lupus Nephritis Patients Juan M. Mejia-Vilet, Arreola Guerra Jose Manuel, Bertha Manuela Cordova Sanchez, Norma O. Uribe-Uribe, Luis E. Morales-Buenrostro, Ricardo Correa-Rotter. *Nephrology, Inst Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico.*

Background: One of the goals of maintenance regimens in lupus nephritis (LN) is to prevent flares while minimizing toxicity. Renal flares have been shown to significantly impact outcome in systemic lupus erythematosus patients.

Methods: We report flare incidence in an 118 mexican-mestizo retrospective cohort of patients with proliferative lupus nephritis that had previously responded to induction therapy. We compared flare incidence by induction/maintenance groups and searched for flare and outcome predictors.

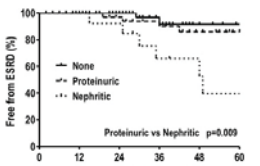
Results: Of 118 patients, 47 (39.8%) presented a renal flare at a median 17 months from time of remission achievement, 34 being classified as proteinuric and 13 as nephritic. Creatinine at initial presentation (HR 1.81 [1.22-2.88], p=0.003), ISN/RPS class IV+V histology (2.19 [1.17-4.09], p=0.014) and azathioprine maintenance therapy (HR 4.13 [1.99-8.60, p<0.001]) were significantly associated with renal flare incidence on multivariate analysis. Flare treatment regimens varied, but clinical response (complete remission 48.9%, partial remission 27.7%) was inferior to that of initial induction therapy. Twelve patients progressed to end stage renal disease (10.2%) on a median 31 months. Age (HR 0.90, [0.82-0.99], p=0.035) and nephritic flare (HR 7.29, [1.46-36.4], p=0.016) were significantly associated with ESRD development.

Figure 1. Proportion of patients with renal flare on follow-up.



IVC-AZA	34	30	28	20	15	10	6
IVC-MMF	17	15	12	9	9	5	4
MMF-MMF	47	44	34	22	14	12	9
AZA-AZA	18	16	15	10	7	3	3

Figure 2. End stage renal disease survival curves for patients with none, proteinuric or nephritic flare.



No Flare	72	60	48	21	10	4
Proteinuric	33	33	31	27	16	5
Nephritic	13	13	12	8	7	4

Conclusions: Mycophenolate mofetil may be superior to azathioprine as maintenance therapy for prevention of renal relapses in mexican-mestizo population. Age and nephritic flares constitute significant predictors of ESRD development.

FR-PO533

Glomerular Necrotizing Lesions and Long Term Outcomes among Patients with Proliferative Lupus Nephritis Abdulkareem Alsuwaida, Sufia Husain, Mohammed A. Al-Ghonaim, Saad S. Alobaili, Jamal S. Al Wakeel, Hala M. KFoury. *College of Medicine, King Saud Univ, Riyadh, Saudi Arabia.*

Background: Although necrotic lesions in lupus nephritis are common in proliferative lupus nephritis (LN), little is known about the impact of these lesions on long-term outcomes. This study was undertaken to investigate the impact of glomerular necrotic lesions on response to therapy and renal outcomes of doubling serum creatinine in patients ISN/RPS class III and IV LN and necrotic lesions.

Methods: 52 patients with ISN/RPS class III or IV LN were enrolled in this retrospective study with mean follow up of 7.4 years. Tuft necrosis was defined as fragmentation of nuclei or disruption of the glomerular basement membrane with fibrin-rich material**.** All patients underwent a repeated biopsy 12-18 months after baseline biopsy.

Results: The prevalence of necrotizing lesions was seen in 24% of those with class III versus 70.4% of class IV (*P*=0.001). The initial median serum creatinine was significantly higher at 92umol/l (Mean 154 umol/l ± 130) in those with necrotizing lesions and 76umol/l (Mean 96 ± 94 umol/l) in those with no necrosis (*P*=0.05). Proteinuria was more severe among those with glomerular necrosis (*P*=0.03). The rate of no remission was seen in 44.0% and 22.2% among those with and without necrosis, respectively. The proportion of doubling of serum creatinine was seen in 32.0% in those with necrosis and 14.8% with no necrosis (*P*=0.1).The chronicity index in the repeated biopsy was significantly worse among those with necrosis. The median chronicity index was 4 (IQR: 1–6) for the group with no necrosis and 7 (IQR: 5–8) for those with necrosis at repeated biopsy (*P*=0.0002).

Conclusions: Glomerular necrosis identifies lupus nephritis patients at greatest risk for progression to renal failure. Proactive intervention and possibly more aggressive inductions therapy in patients with necrotizing lesions may protect the kidneys from developing chronic renal impairment.

Funding: Government Support - Non-U.S.

FR-PO534

Atypical Hemolytic Uremic Syndrome in Setting of Warfarin Withdrawal Neetika Garg,[1] Helmut G. Rennke,[2] Kambiz Zandi-Nejad.[1] *[1]Nephrology, Beth Israel Deaconess Medical Center, Boston, MA; [2]Pathology, BWH, Boston, MA.*

Background: Uncontrolled activation of the alternate pathway of complement system is considered central to the pathogenesis of atypical hemolytic uremic syndrome (aHUS). However, known genetic and acquired defects in the complement system account for only half of the cases. Extensive crosstalk between complement and coagulation-fibrinolysis pathways suggests potential role of the latter in pathogenesis; mutations in PLG (encoding plasminogen) have recently been implicated in some patients. Role of anticoagulation (AC) in such cases is unknown. Here, we describe 2 cases of aHUS that occurred in the setting of AC withdrawal.

Methods: Retrospective chart review of 415 patients from Harvard affiliated hospitals identified 25 cases of aHUS of which 2 developed after AC withdrawal.

Results: Case 1 (2008): 61-year-old male with history of deep venous thrombosis (DVT) on warfarin presented with a traumatic intracranial bleed. Following INR reversal with prothrombin complex concentrate, he developed hemolytic anemia, thrombocytopenia and acute kidney injury (AKI) requiring dialysis. Renal biopsy confirmed an acute thrombotic microangiopathy (TMA). C3 and C4 levels were normal. In the absence of diarrhea and ADAMTS13 activity of 63%, aHUS was diagnosed. Renal function improved with plasmapheresis and AC. No genetic testing was done. Serum creatinine 5 years later is 1.7 mg/dL. Case 2 (2005): 50-year-old male with history of recurrent DVTs and negative hypercoagulable work up on chronic AC developed TMA, confirmed with renal biopsy, in the setting of interruption of AC for a colonoscopy. C3 and C4 levels were normal. With stool negative for E. coli O157:H7, and ADAMTS 13 activity of 59%, aHUS was diagnosed. Following full recovery with AC and steroids, he suffered multiple recurrences in the setting of withholding AC for subsequent procedures/bleeding and ultimately progressed to ESRD. Genetic work up was negative for CFH, CFI and MCP.

Conclusions: Coagulation/fibrinolysis system may play a role in the pathogenesis of aHUS via its interactions with the complement system. Normal C3 level does not necessarily rule out complement activation/consumption given its upregulation as an acute phase reactant.

Funding: Pharmaceutical Company Support - Alexion

FR-PO535

Histological Changes of Lupus Nephritis at the Time of Flare: A Single Center Analysis in Japan Anastasie Kadiombo Tshilela, Hidekazu Ikeuchi, Keiju Hiromura, Kaori Mochizuki, Ken Kayakabe, Noriyuki Sakurai, Toru Sakairi, Yoriaki Kaneko, Akito Maeshima, Yoshihisa Nojima. *Dept of Medicine and Clinical Science, Gunma Univ Graduate School of Medicine, Maebashi, Gunma, Japan.*

Background: Renal flares are common in lupus nephritis (LN). However, limited data are available regarding the histological changes of LN at the time of flare compared to the initial biopsy, especially by using ISN/RPS 2003 classification.

Methods: We retrospectively analyzed 18 LN patients (6 males and 12 females with mean age of 39.2±10.8 years) who received a repeat renal biopsy at the time of flare. All patients were Japanese and received both first and second biopsy at our hospital or affiliated hospitals. Renal histology was classified according to ISN/RPS 2003 classification.

Results: The mean intervals between the first and second biopsy was 6.7±2.4 years. The levels of urinary protein and eGFR were not different between the first and second biopsy: 5.2±4.0 versus 5.6±5.2 g/gCr (p=0.877), 83.1±32.9 versus 73.0±30.8 ml/min/1.73m^2 (P=0.796). In contrast, the level of serum complement activity (CH50) was significantly lower in the first biopsy: 18.4±11.3 versus 32.9±11.6 (P<0.001).The histological changes were shown in Table. Class switches were observed in 6 patients (33%). Five patients changed to mixed type (Class III/IV+V), and 1 patent with mixed type transformed to class III. In addition, chronic lesions (A/C or C by ISN/RPS) were found in 4 patients at first biopsy and in 16 patients at second biopsy.

Table Histological changes in ISN/RPS classifications at first biopsy and second biopsy at flare

Second biopsy at flare	First biopsy					
	II	III	IV	V	Mixed	Total
II	0	0	0	0	0	0
III	0	0	0	0	1	1 (6%)
IV	0	0	7	0	0	7 (39%)
V	0	0	0	1	0	1 (6%)
Mixed	1	1	4	0	4	9(50%)
Total	1 (6%)	0	11 (61%)	1 (6%)	5 (28%)	18 (100%)

Conclusions: Approximately one-third patients had class switches at the time of flare and most of them were a transition to mixed type from class IV. In addition, chronic lesions markedly progressed at flare.

FR-PO536

Remission of Proteinuria Is an Important Prognostic Factor for Mortality in Biopsy-Proven Lupus Nephritis Regardless of Recurrence Ho Jun Chin,[1] Sewon Oh.[2] *[1]Internal Medicine, Seoul National Univ Bundang Hospital, Seong nam, Kyeongkido, Republic of Korea; [2]Internal Medicine, Inje uinversity Ilsan Paik Hospital, Goyang, Kyeongkido, Republic of Moldova.*

Background: Lupus nephritis is one of the most common secondary glomerulonephritis diagnosed by renal biopsy in Korea. We investigated the importance of proteinuric remission on the outcomes in patients with lupus nephritis.

Methods: We selected patients with urine protein to creatinine ratio 0.5 g/g cr or more and biopsy-proven lupus nephritis from 1980 to 2009 in a tertiary referral hospital in Korea. Among 388 patients, we followed 280 patients during 148 ± 95 months until proteinuric remission. Remission of proteinuria was defined as 3 consecutive tests with UPCR < 0.3 g/g cr or proteinuria by dipstick test trace or less.

Results: There were 140 patients who had proteinuric remission. The differences of clinical paramaters at renal biopsy according to proteinuric remission were gender, level of anti-ds DNA antibody, and group of hemoglobin (classfied with criateria of 10.0 g/dl and 12.0 g/dl). There were 108 patients who had experienced recurrence of proteinuria after remission. If the patient would not have proteinuric recurrence until 52.6 months after remission, we could estimate no recurrence with sensitivity 80.0 % and specificity 78.1 % during follow-up period. Patients with remission had 0.169 (95% CI: 0.038-0.756, p=0.020) fold-risk for mortality and 0.476 (95% CI: 0.245-0.923, P=0.028) fold-risk for composite outcome (death or development of end stage renal disease) by Cox's hazard proportional model, however, not for ESRD. Patients with recurrence of proteinuria had similar risk for mortality and composite outcome compared with patients without recurrence.

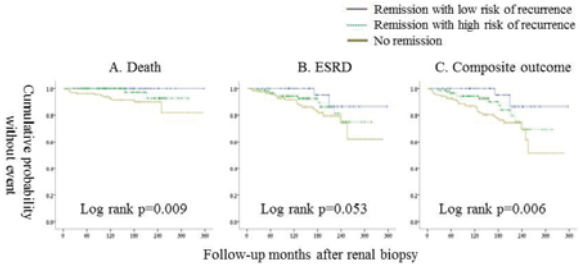


Figure 1. The outcomes of patients grouped by remission and risk of recurrence during follow-up period
Remission with low risk of recurrence: patients with proteinuric remission who did not have recurrence of proteinuria until 50 months after remission. Remission with high risk of recurrence: patients with proteinuric remission who had recurrence within 50 months after remission. ESRD: end stage renal disease, Composite: death or ESRD. Probability of event was estimated by Kaplan-Meier analysis.
P-value was estimated by Log-rank test.

Conclusions: Proteinuric remission in lupus nephritis was an important good prognostic marker for mortality regardless of recurrence after remission of proteinuria.

Funding: Private Foundation Support

FR-PO537

Outcome of Lupus Glomerulonephritis with Impaired Kidney Function at Presentation Jorge L. Castaneda, Juan Carlos Q. Velez, Sally Self. *Medicine, Medical Univ of South Carolina, Charleston, SC; Medicine, Medical Univ of South Carolina, Charleston, SC; Medicine, Medical Univ of South Carolina, Charleston, SC.*

Background: There is paucity of data regarding the rate of response to therapy in patients with lupus glomerulonephritis (LN) who present with impaired kidney function (IKF) at the time of diagnosis. This population is of particular interest due to its highest risk of progression to End Stage Renal Disease (ESRD). Our goal was to report the clinical outcomes of patients with LN + IKF in our institution.

Methods: Medical records of patients (n=51) with biopsy-proven LN, ISN/RPS class III, IV and V and serum creatinine (sCr) ≥ 2 mg/dL at the time of the biopsy were reviewed retrospectively. Response to treatment was defined as decrease in urine protein-creatinine ratio (Up/c) > 50% and < 3 g/day and stabilization of sCr within 25% of baseline.

Results: The mean follow up was 18.2 months. The population was predominantly African-American (86%) female (82%). Baseline mean sCr was 3.5 ± 0.5 mg/dL, mean eGFR was 22.6 ± 2.6 ml/min and mean proteinuria was 5.7 ± 1.2 g/24h. Most patients (68%) received cyclophosphamide for induction therapy. All but 3 subjects (94%) had a proliferative lesion in the biopsy. The mean activity index was 8.2 ± 1.3 and the mean chronicity index was 5.9 ± 0.7. Crescents were seen in 64.7% of the patients. The rate of response to treatment in the first 24 weeks was 13.7%. No patient with sCr ≥ 4 mg/dL responded to treatment (relative risk for failure to respond for those with sCr ≥ 4 mg/dL: 1.21 (95% CI 1.00–1.45, p=0.047). ESRD within 3 years occurred in 51% of the patients. The mortality rate was 15.6% and severe infection requiring hospitalization occurred in 27.5% of the subjects. Among 17 patients who required dialysis at presentation, 5 (29%) were able to discontinue dialysis.

Conclusions: Patients with LN + IKF represent a subgroup with high rate of no response to therapy and progression to ESRD. Higher sCr is associated with worse prognosis. About a third of the patients who require dialysis at presentation may be able to discontinue it even after 20 weeks of treatment.

FR-PO538

Short and Long Term Outcomes of Pure Membranous Lupus Nephritis and the Relevance of Nephrotic Range Proteinuria Jorge L. Castaneda, Juan Carlos Q. Velez, Sally Self. *Medicine, Medical Univ of South Carolina, Charleston, SC.*

Background: Membranous Lupus Nephritis (MLN) represents around 15-20% of clinically significant renal disease in Lupus. MLN has distinct histologic features, clinical manifestations and more favorable outcomes than proliferative forms of lupus Nephritis (LN). Since there is conflicting data regarding the degree of proteinuria than can affect the outcomes, the goal of this study was to describe the rate of response to treatment and to determine the significance of nephrotic range proteinuria.

Methods: The medical records of patients (n=30) with biopsy-proven MLN, ISN/RPS class V were reviewed retrospectively.

Results: The mean follow up was 21.7 months (6-36 months). The population was predominantly African-American (83.3%), female (80%).The mean creatinine was 1.26 (± 0.3) mg/dl. The mean proteinuria was 3.4 g/24h (± 1.4). 20 % of the population presented with hematuria at the time of the biopsy. 97% of the entire population received any form of immunosuppression.Low activity and chronicity index were seen in the entire population 1.3 (± 0.5) and 2.5 (± 1) respectively. The rate of response to treatment at 24 weeks was 43.3%. 8 patients (26.6%) worsened eGFR over the last 3 years and only 1 patient died. Patient and renal survival at 3 years were 96% and 90% respectively. The population was divided into non-nephrotic (n=20) and nephrotic range proteinuria (n=10) at the time of the kidney biopsy. 70% of the population was treated with MMF initially. Patients with nephrotic range proteinuria are at 2.25 times increased risk of non-response to treatment in the first 24 weeks (95% CI 1.3-4,p=0.006). Among non-responders in the first 24 weeks, the rate of CKD progression and development of ESRD/transplantation in the next 36 months were 35.3% and 23.5% respectively. The rate of severe infections that required hospitalization was 23.7%.

Conclusions: MLN represents a condition with a relatively benign course however the development of Nephrotic range proteinuria may represent a risk factor for non-response and worse long-term renal survival. The institution of earlier immunosuppression may change the long-term outcomes in this population.

FR-PO539

Diagnosis, Monitoring and Treatment of Lupus Nephritis: Systematic Review of Clinical Practice Guidelines David J. Tunnicliffe,[1,2] Siah Kim,[1,2] Davinder Singh-Grewal,[3,4,6] Richard K.S Phoon,[5,6] Jonathan C. Craig,[1,2] Allison Tong.[1,2] *[1]School of Public Health, The Univ of Sydney; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney; [3]The Sydney Children's Hospital Network, Sydney; [4]Faculty of Medicine, The Univ of New South Wales, Sydney; [5]Westmead Hospital, Sydney; [6]Sydney Medical School, The Univ of Sydney.*

Background: Lupus nephritis is considered the most serious manifestation of systemic lupus erythematosus. Patients with lupus nephritis experience poor quality of life and are at risk for developing end stage kidney disease. Clinical management is complex and guidelines have recently been developed on the diagnosis, monitoring and treatment of lupus nephritis.This study aimed to compare the quality, scope and consistency of clinical practice guidelines on lupus nephritis.

Methods: MEDLINE, Embase, PsycINFO and CINAHL, guideline organizations and websites of nephrology and rheumatology societies were searched up to April 2014. The Appraisal of Guidelines for Research and Evaluation (AGREE) II instrument and textual synthesis was used to appraise and compare recommendations.

Results: Six clinical practice guidelines and five consensus statements were identified and included. The methodological quality of the guidelines was variable, with the overall mean AGREE II scores ranging from 31% to 75%. Scores were consistently low for applicability with only one guideline scoring above 50%. The guidelines covered five areas: indications for renal biopsy, monitoring, treatment, treatment of difficult disease, reproductive health, and pediatric lupus nephritis. There were inconsistencies in recommendations: class II and V lupus nephritis recommendations were separated by proteinuria range by some, not all guidelines, class III/IV lupus nephritis maintenance therapy duration (1 to 3 years) and variability in recommended doses for induction and maintenance therapy.

Conclusions: International guidelines on lupus nephritis are variable in methodological quality, scope and recommendations. Collaborative and multidisciplinary efforts to develop comprehensive, high-quality evidence-based guidelines are needed to promote best treatment and health outcomes for patients with lupus nephritis.

Funding: Government Support - Non-U.S.

FR-PO540

Experiences and Perspectives of Adolescents and Young Adults Living with Systemic Lupus Erythematosus David J. Tunnicliffe,[1,2] Davinder Singh-Grewal,[3,4,5] Richard K.S Phoon,[3,6] Jeffrey Chaitow,[4] Fiona Mackie,[4,5] Nicholas Manolios,[3,6] H. Patrick McNeil,[5,7] Ming-Wei Lin,[3,6] Sean G. O'Neill,[5,7] Graeme Stewart,[3,6] Angelique F. Ralph,[1,2] Jonathan C. Craig,[1,2] Allison Tong.[1,2] *[1]School of Public Health, The Univ of Sydney, Sydney, NSW, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Sydney, NSW, Australia; [3]Sydney Medical School, The Univ of Sydney, Sydney, NSW, Australia; [4]The Sydney Children's Hospital Network, Sydney, NSW, Australia; [5]Faculty of Medicine, The Univ of New South Wales, Sydney, NSW, Australia; [6]Westmead Hospital, Sydney, NSW, Australia; [7]Liverpool Hospital, Sydney, NSW, Australia.*

Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with potential renal, cardiovascular and musculoskeletal involvement. Disease activity and the treatment burden significantly impair quality of life. We aimed to describe the experiences and perspectives of adolescents and young adults diagnosed with SLE before the age of 18 years.

Methods: Focus groups and face-to-face semi-structured interviews were conducted with 26 patients' aged 14 to 26 years, and thematically analyzed.

Results: Six themes were identified: marring identity (misrepresented self, heightened self-consciousness, sense of alienation); restricted lifestyle and goals (physical limitation, narrowed career options, threat to parenthood); confusion and uncertainty (frustration of delayed or misdiagnosis, ambiguity about cause of symptoms, prognostic uncertainty, exasperation about lack of understanding); resentment of chronic treatment (constant reminder of illness, paradoxical requirement for treatment, debilitating side-effects); gaining resilience (desire for independence, self-reliance); and appreciating personal gains (strengthening of relationships, fortunate in having milder disease).

Conclusions: Young patients with SLE perceive they have limited physical and social capacities and restricted personal and career goals. Psychosocial and educational strategies targeted at improving confidence, self-efficacy, disease-related knowledge, social support, and resolving insecurities may improve treatment and health outcomes of adolescent and young adults with SLE.

Funding: Government Support - Non-U.S.

FR-PO541

Cost-Utility Analysis of Mycophenolate Mofetil versus Intravenous Cyclophosphamide as Induction Therapy for Proliferative Lupus Nephritis

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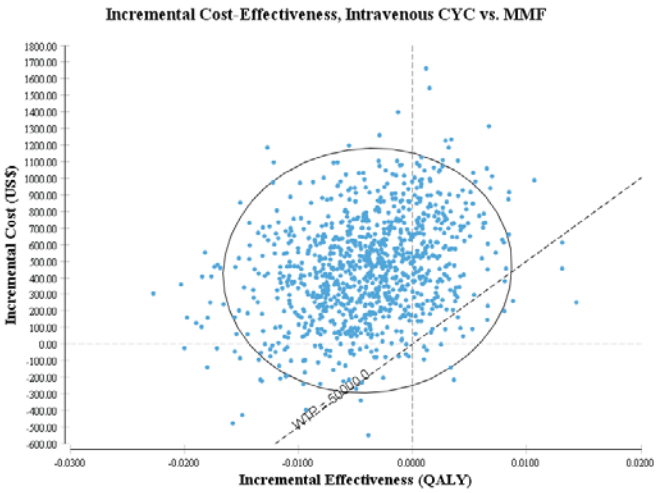
Background: Practice guidelines recommend either mycophenolate mofetil (MMF) or cyclophosphamide (CYC) as initial therapy for patients with Class III and Class IV lupus nephritis (LN). However, the cost-effectiveness of these two induction strategies from a United States perspective has not been reported.

Methods: We constructed a Markov model with a 6-month time horizon to compare the cost-utility of MMF (2gm/day) and intravenous CYC (0.75gm/m2/month) from a societal perspective. We conducted Monte Carlo probabilistic sensitivity analysis to assess parameter uncertainties. We factored in drug adverse events to include ovarian failure and diarrhea. Probabilities of clinical events were drawn mainly from a Cochrane meta-analysis. Utility weights, direct and indirect costs of treatment were based upon published studies. Model outcomes were costs, quality-adjusted life-years (QALY), incremental cost-effectiveness ratios and net monetary benefit. We evaluated model validity according to established guidelines.

Results: MMF (2gm/day) was the dominant strategy compared to CYC ($13,732 versus $16,327 and 0.310 QALY versus 0.306 QALY, MMF and CYC, respectively).



Sensitivity analyses demonstrated that a higher MMF dose (3gm/day) remained dominant and that CYC would be the favored strategy only if its probability of complete remission was 3.9 times higher than MMF at a willingness-to-pay $50,000/QALY.

Conclusions: Our state-transition model over a 6-month time frame suggests that an MMF-based strategy is more effective at a lower cost compared to intravenous CYC as induction therapy for proliferative LN. *The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy of the Department of the Defense, Army, Navy or the U.S. government.*

FR-PO542

Serum Hepcidin in Lupus

Anthony Alvarado,[1] Divya Indrakanti,[1] Xiaolan Zhang,[1] Alice Hinton,[2] Daniel J. Birmingham,[1] Brad H. Rovin.[1] *[1]Nephrology Div, Dept of Internal Medicine, The Ohio State Univ, Columbus, OH; [2]Div of Biostatistics, College of Public Health, The Ohio State Univ, Columbus, OH.*

Background: Hepcidin (Hep) is an inflammation-sensitive iron regulatory protein that mediates the anemia of chronic inflammatory diseases, including chronic kidney disease (CKD). We postulated that Hep increases when systemic lupus erythematosus (SLE) becomes active, and serum hemoglobin (Hg) falls. This was tested by measuring Hep and Hg in serum collected from SLE patients who were undergoing renal flares (RF) and non-renal flares (NRF).

Methods: Serum Hep-25 was measured by ELISA in 38 RF and 25 NRF from 37 SLE patients. For each RF and NRF cycle Hep was measured at baseline (> 6 months from flare), 4 and 2 months before flare, at flare, and 2 and 4 months after flare. Covariates examined were: age, race, and serum creatinine (SCr). Hep levels were normalized by log transformation. Univariate differences were evaluated with t-tests. Mixed models were fit with a random effect for flare and a fixed effect of time. Tukey's HSD was used for multiple comparisons.

Results: We found that at baseline and flare Hg was lower in patients with RF than NRF (11.6±1.2 versus 12.6±0.96 g/dl, P=0.008; 11.4±1.4 versus 12.2±1.2 g/dl, P=0.016, respectively), but there were no differences at baseline or any flare time point in Hep between RF and NRF. Univariate analysis showed that Hep did not increase at RF or NRF compared to baseline or any time point. Between baseline and 4 months pre-flare hep tended to fall in RF (P=0.065). By multivariate analysis race and SCr were significant covariates for Hep in RF, as was SCr in NRF. Multivariate analysis did not show any change in serum hep over the course of RF or NRF. Hg did not change during RF cycles, but in NRF did show a (non-significant) trend to fall relative to baseline in the interval between 2 months pre- and post-flare.

Conclusions: Unexpectedly, these data show that serum hep is not induced, and Hg does not fall, in SLE patients with active disease. The systemic inflammation of active renal or non-renal lupus does not appear sufficient to up-regulate hep, suggesting differences in the nature of the inflammation in SLE and CKD.

FR-PO543

Streptococcus Mutans Strains with Collagen-Binding Protein Are Associated with IgA Nephropathy

Taro Misaki,[1] Shuhei Naka,[2] Keiko Kuroda,[2] Ryota Nomura,[2] Tempei Shiooka,[1] Yoshitaka Naito,[1] Yumiko Suzuki,[1] Hideo Yasuda,[3] Taisuke Isozaki,[1] Kazuhiko Nakano.[2] *[1]Div of Nephrology, Seirei Hamamatsu General Hospital, Hamamatsu, Shizuoka, Japan; [2]Dept of Pediatric Dentistry, Graduate School of Dentistry, Osaka Univ, Suita, Osaka, Japan; [3]First Dept of Medicine, Hamamatsu Univ School of Medicine, Hmamatsu, Shizuoka, Japan.*

Background: IgA nephropathy (IgAN) is the most common primary chronic glomerulonephritis worldwide, however, its precise pathological mechanisms remain unclear. *Streptococcus mutans*,a gram-positive oral streptococcal species, is a known pathogen in dental caries. The association between *S. mutans* strains with the *cnm* gene encoding Cnm, a collagen-binding protein, and infective endocarditis is widely accepted. Recent studies have also revealed an association with other systemic diseases, including ulcerative colitis, non-alcoholic steatohepatitis, and cerebral aneurysm rupture; however, the relationship with IgAN has not been reported. Objective: The aim of this study was to investigate the relationship between *cnm*-positive *S. mutans* strains and IgAN.

Methods: Saliva samples from IgAN patients (n=53) and control subjects (n=32) were collected, and *S. mutans* strains were isolated. Genomic DNA was then extracted from each strain, and PCR was performed to detect *cnm*-positive strains. Collagen-binding assay was also performed for *cnm*-positive strains, with the relative activity of *S. mutans* TW871 set at 100. Renal biopsies from *cnm*-positive and -negative IgAN patients were also compared.

Results: The rates of *S. mutans* isolation in IgAN and control groups were each approximately 84%. In contrast, *cnm*-positive strains were significantly more prevalent in the IgAN group than in controls (32.1% versus 12.5%, $p<0.05$). With regard to collagen-binding assay, the binding rate of *cnm*-positive strains was significantly higher in the IgAN group than in controls (97.0 versus 6.2, $p<0.05$). In addition, segmental glomerulosclerosis score was significantly higher in the *cnm*-positive group than in *cnm*-negative group (0.94 versus 0.57, $p<0.05$).

Conclusions: *S. mutans* strains with collagen-binding protein may be associated with the pathogenesis of IgAN.

FR-PO544

Effects of 1a,25-Dihydroxyvitamin D3 on Memory CD4$^+$T Cells of Focal Proliferative IgA Nephropathy

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Background: To explore the effects of 1a, 25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on memory $CD4^+$ T-lymphocytes in peripheral blood of focal proliferative IgA nephropathy (IgAN) patients.

Methods: Total of twenty incipient focal proliferative IgAN patients (Lee classification: III level) were used as IgAN group and 20 healthy volunteers were used as healthy control group. IgAN group can be divided into two subgroups (proteinuria<1g/d subgroup, proteinuria ≥1g/d subgroup). The serum $1,25(OH)_2D_3$ level was measured by radioimmunoassay (RIA). Peripheral blood mononuclear cells (PBMCs) were stimulated with anti-CD3/anti-CD28 in the absence or presence of various concentrations of $1,25(OH)_2D_3$, Dexamethasone (DEX), and $1,25(OH)_2D_3$ and DEX combined. PBMCs were cultured for 72 hours and the levels of IFN-g, IL-4, IL-17A, Foxp3 were measured by flow cytometry (FCM).

Results: Compared with healthy control group, serum $1,25(OH)_2D_3$ level of IgAN group was significantly lower ($P<0.05$). The levels of IFN-g and IL-17A and the ratios of IFN-g/IL-4 and IL-17A/Foxp3 in IgAN group increased significantly compared with healthy control group, and the level of Foxp3 decreased significantly. The levels of IFN-g and IL-17A and the ratio of IL-17A/Foxp3 in proteinuria ≥1g/d subgroup increased significantly, and the level of Foxp3 decreased significantly, compared with proteinuria <1g/d subgroup and healthy control group. The ratio of IFN-g/IL-4 in proteinuria ≥1g/d subgroup increased

significantly, compared with healthy control group. After treatment with $1,25(OH)_2D_3$, the levels of IFN-g and IL-17A and the ratios of IFN-g/IL-4 and IL-17A/Foxp3 decreased significantly, and the level of Foxp3 increased significantly.

Conclusions: There was a certain extent of vitamin D deficiency in focal proliferative IgAN patients, which may be associated with the severity of proteinuria. $1,25(OH)_2D_3$ had beneficial effects on the immunoregulation of memory $CD4^+$ T-lymphocytes of focal proliferative IgAN patients.

Funding: Government Support - Non-U. S.

FR-PO545

The Significance of Mesangial C4c Deposition in IgA Nephropathy

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Background: In IgA nephropathy (IgAN), activation of the lectin pathway (LP) is associated with more severe renal damage. Mesangial C4d deposition is a marker of activation of the LP and a prognostic factor in renal survival. C4c, the same split products of C4b as C4d, is cut off into liquid phase, while C4d is bound to cell membrane. Therefore, we tried to examine whether C4c is more sensitive prognostic factor in IgAN.

Methods: This retrospective study included total of 104 patients with IgAN who underwent renal biopsy at single center from April 2001 to March 2007, and were followed for at least five years. Patients with IgA vasculitis, systemic lupus erythematosus, liver cirrhosis, or other systemic diseases were excluded. We evaluated baseline age, sex, microscopic hematuria, hypertension, serum creatinine and glomerular filtration rate (GFR), urine protein, and histopathological fincings including mesangial C4c staining by immunofluorescence and, rate of glomerulosclerosis (global and segmental), and active crescent formation by microscopical observation.

Results: 48 patients (46.2%) were C4c positive and 56 patients (53.8%) were C4c negative. The difference of baseline age, serum creatinine, eGFR, microscopic hematuria at renal biopsy was not significant between two groups. Urine protein (g/day) and the rate of eGFR decrease per year (ml/1.73min/m2/year) were significantly higher in C4c positive group (0.63±0.68 versus 1.13±1.68 and 0.85±2.69 versus 1.6±1.8, respectively). Glomeruli showing global sclerosis (%) and segmental sclerosis (%) were also significantly higher in C4c potitive group (15.8±15.5 versus 9.5±11.8 and 10.6±11.9 versus 7.0±7.4, respectively).

Conclusions: These results suggests that mesangial C4c depositon was associated with severe renal damage in IgA nephropathy.

FR-PO546

Distribution of IgA Deposits, and Not Co-Deposition of IgG, C3 or C1q, Is Associated with Crescents or Glomerular Necrosis in IgA Nephropathy

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Background: Although presence of crescents and glomerular necrosis in IgA nephropathy is often interpreted as a feature of activity and has been linked to adverse outcome, factors associated with these lesions are not well elucidated. Because co-deposition of complement molecules or IgG has been associated with worse prognosis, we aimed to study if co-deposition of immunoreactants was associated with the presence of crescents and/or segmental necrosis.

Methods: Consecutive native kidney biopsies at the University of Washington with a diagnosis of IgA nephropathy and no other nephropathy were retrospectively reviewed. Oxford classification scores for mesangial hypercellularity, endocapillary proliferation, segmental sclerosis, interstitial fibrosis, presence of crescents/necrosis, and location and intensity of immunofluorescent (IF) staining of IgA, IgG, IgM, C3, C1q, kappa and lambda light chains, and clinical characteristics were recorded.

Results: Of 110 cases (age 13-77, mean 41 years) reviewed, 48 (44%) had crescents/necrosis. Among parameters examined, only E1 (Chi2=14.6, p =0.0001) and presence of IgA deposits in peripheral capillary walls in addition to mesangial regions were associated with crescents/necrosis (Chi2=4.1, p=0.04). IF intensity of IgA staining was greater in biopsies with M1 than M0 (p=0.008). IF intensities of other immunoreactants were not different in biopsies with or without crescents/necrosis, endocapillary hypercellularity, segmental sclerosis, or interstitial fibrosis. Intensity of IgA, kappa and lambda inversely correlated with age, with more deposits found in younger individuals.

Conclusions: Our results suggest that the distribution of IgA deposits, and not co-deposition of other immunoreactants, influences the progression of IgA nephropathy to crescentic lesions or segmental necrosis. Longitudinal studies are needed to show if presence of subendothelial deposits are of prognostic significance in IgA nephropathy. If so, addition of this parameter into Oxford classification may be warranted.

FR-PO547

Relationship between Ambulatory Blood Pressure and Renal Histopathological Findings by the Oxford Classification in Patients with IgA Nephropathy

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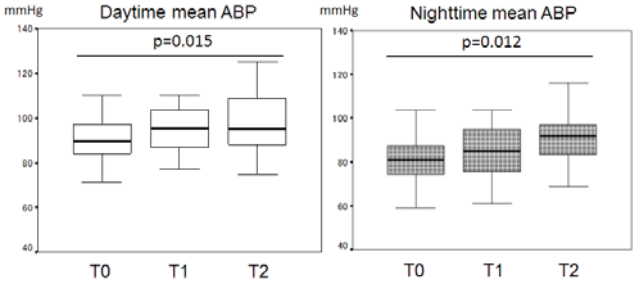
Background: Hypertension is a major risk factor for a poor renal outcome in IgA nephropathy (IgAN) patients. Further, ambulatory blood pressure (ABP) has been shown to be useful in predicting the renal function decline of IgAN. In this study, we have analyzed the relationships between ABP and renal biopsy findings by using the Oxford classification (OC), which is a recently established histopathological criteria for predicting renal outcomes of IgAN patients.

Methods: This cross-sectional study included primary IgAN patients, in whom both renal biopsy and ABP monitoring were performed during the same admission. The histopathological findings were assessed by OC, and analyzed in relation to the ABP.

Results: A total of 111 IgAN patients were analyzed. The mean values were 40 years in age, 64 mL/min/1.73m^2 in eGFR, and 1.2 g/day in urinary protein excretion (UPE), respectively. The score of interstitial fibrosis and tubular atrophy (T) was significantly associated with the mean value of daytime and nighttime ABP. In contrast, the other histopathological findings, including mesangial hypercellularity, endocapillary hypercellularity, and segmental glomerulosclerosis did not show significant association with ABP. In addition, global glomerulosclerosis, extracapillary lesions did not show significant association with ABP. T score was associated with both daytime and nighttime ABP, independent of eGFR, UPE and the use of antihypertensive medications, in multivariate analyses. Any score by OC was not associated with the ratio of nighttime to daytime ABP.



Conclusions: These results suggest that T score by OC is the most relevant renal histopathological parameter associated with ABP in IgAN patients.

FR-PO548

Long-term Impact of Immunosuppressive Therapy for IgA Nephropathy with Moderately Impaired Renal Function: A Single Center Experience in South Korea

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Background: A wide variety of treatment has been attempt to slow progression of IgA nephropathy (IgAN) such as renin-angiotensin system inhibitors and adding corticosteroid for patients with impaired renal function. We compared clinical outcomes of IgAN patients who received immunosuppressive therapy (IST) and who did not received IST, and identified risk factors associated with progression of renal dysfunction.

Methods: Patients with IgAN followed up for at least 36 months and with initial estimated glomerular filtration rate (eGFR) of 30-60 mL/min were retrospectively reviewed. Overall, 74 patients who diagnosed IgAN between 2001 and 2010 were included this analysis.

Results: Median follow-up was 78.5 months. Thirty-two patients received IST (Group 1) and 42 did not (Group 2). Mean age (44.4 versus 45.8 years, P=0.586), mean arterial pressure (MAP, 98 versus 93 mmHg, P=0.103), serum creatinine (1.53 versus 1.42 mg/dL, P=0.141), eGFR (48.1 versus 50.1 mL/min, P=0.269) and median proteinuria (1871 versus 1520 mg/day, P=0.111) at baseline were not significantly different. One patient of Group 1 and 4 of Group 2 progressed to end-stage renal disease (ESRD). ESRD-free survival was not different between two groups (P=0.265). In Group 2, eGFR declined significantly for 3 years (-3.16 mL/min, P=0.038), while that was not significant in Group 1 (-0.21 mL/min, P=0.937). In multivariate linear regression, age at diagnosis (coefficient, 0.546 per 1 year; P=0.015), hypertension (coefficient, -10.507; P=0.029), MAP (coefficient, -0.364 per 1 mmHg; P=0.038), baseline eGFR (coefficient, 1.251 per 1 mL/min; P<0.001) and follow-up duration (coefficient, -2.843 per 1 year; P=0.006) were independent predictors of last-visit eGFR.

Conclusions: IST may have a beneficial effect for slowing progression of IgAN with moderately impaired renal function.

FR-PO549

Mycophenolste Mofetil in IgA Nephropathy with Deteriorating Renal Function Ana Huerta,[1] Evangelina Merida,[2] Eduardo R. Hernandez,[2] Eduardo Gutierrez-Martinez,[2] Enrique Morales,[2] Manuel Praga.[2] *[1]Puerta de Hierro Hospital, Madrid, Spain; [2]12 Octubre Hospital, Madrid, Spain.*

Background: Information about treatment of IgAN showing progressive deterioration of kidney function is scarce. We designed a therapeutic protocol with corticosteroids (CS) + MMF in this type of patients (pts).

Methods: Included in the protocol were 13 pts with biopsy-proven IgAN showing a progressive decline of renal function (GFR decline of 30±9% during the previous 6 months before treatment). All were receiving RAS blockers that were maintained during immunosuppressive therapy. Treatment consisted of oral prednisone (1 mg/Kg/d-1st month, 0.5 mg/kg/d-2nd month, tapering doses during the 3rd month to maintenance therapy with 5-10 mg/d) for 9±4 months, and MMF (1-2 g/d, according to digestive tolerance) for 21±14 months. A linear regression model was used to calculate the slope of GFR, and non-parametrical tests to compare GFR and proteinuria changes after treatment.

Results: All the pts but one (Asian origin) were Caucasians; there were 8 men and 5 women. Age at baseline was 48 ±13 years, serum creatinine 1.81±0.33 mg/dl, and eGFR 40±13 ml/min (CKD-EPI). Mean 24hr urine protein excretion was 2.5±2.6 g/day and all the pts showed microhematuria. A significant change was found when comparing eGFR slope during the 6 month-period before treatment (-2.82±0.93 ml/min/m) with eGFR slope during CS+MMF treatment (+0.24 ±0.74 ml/min/m; p=001). Proteinuria showed a significant decline after treatment (2.5 ±2.6 to 0.71±0.72 g/day, p=0.002). Hematuria disappeared in 11 pts. Follow-up after CS+MMF withdrawal was 34±33 m. eGFR slope during this post-treatment period was +0.07 ±0.32 ml/min/m and proteinuria remained stable in comparison with the treatment period. CS+MMF were well tolerated. Adverse effects included herpes zoster infection in 1 patient and mild gastrointestinal disturbances in 3. No one required treatment withdrawal.

Conclusions: Combination of CS + MMF at relatively low doses for 1-2 years was effective to halt the progression of renal insufficiency in a selected group of IgAN with deteriorating renal function, and was well tolerated. Prospective controlled studies are needed to confirm our results.

FR-PO550

IgA Nephropathy and Kidney Transplantation: Recurrence Rate in the Graft and Long Term Outcomes Sophia Lionaki,[1] Konstantinos Panagiotellis,[1] Ilias Makropoulos,[1] Georgios Vlachopanos,[1] Christos Damaskos,[1] Hariklia Gakiopoulou,[2] Smaragdi Marinaki,[1] George Zavos,[1] Ioannis Boletis.[1] *[1]Nephrology & Transplantation, Laiko General Hopsital, Athens, Greece; [2]Pathology, Univ of Athens.*

Background: To estimate the recurrence rate of IgA Nephropathy (IgAN) in the graft, and also compare the long term outcomes of these patients with those of kidney transplant recipients with other primary diseases.

Methods: We studied all patients who were transplanted in our hospital between 2000 and 2013, had biopsy proven IgAN in the native kidneys, and follow up time longer than 1 year post KTx. Every patient with IgAN was matched with 2 controls, using the following criteria: primary disease, age, gender, time of KTx, and donor source. Patients with Henoch-Schonlein purpura or with a history of non-compliance were excluded. The groups were compared with respect to patient and graft survival, and renal function at the end of follow up. We also recorded the rate of IgAN in the graft and its impact in graft survival.

Results: Between 2000 and 2013, 70 patients with biopsy proven IgAN were transplanted in our center. 67 patients with IgAN and 144 matched controls were included in the study. In a mean follow up time of 65 months, 7 patients (10.4%) were diagnosed with IgAN recurrence in the graft, 39 months post KTx. None of these patients experienced graft loss due to the IgAN. Comparison of outcomes between the IgAN and the control group revealed that patient and graft survival were similar.

Parameter	IgAN, N=65	Controls, N=133	p value
Hemodialysis time (months)	46.2	49.6	0.69
PRA>50% (%)	4.8	9.8	0.22
Delayed Graft Function (%)	27	34.1	0.31
eGFR at 1st discharge (ml/min/1.73m^2)	49.6	53.5	0.17
eGFR at 1st KTx year (ml/min/1.73m^2)	56.6	55	0.59
eGFR at end of follow up (ml/min/1.73m^2)	54.5	58.9	0.21
Induction Rx, anti-CD25 (%)	93.75	91.7	0.32
Maintenance Rx, MPA+CNI+STEROIDS (%)	85.9	85.5	0.11
Incidence of Acute Rejection (%)	9.2	12.7	0.69

Conclusions: KTx in patients with IgAN who end up in ESRD results in patient and graft survival similar to the ones of patients with other primary diseases. Although recurrence of IgAN in the graft is not rare, it is not a cause of graft loss.

FR-PO551

Prognostic Impact of Serum Bilirubin Level on Long-Term Renal Survival in IgA Nephropathy Shigeru Tanaka, Toshiharu Ninomiya, Kosuke Masutani, Masaharu Nagata, Akihiro Tsuchimoto, Kazuhiko Tsuruya, Takanari Kitazono. *Dept of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: Serum bilirubin has been recognized as a novel endogenous antioxidant. The aim of our study was to evaluate the impact of serum bilirubin on kidney prognosis in IgA nephropathy (IgAN).

Methods: We followed retrospectively 694 patients with IgAN diagnosed by renal biopsy between 1982 and 2010. The risk factors for developing end-stage renal disease (ESRD) were estimated using a Cox proportional hazard model. Predictive performance between models with or without serum bilirubin was evaluated by calculating the net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

Results: Seventy-seven patients developed ESRD during the median 4.9 years of follow-up. Estimated glomerular filtration rate (eGFR), proteinuria and histological severity were inversely related to bilirubin levels. In multivariate analysis, serum bilirubin was an independent risk factor for ESRD (hazard ratio for every 0.1mg/dL decrease in serum bilirubin, 1.18; 95% CI, 1.04-1.33). The incidence rate of ESRD decreased linearly with the increases in bilirubin levels (P for trend <0.01). When bilirubin was incorporated into a model with conventional ESRD risk factors, the NRI and IDI were 0.281 (P=0.017) and 0.019 (P=0.012), respectively.

Conclusions: We demonstrated that lower bilirubin levels were significantly associated with higher risk of ESRD in IgAN. In addition, bilirubin provided incremental predictive value in the risk assessment for progression of IgAN beyond that provided by standard risk factors.

FR-PO552

Total Cholesterol Is Independet Risk Factor for Progression of Kidney Disease in IgA Neprhropathy Patients Yasuyuki Nagasawa,[1] Ryohei Yamamoto,[2] Maki Shinzawa,[2] Sayuri Kawada,[1] Aritoshi Kida,[1] Mana Yahiro,[1] Takahiro Kuragano,[1] Tatsuya Shoji,[3] Terumasa Hayashi,[3] Atsushi Yamauchi,[4] Yoshitaka Isaka,[2] Takeshi Nakanishi.[1] *[1]Dept of Internal Medicine, Div of Kidney and Dialysis, Hyogo Medical College, Nishinomiya, Japan; [2]Div of Nephrology and Geriatric Medicine, Osaka Univ Graduate School of Medicine, Suita, Japan; [3]Dept of Nephrology and Hypertension, Osaka General Medical Center, Osaka, Japan; [4]Dept of Nephrology, Osaka Rousai Hospital, Sakai, Japan.*

Background: Ig A nephropathy is one of common primary glomerulonephritis. Recently, several reports indicated that the statin therapy might have favorable effect upon kidney diseases, but there was little information about the effect of hyperlipidemia itself upon progression of kidney diseases, especially in IgA nephropathy patients.

Methods: This study is retrospective cohort study. Subjects were 1205 IgA nephropathy patients who were diagnosed by renal biopsy, and over 15 years old in Osaka University Hospital, Osaka general medical center, Osaka Rosaki Hospital. Outcome was 1.5 times of serum creatinine. Explanatory variables included sex, age, BMI, blood pressure, baseline eGFR, proteinuria, smoking status.

Results: Mean age was 31[23-46] years, BMI was 22.3+-3.5, mean blood pressure was 121.67+-17.1/74.7+-13.0 mmHg, eGFR was 103.9+-33.7ml/min/1.73m2, proteinuria was 0.42[0.19-0.92] g/day. Total cholesterol was 5.03+-1.21mmol/l. Univariate analysis revealed that total cholesterol had significantly risk for progression of renal disease [Hazard Ratio 1.52[1.32-1.72],P<0.001] along with systolic BP (*10mmg), proteinuria (g/day), eGFR (*10ml/min/1.73m2). Multivariate analysis also revealed that total cholesterol was significant risk for progression of renal disease [Hazard Ratio 1.21[1.32-1.72] P<0.007] along with systolic BP (*10mmg) [HR 1.15[1.03-1.29] P=0.013], proteinuria (g/day) [HR 1.25[1.12-1.1.39],p<0.001], eGFR (*10ml/min/1.73m2) [HR 0.82[0.76-0.90],p<0.001]. After the patients were divided into four categories according to total cholesterol, IgA patients with higher cholesterol had poorer prognosis.

Conclusions: Hyper-cholesterol is independent risk for progression of kidney disease in IgA nephropathy patients.

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FR-PO553

Clinicopathological Significance of Monoclonal IgA Deposition in Patients with IgA Nephropathy Hiroshi Nagae,[1] Shota Kawahara,[1] Yukiko Shimomura,[1] Akihiro Tsuchimoto,[2] Kosuke Masutani,[2] Kazuhiko Tsuruya,[2] Ritsuko Katafuchi.[1] *[1]Nephrology, National Fukuoka Higashi Medical Center, Koga, Fukuoka, Japan; [2]Medicine and Clinical Science, Kyushu Univ.*

Background: Nasr et al described proliferative glomerulonephritis with monoclonal IgG deposition (PGNMID), and serum monoclonal protein was detected in 50% of patients with PGNMID. As for IgA nephropathy (IgAN), Lai et al reported that monoclonal IgA-ldeposition was observed in 18 out of 45 patients. However, clinicopathological significance of monoclonal IgA deposition (mIgAd) in IgAN is unclear.

Methods: We retrospectively investigated the prevalence of mIgAd and its clinicopathological significance in 65 patients with IgAN. Pathological parameters included Oxford classification and histological grade proposed by Special IgAN Study Group in Japan.

Results: 38% of patients were male, median age was 40 and median observation period was 31 months. 5 patients (Group M) showed monoclonal IgA-ldeposition and one showed

monoclonal IgA-k deposition. 59 patients (Group P) showed polyclonal IgA deposition. There were no significant differences in urinary protein/creatinine ratio, urinary-RBC and eGFR between Group M and P. TP and albumin were significantly lower in Group M than in Group P. Pathologically, the % of patients with M1 was significantly higher in Group M than in Group P. Only one patient in Group P showed serum monoclonal IgG-l. No patient showed abnormal serum k/l ratio. 75% in Group M and 42% in Group P were treated with steroid. During the follow up period, proteinuria disappeared in all patients in Group M and 68% in Group P. Hematuria disappeared in 67%, and 52% in Group M and P, respectively. 3 patients in Group P progressed end-stage renal disease (ESRD). The frequency of disappearance of proteinuria or hematuria and ESRD was not different between the groups.

Conclusions: The prevalence of mIgAd was 9.2%. Although some parameters differed between the groups, renal outcome were similar in both groups. Thus, IgAN with mIgAd might not be different entity from polyclonal IgA deposition. MIgAd is not likely to be related to hematological disorder. Further investigation is required to clarify the significance of mIgAd in IgAN.

FR-PO554

The Effects of the Pathologic Evidences of Podocyte Injuries on Proteinuria in Patients with IgA Nephropathy Seon Ha Baek, Shin-Young Ahn, Seong Woo Lee, Youn-Su Park, Sejoong Kim, Ho Jun Chin, Ki Young Na, Dong-Wan Chae. *Internal Medicine, Seoul National Univ Bundang Hospital, Korea.*

Background: Although the mesangial cells are primary target of injury in IgA nephropathy (IgAN), pathologic findings suggestive of injury to podocytes (PCs) such as segmental sclerosis (SS, depletion of PCs), crescent formation (CF, activation and proliferation of PCs) and foot process effacement (FPE) are frequently observed in renal biopsies from patients with IgAN. Based on the implications of PC injuries for more widespread inflammation not confined to measangium and the essential role of PC in filtration barrier on proteinuria, we hypothesize that the existence of PC injuries in renal biopsies negatively affect the proteinuria in IgAN.

Methods: We retrospectively assessed the effect of SS, CF, and diffuse FPE on basal proteinuria (BPU) at the time of biopsy and 1 year time averaged proteinuria (TAPU) under the treatment by renin-angiotensin aldosterone system (RAAS) blockade in IgAN patients from Seoul National University Bundang Hospital in Korea.

Results: A total of 294 patients (136 men, 158 women) with biopsy-proven primary IgAN were evaluated. The mean value of eGFR and BPU were 86.8 ml/min/1.73m2 and 1.68 g/g. Diffuse or focal but marked FPE, SS, and CF at kidney biopsy were observed in 29.5%, 72.8% and 25.2% of patients respectively. The presence of FPE (r=.159, P =.007), SS (r =.186, P =.001), and CF (r=.354 P<.001) were significantly associated with the higher BPU in fully adjusted analyses. In 175 patients treated by RAAS blockade without any immunosuppressant, SS and CF were associated with higher 1 year TAPU in fully adjusted analyses. (r =.180, P=.019, r=.310, P<.001 respectively). The patients showing all three features of podocytopathy (namely FPE, SS, and CF) had higher BPU and 1 year TAPU than patients exhibiting lesser number of podocytopathy (r =.427, P<.001, r=.152, P=.047).

Conclusions: Pathologic evidences of PC injuries in IgAN are associated with higher BPU and unfavorable response of proteinuria to RAAS blockade.

FR-PO555

Comparison of Clinical Manifestations Between Henöch-Schönlein Purpura Nephritis and IgA Nephropathy: Analysis of Japan Renal Biopsy Registry (J-RBR) Hiroyuki Komatsu,[1] Shouichi Fujimoto,[1] Norishige Yoshikawa,[2] Hiroshi Kitamura,[3] Hitoshi Sugiyama,[4] Hitoshi Yokoyama.[5] *[1]Univ of Miyazaki, Miyazaki, Japan; [2]Wakayama Medical Univ; [3]Chiba-East Hospital; [4]Univ of Okayama; [5]Kanazawa Medical Univ.*

Background: The clinical presentation of Henöch-Schönlein purpura nephritis (HSPN) in an adequate sample of patients of different ages has not been studied in detail. We therefore surveyed the features of HSPN and differences between HSPN and IgA nephropathy (IgAN) based on data from the Japan Renal Biopsy Registry (J-RBR).

Methods: A cross-sectional survey of 513 patients with HSPN and 5,679 with IgAN registered in the J-RBR between 2007 and 2012 was conducted. Clinical and pathological parameters of blood pressure (BP), and blood and urine laboratory findings at diagnosis were compared between these two groups and among children (≤ 18 years), adults (19-64 years) and elderly persons (≥ 65 years). Factors affecting declining renal function in adult and elderly patients were assessed using multiple regression analysis.

Results: Age distribution considerably differed between HSPN and IgAN although the mean age was 38.6 years in both groups; HSPN had bimodal peaks at 1 - 19 and at 60 - 69 years, whereas IgAN peaked at 30 - 39 years. The clinical features of HSPN were significantly more severe than those associated with IgAN, especially proteinuria (children, 1.28 versus 0.57 g/day; adults and elderly persons, 2.16 versus 1.11 g/day), and hypoalbuminemia (children, 3.72 versus 4.13 g/dL; adults and elderly persons, 3.47 versus 3.95 g/dL). The patients with HSPN had a higher rate (%) of histological classification as endocapillary proliferative or crescentic glomerulonephritis than those with IgAN. More advanced age and increased proteinuria were independent factors associated with decreasing estimated glomerular filtration rates in patients with HSPN and high BP and hypoalbuminemia were added to these in patients with IgAN.

Conclusions: The age distribution of patients with HSPN had bimodal peaks in children and elderly persons, and the clinical features were more severe than those of IgAN across all age groups.

FR-PO556

Long Term Follow-Up of Biopsy Proven Pediatric Henoch Schönlein Purpura Nephritis Tim Ulinski,[1,4] Georges Deschenes,[3] Bilal Aoun.[1] *[1]Pediatric Nephrology, Armand Trousseau Univ Hospital, Paris, France; [2]Pediatrics, CHU Toulouse, Toulouse, France; [3]Pediatric Nephrology, Robert Debré Hospital, Paris, France; [4]Univ Pierre et Marie Curie, Paris, France.*

Background: Henoch Schönlein purpura (HSP) nephritis is responsible for progressive renal disease in 7-50% of patients. Treatment usually depends on the severity of histological lesions.

Methods: Patients from three French centres were retrospectively analysed. The aim of this study was to determine the long-term biological outcome in children with HSP nephritis who underwent a renal biopsy and to identify possible predictive markers for disease development and to examine whether treatment modalities such as intravenous steroid pulses and ACE inhibitor were correlated with a better outcome.

Results: A total of 142 patients with HSP nephritis (graded according to the ISKDC classification) were followed up from 2 to 10.5 years. Mean (± SD) age at presentation was 7.6±2.8 years. Nephrotic range proteinuria was present in 28% with grade II, 60% with grade III and 90% with grade IV histological lesions. Significant proteinuria (>0.5g/L) was found in 9/48 patients three years, in 8/25 patients five years and in 3/14 patients 10 yearspost renal biopsy. There was no correlation between risk for proteinuria at 3, 5, or 10 years with initial histological lesions or treatment modalities such as oral or pulse steroids. Treatment with angiotensin converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARB) was related with lower proteinuria mainly if started precociously.

Conclusions: Even mild forms of HSP nephritis are at risk for significant proteinuria in the long term. There is a risk for under treatment in patients with low grade lesions. Very early introduction of ACEi/ARB may improve long term outcome independent from histological lesions.

FR-PO557

High Serum IgA/C3 Ratio Is a Novel Independent Predictor of Recurrence in IgA Nephropathy After Steroid Pulse Therapy Hoichi Amano,[1] Keita Hirano,[1] Tetsuya Kawamura,[2] Nobuo Tsuboi,[2] Kazushige Hanaoka,[2] Takashi Yokoo.[2] *[1]Nephrology, Ashikaga Red Cross Hospital, Ashikaga, Tochigi, Japan; [2]Nephrology and Hypertension, Jikei Univ School of Medicine, Minato, Tokyo, Japan.*

Background: We previously reported that mesangial hypercellularity in Oxford classification (M) and high histological grades (HGs) in Japanese classification (J Nephrol 2013) predict recurrence in IgA nephropathy (IgAN) after six months-steroid therapy, and tonsillectomy (Tx) combined with the steroid therapy reduces the risk of recurrence (ASN Kidney Week 2013). Other studies have shown that serum IgA/C3 ratio is associated with renal survival in IgAN. However, its association with recurrence is obscure. Therefore, we aimed to identify the independent clinical implication of serum IgA/C3 ratio in recurrence.

Methods: Among 137 patients with IgAN who received six months-steroid pulses (Pozzi et al. J Am Soc Nephrol 2004) from 2004 to 2010 in our affiliated hospitals and whose serum IgA /C3 ratio was determined at the start of treatment, we enrolled 101 patients who achieved a reduction in the urinary protein excretion volume (UPE) to <0.4 g/day at one year following the start of treatment, employing historical cohort design. The endpoint was defined as an increase of UPE >1.0 g/day following the first one year after initiating the steroid therapy.

Results: At the start of treatment, median UPE was 0.81 g/day. Mean of eGFR and IgA/C3 ratio were 72.7ml/min and 3.28, respectively. During a median follow-up of 2.4 years after the first one year following the initiation of treatment, 27 patients (26.7%) reached the end point. The optimal cutoff for serum IgA/C3 ratio predicting recurrence was determined as 2.91 by receiver operating characteristic curve. Of note, high serum IgA/C3 ratio defined as >2.91 was a significant and independent predictor of an increased risk of recurrence in an adjusted model with M, HGs and Tx (HR: 2.58, 95%CI: 1.02-7.85, P=0.044).

Conclusions: Our data indicate that high serum IgA/C3 ratio at baseline is a novel independent predictor of recurrence in IgAN after steroid pulse therapy.

Funding: Clinical Revenue Support

FR-PO558

Dense Deposit Disease in Ireland Carol A. Traynor, John O'regan, Limy Wong, Michelle M. O'Shaughnessy, Anthony M. Dorman, Peter J. Conlon. *Nephrology, Beaumont Hospital, Dublin, Ireland.*

Background: Dense Deposit Disease (DDD), also called membranoproliferative glomerulonephritis Type II, is a rare disease that predominantly affects children and young adults. We describe the clinical characteristics of all cases of DDD diagnosed in Ireland in the period 1976-2011.

Methods: We performed a retrospective review of all native renal biopsies performed in Ireland in the period 1976-2011. The diagnosis of DDD was based on the ultrastructural finding of a transformation of glomerular basement membranes by ribbon-like, highly electron-dense material and predominant immunofluorescence staining for C3. Patients' medical records were reviewed for demographic information, presenting clinical and laboratory findings, treatment, and outcome. Kaplan-Meier analysis was used to determine survival probabilities.

Results: There were 37 patients. Median age at diagnosis was 12.

Characteristic	All Patients (n=37)	ESKD (=20)	No ESKD (n=17)	p value
Age (median)	12.0	14.5	8.5	0.03
Gender male n (%) female n (%)	 16 (43%) 21 (57%)	 11 9	 5 12	ns
creatinine at biopsy median (IQR)	82µmol/L (68-125)	81µmol/L (71-250)	82µmol/L (60-99)	ns
Hypertension	15/33 (45%)	7/17 (41%)	8/15 (53%)	ns
protenuiria 0.5g/24hr 0.5-1g/24hr 1-3g/24hr >3g/24hr	 1/34 5/34 13/34 15/34	 0/18 1/18 8/18 9/18	 1/16 4/16 5/16 6/16	ns
nephrotic syndrome	24/29	14/16	10/13	ns
haematuria	34/35	18/19	16/16	ns
low C3	18/19	8/8	10/11	ns
low C4	2/15	1/7	1/8	ns

describes demographic characteristics. There were 21 females and 16 males affected. Twenty patients (54%) progressed to ESKD. Median time to ESKD was 8.9 years (6.2, 17.1). Patients who developed ESKD were older at presentation (median 14.5 versus 8.5). Two patients had a prior history of acquired partial lipodystrophy (APL). There were 25 transplants in 18 patients. Graft half-life was 9 years and 10 patients developed recurrence of DDD in the graft.

Conclusions: To our knowledge, this is the third largest series to date describing the clinical characteristics and transplant outcomes in patients with DDD. There was a high recurrence rate of DDD in transplanted patients although this did not universally lead to graft loss.

FR-PO559

C3 Glomerulonephritis Associated with Autoimmune Disorders: A Case Series

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Background: C3 glomerulonephritis (C3GN) is a proliferative GN resulting from glomerular deposition of complement factors due to dysregulation of the alternative pathway of complement. This is due to inherited (genetic) or acquired (autoantibodies) abnormalities of the complement regulating proteins (CRP). The autoantibodies presumably cause functional impairment of CRPs. In this study, we set out to determine if C3GN was associated with an underlying autoimmune disease.

Methods: The pathology database was queried for patients (pts) with C3GN, who had serological evidence/clinical diagnosis of an underlying autoimmune disease (AI). Clinical features (fxs), laboratory values, kidney biopsy (Kbx) findings, treatment, and followup were recorded.

Results: 5 pts fulfilled the inclusion criterion. Mean age was 43 yrs, F: M ratio was 3:2, mean serum creatinine was 2.56 mg/dL and mean proteinuria was 4660 mg/day. All pts had positive serology and/or clinical evidence of AI disease. All pts had strongly positive ANA and ds-DNA. In addition, 2 had clinical evidence of AI diseases (1 with Raynauds disease; the other with skin ulcerations and fibromyalgia). C3 and C4 were normal in all, except 1 who had low C3 levels. Kbx showed membranoproliferative (2), mesangioproliferative (2), and endocapillary proliferative (1 patient) GN. IF showed bright C3 staining in all patients. There was mild (1+) staining for IgG in 1 pt and IgM in 4 pts. Tubuloreticular inclusions (TRI) were present in 2 pts. All 5 kbx's showed abundant mesangial and capillary wall electron dense deposits. Treatment included immunosuppressive therapy in 4 (prednisone-4; cyclophosphamide-2; mycophenolate mofetil-1). Follow-up in 2 pts over a mean period of 10 months, showed a mean creatinine of 1.65 mg/dL and mean proteinuria of 1674 mg/day.

Conclusions: This is the first series to show an association between C3GN and autoimmunity. The unusual fxs (positive AI panels, co-immunoglobulin deposits, and TRI's) suggest a true association of two processes. The pathogenic basis for disease might be an aberrant autoantibody directly interfering with CRP function.

FR-PO560

Ten-Year Follow-Up of Patients with Epidemic Glomerulonephritis due to Streptococcus Zooepidemicus

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Background: In 1998 there was a large outbreak of acute glomerulonephritis in the city of Nova Serrana, Southeastern Brazil, caused by Lancefield group C *Streptococcus zooepidemicus*. Epidemiological investigation revealed that patients had consumed a locally produced cheese prepared with contaminated unpasteurized milk. This study describes the follow-up of these patients after a mean time of 10.4 years of the acute episode.

Methods: Of 135 cases identified in 1998, 60 were reexamined and compared with a control group of community individuals (n=48) not affected during the outbreak, matched by age and gender, in a prospective study. Participants had measurements of blood pressure, glomerular filtration rate (estimated by the Modification Diet in Renal Disease equation, eGFR) and albuminuria (radioimmunoassay) expressed as albumin-to-creatinine ratio in a random untimed urine sample. Creatinine was measured using the Jaffé method on an autoanalyzer.

Results: Of the original group of 135 affected subjects, 3 died in the acute phase and 5 (3.7%) required chronic dialysis initiated during the initial disease period. Of the 60 reevaluated cases, all were adults (mean±SD age: 49±15 yr) and 40 (67%) females. Sociodemographic and baseline clinical characteristics were not significantly different between the reevaluated cases and those who were not. At the follow-up examination we found arterial hypertension (blood pressure ≥140/90 mmHg or use of anti-hypertensive drugs) in 33% (n=20) of the cases and 21% (n=10) of the controls (p>0.20), eGFR was <60ml/mim/1.73 m^2 in only 2 cases and 2 controls (3% and 5%, respectively, p=ns); albuminuria >20 mg/g was observed in a similar percentage of cases and controls (19% and 21%, respectively, p=ns); reduced eGFR or increased albuminuria was observed in a similar percentage of cases and controls (n=12 (20%) and n=11 (23%), respectively, p=ns).

Conclusions: After a mean followu-up time of 10.4 years, the prognosis of patients with epidemic poststreptococcal glomerulonephritis due to *S. zooepidemicus* was favorable; rates of abnormal renal function or hypertension were comparable with a healthy control group.

FR-PO561

Long-Term Prognosis of Postinfectious Glomerulonephritis in Adults

Carla Tenório Barros Cisne Pessoa, Camila Barbosa L. Oliveira, Alline S.A. Oliveira, Clarissa Jacob Barros Carvalho, Nathalia K.N. Alecrim, Luis H.B.C. Sette, Gisele Vajgel Fernandes, Lucila Maria Valente, Maria Alina G.M. Cavalcante. *Nephrology, Univ Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Background: Postinfectious glomerulonephritis (PIGN) is uncommon in adults, with a declining incidence over the past decades, particularly in developed countries. The aim of this study is to evaluate the profile, clinical course and outcomes of adult patients with PIGN.

Methods: We conduct a retrospective single center cohort study of adult patients with PIGN diagnosed between 1997-2013. Clinical characteristics, laboratorial data and long-term follow-up were analyzed.

Results: We evaluated 40 patients, 52.5% women with a mean age of 30.7 ± 15.7 years. The most common sites of infection were upper respiratory tract (51.2%), skin (27.5%) and lower respiratory tract (6.9%). Nephritic syndrome was the main clinical presentation (82.5%), followed by rapidly progressive glomerulonephritis (10%) and hematuria and/or non-nephrotic proteinuria (5%). Hypertension was present in 65% of patients. Median serum creatinine (Cr) and Cr clearance (Cl) were 2.0 ± 1.5mg/dl and 53.4 ± 27.7 ml/min/1.73m^2, respectively, and 10% of patients required renal replacement therapy (RRT). Low serum C3 levels were detected in 78.1% and C4 in 22.2%. Biopsy was performed in 14 (35%) patients and 35% of the biopsies had crescents. Mean follow-up time was 29.6 ± 27.5 months. Outcomes are shown in table 1.

Outcome	N=38
Follow up (months)	29.6± 27.5
Serum Cr, mg/dl	0.9 ± 0.3
ClCr, ml/min/1,73m^2 (MDRD)	82.2 ± 13.4
Proteinuria > 300mg/day, %	14.8
Hematuria, %	28.9
Hypertension, %	25
Chronic Kidney Disease, %	28
Doubling Cr, %	0
RRT, %	0

Chronic kidney disease at the end of follow-up was associated with a lower baseline CrCl (36.4 ± 24.0 versus 58.3 ± 27.0ml/min/1.73m^2; p=0.015). Hypertension at the initial presentation (p=0.444), nephrotic proteinuria (p=0.626) and crescents on biopsy (p=0.587) did not increase the risk of chronic kidney disease.

Conclusions: Adult patients with PIGN can have a severe clinical manifestation, demonstrated by baseline renal dysfunction, sometimes with necessity of RRT, and a reasonable risk of chronic kidney disease.

FR-PO562

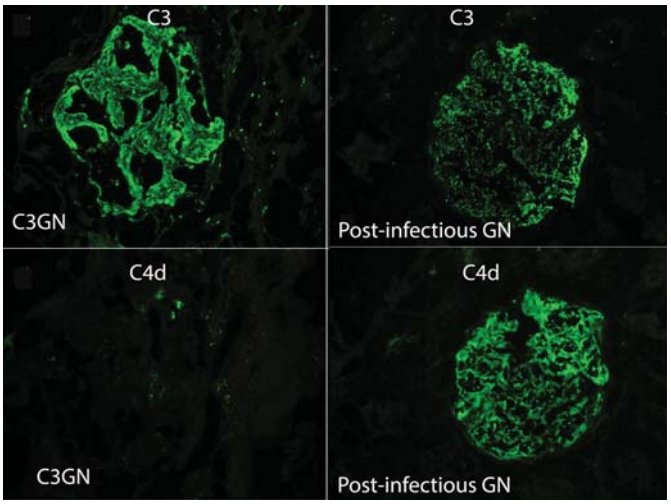
C4d as a Marker for Immune Complex-Mediated Glomerulonephritis versus C3 Glomerulopathy

Sanjeev Sethi, Samih H. Nasr, Fernando C. Fervenza. *Mayo Clinic, Rochester, MN.*

Background: Proliferative glomerulonephritis (GN) is classified as immune complex-mediated (IC-GN) and complement-mediated GN (C3 glomerulopathy). C3 glomerulopathy includes dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). IC-GN results from glomerular deposition of Ig and C3; the C3 in IC-GN is derived from activation of the classical pathway of complement. On the other hand, C3 glomerulopathy results from deposition of C3 and other complement fragments; the C3 is derived from activation of alternative pathway of complement. C4d is a by-product of activation of CP of complement. Thus, we hypothesized that C4d is positive in IC-GN, while it is negative in C3 glomerulopathy.

Methods: IC-GN and C3 glomerulopathy were identified by retrospective review of kidney biopsies. We identified 18 biopsies of IC-GN and 26 biopsies of C3 glomerulopathy. IC-GN included IgA nephropathy, MPGN, membranous nephropathy, lupus nephritis, post-infectious GN, and fibrillary GN. C3 glomerulopathy included 24 cases of C3GN and 2 cases of DDD. C4d staining by IF (0-3+) was done using polyclonal FITC-conjugated antibodies.

Results: C4d was positive in all cases of IC-GN, except for IgA nephropathy. All biopsies of IC-GN showed bright glomerular C4d (2-3+) indicating on-going activation of the classical pathway. On the other hand, C4d was completely negative in 20 (77%) of 26 biopsies of C3 glomerulopathy, and showed only trace/1+ C4d staining in the remaining 6 (23%) biopsies. Importantly, none of the biopsies of C3GN showed the bright (2-3+) C4d staining seen in IC-GN.



Conclusions: IC-GN is characterized by the presence of Ig, C3 and bright C4d. On the other hand, C3 glomerulopathy is characterized by presence of bright C3, negative or weak C4d, and scant or no Ig. Thus, C4d serves a positive marker for IC-GN, while it is negative in C3 glomerulopathy. C4d is a useful ancillary study in determining the etiology of GN.

FR-PO563

Renal Disease in 95 Primary Sjögren's Syndrome with Renal Biopsy

Magali Jasiek,[1] Alexandre Karras,[2] Benjamin Terrier,[2] Rafik Mesbah,[3] Pierre M. Ronco,[4] Philippe Remy,[6] Noemie Jourde-Chiche,[7] Stanislas Faguer,[5] Raphaele Seror,[1] Xavier Mariette,[1] Eric Thervet,[2] Véronique Le Guern,[2] H. Francois.[1] *[1]Nephrology, Bicêtre Hospital, Univ Paris Sud, France; [2]Nephrology and Internal Medicine, Univ Paris Descartes, France; [3]Nephrology, Hôpital Boulogne sur Mer, France; [4]Nephrology, Tenon Hospital, France; [5]Nephrology, Ragueil Hospital, France; [6]Nephrology, Mondor Hospital, France; [7]Nephrology, Marseille Hospital, France.*

Background: Since kidney disease in Primary Sjögren's syndrome (pSS) is rare.

Methods: we conducted a French multicenter retrospective study of biopsy proven nephropathies in pSS patients. Inclusion criteria were patients diagnosed with pSS based on AECG criteria who underwent a kidney biopsy.

Results: We included 95 patients (sex ratio F/M: 9/1, mean age 49,3 years). Renal disease was diagnosed after the first symptoms of pSS in 68,4%. Renal manifestations consisted in renal failure in 86,3% of the patients, proteinuria in 26,3%, electrolyte disturbances alone in 17,9%, lithiasis in 9,5% and nephrocalcinosis in 5,3%. Renal biopsies exhibited acute or chronic tubulointerstitial nephritis in 73 (77%) patients with plasmocytes infiltrate in 69,2%. Glomerular lesions were found in 22 patients (23,2%), and mixed (intersitial and glomerular) in 19. Glomerular disease consisted in cryoglobulinemic glomerulopathy (8), focal and segmental glomerulosclerosis (5), membranous nephropathy (4), minimal change disease (2) or other lesions (3). Of note, 81 patients (85,3%) received an immunosuppressive treatment including steroids (98,8%). 21 patients received another immunosuppressant on top of steroids (mostly rituximab). After a median follow-up of 53 months (1-275), eGFR significantly improved (mean eGFR (MDRD) 39,8 ml/min/1,73m2 at presentation versus 47,3ml/min/1,73m2, p=0,001) and only 3 patients suffered from ESRD. Prognosis was correlated with age, hypergammaglobulinemia and tubulo-interstitial kidney lesions (p<0,05, multivariate analysis) although the overall renal outcome was good.

Conclusions: Tubulo-interstitial nephritis is the most common manifestation found in pSS with frequent plasmocytic infiltrate. In most cases, patients are treated with steroids which allows a good prognosis.

FR-PO564

Renal Dysfunction in Lithium-Treated Patients: A Cross-Sectional Study

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Background: Lithium, the most effective drug for bipolar disorder (BD), is associated with various manifestations of renal injury. In this cross-sectional study, we examined renal concentrating ability and estimated glomerular filtration rate (eGFR) in a cohort of lithium-treated patients.

Methods: Lithium-treated patients were recruited from the outpatient clinics of a secondary and tertiary institution in the Netherlands. Participants were evaluated at the outpatient clinic. Height, weight and blood pressure were recorded and laboratory examinations were performed. Additionally, all participants filled out a comprehensive questionnaire. Moreover, a dDAVP test was performed in a subgroup of patients.

Results: A total of 201 patients – 87 (43.1%) males, median age 51 (range 19-78) yrs, 191 (95.0%) Caucasians, 180 (89.1%) with BD, 13 (6.4%) with unipolar depression and 9 (4.5%) with schizoaffective disorder – were enrolled in an observational registry. The median time on lithium was 8 (range 0-35) yrs. The prevalences of smoking, obesitas (BMI≥30 kg/m^2), hypertension, dyslipidemia, diabetes mellitus en cardiovascular disease were 68.7, 19.9, 20.5, 31.1, 6.0 and 6.4 %, respectively. CKD was present in 41 (20.3%) patients; of these, 16 (7.9%) and 25 (12.4%) patients had CKD stage G1-2 A1-3 and G3 A1-3, respectively. A dDAVP test was performed in a random subgroup of 99 patients. Renal concentrating ability was impaired (<800 mOsmol/kg) in 67 (68.4%) patients with partial NDI (<600 mOsmol/kg) in 17 (17.3%) patients.

Conclusions: This study demonstrates that the majority of lithium-treated patients show an impaired renal concentrating ability or even NDI. Moreover, CKD is present in approximately 20% of patients.

Funding: Private Foundation Support

FR-PO565

Spectrum of Histopathologic Findings on Kidney Biopsy in Individuals with Malignancy Receiving Treatment at a Large Cancer Center

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Background: An increase in serum creatinine and/or denovo proteinuria commonly occurs in individuals with malignancy, but their basis remains poorly characterized. We undertook a systematic study of the pathology noted at kidney biopsy among individuals with malignancy.

Methods: We conducted a chart review of individuals who presented between January 2000 and 2014, and collected demographic, histopathology, treatment, and outcome data. Standard processing of biopsy specimens included light, immunofluorescence and electron microscopy.

Results: We studied 268 individuals. The majority were men, with mean: age of 60 years, serum creatinine at biopsy of 3.08 mg/dl, and urine protein excretion of 4.08 gm protein/gm creatinine. The most common histological finding was glomerular disease (132,49%), followed by tubulointerstitial disease (63,24%), vascular lesions (34,13%), and chronic kidney disease (31,12%). Thrombotic microangiopathy (TMA) was found in 34 individuals; 16 (47%) had a prior stem cell transplant. Chemotherapy was associated with 19 cases of TMA, and carflizomib was the possible etiological agent in one (new). Acute interstitial nephritis in 4 was associated with an experimental chemotherapy agent. We found association of kidney cancer with focal segmental glomerulosclerosis and IgA nephropathy. Of 268, there were 10 membranous nephropathy and 12 minimal change disease. Proliferative glomerular disease was found in 18, 9 of whom had proliferative glomerulonephritis with monoclonal immunoglobulin deposits (PGNMID) which is a recently described entity.

Conclusions: We report the only study to date of the spectrum of kidney diseases in individuals with cancer, and identified several novel associations with anti-cancer therapy. Our systematic investigation emphasizes the importance of kidney biopsy to elucidate the basis for renal dysfunction, and to guide therapeutic interventions in patients with malignancy.

FR-PO566

Glomerular and Interstitial Renal Findings in Liver Cirrhosis

Suman Nayak,[1] Sivaramakrishnan Ramanarayanan,[1] Rajendra Prasad Mathur,[1] Gyan Prakash,[1] Shiv Sarin,[1] Suresh Chandra Tiwari,[2] Archana Rastogi,[1] Chhagan Bihari,[1] Amar Mukund,[1] Chitranshu Vashishtha.[1] *[1]Nephrology, Inst of Liver and Biliary Sciences, New Delhi, Delhi, India; [2]Nephrology, Fortis vasant kunj, New Delhi, Delhi, India.*

Background: The true spectrum of kidney injury in liver disease remains speculative. Since majority of patients are very sick and coagulopathic, there is paucity of literature on renal biopsies and structural renal pathologies in cirrhotics. The pathogenesis is presumed to be the impaired hepatic clearance of immune complexes and their trapping in the kidney, causing the lesions of hepatic immunoglobulin A (IgA) nephropathy and hepatic glomerulosclerosis.

Methods: We reviewed the kidney biopsies of 27 Cirrhotic patients with the clinical suspicion of glomerular disease i.e proteinuria (>500 mg/24 hr), glomerular hematuria or renal dysfunction. Kidney biopsy was done through percutaneous route under real time ultrasound guidance in 24 patients and through transjugular method in 3 patients who were coagulopathic. Biopsy tissues were processed and subjected to light microscopy and immunofluorescence.

Results: Mean age of patients was 50.81±12.49 years. There were 22 (81.5%) male patients and 5 (18.5%) female patients. Basic liver disease was ethanol related in 40.7% patients followed by Hepatitis C (14.8%), NASH (14.8%), Cryptogenic (11.1%), Autoimmune (7.4%), EHPVO (7.4%) and Hepatitis B (3.7%).Mean bilirubin was 1.8±1.42 mg/dl. Renal dysfunction was seen in 85.2% patients. Mean urea and creatinine were 66.66±33.47 mg/dl and 2.41±1.59 mg/dl respectively. Proteinuria was present in all and glomerular haematuria was observed in 66.7% patients. Post procedure one patient had major complication in the form of perinephric hematoma, got managed successfully by coil embolisation. Most common glomerular lesion was IgA Nephropathy (40.7%) followed by CIN (14.8%), MPGN (7.4%), Diabetic Nephropathy (11.1%), FSGS (11.1%), Amyloidosis (3.7%), Nodular glomerulosclerosis (3.7%), granulomatous nephritis (3.7%) and cholemic nephrosis (3.7%).

Conclusions: IgA Nephropathy was the most common glomerular lesion in Cirrhotic patients. Child A Cirrhotics can be taken safely for renal biopsy if there is a strong suspicion of glomerular disease.

Funding: Government Support - Non-U.S.

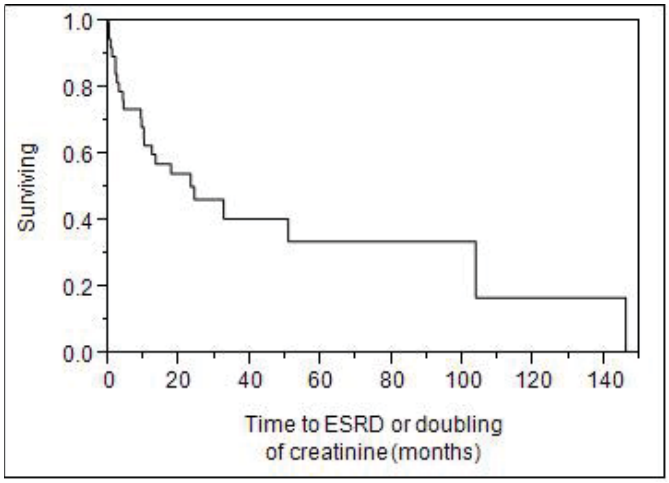
FR-PO567

Progression of Renal Disease and Treatment Outcomes in Proliferative Glomerulonephritis Associated with Non-Organized Monoclonal Immunoglobulin Deposits Gauri Bhutani,[1] Sanjeev Sethi,[2] Samih H. Nasr,[2] Fernando C. Fervenza,[1] Nelson Leung.[1] *[1]Nephrology, Mayo Clinic; [2]Anatomic Pathology, Mayo Clinic, Rochester, MN.*

Background: Prognostic and treatment data are limited for proliferative glomerulonephritis associated with non-organized monoclonal Ig deposits (PGNMID). As initially described, most cases have monoclonal IgG deposits but rare ones show IgM / IgA.

Methods: Pathology database at Mayo Clinic (R) was queried for patients with PGNMID on kidney biopsy between 1/1/2008 and 12/31/2012. A retrospective chart review was conducted in those with native kidney disease. Statistical analysis employed Kaplan-Meier curve, Log Rank test and Cox proportional hazard.

Results: Thirty-eight patients were studied including 34 with monoclonal IgG, 3 IgM and 1 IgA with a median follow-up of 1036 (608 to 1960) days. Median age was 56 (48 - 62) years. Females equaled males and majority were white (87%). Median creatinine and proteinuria at diagnosis were 1.6 (1.4 to 2.9) mg/dl and 6.2 (3-9.3) g/day respectively. Over follow up, there were no deaths and 15 (39%) reached ESRD. At 1 year point, 40% had ESRD or doubling of creatinine. Median time to this combined outcome was 24 (4 - 103) months. Most patients (87%) received treatment ranging from steroids to mycophenolate to chemotherapy agents. Higher initial creatinine was a risk factor for the combined outcome ($p<0.01$; RR 1.14 - 1.84). Normal serum C3 and C4 ($p = 0.10$); mesangioproliferative histology may be protective ($p = 0.09$). No relationship was observed between renal survival and detectability of pathologic Ig or clone, Ig isotype, IgG subtype, light chain, age or gender.



Conclusions: PGNMID has poor renal outcomes even with treatment and especially in those presenting with lower GFR. Subgroup outcome comparisons were limited by small cohort size. Evaluation of individual treatment agents and outcomes after transplant recurrence (n= 14) will be performed.

FR-PO568

Long-Term Prognosis after Kidney Biopsies in Patients with Pre-Biopsy eGFR <15 ml/min/1.73m2 Ann-Merethe Vaagane,[1] Sanjeevan Sriskandarajah,[1] Thomas Knoop,[1] Bjorn Egil Vikse,[1] Anna Reisaeter,[2] Einar Svarstad,[1] Sabine Leh,[1] Rune Bjoerneklett.[1] *[1]Inst of Clin Med, Univ of Bergen, Bergen, Norway; [2]Dept of Transplant Med, Oslo Univ Hospital, Oslo, Norway.*

Background: Kidney biopsies are sometimes performed on patients with pre-biopsy estimated glomerular filtration rate (eGFR) less than 15 ml/min/1.73m2. In this study we have analyzed overall and disease specific long-term End-Stage Renal Disease (ESRD) and mortality risk in such patients.

Methods: All patients registered in the Norwegian Kidney Biopsy Registry 1988-2012 with a specified histopathological diagnosis and a pre-biopsy eGFR <15 ml/min/1.73m2 were included in the study. Deaths and ESRD during follow-up were identified through record linkage with the Norwegian Kidney Registry (ESRD) and Population Registry (deaths). ESRD is defined as commencement of chronic renal replacement therapy (RRT). Only deaths before ESRD are included. Cumulative probability of ESRD free patient survival 1-, 5-, 10- and 20 years was calculated.

Results: 999 pts were identified, 480 progressed to ESRD and 281 died (deaths after ESRD not included).

Diagnoses	N patients	Cumulative patient survival without ESRD (%)			
		1 year	5 years	10 years	20 years
All patients	999	47	31	25	15
Sarcoidosis	8	100	73	73	0
Acute interstitial nephrithis	168	82	68	55	39
Acute endocapillary GN	17	76	76	68	58
Membranous nephropathy	4	75	50	25	25
Chronic interstitial nephrithis	47	64	41	28	10
Acute tubular necrosis	46	59	51	42	29
Trombotic microangiopathy	21	57	57	57	57
Lupus nephrithis	14	50	36	21	0
Benign nephrosclerosis	87	47	18	15	11
ANCA associated GN	166	45	30	22	5
Mesangioproliferative GN	59	42	23	15	9
Henoch Schønleins purpura	5	40	0	0	0
Membranoproliferative GN	23	35	22	22	22
FSGS	17	34	0	0	0
Malignant nephrosclerosis	48	27	17	10	5
IgA nephropathy	56	27	15	15	7
Diabetes nephropathy	44	27	9	9	9
Myeloma cast nephropathy	70	27	9	6	6
Anti GBM nephrithis	37	24	15	15	15
Amyloidosis	62	14	2	2	0

Conclusions: Long-term prognosis in biopsied patients with CKD 5 is overall poor. There is, however, substantial variation in risk of ESRD/death depending on type of renal disease. Kidney biopsies in patients with CKD 5 may therefore be useful for both diagnostic and prognostic aims.

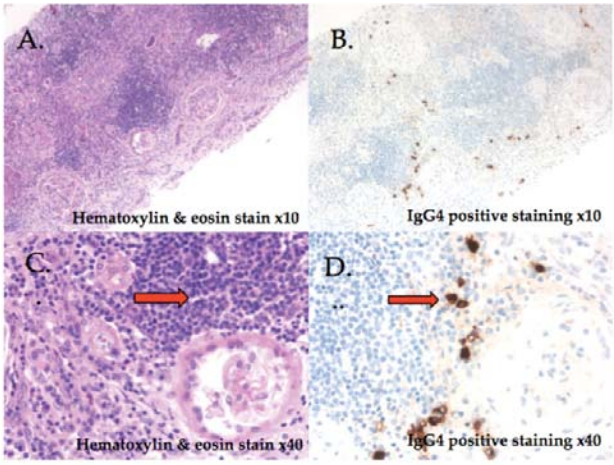
FR-PO569

The Incidence of IgG4-Related Tubulointerstitial Nephritis in Patients with Biopsy Proven Tubulointerstitial Nephritis Kathy Apy Mac, Michael G. Suranyi, Angela Makris. *Dept of Renal Medicine, Liverpool Hospital, Sydney, NSW, Australia.*

Background: Although IgG4 related disease is rare- IgG4TIN is the most common renal manifestation. The disease is usually associated with other organ involvement, renal masses, immune activation and elevated serum IgG4 levels. The incidence of IgG4TIN may be underestimated as staining for IgG4 is not routine.

Methods: We retrospectively reviewed native renal biopsies performed at our center (2002-2012). Renal biopsies with a primary diagnosis of interstitial nephritis were selected. Samples where interstitial nephritis was secondary to a glomerular disease were excluded. The renal tissue was recut and stained using a monoclonal antibody specific to IgG4. Demographic and follow up details were collected. This study was approved by the local ethics committee.

Results: 89 cases of interstitial nephritis from a total 1238 renal biopsies (2002-2012) were selected for further assessment. 12 samples demonstrated staining consistent with the criteria for IgG4-TIN, of which 3 were mildly positively stained, 7 moderately positively stained and 2 were markedly positively stained.



Example of IgG4 positive staining slide

Tubulointersitial nephritis.
x10 power field: A (H&E stain) and B (IgG4 positively stained using immunoperoxidase technique);
x40 high power field: C (H&E stain) showing cluster of lymphocytes infiltration and D (IgG4 positively stained) showing only 13 positively stained plasma cells in the heavy cluster of inflammatory cells.

There were no statistically significant differences in the baseline characteristics between the positive and the negative staining groups.

Conclusions: A clinically important number of IgG4TIN was observed in our population that had been diagnosed as undifferentitated TIN. From our study, IgG4 TIN made up of 1% of all renal biopsies performed over 10 years and 13% all biopsies demonstrating TIN not related to glomerular disease. IgG4 staining should be considered routinely in biopsies demonstrating primary tubulointerstitial nephritis.

FR-PO570

Renal Tubulointerstitial Distribution of Vasohibin-2 and Its Association with Clinicopathological Parameters in Patients with Renal Disorders Yuka Arata,[1] Hiroyuki Watatani,[1] Haruyo Omori,[1] Kana Masuda,[1] Katsuyuki Tanabe,[1] Jun Wada,[1] Hitoshi Sugiyama,[1] Naoki Kanomata,[2] Yasufumi Sato,[3] Yohei Maeshima.[1] *[1]Medicine and Clinical Science, Okayama Univ, Okayama, Japan; [2]Pathology, Kawasaki Med. School, Okayama, Japan; [3]Vascular Biology, Tohoku Univ, Sendai, Japan.*

Background: We recently reported the clinical significance of Vasohibin-1, a negative feedback regulator of angiogenesis, in predicting renal functional deterioration (PLoS ONE, 2014). Vasohibin-2, a homologue of Vasohibin-1, possesses potent pro-angiogenic effects. Study aims were to characterize renal tubulointerstitial distribution of Vasohibin-2 in patients with renal disorders and determine any potential association between the distribution and clinicopathological parameters.

Methods: Immunohistochemistry for Vasohibin-2 was performed in renal biopsy sections from 54 patients (glomerulonephritis, nephrosclerosis, diabetic nephropathy etc.) and six renal sections of healthy tissue from nephrectomized kidneys (renal cell carcinoma). Correlation between clinical/histological parameters and immunoreactivity for Vasohibin-2 in the tubules (semiquantitative score) and vasa recta (the number of vessels with immunoreactivity) was determined by the Spearman's test.

Results: Vasohibin-2 was mainly distributed in distal tubules, peritubular capillaries and vasa recta in subjects with renal disorders. Significant positive correlations were observed between 1) interstitial fibrosis and the tubular Vasohibin-2 score ($r = 0.39$, $P< 0.01$) and 2) tubular atrophy and the tubular Vasohibin-2 score ($r = 0.46$, $P< 0.01$). Significant inverse correlations were observed between 1) proteinuria and the number of Vasohibin-2$^+$ vessels in the vasa recta ($r =-0.41$, $P< 0.01$) and 2) annual reduction of proteinuria and the tubular Vasohibin-2 score ($r = -0.58$, $P< 0.01$). Renal Vasohibin-2 levels were not correlated with angiogenic factors, but were positively correlated with BMI, systolic blood pressure, LDL-C and HbA1c.

Conclusions: These results suggest that Vasohibin-2 may play a role in the progression of renal disorders including tubulointerstitial injuries, and implicates its potential to serve as a novel renal biomarker similar to its homologue, Vasohibin-1.

Funding: Government Support - Non-U.S.

FR-PO571

Clinicopathological Features of Glomerulopathy Associated with Overweight Yusuke Okabayashi, Nobuo Tsuboi, Kentaro Koike, Go Kanzaki, Kotaro Haruhara, Yoichi Miyazaki, Tetsuya Kawamura, Makoto Ogura, Takashi Yokoo. *Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

Background: Obesity-related glomerulopathy (ORG) is defined as a proteinuric renal disease found in patients with BMI of 30kg/m^2 or more, and as an absence of other known renal diseases. In the Japanese population survey, the presence of overweight (OW) with a BMI value of 25≤ BMI <30kg/m^2 was shown to have close relationships to the increased incidence of various diseases. However, little is known regarding the renal complication (s) that can occur in OW patients.

Methods: A total of 27 OW patients with proteinuria without other known renal diseases, were retrospectively analyzed. These patients were compared to the 26 ORG patients. The glomerular density (glomerular number per cortical area, GD) and the glomerular volume (GV) were measured in each patient. Biopsies of the 34 kidney transplant donors (KTD) were used for comparisons of the histological measures as healthy control. Renal outcomes were evaluated by the slope of renal function and the occurrence of progression.

Results: In the OW group, the mean age was 53 years, which was higher than that of the ORG group. Same as the ORG group, the OW group was male predominance (85%) and showed a high incidence of hypertension (80%). The serum values for creatinine or albumin, and the amount of urinary protein excretion were not different to those of the ORG group. The degrees of chronic histopathological lesions were similar to those of the ORG group. In addition, OW patients showed a typical histological characteristic of ORG; i.e., low GD with glomerulomegaly (Tsuboi N, CJASN 2012).

	KTD	OW	ORG
GD (/mm^2)	3.1±1.0	1.6±0.8	1.7±0.6
GV (x10^{6}μm^3)	2.4±0.6	6.1±2.2	6.3±1.8
BMI (kg/m^2)	24.2±3.5	27.1±1.3	32.5±2.9

Both the slope of renal function (-7.7±8.9 versus -5.8±7.1 %/year) and the rate of progression (27 versus 35%) were not different between these two groups.

Conclusions: These results suggest that a glomerulopathy similar to ORG can occur in individuals with OW, even in the absence of obesity. Renal factor (s) other than BMI value may thus underlie the susceptibility to this disease entity.

FR-PO572

The Degree of Proteinuria After Induction Therapy Was More Closely Related to Renal Functional Outcome Than the Clinicopathological Features at Diagnosis in Lupus Nephritis Yusuke Okabayashi, Nobuo Tsuboi, Kentaro Koike, Go Kanzaki, Kotaro Haruhara, Yoichi Miyazaki, Tetsuya Kawamura, Makoto Ogura, Takashi Yokoo. *Div of Nephrology and Hypertention, The Jikei Univ School of Medicine, Tokyo, Japan.*

Background: Heavy proteinuria and renal insufficiency at biopsy are known risk factors for progression in lupus nephritis (LN). However, the significance of the degree of proteinuria achieving during follow-up period has not fully understood. This study therefore investigated whether urinary protein excretion (UPE) after induction therapy as a predictor of progression in LN.

Methods: The impact on the renal outcome was retrospectively examined as to the clinicopathological features at diagnosis and the degree of proteinuria 1 year after diagnosis. Renal biopsy findings were analyzed based on ISN/RPN classification. UPE of <1g/day at 1 year was defined as remission. Progression was defined as 50% decrease in eGFR or the development to ESKD.

Results: Of 113 LN patients in our archive, the 76 patients with follow-up period of more than 1 year were included. All patients received induction therapies, including corticosteroid and/or immunosuppressants. The average values at diagnosis were age of 38 years, eGFR of 91ml/min/1.73m^2, and UPE of 2.1g/day. Class III nephritis was most frequent finding (42%), followed by IV (40%), II (11%), and V (7%). The average value of follow-up period was 8 years and 15 patients underwent progression. Patients without remission were associated with progression in comparison to those with remission (p:0.005). Remission was identified as an independent factor associated with progression by the multivariate analysis. Of note, renal function, UPE and ISN/RPN classification at diagnosis were not associated with progression.

Predictors of progression (Cox proportional hazards model)

	Univariate			Multiivariate		
Variables	Hazard Ratio	95% CI	p-value	Hazard Ratio	95% CI	p-value
eGFR at biopsy (ml/min/1.73m^2)	1.005	(0.99, 1.02)	0.419	1.004	(0.99, 1.02)	0.541
Nephrotic range proteinuria at biopsy	1.795	(0.64, 5.01)	0.264	1.139	(0.30, 4.36)	0.850
Remission at 1year	0.257	(0.09, 0.72)	0.010	0.275	(0.08, 0.99)	0.049
Class IV nephritis	0.844	(0.30, 2.42)	0.752	1.149	(0.38, 3.52)	0.807

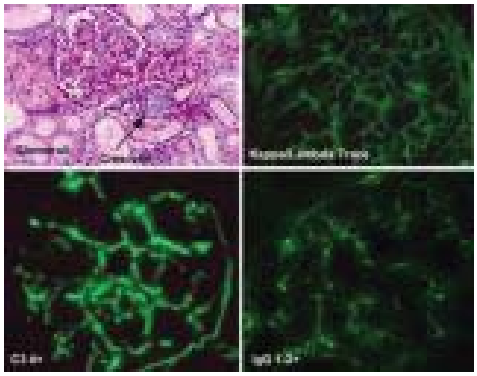
Conclusions: In LN patients, remission of proteinuria achieving 1 year after induction therapy may lead to better renal outcomes.

FR-PO573

Successful Eculizumab Treatment of Atypical Postinfectious Glomerulonephritis/C3 Nephropathy Amita Sharma, Ivy A. Rosales, Samuel V. Gorstein, Aleksandr Vasilyev, Robert B. Colvin, Rex Neal Smith. *Div of Pediatric Nephrology, Massachusetts General Hospital for Children, Boston, MA; Div of Renal Pathology, Massachusetts General Hospital, Boston, MA.*

Background: Acute post streptococcal glomerulonephritis is a common acute GN and most patients recover normal renal function if oligoanuric, with supportive care including dialysis. Patients with prolonged disease (atypical post infectious GN) often have defects in alternate complement pathway.

Methods: We report a 10 year old girl who presented clinically with post infectious glomerulonephritis ASO 683 (0-640) with rapidly progressive glomerulonephritis (peak Cr 6.4 mg/dl).Serum complement C3 was 46 mg /dL (Normal 75-175 mg/d L) with normal complement C4 (Normal 14-40 mg/dL). ANA, ds DNA, ANCA and anti GBM were negative. There was no significant preceding history. Patient continued to be oligo anuric, edematous, severely hypertensive in spite being on diuretics,antihypertensives and steroid pulses. Kidney biopsy showed atypical PIGN/C3 nephropathy with acute crescentic glomerulonephritis with complement C3 predominance but some IgG.



Rather than subjecting her to dialysis, a single dose 1200 mg of Eculizumab followed by a second dose of 900 mg 10 days later was administered, titrating with C5 functional assay.

Results: Gross hematuria cleared within 2 hours after infusion with significant increase in urine output. Serum creatinine normalized in 2 weeks and complement C3 normalized in 10 days, and both remained normal after one year. Complement Factor B stayed normal. Proteinuria and hematuria resolved by 3 months. There were no mutations detected in Complement Factor H, Complement Factor I or MCP.

Conclusions: In this case of acute and rapidly progressive atypical PIGN/C3 nephropathy a single dose of Eculizumab rather than supportive care rapidly reversed the GN and preserved renal function for one year.

Funding: Clinical Revenue Support

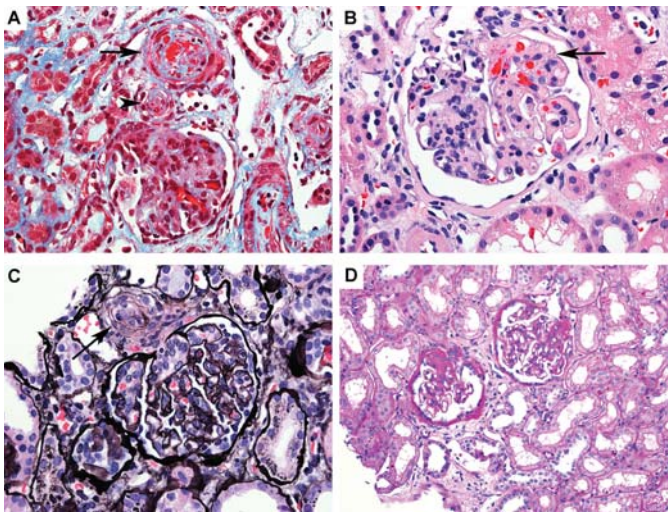
FR-PO574

Treatment with Eculizumab Reverses Thrombotic Microangiopathy Changes in Atypical Hemolytic Uremic Syndrome Carlos E. Araya,[1] Josephine M. Ambruzs,[2] Brenda S. Montane,[1] Robert S. Mathias.[1] *[1]Pediatric Nephrology, Nemours Children's Hospital, Orlando, FL; [2]Pathology, Nephropath, Little Rock, AR.*

Background: Hemolytic Uremic Syndrome (HUS) is a rare and life-threatening disorder. The majority of HUS cases are secondary to infections but 10% of HUS cases are classified as atypical and are caused by a dysregulation in the alternative pathway of the complement system. The treatment of a typical HUS (aHUS) has been challenging. Eculizumab, a humanized monoclonal anti-C5 antibody that prevents the activation of the terminal complement pathway has been found to be effective in patients with aHUS.

Methods: We present a case of a 7 year old female diagnosed with aHUS treated early after her diagnosis with eculizumab. She received one plasmapheresis treatment followed bv eculizumab.

Results: After treatment, the hemolysis decreased, LDH declined from 2345 UI/L to 847 UI/L after 2 weeks, hemoglobin stabilized and platelet count normalized. The serum creatinine normalized within 1 month and the proteinuria decreased to a ratio of 0.5 by 2 months. The findings of the kidney biopsy are shown.



Panel a, b and c- There is an interlobular-sized artery and arteriole with luminal occlusion by endothelial swelling, fibrin and red blood cell fragments consistent with TMA. Glomerular mesangiolysis and an arteriole with endothelial cell swelling and mucoid intimal change resulting in luminal narrowing. Panel d. shows glomeruli post-treatment with segmental sclerosis and hyalinosis with adhesion of the tuft to Bowman's capsule. She has continued on eculizumab for 2 years and her renal function remains normal.

Conclusions: After treatment, we noted resolution of TMA and normalization of renal function, but persistent proteinuria due to FSGS. With eculizumab, no recurrence of aHUS has occurred. This case illustrates the long term favorable outcome of aHUS with early eculizumab treatment.

FR-PO575

Is Bortezomib Useful in Plasmatic Cells Dyscrasias with Renal Affectation? Long Term Follow Up (3.5 Years) in a Reference Hospital Jose L. Lerma, Elena Ruíz, Cristina Lucas, Veronica González, Victoria Mateos. *Nephrology, Hospital Univ, Salamanca, Spain.*

Background: As Standard treatment for blood dyscrasias (BD) with renal affectation offers poor results,new treatment lines tries to improve both overall and renal survival with tolerability.Bortezomib,a proteosome inhibitor,can be useful in CKD.However,few studies evaluate stage 4CKD at diagnosis and its ongoing response to long-term therapy. Objectives:To evaluate Bortezomib's efficacy and safety in patients with BD associated with 4CKD evaluating response in renal function.

Methods: 108multiple myeloma cases were diagnosed at a reference BD Centre over a period of 41 months.Of this population, only 9.25% initially had renal affectation and the average age was 72,males (56%).Average infiltration of plasma cells was 35%.Renal biopsies were performed on 25%.GFR:19ml/min, with urinary Bence Jones (BJ) proteins of 4.5g/24hrs and severe hypercalcaemia in 44%. 100% of patients received Bortezomib as first-line therapy (Haematology porticol).

Results: Creatinine clearance improved from 19.15±12.90ml/min to 37.12±25.31ml/min (MDRD).BJ proteinuria descreased or was eliminated in 83.3%.One case did not respond to therapy.I 83% of cases, hypercalcaemia responded to treatment without requiring bisphosphonates or other measures.One case was refractory to Bortezomib and maintained parameters of myeloma activity.Three patients did not recover renal function and required haemodialysis. One case reached 3.5 year survival without hospital admission.Patients who died were principally elderly with co-morbidities.No clinically significant adverse effects were noted and Bortezomib was very well tolerated.

Conclusions: 1-83% of patients with BD and renal affectation (stage 4 CKD) responded favourably to Bortezomib with a significant reduction in urinary BJ proteins and hypercalcaemia and an improvement in glomerular filtration rate. 2-The medication provoked few side effects and was well tolerated despite the elderly population (72 years). 3-Mortality rate was 22%, particularly affecting the most elderly.78% surpassed 3.5 year survival. 4-Bortezomib can be considered a first-line therapy in the context of BD associated with severe renal involvement.

Funding: Government Support - Non-U.S.

FR-PO576

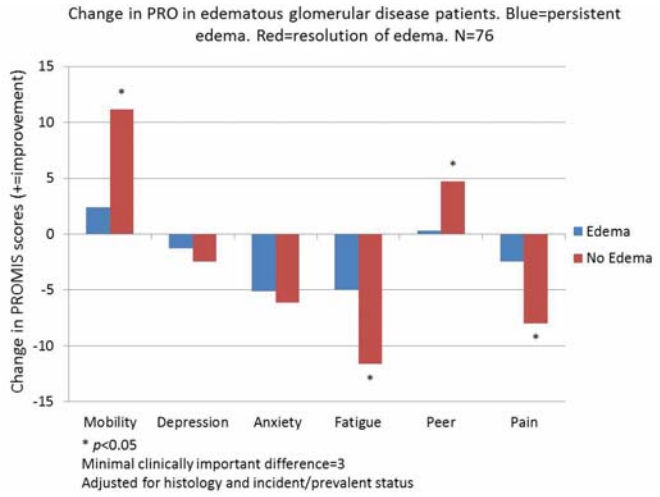
Patient Reported Outcomes in Children and Adults with Nephrotic Syndrome J. Troost,[1] David T. Selewski,[1] Patrick H. Nachman,[2] Susan F. Massengill,[3] Emily G. Herreshoff,[1] Chrysta C. Lienczewski,[1] Alexandra M. Klim,[1] Debbie S. Gipson.[1] *[1]Univ of Michigan; [2]Univ of North Carolina; [3]Carolinas Medical Center.*

Background: Proteinuric kidney diseases adversely affect children and adults beyond the traditional clinical parameters of proteinuria and kidney function.

Methods: We used data from two multisite prospective cohorts, NEPTUNE (U54DK083912-01) and PROMIS NS (U01AR05218106), to assess patient reported outcomes (PRO) over time. 185 adults and 181 children completed the Patient Reported Outcomes Measurement Information System on-line assessment over a median of 12 months. Analyses were stratified by age (8-18 versus ≥18). Distribution of PRO at study enrollment and follow-up was summarized by age, edema status, treatment, and pathology. Among those with edema at baseline we tested the hypothesis that PRO improves with resolution of edema compared to those with persistent edema adjusting for histology and disease duration. For longitudinal analyses, we used generalized estimating equations with an autoregressive correlation structure. Analyses were completed in SAS v9.2.

Results: In children baseline PRO did not differ by edema status or by pathology. Among adults, baseline edema was a strong predictor of physical functioning, fatigue, pain interference, sleep impairment, and social status (|d|=0.34-0.61). Longitudinally, PRO changed over time in response to edema status. Specifically, in children persistence of edema was associated with worse mobility, fatigue, peer relationships, and pain interference compared to those with resolution of edema (Fig). In adults, worse fatigue, pain, and physical functioning were predicted by edema, increasing number of medications and decreasing eGFR.

Conclusions: PROs are associated but not completely predicted by edema status in children and adults with proteinuric kidney diseases. Measurement of PRO may enhance clinical and research assessments.



Funding: NIDDK Support, Other NIH Support - NIAMS: U01AR05218106, Private Foundation Support

FR-PO577

Remission and Progression in the Nephrotic Syndrome Study Network

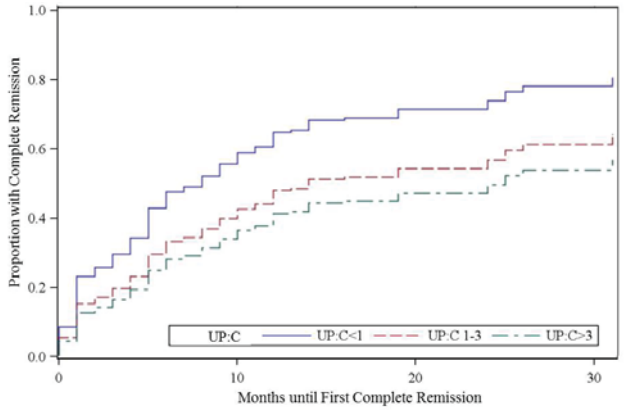
J. Troost,[1] Crystal A. Gadegbeku,[2] Michelle A. Hladunewich,[3] Cynthia C. Nast,[4] Howard Trachtman,[5] Frederick J. Kaskel,[6] Fernando C. Fervenza,[8] Larry A. Greenbaum,[9] Katherine M. Dell,[11] Kevin E.C. Meyers,[12] Marva M. Moxey-Mims,[13] Matthias Kretzler,[1] Debbie S. Gipson.[1] *[1]Univ Michigan; [2]Temple Univ; [3]Univ of Toronto; [4]Cedars-Sinai; [5]New York Univ; [6]Montefiore; [7]Mayo Clinic; [8]Emory Univ; [9]Case Western; [10]CHOP; [11]NIDDK.*

Background: The Nephrotic Syndrome Study Network is an observational cohort of patients with ≥0.5 g/d proteinuria, planned first renal biopsy, with collection of biosamples.

Methods: Subjects were classified into FSGS, minimal change (MCD), membranous (MN) or Other cohorts. Outcome measures, complete remission (CR, UPC <0.3 g/g) and decline in renal function (eGFR <60% baseline or ESKD), were assessed in subjects having ≥8mo observation. Time to event analyses used multivariable Extended Cox Proportional Hazard Models.

Results: 388 individuals (31% FSGS, 24% MCD, 15% MN and 30% Other) are actively enrolled. Prior to renal biopsy, 34% (19% adults) received immunosuppression. 22% of children treated prior to biopsy did not have FSGS, MCD or MN. With a median 18 mo observation period, 61% of children and 47% of adults achieved CR and 16% children and 20% adults had declining kidney function. Multivariate analysis (n=275) demonstrated that CR was least likely with UPC >3 (p<0.01) at biopsy and non-MCD pathology (p<0.01). Among the immunosuppression naïve, post biopsy steroid therapy was associated with CR in FSGS but not with other diagnoses (HR=4.2, CI: 1.3,13.7). ESKD was most likely in patients without a CR (p<0.01) and was negatively correlated with baseline eGFR (<0.05).

Conclusions: CR is common following initial kidney biopsy for nephrotic syndrome. As 22% of pre-treated children had an "Other" pathology diagnosis, earlier diagnostic kidney biopsy may be appropriate. Expansion of epidemiology with molecular profiling is planned to enrich the approach to management of glomerular diseases.



UP:C<1	30	9	1	0
UP:C 1-3	86	49	24	9
UP:C >3	159	88	34	12

Funding: NIDDK Support, Private Foundation Support

FR-PO578

Identification and Characteristics of Population-Based Cohort of Adults with Nephrotic Syndrome: The Kaiser Permanente Nephrotic Syndrome Study

Alan S. Go,[1] Dongjie Fan,[1] Thida Tan,[1] Janet M. Wojcicki,[2] Jingrong Yang,[1] David Law,[1] Juan Daniel Ordonez,[1] Leonid V. Yankulin,[1] Kenneth K. Chen,[1] Glenn M. Chertow,[3] Sijie Zheng.[1] *[1]Kaiser Permanente Northern California; [2]Univ of California, San Francisco; [3]Stanford Univ.*

Background: Few population-based data exist about adults with primary nephrotic syndrome (NS) in the U.S. We identified and characterized a cohort of adults with primary NS within a large integrated healthcare delivery system.

Methods: Between 1996-2012, we identified non-diabetic adult members within Kaiser Permanente Northern California with nephrotic range proteinuria (urine ACR >3500 mcg/mg, PCR >3.5 mg/mg or 24-hr protein excretion >3500 mg) or diagnosed NS (ICD-9 codes 581.x). Patients with secondary causes of NS were excluded. Nephrologists reviewed electronic health records for clinical presentation and relevant lab and biopsy results to confirm primary NS.

Results: Of 5658 reviewed patients, 818 had confirmed NS due to minimal change disease (20%), focal segmental glomerulosclerosis (39%) or membranous nephropathy (41%). Mean age was 49.6 years, 43% were women, 59% were persons of color and 19% Hispanic. Additional comorbidities are shown in the Table, overall and stratified by sex.

	Overall (N=818)	Women (N=350)	Men (N=468)
Mean (SD) age, yr	49.6 (16.6)	49.2 (16.9)	49.9 (16.3)
Race/ethnicity, %			
White	41.3	38.3	43.6
Black	14.1	14.3	13.9
Asian	19.4	21.4	17.9
Other	7.8	8.3	7.5
Unknown	17.4	17.7	17.1
Hispanic	18.6	18.3	18.8
Current/former smoker, %	19.8	14.6	23.7
Baseline medical history, %			
Coronary heart disease	0.9	0.3	1.3
Stroke or TIA	0.5	0.9	0.2
Venous thromboembolism	2.6	1.7	3.2
Peripheral arterial disease	0.7	0.9	0.6
Heart failure	3.3	2.9	3.6
Hypertension	50.6	51.1	50.2
Liver disease	1.7	1.7	1.7
Lung disease	15.0	18.3	12.6
Thyroid disease	15.4	21.1	11.1
Baseline eGFR, ml/min/1.73m², %			
90-150	29.3	34.3	25.6
60-89	19.2	16.9	20.9
45-59	12.0	9.7	13.7
30-44	12.0	11.4	12.4
15-29	11.2	10.9	11.5
<15	4.3	5.1	3.6
Dialysis or transplant	0.4	0.3	0.6
Unknown	11.5	11.4	11.5

Conclusions: We identified a population-based cohort of adults with primary NS that will provide a unique platform for describing the natural history of NS and identifying predictors of adverse outcomes.

Funding: Private Foundation Support

FR-PO579

Glomerulonephritis and Morbid Nephrotic Syndrome Presenting in Pregnancy: A Case Series

Laura Pilozzi-Edmonds,[1] Kainat Shahid,[2] Mark Anthony Cabrera,[3] Michelle A. Hladunewich,[2] Amret T. Hawfield,[3] Geena Joseph,[4] Natasha Michaeloff,[2] Tiina Podymow.[1] *[1]Dept of Nephrology, McGill Univ, Montreal, QC, Canada; [2]Dept of Nephrology, Univ of Toronto, Toronto, ON, Canada; [3]Dept of Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; [4]Dept of Nephrology, McMaster Univ, Hamilton, ON, Canada.*

Background: Nephrotic syndrome due to glomerulonephritis presenting in pregnancy is a challenge to diagnose and treat.

Methods: We present a series of 20 pregnant women from 4 academic centres with glomerulonephritis and morbid nephrotic syndrome (not due to preeclampsia or diabetes), and their demographics, diagnosis, clinical and laboratory features, treatments and outcomes.

Results: 20 women average age 28.8 ± 5.2, of whom 13 were primiparas presented with nephrotic syndrome in pregnancy at gestational age 14.0 ± 6.3 weeks; 16 women presented with edema. Second trimester 24 hour proteinuria ranged from 5.3-50 g/day, serum albumin ranged from 0.9-3.7 g/dl and creatinine ranged 35-316 mean 105 ± 66 µmol/L (1.2 ± 0.8 mg/dl). Third trimester creatinine was 133 ± 80 µmol/L (1.5 ± 0.9 mg/dl). The diagnosis of glomerulonephritis predated pregnancy in 8 patients, 7 women were biopsied during pregnancy, 4 were biopsied postpartum, and 1 patient with reflux nephropathy was not biopsied. Of the 19 biopsied, 8 had FSGS, 4 had class V Lupus, 2 had class IV Lupus, 3 had IgA nephropathy and 2 had hereditary nephritis. During pregnancy, immunomodulatory treatment varied, with 14 receiving oral prednisone with doses ranging 30-120 mg daily, 3 receiving cyclosporine, 4 receiving azathioprine and 2 receiving tacrolimus. Adjunctive therapies included low molecular weight heparin (n=10), aspirin (n=12), and antihypertensives (n=12). Delivery was at gestational age 34.9 ± 3.9 weeks, 10 women had preeclampsia, birth weight at delivery was 2243.4 ± 830.5 g, and 7 infants required NICU admission.

Conclusions: Glomerulonephritis with nephrotic syndrome in pregnancy presents diagnostic, treatment and management challenges with risks to the mother and fetus including morbid edema, kidney injury, immunosuppression, hypertension, preeclampsia, prematurity and low birth weight.

FR-PO580

Mycophenolate in Refractory and Relapsing Lupus Nephritis

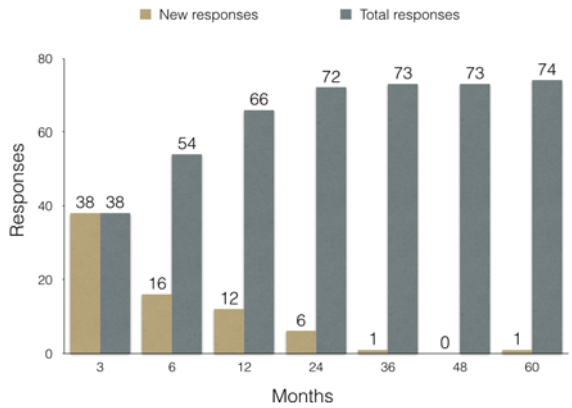
Francisco Rivera,[1] Manuel Praga.[2] *[1]Nephrology, Hospital General Univ de Ciudad Real, Ciudad Real, Spain; [2]Nephrology, Hospital Univ 12 de Octubre, Madrid, Spain.*

Background: Mycophenolate (MF) is effective as induction and maintenance treatment in patients with lupus nephritis (LN). This study evaluates the efficacy and safety of MF in patients with refractory and relapsing LN.

Methods: Data were retrospectively obtained for 85 patients (35 refractory and 50 relapsing) from 11 nephrology departments in Spain. The primary endpoints were the incidence and cumulative number of renal responses and relapses and their relationship with baseline clinical and analytical data. The secondary endpoint was the appearance of side effects.

Results: The main clinical and analytical variables were similar both in refractory and relapsing LN. Most of the patients had received cyclophosphamide, and all of them switched to MF. Seventy-four patients (87%) achieved a response (69% partial, 31% complete).

Figure 1



Age at starting MF, gender, pathological classification, body mass index, blood pressure, baseline renal function, and proteinuria were not associated with achieving response. After stopping MF, 3 of 19 patients (15.7%) relapsed, all at 6 months of follow-up. No differences were found between clinical and analytical variables and number of relapses. Side effects were unremarkable, except for 1 patient, who died of thrombocytopenia and ovarian hemorrhage.

Conclusions: Switching to MF from other immunosuppressive treatments is effective and safe in refractory and relapsing LN.

FR-PO581

Forecasting Renal Prognoses for IgA Nephropathy Using Machine Learning

Hajeong Lee,[1] Junhyug Noh,[2] Jung Pyo Lee,[1] Dong Ki Kim,[1] Yon Su Kim.[1] *[1]Internal Medicine, Seoul National Univ Hospital; [2]Computer Science and Engineering, Seoul National Univ College of Engineering.*

Background: Although various approaches have been applied to renal prognosis prediction for IgA nephropathy (IgAN), well-organized prediction methods are scarce. We aimed to develop new outcome prediction models for IgAN patients using machine learning.

Methods: Among a total of 1,585 patients, we selected IgAN patients who had a follow up ≥10-years and no missing data. The outcome was 10-year renal survival (10YRS) probability. We tested logistic regression (LR) with the Lasso method, a classification and regression tree (CART), and neural networks (NN) for predictive modeling. These results were 5-fold cross validated by random sampling.

Results: A total of 605 patients were included in the final analysis. Initial eGFR was 68.0 ml/min/1.73m^2 and proteinuria 1.63 g/day. Among them, 172 patients reached ESRD within 10-years. In the LR analysis, eGFR, age, male gender, smoking, systolic blood pressure (SBP), hemoglobin and albumin level, proportions of global sclerosis (GS) and segmental sclerosis (SS) were significant predictors for renal outcome (area under the curve [AUC] 0.867; 95% confidence interval [CI], 0.797–0.938; sensitivity [Se] 91.0%, and specificity [Sp] 61.6%). In the CART model, eGFR <48.6 was found to be the most important predictor for 10YRS. In the case of eGFR <48.6, proportion of GS ≥38.2%, serum albumin <3.15 g/dL, and SBP ≥120 mmHg sequentially determined 10YRS. In the eGFR ≥48.6 group, variables such as eGFR <68.4, proportion of SS ≥ 19.0% and age at the time of diagnosis ≥ 44.4 years showed significant relationship with 10YRS sequentially. The performance of the CART model was comparable, with AUC 0.834 (95% CI 0.750–0.918, Se 88.0%, Sp 63.3%). In the NN, 16 variables were selected including eGFR, hemoglobin, interstitial fibrosis, GS, SS, and albumin, sequentially. The model performance was also comparable with other methods (AUC 0.870, 95% CI 0.797–0.943, Se 90.5%, Sp 63.3%).

Conclusions: We have developed and validated LR with Lasso, CART and NN model with good performance to forecast 10YRS in IgAN. These stratified prediction models may be valuable in clinical application.

FR-PO582

Pregnancy in a Patient with Anti-PLA$_2$R Seropositive Membranous Nephropathy

Laith Farah Al-Rabadi, Rivka Ayalon, Ramon G. Bonegio, Jennifer E. Ballard, Alan Fujii, David J. Salant, Laurence H. Beck. *Boston Univ Medical Center, Boston, MA.*

Introduction: There is little information about pregnancy outcomes in patients with active membranous nephropathy (MN). No reports of pregnancy in patients with circulating autoantibodies to PLA$_2$R, the major autoantigen in primary MN (Beck, 2009) are available. Herein, we present the first case of a successful pregnancy in a 39-year old woman with PLA$_2$R-associated MN.

Case Description: In the year prior to pregnancy, the patient developed anasarca, hypoalbuminemia (1.3 - 2.2 g/dL) and proteinuria (29.2 g/24h). Kidney biopsy revealed MN with positive PLA$_2$R staining, and the patient was seropositive for anti-PLA$_2$R. She did not respond to conservative therapy and was treated with rituximab (2 x 1gram IV). Several weeks later, she was found to be 6 weeks pregnant and was closely followed by the maternofetal medicine service without further immunusuppressive treatment. Proteinuria remained in the 8-12 g/24h range. Circulating anti-PLA$_2$R remained positive. At 38 weeks, a healthy baby girl was born, without proteinuria at birth or at her subsequent 6 month postnatal visit. Maternal blood and cord blood was collected, as was a sample of the placental tissue. At the time of birth, the mother still was positive for circulating anti-PLA$_2$R of the IgG1, IgG3, and IgG4 subclasses, although at low titers. A very weak signal for IgG4 anti-PLA$_2$R was found in the cord blood, suggesting possible inefficient transfer across the placenta, even though IgG4 in general is known to be transferred from the maternal to the fetal circulation. IgG eluted from the placental tissue did not contain anti-PLA$_2$R, and PLA$_2$R itself was not detected in the placenta by Western blot or immuofluorescence microscopy.

Discussion: Although the outcome of pregnancy in this case was favorable, we hesitate to suggest that pregnancy is safe in women with active MN and circulating anti-PLA$_2$R because the lack of clinical disease in the baby may have been due to the very low titer of anti-PLA$_2$R in the fetal circulation. Therefore, we recommend close observation of the neonate for proteinuria in future cases.

FR-PO583

Ocular Complications in a Case of Anti-Glomerular Basement Membrane Disease

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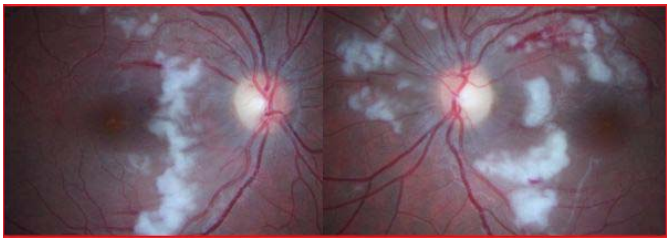
Introduction: Anti-GBM disease occurs as autoantibodies target type IV collagen in glomerular and alveolar basement membranes. This particular collagen is also found in various ocular structures, but case reports of eye pathology in this disease are rare.

Case Description: A 21 year old female presented with haematuria and acute kidney injury. Investigations revealed an anti-GBM antibody titre of 147 u/mL and a crescentic glomerulonephritis. She had no respiratory involvement. IV cyclophosphamide and methylprednisolone were commenced. She received therapeutic plasma exchange for 14 days. She then developed progressive, bilateral visual impairment. Fundoscopy revealed bilateral confluent nerve fibre layer opacities, reminiscent of infarcts, in a curvilinear distribution between the disc and macula of each eye.



Fluorescein and indocyanine green angiography showed multiple choroidal filling defects. She became dialysis dependent, and it was felt that continued immunosuppression would yield no benefit for renal function. The cyclophosphamide was changed to azathioprine, and the steroid dose reduced. On discharge 8 weeks later, visual acuity had improved and the retinal opacities were resolving. She is now starting the work up for transplantation.

Discussion: Retinal involvement in anti-GBM disease is rare and only a handful of case reports are found in the literature. Following her investigations, we postulate that the fundal appearances in our patient may result from a breakdown of the blood-ocular barrier, exposing retinal tissue to autoantibody in the peripheral blood. Treatment of ocular complications of anti-GBM disease mainly involves treatment of the underlying condition, but may require laser therapy. Although these complications are rare, clinicians should be mindful of the possibility of ocular manifestations of vasculitis whilst treating their patients.

FR-PO584

Membranous-Like Glomerulopathy with Masked IgG Kappa Deposits

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Introduction: Membranous-like glomerulopathy with masked IgG kappa deposits is a novel form of glomerular immune complex deposition disease.

Case Description: A 20-year-old Cambodian woman presented with 2 weeks of fevers and flu-like symptoms. Her blood pressure was 170/90, creatinine was 3.6 mg/dl, and albumin was 2.3 g/dl. Urinalysis showed proteinuria, hematuria, and pyuria with a urine protein:creatinine ratio of 9.0. Serologic testing including ANA and sdDNA were negative. C3 and C4 were normal. Kidney biopsy showed a membranous pattern of glomerulonephritis with global basement membrane spikes and subepithelial deposits with several cellular crescents. By immunofluorescence, the deposits stained solely for C3. However, when immunofluorescence was repeated on pronase-digested tissue, the deposits stained dominantly for IgG and kappa. The patient refused immunosuppressive therapy and was treated with losartan and diuretics. Over the next 5 months, her creatinine stabilized at 1.5 mg/dl but nephrotic-range proteinuria persisted, at 6 g/d.

Discussion: MGMID is a recently described immune-complex deposition disease with unique clinical and pathologic characteristics. In the case series of 14 patients that established MGMID as a disease entity, most patients were young women with autoimmune disease or positive autoimmune serologic tests. Pathology showed membranous glomerulopathy with positive staining for IgG and kappa light chains, revealed only after pronase digestion of the paraffin embedded tissue. There is little data on the natural history and treatment of MGMID. Two of the reported cases were treated conservatively with RAAS

inhibitors, but the majority was treated with a variety of immunosuppressive agents. Among 9 cases with follow-up reported, none progressed to end stage renal disease, but most had persistent proteinuria. The long-term clinical outcome of patients managed conservatively, such as the present case, may inform prognosis and therapeutic decision-making for this disease until clinical trial data are available.

FR-PO585

A Case of Membranous-Like Glomerulopathy: What Is Behind the Mask?

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Introduction: Membranous-like glomerulopathy with masked IgG kappa deposits (MGMID) is a new form of glomerular disease that has been recently described. The IgG kappa deposition is not detected by routine immunofluorescense (IF) staining and requires an antigen-retrieval step to be visualized. We present an interesting case of MGMID co-existent with antiphospholipid syndrome (APLS).

Case Description: 22-year-old female presented with hypertension and blurry vision. Physical exam showed a blood pressure of 149/109 mmHg with pitting edema. Fundoscopic examination showed left retinal artery occlusion. Laboratory work-up revealed a creatinine of 1.6 mg/dL, hypocomplementemia, 4.5 g/day of proteinuria and microscopic hematuria. Serologic work-up was negative for ANA, anti dsDNA, MPO, PR-3, ASO and anti-DNase B. There was no evidence of monoclonal gammopathy on serum and urine analysis. Antiphospholipase antibodies were positive. Renal biopsy revealed membranous glomerulopathy with thrombotic microangiopathy. IF showed non-specific low-level positivity for multiple reactants. IF was repeated on pronase digested paraffin and revealed glomerular capillary wall that stained 3+ for IgG, 1+ for C3, 3+ for kappa, and negative for lambda. PLA2R staining was negative. There was a large subepithelial and intramembranous deposition from electron microscopy (EM). These findings correlated with MGMID and APLS. The patient's MGMID was treated with an ACEI, mycophenolate mofetil, and prednisone. Her renal function improved; however, her proteinuria remained in the nephrotic range at 2-month follow up.

Discussion: MGMID is a new clinicopathologic entity of monoclonal immunoglobulin deposition disease. It occurs primarily in young women with poorly defined autoimmune disease. The mechanism of the masking of these deposits under routine IF is unclear. It was hypothesized that the abnormal glycosylation or a quaternary arrangement of IgG molecules impedes antibody blinding. The pronase digestion technique should be applied when deposits are clearly present by EM without corresponding staining by routine IF. Care must be taken when approaching patients who may have MGMID to avoid misdiagnosing them.

FR-PO586

Membranous Nephropathy Associated with Profound Hypothyroidism

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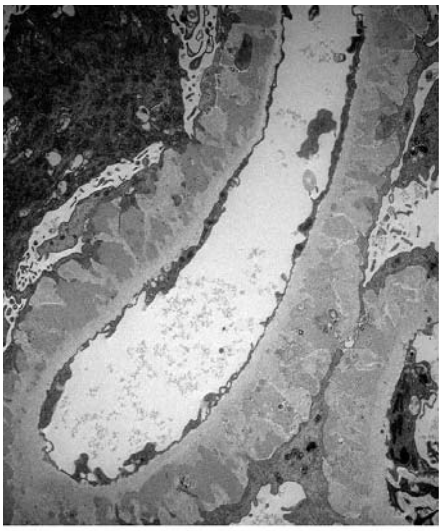
Introduction: Thyroid disorders have been linked to alteration in kidney function and as well as glomerular injury. We report a case of secondary membranous nephropathy associated with untreated profound hypothyroidism.

Case Description: A 64 y/o man with no known past medical history presented with dyspnea, fatigue, and leg edema. Examination was unremarkable except for a BP of 166/87 mmHg and edema of the scrotum and lower extremities. Labs revealed creatinine of 3.1 mg/dL with proteinuria of 4.5 g/day, albumin of 2 g/dL, Hb of 8.9 g/dL. TSH was found to be significantly high at 99.28 mIU/L (normal 0.27-4.20 mIU/L), with low free T4 at 0.52 ng/dL (normal 0.93-1.70 ng/dL) and low total T3 at 45 ng/dL (normal 80-200 ng/dL). Complements were normal as were ANA, ANCA, anti-GBM Ab and thyroglobulin and thyroid peroxidase antibodies. Viral serologies (HIV, HCV, and HBV) were negative and Hb A1c was 5.1%. Ultrasound showed normal sized echogenic kidneys and the echocardiogram revealed dilated cardiomyopathy with EF of 20-25%. A kidney biopsy showed membranous glomerulonephritis (MGN) stage II-III with significant mesangial sclerosis.



The patient was treated with intravenous thyroid hormone replacement that was later changed into oral. ACE-inhibitors and diuretics were also started to reduce volume overload and proteinuria.

Discussion: Thyroid disease has previously been reported to have a link with glomerulonephritis. While autoimmune pathways have been proposed as potential mechanisms underlying this association, so far no precise antibody has been identified to cause simultaneous disorder in the thyroid and the kidney. Interestingly, we did not find any autoimmune cause for profound hypothyroidism in our patient while he presented with MGN. This case, coupled with previous reports, highlights the need to include thyroid studies in the workup of unexplained secondary membranous nephropathy.

FR-PO587

Does the Nephrologist Care If You Have a Cat? Bartonella Infection Associated ANCA Positive Necrotizing Glomerulonephritis

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Introduction: C-ANCA positive immune complex Glomerulonephritis (GN) secondary to Bartonella infective endocarditis has been reported in literature. However, there are no reports of MPO positive immune complex GN secondary to Bartonella infection without endocarditis.

Case Description: We report a 32 y/o male with history of truncus arteriosus s/p mechanical AVR presented with fatigue, intermittent fever, worsening leg edema, non-pruritic rash for several weeks. Examination was remarkable for a petechial rash involving the lower and upper extremities and 2+ lower extremity edema. Labs: BUN 19 mg/dl, Creatinine 5.44 from 1.2 mg/dL twelve weeks ago, Na 134 mEq/L, K 4.2 mEq/L, HCO3 19 mEq/L, LFT WNL, LDH 347 U/L, Haptoglobin <10 mg/dL and pancytopenia. Urinalysis showed dysmorphic RBCs and non-nephrotic range proteinuria. C3 and C4 were low and MPO was strongly positive. Anti-GBM, ANA, HIV, CMV, EBV, Parvovirus, AFB, ASO, Hepatitis panel, SPEP, kappa/lambda, Cryoglobulin and blood cultures were negative. Kidney biopsy showed active segmental crescents and glomerular capillary wall deposition of IgG, IgM, C3, C1q, Kappa and Lamba. Electron microscopy: Glomerular, mesangial, paramesangial and subendothelial deposits suggestive of a secondary process which became more clear as the patient started talking about his cats. Later on, positive bartonella PCR confirmed bartonella infection. TTE/TEE was negative for vegetations. He was started on Rifampin and Doxycycline with clinical improvement but no renal improvement and stays on dialysis.

Discussion: It is well known to rule out infection in any case of RPGN with positive ANCA antibodies, but blood cultures are usually negative in Bartonella infection (a gram negative rod that replicates inside the RBCs). Congenital heart disease, mechanical valve replacement and history of cat scratch are recognized risk factors. It is usually associated with C-ANCA and immune complex deposition in EM. A good social history, high degree of suspicion, early diagnosis with bartonella PCR and treatment with antibiotics is needed to improve the renal prognosis.

FR-PO588

IgA Nephropathy (IgAN) and Pregnancy: The Challenge of Distinguishing between Preeclampsia and Progressive Glomerulonephritis (GN)

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Introduction: Women with chronic GN are at increased risk for progression during pregnancy when the serum creatinine is>1.4 mg/dL. Little is known about factors that hasten progression of IgAN in pregnancy, and knowledge about optimal therapy is limited. We report a case of progressive IgAN in pregnancy.

Case Description: A 22 y.o. woman (G1P0) with a history of CKD of unknown etiology was seen for acute worsening of CKD at 36 weeks gestation.She complained of headache (HA), and had a BP of 160/90. PE showed 3+ leg edema. Serum creatinine was 3 mg/dL, increased from 2 mg/dL at 20 weeks gestation. Lab studies revealed normal C3/C4, albumin 2.0, LDH 465, AST 22, ALT 29, uric acid 8.1, Hgb 9.6, and platelets 202,000. Serologic studies were (-). UP/C ratio was 4.8. UA:3+protein, 3+blood, and glomerular hematuria.The patient underwent urgent C-section after failing induction for suspected preeclampsia (PE). Kidney biopsy 4 days postpartum showed proliferative, sclerosing, and focal necrotizing IgAN with 30% cellular to fibrocellular crescents. There was moderate tubulointerstitial scarring with mild acute tubular injury. She was treated with pulse methylprednisolone followed by an oral prednisone+IV cyclophosphamide 500 mg×1. The patent had progressive kidney dysfunction despite therapy requiring permanent hemodialysis 1 month later.

Discussion: This patient had progressive IgAN complicating pregnancy. The findings of HTN, HA, AKI, and elevated uric acid were concerning for PE meriting emergent delivery. The diagnosis of PE in chronic GN is complicated because there is considerable overlap in clinical findings. The patient had significant tubulointerstitial scarring on kidney biopsy likely explaining the failure of therapy. Earlier referral may have led to more timely diagnosis and therapy and an opportunity for counseling about risks of progressive CKD, premature delivery and PE when the serum creatinine is>1.4 mg/dL. Further research is needed to help define additional biomarkers to distinguish between preeclampsia and progressive GN.

FR-PO589

Diffuse Macrophage-Rich Mesangioproliferative Glomerulonephritis After Antibiotic Exposure Laura R. Kidd,[1] Vanessa Moreno,[1] Geetha Seerangan,[2] Ramdas N. Kumar,[2] William F. Glass.[1] *[1]Pathology and Laboratory Medicine, The Univ of Texas Health Science Center at Houston, Houston, TX; [2]Space City Associates of Nephrology, League City, TX.*

Introduction: The role of macrophages in glomerular disease has been examined in animal models, but human glomerular disease characterized by prominent mesangial infiltration by macrophages appears to largely unrecognized or undescribed.

Case Description: We report the case of a 52 year old female with a past medical history of Hepatitis C, Rheumatoid Arthritis and kidney stones, who presented to an outside hospital with complaints of severe abdominal pain, fever, and pain and tenderness of her left breast. She was found to have pneumonia and was treated with antibiotics, including Vancomycin and cefepime. In the next few days, she developed a skin rash, thrombocytopenia and nephrotic syndrome with a urine protein to creatinine ratio of up to 11 grams and a rising creatinine (up to 1.4 mg/dL). The antibiotics were stopped and she underwent a renal biopsy. Microscopic evaluation showed glomeruli with diffuse mesangial and less prominent endocapillary hypercellularity. The infiltrating cells had an unusual, very slightly foamy appearance, and electron microscopic examination revealed cells abundant phagocytic vacuoles containing cellular debris. Podocyte foot processes were irregular to segmentally effaced and segmental capillaries had mild subendothelial rarefaction and/or loss of fenestrations. Immunohistochemical studies were performed to confirm the lineage of the cells which were found to be CD68 and CD4 positive, indicative of macrophages. No evidence of immune complex deposition was identified. Serologies for a vasculitis were negative for P-ANCA, C-ANCA, and ANA.

Discussion: A search of the literature and the Web (Google) revealed no reports of human biopsy cases with similarly prominent macrophage infiltration. We speculate that the patient's nephrotic syndrome was the consequence of platelet destruction with release of cationic proteins and macrophage infiltration as a consequence of Vancomycin-induced thrombocytopenia a rare antibody mediated reaction.

FR-PO590

Focal Segmental Glomerulosclerosis Associated with Mitochondrial Cytopathy Tingli Wang, Ping Fu, Baihai Su. *Dept of Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

Introduction: Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is a heterogeneous disorder ascribed to a defect in mitochondrial function.An A-to-G transition has been shown at 3243 in tRNALeu (UUR) in MELAS.We present an extremely rare case of mitochondrial cytopathy with focal segmental glomerulosclerosis (FSGS).

Case Description: A 18-year-old boy with mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS) who was diagnosed as having focal-segmental glomerulosclerosis by renal biopsy at our hospital.He also had hypertension,hypothyroidism and non-nephrotic range proteinuria.Kidney biopsy revealed many sclerotic glomeruliand focal segmental glomerulosclerosis (FSGS). A muscle biopsy specimen of the biceps brachii, using Gomori stain, showed some ragged-red fibers,andclustered of type I muscle fiber with ATPase stain.However mitochondrial DNA from the blood didn't show an A-to-G transition at 3243 of transfer RNALeu (UUR), the common point mutation for MELAS.

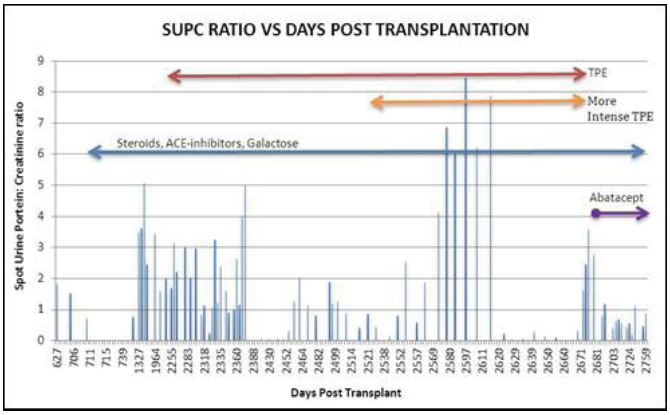
Discussion: The 3243 mutation was originally described only in patients with MELAS but about 16% of patients with MELAS don't have this mutation.Moreover,glomerulopathy is rare in patients with MELAS,and FSGS has been reported only in a fewpatients. Our case is unique in that, thecoexistence of MELAS, hypothalamic hypothyroidism,binocular vision loss, bilateral sensorineural hearing loss and FSGS has not been reported in the past. The present case of mitochondrial cytopathy was characterized by a unique clinical course and rare complications with focal-segmental glomerulosclerosis.The purpose of this report is to increase the awareness of our professionals regarding the manifestations and complications of MELAS.

FR-PO591

Successful Treatment of Proteinuria with Abatacept in Recurrent Focal Segmental Glomerulosclerosis after Transplantation Namita Singh, Marizela Savic, John P. Vella. *Div of Nephrology and Transplantation, Maine Medical Center, Portland, ME.*

Introduction: Abatacept (cytotoxic T-lymphocyte-associated antigen 4-immunoglobulin fusion protein) is a costimulatory inhibitor that targets B7-1 (CD 80). A recent report described the use of abatacept in reducing proteinuria in five patients with focal segmental glomerulosclerosis (FSGS) and B7-1 immunostaining of podocytes in renal tissue. We report the sixth case in the literature.

Case Description: A 32 year-old man with primary FSGS, status-post 2 failed renal transplants due to recurrent FSGS, had recurrence of disease in the third transplant as well. He had nephrotic range proteinuria up to 9 grams per day, that was variably responsive to 3-drug immunotherapy including high dose steroids, angiotensin converting enzyme inhibitors (ACE-inhibitors) and galactose. The patient needed aggressive therapeutic plasma exchange (TPE) treatments up to 3 times a week in order to mitigate the proteinuria. Recurrent attempts to wean either the prednisone or TPE led to prompt, marked proteinuria relapses. Based on the report by Yu et al. (NEJM 11/2013), the patient was started on a trial of abatacept. He received abatacept 10 mg/kg every 2 weeks for 3 doses and thereafter monthly. He has not required further TPE and proteinuria declined to less than 1 gram per day after 3 doses. So far the patient has received 6 doses and continues to have less than half a gram proteinuria.



Discussion: Abatacept induced clinical remission in this case of FSGS leading to discontinuation of TPE treatments and reduced prednisone dosing.

FR-PO592

Collapsing Glomerulopathy Associated with Hemophagocytic Syndrome: A Case Report Inder Patel, Roveena N. Goveas, Chintan Shah, Wihib A. Gebregeorgis. *Dept of Nephrology, Wayne State Univ, Detroit, MI.*

Introduction: Hemophagocytic syndrome (HPS) is an aggressive syndrome of excessive immune activation characterized by bone marrow and organ infiltration by activated, nonmalignant macrophages, which phagocytose blood cells. Very few data are available about the renal complications of HPS.

Case Description: We describe a 60 year old man with NK/T cell nasopharyngeal extranodal lymphoma on radiation therapy who was admitted for dysphagia, fatigue, weight loss and a decline in urine output. He was found to have acute kidney injury with a Cr of 15.2 mg/dl and BUN of 174 mg/dl. He had a baseline Cr of 1.01 mg/dl and BUN 24 mg/dl 3 weeks earlier. Renal ultrasound was unremarkable. Urinalysis showed 3+ protein. He was pancytopenic with WBC count of 1.4 k/cumm, platelet count of 36 k/cumm and hemoglobin of 6.1 gm/dl. His LDH was elevated at 1313 U/Lit and haptoglobin was < 8 mg/dl. There were < 2 schistocytes/HPF on peripheral smear examination. Serology tests for lupus and HIV were negative. ADAMTS13 activity was 55%. He remained oliguric and was initiated on hemodialysis (HD). During the hospitalization, he started having persistent fever of up to 39 °C. Extensive work up failed to disclose infectious etiology for his fever. Kidney biopsy was consistent with collapsing glomerulopathy. The persistent fever, pancytopenia and collapsing glomerulopathy in a patient with lymphoma raised suspicion for HPS and a bone marrow (BM) biopsy was pursued. BM biopsy showed increased hemophagocytic activity with many ingested RBCs, neutrophils and platelets. The presence of fever, pancytopenia, hypertriglyceridemia (triglyceride: 361mg/dl), hypofibrinogenemia (fibrinogen: 123mg/dl), hemophagocytosis on BM biopsy and elevated ferritin level of 7265 ng/ml established the diagnosis of HPS in our patient. Steroid therapy was initiated. He remains HD dependent.

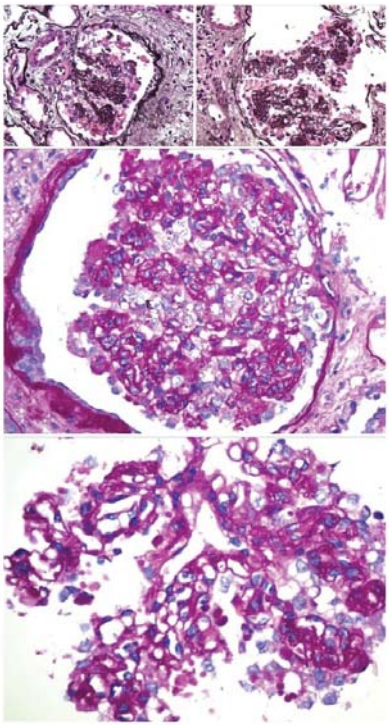
Discussion: Collapsing glomerulopathy associated with HPS is extremely rare. Hypothesized mechanism involves release of pro-inflammatory cytokines resulting in podocyte injury. The diagnosis of collapsing glomerulopathy should raise suspicion and trigger work up for HPS in the right clinical setting. In our case HPS was secondary to NK/T cell lymphoma.

FR-PO593

Collapsing FSGS in a Patient with Acute Malaria Emad Gobran,[1] Leal C. Herlitz,[2] Suzanne E. El Sayegh.[1] *[1]Nephrology, Staten Island Univ Hospital, Staten Island, NY; [2]Pathology, Columbia Univ Medical Center, NY, NY.*

Introduction: Collapsing FSGS has been associated with a various number of infectious etiologies. Here we present a case of Malaria associated FSGS.

Case Description: 72 y/o African M with PMH of HTN and gout presented to the hospital for generalized body aches, fatigue, fever and night sweats for 3 days, history of recent travel to Ghana for 2 wks, he returned to the U.S.A. 10 days prior to symptoms onset. Physical exam was significant for BP 186/99 85, T 102.9F, patient looked in acute distress and shivering. Lab. tests: elevated S. Cr 2.09 (baseline 1.5 in 2012). Hospital course was significant for rapid elevation of S.Cr to 9.5 Proteinuria (7.9 gm) and positive Plasmodium Falciparum on Bl. Smear and was treated with Coartem. He had negative ANA, Anti-dsDNA, Anti-GBM, C3, C4, Hepatitis B and C, ANCA, HIV, Free Light chain, SIF. kidney function continued to deteriorate and became oliguric, on day 7 he was started on HD. had a Lt Kidney bx which revealed 8 glomeruli, 3 of which were globally sclerosed, 4 of the remaining glomeruli displayed lesions of collapsing glomerulopathy which ranged from segmental to global. Collapse of the glomerular tufts and prominent hyperplasia of overlying epithelial cells. Interstitial fibrosis Findings consistent with severe collapsing Glomerulopathy.



Discussion: This Case illustrates a Biopsy proven Collapsing FSGS which carries the worst prognosis among all 5 types of FSGS likely 2ry to Malaria infection with rapid deterioration of kidney function to the point of requiring HD. Literature review revealed only few case reports done in Africa that suggested the possible association of Malaria to secondary form of FSGS.

FR-PO594

Glomerular Filtration Rate Restoration by ACTHAR Gel Therapy in a Patient with Focal, Segmental Glomerulosclerosis with Nephrosis and Acute Renal Failure Vishal Bharat Parekh,[1] Antonio Guasch.[2] *[1]Nephrology, Emory Univ, Atlanta, GA; [2]Nephrology, Emory Univ, Atlanta, GA.*

Introduction: Adrenocorticotropic hormone gel therapy (ACTHAR) induces remission in some patients with Focal, Segmental Glomerulosclerosis (FSGS). Hogan et al (CJASN, 2013) showed small improvement in Cr, and eGFR in FSGS responders; most of responders had relatively preserved renal function. We report a patient who presented with ARF and massive proteinuria due to FSGS that improved markedly with ACHTAR therapy.

Case Description: A 57-year-old Caucasian man with history of FSGS, on no immunosuppression, was admitted with ARF (S. Cr 7.5 mg/dL), anasarca, hypoalbuminemia (S. Alb 1.6 gm/dL), and proteinuria, urine spot protein creatinine ratio (UPCR) 8.8 g/g. He failed previous treatments with prednisone, cyclophosphamide, cyclosporine, tacrolimus and rituximab therapy. He was hypertensive, with no evidence of intravascular volume depletion. A kidney biopsy revealed 25% global and 20% segmental sclerosis and adhesions of tubular pole to proximal tubule in multiple glomerular tufts ("tip lesion"), with interstitial fibrosis. Electron microscopy showed complete foot process effacement with podocyte microvillous transformation. Patient was given pulse solumedrol and started on ACHTAR 80 units SQ thrice weekly for one year with quick improvement in renal function and anasarca. At 6 months, S. Cr 2.23 mg/dL, S. Alb 3.3 mg/dL and UPCR 3.9 g/g. At 1 year, S. Cr 2.2 mg/dL, S. Alb 3.6 g/dL and UPCR was 1.8 g/g. He is edema free.

Discussion: We previously reported (Guasch, AJP-Renal 1991) that ARF in patients with minimal change nephropathy and/or FSGS, there is marked reduction in the glomerular ultrafiltration coefficient (Kf), accounting for low GFR, which correlated with the extent of foot process effacement, suggesting that the loss in the number of filtration slits accounted for ARF. We believe that a similar mechanism resulted in ARF and massive nephrosis in our patient. We postulate that ACTHAR modulates podocyte function, restoring glomerular filtration slits, thereby improving GFR which potentially can treat ARF in patients with FSGS and warrants further evaluation.

FR-PO595

FSGS NOS (Not Otherwise Specified) in an African American Man with HbSC Disease- Sequelae of HbSC Nephropathy or Primary FSGS- A Nephrologist's Dilemma Daniel Taiwo Adeneye, Gary R. Briefel, Suchita J. Mehta. *Nephrology, Univ Hospital at Brooklyn, Suny, Brooklyn, NY.*

Introduction: Focal Segmentalglomerulosclerosis (FSGS) is a clinico-pathogic syndrome that may be idiopathic or secondary to diverse etiologies including sickle cell hemoglobinopathy. Current reports have described 2 types of FSGS in HBSS: the collapsing variant and FSGS with expansile sclerosis. HBSC and HBSS are both hemoglobinopathies, but the former is a milder variant of the disease and the histopathogical features of HBSS may not necessarily represent HBSC disease. No specific histopathogy has been described in HBSC disease and FSGS NOS, has never been reported in HBSC hemoglobinoapthy. Whether the histopathological findings of FSGS NOS; discovered in this case report represents FSGS peculiar to HBSC or primary FSGS is not clear as clearly recorded in the pathogy report of the kidney biopsy. Although a positive supra level would have been supportive of primary FSGS; it is not a sinequanon as it is present in only 70% of patients. Differentiating between the two has therapeutic and prognostic implications especially when making treatment decisions for patients presenting at the early stage of kidney disease. Is there a glomerulopathy specific to HBSC disease?

Case Description: 38 y/o African American man with lower extremity swelling,7G proteinuria; negative serologies and HIV, BUn/Cr 109/15mg.

Biopsy- FSGS NOS.

Discussion: No specific glomerulopathy has been described for HBSC disease and FSGS NOS has prognostic characteristic that is different from other variants of FSGS. 50-60% of primary FSGS respond to immunosuppressive agents including steroids, while FSGS secondary to hemoglobinopathy is best treated with agents that reduce intraglomerular pressure and periodic blood transfusion to reduce the number of sickling cells in the renal medulla. For a patient such as ours, none of this modality would apply as the patient was already end stage.The fact remains that a comprehensive kidney biopsy, more specific biomarkers and study of large sample of HBSC patients to define the specific histopathologic features of HBSC glomerulopathy will be needed to differentiate it from primary FSGS and HBSS glomerulopathy.

FR-PO596

Focal Segmental Glomerulosclerosis in a Patient with Autoimmune Pancreatitis with Elevated IgG4 Ashwin Reddy Ganta,[1] Pramod Kumar Guru.[2] *[1]Dept of Nephrology, Archbold Memorial Hospital, Thomasville, GA; [2]Dept of Nephrology, UPMC, Pittsburgh, PA.*

Introduction: A 65 y/o WF presented to the hospital with abdominal pain and melena. EGD showed esophagitis and Colonoscopy was non contributory. Her bleeding subsided but her abdominal pain continued to worsen. She had similar abdominal pain 3 months ago with elevated pancreatic enzymes that resolved with conservative management and there was no underlying cause identified. Her Scr was 0.5 and her current albumin level was 1.2 (3.3 3 months ago) which was attributed to poor nutrition. Her Amylase and Lipase were again elevated. She did not have h/o alcoholism or Gallstones and CT showed possible inflammatory mass with pancreatic duct abnormality with no e/o Gallstones. A pancreatic biopsy was not attempted given her fragile condition. Nephrology was consulted for anasarca and help with diuresis. Her Urine PCR was 5 gms with + ANA and Nucleolar ANA pattern. Other serology like c3, c4, HIV, HCV, HBV, dsDNA was negative. Ig G4 was elevated at 4x normal. A diagnosis of probable IgG4 Pancreatitis with renal involvement was made based on the Mayo-HISORt criteria and renal biopsy was done. Biopsy showed 26 glomeruli with primary FSGS pattern of injury with IgG, IgM and C3 staining with severe podocyte effacement and no e/o Tubulo-interstitial injury. She was begun on steroids and her abdominal pain improved with reduction in amylase and Ig G4 levels to normal. After 2 weeks of therapy, her Urine PCR dropped to 2.5 gms with improvement in her clinical status. She was lost for follow up and couple of months later she succumbed to sepsis.

Discussion: Discussion: AIP is a new entity that is was first described in 1961 and is more common in Asians. IgG4 related disease (IgG4-RD) refers to the spectrum of AIP with other organ involvement. The kidneys are involved with Tubulo-interstitial pattern of injury with lymphoplasmocytic infiltrate being the most common finding on biopsy. A few isolated cases of MN/MPGN have also been reported. Treatment is with high dose steroids and ours is the first known case in literature with AIP associated 1' FSGS pattern of renal involvement which was steroid responsive.

FR-PO597

Collapsing FSGS Associated with Macrophage Activation Syndrome Revekka Babayev, Shana M. Coley, Andrew S. Bomback. *Columbia Univ Medical Center.*

Introduction: Collapsing FSGS can be primary or secondary to viral infections (eg HIV), drugs (eg interferon) and rarely hemophagocytic lymphohistiocytosis (HLH). HLH can be familial or secondary to infection, malignancy or rheumatic disorders. Diagnosis of secondary HLH requires at least 5 of the following: fever; splenomegaly; cytopenias affecting at least 2 lines; hypertriglyceridemia/hypofibrinogenemia; hemophagocytosis in bone marrow, spleen or lymph nodes; decreased NK-cell activity; markedly elevated ferritin; and soluble CD25 >2400 U/mL. HLH secondary to rheumatic disease is termed macrophage activation syndrome (MAS).

Case Description: A 54 y.o. woman with no prior history presented with 2 weeks of high fevers and painful right lower extremity rash. Exam was notable for hepatosplenomegaly and right lower extremity erythema. Labs showed pancytopenia, transaminitis, and creatinine 1.5 mg/dL. Urinalysis showed 2+ blood, 3+ protein, and protein:creatinine ratio 5.3 g/g. By hospital day 5 creatinine had doubled, and a renal biopsy was performed. Light microscopy revealed collapsing FSGS with focal tubular microcysts and mild tubulointerstitial scarring. Immunofluorescence was negative. Electron microscopy showed diffuse foot process effacement. HIV and parvovirus B19 were negative by PCR. Serologies were positive for ANA, anti-SSA and anti-CCP. Serum ferritin was markedly elevated at >9000 ng/ml. Bone marrow biopsy performed for worsening cytopenias showed hemophagocytosis, supporting a diagnosis of HLH. She was started on pulse dose steroids on hospital day 6 and required 3 sessions of dialysis between hospital days 8-12. She subsequently demonstrated renal recovery and was able to discontinue dialysis.

Hospital day	1	5 (renal bx)	8 (HD initiated)	18 (discharge)
WBC (x10^9/L)	1.7	1.4	1.7	5.6
Hgb (g/dL)	11.2	11.0	11.0	8.3
Platelet count (x10^9/L)	123	105	91	321
Creatinine (mg/dL)	1.52	2.95	5.98	1.2
Albumin (g/dL)	3.1	2.2	2.3	3.5

Cyclosporine was added to oral prednisone for treatment of MAS on hospital day 18. At last follow-up, creatinine was 0.9 mg/dl.

Discussion: A diagnosis of HLH/MAS should be considered in patients with collapsing FSGS and cytopenias. Checking a serum ferritin should be part of the routine work up for such patients.

FR-PO598

A Case of Nephrotic Syndrome Associated with a Hydatidiform Mole

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Introduction: The precise pathogenetic relationship between the hydatidiform mole and glomerulonephritis is not clear, because the reported cases were extremely rare. The most common cause of nephrotic syndrome in pregnancy is preeclampsia. Preeclampsia may have a relation to the molar pregnancy.

Case Description: A 21-year-old woman was referred to our section of nephrology because of generalized edema, hypertension, proteinuria and hematuria. Her obstetric history was G0P0AO. During the investigation, was performed transvaginal ultrasonography that diagnosed incomplete molar pregnancy. The patient denied amenorrhea, but her serum B-HCG was 225.000UI/mL. A renal biopsy was performed. Suction and curettage was performed and a specimen evacuated from the intrauterine cavity was consistent with the diagnosis of hydatidiform mole. Light microscopy of the kidney demonstrated a narrowing the capillary lumen due to swelling of the endothelial, mesangial and epithelial cells with an expansion of the mesangial matrix. The pathologic findings suggested preeclamptic nephropathy. Two weeks later, the edema disappeared and the proteinuria was below 300mg. The patient had completely recovered from the renal manifestations.

Discussion: This is an unusual case of nephrotic syndrome in a young woman. To our knowledge, this is the fourth reported case of nephrotic syndrome associated with molar pregnancy. These several interesting cases linking the pathogenesis of the glomerulonephritis directly to the gestational trophoblastic disease provide a challenge for future research.

Funding: Private Foundation Support

FR-PO599

Collapsing Focal and Segmental Glomerulosclerosis Associated with Neurofibromatosis Type I

Luisa Queiroga Ferreira,[1] Aline Gibson Notaro,[1] Denise Maria do Nascimento Costa,[1,2] Ana Paula Gueiros,[1,2] Rodrigo Amblard Wanderley,[1] Rafaella Barros da Paixão.[1] *[1]Nephrology Dept, Inst de Medicina Integral Prof. Fernando Figueira - IMIP, Recife, Pernambuco, Brazil; [2]Nephrology Div, UFPE, Recife, PE, Brazil.*

Introduction: Neurofibromatosis type 1 (NF1) is a rare autosomal-dominant genetic disorder. Renal involvement in patients with NF1 is rare. The most common manifestation appears to be renal artery stenosis. Glomerulonephritis associated with NF1 were only sporadic.

Case Description: A 21-year-old female with history of generalized edema was referred for evaluation of nephrotic range proteinuria (5.3 g/day), diagnosed 1 month earlier. She had family history of NF1 in her father. Cafe-au-lait spots and neurofibromas scattered across arms and legs. The serum creatinine was 0.8 mg/dL, albumin 1.0 g/dL and urinalysis showed proteinuria 3+ and microscopic hematuria 1+. Serological test for hepatitis B, C, syphilis, HIV were negative. Complement factors were normal; ANA and ANCA were negative. On renal biopsy, one core of renal cortex with 16 glomeruli was examined. One of 16 glomeruli showed global sclerosis and the remaining had segmental collapse associated with the adhesions to Bowman's capsule and hyperplastic and hypertrophic podocytes. There were no crescents and the mesangium was not expanded. There was diffuse and mild interstitial fibrosis and tubular atrophy. Immunofluorescence revealed no deposits. Overall these findings are consistent with collapsing focal and segmental glomerulosclerosis (FSGS). She was treated with enalapril 20 mg/day, furosemide 80 mg/day and simvastatin 40 mg/day. After 7 months she had a gradual decrease of proteinuria (down to 1.89 g/day from 5.3 g/day) and edema disappeared.

Discussion: To our knowledge, this is the first case of collapsing FSGS reported in a patient with NF1. The occurrence of collapsing FSGS with NF1 in our reported patient may also be a coincidence as we have not performed any linkage investigation.

Funding: Private Foundation Support

FR-PO600

Successful Remission of Frequently Relapsing Nephrotic Syndrome due to Minimal Change Disease with Rituximab

Ruchir S. Patel, Susie L. Hu. *Div of Kidney Disease and Hypertension, Warren Alpert Medical School of Brown Univ/Rhode Island Hospital, Providence, RI.*

Introduction: MCD is known to have frequent relapses. We report a case of successful remission of frequently relapsing nephrotic syndrome (FRNS) from Minimal Change Disease (MCD) with Rituximab.

Case Description: 28 year old Caucasian female had FRNS due to MCD (biopsy proven). She was first diagnosed with MCD at age of 14. She had multiple relapses on prednisone and cyclosporine. She was treated with cyclophosphamide PO with remission for a few years. In last 7 years, she has had recurrent disease requiring various combinations of up to 3 immunosupressive agents including prednisone, azathioprine, cyclosporine, mycophenolate or tacrolimus for remission. All medications were stopped in late 2012 while in remission. She subsequently developed 4 relapses the following year. Due to concern for long term steroid exposure, future risk of calcineurin inhibitor induced nephrotoxicity and inadequate response to steroid sparing agents, Rituximab 375 mg/m^2 with 1 month of steroids and transiently overlapping triple therapy was administered. She remained disease free for 6 months. She was given a second dose of rituximab at 6 months while in remission. No further disease activity was noted for an additional 3 months thus far (total 9 months after first dose) and no infectious complications developed. We plan to monitor her disease activity without any additional Rituximab.

Discussion: Rituximab is a potential option for steroid dependent FRNS due to MCD however long term efficacy and delayed adverse effects remain unknown. Small observational studies support the use of Rituximab in FRNS due to MCD in adults however large randomized controlled trials (RCTs) are lacking. Our report also suggests that weekly Rituximab for 3 to 4 weeks may not be needed and thus exposure to Rituximab can be minimized. 2 doses of Rituximab 6 months apart are usually well tolerated. Further RCTs are needed to evaluate long-term efficacy and adverse effects.

FR-PO601

IgG4 Related Tubulointerstitial Nephritis and Membranous Nephropathy Presenting as Nephrotic Syndrome and Acute Kidney Injury

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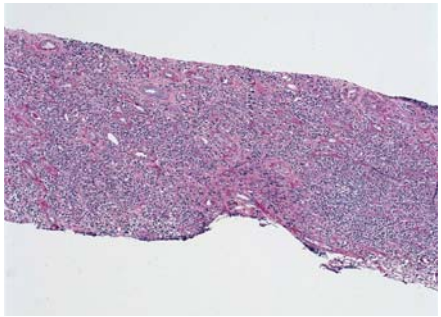
Introduction: IgG4 Related Disease (IgG4-RD) is an increasingly recognized syndrome which usually has multiorgan involvement. We report a rare case of IgG4 related renal disease.

Case Description: 48 year old Asian male with no medical history presented with 2 weeks of leg edema and an 18 lb. weight gain in setting of rare NSAID use. Review of systems is otherwise negative. Physical exam revealed a blood pressure of 141/87 and bilateral leg edema. Laboratory data were significant for creatinine 2.1 mg/dl, BUN 24 mg/dl, eosinophilia of 20%, Albumin 1.5 g/dl, cholesterol 407 mg/dl, bland urine sediment, and urine protein to creatinine ratio of 14 g/g. Serum and urine IFE were negative for monoclonal protein. ANA, HIV, Hep B and Hep C were non-reactive with normal complements. Renal biopsy showed IgG4-related tubulointerstitial nephritis (TIN) and early membranous nephropathy.



Intense lymphoplasmacytic infiltration with positive staining for 147 IgG4+ plasma cell/high power field and IgG4:IgG ratio > 90% were noted. Serum total IgG and IgG4 levels were elevated. Patient was started on prednisone 50 mg/day, lisinopril and furosemide. After 4 weeks of treatment, creatinine improved to 1.27 mg/dl and proteinuria to 7 g/g.

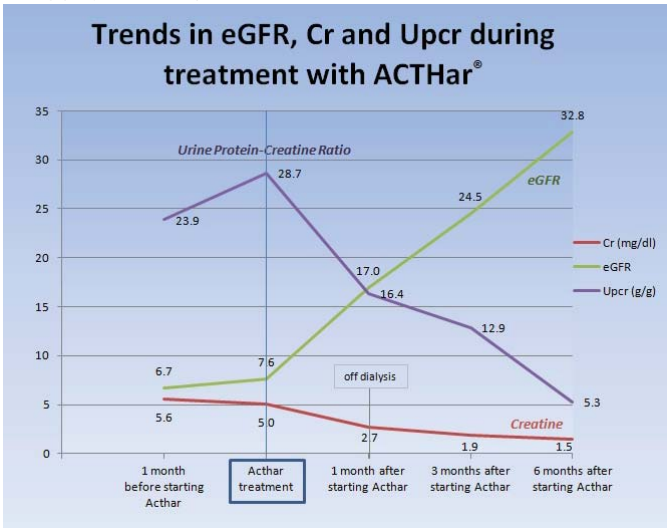
Discussion: Renal manifestations of IgG-RD are mainly in form of TIN, rarely observed with concomitant MN (or MPGN) as seen in our case. Sole kidney involvement is unique. This condition is usually steroid responsive. Long term prognosis of renal disease is not known. Our case highlights the importance of recognizing this rare but potentially treatable renal disease.

FR-PO602

Minimal Change Disease and Dialysis Dependent Acute Kidney Injury Treated with ACTHar and Low Dose Tacrolimus Thet N. Zaw, Robert D. Zenenberg. *Dept of Nephrology, Saint Barnabas Medical Center, Livingston, NJ.*

Introduction: Minimal change disease (MCD) is a major cause of nephrotic syndrome in both children and elderly. MCD may not be as responsive to treatment in elderly. This case report looks at a successful treatment approach in the setting of a resistant MCD with dialysis dependent AKI, and severe steroid side effects, using the combination of an ACTH analogue (ACTHar®) and a low dose calcineurin inhibitor (CNI).

Case Description: The patient is a 78-year-old Caucasian female with a history of borderline hypertension presents with acute onset of worsening lower extremity edema and nephrotic range proteinuria of >20 g/g day. An extensive workup was undertaken including a kidney biopsy, which showed MCD. She was started on oral steroids. Subsequently, the patient had AKI and became dialysis dependent. She had persistent repeat biopsy proven MCD, and developed a severe myopathy, which led to the discontinuation of the steroids. She was switched to a combination very low-dose Tacrolimus and ACTHar® subcutaneous injections. She eventually came off of dialysis and her creatinine stabilized at 1.1 mg/dl, after completing a 6 month course of ACTHar®. Her urine protein excretion had decreased to <6 g/g day. She is doing well on Tacrolimus.



Discussion: It is known that Melanocortin Receptor 1R subtype (MC1R) is expressed on glomerular podocytes. ACTH analogue presumptively exerts through direct MC1R activation on the podocyte, reducing the level of proteinuria by lowering oxidative stress, and improving glomerular morphology. Therefore, specific MC1R agonists such as ACTHar® may lead to fewer side effects and could be used in combination with lower doses of CNI as a new treatment option for nephrotic patients who are unresponsive to the current standard treatment of steroids, particularly in the setting of renal failure, where full dose CNI may be contraindicated.

FR-PO603

Minimal Change Disease with Thrombotic Microangiopathy during Pregnancy Louis R. Spiegel, Dron P. Bhandari, Rimda Wanchoo. *Hofstra North Shore - LIJ School of Medicine.*

Introduction: Preeclampsia is the most common cause of nephrotic range proteinuria in pregnancy and is suspected when proteinuria onset is >20 weeks of gestation. There are several case reports of new onset glomerular disease in pregnancy, mostly post-streptococcal GN and focal segmental glomerulosclerosis, but there are very few cases of minimal change disease (MCD). We describe a case of severe proteinuria, hypertension and acute kidney injury secondary to MCD with superimposed thrombotic microangiopathy during the 3rd trimester of pregnancy.

Case Description: A 21 year old G1P1 presented at 27 weeks of gestation with two weeks of sudden onset edema and anasarca. Her course during pregnancy was unremarkable including no proteinuria and no known prior history of hypertension. Her physical exam revealed a BP of 128/71, HR 71 and rest of the exam significant for bilateral lower extremity edema. Her laboratory data revealed a serum creatinine of 1.4mg/dL and spot urine protein of 20 grams. Her albumin was 1.7mg/dL and uric acid low and liver function tests normal. Based on above data, a clinical diagnosis of MCD was made. Within 24 hours of her evaluation, she became hypertensive with systolic blood pressure of 200 mm of Hg and underwent urgent C-section for atypical pre eclampsia. Several days later, she underwent a kidney biopsy which showed capillary loops with endothelial cell swelling and fibrin thrombi occlusion along with extensive foot process effacement (90%). The findings were consistent with resolving thrombotic microangiopathy (TMA) with superimposed MCD. She was started on oral steroids and discharged home once her renal function returned to normal.

Discussion: De novo MCD developing during pregnancy is very rare and we could find only two cases described in the literature (both during first or second trimester).To our knowledge, our patient is unique in that she had findings consistent with both TMA secondary to pre eclampsia as well as MCD during 3rd trimester of pregnancy, which has not been described before. The differential diagnosis for new onset proteinuria in pregnancy should include MCD. In selected cases, renal biopsy can be used to confirm diagnosis and steroid treatment might be indicated.

FR-PO604

An Unusual Case of Autoinflammatory Renal Amyloidosis Christina Chen, Jonathan S. Hausmann, Arturo Diaz, Stewart H. Lecker. *Beth Israel Deaconess Medical Center.*

Case Description: A 59 year-old man with past medical history of long standing iron deficiency anemia, splenomegaly status post splenectomy, urticarial vasculitis, and history of cocaine use in the distant past, presented to our clinic with CKD. Since 1999, he has been experiencing episodes of fevers and chills that start with extreme thirst, progress to painful hives covering the entire body, and culminate in pitting lower extremity edema. A typical episode resolves within a day. In 2006, his episodes were treated with dapsone with improvement of symptoms. When dapsone was stopped 1.5 years ago due to worsening anemia, his symptoms intensified. For the past year, he had an unintentional 40 lb weight loss due to nausea, vomiting, decreased appetite. On physical exam he was normotensive, appeared undernourished, had no rash, no edema. Labs include creatinine 1.7mg/dL (eGFR 39), potassium 5.7mEq/L, albumin 4.3g/dL, total protein 7.5g/dL, normal SPEP and UPEP, free kappa/lambda 1.79, normal C3/C4, CRP 104.2mg/L, negative ANA, albumin/creatinine 684mg/g. Urine sediment was bland. A colonoscopy with biopsies of the cecum demonstrated amyloidosis. The peptide profile of the amyloidogenic material was consistent with AA type amyloid deposition. Renal biopsy was done showing 12 glomeruli with massive amyloid deposition. Urticarial vasculitis, elevated inflammatory markers and amyloidosis were highly suggestive of an autoinflammatory disease in the cryopyrin associated periodic syndromes (CAPS) group, although subsequent genetic analysis did not allow us to establish a definitive diagnosis. He was started on human anti-IL-1b monoclonal antibody canakinumab with marked improvement in vasculitis and inflammatory markers.

Discussion: Cryopyrin is important in innate immunity and mutations cause spontaneous assembly of the inflammasome and increased production of pro-inflammatory mature IL-1b. A few case studies suggest that canakinumab may reduce proteinuria and stabilize or even improve renal function in patients with CAPS. Although our patient had improvement of his vasculitis, we are awaiting to see if there will be improvement in renal function.

FR-PO605

Spontaneous, Persistent Hypotension as a Sentinel Event for Systemic AL Primary Amyloidosis with Multiple Organ Failure Nader S. Bahri,[1] Raafat Farag Makary,[2] Leighton R. James.[1] *[1]Nephrology and Hypertension, Univ of Florida, Jacksonville, FL; [2]Pathology, Univ of Florida, Jacksonville, FL.*

Introduction: Primary Systemic amyloidosis usually presents with symptoms of cardiac or renal failure. Autonomic neuropathy involvement resulting persistent hypotension is a rare reported presentation.

Case Description: Data was abstracted from an electronic medical record data with review of medical history, examination, lab, pathology and diagnostic studies.

A 60 year-old man with a past medical history of controlled hypertension on multiple medications presented with unexplained symptomatic hypotension. He subsequently developed bilateral lower extremity edema with nephrotic range proteinuria (10 g/day) and acute kidney injury (serum creatinine 2 mg/dl). During the work-up, urine immunofixation revealed a free lambda light chain monoclonal paraprotein; all other serological analyses were negative. Kidney biopsy demonstrated prominent amorphous eosinophilic deposits in the mesangium and GBM, which were congo red positive on light microscopic examination. Fluorescent microscopic studies were negative, while electron-microscopic analysis showed large areas of mesangial and GBM deposits of relatively straight, non-branching fibrils ranging in thickness from 7.65 to 12.09 nanometers, consistent with amyloid fibrils. Bone marrow biopsy and flow cytometric analysis of the bone marrow aspirate revealed abnormal plasma cells which were positive for CD38, CD138, CD56 and lambda light chain C-region and V-region. These findings are diagnostic of plasma cell myeloma causing AL amyloidosis. Due to worsening kidney dysfunction, hemodialysis was initiated. In addition, chemotherapy with Bortezomib was initiated for management of plasma cell myeloma.

Discussion: This case highlights the presentation of systemic AL primary amyloidosis with spontaneous, sustained hypotension, associated with rapid development of multi-organ failure. Autonomic neuropathy is common in primary amyloidosis, as is vascular involvement by the disease. Amyloidosis should be included in the differential diagnosis of new onset persistent hypotension.

FR-PO606

A Case of Transthyretin-Related Familial Amyloid Polyneuropathy Incidentally Found with Abnormal Urinalysis at a General Health Checkup Yasuhiro Yoshimura,[1] Naoki Shiraishi,[1,2] Yutaka Kakizoe,[1] Kenichiro Kitamura,[1] Masashi Mukoyama.[1] *[1]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan; [2]Sakurajyuji Hospital, Kumamoto, Japan.*

Introduction: Familial amyloid polyneuropathy (FAP) is one of hereditary amyloidoses mostly caused by transthyretin (TTR) gene mutations. The typical form of TTR-FAP shows an autosomal dominant inheritance pattern presenting peripheral and autonomic neuropathy at a younger age. Although family pedigrees are usually identified, reports of sporadic TTR-FAP cases are increasing recently. Here, we report a case of asymptomatic TTR-FAP found with abnormality in routine urinalysis.

Case Description: A 69-year-old woman was referred to our hospital because of proteinuria (2.1g/day) and hematuria, which was pointed out at a general health checkup. She revealed asymptomatic and her serum creatinine level was normal. Renal biopsy demonstrated amorphous deposits involving glomeruli and vessels, which was positive for Congo-red stain with characteristic apple-green birefringence. No monoclonal protein was detected by serum or urine immune-electrophoresis, and immunoperoxidase staining for AA protein was negative. Subsequently, immunoperoxidase staining for TTR was performed, which revealed positive. LC-MS/MS analysis from kidney biopsy specimens detected a quantitative value of TTR, and DNA sequencing of the TTR gene showed Val30Met mutation. According to these findings, she was diagnosed as TTR-FAP.

Discussion: Although neuropathy is the principal manifestation of most TTR-associated amyloidosis, urine abnormality may precede as in this case. For AA-negative and AL-negative renal amyloidosis, TTR-FAP should be considered as a possible diagnosis.

FR-PO607

"Normo-Albuminuric" Diabetic Nephropathy: Fact or Myth? Georgi A. Kovachev, Jeffrey M. Turner. *Dept of Internal Medicine, Nephrology Section, Yale Univ, School of Medicine, New Haven, CT.*

Introduction: According to the "classic" paradigm of diabetic nephropathy (DN) the earliest functional marker of renal damage is micro-albuminuria. Since it precedes decline in GFR, it is widely used for screening and prognostication. However, some patients can reach advanced stages or renal failure and remain normo-albuminuric.

Case Description: 60 yo obese female with long-standing but controlled DM type 2 and hypertension has been followed for stable non-proteinuric CKD stage 4 presumably due to controlled hypertension. A renal biopsy obtained for unexplained decline in GFR revealed moderate diabetic nephropathy, RPC class III.

Discussion: Inaccurate GFR and albuminuria estimations, use of ACEI/ARB, non-diabetic renal disease has been used to explain data from large retrospective studies suggesting that 20-30% of diabetic patients with CKD beyond stage 3 are normoalbuminuric. Smaller studies, using isotopic GFR, supervised 24h urine collections and excluding ACEI/ARB users report prevalence of normo-albuminuria with diabetic CKD > stage 3 to be 20%. It seems to be more common in women with type 2 DM. Prospective data also suggest that some diabetics' GFR can decline to ESRD levels without ever becoming albuminuric. Limited data from biopsy studies suggest advanced glomerular lesions are not always accompanied by albuminuria. Some diabetics with CKD and normo-albuminuria may have predominantly vascular and interstitial lesions for unclear reasons. Conversely, non-diabetic renal disease often coexistent with DN, especially in DM type 2, adding to the confusion. A rat model of classic DN without albumiunria and data from human genetic studies supports the intriguing idea that susceptibility to ESRD and albuminuria are controlled by different genes. Future advances in this area may lead to better understanding of DN, improved risk stratification and developing innovative treatment targets.

FR-PO608

Secondary Amyloidosis and IgA Nephropathy in Cystic Fibrosis: A Rare Coincidence and More to Diagnose Sami Alasfar, Srinivas Ramakrishna Gottipati, Derek M. Fine. *Johns Hopkins Univ, Baltimore, MD.*

Introduction: Clinically relevant kidney involvement is rare in patients with cystic fibrosis. Secondary amyloidosis is a recognized but extremely rare complication of cystic fibrosis. IgA nephropathy in cystic fibrosis is even more uncommon and has been described only once in the literature. We report a case of a cystic fibrosis patient with nephrotic syndrome who was found to have both renal secondary (AA) amyloidosis and IgA nephropathy.

Case Description: A 36-year-old Caucasian male with cystic fibrosis presented with several weeks of lower extremity swelling. His home medications included albuterol, azithromycin, multivitamins, and pancreatic enzymes. He had mildly elevated blood pressure at 147/75 mmHg. His weight was 64.7 kg with 8 kg of recent weight gain. He had facial edema and pitting edema of lower extremities. Laboratory evaluation showed a serum albumin of 1.6 g/dL and creatinine of 2.6 mg/dL. Urine microscopic evaluation showed 17 red blood cells per high power field without casts. 24 hour urine collection revealed nephrotic range proteinuria (14.7 gm/24 hour). Other laboratory evaluations were normal including ASO, C3, C4, ANCA, HIV, SPEP, and ANA. He underwent renal biopsy which revealed renal amyloidosis (amyloid-associated protein) and minimally proliferative IgA nephropathy with focal glomerular necrosis and crescent formation. The patient was initiated on oral Prednisone 1 mg/kg/day and after about six weeks of therapy, his creatinine improved to 1.2 mg/dL and proteinuria decreased to 7 grams/24 hours.

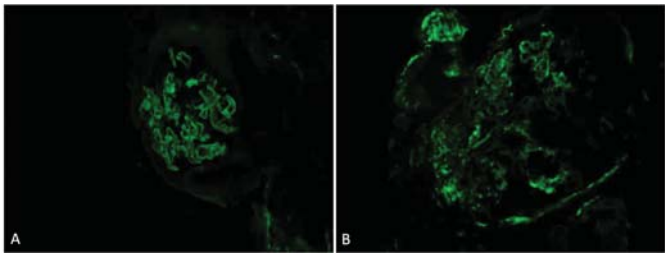
Discussion: Secondary amyloidosis occurs in patients with chronic infectious and inflammatory disease but it is surprisingly a rare complication of cystic fibrosis. Moreover, IgA nephropathy in cystic fibrosis was described only once in the past. Recent advances in the management of cystic fibrosis have led to improvement in survival. It is probable that, in the past, the short life expectancy of patients with cystic fibrosis allowed insufficient time for the development of certain renal complications. Our case suggests that some renal complications and/or manifestations of cystic fibrosis may be seen with increasing frequency in the future as life expectancy improves.

FR-PO609

Fibrillary Glomerulonephritis Masquerading as Anti Glomerular Basement Membrane Disease Juan- Carlos Aycinena,[1] Stuart C. Jennings,[1] Virgilius Cornea,[2] B. Peter Sawaya,[1] Amr Ahmed El-Husseini Mohamed.[1] *[1]Div of Nephrology, Bone and Mineral Metabolism, Univ of Kentucky; [2]Dept of Pathology, Univ of Kentucky, Lexington, KY.*

Introduction: Linear staining (LS) of immunoglobulin (Ig) by immunofluorescence (IF) is characteristic of anti-GBM disease but it has been also described in other entities. However, in patients with clinical presentation of RPGN and pathological diagnosis of crescentic GN with LS of Ig is highly suggestive of anti-GBM disease. Herein we describe a case of fibrillary GN (FGN) presenting as a crescentic RPGN (C-RPGN) with unequivocal LS of IgG and negative circulating anti-GBM antibodies (Ab).

Case Description: A 49 year-old Caucasian female with history of obesity, hypertension (HTN) and microscopic hematuria presented with headache, blurred vision, nausea and vomiting but no hemoptysis. Her blood pressure was 274/152 with no neurological deficit or skin rash. Serum creatinine was 6.4 mg/dl and urinalysis revealed 50 RBC/HPF with no cellular casts. Spot urine for protein/creatinine ratio was 10 mg/mg. The following labs were normal or negative: complement levels, hepatitis B and C profiles, HIV Ab, lupus serologies, ANCA, anti MPO, anti PR3 and anti GBM Ab. A kidney biopsy revealed crescentic GN with fibrinoid necrosis. IF revealed intense LS of GBM for IgG (Fig 1A) and coarse/short LS of C3 (Fig 1B).



The patient received high-dose steroid and plasma exchange without any benefit. Two weeks after admission, EM confirmed the presence of mesangial and intra-GBM deposition of fibrillary structure measuring 8 to 16.5 nm. The patient is currently dialysis-dependent 1 year after the original diagnosis.

Discussion: There are only 3 other reported cases of FGN presenting as C-RPGN with LS by IF. All cases presented with severe HTN and heavy proteinuria, features that are not typical of anti-GBM disease. Prompt diagnosis alters both treatment and prognosis.

Funding: Clinical Revenue Support

FR-PO610

Leukocyte Chemotactic Factor 2 (LECT2) Amyloidosis in First Nations People in British Columbia, Canada: A Case Series Holly L. Hutton,[1] Mari Demarco.[2] *[1]Dept Medicine, Monash Univ, Clayton, Victoria, Australia; [2]Dept Pathology and Laboratory Medicine, St. Paul's Hospital, Vancouver, BC, Canada.*

Introduction: Leukocyte chemotactic factor 2 (LECT2) amyloidosis was first identified in 2008, and has emerged as a frequent type of renal amyloidosis. It is typically reported as being renal limited, and, in the United States, more prevalent in Hispanic patients. We report 4 First Nations people living in Northern British Columbia who were diagnosed with renal LECT2 amyloidosis over the past 4 years.

Case Description: All patients presented with slowly progressive renal impairment and minimal proteinuria. (Table 1)

Age	Sex	Creatinine µmol/L	eGFR (ml/min)	ACR (mg/mmol)
56	F	167	37	1.9
78	M	261	21	64
68	F	223	20	48
62	F	165	27	2.4

Biopsy findings were typical of LECT2 amyloid, with intense congo red staining, and amyloid deposition in the renal interstitium and vasculature as well as glomeruli. After immunohistochemistry for common amyloidogenic proteins did not identify the pathogenic protein, laser microdissection-mass spectrometry was used to make the diagnosis. Although First Nations people comprise only about 4% of the patient population seen by our Nephrology service, all 4 cases of renal LECT2 amyloidosis, diagnosed over the past 4 years, occurred in this ethnic group.

Discussion: The pathogenesis of LECT2 amyloidosis is currently not well understood. Sequencing of the coding region of the *LECT2* gene in patients with LECT2 amyloidosis has revealed a common homozygous single nucleotide polymorphism, indicating a probable genetic component to disease pathogenesis. The fact that our centre has only identified LECT2 amyloidosis in First Nations people adds weight to the hypothesis that there is a genetic contribution to the disease. It may be that a common North American indigenous ancestry of First Nations people and Hispanics accounts for the occurrence of this condition in both populations. LECT2 amyloidosis may be an underdiagnosed cause of chronic kidney disease, as the characteristic minimal proteinuria and slow progression of renal impairment probably result in relatively few patients undergoing renal biopsy.

FR-PO611

Job's Nephropathy Salman Ahmed,[1] Luan D. Truong,[2] Biruh Workeneh.[1] *[1]Div of Nephrology, Baylor College of Medicine, Houston, TX; [2]Pathology, Houston Methodist Hospital, Houston, TX.*

Introduction: Job's syndrome or autosomal dominant hyperimmunoglobulin E syndrome (Hyper-IgE) is a result of STAT3 mutation and leads to a severe immunodeficiency and recurrent infections and what we propose is a renal pattern of injury histologically similar to lupus nephritis rather than classical lupus nephritis.

Case Description: We describe a set of fraternal sisters with autosomal dominant Hyper-IgE (loss of function mutation c.1962_1964delCAT), confirmed by genetic testing, with variable penetrance. The sister affected more aggressively developed renal manifestations as a child; she had AKI with unclear attribution requiring HD, but recovered fully. At age 18 she developed proteinuria (1 gram/day), hematuria and a SCr of 1.6mg/dL, at which time a renal biopsy was performed and revealed immune complex nephritis. Although all classical lupus serologies were negative, the patient was started on prednisone and MMF given her pattern of injury. Unfortunately, her renal disease did not abate and was declared ESRD at age 22. The younger sister who had a lower frequency of serious infections at age 21 also developed proteinuria and hematuria with a normal SCr (1.0 mg/dL) and renal biopsy showed a pattern consistent with lupus nephritis. Her serologies however, showed a positive ANA and anti-Smith and she was started cautiously on an immunosuppressive regimen.

Discussion: Because of how rare this condition is it has been difficult to characterize this association between Job's syndrome and a lupus pattern of injury in the kidneys. We speculate that as a result of recurrent, aggressive infections and tissue exposure that immune complexes form or are trapped in the kidney rather than the classical pathophysiologic pathway that occurs with lupus nephritis. It is not clear whether immunosuppression has a role in this pattern of injury associated with Job's syndrome. Research is linking to renal inflammation and injury, but more analysis is needed to elucidate a mechanism for renal injury.

FR-PO612

An Unusual Case and Treatment of Immunotactoid Glomerulonephritis and Cutaneous Leukocytoclastic Vasculitis with IgMK Monoclonal Gammopathy: A Case Report Andrea Angioi,[1] Giovanni Palladini,[2] Anna Maria Asunis,[1] Giorgio La Nasa,[3] Antonio Ledda,[3] Riccardo Cao,[1] Antonello Pani.[1] *[1]Div of Nephrology, AOBrotzu, Cagliari IT; [2]Fondazione IRCCS-Policlinico San Matteo, Pavia IT; [3]Centro Trapianti Midollo Osseo, ASL8, Cagliari IT.*

Introduction: The treatment of monoclonal gammopathies of renal significance (MGRS) is still debated. We discuss an unusual case of Immunotactoid glomerulonephritis with leukocytoclastic vasculitis (LV) and IgMK spike that we treated with rituximab (RTX), plasmaexchange (PEX), dexamethasone (DEX), cyclophosphamide (CYC) and bortezomib (BOR) despite normal bone marrow biopsy (BMB) to stabilize the renal function.

Case Description: A 50 year-old man was referred in Nov2006 because of isolated microscopic hematuria (ME). Two years earlier he had had MGUS IgMK and LV (immunofluorescence (IF) negative). The requested exams showed decreased eGFR (CKD-EPI:64ml/m/1.73m2) without proteins or Bence Jones (BJ) in urine, while BMB was normal. Cryoglobulins, autoimmunity and infections were excluded. In Nov2009, C3 73 (NV>90 ng/dl), proteinuria (300mg/24h) and BJ (K); eGFR, ME, serum free light chain K, l and K/l were within normal range. Renal biopsy was diagnostic for ITG. IF showed only IgM (3+), k (2+). In Mar2010, eGFR 36ml/m, proteinuria 0.8g/24h, BJ (K) and IgMK 1.2g. A new BMB showed no abnormal plasmacells. We started induction with PEX+DEX 40mg x4 days and CYC+DEX (200/20 mg/day) +RTX 375 mg/m2 every 21 days. After 6 courses, negative BMB**,** eGFR 40ml/m, IgMK 0.12g; maintenance therapy was started with RTX 375/m2 every 3 months. In Nov2012, CYC+RTX for a transient eGFR reduction and in Apr2013 RTX+BOR+DEX due to unexpected albuminuria (2g/24h). Now he is well with stable eGFR (49 ml/m), BJ negative.

Discussion: In this MGRS, the reduction of toxic IgMk resulted in ITG stabilization. Conceptually, our approach is opportune considering the reduced side effects of new courses and the poor renal prognosis if untreated. Supported by indirect clues (here: IF:IgM+/K+; BJ:k), treatment should be considered in selected patients to preserve eGFR. Labeling B-cell clones having mild neoplastic significance but with clear aggressive productive features as being of "undetermined significance" should be avoided.

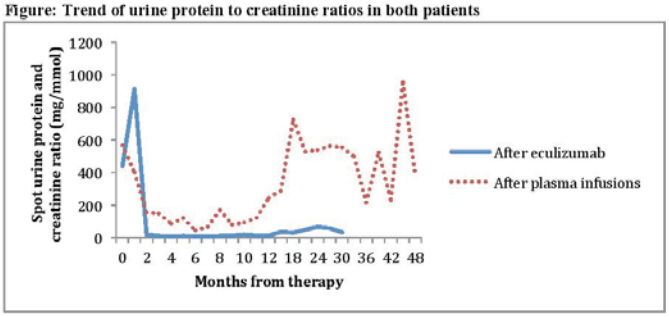
FR-PO613

Complement Targeting Therapies for Refractory C3 Glomerulopathy Rahul Chanchlani, Seetha Radhakrishnan, Diane Hebert, Valerie Langlois, Christoph Licht. *Div of Pediatric Nephrology, Hospital for Sick Children, Toronto, ON, Canada.*

Introduction: Complement targeting therapies including plasma and eculizumab have emerged as disease modifying agents for Membranoproliferative Glomerulonephritis (MPGN), which has recently been re-classified into dense-deposit disease (DDD) and C3 glomerulonephritis (C3GN) and grouped under C3 Glomerulopathy (C3G). There is very little insight on their long-term follow up and safety profile especially in children. We present more than 3 years follow up data with the use of above therapies in 2 children with refractory C3G.

Case Description: Case 1: A 16-year-old girl with MPGN secondary to CFHR1/3 deficiency was started on prednisone and MMF. Due to worsening proteinuria and renal function, hemodialysis and plasmapheresis were also initiated. Four months later, eculizumab was started (900 mg/wk x 4 wks) due to further clinical deterioration and resulted in rapid improvement in neurological status, renal and hematological parameters. Maintenance infusions of eculizumab were continued, 1200 mg q 2 wks for more than 2 years. She currently has mild proteinuria only, normal blood pressure and renal functions despite persistently low C3. **Case 2:** A 7-year-old boy with MPGN secondary to MCP/CD46 mutation continued to have proteinuria despite treatment with various combinations of prednisone, MMF, and tacrolimus over a period of 6 years. Repeat biopsy showed only mild chronic features. Plasma infusions (15 ml/kg of fresh frozen plasma 3 times a week) were started followed by transient improvement in proteinuria. However, despite chronic plasma infusions for almost 4 years (q 2 wks), he continues to have proteinuria with elevated creatinine and normal C3. (Figure)

Figure: Trend of urine protein to creatinine ratios in both patients



Discussion: Eculizumab and plasma infusions are safe and effective options for refractory C3G. Prospective studies in larger cohort are required to prove superiority of one modality over other.

FR-PO614

Chronic Inflammatory Demyelinating Polyneuropathy with Membranous Nephropathy: A Case Report Chetana Rondla, Christine Boumitri, Militza K. Kiroycheva. *Medicine/Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Introduction: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an immune mediated disorder involving spinal nerve roots and peripheral nerves. There have been very few case reports of inflammatory demyelinating polyneuropathies occurring in association with proteinuric kidney diseases mainly membranous nephropathy and focal segmental glomerulosclerosis.

Case Description: A 66 year old Haitian male with history of Atrial fibrillation, Hypertension and Dyslipidemia was referred to the Nephrology clinic for lower extremity edema and proteinuria. Workup was significant for proteinuria of 16gms. Serologic workup was negative. Renal biopsy revealed Membranous Nephropathy with mild tubular atrophy and interstitial fibrosis. He had an extensive workup to rule out secondary causes: CT chest was normal, CT abdomen/ pelvis revealed enlarged lymph nodes with negative subsequent biopsy. PET SCAN and colonoscopy were negative. Complement level was normal. He was started on Steroids and Cyclophosphamide with partial remission of proteinuria. Soon after he was diagnosed with Membranous Nephropathy he complained of progressively worsening bilateral lower extremities weakness. He was evaluated by neurologist. The EMG/Nerve conduction studies were suggestive of demyelinating polyneuropathy with secondary axonal degeneration. Meanwhile weakness worsened and he became wheelchair bound. The rest of his workup came back as follows: CRP: 9.49, ESR: 88, normal Complements, Negative ANA, anti dsDNA, ANCA and anti Jo antibodies. These findings were consistent with inflammatory demyelinating neuropathy and a diagnosis of CIDP was made. Patient had minimal improvement with pulse steroids. He was then started on IVIG treatment following which he had significant improvement of his weakness and is currently able to ambulate with a walker. He is currently on monthly IVIG injections.

Discussion: Extra neural manifestations of chronic inflammatory demyelinating neuropathy have included association with membranous nephropathy. However there have been very few case reports in our literature search. Immunopathogenic association of these two conditions merits further investigation.

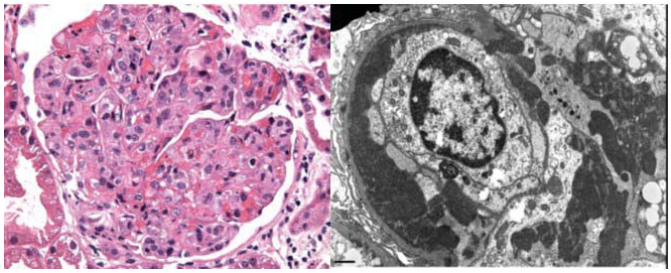
FR-PO615

A Case of Atypical Postinfectious Glomerulonephritis with Cryoglobulinemic Deposits Chetana Rondla, Christine Boumitri, Elie El-Charabaty, Suzanne E. El Sayegh. *Medicine/ Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Introduction: An increasing number of post infectious glomerulonephritis (PIGN) cases with atypical pathologic features have been reported in the last decade. Histologic features of PIGN include diffuse mesangial and endothelial cell proliferation with exudative GN. Coarse immune deposits of C3 and IgG in capillary loops are identified on IF corresponding to the subendothelial deposits on EM.

Case Description: A 55 year old male previously healthy presented for fever, cough and Rt sided pleuritic chest pain of 2 weeks that failed an outpatient course of antibiotics. CT chest revealed RLL pneumonia with loculated pleural effusion. Pleural tap revealed an exudate and patient was started on empiric antibiotics. Admission creatinine was 1.18 and UA revealed trace proteinuria and trace blood. Blood pressure was 139/85. On day 4 creatinine started to increase reaching 10.2 on day 9. 24 hour urine for protein was 1.6g. Complements- C3 and C4 were low (35 and 6 mg/dl) with negative serologies including cryoglobulin with a normal Rheumatoid Factor. All cultures were negative as patient

was already on antibiotics. On day 9 he required hemodialysis. A kidney biopsy revealed diffuse endocapillary and exudative GN with infiltrating monocytes and neutrophils, 3+ subendothelial electron dense deposits (mixed cryoglobulin) and 80% effacement of foot process. The EM findings were typical of cryoglobulinemic GN. On day 14- creatinine started to trend down and he was off dialysis. After 2 weeks of presentation the complement levels were back to normal and at last followup 1 month ago his creatinine was 1.66.



Discussion: Over the last decade pathologically atypical cases of PIGN have been increasingly reported, however PIGN with cryoglobulinemic EM features and negative cryoglobulin is an unusual presentation and to our knowledge has not been reported.

FR-PO616

HIV Associated Membranoproliferative Glomerulonephritis (MPGN) Immune Complex Disease: A Histopathologic Evolution of Kappa Light Chain Monoclonal Gammopathy to a "Lupus Like" Immune Complex Disease

Piangwarin Phaosawasdi, Jose A. Morfin. *Internal Medicine - Div of Nephrology, Univ of California, Davis, Sacramento, CA.*

Introduction: HIV has been associated with different glomerular diseases. Proliferative Glomerulonephritis with Monoclonal IgG Gammopathy (PGNMID) is a distinct glomerular injury without the presence of paraproteinemia. Lupus Like immune complex disease has been linked to HIV. We describe a perplexing case of an HIV patient who demonstrated an evolution and potential link between these two rare entities.

Case Description: A 40 y/o Latino woman with HIV with CD4 counts of 400-600 cells/mm, DM2 and HTN who presented with an episode of fluid overload, serum creatinine of 2.1 mg/dl (baseline 1.1 mg/dl) with nephrotic range proteinuria (3.5 gm/day) and albumin 1 g/l. She had negative serologic studies including normal complement levels, serum and urine electrophoresis, ANA 1:80, negative DS-DNA, RF 27 IU/ml, ANCA, MPO and PR3. Negative Hepatitis panel. Cryoglobulin was non-detected. She underwent kidney biopsy (#1) which showed immune complex-mediated MPGN with glomerular hypercellularity with kappa light chain dominant consistent with PGNMID. A bone marrow biopsy showed no increase in plasma cells. She was started on ACEI. 4 months later, she represented with worsening fluid overload and serum creatinine of 4.5 mg/dl. Repeat serology remained negative. Kidney biopsy (#2) was performed which again showed Immune complex-mediated MPGN, however, there was "full house" immune deposits (lupus like) without kappa restriction and with a focal crescentric formation. She was started on hemodialysis due to anuria with fluid overload.

Discussion: To our knowledge, this is the first case that describes a potential evolution of these two rare and distinct histological glomerular processes, PGNMID and Lupus Like immune complex MPGN. HIV infection is a major risk factor and plausible link leading to derangement and mimicry in the immune system, specifically in CD20 B cell population. Treatment options are challenging given the paucity of data in these entities, but the findings are intriguing to further understand mimicry associated with HIV infection.

FR-PO617

Fibrillary Glomerulonephritis Presenting as Rapidly Progressive Glomerulonephritis with Linear IgA Staining of the Glomerular Capillary Walls

Akiko Endo, Shinya Kaname, Yoshihiro Arimura. *First Dept of Internal Medicine, Kyorin Univ School of Medicine, Tokyo, Japan.*

Introduction: Fibrillary gromerulonephritis (FGN) is a rare glomerular disease characterized by the deposition of randomly arranged fibrils 12 to 30 nm thick in the mesangium and the glomerular basement membranes. On immunofluorescence (IF), the deposits typically stain for polyclonal IgG and complements. The light microscopic features vary, but cellular crescent formation is uncommon in FGN. Here we reported the first case of FGN accompanied by cellular crescent formation with predominantly linear IgA staining of the glomerular capillary wall.

Case Description: A 30-year-old woman was admitted for rapid progressive renal failure (Cr 1.7 mg/dl). Urinalysis showed 50-99 RBC/HPF and proteinuria 3g/day. ANA, MPO-ANCA, PR3-ANCA, anti-GBM antibody, serum and urine immunoelectrophoresis were all negative. Renal biopsy showed crescentic gromerulonephritis (5/37 gromeruli: cellular crescent, 1/37 gromeruli: fibro-cellular crescent). Congo Red staining was negative. IF revealed global linear deposition of IgG (2+), IgA (3+), segmental deposition of IgM (+) and C3 (+) along the glomerular capillary walls. Electron microscopy showed variable thickening of glomerular capillary walls with randomly oriented fibrils measured 21-26 nm in diameters in the capillary walls and the mesangium. She received methylprednisolone pulse, oral predonisone and cyclosporin. Over the follow-up course of 9 months, proteinuria decreased to 0.4g/day and creatinine level remained around 1.5 mg/dl. Re-biopsy showed 1/30 gromeruli with cellular crecentic formation, the same features of IF and partial improvement of thickening of the capillary walls on electron microscopy as compared to those in the first biopsy.

Discussion: In previously reports, 28% cases of FGN were positive for IgA, but glomerular staining for IgA was weaker than IgG. The light microscopic features are heterogeneous, most cases exhibit mesangial expansion and hypercellularity. Crescentic glomerulonephritis was less commonly reported. This is the first case of FGN accompanied by cellular crescent formation with predominantly linear IgA staining of the glomerular capillary walls.

Funding: Private Foundation Support

FR-PO618

An Uncommon Presentation of a Rare Disease: Mesangial Proliferative Glomerulonephritis with Monoclonal IgG Deposits ("Nasr Disease") in a Young Woman

Tanjim Sultana, Maria V. DeVita, Michael F. Michelis, Joshua A. Schwimmer. *Nephrology, Lenox Hill Hospital, NY, NY.*

Introduction: Proliferative glomerulonephritis with monoclonal IgG Deposits (PGNMID) is a rare dysproteinemia-related renal disease first described by Nasr et al. in 2004. It is often characterized by proteinuria, hematuria, and renal insufficiency. There are 4 pathologic patterns, of which the rarest is the mesangial proliferative form (< 3%). We present the 3rd case of this uncommon variant of PGNMID.

Case Description: A 24-year-old African American female was referred for new onset hypertension, increased creatinine (1.4 mg/dL), and albuminuria (924 mg/d) without hematuria. Labs revealed an ANA of 1:160 and a negative serum and urine immunofixation. She was subsequently found to have a positive anticardiolipin IgG, B2 glycoprotein IgG, and lupus anticoagulant. A renal biopsy was performed to exclude anti-phospholipid antibody syndrome involving the kidney or lupus nephritis. Light microscopy revealed 20/41 globally sclerotic glomeruli with old fibrous crescents. The remaining glomeruli had mesangial cell proliferation with glassy eosinophilic deposits. IF revealed diffuse granular mesangial staining for IgG1 only. EM revealed abundant granular, mesangial electron dense deposits without an organized substructure. She was treated with losartan 50 mg daily and no other agents. A bone marrow biopsy showed no evidence of malignancy. Three months later her albuminuria had decreased to 60 mg/d and her creatinine decreased to 1.2 mg/dL.

Discussion: PGNMID is a rare form of glomerulonephritis found in 0.17% of renal biopsies. It presents with proteinuria (100%), hematuria (77%), renal insufficiency (68%), nephrotic syndrome (49%), and a detectable serum or urine paraprotein (30%). There are 4 pathologic variants of PGNMID: membranoproliferative, endocapillary proliferative, membranous, and mesangial proliferative. After a mean of 30 months of followup, 37% of patients with PGNMID developed end stage renal disease or died. After treatment with losartan, our patient with mesangial proliferative PGNMID had microalbuminuria and improved renal function. Optimal therapy for PGNMID is uncertain and further studies are needed.

FR-PO619

IgA-Dominant Postinfectious Glomerulonephritis Mimicking Henoch-Schönlein Purpura in a Child

Ryan C. Mascarenhas,[1] Agnes B. Fogo,[2] Radhakrishna Baliga.[1] *[1]Ochsner Med Ctr, NO, LA; [2]Vanderbilt Univ, Nashville, TN.*

Introduction: IgA-dominant acute post infectious glomerulonephritis (APIGN) is an increasingly recognized morphologic entity typically seen in elderly and diabetics. Henoch-Schönlein purpura (HSP) predominantly occurs in children and is characterized by sudden onset of symmetric purpuric rash of the lower extremities. We report a child with typical initial presentation of HSP whose clinical course, biopsy and outcome were suggestive of IgA-dominant APIGN.

Case Description: A 6-year old AA male was admitted for progressive facial swelling, decreased urine output and difficulty breathing for 3 days. Two weeks prior he had left knee swelling and a purpuric rash over the shins. On exam he was afebrile with facial swelling,nasal flaring and grunting, with a gallop rhythm, and a diffuse excoriated rash on the lower extremity. Lab data:UA 3+ protein,3+ blood with 50RBCs, 13WBCs/hpf, 1.5G protein/24h, BUN/SCr 35/0.8mg/dl, serum cholesterol 104mg/dl, albumin 1.8G/dl, Streptozyme positive, ANA negative and C3 68 (50-180) mg/dl. Echocardiogram (ECHO) indicated decreased function. Viral and bacterial cultures were negative. Kidney biopsy:diffuse endocapillary proliferation with IgA-dominant 3+ mesangial and irregular granular capillary wall staining with lesser IgG and C3;lambda more than kappa. EM:frequent mesangial and subendothelial deposits and subepithelial hump-type deposits. Biopsy was suggestive of IgA-dominant APIGN. Skin biopsy revealed perivascular granular IgA with mild C3. He was discharged on diuretics and ACE inhibitor but returned a month later with acute cardiac decompensation. He received inotropes, IVIG, IV penicillin and steroids. Four months later he is on no medications, with normal ECHO, BUN/SCr 9/0.6mg/dl, C3 104mg/dl, albumin 3.8G/dl, UA 1+ protein, 2+ blood, 28RBCs/hpf, and 142mg protein/24h.

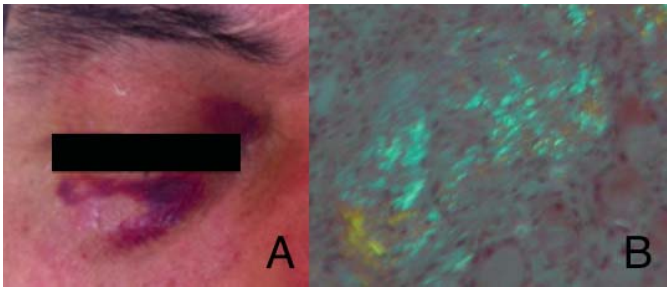
Discussion: The initial clinical presentation including the skin and kidney biopsy findings in IgA-dominant APIGN are typically indistinguishable from HSP. Even though IgA-dominant APIGN mimicking HSP is commonly seen in the adult population it does occur in children. The diagnosis should be made with a high degree of clinical suspicion looking diligently for any underlying infection.

FR-PO620

Renal Amyloidosis Associated with IgA Nephropathy in a Patient with Cirrhosis and Hepatitis B Eduardo Jorge Duque de Sa Carneiro Filho, Lectícia Jorge, Lucia Andrade, Viktoria Woronik. *Univ of São Paulo, São Paulo, SP, Brazil.*

Introduction: We present a cirrhotic patient with an unusual association of IgA nephropaty (IgAN) and renal amyloidosis.

Case Description: A 47-year-old Asian male with cirrhosis diagnosed 10 years ago caused by hepatitis B, treated with lamivudine and tenofovir, was admitted with lower extremity edema and hypotension for last 6 months. Upon physical examination: blood pressure of 76/42 and anasarca. Laboratory results demonstrated normal liver function, positive anti-Hbc, negative HbsAg and anti-Hbs, hemoglobin 11,9 g/dL, rheumatoid fator 11,1 IU/mL, C3 71 mg/dL, C4 16,6 mg/dL, IgA 476 mg/dL, twenty-four-hour urine protein collection with proteinuria of 5.84 g, creatinine 3.4 mg/dL, albumin 2.1 g/dL. Urinalysis showed proteinuria (1 g/L), 3 leukocytes per field and no red cells. During hospitalization, developed bipalpebral atraumatic ecchymoses (figure 1A). Protein electrophoresis, serum and urine immunofixation and bone marrow were normal. Renal biopsy: amyloidosis with nodular and diffuse deposition in glomeruli arteries and arterioles (positive congo-red, figure1B). Immunofluorescence: IgA (3+), C3 (+) in the mesangium and negative for k/l.



Discussion: Viral infections have been uncommonly reported with amyloidosis. Lannergard et al have shown that viral infections, including hepatitis B, result in elevated serum A amyloid protein levels that eventually could precipitate as amyloid. Mesangial IgA deposition observed in our patient is a frequent finding in chronic liver disease with minor glomerular symptoms and non progressive renal disease. It is thought to be a consequence of impaired clearance of IgA by the Kupffer cells and hepatocytes. To summarize, we report a case of nephrotic syndrome due to probably AA renal amyloidosis in a cirrhotic patient with Hepatitis B associated with IgAN probably secondary to cirrhosis.

FR-PO621

Membranous Nephritis with Full House Effect in a Patient with Negative Lupus Serologies Pradeep Vaitla, Ryan C. Mascarenhas, Antonio G. Jimenez, Jorge C. Garces. *Nephrology, Ochsner Clinic Foundation.*

Introduction: Patients with lupus nephritis usually have systemic lupus erythematosus (SLE) diagnosed by clinical and immunological criteria. American college of Rheumatology requires the presence of at least 4 of 11 clinical or laboratory criteria to diagnose SLE and Systemic lupus International Collaborating Clinics (SLICC) requires at least 4 of 11 clinical or laboratory criteria Or biopsy proven lupus nephritis with positive anti nuclear antibody (ANA) or anti deoxyribonucleic acid (DNA) antibody. We report a case of immune complex disease with sub epithelial and intra membranous deposits with IgG, IgA, IgM, C3 and C1q deposition in a patient with nephrotic range proteinuria and negative ANA. The patient had 0 of 4 criteria for ACR lupus criteria and 0 of 11 of SLICC criteria and would not qualify for diagnosis based on the criteria.

Case Description: 33 year old white male with asthma was being evaluated for elevated serum creatinine of 1.3. He did not report skin rash, arthritis,joint stiffness, photosensitivity, use of non steroidal anti inflammatory drugs or recreational drugs. On examination blood pressure was 128/82 mm Hg, rest of examination was unremarkable. Urinalysis showed +3 protein, 9 RBC's/hpf, urine protein to creatinine ratio of 9.5. Investigations were negative for HIV, Hepatitis, ANA, anti DNA and smith antibody, lupus anti coagulant and normal serum complement levels. Kidney biopsy revealed thickened basement membrane with subepithelial and intramembranous deposits, extensive foot process effacement. Immunofluorescence microscopy showed diffuse granular capillary loop staining for IgG (3+), IgA (1+), IgM (2+), C3 (2+) and C1q (1+), stains for phospho-lipase A2 receptor antibody were negative. Tubuloreticular inclusions were not identified.

Discussion: Diagnosis in this patient highlight the deficiencies in diagnostic criteria for SLE as he has no clinical or immunological criteria. Lupus nephritis still presents a challenge in clinical practice and the absence of better serologic markers to predict development of disease may delay appropriate diagnosis and treatment. Kidney biopsy remains the gold standard.

FR-PO622

***Campylobacter* Associated Glomerulonephritis** Kevin C. Roe. *Nephrology, Penn State College of Medicine, Hershey, PA.*

Introduction: *Campylobacter* bacteremia is a rare condition predominately seen in elderly patients, those with liver disease or malignancy. *Campylobacter* infection has been implicated in various inflammatory diseases including Guillain-Barre syndrome, reactive arthritis and myocarditis. We report a case of immune complex mediated glomerulonephritis (GN) in the setting of recurrent *C. fetus* bacteremia.

Case Description: A 78-year-old female was treated for prosthetic valve endocarditis following an ERCP for choledocholithiasis. Treatment required removal of her aortic prosthesis and placement of a new valve. Blood cultures grew *C. fetus* that cleared with ciprofloxacin. Six months later, she presented with generalized weakness, palpable purpura, GI bleeding and rapidly progressive acute renal failure. Urine microscopy was consistent with GN. Blood cultures again grew *C. fetus*. An upper endoscopy revealed a gastric ulcer and poorly differentiated adenocarcinoma on biopsy. Cultures cleared on treatment with meropenem. Echocardiography did not show objective findings of endocarditis. Serologic workup revealed positive ANA (1:80, homogenous pattern), anti-native DNA <20 IU/ml, ANCA directed to proteinase 3, C3 55mg/dL, C4 21mg/dL, serum cryoglobulins were negative and hepatitis serologies were negative. Renal biopsy revealed immune complex mediated GN with IgM and C3 granular glomerular staining on immunofluorescence, subendothelial and subepithelial deposits on electron microscopy.

Discussion: The epidemiology of infection-related GN has changed in the past few years. A significant percentage of cases now target adults (particularly the elderly and immunocompromised) and tend to be associated with nonstreptococcal organisms. *Campylobacter* infection is a potential trigger for immune mediated GN. Case reports of enteric *C. jejuni* infection have been implicated in GN. Molecular mimicry and immune complex activation have been suggested to contribute to *Campylobacter* related disease. Compared with other species, *C. fetus* is the most common isolate to cause bacteremia; however, this is the first reported case of GN associated with *C. fetus* that we are aware of. Our case of *Campylobacter fetus bacteremia* demonstrates notable features of the infection and potential for ensuing GN.

FR-PO623

Membranoproliferative Glomerulonephritis with "Full-House" Immunofluorescence in a Patient with IgG4-Related Disease Yuta Matsukuma,[1] Kosuke Masutani,[1] Kenji Ueki,[1] Akihiro Tsuchimoto,[1] Kiichiro Fujisaki,[1] Kumiko Torisu,[1] Kazuhiko Tsuruya,[1,2] Takanari Kitazono.[1] *[1]Dept of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [2]Dept of Integrated Therapy for Chronic Kidney Disease, Kyushu Univ, Fukuoka, Japan.*

Introduction: Typical renal histopathological finding in immunoglobulin G4 related disease (IgG4-RD) is lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells with fibrosis. Some glomerular lesions such as membranous nephropathy are sometimes evident. We hereby present an unusual glomerulonephritis in a patient with IgG4-RD.

Case Description: A 70-year-old woman was transferred to our hospital because of the microhematuria and proteinuria. The patient showed anemia on conjunctiva and leg edema. Laboratory findings revealed 3+ proteinuria, 2+ hematuria, pancytopenia, hypoproteinemia, and kidney dysfunction. Decreased levels of C3 and C4 were noted, but anti-double strand DNA antibody, anti-nuclear antibody, and cryoglobulin were negative. Serum IgG level was 1,916 mg/dL, and IgG4 1,110 mg/dL. Percutaneous kidney biopsy revealed membranoproliferative glomerulonephritis with subendothelial deposits. On immunofluorescence study, "full-house" depositions of immunoglobulins and complements were found in mesangial area and capillary loop. Electron microscopy revealed endocapillary hypercellularity, mesangial interposition, and electron dense deposits in mesangial and subendothelial area. The lip biopsy revealed diffuse infiltration of IgG4-positive plasma cells in glandular tissue. However, kidney tissue showed focal lymphoplasmacytic interstitial infiltrate with occasional eosinophils, and interstitial fibrosis was mild. In addition, the IgG subclasses in glomeruli were predominant IgG2 and IgG3. We treated this patient with oral prednisolone 30 mg/day. The patient showed decreased urinary protein excretion, gradual improvement of pancytopenia and hypocomplementemia.

Discussion: In this case, we identified IgG4+ plasma cell infiltrate on lip biopsy as well as increased level of serum IgG4, compatible with IgG4-RD. But, IgG subclasses in the glomeruli were predominant IgG2 and IgG3, suggesting the overlapped glomerulonephritis with different pathophysiology.

FR-PO624

IgA-Dominant Post Infectious Glomerulonephritis Presenting as a Fatal Pulmonary-Renal Syndrome Marc M. Saad, Magda Daoud, Patricia Nasr, Elie El-Charabaty, Suzanne E. El Sayegh. *Nephrology, Staten island Univ Hospital, Staten island, NY.*

Introduction: Postinfectious glomerulonephritis has been having over the last decades a major change in its epidemiology, pathophysiology, clinical characteristics and outcomes. We are reporting a case of IgA-dominant post infectious glomerulonephritis presenting as a fatal renal-pulmonary syndrome.

Case Description: An 86 year old Filipino man presented to the Emergency room for worsening dyspnea, hemoptysis, dull chest pain, with lower extremity edema and decrease in urine output over the last two weeks. PMH is significant for HTN, CKD stage III, gout, COPD, and a recent hospitalization 3 weeks ago for pneumonia and treated with IV cefazolin for 2 weeks for MSSA bacteremia. Physical examination was remarkable for HR of 109 bpm and RR of 25/min. There were bibasilar rales in the lungs and bilateral ankle edema. A chest

radiograph showed bibasilar opacifications. Blood work was significant for hemoglobin of 8.3 (baseline 9.7), creatinine of 9.2 (baseline of 1.67). The patient was hypoxic requiring mechanical ventilation. Urinalysis reported RBC casts. Ultrasonography revealed bilateral renal echogenicity compatible with renal disease. On day 2, Patient was started on HD for hypervolemia and on pulse solumedrol 1 g for 3 days, patient's condition precluding kidney biopsy. Serology workup for RPGN was negative with normal complements level. On day 7, bronchoscopy showed alveolar hemorrhage and plasmapheresis was initiated. Renal biopsy revealed Ig A – dominant acute post-infectious glomerulonephritis with endocapillary and focal extracapillary proliferative and exudative features.

Discussion: Previous reports showed that IgA dominant PIGN occurs in elderly with mean age of 60, after time frame of (0-16) weeks, mainly in caucasian (53%) and asian (26%). Staphyloccous was the main infectious agent and diabetes favoring the disease. However, this non diabetic patient had pulmonary-renal syndrome, with normal complement level. Patient had fatal outcome despite steroid pulse and plasmapheresis. Understanding the phathogenesis and identifying the nephrotoxic species of bacteria and the aberrant IgA molecule will open new insights toward prevention and treatment.

FR-PO625

A Case of Infection-Related Glomerulonephritis After Total Knee Arthroplasty Rushi K. Nayak, Germaine Z. Chan, Steven D. Smith. *Nephrology, Mt. Sinai-St. Luke's, New York City, NY.*

Introduction: Infection-related glomerulonephritis (IRGN) is an immune-complex mediated entity that develops in the setting of active infection, commonly due to Staphylococcus or Streptococcus species. In adults, the site of infection can be heterogeneous. We present a case of IRGN due to an infected prosthetic knee joint.

Case Description: A 62 year-old male with hepatitis C presented to the hospital with worsening knee pain at the site of total knee arthroplasty done six years previously. Blood and joint fluid cultures two weeks prior to presentation were positive for methycillin-sensitive Staphyloccus aureus and he was scheduled for knee surgery, but was lost to follow-up. Renal function was normal at that time. Upon return to the hosptial he was afebrile, hypertensive and tachycardic. Right knee was swollen and tender. Lab work revealed acute kidney injury (Cr 4.1 mg/dL), anemia, and urinalysis significant for hematuria and nephrotic-range proteinuria, with evidence of acanthocytes. Serologic testing for HIV and HBV were negative. Complement C3 and C4 levels were normal. Cryoglobulin and rheumatoid factor were absent. He was started on parenteral antibiotics but his renal function continued to deteriorate. Kidney biopsy revealed diffuse mesangial proliferative and focal endocapillary proliferative glomerulonephritis, with focal cellular and fibrous crescents. Immunofluorescence showed granular global mesangial and segmental capillary wall positivity for C3 and IgG, suggestive of IRGN. He underwent revision of his knee arthroplasty with placement of an antibiotic impregnated spacer. He required intermittent hemodialysis which was discontinued two weeks after surgery. Three months after discharge his creatinine was stable at 2.0 mg/dL.

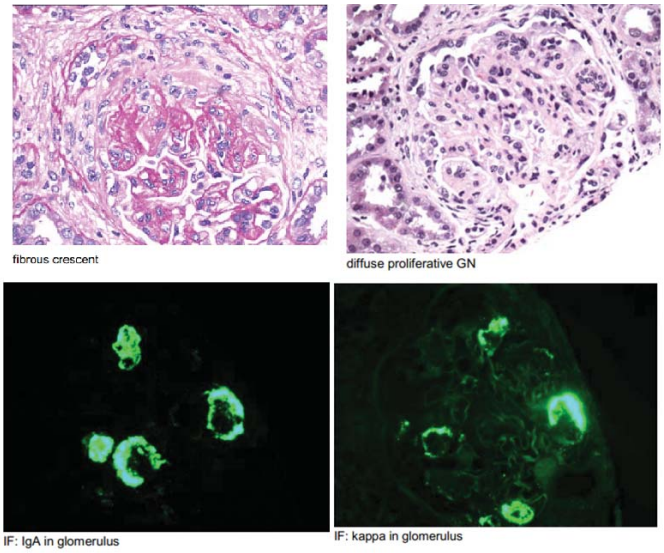
Discussion: This case illustrates a typical presentation of IRGN. HCV-related and C3 GN should be considered, but are less likely in face of ongoing bacterial infection. Hypocomplementemia, absent in this patient, is seen in 35-80% of cases. Treatment considerations include the use of steroids, which were not used given ongoing active infection. The patient experienced a permanent loss of renal function, seen in up to 54% of adult cases of IRGN.

FR-PO626

Chronic Lymphocytic Leukemia (CLL)-Associated Cryoglobulinemic Glomerulonephritis (GN) Sairah Sharif, Farah Daccueil, Naveed N. Masani. *Winthrop Univ Hospital, Mineola, NY.*

Introduction: Glomerular lesions are rare in CLL but when they occur the course may run parallel to CLL suggesting causality. We report a case of patient with CLL who was found to have cryoglobulinemic GN with monoclonal IgA deposits.

Case Description: A 74 year old female with past history of CLL, idiopathic thrombocytopenia and chronic, left hydronephrosis, presented to the emergency complaining of shortness of breath and leg swelling despite an increase in diuretics. Physical exam revealed hypertension 194/84, decreased breath sounds and +3 bilateral lower extremity edema. Workup revealed serum creatinine (SCr) of 3.2 mg/dL (baseline SCr 1.6 mg/dL), a protein:creatinine ratio of 7.9 gm/gm creatinine, and a kappa:lamda ratio of 8.6. A bone marrow biopsy showed normocellular marrow with scattered plasma cells (<10%). Subsequently, a right renal biopsy was done which demonstrated a light microscopic (LM) pattern of diffuse, endocapillary proliferation, active crescents, with membranoproliferative features; immunofluorescence staining was significant for IgA, kappa and C3.



Renal biopsy images courtesy Dr Glen Markowitz, Department of Pathology, Columbia Presbyterian Hospital, New York, USA

Therapy with pulse solumedrol/oral prednisone and ibrutinib was initiated, targeting the underlying CLL, which was ultimately felt to be responsible for the glomerular disease. While the proteinuria remains in the nephrotic range, the SCr has improved to 1.6 mg/dL.

Discussion: Kidney infiltration is seen in CLL up to 90%, but generally subclinical. GN is rare in CLL; where described, LM pattern of membranoproliferative GN is the most common. Most cases get nephrotic syndrome and one third get renal failure. Renal disease maybe related to immune complexes, cryoglobulins or monoclonal protein deposition. This case is significant as chemotherapy lead to improvement of kidney disease and supports use of chemotherapy in CLL-associated glomerulonephritis despite low grade hematological disease.

FR-PO627

Immune Complex Mediated Glomerulonephritis as a Possible Complication of Polycythemia Vera and Myelofibrosis Lisa Aimee Hechanova, Thanh Hoang. *Loma Linda Univ Medical Center, Loma Linda, CA.*

Introduction: Polycythemia vera is a chronic myeloproliferative neoplasm associated with a mutation in the JAK2 V617F domain. This mutation causes activation of the erythropoietin and thrombopoietin receptors, leading to uncontrolled hematopoietic cell growth. Over time, polycythemia vera can progress to myelofibrosis. There have been several published studies which demonstrate an association between myelofibrosis and circulating immune complex formation. Herein we present a case of a patient with polycythemia vera, who was found to have progression to myelofibrosis and immune complex glomerulonephritis.

Case Description: A 50-year-old female with a ten year history of polycythemia vera presented with new onset ascites and peripheral edema. Her polycythemia vera had initially been treated with phlebotomy and low dose aspirin, then splenic radiation for splenomegaly, and more recently ruxolitinib (an oral JAK1/JAK2 inhibitor). She was found to have nephrotic range proteinuria, portal hypertension, leukocytosis, anemia, and thrombocytopenia. She underwent extensive serologic workup, abdominal imaging, bone marrow biopsy, liver biopsy, and renal biopsy. She had creatinine of 1.5mg/dL, proteinuria of 3.5 grams, equivocal ANA, but negative complements, anti-double stranded DNA, anti-Smith antibody, SSA/SSB, hepatitis panel, HIV, SPEP/UPEP, rheumatoid factor and ANCA. Cold agglutinin was positive. She did not have any clinical signs of lupus. Abdominal imaging revealed marked hepatosplenomegaly. Bone marrow biopsy revealed myeloid hyperplasia and marrow fibrosis; liver biopsy showed extramedullary hematopoiesis with centrilobular and sinusoidal obstruction; and kidney biopsy revealed chronic immune complex mediated glomerulonephritis with both subepithelial and subendothelial electron dense deposits, a few tubuloreticular inclusions, as well as positive immunoflurescence staining for IgG, IgM, C3, C1q, fibrinogen, kappa, and lambda.

Discussion: Circulating immune complexes associated with polycythemia vera, particularly after progression to myelofibrosis, may be a rare cause of immune complex glomerulonephritis. We feel, due to the lack of other factors, that this is the most probable etiology in our case.

FR-PO628

First Reported Case of Fibronectin Glomerulopathy in a Hispanic Female Keerti K. Bhanushali,[1] Geeta Kutty,[2] Jane Vernik.[3] *[1]Nephrology, Univ of Florida, Gainesville, FL; [2]Medicine, John Stroger Jr. Hospital of Cook County, Chicago, IL; [3]Nephrology, John Stroger Jr. Hospital of Cook County, Chicago, IL.*

Introduction: Fibronectin glomerulopathy (FNG) is a rare, usually hereditary disease with autosomal dominant inheritance. It is characterized by massive deposition of fibronectin and is Congo-red negative.

Case Description: A 37 yr old Hispanic female with history of hypertension, nephrotic syndrome secondary to membranoproliferative glomerulonephritis diagnosed 8 yrs ago presented with progression of nephrotic syndrome while on optimal medical therapy. No known family history of renal disease. Physical examination was unremarkable except pedal edema. Pertinent laboratory values included normal serum creatinine, serum albumin 2.6gm/dl, serum cholesterol 396mg/dl, 24hr urine protein 15 gm. Viral hepatitis profile, HIV and immunologic panel was negative. Renal biopsy revealed mesangial expansion with PAS positive, Congo red negative homogenous material, minimal cellular proliferation and areas of interstitial fibrosis. Electron microscopy showed massive subendothelial and mesangial electron dense deposits arranged in a fine granular pattern with focal short fibrillary structure (10-16 nm). Immunoflorecence revealed scant reactivity for immunoglobulins and complements. Immunostaining with fibronectin was positive within the mesangial matrix.

Discussion: To our knowledge, no cases of FNG have yet been reported in Hispanic patients. FNG is a hereditary disease that may lead to end-stage renal failure in the second to sixth decade of life. In 40% of families, the disease is caused by heterozygous mutations in the FN1 gene (2q34) encoding fibronectin. However genetic heterogeneity is suspected. Whole genome linkage analysis in a large pedigree showed another disease locus on 1q32, with no specific candidate genes identified so far. Reported experience with treatment using steroids, cytotoxic agents and plasmapheresis is limited and anecdotal. ACE inhibitors or ARBs, diuretics and antihypertensives are used in the management.

FR-PO629

Successful Treatment of IgM Nephropathy with Rituximab

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Introduction: Immunoglobin M Nephropathy (IgMN) is a form of primary glomerulonephritis with unclear etiology and pathogenesis. There is no specific treatment, however, corticosteroids have been tried and are frequently ineffective. We present a case seen for significant proteinuria, diagnosed as IgMN and successfully treated with Rituximab.

Case Description: A 59 year-old man with history of HTN, prostate cancer, COPD, GERD was seen for proteinuria. His medications included amlodipine, metoprolol, ASA, omeprazole and meloxicam. Physical examination was positive for obesity and edema. BMP and CBC were normal. Urine microscopy showed no RBC casts. His urine P/C ratio was 2800 mg/g. ANA, ANCA, C3, C4, ASO titers, HIV, hepatitis, Anti-GBM antibodies were uremarkable. SPEP showed IgM and IgG-lambda and UPEP showed IgG-lambda and free lambda with K/L ratio 0.41. Renal biopsy was performed. LM showed increased mesangial cellularity and matrix and mild interstitial fibrosis. Immunofluorescence (IF) was positive for diffuse global mesangial staining with IgM. EM showed severe foot process effacement with normal basement membrane. He was diagnosed as IgMN with minimal FSGS. Patient received 2 doses of 1 g rituximab, 2 months apart, and started on lisinopril. After the treatment, urine P/C ratio declined and remained less than 0.2 mg/g.

Discussion: The epidemiology of IgMN is deducted retrospectively from renal biopsies due to lack of well-established population based studies. Clinical picture ranges from asymptomatic urine abnormalities to nephrotic range proteinuria. Diagnosis is primarily based on pathology. In LM, morphological changes are seen in different areas. IF is critical in the diagnosis with the characteristic pattern of diffuse and global mesangial IgM positivity. EM shows variable degree of foot process effacement. There are no RCT to date addressing its treatment. Success rate of corticosteroids vary from 0 to 50%. There is limited data on efficacy of immunosuppressive agents. The reported complications of IgMN are renal insufficiency and hypertension. More systematic studies are needed to establish the role of Rituximab in the treatment of IgMN.

FR-PO630

Case Report of a Rare Finding of Sarcoid Granulomatous Glomeruli Nephritis

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Introduction: Renal sarcoidosis commonly presents with acute kidney injury but rarely glomerular disease. The classic findings on biopsy are multinucleated giant cells, normal glomeruli and noncaseating granulomas involving renal interstitium surrounding tubules. We present the first case of glomerular disease from direct glomeruli effacement by renal noncaseating granulomas.

Case Description: This is a case of a 44 year old African-American male with a history of Dermatomyositis referred to Nephrology for acute kidney injury and hyperproteinemia. His studies showed creatinine (Cr) 1.76 mg/dL and gamma globulins without an M spike on serum and urine protein electrophoresis. He returned with increased urinary frequency and thirst, fatigue, Cr 3.42 mg/dL, calcium 12.1 mg/dL, and proteinuria. Any malignant, infectious, drug-induced and auto-immune etiologies were ruled out. CT chest revealed bilateral reticulonodular pulmonary opacities. A renal biopsy revealed near total effacement of renal tubules and glomeruli by numerous small noncaseating granulomas with multinucleated giant cells and extensive fibrosis with patchy chronic inflammation. He was diagnosed with Granulomatous Glomerular and Tubulo-Interstitial Nephritis due to Renal Sarcoidosis and Pulmonary Sarcoidosis stage 3. Therapeutic intervention included Prednisone with improvement.

Discussion: The classic findings on biopsy are noncaseating granulomas involving interstitium. Granulomas are the leading cause of hypercalcemia which adversely causes acute kidney injury but not glomerulopathy. First line therapy is oral prednisone 1mg/kg daily. The patient in this case report had an improvement in both renal function and proteinuria after steroids. Stehle et al. reported lesions of glomerular disease in the setting of sarcoidosis but none with granuloma involvement of glomeruli. Our case was unusual because, in addition to the classic tubulo-interstitial granulomas, there was also direct glomerular involvement and effacement by the granulomas.

FR-PO631

Treatment of Recurrent Mixed Cryoglobulinemia Syndrome

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Introduction: We report a case of recurrent mixed cryoglobulinemia type II in the absence of HCV that responded minimally to cyclophosphamide and plasmapheresis but impressively well to rituximab and plasmapheresis.

Case Description: A 60 year-old Argentinian-American male with history of essential mixed cryoglobulinemia 12 years ago treated successfully with six months of cyclophosphamide and prednisone presented with similar complaint of lower extremities skin rash and swelling. Physical exam was notable for purpuric skin rashes over bilateral legs. He was found to have proteinuria (1.5 gm/day), hematuria, RBC casts, low C3 (35 mg/dL) and C4 (<10 mg/dL), and a creatinine of 2 mg/dL (baseline 1 mg/dL). With the suspicion of recurrent disease, he was started on intravenous solumedrol 1gm daily for 3 days. Before the scheduled biopsy, he developed respiratory failure from diffuse alveolar hemorrhage and combination therapy with oral cyclophosphamide 100 mg/day (total four doses) and daily plasmapheresis was initiated. Labs revealed positive type II cryoglobulins, C3 at 35 mg/dL, C4 at <10 mg/dL, RF at 115 IU/mL, free Kappa/Lambda at 3.53, UPEP with minimal urine protein, SPEP with marked hypogammaglobulinemia; and negative for HIV, HCV, ANA, and ANCA tests. Because of recurrent mixed cryoglobulinemia involving lungs, kidneys, and skin with light chain abnormalities deteriorating rapidly, he was switched to more aggressive therapy with daily plasmapheresis and rituximab 375 mg/m2/week. He was extubated in a week of the first dose of Rituximab and subsequent doses of rituximab (total of 4 doses) were completed as outpatient. Follow up lab studies revealed creatinine at 1.1 mg/dL, urinalysis with 2+ proteinuria and occasional casts, C3 at 68 mg/dL, C4 at 11 mg/dL, free Kappa/Lambda at 2.31 and absent cryoglobulin. His prednisone therapy is tapered and he claims being normal now.

Discussion: Our patient with recurrent type 2 mixed cryoglobulinemia after 12 years of complete remission developed life threatening pulmonary hemorrhage (infrequent complication with worse prognosis) responding extraordinarily well to the rituximab and plasmapheresis suggesting rituximab as superior therapy to cyclophosphamide in such cases.

FR-PO632

Treatment of Recurrent Cryoglobulinemic Nephritis

Eoin D. O Sullivan, Brenda B. Griffin. *Dept of Renal Medicine, Cork Univ Hospital, Cork, Ireland.*

Introduction: Type 1 cryoglobulemia is a rare entity in multiple myeloma[1]. We describe a challenging case of cryoglobulinemic nephritis secondary to multiple myeloma, and the difficulties in treating relapse of renal disease.

Case Description: A 65 year old female with a background of resected ductal carcinoma in situ of breast was referred with a rising creatinine of 550mmol/l. Renal biopsy revealed subendothelial and mesangial immunotactoid deposits suggestive of a cryoglobulemic nephritis. Further investigation revealed IgG of 33 g/l, a paraprotein level of 13.1g/l, and circulating cryoglobulins. A marrow biopsy demonstrated 20% mature plasma cells, confirming a diagnosis of IgG CD138+ multiple myeloma. The patient's creatinine normalised following treatment with pulsed methylprednisolone and oral cyclophosphamide. The patient represented 3 years later with abrupt onset anasarca and nephritic syndrome, a creatinine of 479 mmol/l, urinary PCR of 1148, >500 RBC per HPF. C3 1.14 g/l (0.75 – 1.65), low C4 0.09g/l (0.14- 0.54), and positive cryoglobulins (3430mg/l). Treatment with cyclophosphamide IV and dexamethasone failed to improve symptoms or laboratory values, and a tunneled catheter was sited in anticipation of dialysis. Plasmapheresis was initiated in an attempt to gain control over the cryoglobulinemia.Within 3 sessions the patient's creatinine normalized to 98 mmol/l, and PCR dropped to 63. While the patient's renal function remained stable, 12 months later she represented with diffuse disease requiring radiotherapy and palliation.

Discussion: Recurrence of cryoglobulinemic nephritis can be treated effectively with plasmapheresis but may suggest worsening hematological disease. In the largest case series,presence of renal disease is associated with increased hazard ratio (8.9) [2]. Within a year of renal recurrence our patient presented with diffuse soft tissue plasmacytomas, refractory to therapy. While there is a sparse body of evidence to support plasmapheresis in mixed cryoglobulinemia[345], there are only a number of cases of type 1 cryoglobulinemia having been successfully treated with plasmapheresis, usually in combination with treatment of the underlying hematological malignancy [26].

FR-PO633

Rapidly Recurrent MPGN in a Transplant Patient with Cryoglobulinemia

Akanksha Gupta, Daniel J. Kenan, Harsharan Kaur Singh, Volker Nickeleit. *Div of Nephropathology, Dept of Pathology and Laboratory Medicine, Univ of North Carolina at Chapel Hill, Chapel Hill, NC.*

Introduction: Cryoglobulinemic glomerulonephritis often displays features of Membranoproliferative glomerulonephritis (MPGN) related to glomerular deposition of cryoglobulins.

Case Description: A 49 year old male with hepatitis C presented in 2008 with mixed nephritic and nephrotic syndrome. His serum creatinine was 2.4 mg/dl, urinalysis was notable for 4+ hematuria and 4 gm/day proteinuria. His diagnosis was MPGN type 1

secondary to hepatitis C-related cryoglobulinemia. He developed ESRD and received a cadaveric renal transplant in November 2013. A zero hour renal allograft biopsy showed ATN with no evidence of MPGN. The patient was discharged with a creatinine of 2.95. Two weeks later, he presented with creatinine of 4.2 and was biopsied. The biopsy showed diffuse endocapillary hypercellularity consisting of monocytes and macrophages but no T-cells. There was GBM duplication and lobular accentuation of the tufts. Immunofluorescence showed similar staining of IgM and C3 as seen in native kidney biopsy, together indicating recurrent MPGN two weeks after transplantation. The creatinine gradually improved to a baseline of 1.5. Then in April 2014, he presented again with a rise in creatinine to 3.5, accompanied by hematuria, proteinuria, hypocomplementemia, with associated leukocytoclastic skin rash. Renal biopsy showed only mild acute rejection with no evidence of MPGN. However, the symptoms worsened and he was rebiopsied one week later, demonstrating features of MPGN again, accompanied by electron dense deposits in subendothelial and mesangial areas showing microtubular substructure, supporting the diagnosis of recurrent cryoglobulinemic glomerulonephritis.

Discussion: The median recurrence of MPGN reported in the literature is 4.7 years post-transplant. In this case, MPGN recurred only two weeks post transplant and then disappeared briefly, followed by another recurrence 6 months after transplantation. This case makes the points that 1) Extremely rapid recurrence may occur in cryoglobulinemia and 2) The severity of the MPGN may be related to the normal waxing and waning of the cryoglobulinemic glomerulonephritis.

FR-PO634

Henoch-Schonlein Purpura Nephritis Associated with Staphylococcal Infection Rungwasee Rattanavich, Steven M. Spiegel, Nilubon Methachittiphan Methachittiphan. *Internal Medicine, MetroWest Medical Center, Framingham, MA.*

Introduction: Renal involvement in Henoch-Schönlein purpura (HSP) is more severe in adults. Many etiologies of HSP have been described but Staphylococcal associated with HSP has been rarely reported.

Case Description: 72 year old male with hypertension who presented with rashes bilateral thighs,chills and fatique after underwent open reduction with internal fixation for left ankle fracture 2 weeks prior to presentation.Exams found purplish palpable purpura at bilateral thighs down to toes.The wound was swelling with pus drainage. Incision and drainage was performed.Pus culture revealed Methicillin Sensitive Staphylococcus infection. Blood culture negative. His renal functionwas normal with BUN-26 Cr-0.85 eGFR >60, UA was normal.He was treated empirically with Vancomycin IV before switching the antibiotic based on the sensitivities.During hospitalized his creatinine is progressively increasing to 4.33 on day 14 with present of microscopic hematuria,no proteinuria.Serum electrophoresis showed high level of IgA and IgG, but no M spike was found, C3,C4 complement-normal, P-ANCA, C- ANCA, ASO titer, ANA, DsDNA and cryoglobulin were negative.Renal biopsy was performed showing IgA Nephropathy/Henoch-schonlein purpura with a focal proliferative pattern of glomerular injury.No crescents, glomerulosclerosis. He was treated with Methylprednisolone followed by oral prednisone. His Renal function gradually improved with a creatinine of 1.4 mg/dl approximately one month after treatment was initiated.

Our patient developed Henoch-Schonlein Purpura Nephritis (HSPN) associated with an MSSA infection.There is speculation that enterotoxins from staphylococcus act as super antigens causing renal injury by cytokine burst and immune complex.Renal pathology in HSPN showed IgA-dominant glomerulonephritis as also commonly seen in Staphylococcus infection-associated nephritis.Interestingly a similar clinical presentation can be seen.It is critically important to distinguish between the two.HSPN may need to be treated with corticosteroids and other immunosuppressives while *Staphylococcus* infection-associated glomerulonephritis requires antibiotics and could be worsen from immunosuppressive treatment.

Funding: Private Foundation Support

FR-PO635

Double Positive ANCA/GBM Antibody Glomerulonephritis Presenting as Appendiceal Vasculitis Rungwasee Rattanavich, Sneha Kilari, Steven M. Spiegel. *Internal Medicine, MetroWest Medical Center, Framingham, MA.*

Introduction: Anti-glomerular basement membrane antibody disease usually presents with rapidly progressive glomerulonephritis.25% of cases test positive for both anti-GBM and ANCA antibodies, known as Double positive disease.This abstract describes a rare presentation of this entity.

Case Description: 48 year old woman with hypertension who was diagnosed with acute appendicitis 6 weeks prior to admission. CT abdomen showed a thickened appendix and peri-appendiceal fat stranding. Laparoscopic appendectomy was performed; however, she continued to have refractory abdominal pain,nausea,vomiting and was readmitted, treated with antibiotics without improvement. Her renal function was normal at that time. 5 weeks later she was readmitted.Labs showed BUN-101,Cr-15.56,eGFR-3 with microscopic hematuria. Serology was positive for Anti-IgG; atypical p-ANCA. Anti-MPO,Proteinase 3 antibodies-negative. C3,C4 were normal. Blood and urine cultures-negative.Renal biopsy showed necrotizing and diffuse crescentic glomerulonephritis with linear IgG deposition along glomerular capillaries. Global glomerulosclerosis, tubular atrophy and interstitial fibrosis were absent.Immunofluorescence confirms anti-GBM deposition. Given the clinical course and renal biopsy,the appendiceal tissue was sent for reexamination and showed inflammation and necrosis of small blood vessels within the appendix consistent with vasculitis.Treatment consisted of plasmapheresis, methyprednisolone pulse followed by prednisone, cyclophosphamide.Though there was some improvement in renal function initially,she remains dialysis dependent after 4 months of follow-up.

Discussion: Anti-GBM antibody disease usually presents with glomerular involvement where the inflammation resulting in a rapidly progressive glomerulonephritis. ANCA may be positive along with anti-GBM antibodies, in which case the patient can present with symptoms depending on the site of involvement by vasculitis as we have seen in our patient. Vasculitis should therefore be considered in the differential diagnosis of patients with unusual or persistent abdominal pain and it should also be considered by the pathologist when examining post operative specimens.

Funding: Private Foundation Support

FR-PO636

Red Urine and Elusive Renal Failure Sahil Garg,[1] Nader S. Bahri,[1] Leighton R. James,[1] Raafat Farag Makary,[2] Carmela B. Monteiro.[2] *[1]Dept of Nephrology, UF Health Jacksonville; [2]Dept of Pathology, UF Health Jacksonville.*

Introduction: ANCA associated vasculitis has high morbidity and mortality. This case highlights the importance of timely renal biopsy in patients with high index of suspicion for glomerulonephritis. Immunosuppression with corticosteroids and Cyclophosphamide (Cyc) significantly improves prognosis.

Case Description: A 50 year old female with history of hypertension, chronic obstructive lunge disease, schizoaffective disorder was admitted with complaints of right knee pain and swelling. One year earlier, she developed right tibial osteomyelitis requiring proximal tibial resection but, continued to have repeated infections. On this admission, she underwent incision and drainage of the right knee. Subsequently she developed tea colored urine and acute deterioration in kidney function. Urine sedimentation revealed coarse granular casts (5-10/hpf), few dysmorphic RBC. SPEP, UPEP, ANA, ANCA, dsDNA, Rheumatoid factor was negative, C3 (124.8 mg/dL), C4 (35.5) mg/dL. Hepatitis C Ab was positive but viral particles were undetectable by PCR. 24 hr urine protein excretion was 725 mg. Renal biopsy was delayed for 3 weeks as she was unstable. During this period Hemodialysis was initiated. Subsequently, renal biopsy revealed diffuse, acute proliferative crescentic glomerular lesions with breaks in the glomerular basement membrane, tubular degenerative/regenerative changes with moderate interstitial acute and chronic inflammation. Immunofluorescence and electron microscopy studies were negative for immune complex deposits. She was administered methylprednisone (500 mg daily x 3 doses) and Cyc 0.5 gm/ m2. This was followed by maintenance prednisone 80 mg QD which was tapered by 25 % every 3-4 weeks. Subsequent to initial treatment with Prednisone and Cyc, her renal function exhibited significant improvement. Serum creatinine was 1.8 mg/dL, 2 months later. She is on prednisone and Azathioprine for maintainence immunosupression.

Discussion: Renal Biopsy was indicated because of rapidly worsening renal function and dysmorphic RBCs on urine sedimentation. Induction therapy consists of Cyc and steroids for 3-6 months. Maintenance therapy is with azathioprine or methotrexate.

FR-PO637

Rapidly Progressive Glomerulonephritis Associated with Hodgkin Lymphoma: Case Report Nathalia K.N. Alecrim, Carla Tenório Barros Cisne Pessoa, Camila Barbosa L. Oliveira, Alline S.A. Oliveira, Luis H.B.C. Sette, Maria Alina G.M. Cavalcante, Gisele Vajgel Fernandes, Lucila Maria Valente. *Nephrology, Univ Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Introduction: Glomerulonephritis is a well recognized paraneoplastic manifestation of hematological disease, though it rarely occurs in Hodgkin lymphoma patients. The glomerular abnormalities generally observed are minimal change disease and amyloidosis. We report a patient with rapidly progressive glomerulonephritis associated with Hodgkin lymphoma.

Case Description: A 59 year-old man was admitted to the hospital because of rapid decline in renal function and microscopic hematuria. He had a two months history of left cervical mass, with hard texture and progressive growth. Exams showed serum creatinine of 4.0 mg/dL (baseline: 1.4 mg/dL) and nephrotic range proteinuria. Blood levels of complement (C3 and C4) were normal and testing was negative for antinuclear antibodies, hepatitis B and C and HIV. The patient received methylprednisolone 1g for three days, with partial recovery of the renal function (serum creatinine of 2.8mg/dL) at hospital discharge. Renal biopsy revealed focal and segmental sclerosis with multiples synechiae of the glomerular tuff and fibrotic crescents. Ultrasound of the cervical mass revealed a heterogeneous lymph node conglomerate suspicious of malignancy. The patient underwent excision biopsy, which revealed reactive hyperplasia and immunostaining showed that cells were positive CD30 and CD15 and negative for CD45, consistent with diagnosis of nodular sclerosing classical Hodgkin lymphoma.

Discussion: The association of crescentic glomerulonephritis with Hodgkin's disease has only been described in limited cases. Renal failure in the setting of lymphoma is usually attributed to infiltration of the kidney by malignant cells, obstruction of ureters, amyloidosis or increase in serum calcium or uric acid. Although rare, the possibility of a rapidly progressive glomerulonephritis should be considered, since renal function can be improved with specific treatment and chemotherapy.

FR-PO638

ANCA Negative Pauci Immune Crescentic Glomerulonephritis due to Multiple Myeloma Cyriacus Uzoma Anaele,[1] Weeraporn Srisung,[1] Yvette Corinne Tomacruz,[2] Melvin E. Laski.[1] *[1]Internal Medicine, Texas Tech Univ Health Sciences Center, Lubbock, TX; [2]Internal Medicine, Covenant Medical Center, Lubbock, TX.*

Introduction: Pauci-immune crescentic glomerulonephritis with rapidly progressive glomerulonephritis is most commonly associated with the presence of antineutrophil cytoplasmic antibodies (ANCA). We here report a case of ANCA negative pauci-immune crescentic glomerulonephritis discovered at the time a patient presented with multiple myeloma.

Case Description: A 56 year old Hispanic woman first presented with complaints of nausea, emesis, and fever. Initial serum chemistry showed a creatinine of 9.4 mg/dL, BUN 72 mg/dL, albumin 3.3 gm/dL, total protein 6.5 gm/dL, potassium 5.2 meq/L, and bicarbonate 19 meq/L. All other chemistry was normal. CBC showed normochromic normocytic anemia but WBC counts and platelets were normal. Initial urinalysis showed bland sediment with 100 mg/dl protein, but further studies defined the presence of high concentrations of kappa and lambda light chain in a ratio of 34.89. Subsequent bone marrow biopsy found 13% plasma cells. SPEP did not reveal a spike. ANCA, anti-GBM, ANA, hepatitis panel, and serum complements performed to evaluate the renal disease were all normal. Kidney biopsy revealed chronic sclerosing pauci-immune crescentic glomerulonephritis plus tubular necrosis, severe tubular atrophy, interstitial fibrosis, and severe arteriosclerosis. Congo red stains were negative. EM showed no intraglomerular deposits. The patient was subsequently treated for myeloma with bortezomib and dexamethasone. She was given the diagnosis of ESRD based on the clinical data and pathological findings and remains on outpatient hemodialysis.

Discussion: Renal pathologic manifestations of multiple myeloma often involve glomerular deposition disease, tubulointerstitial disease, or both. Our patient demonstrated neither of these two pathologic findings but did have chronic sclerosing pauci-immune crescentic glomerulonephritis in the absence of ANCA antibodies. Pauci-immune crescentic GN has previously been associated with multiple myeloma but the association remains extremely rare.

Funding: Clinical Revenue Support

FR-PO639

Scleroderma with ANCA Associated Glomerulonephritis: Also a Renal "Crisis" Tauseef A. Sarguroh,[1] Olutayo T. Olabige,[1] Jeffrey D. Wallach,[1] Herman L. Anderson,[1] Leroy Herbert,[1] Vivette D. D'Agati,[2] Sudhanshu Jain.[1] *[1]Nephrology, Harlem Hospital Center, New York, NY; [2]Renal Pathology, Columbia Univ, New York, NY.*

Introduction: Scleroderma renal crisis is historically described as a vasculopathy and is an important cause of morbidity and mortality. We describe a patient with diffuse systemic sclerosis (SSc) presenting with a different renal "crisis" due to acute glomerulonephritis.

Case Description: A 51 year old Hispanic man with hypertension, scleroderma, vitiligo and Raynaud's phenomenon was hospitalized for acute kidney injury. Physical exam showed blood pressure of 155/94, scalp and perioral vitiligo, clubbing and sclerodactyly. Initial workup revealed blood urea nitrogen of 54mg/dl, creatinine of 6.4mg/dl and urine protein/creatinine of 4g/g. Urine microscopy showed dysmorphic RBCs and a RBC cast. Autoimmune workup was positive for p-ANCA (20.1:1), ANA (1: 640;homogeneous), RNP Ab (1.0), Scl-70 (>8) and SSA-Ab Ro (5.3). Other serologies including cryoglobulins, hepatitis B/C and HIV were negative. C3, C4 and CH50 were normal. He was treated with pulse IV methylprednisone for three consecutive days. Renal biopsy showed pauci-immune crescentic glomerulonephritis with 27 of 38 glomeruli showing active cellular crescents. There were no pathological findings to suggest scleroderma vasculopathy or thrombotic microangiopathy. Subsequently, he was started on cyclophosphamide and prednisone. He had partial renal recovery with creatinine down to 3.1mg/dl on discharge. Unlike most other reported cases; our patient did not require dialysis.

Discussion: SSc with ANCA related vasculitis is increasingly reported in literature. Abnormal regulation of T helper cells leading to production of cytokines including IL-1 and IL-17 play important roles in the pathogenesis of both SSc and ANCA associated vasculitis. The presence of anti-MPO (p-ANCA) defines a subset of patients with SSc who are susceptible to crescentic glomerulonephritis. These patients may present in a manner identical to scleroderma renal crisis; yet treatment requirements differ significantly. We propose that the presence of ANCA be routinely investigated in the setting of renal failure in SSc.

FR-PO640

Silica Induced Dual ANCA Positive Vasculitis Nidhi Varma,[1] Rahil Kasmani,[2] Balhinder S. Brar,[2] Deepak K. Malhotra.[1] *[1]Nephrology, Univ of Toledo Medical Centre, Toledo, OH; [2]Nephrology, St. Vincent's Medical Centre, Toledo, OH.*

Introduction: Environmental factors have been reported to trigger autoimmune diseases in genetically susceptible subjects. While case reports of Silica with either ANCA MPO or ANCA PR3 has been seen,the presence of both in one patient has not been established.

Case Description: 53-year old man first presented to the ER with testicular pain, swelling, intermittent hematuria, arthralgia and myalgia.He was diagnosed with epididymitis and treated with ciprofloxacin and later doxycycline.Two weeks later he was admitted with worsening of symptoms and Creatinine of 2.1 mg/dl. He had no past medical history and was not on any meds. He had a 20-pack year history of smoking, no illicit drug use. He had worked as a sandblaster for 30 years. Exam was significant for a BP of 155/72, crackles on auscultation, leg edema and scrotal edema. UA showed 2+ proteinuria, dysmorphic RBCs with acanthocytes. Utox was negative.Proteinuria was 2.5 gms. Hb was 8.5 g/dl. ANCA MPO titer was 283 AU/ml and PR3 titer was 243 AU/ml.ANA, Anti SSA and Anti RMP were also positive. Anti ds DNA, anti Histone, anti Sm, anti GBM ab and HIV was negative. Hepatitis C ab was positive with normal complements. CT chest showed scarring in the upper trachea. Bronchoscopy was normal. Renal usg and Echo were normal. Kidney biopsy showed pauci-immune necrotizing GN with crescents. Methylprednisone 500mg IV and cyclophosphamide 200 mg PO were started. Plasmapharesis was also commenced for a total of 7 cycles. Later oral Prednisone and prophylactic antibiotics was started. Cr continued to rise despite therapy and peaked at 5.4 mg/dl and needed hemodialysis. After about 6 weeks into the illness, the patient presented with pneumonia and septic shock. Immunosuppression was stopped. His clinical condition continued to deteriorate and he died from sepsis and multi-organ failure.

Discussion: Dual ANCA positivity in vasculitides is very rare. Cases with Levamisole (contaminant with cocaine) and infectious endocarditis has been reported.The prognosis of these patients is grim. To the best of our knowledge, this is the only reported case of dual ANCA positive vasculitis induced by exposure to silica.

FR-PO641

Guillian-Barre Syndrome and Lupus Nephritis: Simultaneous Presentation and Remission. A Case for Neuro-Renal Syndromes Sofian Jamal Al-Khatib, Antonio Guasch. *Renal Div, Emory Univ, Atlanta, GA.*

Introduction: Inflammatory demyelinating polyneuropathies (IDP), whether the acute or chronic form, have been reported in association with glomerulonephritis. The glomerular pathologies have mainly included minimal change disease (MCD), membranous nephropathy, or focal segmental glomerulosclerosis (FSGS). Our purpose is to emphasize the frequent association, potential common mechanisms and treatment in this neuro-renal syndrome.

Case Description: A 33-year-old male presented with bilateral lower extremity paralysis, anasarca, and a serum creatinine of 1.76 mg/dL. Electromyography was consistent with Guillian-Barre Syndrome (an acute IDP). Positive serology: ANA 1:160 speckled pattern, dsDNA Antibody (Ab) IgG 65 IU/mL, anti-Smith Ab IgG 77 Units (U), RNP Ab IgG 49 U, SSA/Ro Ab 49 U, Immunoglobulin IgA 509 mg/dL. 24-hour urine collection with 19.3 grams proteinuria. Kidney biopsy revealed lupus nephritis class IV and V. Five days of plasmapheresis failed to improve symptoms. Pulse dose steroids and one dose of cyclophosphamide administered. The following day patient's strength began to improve. Patient discharged with steroids and followed in clinic. Patient received a total of 5 doses of cyclophosphamide, once per month along with a steroid taper. Proteinuria and neurological symptoms resolved simultaneously, with renal function returning to baseline.

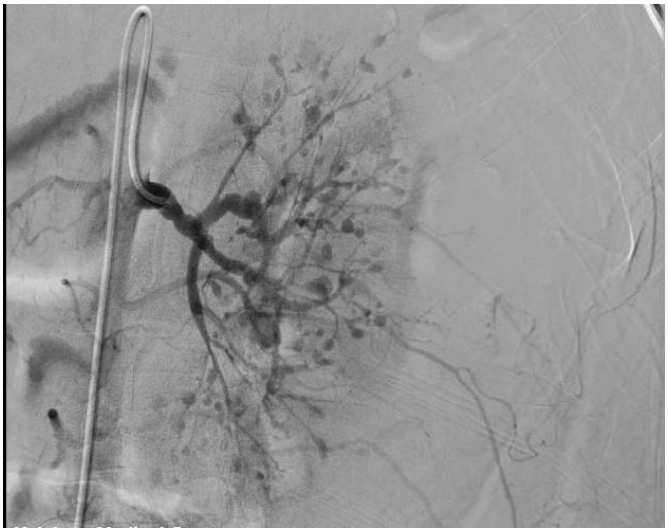
Discussion: We discuss a unique case of an IDP associated with Lupus Nephritis not before described in the literature. In previous reports, there has been an emphasis on minimal change nephropathy, FSGS and membranous nephropathy, suggesting a common etiology/pathophysiology. Experimental disease models, recent evidence proving that INF2 is responsible for cases of Charcot-Marie-Tooth neuropathy associated with FSGS by disrupting distinctly diverse Schwann cells and podocytes, and cases including this one showing a temporal existence and a positive response to immunosuppression for both the neuropathy and nephropathy in IDP's with glomerulopathies are consistent with a common mechanism or a component specific antibody causing both pathologies. This association may lead to further research in this newly described area of neuro-renal syndromes.

FR-PO642

Renal Polyarteritis Nodosa Coexisting with Lupus Nephritis Ramya Vejella, Scott McRight, Neeraj Singh. *Nephrology, LSUHSC Shreveport, Shreveport, LA.*

Introduction: We present a patient with biopsy proven lupus nephritis who presented with spontaneous perinephric hematoma and was diagnosed with polyarteritis nodosa (PAN) on renal arteriogram.

Case Description: A 30 year old African American female was referred to the Nephrology clinic for evaluation for proteinuria and microscopic hematuria. Patient had known history of thrombocytopenia requiring splenectomy, pleuritis and seizure disorder. Her current symptoms included persistent joint aches and weight loss of 15 lbs for 6 months prior to presentation. Further work-up showed serum creatinine of 1.1 mg/dl, 0.6 gm proteinuria on a 24 hour urine, microscopic hematuria, negative dsDNA, ANA > 1:640, Complement 3- 53 (normal range: 90-180), Complement 4- 17.8 (normal range 10-40), negative rheumatoid factor, and negative HIV screen. Patient underwent a left kidney biopsy which was consistent with Class III lupus nephritis. Approximately a month after her renal biopsy, she presented to the emergency room with right flank pain, anemia and hypotension. She denied any history of trauma. A CT scan of abdomen and pelvis without contrast showed a perinephric hematoma. A renal arteriogram was performed and the bleeding vessel was coiled. In addition, the angiogram showed numerous microaneurysms in the kidney consistent with polyarteritis nodosa.



Additional work-up showed a negative hepatitis B and C serologies, AST 28, ALT 10, negative ANCA with PR3 < 0.2 and negative myeloperoxidase at < 0.2. The patient is currently being treated with Cytoxan and high dose prednisone with follow up in our clinic.

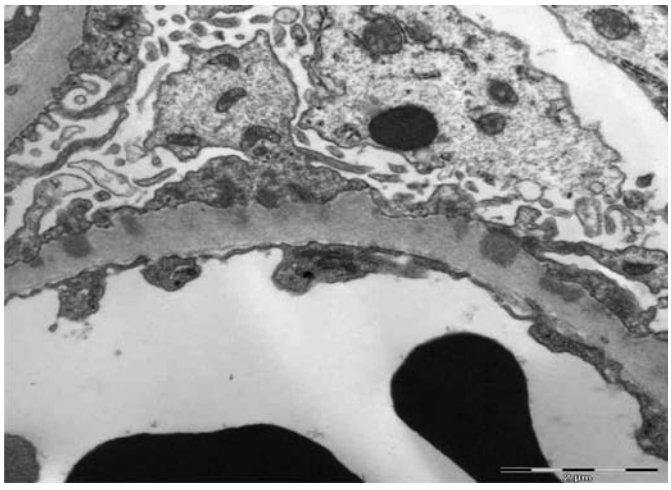
Discussion: The case of lupus nephritis and PAN coexisting in a patient has not been described in the literature before. This case highlights the probable role of lupus in the pathogenesis of medium vessel vasculitis which is typical of PAN.

FR-PO643

Biopsy Proven Lupus Nephritis in a Cocaine User with High ANCA Titers: Coincidence or Correlation? Rohini Prashar, Ginius Pradhan, Sandeep Vetteth. *Univ of Toledo Medical Center, Toledo, OH.*

Introduction: Levamisole-contaminated cocaine is now well recognized to be linked with ANCA Vasculitis. Some reports have also suggested cocaine as a causative factor for Lupus. We report an interesting case of a cocaine user with nephrotic syndrome, positive MPO and PR3 ANCA whose biopsy revealed Membranous Lupus Nephritis.

Case Description: A 37 year old female, habitual cocaine user with history of leg swelling was referred to us for nephrotic range proteinuria, quantified at 6.5 grams. She had lower extremity edema on physical examination and no skin lesions were noted. Investigations revealed normal creatinine, low complements, positive MPO (330 IU/ml) and PR3 (1265 IU/ml) ANCA. Her Anti-nuclear antibody and anti-dsDNA antibody were also positive. Kidney biopsy revealed diffuse thickening of capillary loops without evidence of vasculitis. Immunofluorescence showed diffuse IgA, IgG, kappa, lamda and C3 peripheral granular deposits. Electron Microscopy revealed electron dense deposits consistent with membranous nephropathy.



She stopped using cocaine and therapy with steroids and mycophenolate was initiated resulting in resolution of proteinuria, normalization of anti-ds DNA and reduction in ANCA titers.

Discussion: Despite widespread use of cocaine, there is still paucity of data linking it with autoimmunity and vasculitis. Illicit cocaine, adulterated with levamisole has been associated with ANCA vasculitis with both MPO and PR3 ANCA positivity. In a recent study,the presence of hypocomplementemia with anti-dsDNA antibodies raised the question of drug-induced lupus in a subset of these patients. We hypothesize that our patient might have sub-clinical extra-renal ANCA vasculitis which improved after stopping cocaine, along with the presence of lupus nephritis. Whether the co-existence of these two entities is a coincidence or correlation remains uncertain.

FR-PO644

Myeloperoxidase P-ANCA-Positive Crescentic Glomerulonephritis with Membranous Nephropathy: A Case Series Irfan K. Moinuddin, Bijin Thajudeen, Amy Nicole Sussman, Erika R. Bracamonte. *Div of Nephrology, Univ of Arizona Medical Center, Tucson, AZ.*

Introduction: Rarely, coincident PANCA GN and MN have been described. Idiopathic MN is associated 70% of the time with anti-PLA2R antibodies. The remaining 30% include ab to putative antigens such as neutral endopeptidase. Secondary MN is associated with antigens ranging from hepatitis, lupus, cancers, sphilis and schistosomiasis. Colocalization of MPO antigen and IgG deposits in capillary walls in cases of MN with PANCA vasculitis has been shown. It is postulated that the MPO antigen may promote both neutrophil chemotaxis and immune complex deposition.

Case Description: We report two cases of MN with crescentic GN due to PANCA vasculitis: Case 1 is a 50 year old female who presented for evaluation of rash and renal failure. The rash was a diffuse erythematous macular rash. Patient had a history of cocaine abuse. Patient had a nephritic urinalysis and proteinuria 1600 mg/d. Case 2 is a 30 year old male who presented with 2-3 months history of cold intolerance, weight gain and night sweats. Patient was treated for newly diagnosed Hashimoto's thyroiditis with levothyroxine and renal failure with fluid resuscitation with improvement in both. Renal failure recurred with a persistent nephritic picture, dysmorphic red blood cells and RBC casts.

Both cases had MPO and PANCA positivity. In both cases, the glomeruli showed spike formation on light microscopy and characteristic fine granular capillary loop immunofluorescence staining, predominantly for IgG. In addition, focal segmental necrotizing lesions with crescent formation were seen. Serologic testing for anti-PLA2R antibodies was negative in both cases.

Discussion: The coexistence of PANCA GN and MN supports the hypothesis that MPO may serve as an antigen for IgG deposition in glomerular capillary walls. Further studies are needed to demonstrate colocalization of IgG and anti-MPO, especially in cases of PANCA GN plus MN induced by levamisole and/or hashimoto's thyroiditis.

FR-PO645

Crescentic Glomerulonephritis Associated with Granulocytic Colony-Stimulating Factor (G-CSF): Case Report Carla Tenório Barros Cisne Pessoa, Nathalia K.N. Alecrim, Camila Barbosa L. Oliveira, Alline S.A. Oliveira, Cintia Germana Mergulhão da Costa, Daiane Silva, Luis H.B.C. Sette, Lucila Maria Valente, Gisele Vajgel Fernandes, Maria Alina G.M. Cavalcante. *Nephrology, Univ Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Introduction: Granulocytic colony-stimulating factor (G-CSF) is used as adjuvant therapy in oncologic patients with neutropenia. Stimulation of certain colonies of leucocytes can induce abnormal immune reaction leading to inflammation. Neutrophils are recognized as major cells in the development of crescentic glomerulonephritis. We report a case of crescentic glomerulopathy associated with the use of G-CSF.

Case Description: A 53 year-old woman with breast cancer (ductal carcinoma in situ) received four cycles of neoadjuvant chemotherapy (docetaxel, doxorubicin and cyclophosphamide) associated with G-CSF during four months, with partial remission. After 30 days of the last cycle she was admitted at the hospital with petechiae and purpura on the lower limbs and gross hematuria. Exams showed serum creatinine of 3.9 mg/dL (baseline: 0.6 mg/dL). Blood levels of complement (C3 and C4) were normal and testing was negative for antinuclear antibodies, c-ANCA, p-ANCA, hepatitis B and C and HIV. She had no signs of infection or hypovolemia and no history of nephrotoxic drug use. Renal biopsy showed acute crescentic glomerulonephritis, tubular atrophy with multifocal hyaline casts and moderate interstitial fibrosis. Immunofluorescence was negative. She received methylprednisolone 1g for three days followed by monthly cyclophosphamide and showed improvement of skin lesions and serum creatinine decreased to 0.9 mg/dL.

Discussion: Studies demonstrate that endogenous G-CSF has a role in experimental glomerulonephritis and literature review revealed a few cases of possible association of exogenous G-CSF with acute glomerulonephritis in humans, including two case reports of crescentic glomerulopathy. The aim of this study is describe a case of crescentic glomerulonephritis developed during the use of G-CSF without another triggering factor, highlighting the importance of routine monitoring of renal function in patients using this medication.

FR-PO646

ANCA-Negative ANCA-Associated Crescentic Glomerulonephritis with Concurrent C1q Deposits Richa A. Pandey, Arvind K. Garg, Seth Goldberg, Tingting Li. *Nephrology and Hypertension, Washington Univ School for Medicine, St. Louis, MO.*

Introduction: Anti Neutrophil Cytoplasmic Antibody (ANCA) associated vasculitis (AAV) causes pauciimmune glomerulonephritis (GN), with cellular crescents. Upto 30% patients with AAV lack ANCA. There are rare reports of C1q deposits in the glomerular mesangium in patients with AAV. There is one case report of C1q nephropathy with crescentic GN. We describe a case ANCA negative AAV with strong mesangial C1q deposition.

Case Description: 68 year old caucasian man presented with tea colored urine, hemoptysis and a petechial rash for 14 days duration. Past medical history of type 2 diabetes, hypertension, aortic stenosis. Admission labs showed hemoglobin of 8.1 gm/dl, platelet count 91,000/cumm, serum creatinine 8.5 mg/dl with, potassium 7.1 meq/dl, LDH 450 units/L, reticulocyte count 3.5%, haptoglobin <10 mg/dl, red blood cell casts in the urine sediment, negative ANA and ANCA, normal serum complement C3 and

C4, anti glomerular basement membrane antibody. Bronchoscopy showed evidence of alveolar hemorrhage. He was initiated on hemodialysis for anuric renal failure. Kidney biopsy showed paucimmune crescentic glomerulonephritis, with 3+ C1q staining in the glomerular mesangium on immunofluorescence. Electron microscopy did not show any electron dense deposits. Skin biopsy showed C1q deposition around dermal capillaries. He was treated with intravenous cyclophosphamide and pulse dose steroids. Following initial therapy hematologic parameters, and hemoptysis improved, although he remained hemodialysis dependent.

Discussion: ANCA associated vasculitis is typically pauciimmune, with no evidence of immune complex deposition or complement activation. Recent studies have evaluated the role of complement in pathogenesis of AAV. In a renal biopsy study, 7 cases of AAV had evidence of C1q deposition, only 1 was quantified as 3+. Our patient presented with pulmonary renal syndrome, had negative ANCA levels and normal serum complements. C1q nephropathy is known to present with crescentic GN, but there are no cases with evidence of systemic vasculitis. To the best of our knowledge this is the first reported case of ANCA negative AAV with strong C1q deposition.

FR-PO647

Atypical P-ANCA Associated Crescentic Glomerulonephritis Presenting as Usual Interstitial Pneumonia Kelly Mazurek,[1] Gaurav Alreja,[2] Eric Loman,[1] Ruchir D. Trivedi.[2] *[1]Internal Medicine, Univ of Connecticut Health Center, Farmington, CT; [2]Nephrology, Univ of Connecticut Health Center, Farmington, CT.*

Introduction: Immunofluorescence (IF) positive anti-neutrophil cytoplasmic antibody (ANCA) patterns not fitting the typical description of cytoplasmic and perinuclear are called atypical ANCA. Sera giving rise to such IF patterns target multiple antigens (nuclear, cytosolic and various granular autoantigens) in neutrophils and lack specificity for either myeloperoxidase or proteinase 3 enzymes. Atypical ANCA, although commonly described with non-vasculitic (inflammatory bowel disease, sclerosing cholangitis, connective tissue diseases) and infectious pathologies, is rarely associated with systemic necrotizing small vessel vasculitis. An uncommon case of atypical ANCA manifesting as renal and pulmonary vasculitis is presented.

Case Description: 85-year-old woman with prolonged upper respiratory tract symptoms developed worsening cough and acute kidney injury. Medical history included hypertension, hypothyroidism and an intermittent three-month fever. CT scan of chest revealed diffuse subpleural reticulation and honeycombing of bilateral lower lung lobes consistent with Usual Interstitial Pneumonia. Laboratory data revealed leukocytosis, anemia, and Cr 3.2 mg/dL. Urinalysis showed RBC casts, dysmorphic RBCs and urine protein 1558 mg/g Cr. Autoimmune workup was negative and atypical p-ANCA 1:2560. Kidney biopsy showed segmental fibrinoid necrotizing lesions with cellular crescents and disruption of Bowman's capsule consistent with pauci-immune necrotizing crescentic glomerulonephritis. The patient received aggressive treatment of six cycles of cyclophosphamide with tapering steroids.

Discussion: Majority of ANCA associated crescentic GN and pulmonary fibrosis present with MPO-ANCA. Current literature has not identified atypical ANCA with severe forms of multisystem vasculitis. Our patient had a unique multisystem atypical p-ANCA small vessel vasculitis, with initial presentation as usual interstitial pneumonia a month before onset of renal dysfunction, and eventual renal failure. Both renal and pulmonary function improved substantially with cyclophosphamide and steroids.

FR-PO648

ANCA-Associated Vasculitis Presenting as a Unilateral Adrenal Mass Nyan W. Phyo,[1] Ghayyath Sultan,[1] Sherry L. Werner,[2] Wajeh Y. Qunibi.[1] *[1]Dept of Nephrology, UTHSCSA, San Antonio, TX; [2]Dept of Pathology, UTHSCSA, San Antonio, TX.*

Introduction: ANCA-associated vasculitis commonly presents with pulmonary-renal syndrome but can rarely present with lung or renal mass, malignancy, parotid enlargement or spinal mass. We report here the case of a patient who presented with abdominal pain and a right adrenal mass.

Case Description: A 41 year old Hispanic female was admitted to our hospital with persistent abdominal pain for one week. Laboratory data revealed microscopic hematuria, 3.7 gram proteinuria and creatinine of 4.2 mg/dl. CT scan of abdomen showed large right adrenal cystic lesion (12.3 x 11 cm) which was found nonfunctional. Cyst aspiration revealed bloody drainage with negative cytology. Further work-up showed positive anti-nuclear antibody (titer 1:160) and elevated myeloperoxidase antibody at 64 (normal 0-21 units). Hepatitis B, HCV, HIV, RPR, Anti-dsDNA, and Anti-proteinase-3 antibodies were negative. Complements levels were normal. Kidney biopsy revealed necrotizing crescentic glomerulonephritis with negative immunofluorescence staining establishing a diagnosis of ANCA- associated vasculitis. Patient was treated with plasma exchange, intravenous methyl prednisone, intravenous cyclophosphamide and initiated on hemodialysis due to hyperkalemia and worsening kidney function. She remained dialysis-dependent after 12 weeks despite continuing immunosuppressive drugs.

Discussion: ANCA associated vasculitis rarely presents as a mass lesion. The initial focus on adrenal mass in our patient resulted in delay in diagnosis of vasculitis. Given that early diagnosis and treatment are crucial for improving prognosis, vasculitis should be suspected in patients with hemorrhagic adrenal cysts.

FR-PO649

Treatment-Refractory FSGS in a Patient with Systemic Lupus Erythematosus Kristin Meliambro, Shuchita Sharma, Kirk N. Campbell. *Nephrology, Icahn School of Medicine at Mount Sinai, NY, NY.*

Introduction: Lupus podocytopathy is an increasingly recognized entity in nephrotic patients with systemic lupus erythematosus (SLE) that is distinct from classic immune-complex mediated disease. Case series have described SLE-podocytopathy as typically steroid responsive. Here we present a case of podocytopathy in a patient with SLE with the tip lesion variant of FSGS.

Case Description: This is a 60 year old female with long-standing SLE (20+ years) who presented to nephrology with proteinuria and hypertension. Her physical exam was notable for a malar rash and 1+ bilateral lower extremity edema. She had a urine protein/creatinine (P/C) ratio of 3.06 mg/mg with an eGFR of 42 cc/min and a serum albumin of 2.9 g/dl. Urinalysis revealed 11-25 RBCs/hpf. Mycophenolate Mofetil 500 mg BID had been initiated by rheumatology 4 weeks prior for extrarenal lupus manifestations including arthralgias and the malar rash. She had also been taking Captopril 12.5 mg TID. She did not tolerate hydroxychloroquine due to retinal toxicity. A kidney biopsy was performed that revealed 22 glomeruli, one with segmental sclerosis, tip variant. Electron microscopy (EM) revealed diffuse podocyte foot process effacement. There were no basement membrane or immune deposits by EM or immunofluorescence. Her urine P/C ratio peaked at 14.6, averaging 5-7 g urinary protein per day. The MMF was discontinued due to the development of herpes zoster which was treated with acyclovir. The patient completed 6 month courses of Prednisone 60 mg daily then cyclosporine 2 mg/kg/day with persistent proteinuria of 7-8 grams per day and an eGFR of 30 cc/min. Acthar gel was started at 80 units subcutaneously twice weekly. After 16 months on Acthar her nadir urine protein excretion was 1.3 g. At the time of last follow up, the patient had approximately 4 g of proteinuria and an eGFR of 25 cc/min.

Discussion: This patient's clinical course highlights the potential refractory course of non-canonical lupus-associated renal disease even in the presence of seemingly favorable histologic features. Further studies will be needed to determine the optimal treatment regimen for these patients.

FR-PO650

Unusual Presentation of Wegener's Granulomatosis with Hypercalcemia Samir A. Brahmbhatt, Robert D. Zenenberg. *Internal Medicine and Nephrology, Morristown Medical Center, Morristown, NJ.*

Introduction: Hypercalcemia has been known to be present in granulomatous diseases due to extra-renal production of 1,25-dihydroxy-vitamin D by disease-activated macrophages. It has rarely been known to occur in granulomatosis with polyangiitis (Wegener's) abbreviated as GPA. We present a case of an elderly female who presented with acute renal failure and symptomatic hypercalcemia, diagnosed as GPA. The hypercalcemia workup was non-diagnostic, but the presumption was the same mechanism, and consistent with this, it responded briskly to steroid therapy.

Case Description: 73-year-old female with coronary artery disease, controlled hypertension, ulcerative colitis and hypothyroidism was sent to the ER for hypercalcemia, unintentional 30lb weight loss, anemia and acute renal failure. Initial creatinine was 3.4mg/dl, 52mg/dl BUN, calcium 13.8mg/dl. Urinalysis showed microscopic hematuria, 3+ proteinuria, without red or white cell cast. Imaging studies of the abdomen/pelvis was unremarkable. A paraprotein mediated renal disease workup was negative. The hypercalcemia workup was inconclusive with elevated phosphorus, suppressed iPTH, negative PTHrp, high 25 $(OH)_2D$, but low 1,25 $(OH)_2D$. No signs of malignancy on imaging. No history of calcium supplement usage. Further investigation showed normal TSH, negative QuantiFERON®TB test, no signs of a fungal infection or other granulomatous diseases, but elevated ANA and myeloperoxidase ANCA. A kidney biopsy showed pauci-immune extra capillary proliferative glomerulonephritis consistent with ANCA positive disease. Patient was treated with steroids and anti-CD20 antibody with improvement in her renal insufficiency. Her calcium level normalized to 9.1 within a month.

Discussion: There are only 3 reported cases of hypercalcemia in GPA. They were associated with unregulated production of 1,25 $(OH)_2D$ by inflammatory cells. Although the comprehensive workup for hypercalcemia was largely negative, the presumption that extra-renal production of activated vitamin D was the underlying mechanism is not confirmed, but may be suggested here. This case reinforces that like other granulomatous diseases, GPA should be considered in the differential diagnosis of hypercalcemia.

FR-PO651

A Case of Systemic Lupus Erythematosus with Anti Myeloperoxidase Antibodies, Cerebral Vasculitis and Presumed Lupus Nephritis Responding to Therapeutic Plasma Exchange: A Remarkable Recovery from a Critical Illness Tamara T. Rubenzik, Umut Selamet, Amber P. Sanchez. *Nephrology and Hypertension, Univ of California San Diego, San Diego, CA.*

Introduction: MPO-ANCA antibody (Ab) in the setting of systemic lupus erythematosus (SLE) is uncommon and the significance unclear. We report the case of a patient with newly diagnosed SLE complicated by presumed lupus nephritis, antiphospholipid antibody syndrome (APLA), and persistent seizures despite cyclophosphamide and steroids, who was treated with therapeutic plasma exchange (TPE) with subsequent improvement in neurologic and renal function.

Case Description: A 20 year old male presented with altered mental status, seizures, fevers, polyarthritis, anemia, and rash. Serologic work up revealed positive ANA (>1:640), anti-dsDNA (>200), ANCA (1:1280), MPO Ab (181) and low compliments.

He was diagnosed with SLE and APLA with a cerebral vein thrombosis. The presence of proteinuria, hematuria and an elevated creatinine was consistent with lupus nephritis though the need for uninterrupted anticoagulation precluded a renal biopsy. Despite treatment with cyclophosphamide and steroids, he continued to decline with intractable seizures, renal failure, and a CT angiogram revealing cerebral vasculitis. TPE has been a useful adjunctive therapy in select cases of SLE with central nervous system involvement, as well as in ANCA-associated vasculitis, and was initiated on hospital day 25. Following five TPE treatments his seizures subsided, mental status improved, and creatinine and proteinuria began to downtrend. Treatment with cyclophosphamide and high dose prednisone continued, and he was discharged on hospital day 53. Three months after discharge he maintains normal neurologic and renal function and is independent in activities of daily living.

Discussion: The presence of an MPO-ANCA in the setting of SLE has been associated with crescentic glomerulonephritis in addition to other systemic manifestations similar to ANCA vasculitis. These patients have been reported to respond to treatment with IVIG, tacrolimus, and TPE. TPE proved to be a successful adjunctive therapy in our patient and should be considered in SLE patients with anti MPO antibodies.

FR-PO652

SLE with Fabry's Disease, Is It Too Rare? Pongsathorn Kue-A-Pai,[1] Napat Leeaphorn,[1] Wonngarm Kittanamongkolchai,[2] Raquel M. Rosen.[1] *[1]Internal Medicine, Bassett Medical Center, Cooperstown, NY; [2]Nephrology, Mayo Clinic, Rochester, MN.*

Introduction: Fabry's disease is an uncommon X-linked lysosomal storage disorder. On the other hand, SLE is a common autoimmune disease worldwide. We describe a case of a man with both SLE and Fabry's disease. The relationship between the two conditions and literature review are discussed.

Case Description: A 34-year-old man with history of chronic joints pain who presented with pleuritic-type chest pain, worsening of shortness of breath. Notable exam findings included temperature of 39 degrees Celsius, hypertension and crackles at bases on lung exam. Serum creatinine was 2.9 mg/dL. WBC was 4,800/mm^3. Hemoglobin was 8.9 mg/dL. Urinalysis showed protein 3+ and hematuria. Microscopic urine exam revealed RBC cast. ANA and double stranded DNA were positive. C3 was 70 mg/dL. C4 was 7.3 mg/dL. ESR was 100 mm/hr. Patient underwent renal biopsy which showed diffuse endocapillary proliferative glomerulonephritis with full house immune staining. Electron micrograph revealed Zebra bodies in the podocytes. Diagnosis of SLE nephritis class IV was made. IV steroid and cyclophosphamide were initiated. His condition improved. He was discharged with oral steroid. Subsequent plasma globotriaosylceramide, serum and leukocyte enzyme assay confirmed diagnosis of Fabry's disease.

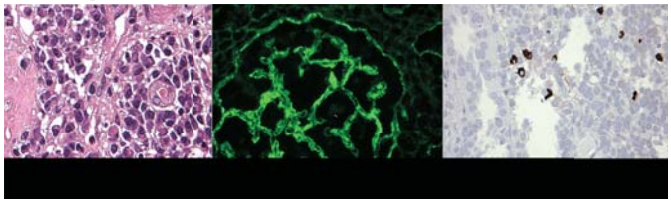
Discussion: Renal involvement of Fabry's disease almost occurs in male hemizygotes and less common in female heterozygote. Approximately 85% of patient with Fabry's disease will have proteinuria which will precedes chronic renal insufficiency but nephrotic-range proteinuria was uncommon. Review of literature revealed 2 previous case reports of lupus nephritis associated with Fabry's disease. This presenting case is the first case in literature who is a man. Although association between these two diseases is not established, recent in vivo study demonstrates lipid metabolism defects including globotriaosylceramide contribute to SLE pathogenesis and also suggest that targeting Glycosphingolipids biosynthesis restores T cell function in SLE. We propose that increase cellular and plasma globotriaosylceramide (Gb3) in Fabry's disease might facilitate in SLE pathogenesis.

FR-PO653

An Overlapping Case of Lupus Nephritis and Immunoglobulin G4-Related Kidney Disease IgG-4 RKD Ahmad Eter,[1] Suzanne E. El Sayegh,[1] Elie El-Charabaty.[1] *[1]Nephrology, Staten Island Univ Hospital, Staten Island, NY; [2]Nephrology, Staten Island Univ Hospital, Staten Island, NY; [3]Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Introduction: The renal pathology can be a dilemma especially in Lupus patients as it mimics different diseases and one of them is IgG-4 RKD, which is a recently recognized disorder defined by dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells with frequently observed elevated antinuclear antibody ANA.

Case Description: We report a 71 year-old Philippino female with PMH of HTN and hypothyroidism who presented with abdominal pain. Physical exam indicated epigastric tenderness. Labs showed BUN100, creatinine 9.27 (baseline 1.2), albumin 1.5, urine microscopy no RBC casts, and TP/Cr 2.6 gr. CT imaging revealed multiple granulomas in rt upper lobe, and inflammatory stranding around the duodenum. Kidney function worsened in the next days and hemodialysis was initiated. Serology was positive for HBsAg, ANA, urine and serum immunofixation indicated free Lambda band and faint bands of IgG, IgG-4 sub-class was normal, total IgG was elevated, and ds-DNA was negative. Bronchoscopy was not diagnostic. Kidney biopsy showed tubulointerstitial nephritis TIN and acute tubular necrosis with abundant tubular basement membrane deposits staining for "full house" thus suggesting IgG-4 related TIN versus membranous and interstitial Lupus Nephritis. Patient did not respond to steroids but responded to mycophenolate mofetil and kidney function improved.



Discussion: This is a challenging case as pathology was inconclusive for definite diagnosis. The full house deposits, positive ANA, and normal IgG-4 levels suggest lupus nephritis, but the findings of IgG-4 positive plasma cells, negative symptoms for systemic lupus, inactive urine sediment, and the negative ds-DNA would suggest a possible diagnosis of IgG-4 related TIN.

FR-PO654

Post Infectious Glomerulonephritis with Positive C-ANCA and Anti PR3 Antibodies Ahmed Zeen Alabedeen Alrifai,[1] Barry M. Wall.[1,2] *[1]Univ of Tennessee Health Science Center, Memphis, TN; [2]VAMC, Memphis, TN.*

Introduction: In the setting of acute glomerulonephritis (GN), the presence of c-ANCA (anti-PR 3) is highly suggestive of granulomatous polyangiitis (GPA). However, similar serologic findings have been described with postinfectious GN.

Case Description: 45 year old male with ulcerative colitis and primary sclerosing cholangitis presented with bloody stool for 5 days and dark colored urine for 1 wk. He had been treated 3 wk prior with clindamycin for root canal infection and 2 wk prior with ciprofloxacin for suspected epididymitis. Laboratory: serum creatinine 5.1 mg/dl (prior value, 0.8 mg/dl). Urinalysis was positive for 2+ protein, 3+ blood with >100 RBCs and WBC, and granular casts in the sediment. Urine protein creatinine ratio was 1.1. (Previous urinalyses were normal). C-ANCA titers were positive: 1:320 with positive anti-proteinase 3: 80 (high positive >30). P-ANCA titers were negative and prior ANCA studies had been negative. Screening tests for anti-nuclear antibodies, ASO titers, HIV, hepatitis B and C were negative. CH50 and C4 were normal, while C3 was low, 54 mg/dl (90- 180 mg/dl). Initial blood and urine cultures were negative. Oral prednisone therapy (1.0 mg/kg) was initiated. Kidney biopsy was suggestive of post-infectious GN; LM- acute proliferative and exudative glomerulonephritis with crescent formation, IF: positive for C3, EM showed scattered subepithelial hump like deposits. Additional blood cultures were positive for Streptococcus viridans and echocardiography was suspicious for valvular vegetation. Prednisone was discontinued and 6 weeks of antibiotics were administered for presumed endocarditis. Blood cultures became negative and renal function gradually returned to baseline without immunosuppressive therapy. During long term follow up serum creatinine is 0.8mg/dl, urinalysis is normal, and ANCA titers are negative.

Discussion: Determining whether acute GN is ANCA *mediated* (as in GPA, requiring immunosuppression) versus ANCA *associated* (as in infection- associated GN, requiring treatment of underlying infection) is crucial in determining appropriate treatment. Kidney biopsy continues to be necessary for making this distinction.

FR-PO655

ANCA Positive Necrotizing Glomerulonephritis Associated with Enterococcal Infective Endocarditis Shriharsha Kallahalli Jayaramu, Yuvaraj Thangaraj, Girish Singhania, Radhika Vemuri, Keerti K. Bhanushali, Gurjit Dhatt. *Nephrology, Univ of Florida, Gainesville, FL.*

Introduction: Crescentic glomerulonephritis secondary to enterococcal infective endocarditis is rare and mostly associated with immune complex deposition in renal biopsy. Here, we report a case of pauci immune crescentic glomerulonephritis associated with enterococcus infective endocarditis.

Case Description: 24 year old male with history of intravenous opioid abuse presented with 20 lbs weight loss, fatigue, nausea, vomiting and hematuria of three months duration. On physical exam, BP 137/91 mmHg, HR of 92/min, RR 20/min, diffuse crackles on lung exam and 2+ pitting edema in bilateral lower extremities. Labs: Serum creatinine of 11 mg/dL, BUN of 110 mg/dL, hemoglobin of 7g/dL, LDH of 204 U/L and haptoglobin of 265 mg/dL. Peripheral smear did not show any schistocytes. Urinalysis showed several dysmorhic RBC and two RBC casts. Serology: Hepatitis and HIV negative, ANA was weakly positive, P-ANCA was positive, C3 was low and C4 complement was normal. Blood cultures were positive for enterococcus 4/4. Transesophageal echocardiogram showed vegetation on posterior mitral leaflet. Ultrasound showed echogenic kidneys. Renal biopsy: Severe necrotizing and crescentic glomerulonephritis without evidence of immune deposits in immunofluorescence and electron microscopy. Infective endocarditis was successfully treated with intravenous antibiotics. He received pulse solumedrol and subsequently developed florid heart failure due to severe mitral regurgitation and underwent mitral valve replacement. At present, the patient continues to be dialysis dependent without any evidence of renal recovery.

Discussion: Diffuse crescentic necrotizing glomerulonephritis is the most common pattern of glomerular injury seen in Infection Related Glomerulonephritis (IRGN) and is often pauci immune. The major differential diagnosis of this clinical picture is ANCA vasculitis but upto 10% of IRGN can show ANCA positivity. Early recognition of IRGN and its varied histological manifestations is imperative for appropriate management.

FR-PO656

Eculizumab Induced Reversal of Dialysis Dependent Renal Failure from Crescentic C3 Glomerulonephritis Melissa Inman,[1] Ginnie Prater,[1] Anant Kharod,[1] Huma Fatima,[2] Eric L. Wallace.[1] *[1]Medicine, UAB, Birmingham, AL; [2]Path, UAB, Birmingham, AL.*

Introduction: Dysregulation of the alternative complement pathway has been implicated in thrombotic microangiopathy, atypical hemolytic uremic syndrome (aHUS), dense deposit disease, and C3 glomerulonephritis (C3GN). Eculizumab is an anti-C5 monoclonal antibody approved for treatment of paroxysmal nocturnal hemoglobinuria and aHUS. Case series have shown eculizumab's potential as a treatment for C3GN but data is limited.

Case Description: A 38 year-old woman with idiopathic urticaria and well-controlled type II diabetes mellitus was sent to the emergency room for a blood pressure of 200/100 mmHg. Laboratory evaluation revealed serum creatinine of 11.0 mg/dL (eGFR 5 mL/min per MDRD formula) up from a baseline creatinine of 1.0 mg/dL. Urinalysis showed 4+ protein and 3+ blood with protein to creatinine ratio of 6.82 g/g. Hemoglobin 6.6 g/dL and platelet count 223 K/mm^3. C3, C4, anti-dsDNA, and ANCA titers were unremarkable. Hemodialysis and empiric steroids were initiated. A kidney biopsy showed 23/49 glomeruli with crescents, immunofluorescence with diffuse mesangial staining for C3 alone, and 10% interstitial fibrosis consistent with C3 glomerulonephritis. The patient failed to improve on steroids and was transferred to University Hospital. Evaluation of the alternative pathway revealed elevated soluble MAC level (0.36 mg/L), positive complement hemolytic assay (6.17%), and two deletion mutations in the CFHR3-CFHR1 gene. Plasma exchange was initiated for 3 treatments. Due to her acute presentation and minimal fibrosis on biopsy, eculizumab was initiated at standard dosing. The patient was discharged on peritoneal dialysis and continued eculizumab. After initiation of eculizumab, the patient's renal function continued to improve. Five months after initiation of therapy, dialysis was discontinued and 24-hour urine showed a creatinine clearance of 34 ml/min.

Discussion: Eculizumab should be considered for treatment of C3GN even in patients who have acutely become dialysis dependent. Adequate time for response to therapy (3 months minimum) should be given as patients can have slow recoveries but be rendered dialysis independent after such time.

FR-PO657

Membranous Nephropathy Associated with Autoimmune Thyroiditis

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Introduction: Membranous nephropathy is the most common cause of nephrotic syndrome in adults and it is well known to be associated with many other conditions, including autoimmune disorders.

Case Description: 24-year-old female with history of Type I Diabetes Mellitus presented with one-month history of generalized edema associated with cold intolerance and hair loss. Her vital signs were normal. Physical exam revealed anasarca. Laboratory data showed iron deficiency anemia, normal serum creatinine, nephrotic range proteinuria, microscopic hematuria, hypoalbuminemia, Hgb was A1C 9.5%, complements were normal, autoimmune serologies were negative, Hepatitis B, Hepatitis C and HIV were negative, RPR was non reactive, TSH was 112.9 mIU/L, FreeT4 0.47 ng/dL, Free T3 was 1.6 ng/dL, Thyroperoxidase antibody (TPO) was 892 mIU/L, Thyroglobulin antibody was 35.3mIU/L, and Thyroid-stimulating IG (TSI) was normal. Renal ultrasound showed normal sized kidneys without abnormalities. Renal veins were patent bilaterally. Renal biopsy showed characteristic features of membranous glomerulopathy with segmental holes and rare spikes of the glomerular basement membrane along with eosinophilic deposits by LM, 2 to 3+ near full-house diffuse global finely granular capillary loops and segmental mesangial staining by IF and frequent small subepithelial immune complex deposits with scattered large mesangial immune complex deposits by EM. PLA2R staining was negative. She was started on thyroid hormone replacement, diuretics, and ACEi. After one month follow up, there was a mild improvement in proteinuria and hypoalbuminemia. Unfortunately, patient failed to follow up thereafter and no long-term follow up is available.

Discussion: To the best of our knowledge, only few cases of coexistent autoimmune thyroiditis with membranous nephropathy have been reported in the literature. The direct pathogenic link between these two diseases is unclear, but a same immune-mediated process affecting both organs is suspected. Optimal therapy for membranous nephropathy in the setting of autoimmune thyroiditis is not well defined. Our case shows a relationship between normalization of thyroid hormones values with decrease in proteinuria.

FR-PO658

Conditional Tissue Specific CTGF Over-Expression Results in Over-Activated Kidney Stroma

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Background: Connective tissue growth factor (CTGF), a member of the CCN gene family, is an extracellular matrix (ECM)-associated heparin-binding protein involved in matrix production. CTGF has been shown to contribute to progression of fibro-proliferative diseases and scarring by modifying of proliferation, migration, and adhesion of fibroblasts. CTGF has also been shown to play a role in ECM remodeling in normal physiological processes including embryogenesis, implantation, and wound healing. However recent new insights into the pathogenesis of fibrosing kidney diseases lead us to re-evaulate CTGF in this context at a cellular level. We therefore generated a mouse model which facilitates tissue specific, conditional over-expression of CTGF using homologous recombination.

Methods: A targeting vector to the ubiquitously expressed Rosa26 locus was cloned harboring a splice acceptor, chicken b-actin promoter followed by a floxed Neo-Stop cassette upstream of *CTGF*, followed by an internal-ribosomal-entry-site (IRES) and EGFP. Targeting vector was sequenced, validated in-vitro, and subsequently electroporated into mouse ES cells. Long-range PCR screens identified correctly targeted clones. Clones were injected into diploid mouse blastocysts and chimeras generated. Founder mice were bred, and offspring with transgene were expanded. To characterize this model, Foxd1 GFPCre/+ mice were utilized to over-express CTGF in kidney stromal populations. Immunostaining and WB was performed. Pericytes from Foxd1GC/+ crosses were isolated.

Results: Analysis of neonatal kidneys from mice derived via Foxd1 GC/+ X Rosa-Caggs-loxp-NeoStop-loxp-CTGF-IRES-GFP crosses displayed over-activated stroma. Kidney sections revealed hyper-cellularity in the interstitium. Immunohistochemistry showed an infiltration of leukocytes. Stromal cells isolated from CTGF over-expressing mice were more proliferative, migratory, and had a significantly higher expression of aSMA than controls.

Conclusions: CTGF over expression restricted to kidney stroma *in vivo* is sufficient to trigger cell activation and myofibroblast transition.

FR-PO659

The Role of Angiopoietin-Like Protein 2 in Renal Fibrosis

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Background: Renal fibrosis is a final common pathway in the progression of CKD. Previously, we reported that abundant expression of Angiopoietin-like protein 2 (ANGPTL2) plays important pathological roles in various non-infectious inflammatory diseases. However, little has been reported about the evidence of ANGPTL2 to be involved in the progression of renal disease.

Methods: We performed Azan-Mallory staining and ANGPTL2 immunohistochemistry in exenterated kidneys from non-invasive ureteral carcinoma patients to verify the localization of ANGPTL2 expression in the pathogenesis of renal fibrosis. Next, we performed UUO to wild type (WT) mice and *ANGPTL2* knockout (KO) mice. Fourteen days after the UUO surgery, those mice were sacrificed and kidney samples were collected.

Results: The immunohistochenical analysis of human kidney showed that ANGPTL2 is marginally expressed in non-fibrotic kidney. On the other hand, in fibrotic kidney, ANGPTL2 expression was increased and was predominantly observed in renal tubule cells. In the animal study, ANGPTL2 mRNA and protein levels were markedly increased in the UUO kidney of WT mice. Histological fibrotic area, mRNA expression of extra cellular martix proteins, including fibronectin, collagen I, III and IV, and protein level of FN and collagen IV were also increased in the UUO kidney of WT mice. In addition, mRNA and protein expression of TGF-β1 and its target molecule CTGF were elevated in the UUO kidney of WT mice. However, the increase of fibrotic markers and pro-fibrotic factors were significantly ameliorated in the UUO kidney of *ANGPTL2* KO mice.

Conclusions: Our current investigation suggested that ANGPTL2 derived from renal tubule cells has a crucial role to promote renal fibrosis. In the pathogenesis of renal fibrosis, ANGPTL2 may regulate TGF-β1 expression and its signaling. These data lead us to consider that ANGPTL2 would be a new therapeutic target against renal fibrosis.

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FR-PO660

Pericyte and Myofibroblast Activation Regulated by the TWEAK/Fn14 Pathway

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Background: Pericytes, mesenchymal cells that partially wrap capillary endothelium and are critical for supporting their integrity, have been shown by genetic fate-mapping to be a major progenitor pool for myofibroblasts in a number of fibrotic contexts including kidney fibrosis.In addition, pericytes provide directional cues that route the innate immune cells extravasating into injured tissue. Previously it was shown that TWEAK, a TNF family cytokine produced largely by leukocytes, promotes macrophage infiltration and fibrosis in the UUO model of renal injury. We hypothesized that TWEAK mediates its effect through its injury-inducible receptor, Fn14. In addition, since Fn14 is expressed by mesenchymal lineage cells and regulates their fate, we hypothesized that TWEAK/Fn14 might be a key molecular axis for pericyte activation in response to tissue injury.

Methods: -UUO: Unilateral Ureter Obstruction surgery -Proliferation and migration of primary cultures of pericytes. Anti-Fn14 and anti Tweak antibodies, Fn14 knockout mice. Disease model characterization-Illumina array and analysis.

Results: We now show that Fn14 deficiency ameliorates myofibroblast appearance and renal fibrosis in the UUO model, phenocopying TWEAK deficient mice. TWEAK activates primary murine cultured renal pericytes through Fn14, inducing low level proliferation, migration and production of proinflammatory mediators, as well as pericyte to myofibroblast transition measured by induction of a-smooth muscle actin expression and stress fiber formation. Differentiated myofibroblasts are also activated by TWEAK/Fn14 signaling, exhibiting higher-level proliferation, migration and cytokine production and cytoskeletal changes. Transcriptional profiling of TWEAK-stimulated pericytes and myofibroblasts supports its multifaceted role and identified TWEAK-response genes.

Conclusions: These findings suggest that targeting the TWEAK/Fn14 pathway is an approach to modulate pericyte and myofibroblast activation and a novel way to target both inflammatory and fibrotic aspects of fibrotic disease.

FR-PO661

Combination of Restoring Glomerular VEGF Expression and ARB Treatment Induces Additional Regression of Glomerulosclerosis in 5/6Nx Mice Ying Wu,[1,3] Shaojun Liu,[2,3] Ji Ma,[4] Haichun Yang,[3] Agnes B. Fogo.[3] *[1]Pediatric Nephrology, Children's Hospital, Jiaotong Univ, Shanghai, China; [2]Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China; [3]Pathology, Microbiology and Immunology, Vanderbilt Univ, Nashville, TN; [4]Pediatric Nephrology, Vanderbilt Univ, Nashville, TN.*

Background: High dose ARB induces glomerulosclerosis regression in part by decreasing matrix accumulation. Increased glomerular capillary growth, which can be stimulated by VEGF, may also contribute to regression of established glomerulosclerosis. In this study, we investigated whether combination of ARB and restoring VEGF could enhance regression of glomerulosclerosis.

Methods: Subtotal nephrectomy (5/6Nx) was performed in podocin-rtTA/TRE-human VEGF (RV) and podocin-rtTA mice (R). Glomerulosclerosis index (SI, 0-4 scale) was assessed by renal biopsy at 8 weeks. From week 8 until 12, doxycycline was added in drinking water to induce VEGF specifically in podocytes. Mice were divided into four groups with equal average SI at week 8: R group (n=11), RV group (n=10), R+ARB group (n=12), RV+ ARB group (n=9).

Results: Human VEGF was expressed on podocytes in RV and RV+ARB, but not other groups. Total glomerular VEGF level was restored to 55% of normal level. ARB significantly reduced blood pressure and proteinuria. Inducing VEGF did not increase proteinuria. There was less glomerular collagen IV accumulation in groups receiving ARB than control. SI, a measure of % tuft occupied by matrix, and thus indicating not only matrix deposition but also capillary loss, decreased from biopsy to autopsy in RV+ARB group (R 71.35±30.94, RV 89.16±36.23, R+ARB 40.73±14.62, RV+ARB -4.88±13.89%). Moreover, 4 of 9 RV+ARB mice showed regression compared to 3 of 12 R+ARB mice and 2 of 11 R mice. More DLL4 positive endothelial cells (tip cell) were detected in RV+ARB (1.07±0.12/glomerulus) and RV (0.79±0.07/glomerulus) than in R (0.48±0.03/glomerulus) and R+ARB (0.7±0.17/glomerulus).

Conclusions: We conclude that ARB induces regression of glomerulosclerosis in part by reducing matrix, and partially restoring VEGF in sclerotic glomeruli induces additional regression by new vessel branches, without deleterious increased proteinuria.

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FR-PO662

PBI-4050 Reduces Pro-Fibrotic Growth Factors and Collagen Expression in Human Proximal Tubule Cells and in the Doxorubicin-Induced Nephrotoxicity Mouse Model of Acute Kidney Injury Martin Leduc, Kathy Hince, François Sarra-Bournet, Pierre Laurin, Brigitte Grouix, Lyne Gagnon. *ProMetic BioSciences Inc., Laval, QC, Canada.*

Background: Many growth factors and their receptors, such as transforming growth factor (TGF)-b, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), have been implicated in the pathogenesis of renal interstitial fibrosis. These growth factors contribute to extracellular matrix production by tubular cells and fibroblasts. The aim of this study was to investigate the effect of PBI-4050, a first-in-class anti-fibrotic compound, on pro-fibrotic growth factors and collagen expression in TGF-β1-stimulated human proximal tubule (HK-2) cells and to corroborate these results *in vivo* in the doxorubicin (Dox)-induced nephropathy model of acute kidney injury.

Methods: mRNA expression was investigated on TGF-β1-stimulated HK-2 cells and in mouse kidney tissue. Nephrotoxicity was induced in BALB/c mice by an intravenous injection of Dox (10 mg/kg) on day 0. Mice were treated with PBI-4050 (100 to 200 mg/kg, oral, once daily) from day -3 to -1 and days 1 to 13. Animals were sacrificed and their kidneys were removed for histological examination (days 7 and 11) or RNA isolation (day 14).

Results: In TGF-β1-stimulated HK-2, PBI-4050 greatly reduced PDGFb, EGF, TGF-β1/2/3 and TGF-β type II receptor expression. Expression of collagens I and III was also significantly inhibited by PBI-4050. *In vivo*, Dox administration led to a significant increase of PDGFa/b, TGF-β1 and collagen I mRNA expression, which was reduced to control levels in PBI-4050-treated mice. Histological examination of renal tissue also showed a significant reduction of Dox-induced lesions (tubules distention, fluid accumulation, necrosis) in PBI-4050-treated mice.

Conclusions: Our results indicate a direct effect of PBI-4050 on human proximal tubule cells as observed by an inhibition of pro-fibrotic growth factors and collagen mRNA expression. The inhibition of the expression of these fibrotic markers was correlated in PBI-4050-treated mice in the Dox-induced nephrotoxicity model, and this was translated to significant reduction of renal histological lesions.

Funding: Pharmaceutical Company Support - ProMetic Life Sciences Inc.

FR-PO663

Pentoxifylline in the Prevention of Peritoneal Fibrosis in Uremic Rats Nelly González,[1] Carmen María del Prado,[1] Virgilia Soto,[2] Marcela Avila,[1] Héctor Isaac Rocha,[3] Ramon Paniagua-Sierra.[1] *[1]Unidad de Investigación Médica en Enfermedades Nefrológicas, Inst Mexicano del Seguro Social, Ciudad de México, DF, Mexico; [2]Dept de Patología, Inst Nacional de Cardiología Dr. Ignacio Chávez, Ciudad de México, DF, Mexico; [3]Escuela Superior de Medicina, Inst Politécnico Nacional, Ciudad de México, DF, Mexico.*

Background: Uremia causes structural and morphological changes in the peritoneum. The inflammatory response in the repair process is responsible of these changes. Pentoxifylline (PTX) suppresses the production of TNF-a and interfere with the synthesis of proinflammatory cytokines.

Methods: An experimental study was performed in male Sprague-Dawley rats weighing 250-300 g. Six groups of animals with at least ten rats each were formed. Group control (C), group 5/6 nephrectomy (5/6Nx) and three groups 5/6 nephrectomy + pentoxifylline (5/6Nx + PTx) (10, 20, 40 mg / kg / day, p.o) and a group with administration of lipopolysaccharide (LPS) (1 mg / kg, i.m). At 8 weeks, were sacrificed and blood samples were taken and peritoneum. Creatinine (sCr), IL-1, IL-6, IL-10, TGF-β and TNF-a in plasma was measured. Peritoneal morphological changes were evaluated by light microscopy.

Results: Serum creatinine levels for group C was 0.41 mg / dL, and 5/6Nx groups was 1.1 mg / dL. The measurement of IL-1 showed a statistically significant difference ($p < 0.03$) between the groups that were given PTx and LPS group. For IL-6, a $p < 0.01$ was found between the groups and 5/6Nx + PTx 5/6Nx compared to group C. Light microscopy showed an increase in the morphological changes in mesothelial and LPS groups 5/6Nx regarding 5/6Nx + PTx C ($p < 0.001$) groups. Increased vascular damage was observed in the groups of LPS and 5/6Nx compared with C and 5/6Nx + PTx ($p < 0.002$) groups.

Conclusions: The data show that the administration of pentoxifylline inhibits peritoneal fibrosis in uremic rats.

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FR-PO664

The Abnormal Expression of miRNA-21 Contributes to the Regulation of Paracrine Function in Cardiac Fibroblasts Xian Yang, Yiwen Li. *Nephrology, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China.*

Background: Cardiac fibroblasts are important not only to normal myocardial function, but also in the pathological cardiac remodeling such as after chronic renal failure (CRF). Cardiac fibroblasts can communicate with myocytes in autocrine, paracrine manner in response to injury. Our previous study dlucidated that a high levels of cytokines IL-1ß, IL-6, TNF-a, growth factors TGF-ß1, VEGFA, HGF and enhanced PI3/AKT, STAT5 and ERK/MAP kinase activities can be seen in CRF mouse model. Meanwhile, recent studies have found that the miRNA-21 of cardiac fibroblasts plays an important role in cardiac remodeling. However, the specific mechanism of miRNA-21 acting on cardiac fibroblasts is unknown.

Methods: Cardiac fibroblasts were separated from adult male mice. Normal medium and medium with indoxyl sulfate were used for the cardiac fibroblasts culture. Afterwards we silenced miRNA-21 level by transgecting siRNA. We observed the expression of cytokines IL-1ß, IL-6, TNF-a, growth factors TGF-ß1, VEGFA, HGF and intracellular signaling protein and miRNA-21 of cardiac fibroblasts before and after silencing miRNA-21 level separately.

Results: In cultured cardiac fibroblasts, uremia toxin indoxyl sulfate induced a higher expression of miRNA-21 accompanied with high levels of cytokines IL-1ß, IL-6, TNF-a, growth factors TGF-ß1, VEGFA, HGF. Meanwhile, it displayed enhanced PI3/AKT, STAT5 and ERK/MAP kinase activities. However, a reduced expression of IL-1ß, IL-6, TNF-a, growth factors TGF-ß1, VEGFA, HGF were observed after silencing miRNA-21. In company with it, the PI3/AKT, STAT5 and ERK/MAP kinase activities fell down.

Conclusions: The abnormal expression of miRNA-21 is involved in the pathological cardiac remodeling by regulating the paracrine manner of cardiac fibroblasts.

FR-PO665

The Inhibitive Effects of Enalapril on NLRP3 Inflammasome Activation and Tubulointerstitial Inflammation in Rats with BSA-Overload Nephropathy Lihong Ding, Dan Liu, Min Xu, Linli Lv, Kun Ling Ma, Bi-Cheng Liu. *Inst of Nephrology, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Proteinuria is not only a common marker of renal disease, but also involved in tubulointerstitial inflammation and fibrosis. The reno-protective effects of angiotensin-converting enzyme inhibitors (ACEI) have been well demonstrated in both animal models and human being previously, which was regarded to delay the progression of renal failure. However, the exact mechanismsfor ACEI against renal failure have not been fully understood.

Methods: After unilateral right nephrectomy, Wistar rats were randomly treated with intraperitoneal injections of 0.9% saline (Control, n=8), BSA (BSA-overload, n=10), or BSA concurrently with intragastric administration of enalapril (Enalapril, n=10) for 9 weeks. The renal lesions were evaluated using histology and immunohistostaining. The NLRP3, caspase-1, IL-1b, and IL-18 expression were analyzed using immunohistostaining, RT-PCR, and Western blot.

Results: BSA-overloading resulted in marked proteinuria and interstitial inflammation with prominent macrophage and lymphocyte infiltration, particularly of B lymphocytes. We found that NLRP3, caspase-1, IL-1b and IL-18 expressed in proximal tubular epithelial cells

(TECs), and in inflammatory cells as well. Furthermore, BSA-overloading rats exhibited a significant increase in renal expression of NLRP3, caspase-1, IL-1b, and IL-18at mRNA and protein level, which was significantly attenuated in rats treated with enalapril.

Conclusions: Proteinuria induced NLRP3 inflammasomeexpression and tubulointerstitial inflammation, and enalapril exerted renoprotective effects possibly associated with thedownregulation of the NLRP3 inflammasome expression and tubulointerstitial inflammation.

FR-PO666

Prostaglandin E2 Modulates Proximal Tubule Cell (MCT) Responses via EP1 and EP4 Receptors Rania Nasrallah, Ramzi Hassouneh, Andrew J. Karam, Jean-Francois Thibodeau, Chris R. Kennedy, Richard L. Hebert. *Kidney Research Centre, Cellular and Molecular Medicine, Univ of Ottawa, Ottawa, ON, Canada.*

Background: Renal prostaglandin E_2 (PGE_2) regulates salt and water transport, and affects disease processes via four EP receptors ($EP_{1\text{-}4}$). PGE_2/EP pathways have been well studied in the glomerulus and distal nephron but very little is known about their role in the proximal tubule. We previously reported that PGE_2 inhibits growth, and increases sodium transporters, fibronectin, and ROS generation in mouse proximal tubule cells (MCT). This study characterizes the specific PGE_2/EP pathways involved.

Methods: First quantitative PCR for $EP_{1\text{-}4}$ and cyclooxygenases (COX-1, COX-2) was performed on total RNA isolated from MCT cells, fresh cultured mouse PT cells (cPT), as well as microdissected proximal tubule (PT), thick ascending limb (TAL) and cortical collecting duct (CD). Next, MCT cells were stimulated for 24 hours with 1 μM and 1 nM PGE_2 or sulprostone (SLP: $EP_{1/3}$ agonist) to study growth (H^3-thymidine and H^3-leucine incorporation), and Western blot expression of sodium potassium ATPase (NKAa1), p27, and fibronectin. To confirm the link to EP receptors, EP_1 and EP_4 were knocked down by siRNA-mediated transfection.

Results: COX-1, COX-2, EP_1 and EP_4 mRNA were detected in all preparations. EP_2 and EP_3 were undetectable in MCT cells, but were expressed in PT and cPT. PGE_2 and SLP reduced H^3-thymidine by 50% and H^3-leucine incorporation by 25-50%. Also, PGE_2 but not SLPincreased immunodetectable p27 by 2-fold, an effect attenuated by EP_4 siRNA. PGE_2 also increased NKAa1 by 2.2-fold and this response was abrogated by EP_4 siRNA. Fibronectin was increased by PGE_2 1.7-fold and EP_1 siRNA decreased the response.

Conclusions: In summary, COX-1, COX-2, and all four EP receptors are detectable in the proximal tubule, but only EP_1 and EP_4 are expressed in MCT. PGE_2 and SLP inhibit growth in MCT cells, but SLP had no effect on p27 levels or NKAa1. PGE_2 increases p27 and NKAa1 via EP_4 in MCT cells, and stimulates fibronectin in MCT cells via EP_1 receptors. EP_1 and EP_4 receptors may prove to be important targets for the management of kidney disease.

Funding: Government Support - Non-U.S.

FR-PO667

Prostaglandin E2 EP3 Receptor Regulates Glomerular Filtration Rate and Urine Output in C57BL/6 Mice Ramzi Hassouneh, Rania Nasrallah, Richard L. Hebert. *Kidney Research Centre, Cellular and Molecular Medicine, Univ of Ottawa, Ottawa, ON, Canada.*

Background: Prostaglandin E_2 exerts diverse functions within the kidney through its four receptors: $EP_{1\text{-}4}$. Renal EP_3 is present in the collecting duct (CD), thick ascending limb, and afferent arteriole. This study explores the physiological role of EP_3 in water and salt handling and glomerular filtration rate (GFR).

Methods: Global $EP_3^{-/-}$ were age (8 wks) and sex matched (male) to wild-type C57BL/6 mice (WT). GFR was estimated by FITC-inulin clearance. Blood pressure was measured using tail-cuff plethysmography. 24-hr urine was collected using metabolic cages and a urinalysis was performed. Blood was collected and plasma analytes were measured. M-1 mouse cortical CD cells were cultured to confluency and stimulated for 4 to 24 hrs in serum-free media using sulprostone (SLP) (EP_3/EP_1 agonist; 0.1nM-1mM) and/or L-798106 (EP_3 antagonist; 10nM-1mM). Protein expression of salt and water channels was quantified by Western blotting. $EP_{1\text{-}4}$ mRNA was quantified using quantitative PCR (qPCR).

Results: The *in vivo* findings are summarized in Table 1. In M-1 cells, aquaporin-2 (AQP2) and a-epithelial Na^+ channel (a-ENaC) were down-regulated by SLP up to 60% and 50%, respectively, and L-798106 reversed this effect. Na^+/K^+ ATPase-a1 was up-regulated up to 70% by SLP, however this effect was unchanged with L-798106. Na^+/K^+ ATPase-b1 and Na^+/H^+ exchanger-1 were unchanged with SLP. qPCR revealed the presence of EP_1, EP_3, and EP_4 in M-1 cells, but no EP_2.

Table 1. Physiological parameters of $EP_3^{-/-}$ and WT mice.

	WT		$EP_3^{-/-}$	
GFR (μL/min/g body wt.)	9.5 ± 0.6		5.85 ± 0.8*	
Blood Pressure (mmHg)	110.9 ± 9/ 75.8 ± 6		111.2 ± 4/ 88.1 ± 4	
Body Weight (g)	21.0 ± 0.5		24.8 ± 0.4*	
Serum Potassium (mM)	7.98 ± 0.3		5.78 ± 0.3*	
Urine Volume (μL/24hr)	429 ± 0.05		979 ± 0.08*	
Urine Osmolality (mOsm/kg)	2470 ± 200		2280 ± 100	
Urinalysis				
	mmol/24hr	*mM*	*mmol/24hr*	*mM*
Sodium	58.5 ± 8	135 ± 8	106 ± 9*	133 ± 5
Potassium	80.1 ± 10	173 ± 7	188 ± 20*	237 ± 10*
Glucose	0.99 ± 0.5	2.1 ± 0.9	3.99 ± 0.6*	5.4 ± 1

Data expressed as means ± SEM; *P< 0.01 using unpaired t-test versus WT; n ≥ 5.

Conclusions: $EP_3^{-/-}$ mice show reduced GFR, increased urine volume, and increased urine output of sodium and potassium compared to WT suggesting an *in vivo* role for EP_3 in transport responses and tubuloglomerular feedback. The data also suggest that EP_3 regulates the expression of AQP2 and a-ENaC in M-1 cells. Further work is needed to ascertain the exact mechanisms responsible for these changes.

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FR-PO668

Antagonism of the Prostaglandin E_2 EP1 Receptor in MDCK Cells Increases Growth through Activation of Akt and the Epidermal Growth Factor Receptor Mary L. Taub. *Biochemistry, Univ at Buffalo, Buffalo, NY.*

Background: Prostaglandin E_2 (PGE_2) stimulates the growth of kidney epithelial cells, and for this reason has been proposed to play a role in cystogenesis in polycystic kidney disease. In this report, the involvement of the G-protein coupled E-prostanoid (EP) receptors in mediating the growth stimulatory effects of PGE_2 are examined. Included amongst the receptors are EP2 and EP4, which activate adenylate cyclase (AC), and EP1, that activates PLC.

Methods: Growth studies, Western blots and genetics were conducted to examine the role of individual EP receptors in controlling MDCK cell growth.

Results: The results indicate that EP2 and EP4 contribute to the growth stimulatory effect of PGE_2, unlike EP1, which is growth inhibitory. Indeed, the EP1 antagonists ONO-8711 and SC51089 increase, rather than inhibit MDCK cell growth. Similarly, ONO-8711 stimulates the growth of the M1 mouse collecting duct cell line, and primary rabbit kidney proximal tubule cells. Consistent with the involvement of EP1 in mediating these effects, the growth stimulatory effects of ONO-8711 and SC51089 are lost in MDCK cells with an EP1 knockdown. The growth stimulatory effects of ONO-8711 and SC51089 are observed in the absence of exogenous PGE_2. This latter effect is prevented by the cyclooxygenase inhibitor ibuprofen, indicating a dependence upon endogenous PGE_2 (and EP1 mediated growth inhibition). Consistent with the hypothesis that EP1 activation inhibits Akt signaling, is the observation that 1) ONO-8711 and SC51089 increase Akt phosphorylation, and 2) MK2206, an Akt inhibitor, prevents the increased growth caused by EP1 antagonists. In addition, 1) SC51089 increases the phosphorylation of the EGF Receptor (EGFR), and 2) AG1478, an inhibitor of the EGFR kinase, inhibits the growth stimulatory effects of ONO-8711 and SC51089.

Conclusions: These results suggest that EP1 receptor antagonism alters EGFR signaling. Consistent with this hypothesis, growth stimulation by ONO-8711 is lost following an EGFR knockdown, and the expression of a dominant negative EGFR. Conversely, EP1 receptor activation would be expected to inhibit EGFR signaling, and thus alter cystogenesis in polycystic kidney disease.

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FR-PO669

Differential Regulation of Inflammation in Human Fluids. A Pilot Study on Patients with Kidney Failure on Dialysis Liliana Monica Bivol, Carl Erik Halvorsen, Rudiger Ganss, Torbjorn Omland, Helge Rosjo. *Medical Dept, Nephrology Div, Akershus Univ Hospital, Lorenskog, Norway.*

Background: Inflammatory mediators (IM) in fluids other than blood and urine from patients ongoing dialysis have not been previously investigated. The aim was to assess inflammation in human fluids (blood, urine, saliva, dialysis fluid) from patients with stage 5 kidney failure versus healthy volunteers.

Methods: Samples from 11 patients ongoing dialysis, and 5 healthy controls were analysed for 7 IM. Multiplex-Luminex detection (MultiELISA, flowcytometry) was used. The results are given in pg/ml. Concentration in the background samples was 0.

Results: Results are shown in the table.

CONTROLS	IL1b	IL6	IL8	MCP1	TGFa	HGF	NGF
Blood	21	26	148	539	67	196	30
Urine	12	40	73	457	57	192	29
Saliva	70	150	727	1295	159	237	16
HD							
Blood	21	53 *	680 *	2648 *	233 *	2999 *	48
Urine	51 *	387 *	11049 *	12944 *	614 *	544 *	311 *
Saliva	638 *	106	15258 *	13472 *	93	472 *	207 *
Dialysis Fluid	10	20	40	226	35	13	12
PD							
Blood	20	78 *	1460 *	3720 *	257 *	2138 *	43
Urine	21	1097 *	11330 *	22200 *	3156 *	1745 *	446 *
Saliva	51	234 *	725	14216 *	46	263 *	171 *
Dialysis Fluid	12	841 **	231 **	4708 **	72 **	302 **	38 **

Table Mean concentration (pg/ml) of Inflammation mediators in human fluids. * p<0.05 compare to controls; ** PD compare with HD fluid

Conclusions: 1. All IM were present in human fluids from volunteers, GFR>105. IL8, MCP1 and HGF were most elevated (<4% of highest detection level) and may play an essential role in renal activation of IM. 2. In dialysis patients, only IL8, MCP1, TGFa, HGF, and IL6 were raised systemic, while in urine all markers were increased, showing higher IM activation renal then systemic. Urine IL6, IL8, MCP1,TGFa and HGF from PD were much higher then in HD patients suggesting differential IM modulation. 3. All inflammation markers were raised in PD versus HD fluid, suggesting that PD activates

local IM, but also that dialysis mode could lead to different regulation pathways in these patients. 4. Almost all markers were elevated in saliva from dialysis patients suggesting that saliva is an important excretion way.

FR-PO670

Rictor-PDCD4 Interaction Controlled by microRNA-21 Contributes to Renal Cancer Cell Invasion Amit Bera,[1] Falguni Das,[1] Nandini Ghosh-Choudhury,[2] Balakuntalam S. Kasinath,[1] Hanna E. Abboud,[1] Goutam Ghosh-Choudhury.[1] *[1]Medicine, UTHSCSA, San Antonio, TX; [2]Pathology, UTHSCSA, San Antonio, TX.*

Background: Oncogenic forces contribute to renal cancer metastasis. Recently, we have identified miR-21 as the oncogenic driver for renal carcinogenesis. However, the mechanism by which miR-21 contributes to metastasis is not known.

Methods: VHL positive and negative renal cancer cells (RCCs) were used.

Results: Increased levels of miR-21 decreased the proapoptotic protein PDCD4 in RCCs. Inhibition of endogenous miR-21 by miR-21 Sponge abrogated RCC migration and invasion, which were restored by siRNAs against PDCD4. Expression of PDCD4 and miR-21 Sponge significantly inhibited phosphorylation of Akt and IKKbeta (IKKb), which regulates NFkappaB-dependent transcription of invasive genes. siPDCD4 prevented miR-21 Sponge-induced suppression of Akt and IKKb phosphorylation. Interestingly, PDCD4 blocked activation of the pro-metastaic kinase mTORC1, which was prevented by constitutively active (CA) IKKb. PDCD4- as well as miR-21 Sponge-induced inhibition of migration and invasion of RCCs was prevented by CA Akt, IKKb and mTORC1. These results suggest that PDCD4 regulates Akt upstream of IKKb to activate mTORC1. mTORC2 is required for full activation of Akt. Importantly, we found that rictor, the exclusive component of mTORC2, formed complex with PDCD4 and this complex formation was significantly elevated in normal proximal tubular epithelial cells than in RCCs due to increased expression of miR-21 and subsequent decrease in PDCD4 in the latter. Finally, we show that increased mTORC2 activity is necessary for mTORC1 activation in RCCs.

Conclusions: Together our results demonstrate a prometastatic function of miR-21 for RCC. We identified a previously unrecognized signaling conduit involving miR-21 to reduce PDCD4-Rictor association, resulting in activation of Akt, IKKb and mTORC1.

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FR-PO671

Functional TRPV1 and TRPV4 Channels Along Different Segments of the Renal Vasculature Lan Chen,[1] Mario Kassmann,[2] Lajos Marko, Dmitry Tsvetkov, Andreas Patzak, Wolfgang Liedtke, Martin Tepel, Maik Gollasch. *[1]Xiamen Zhongshan Hospital, Xiamen Univ; [2]Experimental and Clinical Research Center (ECRC), a joint cooperation between the Charité Medical Faculty and the Max Delbrück Center for Molecular Medicine (MDC).*

Background: Transient receptor potential vanilloid 1 (TRPV1) and vanilloid 4 (TRPV4) cation channels have been recently identified to promote endothelium-dependent relaxation of mouse mesenteric arteries. However, the role TRPV1 and TRPV4 in the renal vasculature is largely unknown. We hypothesized that TRPV1/4 play a role in endothelium-dependent vasodilation of renal blood vessels.

Methods: We studied the distribution offunctional TRPV1/4 along different segments of the renal vasculature. Mesenteric arteries were studied as control vessels.

Results: The TRPV1 agonist capsaicin relaxed mouse mesenteric arteries with an EC_{50} of 9 nM, but not large mouse renal arteries or rat descending vasa recta (EC_{50} >10 μM). This relaxation was endothelium-dependent and absent in vessels of *Trpv1* -/- mice. The TRPV4 agonist GSK1016790A relaxed large conducting renal arteries, mesenteric arteries and vasa recta with EC_{50} of 15 nM, 50 nM and ~10 nM, respectively. These effects were endothelium-dependent and inhibited by a TRPV4 antagonist, AB159908 (10 mM). Capsaicin and GSK1016790A produced vascular dilation in isolated mouse perfused kidneys with EC_{50} of 12 nM and 2 nM, respectively. The capsaicin effects were absent in perfused kidneys of *Trpv1* -/- mice, whereas the effects of GSK1016790A were absent in *Trpv4* -/- kidneys, but present in *Trpv1* -/- kidneys.

Conclusions: Our results demonstrate that two TRPV channels have unique sites of vasoregulatory function in the kidney with functional TRPV1 having a narrow, discrete distribution in the resistance vasculature and TRPV4 having more universal, widespread distribution along different vascular segments. We suggest that TRPV1/4 channels are potent therapeutic targets for site-specific vasodilation in the kidney.

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FR-PO672

Stretch–Activation of Angiotensin II Type 1a Receptors Contributes to the Myogenic Response of Mouse Mesenteric and Renal Arteries Maik Gollasch,[1] Johanna Schleifenbaum,[1] Mario Kassmann,[1] Istvan Andras Szijarto,[1] Hantz C. Hercule,[1] Michael Bader.[2] *[1]Nephrology/Intensive Care and ECRC, Charité Univ Medicine, Berlin, Germany; [2]Max Delbrück Center for Molecular Medicine, MDC, Berlin, Germany; [3]Max Delbrück Center for Molecular Medicine, MDC and FMP, Berlin, Germany.*

Background: Vascular wall stretch is the major stimulus for the myogenic response of small arteries to pressure. The molecular mechanisms are elusive, but recent findings suggest that G protein–coupled receptors can elicit a stretch response. Aims: To determine whether angiotensin II type 1 receptors (AT_1R) in vascular smooth muscle cells exert mechanosensitivity and identify the downstream ion channel mediators of myogenic vasoconstriction.

Methods: We used mice deficient in AT_1R signaling molecules and putative ion channel targets, namely AT_1R, angiotensinogen, TRPC6 channels, or several subtypes of the voltage-gated K^+ (K_v7) gene family (KCNQ3, 4, or 5).

Results: We identified a mechanosensing mechanism in isolated mesenteric arteries and in the renal circulation that relies on coupling of the AT_1R subtype a to a $G_{q/11}$ protein as a critical event to accomplish the myogenic response. Arterial mechanoactivation occurs after pharmacological block of AT_1R and in the absence of angiotensinogen or TRPC6 channels. Activation of AT_1R subtype a by osmotically induced membrane stretch suppresses an XE991-sensitive K_v channel current in patch-clamped vascular smooth muscle cells, and similar concentrations of XE991 enhance mesenteric and renal myogenic tone. Although XE991-sensitive KCNQ3, 4, and 5 channels are expressed in vascular smooth muscle cells, XE991-sensitive K^+ current and myogenic contractions persist in arteries deficient in these channels.

Conclusions: Our results provide definitive evidence that myogenic responses of mouse mesenteric and renal arteries rely on ligand-independent, mechanoactivation of AT_1R subtype a. The AT_1R subtype a signal relies on an ion channel distinct from TRPC6 or KCNQ3, 4, or 5 to enact vascular smooth muscle cell activation and elevated vascular resistance.

FR-PO673

Para-Cresyl Sulfate Impairs Vascular Reactivity and Induces Vascular Remodeling Priscilla Gross,[1] Ziad Massy,[1,2] Lucie Henaut,[1] Cedric Boudot,[1] Tilman B. Drueke,[1] Said Kamel,[1] Isabelle Six.[1] *[1]Jules Verne Univ of Picardie, INSERM Unit 1088, Amiens, France; [2]Chief, Div of Nephrology, Ambroise Paré Hospital, Paris-Ile-de-France-Ouest Univ (UVSQ), Paris, France.*

Background: Vascular dysfunction, by contributing to vascular remodeling, is an important non-traditional cardiovascular (CV) risk factor in chronic kidney disease (CKD). Recent data suggest that uremic toxins may contribute to vascular dysfunction in CKD. Para-cresyl sulfate (PCS), a newly identified uremic toxin, has been associated with the progression of CKD and with CV mortality in this population. Based on its reported vascular pro-inflammatory effects in vitro, we aimed here to asses if PCS induces ex vivo vascular dysfunction and remodeling.

Methods: We studied the effect of PCS on vascular reactivity and remodeling using intact and endothelium-denuded thoracic aorta from C57Bl/6J mice aged 8 weeks. In parralel, we measured the effect of PCS on reactive oxygen species (ROS) production using human vascular smooth muscles cells (VSMCs) and endothelial cells (ECs). The mechanisms of action of PCS were explored using pharmacological inhibitors and western blot technics.

Results: Ex vivo, PCS significantly potentiates phenylephrine-induced contraction in both intact and endothelium-denuded thoracic aorta. Long-term exposure of aorta induces vascular remodeling characterized by a reduction of the surface area of both lumen and media, with no change in media/lumen ratio. In vitro, PCS acutely increases ROS production in both VSMCs and ECs. The pro-contacting effect of PCS is maintained in presence of dimethylthiourea, a ROS scavenger, but totally abolished by Y-27632, a rho-kinase inhibitor. MYPT-1 is a well-known target of rho-kinase. Its phosphorylation increases vascular contraction. Western blot analysis reveals that treatment of VSMCs with PCS leads to MYPT-1 phosphorylation on Threonine-853.

Conclusions: This is the first study which shows noxious effect of PCS on vascular function through rho-kinase activation, leading to vascular remodeling. The vascular effects of PCS could contribute, at least in part, to the CV changes observed in CKD.

Funding: Government Support - Non-U.S.

FR-PO674

Exploring the Nexus of Angiopoietin-Tie2 Signaling by Mass-Spectrometry for Establishing Therapeutic Strategies for Diabetic Nephropathy Tomokazu Souma, Jing Jin, Susan E. Quaggin. *Div of Nephrology, Feinberg Cardiovascular Research Inst, Feinberg School of Medicine, Northwestern Univ, Chicago, IL.*

Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in the U.S. It is characterized by microvascular dysfunction and endothelial instability, *i.e.*, loss of quiescence. Angiopoietin-Tie2 signaling is a major pathway for maintaining endothelial quiescence and survival. Podocytes and mesangial cells express Angiopoietin 1 (Angpt1), a major agonist of Tie2, and is vasculo-protective during diabetes. Angpt 1 binds the Tie2 tyrosine kinase receptor and induces multi-site tyrosine phosphorylation. Although the general functions of Tie2 are well-characterized, the exact phosphorylation sites in response to the various ligands have not been defined, and phosphorylation-site specific functions with regard to *in vivo* endothelial stability are still unclear.

Methods: To identify the phosphorylation sites and characterize signaling cascades for DN phenotypes, we performed phospho-mapping analysis of the Tie1 and Tie2 receptors. We generated flag-tagged full-length Tie1/2, and point mutation variants for kinase dead controls and constitutively active mutants found in vascular malformations in patients. Phosphorylation status of these proteins were analyzed by LC-tandem mass spectrometry. In parallel, we analyzed endogenously isolated Tie2 from the mouse lung to study the effects of a small molecule Tie2 activator (AKB-9785, a gift from Aerpio) on phosphorylation.

Results: We identified five phospho-tyrosine residues, which include 2 novel sites in the juxtamembrane region and the kinase domain, within the intracellular region of Tie2.

By comparison, there is very low baseline phosphorylation of Tie1. Studies to determine interdependence of Tie2 and Tie1 and a pilot study for testing effects of the Tie2 activator on a DN model are underway.

Conclusions: Our mapping results provide a roadmap for the phosphorylation status of Tie2. These data help to delineate the various *in vivo* functions of the receptor for a better understanding of Angpt-Tie signaling, which will aid in the rational design of a targeted therapy to treat microvascular manifestations of DN.

FR-PO675

Exploiting Tie2 Activation to Treat Glomerular Leak Isabel Anna Carota,[1] Chengjin Li,[2] Vera Eremina,[2] Tuncer Onay,[1] Susan E. Quaggin.[1] *[1]Feinberg Cardiovascular Research Inst, Div of Nephrology, Northwestern Univ, Chicago, IL; [2]Samuel Lunenfeld Research Inst, Mount Sinai Hospital, Toronto, Canada.*

Background: Pathologic angiogenesis and microvascular leak play a central role in the development of renal diseases. The Angiopoietin (Angpt)-Tie pathway is critical for the regulation of microvascular permeability and endothelial quiescence. Dysregulation of this pathway is associated with adverse outcomes in vascular diseases, including diabetic nephropathy. Indeed, the induction of diabetes in Angpt1-knockout mice leads to rapid glomerular scarring and renal failure. We hypothesize that loss of Tie-2 activation is a crucial step in the development of glomerular vascular leak under diabetic conditions where its activity is negatively regulated by the vascular endothelial specific tyrosine phosphatase VE-PTP. We hypothesize that restoration of Tie-2 signaling by deletion of VE-PTP is preventive.

Methods: To determine the therapeutic potential of VE-PTP we have examined its expression and function in vivo by studying a VE-PTP-lacZ reporter mouse line and by generating a conditional VE-PTP knockout mouse line.

Results: While VE-PTP is expressed in all blood endothelia, we found highest expression of VE-PTP in specialized microvascular beds including the glomerular capillaries and retinal choriocapillaris, suggesting a possible role in maintenance of fenestrated vascular beds. Additionally we have examined a conditional VE-PTP knockout mouse line, as the conventional knockout of the VE-PTP gene in mice causes embryonic lethality by E10. Mice with global excision of VE-PTP induced from E12.5 onwards are viable and grow to adulthood. By contrast, timed deletion of VE-PTP between E0 and E11.5 leads to embryonic lethality, confirming that VE-PTP is crucial for vascular development and validating our floxed line.

Conclusions: Normalizing Tie-2 activity by targeted inhibition of VE-PTP is an attractive approach to prevent glomerular microvascular leak as it occurs in diabetes. The lack of vascular defects associated with late deletion of VE-PTP suggests that VE-PTP is an ideal therapeutic target for the treatment of vascular complications in renal diseases such as diabetic nephropathy.

FR-PO676

Potential Role of Perivascular Lymphatic Vessels in Preservation of Allograft Function in Kidney Transplant Patients Akihiro Tsuchimoto,[1] Toshiaki Nakano,[1] Shoko Hasegawa,[1] Kosuke Masutani,[1] Hidehisa Kitada,[2] Kazuhiko Tsuruya,[1,3] Takanari Kitazono.[1] *[1]Medicine and Clinical Science, Graduated School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [2]Surgery and Oncology, Graduated School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [3]Intergrated Therapy for Chronic Kidney Disease, Graduated School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: Lymphangiogenesis is often observed in both diseased native kidney and kidney allograft, and correlates with interstitial inflammation. However, there is little information about the clinical significance of lymphatic vessels in kidney transplant patients.

Methods: In this retrospective study, we reviewed 81 kidney transplant patients, who underwent both 3- and 12-month protocol biopsies (PBs). We identified lymphatic vessels by immunohistochemical staining for podoplanin and counted the number of them separately according to their location; perivascular lymphatic vessels (P-Lym) locating around interlobular arteries or veins, and interstitial lymphatic vessels (I-Lym) locating in the interstitium. We analyzed the association between the each lymph vessel density and the kidney graft function.

Results: The densities of P-lym at 3- and 12-month PBs were not different, whereas the densities of I-Lym increased with time; median (interquartile range) densities were 1.7 (0.9-3.1) /mm^2 and 2.6 (1.2-5.1) /mm^2 at 3- and 12-month PBs, respectively (P=0.003). Higher density of P-lym at 3 months was associated with milder IF/TA at 12-month PB (P for trend=0.044), lower decline rate in estimated glomerular filtration rate (eGFR) from 12 to 24 months (r=-0.26, P=0.022), and higher eGFR at 24 months (r=0.29, P=0.010).The favorable associations were still significant even after adjustment for multiple confounders.

Conclusions: Higher density of P-Lym is associated with favorable kidney graft function. Pre-existing lymphatic network may play a role in the inhibition of allograft fibrosis and stabilization of graft function.

FR-PO677

Proteinuria Severity Is Directly Correlated with *Ex Vivo* and *In Vivo* Markers of Thrombotic Capacity in Two Experimental Models of Nephrotic Syndrome Amanda P. Waller,[1] Ruchika Sharma,[1] Melinda A. Chanley,[1] Marvin T. Nieman,[2] William E. Smoyer,[1] Bryce Kerlin.[1] *[1]Clinical & Translational Research, Research Inst at Nationwide Children's, Columbus, OH; [2]Pharmacology, Case Western Reserve Univ, Cleveland, OH.*

Background: Nephrotic syndrome (NS) is characterized by glomerular injury and massive urine protein loss which leads to severe blood coagulation derangements and a high prevalence of life-threatening thrombotic complications. Proteinuria has been epidemiologically linked to thrombotic risk, thus we hypothesized that proteinuria severity is directly correlated with hypercoagulopathy.

Methods: Using puromycin aminonucleoside (PAN) and adriamycin (ADR) induced nephrosis models, we compared established markers of thrombin generation (ETP) and global hemostasis (ROTEM) in blood of male Wistar rats (6/grp) exhibiting a range of proteinuria levels (morning urine [protein:creatinine]). To further delineate the *in vivo* significance of the relationship between proteinuria severity and thrombin activation, IVC ligation was performed in NS and sham rats.

Results: A significant correlation between proteinuria severity and clinically relevant markers such as ETP (R^2=0.845, R^2=0.907; P<0.001), clot formation time (R^2=0.568, R^2=0.377; P<0.001), viscoelastic strength (R^2=0.604;P<0.001, R^2=0.390;P=0.03) and lysis at 60 min (R^2=0.738, R^2=0.638; P<0.001) was present in both the PAN and ADR rat models, respectively. Plasma thrombin/antithrombin complex [TAT] did not change with worsening proteinuria, suggesting that massive proteinuria alone is not sufficient to activate coagulation *in vivo*. In contrast, when an active thrombotic episode was induced by IVC ligation, proteinuric rats exhibited higher plasma [TAT] (P=0.022), which ultimately translated into greater thrombus formation (P=0.019).

Conclusions: These data confirm that proteinuria severity is directly proportional to hypercoagulability in two well-established animal models of NS, as assessed by ex vivo thrombin generation and global hemostasis and by an in vivo thrombosis model. The physiologic relevance of this relationship provides key evidence to further investigate proteinuria as a biomarker for thrombotic risk in persons with NS.

Funding: NIDDK Support

FR-PO678

Sirtuin 1 Ablation in Endothelial Cells Is Associated with Impaired Angiogenesis and Diastolic Dysfunction Julien Maizel,[1,3] Sandhya Xavier,[3] Jun Chen,[3] Chi Hua Sarah Lin,[3] Radovan Vasko,[2,3] Michael S. Goligorsky.[3] *[1]Univ of Picardie, Amiens, France; [2]Univ Medical Center, Gottingen, Germany; [3]New York Medical College.*

Background: The disruption of coordinated myocardial growth and angiogenesis can explain that left ventricular hypertrophy progress toward heart failure with aging. Sirtuin 1 expression (an angiogenesis regulating factor) declines with age, therefore we explored the role played by angiogenesis and Sirtuin 1 in the development of cardiomyopathy.

Methods: We performed transthoracic echocardiographic analyses to compare the cardiac function of 10-15 weeks old (wo), 30-40 wo and 61-70 wo endothelial Sirtuin 1-deleted (Sirt1$^{endo-/-}$) mice and their corresponding knockout-controls (Sirt1$^{Flox/Flox}$).

Results: After 30-40 weeks of age, the Sirt1$^{endo-/-}$ animals presented with a diastolic dysfunction, showed a decreased mRNA expression of Serca2a in the left ventricle (LV) and a decreased capillary density compared to the controls animals, despite a similar VEGFa mRNA expression. However the LV fibrosis and the HIF1a expression were not different between Sirt1$^{endo-/-}$ and control mice. The creation of left ventricular overload - a transverse aortic constriction (TAC)- provoked a more severe diastolic dysfunction and LV fibrosis in the Sirt1$^{endo-/-}$ TAC compared to the control TAC animals. Although the VEGFa mRNA expression was not different and the protein expression of HIF1a was higher in the Sirt1$^{endo-/-}$ TAC, the capillary density remained lower in the Sirt1$^{endo-/-}$ TAC. In human umbilical vascular endothelial cells the administration of Sirtuin 1 inhibitor decreased the mRNA expression of VEGF receptors FLT 1 and FLK 1. The ex-vivo capillary sprouting from aortic explants confirmed the impaired angiogenic response to VEGF in the Sirt1$^{endo-/-}$ mice.

Conclusions: The data model demonstrates the impaired ability of endothelial cells deficient in Sirtuin 1 to create new vessels and adapt to a challenging condition, like hypertension, leading to the development of diastolic dysfunction.

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FR-PO679

Lithium Acts at Contractile Pericytes to Alter Vasa Recta Diameter: Novel Mechanism for Drug-Induced Nephrotoxicity Gary D. Mabbutt, Scott S.P. Wildman, Carol Crawford, Claire M. Peppiatt-Wildman. *Medway School of Pharmacy, Univs of Kent and Greenwich, Kent, United Kingdom.*

Background: Lithium is a widely used medication for the treatment of mental illnesses including manic depression and bipolar disorder. Its long-term use is known to have adverse effects on the kidney and can lead to acquired nephrogenic diabetes, tubular interstitial fibrosis as well as chronic kidney disease [1]. The precise cellular mechanism that precede clinical manifestations are however poorly understood. Previous studies have highlighted a key role for pericyte cells in the regulation of blood flow in the renal medulla, via control of vessel diameter [2]; and dysregulation of medullary blood flow has been shown to contribute to both acute and chronic kidney disease.

Methods: Utilizing a 'rat live kidney slice model' in combination with advanced imaging techniques [2], 'real-time' pericyte-mediated changes in vessel diameter were investigated in response to lithium compounds superfused at therapeutic concentrations.

Results: Data suggest that lithium chloride and lithium citrate [both 1 mM] caused a significantly greater constriction of vasa recta capillaries at pericyte sites (5.6±0.2% and 9.5±1.5% respectively) compared with non-pericyte sites (0.1±0.8%, n=4 and 0.6±0.2%, n=4 respectively; $p<0.01$). Similarly, lithium carbonate [0.1mM] significantly decreased vasa recta diameter by 8.2±0.8% compared with 2.0±0.7% at non-pericyte sites, (n=8, $p<0.001$).

Conclusions: These data indicate that contractile pericytes might represent a novel locus for lithium-induced nephrotoxicity, whether this is as a primary mechanism or secondary to tubular injury requires further delineation. [1] Kishore BKand Ecelbarger CM. (2013) Am J Physiol Renal Physiol 304: F1139–F1149. [2] Crawford, C., et al. (2012) Nephron Physiology, 120 (3), p17-p31.

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FR-PO680

Protective Role of IGF-1R in Endothelial Cells against UUO-Induced Renal Fibrosis Ming Liang,[1] Lauren Elizabeth Woodard,[2] Jinlong Luo,[1] Matthew H. Wilson,[2] William E. Mitch,[1] Jizhong Cheng.[1] *[1]Medicine, Baylor College of Medicine, Houston, TX; [2]Medicine, Vanderbilt Univ, Nashiville, TN.*

Background: The regulation of endothelial cell contact is of central importance for the barrier function of the blood vessel wall. Activated IGF-1 receptor (IGF-1R) can regulate vascular homeostasis and endothelial function.

Methods: Unilateral ureteral obstruction (UUO) was performed in wild type (WT) and in mice with KO or overexpression of IGF-1R specifically in ECs. HUVECs were used to study the mechanisms.

Results: IGF-1R expression was decreased in obstructed kidney after UUO. To assess the role of IGF-1R in endothelial barrier function, we utilized mice with IGF-1R knockout specifically in ECs (IGF-1R EC/KO). After creating UUO in EC-specific IGF-1R KO mice, there was more severe dysfunction of the endothelial barrier by increased inflammatory cell infiltration, leakage of Evans blue dye into the renal interstitium, and increased VE-cadherin phosphorylation when compared to results of WT mice. UUO in IGF-1R EC/KO mice increased the interstitial expression of fibroblast markers and enhanced extracellular protein deposition *versus* results in WT mice. Transendothelial migration in response to hydrogen peroxide (H2O2) was impaired in cultured ECs. Exposure of HUVECs to H2O2 induced phosphorylation of VE-cadherin. In contrast, silencing IGF-1R with small interference RNA enhanced the influence of H2O2 in disrupting the VE-PTP-VE-cadherin interaction. H2O2-induced endothelial barrier dysfunction was suppressed by overexpression of IGF1R. Furthermore, using the PiggBac transposon gene transfer system, we expressed IGF1R in vascular ECs. These mice experienced amelioration of UUO-induced damage, resulting in attenuation of the interstitial expression of SMA-a and collagen I. The expression of IGF1R in ECs also suppressed the inflammatory cells infiltration and renal fibrosis induced by UUO.

Conclusions: Our results indicate that IGF-1R in the endothelium maintains the endothelial barrier function by stabilization of the VEPTP-VE-cadherin complex. Expression of the IGF-1R affects endothelial cell function and the development of chronic kidney disease.

Funding: NIDDK Support

FR-PO681

Foxp3$^+$ Regulatory T Cells Differentiate into IL-17$^+$ T Cells, Contributing to Renal Fibrosis Ching-Yuang Lin. *Div. of Pediatric Nephrology, China Medical Univ Hospital, Taichung, Taiwan.*

Background: CD4$^+$ T cells play a key role in renal fibrosis; which subset looms critical remains unclear. Effector T cell linage shows great plasticity. CD4$^+$ FoxP3$^+$ Treg cells might convert to inflammation-associated Th17 cells that have tissue-fibrotic properties not yet clarified.This study evaluates roles of Treg and differentiation of Treg into Th17 cell contributing to chronic inflammation and renal fibrosis in unilateral ureteral obstruction (UUO) mouse model.

Methods: Study groups included control and UUO for 7, 14, or 21 days.

Results: JG hyperplasia, AT1R expression and lymphocyte infiltration arose in renal tissue after UUO. CD4$^+$FoxP3$^+$T cells increased progressively with presence of FoxP3$^+$IL17$^+$ T cells on Day 14 and then progressively decreased with increasing CD4$^+$IL17$^+$, as verified by double immunostaining in UUO kidney. Progressive renal fibrosis was associated with loss of CD4$^+$FoxP3$^+$IL17$^+$ T cells in splenic single-cell suspension. TGF-β1 mRNA expression positively correlated with IL-17 mRNA level in ligated kidney.FoxP3$^+$IL17$^+$ T cell was generated *in vitro* from CD4$^+$FoxP3$^+$Treg cells with UUO. Trichostatin A induced up-regulation of FoxP3 and down-regulation of RORgt mRNA expression.

Conclusions: Treg cells differentiate into Th17 cells in renal tissue under JG hyperplasia of UUO operation. Th17 cells positively correlate with TGF-β mRNA expression and fibrosis in UUO kidney. TSA had profound negative effect on emergence of Th17 cell from Treg. Data suggest epigenetic modification underlying this phenomenon; such a feature of Treg plasticity can facilitate anti-fibrotic strategy in chronic kidney disease.

FR-PO682

PPARg-Regulated NRF2 Expression Is Protecting from Crescentic Glomerulonephritis. Unexpected Potential Therapy Carole Hénique, Pierre-Louis Tharaux. *Paris Cardiovascular Centre - PARCC, INSERM, Paris, France.*

Background: The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARg) agonists have beneficial effects on renal structure and function in models of diabetes and chronic kidney diseases. However the role of PPARg in acute or subacute model of severe glomerular diseases is unknown. Hypothesizing that podocyte ability to maintain energy supply and defense against reactive oxygen species may help cope with severe immune-mediated insult, we have explored the involvement of podocyte PPARg in crescentic rapidly progressive glomerulonephritis (RPGN).

Methods: RPGN was induced by injection of anti–glomerular basement membrane antiserum (NTS). A gain of function approach was used with administration of Pioglitazone, a PPARg agonist to nephritic mice. We generated mice with specific deletion of *Ppar-gamma* alleles in podocytes (Pod-PPARg mice) for loss of function approaches.

Results: Gain-of-function approach: Pioglitazone, a PPARg agonist, limited renal injury in RPGN. Pioglitazone ameliorated podocyte damage and reduced proteinuria. Pioglitazone administration was effective when performed from the first day of NTS injection or when started in a delayed manner (4 days after NTS injection). Pod-PPARg mice developed more severe glomerular injury and RPGN upon NTS administration. Pod-PPARg mice displayed a 2-fold increase in Albuminuria to Creatinuria ratio at day 10 (2843 +/- 329 versus 1664 +/- 207 g/mol, $p<0.001$) than wild-type mice and higher crescent incidence (35.6 +/- 5.4 versus 15.6 +/- 3.7 %, $p<0.01$). Accordingly, PPARg deletion in podocytes promoted worsening of BUN levels (119.9 +/- 11.6 versus 56.7 +/- 9.0 mg/dl, $p<0.001$). Such aggravation was not due to an effect on proliferation and migration of primary podocytes. We found that Nrf2 transcriptional activity is regulated by PPARg in cultured podocytes and in glomeruli in vivo. Furhermore, Nrf2 -/- mice were found to be prone to markedly aggravated RPGN.

Conclusions: These results suggest that the PPARg-Nrf2 pathway in podocytes is pivotal in protecting the glomerulus from severe inflammatory acute insult and may thus represent a novel therapeutic target for crescentic RPGNs.

FR-PO683

A Serine Protease Inhibitor, Camostat Mesilate, Ameliorates Podocyte Injury Through Its Antiapoptotic Effect in Metabolic Syndrome Model Rat Yoshikazu Miyasato,[1] Teruhiko Mizumoto,[1] Manabu Hayata,[1] Kohei Uchimura,[1] Yutaka Kakizoe,[1] Sakai Yoshiki,[2] Masashi Mukoyama,[1] Kenichiro Kitamura.[1] *[1]Dept of Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan; [2]Research Headquarters, Ono Pharmaceutical Co., Ltd., Osaka, Japan.*

Background: Metabolic syndrome (MetS) frequently complicates kidney injury with proteinuria through podocyte injury, and hypertension is one of the most important components in MetS. Previously we reported that a serine protease inhibitor, camostat mesilate (CM), had beneficial effects against proteinuria and hypertension. Therefore, we investigated the renoprotective mechanism of CM in spontaneously hypertensive rat/ND (SHR/ND) mcr-cp (a rat model of MetS).

Methods: Thirteen week-old rats were divided into following four groups: 1) normal salt fed (NS) group, 2) high salt fed (HS) group, 3) high salt fed and CM treated (HS+CM) group, and 4) high salt fed and hydralazine treated (HS+Hyd) group. Blood pressure measurements and 24-hr urine collections were made during the treatment period. Rats were sacrificed following 4-week treatment period. In addition, we studied the effect of CM on apoptosis in a mouse podocyte cell line (MPC-5).

Results: Proteinuria and hypertension observed in the HS group were attenuated by both CM and Hyd. Although the reduction in BP was similar, the antiproteinuric effect of CM was much greater than that of Hyd. This result indicates that CM has a significant antiproteinuric effect independent of its antihypertensive effect. To clarify this mechanism, we explored the effect of CM on podocyte injury. CM markedly improved the reduction in podocyte specific proteins such as nephrin, podocin, and synaptopodin, and the numbers of podocyte in the HS+CM group were greater than those in the HS group and the HS+Hyd group. Furthermore, TUNEL staining and western blotting analysis demonstrated that CM ameliorated apoptosis of podocytes. In MPC-5, CM remarkably suppressed apoptosis induced by high glucose and aldosterone.

Conclusions: CM showed an antiproteinuric effect by ameliorating podocyte injury through its antiapoptotic effect. Our current results suggest the possibility that CM might be a new class of therapeutic agent against kidney injury in MetS.

Funding: Pharmaceutical Company Support - Ono Pharmaceutical Co., Ltd., Osaka, Japan

FR-PO684

Futhan Ameliorates Ischemia-Reperfusion Induced Renal Injury Dae Eun Choi,[1] Jin Young Jeong,[1] Hyunsu Choi,[2] Ye Jin Kim,[1] Sarah Chung,[1] Yoon-Kyung Chang,[3] Ki Ryang Na,[1] Kang Wook Lee.[1] *[1]Internal Medicine, ChungNam National Univ Hospital, Daejeon, Korea; [2]Inst of Clinical Medicine, Saint Mary Hospital, Catholic Univ, Daejeon, Korea; [3]Internal Medicine, Saint Mary Hospital, Catholic Univ, Daejeon, Korea.*

Background: It has been reported that futhan (nafamostat mesylate) inhibited inflammatory injury via inhibition of complementary activation in ischemic heart, liver and intestine. However, it has been little known that futhan inhibits the apoptosis in ischemia-reperfusion (IR) injured kidney. We investigated whether futhan attenuates IR renal injury and involves apoptosis inhibition.

Methods: We used HK-2 cell and male C57BL/6 mice. C57Bl/6 mice were divided into four groups; Sham, Futhan (Nafamostat mesylate, 2mg/kg) +Sham, IR injury (IR injury; reperfusion 27 minutes after clamping of both renal artery and vein), and Futhan+IR injury. Kidneys were harvested 24hr after IR injury. We performed functional and molecular study and H&E stain and Masson trichrome (MT) stain for histologic examination. For *in vitro* study, HK-2 cell were divided as into three groups; Control, IR-HK-2 (HK-2 cells were incubated for 6 hours with mineral paraffin oil for ischemic injury) and IR-HK-2+Futhan (2nM) groups. Cell survival and the magnitude of apoptosis were evaluated.

Results: BUN**,** serum creatinine level and renal tissue injury score in Futhan+IR injured mice were significantly lower than those of control IR mice (all, $p<0.01$). Futhan treatment significantly improved cell survival in ischemic HK-2 cells ($p<0.01$). Renal Bax protein and mRNA expression were significantly increased in IR injured kidneys and ischemic HK-2 cells. Futhan treatment significantly decreased renal Bax expression ($p<0.05$). Renal Bcl-2 protein and mRNA expression were significantly decreased in IR kidney and ischemic HK-2 cells compared to those of sham and control groups. Futhan treatment increased renal Bcl-2 expression in IR injured kidneys and ischemic HK-2 cells ($p<0.05$). TUNEL positive cells were significantly lower in Futhan+IR injured kidneys, comparing to control IR injured mice ($p<0.05$).

Conclusions: Futhan ameliorates ischemia-reperfusion renal injury via inhibition of apoptosis.

FR-PO685

MicroRNA-328 Inhibits Renal Tubular Cell Epithelial-to-Mesenchymal Transition by Targeting the CD44 in Pressure-Induced Renal Fibrosis Tso Hsiao Chen,[1] Cheng-Hsien Chen,[1] Yung-Ho Hsu.[2] *[1]Div of Nephrology, Dept of Internal Medicine, Wan Fang Hospital, Taipei Medical Univ, Taipei, Taiwan; [2]Div of Nephrology, Dept of Internal Medicine, Shuang Ho Hospital, Taipei Medical Univ, New Taipei City, Taiwan.*

Background: Epithelial-mesenchymal transition (EMT) occurs in stressed tubular epithelial cells, contributing to renal fibrosis. Initial mechanisms promoting EMT are unknown. Pressure force is an important mechanism contributing to the induction and progression of renal fibrogenesis in ureteric obstruction.

Methods: We establish a pressure apparatus to culture rat renal tubular cells (NRK-52E). Sixty mmHg of pressure will be applied on NRK-52E cells for different periods. The expression of EMT markers, such as transforming growth factor-b, connective tissue growth factor (CTGF), fibronectin, a-SMA and Snail will be monitored by Western blotting. Immunoprecipitation and immunofluorescence staining are used to study the interaction between CD44 and ERM.

Results: In our study of cultured rat renal tubular cells (NRK-52E) under 60 mmHg of pressure, we found that the epithelial marker E-cadherin decreased and mesenchymal markers, e.g., a-smooth muscle actin, fibronectin and Snail, increased. Pressure also induced the expression of connective tissue growth factor and transforming growth factor-b. MicroRNA array assays showed that pressure reduced miR-328 at the initial stage of pressurization. We identified a potential target sequence of miR-328 in rat CD44 3'-untranslated regions. In contrast with the miR-328 expression, CD44 expression was up-regulated at the initial pressurization stage. We also found that miR-328 expression decreased and CD44 increased in ureteric obstruction kidneys in the animal study. CD44 siRNA transfection significantly increased E-cadherin expression and inhibited pressure-induced EMT. Both hyaluronan binding peptide pep-1 and osteopontin neutralizing antibody inhibited pressure-induced EMT.

Conclusions: Our results suggest that miR-328-mediated CD44 transient upregulation is an important trigger of the pressure-induced EMT in renal fibrosis.

Funding: Government Support - Non-U.S.

FR-PO686

TRIM72 Binds Phosphatidylserine Receptor in Pre-Apoptotic PTE Cells Prevents Kim-1 Mediated Phagocytosis Pu Duann, Haichang Li, Pei-Hui Lin. *Medicine, OSU Medical School, Columbus, OH.*

Background: TRIM -72, a new member in TRIM family, is originally cloned in striated muscle, recently found with low but appreciable level in quiescent proximal tubular epithelial cells and substantial enhanced after injury. It has unique but potent membrane repair capabilities, was recruited by injured membrane and migrate to and seal membrane defects. Kidney injured molecule (Kim)-1 is a phosphatidylserin (PS) receptor which was absent in healthy but quickly unregulated in injured proximal tubules cells observed in IRI- and CDDP-AKI. PS normally only appeared in inner leaflet of cytoplasm membrane but quickly redistribute to outer surface of dead or dying cells. PS redistribution was generally considered a hallmark event in cell entering apoptosis. As a PS receptor, kim-1 was show to be scavenger receptor in apoptotic cell phagocytosis. By binding to PS-positive apoptotic cells, controversial immune consequence had been observed in different experiment setting. Kim-1 was thus proposed to contain two-faces for its immune role. The kim-1 binding to PS was suggest as harmful because it enhance the immune reaction to cause more PTE toxicity; it may be beneficial that kim-1 mediated phagocytosis helps apoptotic cell clearance therefore reduce autoimmunity.

Methods: We previously report TRIM -72 preferentially bind to synthetic PS dipped on nitrocellulous membrane. Whether TRIM -72 binding to PS suggests an important biological significance in vivo, is not yet studied. To explore whether TRIM -72 and TIM-1 are potential rivalry for competing PS binding, we conducted phargocytosis interference assay.

Results: TRIM 7 2 priming suppress Kim-1 mediated phargocytosis on dexamethasone-induced apoptotic thymocytes. Sonication induced PS-liposome, but not IP-liposome, abolished binding capability of TRIM 7 2 on PS-positive apoptotic cells. TRIM 7 2 may contain a PS binding domain, like PS-pocket in Tim family members, that saturate occupancy therefore blockade Tim-1 up regulation.

Conclusions: Our findings suggeste that TRIM -72 binding to PS-molecules of apoptotic tubule cells prevent Tim-1 expression, Tim-mediated phargocytosis, thereby attenuate ATN injury by reducing inflammatory consequence.

FR-PO687

Ablation of Proximal Tubular Autophagy Prevents Senescence and Fibrosis after Acute Kidney Injury Arpita Baisantry,[1,2] Sagar Bhayana,[1] Song Rong,[1] Hermann G. Haller,[1] Anette Melk,[2] Roland Schmitt.[1] *[1]Dept of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [2]Dept of Pediatric Nephrology, Hannover Medical School, Hannover, Germany.*

Background: Autophagy mediates degradation and turnover of proteins and damaged organelles and is also upregulated during Ischemia-Reperfusion (I/R). Although autophagic activity has been considered a protective mechanism during acute kidney injury (AKI), the functional role of autophagy in long term renal repair and onset of tubular cellular senescence and interstitial fibrosis has not been investigated and constitutes the aim of this study.

Methods: To examine the influence of stress induced autophagy, we generated mice with Tamoxifen inducible Atg5 gene deletion in the proximal tubular S3 segment. After renal I/R GFR was longitudinally assessed and kidneys were harvested at 3 and 30 days for analyzing acute and chronic damage and the development of cellular senescence by histology, immunostaining, Western blot and RT-PCR. In vitro, Atg5 was silenced by siRNA in primary tubular epithelial cells (PTEC) that underwent stress models of cellular senescence induction.

Results: Atg5 deletion in the S3 proximal tubular segment had no significant impact on renal function or kidney histology at 3 days after I/R. However, proximal tubular Atg5-/- mice showed improved GFR recovery and kidneys displayed reduced tubular atrophy and reduced interstitial fibrosis at 30 days. Immunostaining revealed less proliferation of interstitial cells and decreased intra-renal leukocyte infiltration. These changes were paralleled by significantly fewer senescent tubular cells and reduced expression of pro-senescent cell cycle regulators p16 and p19. In vitro, inhibition of the autophagic machinery by Atg5 silencing attenuated the progression towards senescence in PTEC.

Conclusions: Our current results show that ablating autophagy in a restricted tubular compartment prevents chronic damage such as development of cellular senescence and interstitial fibrosis in response to AKI. These findings suggest caution regarding the development of therapeutic strategies for autophagy induction in AKI treatment.

Funding: Government Support - Non-U.S.

FR-PO688

Septin7 Mediates Hyperglycemia-Induced Glomerular Podocyte Apoptosis Through Activating Ca^{2+}/NFAT2 Ruizhao Li, Wei Shi, Li Zhang, Xinling Liang, Chunping Yu, Wei Dong, Yuanhan Chen, Shuangxin Liu. *Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, Guangdong, China.*

Background: Hyperglycemia promotes podocyte apoptosis and plays a key role in the pathogenesis of diabetic nephropathy (DN). However, the mechanisms that mediate hyperglycemia-induced podocyte apoptosis is still far from being fully understood. Recent studies reported that septin7, a kind of cytoskeletal protein, is expressed in podocyte, also in glioma cells. Increasing septin7 protein expression promotes glioma cells apoptosis in vitro. Here, we sought to determine if high glucose (HG) changes septin7 expression in podocyte in vivo and in vitro, whether this leads to podocyte apoptosis. Meanwhile, we also further explore the mechanisms of septin7 mediates HG-induced podocyte apoptosis in DN.

Methods: The normal kidney tissue of three patients with renal cyst surgery and the renal biopsy tissue of three patients with DN were collected. Immortalized mouse podocytes were cultured in media containing normal glucose (NG), HG, HG plus septin7-siRNA, NG plus mannitol (osmotic control), and HG plus 11R-VIVIT (a special inhibitor of NFAT2). The proportion of podocyte apoptosis in every group was observed by flow cytometer. The activation of NFAT2 in podocytes was detected by western blotting. Intracellular Ca^{2+} was monitored in podcocytes using Fluo-3/AM. Septin7 expression in human kidney was detected by immunofluorescence assay. All data were statistical analyzed using spss17.0.

Results: Septin7 protein expression was significantly increased in glomerular podocytes in DN patients than in health control.In mouse cultured podocytes, HG significantly increased septin7 expression, and promoted podocyte apoptosis. Meanwhile, the apoptosis effects induced by HG were also abrogated by silence septin7 expression.We further found that increasing septin7 expression, leading to induce $[Ca^{2+}]i$, subsequent activated NFAT2 and increased nuclear accumulation.

Conclusions: Our results identify a new finding that HG-induced podocyte apoptosis is mediated by septin7/Ca^{2+}/NFAT2 signaling pathway, which may present a promising target for therapeutic intervention.

Funding: Government Support - Non-U.S.

FR-PO689

WWC1 Promotes Podocyte Survival via Stabilizing Slit Diaphragm Protein Dendrin Ting Lin, Wei Shi, Li Zhang, Chunping Yu, Hong Zhang, Yuanhan Chen, Shuangxin Liu, Jianchao Ma, Ruizhao Li, Zhuo Li, Xiaofan Tan, Zongshun Huang, Xingchen Zhao. *Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, Guangdong, China.*

Background: Slit diaphragms (SD) of kidney podocytes form the final barrier to urinary protein loss. Loss or reorganization of SD proteins contributes to abnormalities in podocytes and proteinuria. Dendrin, a constituent of the SD complex, has been found to relocate from the SD to the nucleus in injured podocyte and promote podocyte apoptosis. However, the exact mechanism for nuclear relocation of dendrin is still unclear. WWC1, a kidney and brain associated protein, was identified as an interaction protein with dendrin by a yeast two hybrid screen. But the exact function of WWC1 and correlation with dendrin in podocyte is not well known.

Methods: In vitro, immortalized mouse podocytes were cultured and randomized into three groups: control group, lipopolysaccharide (LPS 50mg·L-1) group, Adriamycin (ADR 0.25 mg·L-1) group. The podocyte apoptosis was determined by Annexin V/PI apoptosis detection kit. WWC1 and nucleus Dendrin expression in podocytes were detected by immunofluorence method and western blotting. In vivo, BALB/c male mice were randomly divided into: normal control group (Con), LPS (10mg/kg) induced group and ADR (12mg/kg) treated group. Mouse urine albumin was tested in different time points.

Results: In this study, we found that WWC1was a key molecular component in stabilizing dendrin protein. WWC1 was significantly decreased in LPS-treated and ADR-treated podocyte. In vivo, loss of WWC1 expression was also been found in podocyte in patients of several nephritic syndromes, or in ADR and LPS mice model. Loss-function assay showed that knockdown of WWC1 promoted podocyte apoptosis and directly induced nuclear relocation of dendrin. In addition, WWC1 directly interacted with dendrin in physiological podocyte.

Conclusions: In summary, our results indicate that WWC1 is a potential anti-apoptotic property via preventing SD protein dendrin from nucleus relocation, and provide a new molecular vision to tackle proteinuric kidney diseases.

Funding: Government Support - Non-U.S.

FR-PO690

Advanced Glycation Endproducts Blocke Formation of Autophagosome in Podocytes Xiaofan Tan,[1,2] Wei Shi,[1] Yuanhan Chen,[1] Xingchen Zhao,[1,2] Yuxiong Lai,[1] Li Zhang,[1] Chunping Yu,[1] Ting Lin,[1] Shuangxin Liu,[1] Jianchao Ma,[1] Ruizhao Li,[1] Zhuo Li,[1] Hong Zhang,[1] Zongshun Huang.[1,2] *[1]Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China; [2]Southern Medical Univ, Guangzhou, Guangdong, China.*

Background: Podocyte dysfunction plays a critical role in the development of diabetic nephropathy. Autophagy is vital for homestasis of podocytes. Here, we report the effect of AGEs on podocytes autophagy flux.

Methods: Conditionally immortalized mouse podocytes were cultrued in normal medium (control) or medium containing AGE-BSA, or co-BSA. Autophagy flux activity was monitored by protein expression of LC3II and P62 through western blotting, tandem Ad-mRFP-GFP-LC3 observing autophagosomes through laser scanning confocal microscope. The autophagososme generation ability was measured by a dynamic model treated with chloroquine (CQ) at different time points. To investigate the mechanism, mTOR activity and Beclin-1 expression was evaluated by western blotting. Rapamycin, the inhibitor of mTOR, were applied further to identify the effect of mTORC1 activity on autophagy in podocytes treated with AGE-BSA. Podocin detected by western blotting was used as a marker to represent podocyte injury under treatments.

Results: AGE-BSA inhibited expression of LC3II and generation of autophagsomes, while controls and co-BSA had no such an effect. There was an obviously lower accumulation of LC3IIwhen added CQ at 2h, 4h, 6h with AGE-BSA compared with controls and co-BSA. Meanwhile, P62 was accumulated in AGE-BSA treatment group, suggesting that the AGE-BSA might inhibite the formation of autophagosomes and so that block autophagy flux. We further found that mTORC1 was abnormally activated and Beclin-1 was downregulated under AGE-BSA stimulation compared with the other groups. On the basis of AGE-BSA treatment, Rapamycin could restore the LC3II protein level and rescue the podocyte injury by upregulating podocin which was downregulated under AGE-BSA treatment only.

Conclusions: AGEs inhibited podocyte autophagy flux though the blockage in the autophagosome fromation, which might be attributed to abnormal mTORC1 activity and inhibiton of Beclin-1 expression.

Funding: Government Support - Non-U.S.

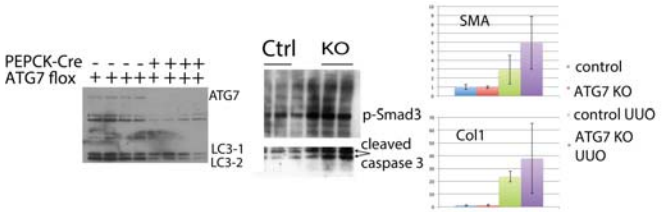
FR-PO691

Absence of Autophagy in the Proximal Tubule Increases Caspase Activation and TGF-Beta Signaling after UUO Jeremy S. Leventhal, Madhav C. Menon, John C. He, Michael J. Ross. *Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Autophagy is a ubiquitous catabolic process whereby cytoplasmic elements are digested in the lysosome after having been first sequestered within a de novo organelle called the autophagosome. Although recent evidence suggests that proximal tubular autophagy protects against acute forms of injury, its effect on chronic renal injury and fibrosis remains poorly understood.

Methods: We performed unilateral ureteral obstruction (UUO) in 8-10 week old mice with conditional knockout of autophagy protein 7 (ATG7) in the proximal tubule and evaluated autophagic capability, parenchymal injury, fibrogenic signaling and gene expression utilizing western blot, qPCR, and histological analysis.

Results: Cortical lysate from mice transgenic for both PEPCK-Cre and floxed ATG7 (ATG7 KO) demonstrated decreased ATG7 and lipidated LC3 compared to control, suggesting impaired autophagy. Three days after UUO, histology demonstrated more severe tubular damage in the cortex of obstructed ATG7 KO kidneys. Ten days after UUO, obstructed ATG7 KO kidneys demonstrated increased caspase 3 activation and Smad3 phosphorylation, suggesting increased apoptosis and TGF-beta signaling. In addition, expression of fibrosis associated genes alpha smooth muscle actin (SMA) and Collagen I (Col1), similar in the unobstructed kidneys of both control and ATG7 KO mice, were higher in the obstructed ATG7 KO kidney compared to control (5.94 versus 2.92 for SMA and 37.3 versus 23.8 for Col1).



Conclusions: In conclusion, loss of autophagy in the proximal tubule worsens injury induced by ureteral obstruction and increases renal TGF-beta activity and fibrotic gene expression, suggesting that intact proximal tubular autophagy is critical to limiting renal fibrosis after injury.

Funding: NIDDK Support

FR-PO692

Thrombospondin1 Induces Podocyte Injury through a CD36 Dependent Pathway Shuxia Wang. *Pharmacology and Nutritional Sciences, Univ of Kentucky, Lexington, KY.*

Background: Thrombospondin1 (TSP1) is a multifunctional matricellular protein and an important player in a variety of kidney diseases. Previous studies from our lab and others demonstrated that glomerular mesangial cells produced excessive amount of TSP1 in response to diabetic stimuli, which stimulated the profibrotic factor-TGF-β activation and the development of glomerulosclerosis. In addition to regulating mesangial cell function, in the current study, we revealed a novel role of TSP1 in regulating podocyte function and contributing to proteinuric kidneydisease.

Methods: In our studies, we utilized immortalized human podocytes as well as a well-established podocyte injury and experimental focal segmental glomerulosclerosis mouse model-adriamycin (ADR) induced nephropathy model.

Results: Our results demonstrated that expression of TSP1 and its receptor-CD36 in podocytes was up-regulated in early stage during podocyte injury induced by ADR in vivo as well as in vitro. Moreover, in vitro data showed that ADR-induced podocyte apoptosis was inhibited by siRNA-mediated TSP1 knockdown. In addition, TSP1 treatment stimulated podocyte actin cytoskeletal disorganization, focal adhesion disassembly and apoptosis. TSP1-induced podocyte apoptosis was not affected by anti-TGF-β pretreatment. However, treatment of human podocytes with anti-CD36 antibody or a peptide to block TSP1/CD36 interaction significantly attenuated TSP1 induced podocyte apoptosis.

Conclusions: Taken together, these data suggest that TSP1-inducted podocyte injury is through a TGF-β independent mechanism and partially mediated by TSP1 interacting with its receptor-CD36.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO693

Proteinuria Causes Dysfunctional Autophagy in Proximal Tubules Angela Nolin, Ramon G. Bonegio, Zhiyong Wang, Steven C. Borkan, John H. Schwartz, Andrea Havasi. *Renal Dept, Boston Univ Medical Center, Boston, MA.*

Background: Proteinuria is a major risk factor for chronic kidney disease progression. Furthermore, exposure of proximal tubular epithelial cells (PTEC) to excess albumin promotes tubular atrophy and fibrosis, key predictors of progressive organ dysfunction. The mechanism by which protein exposure causes tubular cell injury is uncertain. We hypothesize that albumin endocytosis causes tubular cell injury by inhibiting autophagy, a critical process for recycling damaged macromolecules and dysfunctional mitochondria.

Methods: The effect of proteinuria was examined both *in vivo* and *in vitro* experiments. Proteinuria was induced in mice by injection of the of sheep nephrotoxic serum that causes acute immune-complex glomerulonephritis with massive proteinuria by 24-48 hrs. To mimic nephrotic glomerular filtrate, PTECs were exposed to albumin. Autophagy was assessed in tissue samples from mice and primary PTECs. Steady state LC3-II, an autophagy marker was quantified by immunoblot and autophagosomes (APs) were visualized in cell culture and in renal cortical tissue. Autophagic flux was measured in vitro in the presence of bafilomycin, an H^+-ATPase inhibitor that prevents lysosomal LC3-II degradation. Mitochondrial oxygen consumption was measured in primary cells.

Results: Exposure to excess albumin induced defective autophagy and mitophagy *in vitro*. Albumin-exposed cells accumulated damaged mitochondria with altered mitochondrial function. In renal cortices, proteinuria decreased both the number of LC3-II positive APs and the amount of LC3-II detected in cell lysates. Exposure to either recombinant human

albumin or fatty acid free bovine albumin also decreased LC3-I to LC3-II conversion in a concentration-dependent manner and diminished autophagic flux. Albumin exposure decreased the number of APs in cells grown in normal media (basal autophagy) as well as in substrate deficient medium (induced autophagy). Finally, albumin exposure caused mitochondrial dysfunction as evidenced by decreased oxygen consumption rate from coupled ATP production.

Conclusions: Dysfunctional autophagy caused by proteinuria likely contributes to tubular cell toxicity leading to renal progression.

Funding: NIDDK Support, Private Foundation Support

FR-PO694

Angiotensin- (1–7) Attenuates Podocyte Damage Induced by Preeclamptic Serum through MAPK Pathways Jianying Niu,[1] Jimei Tian,[1] Yaping Chen,[2] Yong Gu.[1,3] *[1]Div of Nephrology, The Fifth People's Hospital of Shanghai, Shanghai, China; [2]Div of Gynecology and Obstetrics, The Fifth People's Hospital of Shanghai, Shanghai, China; [3]Div of Nephrology, Huashan Hospital, Shanghai, China.*

Background: Recent evidence indicates that the renin angiotensin system (RAS) is involved in the pathogenesis of preeclampsia. Our previous data shows that decreased Angiotensin- (1-7) [Ang- (1-7)] in the circulation and urine of patients plays an important role in this disease. In this study, we assessed effects of Ang- (1-7) on cultured podocytes incubated with preeclamptic serum and the underlying mechanisms governing the protective role of Ang- (1-7) in preeclampsia.

Methods: Conditionally immortalized human podocytes (provided by Pro.Zhihong Liu, Jinling hospital, Nanjing) were incubated with preeclamptic serum or serum from normal pregnant women (NP) for 12h. The expression levels of nephrin, podocin, WT-1 on cultured podocytes were determined by western blot analysis and Real-time PCR. The change in F-actin expression in the podocytes was assessed by immunofluorescence. The apoptosis of podocytes was determined by flow cytometry. The level of MAPK phosphorylation was evaluated by western blot analysis.

Results: The cultured podocytes incubated with preeclamptic serum undergo the down regulation of podocyte specific proteins (nephrin:0.42±0.04 VS 0.64±0.07,P<0.005; WT-1:0.28±0.05 VS 0.46±0.09, P<0.05), cytoskeletal rearrangement, and apoptosis (8.55±0.68% VS 2.36±1.05%, P<0.001) compared with the control group. Compared with the PE group, the addition of Ang- (1-7) in the PE group increased the expression of nephrin and WT-1 (nephrin:0.57±0.07 VS 0.42±0.04, P<0.05; WT-1: 0.41 ±0.04 VS 0.28±0.05, P<0.05) and decreased the number of apoptotic cells (4.47±0.73% VS 8.55±0.68%, P<0.001). Ang- (1-7) also could attenuate the cytoskeletal rearrangement induced by preeclamptic serum. In addition, Ang- (1-7) partly inhibited the phosphorylation of p38, ERK1/2 and JNK in preeclampsia group.

Conclusions: Our data indicated that Ang- (1-7) serves a protective role on podocytes in preeclampsia. The protective role of Ang- (1-7) in this condition may occur through the downregulation of MAPK phosphorylation.

FR-PO695

Smooth Muscle Cells APOL1 Risk Variants Promote Podocyte Injury in HIV Milieu Xiqian Lan,[1] Hongxiu Wen,[1] Ashwani Malhotra,[1] Karl Leon Skorecki,[2] Pravin C. Singhal.[1] *[1]Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY; [2]Medicine, Rambam Heatth Care Campus, Haifa, Israel.*

Background: APOL1 gene variants (G1 and G2) have been incriminated for higher rates of FSGS in African Americans. In human renal biopsy specimens of FSGS and HIVAN patients, the expression of APOL1 was found to be increased only in smooth muscle cells of arteries and arterioles but not in kidney cells. We recently reported that APOL1 variants induced podocyte injury. Additionally, expression of APOL1 variants enhanced podocyte vulnerability to be damaged by adverse host factors (AHFs) such as HIV. We now hypothesized that HIV enhances smooth cell production of APOL1 G1 and G2 in patients with APOL1 risk variants and enhanced release of APOL1G1 and G2 in blood enhances podocyte vulnerability for injury.

Methods: To dertermine the mediators during AHFs, the effect of IFN-g and TNF-a was evaluated on Human Artery Smooth Muscle Cell (HUASMC) expression of APOL1. Conditioned media (CM) from Vector, overexpressing APOL1 wide type (WT) and variants (Vs) was collected. Human podocytes (HPs) were incubated in serum free media containing CM-Vector, CM-WT and CM-Vs for 24 hours. Subsequently, cells were assayed for lysosomal injury by lucifer yellow and lysosomal tracker, increase in lysosomal permeability (LM) by cathepsin L leak (Magic red staining), and loss of cellular integrity (LCI) by trypan blue staining and lactic dehydrogenase assay. To determine the role of chloride influx, HPs were pretreated with DIDS (a chloride channel inhibitor) and then incubated in CM-WT and CM-Vs. APOL1 was depleted form CMs by using ELISA plates.

Results: CM-Vs enhanced (P<0.01) both lysosomal injury and LM when compared to CM-Vector and CM-WT. CM-Vs significantly increased (P<0.01) podocyte LCI both in control and AHFs. Both HIV infection, and stimulation with TNF-a or IFN-U enhanced the expression of APOL1 in HUASMCs. However, DIDS inhibited CM-Vs-induced lysosomal injury and increase in LM in HPs. APOL1 depleted CMs displayed attenuated podocyte injury.

Conclusions: Human artery smooth muscle cells APOL1 risk variants carry potential to accelerate podocyte injury in general and in HIV milieu in particular.

Funding: NIDDK Support

FR-PO696

Rhein Inhibits Starvation-induced Autophagy in NRK-52E Cells via mTOR/p70S6K and Erk/p38 Pathways Yue Tu,[1] Wei Sun,[2] Yigang Wan.[3] *[1]Nanjing Univ of Chinese Medicine; [2]Jiangsu Provincial Hospital of Chinese Medicine; [3]Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing Univ Medical School.*

Background: The injured/aged renal cells can be eliminated by autophagy, however, whether the role of autophagy is detrimental remains unclear. Under some conditions, suppressing autophagy may protect renal tubular damage. The mTOR/p70S6K pathway is considered one of the key regulatory mechanisms of autophagy. Rhein, as a major renoprotective ingredient from Rhubarb, is frequently used for treating renal tubular-interstitial fibrosis. However, the underlining mechanisms by Rhein in autophagy have not been examined.

Methods: NRK-52E cells were exposed to Rhein in the starvation induced by Hank's balanced salt solution (HBSS) and with or without bafilomycin A1 (autophagy inhibitor). The changes of LC3 conversion and the key protein expressions of mTOR/p70S6K and Erk/p38 pathways were detected. Moreover, NRK-52E cells were exposed to Rhein in HBSS with rapamycin (mTOR inhibitor) or exposed to PD098059 (Erk inhibitor) and SB203580 (p38 inhibitor) in HBSS, and the changes of LC3 conversion were detected. NRK-52E cells were further transfected with EGFP-LC3 plasmid to examine LC3 dots by fluorescence microscopy.

Results: HBSS effectively induced LC3 conversion in NRK-52E cells. This induction was markedly suppressed by Rhein in a dose-dependent manner. Rhein could suppress LC3 conversion induced by bafilomycin A1. The protein expressions of p-mTOR, p-p70S6K, p-Erk and p-p38 were reversely-regulated by Rhein in NRK-52E cells treated with HBSS, respectively. In addition, Rhein reduced LC3 conversion in NRK-52E cells incubating with rapamycin. PD098059 or SB203580 alone also reduced LC3 conversion. Under nutrient-rich conditions, EGFP-LC3 was mostly found to distribute evenly throughout cytoplasm, with few punctuate dots. After starvation, EGFP-LC3 was mostly localized to punctuate structures and these punctuate distribution can be reduced by Rhein.

Conclusions: Autophagy in NRK-52E cells induced by HBSS can be inhibited by Rhein via activating mTOR/p70S6K pathway. In addition, Rhein can abrogate Erk/p38 pathway activation, which suggests Rhein may act as the analogue of Erk and p38 inhibitors.

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FR-PO697

miR-363 Induces Transdifferentiation of Human Tubular Cells to Mesenchymal Phenotype Ryuji Morizane, Toshiaki Monkawa, Shizuka Fujii, Hiroshi Itoh. *Internal Medicine, Keio Univ School of Medicine, Tokyo, Japan.*

Background: micro RNAs (miRNAs) are small non-coding RNAs that act as posttranscriptional repressors by binding to the target mRNAs. On the other hand, mesenchymal-epithelial transition (EMT) is a pathological process of kidney disease such as renal cell carcinoma or kidney fibrosis, and the relationship to miRNAs is becoming recognized as a potential target for therapies.

Methods: We used a human proximal tubule cell line (HKC-8) and a primary culture from human proximal tubule epithelial cells (RPTEC) for the experiments. First, we performed knock-down of DICER-1 which is one of enzymes required for miRNA production, and examined the markers of epithelial and mesenchymal cells. Then, we performed global gene expression analysis using microarray in three experimental models using HKC-8; a EMT model using TGF-beta, a knock-down model of miRNAs using DICER-1 siRNA, and an epithelial model by cell confluency. Based on the result of microarray, we analyzed the function of miR-363 using inhibitor and precursor of miR-363 in HKC-8 and RPTEC.

Results: Knock-down of DICER-1 showed up-regulation of both epithelial and mesenchymal cell markers, suggesting DICER-1 dependent miRNAs were involved in processes of both epithelialization and EMT. To find the miRNAs involved in EMT, we performed microarray of miRNAs using three experimental models, and the result revealed that miR-363 was mildly up-regulated by TGF-beta, and was down-regulated in DICER-1 knock-down samples and a epithelial model. Therefore, we speculated that one of DICER-1 dependent miRNAs, miR-363 has an effect to promote EMT. Over-expression of miR-363 significantly suppressed E-CADHERIN and enhanced VIMENTIN, TWIST1, TWIST2 and MMP9, but not SNAIL1, suggesting that miR-363 promoted EMT via up-regulation of TWIST pathway rather than SNAIL1 pathway.

Conclusions: DICER-1 dependent miRNA, miR-363 promotes transdifferentiation of human tubular cells to mesenchymal phenotype presumably via activation of TWIST pathway.

Funding: Government Support - Non-U.S.

FR-PO698

The Role of Epigenetic Factors and Histone Modifications in Modulation of the Pathogenesis of HIV-Associated Nephropathy (HIVAN) Vasupradha Vethantham, Noopur Goel, Nirupama Chandel, Rivka Lederman, Ashwani Malhotra, Pravin C. Singhal. *Medicine, North Shore LIJ Medical School, Great Neck, NY.*

Background: Development of HIVAN is associated with large scale of changes in expression of genes related to inflammation, fibrosis and those involved in epithelial to mesenchymal transition. Epigenetic changes such as DNA methylation and histone modifications play important roles in regulating changes in gene expression, and are attractive targets for therapeutic intervention because they are potentially reversible.

However, there have been very few studies investigating the role of chromatin modifications in disease state progression in HIVAN. The goal of this study was to investigate changes in epigenetic markers in kidney cells both *in vitro* and *in vivo* models of HIVAN to determine involvement of specific chromatin modifications and/or modifiers in its pathogenesis.

Methods: Protein blots of renal tissues of control and HIV transgenic (Tg26 and Vpr) mice were probed for Histone 4 Lysine 16 acetylation (H4K16Ac) and Histone H3 lysine 4 trimethylation (H3k4me3), pan H3 acetylation, H4 acetylation, and H3k9 trimethylation. *In vitro* studies, protein blots of human podocytes (HPs)-transduced with either empty vector (EV) or HIV were probed for H4K16Ac and H3K4me3, LC3-2 and actin. Morphologic autophagy status was assayed by acridine orange staining. To establish a causal relationship, we evaluated the effect of HIV on HPs depleted of H4K16 acetylation factor (hMof) on autophagy/apoptosis.

Results: Western blot analysis revealed increases in overall levels of H4K16Ac and H3k4me3 levels in Tg26 and Vpr mice kidney tissues versus controls. There were no significant changes in pan H3 acetylation, H4 acetylation, or H3K9 trimethylation levels. Similarly, there was an increase in levels of H4K16Ac and H3K4me3 in HIV/HPs versus EV/HPs. Western blot analysis revealed increased LC3 conversion, autophagosome formation and apoptosis in HIV/HPs. These findings suggest that hMof depletion enhanced autophagosome formation but mitigated apoptosis in HIV/HPs.

Conclusions: Acetylation of H4K16Ac is involvled in the maintenance of podocyte homeostasis in control and HIV milieus.

Funding: NIDDK Support

FR-PO699

Lipidomic Profiling of Young and Aged Wild-Type Mouse Kidneys Reveals Lipid Groups Involved in Kidney Aging Fabian Braun,[1] Bernhard Schermer,[1] Valerie Bartels,[2] Thomas Benzing,[1] Roman-Ulrich Mueller,[1] Christine E. Kurschat.[1] *[1]Dept II of Internal Medicine - Nephrology, Univ Hospital Cologne, Cologne, NRW, Germany; [2]Dept of Cardiology, Univ Hospital Münster, Münster, NRW, Germany; [3]Univ Cologne, CECAD, Cologne, NRW, Germany.*

Background: Facing the worldwide demographic increase in the elderly population, prevention and therapy of aging-related diseases has become a major health concern. Models to study renal aging are currently lacking, although chronic kidney disease with mild to severe impairment of kidney function is common amongst old patients. In order to identify molecular mechanisms involved in kidney aging we analyzed gene expression profiles of young and aged wild-type mouse whole kidney tissue.

Methods: Wild-type C57BL/6 mice were sacrificed at 14 and 96 weeks of age and kidneys were removed. RNA was extracted, reverse transcribed and cDNA was hybridized to Affymetrix microarrays. Statistical analysis was conducted using R/Bioconductor. For GO enrichment and network analyses the DAVID server and NetBox software were used. Lipidomic profiling was done by BIOCRATES, Innsbruck. JMP was used for statistical lipid analysis.

Results: Wild-type C57BL/6 mice were sacrificed at 14 and 96 weeks of age and kidneys were removed. RNA was extracted, reverse transcribed and cDNA was hybridized to Affymetrix microarrays. Statistical analysis was conducted using R/Bioconductor. For GO enrichment and network analyses the DAVID server and NetBox software were used. Lipidomic profiling was done by BIOCRATES, Innsbruck. JMP was used for statistical lipid analysis.

Conclusions: Genes preferentially involved in lipid metabolism, immune response andinflammation were differentially expressed in aged kidney samples. The lipidomic profiling of young versus old kidney tissue confirmed significant differences in three of the investigated lipid groups. Our results will contribute to identifying novel lipid-regulating pathways involved in the process of kidney aging.

Funding: Government Support - Non-U.S.

FR-PO700

FHL2-Drived Molecular Network Mediated Septin2 Knockdown Inducing Apoptosis in Mesangial Cell Xiang-Mei Chen, Yang Lu, Fujian Zhang, Guangyan Cai. *Dept of Nephrology, Chinese PLA General Hospital, Chinese PLA Inst of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center of Kidney Diseases, Beijing, China.*

Background: The apoptosis of mesangial cells (MCs) plays a critical role in the pathological progress of MesPGN. Septin2, a filamentous GTPase, is implicated in the apoptotic progress of MCs in the rat MesPGN model. However, the molecular mechanism of SEPT2 in MCs apoptosis is not clear. Here, we present the FHL2-drived molecular network as the main mechanism of SEPT2-mediated rat primary MCs apoptosis.

Methods: Hochest 33342 and TUNEL staining were applied for apoptosis determination. Label-free LC-MS was applied for proteomic research. siRNA and lentivirus transfection were used for gene function research.

Results: First, we proved that the expression of FHL2 and Septin2 were closely related with MCs apoptosis in anti-Thy1 nephritis model. Then, it was found that FHL2 was a new interaction protein of Septin2 and Septin2 knockdown could induce MC apoptosis by FHL2-mediatied signal pathways including p-ERK1, p-AKT, and NF-kappaB. We applied label-Free quantitative proteomics to identify the mechanism of Septin2/FHL2-regulated apoptosis. Bioinformatics analysis revealed that FHL2-drived molecular network composed of biological functions including glycolysis, oxidative stress, ribonucleotide metabolism, actin cytoskeleton regulation and signaling pathway, was the main mechanism of SETP2-mediated apoptosis. Furthermore, we showed that the effect of Septin2 knockdown on MC apoptosis could be alleviated by the overexpression of FHL2.

Conclusions: This study illustrated the FHL2-drived molecular network controlling SEPT2-mediated apoptosis in MCs and their potential roles in mesangial proliferative nephritis.

Funding: Government Support - Non-U.S.

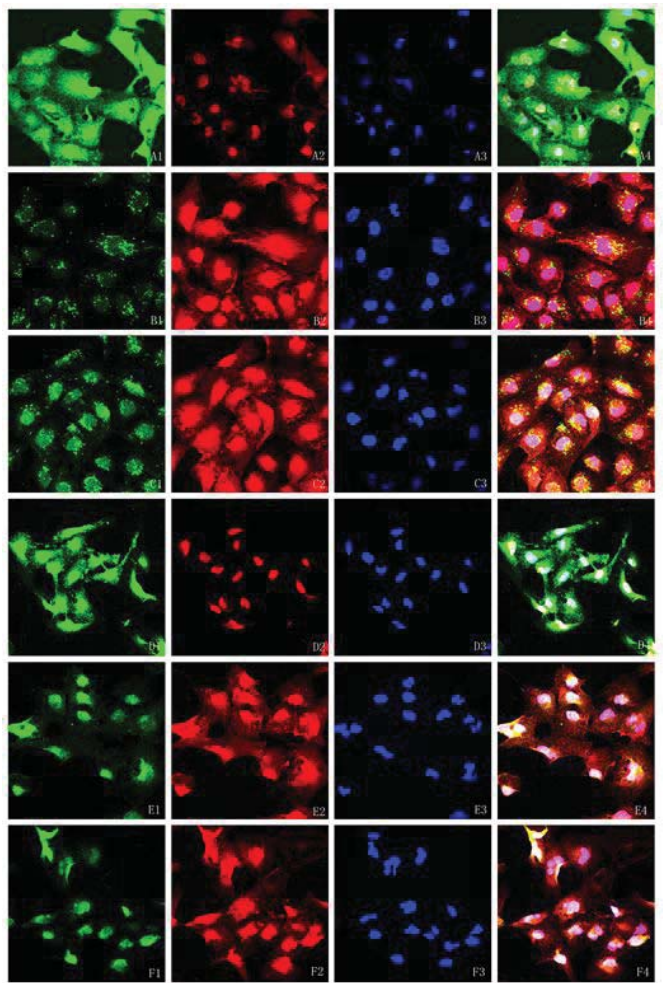
FR-PO701

Hydronidone Suppresses TGF-β1 Induced EMT on Human Kidney Cell Line Zhongyi Zhou, Lanping Jiang, Qunsheng Yuan, Mingxi Li, Xuemei Li, Falei Zheng. *Nephrology, Peking Union Medical College Hospital&Chinese Academy of Medical Science, Beijing, China.*

Background: The analogue of pirfenidone is hydronidone, which is a new drug and the effect of hydronidone on HKC remains unclear. Our animal experiment has confirmed that hydronidone can prohibit the procedure of EMT in renal interstitial fibrosis. HKC was cultured, to observe whether hydronidone influence TGF-β1 induced EMT, and to study the possible mechanism.

Methods: 1) HKC was cultured and treated with hydronidone, TGF-β1 and 5Z-7-oxozeaenol for 48h. 2) The mRNA and protein expression levels of E-cadherin and a-SMA, TGF-β1 in each group were estimated by real time PCR, WB.4) the phosphorylated extent of TAK1 and NF-kB were evaluated by WB.

Results: 1) The double stained immunofluorescence experiment showed that:



compared with the HKC control group, TGF-β1 treated HKC group after 48h significantly expressed lower E-cadherin and higher a-SMA. Compared with the TGF-β1 treatment group, hydronidone 1mM and 2.5mM groups demonstrated higher expression of E-cadherin. 2) Compared with TGF-β1 treatment group, hydronidone 1mM and 2.5mM group displayed a higher level of E-cadherin, a lower level of a-SMA,TGF-β1 in mRNA and protein levels ($P<0.05$).3) Western blot results showed that compared with control group, the protein of p-TAK1,p-NF-kB p65 were increased in TGF-β1 treatment group ($P<0.05$). Compared with TGF-β1 treatment group, the protein of p-TAK1,p-NF-kB p65 were decreased in hydronidone 1mM and 2.5mM groups ($P<0.05$). Compared with the HKC group treated 48h by TGF-β1, the protein of p-TAK1, p-NF-kB p65 were decreased in hydronidone group contain TAK1 inhibitor ($P<0.05$).

Conclusions: 1) TGF-β1 induced HKC proliferation can be suppressed by hydronidone.2) Hydronidone can up-regulate E-cadherin, down-regulate a-SMA and TGF-β1 both in mRNA and protein levels. Besides, it also reduced TAK1 and NF-kB's phosphorylation extent.

FR-PO702

Distinct Mechanisms Involving the Cellular Toxicity to Renal Tubular Cells By Five Protein-Bound Uremic Retention Solutes Ayano Konagai, Takeo Edamatsu, Ayako Fujieda, Atsuko Ezawa, Yoshiharu Itoh. *Pharmaceuticals Div, Kureha Corporation.*

Background: Protein-bound uremic retention solutes are known to be accumulated in the serum of chronic kidney disease patients and progress the disease. Two representative molecules, indoxyl sulfate (IS) and p-cresyl sulfate (PCS), have been well studied regarding renal and vascular functions. However, effects of other solutes such as phenylsulfate (PhS), indoleacetic acid (IAA) and hippuric acid (HA) which have been detected in hemodialysis patients remain unclear. Therefore, we investigated the effects and roles of the five solutes to further understand the toxic mechanisms in renal failure.

Methods: Porcine renal tubular cells, LLC-PK1, were treated with each solute at the concentration which can decrease viable cell number by 50 percent for 2 days. Viable cell number was assessed with WST-8. After synchronization with thymidine or nocodazole, cell cycle progression was analyzed using flow cytometry. Apoptotic cells were detected by Annexin V-FITC and PI staining. Protein and gene expression were determined by western blotting and real-time PCR, respectively.

Results: All solutes reduce cell proliferation. However, treatment of N-acetyl cysteine, an anti-oxidant, reversed the toxic effect by these solutes except for HA. IAA induced apoptosis and HA showed the modest effect. Besides IAA, all the others induced cell cycle delay. IS, PCS and PhS enhanced phosphorylation of p53 and Chk1 or gene expression of ATF4 and CHOP, which are hallmarks of DNA damage or ER stress leading to cell cycle delay.

Conclusions: We clarified the effects of PhS, HA and IAA in addition to the well-known solutes, IS and PCS. Based on their mechanisms, we categorized the five solutes into three groups. First, IS, PCS and PhS induce cell cycle delay through DNA damage relating with reactive oxygen species (ROS) and ER stress. Second, HA slightly causes apoptosis and cell cycle delay through DNA damage without ROS induction. Third, IAA induces apoptosis through ROS but not cell cycle delay. IAA has completely distinct effect from others and might have previously unknown harmful impacts on renal or vascular systems.

FR-PO703

tPA Promotes M1 Macrophage Survival through p90RSK and p38 MAPK Pathway Ling Lin, Kebin Hu. *Medicine, Penn State Univ College of Medicine, Hershey, PA.*

Background: Macrophage accumulation is one of the hallmarks of progressive kidney disease. Resting macrophages have a finite lifespan, but become resistant to apoptosis in response to pathogenic cues, whereas the underlying mechanism remains unknown. Tissue-type plasminogen activator (tPA), a protease up-regulated in the kidneys of chronic injury, has been shown to promote macrophage accumulation and renal inflammation. We hypothesized that tPA may be the endogenous factor that promotes macrophage survival and extends their lifespan that leads to their accumulation in the injured kidneys.

Methods: We examined the role of tPA in macrophage survival and the underlying signaling mechanism.

Results: We found that tPA protected macrophages from both staurosporine and H_2O_2-induced apoptosis. tPA promoted the survival of both resting and lipopolysaccharides (LPS)-induced M1 macrophages, but failed to do so in the interleukin 4 (IL4)-induced M2 macrophages. In the kidneys with unilateral ureteral obstruction (UUO), obstruction-induced M1 macrophages accumulation and M1 chemokine expression were markedly reduced in the tPA-deficient mice in comparison to their wild-type counterparts. The cytoprotective effect of tPA required its receptor, LDL receptor-related protein-1 (LRP-1). tPA induced the phosphorylation of Erk1/2, p90RSK, and p38 in a temporal order. The tPA-mediated macrophage survival was eliminated by PD98059, BI-D1870, or sc68376, the specific inhibitor for Erk1/2, p90RSK, or p38, respectively.

Conclusions: Thus, it is clear that tPA promoted M1 macrophage survival through its receptor LRP-1-mediated a novel signaling cascade involving Erk1/2, p90RSK, and p38, which leads to the accumulation of these cells in the injured kidneys.

Funding: Private Foundation Support

FR-PO704

Nicotine Induces Tubular Cell Apoptosis through the Activation of Renin Angiotensin System (RAS) via Epigenetic Factors Kamesh R. Ayasolla, Nirupama Chandel, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: Cigarette smoking has been incriminated for the progression of hypertension and diabetes associated chronic kidney diseases (CKD). Firstly, kidney cells carry nicotine receptors and have been demonstrated to affect kidney cells directly; secondly, cigarette smoking is known to stimulate systemic activation of RAS and that would modulate the progression of CKD. We hypothesized that nicotine has a potential to stimulate renin generation by kidney cells and thus would activate RAS locally. We further hypothesized that this effect of nicotine is mediated through down regulation of vitamin D receptor (VDR) expression of kidney cells via epigenetic factors. Since epigenetic factors-induced gene expression are reversible, treatment strategies can be developed to reverse the smoking-induced epigenetic alterations and downsream effects.

Methods: Human proximal tubular cells (HPTC) were incubated in variable concentrations of nicotine for 24 and 48 hours and assayed for apoptosis. Protein blots of control and nicotine treated cells were probed for VDR and renin. To establish a causal relationship between nicotine-induced activation of renin angiotensin system and induction of apoptosis, HPTCs were treated with either vehicle or nicotine in the presence or absence of losartan (an angiotensin II type I receptor blocker) /EB1089 (a VDR agonist) and evaluated for apoptosis. To explore relationship between nicotine-induced down regulation of VDR and epigenetic factors, protein blots of control and nicotine-treated HPTCs were probed for DNA methyl transferases (Dnmts), histone trimethylation at K27, and hstone acetylation.

Results: Nicotine enhanced HPTC apoptosis in a dose dependent manner. Nicotine down regulated tubular cell VDR expression but upregulated renin expression. Both vitamin D receptor agonist and losartan attenuated nicotine-induced HPTC apoptosis. Nicotine enhanced tubular cell Dnmt3b and H3 trimethylated K27 but displayed a decrease in histone acetylation.

Conclusions: Nicotine enhances tubular cell apoptosis. This effect of nicotine is mediated through down regulation VDR and activation of the RAS.

Funding: NIDDK Support

FR-PO705

Heparanase Is a Player in Renal Fibrosis by Regulating TGF-β Expression and Activity Valentina Masola,[1] Gianluigi Zaza,[1] Simona Granata,[1] Maria Francesca Secchi,[2] Maurizio Onisto,[2] Giovanni Gambaro,[3] Antonio Lupo.[1] *[1]Renal Unit, Dept of Medicine, Univ-Hospital of Verona, Italy; [2]Dept of Biomedical Sciences, Univ of Padova, Italy; [3]Div of Nephrology and Dialysis, Columbus-Gemelli Hospital, Catholic Univ, School of Medicine, Rome, Italy.*

Background: Epithelial-mesenchymal transition (EMT) of tubular cells is one of the mechanisms which contribute to renal fibrosis and transforming growth factor-b (TGFb) is one of the main triggers. Heparanase (HPSE) is a endo-b-D-glucuronidase that clives heparan-sulphate and takes part to extracellular-matrix remodeling thus regulating the bioavailability of growth factors (FGF-2, TGFb). HPSE controls FGF-2-induced EMT in tubular cells and is necessary for the development of diabetic nephropathy in mice. The aim of this study was to investigate whether HPSE can modulate the expression and the effects of TGFb.

Methods: Several biomolecular strategies (Real time-PCR, immunofluorescence, zymography and ELISA) have been used to assess EMT in WT and HPSE-silenced cells in response to TGF-β and pro-fibrotic factors.

Results: First we proved that the lack of HPSE prevents the increased synthesis of TGF-β by tubular cells in response to pro-fibrotic stimuli such as FGF-2, AGE and albumin. Second, HPSE does not prevent EMT induced by TGF-β although it slows its onset. Indeed TGF-β induces EMT in wt and in HPSE-silenced tubular cells; however in HPSE-silenced cells the acquisition of a mesenchymal phenotype does not develop as quickly as in wt cells, supporting the hypothesis that HPSE facilitates the TGF-β biological activities. Additionally, TGF-β induces an autocrine loop to sustain its signal whereas the lack of HPSE jeopardizes this autocrine loop.

Conclusions: Overall these data confirm that heparanase is a key player in renal fibrosis since it interacts with the regulation and the effects of TGF-β. HPSE is needed for the pathological TGF-β overexpression in response to pro-fibrotic factors such as albuminuria, AGE and FGF-2. Furthermore HPSE modulates TGF-β-induced EMT, the lack of HPSE delays tubular cell transdifferentiation, and impairs TGF-β autocrine loop.

Funding: Government Support - Non-U.S.

FR-PO706

Regulation of p53 Under Hyperglycemia and/or Hyperinsulinmia Conditions in Renal Cells Samy L. Habib,[1,2] Sitai Liang.[2] *[1]Geriatric Research, Education, and, Clinical Center, South Texas Veterans Healthcare System, San Antonio, TX; [2]Cellular and Structural Biology, Univ of Texas Health Science Center, San Antonio, TX.*

Background: Hyperglycemia and insulin resistance are key players in the development diabetes complication. Several evidences suggest that metabolic abnormalities cause renal dysfunction and inflammation, play a major role in precipitating diabetic kidney disease.

Methods: To determine the upstream and downstream target genes of p53, mouse renal proximal tubular cells were exposed to high glucose and/or high insulin concentrations. Several manipulations of downregulation or upregulation IRS1/p-AMPK/tuberin were performed to determine the mechanism of regulation of p53 under hyperglycemia (HG) and/or hyperinsulinmia (HI) conditions.

Results: Under HG+HI or HG exposure, IRS1, AMPK activity and tuberin expression are significantly decreased, while ppS6K expression increased that resulted in significant decrease in p-p53at Ser^{15} expression. On the other hand, insulin treatment resulted in increase expression of tuberin as well as IRS1 and decrease protein expression of p-P53 at Ser^{15}. Downregulation of tuberin by siRNA resulted in decrease AMPK activity and increase p-p53 expression under all treatment conditions. In addition, overexpression of IRS-1 leads to significant increase in AMPK activity, increase in tuberin expression and decrease p-p53. Moreover, downregulation of AMPK by DN-AMPK resulted in significant increase in p-P53 under all treatment conditions. These data suggest that p53 is mainly regulated through IRS1/tuberin/AMPK in cells under hyperglycemia and/or hyperinsulinmia conditions.

Conclusions: Taken together these signals cascade of regulation of p53 through IRS/AMPK/tuberin will be important to improve understanding the mechanism-based therapeutic strategies as a promising option to prevent renal complications in diabetes.

Funding: Veterans Affairs Support

FR-PO707

HPV Oncoproteins Modify DNA Damage Response (DDR) to Explain Inferior Chemotherapeutic Efficacy in Renal Cell Carcinoma
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Background: Better insights into mechanisms of chemotherapy-induced DDR will be relevant for cancer therapies. We determined whether cisplatinum (CP)-induced nephrotoxicity and DDR were recapitulated in renal cells with master switches activated by ATM and related pathways.

Methods: We studied DDR in HK-2 cells, human tubule cells immortalized by HPV16 E6/E7 genes, and HuH-7 cells from human HCC. Cells were treated with CP in IC50 doses with cell viability assays using MTT, gene expression analysis by qRT-PCR arrays, Comet assays for double-strand DNA breaks, cytostaining or westerns for ATM-related genes, ATM promoter activity using a tdT reporter, and FACS for cell cycling or Side Population (SP) assays of stem cells.

Results: In CP-treated HuH-7 cells, typical DDR was observed, including gH2AX expression, Comets, greater ATM promoter activity, fewer cells in S or G2/M and more in G0/G1, and less SP. Fewer ATM-regulated genes were expressed with more p53 expression. In CP-treated HK-2 cells, DDR was incomplete, as gH2AX was expressed and ATM promoter activity increased, but Comets were absent, p53 was expressed less, and SP was present. Next, we determined regulation of MDM2-regulated p53 expression with nutlin-3, which inhibits MDM2, or wild-type p53-induced phosphatase (WIP1) with arsenic trioxide (ATO), which decreases WIP1 expression. Nutlin-3 had no effect on cell viability and did not increase CP toxicity. In HK-2 cells, CP, ATO, or both, affected neither p53 nor WIP1 expression, in agreement with impaired DDR. However, CP and ATO synergistically enhanced cytotoxicity because ATO depolymerized tubulin and also decreased SP in HK-2 cells.

Conclusions: HPV E6/E7 oncoproteins altered DDR in HK-2 cells leading to p53-associated escape. This escape from DDR was overcome by microtubule-disrupting drug, ATO, which increased cytotoxicity and depleted cancer stem cells. Since HPV E6/E7 genes are integrated in ~20% cases of renal cell carcinoma these mechanisms will be clinically significant.

Funding: NIDDK Support

FR-PO708

Involvement of Dysregulated Mitophagy in Albumin-Induced Apoptosis in Proximal Tubule Cells
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Background: Albuminuria, the universal feature of chronic kidney disease, is considered a poor prognostic factor contributes to progression of renal injury. Although it was established that ongoing albuminuria could lead to inflammation, fibrosis, tubular apoptosis and death, the underlying mechanisms remain unknown. Since recent advances in understanding molecular processes contributing to autophagy have provided insight into the relationship between autophagy and apoptosis, we show that dysregulate mitophagy might involve in albumin-induced apoptosis in cultured tubule epithelial cells (NRK-52E).

Methods: NRK-52E cells were exposed to endotoxin-free albumin. Apoptosis was detected by H-33258/PI staining; TUNEL; cleaved-caspase 3 staining; western blot and flow cytometry. Mitophagy were evaluated by western blot; immunofluorescence; GFP-LC3 staining and electron microscopy. Mitochondria function was assessed by oxygen consumption rate, reactive oxygen species, mitochondrial membrane potential and immunofluorescence.

Results: Albumin induced tubular apoptosis in a time- and dose-dependent manner. Increased caspase-3 activity was observed starting at 6 and maximally at 24 to 60 h at 5mg/ml albumin. Transient increase of autophagic flux before 24 h, happening in the early stage, seemed to mediate a compensatory protective response. GFP-LC3–tagged autophagosomes colocalized with mitochondria in tubular cells, suggesting that albumin induces mitophagy. However, sustained albumin ultimately defected mitophagy. Mitophagy-deficient tubular cells exhibit reduced mitochondrial respiratory chain activity and mitochondrial membrane potential and fragmented morphology with marked swelling. Furthermore, rapamycin, an inducer of autophagy, could increase mitochondrial respiratory chain activity and mitochondrial membrane potential and therefore alleviate albumin induced apoptosis.

Conclusions: These findings suggested that basal mitophagy played important roles in regulating apoptosis. Thus, enhancement of mitophagy might hold promise as a novel avenue for preventing albumin induced tubular cell apoptosis and ameliorating tubular injury in proteinuric kidney diseases.

FR-PO709

Hic-5 Regulates Mesangial Cell Proliferation via Altered and Coordinated Expression of Cell Cycle-Related Protein
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Background: We previously reported that expression of Hic-5, a TGF-β1-inducible focal adhesion protein, was associated with glomerular cell proliferation in human and experimental glomerulonephritis (GN). In addition, we demonstrated that Hic-5 might regulate mesangial cell (MC) proliferation after glomerular injury in Habu venom-induced GN in heminephrectomized mice (Kidney Week 2014; TH-PO552).

Methods: To examine the effect of Hic-5 on the glomerular expression of growth factors, PDGF-BB or TGF-β1, or their receptors, immunohistochemistry was performed on both wild type (Hic-5+/+) and Hic-5-deficient (Hic-5-/-) GN mice. In addition, we investigated how Hic-5 regulates MC proliferation and affects cell cycle using cultured Hic-5+/+ and Hic-5-/-MCs.

Results: Glomerular expressions for PDGF-BB or TGF-β1, or their receptors, are enhanced in Hic-5+/+ and Hic-5-/- GN mice on day 7, however, their differences were not observed in both mice. Glomerular cell proliferation in Hic-5-/- GN mice was significantly greater than that in Hic-5+/+ GN mice on day 7, suggesting that Hic-5 might directly regulate MC proliferation. In vitro experiments, PDGF-BB (50 ng/ml) stimulated proliferation in Hic-5+/+ MCs, but TGF-β1 (10 ng/ml) did not. PDGF-BB and TGF-β1 enhanced proliferation of Hic-5-/- MCs compared with Hic-5+/+ MCs. Western blotting for cell cycle-related proteins revealed that PDGF-BB increased the protein levels of cyclins A and D1 in Hic-5+/+ MCs. In contrast, TGF-β1 decreased cyclin A expression in Hic-5+/+ MCs. PDGF-BB and TGF-β1 enhanced the expression of cyclins A and D1 in Hic-5-/- MCs compared to Hic-5+/+ MCs. p21 expression was increased in Hic-5+/+ MCs stimulated with PDGF-BB or TGF-β1. In contrast, p21 expression by PDGF-BB or TGF-β1 was not detected in Hic-5-/- MCs. These results suggested that Hic-5 might be involved in the regulation of MC proliferation through the altered and coordinated expression of cyclins A and D1, and p21.

Conclusions: In conclusion, Hic-5 might regulate MC proliferation by controlling cell cycle.

Funding: Government Support - Non-U.S.

FR-PO710

Role of Calciprotein Particle-Induced Autophagy in Calcification of Human Smooth Muscle Cells
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Background: Recent studies have indicated a protective role of autophagy in calcification of vascular smooth muscle cells, but the mechanisms are poorly understood. Calciprotein particles (CPP) are cytotoxic nanoparticles composed of inorganic calcium-phosphate crystals and mineral binding proteins, such as fetuin-A. CPP circulate in the blood of patients with chronic kidney disease and may contribute to vascular calcification, chronic inflammation and a premature-aging phenotype. However, little is known about the effect of CPP on smooth muscle cells. We studied the role of autophagy in vascular calcification induced by phosphate and CPP.

Methods: We used primary human aortic smooth muscle cells (hAoSMC) to assess CPP-induced autophagy and calcification. We made CPP from Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, 5 mM phosphate, and 10 mM calcium. We also prepared CPP containing Alexa488-labeled Fetuin-A, and observed localization of CPP and LC3 in hAoSMC using confocal microscope. For high phosphate condition, we incubated cells in the presence of 4 mM phosphate. Calcification was analyzed by von Kossa staining. Induction of autophagy was detected by western blot and immunofluorescence.

Results: High phosphate obviously induced endogenous LC3 puncta in hAoSMCs. In electron microscopy, we observed calcium phosphate crystal-like substances in autophagic structures in hAoSMCs under high phosphate. CPP induced calcification of hAoSMC in concentration-dependent manner. Consistent with high phosphate condition, CPP induced autophagy in hAoSMCs. The conversion of LC3-I to LC3-II in western blot and endogenous LC3 puncta formation were induced. Furthermore, we found that a small part of CPP containing labeled fetuin-A localized in LC3-labeled autophagosome under laser confocal microscope.

Conclusions: Phosphate or CPP induce autophagy and calcification in hAoSMC. And some calcium phosphate crystal or CPP localized in autophagosome. Autophagy may regulate CPP transport or dissolution in smooth muscle cells.

FR-PO711

Pressure Applied In Vitro Increases Proliferation of Renal Proximal Tubular Cells
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Background: Increased renal pressure is a common finding in kidneys with obstructed ureters [UO]. In animal models of UO, increased cellular proliferation may contribute to renal damage and/or repair. We investigated the direct effect of pressure on proliferation of renal proximal tubular cells in vitro.

Methods: Human HKC-8 or rat NRK-52E proximal tubule cells were grown at either ambient pressure, or at 60 or 90 mm Hg for 24/48 hr, using a system we designed [Am J Physiol Renal 293:F1877, 2007]. Cells were grown in complete medium [CM; DMEM with

10% FBS] or in starvation media [SM; DMEM]. At 24/48 hr, cells were counted using the Cyquant assay. Western blot and PCR for proliferating cell nuclear antigen [PCNA] and Skp2 were carried out, as was cell cycle analysis by flow cytometry. Media composition was determined in an iSTAT.

Results: HKC-8 grown in CM for 48 hr at 60 mm Hg, demonstrated a 16.5 ± 5.8% increase in cell number compared to ambient pressure. After 48 hr of 60 mm Hg pressure in SM, there was a 39.2 ± 5.1 % increase in cell number. At 90 mm Hg in SM, HKC-8 demonstrated a significant increase in cell number. Similar results were found with NRK-52E. Increased expression of PCNA and Skp2 was found in pressurized cells. Cell cycle measurements demonstrated an increase in HKC-8/NRK-52E in S phase, with a corresponding decrease in cells in G1. Pressure did not significantly affect pH, PCO2 or PO2 of either CM or SM.

Conclusions: We demonstrate direct transduction of pressure into a proliferative response in HKC-8 and NRK-52E cells, measured by cell number, PCNA or increase in cells in S phase. Effects on proliferation were more pronounced in SM, suggesting that pressure may be able to [re-]activate pathways normally turned off during starvation. Increased Skp2 would accelerate degradation of the p27 cell cycle inhibitor, consistent with increased proliferation. Understanding the signaling pathways activated by pressure on epithelial and other cells in the kidney should lead to a better understanding of how pressure contributes to renal damage in UO, or in other diseases characterized by high pressure.

Funding: Other NIH Support - Flow cytometry assays were done in the Translational Technology Core Lab (CTSA, RUCCTS Grant#8 UL1 TR000043) from the National Center for Advancing Translational Sciences (NCATS, NIH), Private Foundation Support

FR-PO712

HIV Induces Dedifferentiation and Eithelial Mesenchymal Tansition (EMT) in Hman Pdocytes: Role of Eigenetic Fctors and ß-Ctenin Pthway

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Background: Patients with HIV kidney infection develop collapsing varinat of glomeurlosclerosis. In this variant, podocytes display proliferative phenotype. This phenotype has been attributed to dedifferentiation of podocytes from its terminally differentiated state through re-entry into cell cycle. In addition, kidney cell infection by HIV has been incriminated for subversion of podocyte machinery and loss of its epithelial phenotype. We hypothesized that HIV would induce dedifferentiation phenotype through enhancing snail expression and associated epigenetic factors leading to down regulation of p-cadherin and nephrin. Additionally, HIV-induced activation of WNT receptors would activate podocyte EMT via ß-catenin pathway. Since epigenitc factors-induced alterations are reversible, thererpeutic strategies can be planned to alter HIV-induced podocyte phenotype.

Methods: cDNA and protein blots of empty vector- (EV/HPs) and NL4-3 without *gag and pol* (HIV/HPs)-transduced human podocytes (HPs) were probed for snail and markers of EMT (a-SMA, vimentin, and FSP-1) (n=3). To explore a causal relationship between hypermethylation and down regulation of p-cadherin and nephrin, EV/HP and HIV/HPs were treated with either buffer or 5-azacytidine (AZAC, a demethylating agent) for 24 hours (n=3) and evaluated for p-cadherin and nephrin expression by Western blot analysis. Binding of snail at the E-boxes was confirmed by chip assay through primers spanning E-boxes for respective genes.

Results: HIV/HPs displayed over expression of snail but down regulation of p-cadherin and nephrin. HIV/HPs displayed 2-3 fold increase in expression of a-SMA, vimentin, and FSP-1. HIV/HPs also displayed enhanced WNT7 mRNA expression and down stream molecules indicating the activation of β-Catenin pathway. AZAC inhibited HIV-induced upregulation of snail and down regulation of p-cadherin and nephrin confirms the role of epigenetics.

Conclusions: HIV induces dedifferentiation and exhibiton of markers of epithelial mesenchymal transitioin in podocytes through epigenetic factors and activation of β-Catenin pathway.

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FR-PO713

Renal Injury Suppresses Claudin-2 Expression to Induce Dedifferentiation of Renal Epithelial Cells

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Background: Epithelial cell dedifferentiation is a feature of rapidly dividing cells under controlled growth, as in recovery of post-renal injury, or non-controlled growth, as in renal cancer. However, mechanisms regulating renal epithelial cells (REC) differentiation remain largely undefined. We have demonstrated that in MDCK cells, EGFR-activation suppresses claudin-2 expression to induce tight junction (TJ) remodeling. TJ dysregulation characterize renal injury and cancer. However, the role of claudin-2 in the regulation of REC morphogenesis remains poorly understood.

Methods: qRT-PCR, Immunoblot and immunostaining analyses were done. Hypoxia was induced in hypoxia chambers. Anti-claudin-2 shRNA was employed.

Results: In mouse kidney subjected to hypoxia, claudin-2 expression decreased significantly ($P<0.01$). Further, chronic loss of claudin-2 expression characterized dedifferentiated MDCK cells, either due to exposure to chronic hypoxia or EGFR-activation ($P<0.001$). This loss of claudin-2 expression was associated with the loss of cilia and preceded decreases in E-cadherin expression, markers of differentiated REC. Further, stable silencing of claudin-2 expression in MDCK cells inhibited markers of terminal differentiation, including dome-formation and cilia growth. By contrast, re-expression of claudin-2 in MDCK^{sol} cells, MDCK cells with constitutive EGFR-signaling and transformed phenotype, reverted the epithelial phenotype. Further studies using protein and transcriptome analyses of more than 200 renal clear cell carcinoma samples and mice renal tumors demonstrated chronic suppression of claudin-2 expression *versus* matched normal kidney ($P<0.001$).

Conclusions: Taken together, our data demonstrate a novel role of claudin-2 in the maintenance of differentiated phenotype of PTE cells. We predict that suppressed claudin-2 expression during renal injury promotes regeneration/repair however its persistent and chronic loss promotes neoplastic transformation under tumorigenic conditions.

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FR-PO714

Exocyst Sec10 Regulates Kidney Tubular Epithelial Cell Wound Healing Through Modulation of Cell Proliferation and Migration

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Background: Migration and proliferation of survived kidney tubule cells after sub-lethal kidney injury play a critical role for the kidney recovery. Exocyst component is involved in cell migration and proliferation. Here, we investigated the role of Sec10 and underlying mechanisms in the wound healing from scratch injury of cultured kidney tubule cells.

Methods: Cultured MDCK cells which over-express or lack Sec10 were scratched and further cultured for the restoration. The rate of growing and wound healing rate was determined.

Results: Sec10 over-expression delayed the wound healing and Sec 10 knock down (KD) vice versa. Mesenchymal transition and proliferation of MDCK cells were inhibited by over-expression of Sec10 and vice versa by KD of Sec10. Activation of ERK in the newly grown cells was lower in Sec 10 over-expressing MDCK cells than control and vice versa in Sec 10 KD MDCK cells. Sec10 over-expressing MDCK cells reduced ruffle formation in the growing edge of the cells, whereas Sec10 KD cells promoted ruffling, indicating that Sec10 modulates migration of tubular cells. Small GTPase Cdc42 mRNA expression was significantly increased in Sec10 KD cells compared with control.

Conclusions: Taken together, the data reveal that Sec10 delays wound healing of kidney tubule cells via inhibition of mesenchymal transition, proliferation, and migration through inhibition of ERK activation and ruffle formation likely by alteration of Cdc42 expression.

FR-PO715

The Roles of Oxidative Stress, Endoplasmic Reticulum Stress and Autophagy in Aldosterone/Mineralocorticoid Receptor-Induced Podocyte Injury

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Background: Podocytes play an important role in the pathogenesis and progression of glomerulosclerosis. Recent studies indicate that aldosterone/mineralocorticoid receptor (MR) is a major contributor of chronic kidney disease (CKD) progression. aldosterone/MR induces glomerular podocyte injury, causing the disruption of the glomerular filtration barrier and proteinuria.

Methods: The expressions of well-known UPR target genes including Bip, grp94 and Chop and podocyte-specific proteins nephrin and podocin were detected by real-time PCR and immunoblotting. we confirmed the induction of autophagy using LysoTracker Red staining and accessing the ratio of LC3II/LC3I to actin. ROS generation was determined using 2,7-dichlorofluorescein diacetate (DCF-DA).

Results: We observed that aldosterone/MR induced ER stress and podocyte injury both in vivo and in vitro. Blockade of ER stress significantly reduced aldosterone/MR-induced podocyte injury. Additionally, we found that ER stress-induced podocyte injury was mediated by Chop. Interestingly, autophagy was also enhanced by aldosterone/MR. Pharmacological inhibition of autophagy resulted in increased apoptosis. Inhibition of ER stress also significantly reduced aldosterone/MR-induced autophagy. Moreover, we observed that the addition of ROS scavenger, N-acetylcystein (NAC), blocked both ER stress and autophagy by aldosterone/MR.

Conclusions: These results suggest that oxidant stress mediated aldosterone/MR-induced podocyte injury via activating ER stress which then triggers both the Chop-dependent apoptosis and autophagy to cope with the injury.

FR-PO716

P53-Mediated Upregulation of MKP-3 Contributes to the Establishment and Maintenance of Renal Tubular Cell Senescence through Inactivation of ERK1/2

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Background: Growth arrest is the essential feature of cellular senescence. At present, the mechanisms responsible for the maintenance of the arrested phenotype of senescent cells are still incompletely understood. Given that extracellular signal-regulated kinases 1 and 2 (ERK1/2) is one of the major kinases controlling cell growth and proliferation, we examined the possible involvement of ERK1/2 in tubular epithelial cell senescence.

Methods: Senescence in normal rat kidney epithelial cells (NRK) was induced by etoposide (ETO), and confirmed by morphology and b-galactosidase (b-gal) activity. Cell proliferation was evaluated by BrdU incorporation. The level of ERK1/2, MEK-1, mitogen-activated protein phosphatase-3 (MKP-3) and p53 was analyzed by Western blot, Northern blot and promoter activity assay. Suppression/downregulation of ERK1/2, p53 or MKP-3 was achieved by using inhibitors and/or siRNA. Oxidative cell injury was induced by free radical donors, and evaluated through the change of cell morphology and viability.

Results: 1) Exposure of NRK cells to ETO induced cellular senescence, as manifested by enlarged cell size, reduced cell proliferation and enhanced b-gal activity. 2) Stimulation of control cells with mitogens resulted in ERK1/2 activation and cell proliferation. Suppression of ERK1/2 significantly blunted cell proliferation. In contrast, senescent cells displayed an impaired activation of ERK1/2 and a loss of cell proliferation. 3) Senescent cells expressed a markedly increased level of p53 and MKP-3. Suppression of the transcription activity of p53 abrogated the elevation of MKP-3 and recovered cellular response to mitogen. Inhibition of MKP-3 increased the basal level of ERK1/2 phosphorylation and cell proliferation. 4) Impaired ERK activation was related to the senescence-associated resistance to oxidative cell injury.

Conclusions: The present results indicate that inactivation of ERK1/2 by p53-mediated upregulation of MKP-3 is a key molecular event implicated in the establishment and maintenance of renal tubular cellular senescence. Our finding could have implications in renal tubular cell repair and regeneration.

FR-PO717

A Western Diet Leading to Maternal Glucose Intolerance and Obesity Elevates Offspring Nephron Endowment without Adverse Effects on Adult Renal Function

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Background: Consumption of a high fat 'western' diet has led to a growing number of pregnancies complicated by maternal glucose intolerance and obesity. Maternal glucose intolerance and obesity have health implications for offspring, yet little is known about their effects on offspring renal development and function. Animal studies report a nephron deficit and reduced renal function in offspring exposed to maternal diabetes, however current literature is limited to models of persistent severe hyperglycemia and fetal growth restriction which do not reflect the typical clinical condition.

Methods: Female C57Bl6 mice were fed a high fat diet (HF, 21% fat) or matched normal diet (NF, 6% fat) for 6 weeks prior to pregnancy and throughout gestation and lactation, producing overweight females with glucose intolerance. Offspring of HF and NF dams were collected prior to birth at embryonic day (E) 18.5 and at postnatal day (PN) 21 for determination of nephron number using unbiased stereology. Glomerular filtration rate (GFR) was assessed at 6 months of age by transcutaneous measurement of FITC-sinistrin clearance.

Results: HF offspring were 5% heavier at E18.5 (p=0.019) but did not differ in bodyweight at PN21 (p=0.107). Compared to offspring of NF dams, offspring of HF dams had approximately 25% more nephrons at E18.5 (p=0.009) and PN21 (p<0.0001). Nephron endowment correlated with maternal glucose tolerance (rho=0.674, p=0.0002) and maternal bodyweight (rho=0.460, p=0.011) prior to pregnancy. There was no difference in offspring bodyweight (p=0.763) or GFR (p=0.248) at 6 months of age.

Conclusions: Offspring born to mothers fed a 'western' diet associated with maternal glucose intolerance and obesity had an elevated nephron endowment, which was established early in nephrogenesis. This increase in nephron endowment was associated with larger offspring at a time of ongoing nephrogenesis. As adults, HF offspring did not show altered GFR. Further studies are required to assess nephron morphology and proteinuria.

FR-PO718

The Effect of Maternal Cigarette Smoke Exposure on Offspring Predisposition to Kidney Disease in Male and Female Mice

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Background: Maternal smoking is associated with long term health consequences in the offspring including obesity, respiratory and cardiovascular diseases. In human, maternal smoking is closely linked to lower fetal kidney volumes during the second and third trimester and lower birth weight. However, it is still unknown whether maternal smoking increased the risk of developing chronic kidney disease in offspring and whether there is any gender difference. The study aims to investigate the effect of maternal smoking on offspring renal development and predisposition to kidney disease in both genders.

Methods: Female Balb/c breeder mice were exposed to either air or cigarette smoke for 6 weeks prior to mating, during gestation and lactation. Female and male offspring were sacrificed at postnatal day (P) 1, P20 (weaning age) and 13 weeks (mature age). Serum and urine were collected and kidneys were harvested. Kidney histological changes were examined. mRNA and proteins expression of renal development and injury markers were determined. Kidney function was assessedand intraperitoneal glucose tolerance test was performed.

Results: At W13, Males offspring from the SE group had decreased kidney/body weight compared to control (p <0.05). Glomerular number was significantly decreased only in male offspring from SE dams at P1, P20 and W13 and glomerular size was significantly increased at P20. mRNA and protein expression of renal development markers such as bone morphogenetic protein (BMP) 4, fibroblast growth factor (FGF) 2, FGF10, glial cell line-derived neurotrophic factor (GDNF) and WNT4 were only upregulated in the kidneys of male offspring from SE dams (p <0.05). Male but not female offspring had a significant increase in urinary albumin/creatinine ratio at W13 (p <0.05). Both genders had increased glucose intolerance at W13 due to maternal SE.

Conclusions: Female mice offspring are less susceptible to renal pathophysiology compared to male offspring. Both gender had metabolic disorders due to maternal SE.

FR-PO719

Furry as a Partner of the Ldb1-Lhx1 Transcriptional Complex for the Specification of the Renal Progenitor Cell Field

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Background: In the vertebrate embryo all renal progenitor cells are derived from the intermediate mesoderm. Lhx1, a LIM-class homeobox transcription factor, is initially expressed throughout the lateral and intermediate mesoderm and is one of the earliest genes to be restricted to the kidney field. We have previously shown that Lhx1 is essential for driving specification of the kidney field, but the molecular mechanism remains undefined.

Methods: By tandem affinity purification we identified the protein Furry (Fry) binding to Lhx1. In *Xenopus*, previous reports indicate that Fry acts as a transcriptional co-repressor of microRNAs preventing message degradation for Spemann organizer genes, temporally controlling their mRNA levels. We established the expression pattern of *Fry* by *in situ* hibridization and analyzed the effects of its depletion in pronephric kidney formation by using morpholinos. To investigate a possible mechanism of action for Fry and Lhx1 in the formation of the kidney field we performed MicroRNA deep sequencing on embryos depleted of the two proteins.

Results: We found that *fry* is expressed in the intermediate mesoderm and developing pronephros. We determined that embryos depleted of *fry* by morpholino injections show almost complete loss of the kidney field, resembling the *lhx1* depletion phenotype. We observed a synergistic effect of these two proteins indicating the requirement of this interaction for the specification of the kidney progenitor cells. We identified miRNAs affected by the depletion of these proteins that might be in part responsible for the phenotype.

Conclusions: These results suggest a role of Fry in the pronephric kidney development associated with the transcriptional activity of Lhx1, possibly through regulation of miRNAs expression.

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FR-PO720

Hedgehog Signaling Controls Renal Capsule Formation and Nephron Number via Cell Autonomous and Non-Autonomous Mechanisms

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Background: Nephrogenesis is controlled by interactions between *Foxd1*+ cortical stromal and adjacent nephron progenitor cells. Underlying mechanisms are poorly defined. Based on expression of Hedgehog signaling effectors, Indian Hedgehog, *Gli1* and *Patched1* in cortical stromal cells, we hypothesize that Hedgehog signalling controls cortical stromal cell functions. Hedgehog ligands signal via SMO to increase levels of GLI transcriptional activators and decrease formation of GLI3 repressor (GLI3R), a processed form of GLI3 protein.

Methods: Hedgehog signalling was modulated in vivo using *Cre*-mediated recombination. Tissue ultrastructure and cell proliferation were analyzed by scanning electron microscopy and in situ BrdU-incorporation, respectively.

Results: *Foxd1-Cre;Smo^{null}* mice (E15.5) demonstrated renal hypoplasia and a discontinuous capsule of cortical stromal cells. Despite initial formation of the capsule, expression of *Gli1*, *Foxd1* and *Raldh2* was markedly decreased with a 54% decrease in capsular stromal cell proliferation by E13.5. Glomerular number was decreased by 42% by E18.5. Surprisingly, *Six2*+ positive cap mesenchyme was expanded with a 41% increase in *Six2*+ cell proliferation at E15.5. Yet, the number of nephrogenic intermediate structures was decreased by 50% starting at the stage of renal vesicle with a specific decrease in *Lhx1* expression. Introduction of conditional and obligate *Gli3R* alleles in *Foxd1*+ cells phenocopied Foxd1-Cre;*Smo^{null}* mice, while homozygous deficiency of *Gli3* rescued the mutant phenotype. Hedgehog functions in *Six2*+ nephrogenic cells were investigated in mice with *Smo* deficiency or *Cre*-mediated Gli3R expression, demonstrating decreased *Six2*+ cell number and proliferation by 70%, and a 60% decrease in nephron number.

Conclusions: We conclude that Hedgehog-Gli signalling acts via cell autonomous and non-autonomous mechanisms to control: (1) stromal cell gene expression and proliferation and nephron number, and (ii) *Six2*+ nephrogenic cell proliferation and nephron formation, in a manner that is dependent on expression of GLI3R.

Funding: Government Support - Non-U.S.

FR-PO721

Nephron Progenitor Networks in Mouse and Man: Novel Targets, Common Threads and Unexpected Differences Lori L. O'Brien,[1] Qiuyu Guo,[1,2] Joo-Seop Park,[3] Anton Valouev,[2] Andrew P. McMahon.[1] *[1]Dept of Stem Cell Biol and Regen Med, USC Keck School of Medicine, Los Angeles, CA; [2]Dept of Prev Med, USC Keck School of Medicine, Los Angeles, CA; [3]Div of Ped Urol and Dev Biol, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: All cells of the nephron are derived from a common progenitor. The nephron progenitor (NP) population of the developing kidney must maintain a balance of self-renewal and differentiation to ensure sufficient nephron endowment. While the mouse kidney generates ~14,000 nephrons over 10 days, around 1 million nephrons are generated over many weeks of human kidney development. Consequently, the lifetime of progenitor programs is quite different in each system.

Methods: Six2 is expressed within NPs where it promotes their self-renewal and prevents ectopic nephrogenesis. Pax2, Hox11 paralogs, Osr1, Sall1, Wt1, and Eya1 are expressed within the NP population and all are required for proper kidney development. Despite their integral roles, little is known about their transcriptional targets and the regulatory interplay amongst these factors. To this end, we have employed ChIP-seq to identify transcriptional targets of Six2, Hoxd11, and Osr1 in mouse and the transcriptional network regulated by SIX2 within the human fetal kidney.

Results: The binding of these three factors and human SIX2 is significantly enriched near genes associated with kidney development such as Sall1, Pax2, Wt1, Itga8, Kif26b, and the three factors themselves, in addition to unique nephrogenic targets within human. We have identified novel targets, including a component of chromatin modifying complexes that is specifically expressed within the NPs; functional analyses are underway.

Conclusions: These data suggest a regulatory circuitry that promotes self-renewal through positive feedback and supports the expression of factors essential for cellular integrity. Our studies reveal shared regulatory actions between mouse and human as well as divergent and interestingly novel regulatory interplay at other nephrogenic regulators. A better understanding here will facilitate efforts to utilize progenitor programs for regenerative strategies.

Funding: NIDDK Support

FR-PO722

The Groucho Associated Phosphatase PPM1B Displaces PTIP to Switch Pax2 from a Transcriptional Activator to a Repressor Sanjeevkumar R. Patel,[1] Saji Abraham.[1] *[1]Internal Medicine, Univ of Michigan, Ann Arbor, MI; [2]Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: In mammals, the Gro/TLE family consists of four proteins of similar molecular weight and structure. These proteins cannot bind to DNA themselves, but modulate the process of transcription by physical interaction with DNA-binding transcription factors. These proteins can down regulate the expression of target genes of transcriptional activators, enhance the transcriptional repression effect of transcriptional repressors, or convert transcriptional activators into repressors. Grg/TLE co-repressors were shown to interact with multiple transcription factors including HES proteins, RUNX proteins and Pax proteins. Pax genes encode developmental regulatory proteins that specify cell lineages and tissues in metazoans. Upon binding to DNA through the conserved paired-domain, Pax proteins can recruit both activating and repressing complexes that imprint distinct patterns of histone methylation associated with either gene activation or silencing. Expression of the Groucho proteins is widespread during development and overlaps partially with the Pax proteins in the neural tube and the kidney. Interaction of the Groucho/TLE members and Pax proteins may repress Pax-regulated genes.

Methods: We identified Grg4 containing complexes from mammalian nuclear lysates by fractionation, immunoaffinity purification, and mass spectrometry.

Results: In this report, we identify the phosphatase PPM1B as an essential component of the Groucho4 repressor complex that is recruited by Pax2 to chromatin. PPM1B can dephosphorylated the Pax2 activation domain and displace the adaptor protein PTIP, thus inhibiting H3K4 methylation and gene activation. Loss of PPM1B, prevents Groucho mediated gene repression.

Conclusions: Thus, PPM1B helps switch Pax2 from a transcriptional activator to a repressor protein. This can have profound implications for developmental regulation by Pax proteins and suggests a model for imprinting specific epigenetic marks depending on the availability of co-factors.

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FR-PO723

Wnt9b Directly Activates beta-Catenin Signaling in the Nephron Progenitor Cells Xinchao Pan, Thomas J. Carroll. *Internal Medicine (Nephrology), Molecular Biology, UT Southwestern Medical Center, Dallas, TX.*

Background: Wnt signals play multiple roles during kidney development. The major mediator of Wnt signaling is the transcription factor beta-catenin. Activation of the Wnt pathway leads to the translocation of β-Catenin from cytoplasm to the nucleus where it acts as a co-factor with members of the LEF/TCF family of DNA-binding transcrlption factors to drive expression of target genes. We recently hypothesized that Wnt9b promotes renewal of the nephron progenitors in a beta-catenin dependent manner. However, several "universal" beta-catenin reporter lines failed to show activity in the nephron progenitors.

Methods: To test whether Wnt9b directly activated beta-catenin activity in the progenitors, we engineered mice driving expression of Lac-z under the control of a putative enhancer element of Tafa5, a Wnt9b target gene. This enhancer element contains conserved Lef/Tcf binding sites.

Results: We found that this Tafa5-LacZ reporter is expressed in the nephron progenitor cells from E11.5 through birth. Wnt9b and β-Catenin are both necessary for expression of this reporter. Further, we have found that the transcription factors C-myc, N-myc and Six2 cooperate with beta-catenin to activate the Tafa5 reporter.

Conclusions: Our data indicate that Six2 and c-myc cooperate with a permissive Wnt9b/beta-catenin program to drive nephron progenitor renewal. We are currently testing how these interactions regulate nephron endowment in vivo.

Funding: NIDDK Support

FR-PO724

Renal Blood Flow and Oxygenation Drive Nephron Progenitor Differentiation Christopher Cain Rymer, Sunder Sims-Lucas. *Pediatric Nephrology, Rangos Research Center, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA.*

Background: During kidney development two discrete, yet simultaneous, vascular processes take place: angiogenesis, vessel growth from a major preexisting vessel, and vasculogenesis, de novo vessel formation from residential endothelial progenitors. Little is known about renal embryonic blood flow and its interactions with renal stem cells. The nephrogenic zone contains the renal stem cells that are thought to exist and proliferate in an oxygen-depleted environments.

Methods: Here, we investigate renal blood flow ontogeny and the role of oxygen concentration on nephron progenitor differentiation. To elucidate blood flow, ultrasound-guided intracardiac microinjection of FITC-tagged tomato lectin (TL) was utilized to map perfusion throughout each embryo. Kidneys were co-stained for vasculature, ureteric epithelium, nephron progenitors, and differentiated nephron structures. Embryonic mice throughout kidney development (E11.5 to E17.5) were used. We also analyzed nephron progenitor differentiation in vitro in normoxia compared to hypoxia.

Results: At E13.5, major vascular branches were perfused, however, smaller-caliber peripheral vessels remained unperfused. By E15.5, glomeruli and peripheral vessels begin to become perfused. While interior kidney vessels were perfused, peripheral vessels within the nephrogenic zone remained unperfused. Prior to an onset of blood flow, vast populations of renal vessels (believed to be vasculogenic) form within the nephrogenic zone. Directly adjacent and internal to the nephrogenic zone, we found differentiated nephron structures surrounded and infiltrated by perfused vessels. In vitro at low oxygen concentration little nephron progenitor differentiation was observed; at higher oxygen concentrations more differentiation of nephron progenitors was induced. This is seen through a formation of Six2 derived aggregates, marking differentiated glomeruli and nephron structures. In hypoxic environments, metanephric cap mesenchyme seems to maintain a higher state of proliferation as compared to normoxia.

Conclusions: From these investigations we concluded that renal blood flow and oxygenation are critical for nephron progenitor differentiation.

Funding: NIDDK Support, Other NIH Support - Vascular Medicine Institute of the University of Pittsburgh

FR-PO725

Metabolic Fitness in Nephron Progenitor Renewal Jiao Liu, Samir S. El-Dahr, Zubaida R. Saifudeen. *Pediatrics, Tulane Univ Health Sciences Center, New Orleans, LA.*

Background: Premature termination of self-renewal rapidly depletes the progenitor population resulting in nephron deficit. Glucose metabolism, ATP production and ROS generation are critical modulators of stem/progenitor cell self-renewal.

Methods: A conditional p53-null mouse model of the Six2+ cap mesenchyme (CM) ($Six2^{p53-/-}$) was generated and used to test the hypothesis that metabolic fitness regulates self-renewal of CM cells. Data was analyzed by immunofluorescence, transcription profiling by RNA-Seq of FACS isolated CM cells, ATP and ROS measurements.

Results: 1. $Six2^{p53-/-}$ kidneys show renal hypoplasia, nephron deficit, loss of Cited1+/Six2+ but not the Cited1-/Six2+ cells, suggesting the NPC prematurely transition to non-self-renewing Six2+/Cited1- cells. 2. While the apoptotic index is unchanged, $Six2^{p53-/-}$ cells show decreased BrdU incorporation and an extended cell cycle. 3. Top down-regulated genes in $Six2^{p53-/-}$cells belong to glucose metabolism pathways - fructose bisphosphatase 1 (*Fbp1*, -82.0), phosphoenol pyruvate carboxykinase 1 (*Pck1*, -14.3) and aldolase b (*Aldob*, -12.0). Oxphos genes are also down-regulated – including genes encoding electron transfer chain (ETC) enzymes and maintenance of mitochondrial membrane potential. 4. As a result of these metabolic derangements, $Six2^{p53-/-}$ cells showed 2.0-fold reduction in ATP levels ($p<0.005$, n=3), 33% decline in ROS levels and diminished mitochondrial immunostaining as detected with an antibody against a mitochondrial protein.

Conclusions: Our data suggest that robust metabolic fitness is required to maintain the stemness of nephron progenitors.

Funding: NIDDK Support

FR-PO726

Renal Dysplasia - unMET Needs? Satyamaanasa Polubothu, Chiara Mari, Karen Price, Paul Winyard. *Developmental Biology & Cancer, UCL Inst of Child Health, London, United Kingdom.*

Background: Renal dysplasia accounts for 50% of paediatric patients with CKD-V. A key step during nephrogenesis is mesenchymal-to-epithelial-transformation (MET), generating the entire nephron from glomerulus to distal tubule. Aberrant MET occurs in dysplasia. We tested whether there is an intrinsic defect in dysplastic mesenchyme which prevents MET or whether the cells can transform if given the right stimuli. Aims: 1) Generate novel human dysplastic mesenchymal cell lines, 2) Assess MET in response to chemical inducers, 3) Evaluate MET in 3-D reconstitution.

Methods: After ethical consent, 6 childhood dysplastic samples (6 months - 5 years) were collected, surgically diced and cultured until confluent. Cells were aliquoted and exposed to MET inducers for 7 days including: Leukaemia-Inhibitory-Factor (LIF; 20ng/ml), Lithium Chloride (LiCl; 1 or 20mM) and BIO (0.1 or 1uM). Phenotypic and molecular conversion was assessed by morphology, Q-PCR and Western blotting. In parallel, fluorescently-labeled cells were grown 1:5 in 3-D reconstitution organ culture with normal human fetal kidney progenitors.

Results: Each culture generated predominantly mesenchymal populations. These postnatal cells express key developmental markers *PAX2*, *HGF*, *GDNF*, *OSR1*, *EYA1* and stem markers *CD24* and *CD133*, but lack other critical factors including *SALL1*, *CITED1*, *SIX2*, *WT1*, *WNT4*, *WNT9b*. Each MET inducer enhanced proliferation c.f. controls, most marked with LIF and LiCl. Sparse islands of epithelial cells also developed but gene expression patterns were not consistently changed by the MET inducers. Recombined dysplastic/normal chimaeras initially re-aggregated but broke down at 48-72 hours, with no evidence of the epithelial differentiation seen in controls.

Conclusions: Our unique human postnatal dysplastic cell lines interestingly express fetal developmental markers but proliferate rather than undergoing MET with inducers, and had inhibitory effects in 3-D culture. Thus far, we cannot distinguish between intrinsic and extrinsic defects in dysplasia. Ongoing work will assess dysplastic versus normal MET. If we can identify why dysplastic cells inhibit 3-D differentiation in vitro, we may be able to target therapies to overcome this in vivo.

Funding: Other NIH Support - UK NIHR Academic Clinical Fellowship (lead author)

FR-PO727

HoxB7.Cre-Mediated mVps34 Deletion Inhibits Nephrogenesis and Causes Nephron Hypertrophy Jinxian Xu,[1] Jianchun Chen,[2] Kaitlyn Werner,[3] Jeffrey G. Dickhout,[3] Jian-Kang Chen.[1] *[1]Cellular Biology & Anatomy and Medicine, GRU, Augusta, GA; [2]Medicine, Vanderbilt Univ, Nashville, TN; [3]Medicine, McMaster Univ, Hamilton, ON, Canada.*

Background: The mammalian homolog of yeast vacuolar protein sorting 34 (mVps34) has been implicated in nutrient sensing, but its physiological role in the renal collecting system is unknown.

Methods: We generated an mVps34-floxed (*mVps34$^{flox/flox}$*) mouse and crossed it with a HoxB7.Cre mouse to produce renal collecting system-specific mVps34 knockout (*KO*) mice and used *mVps34$^{flox/flox}$*;HoxB7.Cre $^{(-)}$ littermates as controls (*Ctrl*).We also bred *mVps34$^{flox/flox}$* mice to a membrane-targeted tomato Red/GFP reporter (mRG) mouse and then to an ImmortoMouse (Im) and established an *mVps34$^{flox/flox}$;mRG^{+};Im^{+}* inner medullary collecting duct (IMCD) cell line.

Results: By E17.5, *KO* mice began to show dilated renal collecting ducts and proximal tubules. At birth, *KO* mice had significantly smaller kidneys and lower kidney-to-body weight ratio (0.21±0.02% versus 0.42±0.18% in *Ctrl*, $P<0.0001$, $n=10$), with indistinguishable body weight but markedly reduced number of DBA- or calbindin-D28K-positive renal tubules. By 2 months of age (long after nephrogenesis is complete), *KO* mice had a 58% reduction in glomerular number (7670 ± 1530 versus 18392 ± 1333 in *Ctrl*, $P<0.001$, $n=5$-7 mice/group) and exhibited unproportionally thinner renal medulla (medulla/whole kidney volume ratios: 22.60 ± 1.84% versus 37.56 ± 3.77% in *Ctrl*, $P<0.05$, $n=4$), with marked glomerular and tubular hypertrophy. 15% of *KO* mice developed glomerulosclerosis and interstitial fibrosis, with elevated albumin/creatinine ratio (278.20 ± 67.27 versus 20.49 ± 2.36 µg/mg in *Ctrl*, $P<0.01$, $n=6$) and increased BUN levels (53.33 ± 8.43 versus 23.75 ± 7.60 mg/dl in *Ctrl*, $P<0.001$, $n=6$). Adeno.Cre-infected *mVps34$^{flox/flox}$;mRG^{+};Im^{+}* IMCD cells showed green fluorescence and mVps34 deletion, with diminished Akt phosphorylation and down-regulated cyclin D1 and cyclin E expression, leading to inhibition of cell proliferation and branching morphogenesis in 3D Matrigel culture.

Conclusions: Our study demonstrates that mVps34 plays an important role in nephrogenesis by promoting cell proliferation and branching morphogenesis of ureteric bud-derived cells.

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FR-PO728

The Effect of Vitamin D Deficient Diet during Pregnancy on Nephron Endowment in Rat Offspring Cagdas Oto,[1] Mehmet Altan,[2] Mehmet Fatih Bozkurt,[3] Caner Bakici,[1] Ozan Ahlat,[4] Alexis Okoh,[5] Sim Kutlay,[5] Rifki Haziroglu,[4] Sehsuvar Erturk.[5] *[1]Anatomy, Ankara Univ, Faculty of Veterinary Medicine, Ankara, Turkey; [2]Medical Oncology, Yale School of Medicine, New Haven, CT; [3]Pathology, Afyon Kocatepe Univ, Faculty of Veterinary Medicine, Afyonkarahisar, Turkey; [4]Pathology, Ankara Univ, Faculty of Veterinary Medicine, Ankara, Turkey; [5]Nephrology, Ankara Univ School of Medicine, Ankara, Turkey.*

Background: Vitamin D deficiency has been suggested to play role in the pathogenesis of various diseases including hypertension. One of the mechanisms responsible for the development of primary hypertension is low nephron number. We hypothesized that inadequate vitamin D intake during pregnancy could influence nephron endowment in the offspring.

Methods: Sprague-Dawley female rats of 21-day old were randomly divided into three groups after weaning and fed with vitamin D-high (6,000 IU/kg), vitamin D-normal (1,500 IU/kg) and vitamin D-low (0.1 IU/kg) diets for 4 weeks. These rats were then mated with Sprague-Dawley male rats fed with vitamin D-normal diet. The offspring were euthanized after their delactation in postpartum 3 weeks and the body weight, heart weight, and kidney weight were measured. Glomerular diameters and nephron numbers were examined stereologically under light microscopy.

Results: The first two days after parturition, the numbers of living litters were 10 in vitamin D-high, 10 in vitamin D-normal, and 4 in vitamin D-low group. The body, heart, and kidney weight and nephron number were lower, while glomerular diameter was greater in the offspring of vitamin D-low group than those in the other groups.

	Vitamin D-high	Vitamin D-normal	Vitamin D-low	p
Body weight (g)	49.7±6.5	52.9±7.3	33.6±2.6	<0.001
Heart weight (mg)	239.6±17.4	224.0±26.1	192.0±18.2	0.006
Kidney weight (mg)	244.1±22.6	282.8±35.3	187.6±9.6	<0.001
Nephron number (n)	26,319±3,417	28,622±3,598	19,525±1,002	0.001
Glomerular diameter (µm)	93.5±1.4	95.0±2.2	98.6±1.4	0.035

Conclusions: Vitamin D deficiency during intrauterine life may have a negative impact on nephron endowment, which might be responsible for the development of hypertension.

Funding: Government Support - Non-U.S.

FR-PO729

Redundant and Distinct Roles of Mdm2 and Mdm4 in Ureteric Bud Branching Morphogenesis Sylvia Hilliard, Samir S. El-Dahr. *Pediatrics, Tulane Univ School of Medicine, New Orleans, LA.*

Background: Murine double minute 4 (Mdm4, also called Mdmx) is a close analogue of the p53-ubiquitin ligase, Mdm2, and a negative regulator of p53 transcriptional activity. Mdm4 dimerizes with Mdm2 to regulate p53 levels and activity in some tissues while exerting functions independent of Mdm2 in others. Deletion of Mdm2 in the ureteric bud (UB) lineage causes renal cystic dysplasia in a p53-depndent manner (Hilliard et al. Dev. Biol. 2011). The role of Mdm4 in UB branching and the functional redundancy of Mdm2 and Mdm4 in kidney development are unknown.

Methods: 1. The temporal expression of *Mdm4* was determined by QRT-PCR. 2. Deletion of *Mdm4* from the UB lineage was achieved by crossing *Hoxb7*-eGFP-*Cre to Mdm4$^{F/F}$* mice. 3. The effects of Mdm4 deletion on UB branching and nephron differentiation were examined by observing GFP fluorescence in vivo, UB tip counting, and IF staining of UB and nascent nephron markers. 4. The functional overlap of Mdm4 and Mdm2 was assessed in UB$^{Mdm4/Mdm2}$ compound mutant mice.

Results: 1. Examination of UB$^{Mdm4-/-}$ kidneys revealed that Mdm4 function is dispensable until the T-stage. 2. Disruption of UB branching starts around E12.5 resulting in fewer UB tips (~42% of wild type), dilated UB ampullae, a few even oriented parallel to the cortical aspect of the kidney. 3. A marker of tip progenitors, p63, is absent from many of the mutant Calbindin^{+} UB, and expression of pan cytokeratin in the tips is less robust than in wild type. 4. The intercalated cell marker, carbonic anhydrase II, is detected in only a few of the UB$^{Mdm4-/-}$ branches. 5. Six2^{+}/Pax2^{+} cap mesenchyme clusters around the mutant UB tips are smaller and less cohesive. 6. UB$^{Mdm4-/-}$ kidneys are endowed with fewer mature glomeruli at birth and are deficient by 1-2 nephron generations. 7. The UB$^{Mdm4-/-, Mdm2+/-}$ kidneys are smaller than the UB$^{Mdm4-/-, Mdm2+/+}$ kidneys, which in turn are smaller than UB$^{Mdm4+/-, Mdm2+/-}$ kidneys.

Conclusions: 1. Mdm4 is required for normal UB branching past the T-stage. Mdm4 inactivation results in renal hypoplasia. Later in development, Mdm4 is required for collecting duct cell differentiation. 2. Mdm2 and Mdm4 cannot fully compensate for each other in UB branching morphogenesis.

Funding: NIDDK Support

FR-PO730

Knockout of the Epithelial Splicing Regulatory Proteins Disrupts Kidney Development and Ureteric Branching Morphogenesis in Mouse
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Background: The Epithelial splicing regulatory proteins 1 and 2 (Esrp1 and Esrp2) coordinate a splicing regulatory network (SRN) of transcripts associated with processes necessary for epithelial function and differentiation. Esrp expression is epithelial cell-type-specific and switch-like changes of several known Esrp regulated alternative splicing (AS) targets are crucial during embryonic development. While transcriptional regulation of epithelial-mesenchymal interactions has been extensively evaluated during development, the contribution of AS to this process is limited. Esrp targets such as the Fgfr2 signaling axis have been shown to be essential for ureteric epithelium (UE) branching and kidney formation. Therefore, we propose that the Esrp SRN converges on pathways crucial for epithelial differentiation and mesenchymal interaction during UE branching and kidney formation.

Methods: To evaluate the requirement of the Esrps in these fundamental processes, we generated mouse knockout alleles for *Esrp1 and Esrp2*.

Results: Esrp1 KO mice are neonatal lethal and present with several developmental defects. Substantially more severe defects are observed in Esrp1/Esrp2 double KO (DKO) mice, some of which phenocopy those observed with disruption in FGF signaling. Evaluation of Esrp KO embryonic kidneys by 3D-reconstruction and histological analysis has revealed higher incidence of renal agenesis, reduced UE branching resulting in smaller kidneys, and dilation of ureteric tips with expanded Ret expression. Splicing analysis in Esrp KO mice indicate transcript-specific splicing susceptibility of Esrp targets, ranging from complete switches by Esrp1 KO while others require DKO.

Conclusions: These results clearly implicate AS regulated by the Esrps in kidney formation and UE branching. Using conditional KO of the Esrps in UE we will evaluate the cell autonomous nature of the UE branching defects, and RNAseq of purified UE will determine the global SRN mediated by the Esrps to guide functional evaluation of AS targets *in vivo*.

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FR-PO731

Specific Claudins Regulate Nephric Duct Elongation and Ureteric Bud Branching Morphogenesis
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Background: The claudin family of tight junction proteins are important for the regulation of renal paracellular transport, but they are also expressed during kidney development. To understand their roles during nephric duct formation and ureteric bud branching morphogenesis, embryonic chick and mouse kidneys were treated with a truncated non-toxic form of the *Clostridium perfringens enterotoxin* (C-CPE) that removes Claudin-3,-4,-6,-7,-8, and -14 from tight junctions.

Methods: Chick embryos and mouse metanephric kidney explants were treated with C-CPE protein (GST fused to amino acids 184-319 of CPE) or with GST alone. CRISPR/Cas mediated gene editing was used to generate homozygous Claudin-3 knockout mice.

Results: Claudin-3 and claudin-4 are expressed in the chick nephric duct, while claudins-3,-4,-6,-7, and -8 are all expressed in the mouse nephric duct and the ureteric bud branching network by whole mount in situ hybridization (WISH). Chick nephric cords were treated with beads saturated with C-CPE protein or GST at E1.5. Adjacent to the C-CPE bead, the nephric duct failed to form as shown by histological analysis and a lack of expression of the nephric duct marker *Lim1* by WISH (15/22 embryos), while the contralateral side and controls (GST beads) showed normal nephric duct formation and elongation (10/10 embryos). Treatment of mouse E11.5 and E12.5 kidney explants from *HoxB7/GFP*$^{+/-}$ transgenic mice with C-CPE caused a marked reduction in the number of ureteric bud tips compared to GST-treated controls (n=5/group, $P<0.05$) that was not due to a defect in apoptosis or proliferation. CRISPR biallelic claudin-3 knockout mice are viable and do not appear to have a defect in kidney development.

Conclusions: Removal of a single claudin, claudin-3, does not affect kidney morphogenesis. However, the selective removal of multiple claudins impairs nephric duct formation and ureteric bud branching morphogenesis. We speculate that the C-CPE-sensitive claudins regulate changes in cell shape that are critical for tubule and branch point formation.

Funding: Private Foundation Support

FR-PO732

Lack of Glycogen Synthase Kinase 3a and b in the Ureteric Bud Leads to Renal Agenesis
Shixin Tao, Reena Rao. *The Kidney Inst, Dept of Internal Medicine, Univ of Kansas Medical Center, Kansas City, KS.*

Background: The Glycogen Synthase Kinase 3 (GSK3) family of serine/threonine protein kinase consists of two isoforms, GSK3a and GSK3b. The GSK3 isoforms regulate cell survival, proliferation, polarization and differentiation, and are critical for embryonic development of the heart and brain. GSK3b plays an important role in urine concentration by the renal collecting ducts and in cyst expansion in polycystic kidney disease. However, the role of GSK3 in renal development is unknown. The current study reports an unexpected finding that suggests that GSK3 plays a critical role in embryonic kidney development.

Methods: GSK3b knockout leads to early perinatal lethality, while global GSK3a knockout is viable. Hence, GSK3a$^{+/-}$, GSKb$^{flox/flox, HoxB7\text{-}Cre}$ mice were bred together. One fourth of the embryos were expected to lack both GSK3a and GSK3b in their ureteric bud, which gives rise to the connecting tubule, collecting ducts and ureters in the adult mouse kidney.

Results: Pups were born in the normal Mendelian ratio. Out of 158 newborn mice, 38 were GSK3a$^{-/-}$, GSKb$^{flox/flox, HoxB7\text{-}Cre}$ representing mice that lacked both GSK3a and GSK3b. However, the GSK3a$^{-/-}$, GSKb$^{flox/flox, HoxB7\text{-}Cre}$ pups died within 32 hours after birth. These mice either completely lacked kidneys or had a very small rudimentary kidney. Hematoxylin and Eosin staining in the rudimentary kidney showed very few glomeruli and tubules. Immunofluorescence staining for Dolichos biflorus agglutinin or Lotus tetragonolobus agglutinin, markers of collecting ducts and proximal tubules respectively revealed a limited number of tubules staining for either marker. The epithelial cells of some of these tubules showed staining for both markers.

Conclusions: The study thus demonstrates that gene deletion of GSK3a and GSK3b in the embryonic ureteric bud leads to renal agenesis and suggests that GSK3 may play a critical role during early development of the kidney.

Funding: NIDDK Support

FR-PO733

Lack of the Prorenin Receptor (PRR) in the Ureteric Bud (UB) Disrupts Developmental Programming of Nephrogenesis
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Background: In this study, we tested the hypothesis that decreased nephron endowment in *PRR*$^{UB-/-}$ mice which lack the PRR in the UB lineage (PLoS ONE, 2013) is due to defects in molecular pathways and cellular mechanisms that control nephrogenesis.

Methods: The number of vesicles (V), comma (C), S-shaped (S), and mature (M) nephrons was counted on P1 from H&E-stained kidney sections. Mesenchymal cell proliferation and apoptosis was assessed on kidney sections (3 sections/kidney) using anti-phosphohistone H3 (pH3) and -cleaved caspase 3 (Ca3) antibodies on embryonic (E) days E12.5 and E18.5. Data were normalized to the total number of DAPI-positive cells (Image J). Expression of key genes that control nephrogenesis was studied by real-time qRT-PCR or *in situ* hybridization (ISH) on E13.5. The intensity of Six2 immunostaining (Abcam, 1:200), normalized for the surface area of the kidney section, was quantitated by Slidebook 4.1 software.

Results: The number of V, C, S and M nephrons was reduced in mutant compared with control kidneys ($p<0.05$). The number of Ca3-positive apoptotic cells was higher (E12.5: 20.7±2.5 versus 4.0±1.4 $p<0.001$; E18.5: 10.4±3.8 versus 2.5±1.2, $p<0.05$). The number of pH3-positive cells did not differ on E12.5 (56.7±6.8 versus 61.250±9.674, $p=0.5$) and was reduced on E18.5 (18.9±2.7 versus 38.7±3.4, $p<0.001$) in the mesenchyme of mutantcompared with control kidneys. qRT-PCR demonstrated decreased ($p<0.01$): Pax2, Pax8, FGF8, Lhx1, Wnt4, Wnt9b and β-Catenin, and increased Six2, Bmp7 and Cited1 mRNA levels in mutant compared to control kidneys. Six2 immunostaining was increased in the mutant versus control kidneys ($p<0.05$). ISH showed apparent reduction in Wnt9b mRNA expression in the mutant UBs.

Conclusions: We conclude that lack of the UB *PRR* disrupts nephrogenesis *via*: 1) Induction of mesenchymal cell apoptosis, 2) Inhibition of mesenchymal cell survival and 3) Aberrant expression of key genes that direct nephrogenesis. We propose that lack of the UB *PRR* disrupts nephrogenesis through reduced Wnt9b-directed canonical Wnt/β-Catenin signaling resulting in decreased renal vesicle formation.

FR-PO734

Abnormal Urinary Tract Formation and Remodeling in the Absence of Hepatocyte Nuclear Factor 1beta
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Background: Congenital Anomalies of Kidney and Urinary Tract (CAKUT) represent 20-30% of prenatal malformations. One of the most prevalent genetic defects responsible for CAKUT is represented by mutations in HNF1B (Hepatocyte Nuclear Factor1 beta), a transcription factor expressed since the first steps of kidney development.

Methods: Analyses of mutant embryos have shown that the absence of HNF1beta leads to renal aplasia, and that this phenotype is due to an absence of mesenchymal to epithelial transition and to abnormal branching of the ureteric bud. However, the development of the urinary tract in these embryos has not been studied.

Results: In wild type embryos, mesonephric tubules are induced in the rostral part of the nephric duct, and are spatially separated from the emergence of the ureteric bud. By contrast, embryos lacking *Hnf1b* are characterized by rudimentary ectopic budding all along the nephric duct. In wild type embryos, at E9.5, the nephric duct connects with the cloaca via the CDN, and then the CND disappears by apoptosis during the following days. In *Hnf1b* mutants, the connection between the nephric duct and the cloaca occurs normally but the CND does not regress and leads to an abnormal connection between the ureter and the bladder.

Conclusions: These observations show that, in addition to its roles during nephrogenesis, Hnf1b plays a crucial role in the formation and remodeling of the urinary tract. This suggests that children with abnormalities of the urinary tract should be tested for mutations in the HNF1B gene.

Funding: Government Support - Non-U.S.

FR-PO735

Deletion of Kelch-Like ECH-Associated Protein 1 (Keap1) in Kidney Epithelial Cells Causes Hydronephrosis in Mice Sanjeev Noel,[1] Lois J. Arend,[2] Samatha Bandapalle,[1] Sekhar P. Reddy,[3] Hamid Rabb.[1] *[1]Dept of Medicine; [2]Dept of Pathology, Johns Hopkins Univ, Baltimore, MD; [3]Dept of Pediatrics, Univ of Illinois, Chicago, MD.*

Background: The Nrf2-Keap1 pathway has an important role in both acute kidney injury (AKI) and chronic kidney disease (CKD). This pathway has a key regulatory function on many antioxidant and stress pathways, including heme oxygenase. We deleted Keap1 in tubular epithelial cells (primarily distal convoluted tubule and collecting ducts) of mouse kidney by Cre-LoxP mechanism to evaluate the specific effect of this pathway on AKI. Surprisingly, these mice had significant developmental defects in the collecting system. This could unveil a key role of this pathway in kidney development, as well as possibly in repair and regeneration.

Methods: Kidney epithelial cell specific Keap1-deficient (Ksp-Keap1-KO) mice were generated by crossing Ksp-Cre with Keap1 floxed ($Keap1^{fl/fl}$) mice. A comprehensive histological examination along with detailed hematological, biochemical and urinary analysis was performed to evaluate these mice.

Results: Kidneys from Ksp-Keap1-KO (10 weeks, male) mice had small visible dilatations on their surface and were slightly larger than $Keap1^{fl/fl}$ kidneys. More significantly, transverse sections through the middle of the kidney revealed moderate to marked renal pelvic expansion and significant compression of medullary parenchyma consistent with hydronephrosis in Ksp-Keap1-KO mice. Ksp-Keap1-KO (n=3) had a significantly higher RBC count (9±0.1 versus 9.6±0.3 M/μL, p=0.04), Hb (12.5±0.2 versus 13.9±0.6 g/dL, p=0.01), hematocrit (42.3±0.6 versus 47.3±2.0%, p=0.02), mean cell volume (46.7±0.5 versus 49±1.0 fL, p=0.02) and mean cell Hb concentration (13.8±0.1 versus 14.4±0.2 g/dL, p=0.003). There was no change in lymphocyte and platelet populations.

Conclusions: These data suggest that absence of Keap1 in renal tubular epithelial cells significantly affects normal kidney development in a fashion consistent with hydronephrosis. There is also an increased hemoglobin. This unexpected data has revealed a novel role for Keap1 mediated signaling pathway in renal development.

Funding: NIDDK Support

FR-PO736

Renal-Specific Inactivation of the Exocyst Sec10 Gene in Mice Causes Lethal Ureteropelvic Junction Obstructions due to Urothelial Defects Noemi Polgar,[1] Amanda J. Lee,[1] Vanessa H. Lui,[1] Kadee-Kalia Tamashiro,[1] Zsanett Jancso,[1] Joshua H. Lipschutz,[2] Ben Fogelgren.[1] *[1]Univ of Hawaii; [2]Univ of Pennsylvania.*

Background: Congenital obstructive nephropathy, the most common cause of pediatric chronic kidney disease and ESRD, is caused by obstruction of the urinary tract during fetal development. The most common cause of congenital obstructive nephropathy is ureteropelvic junction obstruction (UPJ obstruction), when the stenosis is localized to the upper urinary tract where the renal pelvis transitions into the ureter. Despite the high prevalence and medical burden of UPJ obstructions, we have a poor understanding of its molecular and genetic basis, with a scarcity of non-surgical genetic models.

Methods: In previous studies using cell culture models, we have shown the eight-protein exocyst trafficking complex to be critical in maintaining aspects of polarized epithelial cells. To further *in vivo* studies of polarized exocytosis in renal development and disease, we have generated a novel transgenic mouse to conditionally knockout the Sec10 gene, a central component of the exocyst. This is the first conditional mouse strain for any exocyst subunit, and should be valuable in studying the exocyst's role in various tissues and diseases.

Results: Inactivation of *Sec10* in ureteric bud-derived cells using *Ksp1.3-Cre* mice resulted in severe bilateral hydronephrosis and neonatal death. The conditional knockout $Sec10^{FL/FL}$*;Ksp-Cre* embryos developed ureteropelvic junction obstructions between E17.5 and E18.5 as a result of degeneration of the urothelial layers and subsequent overgrowth by surrounding mesenchymal cells. By E16.5, $Sec10^{FL/FL}$*;Ksp-Cre* ureter and pelvic urothelium showed decreased uroplakin-3 protein at the luminal surface of urothelial cells, and complete absence of uroplakin-3 by E17.5. Affected urothelium at the UPJ showed irregular barriers that exposed the smooth muscle layer to urine, suggesting this may trigger the surrounding mesenchymal cells to overgrow the lumen.

Conclusions: Findings from this novel mouse model show Sec10 and the exocyst are critical for maintaining the urothelial barrier in ureters, and failure of this barrier may be a cause of UPJ obstructions.

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FR-PO737

Unilateral Renal Agenesis and Collecting Duct Lumen Defects in *Grainyhead-Like 2*-Deficient Mice Christian Hinze,[1,2] Janett Ruffert,[2] Annekatrin Aue,[2] Max Werth,[3] Katharina Walentin,[2] Zeliha Yesim Yurtdas,[2] Jonathan M. Barasch,[3] Kerim Mutig,[4] Sebastian Bachmann,[4] Kai M. Schmidt-Ott.[1,2] *[1]Nephrology, Charité Universitätsmedizin, Berlin, Germany; [2]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [3]Nephrology, Columbia Univ College of Physicians and Surgeons, New York; [4]Anatomy, Charité Universitätsmedizin, Berlin, Germany.*

Background: Congenital defects of renal branching and luminogenesis are frequent and associated with diseases of high prevalence, including cystic kidney diseases and renal hypoplasia. The molecular and cellular mechanisms governing tubular lumen establishment and branching morphogenesis may provide critical insights into their pathogenesis.

Methods: The transcription factor grainyhead-like 2 (Grhl2) is mostly expressed in epithelial cells of the ureteric bud and the distal nephron. To investigate its role, we generated collecting duct-specific Grhl2 knockout mice (Hoxb7/Cre; $Grhl2^{flox/-}$), which exhibit a deletion of functional Grhl2 protein in most cells of the collecting duct. Littermates were used as controls. We then performed a detailed analysis of the resulting phenotype comprising 3D single cell and collecting duct reconstruction.

Results: Although Hoxb7/Cre; $Grhl2^{flox/-}$ mice were viable to adulthood and kidney function was not impaired, we found a ten-fold higher incidence of unilateral renal agenesis at P30 when compared to control littermates (6.8% versus 0.6%, P=0.0053). Additionally, knockout mice displayed kidneys with a significantly reduced kidney weight compared to control littermates (reduction related to body weight by 16 % in Hoxb7/Cre; $Grhl2^{flox/-}$ mice, P<0.001). A closer examination of the knockout collecting ducts revealed markedly reduced lumen areas in the outer medulla (mean lumen area ± SEM in control mice: 53.2 μm^2 ± 6, n=13; KO mice: 33.3 μm^2 ± 5.5, n=10; P=0.005). Reconstructing the collecting ducts up to single cell level by 3D analysis, we uncovered distinct changes in cell size and cell density constituting this lumen phenotype.

Conclusions: Our data thus indicate an impact of Grhl2 on kidney development and collecting duct lumen formation. This might be relevant for congenital diseases of the kidney, including cystic kidney diseases.

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FR-PO738

A Novel Gene Network Downstream of Grainyhead-Like 2 Regulates Lumen Expansion and Barrier Formation in Renal Epithelial Cells Annekatrin Aue,[1] Christian Hinze,[1,2] Janett Ruffert,[1] Zeliha Yesim Yurtdas,[1] Max Werth,[1] Katharina Walentin,[1] Michael Schumann,[3] Kai M. Schmidt-Ott.[1,2] *[1]Max Delbrueck Center for Molecular Medicine, Berlin, Germany; [2]Dept of Nephrology, Charite, Berlin, Germany; [3]Dept of Gastroenterology, Charite, Berlin, Germany.*

Background: The establishment of a lumen is a fundamental step during organ development. In the nephrons of the kidney, the composition and three-dimensional structure of epithelial tubules are vital to determine body fluid composition. Previous studies of our group identified transcription factor grainyhead-like 2 (Grhl2) being highly expressed in epithelia of the distal tubule and collecting duct of the kidneys, but little is known about its functional relevance in these cells.

Methods: To gain more insights into the transcriptional network downstream of Grhl2 in renal epithelia we performed gene expression analysis and chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) using antibodies against Grhl2 and histone H3 lysine 4 trimethylation (H3K4me3), a histone modification associated with active promoters.

Results: Intersections of genome-wide gene expression analysis and ChIP-seq data resulted in a set of Grhl2 core target genes. Among these genes we identified known and several novel Grhl2 targets like Ovo-like 2 (Ovol2), encoding a zinc finger transcription factor. While downregulation of Grhl2 impaired lumen formation and barrier function of inner medullary collecting duct (IMCD-3) cells, reexpression of Ovol2 induced lumen expansion as well as expression of Grhl2-dependent genes, like the small GTPase Rab25 and claudin 4 (Cldn4), in IMCD-3 Grhl2-defficient cells. Individual overexpression of Cldn4 or Rab25 did not affect the lumen phenotype, but overexpression of Cldn4 together with Rab25 rescued lumen establishment and barrier formation in Grhl2/Ovol2-depleted cells.

Conclusions: These data reveal a novel transcriptional network supporting a Grhl2/Ovol2 feed forward loop network in collecting duct epithelia, which ensures stable expression levels of critical determinants of tubular lumen expansion and barrier function.

FR-PO739

Impaired Kidney Development in Mice with a Mutation in the Planar Cell Polarity Protein Celsr1 Hortensja Lucja Brzoska,[1] Angela Maria D'esposito,[2] Mark Lythgoe,[2] Eugenia Papakrivopoulou,[1] David A. Long.[1] *[1]Developmental Biology and Cancer, UCL Inst of Child Health, London, United Kingdom; [2]UCL Centre for Advanced Biomedical Imaging, London, United Kingdom.*

Background: The planar cell polarity (PCP) pathway plays a critical role in cytoskeletal organization and cell morphology. These processes are vital for kidney development and our previous work has shown that mice with a mutation in *Vangl2*, a core PCP component, have impaired glomerular maturation and ureteric bud branching. Therefore, we hypothesised that mutations in another PCP core molecule, *Celsr1* would also have a profound effect on renal development.

Methods: Using optical projection tomography and quantitative image analysis we examined kidney branching in the *Celsr1^{Crsh}* mutant mouse model. Renal histology and gene expression of PCP genes using quantitative PCR were assessed. *Celsr1* was also knocked down using siRNA in a proximal tubular cell line (HK-2).

Results: During development, in wild-type kidneys Celsr1 was expressed in comma and S-shaped bodies, early glomeruli and in some tubular structures. In adult kidneys Celsr1 expression was detected in the brush borders of proximal tubules and the basal side of distal tubules. At embryonic day (E) 13.5 the total kidney volume of *Celsr1^{Crsh}* mutants was significantly smaller compared to wild-type littermates ($p<0.05$, n=3-5 for each genotype). The kidneys of *Celsr1^{Crsh}* mutants also contained a reduced number of ureteric bud tips ($p<0.05$) but there was no difference in the ureteric bud tip length or angle. At E17.5, some proximal tubules and Bowman's capsules were dilated in *Celsr1^{Crsh}* mice but we did not observe an overt cystic phenotype. At E17.5, there were no changes in the mRNA expression of other PCP genes (*Vangl2*, *Vangl1*, *Scrib*, *Pk1* and *Pk2*) between *Celsr1^{Crsh}* and wild-type littermates. *In vitro* experiments using knock-down of *Celsr1* in HK-2 cells showed significant up-regulation of E-cadherin, an adherens junction molecule involved in cell adhesion and maintenance of tissue structure (n=6, $p<0.0001$).

Conclusions: This data highlights that Celsr1 plays a critical role in kidney tubular branching confirming the importance of the PCP signalling pathway during kidney morphogenesis.

FR-PO740

Bicaudal-C Mutation Induces PKD-Like Phenotypes in Drosophila

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Background: Renal cystogenesis can result from mutations in several genes, including *PKD1, PKD2* or *Bicaudal-C* in human and mice. The highly conserved Bicaudal-C protein was first identified in *Drosophila*. Shown to bind RNA, it down-regulates many targets mRNAs and was implicated in post-transcriptional regulation via cytoplasmic RNA polyadenylation and microRNAs. While it is known that Bicaudal-C mutation in *Drosophila* interferes with embryonic anterior-posterior polarity and development, a potential role in renal function remains to be addressed. Among all tissues, the renal (or Malpighian) tubule of *Drosophila* shows the highest degree of conservation with humans, likely because of functional constraint, making it an excellent model to identify key players in renal tubule function.

Methods: Different allelic combinations of *Bicaudal C* were tested for evidence of renal effects. Dissected Malpighian tubules were analyzed by immunofluorescence, western blot, real-time qPCR and immunoprecipitation.

Results: We found that Bicaudal C was expressed in the *Drosophila* Malpighian tubule with similar distribution to Bicaudal C in the mouse kidney. Furthermore, renal tubules with *Bicaudal C* mutation were >60% larger relative to controls, showed cyst-like structures and frequent tubular branching. *Bicaudal C* mutant flies are also sensitive to salt, indicating poor renal function. Moreover, comparable to *PKD* patients and mouse *Pkd* models, *Bicaudal C* mutant flies have shorter life span (<50%) than controls. Based on these similarities the powerful *Drosophila* model system was used to identify Bicaudal C targets contributing to the cyst-like structures. Implications for signalling pathways and interactions with *PKD* genes in vertebrate cystogenesis will be discussed.

Conclusions: Our study shows for the first time that, similar to vertebrates, *Drosophila* Bicaudal C is necessary for renal function. Moreover, the renal phenotypes of *Bicaudal C* mutants are highly reminiscent of the vertebrate renal cysts, suggesting that analyses of the underlying mechanisms in *Drosophila* may provide insights into the molecular and cellular complexities of renal cystogenesis.

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FR-PO741

Atypical CNT/CD Lacking Detectable Molecular Signature of Principal Cells

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Background: The connecting tubule and collecting duct (CNT/CD) comprise principal cells (PC) and intercalated cells (IC), which bear different molecular signatures and regulate sodium/water and acid/base balance, respectively. Recently, we reported that Aqp2-expressing cells with disrupted *Dot1l* give rise to IC. However, whether such derivation results from *Dot1l* deletion, when and how IC formation occurs, and whether atypical CNT/CD lacking PC exists, remain unaddressed.

Methods: We used loxP-Cre system to generate a new *R^{AC}* mouse model in which Aqp2 lineage is genetically traced by red fluorescence protein. Kidney sections of were subject to double and triple immunofluorescence staining with multiple PC and IC markers and examined with epifluorescence and confocal microscopy.

Results: We identified a new type of CNT/CD structure in which no cells have detectable expression of PC markers (Aqp2 and Aqp3) and some of cells express IC markers (V-ATPase B1B2, Pendrin, and AE1) in adult kidney. Like most IC and all PC in the classical CNT/CD, the cells in the atypical CNT/CD structure are derived from Aqp2$^+$-progenitors. Some of the progenitors become highly differentiated PC at E15.5. Others lose Aqp2$^+$ and sequentially gained V-ATPase B1B2 at E15.5, CAII$^+$ at E16.5, and AE1 and Pendrin at P1.

Conclusions: *R^{AC}* is a new faithful Aqp2-lineage tracing model. Mouse kidney contains a new type of CNT/CD structure that apparently contains IC but not PC. The derivation of IC as well as PC from Aqp2$^+$ progenitors occurs in normal development and progresses in a stepwise manner. The discovery of the atypical CNT/CD structure and Aqp2$^+$ progenitors may facilitate their isolation and functional evaluation.

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FR-PO742

Novel Renal Progenitor Chemical Screen Identifies Prostaglandins as Modulators of Proximo-Distal Segment Fate Choice During Nephrogenesis

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Background: There have been significant recent advances in our understanding of kidney ontogeny, but there are many unanswered questions concerning how different renal cell types arise. The kidney is comprised of segmented functional units called nephrons that consist of a blood filter, tubule and duct. The zebrafish embryo kidney, or pronephros, provides a model to study renal cell type formation due to its high conservation with mammals. Chemical genetics can pinpoint molecules capable of altering physiological processes in the context of the complex humoral environment.

Methods: Previously, we used small molecules to show that retinoic acid (RA) is essential for proximo-distal segment patterning by altering the balance of renal progenitors. To identify other pathways that modulate renal progenitors, we are performing an ongoing novel chemical screen. Zebrafish embryos were incubated during nephron development in each bioactive drug, then evaluated via whole mount *in situ* hybridization using a riboprobe cocktail that labeled alternating nephron segments.

Results: Thus far, a total of 78/480 compounds (16.25%) caused nephrogenesis phenotypes, including RA pathway components. Additionally, prostaglandins (Pgs) emerged as a family of compounds that consistently affected proximo-distal nephron segmentation. Following Pg treatment, the rostral domain of the proximal convoluted tubule (PCT) was diminished. Further, the proximal straight tubule (PST) dramatically expanded at the expense of the distal tubule, such that the distal early (DE) segment was unchanged but caudally shifted, while there was a preferential loss of the distal late (DL) segment.

Conclusions: These data suggest a model in which Pg exposure alters the balance of proximal versus distal nephron cell fates, such that the PCT is reduced, while the PST is promoted at the expense of the DL. Together, these data suggest that both RA and Pg have effects on kidney patterning that may have translational merit for diseases like CAKUT.

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FR-PO743

Nephron Proximal Tubule Pattern Is Regulated By the Transcription Factor *sim1a* and Retinoic Acid

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Background: The mechanisms that establish nephron segments are poorly understood. The zebrafish embryo kidney is a simplified yet conserved model for segmentation because its nephrons contain segments akin to mammals, including the proximal convoluted and straight tubules (PCT, PST), and are associated with the corpuscles of Stannius (CS), endocrine glands that regulate calcium.

Methods: Initial patterning of the renal progenitor field is characterized by the formation of rostral and caudal domains, which is reliant on retinoic acid (RA) signaling. Using gene expression profiling to study nephrogenesis events, we discovered that the transcription factor *single minded homologue 1 (sim1a)* is dynamic in the renal progenitors, first marking the caudal domain, then becoming restricted over time to the proximal tubule segments and finally the CS. *sim1a* knockdown expanded the PCT and abrogated the PST and CS populations. Conversely, overexpression of *sim1a* expanded the PST and CS, while reducing the PCT.

Results: These results suggest that *sim1a* activity is necessary and sufficient to induce the PST and CS fates, and that *sim1a* may negotiate the PCT/PST boundary, possibly by inhibiting PCT fate choice. In addition, *sim1a* expression is responsive to altered levels of RA, which we previously demonstrated is necessary for proximal segment formation. *sim1a* knockdowns treated with RA formed nephrons with predominant PCT segments that lacked the enlarged PST observed in RA treated wildtypes, further indicating that *sim1a* is requisite for PST formation. Alternately, when *sim1a* morphants were chemically treated with the RA synthesis inhibitor diaminobenzaldehyde, distal segments were expanded at the expense of proximal regions.

Conclusions: Taken together, our study reveals novel roles for *sim1a* during proximal tubule formation and reveal that this gene functions downstream of RA during proximo-distal nephron patterning. These findings provide valuable new insights into the genetic pathways that direct nephron development, and may have implications for understanding the causes of renal birth defects.

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FR-PO744

Interplay between the *tbx2a/b* Transcription Factors and Notch Directs Nephron Segmentation

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Background: The zebrafish embryonic pronephros is an excellent model of kidney development, as it possesses remarkable conservation with other vertebrate nephrons, including functionally distinct regions of the proximal convoluted and straight tubule (PCT,

PST), distal tubule segments, and a duct. In addition, endocrine cells called the corpuscle of Stannius (CS) are interpolated between the distal segments. How nephron pattern is established and how the CS forms remains intriguing.

Methods: Using whole mount *in situ* hybridization, we found that transcripts encoding the zebrafish orthologues *tbx2a* and *tbx2b* were spatially restricted to distal renal progenitors during nephron formation. To elucidate the function of these genes in nephrogenesis, morpholino knockdown studies were performed.

Results: *tbx2a* and *tbx2b* single and double morphants exhibited a modest expansion in the proximal segments accompanied by a reduction in the distal domains, indicating that these genes have redundant roles in segment patterning. Noticeably, *tbx2b* morphants formed significantly larger CS clusters, as shown by the elevated expression of the marker *stc1*. Further, in preliminary studies we identified expression of the Notch pathway component *her9* in the developing CS. To study the possible link between *tbx2b* and Notch, DAPT treatment was used to block Notch activity in wildtype embryos and *tbx2b* morphants. DAPT treatment resulted in moderate CS expansion in wildtypes, while DAPT induced further enlarged CS clusters in *tbx2b* morphants. Supporting this result, ectopic activation of Notch signaling in *Tg (hsp70::Gal4; UAS::NICD)* led to a reduced CS post heat-shock induction. In addition, ectopic Notch expanded proximal tubule segments at the expense of distal, reducing *tbx2* expression domains and therefore suggesting that Notch promotes proximal segments in part by inhibiting *tbx* expression.

Conclusions: Taken together, these data reveal that interplay between Notch and *tbx2a/b* mitigates nephron segmentation, and thus provide novel insights into renal genetic regulatory networks.

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FR-PO745

Compensation for Glomerular Damage by Decreasing Regression in Developing Mouse Kidney Jianyong Zhong,[1] Haichun Yang,[2] Ji Ma.[1] *[1]Pediatric Nephrology, Vanderbilt Univ, Nashville, TN; [2]Pathology, Microbiology and Immunology, Vanderbilt Univ, Nashville, TN.*

Background: We previously found a dynamic change in glomerular number in normal neonatal mice, which peaks at P7 then decreases and stabilizes after P18. This is related to regression of superficial glomeruli. In the present study, we tested the hypothesis that, injury in deep glomeruli affects normal maturation of superficial glomeruli which is preserved by podocyte-derived VEGF.

Methods: Nphs1-hCD25 transgene (NEP25) mice express human CD25 in mature podocytes, and primary podocyte injury related FSGS can be induced by immunotoxin (LMB2). By mating NEP25 with Nphs2-rtTA/tetO-hVEGF mouse, we got inducible podocyte VEGF over-expressing mouse (NEP25/rtTA/VEGF) and normal VEGF expression mouse (NEP25/rtTA) on top of NEP25, while rtTA mouse was used as control (CONT). Doxycyclinewas administratedfrom birth (P0), followed by a single LMB2 injection at P4.

Results: At P21, there were significant podocyte injury and decreasing WT1-positive cells in deep cortical glomeruli in NEP25 mouse. Although the number of WT1 positive podocytes in NEP25/rtTA/VEGF mice was not significant different from NEP25/rtTA, the glomerular volume of superficial glomeruli was enlarged (NEP25/rtTA/VEGF 6.79±0.59 $\times 10^4$ versus NEP25/rtTA 5.04±0.44 $\times 10^4$ mm^3, $P<0.05$). There was no significant difference in glomerular number at P21 or P42. To further study the mechanism of VEGF on glomerular development, some mice were studied at P10. Over-expression of podocyte VEGF significantly increased Dll4 expression, a marker of tip endothelial cell (NEP25/rtTA/VEGF 1.19±0.15 versus NEP25/rtTA 0.57±0.10, $P<0.05$). In superficial glomeruli, expression of proliferation marker (Ki67) was significantly increased by enhanced podocyte VEGF (NEP25/rtTA/VEGF 62.29±6.17 versus NEP25/rtTA 42.44±3.22%, $P<0.05$). In parallel, expression of regression marker (TSP-1) was significantly down-regulated (NEP25/rtTA/VEGF 2.63±1.00 versus NEP25/rtTA 3.47±2.00%, $P<0.05$).

Conclusions: We conclude that VEGF-mediated angiogenesis suppresses the normal maturational glomerular regression, which plays important role in the compensatory response to the damage and loss of glomeruli during development.

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FR-PO746

Genetic Analysis of Zebrafish Glomerulus Disease and Development Tomoko Obara, Rebecca Powell. *Cell Biology, Univ of Oklahoma Health Sciences Center, Oklahoma City, OK.*

Background: The treatment of progressive loss of function in the glomerulus filtration barrier is limited, with long-term dialysis and kidney transplantation the only options. The slit diaphragm is a highly specialized intercellular junction between podocyte foot processes that plays a crucial role in a healthy glomerulus filtration barrier. Genes, such as *WT1,* that are essential to podocyte function have been identified, but the mechanism by which mutations in these genes cause glomerular filtration barrier failure is unknown. We used zebrafish to create pathogenic genetic models not readily available in mice. The zebrafish is an important vertebrate model for studying organogenesis and the cellular pathology underlying human diseases. Zebrafish pronephric podocytes undergo dynamic morphological changes analogous to mammalian metanephros development. During the development of the zebrafish glomerulus, immature podocytes accompany the initiation of blood filtration at 40-48 hours post fertilization (hpf). At 96 hpf, the foot process and SD form to complete podocyte maturation.

Results: Due to their known roles in podocyte function, we examined Wtip, a basal body protein, and its interacting molecules (Wt1, Lats2, and Vangl2). Depletion of these resulted in accelerated podocyte maturation and led to a failure of the glomerular filtration barrier. The zebrafish and human ortholog genes rescued these phenotypes. Collectively, our data suggested for the first time that: 1) timing of podocyte maturation is important for healthy glomerulus filtration, 2) podocyte primary cilia are present in zebrafish, and 3) Wtip localizes to the basal body of podocyte cilia. In immature rat podocytes, cilia are present and gradually disappear during development, but their role is unknown. In general, it is known that cilia perform many physiological functions, including movement of fluids, signal transduction, and chemo- and mechanosensing.

Conclusions: Our ultimate goal is to understand how regulatory and signaling molecules work to establish a healthy glomerular filtration barrier, thereby providing a framework to develop interventions to restore lost kidney function.

FR-PO747

Genetic Analysis and Characterization of the Novel Zebrafish Podocyte Mutant *Zeppelin* Paul T. Kroeger, Rachel Miceli, Rebecca A. Wingert. *Dept of Biological Sciences, Univ of Notre Dame, Notre Dame, IN.*

Background: The zebrafish pronephros is highly conserved with higher vertebrates, including mammals, thus making it an excellent model to study kidney formation. The nephron, or functional unit of the kidney, modifies the blood to excrete metabolic waste using a blood filter with specialized epithelial cells known as podocytes. Knowledge about podocyte development is highly relevant to the treatment and prevention of kidney disease, as podocyte injury leads to progressive scarring and nephron atrophy culminating with end stage renal failure, however the pathways that specify the podocyte lineage remain poorly understood. Previous work has demonstrated that expression of *wt1a* and production of retinoic acid (RA) are essential for podocyte formation in zebrafish.

Methods: Through an edema based F3 forward genetic screen, we isolated *zeppelin (zep),* which displays edema at 5 days post fertilization and forms severely reduced podocytes as assayed by *in situ* hybridization with markers such as *wt1a, wt1b, lhx1a*, and *nephrin*.

Results: *zep* mutant embryos are unaffected by RA treatment, indicating that RA acts upstream or in an unrelated pathway with that of *zep.* Interestingly, the interrenal gland of *zep* mutants is increased in size. Preliminary cell death and proliferation assays in *zep* mutants did not show any alterations from wildtypes, suggesting the possibility of a cell fate switch between the podocyte and interrenal lineages. To determine the genetic lesion responsible for *zep*, we utilized a combinatorial strategy of whole genome sequencing (WGS) and meiotic mapping. These techniques narrowed the region to a small interval on chromosome 15, and candidate genes were knocked down with morpholinos (MO). Three independent MOs designed against *breast cancer 2, early onset (brca2)* phenocopied *zep*, recapitulating both the unique late edema phenotype, as well as the vast reduction of podocytes.

Conclusions: This suggests, for the first time, that *brca2* is essential for renal development. Taken together, these findings provide novel insights into the genetic regulatory networks that control podocyte formation in the vertebrate kidney.

Funding: NIDDK Support, Private Foundation Support

FR-PO748

INF2 Regulates Rho/Dia Signaling in the Developing Glomerulus of Zebrafish Hua Sun,[1,3,4] Khaldoun Al-Romaih,[1,3,5] Callum MacRae,[2,3] Martin R. Pollak.[1,3] *[1]Nephrology, Beth-Israel Deaconess Medical Center, Boston, MA; [2]Cardiology, Brigham and Women's Hospital, Boston, MA; [3]Harvard Medical School, Boston, MA; [4]Nephrology, Univ of Iowa Children's Hospital, Iowa City, IA; [5]King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia.*

Background: Mutations in Inverted Formin 2 (INF2), a diaphanous formin family protein that regulates actin cytoskeleton dynamics, cause focal segmental glomerulosclerosis (FSGS) and Charcot-Marie-Tooth Disease (CMT) in humans. In addition to directly remodeling actin filaments in vitro, we have shown that INF2 regulates intracellular actin dynamics and actin dependent cellular behavior by opposing Rhoa/Dia signaling.

Methods: We used a zebrafish model to investigate the in vivo role of INF2 and its interaction with Rho/Dia signaling in the pathophysiology of nephrogenesis and podocyte-mediated diseases. The integrity of the glomerular filtration barrier in zebrafish with INF2 knockdown was examined based on the expression of slit diaphragm proteins, transmission election microscopy, and the functional dextran filtration assay.

Results: Knockdown of the zebrafish homolog of INF2 leads to dysmorphic podocyte and dysfunctional glomerular filtration barrier resulting in an edematous phenotype. These changes correlate with mistrafficking of glomerular slit diaphragm proteins, defective slit-diaphragm signaling, and disinhibited diaphanous formin (Dia) activity. Wild type INF2, but not INF2 harboring human disease mutants, is able to rescue the INF2 deficient phenotype in the zebrafish. The edematous phenotype in INF2 morphants is also rescued by knockdown of RhoA or Dia2.

Conclusions: This in vivo model demonstrates that INF2 is required for normal physiologic regulation of podocyte morphology and function during embryonic development by means of its modulatory effects on Rho/Dia signaling. These data support a model in which diaphanous inhibitory domain (DID) mutants in INF2 interfere with its binding to and inhibition of Dia, leading to uncontrolled Rho/Dia signaling and perturbed actin dynamics. Targeting the uncontrolled Rho/Dia signaling might be a potential therapeutics for human diseases with INF2 deficiency.

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FR-PO749

Stroma MicroRNAs Play a Critical Role in Regulating Nephrogenesis Yu Leng Phua, Sunder Sims-Lucas, Christopher Cain Rymer, Andrew J. Bodnar, Jacqueline Ho. *Rangos Research Center, Children's Hospital of Pittsburgh of UPMC, Univ of Pittsburgh, Pittsburgh, PA.*

Background: MicroRNAs (miRNAs) are small non-coding RNAs that function to fine-tune the expression of mRNA transcripts. While multiple dysfunctional genes have been identified in developmental renal disorders, the role of miRNAs remains largely unknown. Previous studies have shown that the differentiation of nephron progenitors is in part modulated by the renal stroma. Moreover, the stroma also gives rise to various supportive cells within the kidney such as pericytes, renin-expressing cells, smooth muscle cells of the afferent arteriole and glomerular mesangium. In this study, we seek to understand the functional role of miRNAs in the renal stroma.

Methods: We generated a conditional knockout mouse model (FoxD1Cre; Dicerfl/fl) that lacks Dicer, an endoribonuclease required for mature miRNA production in the renal stroma.

Results: Mutant mice developed a multifaceted renal phenotype including an expanded nephron progenitor population and reduced nephron endowment. While differentiation of the stroma into Pdgfrb^{+} pericytes was not impacted by the loss of miRNAs, the kidneys developed fewer renin-expressing cells, less smooth muscle cells around developing arterioles, and mature glomeruli lacked a mesangium. The mesangium defect was not due to defective cellular migration since putative mesangial cells could be seen migrating into early capillary loop nephrons. Instead, increased cellular apoptosis is likely to be a contributory factor. In concordance to the phenotype observed in these transgenic mice, Taqman miRNA profiling of the renal stroma identified a panel of miRNAs that are potentially involved in perivascular development and cell survival.

Conclusions: Stromal miRNAs play an indispensable role in regulating the nephron progenitor niche. While the loss of stromal miRNAs has no overt impact on pericyte differentiation, they are required for maintenance of several perivascular supportive cells including renin-expressing cells, smooth muscle cells of developing arterioles, and the glomerular mesangium.

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FR-PO750

Fgfr2 Is Integral for Bladder Urothelial Development Kenneth A. Walker,[1] Caitlin M. Schaefer,[1] Daniel S. Bushnell,[1] Carlton M. Bates.[1,2] *[1]Pediatrics, Children's Hospital of Pittsburgh, Pittsburgh, PA; [2]Nephrology, Univ of Pittsburgh, Pittsburgh, PA.*

Background: While embryonic bladder urothelium is critical for development of surrounding mesenchyme, little is known about molecular control of urothelial morphogenesis. Fibroblast growth factor receptor 2 (Fgfr2) is highly expressed in embryonic bladder urothelium; however its role in bladder urothelium is unclear. Our hypothesis is that Fgfr2 has critical roles in bladder urothelial development.

Methods: To addresses our hypothesis, we generated a conditional knockout mouse using the *Wnt4cre* and *Fgfr2* floxed mouse lines (termed *Fgfr2$^{Uro-/-}$*). We assessed general histology as well as immunohistochemistry for key markers and for proliferation and apoptosis. We used 3D reconstructive stereology for volumetric analyses. We also performed molecular analysis via quantitative PCR (qPCR).

Results: First, in situ hybridization showed *Fgfr2* deletion from bladder urothelium in *Fgfr2$^{Uro-/-}$* mice. At embryonic day 16.5, 3D reconstructions revealed that *Fgfr2$^{Uro-/-}$* bladders had a significant reduction in total urothelial volume when compared to controls. Furthermore, *Fgfr2$^{Uro-/-}$* bladders also exhibit decreased total muscle volume and increased lamina propria volume when compared with control bladders. Apoptosis was significantly increased in both urothelial and bladder muscle tissues in *Fgfr2$^{Uro-/-}$* bladders, with reciprocal decreases in cell proliferation versus controls. qPCR showed decreased Uroplakin3A and a-smooth muscle actin (aSMA) mRNA expression was detected confirming decreased urothelial and smooth muscle tissues volumes in *Fgfr2$^{Uro-/-}$* bladders, respectively. Increased mRNA expression of immature urothelial markers *Foxa2* and *p63* were detected in *Fgfr2$^{Uro-/-}$* bladders compared to controls. Histological analysis showed urothelial hypoplasia persists in *Fgfr2$^{Uro-/-}$* bladders at post-natal day 21.

Conclusions: In conclusion, urothelial *Fgfr2* appears integral for proper bladder urothelial morphogenesis. Moreover, absence of urothelial *Fgfr2* during development also has non-cell autonomous consequences, leading to improper lamina propria and muscle patterning. Thus, urothelial Fgfr2 is a key mediator of urothelial and overall bladder development.

FR-PO751

Stroke Outcomes and Renal Function in Diabetes: A Meta-Analysis of Randomized Controlled Trials Tomoko Usui, Frank Holtkamp, Jarno Hoekman, Dick de Zeeuw, Hiddo Jan Lambers Heerspink. *Dept of Clinical Pharmacy and Pharmacology, UMC Groningen, Netherlands.*

Background: Recent clinical trials in type 2 diabetes and nephropathy, such as ALTITUDE and TREAT, showed more cases of stroke in the active treatment versus placebo group. To test whether these were chance findings we performed a systematic review and meta-analysis to study which drugs or which type 2 diabetic patients are vulnerable.

Methods: MEDLINE database was searched for randomized controlled trials (RCTs) in type 2 diabetes between 1966 and April 2014. Included RCTs had a mean follow up of at least 1000 patient years and reported stroke outcomes. We extracted baseline characteristics, stroke and cardiovascular (CV) outcomes, and types of intervention from each publication. A multivariable meta-regression model that included age, sex, systolic blood pressure (BP), HbA1c, cholesterol, eGFR, and drug class was used to assess which characteristics modify treatment effect on stroke outcomes.

Results: The meta-analysis included 31 trials involving 18488 patients. Average age ranged from 27 to 69 years, Hba1c ranged from 5.8 to 11.5%, and eGFR from 15 to 122 ml/min/1.73m^2. Type of interventions included HbA1c, cholesterol, BP lowering interventions as well as a platelet inhibitor (aspirin), erythropoiesis stimulator (darbepoietin), and an anti-inflammatory agent (bardoxolone). Across all trials, the effect of the interventions on stroke risk was modified by baseline eGFR (p=0.01) and not by other baseline characteristics. The relative risk of stroke was 0.93 (95%CI 0.88 – 0.98) for trials with mean eGFR≥60 ml/min/1.73m^2, 1.09 (0.90 – 1.32) for eGFR 45 to 59, and 1.38 (1.14 – 1.68) for eGFR<45 (p for heterogeneity=0.009). The treatment effects on other CV outcomes did not differ between eGFR subgroups.

Conclusions: Independent of the type of intervention, patients with type 2 diabetes with a low eGFR face an increased risk for stroke, whereas patients with a higher eGFR are actually protected. These findings are very consistent across the different trials and interventions in these type 2 diabetes populations, and have major implications for trial design (stratification for eGFR!), selection of endpoints, and individual patient care.

FR-PO752

Association of Traditional and Novel Retinal Vascular Parameters with Diabetic Microvascular Complications Charumathi Sabanayagam,[1,2] Carol Y. Cheung,[1,2] Tien Yin Wong,[1,2] Ecosse L. Lamoureux.[1,2] *[1]Singapore Eye Research Inst, Singapore; [2]Duke-NUS Graduate Medical School.*

Background: To examine the association of traditional and novel retinal vascular parameters with diabetic kidney disease (DKD), nephropathy (DN) and retinopathy (DR) in a clinic-based sample of diabetic patients.

Methods: We recruited 425 diabetic patients of Chinese, Malay and Indian ethnicity aged ≥18 years from the Singapore National Eye Centre (2010-2013). Retinal vascular parameters, traditional (arteriolar and venular caliber, arteriole-to-venular ratio [AVR]), novel (fractal dimension [F_d] and branching angle [Ba]) and DR (no, yes) were assessed from fundus photographs. DKD was defined using estimated glomerular filtration rate (eGFR≥90, 60-90, <60 mL/min/1.73m^2), and DN using urinary albumin-to-creatinine ratio (ACR <30, 30-300, ≥300).

Results: The prevalence of DKD, DN, and DR were 18.6%, 42.1% and 63.9% in this clinical sample. In multivariable regression models adjusted for potential confounders, presence and severity of DKD, DN and presence of DR were significantly associated with bigger venular diameter, smaller AVR, smaller F_d and narrower retinal arteriolar Ba.

Table 1: Relationship of mean retinal parameters with DKD, DN and DR

	Mean Retinal parameters*					
eGFR	Arteriole	venule	AVR	F_d	Arteriolar Ba	Venular Ba
≥90	128.7	190.2	0.68	1.41	73.3	76.3
60-90	129.1	192.6	0.67	1.38	70.0	77.5
<60	126.2	195.8	0.65	1.39	69.8	76.9
p	0.2	0.02	0.0006	<0.0001	0.05	0.4
ACR						
<30	128.8	190.6	0.68	1.40	71.4	77.3
30-300	127.1	191.6	0.67	1.39	73.8	74.2
≥300	128.7	197.7	0.65	1.38	69.1	78.9
p	0.07	0.02	<0.0001	<0.0001	0.03	0.2
DR						
No	126.8	185.8	0.69	1.40	71.6	74.4
Yes	128.5	196.1	0.66	1.39	71.5	78.6
p	0.5	0.003	0.0001	0.0002	0.02	0.02

adjusted for age, sex, ethnicity, duration of diabetes, body mass index, smoking, education, systolic blood pressure, HbA1c, cholesterol levels and cardiovascular disease

Conclusions: Novel and traditional retinal vascular parameters were associated with diabetic microvascular complications independent of potential confounders in diabetic patients, suggesting quantification of retinal microvascular parameters might be useful in assessing microvascular damage in diabetic patients.

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FR-PO753

Vascular Stiffness and Chronic Kidney Disease in Patients with Diabetes of Different Ethnic Origin Maria Karaflou,[1,2] Karima Zitouni,[1] Kenneth A. Earle.[1,2] *[1]Clinical Sciences, St. George's, Univ of London, London, United Kingdom; [2]Thomas Addison Unit, St. George's Hospital NHS trust, London, United Kingdom.*

Background: Chronic kidney disease (CKD) is a recognized cardiovascular disease (CVD) equivalent. Its progression to end-stage renal disease has a propensity for patients of African and Asian origin. Vascular stiffness is related to increased CVD risk but its association with CKD is unclear. Therefore, we assessed the relationship between vascular stiffness in type 2 diabetes patients with CKD but without CVD of Caucasian and non-Caucasian heritage.

Methods: We recruited 72 patients of Caucasian (n=28) and non-Caucasian (n=44) origin with type 2 diabetes and eGFR <90 and >45 mL/min/1.73 m^2. Body mass index (BMI) was calculated in kg/m^2. Fasting venous blood was sampled for biochemical profile and haemoglobin A1c (HbA1c). Albumin:creatinine ratio (ACR) was measured in early morning urine samples and vascular stiffness index (SI) was determined using finger plethysmography.

Results: The Caucasian (C) and Non-Caucasian (NC) patients had similar mean [SD] age, HbA1C, eGRF, ACR, systolic blood pressure, diastolic blood pressure, LDL and SI (60.2 [8.3] versus 60.2 [8.0] yrs, p=.847; 57.4 [17] versus 63.7 [18] mmol/mol, p=.067; 66 [16.6] versus 72.1 [15.9] mL/min/1.73 m^2, p=.224; 8.5 [24.9] versus 5.3 [12.5] mg/mmol, p=.214; 138 [16] versus 109[58] mmHg, p=.213; 81[7] versus 80[9] mmHg, p=.940; 1.7 [1.1] versus 1.5 [1.2], p=.658 and 8.6[5.3] versus 8.5[4.3]m/s, p=.575), respectively. The C group had a higher BMI than the NC group (32.4[5.3] versus 28.8 [8.4] kg/m^2, p=.008) and a higher percentage of smokers (80.8% versus 41.5%, chi square p=.002). In the whole population, there was a significant positive correlation between SI and BMI (r=.265, p=.036). This relationship was also found in the patients of Caucasian origin (r=.412, p=.045) and it was not confounded by smoking status, LDL or ACR.

Conclusions: In this study vascular stiffness increased with BMI in patients of Caucasian heritage at high risk of progressive renal disease. SI in this study group is independent of traditional risk factors such as smoking, LDL and ACR. This observation could be relevant in understanding the differential risk of CVD in patients with renal disease.

FR-PO754

The Intima-Media-Thickness of the Carotid Artery Wall and the Progression of Chronic Kidney Disease in Patients with Diabetes Mellitus Type 2

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Background: The presence of albuminuria (UAL) and Chronic Kidney Disease (CKD) are traditional risk factors for cardiovascular disease (CVD) and all-cause mortality. A good predictor of the incidence of CVD is found to be carotid atherosclerosis, which is being evaluated by the Intima Media Thickness of the carotid artery wall (cIMT). The aim of the study was to examine the relationship between the cIMT and the stage of CKD as well as the presence of diabetic nephropathy graded by the UAL in patients with type 2 diabetes mellitus (DM).

Methods: In this study 151 DM type 2 patients (79 males and 72 females) were included. Traditional risk factors (Body Mass Index, blood pressure, serum lipids) as well as cIMT measurements, using B-mode ultrasonography were assessed and correlated to the stage of CKD and the presence of UAL.

Results: The median carotid IMT in stages 1, 2, 3, 4 and 5 was 0.76mm (0.55-1.61), 0.905mm (0.63-1.61), 0.93mm (0.66-1.78), 0.95mm (0.66-1.66) and 0.98mm (0.60-1.60) respectively, and in 35% of the patients the presence of a carotid plaque was revealed. The carotid IMT was significantly increased as the stage of CKD was progressing ($p<0.001$, Kruskal-Wallis test) and was correlated to the eGFR (r=-258, p=0.002) and the presence of UAL (r=301, p=0.004). However, it was not significantly correlated to the glycemic control (assessed by HbA1c). Additionally, hypertriglyceridemia was significantly negatively correlated to the deterioration of eGFR (r=-282, p=0.001) and positively correlated to the presence of UAL (r=373, $p<0.001$).

Conclusions: Increased levels of UAL and reduced eGFR are associated to atherosclerosis of the carotid artery and elevated serum triglyceride levels in type 2 DM patients. This study attenuates the importance of the carotid Intima-Media Thickness as a way of recognition and prediction of GFR's deterioration besides a classic indicator of vascular calcification.

FR-PO755

Low Magnesium Levels and FGF-23 Dysregulation Predict Mitral Valve Calcification as well as Intima Media Thickness in Pre-Dialysis Diabetic Patients

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Background: Mitral valve calcification and intima media thickness (IMT) are common complications observed in chronic kidney disease (CKD) patients and have been implicated with the high cardiovascular mortality incidence observed. **Aims:** The aim of this study isto investigate the implication of serum levels of magnesium and fibroblast growth factor-23 (FGF-23) with mitral valve calcification and IMT in diabetic patients with CKD.

Methods: This is an observational, prospective study involving 150 diabetic patients with mild to moderate CKD, divided into 4 groups according to Wilkins Score' grades of mitral valve characteristics. Carotid echodoppler and transthoracic echocardiography were used to assess features of calcification. Different parameters were analyzed and compared between magnesium levels and IMT values. Statistical tests were used to establish comparisons between groups, to identify risk factors, and to establish cut-off points for prediction of mitral valve calcification.

Results: FGF-23 values continually increased with higher values for both IMT and calcification whereas the opposite trend was observed for magnesium. In addition, FGF-23 and magnesium were found to independently predict both mitral valve calcification and IMT ($p<0.05$). Using Kaplan-Meier analysis, the number of deaths was higher in patients with lower magnesium levels and with poorer Wilkins score. The mean cut-off value obtained for FGF-23 was 117 RU/mL and for magnesium 1.7 mg/dL.

Conclusions: Hypomagnesemia and high FGF-23 levels are independent predictors of mitral valve calcification and IMT and are risk factors for cardiovascular mortality in diabetics with mild to moderate CKD. Thus, FGF-23 and magnesium might be used as diagnostic or therapeutic targets in order to better manage the currently high cardiovascular risk in CKD patients.

FR-PO756

Tubular Injury Is Associated with a High Risk of Ventricular Dysfunction in Subjects with Type 2 Diabetes Mellitus

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Background: Albuminuria has been associated with a higher risk for cardiovascular disease in patients with diabetes and it has yet to be determined whether markers of tubular injury are also associated with a higher risk for CV disease. There are no studies about this association and the aim of the current study was to determine whether markers of tubular injury are associated with a higher risk for echocardiographic abnormalities in subjects with type 2 diabetes without hypertension.

Methods: Subjects aged >20 years old were included, and those with hypertension, albuminuria, antecedent of renal and CVD were excluded. Blood samples were taken after a fasting ≥ 8hrs; the analytical measurements of the biochemical variables were done by standard technique. GFR was estimated using the CKD-EPI equation. Spot urine samples for albumin and a1-microglobulin analysis were collected and the a1M determinations were done by ELISA. Descriptive statistics were calculated and comparisons between groups were done by t-student analysis and chi-square. For variables correlated with A1M excretion a multiple linear regression analysis was used.

Results: A total of 120 subjects with T2DM were included; the mean age was 50.8 ± 7.9 yrs, median time from diagnosis was 8.6 ± 7.2 years and mean BMI was 28.1 ± 4.9. In all the subject we have determination for A1M, of them 10 (8.3%) had AIM higher than 10 µg/gCr; there was no difference in age, GFR and systolic and diastolic blood pressure between the two groups, but C reactive protein (4.45 ± 2.59 versus 2.45 ± 2.28 mg/L p=0.05), glucose (167 ± 43 versus 148 ± 39 mg/dl p= 0.04) and uric acid (6.2 ± 1.3 versus 5.3 ± 1.3 mg/dl p=0.04) were higher in those with AIM excretion ≥10 µg/gCr; 45% of patients with no LMW proteinuria has diastolic dysfunction compared with all of the patients with LMW proteinuria (p=0.002).

Conclusions: Alpha1-microglobulin urinary excretion is associated with a high prevalence of diastolic dysfunction and should be considered as a new risk factor for cardiovascular disease in T2DM patients.

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FR-PO757

Renal Health Is an Important Determinant of Cardiac and Exercise Function in Adolescents with Type 1 Diabetes

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Background: Cardiovascular disease and diabetic nephropathy, the most common causes of mortality in type 1 diabetes, are strongly related in adults with type 1 diabetes, yet little is known about their relationship in youth. We have previously demonstrated that adolescents with type 1 diabetes have exercise and diastolic dysfunction compared to lean non-diabetic controls. For that reason, we hypothesized that renal health, measured by estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR) would be associated with cardiopulmonary fitness and diastolic function in adolescents with type 1 diabetes.

Methods: This cross-sectional study at an academic hospital included 69 adolescents (15.5 ± 2.2 years) with type 1 diabetes. Cardiopulmonary fitness was measured by peak oxygen consumption (VO_2peak) and oxygen uptake kinetics (VO_2kinetics), cardiac function by doppler echocardiography and renal function by eGFR (Bouvet combined creatinine and cystatin C) and ACR.

Results: In adolescents with type 1 diabetes, eGFR by Bouvet was significantly associated with VO_2peak [r = -0.55, R^2 = 30.3%, p=0.002], early (E) mitral inflow velocity (MVE) [r = -0.42, R^2 = 17.6%, p=0.02], ratio of early (E) to late (A) mitral inflow velocities (E/A ratio) [r = -0.42, R^2 = 17.6%, p=0.02] and left ventricular posterior wall thickness in diastole (LVPWd) [r = 0.39, R^2 = 15.2%, p=0.04] univariately and after adjusting for Tanner stage. ACR was not associated with cardiopulmonary fitness or diastolic function.

Conclusions: In adolescents with type 1 diabetes and previously demonstrated exercise and diastolic dysfunction, eGFR by Bouvet but not ACR was significantly associated with markers of cardiopulmonary fitness and diastolic function.

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FR-PO758

Being at High Risk for Obstructive Sleep Apnea Is Associated with More Rapid Loss of Kidney Function in Patients with Diabetic Nephropathy

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Background: Type 2 diabetic nephropathy (DN) is the leading cause of end-stage renal disease in Western countries. Obstructive sleep apnea (OSA) is common in subjects with diabetes. OSA is associated with glomerular hyperfiltration and proteinuria in subjects with normal renal function, raising the question if OSA may be related to chronic kidney disease (CKD) progression. The association between OSA and DN has not been fully investigated. This retrospective cohort study aimed to investigate the prevalence of being at high risk (HR) for OSA among non-dialysis CKD patients with DN and the association of being at HR for OSA with loss of estimated glomerular filtration rate (eGFR).

Methods: Data from the University of Alabama at Birmingham (UAB) CKD Database was retrospectively evaluated. The CKD Database was approved by UAB IRB. We identified 56 subjects with DN who had undergone screening for OSA using the Berlin questionnaire (BQ) during a 9-month period. These patients were divided into a HR and a low risk (LR) for OSA cohort based on the BQ score. Change in eGFR was obtained by calculating the difference between eGFR measurements at the time the BQ was done and when patients were enrolled in Clinic, divided by the total years spent in Clinic.

Results: The prevalence of a HR score for OSA was 61%. There were no significant differences in baseline gender, blood pressure (BP), BMI, use of renin-angiotensin-aldosterone blockers, eGFR, urinary protein/creatinine, and co-morbidities between the HR and LR groups. Subjects with a HR score had a significantly greater loss of eGFR than those with LR score ($P<0.02$; median loss -3.4±1.2 versus 1.5±1.5 ml/min/1.73 m^2 BSA/year for HR versus LR) despite comparable BP and proteinuria on admission to CKD Clinic and similar time spent in Clinic (1.9±0.2 versus 2.1±0.3 years for HR versus LR).

Conclusions: This study shows that a high-risk score for OSA is common in non-dialysis CKD patients with DN and is associated with more rapid loss of renal function. This simple approach identifies patients at higher risk of CKD progression.

FR-PO759

Impact of Blood Pressure and Renal Lesions on the Long-Term Outcomes of Type 2 Diabetic Patients with Biopsy-Proven Diabetic Nephropathy

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Background: Although it is important to determine whether pathological information improves the predictive power when added to clinical information, renal biopsy is not generally indicated in the management of diabetic nephropathy. We investigated the impact of blood pressure (BP) and renal lesions on the outcomes of diabetic nephropathy.

Methods: Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy (n=270) were enrolled. Blood pressure levels at the time of renal biopsy were classified into 4 categories (optimal BP (<120/<80), normal (120-129/80-84) to high-normal (130-139/85-89) BP, grade I hypertension (140-159/90-99), and grade II (160-179/100-109) to III (≥180/≥110) hypertension) according to the criteria of the Guidelines for Management of Hypertension 2014 by the Japanese Society of Hypertension. The outcomes were the first occurrence of renal events (requirement of dialysis, or a 50% decline in estimated glomerular filtration rate (eGFR) from baseline), all-cause mortality, and cardiovascular events (cardiovascular death, nonfatal myocardial infarction, coronary intervention, or nonfatal stroke).

Results: 1) The mean duration of follow-up was 7.9 years. There were a total of 121 renal events, 45 deaths, and 64 cardiovascular events. 2) Higher BP had higher cumulative incidences and the hazard ratios of renal events and all-cause mortality compared to the optimal BP among patients with albuminuria (proteinuria), low eGFR, or advanced vascular lesions. 3) The cumulative incidence of cardiovascular events in grade II to III hypertension was higher than those of other BP levels among patients with advanced vascular lesions. 4) High systolic BP was one of the clinical determinants for renal events, all-cause mortality, and cardiovascular events in multivariate analysis. Advanced arteriosclerosis was one of the pathological determinants for renal events and cardiovascular events.

Conclusions: The available data suggest that the significant impact of BP on the outcomes of type 2 diabetic patients with biopsy-proven diabetic nephropathy was present, particularly in the presence of advanced vascular lesions.

FR-PO760

Poor Glycemic Control Increases in Resistance of Efferent Arteriole and Increase in Intraglomerular Pressure, Not Affecting Resistance of Afferent Arteriole, in Human

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Background: Development and progression of diabetic nephropathy is associated with glomerular hypertension and glomerular hyperfiltration. However, precise glomerular hemodinamic abnormalities in hyperglycemic state have not been demonstrated in human. We examined glomerular hemodynamics in human by measuring both inulcin clearance (C_{in}) and para-aminohypuric acid clearance (C_{PHA}) simultaneously.

Methods: Thirty-one subjects (55.4 ± 14.7 years; 21 type 2 diabetics and 10 non-diabetics) were enrolled in the present study. C_{in} and C_{PAH} were measured simultaneously. C_{in} of all the subjects were more than 60 ml/min. According to Gomez formula (Guidi E, et al. Am J Hypertens 2001), afferent arteriolar resistance (R_a), efferent arteriolar resistance (R_e), glomerular hydrostatic pressure (P_{glo}) and glomerular filtration fraction (FF) were calculated. Association of these values with glycemic control indices was examined.

Results: 1) FF significantly and positively correlated with fasting plasma glucose (FPG), hemoglobin A1c (HbA1c) and glycated albumin (GA) ($r = 0.396$, $p = 0.0303$; $r = 0.587$, $p = 0.0007$; $r = 0.525$, $p = 0.0070$, respectively). 2) P_{glo} significantly and positively correlated with FPG, HbA1c and GA ($r = 0.572$, $p = 0.0008$; $r = 0.535$, $p = 0.0019$; $r = 0.540$, $p = 0.0053$, respectively). P_{glo} was significantly associated with FPG, HbA1c and GA after adjustment of age and serum albumin. 3) Although there was no significant correlation between R_a and glycemic controls of FPG, HbA1c and GA, R_e significantly and positively correlated with HbA1c and GA ($r = 0.499$, $p = 0.0043$; $r = 0.592$, $p = 0.0018$, respectively). R_e was significantly associated with HbA1c and GA after adjustment of age.

Conclusions: These results directly demonstrates, in human, that poor glycemic control is associated with increased R_e, but not with R_a. Increased R_e is suggested to cause increased P_{glo}, leading to increased FF. Thus, hemodynamic abnormalities in poor glycemic control are suggested to be related to glomerular hyperfiltration and hypertention in human.

FR-PO761

Nuclear Angiotensin-2 Expression Increased in Glomerular and Vascular Compartments of Human Diabetic Nephropathy

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Background: Angiotension-2 (Ang2) is a key element involved in renal disorders associated with dysfunctional Renin-Angiotensin-aldosterone (RAA) system such as diabetic nephropathy. Although Ang2 receptors are found in the nuclei of renal cortex and adrenal glands, the nuclear localization of Ang2 in human renal biopsies has been established. In this study, we compared Ang2 expression in the nuclei of diabetic nephropathy (human biopsies) with renal biopsies without glomerular injury as negative controls.

Methods: Renal negative control biopsies (n =14) and 31 renal biopsies with diabetic nephropathy were stained for Ang2 by immunohistochemical method (using a Dako autostainer); sarcoid granulomas as positive controls. Staining intensity of Ang2 was evaluated and scored from 0 to 3+ in the nuclei of podocytes and parietal epithelium of glomeruli, mesangial cells, glomerular endothelium, arterioles and proximal tubules in the two groups. Data were compared using unpaired student t test ($p < 0.05$ was considered statistically significant).

Results: In comparison to the negative control mesengium (0.21±0.09 arbitrary units [AU]), the diabetic group exhibited significantly stronger expression of nuclear Ang2 in the mesangial cells (1.53±0.16 AU; 7 fold increase). In addition, the nuclear expression of Ang2 was significantly upregulated in parietal epithelium (0.90±0.13 AU) and podocytes (0.95±0.12 AU) of glomeruli, glomerular endothelium (1.14±0.43 AU) and arterioles (1.17±0.11 AU) of diabetic group, when compared to the controls (0.21±0.09, 0.39±0.08, 0.43±0.14 and 0.57±0.14 AU, respectively). Nuclear Ang 2 expression was similarly weak in proximal tubules in both groups.

Conclusions: Our study reveals that nuclear expression of Ang2 is present in glomeruli, arterioles and renal tubules, implying a role of nuclear translocation of Ang2 in igniting its cellular activity. Our findings showing the over-expressed nuclear Ang2 in the glomeruli (mainly mesangial cells) and arterioles from diabetic group support that upregulated Ang2 involves in the progression of diabetic nephropathy.

Funding: Clinical Revenue Support

FR-PO762

Effects of Angiotensin II Receptor Blockers and Calciumantagonists on Blood Pressure Variability and Carotid Artery Atherosclerosis in Patients with Type 2 Diabetic Patients with Diabetic Nephropathy

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Background: This prospective, controlled study was to analyze blood pressure variability and carotid intima-media thickness (IMT) changes of ARB combinedwith CCB in the treatment of patients with type 2 diabetic patients with diabetic nephropathy.

Methods: This prospective, sigle-center, cohort study was performed. Patients who presented to the West China Hospital with type 2 diabetic nephropathy were included. This study compared treatment group (Losartan and levamlodipine) and control group (Losartan). The primary outcome was clinical blood pressure, 24-h ambulatory blood pressure (BP) monitoringand the mean of the maximum intima-media thicknesses (IMT) in far walls of common carotids. Secondary outcome measures included evaluated glomerular filtration rate (eGFR), HbA1c, electrolytes and cardiovascular events. All outcome measures were assessed at baseline, 24 weeks and 52 weeks over the 52-week study period.

Results: 80 patients with type 2 diabetic patients with diabetic nephropathy were included and analyzed.After 52 weeks of treatment, Clinic blood pressure reductions were identical with both treatments, No significant difference was found in changes of carotid IMT in both groups, Variance analysis or nonparametric analysis revealed that 24-h systolic BP variability and nighttime systolic BP variability of the control group were significantly higher than those of the combination group. No significant difference between treatments was found in eGFR, HbA1c, electrolytes and cardiovascular events.

Conclusions: Combination of ARB and calcium antagonists in the treatment of patients with type 2 diabetic patients with diabetic nephropathy can reduce 24-h systolic BP variability and nighttime systolic BP variability, carotid IMT changes have been shown to be unrelated to blood pressure reduction.

Funding: Government Support - Non-U.S.

FR-PO763

Comparison of Serum Cystatin C and Creatinine as a Marker for Detection of Microalbuminuria in Diabetic Patients

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Background: Serum cystatin C was introduced as an accurate endogenous glomerular filtration rate (GFR) marker. Microalbuminuria is an early signthat appears before GFR deteriorates and the correlation of cystatin C with microalbuminuria is an important factor in deciding on the usefulness of cystatin C for diabetic patients. In this study, we evaluated the effectiveness of serum cystatin C as a marker for detection of microalbuminuria.

Methods: A total of 3049 type 2 diabetic patients (785 patients with microalbuminuria and 2264 without microalbuminuria) were evaluated with serum creatinine and cystatin C in a single center from 2010 to 2013. GFR was estimated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Associations between albumin-creatinine

ratio (ACR) and two markers were examined by linear regression analysis. Receiver operating characteristics (ROC) analysis of the two filtration markers was performed to determine which marker was most effective for early detection of ACR.

Results: Serum cystatin C (0.9±0.4 versus 0.7±0.2 mg/L), creatinine (1.1±0.4 versus 0.9±0.2 mg/dl), HbA1c (7.6±1.6 versus 7.0±1.2 %) concentrations were significantly higher in patients with microalbuminuria compared those without microalbuminuria. The cut-off values for serum cystatin C and creatinine were 0.95 mg/L and 1.2 mg/dl, respectively. The proportion of abnormal values for cystatin C and creatinine were 25% and 20% in patients with microalbuminuria and 61% and 47% in patients with ACR ≥ 300mg/g. The area under the ROC curve (AUC) of the serum cystatin C curve (0.674, 95 % CI 0.651 – 0.670) was greater than those of serum creatinine (0.625, 95 % CI 0.602 – 0.649). In subgroup analysis, patients with body mass index ≥ 25 kg/m^2, age < 60 years and estimated $GFR_{cystatinC}$ ≥ 60 ml/min/1.73m2 had greater AUC of serum cystatin curve than those of serum creatinine.

Conclusions: The overall relationship between cystatin C and microalbuminuria is stronger than those between creatinine and microalbuminuria.

FR-PO764

The Glomerular Hemodynamic Profile of Normotensive, Normoalbuminuric Type 1 Diabetes Patients Compared to Healthy Controls Marko Skrtic, Yuliya Lytvyn, Ronnie Lok-Hang Har, Paul M. Yip, Melvin Silverman, David Cherney. *Univ of Toronto.*

Background: Renal hyperfiltration in experimental models of early diabetes is associated with increased intraglomerular pressure, thereby promoting the development of diabetic nephropathy. Since direct measurements of intraglomerular pressure cannot be performed in humans, our aim was to characterize the glomerular hemodynamic profile of type 1 diabetes (T1D) patients based on the presence or absence of renal hyperfiltration.

Methods: Glomerular hemodynamic parameters were evaluated in 37 healthy controls (HC) and normotensive, normoalbuminuric T1D patients with baseline hyperfiltration (n=36, T1D-H, GFR≥135 ml/min/1.73m^2) or normofiltration (n=35, T1D-N, GFR 90–134 ml/min/1.73m^2) during euglycemic conditions (4–6 mmol/L). Gomez's equations were used to derive efferent (R_E) and afferent (R_A) arteriolar resistances, glomerular hydrostatic pressure (P_{GLO}) and filtration pressure (DP_F) from inulin (glomerular filtration rate) and paraaminohippurate (renal plasma flow) clearances, plasma protein and estimated ultrafiltration coefficients (K_{FG}). Measurements were repeated during clamped hyperglycemia (9–11 mmol/L).

Results: At baseline during euglycemia, T1D-N had higher R_A (2065±597 versus 1417±740 dyne•sec•cm^{-5}, p<0.001) versus HC, while R_A was lowest in T1D-H (914.2±494 dyne•sec•cm^{-5}, p<0.01 versus HC and T1D-N). In contrast, R_E was similar in the three groups. T1D-H exhibited higher P_{GLO} versus HC and T1D-N (66±6 versus 62±5 versus 55±3 mmHg, respectively, p<0.05) and DP_F was also higest in T1D-H (p<0.05 versus other groups). Similar findings were observed during clamped hyperglycemia. As expected, clamped hyperglycemia increased GFR in T1D-N (p<0.05) compared to T1D-H, but segmental resistances did not change significantly.

Conclusions: Hyperfiltration is primarily related to alterations in R_A rather than R_E in patients with T1D, suggesting that changes in afferent arteriolar resistance related to early T1D may be clinically important. The renal protective potential of novel agents that normalize R_A by impacting tubuloglomerular feedback through increased distal tubular sodium delivery, including sodium glucose cotransport-2 inhibitors, warrants further study.

Funding: Government Support - Non-U.S.

FR-PO765

Glomerular Microvascular Remodeling Cause Polar Vasculosis in Human Diabetic Nephropathy and Advanced Nephrosclerosis: with Three-Dimensional Visualization Noriko Uesugi,[1] Yoshihito Shimazu,[2] Michio Nagata.[1] [1]*Renal Vascular Pathology, Tsukuba Univ, Tsukuba, Ibaragi, Japan;* [2]*Laboratory of Food and Physiological Sciences, Azabu Univ, Sagamihara, Kanagawa, Japan.*

Background: Objects. Neovascularization of small vessels and their aggregation (polar vasculosis (PV)) are frequently found in glomerular vascular poles of diabetes (DM) with nephropathy (DN) from its early stage. Vascular remodeling of afferent arterioles is suspected to cause PV, but not yet be confirmed. To determine their origin will be clue to investigate the pathogenesis of PV. To clarify this, we investigate human DM kidney by three dimensional (D) histological image using approximately 200 serial histological sections.

Methods: We use immunohistochemical method to distinguish arteries from capillaries. Paraffin embedded sections from non-tumor parts of renal carcinoma were double-immunostained by anti-endothelial markers, CD34, and vascular medial marker, smooth muscle actin, followed by PAS staining. The sections were scanned by virtual microscopy and proceeded to reconstruct 3D microvascular structure by RATOC TRI-SRF2 software. We analyzed 11 Japanese cases including 4 DM with/without DN and 7 control cases without DM or hypertension or renal disease.

Results:: PV was observed in two DM cases, one with DN and another without DN (Non-DN) but with marked arteriolar hyalinosis. Non-DN without prominent arteriolar hyalinosis and all control revealed no PV. Glomeruli with PV revealed wide range of histological features, normal to nodular formation and occupied 100% of glomeruli in DN, or 75% in Non-DN. PV vessels often showed marked hyalinosis. 3D image proved that PV vessels were originated from several different efferent arterioles, which were directly connected to glomerular capillaries. The other ends of PV were connected to peritubular capillaries. Wide range of 3D images proved no branching of afferent arterioles after diverged from interlobular arteries and no connection to PV.

Conclusions: PV is caused by remodeling of glomerulr efferent arterioles not afferent ones. These microvascular change might be representative of alteration of diabetic glomerular circulation.

Funding: Government Support - Non-U.S.

FR-PO766

NEW Magnetic Resonance Imaging Methods: Intravoxel Incoherent Motion (IVIM) in the Evaluation of Diabetic Kidney Disease (DKD) Jianxun Wu, Jianteng Xie, Lei Fu, Yangyang Zuo, Yuxiong Lai, Jing Li, Wei Shi, Wenjian Wang. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Intravoxel incoherent motion (IVIM) is a new imaging technique for the estimation of tissue refusion by calculation of diffusivity parameters using multi-b-value diffusion-weighted MR imaging. We investigate the relationship between glomerular filtration rate (GFR) and parameters calculated using intravoxel incoherent motion (IVIM) imaging on the diabetic kidney disease (DKD).

Methods: We studied 17 patients, divided into 4 groups based on eGFR levels (mL/min/1.73m2): group 1, eGFR≥90 (n=5); group 2, eGFR 60–90 (n=3); group 3, eGFR 30–60 (n=4); and group 4, eGFR≤30 (n=5). IVIM imaging was used to acquire diffusion-weighted images at 10 b values. The diffusion coefficient of pure molecular diffusion (D), the diffusion coefficient of microcirculation or perfusion (D*), and perfusion fraction (f) were compared among the groups.

Results: In the renal cortex and medulla, f values were significantly lower in groups 2, 3, and 4 than that in group 1. The D value of renal cortex was significantly low only in group 1. Both in the renal cortex and medulla, there is a correlation between f value and eGFR.

Conclusions: As renal dysfunction of DKD progresses, renal perfusion might be reduced earlier and affected more than molecular diffusion in the renal cortex and medulla. These changes are effectively detected by IVIM MR imaging.

Funding: Government Support - Non-U.S.

FR-PO767

Correlations of BMI with CKD Prevalence in a Cross-Sectional Population Study Sharma S. Prabhakar, Katherine Kam. *Texas Tech Univ Health Sciences Center.*

Background: While the exact prevalence of CKD is unknown, studies estimated that over 10% adult population in the U.S. may have CKD. Diabetes, hypertension and obesity are believed to be major risk factors for CKD. The relationship of obesity to CKD as an independent risk factor is not well investigated. We sought to examine these correlations in a large screening study in Texas.

Methods: Using random digit dialing of an un-selected general population in Texas. a cohort of 1609 subjects was recruited to screen for CKD and its risk factors. Necessary IRB approvals and informed consents were obtained. Detailed history, physical examination, BP, weight and BMI measurements were recorded. Underweight was defined as BMI<18 Kg/m^2, overweight 25-29.9 and obesity class I, II and III were defined as 30-34.9, 35-39.9 and >40 Kg/m^2 respectively. Blood and urine samples were obtained to measure renal function parameters. Estimated GFR (eGFR) was obtained using MDRD and CKD-EPI formulae and CKD staged as per K-DOQI guidelines.

Results: A total of 1579 subjects completed the study, with 279 (17.7%) and 249 (15.8%) having CKD by MDRD and CKD-EPI formulae. Of 1579 subjects, 1255 (79.5%) were overweight and 777 (49.2%) were obese. The prevalence of hypertension and DM exponentially increased with increasing BMI. There was no correlation between the BMI classes and stages of CKD. However there was a bi-modal peaking of CKD at both ends of the BMI spectrum.

BMI class	CKD stage I% (n)	II% (n)	IIIa % (n)	IIIb% (n)	IV% (n)	V% (n)	%ge of Total
Underweight (5)	14.3 (2)	21.4 (3)	0	0	0	0	35.7*
Normal wt. (43)	5.2 (16)	2.9 (9)	3.5 (11)	1.3 (4)	0	1 (3)	13.9
Overweight (54)	2.1 (10)	1.3 (6)	5.6 (27)	2.1 (10)	0.2 (1)	0	11.3
Obesity I (64)	5.2 (19)	4.4 (16)	5.2 (19)	2.7 (10)	0.3 (1)	0	17.8
Obesity II (41)	4.5 (10)	5.0 (11)	5.5 (12)	2.7 (6)	0.5 (1)	0.5 (1)	18.6
Obesity III (41)	9.4 (18)	4.2 (8)	3.6 (7)	3.1 (6)	1 (2)	0	21.4*

p<0.01 vs. normal weight

Conclusions: Obesity is a risk factor for CKD as 59%of CKD subjects were obese. There was a linear increase in the prevalence of CKD from overweight to Class III obesity. While increased prevalence of CKD in obesity in our study confirms previous observations, high prevalence of early CKD in underweight subjects warrants further investigation.

Funding: Government Support - Non-U.S.

FR-PO768

Visceral Fat Is More Closely Associated with Microalbuminuria Than Liver Fat: The NEO Study Renée De Mutsert,[1] Martin Den Heijer,[1,4] Ton J. Rabelink,[2] Frits R. Rosendaal,[1] Hildo Lamb,[3] Aiko P.J. De Vries.[2] *[1]Dept of Clinical Epidemiology, LUMC; [2]Dept of Nephrology, LUMC; [3]Dept of Radiology, Leiden Univ Medical Center (LUMC), Leiden; [4]Dept of Medicine, VUMC, Amsterdam, Netherlands.*

Background: Obesity is a risk factor for microalbuminuria. Especially ectopic lipid accumulation in non-adipose tissue is strongly associated with metabolic disorders. Our aim was to investigate the relative contributions of liver and visceral fat with prevalent microalbuminuria (MA) in the general population.

Methods: This is a cross-sectional analysis of the Netherlands Epidemiology of Obesity study, a population-based cohort of (wo) men aged 45-65 years. MA was defined as morning spot urinary albumin excretion (UAE) of 20-200 mg/ml. Visceral adipose tissue (VAT) was assessed by MRI in combination with ^{1}H-MR spectroscopy of hepatic triglyceride content (HTGC). We estimated associations of VAT and HTGC with UAE using linear regression analyses. Using logistic regression analyses we calculated odds ratios (OR) of MA. Analyses were adjusted for age, sex, ethnicity, smoking, education, alcohol consumption, eGFR (CKD-EPI), and total body fat (%).

Results: Measurements of VAT and HTGC were available in 1911 participants with a mean (SD) age of 55 (6) years, BMI: 26 (4) kg/m², VAT: 87 (54) cm², HTGC: 5.6 (7.7) %, 56% women. Per SD of VAT, UAE increased with 8.9% (95% CI: 3.4, 14.7), and 5.4% (1.3, 9.7) per SD HTGC. In a model including both VAT and HTGC, UAE increased with 7.3% (95% CI: 1.5, 13.5) per SD VAT, and 3.9% (-0.4, 8.4) per SD HTGC. The adjusted OR (95% CI) of MA associated with VAT was 1.75 (1.11, 2.76) and associated with HTGC it was 1.31 (1.08, 1.58). In the joint model the OR (95% CI) of MA associated with VAT was 1.64 (1.03, 2.62) and with HTGC it was 1.22 (0.97, 1.53).

Conclusions: Both liver fat and visceral fat were associated with prevalent microalbuminuria. However, visceral fat was more strongly associated with MA than liver fat in joint models. Visceral fat may be more important in the etiology of MA. Prospective analyses are needed to study associations of VAT, non-alcoholic fatty liver disease and perhaps fatty kidney with microalbuminuria and the development of chronic kidney disease.

FR-PO769

Glomerular Hyperfiltration Is Associated with Obesity and Younger Age in Type 2 Diabetes Bairbre A. McNicholas,[1,2] Andrew Smyth,[1,2] Francis M. Finucane,[3] Matthew D. Griffin.[1,2,3] *[1]REMEDI, School of Medicine, National Univ of Ireland, Galway, Ireland; [2]Nephrology Services, Galway Univ Hospitals, Ireland; [3]Galway Diabetes Research Centre, School of Medicine, National Univ of Ireland, Galway, Ireland.*

Background: Glomerular hyperfiltration (GH) is the first clinical manifestation of nephropathy in type 1 diabetes and is associated with increased future risk for CKD/ESRD. In non-diabetics, severe obesity may cause GH, glomerulomegaly and glomerulosclerosis. However, the clinical significance of GH and the independent influence of obesity on renal outcomes in type 2 diabetes mellitus (T2DM) are not well understood.

Methods: Relationships between obesity (BMI), renal indices [MDRD-eGFR, urine albumin creatinine ratio (ACR), prevalence of CKD (eGFR <60ml/min/SA) and GH (eGFR >130ml/min/SA), change of eGFR over 3-5 years follow-up] and other clinical parameters were analysed in 939 adults with actively-managed T2DM. Cross-sectional analyses at an index time-point and longitudinal analyses over 3-5 yrs follow-up were performed. 87% were receiving ACEi and/or ARB. Subsequently, renal indices were compared between 76 younger (age ≤40 yr) and 500 older (age >40 yr) adults with T2DM and BMI >30 kg/m².

Results: 25/939 (2.7%) subjects had GH at baseline. Compared to all others, these were younger (47±13 v 57±12 yr, p=0.001), were more recently entered into the clinical database (37±43 v 74±85 mth, p=0.03) and had higher BMI (37±11 v 32±6, p=0.001). WHO obesity grade was associated with increased risk for GH [OR 1.07, 95% CI 1.03-1.13 adjusted for time in database, HbA1c and ACR]. The younger obese subset had higher baseline eGFR but similar ACR and HbA1c compared to the older obese subgroup. During follow-up, there was an absolute eGFR increase among the younger obese subgroup (+8.0±18.2 ml/min/SA) compared to a decline among the older obese subgroup (-1.9±18.1, ml/min/SA, p = 0.003) without significant changes in BMI and ACR.

Conclusions: 1. Adiposity increases the prevalence of GH in actively-managed T2DM independently of glycemic control, albuminuria and ACEi/ARB therapy. 2. Younger adults with obesity and T2DM manifest a pattern of increasing eGFR during mid-term follow-up that may presage future risk for CKD.

FR-PO770

The Related Study of α-Klotho and Obesity Related Glomerulonephropathy Yang Min, Guo-Qin Wang, Hong-Liang Rui, Yuan-Yuan Pei, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital, Capital Medical Univ, Beijing, China.*

Background: To investigate the relationship of α-klotho protein and obesity related glomerulonephritis.

Methods: The patient with or without ORG were diagnosed by renal biopsy. The normal and abdominal obesity people were enrolled from the physical examination center. Propensity scoring analysis was done because the four groups of people may differ in important clinical characteristics. The blood and urine samples were detected by ELISA. After the mouse were sacrificed, kidneys were redusted. mRNA expression was detected by real-time quantitative PCR, protein expression was detected by Western blotting.

Results: [circ1] The plasma α-klotho levels were lower in the ORG patients, the CKD patients and abdominal obesity people than normal people, the difference was statistically significant (P<0.05). The plasma α-klotho levels were lower in ORG patients than CKD patients and abdominal obesity people, the difference was statistically significant (P<0.05). [circ2] The urine α-klotho levels were lower in ORG patients than CKD patients, abdominal obesity people and normal people, the difference was statistically significant (P<0.05). There was no difference between CKD patients, abdominal obesity people and normal people. [circ3] Compared with the control mouse, renal tissue α-klotho mRNA and protein levels were both decreased evidently in ORG model mouse.

Conclusions: The plasma and urine α-klotho levels were lower in ORG patients than abdominal obesity people, although both this two groups have the same abdominal obesity, and this maybe owing to the reduction expression of α-klotho in ORG renal tissue.

Funding: Government Support - Non-U.S.

FR-PO771

Structured Exercise in Obese Diabetic Patients with Chronic Kidney Disease: A Randomized Controlled Trial (NCT01036490): Effect of Obesity on Peak Oxygen Consumption David J. Leehey, Eileen Collins, Holly J. Kramer, Cheryl Cooper, Jolene Butler, Conor Mcburney, Christine Jelinek, Anne Garabedian, Susan Oconnell. *Medicine and Research, Hines VA Hospital, Hines, IL.*

Background: Patients with type 2 diabetes, obesity, and chronic kidney disease (CKD) are generally physically inactive, have a high mortality rate, and may benefit from an exercise program. However, there have been no randomized controlled trials to determine the benefits of exercise training in this population.

Methods: We hypothesize that exercise training in obese diabetic patients with CKD will improve proteinuria, stabilize renal function, decrease blood pressure, improve glucose control, improve body composition, decrease inflammation, improve endothelial dysfunction, decrease oxidative stress, and improve health-related quality of life. This is a 52-week randomized controlled study. Inclusion criteria are type 2 diabetes, obesity (body mass index > 30 kg/m²), chronic kidney disease (stage 2-4) and persistent proteinuria (> 200 mg/day for at least 3 months). All subjects receive optimal medical management and dietary counselling. Subjects randomized to exercise also undergo 12 weeks of exercise training followed by 40 weeks of supervised home exercise.

Results: 46 subjects were enrolled, of whom 36 completed at least the 12 week evaluation to date. At baseline, mean values (± SD) were as follows: age 65.9 ± 7.8, body mass index (BMI) 36.7 ± 4.4 kg/m², body fat 40.5 ± 5.8%, glycated hemoglobin (HbA1c) 8.0 ± 1.8%, creatinine clearance (CCr) 57.6 ± 29.3 mL/min, and urinary albumin excretion rate (UAE) 1118 ± 1236 mg/24h. Average treadmill time was 7.8 ± 3.8 minutes and peak oxygen consumption (VO_2 peak) was 13.2 ± 3.4 mL/kg/min. There was a negative correlation between VO_2 peak and age, BMI, percent body fat, and waist circumference, and a positive correlation between VO_2 peak and hemoglobin (Hb). On multivariate regression analysis, when age, race, BMI, HbA1c, CCr, UAE, and Hb were used as covariates, only BMI was predictive of VO_2 peak (P = 0.009).

Conclusions: Obese diabetic subjects with CKD have markedly impaired physical fitness, which is particularly evident in more obese patients.

Funding: Veterans Affairs Support

FR-PO772

Increase of Endothelin Type A Receptor in Local Renal Tissue Correlates with the Progression of Diabetic Nephropathy Huaying Pei,[1] Limin Zhang,[1] Jianrong Wang,[1] Wangxia Yang,[1] Shuxia Fu,[1] Shaomei Li,[1] Lin Yang,[1] Anyu Zhou.[2] *[1]Div of Nephrology, The 2nd Hospital of Hebei Medical Univ, Shijiazhuang, Hebei, China; [2]Rhode Island Hospital and the Warren Alpert Medical School at Brown Univ.*

Background: Diabetic nephropathy (DN) is one of the leading causes of chronic kidney disease but its exact cause of DN is unknown. Increased endothelin-1 (ET-1) has been shown to play an important role in the pathogenesis of DN majorly through activation of one of the ET-1 receptors, ETAR. However, the relationship between the progression of DN and the expression change of the two ET-1 receptors is unknown.

Methods: To explore the role of ET-1receptors in the progression of DN, semi-quantitative analysis was conducted to investigate the expression and localization of ETAR and ETBR in kidney biopsy samples using confocal laser scanning immunofluorescence microscopy. The subjects with DN were assigned to three groups by pathology classification: DN I grade (S1), DN II grade (S2), DN III grade (S3).

Results: Both ETAR and ETBR were observed in the glomerular and proximal tubular in control and DN patients. The expression of both receptors increased significantly in DN patients compared to that of controls. The expression of ETAR was lower than that of ETBR in control subjects. More importantly, the expression of ETAR increased dramatically as the diabetic nephropathy progressing (12.5±1.7 in S3 versus 8.5±1.3 in S2, 5.5± 0.2 in S1 DN patients respectively, P<0.01). However, there was no difference for ETB expression among S3, S2 and S1 groups (P>0.05). The ratio of ETAR and ETBR increased significantly between control and DN patients (P<0.01). The expression levels of ETAR were positively correlated with proteinuria (r=0.730, P<0.01) and the rate of glomerular sclerosis (r=0.783, P<0.01).

Conclusions: Our results suggest that the increase of endothelin receptors in local renal tissue plays critical role in the development and progression of diabetic nephropathy. The increased ration of ETAR and ETBR may be responsible for the progressive damage of kidneys in diabetes patients. Selectively blocking ETA receptor may be critical for developing novel therapeutics to impede the progression of diabetic nephropathy.

Funding: Government Support - Non-U.S.

FR-PO773

Urine Metabolomic Profile Correlates with Renal Function and Changes during Atrasentan Therapy in Patients with Diabetes and Nephropathy

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Background: A panel of 13 urine metabolites (reflecting mitochondrial function) has been previously identified to be reduced in diabetics with kidney disease compared to healthy controls and diabetics with no CKD (*Sharma et al, JASN 2013*). In the recently reported RADAR trial in patients with diabetes and nephropathy (de Zeeuw et al, JASN 2014), the endothelin A receptor antagonist atrasentan improved renal surrogate outcome parameters. We validated the metabolomics profile in RADAR and studied the response of the metabolomic panel to atrasentan.

Methods: RADAR is a placebo controlled double blind trial in patients with diabetes and nephropathy (urine ACR of 300-3500 mg/g and eGFR of 30 to 75 ml/min/1.73m^2) who were randomly allocated to 12 week treatment with placebo (n=50), atrasentan 0.75 mg/d (n=78) or atrasentan 1.25 mg/d (n=83) as adjunct to renin-angiotensin-system inhibition. Targeted urine metabolomics was performed with GC-MS.

Results: 4 out of the 13 metabolites of the panel were below detection. Of the remaining nine, 7 showed reduced values in patients with the reduced eGFR (all p<0.01, with Bonferroni correction for multiple testing). Interestingly, all of the 9 detectable metabolites were improved with atrasentan treatment relative to placebo at 12 weeks of treatment (p=0.0019 for probability of all 9 being elevated with treatment group versus placebo).

Conclusions: We validated and confirmed that a specific panel of urine metabolites is reduced in diabetics with decreased renal function. Treatment with atrasentan for 12 weeks increased the reduced metabolite levels, relative to the placebo group, towards non-CKD values, possibly reflecting stabilization of mitochondrial function.

Funding: Pharmaceutical Company Support - AbbVie

FR-PO774

Mechanism of LDL Reduction during Atrasentan Use in Patient with Diabetes and Nephropathy

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Background: We previously demonstrated that the selective endothelin A receptor antagonist atrasentan reduced albuminuria (UACR) in patients with diabetes and nephropathy (*de Zeeuw et al, JASN 2014*). We also observed a fall in total cholesterol. We therefore evaluated the effect of atrasentan on lipid profile and looked at potential mechanism (s), such as relation with UACR reduction.

Methods: 211 subjects with type 2 diabetes, macroalbuminuria, and eGFR between 30-75 ml/min/1.73 m^2, were enrolled in 2 parallel, multinational, double-blind, randomized, placebo-controlled studies. Subjects were randomized to atrasentan 0.75, 1.25 mg/d or placebo for 12 weeks. Lipid values were evaluated at baseline, after 12 weeks of treatment.

Results: Total-, LDL-, and HDL-cholesterol, triglycerides, and use of fibrates or statins were not different at baseline between groups. A significant placebo corrected reduction in LDL-cholesterol was observed of 12.5 and 14% (0.75 and 1.25 mg/d, respectively). Age, gender, baseline eGFR, serum albumin, UACR, baseline HDL and LDL-cholesterol values, and statin use did not influence the reduction of LDL cholesterol for either atrasentan dose. However, path analysis showed that the reduction of LDL was only partly explained by the reduction in albuminuria: for 37% at the 0.75 mg/d (p=0.008), and for 16% at the 1.25 mg/d dose (p=0.29). UACR responders to 0.75 mg/d atrasentan (defined as >30% albuminuria reduction) showed a significant reduction of LDL (21%) versus non-responders (6%), whereas at 1.25 mg/d LDL reductions were similar in UACR responders (14%) versus non-responders (14%).

Conclusions: Chronic administration of atrasentan reduces LDL-cholesterol significantly. This may partly be the result of altered albuminuria handling, however, at higher atrasentan dose other (undetected) effects appear to play a role.

Funding: Pharmaceutical Company Support - AbbVie

FR-PO775

Predictors of Fluid Retention and Relation with Albuminuria Reduction During Atrasentan Treatment in Diabetics with Nephropathy

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Background: We previously demonstrated that the selective endothelin A receptor antagonist atrasentan reduced albuminuria (UACR) in patients with diabetes and nephropathy (*de Zeeuw et al, JASN 2014*). We also observed a variable, dose dependent degree of fluid retention evidenced by weight gain and decreases in hemoglobin (Hb) and hematocrit (Hct). We thus examined whether the individual response of albuminuria and fluid retention are associated, which could be a major limiting factor in the wide spread use in this patient population. Secondly, we studied potential predictors of fluid retention.

Methods: RADAR was a randomized placebo controlled double blind trial in diabetic nephropathy patients randomly allocated to a 12 week treatment with placebo (n=50), atrasentan 0.75 mg/d (n=78) and atrasentan 1.25 mg/day (n=83) groups as adjunct to renin-angiotensin-system inhibition. For the purpose of this analysis the atrasentan 0.75 and 1.25 mg/d groups were combined. Changes in UACR, weight, Hb, and Hct were analyzed after 2 wks treatment.

Results: No correlation was observed between changes in weight and UACR response (R^2 0.032). Weight increase was similar in UACR responders (defined as >30% reduction in UACR at week 2) and nonresponders (1.1 versus 1.0 kg). Multivariate analysis showed that lower baseline eGFR, HOMA-product, and higher systolic BP, Hba1c, and atrasentan dose predicted weight gain (table). Similar results were obtained when Hb and Hct data were used as proxies for fluid retention.

Conclusions: In patients with type 2 diabetic nephropathy, atrasentan-induced fluid retention is predicted by initial eGFR, blood pressure and glucose control, but not by UACR reduction.These findings suggest potential opportunities for patient selection to optimize antialbuminuria effects and avoiding the potentially important side effect of fluid retention.

Predictors of weight gain	Coefficient	Standard error	p-value
Atrasentan dose	0.59	0.19	0.003
eGFR (10 ml/min/1.73m^2)	-0.21	0.07	0.002
HbA1c (%)	0.27	0.07	<0.001
Systolic BP (10 mmHg)	0.15	0.07	0.024
HOMA-product	-0.19	0.09	0.028

Funding: Pharmaceutical Company Support - AbbVie

FR-PO776

Intra-Individual Variability in Multiple Parameters in Response to Angiotensin Receptor Blockers Determines Ultimate Renal Outcome

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Background: Angiotensin Receptor Blockers (ARBs) are renoprotective and targeted to high blood pressure (BP). However, ARBs have multiple other (off-target) effects which may or may not affect renal outcome. It is unknown whether the on-target and off-target effects are congruent within an individual. If not, this may have major effects on individual long-term renal outcome prediction. We therefore studied intra-individual variability in multiple parameters in response to ARB in type 2 diabetes.

Methods: We used RENAAL trial data, assessing change in systolic BP (SBP) and the off-targets albuminuria, potassium, hemoglobin (Hb), cholesterol and uric acid after 6 months losartan treatment. Response was defined as the 6 months change from baseline. The improvement in predictive performance of renal outcomes (ESRD or doubling Screat) for each individual after adding ARB-induced changes in all risk markers to a renal risk score was assessed by C-index.

Results: Each parameter showed distinct response variability between patients. A response to losartan was observed in SBP (61%), albuminuria (72%), potassium (66%), hemoglobin (72%), cholesterol (61%), and uric acid (47%). The proportion of individuals with off-target responses was similar among patients with or without SBP response, suggesting that off-target responses were independent of SBP response. In addition, no correlations were seen between individual parameter responses within an individual (all r<0.3). Combining individual responses significantly improved renal outcome prediction (increase C-index 0.79 to 0.84, p<0.001). The results were validated in two independent trials with irbesartan: IDNT and IRMA-2.

Conclusions: ARBs have multiple effects which vary between individuals and vary between parameters within an individual. We found no congruency between the different parameter responses within an individual. Combining all ARB responses improves renal risk prediction, highlighting the importance of monitoring and targeting multiple risk markers.

Funding: Government Support - Non-U.S.

FR-PO777

The Effect of 3 Years Insulin Pump Treatment on Albuminuria in Type 1 Diabetes

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Background: We investigated the effect of 3 years insulin pump (CSII) treatment on HbA_{1c}, albuminuria and kidney function compared to multiple daily injections (MDI) in a single center clinical setting.

Methods: We conducted a case-control study of all patients initiating CSII from 2004-10 and followed for at least three years: 193 patients with type 1 diabetes matched (1:2) to 386 patients treated with MDI in the same period. Matching was based on diabetes duration, sex, HbA_{1c} and normo-, micro- or macroalbuminuria at baseline. Urinary albumin creatinine ratio (UACR) was measured yearly and annual change assessed from linear regression. Unpaired t-test compared groups and multiple regression adjusted associations.

Results: At baseline, both treatment groups included 39% men with diabetes duration of (mean±SD) 23±12 years and a frequency of normoalbuminuric of 84%. Patients were (CSII *versus* MDI) 48±12 *versus* 44±11 years old, HbA_{1c} 68±11 *versus* 68±10 mmol/mol, eGFR 100±23 *versus* 101±25 mL/min/1.73m^2, UACR 9 (IQR 6-19) *versus* 9 (6-17) mg/g, and numbers on RAAS inhibition (RAASi) 40 *versus* 38%, (p>0.58 for all; except age: p<0.001). Annual change in UACR in CSII *versus* MDI treated patients was (mean (CI95%))-11.3 (-14.6;-8.0) % *versus* -1.1 (-3.3;1.1) %, (p<0.001). Reduction in UACR was significantly associated to CSII treatment after adjustment for age, sex, diabetes duration and average eGFR, UACR, MAP, HbA_{1c}, cholesterol, RAASi, antihypertensive treatment and smoking (p<0.001). In adjusted analyses of patients on stable RAASi during follow

up (n=465) CSII treatment remained significantly associated to a reduction in UACR (p<0.001). After 3 years HbA_{1c} was 62±11 *versus*69±11 mmol/mol (p<0.001) and eGFR 94±25 *versus*96±26 mL/min/1.73 m^2 (p=0.58).

Conclusions: CSII treatment over 3 years independently reduced HbA_{1c} and UACR compared to MDI, but eGFR remained unchanged. Reduced UACR may be due to less glycaemic variability as the well-known effect of CSII on HbA_{1c} could only partially explain the effect. This cannot be assessed from our data. The effect of CSII treatment on UACR needs confirmation in randomized controlled trials.

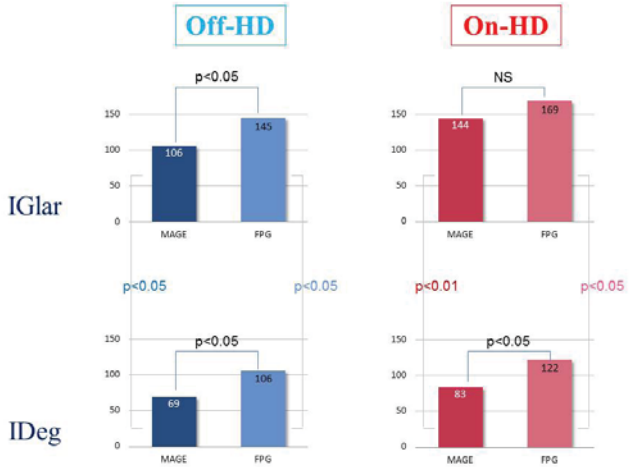
FR-PO778

Superior Glycaemic Control with Insulin Degludec (IDeg) to Insulin Glargine (IGlar) in Patients with Type-2 Diabetes (T2DM) on Hemodialysis (HD) Assessed by Continuous Glucose Monitoring (CGM) Satoshi Funakoshi,[1] Jyunichiro Hashiguchi,[1] Kenji Sawase,[1] Yoshiaki Lee,[1] Hiroshi Ichinose,[1] Osamu Sasaki,[1] Rica Etoh,[1] Masatoshi Hayashida,[1] Yoko Obata,[2] Tomoya Nishino,[2] Takashi Harada.[1] *[1]Nagasaki Kidney Center, Nagasaki, Japan; [2]Nagasaki Univ School of Medicine, Nagasaki, Japan.*

Background: During hemodialysis session plasma glucose decreases steeply, and then increases to hyperglycemic state afterwards in diabetic hemodialysis patients. IDeg, an engineered acylated insulin, was recently reported to form a soluble depot after subcutaneous injection with a subsequent slow release of insulin and an ultralong glucose-lowering and stabilizing effect.

Methods: In this study we evaluated the efficacy of IDegin controlling glycemic variability in diabetic hemodialysis patients comparing to IGlar using CGM system. Eight relatively poor-controlled (HbA1c>8.0 %) diabetic hemodialysis outpatients treated with IGlar were enrolled in this study after appropriate IC. Subjects were then monitored overall 72-hours glycemic control, on both hemodialysis day (HD) and non- hemodialysis days (free day: FD), by CGM. Various doses of IGlarwere converted to the same dose of IDeg, and glycemic controls were compared in each patient.

Results: As shown in Figure 1, the plasma glucose curves under IGlar treatment on HD steeply declined then increased compared to FD.



On the other hand, the plasma glucose variations in IDeg -treated patients were similar between HD and FD. More importantly, the mean amplitude of glycemic excursions (MAGE) under IGlar treatment on HD was significantly higher than in IDeg -treated group (122.3±38.3 mg/dL versus 66.7+22.1 mg/dL, p<0.001).

Conclusions: In our study, IDeg, with its slow release of insulin, could be promising candidate in the treatment in diabetic hemodialysis patients.

Funding: Private Foundation Support

FR-PO779

StepAhead Program Reduces Lower Limb Amputation Rates Among Diabetic End-Stage Renal Disease Patients Stephen D. McMurray,[1] Christine Ordway,[1] Carey Colson,[2] Steven M. Brunelli.[2] *[1]DaVita VillageHealth, Vernon Hills, IL; [2]DaVita Clinical Research, Minneapolis, MN.*

Background: End-stage renal disease (ESRD) patients with diabetes are at high risk of lower limb amputations. Published data have found rates of 4.3 to 13.8 amputations per 100 persons (Eggers et al Kidney Int 1999;56:1524-33; Combe C et al, Am J Kidney Dis 2009;54:680-92). StepAhead is an integrated DM care management program designed to enhance patient self-management and reduce the likelihood of unnecessary complications among diabetic ESRD patients. We examined whether StepAhead was effective in reducing lower limb amputations among diabetic ESRD patients.

Methods: StepAhead was initiated in January 2012, and 447 diabetic ESRD patients from 11 dialysis facilities were enrolled. Patients were followed for 12 months for physician-driven DM management, eye exams, glucometer possession, and glucose education and for 18 months for foot checks. Outcomes included lower limb amputation rates.

Results: Among StepAhead enrollees the mean age was 63 years, 45% female, 45% black, 43% Hispanic, 80% had Medicare as a primary insurer, mean body mass index was 27.9 kg/m^2, and mean Charlson Comorbidity Index was 6.1. Of those who completed 18 months (n=228), 95% had at least 6 foot checks and 80% had at least 12 foot checks after 18 months. Lower limb amputation rates fell from 2.7 to 1.6 amputations per 100 patient-years between the first year and last 6 months in the program (overall rate 2.4 amputations per 100 patient-years).

Conclusions: Versus published normative data, rates of lower limb amputation among StepAhead enrollees was lower. In addition, continued improvement in amputation rates suggests that the full potential of the program in amputation avoidance may take time to be achieved and may not yet have been fully seen.

FR-PO780

Subclinical Kidney Injury before and after Bariatric Surgery in Adolescents with Severe Obesity Nianzhou Xiao, Prasad Devarajan, Michael R. Bennett, Mark Mitsnefes. *Cincinnati Children's Hospital Medical Center, Cincinnati, OH.*

Background: Obesity is an independent risk factor for kidney injury. We have previously shown that severely obese children have increased urinary kidney injury biomarkers (NGAL, KIM-1, IL-18). While an increasing number of obese adolescents attempt to lose weight via bariatric procedure, there are no known studies examining change in kidney status afterward.

Methods: This is a prospective study of 19 severely obese adolescents with no microalbuminuria or decreased GFR at baseline, who had bariatric procedure. Urinary NGAL, IL-18 and KIM-1 were measured at baseline, 6 and 12 months post-surgery. The levels of biomarkers were compared between obese and age-gender-matched lean controls, and between obese children at baseline and after surgery. Data are median (IQR). 95% tile were calculated from 137 healthy adolescents (age 15 to 18 years) using Cincinnati Genomic Control Cohort.

Results: Baseline BMI was 49 kg/m^2 and decreased by 32% (p<0.001) at 1 year after procedure. All urinary biomarkers were significantly increased in the obese cohort in comparison to the lean controls. The levels remained high through 1 year after bariatric surgery. Accordingly, the prevalences of abnormal urine NGAL, KIM1 and IL-18 were significantly higher in obese compared to lean controls at baseline and at 1 year after surgery.

Characteristics	Lean Controls (n = 38)	Obese (n = 19)		
		Baseline	6 m	12 m
Age, year	16 (15, 17)	16 (15,17)		
Gender (M/ F)	4/15	8/30		
BMI, kg/m^2	20 (20, 22)	49 (43, 52)	34 (29, 39)	33 (28, 38)
eGFR, mL/ min/1.73m^2	NA	114 (103, 157)	109 (97, 121)	111 (94, 169)
IL-18, pg/mL >95th tile (>139 pg/ml)	24 (16, 52) 0.02%	113 (51, 847) 43%	142 (78, 354) 50%	112 (42, 7665) 39%
NGAL, pg/mL >95th tile (>75 pg/ml)	20 (7, 39) 0.05%	32 (22,164) 43%	161 (52, 340) 57%	47 (20, 107) 29%
KIM-1, pg/mL >95th tile (>1,876 pg/ml)	430 (209, 831) 0.02%	775 (254, 1187) 20%	2798 (1008, 3294) 71%	974 (624, 2312) 33%

Conclusions: Severe obesity is associated with increased urinary biomarkers of structural and inflammatory kidney injury, despite the absence of microalbuminuria or decreased GFR. The elevation persists at 1 year after surgery, suggesting that close long-term follow up of kidney status is warranted.

FR-PO781

The Influence of Pharmaceutically Induced Weight Changes on Estimates of Renal Function Bernt Johan Illum von Scholten,[1] Frederik I. Persson,[1] Oluf Kristian Højbjerg Hansen,[2] Claus Bo Svendsen,[2] Peter Rossing.[1,3,4] *[1]Steno Diabetes Center, Denmark; [2]Novo Nordisk, Denmark; [3]Univ of Copenhagen, Denmark; [4]Aarhus Univ, Denmark.*

Background: Estimates of renal function based on serum creatinine are derived from cross-sectional studies. If body weight or body composition change over time, this could affect estimates of renal function, if plasma creatinine levels are changed as well. Whether this affects estimated renal function (eGFR) could depend on applied equations as Cockcroft-Gault (CG) which includes creatinine and body weight, whereas the 4-variable MDRD and CKD-EPI equations only include creatinine.

Methods: We compared changes in eGFR based on CG or MDRD in type 2 diabetic patients followed for 26 weeks in the seven phase 3 trials of the glucose- and body weight-lowering GLP-1 analog liraglutide (lira), with that of other glucose lowering medications with less or no weight reducing effects (insulin glargine, glimepiride, exenatide, rosiglitazone and placebo).

Results: The analysis included 5100 patients (3173 patients treated with lira and 1927 treated with comparator). Mean (SD) baseline MDRD eGFR was 88.0 (21.5) and 88.8 (22.1) ml/min/1.73m^2, for lira and comparator respectively. The reduction in body weight (BW) for lira was 1.9 (3.7) kg (with a maximum of 24.7 kg) and for comparator BW increased 0.2 (3.5) kg (maximal reduction of 14.9 kg). Creatinine was unchanged in both groups. In separate lognormal linear regression models with last observation carried forward a 10 % increase in BW corresponded to a 0.8 (-0.4;1.9) % increase in creatinine for lira and a 0.7 (-0.9;2.2) % decrease for comparator, and MDRD eGFR increased 0.24

(-1.0;1.5) % for lira, and 0.55 (-1.2;2.3) % for comparator. A 10 % increase in BW was associated with a 10.2 (9.0-11.5) % increase in CG eGFR for lira, and a 10.5 (8.9;12.2) % increase in CG eGFR for comparator.

Conclusions: In type 2 diabetic patients with normal renal function, weight reduction of 1.9 kg (maximal reduction of 24.7 kg) was not associated with change in plasma creatinine. Accordingly, there was no change in weight independent estimates of GFR (MDRD), whereas weight dependent estimates (CG) were changed.

FR-PO782

The Reno-Protective Effect of Quality Improvement Activity Targeting Better Glycemic Control Keita Hirano,[1,2,6] Sachiko Ohde,[2] Gen Shimada,[3,4] Masami Monden,[5] Yasuhiro Komatsu,[6] Gautam A. Deshpande,[2] Osamu Takahashi,[2,7] Tsuguya Fukui.[2,7] *[1]Dept of Nephrology, Kyoto Univ Graduate School of Medicine; [2]Center for Clinical Epidemiology, St. Luke's Life Science Inst; [3]Dept of General Surgery, St. Luke's International Hospital; [4]Medical information Center, St. Luke's International Hospital; [5]Dept of Endocrinology, St. Luke's International Hospital; [6]Dept of Nephrology, St. Luke's International Hospital; [7]Dept of Internal Medicine, Dept of Internal Medicine.*

Background: Although physicians acknowledge the importance of tight glycemic control to prevent complications such as chronic kidney disease for type 2 diabetes patients, the optimal methods for management are not always clear. We implemented hospital-wide QI activity (quality improvement) which includes monthly monitoring blood glucose levels and prescribing medications, and medical education by specialists. Purpose of this study is to investigate the effectiveness of QI activity by evaluating kidney function of type 2 diabetic patients between pre and post QI period.

Methods: This retrospective open cohort study included type 2 diabetic patients with baseline eGFR ≥60 ml/min/1.73m^2 at the St. Luke's International Hospital. All patients seen at least twice between 2005 and 2012 were stratified into two groups based on their first visit year: 2005-2008 year group (pre-QI period) versus 2009-2012 year group (QI period). We used linear random effects models with individual intercepts (initial eGFR level) and individual gradients (change in eGFR per year), to derive parameters describing the progression of kidney function.

Results: A total of 6,875 patients were included in the study (mean age of 62.1 years, SD 13; 66.3% male). According to the linear random effects models, eGFR decreased by 1.0 ml/min/1.73m^2 per year among the 2005-2008 year group, while decreasing by 0.2 ml/min/1.73m^2 among the 2009-2012 year group. After QI activity implementation, the prescription of metformin and DPP-4 inhibitors dramatically increased and better glycemic control was achieved.

Conclusions: According to our results, QI activity have succeded in preventing rapid decrease in kidney function in type 2 diabetic patients.

Funding: Pharmaceutical Company Support - Merck & Co., Inc.

FR-PO783

Trained Breathing-Induced Oxygenation Reverses Cardiovascular Autonomic Dysfunction in Patients with Type 2 Diabetes and Renal Disease Pasquale Esposito,[1] Teresa Rampino,[1] Per-Henrik Groop,[2] Luciano Bernardi,[3] Antonio Dal Canton.[1] *[1]Nephrology, Dialysis and Transplantation, Fondazione IRCCS, Pavia, Italy; [2]Dept of Nephrology and Folkaelsan Research Center, Univ of Helsinki, Finland; [3]Dept of Internal Medicine, Fondazione IRCCS, Pavia, Italy.*

Background: Cardiovascular autonomic neuropathy (CAN) is a risk factor for cardiovascular disease in diabetic and renal patients but its pathogenesis is obscure. This study was focused to understanding whether hypoxia is a functional determinant of CAN in diabetic patients and the extent to which hypoxia and CAN may be reversed by trained breathing blood oxygenation.

Methods: 26 type II diabetic patients (age 61±0.8 years) were enrolled. Diabetes duration was 10.5±2 years, GFR was 68.1±5.5 ml/min. 24 healthy subjects (age 58.5±1) were the controls. CAN was assessed by the measurement of arterial baroreflex sensitivity (BRS) obtained recording the RR interval and blood pressure during spontaneous, normal (15 breaths/min) or slow (6 breaths/min) controlled breathing in conditions of normoxia or hyperoxia (5 L/min oxygen).

Results: During normal controlled breathing, diabetic patients presented higher heart rate (HR) and lower BRS compared with the control participants (5.5±0.6 versus 11.8±2.0 ms/mmHg, p<0.005).Slow breathing and oxygen administration significantly reduced HR and increased BRS in both diabetic patients and the control group (9.6±1.9 and 15.3±2.3 ms/mmHg, respectively, p<0.05).

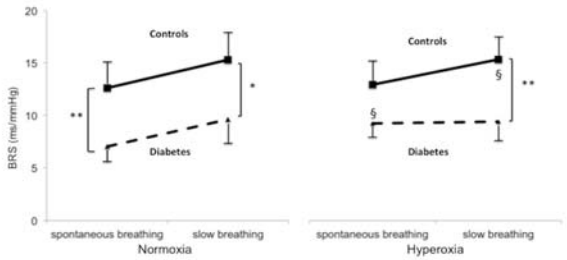


Figure 1. Effect of oxygen and slow breathing on BRS.

Data are expressed as means+SEM. In normoxia BRS was depressed in type 2 diabetes, while slow breathing increased BRS in both the groups of subjects.
In hyperoxia diabetic patients presented an improved BRS, that was unaffected by slow breathing, indicating that the two effects are probably related.
Instead, in healthy subjects hyperoxia induced a further increase in BRS during slow breathing.
* p<0.05, * * p <0.005, § p<0.05 vs spontaneous breathing

Similarly, also patients affected by chronic kidney disease, presented a significant improvement of BRS during deep breathing and hyperoxia (9.8±3.7 and 10.7±3.4 ms/mmHg, respectively, p<0.05 versus spontaneous breathing).

Conclusions: CAN affecting type II diabetic patients can be partially reversed by increasing oxygen supply through controlled breathing, thus suggesting hypoxia as a cause of cardiovascular risk potentially prevented by trained breathing.

Funding: Pharmaceutical Company Support - We received a research grant from the Italian Kidney Foundation (FIR), Private Foundation Support

FR-PO784

Effect of Dietary Protein Restriction and Uric Acid on Progression of Diabetic Nephropathy Sascha Pilemann-Lyberg,[1] Morten Lindhardt,[1] Frederik I. Persson,[1] Henrik Post Hansen,[2] Peter Rossing.[1,3,4] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Dept of Nephrology, Herlev Univ Hospital, Denmark; [3]Aarhus Univ, Denmark; [4]Univ of Copenhagen, Denmark.*

Background: Diabetic nephropathy (DN) is a major cause of end stage renal disease (ESRD). Evidence suggests that serum uric acid (UA) is a risk factor for development and progression of chronic kidney disease and early loss of kidney function in patients with diabetes. We aimed to investigate whether a high level of UA is associated with GFR decline, ESRD or death in patients with type 1 diabetes.

Methods: We conducted a *post hoc* analysis of 82 patients included in a prospective, randomized, controlled trial with allocation to either low versus normal protein diet for a median of 4 years. Patients were selected with progressive DN (prestudy GFR decline of ≥ 2 ml/min/year). UA was measured at baseline. The primary endpoint was a combined endpoint of death or ESRD and the secondary endpoint was decline in GFR (yearly measurement of Cr51EDTA plasma clearance). A cox regression model was built to evaluate the effect of UA on the primary endpoint. General linear model was used to evaluate association to decline in GFR. Both models were performed with and without adjustment for progression promoters (HbA1c, systolic blood pressure, cholesterol, baseline GFR, baseline urinary albumin excretion rate (UAER)) and diet allocation with backward elimination.

Results: Mean baseline UA was 6.5 mg/dL (SD±2.1), GFR 68mL/min/1.73m^2 (SD±31), geometric mean of UAER 772mg/24h (IQR, 415 to 1510). As previous shown low protein diet significant effect on the primary endpoint (log rank test, p=0.042). Level of UA was similar in patients reaching the endpoint compared to those who did not (p=0.88) and had no effect in the cox model before and after adjustment (p=0.91). Level of UA was not associated with change in GFR (r^2=0.04, p=0.11). Each doubling of baseline UAER was associated with an annual decline in GFR by 0.7mL/in (p=0.03).

Conclusions: Serum uric acid did not predict the endpoint of ESRD or death and was not associated with decline of GFR. As these results are in contrast with previous finding further evaluation of the role of UA as a progression marker is needed.

Funding: Private Foundation Support

FR-PO785

Benefits of Lifestyle Modification Program in Obese Chronic Kidney Disease Patients Sankar D. Navaneethan,[1] Ciaran E. Fealy,[2,3] Amanda R. Scelsi,[2] Hazel Huang,[2] Steven K. Malin,[2] John P. Kirwan.[2] *[1]Nephrology, Cleveland Clinic; [2]Pathobiology, Lerner Research Inst; [3]Kent State Univ.*

Background: Obesity is an independent risk factor for the development and progression of chronic kidney disease (CKD). However, the feasibility and benefits of lifestyle modification on weight loss in people with CKD is less well understood. Therefore, we examined the effects of a diet and exercise intervention on body composition, exercise capacity, insulin resistance, inflammation, adipokines and kidney function in obese subjects with CKD.

Methods: Nine subjects (2M/7F, age: 57 years, median BMI 43.9 kg/m^2, iothalamate glomerular filtration rate [iGFR] 62 ml/min) underwent a 12-week lifestyle modification intervention program. This included dietary modification (500-kilocalorie deficit diet) by a registered dietitian based on measured resting energy expenditure assessment and a supervised arobic exercise (i.e. ~ 85% HR_{max}) program at our Clinical Research Unit. Body composition (iDXA), exercise capacity (VO_{2max}), insulin resistance (Matsuda Index), inflammation (hs-C-Reactive Protein), adipokines (leptin) and kidney function (iGFR) were measured before and after the intervention. Changes in parameters were compared using Wilcoxon signed rank test.

Results: After the 12-week program, there was a significant decrease in BMI and fat mass. There was also a significant increase in exercise capacity, along with improvements in insulin sensitivity and leptin. However, there was no significant change in inflammation or kidney function after the intervention.

Variable	Median (Inter-quartile range)	p-value
Change in BMI (kg/m^2)	-1.9 (-2.6, -1.4)	0.004
Change in Fat mass (kg)	-4.9 (-5.9, -3.0)	0.03
Change in Matsuda index	0.55(0.43, 1.2)	0.02
Change in Leptin	-5.1 (-14.5, -3.3)	0.008
Change in Log CRP	0.01 (-0.04, 0.14)	0.46
Change in Iothalamate GFR (ml/min)	-2.00 (-6.00, 0.10)	0.37

Conclusions: Weight loss with 12-weeks of diet and exercise is feasible in a CKD population. The effects on body weight also related to improvements in exercise capacity, insulin resistance and leptin. Whether lifestyle-induced weight loss can be sustained in CKD patients together with improvements in kidney function merits further study.

Funding: Other NIH Support - National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health (Grant #TR000440)

FR-PO786

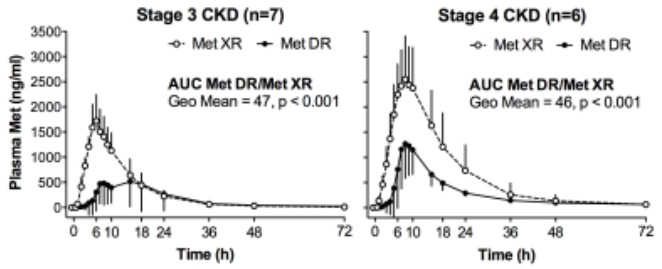
Effects of New Metformin Formulation in Stage 3 and 4 CKD: A Pilot Study

George L. Bakris,[1] Sunder Mudaliar,[2] Terri Kim,[3] Colleen Burns,[3] Sharon D. Skare,[3] Mark Fineman,[3] Alain Baron.[3] *[1]The Univ of Chicago Medical Center, Chicago, IL; [2]VA Medical Center, San Diego, CA; [3]Elcelyx Therapeutics, San Diego, CA.*

Background: Metformin (Met) is contraindicated in patients with Stage 3 or higher CKD due to increased risk of lactic acidosis. Low-dose Met is sometimes used off-label in patients with Stage 3 CKD to limit exposure. Delayed release (DR) Met targets delivery to its site of action in the lower bowel where absorption is poor, resulting in greater glucose-lowering efficacy and lower plasma Met for a given dose. We previously showed that 1000 mg Met DR produces a greater reduction in glucose and lower plasma Met levels compared to 1000 mg extended release (XR) Met in patients with normal renal function (RF). Thus, Met DR may be safer and more effective than currently available Met in patients with Stage 3 or higher CKD.

Methods: This randomized crossover study assessed the plasma PK of a single dose of Met DR, Met XR and placebo in 31 patients with T2DM and normal RF (eGFR ≥90mL/min/1.73m^2) or CKD: Stage 2 (60-89), Stage 3 (30-59) and Stage 4 (15-29).

Results: Patients were mostly white males (77%) with mean±SD BMI 32±4kg/m^2, 10±8yr duration of T2DM, fasting glucose 157±49mg/dl, and A1C 7.5±1.2%. Compared to Met XR, Met DR was associated with a 48, 26, 53 and 54% reduction in Met exposure in patients with normal RF, and Stage 2, 3 and 4 CKD. Figure 1 shows Met exposure in patients with Stage 3 and 4 CKD.



Plasma lactate and anion gap were not increased in any group. Adverse event rates were low and similar across groups.

Conclusions: In patients with T2DM and Stage 3 or higher CKD, Met DR was associated with lower Met than the same dose of currently available Met XR without increased lactate or anion gap. Together with previous efficacy data, these data support further study of Met DR in patients with T2DM and advanced CKD.

Funding: Pharmaceutical Company Support - Elcelyx Therapeutics, Inc.

FR-PO787

The Efficacy and Safety of Dipeptidyl Peptidase-4 Inhibitors in the Treatment of Type 2 Diabetes Mellitus in Patients with Chronic Kidney Disease: A Meta-Analysis of Randomized Clinical Trials

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Background: Glucose-lowering treatment options are limited for uncontrolled type 2 diabetes mellitus (T2DM) patients with advanced chronic kidney disease (CKD).The present meta-analysis is aimed to assess the long-term glucose-lowering efficacy, safety and tolerability of dipeptidyl peptidase-4 (DPP-4) inhibitor agents compared with placebo or other anti-diabetic medications in patients with T2DM and CKD.

Methods: We searched MEDLINE, EMBASE and Cochrane CENTRAL (2005 through Dec 2013) for relevant abstract and publications. Studies were eligible if they were randomized controlled trials (RCTs) that compared a DPP-4 inhibitor with a placebo in patients with T2DM and CKD (estimated GFR < 60 ml/min or ESRD). Included studies had information on stage of chronic kidney disease (CKD), glycemic control by HbA1C measurement, dose and type of DPP-4 inhibitor, as well as duration of T2DM and other anti diabetes medication.

Results: We screened 3,244 abstracts for inclusion in our meta-analysis. Among 151 potentially relevant studies, 6 RCTs enrolling 1082 patients with a mean follow up of 54 weeks were included in the meta-analysis. Compared to placebo or active control, treatment with a DPP 4 inhibitor was associated with a -0.53 % change in HbA1C. Rates of adverse events including hypoglycemia, death or pancreatitis were similar in placebo and treatment groups.

Conclusions: In patients with Type 2 diabetes and CKD, treatment with DPP 4 inhibitors appears to be safe and effective. Longer term follow up including post marketing surveillance studies are needed to verify the safety of these medications in the CKD populations.

FR-PO788

Systematic Literature Review of Clinical Trials of Dipeptidyl Peptidase-4 Inhibitors in Patients with Type 2 Diabetes and Renal Impairment

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Background: DPP-4 inhibitors (DPP-4i) have demonstrated utility in patients (pts) with normal renal function. However, more information is required on efficacy and safety of DPP-4i in pts with moderate to severe renal impairment (RI). We performed a systematic literature review of long-term efficacy and safety of DPP-4i in T2DM pts with RI.

Methods: We searched EMBASE, MEDLINE and Cochrane Central Register of Controlled Trials, from database inception to Nov 2013, to identify randomised, placebo (PB)-controlled trials (CTs) of DPP-4i in T2DM pts with RI. Eligible trials were of ≥12 weeks' (wks) duration with ≥50 pts. Change in HbA1c and incidence of hypoglycemic events (HEs) were evaluated.

Results: Four PB CTs, of vildagliptin (Vilda), saxagliptin (Saxa), linagliptin (Lina) or sitagliptin (Sita), were included; the first two had 52-wk extensions. In Vilda, Saxa and Lina trials, 74–82% pts received insulin at baseline (BL), while 10% received insulin in PB CT with Sita. After 52 wks, Vilda, Saxa and Lina all significantly reduced HbA1c by 0.4–0.7% (BL 7.7–8.7%) compared to PB. HbA1c lowering was similar at 12 and 52 wks in these studies, implying sustainable effects in this setting. In a12-wk study, a significant HbA1c reduction of 0.4% (BL 7.7%) was observed in Sita-treated pts compared to PB. Vilda-treated pts reported a similar incidence of HEs compared to PB (23% versus 17%). Similarly, rate of HEs with Saxa was comparable to PB (28% versus 29%). Lina-treated pts reported a numerically higher rate of HEs compared to PB (63% versus 49%), possibly indicating an overall higher HE rate. However, between-treatment differences in HEs were not significant for any of these molecules. No adverse effects on renal function, fluid retention or weight were observed.

Conclusions: Many pts with T2DM have RI, which makes diabetes management complicated and costly. These studies suggest DPP-4i have the potential to improve glycemic control in this setting, without increased HEs or other adverse events.

Funding: Pharmaceutical Company Support - Novartis

FR-PO789

Efficacy of Dipeptidyl Peptidase-4 Inhibitors in Hemodialysis Patients with Type 2 Tomomi Ishikawa,[1] Michihiro Hosojima,[2] Ryohei Kaseda,[2] Hideyuki Kabasawa,[1] Shoji Kuwahara,[3] Noriaki Iino,[1] Yoshiki Suzuki,[4] Ichiei Narita,[1] Akihiko Saito.[3] *[1]Clinical Nephrology, Niigata Univ, Japan; [2]Clinical Nutrition Science, Niigata Univ, Japan; [3]Applied Molecular Medicine, Niigata Univ, Japan; [4]Health Administration Center, Niigata Univ, Japan.*

Background: Blood glucose profile of dialysis patients remains precisely unclear. Few studies reported about the efficacy and safety of dipeptidyl peptidase-4 (DPP-4) inhibitors in diabetic dialysis patients. Our aim of this study is to monitor blood glucose levels estimated by using continuous glucose monitoring (CGM) and clarify the effect of DPP-4 inhibitors in Japanese hemodialysis (HD) patients with type 2 diabetes.

Methods: Thirty diabetic and 17 non-diabetic patients on HD underwent CGM for 48-168 h. The following parameters were measured: 1) mean 24-h glucose levels, 2) standard deviation of glucose levels (SD), 3) mean amplitude of glycemic excursion (MAGE), 4) area over the curve (AOC) for glucose levels <70 mg/dL (AOC<70) for 24 h (8am to 8am) or night time (8pm to 8am) in HD days, and 5) AOC for glucose levels under the initial levels during HD for 4h (AOC<I).

Results: CGM-estimated blood glucose levels were decreased dramatically during HD in both diabetic and non-diabetic patients. Using DPP-4 inhibitors ameliorated this decrease in diabetic patients (AOC <I: 109.8 ± 64.5 h·mg/dl versus 175.4 ± 95.3 h·mg/dl in DPP-4 inhibitors group versus non-DPP-4 inhibitor group, respectively, $p<0.05$). Asymptomatic hypoglycemia was also recorded by CGM in several diabetic patients who did not use DPP-4 inhibitors. Using DPP-4 inhibitors lessened nocturnal hypoglycemia in HD days (AOC <70: 0.9 ± 3.9 versus 14.8 ± 33.1 h·mg/dl, DPP-4 inhibitor group versus non-DPP-4 inhibitor group, respectively, $p<0.05$). Furthermore, DPP-4 inhibitors also ameliorated glucose variability (SD and MAGE) in both HD and non-HD days.

Conclusions: Our CGM study showed great glucose variability and hypoglycemic events in HD patients. We propose that using DPP-4 inhibitors is promising therapy for lowering the glucose variability and hypoglycemic events in diabetic HD patients.

FR-PO790

Effect of DPP-4 Inhibition on the Progression of Renal and Cardiovascular Disease on Type-2 Diabetic Patients María Marques Vidas, Felipe E. Zalamea, Jeanette Nora Fernandez C., Sofia Karsten, Paula López, Maria Rosario Llopez Carratala, Esther Rubio Gonzales, Jose Portoles. *Nephrology, H Puerta de Hierro Majadahonda, Madrid, Spain.*

Background: The role of the dipeptidyl peptidase-4 (DPP-4) in modulating immunity and the interaction of this system with several mechanisms that control blood pressure has risen the possibility of the existence of pleiotropic effects of the DPP4-inhibitors (DPP4i) on the cardiovascular system and renal function beyond their effect on blood glucose control. The aim of this study was to test the effect of the treatment with DPP4i on the progression of renal and cardiovascular disease.

Methods: We retrospectively analyzed the cardiovascular and renal outcomes of type-2 diabetic patients that had been on DPP-4 i (DPP4i group, n 48), other oral antidiabetic drugs (OAD, n 21) or insulin (n 51). Patients with less than one year of stable treatment, MDRD-eGFR < 10 ml/min/1.73 m² and renal disease of non-diabetic origin were excluded. All patients were on maximum dose tolerated of RAAS blockers according local practice.

Results: The median follow-up was 3.55 ± 0.71 years and there were no significant differences on demographic data nor on time of diabetes-onset between groups. We found no significant modifications from baseline on serum creatinine in any treatment group. However, eGFR MDRD was significantly reduced on DPP4i (-15,3 %) and insulin (-10,7 %) groups with respect to OAD (24,8 %), *p< 0.05*. Urinary albumin/creatinine ratio increased along time in all the groups, but the increase average was lower on the DPP-4i group compared to OAD and insulin: 47% versus 85% versus 103%, *p ns*. There were no significant differences on cardiovascular outcomes between groups: 72,5% of the DPP-4i group, 84.6% of the OAD group and 72.9% of the insuline group were free from cardiovascular events. Total and LDL-cholesterol, uric acid and HbA1 levels showed no significant modifications in any group.

Conclusions: We conclude that treatment with DPP-4i showed a beneficial effect on the progression of albuminuria on type-2 diabetic patients independently of glycemic control. The possible RAAS-blockade-like effect of these drugs on systemic and glomerular hemodynamics needs to be further elucidated.

FR-PO791

Efficacy and Safety of Liraglutide versus Placebo by Age Subgroup in Subjects with Type 2 Diabetes and Moderate Renal Impairment (LIRA-RENAL): A Randomized Trial David Scott,[1] Guillermo Umpierrez,[2] Stephen Atkin,[3] Stephen Bain,[4] Peter Rossing,[5] Minara Shamkhalova,[6] Heidrun Bosch-Traberg,[7] Annika Syrèn,[7] Melanie Davies.[8] *[1]Diabetes, Clinical Research Development Associates, New York, NY; [2]Emory Univ, Atlanta, GA; [3]Weill Cornell Medical College Qatar, Doha, Qatar; [4]Inst of Life Science, Swansea Univ, Swansea, United Kingdom; [5]Steno Diabetes Center, Gentofte, Denmark; [6]Endocrinology Research Centre, Moscow, Russian Federation; [7]Novo Nordisk A/S, Søborg, Denmark; [8]Univ of Leicester, Leicester, United Kingdom.*

Background: To determine the effects of adding liraglutide 1.8 mg (lira) or placebo (PBO) to existing oral antidiabetic agents and/or insulin therapy to subjects with moderate renal impairment (eGFR 30-59 mL/min/1.73 m²; MDRD), we evaluated age-dependency (18-64; 65-74; ≥75 yr) on selected efficacy and safety parameters.

Methods: In this 26-week, placebo-controlled, double-blind trial, adults with T2DM and moderate renal impairment, BMI of 20-45 kg/m², HbA_{1c} of 7.0-10.0% and on stable diabetes medication were randomized 1:1 to receive either once-daily lira or PBO. The primary endpoint was HbA_{1c} change from baseline (BL) to Week 26.

Results: 277 subjects were exposed to lira or PBO and included in the analysis. Lira demonstrated a significant reduction in HbA_{1c} across all age subgroups compared to PBO (Table). More GI AEs (mostly nausea and vomiting) were reported in all lira age subgroups compared to PBO. No pattern for hypoglycemic events was noted by age subgroup. No differences were seen in either systolic (p=0.2511) nor diastolic (p=0.8907) blood pressure between lira and PBO for the trial population.

Conclusions: In subjects with T2DM and moderate renal impairment, lira showed superior HbA_{1c} reduction across all age subgroups compared to PBO. Overall, there was no difference in the safety profile of lira between age subgroups over 26 weeks.

Age Subgroup	18-64 yr		65-74 yr		≥75 yr	
Treatment Group	Lira n=38	PBO n=55	Lira n=72	PBO n=66	Lira n=30	PBO n=16
HbA_{1c}, BL, mean % (SD)	8.15 (0.79)	8.09 (0.87)	8.13 (0.79)	7.94 (0.85)	7.87 (0.79)	7.97 (0.83)
Change from BL at Week 26, estimated means	-1.04	-0.31	-0.92	-0.40	-1.37	-0.59
Treatment difference, estimated; p-value	-0.72 p=0.0005		-0.52 p=0.0031		-0.78 p=0.0135	
Subgroup by treatment interaction	p=0.8392					
AE, % subjects	73.7	69.1	75.0	72.7	83.3	50.0
SAE, % subjects	10.5	3.6	9.7	15.2	10.0	18.8
GI AE, % subjects	36.8	14.5	31.9	16.7	43.3	31.3
Confirmed hypo, % subjects	10.5	10.9	12.5	22.7	6.7	12.5

BL=baseline; SD=standard deviation; AE=adverse event; SAE=serious adverse event; GI AE=gastrointestinal adverse event; hypo=hypoglycemia

Clinical trial reg. number: NCT01620489

Funding: Pharmaceutical Company Support - Novo Nordisk A/S

FR-PO792

Patiromer Lowers Serum K^+ and Prevents Recurrent Hyperkalemia in Patients with Diabetes and CKD on RAAS Inhibitors: Subgroup Results of a Phase 3 Trial Matthew R. Weir,[1] George L. Bakris,[2] David A. Bushinsky,[3] Martha Mayo,[4] Dahlia Garza,[4] Yuri Stasiv,[4] Yingxin Hou,[5] Heidi Christ-Schmidt,[5] Lance Berman.[4] *[1]Univ of Maryland, Baltimore, MD; [2]Univ of Chicago, Chicago, IL; [3]Univ of Rochester, Rochester, NY; [4]Relypsa, Redwood City, CA; [5]Statistics Collaborative, Washington, DC.*

Background: RAAS inhibitors (RAASi) have proven cardiorenal benefits in patients (pts) with CKD and Type 2 diabetes (T2DM), yet hyperkalemia (HK) often limits RAASi therapy. Patiromer, a nonabsorbed polymer with high K^+-binding capacity and good GI tolerability, normalized serum K^+ ($s\text{-}K^+$) and prevented HK recurrence in a large study in CKD pts with HK on RAASi. Here we present results in pts with T2DM.

Methods: Pts with BL $s\text{-}K^+$ 5.1 to <6.5 mEq/L received patiromer (4.2 or 8.4 g BID to start) in a 4-wk Initial Treatment Phase; pts with BL $s\text{-}K^+$ 5.5 to <6.5 mEq/L were eligible to continue into an 8-wk placebo (PBO)-controlled Randomized Withdrawal Phase. Primary and secondary endpoints, respectively, were: change in $s\text{-}K^+$ from BL to Wk 4 and % of pts with $s\text{-}K^+$ within target at Wk 4 [Initial Treatment Phase]; between-group difference in $s\text{-}K^+$ change over the 1st 4 wk of the phase and % of pts with recurrent HK [Randomized Withdrawal Phase].

Results: Of pts treated in the 1st and 2nd phases, respectively, 57% and 63% had T2DM. Consistent with overall results, primary endpoints were significant for pts with T2DM (Table).

Table. Primary Endpoint Results in Both Study Phases			
Primary Endpoint Results in the Initial Treatment Phase			
Population	Mean ± SE Baseline Serum K^+, mEq/L		Mean Change ± SE in Serum K^+ (95% CI), mEq/L [p value]
Overall (n=237)	5.58 ± 0.03		-1.01 ± 0.03 (-1.07, -0.95) [p < 0.001]
Diabetes (n=138)	5.61 ± 0.05		-1.00 mEq/L ± 0.04 [p < 0.001]
Primary Endpoint Results in the Randomized Withdrawal Phase			
Population	Median Change (25th, 75th Percentile) in Serum K^+ from Baseline to Week 4 of Phase, mEq/L		Between-Group Difference in Median Change in Serum K^+ (95% CI), mEq/L [p value]
	Placebo	Patiromer	
Overall (n=107)	0.72 (0.22, 1.22)	0.00 (-0.30, 0.30)	0.72 (0.46, 0.99) [p < 0.001]
Diabetes (n=67)	0.69 (0.19, 1.29)	0.03 (-0.20, 0.30)	0.66 (0.28, 1.03) [p < 0.001]

Overall and in pts with T2DM, >75% of pts had normalized s-K^+, and significantly ($p<0.001$) more PBO pts developed recurrent HK. Overall, patiromer was well tolerated; mild-to-moderate GI symptoms were the most common AEs. Rates of other AEs with patiromer were similar to, or lower than, PBO.

Conclusions: After controlling s-K^+, patiromer significantly decreased HK recurrence compared to PBO in CKD pts with T2DM on RAASi, with a tolerability profile that may allow continuous management of s-K^+ in these pts.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

FR-PO793

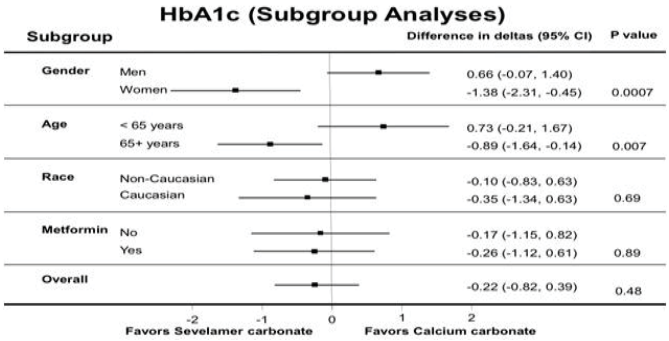
Sevelamer Carbonate Reduced Advanced Glycation Endproducts, Inflammation, and Glycated Hemoglobin in a Randomized Controlled Trial of Patients with Diabetic Kidney Disease

Nikolas B. Harbord,[1] Jaime Uribarri,[2] Elena M. Yubero-Serrano,[3] Mark Woodward,[4] Agustin Busta,[1] Leonid Poretsky,[1] Helen Vlassara,[2] Gary E. Striker.[2] *[1]Mount Sinai Beth Israel, New York, NY; [2]Mount Sinai Hospital, New York, NY; [3]Lipid & Atherosclerosis Research Unit, IMIBIC/Reina Sofia Univ Hospital/Univ of Cordoba, Cordoba, Spain; [4]The George Inst for Global Health, Camperdown, NSW, Australia.*

Background: Oxidative stress and inflammation (OS/Infl) underlie the complications of diabetics with kidney disease (DKD). Glycated hemoglobin (HbA1c) is a therapeutic target and correlates with progression of DKD. Dietary advanced glycation endproducts (AGEs) contribute to OS/infl, and sevelamer carbonate (SC) can bind and prevent their absorption. In a cross-over trial, we demonstrated reduction of AGEs, HbA1c, and OS/Infl with SC in DKD. This study further examined these effects in a larger DKD population.

Methods: In a two center, randomized, intention to treat, open label study, we enrolled 117 subjects with stage 2-4 DKD, HbA1c >6.5% and ACR >200mg/g to either SC (1600mg TID with meals) or CaCarbonate (1200mg TID with meals). Treatment continued for 6 months without changes to medications or diet.

Results: AGE intake did not change. SC reduced serum and cellular AGEs (MG and CML, p=<0.001-0.002), pro-inflammatory TNFR1 (p=0.04) and RAGE (p=0.0004); and increased anti-inflammatory adiponectin (p=0.01) and Nrf2 (p=0.0003), AGER1 (p=0.003) and SIRT1 (p<0.06) (trend). SC did not lower HbA1c in the overall population, but in women (p=0.0007) and subjects >65 years of age (p=0.007). Neither concomitant Metformin use nor race affected HbA1c response to SC.



Conclusions: SC reduces AGEs and inflammation in patients with DKD, and improves HbA1c in women and older patients. Our finding of different metabolic response among diabetic women and older patients with DKD to lowering of AGEs and OS/Infl deserves further investigation.

Funding: Pharmaceutical Company Support - Sanofi-Genzyme

FR-PO794

Alterations in a Panel of Serum Biomarkers Precede the Development of Microalbuminuria (MA) in Patients with Diabetes Mellitus Type II

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Background: The aim of the study was to investigate if specific serum biomarkers indicative of vascular inflammation and endothelial dysfunction can predict the development of microalbuminuria in patients with diabetes mellitus type II.

Methods: Data from the ROADMAP study were used. 65 patients developed microalbuminuria after first sampling. A control group of 86 patients was generated by matching for age, sex, BMI, HbA1c, duration of diabetes, baseline urinary albumin creatinine ratio (UACR), baseline GFR, systolic blood pressure and LDL. The proinflammatory biomarkers sTNFR-I, sTNFR-II, ST2 (IL-33 receptor), VAP-1 (vascular adhesion protein-1), CXCL-16, S100A8 (calgranulin A) and Galectin-3, the anti-inflammatory mediators C1qR1 (receptor of C1q) Thrombomodulin, the endothelial stress marker Copeptin and the collagen degradation product Endostatin, were compared in baseline samples of cases and controls. Results of the univariate analysis are presented here.

Results: sTNFR1, S100A8, VAP-1, CXCL-16, Galectin-3 and Endostatin did not differ between future MA patients and controls. sTNFR-II and soluble ST2 were significantly increased in versus 1569±66 patients with future microalbuminuria (3183±145, versus 2858±90 pg/ml; p =0.047 and 14450±800 versus 12464±568 pg/ml; p=0.039). C1qR was significantly reduced in patients with future microalbuminuria (34±2.1 versus 28±2.0 ng/ml; p=0.047). Thrombomodulin and Copeptin were significantly increased in patients with future MA (4415±161 versus 4067±96 pg/ml;p=0.054 and 15±1,1 versus 11±0.48 pg/ml; p=0.003). Especially the results for copeptin were highly significant.

Conclusions: The alterations of serum inflammatory and endothelial stress markers prior to the development of microalbuminuria in patients with diabetes type II, adds to the evidence supporting its significance as a biomarker reflecting vascular inflammation and early vascular damage.

FR-PO795

A Predictive Model for All-Cause Mortality among Older Adults with Chronic Kidney Disease

Ronit Katz,[1] Nisha Bansal,[1] Ian H. de Boer,[1] Carmen A. Peralta,[2] David Siscovick,[1] Dena E. Rifkin,[3] Tamara Harris,[7] Stephen Kritchevsky,[5] Mark J. Sarnak,[4] Michael Shlipak,[2] Joachim H. Ix.[3] *[1]U Washington; [2]UCSF; [3]UCSD; [4]Tufts; [5]WFMC; [6]NIA.*

Background: Chronic kidney disease (CKD) is associated with increased mortality. Accurate prediction tools for mortality prior to ESRD may guide clinical decision making, particularly among elderly persons with CKD.

Methods: We developed a prediction equation for 5-year risk of death among participants with CKD in the Cardiovascular Health Study. Twenty-two candidate predictor variables were explored which included demographics, physical examination measures, comorbidity, medication use, kidney function (estimated glomerular filtration rate [eGFR] calculated from serum creatinine and the CKD-EPI equation; urine albumin to creatinine ratio [ACR]) and novel biomarkers (C-reactive protein, fibroblast growth factor-23, and cystatin C). Models were developed using Cox regression and evaluated using C statistics and net reclassification improvement (NRI). A final parsimonious model was externally validated in an independent cohort of community-living elders, the Health Aging, and Body Composition Study.

Results: The development cohort included 828 participants who had a mean age of 80 (±5.6) years, eGFR of 47 (±11) ml/min/1.73 m^2 and median ACR was 13 [IQR 6,51] mg/g. The best model for 5-year risk of death included age, gender, race, eGFR, urine ACR, systolic blood pressure, smoking, diabetes mellitus, heart failure, and stroke (C-statistic 0.73 p<0.0001) (**Table**). Addition of weight change, self-reported health or novel biomarkers did not significantly improve NRI. The model performed well in external validation (C-statistic 0.69).

Conclusions: A simple prediction tool using 10 readily available clinical variables can assist in predicting 5-year risk of death in elderly CKD patients, which may be useful in counseling patients and clinical decision making.

Table. Final multivariable model for 5 year risk of mortality for participants with CKD in CHS		
	Estimated β	HR (95% CI)
Age (per 5 years older)	0.427	1.53 (1.38, 1.70)
Male	0.275	1.32 (1.04, 1.68)
Black	-0.313	0.73 (0.52, 1.03)
eGFR-Cr (per SD= 11 lower)	0.104	1.11 (0.99, 1.25)
UACR (per doubling)	0.143	1.15 (1.09, 1.22)
SBP		
<110		1.00
110 – 139	-0.177	0.84 (0.55, 1.27)
≥ 140	-0.271	0.76 (0.50, 1.17)
Diabetes	0.303	1.35 (1.02, 1.80)
Current smoker	0.515	1.67 (1.08, 2.59)
Prevalent CHF	0.881	2.41 (1.86, 3.14)
Prevalent Stroke	0.297	1.35 (0.96, 1.88)
CHS equation: 5-year mortality risk prediction equation: $1-0.8637529^{\exp(\beta \cdot x - 5.4387275)}$ where β is the regression coefficient and x is the level for each risk factor.		

FR-PO796

Modelling Progression of Chronic Kidney Disease in the Irish Health System Using State Transition Probabilities Austin G. Stack,[1,2] Mohamed Elsayed,[1,2] Waleed Mohammed,[1,2] Ailish Hannigan,[2] John P. Ferguson.[2] *[1]Nephrology, Univ Hospital Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Limerick, Ireland.*

Background: Knowledge of transition probabilities from one stage of Chronic Kidney Disease (CKD) to another may be useful in deciding how to treat and monitor patients with CKD. We determined 1-year transition probabilities for patients with CKD in the Irish health system.

Methods: Demographic and laboratory data were provided from a regional laboratory database and merged with a patient information system between 2005 and 2011 (n=174,786). CKD stage for each patient was defined by median eGFR for each calendar year. Estimated glomerular filtration rates (eGFR) were determined using the CKD-EPI equations from standardised serum creatinine values, excluding episodes of acute kidney injury (AKI). Transition probabilities between CKD stages were defined as the number of patients classified as stage i in the previous year, and stage (i+1) in the current year, divided by total number of patients classified as stage i in the previous year and remained in the health system in the current year. Discrete multistate models determined transition rates across stages by demographic group and AKI presence.

Results: The multistate model found that age, gender and occurrence of AKI influenced progression to a worse stage of CKD. While the probability of transitioning from Stage 2 to Stage 3 and Stage 3 to Stage 4 CKD increased with age, the transition probability from Stage 4 to Stage 5 showed the converse

	Odds Ratios (95% Confidence Intervals) for CKD Progression in the Irish Health System				
CKD Stage Transition	# in Start Stage	# Transitions	Women (vs men)	Age (per 5 yrs)	AKI (vs no AKI)
1->2	91, 569	15, 618	1.00 (0.97-1.04)	1.35 (1.34-1.36)	1.36 (1.21-1.51)
2->3	109, 087	8, 318	1.14 (1.09-1.19)	1.41 (1.40-1.43)	2.65 (2.140-2.91)
3->4	32, 257	1,249	0.99 (0.88-1.11)	1.19 (1.15-1.23)	2.66 (2.30-3.07)
4->5	2, 733	195	0.65 (0.48-0.88)	0.88 (0.84-0.92)	1.75 (1.29 (2.37)

Conclusions: State transition models that model progression of CKD have the potential to identify patient groups with the greatest risk of disease progression and for whom intensive monitoring and treatment is required.

Funding: Government Support - Non-U.S.

FR-PO797

Advanced Chronic Kidney Disease Is a Strong Risk Factor for *Clostridium difficile* Infection Sun Chul Kim, Hyojeong Chang, Myung-Gyu Kim, Sang-Kyung Jo, Won-Yong Cho. *Div of Nephrology, Dept of Internal Medicine, Korea Univ Anam Hospital.*

Background: It has been suggested that chronic kidney disease (CKD) is a risk factor for *Clostridium difficile* infection (CDI) and is associated with increased mortality among patients infected with *C. difficile*. However, recent studies about the clinical impact of CKD on CDI in Asian countries are still controversial.

Methods: This was a single-center, retrospective case-control study. A total of 171 patients with CDI were included as cases and 342 age- and sex-matched patients without CDI were used as controls. We compared the prevalence of CKD in the study sample and identified independent risk factors that could predict the development and prognosis of CDI.

Results: Patients with CDI had a higher prevalence of ICU admission, CKD, use of any antibiotics, and gastric acid reducing drug use. The prevalence of ESRD requiring dialysis and pre-dialysis CKD stage 4, 5 was significantly higher among CDI patients than among patients without CDI. Patients with CDI had a significantly longer length of hospital stay and increased in-hospital mortality than patients without CDI. In a binary logistic regression model to identify the risk factors associated with CDI, predialysis stage 4, 5 CKD (OR, 2.87; $p = 0.039$), ESRD requiring dialysis (OR, 4.00; $p = 0.001$), ICU admission (OR, 2.54; $p < 0.001$), and use of any antibiotics (OR, 4.31; $p < 0.001$) were shown to be independent risk factors for CDI. The previous use of 2 or more antibiotics (OR, 4.48; $p = 0.007$) or vancomycin (OR, 5.02; $p = 0.060$), the presence of PMC (OR, 4.34; $p = 0.003$), and more advanced CKD (OR, 3.76; $p = 0.013$) were independent risk factors for treatment resistance to initial metronidazole therapy.

Conclusions: Advanced CKD is an independent risk factor for CDI and associated with higher in-hospital mortality and poor treatment response in CDI patients. Therefore in CKD patients, careful attention should be paid to the occurrence of CDI and its management in order to improve the outcome of CDI.

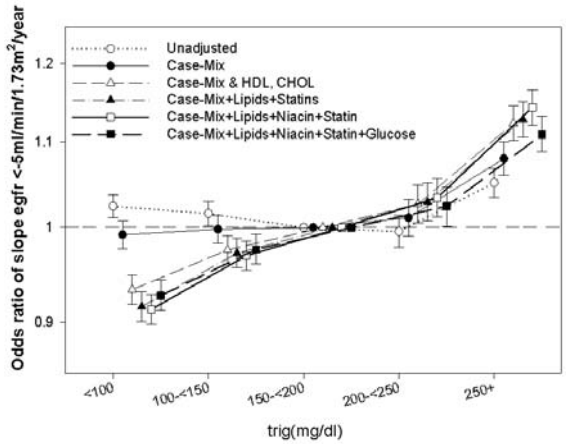
FR-PO798

Association of Baseline Triglyceride Levels with Progression of Chronic Kidney Disease in over 2 Million U.S. Veterans Elani Streja,[1] Kamyar Kalantar-Zadeh,[1] Hamid Moradi,[1] Miklos Zsolt Molnar,[2] Jun Ling Lu,[2] Csaba P. Kovesdy.[2] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Memphis VAMC, Memphis, TN.*

Background: It has been suggested that elevated lipoproteins can contribute to deterioration of kidney function. The association of serum triglyceride levels with development of chronic kidney disease (CKD) is unclear.

Methods: In a cohort of 2.6 million U.S. veterans with normal baseline eGFR in 2005-2006 we examined the association of baseline triglyceride with slopes of eGFR over median follow up of 8.0 years (6.8, 8.4). Triglyceride (mg/dL) levels were categorized into five groups (<100, 100-<150, 150-<200, 200-<250, 250+). Associations were examined in crude and adjusted logistic regression models (for slopes <-5ml/min/1.73m^2/year), with adjustments for demographics, comorbidities including diabetes, HDL, total cholesterol, glucose and use of niacin and statins.

Results: Patients were 61±13 years old, 6% female, 16% African-American, and 26% diabetic with a mean baseline eGFR 83±13 mL/min/1.73m^2. In the total cohort, 8.9% of patients had a slope <-5 mL/min/1.73m^2/year. Higher triglycerides were linearly associated with rapid decline of kidney function independent of confounders.



Conclusions: Baseline triglyceride levels are linearly associated with faster progression of CKD. Studies evaluating the effect of treatment of hypertriglyceridemia on progression of CKD are warranted.

Funding: NIDDK Support

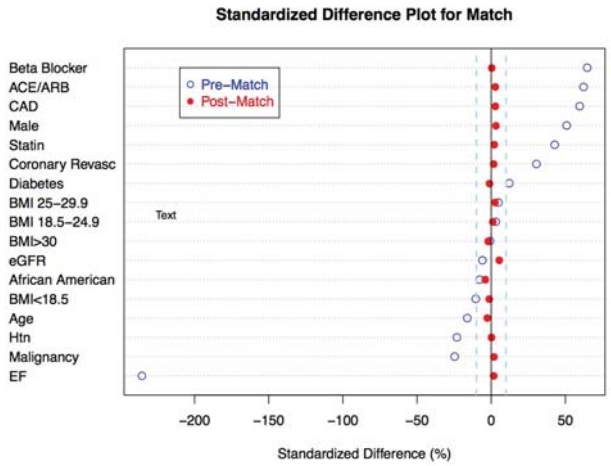
FR-PO799

Implantable Cardioverter-Defibrillators (ICD) and Mortality in Patients with Chronic Kidney Disease (CKD) Georges Nakhoul, Jesse D. Schold, Susana Arrigain, Serge Harb, Joseph V. Nally, Sankar D. Navaneethan. *Hypertension and Nephrology, Glickman Urological and Kidney Inst, Cleveland, OH.*

Background: CKD is associated with increased cardiovascular mortality, particularly from sudden cardiac death (SCD). The benefit of ICDs in prevention of SCD among the general population is proven. However, major clinical trials excluded patients with advanced CKD and thus the benefit of ICD remains unclear in this population.

Methods: We conducted a propensity-matched analysis using our pre-existing Electronic Health Record (EHR)-based CKD registry. We developed a propensity score of the likelihood of having an ICD with the following variables: age, gender, race, hypertension, diabetes, eGFR, ejection fraction, coronary artery disease, revascularization, BMI, malignancy, ACE/ARB, statin, and beta blocker. We used one-to-one greedy matching with 0.1 caliper width to match patients with ICD to those without. We used a Kaplan-Meier curve and Cox proportional hazards model to examine the survival of matched patients with and without ICD.

Results: 1,770 patients with ICD and 9,477 potential controls (CKD patients without ICD) had ECHO data.



We were able to match 983/1770 (56%) ICD patients to the control group with 1015 (51.6%) patients having eGFR of 45-59 ml/min/1.73 m^2 and 951 (48.4%) with eGFR < 45 ml/min/1.73 m^2. Kaplan-Meier survival estimates at 2 years were 80% (95% CI: 77, 82) for ICD and 69% (95% CI: 66, 72) for the control group. After adjusting for covariates

including albumin and hemoglobin, the hazards of mortality among propensity matched patients for the ICD group compared with the non ICD group was 0.78 (0.68, 0.90). There was no difference in the risk for death based on stage of kidney disease.

Conclusions: ICD therapy is associated with a survival benefit in patients with non-dialysis dependent CKD, similar to the reports in general population.

Funding: Pharmaceutical Company Support - Development of CCF CKD registry was supported by an unrestricted educational grant to the Department of Nephrology and Hypertension from Amgen

FR-PO800

Acidemia Is Associated with Decreased Physical Capacity and Activity Levels in Patients with Chronic Kidney Disease

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Background: Metabolic acidosis is often a feature of chronic kidney disease (CKD) and is integral to the pathogenesis of skeletal muscle wasting and protein catabolism, which is associated with increased morbidity and mortality. Exercise has been suggested as a way of mitigating skeletal muscle wasting but there is little data on the effects of acidosis on physical capacity and activity levels in CKD.

Methods: 916 patients with CKD not on dialysis completed Duke Activity Status Index (DASI) and Leisure Time Exercise Questionnaire (LTEQ) to assess functional capacity and activity levels respectively. Responses were correlated with haematological and biochemical data and co-morbid diseases. 49 patients were found to have venous bicarbonate <21mmol/L, and a group of 49 patients matched for age and eGFR with venous bicarbonate >21mmol/L were selected without knowing DASI or LTEQ scores.

Results: Groups were well matched for age, sex, haemoglobin, albumin, eGFR and medical co-morbidity, with no statistically significant differences between groups. Mean DASI for the group bicarb<21 was 26.4 versus 35.2 for the group with bicarb>21 (p=0.006) and median LTEQ for the group bicarb<21 was 3 versus 18 for the group with bicarb>21 (p<0.001). Using Spearman's correlation LTEQ versus bicarb: rho=0.312 (p=0.002) and DASI versus bicarb: rho=0.209 (p=0.039).

Conclusions: Our results suggest that patients with CKD and acidaemia have significantly decreased activity and functional capacity compared to patients with normal acid base balance. Whilst correction of acidemia with enteral sodium bicarbonate may improve muscle protein wasting and inflammation, exercise training is also a potentially beneficial, underused, intervention. More research is needed to define the effects of acidosis on muscle function, the effects of exercise on acidosis, and how correcting acidosis may improve exercise tolerance and quality of life.

Funding: Private Foundation Support

FR-PO801

Prevalence, Predictors and Outcomes of Pulmonary Hypertension in Chronic Kidney Disease: A Report from the Chronic Renal Insufficiency Cohort (CRIC) Study

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Background: Pulmonary hypertension (PH) is generally associated with poor outcomes; however, the impact of PH in chronic kidney disease (CKD) is unknown. We evaluated the prevalence and predictors of PH and its impact on long term clinical outcomes in non-dialysis dependent CKD.

Methods: CRIC participants who had echocardiography at year 1 of the study were studied. PH was defined as estimated pulmonary artery pressure (PAP) >35 mm Hg and/or tricuspid regurgitant velocity (TRV) >2.5 m/sec. The risk factors for PH were examined using logistic regression, and its associations with cardiovascular events (adjudicated hospitalized heart failure, myocardial infarction and stroke), renal events (50% decline in eGFR and ESRD) and death were examined using Cox-proportional hazard models. We also examined these associations by considering PAP and TRV as continuous measures.

Results: Out of 2959 patients, 21% (n=623) had PH. Old age, non-hispanic blacks, lower eGFR and higher proteinuria were associated with higher risk for PH in CKD. After covariate adjustment, presence of PH, elevated PAP and TRV were associated with an increased risk for death, cardiovascular and renal events.

	All-cause mortality * (n=497)	Cardiovascular events * (n=575)	Renal events * (n=705)
	Hazard Ratio (95% CI)		
PH present	**1.38 (1.09, 1.74)**	1.11 (0.89, 1.39)	1.13 (0.92, 1.40)
PAP (each SD increase)	1.11 (0.96, 1.28)	**1.21 (1.04, 1.39)**	1.13 (0.98, 1.31)
TRV each SD increase	**1.16 (1.02, 1.32)**	**1.21 (1.06, 1.38)**	**1.14 (1.00, 1.29)**

PH: Pulmonary hypertension; PAP- Pulmonary artery pressure; TRV- tricuspid regurgitant velocity; SD- standard deviation

* Adjusted for demographics, smoking, diabetes, hypertension, previous cardiovascular disease, COPD, BMI, albumin, hemoglobin, LDL cholesterol, use of RAS blockers, diuretics, statins, beta-blockers. LVEF, diastolic dysfunction, EGFR, and proteinuria

Conclusions: This cohort of CKD patients had a higher prevalence of PH (PAP>35 mm Hg and/or (TRV) >2.5 m/sec) than that reported in the general population. Further, PH indices were independently associated with death, cardiovascular and renal events in CKD patients.

Funding: NIDDK Support

FR-PO802

Early GFR Decline as a Predictor of Diabetic Retinopathy in Non-CKD

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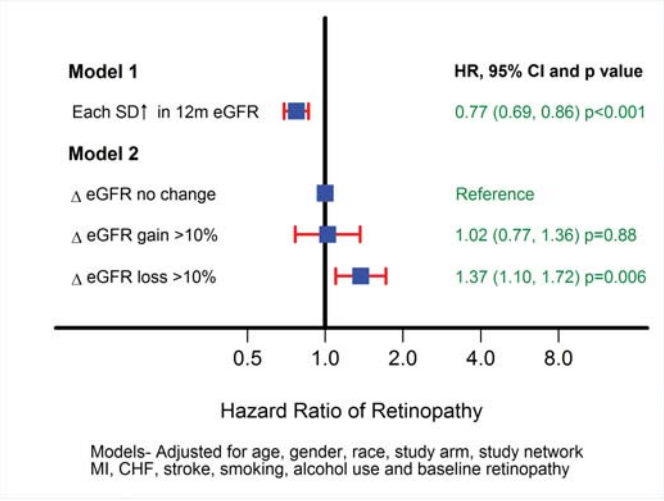
Background: Early GFR decline in the non-CKD diabetic population might reflect systemic microvascular disease and hence, predict progression of diabetic retinopathy.

Methods: We examined this hypothesis in a secondary analysis of the ACCORD study (a RCT conducted by NHLBI to examine the effects of glycemic control, BP control and fibrates on CV outcomes in type 2 DM). The cohort for this analysis consisted of those with a). baseline and 12< CKD-EPI eGFR b). 12M eGFR ≥ 60ml/min1.73m^2. Based on the baseline and 12M eGFR, DeGFR groups (no change: -10% to +10%, >10% loss: DeGFR <-10% and >10% gain: DeGFR>+10%) were defined. Separate Cox regression models were used to relate DeGFR gropus and 12M eGFR with subsequent incident retinopathy (laser Rx or vitrectomy) events.

Results: 7574 diabetics without CKD at 12M were included. The mean age was 62.8±6.2 yrs, 62.6% were men, 61.9% were Caucasian. The mean ± SD 12M eGFR was 86.3±14.2ml/min/1.73m^2. The mean DeGFR in the no change, DeGFR loss and DeGFR gain groups were -1.2±3.8, -17.7±7.5, 15.7±7.8ml/min/1.73m^2, respectively. There were 396 retinopathy events over 27495 patient-yrs of follow-up.



Each SD ↑ in 12M eGFR was strongly associated with lower hazard of retinopathy events (Fig). Compared to the group DeGFR no change group, DeGFR loss >10% group had higher hazard of retinopathy (Fig). DeGFR and 12M eGFR were strongly correlated (r=0.53, p<0.001) and when both were included in the same model, neither had significant association with subsequent retinopathy.

Conclusions: Both current eGFR and decline in eGFR in the past 12 m predict subsequent retinopathy in diabetic non-CKD population. Early decline in eGFR in the non-CKD diabetic patients might be a marker of systemic microvascular disease.

Funding: NIDDK Support

FR-PO803

Illness Perceptions, Medication Beliefs and Adherence in CKD Patients with Comorbid HIV Ioannis Konstantinidis, Jeffrey Weiss, Christina M. Wyatt. *Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: CKD is often accompanied by complex comorbidities that impose a significant medical and adherence burden. Patients often prioritize such conditions over CKD, but data on adherence in this setting are limited. We aimed to assess perceptions about CKD and HIV and to investigate the relationship between illness perceptions, medication beliefs, and medication adherence in patients with both CKD and HIV.

Methods: Inclusion criteria were CKD on medications to delay CKD progression or manage complications, HIV-positive on antiretroviral therapy, and planning to continue current therapy for ≥3 months. Patients on hepatitis C therapy were excluded. Participants reported adherence for each prescribed medication with a Visual Analog Scale and completed the Illness Perception Questionnaire-Revised and the Beliefs About Medicines Questionnaire for both CKD and HIV.

Results: Among 32 eligible participants, mean age was 53±8 yrs, 53% were male, and 66% were non-Hispanic black. Mean education was 12±2 yrs. Participants were prescribed a mean of 11±2 medications. Most were CKD Stage 3-5 (88%), all had undetectable HIV viral load, and mean CD4 cell count was 603±244. Patients were diagnosed with HIV (16.4 yrs) and treated for HIV (12.3 yrs) significantly longer than for CKD (5.7 and 4.2 yrs, respectively) [all p <0.001]. 56% considered CKD their primary health concern compared to 22% for HIV. Greater than 95% adherence was reported by 78%, 72% and 66% of patients for CKD, HIV, and both CKD and HIV medications, respectively. There was a trend for patients to experience more personal control over HIV than CKD (p=0.06), have more concerns about HIV medications (p=0.07), and view them as more necessary than for those for CKD (p=0.06).

Conclusions: In these patients with well-controlled HIV, CKD is a dominant health concern. Despite a perception of less personal control over CKD, self-reported adherence to CKD and HIV medications was similar. Ongoing data collection will evaluate adherence to one CKD and one HIV medication with Medication Event Monitoring System and pill counts to better elucidate the relationship between illness perceptions, medication beliefs, and adherence.

Funding: Private Foundation Support

FR-PO804

Endothelial Ischaemia Reperfusion Injury Is Not Increased in Chronic Kidney Disease Kristin Vibeke Veighey,[1,2] Nichola Hawkins,[2] David C. Wheeler,[1] Raymond Macallister.[2] *[1]Centre for Nephrology, Univ College London, London, United Kingdom; [2]Centre for Clinical Pharmacology and Therapeutics, Univ College London, London, United Kingdom.*

Background: Arterial endothelial function in humans declines with advancing CKD. We have previously demonstrated a reduction in brachial artery flow mediated dilatation (FMD) following experimental IR injury in healthy volunteers. We therefore postulated that FMD would decrease and IR injury would increase with declining GFR in CKD patients.

Methods: 47 healthy volunteers (mean age 36±5) and 11 CKD patients (mean age 38±11) underwent FMD measurements before and after IR injury, induced by 20min upper arm cuff inflation to 200mmHg followed by 20min cuff deflation. Patients had CKD 4-5 but had not commenced RRT (mean eGFR 17±8), and had no known cardiovascular disease/ diabetes. All vasoactive medications (e.g beta blockers, ACE/ARBs, nitrates, nicorandil) were withheld for at least 24 hours prior to the study.

Results: There was no significant difference between mean baseline FMD in the healthy cohort compared with the CKD cohort (11.27±1.2 versus 9.75±0.8, p=0.33). There was a statistically significant correlation between decreasing eGFR and decreasing FMD (R^2=0.39 p=0.04). In healthy volunteers, there was a statistically significant decrease in FMD following IR injury (9.80±5.36 versus 5.35±0.60, p<0.0001). In CKD patients however, there was no difference (11.27±1.20 versus 10.62±1.83, p=0.64). The mean % reduction in FMD in CKD patients was significantly less than in the healthy cohort (5.23±15.91% versus 31.54±5.48%, p=0.04). Matching age, sex and BP made no difference to this observation (CKD - 11.04±3.39 versus 10.43±5.01 p=0.76, healthy - 10.30±5.19 versus 7.07±4.46 p=0.003, n=7). Cholesterol, glucose, calcium and phosphate were not significantly different between the two groups.

Conclusions: Although a reduced GFR is associated with decreased FMD, there was no corresponding increase in IR injury. This may be attributable to the effects of vasoactive medications which may protect against endothelial injury in these patients. This may also account for the failure to translate animal IR studies to patients.

FR-PO805

Placental Growth Factor as a Predictor of Cardiovascular Events in Patients with Chronic Kidney Disease from the Novel Assessment of Risk Management for Atherosclerotic Diseases in Chronic Kidney Disease (NARA-CKD) Study Masaru Matsui, Shiro Uemura, Yukiji Takeda, Katsuhiko Morimoto, Ken-Ichi Samejima, Yasuhiro Akai, Yoshihiko Saito. *First Dept of Internal Medicine, Nara Medical Univ, Kashihara, Japan.*

Background: Chronic kidney disease (CKD), of which prevalence is increasing in aged society, is a strong risk for cardiovascular events, but the risk stratification has not been well established. Placental growth factor (PlGF) plays a critical role in atherogenesis through vascular inflammation and destabilization of plaques. Whether circulating levels of PlGF are associated with mortality and cardiovascular disease in CKD is unknown.

Methods: A prospective cohort study of 1351 consecutive participants with chronic kidney disease who were enrolled in the Novel Assessment of Risk management for Atherosclerotic diseases in Chronic Kidney Disease (NARA-CKD) study between April 1, 2004 and December 31, 2011, aimed to determine the impact of PlGF on all-cause mortality and cardiovascular events in patients with CKD.

Results: During a median follow-up of 3 years, 199 participants died and 383 had cardiovascular events, defined as atherosclerotic disease or heart failure requiring hospitalization. In the adjusted analyses, mortality and cardiovascular risk were higher in each successive quartile of PlGF; hazard ratios (HRs) [95% confidence intervals (95% CIs)] were 1.59 [0.83–3.16] and 1.55 [0.92–2.66] for the second quartile, 2.97 [1.67–5.59] and 3.39 [2.20–5.41] for the third quartile, and 3.87 [2.24–7.08] and 8.42 [5.54–13.3] for the fourth quartile, respectively. The composite endpoint of mortality and cardiovascular events occurred during the study period in 76.4% of patients in the highest PlGF quartile (≥ 19.6 pg/mL) and the lowest eGFR tertile (< 30 mL/min/1.73m²). The association between PlGF and both mortality and cardiovascular events was not attenuated when participants were stratified by age, sex, traditional risk factors, and eGFR.

Conclusions: Elevated PlGF is an independent risk factor for all-cause mortality and cardiovascular events in patients with CKD.

FR-PO806

Prospective, Randomized, Controlled Trial: Comparison of Total Parathyreoidectomy without Autotransplantation and without Thymectomy versus Subtotal Parathyreoidectomy with Thymectomy for Renal Hyperparathyreoidism Sylvia Stracke,[1] Doris Henne-Bruns,[3] Peter Wuerl,[3] Frieder Keller.[2] *[1]Nephrology, Internal Medicine A, Univ Medicine Greifswald, Greifswald, Germany; [2]Nephrology, Internal Medicine 1, Univ Hospital Ulm, Ulm, Germany; [3]General Surgery, Univ Hospital Ulm, Ulm, Germany.*

Background: In patients with chronic renal failure, secondary hyperparathyroidism (sHPT) sometimes requires parathyroidectomy. Prospectively, we compared total parathyroidectomy without autotransplantation and without thymectomy (tPTX) with subtotal parathyreoidectomy with thymectomy (sPTX) for renal hyperparathyreoidism.

Methods: From 11/2004 to 11/2008, 43 consecutive patients with sHPT were prospectively randomized to receive either tPTX (22 patients) or sPTX (21 patients). Patients were followed for a median of 36 months (11-62 months). Outcome parameters were parathyroid hormone (PTH), calcium, phosphate, alkaline phosphatase (AP), vitamin D use, bone mineral density (DXA) and coronary calcification (Agatston score).

Results: tPTX reduced PTH significantly lower than sPTX (17 versus 71 pmol/L; p=0.03). 36 months (11-62) after tPTX, serum calcium was measured with 2,15±0,23 mmol/l versus 2,31±0,24 mmol/l (p=0.04) after sPTX. In the direct postoperative period, the need for active vitamin D supplementation was higher after tPTX (12,20±9,24 µg/week) compared to sPTX (6,67±6,95 µg/week). There was no difference in reduction of AP, serum phosphate, postoperative mortality, postoperative longterm dose of vitamin D, bone mineral density nor the Agatston score of the coronary arteries. Bone mineral density improved similarly in both groups. The preoperative Agatston-Score was high in both groups, but stayed unchanged throughout the whole observation period: tPTX preOP 1869±2458, postOP 2865±5865; sPTX preOP 6909±11758, postOP 6854±15414.

Conclusions: tPTX is as safe as sPTX. tPTX is more effective than sPTX in reducing PTH. Directly after PTX, serum calcium is lower after tPTX requiring a higher dose of active vitamin D. After both operations, tPTX as well as sPTX, bone mineral density increased and the otherwise expected progression of coronary calcification did not occur. Parathyroidectomy may stop vascular calcification.

FR-PO807

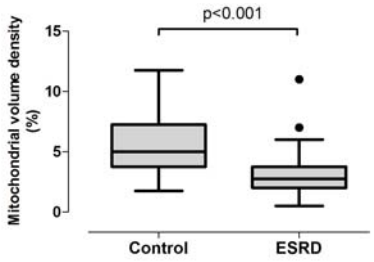
Skeletal Muscle Mitochondrial Volume Density and Morphology in Patients with End-Stage Renal Disease (ESRD) on Maintenance Hemodialysis (MHD) Jorge Gamboa, Chelsea Marie Derer, Charles D. Ellis, Edward D. Siew, T. Alp Ikizler. *Medicine, Vanderbilt Univ Medical Center.*

Background: Oxidative stress and inflammation correlate with cardiovascular events in patients with ESRD. Mitochondrial dysfunction may be the cause of oxidative stress and inflammation. Patients with ESRD on MHD exhibit mitochondrial abnormalities in peripheral blood mononuclear cells (PBMCs) and ultra-structural abnormalities in skeletal muscle. It is still unknown, however, if mitochondrial dysfunction results from a decreased number of mitochondria or from intrinsic mitochondrial impairment. Thus, we hypothesized that MHD patients will have lower mitochondrial DNA (mtDNA) copy number and lower mitochondrial volume density in skeletal muscle than healthy individuals.

Methods: We obtained PBMCs and vastus lateralis muscle biopsies from healthy individuals and patients with ESRD. mtDNA copy number was evaluated with PCR. Thin sections (~ 40) per subject were used for the calculation of mitochondrial volume density using the stereological point counting method.

Results: Patients with ESRD have lower mtDNA copy number (median 1.75, IQR 1.09-2.02) than healthy controls (2.16, IQR 1.51-3.01, p=0.041). In patients with ESRD mitochondria demonstrated signs of cristae swelling, and double-membrane structures compatible with mitophagy. Lipofuscin granules were also abundant in MHD patients, indicating oxidative damage in mitochondria and lysosomes. Mitochondrial volume density was higher in healthy controls compared to patients with ESRD **[Figure 1]**.

Figure 1.



Conclusions: The implications of these findings are that muscle quality is an important factor that might influence physical functioning in MHD patients. Further studies are required to evaluate mitochondrial function and the correlation between morphological and functional changes in patients with ESRD, especially in response to interventions such as exercise.

Funding: NIDDK Support

FR-PO808

An Increased Ultrasonographic Resistive Index Is a Risk Factor for Hypoalbuminemia in Chronic Kidney Disease, Independently of Proteinuria and Renal Function Ryuta Fujimura, Kaori Takaori, Yoko Shima, Chisako Nakano, Masafumi Yamato, Akira Wada, Takahito Ito. *Nephrology, Osaka National Hospital, Osaka, Osaka, Japan.*

Background: Lower serum albumin concentration (ALB) is a risk factor for cardiovascular disease and worsening kidney function. Inflammation, malnutrition, proteinuria, and decreased glomerular filtration rate (GFR) are associated with hypoalbuminemia in chronic kidney disease (CKD) patients. However, the role of kidney damage in hypoalbuminemia remains unknown. We studied an association of CKD and ALB by ultrasonography.

Methods: We had 199 consecutive patients who underwent a Doppler-ultrasonography from January 1th, 2013 to December 31th, 2013. We analyzed 110 patients (61.4±15.7 year-old, 83 males) after 89 patients had been excluded because of polycystic kidney disease, hepatic virus infection, single kidney, or insufficient data of blood chemistry.

Results: ALB (3.90 ± 0.73 g/dl) correlated with eGFR (43.3±23.8 ml/min/1.73m^2, r=0.248, p=0.0089), log[urinary protein (UP)] (-0.50 ± 1.83 g/gCr, r=-0.665, p<0.0001), serum cholesterol concentration (CHO) (201.9 ± 52.5 mg/dl, r=-0.295, p=0.0017), and body mass index (BMI) (23.43±4.29, r=0.275,p=0.0037), but not with age or C-reactive protein. The average of the right and left renal resistive indexes (RI) (0.706±0.079) correlated with ALB (r=-0.345, p=0.0002), Log[UP] (r=0.269, p=0.0044), eGFR (r=-0.470, p<0.0001), age (0.506, p<0.0001), and CHO (r= -0.207, p=0.0298). Age correlated with eGFR (r=-0.422, p<0.0001). On multivariate analysis with ALB as the dependent variable, we found that an increased RI was associated with hypoalbuminemia, independently of UP and GFR.

Model	R2	Dependent Variable	Standard Beta	95%CI
1	0.457	mRI	-0.176	-0.309 - -0.134
		eGFR	0.008	-0.004 - 0.005
		log[urinary protein]	-0.615	-0.302 - -0.185
2	0.487	mRI	-0.237	-3.537 - -0.816
		cholesterol	-0.189	-0.004 - 0.000
		log[urinary protein]	-0.547	-0.277 - -0.157
3	0.488	mRI	-0.208	-3.364 - -0.461
		eGFR	0.086	-0.002 - 0.007
		cholesterol	-0.218	-0.005 - 0.000
		log[urinary protein]	-0.525	-0.270 - -0.145

Conclusions: An increased ultrasonography RI accounts for hypoalbuminemia in CKD. RI might be a practical marker for renal mishandling of amino acid recycling.

Funding: Private Foundation Support

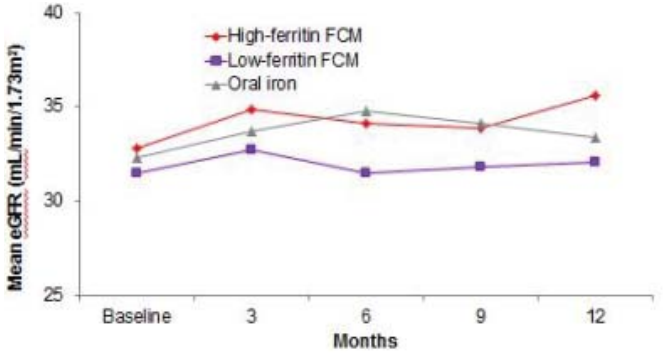
FR-PO809

Iron Administration to Non-Dialysis CKD Patients for One Year Does Not Cause Worsening of Renal Function: Results From the FIND-CKD Trial Iain C. Macdougall,[1] Andreas H. Bock,[2] Fernando Carrera,[3] Kai-Uwe Eckardt,[4] Carlo A. Gaillard,[5] David B. Van Wyck,[6] Bernard Roubert,[7] Jacqueline G. Nolen,[7] Simon D. Roger.[8] *[1]King's College Hospital, UK; [2]Kantonsspital, Switzerland; [3]Eurodial, Portugal; [4]Erlangen-Nuremberg, Germany; [5]Groningen, Netherlands; [6]Davita, US; [7]Vifor Pharma, Switzerland; [8]Gosford, Australia.*

Background: Laboratory data suggest that intravenous (IV) iron may exacerbate oxidative stress and renal tubular toxicity but robust scientific evidence that this translates into worsening renal function in non-dialysis CKD (ND-CKD) patients is lacking. One of the secondary endpoints in the FIND-CKD study was renal function measured by eGFR and this allowed further elucidation of this controversy.

Methods: 626 ND-CKD patients with iron deficiency anaemia (Hb 9–11g/dl; ferritin<100µg/l or <200µg/l and TSAT<20%; eGFR≤60ml/min were randomized to 1 of 3 strategies of iron administration (IV ferric carboxymaltose [FCM] aiming for a ferritin of 400–600µg/l, versus IV FCM aiming for a ferritin of 100–200µg/l, versus oral ferrous sulphate 200mg iron daily). Renal function (eGFR, MDRD-4) was measured every 3 months for 1 year.

Results: Over the 1-year study patients received a mean total dose of 2685 and 1040mg in the high-ferritin FCM and low-ferritin FCM groups, respectively. Mean eGFR (ml/min/1.73m^2) are shown for all 3 groups.



Conclusions: This study is the longest and largest to compare oral versus IV iron in ND-CKD patients. The results demonstrate that the administration of IV FCM in doses that maintain ferritin levels of 100-200 or 400–600µg/l over 1 year do not negatively impact renal function as measured by eGFR (MDRD-4). While this study did not directly measure true GFR, assess proteinuria or tubular damage markers, it nevertheless provides reassuring data on the absence of any overt renal toxicity following IV iron administration in ND-CKD patients over the course of 1 year.

Funding: Pharmaceutical Company Support - Vifor Pharma

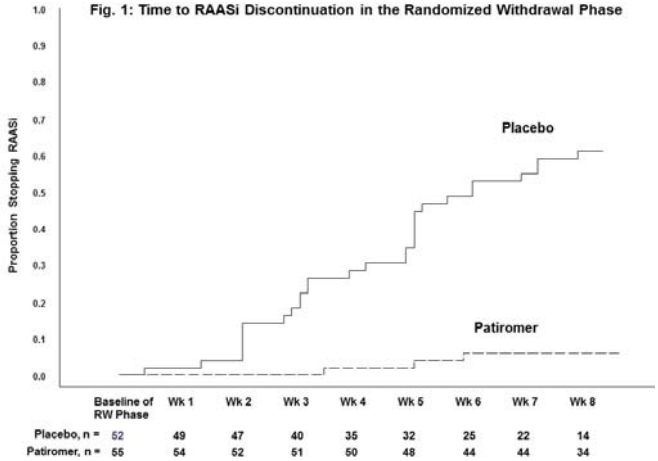
FR-PO810

Patiromer Reduced RAASi Dose Discontinuations in CKD Patients with Moderate-to-Severe Hyperkalemia Matthew R. Weir,[1] George L. Bakris,[2] David A. Bushinsky,[3] Bertram Pitt,[4] Martha Mayo,[5] Dahlia Garza,[5] Yuri Stasiv,[5] Janet Wittes,[6] Heidi Christ-Schmidt,[6] Lance Berman.[5] *[1]Univ of Maryland, Baltimore, MD; [2]Univ of Chicago, Chicago, IL; [3]Univ of Rochester, Rochester, NY; [4]Univ of Michigan, Ann Arbor, MI; [5]Relypsa, Redwood City, CA; [6]Statistics Collaborative, Washington DC.*

Background: RAAS inhibitors (RAASi) have cardiorenal benefits in patients (pts) with CKD, yet hyperkalemia (HK) often limits RAASi therapy. Patiromer, a nonabsorbed, metal-free polymer with high K^+-binding capacity, controlled serum K^+ (s-K^+) and decreased recurrent HK versus placebo (PBO) with good GI tolerability in a two-part Phase 3 trial in CKD pts with HK on RAASi. We present additional endpoints from the PBO-controlled Randomized Withdrawal Phase.

Methods: Pts with s-K^+ 5.1 to <6.5 mEq/L received patiromer (4.2 or 8.4 g BID to start) in a 4-wk Initial Treatment Phase; pts with BL s-K^+ 5.5 to <6.5 mEq/L were eligible to continue into an 8-wk PBO-controlled Randomized Withdrawal Phase. Additional endpoints included % of pts requiring an intervention for recurrent HK (RAASi dose ↓/discontinuation [PBO]; patiromer dose ↑/RAASi discontinuation[patiromer]) and time to RAASi discontinuation.

Results: Overall (n=237) after having controlled s-K^+ in the Treatment Phase (BL mean s-K^+ = 5.58 ± 0.03 mEq/L, D from BL = -1.01 ± 0.03 mEq/L; p<0.001), 55 pts were randomized to continue patiromer and 52 were switched to PBO in the 2nd phase. As a result of recurrent HK (s-K^+ ≥ 5.5 mEq/L; PBO = 60%, patiromer = 15%; p< 0.001) more PBO (62%) than patiromer (16%) pts required an intervention to manage s-K^+. By Wk 8, significantly (p<0.001) more patiromer (94%) than PBO (39%) pts were still on RAASi therapy. Figure 1 shows time to RAASi discontinuation.



Conclusions: After controlling their s-K^+ in this Phase 3 study, significantly fewer CKD pts with moderate-to-severe HK on patiromer had recurrent HK and more stayed on RAASi therapies versus PBO.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

FR-PO811

Identification of Potential New Actors Associated with Chronic Kidney Disease Complications Using Plasma Proteome Analysis

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Background: Systemic complications including cardiovascular events and infections are the major cause of death in patients with chronic kidney disease (CKD). The exact mechanisms are not fully understood. In the present study, we investigated the plasma proteome changes in CKD patients in order to identify new mechanisms and factors that could be associated with CKD-related complications.

Methods: Using LC-MS/MS for discovery and ELISA for validation, we analysed the plasma proteome of patients with stage 2-3 CKD (n=14) and stage 5 CKD with hemodialysis (HD) (n=16). A validation cohort of 40 patients with different CKD stages with or without HD was also assessed.

Results: We identified 2054 proteins, of which 333 were significantly modified during progression of CKD, mainly involved in altered hemostasis and acute phase response. We identified and validated complement factor D (Cfd), lysozyme C (Lyz) and leucine-rich alpha-2-glycoprotein (Lrg1) as new potential actors of CKD-associated complications, in addition to alterations of known factors associated with the development of vascular damage and inflammation in CKD such as beta-2-microglobulin and prostaglandin-H2 D-isomerase.

Conclusions: Using plasma proteome analysis, we have identified Cfd, Lyz and Lrg1 as new potential actors in the complications of CKD. This will help to understand the mechanisms of these complications, and monitoring of plasma levels of these potential targets in CKD patients might help to prevent the occurrence of systemic complications.

FR-PO812

Association of Variants in Genes Belonging to the Angiogenesis/Wound Repair Pathway with Lower Extremity Amputation: Findings from the CRIC Study

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Background: Diabetes is the major risk factor for non-traumatic lower extremity amputation (LEA). The role of genetic polymorphisms in predisposing diabetics to impaired wound healing leading to amputation has not been sufficiently explored. We investigated the association of variants in a set of genes belonging to the angiogenesis/wound repair pathway with LEA in the Chronic Renal Insufficiency Cohort (CRIC).

Methods: This study was conducted on 3772 CRIC subjects who were genotyped on the ITMAT-Broad-CARe array (IBC) chip. A total of 1017 SNPs in 22 genes belonging to the angiogenesis/would repair pathway were investigated. LEA was determined from patient self-report. The association between genetic variants and LEA status was examined using logistic regression under additive genetic models as implemented in the software PLINK. Separate analyses were performed for non-Hispanic white, non-Hispanic Black and Hispanic subjects. Further stratification was based on diabetic status. Unadjusted analyses as well as analyses adjusted for age, sex, eGFR, BMI, peripheral vascular disease, HbA1C and population stratification were conducted.

Results: In non-Hispanic white subjects with diabetes, rs11938826, an intronic SNP in the gene Basic Fibroblast Growth factor (*FGF2*),was significantly associated with LEA in covariate-adjusted analysis {OR: 2.83 (95% Confidence Intervals: 1.73, 4.62); p-value: 0.000034; Bonferroni adjusted p-value: 0.02}. In the same sub-group, another *FGF2* intronic SNP, rs1960669, had a similar effect estimate and a p-value of 0.00095, but did not withstand the Bonferroni adjustment. No other significant association could be identified in the other ethnic groups.

Conclusions: Variant/s in *FGF2* can predispose diabetics with chronic kidney disease to LEA. Dysregulation of the *FGF2* gene represents an opportunity to understand further, and possibly intervene upon, mechanisms of wound healing in diabetics with chronic kidney disease.

Funding: NIDDK Support

FR-PO813

Acupuncture Therapy for Symptoms of Hemodialysis Patients

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Background: Hemodialysis (HD) patients have various symptoms related to uremia, and some of them are intractable. The purpose of this study is to examine the effectiveness and safety of acupuncture for symptom management of HD patients.

Methods: Subjects were 45 patients who undergoing HD regularly, 15 males and 30 females, with a mean age of 64±9.8 years, and a mean dialysis history of 11±9.7 years. Eleven patients were complicated with diabetes mellitus (DM). The objected symptoms in this study are 1) pain caused by various reasons including uremic polyneuropathy, 2) itching, 3) muscle stiffness, 4) hypesthesia, 5) fatigue and 6) sleeplessness. Acupuncture therapies were operated at bedside by the acupuncturist during HD sessions, and at home by the self-treatment of patients under directions of the acupuncturist. Needles with guide tube (0.16×40 mm) were used for treatments by the acupuncturist, and press tack needles to stick on skin (0.2×0.6 mm) for both treatments. The number of treatments was different in each case, ranging from 4 to 43. Each symptom was evaluated before and after treatment by a visual analogue scale (VAS) and a faces pain scale (FS).

Results: Comprehensively, the acupuncture improved the VAS score significantly from 66.1±25.1 pre-treatment to 41.6±26.0 post-treatment ($P<0.05$). The improvement of itches was the most effective symptom, which VAS scale was decreased from 48.1±27.9 to 24.0±27.0 ($P<0.05$). The acupuncture therapies also significantly improved pain (VAS scale from 72.2±21.2 to 41.6±26.0), muscle stiffness (from 64.3±28.3 to 43.2±23.5) and hypesthesia (from 67.1±19.1 to 45.9±21.2), while no clear effects on fatigue and sleeplessness were observed. The effect for itching was remarkable in DM patients, while that for pain was observed in both DM and non-DM patients. The FS results improved in 17 patients, remained the same in 20, and were worse in 8. No severe complications including hemorrhage were observed.

Conclusions: Acupuncture is an effective and safe treatment for symptom managements of HD patients. Refractory itches, pains and muscle stiffness are promising therapeutic targets for acupuncture in HD patients.

Funding: Government Support - Non-U.S.

FR-PO814

Cardiac Functions Improve after Kidney Transplantation in Children with Chronic Kidney Disease

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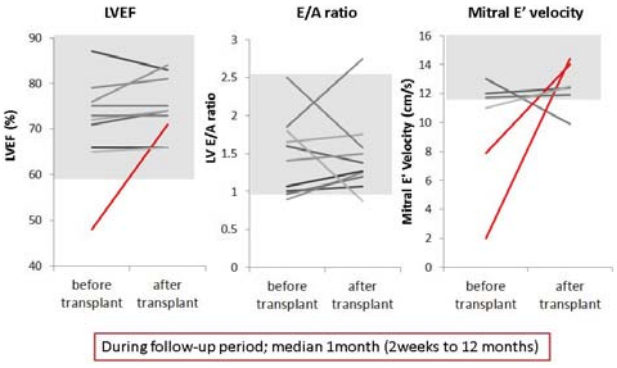
Background: Current studies indicate that cardiac function deteriorates during chronic kidney disease (CKD). Although kidney transplantation (KTx) has improved survival in children with CKD, the prevalence of cardiac dysfunction after KTx has been unknown.

Methods: This was a retrospective cohort study using clinical data from patients with CKD who underwent KTx or had scheduled the operation. Cardiac functions (left ventricular ejection fraction (LVEF), left ventricular E/A ratio, mitral E' velocity) were evaluated in patients with CKD by echocardiography comparing to age-matched healthy controls, and correlation between estimated GFR (eGFR) and echocardiographic parameters was assessed. The patients were then grouped as receiving or not receiving dialysis, and cardiac functions were analyzed. Additionally, cardiac function was evaluated before and after KTx in patients for whom follow-up echocardiographic data were available.

Results: Thirty three patients (20 boys) were included. Median age at measurement of basic cardiac function in our hospital was 10 years. E/A ratio and E' velocity in patients with CKD were significantly lower than in controls ($p<0.05$). E' velocity showed linear correlation with eGFR ($r=0.37$, $p<0.05$). Twenty patients (60%) underwent peritoneal dialysis (PD). E/A ratio in patients with PD was significantly lower than in those without PD ($p<0.05$). KTx was performed in 28 patients and 10 patients received follow-up echocardiography. Their cardiac functions improved after KTx.

Figure 1, Cardiac functions after kidney transplantation



Conclusions: This study indicated left ventricular diastolic dysfunction was prevalent in children with CKD; however, these echocardiographic abnormities may be reversible after KTx.

FR-PO815

Signaling Involved with Cbfa1 in VSMC Calcification Induced by High Phosphate

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Background: To observe the possible molecular signaling that regulates Cbfa1 on high phosphate induced vascular smooth muscle cells (VSMC) calcification.

Methods: Passage 3 to 5 of VSMC were used for experiments. VSMC were divided into two groups: normal phosphate group (Pi 1.3 mmol/L) and high phosphate group (Pi 2.6 mmol/L). Calcium deposition was visualized by Alizarin stain method at day 7. Cbfa1 and OPN mRNA levels were determined by Real-Time PCR. p-AKt (ser473), Cbfa1 and OPN protein expressions were quantified by Western Blot. Then, VSMC were treated with AKt inhibitor, Wortmannin. VSMC were divided into four groups: high phosphate group (Pi 2.6 mmol/L); high phosphate+ Wortmannin (10 nmol/L, 50 nmol/L and 100 nmol/L). After 24h, Cbfa1 and OPN mRNA levels were determined, and p-AKt (ser473), Cbfa1 and OPN protein expressions were quantified.

Results: After 7 days, compared with normal phosphate group, calcium deposition was obvious in high phosphate group;Cbfa1 and OPN mRNA expressions were significantly increased and the expressions of p-AKt, Cbfa1 and OPN protein were significantly increased in high phosphate group (P<0.05). After treated with Wortmannin 24h, compared with high phosphate group,calcium deposition was not decreased in high phosphate+ Wortmannin (10nmol/L) group. But calcium deposition was significantly decreased in high phosphate+ Wortmannin (50nmol/L, 100nmol/L) groups. Compared with high phosphate group, Cbfa1 and OPN mRNA expressions were not decreased in high phosphate+ Wortmannin (10nmol/L) group, but Cbfa1 and OPN mRNA expressions were significantly decreased in high phosphate+ Wortmannin (50nmol/L, 100nmol/L) groups (P<0.05). Compared with high phosphate group, p-AKt (ser473), Cbfa1 and OPN protein expressions were not decreased in high phosphate+ Wortmannin (10nmol/L) group, but p-AKt (ser473), Cbfa1 and OPN protein expressions were significantly decreased in high phosphate+ Wortmannin (50nmol/L, 100nmol/L) groups (P<0.05).

Conclusions: AKt inhibitor, Wortmannin may dose-dependently inhibit VSMC calcification and expressions of p-AKt (ser473), Cbfa1 and OPN. AKt is involved in VSMC calcification induced by high phosphate and AKt may work by activating Cbfa1.

Funding: Government Support - Non-U.S.

FR-PO816

Treatment of the CKD-MBD with a Ligand Trap for the Activin Receptor Type 2A

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Background: The CKD-MBD consists of vascular calcification, an osteodystrophy, and stimulation of skeletal osteocyte FGF23 secretion at its inception, and hyperphosphatemia, develops later in the course to further stimulate vascular calcification. Here we demonstrate that inhibition of activin, a member of the TGFb superfamily signaling through the activin type 2A receptor (ActR2A), induced by CKD inhibits vascular calcification and prevents cardiac hypertrophy.

Methods: CKD with hyperphosphatemia and 60% reduction in GFR (CKD-3) was induced at 14 weeks of age in our *ldlr-/-* high fat fed model vascular calcification. Some CKD mice were treated with RAP-011 (an ActR2A ligand trap), injected IP weekly beginning at 22 weeks of age and studied at 28 weeks. Aortic Ca levels, expression of osteoblastic and vascular smooth muscle proteins, skeletal histomorphometry and microCT imaging, serum chemistries and FGF23 and PTH levels were measured. Activin, Follistatin and Inhibin levels were measured by elisa, RT-PCR and westerns.

Results: Activin levels were increased in the vasculature and the circulation without changes in follistatin. CKD stimulated vascular calcification which was reduced below 22 week levels by. RAP-011 treatment, and cardiac hypertrophy was prevented. CKD induced expression of aortic Runx2, osterix, and alkaline phosphatase message and protein, and these were reversed by RAP-011 treatment. CKD reduced smooth muscle 22a (sm22a) message and protein, and RAP-011 treatment normalized sm22a expression. Bone volume was increased by RAP-011, and osteoclast pit surface was decreased, but bone formation rates and osteoblast surfaces were not affected. Hyperphosphatemia, and FGF23 levels were not changed by RAP-011.

Conclusions: Treatment with an activin receptor type 2A (ActR2A) ligand trap, RAP-011, inhibited bone resorption and increased bone volume. RAP-011 inhibited Smad dependent signaling, blocked aortic osteoblastic transition, increased vascular smooth muscle protein levels and decreased CKD stimulated vascular calcification and cardiac hypertrophy.

Funding: NIDDK Support, Pharmaceutical Company Support - Celgene

FR-PO817

Effect of Valvular Calcifications on Echocardiography on Cardiovascular Outcomes in Hemodialysis Patients: A Meta-Analysis of Prospective Studies

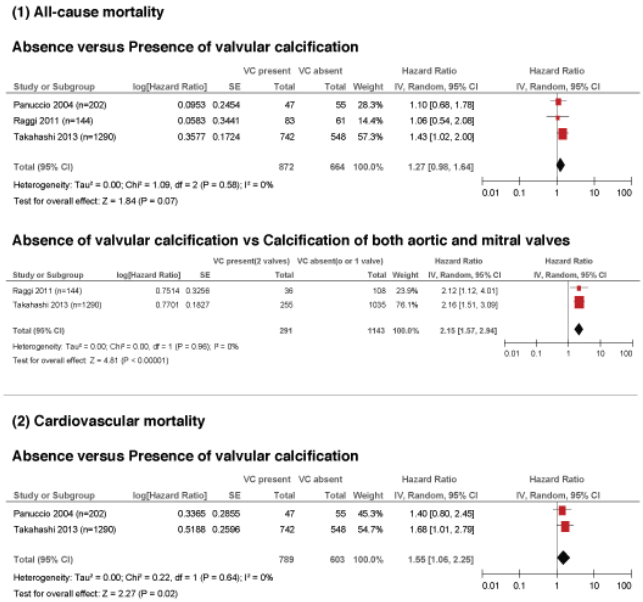
Ioannis Konstantinidis,[1] Achint Patel,[1] Spyridon N. Papageorgiou,[2] Alexandre Benjo,[3] Shiv Kumar Agarwal,[4] Narender Annapureddy,[5] Girish N. Nadkarni,[1] Joseph A. Vassalotti.[1] *[1]Icahn School of Medicine at Mount Sinai; [2]Univ of Bonn; [3]Ochsner Clinic; [4]Univ of Arkansas; [5]Rush Univ.*

Background: Valvular calcification (VC) is associated with all-cause/cardiovascular (CV) mortality in hemodialysis (HD) patients. The 2009 KDIGO guidelines suggest but do not recommend echocardiography (echo) to detect VC. We assessed the impact of VC identified by echo in HD patients, including studies after the KDIGO guidelines.

Methods: We performed a systematic review/meta-analysis of prospective studies that examined the relationship between VC on echo and all-cause/CV mortality in HD patients. MEDLINE, EMBASE, CENTRAL, CINAHL, Web of Knowledge, LILACS, and ClinicalTrials were searched until May 2014. Two authors performed study selection and data extraction. Hazard ratios (HR) were estimated with random-effects model using RevMan 5.2.

Results: Of 6,711 initially identified studies, three (n=1,636) were included; all assessed VC of the aortic and mitral valve. For all-cause mortality, three reported adjusted HRs (n=1,636) for absent versus present VC; pooled HR was 1.27 (95% CI, 0.98-1.64). Two studies reported adjusted HR (n=1,434) for absent VC versus VC presence at both aortic and mitral valve; pooled HR was 2.15 (95% CI, 1.57-2.94). For CV mortality, 2 studies reported adjusted HRs (n=1,492) for absent versus present VC; pooled HR was 1.55 (95% CI, 1.06-2.25). Range of follow-up was 44-67 mos. There was no significant heterogeneity between studies for either all-cause or CV mortality.

Figure 1: Forest plots for impact of valvular calcification on all-cause and cardiovascular mortality



Conclusions: VC of both aortic and mitral valves but not individually is associated with significantly higher all-cause and cardiovascular mortality. VC identified by echo could be used to identify high risk patients for aggressive modification of risk factors.

FR-PO818

Effect of Vascular Calcifications on Plain Radiography on Outcomes in Hemodialysis Patients: A Meta-Analysis of Prospective Studies

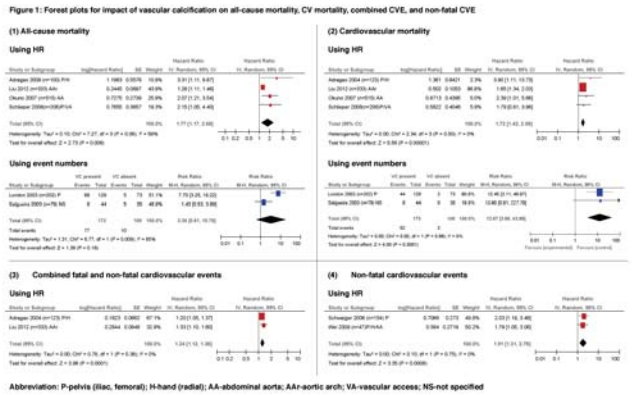
Ioannis Konstantinidis,[1] Achint Patel,[1] Spyridon N. Papageorgiou,[2] Alexandre Benjo,[3] Madhav C. Menon,[1] Rabi Yacoub,[1] Girish N. Nadkarni,[1] Joseph A. Vassalotti.[1] *[1]Icahn School of Medicine at Mount Sinai; [2]Univ of Bonn; [3]Ochsner Clinic.*

Background: Vascular calcification (VC) is associated with all-cause/cardiovascular (CV) mortality in hemodialysis (HD) patients. The KDIGO 2009 guidelines suggest but not recommend abdominal radiograph for VC based on low quality evidence. We assessed the impact of VC on plain radiography (XR) in HD patients.

Methods: This systematic review/meta-analysis of prospective studies examined the relationship between VC on XR and all-cause/CV mortality and cardiovascular events (CVE) in HD patients. MEDLINE, EMBASE, CENTRAL, CINAHL, Web of Knowledge, LILACS, and ClinicalTrials were searched until May 2014. Two authors performed study selection and data extraction. We used RevMan 5.2 to estimate hazard ratios (HR) and risk ratios (RR) with a random-effects model.

Results: Of 6,711 studies, nine (n=1,759) were included. For all-cause mortality, four reported HRs (n=1,154) and two reported events (n=281) with mean f/u 44/18 mo; pooled HR and RR were 1.77 and 3.38 (not significant). For CV mortality, four reported HRs

(n=1,177) and two reported events (n=281) with mean f/u 46/18 mo; pooled HR and RR were 1.72 and 12.67. For combined fatal/non-fatal CVE, 2 studies reported HRs (n=201; mean f/u 45 mo); pooled HR was 1.91. For non-fatal CVE, 2 studies reported HRs (n=456; mean f/u 41 mo); pooled HR was 1.24. Significant heterogeneity existed for all-cause mortality but not CV mortality/CVE.



Conclusions: VC is associated with higher all-cause/CV mortality and CVE in HD patients. VC on XR could be used to identify high risk patients for aggressive CV disease detection/management. Interpretation of pooled estimates for all-cause mortality requires caution due to heterogeneity. Assessment of interventions' impact on VC is planned.

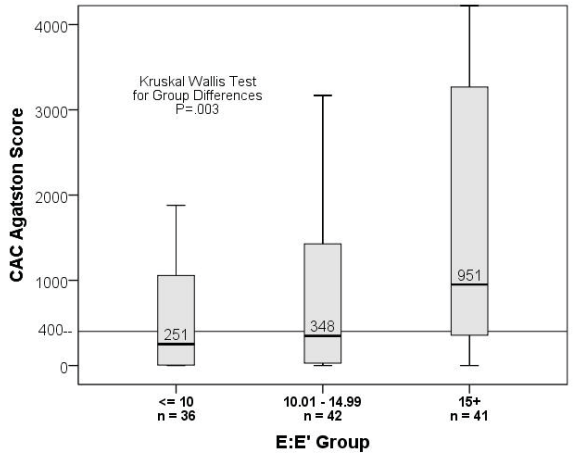
FR-PO819

Global Cardiac Function Is Negatively Affected by Coronary Artery Calcification in CKD-5D Patients Paul Anaya,[1,2] Gustav A. Blomquist,[3] Daniel Davenport,[4] Vincent L. Sorrell,[1,2] Marie-Claude M. Faugere,[5] Hartmut H. Malluche.[5] *[1]Cardiovascular Medicine, Univ of Kentucky, Lexington, KY; [2]Internal Medicine, Univ of Kentucky, Lexington, KY; [3]Radiology, Univ of Kentucky, Lexington, KY; [4]Surgery, Univ of Kentucky, Lexington, KY; [5]Nephrology, Univ of Kentucky, Lexington, KY.*

Background: Coronary artery calcification (CAC) is common in CKD-5D patients (pts). Its impact on cardiac function is not known. This study evaluates cardiac functional implications of CAC in CKD-5D.

Methods: Pts were from 37 Kentucky dialysis centers. Blood was analyzed for traditional and novel bone biomarkers. 2-D and Doppler echocardiography (ECHO) was performed and CAC was measured using 64-slice MSCT for Agatston scores (AgS). Relationships between CAC, bone markers, and ECHO results of left ventricular (LV) function were analyzed.

Results: There were138 pts: 58.6% men, 46% Caucasian. Median age 55.5 yrs (range 21-84), dialysis 49.5 mos (range 3-292). AgS > 100 in > 70% of pts with AgS > 400 in > 50%. There were relationships between CAC and LV diastolic dysfunction (DiaDf) measured by E/E' (r=0.33, p<0.01), speckle-derived global longitudinal strain (GLS) (r=0.29, p=0.03), and LV systolic velocity (r= -0.22, p=0.01). Pts with the most severe CAC had E/E' values indicating severe DiaDf. (Fig.) Multivariate regression identified CAC as an independent predictor of E/E' (r= 0.34, p< 0.001). Valvular calcification (ValvCalc), based on a visual scoring scale, varied linearly with CAC, E/E', and GLS (p<0.05), and inversely with LV systolic velocity (p<0.05). Only FGF-23 correlated linearly with GLS.



Conclusions: CAC adversely impacts LV systolic and DiaDf in CKD-5D. CAC correlates strongly with ValvCalc, suggesting that (a) the pathologic mechanism leading to CAC may also affect heart valves (b) ValvCalc identified on routine ECHO may be a marker of CAC.

Funding: NIDDK Support, Private Foundation Support

FR-PO820

A Decreased Level of Serum Solubleklotho Is a Biomarker Associated with Vascular Calcification in Peritoneal Dialysis Patients Qiuling Fan. *Dept of Nephrology, China Medical Univ, Shenyang, Liaoning, China.*

Background: Low serum Klotho levels are related to the prevalence of cardiovascular diseases in community-dwelling adults. However, it is unclear whether the serum Klotho levels are associated with vascular calcification in peritoneal dialysis patients.

Methods: We determined the levels of serum solubleKlotho in 58 continuous ambulatory peritoneal dialysis (CAPD) patients using ELISA and investigated the relationship between the level of Klotho and markers of CKD-mineral and bone disorder (CKD-MBD) and the abdominal aortic calcification score (AAC), a marker of vascular calcification.

Results: Fifty CAPD patients (86.2%) had abdominal aortic calcification. The serum Klotho level significantly correlated with the 25-hydroxyvitamin D level and inversely correlated with AAC, serum phosphorus and FGF23, not correlated with the parathyroid hormone, bone-specific alkaline phosphatase (BAP), and serum calcium levels. There were significant decreases in serum Klotho in patients with abdominal aortic calcification of ACI>8. Multivariate Logistic regression analysis showed that lower serum soluble Klotho level and FGF23 were independent risk factor for abdominal aortic calcification. ROC-AUC of serum soluble Klotho for abdominal aortic calcification was 0.81 (cut-off 153.78 pg/mL, accuracy 87.5 %, specificity58.0%).

Conclusions: Decreases in the serum solubleKlotho levels are independently associated with vascular calcification in peritoneal dialysis patients. Further research exploring whether therapeutic approaches to maintain or elevate the Klotho level could improve vascular calcification in peritoneal dialysis patients is warranted.

FR-PO821

Skin Autofluorescence - A Novel Risk Marker or Risk Factor for Aortic Valve Calcification in Chronic Kidney Disease? Angela Yee Moon Wang,[1] Ck Wong,[2] Yat Y. Yau,[3] Iris Chan,[4] Christopher W. Lam.[5] *[1]Medicine, Queen Mary Hospital, Univ of Hong Kong, Hong Kong, Hong Kong; [2]Chemical Pathology, Chinese Univ of Hong Kong, Prince of Wales Hospital, Hong Kong, Hong Kong; [3]Pathology, United Christian Hospital, Hong Kong, Hong Kong; [4]Radiology, Biomedical Imaging Center, Hong Kong, Hong Kong; [5]Macau Inst for Applied Research in Medicine and Health, Macau Univ of Science and Technology, Macau, Macau.*

Background: Experimental data suggest advanced glycation endproduct (AGE) may induce vascular smooth muscle cells calcification. We hypothesized that skin autofluorescence (AF), a proxy of tissue AGE, may be associated with aortic valve calcification (AVC) in chronic kidney disease (CKD).

Methods: 300 stages 3–5 non-dialysis CKD patients (age:60±10yrs, 56%men) underwent plain multislice computed tomography to estimate aortic valve calcium score (AVCS) and had skin AF assessed using AGE Reader.

Results: Intact parathyroid hormone (iPTH) (P<0.001) displaced estimated GFR as the third most significant factor associated with skin AF after age and diabetes in multiple regression analysis. Using multinomial logistic regression, skin AF retained significance in predicting AVCS≥400 [OR, 8.80, 95% CI, 1.87–41.3, P=0.006] when adjusting for age, gender, serum calcium, phosphate, eGFR, albumin, inflammation, lipids and blood pressure. Its significance was well retained when further adjusting for iPTH [P=0.01], fasting glucose [P=0.01], glycosylated hemoglobin [p=0.01] and diabetes [OR, 11.60, P=0.02] in stepwise multinomial regression. Notably, combination of diabetes and high iPTH was associated with the highest skin AF and AVCS. Using receiver-operator characteristics curve analysis, skin AF showed highest predictive value for AVCS [area under the curve, 0.72, 95% CI, 0.65–0.80; p<0.001] compared to other biochemical parameters of mineral bone disease (MBD).

Conclusions: Skin AF showed an independent novel relationship with AVCS in CKD beyond inflammation and other biochemical parameters of CKD-MBD. These data suggest skin AF is useful in identifying CKD patients with significant AVC. Tissue AGE may represent a novel culprit linking CKD to an increased risk of heart valve calcification that warrant further investigation.

Funding: Pharmaceutical Company Support - Unrestricted grant from Sanofi

FR-PO822

Indoxyl Sulfate Contributes to Vascular Calcification in Rats with Chronic Renal Failure Ellen Neven,[1] Rida Bashir-Dar,[1] Rita L.M. Marynissen,[1] Bjorn Meijers,[2] Pieter Evenepoel,[2] Patrick C. D'Haese.[1] *[1]Pathophysiology, Univ of Antwerp, Antwerp, Belgium; [2]Nephrology, Univ Hospitals Leuven, Belgium.*

Background: Chronic renal failure (CRF) patients develop extensive arterial calcifications, having a significant impact on mortality. Besides a disturbed mineral metabolism, protein bound uremic retention solutes, particularly indoxyl sulfate (IS) and p-cresyl sulfate (PCS), have also been associated with cardiovascular disease and mortality in these patients. To clarify the potential causal role of IS and PCS in the development of vascular calcification, the impact of both uremic toxins on arterial calcification was studied in CRF rats.

Methods: To induce CRF, all study groups were daily gavaged with 600 mg/kg adenine sulfate for 10 days and received a 1.2% P diet for 7 weeks.Two groups of CRF rats were exposed to 200 mg/kg IS (high IS) or PCS (high PCS) via the drinking water for 1 week from the start of CRF induction onwards and continued exposure to 150 mg/kg IS

or PCS from week 3 to7. Two other CRF groups were exposed to 150 mg/kg IS (low IS) or PCS (low PCS) after adenine administration was stopped, from week 3 to 7. Control CRF animals received vehicle. Calcification in the aorta, carotid and femoral arteries was measured by histomorphometric analysis on Von Kossa stained sections and by determining the calcium content of the vessels.

Results: Adenine administration led to the induction of CRF in all study groups, as evidenced by a significant decrease of the creatinine clearance, and hyperphosphatemia. No additional effect of toxin exposure on renal dysfunction was found. The calcium content of the aorta and the carotid artery was significantly increased in the CRF group exposed to the high IS dose as compared to vehicle treated CRF animals. No effect of either low or high PCS and low IS on the development of vascular calcification was observed.

Conclusions: The current experimental study shows that the uremic toxin IS accelerates the development of arterial calcification in chronic kidney disease. The potential effect of this toxin on phenotypic changes of vascular smooth muscle cells in the vessels will be further explored.

FR-PO823

Can Established Vascular Calcification Be Reversed by Pyrophosphate or Sevelamer in an Animal Model of Chronic Renal Failure? Rida Bashir-Dar,[1] Geert J. Behets,[1] Bruce L. Riser,[2,3] Patrick C. D'Haese,[1] Ellen Neven.[1] *[1]Biomedical Sciences, Univ of Antwerp, Wilrijk, Belgium; [2]Physiology and Biophysics, Rosalind Franklin Univ of Medicine and Science, Chicago, IL; [3]Medicine, Chicago Medical School, Chicago, IL.*

Background: Vascular calcification (VC) is commonly observed in CKD patients and is thought to contribute significantly to the cardiovascular morbidity and mortality in this population. In previous experimental studies, both Sevelamer (Sev) and pyrophosphate (PPi) have proven to be effective in preventing VC in chronic renal failure (CRF), the former by controlling phosphate absorption, the latter by directly interfering with the hydroxyl apatite crystal formation. However, since most patients present with established VC, it is of utmost importance to evaluate whether these compounds may also reverse established VC.

Methods: CRF was induced in all rats by subjecting them to a protein restricted high adenine diet for 4 weeks. Treatment (Tx) with PPi (30 or 120 µmol/kg/day via peritoneal infusion), Sev (1500 mg/kg/day via gavage) or vehicle was then started for 3 weeks. An additional CRF control group was sacrificed before initiation of Tx. VC was assessed by in vivo µ-CT before and after Tx, and by total calcium measurement and Von Kossa staining of the aorta at sacrifice. Static bone parameters were measured on Goldner stained slides.

Results: Hyperphoshatemia and VC had developed in all animals at the end of adenine administration (i.e prior to Tx). Whilst serum calcium was decreased in all groups inherent to CRF, the Sev group showed relative hypercalcemia. Neither doses of PPi or Sev were able to significantly reverse established VC. While PPi treatment induced no effects on static bone parameters, an increased amount of osteoid was seen in the Sev group, likely due to a relative phosphate depletion inherent to the potent phosphate binding of the compound.

Conclusions: Whilst previous experimental studies demonstrated that both PPi and Sev are able to prevent the initial development of VC, under the conditions tested, neither compound reversed established medial VC.

Funding: Pharmaceutical Company Support - Baxter Healthcare

FR-PO824

Alternative Model of Chronic Renal Failure Allowing Concomitant Evaluation of Vascular Calcification and Bone Pathology Rida Bashir-Dar, Geert Dams, Anja Verhulst, Geert J. Behets, Patrick C. D'Haese, Ellen Neven. *Pathophysiology, Univ of Antwerp, Antwerp, Belgium.*

Background: The 0.75% adenine/low protein rat model is commonly used to study chronic renal failure (CFR)-related vascular calcification (VC). However, as it shows consistent but excessive VC and chaotic and immeasurable bone mineralization due to excessive bone turnover, it is less suited to study the bone-vascular axis in one and the same animal. Moreover, with the conventional model partial restoration of the CRF after stop of adenine administration complicates evaluation of progression/reversal of established VC in intervention studies. Since VC and bone mineralization are related to each other, an animal model in which both pathologies can be studied concomitantly would be an added value.

Methods: Rats were maintained on either a low adenine/ low vitK diet to induce CRF and VC or a control diet without adenine. To follow the onset and development of VC and the evolution of bone pathology over time, rats were killed at week 4, 8, 10, 11 and 12. Static and dynamic bone parameters were measured after double tetracycline labeling. VC was evaluated by Von Kossa (VK) staining and measurement of the arterial Ca content.

Results: A severe CRF as indicated by an 8-fold increase in serum creatinine levels, hyperphosphatemia (± 18 mg/dl) and hypocalcemia was achieved after 4 weeks which remained stable until week 12. Mortality was negligible. Aortic calcification as assessed by total Ca measurement was seen in all animals from week 4 onwards, whilst Ca levels in carotid and femoral artery were significantly increased from week 8 onwards. VK staining revealed a similar pattern. Four and 8 weeks after CRF induction, both static and dynamic bone parameters were measurable in all animals, thereby presenting typical features of hyperparathyroid bone disease. However, from week 10 onwards, dynamic bone parameters were no longer measurable in about 50% of the animals due to chaotic tetracycline incorporation.

Conclusions: A low adenine/low vitK diet resulted in a stable CRF, moderate VC and quantifiable bone pathology after 8 weeks. This rat model is the first one that lends itself to study these two main complications in CRF concomitantly.

FR-PO825

Aortic Calcification Was Accelerated After Radical Nephrectomy: Clinical Impact of GFR Decline for Vascular Calcification Yoshinari Yasuda,[1] Akitaka Suzuki,[2] Ryohei Hattori,[2] Yasuhito Funahashi,[1] Shoichi Maruyama,[1] Seiichi Matsuo.[1] *[1]CKD Initiatives/Nephrology/Urology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan; [2]Urology, Japan Red Cross Nagoya Daiichi Hosp, Nagoya, Japan.*

Background: Chronic kidney disease (CKD) is one of serious risk factors for cardiovascular diseases (CVD) and ectopic calcification in aorta plays significant role in high mortality among CKD patients. However, chicken or egg problem remains unsolved. In this study, aortic calcifications before and after surgical GFR loss were analyzed among patients who underwent radical or partial nephrectomy, whose comorbidities associated with vascular calcification was comparable except GFR.

Methods: Study subjects were 102 and 61 consecutive patients who underwent radical (R) and partial (P) nephrectomy from 2004 to 2008 in Nagoya University and affiliated hospitals, and were followed-up more than 5 years without recurrences. As control (C), 94 consecutive gastric cancer patients underwent gastrectomy in 2008 were analyzed. Aortic calcification index (ACI) was evaluated using pre- and 5-year postoperative CT scan images, and DACI (post -pre ACI) was calculated.

Results: Patients characteristics, age, gender, HTN, DM, dyslipidemia, previous CVD, eGFR and ACI, before operation were comparable among 3 groups. Postoperative eGFR was the lowest in R and the highest in C group. DACI was significantly greater in R compared to P and C groups, and trend of higher DACI in P was shown compared to C group.

	radical nephrectomy	partial nephrectomy	gastrectomy
preoperative eGFR (ml/min/1.73m^2)	74.1±15.4	76.7±17.5	70.8±12.9
postoperative eGFR (ml/min/1.73m^2)	50.5±11.8	66.6±16.7	70.4±12.9
preoperative ACI	9.16±8.63	8.54±10.8	8.42±7.29
postoperative ACI	16.7±13.7	11.3±12.1	10.8±8.56
ΔACI	7.49±6.38	2.75±2.82	2.34±2.38

In multivariate analysis, 6-month postoperative eGFR ($p<0.001$), preoperative ACI ($p<0.001$), previous CVD ($p=0.020$) and dyslipidemia ($p=0.037$) were significantly associated with DACI.

Conclusions: Aortic calcification was accelerated after radical nephrectomy, which strongly suggested direct link between GFR loss and ectopic calcification.

Funding: Government Support - Non-U.S.

FR-PO826

Wnt/β-Catenin Signaling Promotes Transdifferentiation and Calcification of Vascular Smooth Muscle Cells in a High-Phosphate Environment Danqin Sun, Ping Wen, Lei Jiang, Chunsun Dai, Junwei Yang, Weichun He. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

Background: Vascular calcification is extremely prevalent in patients with chronic kidney disease and contributes to increased risk of cardiovascular events and mortality. Osteoblastic differentiation of vascular smooth muscle cells (VSMCs) is involved in the pathogenesis of vascular calcification. Wnt/β-Catenin signaling plays a crucial role in osteogenesis and resent studies implicate it in vascular calcification.

Methods: Here, we sought to determine the role of Wnt/β-Catenin signaling in VSMCs transdifferentiation and calcification induced by high-phosphate.

Results: High-phosphate stimulated β-Catenin activation in VSMCs, along with Runx2 induction and calcium deposition. Activation of β-Catenin, through either Wnt3a or ectopic expression of stabilized β-Catenin, induced Runx2 expression in VSMCs. Upregulation of β-Catenin activity by Wnt3a also promoted calcification of VSMCs, whereas blockade of Wnt/β-Catenin signaling by Dickkopf-1 inhibited calcium deposition induced by high-phosphate. In VSMCs, a functional T cell factor/lymphoid enhancer-binding factor-binding site was identified in the promoter region of the Runx2 gene, which interacted with T cell factor upon β-Catenin activation. In rat with uremic vascular calcification, β-Catenin was dramatically accumulated in the cytoplasm and nuclei of VSMCs of aortic tunica media, meanwhile, the amount of activated β-Catenin in arterial wall was increased, accompany with Runx2 induction, followed by arterial medial calcification.

Conclusions: Collectively, these studies suggested that through direct downstream target Runx2, the activation of Wnt/β-Catenin signaling induced by high-phosphate could play a role in promoting osteoblastic differentiation and calcification of VSMCs.

Funding: Government Support - Non-U.S.

FR-PO827

MicroRNAS 29b, 133b and 211 Regulate Vascular Calcification Mediated By High Phosphorus Sara Panizo,[1] Manuel Naves,[1] Natalia Carrillo-Lopez,[1] Jose L. Fernandez-Martin,[1] Maria P. Ruiz-Torres,[2] Jorge B. Cannata-Andia,[1] Isabel Rodriguez.[1] *[1]Bone and Mineral Research Unit, IRSIN, REDinREN from ISCIII, HUCA, Oviedo, Spain; [2]Dept of Systems Biology. REDinREN from ISCIII. Univ of Alcalá, Madrid, Spain.*

Background: Vascular calcification (VC) is associated with morbidity and mortality. Few recent studies have suggested that some microRNAs (miRNAs) might have a role in the transdifferentiation of vascular smooth muscle cells (VSMC) in osteoblast-like cells.

The aim of this study was to investigate *in vivo* and *in vitro*, whether several miRNAs already implicated in osteoblast differentiation and bone formation can have any regulatory role in the process of VC.

Methods: To induce VC, 7/8 nephrectomized rats were fed a high phosphorus diet, and sacrified at 12 and 20 weeks. Eight miRNAs with a known role in the osteogenic differenciation in bone (miR-29b, miR-125, miR-133b, miR-135, miR-141, miR-200a, miR-204, and miR-211) were analyzed in the aortas.

Results: Decreased levels of miR-133b and miR-211 correlated with increased RUNX2 expression, as well as increased levels of miR-29b correlated with decreased expression of several inhibitors of osteoblastic differentiation (ACVR2A, CTNNBIP1 and HDAC4). The same trend of results were observed in two *in vitro* models (VSMC cultured with uremic serum and with high calcium and phosphorus media). The results obtained with the transfection of VSMCs with antagomir to miR-29b to inhibit its expression, or with pre-miR-133b or pre-miR-211 to increase the expression of these miRNAs, confirm their role in VSMC calcification.

Conclusions: In conclusion, calcifying stimuli promoted by high phosphorus downregulated miR-133b and miR-211 and upregulate miR-29b modifying the expression of several factors involved in the phenotypic modulation and osteogenic differentiation of VSMCs. The results suggest the three miRNAs studied may play a regulatory role in the process of VC.

Funding: Government Support - Non-U.S.

FR-PO828

A New Role for Lamin A in the Process of Vascular Calcification

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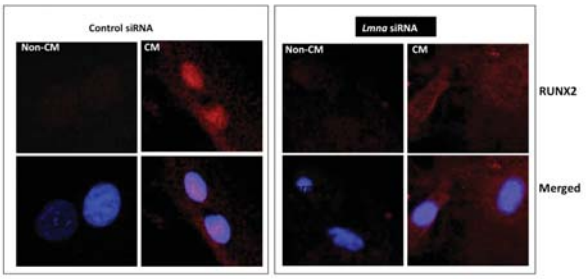
Background: Hyperphosphatemia, one of the main components of the CKD-MBD, and defects in the proccesing of Lamin A have been associated with vascular calcification (VC) and accelerated ageing. Thus, the aim of this "*in vivo and in vitro*" study was to investigate the role of Lamin A in the VC promoted by phosphate load.

Methods: A rat model of CKD-MBD was used. *In vivo* VC and protein expression were analized by von Kossa and 2D-DIGE, respectively. Primary rat vascular smooth muscle cells (VSMCs) were exposed to control or calcifying media (CM). In vitro VC was determined by calcium content and alizarin red. qPCR, western blot and immunoprecipitation (IP) were performed according to standard protocols. Knockdown of Lmna was performed in A7r5 VSMC line, using siRNA. Nuclear morphology and IF were assessed using FITC secondary antibodies.

Results: Rats with VC showed a 2.4-fold increase in Lamin A. VSMCs cultured in CM showed positive alizarin red staining, significant increases in calcium content (60%), Runx2 gene expression (2.3 fold), Lamin A protein levels (1.8 fold) and nuclear deformities (43%) compared to control. Knockdown of Lamin A prevented the increase of Runx2, Ostepontin, and Osteocalcin, preventing VSMC calcification. Co-IP showed that Runx2-Lamin A interaction increased in calcifying VSMCs. IF showed that the prevention in mineralization may be due to Runx2 down-regulation and impairment in its translocation to the nucleus



Conclusions: These results suggest that in VC there is an increase of Lamin A, osteogenic markers and nuclear shape abnormalities and the Runx2 recruitment to the nucleus is associated to the increase in Lamin A. Taken together, these results suggest that the nuclear envelope biology may play a role in VC, trough facilitating the role of Runx2.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO829

Characterisation of the Vitamin D Hormonal System in Healthy and CKD Artery

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Background: Vitamin D deficiency is associated with increased cardiovascular (CV) risk in the general population and in CKD, although the precise mechanisms remain unclear. We characterised arterial expression, activity and response to VDRa of the vitamin D signaling system in human aortic smooth muscle cells (HAoSMC), and healthy and CKD artery.

Methods: Artery biopsies were obtained from patients with normal renal function (n=9) or CKD (n=9). Gene expression for the VDR, 1a-hydroxylase (CYP27B1), 24-hydroxylase (CYP24A1), osteocytic markers RUNX2 and osteocalcin, and matrix metalloproteinases (MMPs) were explored by PCR, and protein expression by Western blot and immunohistochemistry. Artery rings were cultured with the VDRa's calcitriol or paricalcitol, and calcitriol synthesis measured by EIA.

Results: VDR, CYP27B1 and CYP24A1 were expressed in HAoSMC and healthy artery, and HAoSMC synthesized calcitriol when exposed to 25-hydroxyvitamin D. In CKD arteries, basal VDR and CYP27B1 protein expression was reduced and CYP24A1 increased by immunohistochemistry. Congruently, calcitriol synthesis was lower from tissue lysates (4 versus 8 pmol/mg/h, p=0.058) and culture supernatant (13 versus 29 pmol/mg/h, p=0.004) of cultured CKD arteries. Exposure of normal or CKD artery to 10nM calcitriol resulted in increased VDR mRNA expression without altering CYP27B1. However in CKD arteries, calcitriol induced a > 1,000 fold increase in CYP24A1 expression compared to control. Incubation with calcitriol or paricalcitol increased basal VDR and CYP24A1 expression but, in CKD arteries, VDR induction was attenuated, CYP27B1 reduced, and CYP24A1 greatly increased ($p<0.01$). Further, VDRa exposure induced RUNX2, osteocalcin and MMP2/9 in CKD arteries (calcitriol > paricalcitol) but not control.

Conclusions: Human arteries express all components of the vitamin D signaling system and synthesize calcitriol. In CKD, the normal response to VDRa is perturbed, notably with marked CYP24A1 induction and increased expression of markers of osteogenic transformation.

FR-PO830

Cholesterol Sensor SCAP Mediates Phosphate Induced Vascular Calcification via Lipid Independent Pathway

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Background: Dyslipidemia is one of the risk factors contributing to vascular calcification in CKD patients. However, the metabolism by which lipids interact with mineral metabolism remains unknown. We propose that Sterol Regulatory Element Binding Protein (SREBP) Cleavage Activating Protein (SCAP), a central controller of lipid metabolism, plays a role in vascular calcification.

Methods: Calcium deposition in human primary culture of vascular smooth muscle cells (VSMCs) was analyzed by Alizarin Red S staining and quantititive assay. The expression of the related genes and proteins were analyzed by Real-time RT-PCR, Western Blotting, Confocal microscope and Flow Cytometer. Cell proliferation, migration and apoptosis were monitored by cell cycles analysis, scratch-wound and TUNEL assay.

Results: We demonstrated that phosphate stimulated the expression of SCAP in a dose dependent manner in VSMCs. We established a VSMCs calcification model in vitro, in which SCAP expression was positively associated with the expression of osteogenic marker cbfa1. Over-expression SCAP in VSMCs led to more calcium deposition, accompanying with an increased matrix vehicle (MV) secretion, up-regulation of cbfa1, BSP and ALP while VSMCs maker a-SMA was down-regulated. These effects were blocked by knocking down SCAP using siRNA. Further, SCAP induced calcification was not affected by knocking-down SREBP2, a SCAP target gene for lipid homeostasis, suggesting that the enhanced vascular calcification by SCAP is via a lipid independent pathway.

Conclusions: Our data strongly suggest that SCAP is a novel mediator of phosphate induced vascular calcification, and its pro-calcification effect is independent of its traditional regulatory function in lipid metabolism.

FR-PO831

Spironolactone Prevents the Upregulation of Pit1 and Vascular Calcification in Experimental CKD

Victor Manuel Barrientos,[1,2] Magdalena Gonzalez,[1,2] Luis F. Michea.[1,2] *[1]CEMC, U de Chile; [2]Millennium Inst on Immunology and Immunotherapy, ICBM, Facultad de Medicina, U of Chile, Chile.*

Background: Vascular calcification (VC) is a major risk factor for mortality in end stage renal disease and correlates with hyperphosphatemia. Phosphate-dependent mineralization of vascular smooth muscle cells (VSMC) depends on the upregulation of the Na-dependent phosphate cotransporter Pit1 and osteochondrogenic (OC) factors that mediate transdifferentiation. Mineralocorticoid receptor (MR) pharmacological blockers ameliorate experimental atherosclerosis and vascular calcification in klotho null mice. We hypothesize that vascular MR activation contributes to upregulation of Pit1, osteoblastic differentiation and calcification of VSMC.

Methods: 5/6 nephrectomy (NPX) rats under high phosphate diet (HP;1.2%) or HP plus vitamin D (VD;80ng/kg/day) or vehicle were given spironolactone (Spiro) (15mg/kg/day) in the food. Sham-operated rats in HP diet were controls. After 5 weeks, we performed biochemical analysis (blood, urine), calcification, Pit1 upregulation and OC factors in aorta. In Aortic VSMC (A7r5) we tested if Spiro prevented 2.5mM HP7-induced calcification.

Results: Aortic sections from NPX+HP rats showed granular calcification in the media and NPX+HP+VD rats presented large areas of calcification that was prevented by Spiro. Measurement of aortic tissue calcium content in all groups confirmed that Spiro treatment prevented calcification. VC was associated with upregulation of Pit1, cbfa-1 and Sox-9 transcripts. Genes potentially involved in VC were also upregulated (TNF-alpha, Nox-1 and Nox-4 (n=5;$P<0.05$;SH *versus* NPX-HP (VD)). Spiro prevented upregulation of all these genes (n=$P<0.05$;NPX+HP (VD) *versus* NPX+HP (VD) +Spiro). Finally, HP caused calcification (Alizarin Red) on VSMC, concomitant with the upregulation of Pit1, cbfa-1,

Sox-9 and Nox-1 (n=4;P<0.05). The treatment with aldosterone 100nM+HP increased the calcium deposition and the abundance of all these transcripts. Spiro (10uM) prevented calcium deposition and gene induction.

Conclusions: We conclude that vascular activation of MR contributed to upregulation of Pit1 *in vivo* and *in vitro* in CKD conditions. Supported: CONICYT/FONDECYT/Regular/ Nº1130550, IMII P09-016-F.

Funding: Government Support - Non-U.S.

FR-PO832

Warfarin Accelerates Uremic Vascular Calcification, From Clinical to Bench Szu-Yuan Li,[1] Jia-Sin Liu,[2] Chih-Cheng Hsu,[2] Der-Cherng Tarng,[1] Chih-Ching Lin,[1] Wu-Chang Yang,[1] Chi-Ting Huang.[2] *[1]Nephrology, Taipei veterans General Hospital, Taiwan; [2]Inst of Population Health Sciences, National Health Research Insts, Taiwan.*

Background: Mesenchymal stem cells differentiate to adipocytes, osteoblasts, chondrocytes and vascular smooth muscle cell (VSMC), in the setting of kidney disease, these VSMC can transform into osteocyte-like cells by upregulation of transcription factors such as RUNX-2 and MSX2. These transcription factors are critical for normal bone development and thus their upregulation in VSMC is indicative of a phenotypic switch. These osteoblast-like VSMC then become calcified in a process similar to bone formation. There are several locally expressed calcification inhibitors; including Matrix Gla protein (MGP), osteopontin, and pyrophosphate protect vascular system from calcification. (Fig 1) MGP is a Vit-K dependent protein, and its role in vascular biology is just beginning to be understood. Unlike the coagulation factors, which undergo hepatic carboxylation, MGP is carboxylated within the vasculature. This peripheral carboxylation process is distinct from hepatic carboxylation, yet both are inhibited by warfarin.

Methods: We used Taiwan National Health Insurance database to conduct a propensity score- matched cohort to analyzed the CV effects of warfarin in dialysis atrial fibrillation (Af) patients. *In vitro* experiments was used to explore mechanism; and micro-CT scan was used to follow up series vascular changes in uremic animals with or without warfarin.

Results: In dialysis-Af patients, warfarin administration was associated with a higher risk of developing CHF (HR 1.51, p<0.05) and PAOD (HR 2.43, p <0.01). Warfarin accelerates VSMC transform to osteocyte-like cells, (Fig 2) and warfarin accelerates aorta calcification in uremic animal model. (Fig 3)

Conclusions: Warfarin is able to promote uremic vascular calcification and may be harmful to dialysis population.

Fig 1

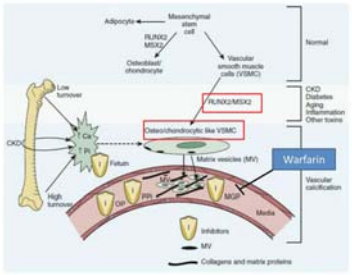


Fig 2

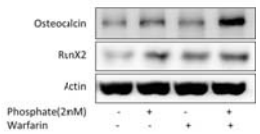
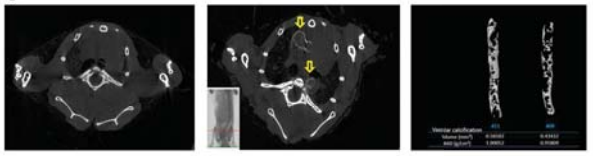


Fig 3



Funding: Government Support - Non-U.S.

FR-PO833

Calcifying Matrix Vesicles (MV) Derived From Vascular Smooth Muscle Cells (VSMC) Exposed to High Phosphorus Can Induce Calcification and Upregulate Gene Expression in Neighboring VSMC Kalisha O'Neill,[1] Sharon M. Moe,[1,2] Shannon Roy,[1] Neal X. Chen.[1] *[1]Indiana Unviersity School of Medicine; [2]Roudebush VAMC, Indianapolis.*

Background: Hyperphosphatemia is associated with arterial calcification in patients with CKD and incubating VSMC with high phosphorus induces the production of calcifying MV. We tested the hypothesis that calcifying MV may play a role in cell to cell communication as a mechanism to propagate calcification.

Methods: We compared MV from VSMC incubated with high phosphorus (calcifying MV) to MV from VSMC incubated with normal phosphorus (control MV). We utilized bovine VSMC and compared VSMC from normal and CKD rats, the latter a model of CKD-MBD that spontaneously develops arterial calcification. MV were co-cultured with VSMC to assess the change in calcification and expression of osteoblast genes in VSMC determined by qPCR. MV miRNA expression was analyzed by qPCR, and MV endocytosis was examined by confocal microscopy using the fluorescent dye PKH-26.

Results: Calcifying MV induces a 3 fold increase in mineralization of a cell free collagen matrix compared to control MV, regardless of whether they were isolated from VSMC from bovine, CKD or Normal rats. The addition of rat or bovine calcifying MV to rat or bovine VSMC accelerated calcification of each VSMC compared to control MV. Compared to control MV, calcifying bovine MV induced the expression of RUNX2 and transglutaminase 2 in BVSMC when co-cultured. To determine if these differences were due to altered cellular uptake, we analyzed the uptake of both calcified and control MV in VSMC by confocal live cell imaging. Both forms of MV were similarly endocytosed, indicating that it was the content, not the uptake of MV that triggered gene expression. To understand the mechanism of triggered gene expression, we compared the miRNA content of calcifying versus control MV and found differences in multiple miRNAs thought to control expression of genes known to be involved in calcification.

Conclusions: Calcifying MV isolated from VSMC exposed to high phosphorus are endocytosed and can modify the gene expression and calcification potential of adjacent VSMC. Transfer of miRNA may be one mechanism by which this occurs.

Funding: Other NIH Support - NIAMS, Veterans Affairs Support

FR-PO834

Ca Load Induces Vascular Smooth Muscle Cell Mineralization Partly Mediated by TRPV Masahide Mizobuchi,[1] Takashi Inoue,[1] Hiroaki Ogata,[2] Fumihiko Koiwa,[3] Tadao Akizawa,[1] Takanori Shibata.[1] *[1]Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine; [2]Dept of Internal Medicine, Showa Univ Northern Yokohama Hospital; [3]Div of Nephrology, Dept of Medicine, Showa Univ Fujigaoka Hospital.*

Background: Vascular calcification has been shown to be a complicated process including the transformation of vascular smooth muscle cells (VSMCs) into osteoblastic-like cells, apoptosis, elastin degradation, and loss of vascular calcification inhibitors. Although hypercalcemia, as well as hyperphosphatemia, is a risk factor for vascular calcification, little is known about the involvement of calcium (Ca) in the process of vascular calcification. We studied the effect of Ca load on VSMC mineralization.

Methods: Human aortic VSMCs were incubated in a low Ca media (0.4 mM: LCa), normal Ca media (1.8 mM: NCa), and a high Ca media (5.0 mM: HCa). All the media had normal phosphate concentration (0.8mM). VSMC mineralization, and gene expression of markers for osteogenic process and a Ca channel (transient receptor potential vanilloid-2: TRPV-2) was determined by real-time RT-PCR. RNA interference for TRPV-2 was performed to study the effect of TRPV2 on the mineralization.

Results: VSMC mineralization was induced in a dose dependent manner (P <0.05: LCa versus MCa, P<0.05: MCa versus HCa) with the induction of an osteogenic process which was confirmed by the increase in Runx2 mRNA and ALP mRNA expression (P<0.05: LCa versus HCa). In parallel with the osteogenic process, we confirmed the up-regulation of a Ca channel, TRPV-2 in the HCa (P<0.05: LCa versus HCa). When TRPV-2 mRNA in VSMC was knocked-down by RNA interference, the mineralization was significantly suppressed in the high Ca media (P<0.05).

Conclusions: These results suggest that Ca load induced VSMC mineralization which is partly mediated by TRPV2. The mineralization is induced in normal phosphate concentration. Thus in addition to phosphate, Ca load is a considerable factor for the process of VSMC mineralization. The precise mechanism by which Ca load induces VSMC mineralization is under investigation.

FR-PO835

Role of RAGE Signalling in Uremic Vascular Calcification Process Fatouma Toure,[1] Karim Belmokhtar,[1] Jeremy Ortillon,[1] Stephane Jaisson,[1] Agnes Boullier,[2] Jean-Marc Chillon,[2] Marie Daniele Diebold,[1] Guenter Fritz,[3] Philippe Gillery,[1] Ann Marie Schmidt,[4] Ziad Massy,[2] Philippe Rieu.[1] *[1]CNRS UMR URCA 7369 and CHU, Reims, France; [2]INSERM U1088, Amiens, France; [3]Univ of Frieburg, Frieburg, Germany; [4]Diabetes Research Program, NYU, New York.*

Background: ESRD is associated with increased cardiovascular morbi-mortality related to increased vascular calcification process. The receptor for advanced glycation end products (RAGE) is an ubiquitous, pro-inflammatory receptor involved in vascular remodelling during diabetes but the role of RAGE signalling in uremia induced vascular calcifications has not been extensively studied.

Methods: Uremia was induced in 8 weeks ApoE-/- mice. Control group was ApoE-/- sham operated animals. Serum and aortas were collected in both groups after 12 weeks of uremia for measurement of RAGE ligand (Carboxymethyllysine, CML) using LC/MS/MS and quantification of RAGE expression by qRT-PCR. Primary cultures of Vascular smooth muscle cells (VSMC) were established from aortas of WT or RAGE deficient mice. Inorganic Phosphate (Pi) and RAGE ligands (CML and S100A12) were used to study in vitro calcification processes including i) quantification of calcifications ii) mRNA and protein expression of cellular markers of osteodifferenciation (Runx2, BMP2, collagen1) iii) analysis of apoptosis.

Results: After 12 weeks of uremia, CML concentration was significantly increased in serum and aortas of uremic animals compared to non uremic controls. RAGE expression was also significantly increased in the aortas of uremic mice. In vitro stimulation of WT VSMC with RAGE ligands, induced the formation of calcifications, osteogenic transformation of the cells, and increased apoptosis. These processes were significantly inhibited in RAGE deficient VSMC. RAGE deletion was also protective against Pi induced vascular calcification processes. In vivo confirmation of these pathways in ApoE-/- RAGE-/- mice is in progress.

Conclusions: Uremia increases RAGE and RAGE ligands expression in the aortic wall. RAGE signalling induces VSMC dependant mineralization processes initiated not only by RAGE ligands but also by Pi. Therefore, RAGE may play a central role in the pathogenesis of uremic vasculopathy.

FR-PO836

Uremic Serum Induces Calcification in Human Aortic Smooth Muscle Cells Violeta Cazaña,[1] Teresa Giráldez,[2] Carmen Mora,[1] Mercedes Muros de Fuentes,[3] Ernesto Martín,[1] Javier Donate,[1] Juan F. Navarro-Gonzalez.[1,4] *[1]Research Unit, Univ Hospital Nuestra Señora de Candelaria (HUNSC), Tenerife, Spain; [2]Physiology, Univ of La Laguna, Tenerife, Spain; [3]Clinical Biochemistry, HUNSC, Tenerife, Spain; [4]Nephrology, HUNSC, Tenerife, Spain.*

Background: Uremic patients have a significant vascular damage characterized by artery calcification. It has been reported that uremic serum induces expression of key proteins in the osteogenic transformation of vascular smooth muscle cells. The aim of the study is to characterize the calcification process in a human *in vitro* model of smooth muscle cells of the vessel wall under conditions of uremia.

Methods: Primary human aortic smooth muscle cells (HASMC) were cultured in medium supplemented with pooled sera (20%) from either healthy or dialysis patients. Cells cultured in medium supplemented with 2.5mMP_i+2mMCa (P_i-Ca) were used as positive controls. After 2, 3, 5, 7 and 10 days, calcification was identified by Alizarin red staining, and alkaline phosphatase (ALP) activity was quantified. The expression of the phosphate transporter Pit-1, Transgelin (TAGLN), transcription factor RUNX2 and ALP was evaluated by qPCR.

Results: For each day of the assay, HASMC incubated with uremic serum showed significant calcium deposits compared to pooled control serum, with the appearance of abundant mineralized nodules. The addition of uremic serum to HASMC culture increased significantly the activity and the expression of ALP compared to cells cultured with Pi-Ca. Exposure of HASMC to growth media containing uremic serum did not cause a significant upregulation of RUNX2 compared with HASCM in medium with P_i-Ca. However, cells treated with Pi-Ca had an increased expression of Pit-1 compared to uremic serum, and a reduceed expression of TAGLN (a specific smooth muscle gene that is lost in the process of phenotypic modulation).

Conclusions: Uremic serum induces HASMC calcification and increased expression of alkaline phosphatase activity, even in the presence of lower P concentrations (<0.5 mmol/L). However, an increased expression of Pit-1 and RUNX2 was not observed, as compared to Pi-Ca-stimulated cells, suggesting a different mechanism from that of hyperphosphatemia-induced calcification.

Funding: Private Foundation Support

FR-PO837

The Direct Inhibitory Effect of Etidronate Disodium Hydrate on Progression of Phosphate-Induced Calcification in Human Vascular Smooth Muscle Cells Masanori Tokumoto,[1] Shunsuke Yamada,[1,2] Tomoe Fujino,[1] Kazuhiko Tsuruya,[3] Takanari Kitazono,[2] Hiroaki Ooboshi.[1] *[1]Div of Internal Medicine, Fukuoka Dental College, Fukuoka, Japan; [2]Dept of Medicine & Clinical Science, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan; [3]Dept of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: Vascular calcification is a life-threatening pathophysiological abnormality, especially in chronic kidney disease (CKD). The main contribution factor for vascular calcification is phosphate (P), but it is difficult to control P retention completely in CKD. Therefore, we examined direct effects of etidronate disodium hydrate (EDH), a bisphosphonate, on P-induced calcification in vascular smooth muscle cells (VSMCs).

Methods: In experiment 1, human VSMCs were cultured in medium containing 2.9mM P with 0, 1, 5, or 10mM EDH for one or two weeks. In experiment 2, following one week incubation with 2.9mM P, human VSMCs were treated with 0, 1, 5, or 10mM EDH in medium containing 0.9 or 2.9mM P. The precipitated calcium contents were evaluated.

Results: 5 or 10mM EDH inhibited P-induced calcification completely for a week and by about 70 or 100% for two weeks, and 1mM EDH decreased by about 50% for a week ($p<0.01$), but did not for two weeks. Even after the culture with 2.9mM P medium for a week, 10mM EDH treatment completely suppressed the progression of P-induced calcification, but 5mM EDH reduced the progression by only 20% ($p<0.01$), and 1mM EDH did not. The elimination of excess P load without EDH decreased the progression of calcification by about 80% ($p<0.01$), and the addition of 1mM EDH did not have an additional effect. However, 5 or 10mM EDH administration without excess P load completely stopped further calcification ($p<0.01$).

Conclusions: The therapy with EDH, a bisphosphonate, can directly inhibit initiation as well as progression of P-induced calcification in human VSMCs.

FR-PO838

Inhibition of Matrix Metalloproteinases Attenuates the Calcification of Vascular Smooth Muscle Cells Uwe Querfeld,[1,2] Nadja Kretzschmar,[1] Eva Hecht,[1] Christian Freise.[1] *[1]Pediatric Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Center for Cardiovascular Research, Charité Universitätsmedizin Berlin, Berlin, Germany.*

Background: Vascular calcification involves transition of contractile vascular smooth muscle cells (VSMCs) into an osteoblast-like phenotype as part of a coordinated process of vascular remodeling resembling bone mineralization. Matrix metalloproteinases (MMPs) have important physiological roles in bone and vascular tissue remodeling. We performed in-vitro studies to characterize the individual contributions of the gelatinases MMP-2 and MMP-9 to the process of VSMC calcification.

Methods: VSMC (murine MOVAS-1) calcification was induced by incubation with a calcification medium (CM) containing elevated levels of calcium (2.7 mM) and phosphorous (2.8 mM). Calcification was quantified by calcium measurements (o-cresolphtalein method) and by alizarin-red staining. MMP activities were determined by specific substrate assays.

Results: After 9 days, CM-treated VSMC cultures exhibited distinct calcifications, which were further enhanced by the presence of recombinant MMP-2 or MMP-9 (~1.6-fold and ~2.2-fold, respectively) and accompanied by enhanced secretion of both gelatinases by VSMC. Vice versa, incubation with a specific inhibitor of MMP-2 or MMP-9 or the selective (for both gelatinases) inhibitor Ro28-2653, respectively, significantly decreased VSMC calcifications. Addition of the MMP-inhibitors decreased total gelatinolytic activity in VSMC supernatants, indicating a (negative) feedback loop for the secretion of the gelatinases under these conditions. Selective knockdown of MMP-2 and MMP-9 mRNA expression in the VSMC provoked reduced calcifications of the CM-treated cells.

Conclusions: Inhibition of enzymatic activity of MMP-2/-9 and transient knockdown of mRNA expressions prevented calcification of VSMC while addition of recombinant MMPs enhanced VSMC calcifications. Thus, both gelatinases provide extracellular calcification signals, and their effect is mainly due to their enzymatic activity, as indicated by the effect of inhibitors. Inhibition of arterial MMP-2/-9 may be a therapeutic target for the prevention of vascular calcifications (in CKD).

Funding: Private Foundation Support, Clinical Revenue Support

FR-PO839

Association between Epicardial Adipose Tissue and Vascular Calcification in Hemodialysis Patients Xoana Barros,[2] Nadine Kaesler,[1] Thilo Krueger,[1] Markus Ketteler,[3] Jürgen Floege,[1] Vincent Brandenburg.[1] *[1]Uniklinikum RWTH Aachen, Germany; [2]Hospital Clinic Barcelona, Spain; [3]Klinikum Coburg, Germany.*

Background: Epicardial adipose tisse (EAT) is associated with coronary artery calcification (CAC) and atherosclerosis in the general population. EAT is thought to trigger coronary artery disease by paracrine mechanisms. Recently it was speculated that large amounts of EAT may predict mortality in incident HD patients. Our aim was to assess the potential association of EAT with vascular calcification (VC) in chronic HD patients and compare for the first time the progression of EAT and VC over time.

Methods: Prospective study of 59 chronic HD pts (59±15 yrs; 49% male, 25% DM, HD vintage 57±55 m) who underwent non-enhanced multi-slice computed tomography (CT) at baseline; 37 pts repeated CTs after 24±5 m. Two radiologists assessed independently EAT, CAC and aortic valve calcification (AVC). EAT was presented as volume and CAC and AVC as Agatston scores. 30 healthy volunteers without VC and without CKD served as controls for EAT.

Results: Baseline EAT was 128±61 cm≥ and significantly higher than in the control group (94±46 cm≥; $p<0.05$). Median CAC was 329 (IQR 23-1181, prev 90%) and AVC was 0 (IQR 0-25.3, prev 37%). No significant differences were detected in EAT between genders or diabetics versus non-diabetics. There was a significant positive correlation between baseline EAT and age ($r=0.386$; $p=0.003$), BMI ($r=0.314$; $p=0.016$), CAC ($r=0.278$; $p=0.03$), and AVC ($r=0.282$; $p=0.03$). No correlation between EAT and biochemistry (fetuin-A, adiponectin, C-peptide, phosphate, CRP, Hb, PTH) was detected. During follow-up the amount of CAC significantly increased (annual change +19% (IQR 2-35%), Wilcoxon paired test, $p<0.005$). In contrast, the change of EAT was not significant (EAT at follow-up 133±57 cm≥, t-paired test, $p=0.066$). 35% of pts showed regression of EAT.

Conclusions: EAT correlated significantly with CAC and AVC in chronic HD patients. Rapid and homogeneous progression on CAC contrasts with EAT development over 2 years, where a third of the patients exhibited EAT regression. Larger studies need to assess a potential causality between EAT and VC as well as the EAT effects on the high cardiovascular risk in ESRD.

Funding: Clinical Revenue Support

FR-PO840

Pulse Wave Velocity Is Superior Over Ankle Brachial Index for Screening Vascular Calcification in Hemdialysis Patients Daqing Hong, Li Wang. *Renal Dept, Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: Vascular calcification is prevalent in hemodialysis patients, accounting for an inevitable impact on patient survival. Pulse wave velocity (PWV) and ankle brachial index (ABI) was reported to be associated with vascular calcification, while no studies focused on the relationship between PWV or ABI and different vascular calcification. The objective of this study is to compare the difference of PWV and ABI for screening vascular calcification.

Methods: 118 hemodialysis patients were enrolled. Coronary artery CT and radiographs of lateral abdominal, pelvis, and hands were measured to evaluate vascular calcification of different arteries. PWV and ABI was measured as a screening method. ROC curves was used to analyze the relationship between PWV or ABI and vascular calcification by CT or X-ray radiographs.

Results: PWV was positively associated with coronary artery score and X-ray vascular calcification score ($P<0.05$). ROC curves showed an AUC>0.5 for PWV and an AUC<0.5 for ABI in diagnosis of different artery calcification.

Conclusions: Pulse wave velocity is superior over ankle brachial index for screening vascular calcification in different anatomical regions.

Funding: Clinical Revenue Support

FR-PO841

Accelerated Progression of Vascular Calcification in Children with CKD Is Associated with Fetuin-A Levels and Vessel Characteristics

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Background: Vascular calcification is thought to begin early in CKD and progress rapidly on dialysis. We obtained arterial biopsy samples to determine a quantitative and histological assessment of the vessel calcium load and compared this with longitudinal changes in the vessels by imaging studies.

Methods: 48 children (13 CKD4-5, 28 dialysis and 7 failed transplants) had vascular imaging (carotid intima-media thickness [cIMT], pulse wave velocity [PWV] and coronary artery calcification [CAC] on CT scan), biomarker analyses and an arterial biopsy (at the time of renal transplantation or PD catheter insertion). The Ca load in the vessel wall was quantitated and histology for hydroxyapatite deposition, vascular smooth muscle cell apoptosis and osteogenic differentiation was performed. 41 children (20 dialysis and 21 transplants) had a second set of imaging after 14.2 ±3.9 months.

Results: The baseline vessel Ca load strongly correlated with cIMT in dialysis patients ($p=0.005$) whereas 11 of 16 pre-dialysis patients had normal cIMT. Dialysis patients had a significant annualised increase in cIMT and PWV standard deviation scores ($p<0.005$ and $p=0.03$). CAC increased in 5 children with baseline CAC and was found in 3 others. cIMT progression showed a close correlation with the vessel Ca load ($p=0.002$, $r^2=0.33$). Patients with cIMT progression had the highest apoptotic index implying vascular smooth muscle cell loss and greater osteogenic differentiation. The baseline cIMT ($r=0.31$) and Fetuin-A levels ($r=0.41$), but not FGF-23, soluble klotho or 25-hydroxyvitamin D associated with cIMT progression. Changes in PWV and CAC did not correlate with vessel Ca load.

Conclusions: In children on dialysis vascular calcification is rapidly progressive and strongly correlates with baseline vessel wall characteristics and Fetuin-A levels. No association was found between vascular measures and FGF-23 or soluble klotho levels. Fetuin-A may be a useful biomarker to predict rapid progression of vascular calcification in CKD.

FR-PO842

Expression of Xyloslytransferase (XYLT1) in Human Arteries Is Correlated with Serum Phosphate

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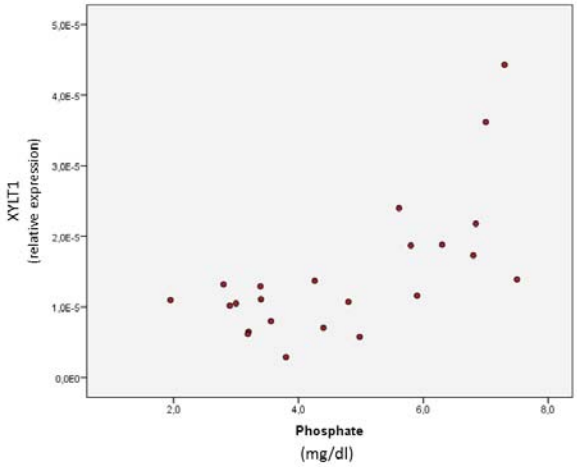
Background: We have previously shown that inter-individual and highly variable tissue concentrations of sodium can be found in human arteries. There is evidence suggesting that this high variability is due to osmotically inactive sodium, bound to glycosaminoglycans. Glycosaminoglycan synthesis is initiated by XYLT1 which correlates with tissue sodium concentrations. We now sought to better characterize this regulation of XYLT1 expression in human arteries.

Methods: We studied 27 patients on dialysis. 21 live kidney donors served as healthy controls. All patients were free of clinically detectable edema. During transplant surgery, abdominal muscle and arteries were biopsied. Expression of XYLT-1 and Na-K-ATPase1 were determined in arterial tissue by real-time PCR (n=31), standardized to s18 RNA and correlated with clinical data.

Results: On univariate analysis, XYLT1 expression in human arteries was significantly correlated with serum sodium ($r = 0.456$; $p = 0.022$), calculated serum osmolality ($r = 0.558$; $p = 0.004$), serum phosphate ($r = 0.664$; $p = 0.001$), serum bicarbonate ($r = -0.523$; $p = 0.031$), and arterial Na-K-ATPase1 expression ($r = 0.459$; $p = 0.021$). On multivariate analysis, XYL1 expression was significantly correlated with serum phosphate ($p = 0.004$).



Conclusions: XYLT1 expression in human arteries is correlated with serum phosphate concentrations. This may form a pathophysiologic basis for the development of arteriolopathy in patients with hyperphosphatemia, e.g. in advanced renal failure.

FR-PO843

A Newly Developed Chronic Kidney Disease–Mineral-Bone Disorder Rat Model

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Background: Chronic kidney disease–mineral bone disorder (CKD-MBD) is associated with all-cause and cardiovascular mortality in CKD patients. Thus, elucidating its pathophysiological mechanism is essential for improving morbidity and mortality of these patients. In this study, we investigated CKD-MBD using a newly developed rat model.

Methods: In the present study, we used male Sprague Dawley (SD) rats and Spontaneously Diabetic Torii (SDT) rats, which are and non-obese rat models with type 2 diabetes. CKD was induced by 5/6 nephrectomy (Nx). At 10 weeks, they were classified into seven groups and were administered vehicle (V), low-dose paricalcitol (LP, 0.1 mg/kg), and high-dose paricalcitol (HP, 0.3 mg/kg) three times a week, except in the control group (the control, SD-Nx+V, SDT-Nx+V, SD-Nx+LP, SDT-Nx+LP, SD-Nx+HP, and SDT-Nx+HP group). At 20 weeks, the rats were sacrificed and urinary and blood biochemical analyses and histological analysis of the aorta were performed in all the groups.

Results: At 20 weeks, there were no significant differences in HbA1c levels, blood pressure, and renal function among the six groups, except the control group. Despite comparable doses of paricalitol, serum calcium and phosphate levels and the urinary excretion of calcium and phosphate were higher in the SDT rats compared with the SD rats. Serum PTH levels were decreased and serum FGF23 levels were elevated after administering paricalcitol in both groups of rats; however, serum PTH and FGF23 levels were higher in the SDT rats compared with the SD rats. Further histological and biochemical analyses revealed that the degree of vascular calcification of the aorta was more severe and the calcium content of the aorta was also higher in the SDT rats compared with the SD rats.

Conclusions: Our study suggested that CKD-MBD of 5/6 nephrectomized SDT rats was greatly influenced by vitamin D. Thus, this can be a suitable CKD-MBD model to examine the pathophysiology of CKD-MBD.

FR-PO844

Vitamin D Influences Drug Metabolism and Transport Pathways in Human Kidney Cells

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Background: Despite vitamin D (VitD) deficiency and subsequent treatment in chronic kidney disease (CKD), limited information is available to inform about potential drug interactions. The goal of the current study was to determine the influence of VitD on expression and function of drug metabolism and transport pathways.

Methods: Human proximal tubular epithelial cells (hPTCs;ScienCell) were grown to confluence in 100x20 mm/well plates and seeded at 5000 cells/cm^2. hPTCs were incubated for 6 days with cholecalciferol ($VitD_3$) or calcitriol ($1,25\ (OH)_2D_3$) 100nM and 240nM in 0.1% ethanol *versus* vehicle in the presence and absence of 10% uremic serum, followed by determination of gene/protein expression of drug transporters and enzymes. P-glycoprotein (P-gp) function was assessed in transwells with [^{3}H]Digoxin+VitD (100nM) *versus* vehicle for 3 and 6 days. CYP2R1 function was assessed by evaluating metabolic ratios (MRs) of 25-OH VitD to VitD in the presence and absence of uremic serum. Gene and protein expression were determined with RT-PCR, western blots, and/or immunofluorescence.

Results: Transporter gene expression was altered with VitD: P-gp/*ABCB1* increased with all treatments (317-347%), MRP2/*ABCC2* increased 15% with 100nM VitD, and MATE1/*SLC47A1* decreased up to 220% for all treatments. *CYP3A5* and *CYP2B6* expression

increased with all treatments. With uremic serum, P-gp/*ABCB1*, MRP2/*ABCC2*, OATP4C1/*SLC04C1*, *ALDH1A1* and *CYP2B6* increased and *CYP24A1* expression decreased. Protein expression was consistent with RT-PCR data. A 10% increase in basolateral to apical transport of [^{3}H]Digoxin *versus* vehicle was seen with VitD exposure. Decreased conversion of VitD to 25-OH VitD (MRs 0.002 *versus* 23.5) in the presence and absence of uremic serum, respectively was noted.

Conclusions: Exposure of hPTCs to VitD resulted in altered expression and function of metabolism enzymes and transporters. Uremia can further modulate the influence of VitD exposures. The extension of these data to other cell types and humans with CKD requires further study.

FR-PO845

Excess of 25-Hydroxyvitamin D Exacerbates Tubulointerstitial Fibrosis in Mice Yasuo Kusunoki,[1] Isao Matsui,[1] Takayuki Hamano,[2] Akihiro Shimomura,[1] Daisuke Mori,[1] Sayoko Yonemoto,[1] Yoshitsugu Obi,[1] Yoshiharu Tsubakihara,[2] Yoshitaka Isaka,[1] Hiromi Rakugi.[1] *[1]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Comprehensive kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

Background: In most of the observational studies, both deficiency and excess of serum 25-hydroxyvitamin D (25 (OH) D), but not active vitamin D (1,25 (OH) $_2$D), are strongly associated with poor prognosis. Those results suggest that 25 (OH) D *per se* may have some direct biological effects.

Methods: Using unilateral ureteral obstruction (UUO) model, we examined the effects of 25 (OH) D on renal fibrosis. To exclude the effects of 1,25 (OH) $_2$D hydroxylated from 25 (OH) D, we used 25 (OH) D-1a-hydroxylase systemic knockout mice (CYP27B1 KO mice). Mice were randomly divided into three groups: group vehicle, moderate-25 (OH) D (6.25ng/gBW), and high-25 (OH) D (100ng/gBW). Vehicle or 25 (OH) D was injected three times a week prior to the UUO operation. Effects of 24,25 (OH) $_2$D, another metabolite of 25 (OH) D, were also analyzed in a similar way. To explore the underlying mechanisms how 25 (OH) D exerts its own effects, we analyzed the effects of 25 (OH) D on CYP27B1/VDR double KO mice and on macrophage-depleted CYP27B1 KO mice.

Results: Serum 25 (OH) D levels in group vehicle, moderate-25 (OH) D, and high-25 (OH) D, were 7.78±0.23, 38.45±5.67, and 334.54±47.10 ng/mL, respectively. Both histological analyses and real time PCR demonstrated that high-dose 25 (OH) D exacerbated tubulointerstitial fibrosis in CYP27B1 KO mice, while moderate-dose 25 (OH) D had no effect. Oxidative stress, assessed by gH2AX-staining and Western blotting for nitrotyrosine, was aggravated in group high-25 (OH) D. Because high-dose 24,25 (OH) $_2$D did not exacerbate tubulointerstitial fibrosis, it was suggested that 25 (OH) D *per se* has direct biological effects. The profibrotic effect of high-dose 25 (OH) D was not observed in CYP27B1/VDR double KO mice or macrophage-depleted CYP27B1 KO mice.

Conclusions: Excess of 25 (OH) D exacerbated tubulointerstitial fibrosis in a VDR-dependent manner. The aggravated oxidative stress in group high-25 (OH) D might be derived from phenotypic changes in kidney-infiltrating macrophages.

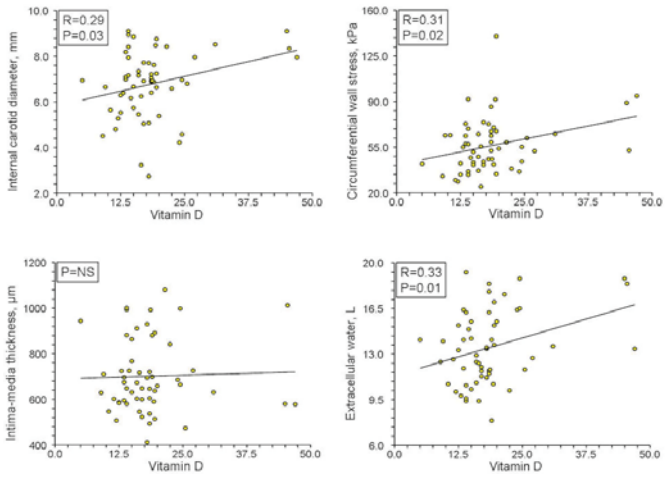
FR-PO846

Outward Vascular Remodeling and Hyperhydration Are Associated with Elevated Vitamin D Levels Paolo Lentini,[1] Luca Zanoli,[2] Massimo de Cal,[1] Stefania Rastelli,[2] Anna Basso,[1] Andrea Contestabile,[1] Antonio Granata,[3] Roberto Dell'Aquila.[1] *[1]Nephrology, S. Bassiano Hospital, Bassano Del Grappa, Italy; [2]Internal Medicine, Univ of Catania, Catania, Italy; [3]Nephrology, S.Giovanni di Dio Hospital, Agrigento, Italy.*

Background: Chronic inflammation,vascular calcification and fluid overload are common causes of cardiovascular events in hemodialysis [HD] patients [pts]. Compare to hypertensive pts, in which there is an inward remodeling (higher intima-media thickness (IMT) and higher internal carotid diameter [D]), outward carotid remodeling (unmodified IMT) is a common feature of CKD. Several studies reported that Vitamin D [VitD] might play a key role in endothelial dysfunction and in the atherosclerotic plaque structure; however, it is not clear whether in HD pts VitD levels may act the induction of vascular calcification. Echotracking system is the reference non-invasive technique to assess arterial structure and function, including IMT, an established independent predictor for future cardiovascular events, and D. Overhydration [OH] is a danger common feature among HD pts. AIM: To evaluate the link between VitD, arterial parameters and body composition in HD pts.

Methods: Arterial parameters were measured with a high-definition echotracking system paired with an high resolution (13 MHz) probe. Bioimpedance spectroscopy (BIS) was performed and overhydration (OH) and extracellular water (ECW) were used as volume indices.

Results: 60 HD pts were enrolled (age 64±16 yrs, males 55%). Elevated VitD levels were associated with outward vascular remodeling (with enlarged D but not with enlarged IMT). Consequently, higher the VitD, higher the circumferential wall stress.VitD was also positively associated with ECW and OH detected with BIS.



Conclusions: Elevated vitamin D levels may lead to outward vascular remodeling and OH: these alterations may be non-invasively detected with BIS and echotracking systems.

FR-PO847

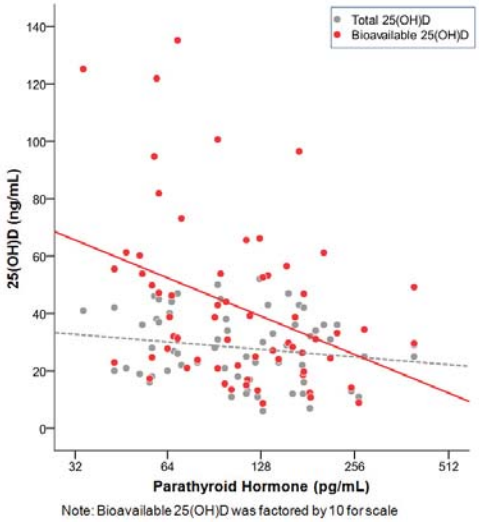
Bioavailable 25 (OH) D Is a Better Predictor of PTH and eGFR than Total 25 (OH) D in CKD Patients Anders H. Berg,[1] Julia Beth Wenger,[2] Ishir Bhan,[2] Hector Tamez,[2] S. Ananth Karumanchi,[1] Ravi I. Thadhani.[2] *[1]BIDMC, Boston, MA; [2]MGH Nephrology, Boston, MA.*

Background: An inverse correlation between 25 (OH) D and parathyroid hormone (PTH) is reported in both the general and CKD population. However, the strength of this relationship is modest; hyperparatyhroidism is not universal among patients with low levels of total 25 (OH) D. We previously suggested that bioavailable vitamin D ($B_{av}D$) is a more appropriate measure of vitamin D status in the general population (NEJM 2013). We now examine this in patients with CKD.

Methods: From a previously completed randomized placebo controlled trial of patients with stage 3-4 CKD (PRIMO, JAMA 2012), we measured total 25 (OH) D, PTH, estimated glomerular filtration rate (eGFR), and calculated concentrations of $B_{av}D$ using genotype-specific binding affinity constants and circulating levels of vitamin D binding protein.

Results: In 64 CKD patients, median (Q1, Q3) levels of total 25 (OH) D and $B_{av}D$ were 28 ng/ml (20, 38) and 3.3 ng/ml (2.2, 5.4), respectively. $B_{av}D$ was significantly associated with PTH (r_s= -0.34, p=0.005) while total 25 (OH) D was not (r_s= -0.14, p=0.26). Similar associations were also found between $B_{av}D$, total 25 (OH) D, and eGFR ($B_{av}D$ r_s= 0.28, p=0.02; total 25 (OH) D r_s= 0.08, p=0.55). In multivariable linear regression models adjusting for age, corrected calcium, and eGFR, $B_{av}D$ remained a significant predictor of PTH (b= - 0.06, p=0.01) while total 25 (OH) D remained unrelated to PTH (b= - 0.006, p=0.23). Correlations strengthened further when the analysis was restricted to homozygous patients (in whom affinity constants could more accurately be determined).

Conclusions: Bioavailable Vitamin D may be a more appropriate measure of vitamin D status in patients with CKD. If confirmed, $B_{av}D$ measures in CKD may better direct supplemental inexpensive nutritional vitamin D to control PTH.



Funding: NIDDK Support

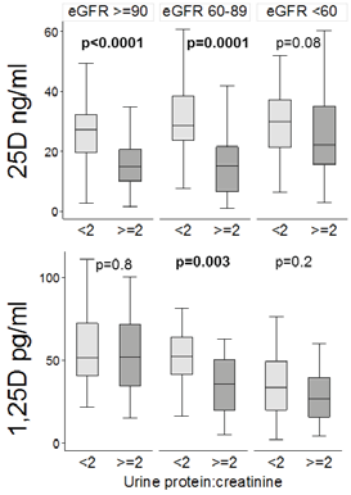
FR-PO848

Vitamin D Metabolism in the Nephrotic Syndrome Study Network (NEPTUNE) Michelle Denburg,[1,2] Myles S. Wolf,[3] Ian H. de Boer,[4] Andrew N. Hoofnagle,[4] Samir Sayed,[1] L. Barisoni,[5] Lawrence B. Holzman,[2] Mary B. Leonard.[1,2] *[1]Children's Hospital of Philadelphia; [2]U. Pennsylvania; [3]Northwestern U.; [4]U. Washington; [5]U. Miami.*

Background: 25-hydroxy and 1,25-dihydroxyvitamin D (25D and 1,25D) circulate bound to vitamin D-binding protein (DBP) or albumin. Studies of vitamin D (VitD) in nephrotic patients are limited. We sought to determine correlates of VitD levels and the relative impact of nephrotic-range proteinuria (NRP) and GFR.

Methods: Plasma 25D, 1,25D, DBP, PTH, and FGF23 were measured in 249 NEPTUNE participants (pts): 21% minimal change, 33% FSGS, 17% membranous, 29% other. Multivariable linear regression was used to assess correlates of VitD metabolites.

Results: 43% of pts were VitD deficient (25D <20 ng/ml). NRP was associated with lower plasma DBP, independent of eGFR (p=0.001). Adjusted for albumin, DBP, and VitD supplementation, NRP was independently associated with lower 25D (Model 1). The significant impact of NRP on 25D was noted in earlier CKD stages, but not in pts with eGFR <60 ml/min/1.73m². Traditional risk factors (black race, season) and histopathology were not independently associated with 25D. Despite substantially lower 25D, NRP did not result in lower 1,25D among pts with normal eGFR. In contrast, 1,25D was lower in pts with NRP and eGFR of 60-89. 1,25D was similarly reduced in the setting of an eGFR <60 with versus without NRP. Adjusted for the reciprocal effects of PTH and FGF23, NRP was associated with lower 1,25D, but this was explained by lower substrate 25D (Model 2).



Model #1 R^2 = 0.50	25D % Difference	p
Albumin (g/dl)	45.4	<0.001
DBP (mg/dl)	1.2	0.004
Vit D supplement	63.1	<0.001
Up/c ≥2	-17.8	0.04

Also adjusted for age

Model #2 R^2 = 0.58	1,25D % Difference	p
PTH (per 10%)	2.2	<0.001
FGF23 (per 10%)	-5.0	<0.001
eGFR vs. ≥90		
60 – 89	-16.6	0.02
<60	-40.3	<0.001
25D (per 10%)	3.4	<0.001

Also adjusted for age, sex, race

Conclusions: NRP was associated with 1) lower 25D in pts with eGFR ≥60 independent of binding proteins, 2) lower DBP independent of eGFR, and 3) lower 1,25D explained by lower 25D. The impact of NRP on VitD was greatest in CKD stage 2, likely due to preserved filtration/urinary 25D loss with inadequate 1a-hydroxylation to compensate for less substrate. Supported by U54DK083912.

Funding: NIDDK Support, Pharmaceutical Company Support - Genentech, Private Foundation Support

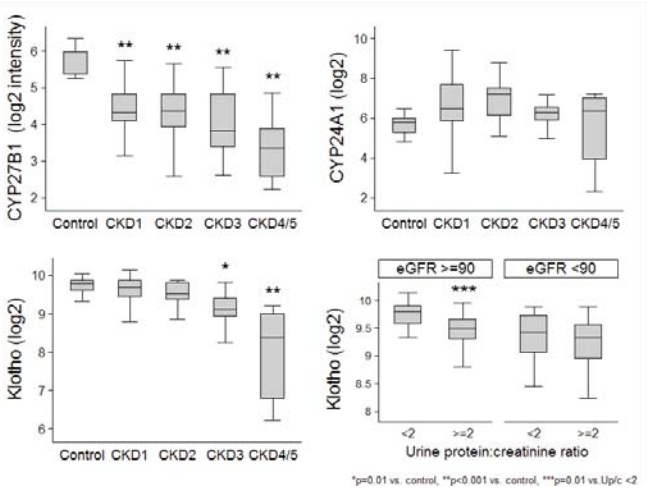
FR-PO849

Mineral Metabolism and Tubulointerstitial Gene Expression in the Nephrotic Syndrome Study Network (NEPTUNE) Michelle Denburg,[1,2] Sebastian Martini,[3] Myles S. Wolf,[4] Ian H. de Boer,[5] Andrew N. Hoofnagle,[5] L. Barisoni,[6] Lawrence B. Holzman,[2] Matthias Kretzler,[3] Mary B. Leonard.[1,2] *[1]Children's Hospital of Phila.; [2]U. Pennsylvania; [3]U. Michigan; [4]Northwestern U.; [5]U. Washington; [6]U. Miami.*

Background: The objectives were to assess tubular mRNA expression of vitamin D (VitD) hydroxylases (CYP27B1 and CYP24A1), the vitamin D-receptor (VDR), and Klotho and their relations to measures of mineral metabolism in NEPTUNE.

Methods: Transcriptomic profiles of the tubulointerstitium from microdissected renal biopsies of 94 NEPTUNE participants (pts) were generated using the Affymetrix 2.1 ST micro-array platform and compared to 5 control live donor samples (CO). Plasma VitD metabolites (25D, 1,25D, 24,25D), PTH, and FGF23 were measured in NEPTUNE pts.

Results: CYP27B1 expression was 61-82% lower in NEPTUNE pts versus CO (p<0.001) and was lower with increasing CKD severity. No differences in CYP24A1 expression were found versus CO or by CKD severity. Compared to CO, Klotho expression was lower in CKD stages 3 (39%, p=0.01) and 4/5 (70%, p<0.001). Among pts with eGFR >90, nephrotic-range proteinuria was associated with 17% lower Klotho expression (p=0.01). VDR expression was reduced in CKD stages 3 (33%, p=0.02) and 4/5 (50%, p=0.001) versus CO. Adjusted for 25D, age, race, and eGFR, CYP24A1 was negatively associated with 1,25D (p=0.001), while CYP27B1 was positively associated (p<0.001) and more so with lower eGFR (interaction p<0.001). Adjusted for 25D, age, race, and eGFR, 10% greater CYP24A1 expression was associated with 2% higher 24,25D concentrations (p=0.001). Independent of phosphate, 10% lower Klotho expression was associated with 9% higher FGF23 (p<0.001).



Conclusions: Glomerular disease is associated with substantial differences in tubular expression of genes regulating mineral metabolism, and their expression correlates with circulating VitD metabolites and FGF23. Supported by U54DK083912.

Funding: NIDDK Support, Pharmaceutical Company Support - Genentech, Private Foundation Support

FR-PO850

Vitamin D Pathway Genes in Relation to Age at Renal Replacement Therapy Onset Alicja E. Grzegorzewska,[1] Grzegorz Ostromecki,[2] Adrianna Mostowska,[3] Anna Sowinska,[4] Pawel P. Jagodzinski.[3] *[1]Dept of Nephrology, Poznan Univ of Medical Sciences (PUMS), Poznan, Poland; [2]DaVita Dialysis Center, Piala, Poland; [3]Dept of Molecular Biology, PUMS, Poznan, Poland; [4]Dept of Statistics, PUMS, Poznan, Poland.*

Background: Vitamin D deficiency is associated with life-threatening diseases. We aimed to show a frequency distribution of vitamin D pathway genes (VDPG) and their associations with clinical-laboratory data of ESRD patients starting renal replacement therapy (RRT) at young, middle and advanced age.

Methods: Group I started RRT at age ≤40 years (n=172, 62% M), group II at 41–70 years (n=676, 59% M), and group III at >70 years (n=326, 48% M). Healthy persons aging ≤40 years (group Ia, n=180) and 41–70 years (group IIa, n=476) were controls. All subjects underwent genotyping for polymorphic variants of genes encoding vitamin D binding protein (*GC*): rs2298849 (HRM), rs7041 (RFLP), rs1155563 (HRM), vitamin D receptor (*VDR*): rs2228570 (RFLP), rs1544410 (RFLP), and retinoid X receptor A (*RXRA*): rs10776909 (HRM), rs10881578 (HRM), rs749759 (RFLP). Clinical (CAD/MI, PTX, treatment with cinacalcet) and laboratory (Ca, P, ALP, PTH, 25 (OH) D) data were related to VDPG polymorphisms shown in age categories.

Results: Groups I-Ia differed in polymorphism of rs2228570 (P_{trend} =0.031); groups II-IIa – rs2228570 (P_{trend} =0.011), rs10776909 (P_{trend} =0.027), rs749759 (P_{trend} =0.035). MAFs of these genotypes were greater in groups I-II than those in groups Ia-IIa, resp. Distribution of rs7041 polymorphic variant differed between groups I-II (P_{trend}=0.049) and II-III (P_{trend}=0.032). The younger group the higher MAF of rs7041 was observed (I - 44%, II – 43%, III – 38%; OR 1.24, 1.02-1.52 for II versus III, P=0.034). The Bonferroni corrected and gender adjusted, significant (P<0.017) VDPG associations with phenotypic features were shown in all groups: I - PTH (rs10881578), Ca (rs1155563), II - MI (rs10881578), Ca (rs10776909), III: 25 (OH) D (rs749759).

Conclusions: *VDR* and *RXRA* polymorphisms are associated with susceptibility to ESRD. The rs7041 allele T is associated with a need of RRT start at younger age. The association of VDPG with clinical/laboratory features is mediated by age at RRT onset.

Funding: Pharmaceutical Company Support - This study was funded as the blindly reviewed winning project by the scientific grant of Baxter Company allocated by the Chapter of Polish Society of Nephrology, grant number 504-04-02225363-00013-03071

FR-PO851

Genetic, Environmental and Disease-Associated Determinants of Vitamin D Status in Children with Chronic Kidney Disease: Findings from the 4C Study Anke Doyon,[1] Bettina Schmiedchen,[2] Anja Christine Sander,[3] Aysun Karabay Bayazit,[4] Anna Kottgen,[5] Matthias Wuttke,[5] Uwe Querfeld,[6] Franz S. Schaefer.[1] *[1]Univ Children's Hospital, Heidelberg, Germany; [2]German Inst of Human Nutrition, Potsdam, Germany; [3]Inst of Medical Biometry, Univ of Heidelberg, Germany; [4]Cukurova Univ, Adana, Turkey; [5]Renal Divison, Univ Hospital Freiburg, Germany; [6]Charité Children's Hospital, Berlin, Germany.*

Background: We investigated the impact of common variants in Vitamin D regulating genes, relative to environmental and disease-associated factors, on the vitamin D status of children with CKD.

Methods: Serum 25-hydroxy-vitamin-D, 1,25-dihydroxy-vitamin-D and 24,25-dihydroxy-vitamin-D were measured in 521 children (6-18 years, CKD stages III-

IV) from 12 European countries. Subjects were genotyped for SNPs in the genes encoding for 25-hydroxylase (CYP2R1), D-binding protein (GC), 7-dehydroxycholesterol-reductase (DHCR7) and 24-hydroxylase (CYP24A1). Associations of genetic status, season, local solar radiation, oral vitamin D supplementation, and disease-associated factors with vitamin D status were assessed.

Results: Serum 25 (OH) D levels were positively associated with vitamin D supplementation and summer season, and inversely with glomerulopathy diagnosis and, unexpectedly, residence in a region with higher solar exposure. When adjusting for these factors, lower serum 25 (OH) D was independently associated with genetic variations in DHCR7 (rs4945008), GC (rs7041), 25-hydroxylase (rs12794714) and 24-hydroxylase (rs3886163). The effect was strongest for patients homozygous for the SNP in DHCR7, who showed 32% lower 25 (OH) D levels than carriers of the major allele. The minor allele frequency for this SNP was significantly higher in subjects of Turkish origin (41.7% versus 26.9%). Serum 24,25 (OH) $_2$D was positively associated with 25 (OH) D, 1,25 (OH) $_2$D and FGF-23 serum levels, and decreased with declining renal function and rising serum iPTH. Minor allele homozygosity for rs7041 of GC was independently associated with higher 24,25 (OH) $_2$D levels.

Conclusions: Genetic variation significantly impacts on vitamin D turnover, adding to seasonal, geographic and disease-related factors and nutritional supplementation.

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

FR-PO852

Impact of the Dialysis Membrane on the Vitamin D Metabolism Markers

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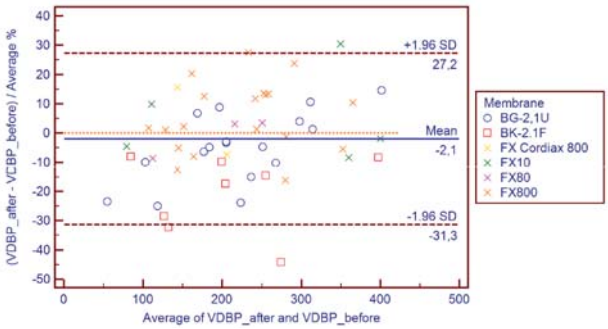
Background: KDIGO guidelines recommend vitamin D supplementation in hemodialyzed patients as to monitor 25 (OH) D levels. Patient-to-patient inconsistency can however be observed in response to the treatment. In this study, we aimed to evaluate the impact of the dialysis membrane on 25 (OH) D, albumin (ALB) and vitamin D binding protein (VDBP), the major players of vitamin D transport and storage.

Methods: ALB (Roche Cobas), VDBP (Elisa R&D) and 25 (OH) D (LCMS/MS) were measured in 54 patients before and after a 4-hours dialysis session. Six dialysis membranes were used: FX Cordiax 800 (n=2), FX10 (n=5), FX80 (n=3), FX800 (n=19), BK-2.1F (BK:n= 8) and BG-2.1U (BG: n=17). Wilcoxon test was used to compare the results before/after the session, p<0.005 considered as significant.

Results: After dialysis session, ALB levels significantly increased by 9.5% (p<0.0001) with the FX membranes but not with BK or BG ones. VDBP levels significantly decreased by 13% (p=0.0078) with BK, but remain unchanged with BU or FX membranes. As expected, 25 (OH) D levels followed VDBP and decreased by 13% (close to significance level) with BK but remained unchanged with the other membranes.



Conclusions: This is the first study showing that VDBP is dialyzed by a membrane with high cut-off (BK-2.1F). This contributes to a trend in decreasing 25 (OH) D levels. Even if these results need to be confirmed in a higher number of patients, this observation can partially explain the patient-to –patient response to the same vitamin D dose.

FR-PO853

Ergocalciferol versus Cholecalciferol for Nutritional Vitamin D Replacement in CKD

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Background: It is unknown whether supplementation with cholecalciferol is more effective than with ergocalciferol in chronic kidney disease (CKD) patients with nutritional vitamin D [25 (OH) D] deficiency.

Methods: A retrospective analysis of CKD patients (n = 60) was conducted to assess the relative effectiveness of ergocalciferol versus cholecalciferol on serum 25 (OH) D levels. Patients had 25 (OH) D levels assessed at baseline, after attempted repletion with ergocalciferol, and then after attempted repletion with cholecalciferol; patients served as their own controls. Absolute and dose-standardized changes were calculated. The relative paired differences of the effects of the drugs were compared using t-tests. Multiple regression modeling was used to determine the factors significantly associated with differential responsiveness to the drugs.

Results: Mean age was 66.3 years; 61.7% were female. Mean baseline 25 (OH) D level was 15.2 ng/mL, mean parathyroid hormone (PTH) was 57.3 pg/mL, and mean proteinuria was 970 mg/day. After 6 months of therapy, increase in 25 (OH) D levels was over 2-fold higher for cholecalciferol than for ergocalciferol, 2.6 ± 0.3 ng/mL per 100,000 units versus 1.1 ± 0.3 ng/mL per 100,000 units (p < 0.0001). In terms of paired differences, cholecalciferol was strongly favored over ergocalciferol in absolute (6.1 ± 1.1 ng/mL), and relative (1.5 ± 0.3 ng/mL, per 100,000 units) average differences. After multivariable adjustment, higher baseline 25 (OH) D (p = 0.01), lower baseline estimated glomerular filtration rate (p = 0.03), and lower baseline PTH (p = 0.001) were associated with significantly better response to cholecalciferol than to ergocalciferol.

Conclusions: Cholecalciferol appears to be more effective than ergocalciferol for nutritional vitamin D deficiency in CKD. Guidelines, such as those of the National Kidney Foundation, may need to be revised.

FR-PO854

Estimation of Optimal Serum Concentrations of 25-Hydroxyvitamin D for Progression and Mortality in Chronic Kidney Disease

Pablo Molina,[1,2] Jose L. Gorriz,[1,2] Ana Peris Domingo,[1] Andres Antolin Carinena,[1] Ricardo Mouzo,[1] Alberto M. Martinez-Castelao,[1] Del Pino Pino Maria Dolores,[1] Sandra Beltrán,[2] Belen Vizcaino,[2] Luis M. Pallardo.[2] *[1]OSERCE-2 Study Investigators, Spain; [2]Nephrology, H. U. Dr Peset, Valencia, Spain.*

Background: Based on levels of 25-hydroxyvitamin D[25 (OH) D] required to suppress parathyroid hormone,guidelines have defined optimal vitamin D status at 25 (OH) D≥30ng/ml.Aware of the weakness of this recommendation,we conducted this post-hoc analysis using data from the OSERCE-2 study to evaluate thresholds for serum 25 (OH) D concentrations in relation to hard end-points such as death,kidney progression and hospitalization.

Methods: We studied 470 subjects participating in OSERCE-2 study, a prospective,multicenter study which enrolled 742 non-dialysis 3-5 stages CKD patients. In this re-analysis we excluded patients on treatment with active vitamin D,so 25 (OH) D levels reflected the effect of the exposure to vitamin D.Subjects were classified further into 3 groups by baseline 25 (OH) Dlevel:<20,21-29 and ≥30ng/mL,considering 25 (OH) D 21-29ng/ml as reference group.Deaths,kidney progression and hospitalizations were prospectively gathered over a 3-year period.To identify 25 (OH) Dlevels at highest risk for outcomes,ROC curves were performed.

Results: Over 29±12 months of follow up 46 (10%) patients dead,156 (33%) showed kidney progression,and 126 (27%) were hospitalized. After multivariate adjustment,Cox regression analysis showed 25 (OH) D<20 as an independent predictor of all-cause mortality[HR=2.327 (95%CI:1.102-4.912);p=0.027] and kidney progression[HR=2.456 (95%CI:1.628-3.706);p<0.001],whereas the group with 25 (OH) D≥30ng/ml did not have a different hazard for outcomes from the reference group.ROC curves identified 25 (OH) D levels at highest risk for death,kidney progression and hospitalization,at 17.4,18.6 and 19.0ng/ml, respectively.

Conclusions: 25 (OH) D<20ng/ml was an independent predictor of death and progression in patients with stage 3-5 CKD, with no additional benefits when patients reached the suggested levels by CKD guidelines. In the absence of clinical trials, and in line with the Institute of Medicine recommendations, our results suggest that 25 (OH) D levels >20ng/ml could be sufficient for CKD subjects.

Funding: Pharmaceutical Company Support - Abbvie, Private Foundation Support

FR-PO855

Vitamin D Status Determines the Dominance between FGF23 and PTH, which Affects Different Pattern of 1,25 (OH) $_2$D Decline in the Course of CKD Progression

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Background: Rise of FGF23 was found to precede that of PTH in the course of CKD progression, but many studies did not account for the confounding effect of vitamin D (25D) status. In this cross-sectional study, we studied the influence of 25D status on the dominance of two hormones, and consequently showed the different pattern of 1,25 (OH) $_2$D decline with the decrease in eGFR.

Methods: The study population consisted of 738 predialysis outpatients of nephrology department of two hospitals in Japan. Patients were classified as 25D insufficient (serum 25D levels <20ng/mL) and 25D replete (>=20ng/mL) groups. We utilized restricted cubic spine analysis to estimate the thresholds of eGFR at which each CKD-MBD marker increased or decreased.

Results: Most patients (87.5%) were in CKD stage 3 to 5. The median of age was 64, and the proportions of patients with diabetes mellitus and 25D insufficiency were 19.2% and 22.9%, respectively. Only 5.8% of patients received active vitamin D. In the state of 25D insufficiency, the dominant increase of PTH over FGF23 with the eGFR decline (each threshold of eGFR was about 50 and 30mL/min/1.73m$_2$, respectively) lead to stable 1,25 (OH) $_2$D levels in early CKD, while in 25D replete patients, the dominant increase in FGF23 over PTH (the slope of FGF23 was constantly positive across all CKD stages, while the threshold of eGFR of PTH elevation was about 60mL/min/1.73m^2) resulted in decrease in 1,25 (OH) $_2$D levels in early CKD. Vitamin D status did not affect 1,25 (OH) $_2$D levels in patients with eGFR<30mL/min/1.73m^2.

Conclusions: The 1,25 (OH) $_2$D level was stable in early CKD in the 25D insufficient group, while it decreased in the 25D replete group. This might be attributed to different patterns of elevation in two phosphatonins in CKD patients, and its clinical relevance remains to be elucidated.

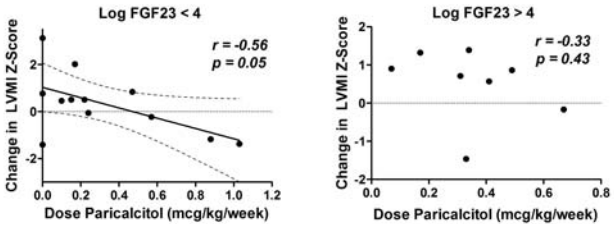
FR-PO856

Elevated FGF23 Levels Antagonize Paricalcitol Mediated Cardioprotection in Young Hemodialysis Patients Wacharee Seeherunvong,[1] Chryso P. Katsoufis,[1] Nao Sasaki,[2] Alcia D. Edwards- Richards,[1] Jayanthi Chandar,[1] Gaston E. Zilleruelo,[1] Phillip Ruiz,[3] Carolyn L. Abitbol,[1] Michael Freundlich.[1] *[1]Pediatric Nephrology, Univ of Miami/Holtz Children's Hospital, Miami, FL; [2]Pediatric Cardiology, Univ of Miami/Holtz Children's Hospital, Miami, FL; [3]Pathology, Univ of Miami, Miami, FL.*

Background: Elevated FGF23, a putative pro-hypertrophic and cardiotoxic hormone, associates strongly with left ventricular hypertrophy (LVH). Whereas administration of the calcitriol analog paricalcitol (Pc) attenuates cardiac hypertrophy, it also stimulates bone production of FGF23. We hypothesized that this paradoxical antagonism between the 2 hormones could be mediated through a therapeutic threshold depending on the degree of FGF23 elevation.

Methods: Twenty young HD patients (age 16±4yrs; dialysis vintage 30±16mos), receiving intravenous Pc, underwent sequential echocardiography (Echo) with assessment of LV mass index (LVMI) and LVH. C-terminal plasma FGF23 levels (RU/ml) were expressed as log-transformed values, and all other parameters were averaged over 6 months before each Echo (E1 and E2 time points).

Results: Between E1 and E2, log FGF23 remained unchanged (3.84 and 3.79, respectively) and correlated with serum phosphorus ($p<0.01$) and very robustly with calcium (both time points $r= 0.6$, $p<0.01$). LVH was unchanged in 50% of patients, with a 24% reduction in LVMI in 6/20 (30%) ($p<0.05$) between E1 and E2. The salutary effect of Pc in reducing LVMI was observed only when the log FGF23 levels were <4 ($r=-0.56$; $p=0.05$). This therapeutic effect was markedly blunted when FGF23 levels were >4.



Conclusions: In young HD patients, Pc treatment is cardioprotective within the therapeutic constraints of FGF23 stimulation and only when the log FGF23 level remains <4. Our findings provide a plausible explanation for this clinical paradox of hormonal antagonism.

FR-PO857

Effects of Active Vitamin D on Fibroblast Growth Factor-23 in Patients with Chronic Kidney Disease; Subgroup Analysis of the PACE Trial Seth Goldberg,[1] Daniel W. Coyne,[1] Mark D. Faber,[2] Stuart M. Sprague.[3] *[1]Washington Univ, St. Louis, MO; [2]Henry Ford Hospital, Detroit, MI; [3]NorthShore Univ HealthSystem -- Univ of Chicago, Chicago, IL.*

Background: In the development of chronic kidney disease-mineral bone disorder (CKD-MBD), dysregulated mineral metabolism with hyperphosphatemia accompanies the elevation in parathyroid hormone (PTH) concentrations. Fibroblast growth factor-23 (FGF-23) increases with progressive CKD, contributing to lower calcitriol levels and secondary hyperparathyroidism (SHPT). Low calcitriol levels, higher PTH, and higher FGF-23 are associated with increased mortality in CKD. Few human studies have compared the effect of active vitamin D analog treatment on FGF-23 levels in stage 3 and 4 CKD patients.

Methods: Data for the present study were from an open label phase 4 study of paricalcitol versus calcitriol for suppression of PTH in CKD stages 3 and 4 (Clinical Trial registry, NCT00823303). Levels of FGF-23 (Immunotopics 2nd generation C-terminal assay) were obtained at screening, 12 weeks, and 24 weeks. FGF-23 levels and their relationship to other markers of SHPT including PTH, albumin-corrected calcium, phosphorus, and urinary phosphorus, were analyzed.

Results: A total of 110 patients were randomized to receive study drug. Of these, 79 had at least one level of FGF-23 for analysis. The median (interquartile range) FGF-23 concentration increased from 240 (154, 382) RU/mL at baseline, to 392 (214, 555) RU/mL at 24 weeks ($p=0.002$). There were no significant differences in FGF-23 levels between groups at 24 weeks (366 (181, 536) RU/mL with paricalcitol, 423 (239, 566) RU/mL with calcitriol, $p=0.63$). FGF-23 levels correlated directly with the serum phosphorus ($p<0.001$) and urinary phosphorus/Cr excretion ($p<0.001$). There was no correlation between the FGF-23 levels and PTH at baseline, 12 weeks, or 24 weeks.

Conclusions: Treatment of SHPT with calcitriol and paricalcitol for 24 weeks increased FGF-23 levels similarly, with similar suppression of PTH (40-60%). As both agents treat CKD-related calcitriol deficiency and SHPT, while increasing FGF-23 and serum calcium, the effect of these agents on morbidity and mortality requires further trials.

Funding: Pharmaceutical Company Support - Abbott Pharmaceuticals

FR-PO858

Predictors of Circulating Cathelicidin during Acute Infection in Hemodialysis Patients Kristin M. Corapi,[1] Joseph James DeFerio,[1] Julia Beth Wenger,[1] Niels Borregaard,[2] Ishir Bhan.[1] *[1]Nephrology, Massachusetts General Hospital, Boston, MA; [2]Hematology, Univ of Copenhagen, Copenhagen, Denmark.*

Background: Plasma cathelicidin is a vitamin D regulated antimicrobial peptide that predicts mortality in end-stage renal disease (ESRD). Levels correlate with 25 (OH) vitamin D (25OH D) in healthy individuals. ESRD-associated alterations in vitamin D metabolism have the potential to alter the expression of cathelicidin. We performed an observational study of hemodialysis (HD) patients with and without acute infection to determine if cathelicidin associates with 25OH D or other markers of mineral metabolism in ESRD.

Methods: Patients were enrolled upon hospital admission. Blood was collected at the start of the first inpatient HD session. Cases were patients admitted with an acute infection and controls were admitted with a non-infectious illness. Demographics, routine laboratory parameters, and dialysis details were compared using univariate analyses. Spearman correlation was used to identify correlates of cathelicidin. Multivariable linear regression was used to adjust for possible confounders.

Results: Enrollment included 30 cases and 28 controls. The majority of subjects were male (66%) with a mean age of 65 years. Analyzing the entire sample, fibroblast growth factor-23 (FGF-23; $r=0.31$, $p=0.03$), vitamin D binding protein (DBP; $r=0.35$, $p=0.008$) and bioavailable vitamin D (BioD; $r= -0.35$, $p=0.007$), but not 25OH D ($r=-0.10$, $p=0.47$), were found to have significant correlations with plasma cathelicidin. Only DBP ($r= 0.53$, $p=0.003$) and BioD ($r= -0.39$, $p=0.03$) had a significant correlation with cathelicidin amongst the 30 cases with infection. In a multivariable linear regression model, DBP, but not BioD, remained a significant predictor of cathelicidin after adjusting for white blood cell count and FGF-23 level.

Conclusions: In ESRD, plasma cathelicidin is not associated with 25OH D levels, but correlates strongly with DBP, particularly in the setting of infection. Given DBP's recognized role as a potential immunomodulator, this may represent a novel downstream effect of DBP.

Funding: Pharmaceutical Company Support - Sanofi

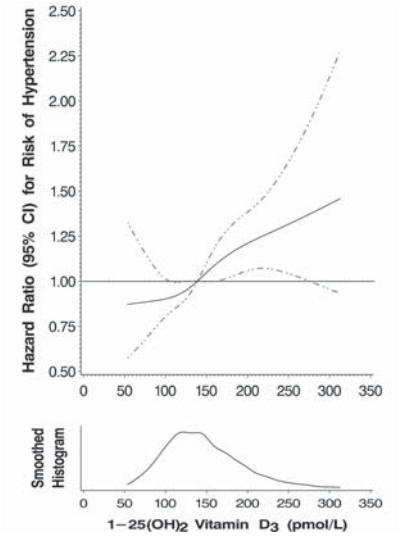
FR-PO859

Active Vitamin D 1,25-Dihydroxyvitamin D3 Is Associated with Risk of Hypertension Adriana J. van Ballegooijen,[1] Hiddo Jan Lambers Heerspink,[2] Dick de Zeeuw,[2] Marjolein Visser,[1] Ingeborg A. Brouwer,[1] Martin H. De Borst,[4] Ron T. Gansevoort,[4] Stephan J.L. Bakker,[4,5] Michel M. Joosten,[4,5] Ido Peter Kema.[3] *[1]Health Sciences, VU Univ, Amsterdam, Netherlands; [2]Dept of Clin Pharmacol, UMC Groningen, Netherlands; [3]Dept Clin Chem, UMC Groningen, Netherlands; [4]Dept Nephrol, UMC Groningen, Netherlands; [5]Top Inst Food and Nutrition, Wageningen, Netherlands.*

Background: Previous observational studies on the vascular effects of vitamin D have predominantly relied on its precursor -25-hydroxyvitamin D- while the active metabolite 1,25-dihydroxyvitamin D (1,25D) may be of more physiological relevance.

Methods: We prospectively studied the association between 1,25D and hypertension risk (blood pressure ≥140/90 mmHg, or initiation of blood pressure-lowering drugs) in 5,066 subjects aged 28-75 years, free of hypertension at baseline from the PREVEND Study, a well-defined, general population-based cohort with serial follow-up. We measured plasma 1,25D using liquid chromatography-tandem mass spectrometry.

Results: Mean±SD plasma 1,25D levels were 145±47.0 pmol/L. Lower 1,25D levels were more prevalent during winter and associated with lower 24h urinary calcium excretion, and estimated glomerular filtration rate (eGFR). During a median follow-up of 7.4 years (range 1.7-11.2), 1,036 participants (20.5%) developed hypertension. Each 1-SD increment (47.0 pmol/L) in plasma 1,25D was associated with a 11% greater risk of hypertension (HR 1.11; 95%CI, 1.04-1.19) after adjustment for potential confounders including eGFR and 24h urinary albumin excretion.



Conclusions: In contrast to previous inverse associations between its inactive precursor and hypertension risk, plasma 1,25D was positively associated with risk of hypertension. We hypothesize that higher circulating levels of active vitamin D may promote vascular calcification, which in turn could lead to increased arterial stiffness and a greater hypertension risk.

Funding: Government Support - Non-U.S.

FR-PO860

Sucroferric Oxyhydroxide Does Not Affect Oral Vitamin D Agonists: Post Hoc Analysis of Phase 3 Data Stuart M. Sprague,[1] Jaco Botha,[2] Viatcheslav Rakov,[2] Jürgen Floege.[3] *[1]NorthShore Univ Health System; [2]Vifor Pharma, Switzerland; [3]RWTH Univ Hospital Aachen, Germany.*

Background: This analysis evaluated potential interactions of sucroferric oxyhydroxide (SFO; VELPHORO®/PA21) and oral vitamin D receptor agonists (VDRAs) during a Phase 3 study of SFO versus sevelamer carbonate (SEV) and its extension study, using intact parathyroid hormone (iPTH) as a surrogate pharmacodynamic marker of VDRA activity.

Methods: 1,059 patients were randomized to SFO (1.0–3.0 g/day) or SEV (2.4–14.4 g/day) for 24 weeks. Eligible patients enrolled in a 28-week extension study. Three populations were analyzed: patients taking concomitant oral VDRAs (Pop. 1), those not taking concomitant VDRAs, either orally or intravenous (Pop. 2), and those treated with intravenous paricalcitol (Pop. 3). Populations were compared using a mixed-effects model to obtain least squares mean change in iPTH from baseline to study endpoint (Week 52).

Results: 505 patients were included. In Pop. 1 (oral VDRA), iPTH levels decreased from baseline to Week 52 in the SFO group, but increased in the SEV group (Table). In Pop. 2 (no VDRA) iPTH levels increased to a similar extent in both treatment groups. In Pop. 3, (intravenous paricalcitol) iPTH decreased similarly in both treatment groups (n=68; data not shown). Overall (Pop. 1 and Pop. 2 combined), changes in iPTH levels were not affected to a significant extent by any of the covariates analyzed (Table).

Table: Mixed-effect model analysis of change in iPTH from baseline to endpoint (Week 52) for Pop. 1 (N=187) vs Pop. 2 (N=250).

	Least squares mean [95% CI] (pmol/L)	P-value
SFO		
Pop. 1 ΔBL	−3.3 [−9.1; 2.6]	
Pop. 2 ΔBL	5.1 [0.3; 9.9]	
Pop. 1 vs Pop. 2	8.3 [1.1; 15.6]	0.024
SEV		
Pop. 1 ΔBL	8.6 [−0.4; 17.5]	
Pop. 2 ΔBL	4.9 [2.2; 12.1]	
Pop. 1 vs Pop. 2	−3.7 [−14.8; 7.5]	0.518
SFO vs SEV (Pop. 1 [oral VDRA])	−11.8 [−22.3; −1.4]	0.027
SFO vs SEV (Pop. 2 [no oral VDRA])	0.18 [−7.9; 8.3]	0.965
Covariates		
BL iPTH		0.689
BL phosphorus		0.088
Treatment		0.084
Prior calcium-based phosphate binder		0.385
Use of oral VDRA		0.420
Interaction between treatment and use of oral VDRA		0.074

BL, baseline; CI, confidence interval.

Conclusions: In contrast to SEV, results showed no apparent interaction between SFO and oral VDRAs as indicated by changes in iPTH.

Funding: Pharmaceutical Company Support - Vifor Pharma

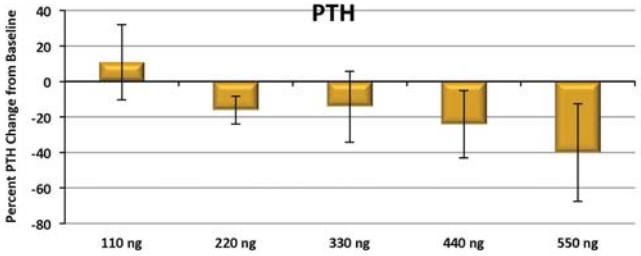
FR-PO861

2-Methylene-19-nor- (20S)-1a,25-Dihydroxyvitamin D3, a Promising New Therapeutic for Secondary Hyperparathyroidism Hector F. Deluca. *On Behalf of Phase 2 Study Sponsor and PI's, Deltanoid Pharmaceuticals, Inc., Madison, WI.*

Background: Vitamin D analogs alone or together with a calcium mimetic are used successfully to control secondary hyperparathyroidism in dialysis patients. However, both pharmaceuticals have significant side effects. Vitamin D compounds can cause unwanted hypercalcemia. Conversely, calcimimetics can produce hypocalcemia. We are developing a vitamin D analog (2-methylene-19-nor- (20S)-1a,25-dihydroxyvitamin D3 (2MD, DP001) that specifically localizes in the parathyroid glands and osteoblasts. At doses at least 10X lower than currently used vitamin D analogs, 2MD suppresses serum PTH in 5/6 nephrectomized rats and in post-menopausal women without a change in serum calcium.

Methods: A Phase 2A open label dose-finding trial was conducted in dialysis patients. Oral doses from 110 ng to 550 ng were administered after each dialysis session 3X/week for one month to 4-6 patients at each dose level. A second Phase 2B, randomized, placebo-controlled, double-blind study was conducted in 62 dialysis patients previously taking a vitamin D analog with or without calcimimetic co-therapy (Phase 2B). Each enrollee received either placebo or 2MD orally thrice weekly for 3 months. PTH, serum calcium and phosphorus levels were regularly measured throughout both studies.

Results: The Phase 2A study results showed that 2MD suppressed PTH in a dose-dependent manner at doses of 220 ng to 550 ng when given orally 3X/week at dialysis without any significant changes in serum calcium or phosphorus.



The in-life portion of the Phase 2B study has been completed and is awaiting database lock. Initial blinded results indicate no safety concerns. The results of these two trials will be reported.

Conclusions: Data to-date show 2MD is safe and will benefit dialysis patients with secondary hyperparathyroidism.

Funding: Pharmaceutical Company Support - Deltanoid Pharmaceuticals, Inc.

FR-PO862

A New Vitamin D Analogue VS-105 Regulates Parathyroid Hormone Secretion and Gene Expression in Human Parathyroid Cells Masafumi Fukagawa, Kaichiro Sawada, Genta Kanai, Takatoshi Kakuta. *Div of Nephrology, Endocrinology and Metabolism, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

Background: Active vitamin D sterols are almost routinely used for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease, however, development of hypercalcemia by the therapy causes serious problems such as vascular calcification. VS-105 (20R,24S-24-Methyl-2-methylene-19-nor-1a,25 (OH) 2-22-oxa-D3) is a synthetic new vitamin D analogue developed for the treatment of secondary hyperparathyroidism. We evaluated effects of VS-105 on gene expressions in cultured human parathyroid cells.

Methods: Parathyroid cells were prepared from surgically removed parathyroid glands from dialysis patients with informed consent. Mini-glands (spheroids) were formed with 10^5-10^6 cells (Kidney Int, 2009). VS-105 (10, 50 and 100 nM) and Paricalcitol (10, 50 and 100 nM) was added to wells respectively. The culture media containing reagents were exchanged every 24 hours for three days. We measured parathyroid hormone (PTH) levels secreted from the mini-glands to supernatants by ELISA. Expressions of mRNA of PTH, calcium-sensing receptor (CaR), vitamin D receptor (VDR) and CYP24A1 were analyzed by RT-PCR.

Results: VS-105 suppressed intact PTH secretion and PTH mRNA expression more effectively than Paricalcitol. The mean values and S.D. of secreted intact-PTH from cells treated with VS-105, Paricalcitol and Calcitriol were 2922.6 +- 826.6, 4218.3 +- 1484.5 and 6655.6 +- 1641.6 pg/ml/DNA microgram, respectively. Messenger RNA expression of CaR increased almost 1.8-folds by VS-105 and 1.5-folds by Paricalcitol as compared with vehicle. VS-105 did not significantly modified VDR mRNA expression, in contrast to Paricalcitol. VS-105 increased mRNA expression of CYP24A1 almost 1.9-folds, more efficiently than Paricalcitol.

Conclusions: VS-105 can be a potent therapeutic agent for severe hyperparathyroidism. Such different profiles of parathyroid gene expression may be helpful for developing more potent and safer agents for severe hyperparathyroidism in patients with chronic kidney disease.

Funding: Private Foundation Support

FR-PO863

A Novel Vitamin D Receptor Modulator, VS-105, Improves Bone Mineral Density in 5/6 Nephrectomized Uremic Rats J. Ruth Wu-Wong,[1] Yung-Wu Chen,[1] Jerry Wessale,[1] Maysaa Oubaidin,[2] Phimon Atsawasuwan.[2] *[1]Vidasym, Chicago, IL; [2]Univ of Illinois, Chicago, IL.*

Background: Mineral and bone disorder (MBD) begins early in the course of chronic kidney disease (CKD). Vitamin D is essential for bone health and vitamin D receptor modulators (VDRMs) are also commonly used to manage hyperparathyroidism secondary to CKD. Previous studies on VDRMs have often attributed their bone effects to enhanced intestinal calcium absorption and parathyroid hormone (PTH) suppression. It is not well studied whether VDRMs at non-hypercalcemic doses have effects on bone mineral density (BMD).

Methods: The 5/6 nephrectomized (NX) male Sprague Dawley rat with established uremia exhibits MBD with elevated PTH and reduced BMD. Previously we have shown that after 2-weeks of treatment (i.p. or p.o.) in 5/6 NX rats, VS-105 at non-hypercalcemic doses suppressed PTH and reduced cardiac fibrosis. In this study, the effect of VS-105 on BMD was evaluated in sham-operated and 5/6 NX rats.

Results: Treatment of 5/6 NX rats by VS-105 at two doses (0.05 and 0.2 μg/kg, i.p., 3x/week, for 8 weeks) significantly improved BMD at the lumbar vertebra (sham: 347 ± 21 mg/cm^2; NX/vehicle: 318 ± 7 mg/cm^2; VS-105 at 0.05 μg/kg: 348 ± 11 mg/cm^2; VS-105 at 0.2 μg/Kg: 377 ± 9 mg/cm^2). VS-105 at both doses significantly suppressed serum PTH even after dosing for one day, and the effect was maintained throughout the treatment period. Neither dose of VS-105 affected serum calcium, which was likely due to its lack of effects on inducing the expression of intestinal calcium transporter genes such as Calb3 and

TRPV6, and on stimulating intestinal calcium transport. In a mouse calvaria bone primary organ culture system, VS-105 was ~2-fold less effective than calcitriol in stimulating net calcium release from calvaria (a measurement of osteoclast activity).

Conclusions: These results demonstrate that VS-105 is effective in suppressing PTH and improving BMD in a dose range that does not affect serum calcium in uremic rats, and the improvement in BMD is not attributable to enhanced intestinal calcium absorption. The overall preclinical profile of VS-105 supports clinical development for its use in treating MBD in CKD patients.

Funding: Other NIH Support - The project was supported by grant number SBIR 1R43AR065247 from the NIH

FR-PO864

Cardiac Effect of Vitamin D Receptor Modulators in Uremic Rats

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Background: Vitamin D receptor (VDR) modulators (VDRMs) are commonly used to control secondary hyperparathyroidism (SHPT) associated with chronic kidney disease, and have been associated with beneficial cardiovascular disease outcomes. In this study we compared the cardiac effect of VS-105, a novel VDRM (Br J Pharm 164: 551, 2011), with that of paricalcitol in 5/6 nephrectomized (NX) uremic rats.

Methods: Male Sprague-Dawley rats were 5/6 nephrectomized and fed a normal diet for 4 weeks to establish uremia. Rats were then treated (i.p. 3 times/ week) with vehicle (propylene glycol), paricalcitol (0.025 mg/kg and 0.15 mg/kg), or VS-105 (0.05 mg/kg and 0.3 mg/kg) for 4 weeks.

Results: In uremic groups, both VDRMs (low and high doses) did not alter- serum creatinine and phosphorus levels. Serum calcium levels were significantly higher in the high dose paricalcitol group compared to sham. PTH levels were significantly decreased in the low dose paricalcitol and VS-105 groups, and were further reduced in the high dose groups. Interestingly serum FGF23 levels were significantly higher in the high dose paricalcitol group compared to sham whereas VS-105 did not show a significant effect on FGF23 levels. Left ventricle (LV) weight, and LV mass index determined by echocardiography was significantly suppressed in the high dose group for both VDRMs. The suppression was more evident in VS-105 treated animals. Western blotting showed significant decreases in fibrosis markers (TGF-β, type 1 collagen, and fibronectin) in the high dose group for both VDRMs (versus the NX-vehicle group). Masson trichrome staining showed a significant decrease in cardiac fibrosis in the high dose group for both VDRMs.

Conclusions: These results suggest that VS-105 is less hypercalcemic than paricalcitol with favorable effects on SHPT and cardiac parameters similar to paricalcitol in uremic rats. The anti-cardiac fibrosis effect was one of the noteworthy characteristics for VS-105.

Funding: Government Support - Non-U.S.

FR-PO865

High Phosphate Induce Hyperparathyroidism through COX2-PGE2-EP2 Pathway

Shensen Li, Qian Zhang, Haiming Li, Jing Chen. *Div of Nephrology, Huashan Hospital, Shanghai Medical College, Fudan Univ, Shanghai, China.*

Background: Secondary hyperparathyroidism (SHPT) has been proved to play a central role in CKD-MBD, but its pathogenesis is still not fully understood. The purpose of current study was to elucidate whether prostaglandin E2 (PGE2) and its type 2 receptor (EP2) mediate the pathogenic role of COX2 in uremic parathyroid gland and to identify the potential therapeutic targets in SHPT.

Methods: PTG samples were obtained from parathyroidectomy surgery of uremic patients. *In vitro*, freshly excised PTG tissue were incubated in following groups: (1) normal phosphate (1mM), (2) high phosphate (HP) (2-8mM), (3) HP (4mM) with COX2 inhibitor (NS398 10^{-6}mM) or EP2 receptor antagonist (AH6809 10^{-7}mM), (4) PGE2 (10^{-5}-10^{-7}mM), (5) EP2 receptor agonist (butaprost 10^{-5}-10^{-7}mM). *In Vivo*, experiments were conducted in male SHPT Sprague-Dawley rats. By means of a small neck surgery, lentiviral carried EP2 shRNA ($1x10^{7}$ IU/ml) were implanted in the parathyroid of SHPT rats.

Results: Compared to normal PTG, the obviously enhanced immunoreactivities of COX2, mPGES1 and EP2 were detected in hyperplastic PTGs from 20 uremic patients. In cultured PTG tissues, COX2 inhibitor or EP2 antagonist significantly inhibited HP-induced iPTH secretion to 62±0.9% or 67±1.3%, and PCNA protein expression to 64±2.2% or 70±3.4%, respectively ($P<0.05$). Meanwhile, PGE2 or butaprost directly induced a dose-dependent high expression of iPTH and PCNA. In vivo study,EP2 shRNA lentivirus down-regulated EP2 gene expression by 67.7±4.5% in PTG, and significantly reduced serum iPTH levels to 21.8±5% and PTGs size to 72±4.8% ($P<0.05$). No significant differences were found in serum levels of creatinine, BUN, calcium, phosphorus or end-products of prostaglandins metabolism between each group of uremic rats.

Conclusions: Inhibition of COX2-PGE2-EP2 pathway significantly reduced the secretion of PTH and PTG cell proliferation *in vitro*, while PGE2 and EP2 agonist directly stimulated such reactions. Knocking down EP2 receptor expression in parathyroid significantly attenuated serum iPTH elevation and PTG proliferation in uremic rats. These data suggested that the COX2-PGE2-EP2 pathway promoted SHPT and parathyroid hyperplasia in ESRD.

Funding: Government Support - Non-U.S.

FR-PO866

Primary Hyperparathyroidism in Mice due to Deletion of the Gene Encoding the Transient Receptor Potential Canonical Type 1 (TRPC1) Channel: A Model of Metabolic Syndrome with Chronic Kidney Disease (CKD)

Bonnie Eby,[1] Alexander Lau,[1] Lindsay J. Barron,[1] Leonidas Tsiokas,[2] Kai Lau.[1,3] *[1]Medicine, Univ of Oklahoma Health Sciences Center, OKC, OK; [2]Cell Biology, Univ of Oklahoma Health Sciences Center, OKC, OK; [3]Medicine, VAMC, OKC, OK.*

Background: In parathyroid glands, the TRPC1 protein complexes with stromal interacting molecule 1 (STIM1) and ORAI1, the Ca release activated Ca channel modulatory protein 1, to mediate store-operated Ca entry (SOCE). We and others reported that activating mutations of STIM1 or ORAI1 produce hypocalcemia and the Stormorken syndrome. Inactivating mutations of STIM1, ORAI1, or TRPC1 could produce the opposite. We tested the hypothesis that TRPC1 deficiency impairs SOCE, reduces cell Ca, stimulates PTH and causes hypercalcemia.

Methods: In male littermates born to +/- parents, metabolic, clearance and ultrasound studies were done. Ca and lipids were measured by published methods, creatinine by HPLC, and PTH by a mouse 1-84 ELISA Kit.

Results: Nulls were 10% fatter versus +/- and wt from 4 to 30th week. Fasting triglyceride was 40% higher in null versus wt. Fasting cholesterol was 65% higher in null and +/-. From 5th-10th mon, fasting serum (S) Ca was 6-7% higher in both null and +/- ($p<0.03$ for both). At 12.5 mon, non-fasted SCa was higher in null (11.4 mg%) and +/- (11.2 mg%) versus wt (10.2 mg%) ($p<0.001$ for both). Despite hypercalcemia, [PTH] (pg/ml) was higher in null than wt (310 versus 218, $p<0.05$) and not suppressed in +/- (259). Hematocrit was lower in null (11%) and +/- (8%). SP was similar at 9 and 12 mon for all 3 groups. At 12 mon, liver density was 30% higher in null versus +/- and wt. Albuminuria was 65% heavier in null. Kidney volume was 20% lower. At 16 mon, creatinine clearance was down by 44% in null ($p<0.02$) and 40% in +/- ($p<0.05$).

Conclusions: 1.TRPC1 null mice exhibit the metabolic syndrome (obesity, fatty liver and dyslipidemia shown here and insulin resistance and glucose intolerance shown earlier). 2. Haploid deficiency produces hypercalcemia and impairs PTH suppression as diploid deficiency produces hypercalcemia and excess PTH, data in support of the stated hypothesis. 3. Renal failure emerges by 16 mon in both haploid and diploid deficiency, making them useful CKD models.

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

FR-PO867

mTOR Pathway Activation Is Crucial to Parathyroid Cell Proliferation in Both Experimental Uremia and Acute Kidney Injury and Is Enhanced in the Parathyroids of CKD Patients

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Background: Parathyroid cell proliferation is central to secondary hyperparathyroidism (SHP). The signal transduction pathways for parathyroid cell proliferation are not defined. We have previously presented that the mTOR pathway is activated in the parathyroids of SHP rats and that the mTOR inhibitor rapamycin decreases parathyroid mTOR activation and prevents parathyroid cell proliferation. Rapamycin also decreased parathyroid cell proliferation in vitro in parathyroid organ cultures. Ribosomal protein S6 (rpS6) is a downstream target of the mTOR pathway. We now show that mTOR activation is essential for the high levels of PTH in folic acid induced acute kidney injury (AKI). We also show for the first time that mTOR is activated in human parathyroids from patients with SHP.

Methods: Knock-in mice, whose rpS6 is unphosphorylatable due to substitution of all five phosphorylatable serines to alanines ($rpS6^{P-/-}$) and wild type ($rpS6^{P+/+}$) mice were injected with folic acid to induce AKI. Archival human parathyroid sections from dialysis patients with SHP and control parathyroids removed at thyroparathyroidectomy were studied by immunofluorescence for phosphorylated rpS6 and proliferation markers, PCNA and Ki-67.

Results: rpS6 is a downstream target of the mTOR pathway. Knock-in $rpS6^{P-/-}$ and wild type mice were injected with folic acid to induce AKI. AKI led to the expected increase in serum PTH in $rpS6^{P+/+}$ wild type mice but significantly less so in the $rpS6^{P-/-}$ mice demonstrating the mTOR activation is essential for the pathogenesis of SHP. Parathyroids from CKD patients with SHP showed increased staining for phosphorylated rpS6 compared to control parathyroids. Moreover, phosphorylated rpS6 co-localized with PCNA, a marker of cell proliferation.

Conclusions: mTOR is an essential regulator of parathyroid cell proliferation and it exerts this effect through rpS6 phosphorylation. Importantly, mTOR is activated in human CKD parathyroids and co-localizes with PCNA in proliferating cells. Therefore, mTOR is central to the pathogenesis and regulation of parathyroid cell proliferation in SHP.

Funding: Government Support - Non-U.S.

FR-PO868

Characterization of the PTH-Responsive Phosphoproteome in the Proximal Tubule

Rebecca Murray,[2] Michael Merchant,[3] Syed J. Khundmiri,[2,3] Barbara Clark,[4] Eleanor D. Lederer.[1,2,3] *[1]Robley Rex VAMC, Louisville, KY; [2]Physiology & Biophysics, Univ of Louisville, Louisville, KY; [3]Medicine, Univ of Louisville, Louisville, KY; [4]Biochemistry, Univ of Louisville, Louisville, KY.*

Background: PTH is a key regulator of the expression and function of the type IIa sodium phosphate cotransporter (Npt2a), the protein responsible for regulated renal phosphate reabsorption. We have previously shown that PTH induces rapid destabilization

of Npt2a mRNA through both PKA- and PKC-dependent mechanisms. We hypothesize that PTH regulates Npt2a mRNA through phosphorylation of an RNA-binding protein (RBP), resulting in enhanced Npt2a mRNA degradation.

Methods: To address this aim, we treated opossum kidney (OK) cells, a PTH-sensitive proximal tubule cell culture model, with 100nM PTH for 30m and 2h, followed by mass spectroscopy characterization of the PTH-stimulated phosphoproteome.

Results: We identified 1083 proteins whose phosphorylation status significantly changed in response to PTH, including several RBPs. Through computational analysis, we identified two phospho-RBPs (Roquin-2 and KHSRP) with predicted binding sites for the 3'-UTR of Npt2a mRNA. Roquin-2 phosphorylation was significantly increased at both the 30m and 2h time points, whereas KHSRP phosphorylation was significantly increased only at the 2h time point. Western blot analysis confirmed expression of both proteins in OK cells and showed increased expression of both of these proteins in response to PTH, as well as decreased expression in response to treatment with low phosphate media, an upregulator of Npt2a expression. Immunoprecipitation of KHSRP from control and PTH-treated OK cell lysates, followed by RNA isolation and qRT-PCR, showed Npt2a mRNA was pulled down with KHSRP.

Conclusions: We conclude that Roquin-2 and KHSRP are potential mediators of PTH-induced changes in Npt2a expression. Funding provided by VA to EDL.

Funding: Veterans Affairs Support

FR-PO869

An Immuno LC MS/MS Assay for Parathyroid Hormone Demonstrates Variable Oxidation of PTH Peptides: Implications for PTH Assay Li Cui,[1] Gavin E. Reid,[1,2] Fabrizio Bonelli,[3] John Wall,[3] Angela L. Podgorski,[3] Marie D. Philipneri,[4] Anjana S. Jagalur,[4] Amy L. Krieg,[4] Mustafaa S. Mahmood,[4] Kevin J. Martin.[4] *[1]Dept of Chemistry, Michigan State Univ, East Lansing, MI; [2]Dept of Biochemistry and Molecular Biology, Michigan State Univ, East Lansing, MI; [3]Diasorin Inc, Stillwater, MI; [4]Div of Nephrology, Saint Louis Univ, St. Louis, MO.*

Background: Assay of PTH has been historically problematic due to variable standardization, variable cross reactivity with PTH fragments and variable correlations with biological parameters. A recent demonstration that PTH peptides may be oxidized further complicates these issues since oxidized PTH has no biological activity.

Methods: To characterize this issue further an immuno-LC MS/MS assay was developed using stable isotope labelled PTH and oxidized synthetic peptide internal standards to analyze the nature of circulating PTH peptides and their degree of oxidation and to examine the effects of PTH oxidation on immunochemiluminescent PTH assays. Two series of samples from patients on hemodialysis were obtained and analyzed.

Results: In Set A, oxidized PTH 1-84 and oxidized PTH 7-84 were commonly observed. The oxidized peptides ranged from 0 to 80% of the total PTH peptides. Of 41 samples, 34 of 41 had more than 10% oxidized PTH peptides. In general, there was more oxidized PTH 7-84 than oxidized PTH 1-84. In contrast, in Set B, only minimal amounts of oxidized PTH peptides were observed and only 3 of 29 patient samples had more that 10% oxidized PTH peptides.

Conclusions: The marked difference in the amount of oxidized PTH peptides between Set A and Set B suggests that oxidation of PTH may be occurring ex-vivo. Further studies are required to fully define the possibility of ex-vivo oxidation and the contributing factors. The immuno-LC MS/MS results correlated extremely well with the assay of PTH 1-84 and less well with other "intact PTH" assays. The effects of oxidation of PTH peptides on the assay of PTH by currently used intact PTH assays may represent an additional factor contributing to the variability of PTH assay results and their correlation with biological parameters.

Funding: Pharmaceutical Company Support - Diasorin

FR-PO870

Common Genetic Variants Associate with Circulating Parathyroid Hormone Concentration Cassianne Robinson-Cohen,[1] Pamela L. Lutsey,[2] Yuri Milaneschi,[4] Carrie M. Nielson,[3] Braxton D. Mitchell,[5] Elizabeth Selvin,[6] Josef Coresh,[6] Caroline S. Fox,[7] Ian H. de Boer,[1] Bryan R. Kestenbaum.[1] *[1]Kidney Research Inst, U of Washington; [2]U of Minnesota; [3]OHSU; [4]VU Univ Medical Center/GGZ inGeest; [5]U of Maryland; [6]Johns Hopkins Univ; [7]NIH/NHLBI.*

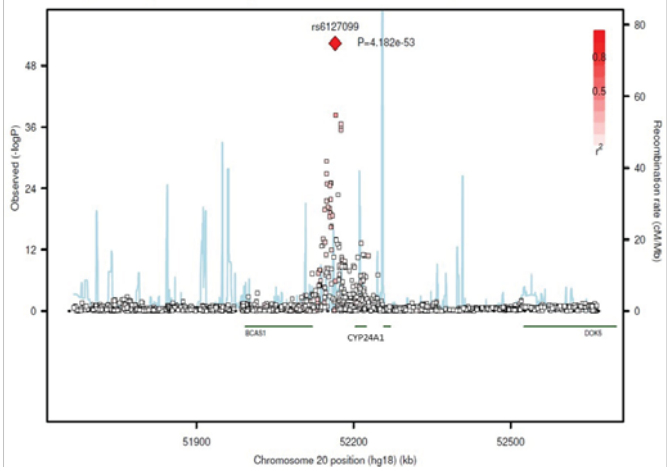
Background: Parathyroid hormone (PTH) is commonly elevated in CKD patients and is associated with uremic bone disease. PTH is regulated by calcium, phosphate, and 1,25-dihydroxyvitamin D; however, substantial inter-individual variation in serum PTH levels remains unexplained. We conducted the first genome wide association study to investigate associations of common genetic variants with serum PTH concentrations.

Methods: We studied 22,650 participants of European ancestry from nine population-based cohort studies. We excluded participants with eGFR <30 ml/min/1.73m^2. We used a log-based model to account for differences in PTH assays across studies and we adjusted for age, sex, and principal components of ancestry.

Results: Loci from 5 independent regions reached genome-wide significance (P <5 x 10^{-8}), for associations with serum PTH, including loci near CYP24A1 (rs6127099, P=4.2E-53), SLC34A1 (P=6.6E-17), CLDN14 (P=3.5E-16), RTDR1 (P=8.7E-9) and CASR genes (P=4.8E-8). Identified loci had minor allele frequencies between 7-40%. For the top single nucleotide polymorphism near CYP24A1, each additional copy of the minor allele was associated with an estimated 7% lower serum PTH concentration. Independent replication efforts and further characterization of these loci for associations with clinical hyperparathyroidism are ongoing.

Conclusions: Common genetic variants located near vitamin D metabolism, calcium sensing, and phosphate transport genes are associated with differences in serum PTH concentrations. The functional relationship of other identified loci with PTH metabolism awaits identification.

Figure 1. Regional association plot of rs6127099 and surrounding variants with serum PTH



Funding: NIDDK Support, Other NIH Support - NHLBI, Government Support - Non-U.S.

FR-PO871

Analysis of Hyperparathyroidism and Its Treatment in Patients with Non-Dialysis Chronic Kidney Disease Thilini Nishani Abeygunaratne, Darren Green, Diana Chiu, Philip A. Kalra. *Salford Vascular Research Group, Univ of Manchester, Manchester, United Kingdom.*

Background: Abnormalities of chronic kidney disease-mineral bone disease (CKD-MBD) occur in early stages of CKD and guidelines remain vague as to its treatment. Aim of this study was to observe the prevalence of CKD-MBD abnormalities in a non-dialysis CKD population according to the NKF- KDOQI guidelines, and to assess frequency of use of phosphate binders and vitamin D analogues in its treatment.

Methods: A sub study of a prospective longitudinal cohort study of non-dialysis patients requiring CKD management in secondary care. HPTH was defined according to NKF KDOQI guidelines (stage 3: 35-75 pg/ml:stage 4: 80-100 pg/ml: stage 5: 150-300 pg/ml). Given some patients had treated HPTH, patients within "normal PTH range" were further evaluated.

Results: The 1667 patients had a mean age of 64.6±14.3 years, eGFR 32.5 ±16ml/min/1.73 m^2,65% males. The mean PTH was 95±89.4 pg/ml and higher PTH and HPTH prevalence was observed in more advanced CKD.

	All Patients	Stage 3a	Stage 3b	Stage 4	Stage 5
N	1667	276 (17%)	485 (29%)	621 (37%)	285 (17%)
Age (yrs)	64.6±14.3	62.6±13.6	65±13.8	66.4±13.9	62.8±15.5
eGFR (ml/min/1.73^2)	32.5±16	51.1±4	36.6±4.18	21.9±3.9	11.7±4.5
PTH (pg/ml)	95±89.4	49±34.1	64±47.3	113±97.6	153±115.9
Phosphate binders: all	8.5%	5.8%	4.3%	9%	16.8%
Ca containing (%)	7.7%	5.4%	4.1%	8.2%	14.7%
Vitamin D Analogues	21.5%	8%	13.2%	27.9%	35.1%
PTH:Below Range	699 (41.9%)	120 (43.5%)	139 (28.6%)	273 (44%)	167 (58.6%)
EuPTH:within range	464 (27.8%)	105 (38%)	186 (38.4%)	85 (13.6%)	88 (30.9%)
HPTH	504 (30.3%)	51 (18.5%)	160 (33%)	263 (42.4%)	30 (10.5%)

Conclusions: This observational study of an unselected cohort of patients at different stages of CKD shows that phosphate binder use was low (<10%) except in stage 5 CKD patients (17%); vitamin D analogue use increased progressively across the stages. These results only represent a cross sectional analysis, and therefore not possible to define HPTH according to KDIGO, which depends upon a PTH above the upper limit of normal as well as a rising PTH. Further analysis with serial PTH measurements will increase the value of the study.

FR-PO872

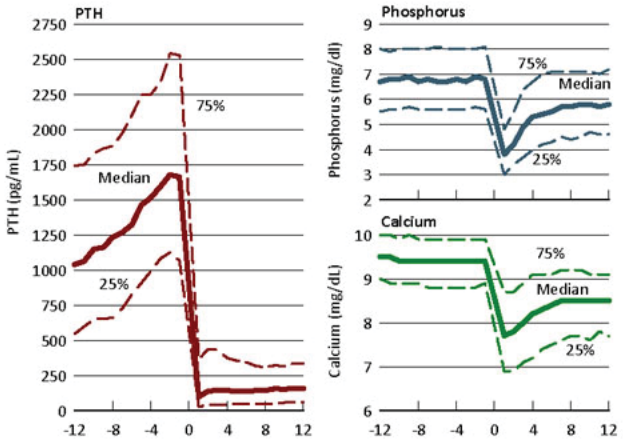
Changes in PTH, Calcium, and Phosphorus Levels after Parathyroidectomy in Patients on Hemodialysis James B. Wetmore,[1] Jiannong Liu,[1] Thy P. Do,[2] Kimberly Lowe,[2] Areef Ishani,[3] Brian D. Bradbury,[2] Geoffrey A. Block,[4] Allan J. Collins.[1] *[1]Chronic Disease Research Group, Minneapolis, MN; [2]Center for Observational Research, Amgen, Inc., Thousand Oaks, CA; [3]Minneapolis VA Health Care System, Minneapolis, MN; [4]Denver Nephrology Clinical Research Div, Denver, CO.*

Background: Little is known about how levels of parathyroid hormone (PTH), calcium (Ca), and phosphorous (P) change after parathyroidectomy (PTX) in hemodialysis patients.

Methods: Medicare Parts A/B claims were combined with monthly lab data from DaVita, Inc., to assess PTH, Ca, and P levels in the years before and after PTX. The study cohort comprised all patients who underwent PTX in 2007-09, were aged ≥ 18 yrs, had Medicare Parts A/B as primary payer, and had been receiving HD for ≥ 1 year before PTX. PTX was identified from ICD-9 inpatient procedure codes.

Results: Among 1402 PTX patients, mean (±SD) age was 48.9 ± 13.0 years, 52.4% were male, 58.8% were African-American, and mean dialysis duration was 7.5 ± 4.6 years. PTH increased over the year before PTX from a median of 1039 pg/mL to 1661 pg/mL; levels were > 2500 pg/mL for 25% immediately prior to PTX. After PTX, PTH decreased to a median of 98 pg/mL at 1 month, but values were highly skewed, with a mean of 326 ± 535 pg/mL. Ca fell substantially after PTX, from a median of 9.4 mg/dL to 7.7 mg/dL at 1 month post-PTX; levels were ≤ 6.9 mg/dL for 25%. At 3 months, median Ca was 8.0 mg/dL; levels were ≤ 7.1 mg/dL for the lowest 25%. Median P immediately prior to PTX was 6.8 mg/dL, stabilizing at a median of 5.8 mg mg/dL one year after PTX.

Conclusions: Patients had very high PTH levels at the time of PTX. After PTX, levels fall below recommended goals for many patients, but also remain high for many. A substantial number of patients experienced strikingly low Ca levels after PTX, which can be sustained for several months.



FR-PO873

Risk Factors and Long-Term Outcome Among Dialysis Patients with Parathyroidectomy: A Population-Based Cohort Study in Taiwan Wei-Chih Kan,[1,2] Chih-Chiang Chien.[1,2] *[1]Nephrology, Chi-Mei Medical Center, Tainan, Taiwan; [2]Medical Laboratory Science and Biotechnology, Chung Hwa Univ of Medical Technology, Tainan, Taiwan.*

Background: Parathyroidectomy (PTX) is a common-used surgery in dialysis patients with severe hyperparathyroidism. Risk factors for high-risk group of PTX and their long-term outcome are inconclusive. The study aims to identify the risk factors for PTX in dialysis patients and survey for their long-term outcome after PTX.

Methods: We conducted an observational cohort study to investigate the risk factors and long-term outcome in dialysis patients with PTX, based on Taiwan's National Health Insurance claim data between 1999 and 2008, and 35,162 dialysis patients was enrolled for this study. Several risk factors possibly affecting mortality were analyzed with Cox proportional hazards models. Kaplan-Meier method was used to calculate the incidence of PTX and the survival rate after PTX.

Results: In Taiwan, The PTX rate was 8.10 per 1,000 patient-years between 1999 and 2008. Stratified on the basis of sex and diabetic mellitus (DM), the highest incidence rate of PTX was in the group of females without DM. After stratified by age and DM, the highest incidence rate of PTX was in those patients with aged 18-44 years without DM. The risk factors for PTX were younger age, female (hazard ratio (HR) 1.409, DM (HR 0.479), peritoneal dialysis (HR 1.657) and hypertension (HTN) (HR 1.317). The cumulative survival rates after PTX were 97.1%, 94.5%, 82.8% and 77.4% at the 1st, 2nd, 5th and 7th year, respectively. Only age was significantly associated with higher mortality after PTX via multivariable analysis.

Conclusions: High-risk group of PTX in dialysis patients were female, young-aged, non-DM, HTN, and patients on peritoneal dialysis. Older age was associated with a higher mortality after PTX.

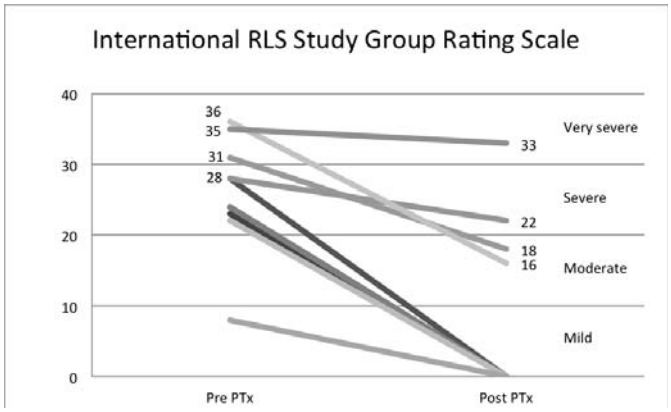
FR-PO874

Effect of Parathyroidectomy on Sleep Disorders: Is There Any Improvement? Roberto S.S. Santos,[1] Fernando Coelho,[2] Bruno C. Silva,[1] Vanda Jorgetti,[1] Rosa M.A. Moyses,[1] Rosilene M. Elias.[1] *[1]Nephrology, Univ de São Paulo, São Paulo, Brazil; [2]Neurology, Univ Federal de São Paulo, São Paulo, Brazil.*

Background: Secondary hyperparathyroidism (SHPT) is associated with complications that are not limited to the skeleton and include sleep disorders. However, there is no data based on polysomnography in these patients. In addition, little research has been made into the effects of parathyroidectomy (PTx) on sleep disorders. This prospective study was devised to investigate whether PTx can influence sleep disorders in end-stage renal disease patients on hemodialysis with SHPT.

Methods: We evaluated 18 patients (6 men, aged 48±11 years) who underwent standard PSG immediately pre and within 12 weeks post PTx. Restless Leg Syndrome (RLS) diagnosis and rating scale were accessed using the International RLS Study Group.

Results: There was a decrease in serum parathyroid hormone (PTH), calcium, phosphate, and alkaline phosphatase after PTx. Ten patients (56%) had RLS before surgery and only 4 (22.2%) remained with this diagnosis post PTX (p=0.04), accompanied by a decrease in rating scale, figure1. Patients with RLS presented higher phosphate and higher severity of periodic leg movements of sleep/h (PLMI) at baseline (p=0.008 and 0.004, respectively). PSG data are shown in table1. There was no improvement in apnea-hypopnea index (AHI) or any other sleep parameter.



PSG data	Pre PTx	Post PTX	p
Total sleep time, min	328±75	342±73	0.505
NREM, %	83 (79, 88)	83 (78, 86)	0.164
REM %	16 (12, 21)	17 (14, 22)	0.264
AHI	8.9 (2.3, 20.6)	12 (5.4, 20.4)	0.378
PLMI	6.1 (0,29)	14.7 (0,43)	0.098

Conclusions: PTx did not improve sleep architecture or sleep-disordered breathing. However, PTx provided an opportunity to improve RLS, a disabling condition for which usually only symptomatic treatment is available. Whether RLS may be improved by reduction of serum phosphorus or PTH merits further investigation.

Funding: Government Support - Non-U.S.

FR-PO875

The Calcimimetic R568 Effectively Prevents the Loss of Choroid Plexus and Parathyroid Klotho in Chronic Kidney Disease M. Vittoria Arcidiacono, Sandra De la Fuente, Petya Valcheva, Sabrina De Gaspari, Adriana S. Dusso. *IRBLleida, Spain.*

Background: Progressive loss of renal and parathyroid (PT) Klotho is a main determinant of the severity of renal and vascular lesions in chronic kidney disease (CKD). However, the impact of CKD on choroid plexus (CP) Klotho, essential for neuronal and cognitive function, is unknown. Our prior studies in mouse CKD showed that the calcimimetic R568 improved motor-neuron function by preventing CKD-induced increases in monocyte ADAM17, the cause of TNFa-driven inflammation. Since inflammation is a CKD-independent suppressor of Klotho expression and vitaminD is an inducer of the Klotho gene, this work compared R568 and vitaminD efficacy to prevent CKD-induced Klotho loss.

Methods: Two months after 75% nephron reduction (NX), mice received either R568 (s.c. 60 mg/g body weight), 25-hydroxyvitaminD (25D, i.p. 80 ng weekly) + paricalcitol (P, i.p. 16 ng thrice weekly) or the combination for 6 weeks. Tissue Klotho was measured by immunohistochemistry.

Results: Similar to renal and PT Klotho, CP Klotho was markedly reduced in untreated uremic mice. Surprisingly, R568, which had no effect on renal Klotho levels, completely prevented CKD-induced reductions in PT ($p<0.02$) and CP ($p<0.015$) Klotho at a dose ineffective to suppress PTH. Also, 25D+P, which effectively prevented CKD-induced elevations in serum PTH ($p<0.01$) and reductions in renal Klotho ($p<0.02$), had no effect on PT or CP Klotho. CKD-induced PT hyperplasia, the cause of reductions in vitaminD receptor (VDR), could partially account for vitaminD inefficacy to maintain PT Klotho. To test this possibility, we compared PT Klotho content after 14 weeks of NX in wild type mice and transgenic littermates with a PT specific EGFR-inactivation that prevents both PT gland enlargement and VDR loss. PT Klotho was similar in mice of both genotypes, supporting an abnormal vitaminD induction of PT Klotho in CKD.

Conclusions: In CKD, loss of CP Klotho may contribute to impair neuronal and cognitive function. The distinct efficacy of vitaminD and R568 treatment in preventing renal or PT/CP Klotho suggests the importance of revisiting current strategies to attenuate CKD-accelerated aging also beyond mineral bone disorders.

FR-PO876

Treatment with Cinacalcet Increases Plasma Adiponectin Concentration in Hemodialysed Patients with Chronic Kidney Disease and Secondary Hyperparathyroidism Marcin Adamczak,[1] Piotr Kuczera,[1] Grzegorz Machnik,[2] Boguslaw Okopien,[2] Andrzej Wiecek.[1] *[1]Dept of Nephrology, Endocrinology and Metabolic Diseases, Medical Univ of Silesia, Medical School in Katowice, Katowice, Poland; [2]Dept of Internal Medicine and Clinical Pharmacology, Medical Univ of Silesia, Medical School in Katowice, Katowice, Poland.*

Background: Cinacalcet increases the sensitivity of calcium receptor (CaR) to circulating serum calcium. CaR is expressed among others also in adipocytes. Adiponectin is a beneficial adipokine with antiatherogenic and insulin-sensitizing properties. The aim of this study was to assess the influence of 3-month cinacalcet therapy on plasma adiponectin concentration in hemodialysed patients with chronic kidney disease (HDP) and secondary hyperparathyroidism (sHPT).

Methods: In 65 HDP [38 males, 27 females; mean age 53.6 (50.0-57.1) years] with sHPT treated with cinacalcet (30-120 mg/day) plasma adiponectin, advanced oxidation protein products (AOPP), serum interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations were assessed before the first dose of cinacalcet and after 3 months of treatment. The results are shown as means and 95% confidence index.

Results: A significant decrease in serum parathormone (PTH) concentration - from 1089 (891-1286) pg/ml to 775 (574-976) pg/ml was found after 3 months of treatment with cinacalcet ($p<0.0001$). The treatment was associated with a significant ($p=0.048$) increase in plasma adiponectin concentration from 16.9 (14.4-19.5) µg/ml to 17.8 (15.0-20.6) µg/ml. Significant ($p=0.03$) reduction of plasma AOPP concentration was observed – from 186.7 (156.7-216.7) pg/ml to 162.6 (141.2-183.9). BMI and mean concentration of serum markers of inflammation were stable during the entire treatment period.

Conclusions: 1. Three months treatment with cinacalcet in haemodialysed patients with sHPT leads to increased plasma adiponectin concentration. 2. Increased adiponectinemia may be related to the reduction of oxidative stress and may lead to reduction of cardiovascular mortality and morbidity.

Funding: Government Support - Non-U.S.

FR-PO877

Cinacalcet Hydrochloride Promotes G1 Phase Transition From G0 Quiescent State and Lead to Apoptotic Pathway of Parathyroid Cells Takayo Miyakogawa, Kaichiro Sawada, Genta Kanai, Ryoko Tatsumi, Takatoshi Kakuta, Masafumi Fukagawa. *Div of Nephrology, Endocrinology and Metabolism, Tokai Univ School of Medicine, Isehara, Japan.*

Background: Recent studies reported that cinacalcet promotes apoptosis of parathyroid cells. We previously reported that, compared to patients treated without cinacalcet, frequencies of both apoptosis (TUNEL positive) and proliferation (Ki-67 positive) of parathyroid cells increased significantly in parathyroid glands (PTGs) of patients treated with cinacalcet.

Methods: Paraffin sections of PTGs from patients treated with/without cinacalcet were reacted with anti-c-myc and anti-p27Kip1 antibodies. RNAs were recovered from sections and used for real time RT-PCR with primers for c-myc and $p27^{Kip1}$. Parathyroid cells were collected from PTGs and cultured with media containing 100 nM cinacalcet hydrochloride. Real time RT-PCR was performed as above with RNAs from a part of cultured cells. Remaining cells were stained with PI and DNA contents were analyzed by flow cytometry.

Results: We found that cells expressing c-myc appeared and cells expressing $p27^{Kip1}$ decreased its amount in PTGs from cinacalcet treated patients. As these genes are known to control G0-G1 phase transition in cell cycles, our findings suggested that cinacalcet treatment may activate cell cycles by accelerating the transition of cells from G0 phase into G1 phase. These changes of expressions of c-myc and $p27^{Kip1}$ were reproduced in *in vitro* parathyroid cells cultured with cinacalcet. However, the flow cytometry analysis of these cultured cells revealed only a little increase of ratios of cells in S phase (from 3.51% to 4.69%) and G2 phase (from 1.86% to 2.66%).

Conclusions: Although cinacalcet promotes transition of parathyroid cells from G0 phase (Ki-67 negative) into G1 phase (Ki-67 positive), we estimate that most of cells in G1 phase could not get over some checkpoints of cell cycles before mitosis, and they are driven to die (TUNEL positive). Transitory entry into G1 phase may be necessary for parathyroid cells in G0 phase to proceed to apoptosis pathway. Volumes of PTGs may be controlled with these mechanisms through signals from calcium-sensing receptors.

FR-PO878

Cinacalcet for Hypercalcemic Hyperparathyroidism after Renal Transplantation: A Long-Term Prospective Self-Controlled Study Ursula Thiem,[1] Kyra Borchhardt.[1,2] *[1]Nephrology, Med Univ of Vienna, Vienna; [2]Dialysis Inst Klagenfurt, Austria.*

Background: Several short-term studies demonstrated successful control of hypercalcemic hyperparathyroidism after renal transplantation with cinacalcet. As long-term studies in kidney transplant recipients (KTRs) are lacking, we herein describe our long-term experience with this drug.

Methods: In this prospective open-label self-controlled study, 44 stable KTRs with hypercalcemic hyperparathyroidism were treated with cinacalcet and its effects on biochemical parameters of mineral metabolism were assessed by comparing pre- and post-treatment periods using summary statistics. For each patient, a post-treatment median was calculated that summarizes all repeated measurements of a parameter of interest up to four years after cinacalcet initiation. This was compared to each patient's pre-treatment median that summarizes all repeated measurements obtained between the date of initial hypercalcemia following transplantation and cinacalcet initiation (median time 1.6 (IQR 0.6-3.8) years). Results are reported as mean differences (95% CI) between pre- and post-treatment periods.

Results: Cinacalcet was initiated after a median of 1.8 (0.8-4.7) years after transplantation and was maintained for 6.2 (3.9-7.6) years. Total serum calcium levels significantly decreased (-0.30 (-0.34 to -0.26) mmol/l, $p<0.001$), which was accompanied by an increase in urinary fractional calcium excretion (0.24 (0.06-0.42) %, $p<0.05$). Serum phosphate levels (sPh) and renal tubular reabsorption of phosphate corrected for glomerular filtration rate (TmP/GFR) simultaneously increased within the first year after treatment start, then reaching a plateau in the lower normal range (sPh: 0.19 (0.15-0.23) mmol/l, $p<0.001$; TmP/GFR: 0.20 (0.16-0.23) mmol/l, $p<0.001$). Intact parathyroid hormone levels significantly decreased over time (-79 (-103 to -55) pg/ml, $p<0.001$). We did not observe deterioration of graft function following treatment with cinacalcet. Side-effects included minor gastrointestinal symptoms, which were reversible after dose reduction or switch to twice-daily administration.

Conclusions: Cinacalcet effectively controlled hypercalcemic hyperparathyroidism in KTRs in the long-term.

Funding: Government Support - Non-U.S.

FR-PO879

Cinacalcet (CM) versus Paricalcitol (PC) in Renal Transplant (TX) Patients with Hypercalcemic Persisting Secondary Hyperparathyroidism (HPSH). Pilot Study Marzia Pasquali,[1] Lida Tartaglione,[1] Silverio Rotondi,[1] Carlo Massimetti,[2] Sandro Mazzaferro.[1] *[1]Sapienza Univ, Rome, Italy; [2]Bel Colle Hospital, Viterbo, Italy.*

Background: Post TX HPSH results, at least partly, from decreased Ca- (CaSR) and VitD- (VDR) receptors expression on parathyroids. Receptor activators like CM or PC could increase expression of both.We hypothesized that, in post TX HPSH, after serum Ca normalization with CM, PC could maintain PTH suppression without hypercalcemia if CaSR were increased.

Methods: We prospectively enrolled 19pts with HPSH to receive CM (Phase I, 1 month), dose-titrating for normal sCa. Responders were randomized (Phase II, 3 months) to continue CM (Group A) or shift to PC (Group B). Serum Cr,Ca,Pi,PTH, BALP, 1,25D,FGF23 and Urine Ca/Cr were checked basally and monthly.

Results: 16 pts (54±7y.o.; eGFR 58±20ml/min, TX since 7±5yrs, sCa 11,1±0,6mg/dl, Pi 2,7±0,4mg/dl, PTH 133±43pg/ml, BALP 35±36U/l, FGF23 81±25pg/ml) completed the study. During Phase I, CM therapy (mean dose 35±12mg/day) reduced sCa ($p<.0001$), PTH ($p<.001$) and FGF23 ($p<.0001$) and increased sPi ($p<.0001$). During Phase II: in Group A (8 pts; CM mean dose 41±15mg/day) there was no further change; in Group B (8 pts; PC mean dose 0.8±0.3mg/day) there was: increment of sCa (back to basal, pre-treatment, values) ($p<.0001$) and FGF23 ($p<.005$); reduction of BALP ($p<.002$) and Pi (ns, $p<.06$); persistence of PTH suppression.

Conclusions: We confirm the lowering effect of CM on Ca and PTH in HPSH and report that, in TX pts, FGF23 can decrease contemporarily to sPi increments. PC maintain the reached suppression of PTH but with loss of the (hypo) calcemic effect, as if CaSR stimulation by CM were more relevant than increased CaSR expression. PC therapy increase FGF23 and this is potentially implicated with suppression of PTH and tendency to sPi reduction. BALP and FGF23 changes point to direct effects of PC on bone. In conclusion, with a short-term period of therapy, the role of receptors expression seems secondary to the direct effect of drugs. From a clinical point of view, contemporary administration of CM and PC could be advantageous to control HPSH in TX.

Funding: Pharmaceutical Company Support - ABBVIE, Government Support - Non-U.S.

FR-PO880

Impact of Cinacalcet Pre-Transplant on Mineral Metabolism in Renal Transplant Recipients Ashish K. Sharma,[1,2] Sven-Jean Tan,[1,2] Stephen G. Holt,[1,2] Nigel David Toussaint.[1,2] *[1]Dept of Nephrology, The Royal Melbourne Hospital, Parkville, VIC, Australia; [2]The Univ of Melbourne, Parkville, VIC, Australia.*

Background: Cinacalcet, a calcimimetic, was approved in Australia in November 2007 for treating secondary hyperparathyroidism in dialysis patients. Reports on biochemical profiles and clinical outcomes in patients discontinuing cinacalcet at the time of renal transplantation are limited.

Methods: A single-centre retrospective analysis over 10 years was undertaken to study markers of mineral metabolism in renal transplant recipients from January 2002 to December 2011. We assessed changes in biochemical parameters with the introduction of cinacalcet, and compared patients discontinuing cinacalcet at the time of transplantation with cinacalcet-naïve patients.

Results: A total of 696 renal transplants were performed over 10yrs. Mean age of patients at transplant was 47.4yrs (64.8% male) with 29 (4.2%) deaths, 94 (13.5%) graft loss and 16 (2.3%) parathyroidectomies post-transplant (all in cinacalcet-naïve patients). Since November 2007, 377 patients received transplants, 19.9% on cinacalcet pre-transplant. No significant differences were seen in markers of mineral metabolism at 12 months post-transplant in pre and post-cinacalcet eras. At time of transplantation, parathyroid hormone (PTH) levels were higher in cinacalcet versus cinacalcet-naïve patients (61.5+/-60.5 versus 36.0+/-30.7pmol/L, $p<0.001$). Twelve months post-transplant PTH was higher (15.1+/-11.2 versus 9.6+/-8.4pmol/L, $p<0.001$) and calcium was higher (2.49+/-0.17 versus

2.44+/-0.17mmol/L, p=0.009) for those administered cinacalcet just prior to transplantation. There was no difference in renal function at 12 months (mean eGFR 53.6+/-17.4ml/min/1.73m^2) between the two groups.

Conclusions: Biochemical profiles suggest minimal changes to markers of post-transplant mineral metabolism with the introduction of cinacalcet. Renal transplant recipients discontinuing cinacalcet at the time of transplantation had statistically significant PTH and serum calcium at 12 months although this may not be clinically significant.

FR-PO881

Fibroblast Growth Factor-23 Vit D and Mineral Metabolism in Renal Transplant Recipients Raj K. Sharma, Sonia Mehrotra, Narayan Prasad, Amit Gupta, Anupama Kaul. *Nephrology, Sanjay Gandhi Post Graduate Inst of Medical Sciences, Lucknow, UP, India.*

Background: Mineral bone disorder related to chronic kidney disease persists after kidney transplantation.

Methods: Fifty chronic kidney disease patients on dialysis going for transplantation were prospectively studied before and after renal transplantation for parameters like FGF23, vit D receptor (VDR), 25 (OH) D, 1,25 (OH) 2 D, PTH, serum Ca, S. Po4, alk PO4, eGFR.

Results: The prevalance of hypophosphatemia after transplantation was high 54%, hypercalemia 27.3%, elevated intact PTH levels 78.3% (at the median duration of 3 months after transplant). While before transplant 54% had hyperphosphatemia, hypercalcemia in 2.9%, elevated PTH in 97% as compared to control healthy population. FGF 23 levels were high in 82.4% before transplant (79.36+92.4 pg/ml) and decreased to normal level at 3 month post transplant (3.74+1.31 pg/m) (normal control 3.17+0.9 pg/ml), 25 (OH) D levels showed vit D deficiency in 28.6% before transplant (28.56+12.69 ng/ml), after transpalnt vit D deficiency seen in 43.9% (20.3+6.19 ng/l). 1,25 (OH) D levels before transplant were 136.36+96.14 pmol/L which was less than control (176.1+107 pmol/L) and decreased further to 45.65+34.47 pmol/ml at 3 months post transplantation. VDR activity before transplant was higher (1.91+2.73 ngm/ml) than control (1.52+0.8 ng/ml) and showed further increase to 2.25+2.19 ng/ml after transplantation. There was no difference in the eGFR in transplant patients with or without vit D deficiency (MDRD eGFR 74.4+20.6ml/ and 73.4+18.6 ml/min). Serum intact PTH levels were 316+251.8 pg/ml before transplant and came down to 91.6+58.2 at 3 months post transplantation.

Conclusions: There was high incidence of vit D deficiency or insufficiency in ESRD patients (62.9%) which increased further to 95.7% at 3 month after kidney transplant. 1,25 (OH) 2 D3 levels also decreased after transplant with concomitant increase in VDR activity. FGF 23 levels quickly came down to normal levels by 3 months post transplant. There was normalization of S.PO4 levels by 3 months post transplant. The dysregulated mineral metabolism may continue after kidney transplant despite improvement in renal function and normalization of FGF 23.

FR-PO882

Effects of Inflammation on Serum Levels of SOST in Hemodialysis Patients (HD) Silverio Rotondi, Marzia Pasquali, Lida Tartaglione, Sandro Mazzaferro. *Sapienza, Rome, Italy.*

Background: Produced by osteocytes, Sost inhibits osteoblasts Wnt pathway, thus affecting bone turnover. Recently Sost levels have been reported to be increased in HD, with a possible negative effect on bone disease. Moreover, in experimental osteoporosis, Sost have been shown to positively correlate with inflammatory cytokines. Since HD suffer with chronic inflammation, we sought to evaluate if inflammation affects Sost in HD.

Methods: 41patients (59±16y.o.) on HD since 5,9±4,8 y were sampled for parameters of inflammation (IL1,IL6,IL10,TNFa) and of mineral metabolism (Ca,P,PTH,Vit D). 30healthy subjects (34±12y.o.; eGFR95±19ml/min) as control.

Results: HD population showed a moderate degree of secondary hyperparathyroidism ((Mean values: Ca 8,9±0,9; P 4,7±1,5;PTH 343±363;vitamin D 11±6) and a significant increment of all standard inflammatory cytokines and of OPG and RANKL.

	Sost nmol/l	OPG pg/ml	RANKL mcg/ml	IL 1 pg/ml	IL 6 pg/ml	IL10 pg/ml	TNFα pg/ml
HD (41)	59±16	5±3	0.4±0.7	0.27 ±0.58	12±19	9.5±11.9	13±11
Control (30)	28 ±10	0.1±0.2	0.1±0.05	0.01 ±0,01	0.3 ±0.3	4.3±1.3	3 ±2
p<	.0001	.0001	.0001	.0001	.0001	.0001	.0001

Sost mean levels were twice times higher than normal. Sost correlated positively with age (r.503p.001),OPG (r.424p.01),IL6 (r.419p.01) and TNFa (r.329p.05) and negatively with phosphate (r.498 p.001) and PTH (r-.347 p.05).

Conclusions: Our data confirm the increment of Sost in HD in a range similar to what has been already reported. The negative correlations with P and PTH are in agreement with a role of Sost on bone:higher Sost-lower bone turnover. The positive relationship between Sost and cytokines points to a role of inflammation on bone, exerted through Sost.Inflammation, increasing Sost, could affect bone turnover, a modulatory effect already described in HD. Further,the positive correlation of Sost with age, OPG and cytokines suggest a link with artheriosclerosis. In fact, Sost have been shown to correlate with vascular calcificationsin HD. So the increase of Sost occurring in HD could be secondary, in part, to inflammation. Sost in this population can negatively affectbone turnover thus closing a circle linking inflammation andbone disease.

FR-PO883

Effects of Nephrologists Care in Late Stage CKD Patients with Anemia on Long-Term Dialysis, Cardiovascular Disease Event and Mortality Jia-Sin Liu,[1] Yu-Kang Chang,[1] Szu-Yuan Li,[2] Chih-Ching Lin,[2] Der-Cherng Tarng,[2] Wu-Chang Yang,[2] Chih-Cheng Hsu.[1] *[1]Div of Geriatrics and Gerontology, Inst of Population Health Sciences, NHRI, Miaoli, Taiwan; [2]Taipei Veterans General Hospital, Taipei, Taiwan.*

Background: It is undetermined whether and to what extent the professional nephrologist care for late stage chronic kidney disease (CKD) patients could delay disease progression to long-term dialysis or reduce risks of cardiovascular event or mortality.

Methods: We set up a nationally representative population-based cohort of the stage 5 CKD patients with anemia using the national insurance claim database in Taiwan from 2000 to 2009. There were 53,023 subjects meeting the inclusion criteria: serum creatinine higher than 6 mg/dL, without any dialysis record and under the erythropoiesis-stimulating agent (ESA) treatment (hematocrit less than 28 %). The study cohort was followed until 2009/12/31 or death. The mean follow-up time was 3.2 years. We divided our study subjects into 3 groups: never (n=12,295), sporadic (n=15,215), and continuing (n=25,513) nephrologist care for those who had never, 1-5, and >=6 nephrologist visits, respectively, in the previous 3 years before the ESA use.

Results: Compared to the never group, the multivariable Cox proportional hazards models showed those in the continuing group were more likely to survive long enough to develop chronic dialysis (HR 1.05, 95%CI 1.02-1.08), but less likely to be hospitalized in the ICU (HR 0.86, 95%CI 0.83-0.89), and also less likely to develop heart failure (HR 0.79, 95%CI 0.71-0.88) or die including the mortality before dialysis (HR 0.58, 95%CI 0.55-0.60) and after dialysis (HR 0.78, 95%CI 0.74-0.82).

Conclusions: In conclusion, the continuity of nephrologist care for the advanced CKD patients can significantly prolong patients' survivorship, reduce cardiovascular events and prevent pre-mature death. The survival benefit in the group receiving persistent nephrologist care can sustain even after dialysis commencement. Yet, the policy makers also have to be aware that the prevalence of dialysis may temporally be increased because of the reduction of pre-mature death in the well-cared advanced CKD patients.

FR-PO884

Iron Supplementation Is Associated with Low Mortality in Pre-Dialyzed Advanced Chronic Kidney Disease Patients Receiving Erythropoiesis-Stimulating Agents – A Nationwide Database Analysis Ko-Lin Kuo,[1] Jia-Sin Liu,[2] Szu-Chun Hung,[1] Chih-Cheng Hsu,[2] Der-Cherng Tarng.[3] *[1]Taipei Tzu Chi Hospital, Taiwan; [2]National Health Research Insts, Taiwan; [3]Taipei Veterans General Hospital, Taiwan.*

Background: Iron supplementation is associated with adverse outcomes in patients undergoing hemodialysis; however, a risk/benefit analysis of iron supplementation in pre-dialysis advanced chronic kidney disease (CKD) patients has not been conducted.

Methods: To assess the effectiveness and the safety of iron supplementation in patients with CKD stage 5 who have not yet received dialysis (CKD 5 ND), a prospective cohort study was conducted based on the Taiwan National Health Insurance Research Database. From January 1, 2000, to June 30, 2009, we enrolled 31 971 adult patients who had serum creatinine levels >6 mg/dL and hematocrit levels <28% and who were treated with erythropoiesis-stimulating agents (ESAs) but not yet received renal replacement therapy. All patients were further divided into two groups with or without iron supplementation within 90 days after starting ESA therapy. Patient followed-up took place until dialysis, pre-dialysis death or December 31, 2009. The primary outcomes were pre-dialysis death, hospitalization before death or chronic dialysis.

Results: After propensity score-matching, the patients who received iron supplementation were associated with a lower risk of all-cause death (HR, 0.85; 95% CI, 0.80-0.90) compared with nonusers in a median follow-up visit of 8 months. Moreover, compared with the nonusers, the iron users were associated with a lower risk of overall hospitalizations (HR, 0.97; 95% CI, 0.94-0.99) although they exhibited a higher risk of chronic dialysis (HR, 1.05; 95% CI, 1.01-1.08).

Conclusions: Iron supplementation has a 15% decreased risk ofdeath in CKD 5 ND patients who received ESA treatment. However, physicians should be aware of an increased risk of chronic dialysis in patients who receive iron supplementation.

FR-PO885

Potential Renal Protective Effect of Telbivudine in Patients with Chronic Hepatitis B Infection Compared to Other Antivirals Vibhakorn Permpoon,[1] Krit Pongpirul,[2,3] Sira Sooparb,[4] Sinn Anuras.[1] *[1]Digestive Disease Center, Bumrungrad International Hospital, Bangkok, Thailand; [2]Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn Univ, Bangkok, Thailand; [3]International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [4]Nephrology Center, Bumrungrad International Hospital, Bangkok, Thailand.*

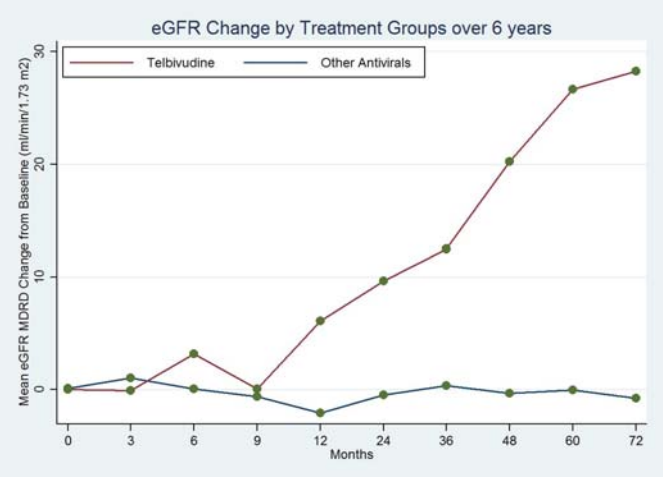
Background: This study is aimed to comparatively explore potential protective effect of Telbivudine and other antivirals on renal function.

Methods: Demographic and clinical information from medical records of 1,065 CHB patients between January 2008 and December 2013 were analyzed. Analysis of covariance model with treatment and ethnicity covariates was used to test the difference in eGFR between Telbivudine and other antivirals. Means were calculated using the least square method.

Results: Majority of the 966 patients received antivirals were Asian male with mean age of 47.57 years. Telbivudine was used in 176 cases (18.22%), of which 81 cases (46%) were combined with other antivirals. Baseline eGFR were comparable (p=0.6885). Renal function steadily improved in Telbivudine group but declined in non-Telbivudine group, with statistically significant difference shown at 12 months after treatment (p=0.0087).

Months	Telbivudine	Other Antivirals	P value*	N
Baseline	95.4244	95.6384	0.6885	577
3	101.0787	101.5174	0.8422	361
6	96.8450	94.1646	0.3383	320
9	95.0195	93.0158	0.4654	286
12	99.8380	91.1020	0.0087	295
24	105.2184	93.7611	0.0000	375
36	109.9087	95.7521	0.0000	299
48	110.0040	89.8663	0.0000	209
60	111.6309	82.3646	0.0000	145
72	113.7585	90.0600	0.1081	67

* P value was derived from analysis of covariance modelling, including treatment, ethnicity, and baseline value as covariates.



Conclusions: Potentially superior renal protective effect of Telbivudine to other antivirals for chronic hepatitis B infection exists and starts after twelve months.

FR-PO886

The Association between Renal Outcome and Use of Erythropoiesis Stimulating Agents in Patients with Advanced Chronic Kidney Disease in Taiwan

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Background: Animal studies have shown the renoprotective effects associated with exogenous erythropoiesis stimulating agents (ESAs) at a dose as low as not to raise the hemoglobin (Hb) level. In Taiwan, the maximal ESA dose is restricted to 100mcg darbepoetin alfa or 20000U epoetin beta monthly by the National Health Insurance program. We analyzed the association between renal outcome and the use of ESAs in advanced CKD patients in Taiwan.

Methods: An observational retrospective cohort study of 455 patients with estimated glomerular filtration rate (eGFR) less than 15mL/min/1.73m^2 conducted at a single center. The patients were enrolled from 2007/1/1 to 2011/12/31 and followed until 2012/12/31. The use of ESAs was recorded every 3 months. The primary outcome was the initiation of dialysis. The secondary outcome was the annual decline rate of eGFR.

Results: The average dose of ESA received by the ESA users was around 63mcg darbepoetin alfa or 12600U epoetin beta monthly. During the first year, the Hb level of ESA non-users decreased from 10.0±1.8 to 9.7±1.8g/dL (p=0.07), while the Hb level of ESA users increased from 9.4±1.5 to 9.6±1.3g/dL (p=0.05). The factors independently associated with dialysis in the time-dependent Cox proportional hazard model were listed in table1. Of note, the use of ESA was independently associated with decreased risk for dialysis. The annual eGFR decline rates were 1.7±3.9 in ESA non-users and 2.5±3.0mL/min/1.73m^2 in ESA users (p=0.05).

Predictors	*Univariate analysis*		*Multivariate analysis*	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Albumin	0.31 (0.24-0.40)	<0.001	0.31 (0.22-0.45)	<0.001
eGFR	0.83 (0.80-0.87)	<0.001	0.76 (0.73-0.80)	<0.001
ESA use	0.38 (0.29-0.50)	<0.001	0.26 (0.19-0.36)	<0.001
Log UPCR	5.60 (3.93-7.98)	<0.001	3.87 (2.41-6.22)	<0.001
Uric acid	1.14 (1.08-1.20)	<0.001	1.12 (1.05-1.20)	0.001
Male	1.40 (1.12-1.75)	0.003	1.54 (1.16-2.03)	0.002
Age	0.99 (0.98-1.00)	0.042	0.98 (0.97-0.99)	0.007
BMI	0.99 (0.97-1.02)	0.62	0.96 (0.94-0.99)	0.008

Conclusions: The use of ESAs in Taiwan was associated with delayed initiation of dialysis in patients with advanced CKD.

FR-PO887

What Are the Most Important Targets for the Management of Progression in CKD?

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Background: KDIGO guidelines for management of CKD recommend interventions on blood pressure, proteinuria, glycemic control, salt intake, lifestyle, anemia and calcium-phosphorus metabolism. We previously showed that close adherence to these guidelines reduced the risk of progression in CKD (Peeters, JASN, 2014). However, simultaneously addressing all aspects of CKD management can be challenging for doctor and patient. The purpose of this study was to identify the most important treatment factors, so that interventions may be prioritized.

Methods: The MASTERPLAN study was a RCT performed in 9 Dutch hospitals. The intervention consisted of 14 targets based on guidelines. In total 788 patients with eGFR between 20-70 ml/min per 1.73m2 were included. The outcome was a composite of death, end stage renal disease, and 50% increase in serum creatinine. The total effect of the MASTERPLAN can be split up in a direct and indirect effect. The indirect effect is defined as the proportion of the total effect explained by a change in treatment target at one year follow-up. It can be determined with mediation analysis (Imai, APSR, 2011).

Results: We were able to explain 52% of the risk reduction in the intervention arm through a reduction in blood pressure, proteinuria and LDL-cholesterol. However, a substantial proportion of risk reduction due to nurse practitioner aided care remained unexplained.

	TE	95% CI	IE	DE	Mediated (%)
Log proteinuria	-227	-468-17	-55	-171	24
LDL cholesterol	-264	-481--50	-34	-231	13
Blood pressure	-210	-425-4	-31	-181	15
Log parathyroid hormone	-266	-489--47	-1	-207	0
Total					52

The estimates for total effect (TE), indirect effect (IE) and direct effect (DE) can be interpreted as the decreased number of patients with a composite endpoint per 10.000 patients a year.

Conclusions: The reduced risk due to the MASTERPLAN intervention was mediated through lower protein creatinine ratio, LDL cholesterol and blood pressure, which may thus be priorities for a more focused intervention.

FR-PO888

Screening and Management for Chronic Kidney Disease and Risk Factors – Community-Based Program in Resource Poor Setting

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Background: A community-based program to screen and manage hypertension, diabetes, kidney disease and its risk factors were developed in Eastern Nepal. The outcome data of such program are reported here.

Methods: A community-based screening on 20,811 people ≥20 years was performed under the coordination of BP Koirala Institute in Dharan with a collaborating network of community-based volunteers, medical students, nursing students and local leaders. Screening and intervention for chronic kidney disease, and its risk factors such as hypertension and diabetes were performed. An organization was developed to follow-up screened positive individuals in primary or equivalent health centers. Referrals were made to tertiary care centers as needed. Health status, lifestyle habit, physical examination and blood pressure were evaluated. Spot urine was examined for proteins by dipstick. Fasting blood glucose and serum creatinine were measured.

Results: Impaired kidney function (estimated GFR <60 mL/min/1.73m^2 using MDRD - 4 variables equation), dipstick proteinuria >1+, hypertension, diabetes were present in 10.6%, 5.4%, 22%, and 8%, respectively. The control of blood pressure or diabetes at baseline was 14% and 8% in respectively. 4471 participants positive at screening entered an intervention program by combining lifestyle modifications and pharmacological management with cheap drugs. At the end of three years follow up blood pressure control (<140/90 mmHg) was achieved in 53% (SBP) and 63% (DBP) of individuals, whereas glycemia target (<126 mg/dl) in 55%. Normalization of dipstick proteinuria occurred in 63% of individuals, while renal function improved in 48% of subjects during the same period.

Conclusions: The integrated care for chronic kidney disease and its risk factors is possible in low-resource settings like Nepal. The significant control of hypertension, diabetes, proteinuria and eGFR achieved in this program is expected to translate in less need of renal replacement therapy and reduced CV mortality on the long-term.

FR-PO889

Association of Drug Effects on Biochemical Endpoints (Serum Parathyroid Hormone, Calcium, and Phosphorus) with All-Cause and Cardiovascular Mortality: A Meta-Analysis

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Background: Treatments to lower elevated serum parathyroid hormone and phosphorus levels are prescribed nearly universally for patients with advanced chronic kidney disease. We aimed to investigate whether drug effects on these biochemical endpoints are correlated with mortality outcomes in nephrology trials.

Methods: We did a systematic review and meta-analysis of randomized controlled trials in chronic kidney disease that reported drug effects on both biochemical endpoints (serum parathyroid hormone, calcium and phosphorus) and all-cause or cardiovascular mortality. We calculated correlated effect sizes for biochemical and mortality outcomes using bivariate random effects meta-analysis.

Results: We identified 28 studies involving 6473 participants reporting biochemical and mortality outcomes. Associations between treatment effects on surrogate endpoints and the corresponding risks of mortality were weak and imprecise. All correlation coefficients were ≤0.6 and 95% credible intervals were generally wide and overlapped with zero, consistent with the possibility of no association. The exception was an inverse correlation between drug effects on serum parathyroid hormone levels and all-cause mortality, which was nominally significant (-0.60, 95% credible interval -0.85 to -0.16), but very uncertain. Risks of bias within randomization processes, allocation concealment and loss of randomized participants from analyses were high, further reducing confidence in the summary correlations.

Conclusions: Drug effects on serum parathyroid hormone, phosphorus, and calcium are weakly and imprecisely correlated with all-cause and cardiovascular death in the setting of chronic kidney disease. Risks of mortality cannot be inferred from treatment-induced changes in serum biochemical values in nephrology trials.

Funding: Government Support - Non-U.S.

FR-PO890

Comparative Efficacy and Safety of Blood Pressure Lowering Drugs in Diabetic Kidney Disease: A Network Meta-Analysis

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Background: The comparative efficacy of blood pressure lowering agents in diabetic kidney disease remains uncertain because few active comparator trials have been conducted.

Methods: We did a network meta-analysis, which uses both direct and indirect drug comparisons from randomized trials within a single analytical framework. We included data in adults with diabetes and kidney disease. Primary outcomes were all-cause mortality and ESKD. We also examined cardiovascular events, regression of albuminuria and doubling of serum creatinine.

Results: We identified 190 studies involving 41,598 participants. An ACE inhibitor (ACE) plus an angiotensin-receptor blocker (ARB) was the best intervention for preventing ESKD (OR 0.50; 0.31-0.79). The only other drug to significantly prevent ESKD was an ARB alone (0.77; 0.65-0.91), while point estimates suggested beneficial effects from ACE alone (0.71, 0.47-1.08) and endothelin inhibitor (0.71, 0.44-1.14) treatment. Point estimates suggested combination ACE and ARB were superior to ARB alone (0.64, 0.41-0.99) and ACE alone (0.69, 0.34-1.24) for preventing ESKD. ACE or ARB both alone or in combination with each other or another drug (CCB, diuretic) were the best interventions for inducing regression of albuminuria (mean ORs 2.25 to 9.87), whereas calcium channel blockers and diuretics were not beneficial. Endothelin inhibitors (0.32, 0.11-0.91), ACE (0.71, 0.55-0.93) and ARB (0.75, 0.64-0.88) were the best drugs for preventing doubling of serum creatinine whilst renin inhibitors and beta blockers were potentially harmful. No antihypertensive drug was significantly more effective than placebo or any other drug for reducing all-cause mortality or myocardial infarction.

Conclusions: No single blood pressure lowering strategy improved survival in diabetic kidney disease. An ACE inhibitor combined with ARB was the best intervention for preventing ESKD.

Funding: Private Foundation Support

FR-PO891

Comparative Effectiveness and Safety of Erythropoiesis-Stimulating Agents for Anemia in Adults with CKD: A Cochrane Network Meta-Analysis

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Background: While clinical and policy decisions about anemia management in chronic kidney disease depend on the comparative effectiveness of erythropoiesis-stimulating agents (ESAs), large head-to-head trials are lacking.

Methods: We did a network meta-analysis of 56 randomized trials involving 15,596 participants. We included trials that evaluated epoetin alfa, epoetin beta, darbepoetin alfa, methoxy polyethylene glycol-epoetin beta or a biosimilar ESA. Primary outcomes were blood transfusion and all-cause mortality.

Results: In moderate to low quality evidence, epoetin alfa, epoetin beta, darbepoetin alfa and methoxy polyethylene glycol-epoetin beta were superior to placebo for preventing blood transfusions (mean odds ratios 0·18, 0·09, 0·17, and 0·15). In very low quality evidence, biosimilar ESAs were possibly no better than placebo (95% CI, 0·05 to 1·47) for prevention of blood transfusions with considerable imprecision in the estimates. Head-to-head comparisons between ESAs were inconclusive and imprecise. Epoetin alfa, epoetin beta, darbepoetin alfa and methoxy polyethylene glycol-epoetin beta all increased the odds of hypertension (2·31, 2·57, 1·83 and 1·96, respectively) but the treatment risk for biosimilar formulations was inconclusive (1·18, 95% CI, 0·47 to 2·99) and head-to-head comparisons between all ESAs were imprecise. The comparative effects of ESAs on mortality, myocardial infarction, stroke, and vascular access thrombosis were ill-defined and network analyses for fatigue, breathlessness, major cardiovascular events and end-stage kidney disease were not possible.

Conclusions: In the setting of chronic kidney disease, there is insufficient evidence in randomized trials to show clinical differences between ESA formulations. The efficacy of biosimilar ESAs against placebo is unproven. Additional comparative data for ESAs measuring patient-important outcomes are needed to inform prescribing.

Funding: Government Support - Non-U.S.

FR-PO892

Statins for Primary or Secondary Prevention Improve Survival in Chronic Kidney Disease Stages 3-5

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Background: We examined the relationship between statin use for primary (PP) or secondary prevention (SP) and all-cause mortality in the Ayrshire CKD Epidemiology Study (ACES) cohort.

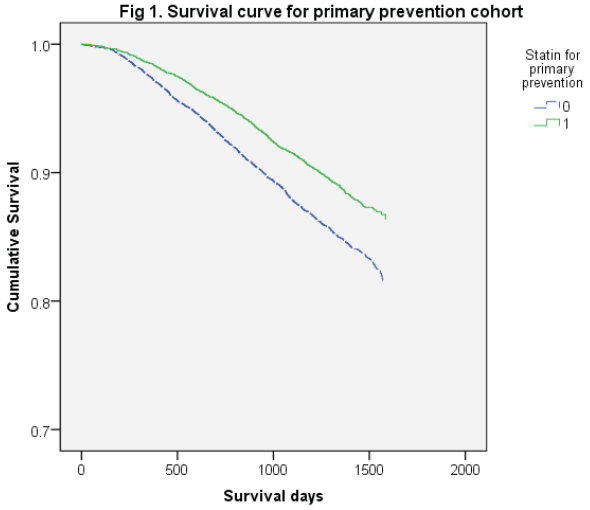
Methods: Adult population from 07/01/2009-06/30/2010 was 312,943. Using laboratory data, we identified all patients with CKD 3-5 with an algorithm to ensure chronicity. Community dispensing data, hospitalisations, and date of death for the following 4.5 years was obtained from Information Services Division Scotland. Multivariate Cox models were generated.

Results: CKD prevalence was 5.8% (n=18,113). Those with nephrotic range proteinuria, non-statin lipid therapy or renal replacement therapy were excluded (n=950). Lipid profiles were tested in 73%. LDL cholesterol was 39 (37-40) mg/dL lower in the 45% who were on statins. Table 1 details the characteristics between those on and not on statins for PP or SP.

	Primary (12,104)		Secondary (5,059)	
Statin	Yes (38%)	No (62%)	Yes (63%)	No (37%)
Age (ave±SD)	73±9	74±12	75±9	79±9
eGFR (ave±SD)	50±12	50±12	48±13	48±13
Female	64%	70%	54%	63%
Chol	165±35	205±43	155±36	187±44
HDL	52±15	54±17	49±15	51±17
LDL	82±28	121±36	75±28	107±36
TG	161±88	152±78	154±85	142±78
Coronary heart disease			86%	82%
Cerebrovascular disease			27%	28%
Hypertension	30%	28%	66%	62%
Diabetes	17%	5%	27%	13%
Peripheral Arteriosclerosis	3%	1%	9%	7%
Proteinuria	24%	15%	27%	19%

After adjustment for factors in table 1, statins was associated with HR of 0.72 (0.66,0.79) and 0.73 (0.66,0.81) for PP and SP respectively.

Fig 1. Survival curve for primary prevention cohort



Conclusions: We demonstrated a significant mortality benefit associated with statin use in CKD. Despite being an evidence-based intervention, only 45% of patients were receiving a statin.

Funding: Private Foundation Support

FR-PO893

Association between Prescription Narcotic Use and Markers of Kidney Disease in U.S. Adults

Celestina Barbosa-Leiker,[1] Sterling Mcpherson,[1] Kenn B. Daratha,[1,2,3] Radica Z. Alicic,[2] Robert Short,[1,2] John M. Roll,[1] Katherine R. Tuttle.[2,3] *[1]College of Nursing and Program of Excellence in the Addictions Research, Washington State Univ; [2]Providence Health Care; [3]School of Medicine, Univ of Washington.*

Background: Analgesic use has been associated with increased risk of chronic kidney disease (CKD). In a general population cohort, habitual *non-narcotic* analgesic use was not linked to evidence of CKD. The purpose of this study was to determine relationships between *prescription narcotics* and *non-steroidal anti-inflammatory drugs* (NSAIDs) use, as well as any analgesic use, with albuminuria and estimated glomerular filtration rate (eGFR) in a representative sample of U.S. adults.

Methods: Participants in the National Health and Nutrition Examination Survey (2009-2010) with serum creatinine, urinary albumin, and covariate data (n=3980) were included. Use of prescription narcotics, NSAIDs, and any analgesics were compared to referent groups with use of no medication or use of non-analgesic medication. Urine albumin-to-creatinine ratio (UACR) and eGFR were analyzed as continuous and binary variables (UACR ≥30 mg/g or eGFR <60 mL/min per 1.73m^2 by CKD-EPI). Regression models controlled for sex, age, poverty/income ratio, race, diabetes, hypertension, and smoking status.

Results: Frequencies of use were: prescription narcotics 4.4%; NSAIDs 5.6%; any analgesic 12.7%; non-analgesic medication 38.7%; no medication 48.6%.Narcotic use (b=0.22, p=0.01) and analgesic use (b=1.73, p=0.02) were associated with higher UACR, while NSAIDs use was not (b=0.17, p=0.11). Only narcotic use was related to risk of UACR ≥ 30 mg/g (OR=1.54, 95%CI=1.03-2.30, p=0.04), where 13.8% of those taking narcotics had high UACR compared to 8.1% of those taking non-narcotics or no medications. No type of analgesic use was related to eGFR.

Conclusions: Among U.S. adults, prescription narcotic users have increased risk of elevated albuminuria. Use of any analgesic, but not NSAIDs, was associated with albuminuria indicating that narcotics drive this relationship. Prescription narcotic use is associated with numerous adverse health consequences including albuminuria, and possibly related sequelae, such as CKD.

FR-PO894

Vitamin D Analogs Reduce the Progression of Chronic Kidney Disease: Chronic Kidney Disease Research of Outcomes in Treatment and Epidemiology Study (CKD-ROUTE Study)

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Background: The use of vitamin D analogs has been generally recommended for the treatment of mineral and bone disorder in chronic kidney disease (CKD). However, the suppressing effect of vitamin D therapy on the progression of CKD has not yet been established.

Methods: A prospective cohort derived from one-year follow-up data of the CKD Research of Outcomes in Treatment and Epidemiology study. Participants aged over 20 years with pre-dialysis CKD stage 2-5 were eligible. The primary outcome was composite of end-stage renal disease (ESRD) or a 50% reduction in estimated glomerular filtration rate (eGFR). A Cox proportional hazards model was used to evaluate the association between the use of vitamin D analogs and the primary outcome. Furthermore, we conducted an additional analysis matched for a propensity score for the vitamin D analogs use between patients with and without the use of vitamin D analogs.

Results: We enrolled 735 patients (male 69%). The numbers of patients with and without the use of vitamin D analogs were 99 and 636, respectively. The primary outcome was observed in 108 patients (ESRD, 81; a 50% reduction in eGFR, 27) and 32 patients died. The mean age was 67±13 (SD) years; eGFR, 31±18 ml/min/1.73m^2. Multivariate Cox proportional hazard models adjusted for baseline characteristics showed that the use of vitamin D analogs independently decreased the risk of the primary outcome; hazard ratio adjusted for patients' characteristics (aHR) 0.57, 95% confidence interval (CI) (0.33, 0.97). The matched analysis also showed similar tendencies; aHR 0.43, 95% CI (0.22, 0.84).

Conclusions: Administration of vitamin D analogs for patients with pre-dialysis CKD reduces the risks for CKD progression.

FR-PO895

Association Between Serum Potassium and Discontinuation of Renin-Angiotensin Aldosterone System Blocker Medication in Patients with Chronic Kidney Disease

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Background: In states of health, the kidneys maintain serum potassium (K) concentration between 3.5 and 5mEq/L. Patients with chronic kidney disease (CKD) are predisposed to hyperkalemia, which may be aggravated by drugs that increase serum K, such as RAAS blockers: angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor antagonists. While the risk/benefit threshold is unclear, RAAS blockers may be discontinued in response to hyperkalemia, which in turn denies patients potential cardio- and reno-protective benefits of these agents. We studied the association between serum K and discontinuation of RAAS blockers stratified on CKD severity.

Methods: We assembled a retrospective cohort of CKD (eGFR<60mL/min/1.73 m^2) patients with available serum K data between Jan-2009 and June-2013 (N=55,266). Serum K was considered as a time-varying exposure, updated at the time of each measurement. We considered all intervals in which the patient began on a RAAS blocker (ie, was at-risk for discontinuation) between study entry and end of study (Jun-2013), death, or loss to follow up.

Results: At baseline, 29.8% of patients were prescribed a RAAS blocker. Across all strata of eGFR, there was a U-shaped association between serum K and RAAS blocker discontinuation, which was statistically significant (p<0.001 in each stratum).

eGFR mL/min/ 1.73m^2		K <3.5 mEq/L	K 3.5 - 3.9 mEq/L	K 4.0 - 4.4 mEq/L	K 4.5 - 4.9 mEq/L	K 5.0 - 5.4 mEq/L	K 5.5 - 5.9 mEq/L	K ≥6.0 mEq/L	P value
< 30	Pt-year	24	168	620	1,016	706	243	51	
	# discont	44	176	587	893	636	290	86	
	Adjusted IRR[a] [95% CI]	2.07 [1.52, 2.81]	1.19 [1.01, 1.41]	1.10 [0.99, 1.22]	Ref	1.00 [0.90, 1.11]	1.31 [1.15, 1.49]	1.81 [1.45, 2.26]	< 0.001
30 - 39	Pt-year	48	485	1,877	2,488	1,409	367	60	
	# discont	48	354	1,146	1,440	894	303	63	
	Adjusted IRR[a] [95% CI]	1.69 [1.26, 2.26]	1.28 [1.14, 1.44]	1.07 [0.99, 1.16]	Ref	1.08 [0.99, 1.17]	1.38 [1.22, 1.57]	1.71 [1.33, 2.20]	< 0.001
40 - 49	Pt-year	101	1,071	3,900	4,544	2,027	369	47	
	# discont	72	563	1,916	2,190	1,058	269	53	
	Adjusted IRR[a] [95% CI]	1.50 [1.19, 1.91]	1.13 [1.03, 1.25]	1.05 [0.98, 1.11]	Ref	1.06 [0.99, 1.15]	1.45 [1.28, 1.65]	2.21 [1.68, 2.90]	< 0.001
50 - 59	Pt-year	129	1,441	5,103	5,171	1,911	313	28	
	# discont	78	712	2,254	2,339	902	203	23	
	Adjusted IRR[a] [95% CI]	1.37 [1.09, 1.72]	1.13 [1.03, 1.23]	1.00 [0.94, 1.06]	Ref	1.03 [0.95, 1.11]	1.39 [1.20, 1.61]	1.70 [1.13, 2.56]	< 0.001

Abbreviations: CI, confidence interval; discont, discontinued; IRR, incidence rate ratio; pt, patient; Ref, reference.
[a]Multivariable Poisson model adjusted for age, sex, race, diabetes, heart failure, coronary artery disease, cerebral vascular accident, beta blocker use, centrally acting calcium channel blocker use, loop diuretic use, and thiazide diuretic use.

The pattern of association was similar across eGFR strata (p-interaction=0.66). Compared to serum K 4.5-4.9mEq/L, K ≥6.0mEq/L was associated with a 1.7-2.2-fold greater rate of discontinuation; K 5.5-5.9mEq/L was associated with a 1.3-1.5-fold greater rate.

Conclusions: Across each cohort of CKD stage patients (stage 3, 4, 5), serum K ≥5.5mEq/L is consistently associated with greater rate of RAAS blocker discontinuation.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

FR-PO896

Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial of Once-Daily ZS-9 for Treatment of Hyperkalemia: Achievement and Maintenance of K^+ in Subgroup Analysis of Patients with Significant Renal Impairment

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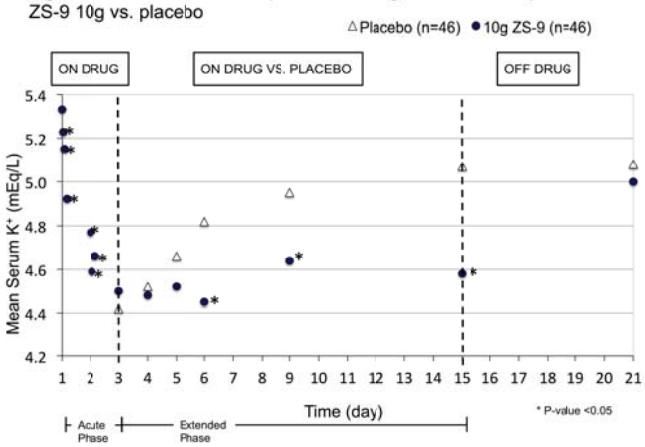
Background: Hyperkalemia (HK) increases risk of mortality and limits RAAS therapy in pts with heart failure and CKD. ZS-9, a nonabsorbed cation exchanger specifically designed to trap excess K^+ in the gut, was evaluated in a subgroup of HK pts with significant renal impairment (eGFR<60) from a large Phase 3 trial.

Methods: Pts (N=753) with K^+ 5.0-6.5 mEq/L were randomized (1:1:1:1:1) to ZS-9 (1.25, 2.5, 5 or 10g) or placebo (PBO) orally 3x daily for 48hr (acute phase). Following this phase, pts with K^+ 3.5-5.0 mEq/L (n=542) were randomized 1:1 to the same ZS-9 acute phase dose or PBO 1x daily on Days 3-15 (extended phase). RAASi were maintained during the study. Unpaired t-tests were used to compare serum K^+ in pts treated with ZS-9 versus PBO.

Results: Of 753 pts, 561 (75%) had baseline eGFR<60. Mean baseline K^+ was 5.4 mEq/L. At 48hr, significantly greater decreases in K^+ were seen with 2.5g (n=101), 5g (n=115) and 10g (n=102) ZS-9 (-0.43, -0.59, and -0.84 mEq/L, respectively) compared to PBO (n=120, -0.22 mEq/L; p=0.022, p<0.001, and p<0.001, respectively). In the extended phase, baseline K^+ was similar in the 10g ZS-9 (n=46, 4.5 mEq/L) and PBO groups (n=46, 4.4 mEq/L). Pts on ZS-9 10g maintained normokalemia during the extended phase (4.6 mEq/L at Day 15; p<0.0001) whereas K^+ increased with PBO (5.1 mEq/L at Day 15). Similar results were seen with 5g ZS-9 (p<0.0001).

Figure. Mean K+ over time in patients with significant renal impairment on ZS-9 10g vs. placebo



Conclusions: ZS-9 may fulfill an important clinical unmet need in hyperkalemic pts with significant renal impairment through the restoration and maintenance of normokalemia.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

FR-PO897

Reducing the Uremc Toxins Protects Kidney Function Deterioration in Patients with Chronic Kidney Disease Ran-Hui Cha,[1] Jae Hyun Chang,[2] Jung-Hwan Park,[3] Dae R. Cha,[4] Chun Soo Lim,[5] Ki Young Na,[6] Sang Youb Han,[7] Yon Su Kim.[8] *[1]Dept of Internal Medicine, National Medical Center; [2]Dept of Internal Medicine, Gachon Univ Gil Medical Center; [3]Dept of Internal Medicine, Konkuk Univ Hospital; [4]Dept of Internal Medicine, Korea Univ Ansan Medical Center; [5]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center; [6]Dept of Internal Medicine, Seoul National Univ Bundang Hospital; [7]Dept of Internal Medicine, Inje Univ Ilsan Hospital; [8]Dept of Internal Medicine, Seoul National Univ College of Medicine.*

Background: The notion that AST-120 may delay clinical outcome has not been evaluated thoroughly. In this study, we aimed to find the long-term effect of AST-120 on the renal disease progression [(doubling of serum creatinine (SCr), eGFR decrease more than 50%, or initiation of renal replacement therapy)] in patients with advanced chronic kidney disease (CKD).

Methods: We prospectively recruited 579 patients (CKD stage 3 and 4) from 11 medical centers in Korea and randomized them into AST-120 and control arm. A total of six gram of AST-120 in three divided doses was given to patients in AST-120 arm along with the standard conventional treatment. Primary analysis was done in 465 Per-Protocol participants.

Results: Initial mean SCr and eGFR levels were 248.2 μmol/l and 26.8 ml/min/1·73m^2, respectively. There was no significant difference in the occurrence of composite primary outcomes between two arms (Log-rank P=0.41). AST-120 was beneficial for the preservation of GFR in patients without composite primary outcome and with diabetic nephropathy. And the slope of 1/SCr was significantly attenuated in both two arms and the value of AST-120 arm was less than that of control arm (P=0·046). The magnitude of serum indoxyl sulfate (IS) decrement in one year by AST-120 was reversely correlated with the occurrence of composite primary outcomes (P=0.014, 0.033).

Conclusions: Long-term use of AST-120 has the potential of renal protection, and the change of serum IS level may be used as a surrogate marker for delineating the patient subgroups who would be beneficial by the use of AST-120. (Clinicaltrials.gov: NCT00860431).

FR-PO898

A Multiple Parameter Response Efficacy (PRE) Score to Predict the Effect of Atrasentan on Renal and Cardiovascular Outcomes Bauke Schievink,[1] Dick de Zeeuw,[1] Jarno Hoekman,[1] Hiddo Jan Lambers Heerspink,[1] The RADAR Study Group.[2] *[1]Univ Medical Center Groningen, Groningen, Netherlands; [2]Multiple Affiliations.*

Background: In the RADAR trial (*de Zeeuw et al,JASN 2014*), we showed that 12 weeks treatment with the selective endothelin A receptor antagonist atrasentan (0.75 and 1.25 mg/d) reduced albuminuria (UACR), blood pressure, cholesterol, hemoglobin and increased body weight in patients with diabetes and nephropathy. UACR reduction ≥30% was achieved by 51% and 55% of patients. We previously developed an algorithm (PRE score,*Smink et al,CPT 2014*) that translates the shortterm effects of a drug to longterm hard outcomes. We applied the PRE score to the RADAR data to test the potential of both atrasentan doses for longterm renal/cardiovascular protection.

Methods: PRE scores were calculated by applying a multivariable Cox model, derived from a background dataset of completed clinical trials, to the baseline and week 12 risk marker levels to calculate renal and heart failure (HF) risk change for atrasentan 0.75 or 1.25 mg/d.

Results: The PRE score indicated a renal risk change of -23% and -30% for atrasentan 0.75 and 1.25 mg/d, respectively (Table). PRE scores indicated a smaller increase in HF risk for the low atrasentan dose. We could dramatically improve the beneficial outcome (almost 50% renal risk reduction) by selecting patient that achieved ≥30% UACR reduction following atrasentan treatment (responders). The nonresponder group showed PRE scores indicating increased renal and HF risk.

Conclusions: In the overall RADAR population, the PRE score predicts that low dose atrasentan will decrease longterm renal risk without increased risk of HF. However, within this population UACR responders are actually contributing to the positive prediction, whereas nonresponders show a predicted increased risk. Patients with UACR response should be selected in a future hard outcome trial.

Population-Dose	Renal	Heart failure
Overall		
0.75mg	-23% (-47 - +1)	+2% (-16 - +20)
1.25mg	-30% (-55 - -6)	+7% (-13 - +27)
Responders		
0.75mg	-47 (-71 - -23)	-9 (-29 - +11)
1.25mg	-47 (-71 - -22)	+5 (-19 - +29)
Nonresponders		
0.75mg	+4 (-22 - +31)	+12 (-10 - +33)
1.25mg	+4 (-31 - +39)	+10 (-17 - +36)

Funding: Pharmaceutical Company Support - Abbvie

FR-PO899

Effect of CTP-499 on Biomarkers Associated with Chronic Kidney Disease in Patients with Type 2 Diabetes Ara Aslanian, Kristine Hogan, Philip B. Graham, LuAnn A. Sabounjian, Lijun Wu. *Concert Pharmaceuticals, Lexington, MA.*

Background: The progression of CKD is intimately related to the pathophysiology of tubulointerstitial damage, as the severity of tubule injury predicts long-term clinical outcomes. Tubule cell dysfunction is mediated by pro-fibrotic and pro-inflammatory factors, thus therapeutic intervention that ameliorates these processes may be beneficial. CTP-499 is a novel, deuterium-containing, multi-subtype selective PDE inhibitor that had protective effects on renal function in patients with type 2 diabetic kidney disease in a 48-week Phase 2 clinical study. We have shown in non-clinical studies that CTP-499 has anti-fibrotic and anti-inflammatory activities consistent with inhibition of specific PDE subtypes. To investigate the mechanism of CTP-499 in diabetes-induced CKD, we examined a panel of biomarkers from this Phase 2 clinical trial patient population.

Results: At 48 weeks, biomarkers associated with the severity of tubulointerstitial damage were significantly reduced in patients receiving CTP-499 compared to placebo, including IL-18 and clusterin (25% and 42%, respectively, P < 0.05; N = 65 CTP-499, 57-58 Placebo). In general, treatment-dependent changes were more prominent in patients with a baseline UACR ≥ 850 mg/g, a group that shows more rapid decline in renal function than patients with lower baseline UACR. In this subgroup, the tubule injury markers MCP-1 and N-OPN were reduced by 34% and 55%, respectively (P < 0.05, N = 32 CTP-499, 27 Placebo) in addition to IL-18 and clusterin. Also, the extracellular matrix proteins fibronectin, collagen IV, and laminin, which undergo increased deposition in CKD, were significantly reduced in CTP-499-treated patients compared to placebo (13-68%; P < 0.05, N=65 CTP-499, 57-58 Placebo). These data are consistent with results showing that CTP-499 reduced tubule cell apoptosis and collagen deposition in the rat UUO kidney fibrosis model.

Conclusions: Collectively, these data suggest CTP-499 has protective effects on renal tubules in diabetic kidney disease, and that anti-fibrotic mechanisms may contribute to this activity. These results further support continued development of this novel agent for the treatment of CKD.

FR-PO900

Composite Creatinine-Based Outcomes for Trials in DKD Jamie P. Dwyer,[1] Tom Greene,[2] Hiddo Jan Lambers Heerspink,[4] Nan Hu.[2] *[1]Nephrology, Vanderbilt, Nashville, TN; [2]Biostatistics, Univ of Utah, Salt Lake City, UT; [3]Nephrology, Rush Med Ctr, Chicago, IL; [4]Clin Pharm, Univ Groningen, Groningen, Netherlands.*

Background: FDA accepts doubling of creatinine (SCr) as an endpoint in trials, but it is a late event. Surrogate endpoints based on lesser SCr ↑ may reduce *N* and follow-up for trials. Lesser SCr ↑ can be affected by acute hemodynamic effects which hide the long-term treatment effect. We investigated composite endpoints based on 25% to 45% SCr ↑ at discrete time points in the IDNT and RENAAL trials, where ARB intervention led to acute increases in SCr.

Methods: We assessed the association of SCr events with ESRD using time-dependent ROC curves, and evaluated power to detect treatment effect by Z-scores indicating the ratio of the estimated treatment effect to the standard error representing the precision of the analysis, with Z-score <-1.96 indicating a statistically significant treatment benefit.

Results: In IDNT the time-dependent area under the curve for determining ESRD by month 33 based on %change in SCr to 18 mos was 0.66 without confirmation of SCr events, and was 0.76 with. Percent of pts with events (Evt%), (time-dependent sensitivity/specificity for ESRD by 33 mos), and Z-score for the composite, defined by designated confirmed changes in SCr at either 12 or 18 months are:

	%ΔSCR Over 18 Months		
%ΔSCR Over 12 Months	25%	35%	45%
25%	EVT% = 52.3 (56.5%/86.5%) Z = -0.46	EVT% = 45.7 (51.9%/88.1%) Z = -0.15	EVT% = 40.5 (51.3%/89.7%) Z = -0.49
35%	EVT%=39.6 (54.7%/88.1%) Z=-0.75	EVT% = 39.2 (44.9%/94.6%) Z= - 0.93	EVT% = 33.1 (43.7%/95.1%) Z = -1.67

The performance of the composite defined by a confirmed %DSCR of 35% at 12 months and of 50% at 18 months was externally validated using the RENAAL data base (Z = -1.29), although statistical significance of the treatment effect on the endpoint was not reached.

Conclusions: A confirmed 35%↑ in SCr by 12 months or 45% by 18 months was moderately robust to the acute effect seen in IDNT and RENAAL and predictive of long-term incidence of ESRD. This outcome would demonstrate improved performance if the intervention did not have an acute effect on SCr.

Funding: Pharmaceutical Company Support - NephroGenex, Inc

FR-PO901

Study of Diabetic Nephropathy with AtRasentan (SONAR); a New Enrichment Study Design Dick de Zeeuw,[1] Hans-Henrik Parving,[2] Blai Coll,[3] Dennis L. Andress,[3] John J. Brennan,[3] The SONAR Steering Committee.[4] *[1]Univ Medical Center Groningen, Netherlands; [2]Univ Hospital Copenhagen, Denmark; [3]AbbVie, North Chicago; [4]Multiple.*

Background: Diabetes patients with nephropathy are at high risk for renal/CV events. Recent clinical trials of novel treatments have been negative. One reason for treatment failure may have been suboptimal patient selection. The selective endothelin A receptor antagonist, atrasentan, may be a new protective drug, since it appeared to produce a strong effect on albuminuria lowering: -35% (-24 to -45,95% CI), and in 51% of participants achieving >30% reduction (*de Zeeuw et al,JASN 2014*). To test this, we designed the SONAR study, selecting diabetic nephropathy patients with an initially favorable response to atrasentan, subsequently randomized to compare atrasentan with placebo for a primary composite renal endpoint consisting of time to doubling of serum creatinine or onset of ESRD. Secondary endpoints: CV morbidity and mortality, UACR, changes in estimated glomerular filtration rate (eGFR), and quality of life.

Methods: A prospective, randomized, double-blind, enriched-population, placebo-controlled, multinational study in 4148 subjects, with CKD stages 2-4 and macroalbuminuria on maximum tolerated dose of RAS inhibitor. After 6 weeks exposure to 0.75mg/d atrasentan (enrichment period), 3148 patients with >30% UACR decrease (responders) and no signs of major side effects (e.g. fluid retention, heart failure, and BNP<300 pg/ml) are randomized, stratified according to geographical region, baseline albuminuria and albuminuria response (30-45, 45-60 and >60% reduction). Diuretic therapy is optimized during run-in and enrichment. Study power: 425 distinct primary events; a 27% hazard reduction with 90% power,p<0.05. Additionally, 1000 nonresponders will be randomized and followed.

Conclusions: The SONAR trial will test whether we can lower the high residual renal and CV risk in type 2 diabetic nephropathy patients using atrasentan treatment on top of guideline-recommended therapies. The selection and new enrichment design will not only set the next stage for this particular population, but will also define the future role of albuminuria as a target.

Funding: Pharmaceutical Company Support - AbbVie

FR-PO902

Reducing Albuminuria Confers Renoprotection: A Systematic Review and Meta-Analysis of Randomized Controlled Trials Tobias Felix Kröpelin,[1] Hiddo Jan Lambers Heerspink,[1] Jarno Hoekman,[2] Dick de Zeeuw.[1] *[1]Dept Clinical Pharmacy & Pharmacology, Univ Medical Center Groningen, Netherlands; [2]Div of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Univ, Netherlands.*

Background: Albuminuria has been proposed as a surrogate endpoint in randomized clinical trials (RCT's) of renal disease progression. Most evidence is based on observational analyses showing that a treatment induced short-term change in albuminuria correlates with risk change for end-stage renal disease (ESRD). However, these studies are prone to bias and residual confounding. To avoid this type of bias we performed a meta-analysis of RCT's to determine and correlate the placebo corrected drug effect on albuminuria and ESRD in order to reliably examine whether albuminuria is a valid surrogate endpoint.

Methods: MEDLINE and EMBASE were searched without language restriction for RCT's reported between 1950 and April 2014. Included RCT's had a mean follow up of at least 1000 patient years, reported ESRD outcomes, and measured albuminuria at baseline and during follow-up. Meta-regression was performed to assess the association between drug effects on albuminuria and ESRD.

Results: Twenty-one RCT's involving 78,342 patients and 4,183 ESRD events were included. Median time to first albuminuria measurement was 6 month. Fourteen trials tested the effect of renin-angiotensin-aldosterone-system inhibitors (RAASi) and 7 trials tested other interventions. We observed a wide variability across trials in the treatment effect on albuminuria (range -1.3% to -32.1%) and ESRD (range -55% to +35 relative risk change). Meta-regression revealed that the treatment effect on albuminuria significantly correlated with the treatment effects on ESRD: for each 30% reduction in albuminuria the risk of ESRD decreased by 23.7% (95%CI 11.4 to 34.2).The association was consistent regardless of RAASi drugs or non-RAASi drugs (p interaction 0.73) or other patient or trial characteristics.

Conclusions: The significant association between drug effects on albuminuria and ESRD and the consistency across drug classes and patient characteristics suggests that albuminuria is a valid substitute for ESRD in a variety of circumstances.

FR-PO903

Prognostic Utility of Estimated Albumin Excretion Rate (eAER) in Chronic Kidney Disease (CKD): Results from the Study of Heart and Renal Protection (SHARP) Marion Mafham, Natalie Staplin. *On Behalf of the SHARP Collaborative Group, Clinical Trial Service Unit and Epidemiological Studies Unit, Univ of Oxford, Oxford, United Kingdom.*

Background: eAER estimates 24-hour albuminuria more accurately than urine albumin/creatinine ratio (ACR), but whether eAER better predicts end-stage renal disease (ESRD) and vascular events (VEs) is unknown.

Methods: 5552 participants in SHARP (a randomized trial of LDL cholesterol lowering in 9270 patients with CKD) who were not on dialysis, and had estimated glomerular filtration rate (eGFR) and urine ACR measured at baseline, were included. eAER was calculated from ACR and creatinine excretion rate (CER) estimated using a formula from the MDRD study which includes age, sex and race. Relative risks (RR) of ESRD and VEs were estimated using Cox regression and improvements in risk prediction were based on the likelihood ratio test.

Results: During a median follow-up of 4.8 years, 1959 participants progressed to ESRD and 1204 had a VE. Given age, sex and eGFR, both ACR and eAER substantially improved ESRD risk prediction (both increasing the log-likelihood ratio statistic by 436) with similar adjusted RR of ESRD per 10-fold higher level of 2.10 (95% CI 1.96 to 2.26) for ACR and 2.10 (95% CI 1.95 to 2.26) for eAER. Neither ACR nor eAER provided any further predictive information over and above the other. Results were similar among individuals with eGFR <30 and ≥30 ml/min/1.73m^2 and when those with glomerulonephritis, diabetic nephropathy, cystic kidney disease or other renal diagnoses were examined separately. For the prediction of VEs, the addition of ACR (or eAER) to a model including known cardiovascular risk factors and eGFR resulted in a modest improvement in model fit (increasing the log-likelihood ratio statistic by 30), with identical adjusted RRs per 10-fold higher level of 1.26 (95% CI 1.16 to 1.37) for both ACR and eAER. Again, neither ACR nor eAER provided any further prognostic information over and above the other. Results were similar using alternative CER formulae which include weight.

Conclusions: Among individuals with moderate to severe CKD, eAER does not improve risk prediction of either ESRD or VEs beyond that provided by ACR.

Funding: Pharmaceutical Company Support - The Study of Heart and Renal Protection (SHARP) was coordinated by the Clinical Trial Service Unit. The University of Oxford is the trial sponsor with financial support from Merck Sharp & Dohme

FR-PO904

The Discrepancy of Clinical Significance Between Diabetic and Hypertensive Retinopathy in Patients with Chronic Kidney Disease Hyeon Seok Hwang,[1] Se Young Kim,[1] Yoon-Kyung Chang,[1] Chul Woo Yang,[2] Suk Young Kim,[1] Hye Eun Yoon.[2] *[1]Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Daejeon, Korea; [2]Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

Background: Chronic kidney disease (CKD) and retinopathy shares a common pathophysiology of microvascular dysfunction. However, the different clinical significance of diabetic retinopathy (DMR) and hypertensive retinopathy (HTNR) is undetermined in CKD patients.

Methods: We included 501 diabetic and 191 non-diabetic patients with nondialysis-dependent CKD stages 3–5. The clinical significance of DMR and HTNR was evaluated in terms of rate of renal function decline and composite of any cardiovascular event or death after the index retinopathy examination.

Results: DMR was observed in 269 (53.7%) diabetic patients with CKD, and HTNR were found in 44 (23.0%) non-diabetic patients with CKD. The rate of renal function decline was significantly steeper in diabetic patients with DMR than those without DMR (-7.4 ± 9.8 mL/min/1.73 m^2/y versus -2.4 ± 7.6 mL/min/1.73 m^2/y; P < 0.001). Patients with proliferative DMR showed more rapid renal function decline than patients with non-proliferative DMR (P = 0.015). However, HTNR was not associated with rapid decline of renal function (-3.1 ± 9.0 mL/min/1.73 m^2/y versus -2.9 ± 9.9 mL/min/1.73 m^2/y; P = 0.769). In a multivariate analysis, DMR was an independent predictor for renal function decline (b = –2.16; P = 0.014). Diabetic patients with DMR showed lower composite event-free survival rate than those without DMR (P = 0.048), while HTNR did not increase the risk for composite event. In the multivariate hazards model, retinopathy was independently associated with any cardiovascular events or death (hazard ratio, 1.59; P = 0.026) in diabetic CKD patients.

Conclusions: DMR is an independent risk factor for rapid renal function decline and composite of cardiovascular event or death in diabetic patients with CKD. However, HTNR was not associated with renal and cardiovascular outcome in non-diabetic patients with CKD.

FR-PO905

Chronic Kidney Disease of Unknown Origin and Proposed Risk Factors Prevalence in Hemodialysis Patients from Southwest Guatemala
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Background: An epidemic of chronic kidney disease of unknown origin (CKDu) has been documented in southwest El Salvador and Nicaragua, yet no data is available from neighboring Guatemala. Therefore, we sought to determine the CKDu and proposed risk factors prevalence in hemodialysis patients from southwest Guatemala.

Methods: The Social Security healthcare is the largest provider of hemodialysis in southwest Guatemala. All patients on hemodialysis were screened for diabetes. Non-diabetics were interviewed (and charts reviewed) asking for traditional and proposed CKD risk factors. Analyses were done in SPSS.

Results: Most patients (74%) were non-diabetic (178/242). Obesity was the most prevalent known risk factor. Many of CKDu's proposed risk factors were present in more than half the patients, including male gender and agricultural work. Almost one third were sugar cane workers. Upon dialysis initiation, only hyperuricemia was common. About 20% (45/242) possibly had CKDu. About 20% (45/242) of participants did not have a history of hypertension and overweight/obesity and could be considered possibly affected by CKDu.

Known and proposed CKD risk factor prevalence in non-diabetics from Southwest Guatemala

	n = 178 (%)
Known Risk Factors	
Hypertension	99 (55.6%)
Overweight/Obese	59 (33.1%)
Not Hypertensive or Overweight	45 (25.3%)
Family History -Renal Disease -Hypertension -Diabetes	 29 (16.3%) 51 (28.8%) 47 (26.4%)
Proposed Risk Factors	
Age < 50 years old	106 (59.6%)
Age at Diagnosis ≤ 40 years old	99 (55.6%)
Male	144 (80.9%)
≤ 6 years of schooling	127 (71.3%)
Possible Leptospirosis	15 (8.4%)
Ever Use -NSAIDs -Alternative Medicine	 154 (86.5%) 99 (55.6%)
Agricultural Occupation - > 20 years work - Age ≤ 15 when started	113 (63.5%) 51 (45.1%) 73 (64.6%)
Sugar Cane Worker	52 (29.2%)
Alcohol Consumption	128 (71.9%)
Hypokalemic (n=177) *	24 (13.6%)
Hyperuricemic (n=162) *	87 (53.7%)
* At first dialysis	

Conclusions: Several of the proposed risk factors for CKDu were highly prevalent and require additional research to establish causality.

Funding: Private Foundation Support

FR-PO906

Prevalence of Glomerulonephritis in the U.S. Medicare Population
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Background: Glomerulonephritis (GN) is a serious disorder that can lead to death, end-stage renal disease, and other morbid events. However, the prevalence of GN in the Medicare population is unknown.

Methods: A retrospective observational study was conducted to ascertain the prevalence of GN using the 20% Medicare sample from 2007-11. A primary GN was established by the appearance of ≥ 2 ICD-9 codes for a GN > 30 days apart, plus ≥ 1 code for resultant renal disease occurring on or after the first GN code; a systemic immunologic disorder resulting in a GN required ≥ 3 relevant ICD-9 codes ≥ 3 days apart, plus ≥ 2 subsequent ICD-9 codes for a GN or resultant renal disease ≥ 30 days apart. We calculated the prevalence of GN's per 100,000 patients by dividing the number of GN cases by the total number of individuals in the database from 2007-11, and determined hospitalization and death rates per 1000 patient-years.

Results: A total of 8,276,664 individuals were studied. Mean ± SD age was 68.2 ± 12.9 years and 55.1% were female. Period prevalence from 2007-11 was 433 cases of primary GNs per 100,000 individuals, representing 35,870 total cases, while there were 878 cases of GN resulting from systemic diseases per 100,000 individuals, representing 72,666 total cases. Incidence rates were 86.7 and 136.1 per 100,000 patient-years, respectively. Total hospitalization rates were 2083 and 2093 hospitalizations per 1000 patient-years, respectively, while death rates were 136 and 195 per 1000 patient-years.

Conclusions: There are likely >100,000 cases of GNs in the Medicare population alone, representing a substantial burden to patients, payers, and the nation. GNs appear to be associated with high hospitalization and death rates, but further study is required.

FR-PO907

Impact of Elevated Blood Pressure on CKD Prevalence and Staging: The Texas Kidney Study
Sharma S. Prabhakar, Katherine Kam. *Internal Medicine, Texas Tech Univ Health Sciences Center.*

Background: Recent literature points to high estimates of of chronic kidney disease (CKD) in general population although true incidence and prevalence of CKD are unknown. The Texas Kidney study was designed to address the epidemiological issues of CKD in Texas and we report here the impact of uncontrolled and undetected hypertension on prevalence and stages of CKD in a random screening study of general population.

Methods: A cohort of 1606 subjects was recruited through random digit dialing of an unselected general population to participate in a study to evaluate the prevalence and risk factors for CKD. A total of 1579 subjects completed the study. IRB approvals and informed consents of all subjects were obtained. Detailed history and clinical parameters including two separate measurements of blood pressure were obtained during the screening. Subjects with elevated BP (systolic >140 and/or diastolic >90 mm/Hg) were analyzed separately. Blood and urine samples were obtained to measure the renal function. Both MDRD and CKD-EPI formulae were used separately to estimate the prevalence of CKD using K-DOQI guidelines.

Results: The overall prevalence of CKD in this cohort (n=1579) was 17.7% by MDRD and 15.8% by CKD-EPI formula while in the high BP group (n=133) it was higher at 22.6% ($p<0.05$) by both formulae. About 38% in the latter group were unaware of their hypertension. Other co-morbid conditions in this group were obesity (22%) Diabetes (20%), stroke (6%) and smoking (16%).

Group (n)	CKD prevalence MDRD CKD EPI	Males (%) with CKD	Blacks (%) with CKD	High alb/ creat ratio >100mg/G	CKD stage I-II MDRD CKD-EPI	CKD Stage III-V MDRD CKD EPI
Elevated BP Group (133)	22.6% (30) 22.6% (30)	48.9*	12.8*	8.27%*	12.8% 13.6%*	9.8% 9.1%*
Total Study Group (1579)	17.7% (279) 15.8% (249)	38.1	6.4	3.62%	7.8% 8.1%	9.9% 7.7%

*P<0.05 vs. total study group

Conclusions: We conclude that the prevalence of CKD is 16-18% (depending on the methodology) in general population in Texas which is higher than the previously reported data. However the prevalence of CKD in uncontrolled or previously undetected hypertension is much higher (22.6%) with high prevalence of heavy proteinuria, which underscores the importance of hypertension in CKD progression.

Funding: Other U.S. Government Support

FR-PO908

Association of Serum 25-Hydroxy Vitamin D Levels with Chronic Kidney Disease Progression
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Background: Experimental studies have shown that vitamin D serves as an immune modulator and a suppressor of interstitial fibrosis. However, a relationship between vitamin D and chronic kidney disease (CKD) progression in patients is uncertain. We investigated whether 25-hydroxy vitamin D [25 (OH) D] deficiency influences the progression of CKD.

Methods: In this prospective study, serum levels of 25 (OH) D were obtained from 135 patients with chronic kidney disease in the winter of 2009. The primary composite endpoints was end stage renal disease (ESRD) or kidney transplant (KT). The secondary composite endpoint was a >50% decline from baseline in estimated glomerular filtration rate (eGFR). Data were collected over the subsequent four years.

Results: The mean age of the study population was 61.3 ± 12.4 years, and the mean eGFR was 32.6 ± 13.5 ml/min per 1.73 m^2. A total of 42.2% of patients had diabetes. Vitamin D deficiency,as defined by <15 ng/ml serum 25 (OH) D, was observed in 60% of the patients (n = 81). The mean 25 (OH) D serum level was 14.7 ± 7.8 ng/ml. During the four-year follow-up period, 27 patients reached the primary end point (21 patients with 25 (OH) D deficiency and 6 patients without 25 (OH) D deficiency, P = 0.03). Fifteen and six patients reached the secondary end point in the 25 (OH) D deficiency and no 25 (OH) D deficiency groups, respectively (P = 0.2). The annual rate of eGFR change was greater in the 25 (OH) D deficiency group (-1.6 ± 2.5 versus -0.7 ± 2.0 ml/min per 1.73 m^2. P = 0.03). Kaplan-Meier analysis revealed that the risk of ESRD or KT was significantly higher in CKD patients with 25 (OH) D deficiency (log rank test; P = 0.04). Multivariate Cox proportional hazards models also demonstrated that the risk of ESRD or KT was significantly higher in CKD patients with 25 (OH) D deficiency (hazard ratio [HR] 7.65, 95% confidence interval [CI] 2.1-27.4; P=0.02).

Conclusions: These results demonstrate that 25 (OH) D deficiency is independently associated with a higher risk of the composite outcome in patients with CKD.

FR-PO909

Renal Outcomes of Patients with Acute Renal Infarction
Jihyun Yang, Hyojeong Chang, Myung-Gyu Kim, Sang-Kyung Jo, Won-Yong Cho. *Korea Univ, Seoul, Republic of Korea.*

Background: Renal infarction (RI) is uncommon disease. Although underlying hypercoagulable state is known to be a risk factor and the consequent renal mass reduction can affect its renal outcomes, little is known about the clinical characteristics or longterm renal outcome.

Methods: This is a single center retrospective study including 86 patients with newly diagnosed acute RI between January 2002 and March 2014. Their clinical features, possible etiologies and longterm renal outcome data were reviewed.

Results: The mean age was 63.5 ± 15.5 years and the prevalence of diabetes or hypertension was 24.7 or 43.0%. Bilateral involvement was shown in 18.6%. Of the underlying diseases, cardiovascular origin including atrial fibrillation, valvular heart disease, infective endocarditis were most common (39.5%), and renal vascular injury and hypercoagulable status were found in 14% and 3.5%. Idiopathic RI comprised 20% of cases. For the treatment, anti-platelet therapy, anticoagulation or thrombolysis were used in 53.5%, 57.6% and 3.5%, respectively. At the time of diagnosis, acute kidney injury (AKI) was accompanied in 30.2% of patients. In univariate analysis, male sex, alcohol, smoking, high CRP, leukocytosis and the presence of more than two underlying causes were significant risk factors for the development of AKI. In multivariate analysis, only the presence of two or more underlying causes was a risk factor for predicting the development of AKI. During follow up (55.9±35.2 months), chronic kidney disease (CKD) with eGFR<60 mL/min/1.73m² developed in 30.2%. In univariate analysis, development of CKD was associated with old age, hypertension, smoking, leukocytosis, history of AKI and the presence of more than two underlying causes of RI. In cox-regression analysis, old age and the presence of more than two causes of RI were the independent risk factors for the development of CKD.

Conclusions: We found that idiopathic RI comprised about one fifth of overall cases and also that both AKI and CKD were relatively common complications of RI. Because old age, presence of more than two causes of RI were associated with the development of AKI and also with CKD, more careful attention should be paid for the improvement of patients outcomes.

FR-PO910

Long-Term Predictors for End-Stage Renal Disease Emanuel Zitt,[1] Constanze Pscheidt,[2,3] Reinhard Kramar,[4] Gabriele Nagel,[2,3] Karl Lhotta.[1] *[1]Nephrology and Dialysis, Academic Teaching Hospital Feldkirch, Austria; [2]Agency for Preventive and Social Medicine, Austria; [3]Inst of Epidemiology and Medical Biometry, Ulm Univ, Germany; [4]Austrian Dialysis and Transplant Registry, Austria.*

Background: Aim of this observational study was to identify anthropometric and metabolic long-term risk factors for the development of end-stage renal disease (ESRD).

Methods: The population-based surveillance/screening programme "Vorarlberg Health Monitoring and Promotion Programme" has collected information on cardiovascular risk factors in more than two-thirds of the population of Vorarlberg, the western-most part of Austria with approximately 370.000 inhabitants. From 1988 to 2005, the following data were collected prospectively during regular health examinations in 185.341 persons (M/F 85.460/99.881): age, smoking status, body-mass-index (BMI), blood glucose, systolic (BPsys) and diastolic (BPdia) blood pressure, total cholesterol (TC), triglycerides (TG) and gamma-glutamyltransferase (GGT). These data were merged with the Austrian Dialysis and Transplant Registry. To determine the long-term risk for ESRD multivariable-adjusted Cox regression models were used to estimate hazard ratios (HR), additionally stratified by renal disease and by 5-year time slots of follow up after the first health examination.

Results: Mean age at first health examination was 41.6 years. During a mean follow-up of 17.5 years 403 patients (M/F 245/158) reached dialysis dependent ESRD. Significant long-term predictors for ESRD were: age (per year) HR 1.02 (95% CI: 1.01-1.03), male sex 1.72 (1.36-2.16), smoking 1.33 (1.06-1.66), BMI (per 1 kg/m²) 1.04 (1.01-1.06), glucose (per 1 mmol/L) 1.09 (1.05-1.12), BPsys (per 5 mmHg) 1.10 (1.07-1.14), BPdia (per 5 mmHg) 1.09 (1.03-1.15), TG (per 1 mmol/L) 1.07 (1.02-1.13) and TC (per 1 mmol/L) 1.22 (1.13-1.32). Time slot analyses identified age, male sex, BMI, glucose, BPdia and GGT as long-term predictors after more than 15 years of follow-up. Stratifying by renal disease disease-specific patterns of metabolic risk factors could be determined.

Conclusions: Already at a very early stage, specific anthropometric and metabolic factors are associated with an increased risk for ESRD.

FR-PO911

Association between Smoking Habits and Urinary Excretion of Albumin in Patients with Advanced Chronic Kidney Disease. CERCA-DIABETES Study Elvira Bosch, Celia Lopez, Fatima Batista, Mar Lago, Agustin Toledo, Cesar Garcia-Canton. *Dept of Nephrology, Hospital Univ Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain.*

Background: To evaluate the effects of smoking habits on the prevalence of albuminuria and proteinuria in patients with chronic kidney disease (CKD) and to assess possible modulatory effects of factors such as gender, diabetes, control of blood pressure (BP) or antyhipertensive treatment.

Methods: Transversal study with 259 patients with stage 4 and 5 CKD. Patients were classified according to their smoking habits into: smoker, non-smoker and ex-smoker. Demographic and clinical variables, as well as kidney function and proteinuria at the beginning of the study were analyzed.

Results:

	Non-smokers (n:111)	Ex-smokers (n:104)	Smolers (n:43)	p
Age (years)	68.8 ± 12.2	67.6 ± 10.9	57.6 ± 13.2	0.000
Sex (%)	♀ 77.5 /♂ 22.5	♀ 18.3/ ♂ 81.7	♀ 20.9 / ♂ 79.1	0.000
DM-2 (%)	57.7	69.2	60.5	ns
HTA (%)	97.2	95.1	97.7	ns
Ischemic c. (%)	22.5	25	19	ns
BMI (kg/m²)	34 ± 17	30.1 ± 5.3	27.9 ± 5.6	0.004
ACEinhibitor/ARAII (%)	45.9	54.8	35.7	ns
GFR (ml/min)	22.6 ± 6.7	22.5 ± 6.7	21.7 ± 4.7	ns
Proteinuria (g/24h)	0.9 ± 1.6	2.1 ± 3.6	3.5 ± 6	0.000
MAU (mg/dl)	23.3 ± 44.8	38.9 ± 61.9	81,7 ± 96.6	0.000
A/C ratio (mg/g)	422.7 ± 846.7	636.9 ± 989.4	1428 ± 1642.2	0.000

shows these variables for the three groups of patients. Smokers and ex-smokers presented higher proteinuria and albuminuria than non-smokers (p<0.005). No differences were found in the prevalence of DM-2, treatment with renin-angiotensin-aldosterone system inhibitors (RAASI) or glomerular filtration. After adjusting by age, DM, BMI, BP, glomerular filtration or RAASI treatment, smoking habit was found to be a risk factor for the development of albuminuria (HR:2.9,CI95%: 1.09-7.7;p:0.032) and proteinuria (HR:3.6,CI95%:1.09-11.8;p:0.035).

Conclusions: The prevalence of proteinuria and urinary excretion of albumin is higher in smoker and ex-smoker patients than in non-smoker ones. Differences cannot be accounted for by other variables such as gender, diabetes, BMI, BP or RAASI treatment.

FR-PO912

Measures of Carbohydrate Metabolism and Clinical Outcomes in Non-Diabetic Chronic Kidney Disease: Findings From the Chronic Renal Insufficiency Cohort (CRIC) Study Amanda Hyre Anderson,[1] Francis Perry Wilson,[1] Raymond R. Townsend,[1] Jing Chen,[2] L. Lee Hamm,[2] J. Richard Landis,[1] James P. Lash,[2] Daniel J. Rader,[1] Bruce M. Robinson,[2] Harold I. Feldman.[1] *[1]Univ of Pennsylvania; [2]The CRIC Study.*

Background: Disturbances of carbohydrate metabolism are common in chronic kidney disease (CKD), but their relationships to CKD progression, cardiovascular disease (CVD) and death in this setting remain unclear.

Methods: Data from a subset of CRIC Study participants with non-diabetic CKD (N=1,865, average follow-up 5 years) were analyzed in Cox proportional hazards models to examine the association of homeostasis model assessment-estimated insulin resistance (HOMA-IR), fasting plasma glucose, hemoglobin A1c (HbA1c), and C-peptide with risk of a renal composite endpoint (end-stage renal disease or halving of estimated glomerular filtration rate [eGFR]), atherosclerotic CVD (MI/stroke/PAD) and death with adjustment for demographics, anthropometrics, comorbidities and clinical and biochemical measures.

Results: The mean (IQR) age was 59 (49-65) years, and mean (SD) eGFR was 49 (18) mL/min/1.73m². All carbohydrate metabolism measures, whether examined continuously on the log scale or across quartiles, were not significantly associated with the renal composite, atherosclerotic CVD, or death in multivariable-adjusted models (Table). Effect modification by level of kidney function or race was not detected.

	Hazard Ratio	95% CI
Event: Renal composite		
HOMA-IR	1.04	(0.91,1.19)
Glucose	0.92	(0.80,1.04)
HbA1c	0.96	(0.85,1.08)
C-peptide	1.08	(0.94,1.24)
Event: Atherosclerotic CVD		
HOMA-IR	1.00	(0.86,1.15)
Glucose	1.11	(0.91,1.32)
HbA1c	1.09	(0.92,1.29)
C-peptide	1.03	(0.83,1.28)
Event: All-cause death		
HOMA-IR	1.10	(0.98,1.22)
Glucose	1.01	(0.86,1.17)
HbA1c	1.01	(0.86,1.17)
C-peptide	1.10	(0.92,1.30)

Conclusions: Previously reported associations of carbohydrate metabolism with clinical outcomes in the setting of kidney disease were not detectable in this study of individuals with reduced levels of kidney function.

Funding: NIDDK Support

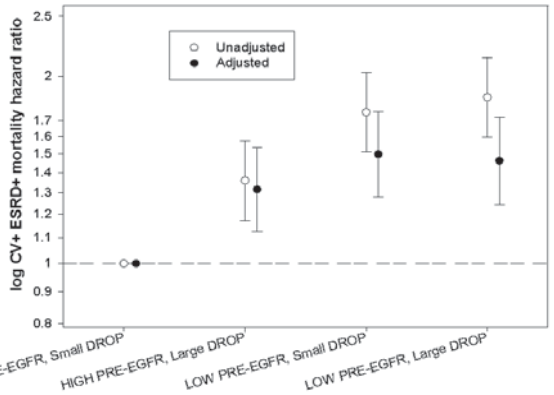
FR-PO913

Association of Glomerular Filtration Rate and Post-Nephrectomy Outcomes in U.S. Veterans Elani Streja,[1] Kamyar Kalantar-Zadeh,[1] Lin Li,[1] Miklos Zsolt Molnar,[2] Jun Ling Lu,[2] Csaba P. Kovesdy.[2] *[1]Harold Simmons, UC Irvine MC, Orange, CA; [2]Memphis VAMC, Memphis, TN.*

Background: Radical nephrectomy has been considered the standard of care for localized renal malignancy, but results in variable loss of kidney function. It is unclear if the degree of kidney function loss is associated with the post-nephrectomy incidence of ESRD, survival, and cardiovascular events.

Methods: We identified 7,448 U.S. Veterans that had either a total or partial nephrectomy between Oct. 2004-Sept. 2012. EGFR prior to admission for nephrectomy and percentage drop in EGFR from pre-nephrectomy to 31 days post-nephrectomy were estimated from creatinine measurements using CKD-EPI equations and medians were calculated. Patients were categorized into 4 groups based on whether they were less than or greater than or equal to the median for the pre-nephrectomy EGFR and percent drop. Associations between these 4 groups with a combined risk of all-cause mortality, incident ESRD and cardiovascular event were evaluated in crude and case-mix adjusted cox models.

Results: Patients were 60±10 yrs old, 4% female, 18% African-American, and 29% diabetic. Median (p25, p75) values for pre-admission EGFR and percentage drop were 79 ml/min/1.73m^2 (65, 94) and 26% drop (8%, 39%) respectively. There were 1,631 deaths, 59 ESRD outcomes, and 310 coronary events. Using patients with combined high pre-admission EGFR, and small percent drop as the reference, patients with a combined large percent drop and high EGFR had a 32% increased risk of combined outcomes. Patients with low pre-admission EGFR had a 50% increased risk, with similar risk of outcomes for patients with small or large percent drop.



Conclusions: Loss of kidney function after nephrectomy is associated with adverse outcomes. Further studies are needed to determine if nephron-sparing surgery can improve clinical outcomes.

Funding: NIDDK Support

FR-PO914

Muscle Mass Assessed by Urinary Creatinine Excretion and Chronic Kidney Disease Outcomes Benedicte Stengel,[1] Elena Tynkevich,[1] Jean-Philippe Haymann,[2] Marie Metzger,[1] Pascal Houillier,[3] Martin Flamant.[4] *[1]Inserm Unit 1018, CESP, Villejuif, France; [2]Dept of Physiology, AP-HP, Tenon Hospital, Paris, France; [3]UMRS 775, Paris 5 Univ, Paris, France; [4]Dept of Physiology, AP-HP, Bichat Hospital, Paris, France.*

Background: Muscle wasting predicts mortality in patients with end-stage renal disease (ESRD), but its role in the progression of chronic kidney disease (CKD) is uncertain. We studied CKD outcomes associated with low muscle mass, assessed by urinary creatinine excretion.

Methods: The NephroTest cohort included 1,429 patients with CKD stages 1 to 4 and both measured (mGFR) (by ^{51}Cr-EDTA) and estimated (eGFR) (by CKD-EPI equation) glomerular filtration rates. We estimated adjusted hazard ratios (HR, 95% confidence interval) for ESRD and pre-ESRD death associated with urinary creatinine quartiles defined by gender-specific thresholds (11.3, 13.3, and 15.5 mmol/24 h in men and 7.5, 9.0, 10.5 in women).

Results: Mean urinary creatinine excretion was 13.6 ± 3.2 mmol/24 hour (0.17 ± 0.05 mmol/kg/24 h) in men and 9.2 ± 2.1 mmol/24 hour (0.14 ± 0.05) in women. Over a median follow-up of 3.6 years, 229 patients developed ESRD and 113 patients died before ESRD. Compared with patients in the highest urinary creatinine quartile, those in the lowest quartile had a higher HR for pre-ESRD mortality: 2.0 [0.98-4.1], independent of demographic variables, mGFR, cardiovascular risk factors, body mass index, and several biomarkers of nutritional status, and inflammation. In contrast, their ESRD risk was not higher either before or after adjusting for confounders including mGFR: crude HR, 0.99 [0.68-1.4] and adjusted HR, 0.69 [0.46-1.0]. Interestingly, adjustment for eGFR instead of mGFR distorted this relation by producing a significantly higher HR for ESRD in the lowest quartile: 1.6 [1.1-2.5].

Conclusions: Muscle wasting in early stage CKD, reflected by lower urinary creatinine excretion, is associated with increased mortality, but not with progression to ESRD. Previous studies showing greater ESRD risk with lower urinary creatinine excretion may have been confounded by inappropriate adjustment for creatinine-based estimated GFR.

Funding: Pharmaceutical Company Support - Roche, Government Support - Non-U.S.

FR-PO915

The Association of Kidney Function with Repetitive Breath-Hold Diving Activities of Haenyeo, Korean Women Unassisted Diver Yun Jung Oh,[1] Su Mi Lee,[2] Dongyeol Lee,[2] Chungsik Lee.[1] *[1]Internal Medicine, Cheju Halla General Hospital, Jeju, Republic of Korea; [2]Internal Medicine, Donga Univ Hospital, Pusan, Republic of Korea.*

Background: The effects of repetitive deep breath-hold diving on cardiovascular, respiratory, and metabolic adjustments have been reported in previous studies, which include bradycardia, vasoconstriction of selected vascular beds, depressed metabolism, and decreased respiratory exchange ratio. However, renal response to high-pressure and hypoxic environment associated with diving has not investigated yet. We aimed to examine the association between kidney function and breath-hold diving activities of Korean women divers, haenyeo who traditionally harvest marine products controlling breathing without any apparatus in Jeju province.

Methods: A cross-sectional study was performed using the medical records of 1,920 women divers and 3,220 non-divers women who presented to a hospital for health check-up. Using propensity score matching, 647 women diver/non-diver were paired and the prevalence of chronic kidney disease (CKD; estimated GFR<60ml/min/1.73m^2) was investigated in both diver and non-diver groups.

Results: Women divers were older and showed higher prevalence of comorbidities (hypertension, cardiovascular disease, and diabetes) than non-divers in unmatched cohort. Women diver group showed an increased prevalence of CKD compared to non-diver group after propensity score matching (11.6% versus 7.1%; P=0.006). In multivariable analysis, women diver group remained a significant association with prevalence of CKD in unmatched cohort (OR, 1.971; 95% CI, 1.478-2.627; P<0.001). In the matched cohort, women diver group also significantly associated with a higher adjusted OR of CKD (OR, 1.898; 95% CI, 1.204-2.993; P=0.006).

Conclusions: Kidney function was more decreased in women divers compared to non-divers, suggesting that prolonged repetitive breath-hold diving may exert adverse influence on kidney function.

FR-PO916

The Association between Periodontal Disease and Kidney Function Decline in African Americans: The Jackson Heart Study Vanessa Grubbs,[1,4] Eric Vittinghoff,[1] Michael Griswold,[2] Neil R. Powe,[1,4] Kirsten Bibbins-Domingo,[1,4] James D. Beck,[5] A. V. Kshirsagar,[5] Wei Wang,[2] Adolfo Correa,[2] Bessie A. Young.[3] *[1]UCSF; [2]UMMC; [3]VA Puget Sound; [4]SFGH; [5]UNC Chapel Hill.*

Background: Chronic kidney disease (CKD) remains a prevalent public health problem that disproportionately affects African Americans, despite efforts targeting traditional risk factors. While periodontal disease (PD), a chronic bacterial infection of the oral cavity, is both common and modifiable and implicated as a novel potential risk factor for CKD in cross-sectional studies, the association between PD and longitudinal decline in kidney function has not been fully explored.

Methods: We conducted a longitudinal analysis of 699 African American participants who underwent complete dental examinations as part of the Dental-Atherosclerosis Risk in Communities study (baseline, 1996-1998) and subsequently enrolled in the Jackson Heart Study (follow-up, 2000-2004). CKD-EPI estimated glomerular filtration rate (eGFR) ml/min/1.73m^2 was calculated at baseline and follow-up. Among participants with baseline eGFR>60ml/min/1.73m^2, incident CKD was defined as incident eGFR<60ml/min/1.73m^2 accompanied by a 5% annualized eGFR decline from baseline. Multivariable Poisson regression was used to examine the association between PD status (defined by Centers for Disease Control/American Academy of Periodontology consensus criteria), and incident CKD adjusting for age, sex, diabetes, hypertension, smoking, and income.

Results: Among study participants, mean age at baseline was 60.5 years (SD 5.2), median eGFR was 95.7 (IQR 83.2, 110.3) ml/min/1.73m^2, and 114 (16.3%) had severe PD. During a mean follow-up of 4.8 years (SD 0.6), there were 21 (3.0%) incident CKD cases. Participants with severe PD had a 4.2-fold greater incidence of CKD after adjusting for covariates compared to those without severe PD [IRR 4.18, 95% CI (1.69, 10.39), p=0.002].

Conclusions: Severe periodontal disease is common among a population at high-risk for CKD and is associated with clinically significant kidney function decline. Further research is needed to determine if the association is causal and, if so, modifiable.

Funding: NIDDK Support, Private Foundation Support

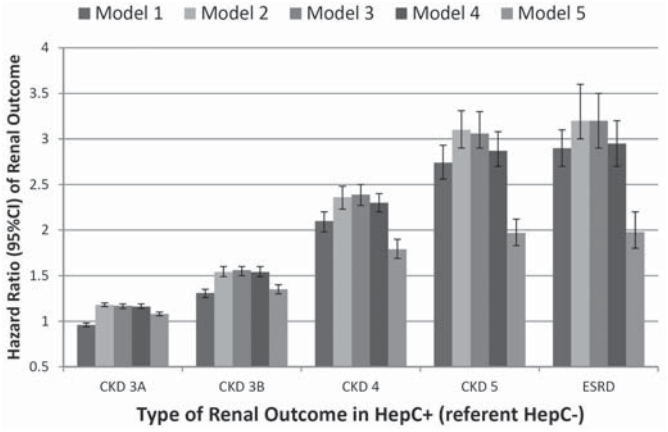
FR-PO917

Association of Hepatitis C Virus Infection with Progressive CKD and Mortality in a Large Cohort of U.S. Veterans Hazem M. Alhourani,[1] Barry M. Wall,[1,2] Miklos Zsolt Molnar,[1] Jun Ling Lu,[1] Robert B. Canada,[1] Jennie Z. Ma,[3] Elani Streja,[4] Kamyar Kalantar-Zadeh,[4] Csaba P. Kovesdy.[1,2] *[1]Univ of Tennessee; [2]Memphis VAMC; [3]Univ of Virginia; [4]Univ of California Irvine.*

Background: An estimated 4 million Americans have been exposed to the hepatitis C virus (HCV). The risk of incident CKD and mortality in patients with normal kidney function infected with hepatitis C (HCV) is unclear.

Methods: In a nationally representative cohort of 1,021,049 U.S. Veterans (n=100,518 HCV+ and 920,531 HCV-) with normal baseline eGFR we examined the association of HCV infection with: (1) all-cause mortality, (2) incidence of CKD stages 3a, 3b, 4, 5 and ESRD, and (3) the slopes of eGFR. Associations were examined in crude and adjusted Cox models and logistic regression models (for slopes), with sequential adjustments for demographics (model 1), eGFR (model 2), comorbidities (model 3), BP and BMI (model 4), and markers of socioeconomic status, adherence with medical interventions and medication use (model 5).

Results: The mean (SD) age was 54.5 (13.1), 22% were black, 92% were male, and the mean (SD) baseline eGFR was 88 (16) ml/min/1.73m^2. Outcomes occurred in 116,935 (deaths); 159,872 (CKD3a); 39,518 (CKD3b); 10,578 (CKD4); 4,748 (CKD5); 3,383 (ESRD) and 70,989 patients (slopes steeper than -5 ml/min/1.73m^2/year). HCV infection was associated with higher mortality (fully adjusted HR, 95%CI: 2.17 [2.13-2.21]), with higher risk of steeper slopes (fully adjusted OR, 95%CI: 1.45 [1.41-1.48]) and with higher incidence of various CKD stages (Figure). The risk of incident CKD increased with more advanced stages of CKD end points (Figure).



Conclusions: HCV infection is associated with higher mortality and with progression of CKD. Randomized controlled trials are indicated to determine if treatment of HCV infection can prevent the development and progression of CKD and improve survival.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO918

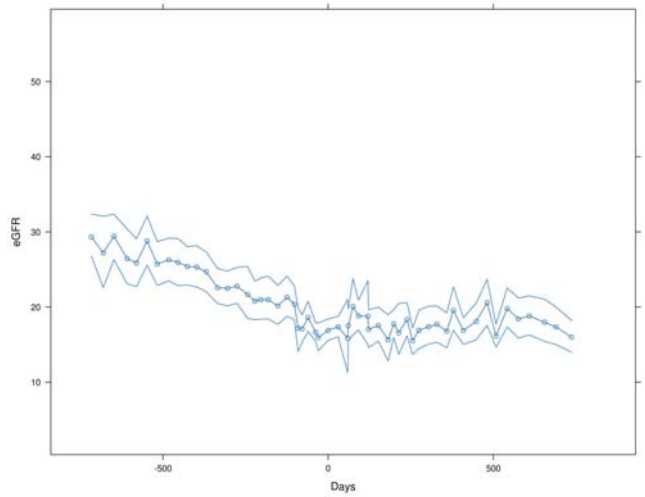
Arterio-Venous Fistula (AVF) Creation May Slow Estimated Glomerular Filtration Rate (eGFR) Trajectory

Thomas A. Golper, Phillip Matthew Hartle. *Medicine/Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

Background: The KDOQI Vascular Access guidelines prompted a re-awareness to timely create AVFs, a policy practiced at our institution. We have often observed a slowing of the decline in kidney function (eGFR) after AVF creation, prompting the present retrospective observational analysis of the eGFR trajectory before and after the AVF creation.

Methods: We identified 123 subjects between 2005 and 2010 with at least two eGFR determinations (IDMS-traceable MDRD Study equation) for 2 years before and 2 years after AVF creation. Inclusion eligibility was that the fistula was maturing by the nephrologist's subsequent post creation exams, as noted in the medical record. Termination events were death, starting dialysis or transplantation. Each subject served as his/her own control for the before and after AVF creation eGFR measurements so size normalization was not performed.

Results: Subject characteristics are mean age of 68 years, 41% female, 56% diabetic, 97% hypertensive. The rate of change of eGFR for the two years prior to AVF creation was 5.9 mL/min/year (IQR 6.5, 5.3) and for up to 2 years after AVF creation 0.5 mL/min/year (IQR 1.1, 0.1) (p< 0.001).



Conclusions: Agreeing to timely AVF creation selects patients in an otherwise typical population. Observational studies suggest trajectory can accelerate as eGFR declines. We observed the opposite. There are biologically and physiologically plausible explanations as to how an AVF creation might alter kidney function, but this clinical observation has been attributed to improved compliance and/or divine intervention. A prospective observational study is indicated because, if the present observation is confirmed, AVF creation in and of itself may delay the initiation of dialysis, reducing morbidity and costs.

Funding: Pharmaceutical Company Support - Dialysis Clinics, Incorporated

FR-PO919

Contemporary Rates and Predictors of Loss of Kidney Function and End-Stage Renal Disease in Adults with Chronic Kidney Disease

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Background: Identifying CKD patients at high-risk for short-term adverse renal outcomes is challenging. Within a very large, diverse community-based CKD cohort, we evaluated contemporary rates and predictors of CKD progression.

Methods: Within Kaiser Permanente Northern California, we identified adult members with Stage 3/4 CKD (eGFR 15-59 min/1.73m^2 by CKD-EPI) between 2008-2012 and no prior dialysis or transplant. Through 2012, we calculated the rate and identified predictors of the composite outcome of ESRD, reaching eGFR <15 ml/min/1.73m^2, or >50% reduction from baseline eGFR. In sensitivity analyses, we used an alternative composite outcome that included >40% eGFR reduction. Demographic and clinical characteristics, plus baseline medication use were obtained from health plan databases.

Results: Among 149,487 Stage 3/4 CKD patients, mean age was 73 yr, 57% were women, 23% were persons of color and 29% were diabetic. Over mean follow-up of 3.5±1.5 years, the overall annual renal composite outcome rate was 2.14 (2.10-2.18), and higher in diabetics (3.86) versus non-diabetics (1.45). The strongest multivariable predictors of CKD progression are shown in the table.

Characteristic	Adjusted Hazard Ratio (95% CI)
eGFR (vs. 45-59 ml/min/1.73 m2)	
30-44	1.07 (1.02-1.13)
15-29	2.85 (2.69-3.02)
Proteinuria	2.63 (2.42-2.64)
Hemoglobin (vs. ≥13 g/L)	
12-12.9	2.31 (2.16-2.46)
11-11.9	3.71 (3.48-3.96)
10-10.9	6.12 (5.71-6.56)
9-9.9	8.67 (7.92-9.50)
<9	12.57 (11.23-14.08)
Male gender	1.17 (1.11-1.24)
Asian	1.25 (1.17-1.33)
Hispanic	1.13 (1.06-1.20)
Current/former smoking	1.09 (1.04-1.14)
Diabetes	1.12 (1.05-1.20)
Systolic BP (vs. ≤120 mmHg)	
121-129	1.16 (1.09-1.23)
130-139	1.29 (1.22-1.37)
140-149	1.36 (1.27-1.45)
150-159	1.70 (1.56-1.86)
160-179	2.00 (1.78-2.25)
≥180	1.36 (1.19-1.75)

When using >40% eGFR reduction in the composite outcome, the rate was 3.58 (3.53-3.63) and predictors were similar.

Conclusions: In a large, contemporary CKD population, significant progression affected 1 in 47 patients annually. A combination of readily available patient features can identify the subset of high-risk CKD patients.

Funding: Pharmaceutical Company Support - AstraZeneca

FR-PO920

Predictors of Fast Progression in a Community-Based Population with Stage 3 Chronic Kidney Disease

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Background: Few studies have systematically evaluated a wide range of potential risk factors for accelerated progression of CKD in representative "real world" populations. We examined a large, diverse community-based CKD cohort to identify predictors of fast progression during the first two years of follow-up.

Methods: Within Kaiser Permanente Northern California, a large integrated health care delivery system, we identified adult Stage 3 CKD patients (eGFR 30-59 ml/min/1.73m^2 by CKD-EPI) between 2008-2010, no prior dialysis or transplant, had outpatient serum creatinine values spaced 10-14 months apart and did not initiate renal replacement therapy, die or disenroll during the first 2 years of follow-up. Through 2012, we calculated the annual rate of change in eGFR and classified patients as fast progressors if they lost >4 ml/min/1.73m^2 per year.

Results: We identified 30,739 eligible adults with Stage 3 CKD (mean entry eGFR 48±9, mean age 73 yr, 57% women, 35% persons of color and 28% diabetic). Annual incidence of fast progression was 14.6% (14.2-15.0) and higher in diabetics (18.2%) versus non-diabetics (12.6%). The strongest multivariable predictors of fast CKD progression are shown in the Table.

Characteristic	Adjusted Hazard Ratio (95% CI)
Age >80 (vs. 18-49 yr)	1.49 (1.15-1.92)
Heart failure	1.37 (1.20-1.55)
Dementia	1.37 (1.10-1.69)
Systolic BP (vs. <120 mmHg)	
130-139	1.21 (1.10-1.33)
140-159	1.36 (1.22-1.51)
160-179	1.97 (1.69-2.29)
≥180	2.18 (1.66-2.86)
Proteinuria	1.76 (1.60-1.94)
Hemoglobin (vs. ≥13 g/L)	
12-12.9	1.33 (1.21-1.46)
11-11.9	1.73 (1.55-1.93)
10-10.9	1.91 (1.62-2.26)
<9	1.75 (1.07-2.85)
Total cholesterol >240 (vs. <200 mg/dL)	1.34 (1.08-1.67)
HDL cholesterol (vs. ≥60 mg/dL)	
50-59	1.18 (1.06-1.31)
40-49	1.20 (1.08-1.34)
35-39	1.16 (1.02-1.32)
< 35	1.19 (1.03-1.38)

Conclusions: In a large, contemporary population of adults with Stage 3 CKD, accelerated progression of kidney dysfunction affected ~1 in 7 patients. Older age, selected morbidities, high blood pressure, proteinuria, anemia, high total cholesterol and lower HDL were the strongest predictors of rapid progression of CKD.

Funding: Pharmaceutical Company Support - AstraZeneca

FR-PO921

Progression of Chronic Kidney Disease in Primary Care over 1 Year

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Background: Most people with CKD stage 3 are elderly and managed in primary care but much of the literature comes from studies in specialist nephrology centres. Questions remain regarding optimal frequency of follow-up and risk of progressive disease in this population. The Renal Risk in Derby (RRID) study aims to evaluate decline of renal function in a cohort of people with CKD 3 recruited from general practices in Derbyshire, UK.

Methods: Data from 1621 of 1741 participants in the RRID cohort were included in this analysis. Participants were recruited from primary care practices. All had eGFR 59-30ml/min on 2 occasions prior to inclusion. At baseline and 1 year visits, participants underwent clinical assessment, urine and serum biochemistry. Progression of CKD was defined using KDIGO criteria (25% loss of GFR and an increase in GFR category or an increase in albuminuria category).

Results: 38 participants died before the year 1 assessment. Overall, there was a small but statistically significant decline in the mean eGFR from baseline to Year 1 (52.7ml/min to 52. 3ml/min p=0.025). 140 out of 1621 participants (8.6%) demonstrated progression in either albuminuria or GFR category. Progression largely occurred separately in these two groups.

	No Albuminuria Progression n (%)	Albuminuria Progression n (%)
No GFR Progression n (%)	1481 (91.3%)	87 (5.4%)
GFR Progression n (%)	47 (2.9%)	6 (0.37%)

Significant univariate associations with progression were age (HR=1.03), diabetes (HR=2.4), haemoglobin (HR=0.72), albumin (HR=0.94), bicarbonate (HR=0.90) and GFR (HR=0.978). Multivariable logistic regression analysis identified haemoglobin (HR=0.85; 95% CI 0.73-0.99 p=0.041), albuminuria (HR 1.28; 1.11-1.47 p=0.001) and a definite or possible episode of AKI (HR 4.60; 2.75-7.90 p<0.001) as independent determinants of progression.

Conclusions: Over 1 year, a significant minority showed progression in either albuminuria or GFR category. Progression was independently associated with baseline haemoglobin, albuminuria and AKI. Our results support annual follow-up except in those with albuminuria or following AKI. Interventions to prevent AKI will likely also reduce CKD progression.

Funding: Private Foundation Support

FR-PO922

Progression to Next Stage of Chronic Kidney Disease (CKD) Using Modified KDIGO (eGFR/Proteinuria) Classification in Children

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Background: The combination of eGFR and proteinuria in the latest KDIGO classification guideline has not been applied in children with CKD to inform the frequency of follow-up visits. Using data from the Chronic Kidney Disease in Children (CKiD) study, we assessed the percentage of individuals in a given stage who progressed to another stage within 1.5 yrs of follow up.

Methods: Accelerated failure time models were used to classify subjects into categories of eGFR and urine protein-to-creatinine ratio (uP/c) with unique risk for developing an event: either (i) 50% reduction of baseline GFR (ii) initiation of RRT or (iii) death during follow-up. Seven unique risk stages were identified. At annual follow-up visits, the proportion at each stage remained or transitioned to a different stage of CKD in 1.5 years was then assessed.

Results: 854 subjects (71.2% non-glomerular and 29% glomerular causes) with 2591 patient-years follow-up experienced 219 events (defined above). Median (IQR) age of the cohort at baseline was 11 (8, 15) yrs. Among the 1841 (82%) patient visits with original eGFR ≤60ml/min/1.73m^2, around one third of them transitioned to a higher risk stage or experienced an event within 1.5 yrs. (table). Risk of an event for individuals in stage A-D was low, while 20-56%of individuals in stages E-G experienced an event in this time frame.

		Percentage remaining or transitioning to alternate stage in 1.5 yrs							
N	Original stage	A	B	C	D	E	F	G	Event
408	A	78	14	7	0	<1	<1	0	<1
422	B	13	57	25	2	<1	1	0	2
575	C	6	15	50	15	7	5	<1	2
269	D	1	2	19	41	17	9	3	8
276	E	0	<1	7	6	54	1	13	20
177	F	1	<1	8	11	4	39	15	21
122	G	0	0	0	1	13	1	30	56

eGFR category (ml/min/1.73m^2): G1 ≥90; G2 60-90; G3a 45-60; G3b 30-45; G4 15-30
A: eGFR G1 and uP/C <2.0 or eGFR G2 and uP/C <0.5
B: eGFR G3a and uP/C <0.5
C: eGFR G2/G3a and uP/C 0.5-2.0 or eGFR 3b and uP/C <0.5
D: eGFR G3b and uP/C 0.5-2.0
E: eGFR G4 and uP/C <2.0
F: eGFR G1/G2/G3a/G3b and uP/C >2.0
G: eGFR G4 and uP/C >2.0

Conclusions: Based on observation of progression in CKiD using eGFR and uP/C, children with eGFR >30ml/min/1.73m^2 and uP/C<2.0 are at low risk of an event, despite progression over a 1.5 yr period, while children with uP/C>2.0 or eGFR≤30ml/min/1.73m^2 are at substantially higher risk and more frequent follow up is suggested.

Funding: NIDDK Support

FR-PO923

Decline in Kidney Function with Aging: Mathematical Modeling of Serial eGFR Measurements

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Background: Decline in kidney function in the elderly is thought to be a consequence of normal aging. However, long-term prospective studies of kidney function in the general population are lacking. The purpose of this study was to characterize the changes in estimated glomerular filtration rate (eGFR) over time in a large cohort of Icelandic subjects.

Methods: For participants aged 33-75 years in the Reykjavik Study (RS), conducted between 1967 and 1995, we identified all subsequent measurements of serum creatinine (SCr) from clinical laboratories in the Reykjavik area. The MDRD Study equation was used to calculate eGFR and various statistical models for longitudinal data were applied to characterize changes in eGFR over time. The analysis was made separately for men and women.

Results: At least one SCr value was identified for 14,572 participants (85.4%), of whom 51.6% were women. Mean follow-up time was 28.4 years. Decline in eGFR with age was observed to be non-linear and best represented by the class of generalized additive mixed models. A sharper decline in eGFR after age 70 years was noted. Age, age at first entry and diabetes were significantly associated with eGFR decline in both men and women, and diastolic blood pressure and proteinuria associated with eGFR in women. At age 70 years, eGFR <60, <45 and <30 mL/min/1.73 m^2 was observed in 29.5%, 6.6% and 1.3% of subjects, and at age 80 years in 45.1%, 17.9% and 4.3% of subjects, respectively.

Conclusions: Kidney function declines with age in a non-linear fashion. Assessment of the change in eGFR over the lifespan and into older age requires flexible modeling approaches. Major cardiovascular risk factors were associated with the change in eGFR with age.

Funding: Government Support - Non-U.S.

FR-PO924

Usefulness of the CKD Classification in Targeting of HIV Individuals at High Risk of Poor Outcomes

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Background: Chronic kidney disease (CKD) is now epidemic among HIV-infected populations and a likely determinant of their prognosis.

Methods: A total of 1,975 HIV-infected subjects (1,852 men, 124 women, mean age: 44.5 ± 11.5 years) who regularly visited one of 5 tertiary hospitals were classified into 4 risk categories, using the combination of 5 stages of eGFR and 3 grades of ACR (urinary albuminuria-creatinine-ratio) (categories 0 to 3). Among 1,975 HIV subjects, 661 were

prospectively followed-up for 4 years to determine incidence of composite outcomes, including all-cause mortality, cardiovascular disease and a decline over 25% from baseline in eGFR. The cumulative incidence of the outcomes was analyzed with Kaplan-Meier method, and hazard risk (HR) of the categories for the outcome incidence was calculated using multivariable proportional hazards regression analysis, adjusted for age, gender, infection markers, and presence or absence of comorbidities.

Results: The frequency of each CKD category was shown in Table. The Kaplan-Meier estimates were significantly increased over time in the risk categories 2 *plus* 3, compared with the categories 0 *plus* 1. The HR of risk categories 2 *plus* 3 was 2-fold greater (HR = 2.00; its 95% confidence interval, 1.08 - 3.57; P = 0.0277), as compared to risk categories 0 *plus* 1.

Conclusions: The CKD classification may serve as a new tool for highlighting HIV-infected individuals at increased risk.

Table Distribution of HIV individuals according to the CKD risk categories				
GFR stage	eGFR	ACR <30	ACR 30-300	ACR >300
G1	>90	category-0:597 (28.0%)	category-1: 41 (1.9%)	category-2: 0 (0.0%)
G2	60-90	category-0:1200 (56.2%9	category-1: 89 (4.2%)	category-2: 4 (0.2%)
G3A	45-60	category-1:135 (6.3%)	category-2: 23 (1.1%)	category-3: 3 (0.1%)
G3B	30-45	category-2: 10 (0.5%9	category-3: 11 (0.5%)	category-3: 3 (0.1%)
G4	15-30	category-3: 3 (0.1%)	category-3: 4 (0.2%9	category-3: 4 (0.2%)
G5	<15	category-3:0 (0.0%)	category-3 0 (0.0%)	category-3: 8 (0.4%9

FR-PO925

Characterizing Patterns of Kidney Function Decline Associated with *APOL1* High-Risk Variants: The African American Study of Kidney Disease and Hypertension (AASK) Adrienne Tin,[1] M. Grams,[1] Michelle M. Estrella,[1] Michael S. Lipkowitz,[2] Liang Li,[3] Tom Greene,[4] Wen Hong Linda Kao,[1] Lawrence J. Appel.[1] *[1]Johns Hopkins Univ; [2]Georgetown Univ Medical Center; [3]Cleveland Clinic; [4]Univ of Utah.*

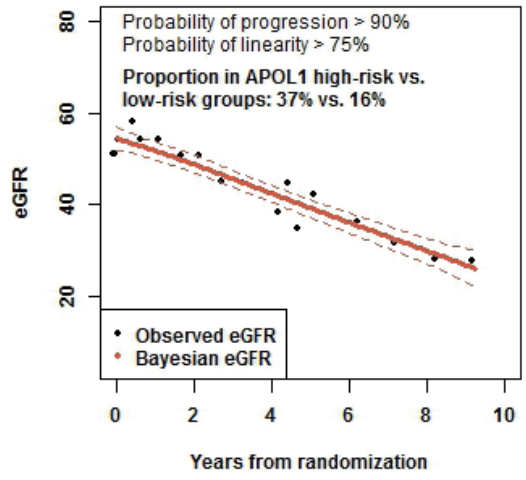
Background: *APOL1* high-risk variants have been associated with faster chronic kidney disease (CKD) progression. Whether trajectories of estimated glomerular filtration rate (eGFR) in patients with *APOL1* high-risk status (≥2 risk alleles) manifest as a steady decline or punctuated decrements in eGFR is unknown.

Methods: Among participants in AASK, we used Bayesian estimates of the probabilities of (a) eGFR progression and (b) linearity to characterize eGFR trajectories. A steady decline in eGFR was defined as the probability of progression over 90% and the probability of linearity over 75% (figure). Logistic regression was used to estimate the odds ratio of steady eGFR decline associated with *APOL1* high-risk status.

Results: Over a mean follow-up of 9 years, 128 of the 622 participants with eGFR trajectory and *APOL1* genotyping data had eGFR trajectories characterized by steady decline. Compared to participants without a steady eGFR decline, those with a steady eGFR decline had lower baseline levels of serum albumin (4.17 versus 4.28 g/dl, p=0.002) and eGFR (45 versus 51 ml/min/1.73 m^2, p<0.001), and a higher proportion with proteinuria (52% versus 19% with urine protein:creatinine >0.22, p<0.001). Participants with a steady GFR decline were also more likely to be of *APOL1* high-risk status (37% versus 16%, p<0.001). This association persisted after adjustment for gender, trial treatment, and baseline age, eGFR, proteinuria, blood pressure, and serum albumin (OR 2.03, p=0.004).

Conclusions: These results showed that more than a third of patients with CKD attributed to hypertension and the *APOL1* high-risk genotype experienced unremitting steady decline in eGFR.

Figure 1. An example of steady eGFR decline



Funding: NIDDK Support

FR-PO926

Family History as a Predictor of Renal Outcomes: Results from the Michigan Kidney Translational Core Center Crystal A. Gadegbeku,[1] J. Troost,[2] Jennifer Joyce Hawkins,[2] Susan P. Steigerwalt,[3] Kalyani Perumal,[4] Zeenat Yousuf Bhat,[5] M. Sampson,[2] Frank C. Brosius,[2] Matthias Kretzler,[2] Debbie S. Gipson.[2] *[1]Temple Univ, Philadelphia, PA; [2]Univ of Michigan, Ann Arbor, MI; [3]Renaissance Renal Research Inst, Detroit, MI; [4]Univ of Illinois, Chicago, Chicago, IL; [5]Wayne State Univ, Detroit, MI.*

Background: Epidemiologic studies have identified familial clustering in the ESRD population. However, few studies characterize the impact of family history on progression of renal disease.

Methods: History of kidney disease in first-degree relatives (FHx) was obtained from consented participants in the Clinical Phenotyping Resource and Biobank Core (C-PROBE) of the Michigan Kidney Translational Core Center (2P30-DK-081943). This multi-site core collects clinical, demographic, and social data along with biospecimens from participants with a broad range of nephropathies to facilitate translational research. FHx, eGFR (CKD-Epi equation), ESRD and vital status were analyzed in participants with ≥ 1 annual follow-up visit. Renal outcomes are defined as 40% reduction in eGFR, ESRD (eGFR < 15 ml/min on 2 consecutive visits, dialysis, or kidney transplantation) and/or death. Data are reported as mean (sd). Outcomes were modeled using an unconditional logistic regression (SAS v9.2).

Results: 97 of 433 participants (22%) had a FHx and, overall, were older with a lower eGFR and more likely to have non-glomerular diseases and diabetes. In multivariate regression, adjusting for age, eGFR, diabetes status, FHx was associated with an OR of 1.8 for 40% reduction in eGFR but no difference in ESRD/death in this time interval.

C-PROBE Cohort	FHx	No FHx	p-value
N	97	346	
Mean Follow-Up, yrs	2.3 (1)	2.1 (1)	0.16
Age, yrs	59 (14)	51 (18)	<0.01
Sex (% women)	56%	62%	0.29
Black/African American	43%	40%	0.55
Hispanic	8%	7%	0.73
Baseline eGFR (ml/min)	47 (26)	58 (33)	<0.01
Glomerular Disease	43%	60%	<0.01
Diabetes Status (Yes)	31%	21%	0.03
40% Reduction in eGFR	52%	34%	<0.01
ESRD or Death	15%	10%	0.17

Conclusions: These observations extend the data linking FHx to worse renal outcomes. Future multi-and trans-disciplinary translational research is required to explore complex mechanisms of disease susceptibility and risk.

Funding: NIDDK Support

FR-PO927

A Retrospective Study of Safety and Efficacy of 24-Hour SLED-RCA in Critically Ill Patients Sergio Trevino, John Manllo, Jian Li, Jerry Yee, Stanley Frinak, Lenar T. Yessayan, Balazs Szamosfalvi. *Div of Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Background: Regional citrate anticoagulation (RCA) use is limited by concerns of electrolyte complications. The aim was to evaluate the safety and efficacy of 24-hour sustained low efficiency dialysis (SLED) with RCA.

Methods: Continuous SLED-RCA was performed using a Fresenius 2008 K or K2 hemodialysis machine at blood flow 60 ml/min and dialysate flow 400 ml/min using dialysate Na 140, K 4, Ca 0 and HCO3 32. Acid citrate dextrose-A solution (115 mmol/L) was infused into the arterial limb of the extracorporeal circuit at 150 ml/hr. Calcium chloride solution (136 mmol/L CaCl2 and 19.7 mmol/L MgCl2) infusion rate was adjusted according to a dosing table based on circuit hemoglobin levels as measured by a hemoglobin sensor and daily serum albumin. Laboratory measurements were obtained at baseline and every 12 hours. Age, gender, comorbidity and reason for kidney support were collected. RCA effectiveness was measured in terms of catheter+circuit patency. Electrolyte trends and complications for the first 48 hours are reported using standard boxplots. Complications related to RCA were defined as hypocalcemia with ionized calcium (iCa) <0.9 mmol/L, hypercalcemia iCa >1.3 mmol/L, hypernatremia Na^+ >148 mEq/L and greater than 5 mEq/L rise in Na^+ from baseline, metabolic alkalosis with HCO3 >30 mEq/L, and clinically significant hypophosphatemia as P <2.0 mg/dl.

Results: 187 48-hour sessions of SLED-RCA were analyzed. We did not preclude any patient from receiving SLED-RCA due to liver failure. The percentage of catheters and/or circuits with clotting within 12 h, 24 h and 48 h was 8/187 (4%), 8/187 (4%) and 15/187 (8.5%), respectively. There were no cases of hypernatremia or metabolic alkalosis. Hypophosphatemia developed in 18/555 (3%), hypercalcemia in 9/526 (1.7%; highest iCa=1.39), hypocalcemia in 2/526 (0.3%; lowest iCa=0.74).

Conclusions: SLED-RCA reduces circuit clotting and carries a very small risk of clinically significant hypo- or hypercalcemia. The risk of hypernatremia or metabolic alkalosis is entirely abrogated. Clinically significant hypophosphatemia is mostly avoided by intravenous phosphate replacement.

FR-PO928

A Regional Intensive Care Unit Transition From Heparin to Citrate Anticoagulation as First-Line- Safety, Filter Down-Time, and Cost
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Background: Regional citrate anticoagulation (RCA) is being increasingly used for continuous renal replacement therapy (CRRT). Evidence suggests that compared to heparin, RCA prolongs filter life, and may reduce bleeding risk, but there is little data on how this translates into more relevant outcomes such as time on filter or cost. The aim of this study was to establish if a change from heparin to RCA resulted in more achieved time on filter, and calculate any cost difference. Safety parameters were a secondary endpoint.

Methods: A single-center, retrospective observational study from 2006-12 during which a transition from heparin to RCA occurred. Both methods were protocolised, with the RCA protocol aimed at simplicity, using either 14 or 18 mmol (diluted) citrate bags depending on availability. Case note demographic and dialysis data, pathology results and costings were obtained.

Results: 188 patients had 992 dialysis days (heparin 334 versus RCA 658). Demographics were well matched. The RCA group used less filters per day (p=0.03), had more days when prescribed dialysis was achieved (85% versus 60%, p<0.001), had less dialysis days with "down-time" (15% versus 40%, p<0.001), and less time off the filter on those "down-time" days (2.4 versus 6.1 hours, p=0.02). RCA was estimated to cost AU$495 per day, compared to Heparin at $440 per day. There was no statistical difference in clinically significant safety events between the 2 groups, although 2 catastrophic bleeding events in the heparin group were the impetus for the transition, and there was more metabolic alkalosis in the citrate group depending on which formulation was used.

Conclusions: Citrate anticoagulation safely provides less filter down-time, allowing for improved delivery of prescribed dialysis dose, and uses less filter circuits. The cost difference per day favours heparin, but at $55 per day is relatively small.

FR-PO929

Regional Citrate Anticoagulation (RCA) Reduces Blood Transfusion Requirement in Surgical Critically Ill Patients Undergoing Continuous Renal Replacement Therapies (CRRT)
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Background: The use of RCA-CRRT in critically ill patients (pts) requiring dialysis is improving. We utilize RCA-CRRT in >50% of about 1000 treatment performed yearly. Blood transfusion requirement is important both for clinical and economical reasons, but available literature on this topic, comparing different types of CRRT, is poor. This retrospective study, focused on critically ill pts who underwent major surgery, has the aim to evaluate the impact of RCA-CRRT on blood transfusions.

Methods: Between January 2011 and December 2012, we treated 293 pts with CRRT. We excluded: pts treated with alternated standard dialysis (STD-CRRT, with heparin or without anticoagulation) and RCA-CRRT; pts with CRRT duration ≤ 2 days. We included 63 pts, who underwent cardiac, vascular or abdominal surgery, treated exclusively with RCA-CRRT or STD-CRRT. Indication to RCA-CRRT was given on clinical basis. Age, length of hospital stay (LOS), CRRT duration, hospital mortality (HM), haemoglobin value at start and end of CRRT period (Hb-S and Hb-E), blood transfusion/CRRT days ratio (CRRT-BT) and blood transfusion/hospital days ratio (tot-BT) were evaluated for each patient. Statistical analysis was performed with Student's *t test.*

Results: RCA-CRRT pts were 50, mean age 72.8 years, male 82%; mean LOS 35.9 days; mean CRRT duration 10 days, 500 total CRRT days; HM 40%; mean Hb-S and Hb-E 9.36 g/dl and 9.43 g/dl respectively (p=NS), mean CRRT-BT 0.56, mean tot-BT 0.45 STD-CRRT pts were 13, mean age 68.5 years, male 77%, mean LOS 26.7 days; mean CRRT duration 10.2 days, 132 total CRRT days; HM 69%, mean Hb-S and Hb-E 10.4 g/dl and 9.2 g/dl respectively (p<0.05), mean CRRT-BT 1.08, mean tot-BT 1.48. Tot-BT and CRRT-BT were significantly lower in RCA-CRRT pts than in STD-CRRT pts.

Conclusions: We observed that RCA-CRRT is associated with significantly lower need of blood transfusions, and higher stability of Hb levels during CRRT. Moreover, HM is lower in RCA-CRRT pts than in STD-CRRT pts, without differences of LOS and dialysis duration.

FR-PO930

Effect of Nafamostat Mesilate as an Anticoagulant during Continuous Renal Replacement Therapy
Min-Jee Han, Chae Rim Kim, Do Hyoung Kim, Su Hyun Kim. *Dept of Internal Medicine, Chung-Ang Univ Hospital, Seoul, Republic of Korea.*

Background: The use of continuous renal replacement therapy (CRRT) is considered the favored renal replacement therapy modality in patients with hemodynamic intolerability. An important limitation of CRRT is the need for prolonged anticoagulation to prevent extracorporeal filter clotting and failure. Nafamostat mesilate (NM), a serine proteinase inhibitor, is characterized by short half life resulting in little systemic anticoagulation effect. The aim of this study was to evaluate the effect of NM on circuit patency of CRRT and survival rate of patients receivinging CRRT with acute kidney injury (AKI).

Methods: We retrospectively reviewed 115 patients with AKI treated with CRRT between March 2005 and December 2012. We devided the patients into 2 groups according to the anticoagulants used during CRRT. Among 115 patients, 85 patients (73.9%) were treated with continuous NM infusion and remaining 30 patients (26.1%) were treated with unfractinated heparin for anticoagulation.

Results: The median filter survival with NM was significantly greater than heparin (24.2 versus 17.4 hours, p=0.021) and Kaplan-Meier survival plots revealed the longer survival of the circuits using NM than heparin (p=0.014). In cox proportional hazard models, NM predicted longer filter survival (hazard ratio 0.59, 95% confidence interval 0.38-0.92, p=0.020). And NM group showed a significantly lower 30 days mortality rate compared to heparin group (40.0% versus 63.3%, p=0.028). In multivariate Cox analysis, patient survival was significantly higher in the NM group than heparin group (hazard ratio 0.46, 95% confidence interval 0.25-0.84, P=0.011).

Conclusions: As compared with heparin, NM was associated with prolonged filter survival and 30 days patient survival. These data suggest that NM is a good choice for anticoagulant during CRRT in critically ill patients. NM can be used as a safe and effective regional anticoagulant for CRRT.

FR-PO931

Ability of Nafamostat Mesilate to Prolong Filter Patency during Continuous Renal Replacement Therapy in Patients with a High Risk of Bleeding: A Randomized Controlled Study
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Background: Continuous renal replacement therapy (CRRT) is considered as an effective modality for renal replacement therapy in hemodynamically unstable patients in intensive care units (ICUs). However, role of heparin anticoagulation, which is used to maintain circuit patency, is equivocal due to the risk of bleeding and morbidity. Futhan is an effective anticoagulant for patients prone to bleeding. Hence, we conducted a prospective, randomized controlled study investigating the effect of futhan on CRRT filter life span and the adverse events in the patients prone to bleeding.

Methods: Patients were randomized into either the futhan or no-anticoagulation group. The futhan group received futhan, while the no-anticoagulation group received no anticoagulation medication. Baseline characteristics and appropriate laboratory tests were taken from each group.

Results: Filter life span and numbers of filters used during CRRT were similar in both groups. For the futhan group versus the no-anticoagulation group, the overall number of filters used during CRRT (2.71 ± 2.12 versus 4.50 ± 3.25; $p<0.05$) and the number of filters changed due to clots per 24 hours (1.15 ± 0.81 versus 1.90 ± 1.60; $p<0.05$) were significantly different. When filter life span was subdivided into below and over 12 hours, the number of filters functioning over 12 hours was significantly higher in the futhan group than the no-anticoagulation group ($p<0.05$, odds ratio 1.84). There were no significant differences for transfusion, mortality, or survival between the two groups, and no adverse events related to futhan were uncovered.

Conclusions: Futhan may be used as an effective and safe anticoagulation treatment, without increasing major bleeding complications, in patients prone to bleeding.

Funding: Pharmaceutical Company Support - SK chemicals, Seoul, Republic of Korea

FR-PO932

Survival Benefit of Polymyxin B Hemoperfusion in Septic Shock Patients Receiving Continuous Renal Replacement Therapy
Masao Iwagami,[1] Kent Doi,[2] Hideo Yasunaga,[3] Masaomi Nangaku,[2] Eisei Noiri.[2] *[1]London School of Hygiene and Tropical Medicine; [2]The Univ of Tokyo Hospital; [3]The Univ of Tokyo.*

Background: While endotoxin adsorption by polymyxin B hemoperfusion (PMX) has been suggested to improve survival in septic shock (JAMA. 2009), our retrospective analysis showed that postoperative PMX had no survival benefit for patients with lower gastrointestinal tract perforation (Crit Care Med. 2014). However, our cohort had lower mortality. Previous studies have suggested that PMX may be effective for critically ill patients at higher risk of mortality. Therefore, this study was conducted to examine the survival benefit of PMX in septic patients requiring continuous renal replacement therapy (CRRT) who are known to have increased mortality.

Methods: Patients aged 18 years or older in the Japanese Diagnosis Procedure Combination Database satisfying the following criteria were enrolled: hospitalized during 39 months between 2007 and 2012; started CRRT under the diagnosis of sepsis (except Gram-positive bacteremia); and required noradrenaline and/or dopamine. Exclusion criteria included end-stage renal disease, suspected cardiogenic shock, intermittent dialysis, plasma exchange, and not starting PMX on the same day as CRRT. Propensity scores were generated from age, sex, admission diagnosis category, surgical procedure, time from admission to CRRT initiation, vasoactive drugs, ventilator, transfusion, hospital volume and type. After one-to-one matching from those with and without PMX, 28-day mortality counting from CRRT initiation was compared.

Results: Of 4,027 eligible patients, 1,116 received one or two PMX sessions. Propensity score matching created a matched cohort of 1,115 pairs. The 28-day mortality was 40.8 % in the PMX group and 47.0 % in the control group (P=0.003). Logistic regression analysis also showed an association between the use of PMX and 28-day mortality with an adjusted odds ratio of 0.76 (0.63–0.90). Two PMX sessions led to lower 28-mortality than one session.

Conclusions: Although unmeasured confounders need to be carefully considered, this retrospective study suggests that septic shock patients requiring CRRT may be an appropriate target population benefitting from PMX.

Funding: Government Support - Non-U.S.

FR-PO933

Acute Cast Nephropathy in Multiple Myeloma: Effects of Intensive Polymethylmethacrylate-Enhanced Adsorption Dialysis on Renal Survival

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Background: Renal recovery in multiple myeloma (MM) cast nephropathy is correlated to an early reduction of sFLC concentrations obtained through chemotherapy combined to extracorporeal sFLC removal. We previously reported that polymethylmethacrylate (PMMA) membranes (BK-F; Toray Inc., Japan) can adsorb high quantities of sFLC especially with a new dialysis procedure called "enhanced adsorption dialysis " (EAD). During 4 hours EAD a specific designed circuit allows to safely use 2 dialyzers sequentially without treatment interruption. Nevertheless no clinical data are available on EAD efficacy in renal recovery. We report our experience of EAD treatment in incident MM patient with dialysis dependent renal failure.

Methods: we treated 12 incident patients with dialysis-dependent renal failure and high levels of sFLC. All patients had highly suspicious (n=8) or biopsy proven (n=4) cast nephropathy. In all patients a bortezomib based chemotherapy was started together with daily EAD treatment to reduce sFLC levels. Through daily sFLC measurement we scheduled dialysis treatments aiming to the greatest sFLC reduction in the shortest time. Renal outcome was measured as dialysis independence at 1, 6 and 12 months. Results are reported as average and (min-max) values.

Results: At presentation eGFR and sFLC levels were 9,7 ml/min (4,3-31,8) and 7868,5 mg/l (2300-25118) respectively. We performed on average 5 (1-8) EAD sessions for patient obtaining a sFLC removal of 36,7% /session (-13 to 71%). sFLC were reduced by 59,2% (0-92%) at 12 days and by 70,2% (0-99%) at 21 days. At present 11 out of 12 patients completed 1 and 6 months follow up while one patient died. At 1 and 6 month follow up 50% and 83,5% of patients recovered renal function respectively. Only 9 patients completed one year of follow up and we registered 78% of dialysis independence (1 dead and 1 ESRD).

Conclusions: PMMA enhanced adsorption dialysis together with bortezomib based chemotherapy was able to efficiently reduce sFLC levels and to promote an high rate of persistent dialysis independence.

FR-PO934

Is the CARPEDIEM a Promising Machine to Treat Neonatal and Pediatric Patients with Acute Kidney Injury?

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Background: Among neonatal and pediatric patients admitted on intensive care, 8-20% develop acute kidney injury (AKI). With the current hemodialysis machines, non-accurate weight loss and large extracorporeal blood volumes imply important risks. Recently, CARPEDIEM™ (Bellco, Italy) has become available for Slow Continuous Ultrafiltration (SCUF) or Continuous Veno Venous Hemofiltration (CVVH) in pediatric patients weighing 2.5 to 10kg.

Methods: Three roller pumps guarantee accurate flows for blood (2-50mL/min), infusion and ultrafiltration (0-1000mL/h). Fluid balance is controlled by infusion and effluent scales. Three kits are available: HCD0075/015/025 with a polysulfone dialyzer of 0.075/0.15/0.25m² and 27/33/41mL priming volume, and a maximum ultrafiltration of 2.5/4/17mL/min.

Results: In our hospital, we treated 2 patients: a male (M) (14 weeks, 5.2kg, multiple organ failure and cytomegalovirus infection) and a female (F) (30months, 13kg, AKI). A right (M) and left (F) jugular 6.5Fr double lumen catheter was inserted, and the HCD025 kit was used once (M) and twice (F). The kit was primed with albumin (SOPP 4%) (M) and with NaCl and 10mL 20%HA (F). A bolus of heparin was given at start [150IU (M) and 300IU (F)] and during the session [75IU/h (M) and 200IU/h (F)]. Predilution CVVH (substitution 4-4.3mL/min) was performed with blood flow 20-30mL/min (M) and 30-45mL/min (F), and ultrafiltration 500mL/24h. Continuous CVVH however needed to be stopped already after 10h due to hemodynamic instability (M) and after 9h and 8h due to technical problems related to ultrafiltration, respectively, anticoagulation problems while using heparin (F).

Conclusions: CARPEDIEM™ is certainly a promising machine to treat pediatric and neonatal patients with AKI when only ultrafiltration is needed, but offers so far not a solution either when higher dialysis efficiency or citrate dialysis is needed, or when lactate substitution solution should be avoided. Hence, modifications are necessary to fulfill neonatal dialysis needs.

FR-PO935

Successful Treatment of Life Threatening Diphenhydramine Intoxication By Intermittent Hemodialysis Using a High Cut-Off Membrane

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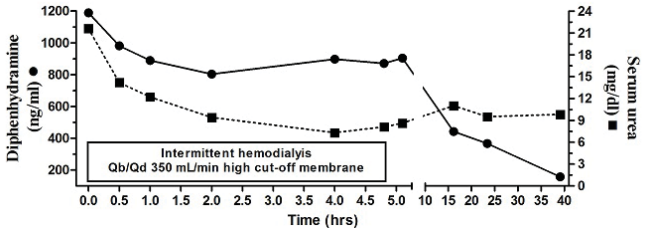
Background: Diphenhydramine (DPH) is a first-generation H1-antihistamine of the ethanolamine type. Overdose can result in ataxia, fever and coma. DPH has a molecular weight of 291 D. After oral administration 60 % of the drug is absorbed. Its volume of distribution is 4.5 L/kg and the elimination half-life 8.4 hours. Hemodialysis is the extracorporeal treatment of choice for various life threatening intoxications, except for highly protein bound substances, which are preferably removed by charcoal hemoperfusion. This technique is however limited by its availability and its significant side-effects.

Methods: A 19 year-old Caucasian woman was admitted to the emergency department seven hours after ingesting 500 mg of diphenhydramine in a suicide attempt. Due to the initial diphenhydramine level of 1189 ng/ml and in an attempt to increase body temperature an intermittent haemodialysis using the GENIUS® dialysis systemwas initiated. A 1.8 m² high cut-off polysulfone filter was used.

Results: The initial dialyser clearance of diphenhydramine at the start of a 4.8 hour treatment (blood and dialysate flow of 350 mL/min) was 91 mL/min. After 3 hours of hemodialysis the patient quickly recovered clinically and gained consciousness allowing stating the existence and location of a second poisoned victim. Using a 4.8 hour intermittent hemodialysis resulted in a reduction of the initial diphenhydramine levels by 27 %.



Conclusions: Timely detoxification resulted in rapid gain of consciousness allowing the patient to state the existence and location of a second poison victim.

FR-PO936

Evaluation of a Dialysate Delivery System for Passive-Flow Hemodialysis

Bridger W. Bach, Benjamin Harris Timmins, Martin C. Gregory. *Medicine, Univ of Utah, Salt Lake City, UT.*

Background: Hemodialysis is life-saving treatment for acute renal failure that is economically beyond the reach of many who may benefit from it worldwide. Passive-flow dialysis is a simple method of providing dialysis without the need for external sources of energy, thus potentially making hemodialysis available at low cost for areas without reliable electricity.

Methods: A dialysate delivery system was constructed from 2 concentric closed cylinders of 1/8" acrylic. The space above the inner cylinder was filled with 46 L of water. Tubing connected the top of the space above the inner cylinder via a heat exchanger and dialyzer to the bottom of the space below the inner cylinder. A T-junction in the tubing after the dialyzer and heat exchanger permitted withdrawal of fluid. Ultrafiltration control was achieved by clamping the effluent tubing in a pinch clamp mounted on a scale actuated by collected fluid and controlled by a moveable counterweight.

Results: When the tubing was unclamped the inner cylinder (float) rose and displaced fluid from above the inner cylinder to below it. This fluid flowed through the heat exchanger and dialysate compartment of the dialyzer. Dialysate flow was linear with time at a rate of 634 mL/minute ($R^2 = 0.9996$). Lower flow rates are readily achievable by constricting the dialysate flow path. The ultrafiltration controller removes up to 3 kg of fluid from the circuit in controllable increments. Because the volume of dialysate contained in the space between the 2 cylinders is constant, fluid removed from the T-junction equals ultra filtration across the dialyzer. The performance of the heat exchanger has yet to be fully characterized.

Conclusions: The prototype shows the feasibility of driving dialysate passively at constant flow, and of controlling ultrafiltration with a simple device that does not require external energy beyond that required from the operator to fill the cylinder and to adjust ultrafiltration periodically. For dialysis to be feasible, a method of driving blood through the dialyzer is necessary: this could be obtained from the patient's own blood pressure using a Scribner shunt, as was done many years ago with Kiil dialyzers.

Funding: Private Foundation Support

FR-PO937

The Benefit of Specialized Team Approaches in Patients with Acute Kidney Injury Undergoing Continuous Renal Replacement Therapy: Propensity Score-Matched Analysis Eunyoung Lee, Seung Gyu Han, Mi Jung Lee, Hyung Jung Oh, Shin-Wook Kang, Tae-Hyun Yoo. *Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea.*

Background: Continuous renal replacement therapy (CRRT) has been widely used in critically ill patients with acute kidney injury (AKI). To maintain the quality of care for these patients, some centers operate a specialized CRRT team (SCT) composed of physicians and nurses. The aim of this study was to validate the beneficial impact of SCT approach on the clinical outcomes in AKI patients undergoing CRRT.

Methods: A total of 334 patients, who started CRRT for severe AKI between 2007 and 2009 at Yonsei University Health System, were included. Subjects were dichotomized based on SCT application and matched with a propensity score. Kaplan-Meier (KM) analysis was conducted to compare the 28- and 90-day all-cause death rates according to SCT approach. Cox proportional hazard analysis was performed to determine the consequence of SCT approach on the 28- and 90-day mortalities.

Results: During the study period, the 28- and 90-day all-cause death rates were significantly decreased after SCT approach. In addition, down-time per day, lost time per filter-exchange, and RBC-transfused number during CRRT were significantly reduced after SCT approach. Moreover, KM plots showed that the 28- and 90-day all-cause mortality rates were also significantly decreased after SCT approach (log-rank test, P=0.028 and P=0.033, respectively). Furthermore, multivariate Cox regression analysis revealed that SCT approach significantly reduced the 28- and 90-day all-cause mortality rates, after adjusting for age, gender, mean arterial pressure, APACHE II and SOFA scores, RIFLE criteria, and biochemical parameters [28-day mortality, hazard ratio (HR) =0.897, 95% confidence interval (CI) =0.681-0.982, P=0.040; 90-day mortality, HR=0.927, 95% CI=0.725-0.997, P=0.042].

Conclusions: A well-organized and specialized CRRT team could be beneficial for improving patient survival in AKI patients requiring CRRT.

FR-PO938

Patients with Multiple Myeloma Who Recover from Dialysis Dependent Acute Kidney Injury Have Good Long-Term Outcomes Punit Yadav,[1] Colin A. Hutchison,[2] Stephanie J. Stringer,[1] Mark David Jesky,[1] Lesley B. Fifer,[1] Mark Cook,[1] Paul Cockwell.[1] *[1]Univ Hospital Birmingham, Birmingham, United Kingdom; [2]Hawke's Bay Hospital Soldiers' Memorial, Hastings, New Zealand.*

Background: Over 70% of patients with multiple myeloma (MM) and dialysis dependent acute kidney injury (AKI) remain on dialysis and have poor long-term survival (< 1-year). However the overall survival and determinants of survival of patients with MM and AKI who recover independent renal function are unknown.

Methods: This is a single-centre retrospective study of patients with MM and dialysis dependent AKI who recovered renal function between January 2005 and December 2012. We assessed survival and long-term renal outcomes. Cox regression modelling was used to assess determinants of survival.

Results: 27 patients fulfilled criteria for inclusion. The mean age was 61.4 years; 70.4% were males. The median follow-up was 57.5 months. Light chain (LC) only myeloma was seen in 11 (40.7%) patients; IgG in nine (33.3%), IgA in four (14.8%), IgD in two (7.4%) and IgM in one (3.7%). A kappa free light chain (FLC) clone was present in 14 (51.9%) and a lambda FLC clone in 13 (48.1%) patients. 23 of 24 patients with an adequate kidney biopsy had cast nephropathy. All patients were treated with high dose dexamethasone; 25 were treated with regimens including thalidomide or bortezomib. The median time on dialysis was 27 days (IQR 16-53). 51.8% of patients were alive at analysis; the overall median survival was 70 months. 25 patients maintained renal recovery; two restarted dialysis at a median time of 37.5 months and died at a median of 3 months after restarting dialysis. The independent determinants of worse survival were a known history of CKD (P=0.021) and the presence of a lambda FLC clone (P=0.022). Shorter length of time on dialysis and higher percentage clonal serum FLC reduction from baseline at day 21 predicted a better eGFR at 6 months (p 0.029, R^2 0.332).

Conclusions: In this series 92.5% of patients with MM and dialysis dependent AKI who recover renal function have no requirement for further dialysis. Survival following recovery of renal function is good. Early variables are independently associated with renal function and survival.

FR-PO939

Outcomes following Acute Kidney Injury Requiring Renal Replacement Therapy in Wounded Warriors during the Iraq and Afghanistan Conflicts Jonathan Alexis Bolanos, Christina M. Yuan, Dustin J. Little, Kevin C. Abbott, Stephen W. Olson. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: AKI requiring renal replacement therapy (RRT) is associated with 50-65% in-hospital mortality and with subsequent CKD. We performed a retrospective, descriptive study of post-traumatic AKI requiring RRT in military beneficiaries injured in operations in Iraq and Afghanistan between 2001 and 2013, who were evacuated to the National Capital Region.

Methods: Fifty-one subjects were identified using electronic medical records and nephrology procedure records. Those who required care at a subspecialty burn unit were excluded, as this data has been previously published.

Results: Mean age at injury was 27 ± 7 years. 50 subjects were male; 18% were black, and 57% were white. The presumed cause of AKI was rhabdomyolysis in 67%; the remainder had ATN thought secondary to contrast, nephrotoxic medications, shock, and/or sepsis. 84% of injuries were due to blast or projectiles. Mean days of RRT were 19 ± 11. Eleven patients died in the hospital; an in-hospital mortality rate of 21.6% (95% CI 12.3-34.8%). This was significantly less than the predicted mortality rate of 50% (p<0.0001, Binomial distribution). Of the 40 who survived hospitalization, 1 had end-stage renal disease due to cortical necrosis (demonstrated by biopsy). Of the remaining 39, 1 died subsequently. Median time to the last follow-up creatinine for the 39 subjects discharged from the hospital was 32 months (range 1-110 months). Serum creatinine at last follow-up was not significantly different from discharge creatinine (0.87 ± 0.25 versus 0.82 ± 0.36 mg/dL, p=0.26, paired t-test), and eGFR by CKD-EPI was >60 ml/min/1.73m^2 in all subjects (116 ± 24 ml/min/1.73m^2). However, eGFR in this population may not be accurate due to the great frequency of multiple amputations.

Conclusions: Despite their severe injuries, these subjects had significantly better in-hospital survival than would have been predicted for AKI requiring RRT, possibly because of their youth and lack of pre-existing conditions.

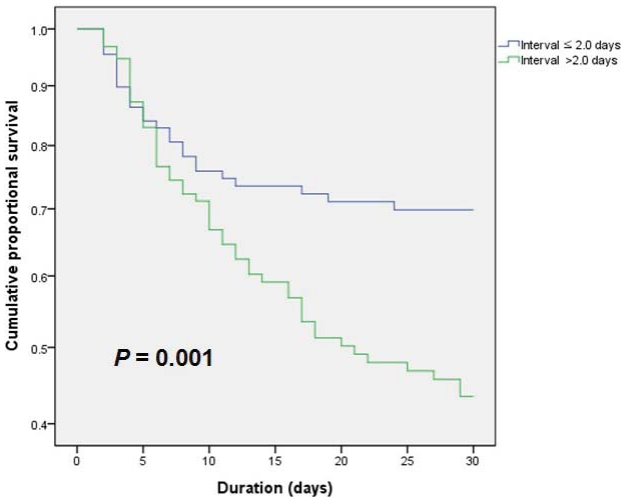
FR-PO940

Early Initiation of Continuous Renal Replacement Therapy May Improve Patient Survival in Acute Kidney Injury Ho Sik Shin,[1] Gyung-Hoon Kang,[1] Ye Na Kim,[1] Yeonsoon Jung,[1] Hark Rim,[1] Hyun Yul Rhew.[2] *[1]Internal Medicine, Kosin Univ College of Medicine, Busan, Korea; [2]Urology, Kosin Univ College of Medicine, Busan, Korea.*

Background: the effects of the timing of CRRT initiation and the characteristics of the infectious process on the clinical outcomes in sepsis patients seem to be controversial. In this study, we tried to elucidate whether the timing of CRRT application, based on the interval between the start time of vasopressors infusion and CRRT initiation, was an independent predictor for mortality in critically ill patients with AKI.

Methods: We evaluated patients with AKI who were treated in ICU of Kosin University Gospel Hospital from January 1, 2010 to December 31, 2011. A total of 200 consecutive patients were included over a 48 month period. Predictors of all-cause death were examined using the Kaplan-Meier and Cox proportional hazards analyses in both treatment groups.

Results: The main contributing factors of AKI were sepsis (38%) and cardiac dysfunction (40%). 28-day overall mortality rates in the early CRRT group were significantly lower than those in the late CRRT group (P = 0.001). Furthermore, early CRRT treatment was independently associated with a lower mortality rate even after adjustment for age, sex, DM, and number of failed organ (P = 0.023).



Conclusions: Early initiation of CRRT may be of benefit.

FR-PO941

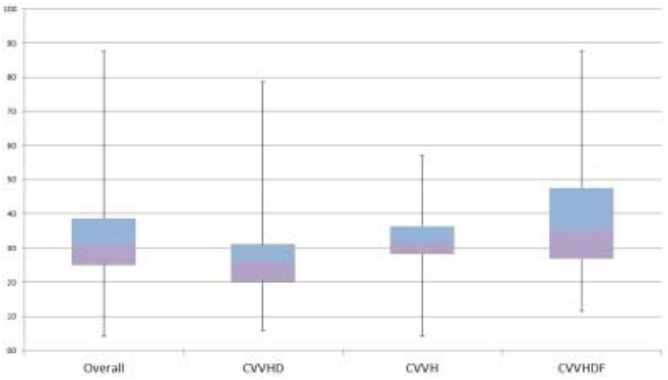
The DoReMIFA (Dose Response Multicenter Investigation Fluid Assesment) Trial: Why a So High Delivered Dose? Francesco Garzotto,[1] Jonah G. Powell-Tuck,[3] David Benavente,[4] Manuel E. Herrera-Gutiérrez,[5] Ciro Tetta,[2] Claudio Ronco.[1] *[1]St. Bortolo Hospital and IRRIV; [2]Fresenius Medical Care; [3]Guy's and St. Thomas' NHS Foundation TrustGuy's and St. Thomas' NHS Foundation Trust; [4]Clinical las condes; [5]Hospital Carlos Haya.*

Background: In the last decade many trials have investigated the potential benefit of more intensive renal replacement therapy (RRT) in critically ill patients with acute kidney injury compared with less-intensive strategies. No improvements have been found in patient survival or recovery of renal function. Our aim was to evaluate the effect of these results on delivered dose in common clinical practice.

Methods: We developed an observational, prospective, multicenter trial (Doremifa) on critically ill patients. Only the RRT section (160 patients) of the entire cohort was analyzed for this purpose. Due to difficulties in carrying out a formal comparison of treatment intensities, we only performed the analysis on continuous RRT (43.1% of the total 1083 records) excluding intermittent RRT and SLED. 172 records were incomplete

and were not considered. Dose was calculated using total effluent (the sum of the dialysate and ultrafiltrate) with correction for percentage pre/post dilution, and expressed as ml/kg/hour. Actual duration was also considered.

Results: Of the 1083 treatments performed, 33.8% were continuous veno venous (CVV) hemofiltration (CVV-H), 25.9% CVV-HD (hemodialysis) and 39.3% CVV-HDF (hemodiafiltration).



The median delivered dose was 31.2 (IQR 28.2 to 36.4), 25.9 (IQR 20.2 to 31.1) and 35.0 (IQR 26.8 to 47.6) ml/kg/h respectively. Overall delivered dose was 31.1 (IQR 24.9 to 38.8). Main reason for RRT discontinuation was Filter/Circuit clotting in 57% of the cases.

Conclusions: Despite the relationship between patient outcome and treatment dose being explored in many studies with conflicting results; 5 years later our data surprisingly showed a 14.7% increase in the delivered dose (31.1 versus 27.1), when compared with the doremi trial.

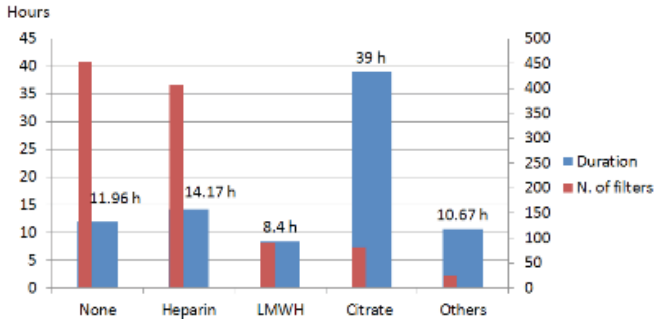
FR-PO942

The (DoRe MIFA) Dose Response Multicenter Investigation Fluid Assesment Trial: A Description of the CRRT Modalities Francesco Garzotto,[1] Anna Lorenzin,[1] David A. Martin-Langerwerf,[2] Lucia L. Cachafeiro,[3] Rene Robert,[4] Jie Teng,[5] Anibal Marinho,[6] Claudio Ronco.[1] *[1]Nephrology IRRIV, St. Bortolo Hospital, Italy; [2]Hospital del Vinalopo, Spain; [3]Hospital Univ La Paz, Spain; [4]CHU de Poitiers, France; [5]Zhongshan Hosp, China; [6]Centro H. Do Porto, Portugal.*

Background: Acute kidney injury (AKI) is an independent risk factor for mortality in critically ill patients.A condition of AKI is defined when serum creatinine concentration is higher than 2mg/dl or the daily increase is 0.3mg/dl;in this settings,AKI patients often need renal replacement therapy (RRT).However,there is not a uniformal worldwide consensus on the management of RRT.We performed a prospective observational multicenter study designed to evaluate how RRT is managed in the intensive care units (ICU) all over the world.

Methods: We developed a web-system for data-entry in order to collect data from 20 ICU centers around the world.On a follow-up of 1415 patients,we considered RRT starting reason,modality,prescribed dose versus actual dose and fluid overload.

Results: We observed 613 AKI patients over 1415,whose 160 (26%) started RRT.The reasons of RRT initiation were divided in:40%azotemia and/or electrolites imbalance,19%fluid overload,34%both.AKI patients started RRT after a mean of 6.8 days after admission.The prescribed RRT are 67%continuous (21%CVVH,20%CVVHD,25%CVVHDF) and 33% Intermittent. The actual duration of the therapy was 90% of the prescribed one. Figure 1 shows time, filters' number and anticoagulation modality used in RRT



Conclusions: We developed a multicentre database to understand more deeply the RRT management over the world.From the data we have collected until now, we can deduce:the actual RRT duration is similar to the prescribed one meaning that downtime is relatively short;citrate anticoagulation therapy allows higher filter's lifetime than others modality;1/3 of RRTs prescribed in ICUs is intermittent instead of continuous.

FR-PO943

Investigation of Carnitine Deficiency in Children Receiving Continuous Renal Replacement Therapy Kristen Sgambat, Asha Moudgil. *Children's National, Washington DC.*

Background: Carnitine deficiency is common in patients receiving chronic hemodialysis (HD) due to inadequate intake, decreased production, and removal during dialysis. The effect of continuous renal replacement therapy (CRRT) on carnitine homeostasis has not been previously studied. We hypothesized that children receiving CRRT may be at risk for carnitine deficiency due to continuous removal, absent intake, and comorbidities related to underlying critical illness.

Methods: Medical records of all patients receiving CRRT at Children's National between 2011 and 2014 were reviewed for total carnitine (TC) and free carnitine (FC) levels. Prevalence of carnitine deficiency at baseline, 13, and 21 days duration on CRRT was determined. Correlation of carnitine level with days on CRRT was assessed by Pearson correlation. Mean TC and FC levels of children on CRRT were compared to those of a prospective comparison group of children on chronic HD for > 3 months by Student's t test.

Results: The study group included 36 CRRT patients with mean age 8.7±1.2 years. At initiation of CRRT, 37.5% of children were TC deficient. After 13 days on CRRT, 65.5% were deficient; prevalence of deficiency significantly increased to 83.3% by day 21 (p=0.03), and 100% of children on CRRT for >21 days were TC deficient (p=0.03). Prevalence of FC deficiency similarly increased with duration of CRRT from 50% at baseline to 71.4% by day 13 and 91.7% by day 21 (p=0.03). There was a significant negative correlation between levels of TC (r=-0.43, p=0.002) and FC (r=-0.41, p=0.003) with number of days on CRRT. Mean carnitine level of children on CRRT for 0.5 week was lower compared with levels of 9 children on chronic HD for mean duration of 9.3±6 months (TC 26.7±3.1 versus 49±1.7, p=0.0001 and FC 18.1±2.4 versus 29±1.2 µmol/L, p=0.006).

Conclusions: Carnitine levels significantly decrease with longer time on CRRT, and the majority of children on CRRT for 1 week are carnitine deficient. Deficiency is more severe after only 0.5 week on CRRT in comparison to children on chronic dialysis for a mean of 9.3 months. Consequences of carnitine deficiency and possible benefits of supplementation in the critically ill pediatric CRRT population should be investigated.

FR-PO944

Use of Arterio-Venous Access in ESRD Patients Receiving Continuous Renal Replacement Therapy Vanya Grover, Aleksey Etinger, Adebayo Shakir Adewale, Sheila Mary Donaghy, Nand K. Wadhwa. *Nephrology, Stony Brook Univ Hospital, Stony Brook, NY.*

Background: Continuous renal replacement therapy (CRRT) is increasingly used in end stage renal disease (ESRD) patients with multi-organ failure admitted to intensive care units (ICU). We report our experience on safety of the pre-existing AV access in ESRD patients as an alternative to a dual lumen catheter for CRRT.

Methods: The AV access was used in all 13 ESRD patients (7 males, 6 females; 10 AV fistulas, 3 AV grafts; mean age 57, range 32-76 years) for CRRT from January 2012 to December 2013. Policy and procedure was established before using AV access for CRRT. Dialysis nursing staff placed 16 gauge plastic angiocaths in AV access, connected using 30" extension tubing (tubing secured with an anchor) to the arterial and venous tubing of M100 set with AN69 hemofilter using Prisma/Prismaflex CRRT system. Angio-catheters were changed every 72 hours with the change of M100 set with AN69 hemofilter or earlier if CRRT was discontinued. PRISMASATE BGK 4/2.5 was delivered at 500-1000 ml/hr as a dialysate and PRISMASOL BGK2/0 or 4/0 was infused at 1500-2000 ml/hr as replacement fluid. All patients received citrate anticoagulation. Data were collected retrospectively.

Results: The indication for CRRT included septic shock in 6, hemorrhagic shock in 4 or cardiogenic shock in 3. The mean blood flow rate was 127 ± 26 ml/min. Minimum effluent rate of 20-25 ml/kg/hour was achieved in each patient. Total duration of CRRT was 892 hours over 49 days. Mean duration of CRRT was 18.8 ± 5.1 hrs/day. All AV accesses were functioning after the CRRT was discontinued. Seven patients survived and received intermittent HD using AV access without complications. No AV access bleeding, infection or technical problems were observed.

Conclusions: Our experience suggests that use of AV fistula or graft as access with an angio-catheter (connected via an extension tube while secured with a tubing anchor) for CRRT can be used safely and obviates the need for a double lumen catheter for a venous access and its potential complications. In addition, the use of AV access may keep fistula or graft patent in these patients.

FR-PO945

Pediatric Nephrology Follow-Up after Continuous Renal Replacement Therapy for Acute Kidney Injury Nicole Christin, Margret E. Bock, Farah N. Ali. *Kidney Diseases, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL.*

Background: Acute kidney injury (AKI), in its most severe form, requires renal replacement therapy. Outcome studies of pediatric AKI show that evidence of chronic kidney disease is commonly present at long-term follow-up. Rates of outpatient Pediatric Nephrology (PN) follow-up after discharge are low and late referral to PN is associated with worse outcomes. We hypothesize that factors contributing to low PN follow-up after AKI include: lack of a scheduled appointment with PN at time of discharge, lack of discharge instructions recommending PN follow-up, non-nephrology primary diagnosis, and lack of a primary medical doctor (PMD).

Methods: A retrospective chart review of patients who received CRRT at our institution from January 1, 2008 to December 31, 2012 was performed.

Results: 115 patients required CRRT; 73 patients were excluded (44 died, 25 had CKD, 2 were adults, 2 had ingestions). 42 pediatric patients with AKI were included; median age was 6.8 years (IQR 3.2-14.3), 64% were male, 38% were Caucasian, 19% African American and 36% Hispanic. 93% had an identified PMD and 62% had public health insurance. 71% of patients requiring CRRT presented for follow-up. At discharge, 62% had instructions to be seen by PN. 74% had a scheduled appointment with PN and 86% of those came to the visit.There was a significant association between presenting for follow-up and having discharge instructions that stated the need for follow-up, eGFR at start of CRRT, and a nephrology primary diagnosis ($p < 0.05$).No association was found between presenting for follow-up and age, gender, race, insurance type or having a PMD. Moreover, primary diagnosis, eGFR at discharge, time on CRRT, dialysis-free interval prior to discharge were not associated with PN follow-up rates.

Conclusions: eGFR at the time of dialysis initiation, non-nephrology primary service and written discharge instructions recommending PN follow-up were linked to higher follow-up rates. Identifying these factors prior to discharge may improve future PN follow-up after severe AKI requiring CRRT and therefore potentially identify and slow the progression of chronic kidney disease.

FR-PO946

Continuous Venovenous Hemodiafiltration to Obtain Therapeutic Hypernatremia in Patients with Renal Failure: A Case Series

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Background: Hypertonic saline is used to induce serum hyperosmolality by targeting a sodium (Na) concentration of 145-155 meq/L in patients with elevated intracranial pressure (ICP). Acute kidney injury (AKI) or end-stage renal disease (ESRD) patients with elevated ICP who require continuous renal replacement therapy (CRRT) often require adjustment to the composition of the CRRT solutions to maintain the target Na concentration. We describe a simplified CRRT protocol using 3% saline as the post-filter replacement fluid (RF) to maintain the targeted Na concentration without using customized CRRT solutions.

Methods: We retrospectively analyzed 9 patients with AKI or ESRD and elevated ICP treated with continuous venovenous hemodiafiltration (CVVHDF) using 3% saline as the post-filter RF. Our standard protocol involves performing CVVHDF using the Prismaflex device with a blood flow rate of 200 ml/min and an effluent rate of 25-30 ml/kg/hr. Our standard pre-filter RF and dialysate contain a Na concentration of 140 meq/L and are each delivered at a rate of 1,000-1,500 ml/hr. Normal saline (NS) at 200 ml/hr acts as the post-filter RF as required with the Prismaflex to prevent clotting of the de-aeration chamber. In patients who require therapeutic hypernatremia, we simply change the post-filter RF from NS to 3% saline and deliver it at a rate of 100-150 ml/hr.

Results: Mean age was 45.2 ± 15.5 years. Mean weight was 80.9 ± 20.0 kg. Six had AKI and 3 had ESRD. The most common indications were cerebral edema (5/9) followed by intracerebral hemorrhage (4/9). Median total CVVHDF duration was 45 (range 10-91) hours. Median serum Na concentration prior to the start of 3% saline was 140 meq/L. All patients achieved a therapeutic Na level by a median of 19 (range 0-30) hours of treatment. Serum Na remained between 145-155 meq/L in all patients for the duration of use of 3% saline as the post-filter RF.

Conclusions: We demonstrate a simple method to maintain serum Na levels in the therapeutic range by using 3% saline as post-filter RF with CVVHDF, obviating the need for customized replacement and dialysate solutions with adjusted Na concentrations.

FR-PO947

Determination and Clinical Validation of Half-Life Based Estimation of Dialysis Duration in Methanol Poisoning

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Background: Treatment of methanol poisoning (MP) by hemodialysis (HD) requires monitoring of serum methanol levels (ML) until a level of < 5 mmol/L is achieved. ML are not readily available and delay in obtaining the results may result in longer duration of dialysis. We hypothesize that based on methanol's half-life ($T_{1/2}$) during HD, dialysis duration can be safely estimated. The objectives of the present study are to 1) define the $T_{1/2}$ of methanol during HD in incident cases of MP (training set) and 2) to validate the $T_{1/2}$ approach in a second cohort of patients with MP (validation set).

Methods: This is a retrospective single center study of incident methanol poisoning from 1997 to 2013 referred to CHU de Québec (Quebec, Canada) for HD. The training set included MP with ≥ 5 measurements of ML during dialysis (n= 27). Dialysis was performed by various filters with Qb of 350- 400 ml/min and a QD of 750 ml/min. A one phase decay exponential regression analysis with Robust fitting method and a plateau constrain to a value of >0 was used to determine $T_{1/2}$. The 95th percentile $T_{1/2}$ was determined. A target ML that will allow the remaining 5% patients to end with a ML < 5 without unduly increasing dialysis duration was determined. These findings were confirmed in the validation set (n=29).

Results: In the training set, the median $T_{1/2}$ was 129 (108,139) minutes and the 95th percentile was 150 min. Targeting ML of < 4 yielded ML < 5 in all patients. In the validation set, the median $T_{1/2}$ was 126 (112,137) minutes and the 95th percentile was 155 minutes. Using a half-life of 150 minutes and targeting ML < 4 yielded a ML < 5 in all patients. The median difference between estimated and observed dialysis duration was - 16 [-75,34] minutes.

Conclusions: In patients with MP, estimation of dialysis time based on a methanol $T_{1/2}$ of 150 minutes and targeting ML level < 4 is safe and does not lead to unduly extension of dialysis duration.

FR-PO948

Platelet Loss in Membrane Therapeutic Plasma Exchange (mTPE)

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Background: Membrane therapeutic plasma exchange (mTPE) using dialysis equipment is gaining popularity amongst nephrologists. A decrease in the platelet count of 30-53% per treatment has been reported with centrifuge-based therapeutic plasma exchange (cTPE), however, little is known about the effects of mTPE on the patient's platelet count. We performed a retrospective analysis to study the effect of mTPE on the platelet count.

Methods: Data was retrospectively collected on all patients receiving inpatient mTPE at our institution from January 2013 to April 2014. Procedures were performed using the Asahi Plasmaflo™ OP filter with the NxStage® System One™ Machine. No anticoagulation was used. Patients with thrombotic thrombocytopenic purpura (TTP) who are expected to have increasing platelets with plasma exchange were excluded. Pre and post treatment platelet counts were measured on the morning of and the morning after each treatment respectively. Filtration fraction (FF) and transmembrane pressure (TMPa) for each treatment were collected. Bleeding complications were investigated. Paired student's t-test was used for statistical analysis.

Results: A total of 597 mTPE treatments were performed on 141 patients (average 4.2 treatments). Of these, data analysis was performed on 396 treatments (117 patients) for whom platelet counts were available. There was a significant difference in the platelet count before (211 ± 99 thousand) and after (192 ± 96 thousand) each treatment ($p < 0.0001$) with a mean absolute platelet count drop of 18.6 thousand (8.2%) per treatment. The mean absolute drop in the platelet count at the end of the treatment course was 59 thousand (22.9 %); $p < 0.0001$. There was no correlation between FF, TMPa and the change in the platelet count using simple linear regression. There were a total of nine bleeding complications, none related to drop in the platelet count.

Conclusions: Platelet count decreased significantly (8.3% per session, 22.3% per treatment course) in patients undergoing mTPE. No major bleeding complications were correlated to the platelet loss. The platelet count should be monitored during a course of treatment as cumulative drop increases with multiple treatments.

FR-PO949

Pharmacokinetics of Intraperitoneal Cefalothin and Cefazolin in Patients Being Treated for Peritoneal Dialysis-Associated Peritonitis

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Background: The standard treatment of peritoneal dialysis (PD)-associated peritonitis (PD-peritonitis) is intraperitoneal (IP) administration of antibiotics. Antibiotic clearance may vary between individuals on PD. Only limited data on the pharmacokinetics (PK) of IP cephalosporins exists and as such it is unclear how appropriate contemporary dose recommendations are for this class of antibiotics. The aim of this study was to describe the PK of IP cefalothin (CLN) and cefazolin (CFZ) in patients treated for PD-peritonitis.

Methods: IP CLN or CFZ was administered with gentamicin in a six hour dwell to 19 patients with PD-peritonitis during routine care. Serial plasma and PD effluent samples were collected for 24 hours. Antibiotic concentrations were quantified using a validated chromatographic method with PK analysis performed using a non-compartmental approach.

Results: 8 patients were administered CLN and 11 CFZ. The median bioavailability for both antibiotics exceeded 92%, but marked interindividual PK variability was observed. Both drugs and dosing regimens achieved high plasma and PD effluent concentrations throughout the antibiotic dwell. The median trough total antibiotic concentrations for CFZ and CLN during the dwell were (plasma 49 versus 8 mg/L, $p<0.01$; PD effluent 37 versus 38 mg/L, $p=0.34$) despite administration of a similar dose (median 1.2 versus 1.1 gram, $p=1.0$), and larger (but highly variable) volume of distribution (35 versus 14 L, $p=0.0506$), respectively. However, antibiotic concentrations were low during PD dwells not containing antibiotic, particularly CLN which was frequently undetectable in plasma and PD effluent.

Conclusions: When IP CLN or CFZ is allowed to dwell for 6 hours, sufficient plasma and PD effluent concentrations are present for common pathogens. There is a risk of subtherapeutic antibiotic concentrations with once daily IP cephalosporins, in particular for CLN for which more frequent dosing may be required.

Funding: Government Support - Non-U.S.

FR-PO950

Intradialytic Antibiotic Concentrations Predict Pharmacokinetics Between Dialysis Treatments

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Background: It is important to dose antibiotics accurately. Underdosing risks treatment failure and emergence of antibiotic resistance while overdosing risks unwanted side effects. The prevalence of advanced age and comorbid organ failures in dialysis patients poses a special challenge for drug dosing. An approach to individualizing pharmacokinetics based on intradialytic sampling would simplify dose personalization. We developed a computational approach based on signal processing techniques to use measurements obtained during a dialysis session to predict plasma drug concentrations between sessions.

Methods: A discrete-time low-pass filter solution to the ordinary differential equations describing two-compartment linear pharmacokinetics was coded in MATLAB. In an IRB-

approved study, patients receiving intermittent dialysis and a b-lactam antibiotic were sampled every five minutes during 30 minutes of hemodialysis followed by 30 minutes of isolated ultrafiltration, and then at 0, 30, 60, 120, and 240 minutes after an antibiotic dose on a day without dialysis. Antibiotic concentrations were measured by HPLC. Filter parameters were fitted to the intradialytic data using a steepest-descent followed by quorum voting approach. The filter then predicted response to a subsequent antibiotic dose. Predicted and actual concentrations were compared.

Results: We report results for the first three subjects. A best fit model could be obtained in each case. Mean error between predicted and observed concentrations was 9.7%.

Conclusions: We have developed a novel computational tool that can individualize drug dosing using only measurements obtained during a hemodialysis treatment. Pilot clinical trials are needed to determine clinical utility of this approach.

FR-PO951

Prospective Single and Multiple Dose Pharmacokinetic Study of Intravenous Colistin in Critically Ill Patients with Acute Kidney Injury Undergoing Extended Daily Dialysis

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Background: The lack of new antibiotics for the treatment of multidrug-resistant bacteria renewed interest in antibiotics like colistin. Due to scarce data, we aimed to perform a prospective, observational clinical study on dosing of IV colistin in critically ill patients with anuric acute kidney injury (AKI) undergoing extended daily dialysis (EDD) to develop dosing recommendations.

Methods: So far four subjects were enrolled. Pharmacokinetics on the first day and between the 5th and 9th day of treatment were performed. Blood samples were collected before and at the end of IV administration of CMS (prodrug) and throughout the dialysis session [average:8.1 hrs, polysulfone high-flux dialyzer (1.3 m^2; mean blood and dialysate flow:200/180 ml/min)]. Dialyzer clearance was calculated and samples from the spent dialysate were taken.

Results: Peak levels of colistin after a loading dose (6 million units) were 3.83-10.01µg/ml (CMS: 13.14-24.76µg/ml). Even after five to nine days of treatment with 3 million units q 8 hrs, there was neither an accumulation of colistin (peak level day#5-9: 8.96-10.71µg/ml) nor an accumulation of CMS (7.68-14.07µg/ml). Dialyzer plasma clearances of colistin ranged between 28-88ml/min (CMS: 26-158ml/min). After dialysis, 108-246mg colistin could be recovered in the spent collected dialysate on day#1 and 158-204mg on day#5-9.

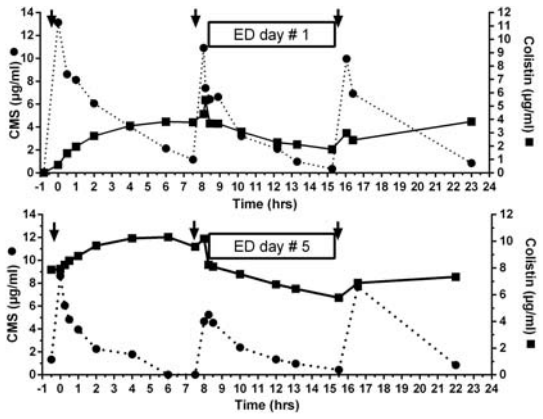

Figure: Plasma concentrations of colistin (•) and CMS (•) in a patient with acute kidney injury undergoing extended dialysis (duration depicted by box size) on day # 1 and day # 5 of colistin treatment (first dose 6 million units, thereafter 3 million units q 8 hours, indicated by arrows)

Conclusions: Our data suggest that EDD eliminates colistin effectively and to a larger extent than reported for IHD. Thus, dosing colistin as recommended for a regular hemodialysis is inadequate and would result in a significant under-dosing. After a loading dose of 6 million units, a dose of 3 times 3 million units yields therapeutic drug levels on EDD.

Funding: Clinical Revenue Support

FR-PO952

Hemodialysis Has Minimal Impact on the Pharmacokinetics of AKB-6548, a Once-Daily Oral Inhibitor of Hypoxia Inducible Factor Prolyl-Hydroxylases (HIFPHs) for the Treatment of Anemia Related to Chronic Kidney Disease (CKD)

Akshay Buch,[1] Tasha M. Farmer,[1] Charlotte Hartman,[1] Harry Alcorn,[2] Robert Shalwitz.[1] *[1]Akebia Therapeutics, Inc., Cambridge, MA; [2]DaVita Clinical Research, Minneapolis, MN.*

Background: AKB-6548 is a novel once-daily, oral therapy in development for the treatment of anemia related to CKD (pre-dialysis and patients requiring dialysis). The completed clinical studies have demonstrated its effectiveness for increasing hemoglobin in a dose-responsive manner in CKD patients not on dialysis.

Methods: The safety, tolerability and pharmacokinetics of AKB-6548 were evaluated in an open-label, randomized, crossover study in 12 subjects with anemia secondary to end stage renal disease (ESRD) undergoing chronic hemodialysis (HD). Single doses (450 mg) were administered 4 hours prior to starting a HD session (PRE-HD) or 2 hours after completing a HD session (POST-HD). Plasma samples, collected over 48 hours after each dose, were analyzed for AKB-6548 and two metabolites (phenolic glucuronide and acyl-glucuronide) using validated bioanalytical methods. In addition, dialysate samples were collected at pre-specified time points for the PRE-HD dose.

Results: AKB-6548 was well tolerated by subjects with no subjects discontinuing the study due to an adverse event (AE). Seven subjects reported a total of 7 AEs during the study. All AEs were mild in intensity, except for one of moderate intensity (abdominal pain), and only GI-related AEs were considered related to study drug. All AEs were resolved by the end of the study. No serious adverse events were reported during the 48-hours in-house observation period after the dose administrations. The pharmacokinetic results indicate that administration of the dose before or after the HD session did not have a marked effect on the PK parameters of AKB-6548 or the two glucuronide metabolites.

Conclusions: The exposure based on Cmax and AUC for the major metabolite (O-glucuronide) was 30-50% of AKB-6548 and the exposure to acyl-glucuronide was extremely small (<1%). The terminal half-life of AKB-6548 and O-glucuronide were approximately 9-10 hours. The HD procedure had minimal impact on the clearance of AKB-6548.

Funding: Pharmaceutical Company Support - Akebia Therapeutics, Inc.

FR-PO953

Pharmacokinetics of Intravenous Triferic in Healthy Volunteers

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Background: Triferic™ (soluble ferric pyrophosphate citrate) is an investigational iron salt, administered via the dialysate to replace dialytic iron losses and maintain hemoglobin levels. Triferic conceals the iron (III) core by covalent bonding with pyrophosphate and citrate. Triferic donates iron and pyrophosphate to human apotransferrin directly, thereby bypassing the reticuloendothelial system.

Methods: Triferic administration via IV infusion over 4-hour period has been studied in healthy volunteers in a double-blind, placebo controlled single ascending dose study.

Results: Placebo subjects showed a pronounced diurnal variation in serum iron, necessitating a baseline correction for accurate quantitation of total iron. Single Triferic doses from 2.5 mg to 10 mg iron over 4 hours result in dose proportional increases in serum iron concentration, transferrin bound iron (TBI) and TSAT. The baseline corrected non-compartmental PK parameters are summarized in the table.

	0 mg	2.5 mg	5.0 mg	7.5 mg	10.0 mg
λ_z (1/hr.)	-	0.544	0.668	0.711	0.917
$t_{½}$ (hr.)	-	1.3	1.2	1.3	1.0
T_{max} (hr.)	-	4.9	4.3	4.75	4.33
C_{max} (mg/dL)	62.6	113	151	228	261
$AUC_{0\text{-}4}$ (h*mg/dL)	126	235	329	471	590

Triferic iron is rapidly cleared from the circulation with a $t_{½}$ of approximately 1.2 hours. Measurements of TBI across the dose range demonstrated no non-transferrin-bound iron (NTBI). In CKD-HD patients receiving 2 µM Triferic-iron via dialysate (110 µg/L), the mean incremental pre to post dialysis iron concentration is 115 mg/dL. In healthy volunteers, a 10 mg dose of Triferic yields an incremental increase of 210 mg/dL. Based on these measurements, approximately 5.4 mg of iron is delivered with each hemodialysis treatment. Triferic IV was well tolerated with a safety profile similar to that of placebo.

Conclusions: Triferic is a novel iron replacement product delivered via the hemodialysate and designed to replace dialytic iron losses. The healthy volunteer intravenous clinical pharmacology results demonstrate linear PK without NTBI. The results support the efficacy of Triferic dialysate in Phase 2 and 3 clinical studies in CKD-HD patients demonstrating maintenance of iron balance and hemoglobin levels while reducing the requirements for ESAs.

Funding: Pharmaceutical Company Support - Rockwell Medical

FR-PO954

Population Pharmacokinetics of AMG 416, a Novel Calcimimetic Peptide, in Patients with Secondary Hyperparathyroidism Receiving Hemodialysis

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Background: Activation of the calcium-sensing receptor (CaSR) on the parathyroid gland (PTG) reduces parathyroid hormone (PTH) synthesis and secretion from the chief cells of the PTG. AMG 416 is a novel calcimimetic peptide that is currently being evaluated as a treatment in patients with secondary hyperparathyroidism (SHPT) receiving hemodialysis (HD). The purpose of this analysis is to characterize the population pharmacokinetics (PK) of AMG 416 in patients with SHPT receiving HD.

Methods: A total of 1649 plasma concentrations from 143 patients enrolled in three clinical studies were pooled for this analysis. AMG 416 was administered as single or multiple intravenous bolus doses at the end of hemodialysis at doses ranging from 2.5 to 60 mg. The time course of intact AMG 416 plasma concentration was analyzed with non-linear mixed effect modeling using NONMEM 7.2. The influence of sex, race, and baseline characteristics (age, weight, creatinine, albumin, total bilirubin, liver enzymes, intact PTH, phosphorus, corrected calcium, vitamin D supplements, phosphate binders, and calcium supplements) on PK parameters was explored. The predictive ability of the final model was evaluated using standard diagnostic plots, bootstrapping and predictive checks.

Results: An open three-compartment linear PK model that accounts for the AMG 416 clearance due to HD best described the data.Plasma clearance (inter-subject variability)

was estimated as 0.564 L/h (14.0%). The HD clearance was estimated to be 22.2 L/h. The volume of distribution at steady-state was approximately 624 L (82%). Within the range of covariates studied,no statistically significant associations between the covariates and PK parameters were found. Bootstrapping and predictive checks indicated that the model adequately described the time course of AMG 416 plasma concentrations in patients with SHPT receiving HD.

Conclusions: The integrated analysis of phase I/II PK data demonstrated that AMG 416 exhibits linear and stationary PK within the range of doses evaluated. This analysis suggests that no additional considerations for dose adjustment are warranted on the basis of PK.

Funding: Pharmaceutical Company Support - Amgen Inc.

FR-PO955

Effect of End Stage Renal Disease on the Expression and Function of Hepatic Reductase Drug Metabolizing Enzymes

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Background: The expression and function of hepatic drug metabolizing enzymes and transport proteins is altered in end-stage renal disease (ESRD). Hepatic reduction is an important pathway for the metabolism of many drugs and endogenous compounds. Yet to date, the effect of ESRD on hepatic reduction has not been investigated. The aim of this study was to evaluate the effect of ESRD on the expression and function of hepatic reductases in human liver tissue.

Methods: Liver tissue was collected from deceased ESRD patients (n=10) and deceased control patients (n=11), and cytosolic and microsomal cellular fractions were isolated from each tissue sample. Gene (mRNA) and protein expression of multiple hepatic reductases were determined by qRT-PCR and Western blotting, respectively. Metabolic activity was assessed using warfarin as a pharmacological probe substrate, with the formation rate of warfarin alcohols reflecting overall reductase activity. Warfarin alcohols were determined using liquid chromatography-tandem mass spectrometry.

Results: Gene expression of carbonyl reductase 1 (CBR1) and aldo-keto reductases (AKR) 1C1, AKR1C2, AKR1C3, and AKR1C4 was decreased up to 67% in ESRD livers compared to controls (P=NS for all). A significant (65%) decrease in CBR1 protein expression was observed in ESRD livers relative to controls (P<0.05). AKR protein expression was unchanged. The formation rate of *RS/SR*-warfarin alcohol was decreased by 22% and 27% in cytosol and microsomes, respectively of ESRD livers versus controls (P=NS).

Conclusions: Our results demonstrate a trend toward decreased expression and function of selective hepatic reductases in livers of ESRD patients. Given the large interindividual variability observed, future studies with larger sample size are warranted to confirm our findings. The results may provide a mechanism for altered non-renal drug clearance in ESRD.

FR-PO956

Effect of Experimental Kidney Disease on the Functional Expression of Hepatic Reductases

Osama Y. Alshogran,[1] François A. Leblond,[2] Vincent Pichette,[2] Thomas D. Nolin.[1] *[1]School of Pharmacy, Univ of Pittsburgh, Pittsburgh, PA; [2]Service de Néphrologie et Centre de Recherche, Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Background: Chronic kidney disease (CKD) affects the nonrenal clearance of drugs by modulating the functional expression of drug metabolizing enzymes and transporters. The impact of CKD on oxidative and conjugative metabolism has been extensively studied. However, the effect of CKD on hepatic reduction, an important drug metabolism pathway, has not been investigated. Carbonyl reductase 1 (CBR1) and aldo-keto reductase 1C3 (AKR1C3) are important cytosolic reductases, while 11b-hydroxysteroid dehydrogenase type 1 (11b-HSD1) is a key microsomal reductase. Warfarin is reduced stereoselectively by these enzymes to form *RS/SR*- or *RR/SS*-warfarin alcohol. We aimed to assess the effect of experimental CKD on hepatic reduction using warfarin as a pharmacological probe substrate.

Methods: Cytosolic and microsomal cellular fractions were isolated from liver tissue harvested from 5/6th-nephrectomized and control rats (n=10 per group). The enzyme kinetics for warfarin reduction were evaluated in both fractions under optimized conditions. Warfarin alcohol metabolites were measured using LC-MS, and formation of warfarin alcohols was used as an indicator of hepatic reductase activity. Selective inhibitors were employed to identify reductases involved in warfarin reduction. Gene expression of reductases was quantified using qRT-PCR.

Results: Metabolic formation of *RS/SR*-warfarin alcohol was decreased by 39% (P<0.001) and 43% (P<0.01) in cytosol and microsomes, respectively in CKD rats compared to controls. However, *RR/SS*-warfarin alcohol formation was unchanged. The mRNA expression of CBR1, AKR1C3 and 11b-HSD1 was significantly reduced by 34%, 93%, and 35% (P<0.05), respectively in CKD rats compared to controls.

Conclusions: These results suggest that the activity of hepatic reductases is selectively decreased in kidney disease, secondary to the reduced mRNA expression. The findings may explain one mechanism for altered nonrenal clearance, exposure, and response of drugs in CKD patients.

FR-PO957

Apical Shear Stress Enhances Organic Cation Transport in hOCT2/hMATE1 Transfected MDCK Cells

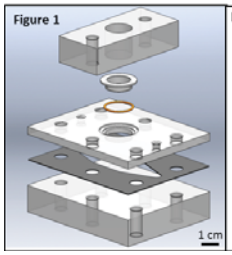
Aishwarya Jayagopal,[1] Peter Soler,[1] Nicholas J. Ferrell,[2] Paul R. Brakeman,[3] Deanna L. Kroetz,[1] William Fissell,[2] Shuvo Roy.[1] *[1]Bioengineering, UCSF; [2]Nephrology, Vanderbilt Univ; [3]Pediatrics, UCSF.*

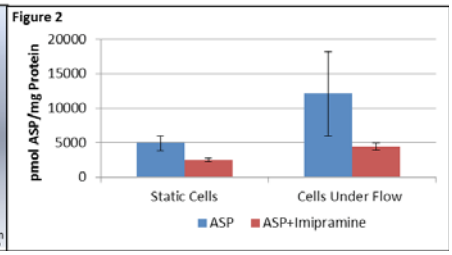
Background: Active transport by renal proximal tubules plays a significant role in human drug disposition and is therefore important to model when developing drugs. However, current *in vitro* drug testing methods fail to mimic important physiological cues, such as flow induced shear stress. In this study, the effect of shear stress on active transport was investigated using a parallel plate bioreactor cultured with MDCK cells expressing human organic cation transporters.

Methods: A polycarbonate parallel plate bioreactor compatible with Snapwell inserts was used in these experiments (Fig. 1). The device provides a flow path across the apical side of the cells and a static media reservoir on the basal side. MDCK cells transfected with a pair of uptake and efflux transporters (hOCT2/hMATE1) were grown on Snapwells under static conditions until confluence and then placed in the bioreactor. Media flow was increased over 7 days until shear stress of 0.2 dynes/cm^2 was reached. Uptake of 25mM 4- (4-dimethylamino) styryl-N-methylpyridinium (ASP+), a fluorescent OCT2/MATE1 substrate, was measured for 1 hour with or without pretreatment (30 minutes) with 500mM imipramine, an OCT2/MATE1 inhibitor. Control cells were cultured under static conditions.

Results: Cells maintained under flow showed a 2.2 fold increase in protein concentration over static controls, confirming previous observations of shear stress effects. Furthermore, cells cultured under shear stress showed a 2.4 fold increase in ASP+ uptake when compared to cells cultured under static conditions and a 63.4±3.7% inhibition with imipramine compared to 48.6±5.5% inhibition in static cells (Fig. 2).

Conclusions: These results indicate that exposure to shear stress increases uptake of the active transport substrate ASP+ compared to static growth conditions.





FR-PO958

Comparison of Pharmacokinetic-Pharmacodynamic Relationship of Atrasentan between Asian Population and Caucasian Population

Hirofumi Makino,[1] Dick de Zeeuw,[2] Hiddo Jan Lambers Heerspink,[2] The RADAR Study Group.[3] *[1]Okayama Univ Hospital, Japan; [2]Univ Medical Center Groningen, Groningen, Netherlands; [3]The RADAR Study Group.*

Background: Previously, we showed that the selective endothelin (ET) A receptor antagonist atrasentan lowers albuminuria both in an Asian and Caucasian diabetic nephropathy population (*de Zeeuw et al, JASN 2014*). As drug responses for many drugs may differ between Asian and Caucasian populations, we compared pharmacodynamics and pharmacokinetics (PK) of atrasentan between Asian population (Japan and Taiwan; n=71) and Caucasian population (U.S. and Canada, n=140).

Methods: We conducted a 12 week randomized, double-blind, placebo-controlled trial, which consists of 3 arms (placebo, atrasentan 0.75 mg, atrasentan 1.25 mg) in type 2 diabetic patients with macroalbuminuria (UACR: ≥300 mg/g) and decreased renal function (eGFR: 30-75 mL/min/1.73 m^2), receiving maximum tolerated labeled dose of a RAS inhibitor (ARB or ACEi). The primary efficacy endpoint was UACR (urinary albumin creatinine ratio). Blood collection for PK analysis was conducted at Weeks 2, 4, 6, 8, 10 and 12 visits.

Results: UACR response was higher in Asian compared to Caucasian patients:, -35.9% versus -25.1% (0.75 mg) and -50.6% versus -27.7% (1.25 mg). Similarly, atrasentan plasma concentration was higher in Asian: 2.2 versus 1.6 (0.75mg), and 4.9 versus 3.4 ng/mL (1.25mg). A strong correlation was observed between plasma concentration and UACR effect at populations/dose level (R^2 0.98; p<0.01). The difference in plasma levels could be partly explained by the significant differences in body weight for both dose groups (67 versus 95 and 65 versus 95kg): correction by body weight left no significant difference in plasma concentration between Asian and Caucasian: 0.06 ng/ml (0.75mg; p=0.92), and 1.05 ng/mL (1.25 mg; p=0.069).

Conclusions: Asian patients show an enhanced antialbuminuric (dose) response to atrasentan compared to Caucasian patients. This difference is correlated with differences in plasma drug concentrations, and in turn partly explained by the difference in body weight/body surface area.

Funding: Pharmaceutical Company Support - AbbVie

FR-PO959

Gene Expression Analysis of Tacrolimus-Induced Tubulointerstitial Fibrosis After Ischemia/Reperfusion Injury Haruka Shinke,[1] Yuka Tamura,[1] Shunsuke Fujita,[2] Shunsaku Nakagawa,[1] Takahisa Yano,[3] Kazuo Matsubara,[1] Satohiro Masuda.[1,2,3] *[1]Dept of Clinical Pharmacology and Therapeutics, Kyoto Univ Hospital, Kyoto, Japan; [2]Dept of Clinical Pharmacology and Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kyushu Univ, Fukuoka, Japan; [3]Dept of Pharmacy, Kyushu Univ Hospital, Fukuoka.*

Background: Tacrolimus-induced kidney injury is a common and unavoidable adverse effect in patients after solid organ transplantation. In this study, we used isolated proximal tubules to examine the trascriptome of a rat model of tubulointerstitial fibrosis induced by tacrolimus administration after ischemia/reperfusion (IR) injury.

Methods: Male Wistar/ST rats (8-week-old) were subjected to IR of both kidneys. Tacrolimus (5 mg/kg/day) or vehicle was administered on consecutive 14 days, starting from the day before IR. All protocols were approved by the Animal Research Committee, Kyoto University.

Results: Immunofluorescence analysis showed that the expressions of the Kim-1 and a-SMA were significantly increased in tacrolimus-treated rats. Hence, these rats were established as rat models of tacrolimus-induced tubulointerstitial fibrosis. The total clearance of intravenously administered metformin, a typical substrate for tubular organic cation transporters, OCT2 and MATE1, decreased to 66 % in tacromilus-treated rats than in the vehicle-treated rats ($p = 0.003$), indicating that tacrolimus-induced tubulointerstitial fibrosis causes the depression of tubular detoxification by drug transporters. Furthermore, microarray analysis (Whole Rat Genome Microarrays, Agilent Technologies) using isolated proximal tubules indicated that the signals for 53 probes, including early growth response-1 (egr-1), increased to more than 2-fold that of vehicle treated rats. It is suggested that the upregulation of these genes is associated with tacrolimus-induced tubulointerstitial fibrosis.

Conclusions: A rat model of tacrolimus-induced tubulointerstitial fibrosis was successfully established, and several genes that are closely associated with the pathology of tacrolimus-induced tubulointerstitial fibrosis were identified.

Funding: Government Support - Non-U.S.

FR-PO960

Comparison of Mycophenolic Acid Exposure and Gastrointestinal Adverse Effects in Stable Renal Transplant Recipients: Association to Calcineurin Inhibitor Therapy Calvin J. Meaney,[1,2] Rocco C. Venuto,[3] Gregory E. Wilding,[4] Joseph D. Consiglio,[4] Kathleen M. Tornatore.[1,2,3] *[1]Immunosuppressive Pharmacology Research; [2]NYS Center of Excellence, School of Pharmacy; [3]School of Medicine; ECMC; [4]School of Public Health; Univ at Buffalo, Buffalo, NY.*

Background: International consensus guidelines for therapeutic drug monitoring (TDM) of mycophenolic acid (MPA) recommend an area under the concentration-time curve 0-12 hours (AUC) of 30 to 60 mg•h/L for renal transplant recipients (RTR) on cyclosporine (CYA) and mycophenolate mofetil (MMF). There are minimal data to define a target MPA AUC for tacrolimus (TAC) and mycophenolate sodium (ECMPS). This analysis compared MPA AUCs with a novel GI adverse effect (AE) score in RTR receiving CYA-MMF or TAC-ECMPS.

Methods: A prospective cross-sectional analysis of 12 hour pharmacokinetic studies in 147 stable RTR (time post-transplant:4.0±3.2 years) was completed in 80 RTR on CYA-MMF and 67 RTR on TAC-ECMPS without routine MPA TDM. MPA was analyzed by mass spectrometry and MPA AUC was determined by non-compartmental analysis. RTR were assessed for GI AE using a validated scale (diarrhea, dyspepsia, vomiting, acid suppressives) yielding a GI AE score (GI AE). General linear models compared MPA AUC < 30; 30 to 60 and >60 mg•h/L.

Results: Table 1 summarizes the data. RTR on TAC-ECMPS had higher MPA AUCs with 2-fold greater GI AE.

Table 1	CYA-MMF (n=80)	TAC-ECMPS (n=67)	P-value
MPA Dose (mg)	693±215	570±179	<0.001
MPA AUC >60 mg•h/L[+]	71.9±11.8 (13%)	89.9±23.4 (57%)	0.024
MPA AUC 30-60 mg•h/L[+]	44.8±7.2 (56%)	46.1±7.9 (34%)	0.475
MPA <30 mg•h/L[+]	21.3±6.7 (31%)	23.3±3.9 (9%)	0.486
GI AE Score	1.1±1.0	2.5±1.8	<0.001
e-GFR ml/min/1.72m^2	48.8±18.0	55.2±15.7	0.024
Calcineurin inhibitor trough (ng/ml)	129±46.5	7.20±1.89	NA
[+] mean ± standard deviation with (%) RTR in respective AUC range			

Conclusions: The majority of RTR receiving TAC-ECMPS exceeded the recommended MPA AUC range and had more GI adverse effects compared to CYA-MMF. A prospective comparison using TDM is needed to define the optimal 12 hour MPA AUC range that provides immunosuppression with less AE.

Funding: NIDDK Support, Pharmaceutical Company Support - Novartis Pharmaceuticals

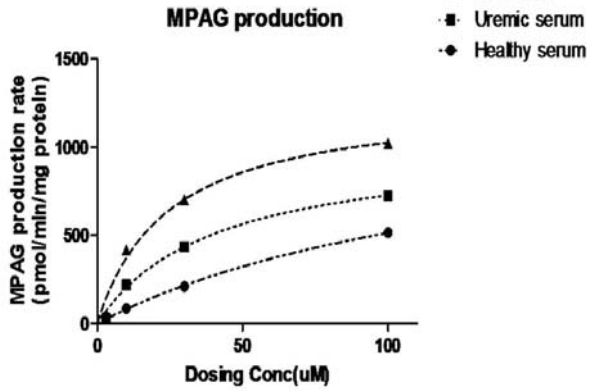
FR-PO961

Explaining Unexpected Drug Toxicities: How Uremic Toxins Alter Hepatic Mycophenolic Acid Disposition Lynda A. Frassetto,[1] Leslie Benet,[1] Jason Baik,[1] Ji Li,[2] Janel Long-Boyle.[1] *[1]UCSF, San Francisco, CA; [2]China Pharmaceutical U, China.*

Background: We have previously studied the effects of uremic serum and specific uremic toxins on uptake and efflux transporters and Phase I metabolizing enzymes (e.g., the cytochrome P450 system), in particular on drug interactions in HIV-infected kidney and liver transplant patients. We now study uremic toxin effects on Phase II metabolizing enzymes, using the glucuronidation of mycophenolic acid (MPA) as our model. MPA clearance can be highly variable; renal function is a strong predictor of unbound (i.e., available) MPA clearance. MPA is taken up in the liver by the organic anion transporter OATP, and glucuronidated to the primary inactive metabolite, MPA 7-O-glucuronide (MPAG).

Methods: Rat hepatocytes and microsomes were incubated with no serum, healthy and uremic human serum, to evaluate MPA uptake and metabolism to MPAG. Rifampin, an inhibitor of OATP was used as a positive control. MPA and MPAG were measured by LC/MS/MS.

Results: MPA uptake by rat hepatocytes was inhibited by 18% in uremic serum ($P<0.05$) and 47% in control serum with rifampin ($P<0.01$) compared with control (healthy) serum. Unexpectedly, microsome studies showed that uremic serum activated metabolism; MPAG production increased and intrinsec clearance increased from 8.7±5.6 to 25.2±10.7 µL/min with the uremic serum compared to the healthy serum, ($p=0.03$, see figure below). There was also a two-fold decrease in MPA protein binding in the presence of uremic serum compared to healthy serum.



Conclusions: Uremic serum plays an important role in MPA disposition through three processes; effects on drug transport, on protein binding and on metabolic enzyme activation leading to increased parent drug clearance. Further studies in human hepatocytes and microsomes are ongoing.

Funding: Clinical Revenue Support

FR-PO962

Uremic Toxins p-Cresol Sulfate and Trimethylamine N-oxide Inhibit the Function of Renal Drug Transporters OAT1, OAT3, and MATE1 Catherine K. Yeung,[1,2] Danny D. Shen,[1,3] Jonathan Himmelfarb.[2] *[1]Dept of Pharmacy, Univ of Washington, Seattle, WA; [2]Kidney Research Inst, Univ of Washington, Seattle, WA; [3]Dept of Pharmaceutics, Univ of Washington, Seattle, WA.*

Background: Renal tubular secretion of drugs and endogenous uremic solutes requires coordinated uptake transport at the basolateral membrane and efflux transport at the apical membrane to effect vectorial movement of drugs from the peritubular vasculature, across the proximal tubular epithelium, and into the tubular lumen for excretion. Inhibition of drug transporter function by circulating uremic toxins leads to altered renal clearance and pharmacokinetic unpredictability in patients with chronic kidney disease, resulting in sub- or supra-therapeutic drug dosing.

Methods: This study evaluated the inhibitory potential of p-cresol sulfate and trimethylamine n-oxide, uremic toxins both associated with cardiovascular disease, on renal uptake (OAT1/3, OCT2) and efflux (MATE1/2K, P-gp, MRP2/4) transporters, using MDCK-II cells expressing recombinant OAT1, OAT3, OCT2, MATE1, MATE2K, or P-glycoprotein and Sf9 vesicles transfected with MRP2 or MRP4.

Results: At 1000 µM p-cresol sulfate, 78.2% ± 1.90% inhibition ($p<0.0001$) was seen for OAT1 and 87.8% ± 17.2% inhibition ($p=0.0027$) was seen for OAT3. Small but statistically significant inhibition was observed for MATE1 (15.0% ± 1.72%, $p=0.0018$) and MRP4 (14.9% ± 5.91%, $p=0.0129$). At a concentration of 1000 µM trimethylamine N-oxide, 12.9% ± 4.96% inhibition ($p=0.0160$) was seen for OAT1 and 22.1% ± 3.88% inhibition ($p=0.0015$) was seen for MATE1. No change was observed in transporter activity for OAT1B3, OAT3, OCT2, or BCRP.

Conclusions: We have shown that p-cresol sulfate and trimethylamine n-oxide inhibit the in vitro activity of multiple renal drug transporters at physiologically relevant concentrations. These alterations in transporter activity may explain the slower and more variable renal drug clearance in subjects with chronic kidney disease. A clearer

understanding of the effects of uremic toxins on proximal tubular drug secretion would allow a more informed strategy for medication selection and dosing in patients with renal impairment.

Funding: Other NIH Support - NCATS KL2 TR000421; NCATS 1UH2 TR000504, Private Foundation Support

FR-PO963

The *CYP3A4*22* Allelic Variant Increases the Risk of Tacrolimus Overexposure in Kidney Transplant Recipient

Nicolas Pallet, Eric Thervet. *Hopital Europeen Georges Pompidou.*

Background: A recently described allelic variant of cytochrome P450 3A4, the *CYP3A4*22* allele, is associated with a decreased expression in the liver and lower global metabolic activity. Carriers of this polymorphism have a slower Tac metabolism, and may be at risk of Tac overexposure.

Methods: We tested this hypothesis in a population of 280 kidney transplant recipients.

Results: The frequency of the *CYP3A4*22* allele in the entire cohort was 7.7%, and the vast majority (90%) of the *CYP3A4*22* carriers were non-expressors for the CYP3A5 (*CYP3A5*3/*3*). The risk of Tac overexposure after 6 Tac doses was 12 fold higher in the *CYP3A4*1/*22* patients, and the median serum creatinine concentration was 191 mmol/l in the *CYP3A4*1/*22* patients versus 146 mmol/l, in the *CYP3A4*1/*1* patients, p=0.03. Two months after transplantation, the median Tac dose to reach target concentrations in *CYP3A4*1/*22* patients was 30% lower than in the *CYP3A4*1/*1* patients: 0.08 mg/kg (0.05-0.11) versus 0.11 mg/kg (0.07-0.14), respectively, *p*=0.03.

Conclusions: In conclusion, the presence of the *CYP3A4*22* allele, is associated with a high risk of Tac overexposure 10 days after transplantation, and carriers of this defective allele require 30% lower Tac dose to reach target concentrations.

FR-PO964

Chronic Kidney Disease Serum Inhibits Transcription of CYP3A4 in a Human Hepatocyte Model

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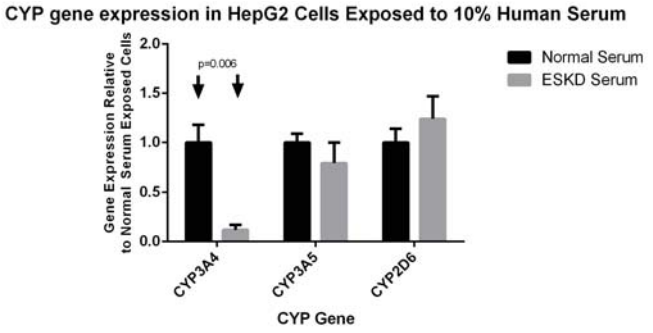
Background: Hepatic drug metabolism by cytochrome P450 (CYP) is reduced in end stage kidney disease (ESKD). The underlying mechanisms are uncertain. Previous work demonstrated transcriptional and translational inhibition of rodent *CYP3A*, exposing rat hepatocytes to serum from dialysis patients. In the light of known specie-specific differences in CYP regulation, we investigated changes to *CYP3A4, CYP3A5* and *CYP2D6* transcription and protein expression by serum from dialysis patients using a human hepatocyte model.

Methods: Serum was obtained from 10 adults with ESKD immediately prior to haemodialysis and from 6 healthy adults. Pooled serum (1,3,10,30% concentrations) was applied to HepG2 cells (human hepatoma cell line) for 24h. *CYP3A4, 3A5* and *2D6* gene expression was examined by RT-qPCR. CYP protein expression was determined by Western blotting.

Results: Demographics of ESKD patients and controls were similar. There were no significant differences in cell confluence. *CYP3A4* gene expression was suppressed in cells exposed to 10% and 30% ESKD serum, compared to control serum (p=0.006). There was no change in *CYP3A5* or *CYP2D6* transcription.



There was no significant change in CYP3A4 or 3A5 protein abundance with serum type at 24h. CYP2D6 is expressed at low concentration, so could not be detected by Western blotting.

Conclusions: Transcription of *CYP3A4*, but not *CYP3A5* or *CYP2D6,* is suppressed in HepG2 cells following exposure to serum from patients with ESKD, compared to healthy volunteers. Protein abundance is unchanged after 24h serum exposure, longer exposures are under investigation. The responsible molecular pathways have yet to be determined for this pharmacologically important observation.

Funding: Private Foundation Support

FR-PO965

Tenapanor, a Minimally Absorbed Small-Molecule Inhibitor of NHE3, Reduces Absorption of Sodium and Phosphorus in Healthy Japanese Volunteers

Susanne Johansson,[1] David P. Rosenbaum,[2] Mikael Knutsson,[1] Maria Leonsson Zachrisson.[1] *[1]AstraZeneca R&D, Mölndal, Sweden; [2]Ardelyx Inc., CA.*

Background: Tenapanor (AZD1722) is a first-in-class, small-molecule inhibitor of the Na^+/H^+ exchanger isoform 3 (NHE3) in clinical development for the treatment of patients with kidney disorders. Preclinical and early clinical studies show that tenapanor reduces intestinal absorption of sodium (Na) and phosphorus (P). Here, we report on the safety, pharmacokinetics and pharmacodynamics of tenapanor in Japanese volunteers.

Methods: In this phase 1, double-blind, placebo-controlled study (D5611C00005), healthy Japanese adults received 180 mg single-dose tenapanor hydrochloride or 7 days of tenapanor hydrochloride at one of four doses (15, 30, 60, 90 mg, all bid) or placebo. Participants received a standardized diet across all cohorts. Pharmacodynamic measures included 24-hour stool and urine Na and P.

Results: All reported adverse events were mild, and there were no serious adverse events. Plasma concentrations of tenapanor were above the limit of quantification (0.5 ng/mL) in just three of 516 samples; the highest concentration measured was 0.7 ng/mL. In the single-dose cohort, tenapanor provided numerically higher stool Na and P levels than placebo, in conjunction with lower urine Na and P levels than placebo (table). Similar trends were observed over 7 days in the repeated-dose cohorts.

24-hour excretion, mmol/day, mean (SD)						
	180 mg (n=6)[a]	15 mg bid (n=12)[b]	30 mg bid (n=12)[b]	60 mg bid (n=12)[b]	90 mg bid (n=12)[b]	Placebo (n=13)[c]
Stool						
Na	41.9 (25.5)	21.3 (17.8)	32.2 (16.8)	28.4 (23.4)	30.8 (15.6)	4.1 (3.1)
P	31.0 (11.5)	21.5 (6.5)	23.4 (7.0)	17.6 (7.5)	24.8 (8.6)	16.8 (7.9)
Urine						
Na	110.4 (49.3)	104.8 (17.9)	111.5 (30.7)	105.3 (33.1)	101.2 (32.0)	135.2 (31.8)
P	18.7 (4.4)	17.5 (4.4)	19.4 (3.5)	15.5 (3.9)	15.3 (4.5)	23.5 (4.5)

[a]Single dose; [b]Daily mean over 7 days; [c]Combined data from participants receiving single (n=1) and repeated bid (n=12) doses.

Conclusions: Tenapanor was well tolerated, minimally absorbed and provided reductions in intestinal Na and P absorption in healthy Japanese volunteers. This promising risk–benefit profile suggests that further clinical development of tenapanor is warranted.

Funding: Pharmaceutical Company Support - AstraZeneca

FR-PO966

Reduction of Sodium and Phosphorus Absorption Provided by Tenapanor Is Not Affected by Co-Administration of Sevelamer in Healthy Volunteers

Susanne Johansson,[1] David P. Rosenbaum,[2] Maria Leonsson Zachrisson,[1] Mikael Knutsson,[1] Dennis Ruff.[3] *[1]AstraZeneca R&D, Mölndal, Sweden; [2]Ardelyx Inc, Fremont, CA; [3]ICON Development Solutions, San Antonio, TX.*

Background: Tenapanor (AZD1722) is a minimally absorbed small-molecule inhibitor of the Na^+/H^+ exchanger isoform 3 (NHE3) that reduces absorption of dietary sodium (Na) and phosphorus (P). Tenapanor is under investigation for treating patients with chronic kidney disease, who often require therapy with phosphate binders, such as sevelamer, to help to control hyperphosphatemia. *In vitro* studies showed binding between tenapanor and sevelamer, but this finding was not confirmed *in vivo* in rats. Here, we report a human study investigating whether co-administration of sevelamer with tenapanor impacts on the pharmacodynamics of tenapanor.

Methods: In this open-label 2-way crossover study (D5611C00006), 16 healthy adults (median [range] age, 47 [27–58] years; 11 men) were randomized to receive tenapanor hydrochloride 15 mg bid alone for 4 days or tenapanor hydrochloride 15 mg bid and sevelamer carbonate 800 mg tid for 4 days, with a 2-day washout between treatments. A diet standardized for Na content was provided with similar menus in both treatment periods. Pharmacodynamic measures included 24-h stool/urine Na and P levels.

Results: Tenapanor treatment resulted in comparable stool Na and P levels whether administered alone or combined with sevelamer (table). Stool frequency, consistency and weight were also comparable between the regimens. Treatments were well tolerated with no discontinuations due to AEs or serious AEs.

Excretion, mmol/day, mean (SD)			
	Day −1 (n=16)	Tenapanor (n=16) [a]	Tenapanor+sevelamer (n=16) [a]
Stool Na	3.6 (6.2)	29.2 (19.3)	25.7 (17.9)
Urine Na	127.4 (39.8)	133.3 (27.6)	137.2 (30.0)
Stool P	17.1 (9.8)	37.4 (11.4)	37.7 (7.5)
Urine P	31.3 (6.7)	26.2 (5.2)	25.2 (4.9)
[a]Daily mean over 4-day treatment period			

Conclusions: The reductions in intestinal Na and P absorption provided by tenapanor were not affected by co-administration of sevelamer. This suggests that the binding between sevelamer and tenapanor observed *in vitro* does not translate into humans to an extent that is clinically meaningful, if at all.

Funding: Pharmaceutical Company Support - AstraZeneca

FR-PO967

The Effect of Sodium Nitrite on Blood Pressure, Glomerular Filtration Rate and Fractional Sodium Excretion in Healthy Subjects Jeppe B. Rosenbaek, Safa Al Therwani, Janni Majgaard Jensen, Frank H. Mose, Erling B. Pedersen, Jesper N. Bech. *Univ Clinic in Nephrology and Hypertension, Dept of Medical Research, Holstebro Regional Hospital and Aarhus Univ, Holstebro, Denmark.*

Background: Recent research has shown that sodium nitrite is readily converted to nitric oxide (NO) by enzymes in vivo and exerts a vasodilating effect. The purpose of the present study was to examine the effects of sodium nitrite on blood pressure, heart rate, GFR and fractional sodium excretion.

Methods: In a single blinded, crossover, placebo controlled dose-response study 12 healthy subjects were treated, in a randomized order, with placebo (isotonic NaCl) or one of three doses of sodium nitrite 40, 120 or 240 mg/kg/hour for two hours. Each examination was preceded by 4 days standardized diet**.** The subjects were supine and water loaded throughout the day. Before, during and after sodium nitrite administration we measured diastolic, systolic and mean arterial blood pressure (DBP, SBP and MAP), heart rate, GFR by chrome-EDTA clearance and fractional sodium excretion.

Results: Preliminary data shows no significant change in DBP, SBP, MAP, heart rate, GFR or fractional sodium excretion on either dose of sodium nitrite compared to placebo.

Conclusions: In supine, water loaded subjects the given doses of sodium nitrite exert no hemodynamic or renal effects on the measured parameters. Either the doses are too low, or sodium nitrite has only a marginal effect on renal NO availability in healthy subjects.

Funding: Government Support - Non-U.S.

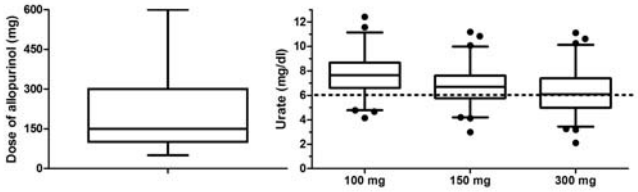
FR-PO968

Frequency and Dosing of Uric Acid Lowering Therapy in Patients with CKD 3 – Baseline Data of the German Chronic Kidney Disease (GCKD) Cohort Jan T. Kielstein, Markus Heisterkamp, Stephanie Titze, Johan M. Lorenzen, Martin Busch, Kai-Uwe Eckardt, Anna Kottgen. *GCKD-Study Investigators.*

Background: Uric acid (UA) is a prognostic marker of chronic kidney disease (CKD) progression. Preclinical studies support an independent pathophysiological role of UA in this process. ULT in CKD is challenging as the most frequently used xanthine oxidase inhibitor allopurinol requires dose reduction with reduced GFR. Aim of the study was to analyze the prevalence of ULT and adherence to dose suggested adjustments.

Methods: Baseline data and drug prescription patterns were analyzed in a 1965 patient subset of the GCKD Study, a prospective observational CKD cohort study that enrolled more than 5200 patients with CKD of various aetiologies between 2010 and 2012. At enrollment patients had an estimated glomerular filtration rate (eGFR) of 30-60 mL/min/1.73 m² or overt proteinuria in the presence of a higher eGFR.

Results: Of the 1965 patients (age 62 ± 13 years) investigated 551 (28.1%) received ULT. Median serum urate concentrations in patients without and with ULT were 7.22 [5.98-8.34] and 6.81 [5.72-6.81] mg/dl. The proportion of patients with plasma urate concentrations below a target level of 6 mg/dL was 25% and 31%, resp. Most of the patients with ULT received allopurinol (93.2%). Median allopurinol dose was 150 mg/d. 30.2% of the allopurinol treated patients received a dose of 300 mg/d. Compared with an allopurinol dose of 100 mg/d patients receiving 150 or 300 mg/d had significantly lower urate levels.



Conclusions: Hyperuricemia is frequent in CKD 3 but only less than a third of patients is treated. Treatment frequently fails to lower urate levels below 6 mg/dl. In a third of the patients treated with allopurinol the dose was higher than recommended. Our data indicate that the treatment of hyperuricemia in CKD could be improved in both frequency of treatment and dose adjustment.

Funding: Other NIH Support - The GCKD Study is funded by the Federal Ministry of Research and Education (BMBF) and the nonprofit KfH Foundation of Preventive Medicine. It is conducted under the patronage of the German Society of Nephrology (DGfN)

FR-PO969

An Extended Release Formulation of Torsemide Is More Effective in Enhancing Sodium and Fluid Excretion in Normal Subjects Wen Shen,[1] Salim Shah,[2] Fatima S. Khwaja,[3] Bertram Pitt,[4] Christopher S. Wilcox.[2] *[1]Div of Hypertension & Nephrology, Georgetown Univ Hospital; [2]Georgetown Univ; [3]Sarfez Pharmaceuticals, Inc; [4]Univ of Michigan.*

Background: Despite outstanding acute natriuresis, loop diuretics have limited therapeutic efficacy due to the compensatory renal sodium reabsorption after the diuretic effects wear off. We hypothesized that an extended relases (ER) formulation would enhance salt and water loss.

Methods: Ten normal subjects were randomized to 20mg immediate release (IR) or ER formulation torsemide, and were crossed over to receive the other formulation after 3 weeks. They consumed a fixed diet with 300 mmol·day^{-1} of Na^+. The ER formulation released the drug into solution over 12 hours.

Results: Both formulations increased peak Na and fluid excretion similarly. Natriuresis returned to baseline after 3hrs with IR, but remained elevated for 8-12hrs in ER. This resulted in doubling of fluid (1634 ± 385 versus 728 ± 445 mL; $P < 0.02$) and sodium loss (98 ± 15 versus 42 ± 17 mmol; $P < 0.05$) despite an 18% reduction in torsemide bioavailability with ER. Body weight (-0.5 ± 0.2 kg; $P < 0.05$) and blood pressure (-2.9 ± 1.8 versus +1.3± 1.2 mmHg; $P<0.05$) were reduced only after ER but GFR was reduced only after IR (C_{CR}; -24.5 ± 6.6 ml•min^{-1} per 1.73 m^{-2}; $P < 0.005$). Potassium excretion was unchanged in both groups. The increase of plasma and urine torsemide was delayed and extended after ER, resulting in a 3.5 fold prolongation of Tmax. Increase in sodium excretion was a hysteretic function of the log renal torsemide excretion (an index of delivery to the active site), but the up-trending phase of Na excretion was twice as long with ER.

Conclusions: An ER formulation of torsemide led to weight reduction despite a very high salt intake. It doubled the fluid and sodium loss while maintaing GFR. The enhanced efficacy with ER was related to a reduced time for post-diuretic renal Na^+ retention, a more efficient diuretic effect and a delayed development of within-dose tolerance. This suggests that torsemide ER may be superior to IR under the condition of high salt diet and may be more predictively effective in treating edema and hypertension.

Funding: Other NIH Support - SBIR

FR-PO970

Different Associations of Wall Shear Stress with Lumen Remodeling in Artery and Vein following Arteriovenous Fistula Creation in a Porcine Model Yan-Ting E. Shiu,[1] Daniel B. Pike,[1] Christi M. Terry,[1] Michelle Fitts,[1] Ilya S. Zhuplatov,[1] Alfred K. Cheung.[1,2] *[1]U of Utah, SLC, UT; [2]VASLCHCS, SLC, UT.*

Background: Arteriovenous fistula (AVF) maturation requires the dilation or outward remodeling of the AVF vein. Hemodynamic wall shear stress (WSS) is known generally to affect vascular wall remodeling. However, it is unclear if the relationships between WSS and subsequent lumen area changes are different between the artery and vein of the AVF. We explored these associations in a pig AVF model as a potential explanation for the differential wall remodeling of artery and vein.

Methods: At 1, 2, 6, or 12 wk after the creation of a carotid-jugular AVF in pigs (n=4 at each wk), MR images were acquired to determine the AVF lumen geometry and blood flow rates, which were then used to derive detailed spatial profiles of WSS values (by computational fluid dynamics) and lumen cross-sectional areas. Early WSS values were correlated with later lumen area changes (Darea) over a length of 4 cm, starting from the AV anastomosis.

Results: In both the artery and vein, average lumen area increased from wk1 to wk6 (Table 1); area remained steady thereafter. The absolute and percentage area increases were larger in vein than artery. Average WSS at wk1, wk2, and wk6 was also higher in vein than artery. Importantly, in the artery, WSS at wk1 and Darea from wk1 to wk6 had a *positive* exponential correlation (r^2=0.41, $p<0.0001$) with Darea *increasing* with *increasing* WSS at wk1. In contrast, WSS at wk1 and Darea from wk1 to wk6 had a *negative* exponential correlation (r^2=0.27, $p<0.0001$) in the vein, with Darea generally *decreasing* with *increasing* WSS at wk1.

Table 1: WSS, Area, and Δarea in AVF Artery and Vein

	WSS (dyn/cm²)			Area (mm²)			Δarea wk1 to wk6	
	wk1	wk2	wk3	wk1	wk2	wk3	(mm²)	(%)
Artery	97±40	209±114	272±335	23±3	24±7	44±6	21±7	91±29
Vein	122±67*	383±399†	871±550‡	21±9	69±22‡	64±19‡	43±21‡	205±100‡

Values ± standard deviation.
All comparisons between artery and vein; *p<0.05, †p<0.001, ‡p<0.0001.
Presurgical WSS is 14±3 dyn/cm² in artery and 7±2 dyn/cm² in vein.
Presurgical area is 20±1 mm² in artery and 19±2 mm² in vein.

Conclusions: Our finding of WSS-Darea associations with opposite directions between artery and vein is novel. This difference may partly explain the differences in arterial and venous long-term remodeling observed clinically.

Funding: NIDDK Support

FR-PO971

Effect of Oscillating Shear Stress Derived from Vascular Access on Protein and Gene Expression in Endothelial Cells In Vitro Andrea Remuzzi,[2] Irene Cattaneo,[1] Lorena Longaretti,[1] Marina Figliuzzi,[1] Bogdan Ene-Iordache.[1] *[1]IRCCS Mario Negri Inst, Bergamo; [2]Univ of Bergamo, Dalmine, Italy.*

Background: Failure of arteriovenous fistulas (AVF) for hemodialysis patients is strongly related to progressive stenosis caused by of intimal hyperplasia (IH). Using idealized models of end-to-side AVF we suggested that areas prone to develop IH are exposed to oscillating wall shear stress (WSS) [NDT 2012]. Here we investigated in vitro the effects of oscillating WSS, calculated at the inner wall of the venous segment of AVF, on activation of human umbilical vein endothelial cells (HUVEC), as compared to pulsatile and unidirectional WSS calculated for areas not prone to IH.

Methods: Confluent monolayers of HUVEC were exposed for 48h to oscillating and to pulsatile WSS (range -2.6 to 1.1 and 1.2 to 2.3 Pa, respectively), using a cone-and-plate device. Culture medium was changed each 12 hrs. At 48h we quantified cell morphology. We also quantified MCP1 production (ELISA) by HUVEC and we evaluated mRNA expression of Krüppel-like factor 2 (KLF2, a transcription factor that mediates flow induced anti-inflammatory effects on EC) by real time-PCR.

Results: HUVEC exposed to oscillating WSS did not align nor elongate with flow while pulsatile WSS strongly elongated and aligned cells with flow. MCP1 production was higher ($p<0.05$) in HUVEC exposed to oscillating WSS, as compared to pulsatile flow (5.0+0.7 versus 2.1+0.5 ng/hr/10^6 cell, n=3). In HUVEC exposed to oscillating WSS, KLF2 mRNA levels were comparable to those estimated in static cultures (1.4 fold increase, NS) but they were significantly increased in HUVEC exposed to pulsatile WSS (3.7 fold increase, $p<0.01$).

Conclusions: Our findings indicate that exposure to oscillating WSS importantly affect EC structure and pro-inflammatory state. Thus, disturbed flow importantly increased MCP1 production and decreased KLF2 expression in EC, at variance to unidirectional flow. This pro-inflammatory condition may selectively induce monocyte recruitment in AVF areas subjected to oscillating flow. These changes in protein and gene expression may be responsible for local development of IH and subsequent AVF stenosis.

FR-PO972

Arteriovenous Fistula Remodeling in a Rat AVF Model Timmy C. Lee, Maheshika Srimali Somarathna, Lingling Guo. *Medicine, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Neointimal hyperplasia development and poor vascular remodeling are the most common etiologies of arteriovenous fistula maturation (AVF) failure. The aim of this study was to evaluate in a rat AVF model: (1) serial arterial and venous morphometric changes and (2) changes in expression in the artery and vein of key remodeling proteins, matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9).

Methods: AVFs were created using an end to side anastomosis between the femoral vein and artery in 16 week old Sprague-Dawley rats. These rats were sacrificed at 7, 14, 21, and 28 days after creation. Morphometric analyses of the arterial and venous anastomosis were performed. Immunohistochemical analysis from the vein and arterial anastomosis was performed to evaluate expression of MMP-2 and MMP-9.

Results: A progressive increase in average intima/media area was seen at both the arterial and venous anastomosis, respectively, at 7, 14, 28, and 42 days. MMP-2 expression in both the vein and artery peaked at 7 days with a secondary peak again at 21 days. MMP-9 expression in the vein and artery remained elevated from 7 days to 21 days, with a decline in expression at 28 days.

	7 Days	14 Days	21 Days	28 Days
Average Intima/media area (vein)	2.37	4.17	4.06	6.77
Average Intima/media area (artery)	0.258	0.130	0.521	0.545
MMP-2- % stain (Vein)	26.5%	7.34%	19.9%	14.4%
MMP-2- % stain (artery)	10.6%	4.85%	12.5%	3.5%
MMP-9- % stain (Vein)	50.7%	29.1%	31.1%	19.3%
MMP-9- % stain (Artery)	20.9%	21.8%	22.7%	16.7%

Conclusions: Our results suggest a progressive remodeling pattern in both the vein and artery after AVF creation. MMP expression is substantially elevated early after AVF creation suggesting an initial vascular remodeling response with a decline in expression at later time points. Further pathophysiologic insight of the role MMPs play in AVF remodeling after AVF creation may improve our understanding of AVF maturation failure.

FR-PO973

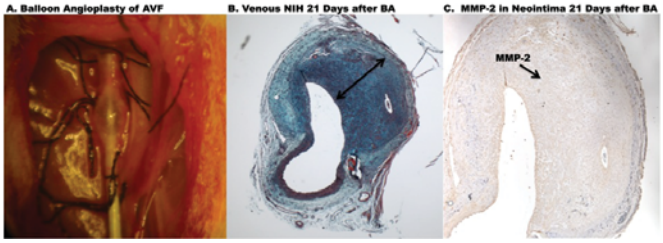
A Novel Model of Balloon Angioplasty Injury in Rat Arteriovenous Fistula Timmy C. Lee, Maheshika Srimali Somarathna, Lingling Guo. *Medicine, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Balloon Angioplasty (BA) remains the primary therapy to treat arteriovenous fistula (AVF) maturation failure and AVF stenosis. However, BA procedures injure the vascular endothelium, leading to recurrent and aggressive neointimal hyperplasia (NIH) development. The lack of a rodent model to study BA injury is a major gap in the field as it hinders insight into the pathogenesis of BA injury in AVF and development of better therapies to prevent and treat BA injury to prolong AVF patency. The aim of this study was to develop a rat model of BA injury in AVF.

Methods: BA of 16 week old Sprague-Dawley rats were performed: (1) in the femoral vein immediately prior to creation of AVF (end to side anastomosis of the femoral vein to femoral artery) (group 1) and (2) in AVFs at the juxta-anastomosis 14 days after creation without pre-AVF creation angioplasty (group 2). We assessed for changes in NIH development and expression of key vascular remodeling proteins, matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) at serial time points following BA.

Results: In group 1, the mean intima/media area of the venous anastomosis at 14 and 21 day post-angioplasty and AVF creation was 1.99 and 9.27, respectively, with a progressive increase in staining primarily within the neointima for MMP-2 and 9 from 14 to 21 days. In group 2, the mean intima/media area of the venous anastomosis was 2.80 and 11.7 at 7 and 14 days after BA, respectively, with also a progressive increase in MMP-2 and 9 staining primarily within the neointima from 7 to 14 days.

Conclusions: Our novel rat model of BA injury in AVF displays similar features to BA injury in humans. Rats developed progressive NIH with early remodeling following BA injury. Our BA model will allow for future studies to evaluate the pathophysiologic mechanisms of BA injury in AVF and test therapies to prolong AVF survival after BA procedures.



FR-PO974

Assessment of Novel Antithrombotic Fusion Proteins for Inhibition of Arteriovenous Graft Stenosis Christi M. Terry,[1] Ilya S. Zhuplatov,[1] Yuxia He,[1] Daniel Orme,[1] Alfred K. Cheung,[1] Tze-Chein Wun.[2] *[1]Internal Medicine, UofUT, SLC, UT; [2]EVAS Therapeutics, LLC, Ballwin, MO.*

Background: Coagulation generates activated platelets, fibrin and mural thrombi that may promote neointimal hyperplasia (NH) and stenosis of arteriovenous grafts (AVG). Two novel proteins, A6L15 and TAPANV, contain Kunitz protease inhibitor domains that target the tissue factor/VIIa and FVa/Xa coagulation complexes, respectively. The proteins are fused to annexin V domains that target the proteins to thrombogenic membranes to locally inhibit thrombosis. We tested these proteins for efficacy against NH in a pig model of AVG stenosis.

Methods: TAPANV was added to blood (20 mg/mL) and activated clotting times (ACT) were assessed *in vitro* (n=8). Pigs received an i.v. bolus of either protein (300 mg). The synthetic graft lumen and a segment of isolated carotid artery and jugular vein were irrigated with 100 mg/mL of A6L15 (n=11) or TAPANV (n=10) for 5 min during AVG creation. Control pigs with AVG received systemic heparin (n=14) or nothing (n=1). At 4 wks, pigs underwent MRI of the AVG, the AVG was explanted and vein-graft tissue was used for histological assessment of NH. Blood was collected for ACT, prothrombin times (PT) and activated partial thromboplastin times (aPTT) before and after surgery.

Results: TAPANV prolonged ACT *in vitro* compared to no drug (160.5±36 versus 97.9±5.7 sec, $p<0.05$). The AVG of the untreated pig clotted at surgery. Post-surgical ACT and aPTT were unchanged from pre-surgical values with either protein, or with heparin. Post-surgical PT were prolonged with A6L15 and heparin treatments (A6L15: 10.5±0.5 versus 10.8±0.5 sec, $p<0.05$, n=11; Heparin: 10.4±0.3 versus 10.7±0.4 sec, $p<0.05$, n=12) but not with TAPANV ($p=0.254$; n=10). No delays in healing or overt bleeding episodes were observed during follow-up. There was no significant difference between the protein-treated and control groups in NH development at the vein-graft anastomoses, as determined by MRI or histology.

Conclusions: TAPANV or A6L15 were safe and inhibited perisurgical AVG thrombosis yet did not inhibit AVG NH when applied at time of surgery. These results suggest acute localized inhibition of thrombosis does not inhibit NH in AVG.

Funding: Other NIH Support - NHLBI

FR-PO975

Outcomes following Percutaneous Thrombectomy for Polytetrafluoroethylene and Bovine Hemodialysis Grafts Karthik Karanam, Theodore F. Saad. *Section of Renal & Hypertensive Diseases, Christiana Care Health System, Newark, DE.*

Background: Percutaneous thrombectomy (PT) is commonly performed for treatment of thrombosed hemodialysis grafts. Data on PT success and patency for polytetrafluoroethylene (PTFE) versus bovine grafts are limited. Our study purpose was to compare clinical success of PT for PTFE versus bovine grafts and to assess post-PT patency based upon time from surgical graft placement to 1st thrombosis.

Methods: We retrospectively reviewed all PT procedures done by interventional nephrologists for 1st graft thrombosis from 2009-2013. Cases were identified and data collected from a prospectively maintained database containing all procedure outcomes and interventions. We recorded: date of graft placement, graft material (PTFE versus bovine), surgeon, date of 1st thrombosis, procedure clinical success (graft usable for next dialysis treatment), primary patency (PP), secondary patency (SP), and rate of interventions performed to maintain graft patency. Outcomes were determined for all cases combined, and for sub-groups of PT performed at 0-45, 46-90, and >90 days post graft placement.

Results: 302 PT procedures for 1st thrombosis were identified. PT was successful in 204/226 (90.3%) PTFE and 68/76 (89.5%) bovine grafts. For PTFE versus bovine, success

rates were 25/31 (80.6%) versus 10/11 (90.9%) at 0-45 days, 30/35 (85.7%) versus 10/14 (71.4%) at 46-90 days, and 149/160 (93.1%) versus 48/51 (94.1%) at >90 days respectively. Post-PT patency rates are reported in the table:

Kaplan-Meier graft patency % (PTFE vs. bovine)				
Days post graft	6 month PP	6 month SP	12 month SP	24 month SP
0-45	25.8 vs. 0	61.3 vs. 72.7	51.6 vs. 45.5	45.2 vs. 27.3
46-90	22.5 vs. 43.8	74.3 vs. 64.3	71.4 vs. 50.0	68.6 vs. 42.9
>90	35.6 vs. 33.3	81.9 vs. 74.5	78.1 vs. 72.5	74.4 vs. 62.7
ALL	31.9 vs. 31.6	77.7 vs. 72.4	73.2 vs. 68.4	69.2 vs. 60.5

The mean number of interventions to maintain graft patency was 2.6/yr for PTFE and 2.5/yr for bovine grafts. No comparisons achieved statistical significance.

Conclusions: PT clinical success and post-PT primary and secondary patency rates are no different for PTFE versus bovine grafts treated for thrombosis at any time interval post graft implantation. Intervention rates are the same for PTFE and bovine grafts.

FR-PO976

Endovascular Salvage of Immature Hemodialysis Arteriovenous Fistula

Venkatesh Rajkumar Sridharan, Yong-Soo Kim. *Div of Nephrology, Dept of Medicine, The catholic Univ of Korea Seoul St. Mary's Hospital, Seoul, Korea.*

Background: The rising CKD population along with the understanding that arteriovenous fistula (AVF) is the closest to an ideal vascular access has resulted in the creation of a lot of AVF including a number of marginal cases. This has resulted in immature fistulas as one of the common problems associated with AVF.

Methods: A retrospective analysis over a three year period (Jan 2011- april 2014) of all the cases of immature AVF referred to a large tertiary care hospital in seoul,south korea. We have analysed the anatomical causes for immaturity and the success rate of endovascular salvage procedures done.

Results: There were 25 radiocephalic,25 brachiocephalic and 2 transposed brachiobasilic fistulas.Mean interval from access creation to referral for angiography was 103+/-67 days (44-349days).Anatomical problems were identified in all the cases.The causes of immature AVF were stenosis (67.3%),accessory veins (9.6%),both (17.3%) and others (5.8%).Inflow stenosis predominated in forearm AVF whereas in the upperarm AVF outflow stenosis was marginally higher than inflow stenosis.The technical and clinical success rates were 94.2% and 90.4% respectively.

Conclusions: The referral for immature fistulas is late. Close follow up of created AVF is needed to detect immature ones at the earliest and endovascular salvage of them can be done with a good success rate.

FR-PO977

Outcomes of Incisional Percutaneous Thrombectomy for Aneurysmal Arteriovenous Fistulae

Theodore F. Saad.[1] *[1]Section of Renal & Hypertensive Diseases, Christiana Care Health System, Newark, DE; [2]Nephrology Associates, PA, Newark, DE.*

Background: Percutaneous thrombectomy (PT) is commonly performed for treatment of thrombosed hemodialysis fistulae using various methods and devices. Dilated aneurysmal fistulae present a particular challenge and conventional percutaneous methods may not be effective. We describe results of a modified PT technique utilizing a small incision for direct thrombus extraction.

Methods: The incisional PT technique involves bidirectional percutaneous access with balloon occlusion of arterial inflow and venous outflow. A 1 cm incision is made over the fistula, thrombus extracted using Kelly forceps and manual maceration, and the incision sutured closed. All stenoses are treated with angioplasty or stenting as required. We retrospectively reviewed all incisional PT procedures done for fistula thrombosis from 2009-2014. Cases were identified and data collected from a prospectively maintained database containing all procedure outcomes and interventions. We recorded: clinical success (fistula usable for next dialysis session), primary and secondary patency, and interventions performed to maintain fistula patency.

Results: 44 incisional PT procedures were performed in 38 patients (6 had procedure twice). PT was successful in 42/44 (95.5%). Post-PT patency rates following 38 first incisional PT are reported in the table:

Patency				
	3 month	6 month	12 month	24 month
Primary	53%	29%	24%	24%
Secondary	74%	63%	55%	53%

The mean number of interventions to maintain fistula patency was 1.8/yr. There were no immediate procedure complications. 3 required stents at the index procedure and 3 at subsequent procedures. Two subjects required surgical ligation <30 days post thrombectomy due to bleeding and infection from fistula ulcerations, unrelated to the thrombectomy incision.

Conclusions: Incisional PT can be performed with high clinical success for aneurysmal fistulae considered unsalvageable using conventional PT techniques. Primary patency rates are low, in part due to low threshold for early re-intervention. Acceptable secondary patency can be achieved with intervention rates similar to other reports. Ulcerated fistula aneurysms may pose higher risk for infection and hemorrhage post thrombectomy.

FR-PO978

Annual Decline in Pentraxin 3 Is a Risk of Vascular Access Troubles in Hemodialysis Patients

Kei Nagai,[1] Toshiaki Usui,[1] Atsushi Ueda,[2] Chie Saito,[1] Kunihiro Yamagata.[1] *[1]Nephrology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Nephrology, Univ of Tsukuba, Hitachi Medical Education and Research Center, Hitachi, Ibaraki, Japan.*

Background: Pentraxin 3 (PTX3), a multifunctional modulator of the innate immuno-inflammatory response, is higher in patients undergoing hemodialysis than healthy control. Our study focused on annual change in plasma PTX3 levels in patients with chronic hemodialysis, because regularly undergoing hemodialysis for many years modify vascular inflammatory status.

Methods: To demonstrate whether annual change in PTX3 is associated with vascular events, we measured blood levels of pentraxins (PTX3 and high-sensitivity C-reactive protein [hsCRP]) at baseline and in the next year in 76 hemodialysis patients and observed 20 patients with vascular access troubles during follow-up years.

Results: The annual decline in plasma PTX3, but not serum hsCRP, is a significant risk of the incidence of vascular access trouble that is a critical and specific complication for hemodialysis patients (hazard ratio; 0.732 per +1 ng/mL/year in PTX3, **P*=0.039).

	Unadjusted			Adjusted by DM, sex, age, dialysis-period and BMI		
	Hazard Ratio	95% C.I.	*P*-value	Hazard Ratio	95% C.I.	*P*-value
Baseline PTX3	1.006	0.836 - 1.211	0.951	1.237	0.936 - 1.635	0.136
ΔPTX3	0.849	0.680 - 1.061	0.151	0.732	0.544 - 0.985	*0.039
Baseline hsCRP	3.644	0.469 - 28.30	0.216	2.713	0.389 - 18.92	0.314
ΔhsCRP	9.486	0.108 - 833.2	0.324	3.605	0.033 - 394.7	0.592

The incidence of vascular access troubles during follow-up years were set as the outcome. Co-variants examined in Table.II were used for adjustment. * P < 0.05. Abbreviations: C.I., Confidential Intervals: ΔPTX3, Annual change in pentraxin 3 (ng/mL per year): ΔhsCRP, Annual change in high-sensitivity C-reactive protein (mg/dL per year).

Conclusions: This study is the first to focus on the annual change of pentraxins in a hemodialysis cohort.

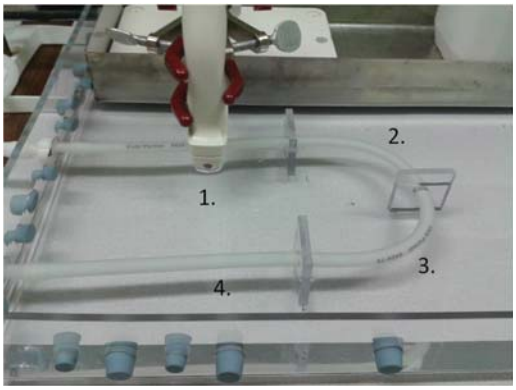
FR-PO979

Re-Introducing Spiral Flow in a Vascular Access Graft Using a Spiral Inducing Stent

Shona Matthew,[1,2] Alexandra Webb,[2] Efstratios Kokkalis,[1] Malcolm Harry Dunn,[2] John Graeme Houston.[1,3] *[1]CVDM, Univ of Dundee, Dundee, United Kingdom; [2]Physics, Univ of St. Andrews, St. Andrews, United Kingdom; [3]Radiology, NHS Tayside, Dundee, United Kingdom.*

Background: Single spiral flow (SSF) patterns observed in arteries are thought to be initiated by the contraction of the heart and maintained by the geometry of the aortic arch. The turbulent flow patterns found around haemodialysis (HD) vascular access (VA) points have been linked to stenosis and are thought to contribute to VA failure at the distal anastomosis (DA). The reintroduction of SSF in vascular grafts has been linked to improved graft patency. This study investigated flow patterns through a U-bend of similar geometry to a HD VA graft before determining the impact of a SSF inducing stent (SSFiS) on flow patterns through the DA and beyond the graft.

Methods: A flow-rig consisting of an acrylic tank, 8mm C-flex vessel-mimic (VM) (Cole-Parmer, UK), SSFiS (Vascular Flow Tech, Ltd, UK), and a UHDC flow-pump (Shelley, Canada). The tank and tubing were filled with tissue-mimic (TM) (9% glycerol in distilled water) and blood-mimic (BM) (Model 707, ATS, U.S.A.).



Colour Doppler Images of flow patterns were acquired using an HDI 5000 ultrasound scanner (ATL US, U.S.A.).

Results: When the SSFiS was placed before the U-bend (brachial artery) the geometry of the vessel-mimic rapidly destroyed the SSF pattern created by the stent. When the

SSFS was placed after the u-bend, (distal graft) the spiral stent consistently re-ordered the flow pattern created by the U-bend into a SSF pattern that propagated through the DA and 20cm downstream.

Conclusions: This study demonstrates that to reintroduce spiral flow in a u-shaped conduit such as VA graft a SSFiS should be inserted in the distal limb of the graft. Although grafts are now being made which induce SSF (VFT), it may be appropriate to consider placement of a SSFiS in distal grafts to re-introduce SSF in the DA.

FR-PO980

Improving Blood Flow Parameters in Central Vein Catheter Dialysis Shona Matthew,[1,2] Alexandra Webb,[2] Efstratios Kokkalis,[1] Malcolm Harry Dunn,[2] John Graeme Houston.[1,3] *[1]CVDM, Univ of Dundee, Dundee, United Kingdom; [2]Physics, Univ of St. Andrews, St. Andrews, United Kingdom; [3]Radiology, NHS Tayside, Dundee, United Kingdom.*

Background: Blood flow in human arteries and veins has been widely reported as single spiral flow and is thought to reduce turbulence that causes stenosis. Turbulent flow is found around central vein catheters (CVC) used for haemodialysis (HD) and has been linked to the formation of a stenosis due to disturbance of the central vein endothelial cells. We investigate the effects of CVC insertion on flow patterns within a vessel during and between simulated HD sessions.

Methods: An in vitro flow-rig consisting of an acrylic tank, 8mm C-flex vessel-mimic (VM) (Cole-Parmer, UK) in an anatomically relevant geometry, with and without a spiral inlet profile (SIP) stent (Vascular FlowTechnologies, UK), and a UHDC flow-pump (Shelley Medical Imaging, Canada). The tank and VM were filled with tissue-mimic (TM) (9% glycerol in distilled water) and blood-mimic (BM) (Model 707, ATS, U.S.A.) respectively. A 25cm HD catheter (Cannon II Access) was inserted into the VM and attached to a HD machine (Fresenius, Germany). Images of flow patterns were acquired using an HDI 5000 ultrasound scanner (ATL Ultrasound, U.S.A.).

Results: When BM flowed at 330ml/min through VM with a laminar inlet profile, Poiseuille flow was observed. When BM flowed through VM with a SIP, a flow pattern suggestive of a single spiral flow was observed to propagated 17.5cm downstream. On insertion of a CVC to VM with SIP, single spiral flow was observed to propagated 15cm downstream of the catheter. Connecting the CVC to the HD machine and passing BM through the CVC at 320ml/min destroyed the spiral flow creating a turbulent profile.

Conclusions: This preliminary study has demonstrated that using a spiral inducing stent before a CVC propagates spiral flow in the vessel 15 cm beyond the end of a CVC in keeping with normal central vein flow which is associated with reduced turbulence. When flow is initiated in the catheter a turbulent pattern is observed, regardless of the inlet profile. These findings may suggest a means of reintroducing normal spiral flow in central veins with a CVC catheter in place in order to reduce turbulence.

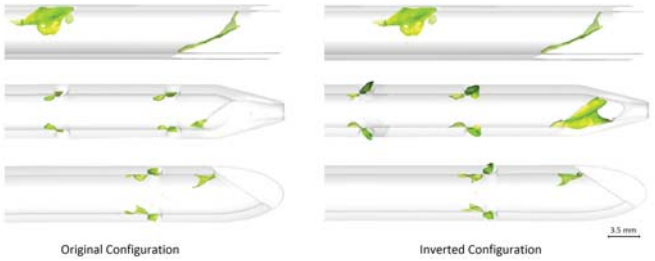
FR-PO981

Comparison of Flow Velocity Reversal among Symmetrical Tip Dialysis Catheters Using Computational Flow Dynamics Timothy Clark,[1] Giuseppe Isu,[2] Diego Gallo,[2] Pascal Verdonck,[3] Umberto Morbiducci.[1] *[1]Radiology, Univ of Pennsylvania, Philadelphia, PA; [2]Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy; [3]Inst Biomedical Technology, Ghent Univ, Gent, Belgium.*

Background: Thrombosis remains a failure mode of all chronic dialysis catheters, in part from flow stagnation from reversal of blood velocity opposite to the main direction of flow. We compared reversed blood velocity among three symmetrical tip catheters using computational flow dynamics (CFD).

Methods: A validated CFD model simulated three symmetrical tip dialysis catheters (Palindrome, Glidepath and VectorFlow) at 400 mL/min within the superior vena cava. To characterize the velocity of blood entering the catheters through the terminal tip and sideholes (before purely laminar, unidirectional flow) we partitioned arterial lumen blood into finite volume elements 5.0 cm distal to the last sidehole, and calculated the volume of blood with reversed velocity at any moment in time with respect to the main direction of flow. Each catheter was run in forward and inverted directions.

Results: The Glidepath showed the highest percentage of reversed velocity within the arterial lumen (6.7% forward, 6.8% inverted) followed by Palindrome (5.6% forward, 5.6% inverted) and VectorFlow (3.3% forward, 3.7% inverted). Blood pathline analysis showed the reversed velocity blood flow in the Glidepath was from geometric asymmetry of the distal guidewire aperture producing flow perturbation when run in inverted configuration. Flow stagnation zones were found most prominently around catheter sideholes and terminal apertures



[Figure, zones shown in green], where laminar flow entering from the catheter tip becomes interrupted by sidehole inflow with resultant areas of velocity reversal.

Conclusions: Blood velocity reversal was seen within each symmetrical tip design which was lowest within the VectorFlow catheter.

FR-PO982

The Relationship between Doppler Parameters and Autologous Arteriovenous Fistulae Dysfunction Xiaotong Xie, Xiaoliang Zhang, Hong Liu. *Nephrology, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Autologous arteriovenous fistulae (AVF) dysfunction is one of the most common complications in maintenance haemodialysis patients (MHD). Doppler ultrasonography (DU) has been considered as a useful technique to access the function of AVF, whereas the characteristics of doppler parameters are not established. The current study is aimed to reveal the relationship between doppler parameters and AVF dysfunction.

Methods: MHD patients with AVF treated in this hospital were included in this study. DU was used to examine the AVFs in MHD patients. They were grouped according to fistulae's function. Fistula dysfunction was defined as: the blood flow was less than 200 ml/min, except technical and hemodynamic reasons during dialysis. In this study, we analyzed the characteristics of doppler parameters and the relationship between these parameters and arteriovenous fistulae dysfunction.

Results: 1) 145 patients were concluded in this study, aged 23 to 88 years old. There were 126 cases with wrist fistula and 19 cases with elbow fistula. 2) According to the function of AVF, patients were divided into well-functional group (115 cases) and dysfunctional group (29 cases). The blood flow volume and anastomosis diameter showed a significant reduction in dysfunctional group compared with well-functional group (803.84 ± 108.91ml/min *versus* 1150.62 ±63.6ml/min and 0.47±0.02cm *versus* 0.37±0.03cm, respectively, $P<0.05$), the patients' age and the resistance index (RI) showed a significant increase in dysfunctional group compared with well-functional group (66±3years old *versus* 56±1years old and 0.55±0.03 *versus* 0.48±0.01, respectively, $P<0.01$). 3) We further grouped patients according to the quarterback of blood flow, RI, anastomosis diameter and analyzed the incidence of fistula dysfunction within each group. Finally, we found out that the threshold indices for blood flow volume is 641.3 ml/min, the RI 0.41 and the anastomosis diameter 0.39 cm.

Conclusions: DU examination is helpful to early detect AVF dysfunction. The threshold doppler indices provide important experimental evidence for early intervention.

Funding: Government Support - Non-U.S.

FR-PO983

Morphometric and Histologic Parameters in Veins of Diabetic Patients Undergoing Fistula Placement Ivana Lazich,[1] Anthony Chang,[2] Sydeaka Watson,[3] Mary S. Hammes.[1] *[1]Dept of Medicine, Section of Nephrology, Univ of Chicago; [2]Dept of Pathology, Univ of Chicago; [3]Dept of Health Studies, Univ of Chicago.*

Background: Diabetic patients have higher rates of arteriovenous fistula (AVF) failures due to inadequate vascular remodeling. Upper arm AVFs, such as brachiocephalic fistula (BCF) have superior outcomes in this population, when compared to lower arm AVFs, for unclear reasons. The aim was to compare indicators of vascular remodeling and endothelial dysfunction in veins of patients with or without diabetes at the time of surgical placement.

Methods: Vein samples were collected from patients at time of BCF creation. Morphometric measurements were obtained using Image J software for percentage of luminal stenosis and intimal to medial area and thickness ratios. Histological analysis, for extent of intimal and medial fibrosis, level of endothelial nitric oxide synthase (eNOS) expression and degree of intimal presence of myofibroblasts, was done by immunohistochemical staining and scored in a semi-quantitative manner. Comparison of diabetics and nondiabetics was done using Wilcoxon rank sum and Fisher's exact tests.

Results: 18 patients were included; 10 were diabetics. There were no differences in age, B/P control, phosphorus levels and days spent on dialysis the year preceding BCF placement. There was a significant difference in the measurement of luminal and medial area between groups, with diabetics having a larger luminal area of average 832001.18 μm^2 (317582.17-3695670.36) $p<0.05$. The maximal intimal to medial thickness ratio was higher in diabetic veins (0.24 versus 0.71, $p<0.05$) along with higher maximal intimal thickness (115.14 versus 312.12 µm, $p<0.05$). Degree of eNOS expression, fibrosis and degree of myofibroblast predominance in the intima of diabetic veins, was not found to be different ($p>0.05$).

Conclusions: There is a significant difference in vascular wall remodeling between diabetic and nondiabetic patients veins at the level of the cephalic vein at the time of BCF placement. The unexpected finding of a larger luminal area in diabetic veins could be a major factor positively affecting BCFs outcomes in a patient population with otherwise impaired remodeling.

Funding: NIDDK Support, Other NIH Support - RO1DK090769

FR-PO984

Significant Heterogeneity Exists in the Conformation of Native Arteriovenous Fistulae Richard W. Corbett, Lorenza Grechy, Paul Elliot Herbert, Jeremy Crane, Francesco Iori, Wladyslaw M. Gedroyc, Colin Caro, Peter E. Vincent, Neill D. Duncan. *Imperial College London, London, United Kingdom.*

Background: Native arteriovenous fistulae (AVF) for haemodialysis are susceptible to non-maturation due to the formation of peri-anastomotic neointimal hyperplasia (NIH). Features of the flow field are believed to be implicated in the formation of this adverse pathology. Computational fluid dynamic simulations of idealised blood flow highlight the

importance of local geometry, in particular curvature of the native artery. Furthermore, CFD and animal models predominantly consider the fistula as a two-dimensional (planar) structure but little is understood about the three-dimensional geometry of native fistulae in patients.

Methods: As part of a continuing ethically approved observational cohort study, 11 patients underwent creation of an end-to-side radio- or brachio-cephalic fistula within our centre following current surgical best practice. Phase-contrast MRI was performed on the day of surgery to obtain AVF luminal geometry along with Doppler ultrasound to measure bulk flow within each vessel. MRI data was segmented and then reconstructed for use in individualised CFD studies and qualitative analysis of local geometry.

Results: A wide variation in arterial geometry and conformation was noted, with the venous anastomosis arising from both the inner and outer curvature of upper arm arteries irrespective of AVF site. There was a significant heterogeneity in the planarity of both the venous limb and the native artery.

Conclusions: AVF are not planar structures but demonstrate significant non-planarity and variations in arterial curvature within current practice. Non-planarity may have significant theoretical benefits promoting favourable flow conditions. The association of patient-specific CFD studies with clinical outcomes from a larger cohort study may help define the correlates of successful AVF surgery and allow the identification of strategies to minimise the unacceptably high rate of non-maturation in native AVF.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO985

Intraoperative Measurement of Vessel Wall Oxygenation During Formation of Native Arteriovenous Fistulae Richard W. Corbett, Neil T. Clancy, Paul Elliot Herbert, Jeremy Crane, Peter E. Vincent, Colin Caro, Neill D. Duncan, Daniel Elson. *Imperial College London, United Kingdom.*

Background: Native arteriovenous fistulae (AVF) for haemodialysis are susceptible to non-maturation due to the formation of peri-anastomotic neointimal hyperplasia (NIH). Animal studies have demonstrated the presence of arterial wall hypoxia immediately following the creation of a fistula and a temporal correlation with cellular proliferation, suggesting a causative role for hypoxia in NIH development. This study was designed to assess the feasibility of intraoperative measurement of vessel wall oxygenation.

Methods: As part of a continuing, ethically approved observational cohort study, 11 patients underwent creation of an end-to-side radio-or brachio-cephalic arteriovenous fistula following current surgical best practice. Optical evaluation was made using a novel multispectral imaging system based around a white light source and a liquid crystal tunable filter attached to a monochrome camera. A correlate of oxy- to deoxy-haemoglobin ratio along with indicators of total haemoglobin and oxygen saturations were measured in the peri-anastomotic area with the vessels flushed and after re-perfusion with blood.

Results: Comparison of the calculated correlate of total haemoglobin across the visible spectrum revealed a divergence in the red end of the spectrum (over 580nm) when the anastomosis was flushed free of blood in comparison to the fully perfused anastomosis, suggesting that the blue-green signal was influenced mostly by the optical properties of the wall. This could allow the creation of patient specific maps of peri-anastomotic wall oxygenation.

Conclusions: Rapid changes in vessel wall oxygenation may occur after creation of AVF due to impaired luminal mass transfer of oxygen resulting from adverse changes in the flow field. Multispectral imaging could offer a non-invasive technique to differentiate between vessel and luminal oxygenation at the time of surgery, in turn allowing the evaluation of the role of hypoxia in the development of NIH and deleterious clinical outcomes.

Funding: Private Foundation Support, Government Support - Non-U.S.

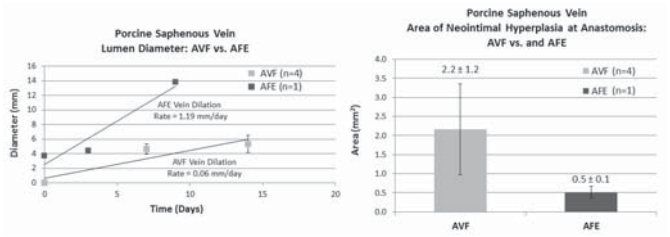
FR-PO986

Improved Vein Maturation in Porcine Model Using Rotary Blood Pump System when Compared to AVF Nicholas Franano,[1] Howard M. Loree,[2] Mark R. Cunningham,[3] Dale M. Groth,[3] Lesley A. Szenay,[3] Barrett S. Hutto,[4] James Lee,[4] Steve P. Woodard,[4] Geoff D. Tansley,[5] Bradley S. Dixon.[6] *[1]Flow Forward Medical, Olathe, KS; [2]Flow Forward Medical, Lowell, MA; [3]Surpass, Osceola, WI; [4]CIRTEC Medical Systems, Los Gatos, CA; [5]School of Engineering, Griffith Univ, Gold Coast, QLD, Australia; [6]Carver College of Medicine, Univ of Iowa, Iowa City, IA.*

Background: The Arteriovenous Fistula Eligibility (AFE) System™ is designed to rapidly dilate peripheral veins prior to AVF surgery by increasing vein wall shear stress (WSS), thereby enhancing AVF eligibility and maturation. Pilot *in vivo* studies compared rates of outflow saphenous vein (SV) dilation and amount of neointimal hyperplasia (NIH) at the venous anastomosis in AFE System and AVF treated pigs.

Methods: The prototype device comprised an extracorporeal centrifugal blood pump, cuffed and heparin-coated inflow/outflow conduits, and a benchtop power unit. It was implanted in a 25 kg pig in a left external jugular vein-to-left SV configuration and the SV was treated for 9 days at a target WSS level of 4 Pa. In two other pigs, bilateral SV-to-femoral artery AVFs were created and allowed to mature for 4 weeks (n = 4 AVFs). Vein dilation and anastomotic NIH area were assessed by angiography and histology, respectively.

Results: Angiography showed a significant increase in the rate of outflow vein dilation with the AFE System (1.19 mm/day) compared to AVF treatment (0.06 mm/day). Histology displayed an important reduction in NIH in veins with the AFE System (0.5±0.1 mm^2) compared to AVF treatment (2.2±1.2 mm^2).



Conclusions: These studies showed 75% less NIH at the venous anastomosis and a 20x greater rate of outflow vein dilation with the AFE System compared to a standard AVF. Use of the AFE System to rapidly dilate veins prior to AVF creation could enable routine use of smaller veins and reduce failure rates.

Funding: Pharmaceutical Company Support - Flow Forward Medical, Inc.

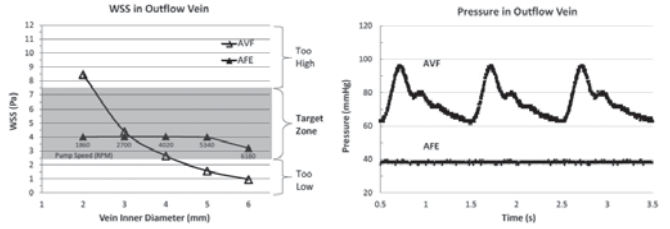
FR-PO987

In Vitro Study of Blood Pump System to Enhance AVF Eligibility and Maturation Howard M. Loree,[1] George Agyapong,[1] Gwendolyn A. Ngai,[1] Geoff D. Tansley,[2] Bradley S. Dixon,[3] Nicholas Franano.[4] *[1]Flow Forward Medical, Lowell, MA; [2]School of Engineering, Griffith Univ, Gold Coast, QLD, Australia; [3]Carver College of Medicine, Univ of Iowa, Iowa City, IA; [4]Flow Forward Medical, Olathe, KS.*

Background: Prior work suggests that outflow vein wall shear stress (WSS) and pulsatility are key factors in AVF failure. The Arteriovenous Fistula Eligibility (AFE) System™ is a small, temporary, wearable rotary blood pump system designed to rapidly dilate peripheral veins prior to AVF creation, enabling AVF placement in patients with small veins, and reducing AVF maturation failure. The AFE System directs non-pulsatile blood flow from the central venous (CV) circulation to a peripheral vein at an adjustable rate. This obviates cyclic vein wall stretching, provides more optimal and consistent WSS levels, and offers better conditions for WSS-mediated remodeling. A mock circulatory loop was used to model hemodynamics after AVF or AFE System placement in the human forearm.

Methods: For the radiocephalic AVF model, a pulsatile VAD and air-charged compliance chamber provided flow to the outflow vein. For the AFE System model, a centrifugal pump provided flow to the outflow vein. An open reservoir represented the CV circulation. Flexible PVC tubing simulated arteries and veins of various diameters, and glycerin blood analog was used. Pressure and flow measurements were made and mean WSS values calculated at various points in the loops.

Results: Outflow vein WSS varied across the range of vein diameters in the AVF but was maintained with the AFE System by adjusting pump speed (**Fig. A**). Outflow vein pressure was pulsatile in the AVF but was nonpulsatile with the AFE System (**Fig. B**).



Conclusions: In contrast to an AVF, the AFE System provides nonpulsatile flow and consistent outflow vein WSS levels, with potential to optimize pre-surgical vein diameter and possibly improve AVF maturation.

Funding: Pharmaceutical Company Support - Flow Forward Medical, Inc.

FR-PO988

Determinants of Intraoperative Blood Flow of Autologous Radiocephalic Arteriovenous Fistula at the Time of Fistula Construction Myung Jin Choi,[1] Jiwon Ryu,[1] Jwa-Kyung Kim,[1] Ja-Ryong Koo,[1] Jung-Woo Noh,[1] Yeo Jin Bang,[2] Miji Jeong.[2] *[1]Dept of Internal Medicine, Collage of Medicine, Hallym Univ & Hallym Kidney Research Inst, Chuncheon, Republic of Korea; [2]Hemodialysis Center, Chuncheon Sacred Heart Hospital, Chuncheon, Republic of Korea.*

Background: The native radiocephalic arteriovenous fistula (RCAVF) is recommended first choice of hemodialysis access, but 20-60% of RCAVF fail to mature adequately to perform dialysis therapy. Established risk factors for access maturation failure include arterial diameter, venous diameter, presence of diabetes, and race. Recently, several studies reported that intraoperative blood flow is associated with early failure of RCAVF. This study was designed to determine the predictor for intraoperative blood flow measurements.

Methods: From March 2006 to November 2013, 103 consecutive patients who performed autologous RCAVF creation were enrolled. Before surgery, all patients underwent arm venography for vascular mapping and ankle-brachial index (ABI) measurement. Intraoperative blood flow was measured within 5 to 10 minutes of completion of the vascular anastomoses.

Results: Of the 103 patients, the mean age was 61.7 ± 12.6 years and 61.2 % of patients were male. Seventy-one (68.9 %) patients had diabetes and 93 (90.3 %) patients

had hypertension. Mean arterial and venous diameter were 2.85 ± 0.78 and 2.92 ± 0.78 mm, respectively. Mean intraoperative flow was 307.9 ± 150.9 ml/min. Intraoperative blood flow showed a significantly negative association with baPWV and a positive association with artery size and vein size in the univariate analysis. In the multivariate analysis, intraoperative blood flow was independently associated with BaPWV (b=0.070, p=0.026) as well as artery size (b=67.614, p <0.001) and venous diameter (b=46.139, p=0.013).

Conclusions: BaPWV is an important determinant of intraoperative blood flow in autologous RCAVF. Considering the importance of early recognition of AVF maturation failure and timely intervention, preoperative screening by means of ABI and vascular mapping may help to detect a high risk patients for early access failure.

FR-PO989

Retrospective Analysis of High Arteriovenous Fistula Flow Effects on Cardiac Biomarkers Emily Lu, Amanda Jo DeMauro, Madeline R. Sterling, Daniel Edmonston, Cathy Jalali, Jeffrey I. Silberzweig. *Dept of Medicine and Div of Nephrology & Hypertension, Weill Cornell Medical College, New York, NY.*

Background: High A-V fistula (AVF) flow can be associated with increased cardiac output and heart failure. Prior work correlated AVF flow with myocardial wall stress and brain natriuretic peptide. We retrospectively analyzed the association between flow and other cardiac biomarkers (left ventricular hypertrophy (LVH), myocardial injury, atrial fibrillation (Afib), sudden cardiac death (SCD)) to assess the risk profile of high-flow AVF.

Methods: Via e-database, we identified 204 patients (pts) with at least one AVF flow ≥1 L/min measured by Transonic. We collected data on age, gender, fistula flow, fistula duration, QTc, LVH by EKG or echocardiogram, Troponin >0.5, Afib and SCD. Associations between flow parameters, fistula duration and cardiac biomarkers were tested by Pearson correlation coefficient (Pearson); Chi-square; Student's T-test; and linear or logistic regression models (confidence interval (CI) 95%).

Results: We had 58% men and 42% women of mean age 68.2 years with mean flow rate 978 mL/min; maximum (max) flow rate 1769 mL/min; mean flow range 1299 mL/min; mean fistula duration 1679 days; mean QTc 476.5 ms in 118 pts; LVH in 35.7% of 126 pts; Troponin >0.5 in 39.1% of 105 pts; Afib in 19.8% of 202 pts; and SCD in 30.5% of 105 deceased pts. Mean flow rate correlated with max flow rate (Pearson 0.60, p<.001) and mean flow range (Pearson 0.76, p<.001). Max flow rate and mean flow range are correlated (Pearson 0.96, p<.001) and modeled (b=0.92, CI: 0.89, 0.94, p<.0001). We found no statistically significant associations between fistula duration and flow parameters; flow parameters and cardiac biomarkers; or fistula duration and cardiac biomarkers.

Conclusions: Although prior studies led to concern that high AVF flow may be associated with cardiac complications, we demonstrated no association with any of our cardiac biomarkers. The flow parameters' degree of correlation and statistical significance show the high quality of our data. Our retrospective, single-institution study suggests that high AVF flow does not raise cardiac risk, but a larger prospective study may be warranted.

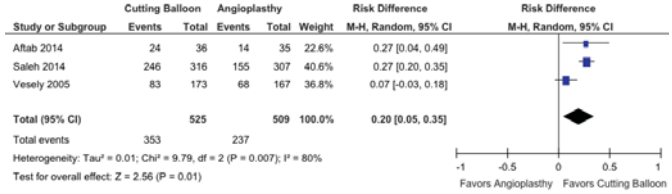
FR-PO990

Comparison of Six Month Patency in Cutting Balloons versus Conventional/ High Pressure Angioplasty for Hemodialysis Vascular Access: A Meta-Analysis of Randomized Controlled Trials Girish N. Nadkarni,[1] Alexandre Benjo,[2] James Jenkins,[2] Neil R. Patel,[1] Ioannis Konstantinidis,[1] Achint Patel,[1] Shiv Kumar Agarwal,[3] Narender Annapureddy,[4] Georges El Hayek,[1] Tyrone Collins,[2] Damodar Kumbala.[2] *[1]Mount Sinai, New York, NY; [2]Oschner Clinic, New Orleans, LA; [3]Univ of Arkansas Medical Sciences, Little Rock, AR; [4]Rush Univ, Chicago, IL.*

Background: Hemodialysis (HD) access failure is a common cause of increased morbidity and healthcare cost in patients with end stage renal disease (ESRD). Six-month restenosis rates with high balloon angioplasty (HPB) remain high. Interventions with cutting balloons (CB) are a potential alternative tool. We aimed to pool existing data from randomized controlled trials (RCT's) to assess difference in 6-month patency between CB and HPB.

Methods: MEDLINE, EMBASE, CENTRAL, Web of Knowledge, and ClinicalTrials were searched through June 2014 for RCTs comparing PTA with HPB and CB. Two authors independently reviewed the studies and extracted the data. RevMan 5.2 was used to estimate risk difference; since there was significant heterogeneity a random-effects model was used.

Results: Of 34 studies, 3 (n=1034) were included. The 6-month patency was higher in CB group as compared to HPB [353/525 (67%) versus 237/509 (46.5%); p<0.01]. There was significantly lower risk of restenosis in patients treated with CB compared to HPB groups (RD 0.20; 95% Confidence Interval: 0.05-0.35; p=0.01) with a number needed to treat of 5 for the CB group.



Conclusions: PTA with CB significantly reduces the incidence of stenosis at 6 months in comparison to the conventional HPB. PTA with CB should be considered routinely as a first-line therapy in ESRD patients with HD vascular access stenosis.

FR-PO991

Measuring Access Blood Flow (Qa) in Arteriovenous Fistulae (AVF): Further Insight on the Relationship Between the Hemoglobin Dilution (HbQa) and the Ultrasound Dilution (UQa) Technique Nicola Tessitore, Valeria Bedogna, Alessia Pendino, Giuseppina Giu Pessolano, Antonio Lupo. *Hemodialysis Borgo Roma, Renal Unit, Verona, Italy.*

Background: Current guidelines recommend routinely screening AVF for stenosis by measuring Qa. The most validated and accepted method is the UQa technique, which requires a dedicated equipment making it expensive and unavailable in all hemodialysis (HD) units. In 2008, Tiranathanagui et al described an alternative methods for measuring Qa based on haemoglobin dilution (HbQa), which has the advantage of being cheaper and accessible to all HD units, and compared well with UQa in a small series of 20 AVF.

Methods: The aim of our pilot study is to assess the interassay variability and diagnostic performance for stenosis of HbQa in 47 HD patients (29 males, age 64±16 y) with an AVF (24 in the lower forearm and 23 more proximally located) by comparison with UQa (Transonic HD03). All patients had Qa measured by both methods in the same HD session and underwent fistulography. In 22 AVFs Qa measurements were repeated 1-4 week later to calculate the interassay coefficient of variation.

Results: Mean [95%CI] Qa values were 962[840-1084] for HbQa and 890[773-1008] ml/min for UQa, with a mean Qa overestimation of 72[21-123] ml/min by HbQa (p=0.007). The two Qa methods were highly correlated (R=0.910,p<0.001) and showed the same interassay coefficient of variation (15±10 for HbQa and 13±7 % for UQa, p=ns). Fistulography identified a significant (>50%) stenosis in 22 AVF. The area under curve for stenosis was 0.929[0.861-0.997] for HbQa and 0.887[0.792-0.982] for UQa (p=ns). A Qa<630 ml/min with HbQa and a Qa<560 ml/min with UQa ensured a similar high positive predictive value (PPV 100%; sensitivity 54%[32-75]). A Qa<1000 with HbQa and a Qa<1150 ml/min with UQa ensured a similar high sensitivity (SE 95%[77-100]; PPV 78%[58-91] and 62%[43-78], respectively).

Conclusions: Our study shows that in AVF HbQa and UQa have similar interassay variability and diagnostic performance for stenosis, suggesting that HbQa is a valid alternative to UQa, bearing in mind that higher Qa thresholds (of approximately 70 ml/min) should be used if one favours PPV and lower Qa thresholds (of approximately 150 ml/min) if one favours SE.

Funding: Government Support - Non-U.S.

FR-PO992

Sources of Variation in Dynamic Dialysis Venous Pressure (DVP) Measurements: Limitations and Opportunity John Jason White,[1] Anatole Besarab,[2] William D. Paulson.[1] *[1]Medicine, Georgia Regents Univ, Augusta, GA; [2]Medicine, Stanford Univ School of Med, Palo Alto, CA.*

Background: DVP is measured with the blood pump set at prescribed speed Qb, and is widely used by clinicians during rounds as a crude indicator of whether access stenosis should be suspected. However, the relative influence of various factors that determine DVP is unknown. This study determined this influence, and how these factors can be used to predict static VP (SVP, Qb = 0), which is suitable for surveillance.

Methods: We studied 55 patients with native fistulas (n = 43) or grafts (n = 12). DVP and SVP were measured in the first 1/3 of a session. We used multiple regression analysis to determine relations among these variables: DVP (corrected and uncorrected for drip chamber height), SVP (height corrected), Qb, needle gauge, hemoglobin level (Hgb), drip chamber height, mean arterial pressure (MAP).

Results: The multiple correlation coefficient for the relation of DVP (height uncorrected) with 6 independent variables (SVP [height corrected], Qb, needle gauge, Hgb, drip chamber height, MAP) was 0.848 (P<0.001). Thus, 72% of DVP variation was due to its relation with the 6 variables ($0.848^2 = 0.72$). We computed the correlation of DVP with each variable after controlling for the effects of the other 5 variables (R^2 values shown):

Dependent variable	Qb	SVP (height corrected)	Needle gauge	Hemoglobin level	Height	MAP
DVP (height uncorrected)	0.573	0.438	0.344	0.179	0.153	0.058
P value	<0.001	<0.001	<0.001	0.002	<0.001	0.09

Thus, Qb had the largest effect on DVP whereas MAP was not statistically significant. Finally, SVP correlated with DVP (both height corrected), Qb, needle gauge, and Hgb (multiple $R^2 = 0.485$, P<0.001).

Conclusions: This study shows that DVP uncorrected for height (i.e. rounding VP) is dominated by blood pump speed and other factors that are unrelated to intra-access pressure, indicating that DVP provides little access information. However, regression analysis shows that DVP can be used to estimate SVP when additional factors are taken into account. This study supports the concept that changes in estimated SVP can be used in surveillance to determine whether access stenosis should be suspected.

Funding: Clinical Revenue Support

FR-PO993

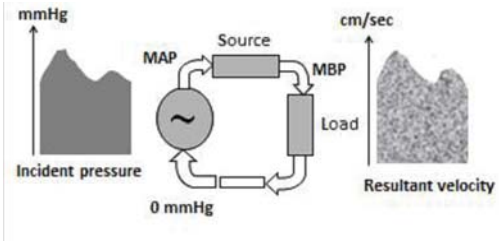
Non-Invasive Arteriovenous Fistula Pressure Mapping Using Pressure from Flow Theory David H. King,[1] William D. Paulson,[2] Mo Al-Qaisi,[1] Abdelgalil Abdelrahman Ali.[1] *[1]Renal Unit, Broomfield Hospital, Chelmsford, Essex, United Kingdom; [2]Dept of Medicine, Regents Univ, Augusta, GA; [3]Cardiovascular Sciences, St. George's Hospital, Tooting, London, United Kingdom.*

Background: Pressure from Flow theory[1] is an unconventional approach pressure estimation independent of anatomical variation and suitable for AVF surveillance.

Methods: Figure 1 is a vascular model. Source ***S*** and Load ***L*** represent the impedance of transit artery and recipient organ, ***Vff*** = pulsatile flow velocity/mean velocity, ***Pff*** = ***(Systolic – Diastolic pressure) /MAP*** measured centrally. ***MBP*** = organ perfusion pressure. The matched state ***S/L=1*** is a unique condition where no reflected waves exist so incident pressure and resultant flow waveforms are identical and ***Pff/Vff =1***. It can also be shown that ***Pff/Vff = S/L*** when ***L*** is infinite or zero. $MBP_{direct} = MAP/(1+S/L)$, therefore $MBP_{estimated} = MAP/(1+Pff/Vff)$.

Results: Femoral artery pressure and blood velocity waveforms were sampled in an anaethetised dog. ***S*** and ***L*** were altered following peripheral vasodilation then iliac artery constriction, resulting in a series of direct (79 to 41mmHg) and estimated mean pressure readings. AVF data was obtained from 14 human volunteer dialysis patients by sampling static needle pressure and Doppler velocity waveforms at the same location. The AVF pressure data (55 to 19mmHg) lies on the same line of identity as the dog data showing the algorithm can be applied anywhere in a vasculature, even with the vascular bed bypassed. All data combined resulted in a best fit straight line with a slope close to 1 and intercept close to the origin, $MBP_{estimated} = 0.907*MBP_{direct} + 0.9368$, $r^2 = 0.920$

Conclusions: This study shows that the 'Pressure from Flow' algorithm may be sufficiently robust to use in studies on fistulae as well as normal vasculature *1.International Patent/ Application No. PCT/GB2010/000436 King and Al-Qaisi.*



FR-PO994

Regular Ultrasound Surveillance of Arteriovenous Fistula with One-Day Management System: A 6-Year Experience Ji In Park, Nara Shin, Eunjin Bae, Sunhwa Lee, Hajeong Lee, Dong Ki Kim, Yon Su Kim, Kwon Wook Joo. *Internal Medicine, Seoul National Univ Hospital, Seoul, Republic of Korea.*

Background: Recent studies revealed elective intervention of subclinical stenosis in arteriovenous fistulas (AVFs) reduces the risk of access loss. Regular surveillance of AVFs with ultrasound seems to be an emerging strategy. Here, we report our 6 year-experience of surveillance of AVFs with ultrasound.

Methods: We set up the AVF surveillance program as an outpatient clinic in Seoul National University Hospital. Patients with AVFs or arteriovenous grafts (AVGs) were recommended to examine the patency with ultrasound every 3 months and whenever they have emergent vascular problem. If significant stnosis or thrombosis were detected, they underwent percutaneous trasluminal angioplasty (PTA) or surgery at the day. We retrospectively reviewed data between 2008 and 2013.

Results: Total of 1752 patients visited the program, of whom 84.2% patients had have the AVF or AVG operation at Seoul National University Hospital. Men were 60% and mean age was 62.2 years. Prevalence of hypertension and diabetes mellitus were 72.4% and 47.4%, respectively. AVFs were dominant (81.4%) compared to AVGs (18.6%). Each patient visited the surveillance clinic for average 5.7 times. Among total of 9972 vitis, 827 (5.3%) cases underwent PTA and 180 (2.1%) did surgery. When we compared AVFs to AVGs, patients with AVFs were significantly younger (61.5 versus 66.1 years), visited less (5.5 versus 6.9 times), underwent intervention less (0.4 versus 1.4 times), and took longer time to first use (88.4 versus 54.5 days) ($P < 0.001$).

Conclusions: The AVF surveillance program found considerable cases who needed PTA or surgery. Especially patients with AVGs visited and underwent interventions more frequently.

FR-PO995

Physician Visit Frequency and Vascular Access Interventions in Hemodialysis Kevin F. Erickson,[1] Matthew Mell,[2] Wolfgang C. Winkelmayer,[1] Glenn M. Chertow,[1] Jay Bhattacharya.[1] *[1]Medicine, Stanford Univ, Palo Alto, CA; [2]Surgery, Stanford Univ, Palo Alto, CA.*

Background: Medicare reimbursement policy encourages frequent provider visits to patients with end-stage renal disease (ESRD) undergoing hemodialysis. Patients survive longer when receiving dialysis through an arteriovenous (AV) fistula or graft compared to a central venous catheter. We hypothesized that more frequent face-to-face provider (physician and advanced practitioner) visits leads to more procedures and therapeutic interventions aimed at preserving AV fistulas and grafts, improved vascular access outcomes, and fewer hospitalizations.

Methods: We used multivariable regression to evaluate the association between provider (physician and advanced practitioner) visit frequency and interventions aimed at preserving vascular access, vascular access survival, hospitalization for vascular access infection, and outpatient antibiotic use in a cohort of 63,082 Medicare beneficiaries receiving hemodialysis in the United States.

Results: One additional provider (physician and advanced practitioner) visit per month was associated with a 13% increase in the odds of receiving an intervention to preserve vascular access (95% CI 11% to 14%), but was not associated with vascular access survival (HR 1.01, 95% CI 0.99 to 1.03). One additional provider visit was associated with decreased hospitalization for vascular access infection (OR 0.90, 95% CI 0.86 to 0.96) and an increase in outpatient intravenous antibiotic administration (OR 1.09, 95% CI 1.05 to 1.13), although the associated changes in absolute probabilities of hospitalization and antibiotic administration were small.

Conclusions: More frequent face-to-face provider (physician and advanced practitioner) visits are associated with more procedures and therapeutic interventions aimed at preserving vascular accesses, but not with prolonged vascular access survival and only a small decrease in hospitalization for vascular access.

Funding: NIDDK Support, Other NIH Support - Agency for Healthcare Research and Quality

FR-PO996

Characteristics of Dialysis Venous Pressure (VP): Criteria That Assist in Deciding Whether to Refer for Evaluation of Stenosis Usman Afzal,[1] John Jason White,[1] Eduard R. Fatakhov,[1] Anatole Besarab,[2] William D. Paulson.[1] *[1]Medicine, Georgia Regents Univ, Augusta, GA; [2]Medicine, Stanford Univ School of Med, Palo Alto, CA.*

Background: Dialysis VP measurements are an important method for determining whether an access should be referred for correction of stenosis. This study determined variation in VP from session to session and applied these results to a practical approach for referral.

Methods: Fifty-five patients with arteriovenous fistulas (n = 43) or synthetic grafts (n = 12) underwent VP measurements in 3 sessions within 2 wks. Sessions were divided into equal thirds, and VP was measured with blood pump speed (Qb) = 0 (static VP: SVP) and at prescribed Qb (dynamic VP: DVP). VP was adjusted for mean arterial pressure (VP/MAP). We determined whether timing of measurements in a session affects VP, and used the student t distribution to determine thresholds for a significant change in VP; all tests were 2-tailed. The SD used in the t distribution was for the difference between 2 VP/MAP values from different sessions.

Results: The median Qb = 450 ml/min; range from 260 to 600 ml/min. The pooled within-patient SD for differences between 2 VP/MAP's from the same third of different sessions are shown below:

Pooled within-patient SD for difference between 2 measurements	First third	Middle third	Last third
SVP/MAP (Qb = 0)	0.156	0.178	0.181
DVP/MAP (Qb prescribed)	0.610	0.611	0.547

Results for grafts and fistulas were not significantly different and are combined in the table. We found that the change in SVP/MAP in in the first third of a session must be >0.313 to be significant at $P<0.05$; the change in DVP/MAP must be >1.222. The values of DVP are higher than SVP, which thereby yields a higher SD. The change must be somewhat larger for SVP/MAP in the middle and last thirds of a session. If measurements are taken any time in a session, then the change in SVP/MAP must be >0.340.

Conclusions: This study defines the variability of VP and provides criteria for determining whether VP has increased or decreased for both static and dynamic VP. These criteria can be a useful aid in assessing whether to refer an access for possible correction of stenosis.

Funding: Clinical Revenue Support

FR-PO997

Arteriovenous Fistula Physical Exam: Can It Be Taught? Alexander S. Yevzlin,[1] Amanda M. Valliant.[2] *[1]Nephrolgoy, Univ of Wisconsin, Madison, WI; [2]Nephrolgoy, Univ of Wisconsin, Madison, WI; [3]Nephrolgoy, Univ of Wisconsin, Madison, WI.*

Background: Physical examination (PE) has been shown to be an excellent means of prediction of arteriovenous fistula (AVF) dysfunction. Although quick and inexpensive, PE is seldom used by dialysis staff. We hypothesized that the physical exam can be taught to a non-medical professional, and that, with time, it would be comparable to the physical exam performed by an expert.

Methods: A pre-medical student and an interventional specialist (MD) examined AVF for dysfunction in a tertiary care hospital over a six month period on patients who were referred for due to access dysfunction. Physical exam findings were categorized as inflow, outflow, both or neither. Data was blindly recorded and compared to the gold standard of angiography. Potential confounding variables, including age, gender, diabetic status, and location of AVF were collected. Outcomes were compared using ROC curves. Logistic regression was used to assess the impact of confounding variables on the accuracy of the student's physical exam.

Results: Forty-nine subjects were evaluated with an average age of 58.78. The majority of the subjects were male (61.2%), diabetic (53.1%), and had an upper arm AVF (75.5%). In the first three months of the study the student performed the AVF exam with an AUC of 0.700, a sensitivity of 66.67% and a specificity of 73.33%, compared with the specialist (AUC 0.911, a sensitivity of 88.89% and a specificity of 93.33%), P = 0.0869. In the next

and final 3 months of the study, the student performed the AVF exam with an AUC of 0.883, a sensitivity of 90.00% and a specificity of 86.67%, compared with the specialist (AUC 0.967, a sensitivity of 100.00% and a specificity of 93.33%), P = 0.2817. Among the potential confounding variables of age, gender, diabetes, and AVF location, only wrist AVF were associated with a decrease in PE accuracy (odds ratio 0.1680, P = 0.0233).

Conclusions: AVF exam can be taught to a non-medical professional in a short duration of time and the predictive value of the exam can be similar to that of an interventional specialist.

FR-PO998

Post-Operative Hand Grip Exercise for Arteriovenous Fistula Maturation Danielle L. Kirkman,[1,2,3] Rebecca-Jane Law,[2] Mick John Kumwenda,[3] Mahdi M. Jibani,[3] Jamie Hugo Macdonald.[2] *[1]Univ of Delaware, Newark, DE; [2]Bangor Univ, Bangor, United Kingdom; [3]Betsi Cadwaladr Univ Health Board, United Kingdom.*

Background: More than half of arteriovenous fistulae fail to mature for hemodialysis use, accentuating the need for interventions to enhance fistula maturation. This pilot study aimed to investigate the effect of post operative forearm exercise on fistula vessel diameters, early dialysis suitability failure and maturation time.

Methods: In this controlled trial, 31 participants were randomly allocated to either an EXERCISE (Mean (SD): 52 (14) years) or a CONTROL (60 (11) years) group following fistula surgery. EXERCISE received a home based progressive resistance hand grip exercise program over 8 weeks. Exercise was prescribed four days per week for 30 minutes at an intensity perceived as 'somewhat hard' to 'hard'. CONTROL received routine care. Primary outcomes included fistula feeding artery and draining vein diameters by ultrasound, fistula failure and maturation time. Secondary outcomes were maximal isometric handgrip strength and forearm muscle circumference. Outcomes were measured one day, four and eight weeks post surgery.

Results: Compared to CONTROL, EXERCISE had no effect on arterial diameter (mean change [95% CI], -0.24 [-1.12; 0.51] mm) or venous diameter (0.16 [-1.84; 1.24] mm) with small to trivial effect sizes reported. Nineteen percent and 33% of fistulae failed in EXERCISE and CONTROL, respectively. EXERCISE did not affect maturation time (1 [-16; 28] days). EXERCISE increased handgrip strength (4.5 [0.5; 8.0] kg) but not forearm muscle circumference (0.8 [-1.9; 3.4] cm).

Conclusions: This study infers post operative forearm exercise to have no effect on fistulae vasculature diameters, maturation time or failure rate. A similar protocol implemented *before* arteriovenous fistula creation, however, is hypothesised to reduce immediate vascular access failure and enhance maturation for successful hemodialysis use. Registered clinical trial *NCT01061008.*

Funding: Government Support - Non-U.S.

FR-PO999

Associations between Preoperative Arterial Function Tests (AFTs) and Postoperative Arteriovenous Fistula Flow in the Hemodialysis Fistula Maturation (HFM) Study Michael Allon, Tom Greene, The HFM Study Group. *NIDDK, NIH.*

Background: Arteriovenous fistula (AVF) maturation requires vascular dilation to permit increases in AVF blood flow. Preoperative functional properties of the vasculature may be determinants and predictors of postoperative vascular remodeling.

Methods: Patients in HFM underwent 4 preop AFTs: (1) Brachial artery flow-mediated dilation (FMD); (2) Brachial artery nitroglycerin-mediated dilation (NMD); (3) Carotid-femoral pulse wave velocity (CF-PWV); and (4) Carotid-radial (CR-) PWV. They also underwent preop ultrasound (US), and US of the AVF at ~1 day, and 2 and 6 weeks postop. We fit regression models of postop AVF flows examining individual AFTs as predictors.

Results: After adjustment for AVF location and preop US variables, there were significant associations between 6-week postop AVF flow and each preop AFT (Table 1). Age, sex, race, diabetes, CAD, PAD, and preop arterial calcification were also individually predictive of postop AVF flow ($p<0.01$). After adjustment for these baseline features, associations of 6-week AVF flow with FMD%, NMD%, and CF-PWV were attenuated and no longer statistically significant.

Table 1: Regressions of 6-week AVF flows on individual AFT predictors

	Controlling for AVF location + preop US variables (art diameter, vein diameter, and upper arm flow)		Controlling for AVF location + preop US variables + baseline patient characteristics *	
Predictor AFTs	Diff in AVF flow (95% CI)	p-value	Diff in AVF flow (95% CI)	p-value
Brachial NMD% (n=361)	17.91 (7.41, 29.45)	0.001	7.28 (-2.23, 17.72)	0.14
Brachial FMD% (n=428)	14.12 (3.10, 26.32)	0.01	4.33 (-5.41, 15.07)	0.40
CF-PWV (n=349)	-8.05 (-14.27, -1.38)	0.02	0.90 (-6.36, 8.71)	0.81
CR PWV (n=350)	17.19 (2.79, 33.60)	0.02	15.49 (1.94, 30.84)	0.02

* Pt age, sex, race, diabetes, CAD, PAD, and arterial calcification

Conclusions: Preop AFTs were associated with 6-week postop AVF flow when adjusted for preop US variables, but only a somewhat paradoxical association with carotid-radial PWV remained statistically significant after adjustment for baseline features. Baseline demographics, vascular US measures, and other clinical factors may confound predictive relationships of preop AFTs to postop AVF flow.

Funding: NIDDK Support

FR-PO1000

Arteriovenous Graft Outcomes Are Not Affected By Failure to Mature Scoring Risk Group Egbert C. Liquete, Rebecca Bajoka, Ruchira Sengupta, Sagger Mawri, Jerry Yee, Lenar T. Yessayan, Lalathaksha Murthy Kumbar. *Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Background: The failure to mature (FTM) was developed to identify patients with high risk for arteriovenous fistula (AVF) maturation failure. Patients at very high risk for AVF failure by FTM scoring are often recommended to alternatively undergo arteriovenous (AVG) constructions. Because the literature on AVG outcomes in these patients is limited, we determined whether the preoperative FTM score risk profile predicts AVG outcomes.

Methods: Retrospective analysis of earliest documented, upper extremity AVG constructions from January 2009 to November 2013. Demographics and data on comorbidities, prior access, subsequent access interventions, and medications were collected. AVGs were classified into 4 groups based on FTM score: Low (<2), Medium (2-3), High (3.1-7.9), and Very High (>8). Primary (abandonment), secondary (thrombosis, angioplasty) outcomes, primary patency, assisted primary patency, and survival probability were collected at 6 and 12 months. Variables of interest were compared among risk groups by Fisher Exact testing; survival by the lifetest method; and times-to-event compared by log-rank tests (SAS 9.4, SAS Inc, Cary, NC, U.S.A.).

Results: Analysis of 169 AVGs was conducted. 85% of patients were classified as medium and high FTM risk (41% versus 45%). The most common procedures among all AVGs were angioplasty (46.8%), thrombectomy (24.9%) and tunneled, catheter placements for any reason (24%). There were no significant differences in primary, assisted primary patency or survivability at 6 and 12 months among various risk groups. However, between the very high risk compared to the low risk group, a trend toward higher cumulative survival at 12 months (91% versus75%) and longer median time to first angioplasty (6.8 versus5.3 months) and thrombectomy (6.4 versus 5.7 months) was noted.

Conclusions: AVG outcomes are not affected by the preoperative FTM score risk profile. AVGs should be the access of choice in patients at very high risk for AVF maturation failure by FTM scoring, in order to minimize hemodialysis catheter prevalence and probable, futile AVF maturation attempts.

FR-PO1001

Can Augmentation Procedures Promote AVF Use without Compromising Access Long Term Patency? Hoon Suk Park, Kyungyoon Chang, Bum Soon Choi, Cheol Whee Park, Chul Woo Yang. *Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

Background: Native arteriovenous fistula (AVF) is superior to arteriovenous graft and central venous catheter due to its favorable long term patency and minimal complication rates associated with its use. To increase the rate of AVF use, salvage therapies for augmentingng immature fistulae are strenuously performed these days. But, there are some controversies that AVF matured by these salvage therapies requires frequent intervention for maintaining its patency and may have resultant decreased long term patency. Therefore, we investigated the effects of salvage therapies on patencies after starting using fistulae.

Methods: From total 361 patients with native fistulae, 266 with AVF matured by salvage therapies and 95 with AVF matured spontaneously without intervention were compared and we investigated the factors that may influence on patencies of AVF.

Results: The group with salvage therapies were older (years, 65 versus 59; $p = 0.005$) and decreased preoperative venous diameter (mm, 2.74 versus 3.18; $p < 0.001$) compared with the other group without it while the other parameters including preoperative arterial diameters, sex, diabetes, presence of coronary artery disease and left ventricular ejection fraction were comparable between two groups. Primary patency was decreased in the group with salvage therapies compared with the other group without it ($p = 0.021$). However, primary assisted patency and secondary patency were comparable between two groups ($p = 0.517$ and $p = 0.127$). In multivariate Cox regression analysis, the presence of salvage therapies before use was not significantly associated with access survival (odds ratio [OR] 0.548, 95% confidence interval [CI]: 0.249–1.205; $p = 0.135$) whereas spontaneous maturation (OR 0.326, 95% CI: 0.159–0.667; $p = 0.0026$), history of thrombosis during use (OR 25.051, 95% CI: 11.015–56.970; $p < 0.001$) and preemptive intervention (OR 0.313, 95% CI: 0.108–0.910; $p = 0.033$) were significantly associated with it.

Conclusions: Augmentation procedures can salvage immature fistulae without compromising access long term survival.

FR-PO1002

Exercise Training in Haemodialysis and Renal Transplant Patients Laura Mason,[1] Michael James Lewis,[1] Ashraf I. Mikhail.[2] *[1]A-STEM Research Centre, Swansea Univ; [2]Morriston Hospital, Swansea, United Kingdom.*

Background: Poor physical functioning is associated with morbity, mortality and poor QOL in haemodialysis (HD) patients. Renal transplant recipients (RTR) with reduced physical function are also more likely to suffer allograft failure and cardiovascular events. Exercise training may help to ameliorate many of the factors which lead to poor physical functioning in these groups. The aim of this study was to compare the effects of a 24-week exercise training program on physical functioning and body composition in HD and RTR patients.

Methods: Thirteen mobile HD patients (F=4,M=9, age:49±18yrs, mass:76±14kg, height:1.70±0.10m) and 12 RTR patients (F=8,M=4, age:45±15yrs, mass:92±28kg, height:1.64±0.08m) volunteered for the study. All participants completed 24 weeks of individualised exercise training on 3 days per week. Physical functioning was assessed during an incremental exercise test and body composition was measured using air displacement plethysmography. Measurements were taken at 0, 12 and 24 wks.

Results: 5 HD and 7 RTR completed 24 wks of training. Peak work rate achieved on the incremental cycle was significantly improved following the training period (HD: 0wks=89±11, 24wks=121±15 W, RTR: 0wks=72±18, 24wks=97±17 W, P≤0.001) as was peak aerobic capacity (HD: 0wks=18.4±3.8, 24wks=21.8±4.2ml·kg^{-1}·min^{-1}, RTR: 0wks=14.5±4.1, 24wks=18.3±5.1ml·kg^{-1}·min^{-1}, P=0.015) with no differences between HD and RTR for either variable (P=0.294, P=0.136 respectively). No significant differences were found in body mass in either group (P=0.137) however percentage body fat significantly decreased in both groups (HD: 0=30.0±15.7, 24=26.5±15.0%, RTR: 0=44.4±11.3, 24=40.0±9.9%, P=0.022). Significant negative correlations were also found at each time point between percentage body fat and peak aerobic capacity (R≥0.689, P≤0.019).

Conclusions: These results show that 24 wks of exercise training can significantly improve physical functioning in both HD and RTR patients, and can reduce percentage body fat in the absence of dietary intervention. This study provides further evidence that regular exercise training should be encouraged both prior to and following kidney transplantation.

FR-PO1003

Effects of a 6 Months Physical Activity during Hemodialysis on Cardiovascular Parameters: Anemia Control, HDL-C, HTA Control...

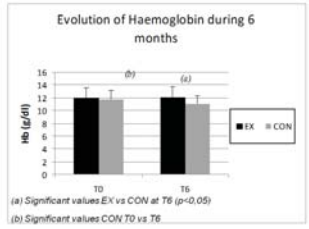
Myriam Rouchon Isnard, Céline Coutard, Marie-Hélène Mosnier, Nathalie Ramade, François Jamy. *Aura Auvergne, Chamalieres, France.*

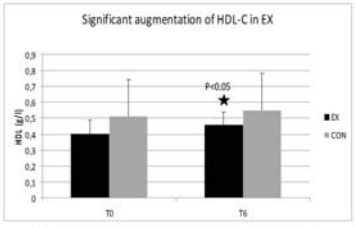
Background: Cardiovascular diseases are the main cause of morbidity and mortality in hemodialysis patients. Chronic Kidney Disease is a Cardiovascular Risk Factor (CVRF) associated with Hypertension (HTA), Dyslipidemia (low HDL), Haemoglobin (Hb) variation,etc. Physical Activity (PA) is well known in cardiology to allow a control of the CVRF and a better survival. The impact of PA on blood pressure levels, dyslipidemia and Hb variation has been investigated with intervention studies of per dialysis exercise programs. The aim of our study was to determine if PA per dialysis can decrease CVRF and cardiovascular hospitalizations.

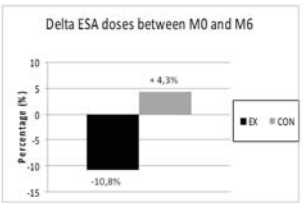
Methods: We studied 84 haemodialysis patients in AURA Auvergne hemodialysis centers. They were voluntarily assigned to either intradialytic exercise training (cycling) group (EX; n=42) or a control group (CON; n=42). Baseline characteristics of the patients:

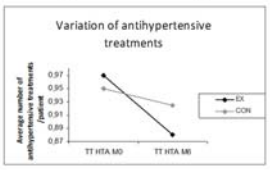
GROUPS	Number	%Male	Age	Time in Dialysis (year)	Diabetes %	Cardiovascular Risk Factors %
EX	42	71.42	65,11 ± 15,76	4.47 ± 3.94	28.5	95
CON	42	78.5	63,6 ± 16,05	4.732 ± 24.56	28.5	92.6

Results: Statistics comparisons: ANOVA 2 and post-hoc with Newman Keuls.









	Cardiovascular Hospitalizations	Total Hospitalizations
EX	2	19
CON	3	8

In EX Hb remains stable D=0.11g/dl, with lower ESA dose: -10.8%; on the contrary, Hb in CON do not remain stable D=-0.76g/dl (p<0.05). HDL-C rise up significantly in EX: 13.13% (p<0.05) and is stable in CON. The average of antihypertensive treatment is lower in EX after 6 months exercise period. Despite a higher number of total hospitalizations in EX, there is less cardiac causes compared to CON.

Conclusions: Our results show that per dialysis cycling improves HTA and dyslipidemia control. Hb is more stable. So we can conclude that PA lowers CVRF: with a longer follow-up, the cardiovascular events may be reduced. But certainly randomised studies are needed on the subject.

FR-PO1004

Arterial and Cardiac Alterations and Their Relationships to Exercise Intolerance in Maintenance Hemodialysis Patients

Jin Hee Jeong,[1] Pei-Tzu Wu,[2] Peter J. Fitschen,[1] Brandon Kistler,[1] Annabel Biruete,[1] Hyunwoo Hank Park,[1] Mohamed Ali,[3] Bo Fernhall,[3] Ken Wilund.[1] *[1]Kinesiology, Univ of Illinois, Urbana, IL; [2]Translational Science Section, Univ of California at Los Angeles, Los Angeles, CA; [3]Kinesiology, Univ of Chicago, Chicago, IL.*

Background: Maintenance hemodialysis (MHD) patients experience impaired physical function which significantly reduces their quality of life. Cardiovascular (CV) structural and functional alterations may contribute to the limited functional capacity observed in this population. However, the relationship between arterial and cardiac impairments and exercise intolerance in MHD patients is not fully understood. Identification of the most relevant CV risk factors for impaired exercise capacity could help to direct future research efforts to improve exercise capacity and, ultimately, quality of life in MHD patients.

Methods: 112 MHD patients (age =55.5± 11.4y) were tested for arterial wave reflection (augmentation index (Aix75)), arterial stiffness (carotid-femoralpulse wave velocity (PWV) and b-stiffness)), carotid artery lumen diameters (D), carotid intima-media thickness (cIMT), cardiac systolic function (ejection fraction (EF)), and left ventricular mass index (LVMI) on a non-dialysis day. An incremental shuttle walk test was conducted to estimate aerobic capacity.

Results: cIMT, D, Aix75, PWV, andb-stiffness were positively associated with age (p<0.01 for all). D was positively correlated with PWV and b-stiffness and negatively correlated with EF (p<0.05 for all). By univariate analysis, Aix75, PWV and b-stiffness were all inversely associated with shuttle walk time (p< 0.05 for all). In contrast, there were no significant correlations between shuttle walk time and cIMT, D, EF and LVMI.

Conclusions: Arterial function measures were more closely associated with walking performance than with cardiac structure or function in patients with MHD. These data suggest that peripheral rather than central limitations may be important determinants of exercise intolerance and therefore an important therapeutic target in MHD patients.

Funding: NIDDK Support

FR-PO1005

Exercise Training During Hemodialysis Is Not Associated with Intradialytic Hypotension

Kelvin Leung,[1] Pietro Ravani,[1,2] Kristen Parker,[3] Nathalie Tang,[3] Stefan Mustata,[1] Tanvir Chowdhury Turin,[2] Zhihai Ma,[2] Jennifer M. MacRae.[1] *[1]Medicine, Univ of Calgary, Calgary, AB, Canada; [2]Community Health Sciences, Univ of Calgary, Calgary, AB, Canada; [3]Southern Alberta Renal Program, Alberta Health Services, Calgary, AB, Canada.*

Background: Intradialytic exercise is associated with improved hemodialysis (HD) efficacy, physical function, and quality of life. However, concerns regarding exercise induced intradialytic hypotension (IDH) have limited its widespread use.

Methods: We performed a 12-week observational study in 48 maintenance HD patients. Patients performed intradialytic exercise (cycle ergometer) up to 3-times per week at moderate intensity (Borg scale 5), with duration at the patient's discretion. Hemodynamic parameters, fluid removal, exercise duration, single-pool urea kinetics (spKt/V), a numerical count of symptomatic (chest pain, shortness of breath, nausea/vomiting, dizziness, fatigue, cramps or anxiety) and asymptomatic IDH episodes were recorded. IDH as defined by an abrupt drop in the systolic blood pressure of ≥20mm Hg. The duration of exercise (minutes exerted and in stratums [presence or absence of exercise; no exercise, <30 minutes, ≥30 minutes]) and IDH were analyzed using a random-effects model.

Results: A total of 1727 HD sessions were recorded with 827 involving exercise. Symptomatic and asymptomatic IDH occurred in 8.3% and 33.5% of all HD sessions, respectively. The rates of symptomatic and asymptomatic IDH were similar with intradialytic exercise (Table 1). Symptomatic IDH and asymptomatic IDH were not affected by intradialytic exercise after adjustments for demographics, comorbidities, and intradialytic fluid removal (<0.01 symptomatic IDH episode per 10 minute exercise, P>0.10). The results were consistent when analyzed across the stratums. The dialysis efficacy was not affected by the presence or duration of intradialytic exercise.

Conclusions: Intradialytic exercise is not associated with hemodynamic instability or IDH.

Table 1: Rates of Symptomatic and Asymptomatic IDH

	Symptomatic IDH	Asymptomatic IDH
No Exercise	13.2 per 100 HD sessions	98.9 per 100 HD sessions
Intradialytic Exercise	9.07 per 100 HD sessions	97.6 per 100 HD sessions

Funding: Government Support - Non-U.S.

FR-PO1006

Achievement of Prescribed Ultrafiltration and Clearance Goals and Incidence of Intradialytic Hypotension among Hospitalized Patients

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Background: Intradialytic hypotension (IDH) is a common complication in outpatient hemodialysis (HD), but its incidence is not well studied in the acute setting. We aim to characterize the frequency of IDH among hospitalized ESRD and AKI patients, as well as ascertain the rate of achievement of prescribed ultrafiltration (UF) goals.

Methods: We conducted a retrospective review of all intermittent HD treatments performed on inpatients in an academic center from December 2013-March 2014. IDH was

defined as the systolic or diastolic blood pressure threshold prespecified by the nephrologist at which a supportive intervention (e.g. saline, decreased UF) should occur. Characteristics of sessions with and without IDH were compared.

Results: A total of 755 HD sessions (ESRD 62%, AKI 38%) were performed in 173 inpatients, with a median of 3 (IQR 1, 5) sessions/patient, and a median of 3h/session. Prescribed UF per session was 3 (IQR 2, 3) L or 9.6 (IQR 4.2, 10.7) ml/kg/hr. Achieved UF per session was 2 (IQR 1, 3) L or 7.3 (IQR 4.2, 10.7) ml/kg/hr. Prescribed UF goals were achieved in 39% of sessions; the median achieved/prescribed UF (APUF) ratio was 0.86 (IQR 0.67, 1.00). IDH complicated 251 (33.2%) sessions; IDH occurred more commonly in sessions in the ICU, and in AKI patients.

	IDH n=251	No IDH n=504
ICU, n (%) **	78 (31)	79 (16)
AKI patients, n (%) *	115 (46)	174 (34)
Actual HD duration (min)	210[185,210]	210[210,210]
Prescribed UF, ml/kg/h	8.9[5.7,12.6]	9.7[6.2, 13.3]
Achieved UF, ml/kg/h**	6.0[3.5,8.3]	8.2[5.2,11.5]
Achieved/prescribed UF ratio**	0.7[0.48, 0.90]	0.95[0.75,1.0]
Kt/V	1.2[0.9, 1.6]	1.3[1.0, 1.5]

* p<0.05; **p<0.001. Data presented as medians and IQ range

The APUF ratio was significantly lower in sessions with IDH. There was no difference in prescribed UF, blood flow, dialysate flow, Kt/V, prescribed or actual duration of HD between the two groups.

Conclusions: Intradialytic hypotension is common among hospitalized patients, and more commonly encountered in those with AKI. This adversely affects the ability to achieve prescribed UF goals, but does not appear to reduce Kt/V.

FR-PO1007

Cardiomegaly Modifies the Effect of Ultrafiltration Rate on Mortality in Incident Hemodialysis Patients

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Background: Higher ultrafiltration rate (UFR) is associated with mortality in hemodialysis patients. The rapid fluid removal could result in intradialytic hypotension, cardiac injury and sympathetic overactivity. These detrimental effects are theoretically more prominent in heart failure. We hypothesized that cardiomegaly, measured by routine chest radiograph, could modify the association between ultrafiltration rate and mortality in hemodialysis patients.

Methods: We studied 2615 incident hemodialysis patients in southern Taiwan from 1997 to 2009. After dialysis for 3 months, the baseline data were collected and they were divided by cardiothorathic ratio (CTR) and UFR.

Results: In the cohort, the mean age was 59.1 ± 14.2 years, 50.4% were women and 48.7% were diabetes.The mean UFR was 9.6 ± 3.8 ml/h/kg and the mean CTR was 49.4 ± 6.5 %. For all population, UFR > 12.5 ml/h/kg group had higher risks for all-cause mortality and cardiovascular (CV) mortality after a median follow-up of 5 years. In the CTR > 50% group (962 patients), both UFR10~12.5 ml/kg/hr and > 12.5 ml/h/kg groups had significantly higher risks for all-cause mortality with adjusted HR 95% CI: 1.37 (1.05 to 1.77) and 1.57 (1.19 to 2.08), respectively, which was not seen in the CTR ≤ 50% group (p for interaction < 0.05). In the CTR > 50% group, UFR > 12.5 ml/h/kg group had a higher risk for CV mortality with adjusted HR 95% CI: 1.50 (1.01 to 2.24), which was not seen in the CTR ≤ 50% group (p for interaction < 0.05).

Conclusions: Our study showed Asian hemodialysis patients with cardiomegaly (CTR > 50%) and higher UFR were associated with higher risks for all-cause mortality and CV mortality while those without cardiomegaly could tolerate higher UFR.

FR-PO1008

Serial Measurements of Pre Dialysis N Terminal Probrain Type Natriuretic Peptide (NT-proBNP), Correlations with Extracellular Water Determined by Bioelectrical Impedance Assessments in Hemodialysis Patients

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Background: Increased cardiovascular mortality of hemodialysis patients is linked to volume overload and failure to achieve dry weight. Multifrequency bioelectrical impedance assessments (MF-BIA) can aid clinical examination in assessing volume status. However not all patients are suitable for MF-BIA measurements, and so we investigated whether serial N-terminal proBrain Natriuretic Peptide (NT-proBNP) measurements correlated with MF-BIA.

Methods: We prospectively measured pre-midweek NT-proBNP and MF-BIA in 186 stable hemodialysis outpatients, six months apart. The correlations between quartiles of water excess and quartiles of NT-proBNP changes were studied.

Results: We performed total 470 measurements in 186 patients, mean age 62.6±15.7yr, 33.3% male, 42.5% diabetic, 38.7% Caucasoid, 17.2% with a history of myocardial infarction, 9.7% coronary artery bypass surgery. Median predialysis NT-proBNP was 281.0 (129.5-890) pmol/l, and mean predialysis extracellular water (ECW) /total body water (TBW) ratio was 0.396±0.016. Median NT-proBNP change was 127.5 (36-397) pmol/l, and ECW/TBW change 0.5% (-0.45% to1.63%). There was no correlation between predialysis systolic blood pressure and ECW/TBW (r = 0.076, p = 0.305) or ECW excess (r = 0.079, p = 0.289). Patients were then divided into quartiles of ECW/TBW change and ECW excess. The absolute changes in NT-proBNP were significantly higher in the quartile with the greatest increase in ECW/TBW in comparison with all the lower quartiles for both ECW/TBW changes (1231±2044 versus 389±626 and 372±752 and 170±79 respectively, p < 0.05) and ECW excess (1180±2006 versus 518±839 and 232±481 and 194±412 respectively, p < 0.05).

Conclusions: Our data suggests that changes in serial NT-proBNP can help guide clinicians in determining target weight, especially for those patients unsuitable for MF-BIA.

Funding: Government Support - Non-U.S.

FR-PO1009

Relationship of Heart Rate Variability (HRV) and Heart Rate to Fluid Load in Hemodialysis (HD) Patients: Preliminary Results from the Monitoring in Dialysis (MiD) Study

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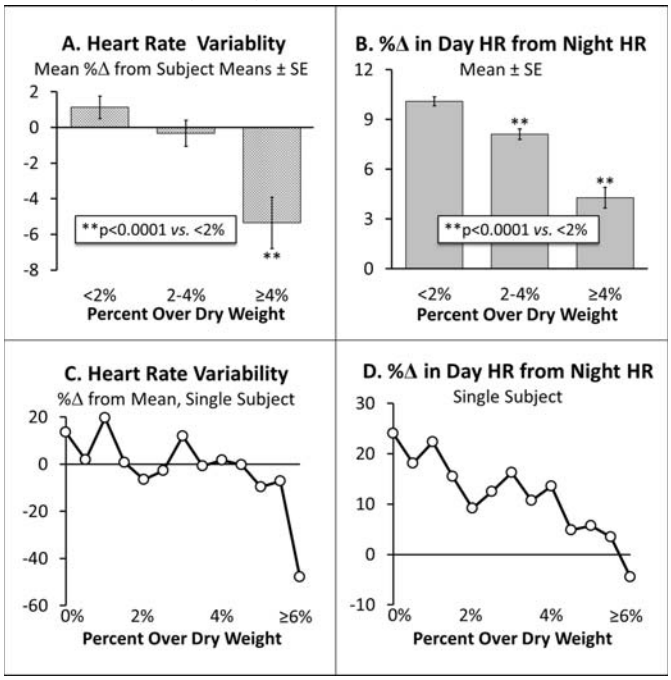
Background: Chronic fluid overload is linked to increased hospitalizations and death in ESRD. Volume status affects heart rate and HRV, but continuous monitoring of these parameters has not been explored as a potential fluid management tool.

Methods: MiD is a prospective multi-center study to characterize arrhythmias in 3x weekly HD patients with an implantable continuous cardiac monitoring device, the Medtronic Reveal® XT. HD treatment data are collected concurrently during the initial 6 months. In addition to arrhythmias, Reveal records HRV (SD of normal-normal RR intervals) and mean night- and daytime heart rates for each calendar day. Reveal HRV and heart rate data were analyzed in relation to fluid load. The fluid measure used was the mean percentage over clinical chart dry weight on each calendar day, estimated using observed pre- and post-HD weights and linear interpolation between adjacent weight observations. The sample was restricted to weeks with 3 HD sessions.

Results: The analysis sample included 4976 days of Reveal data from 43 of 45 implanted MiD subjects with concurrent HD session data. Higher excess fluid levels were associated with statistically significant reductions in HRV (Figure A) and compression of daytime heart rate toward nighttime heart rate (Figure B). This pattern was also apparent in data for individual subjects (Figures C and D).



Conclusions: Reduced HRV recorded by implantable continuous cardiac monitoring devices has the potential as a tool to improve fluid management in HD patients which may improve long term cardiovascular outcomes of ESRD patients.

Funding: Pharmaceutical Company Support - Medtronics, Inc

FR-PO1010

Dialysate Sodium Concentration Reduction to Reduce Haemodialysis Induced Myocardial Stunning: An Initial Randomised Controlled Trial

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Background: Large interdialytic weight gains in haemodialysis patients have been linked to higher mortality.Consequent increased ultrafiltration requirements drive HD induced cardiac injury.Reduction of dialysate sodium can reduce IDWG but concerns about tolerability remain.We investigated the effects of stepwise reduction in dialysate sodium on IDWG and HD induced myocardial stunning.

Methods: 19 patients receiving hospital HD underwent stepwise reduction in dialysate sodium (DiNa) over 8 weeks.Patients were randomized into 2 groups with one returning to standard treatment (140mmol/L) and the other continuing at the lowest tolerated sodium level.Studies were conducted at baseline,8 and 20 wks using pre and peak stress echocardiography analysed using 2D speckle tracking software to assess cardiac response.A stunned segment was defined as undergoing a 30% reduction in longitudinal strain.

Results: At 8 wks there was a significant reduction in mean IDWG (2.28kg±0.16 versus 1.61kg±0.17 p=0.008) for all patients but no significant difference in mean number of stunned segments (3.64±2.1 versus 3.71±1.1) or pre-dialysis global longitudinal strain (14.93±3.6 versus 15.14±2.78).Mean lowest tolerated DiNa was 133mmol/L (control) and 135mmol/L (intervention).Pre-dialysis serum sodium was higher in controls *(*138±4.3) than the intervention arm (135±4.6). At 20wks reduction in IDWG was sustained in the intervention group (2.65±0.75 versus 2.05±0.63 versus 1.84±0.65) but not the group returning to standard treatment (1.90±0.33 versus 1.17±0.44 versus 1.41±0.43).After increased exposure to reduced DiNa there was a modest reduction in number of affected segments (2.6±1.51 versus 3.5±0.54) and a small improvement in pre-dialysis GLS (13.58±4.06 at baseline versus 14.62±3.78 at 20wks).There was no difference between pre-dialysis systolic BP or post-dialysis systolic BP nor in episodes of hypotension during HD.

Conclusions: Stepwise reduction of dialysate sodium is a safe and effective method to reduce IDWG and ultrafiltration volumes and results in a modest improvement in cardiac tolerability.

FR-PO1011

Conversion of Facility Standard Dialysate Sodium to ≤138 mEq/L Is Associated with Reduction in Excessive Interdialytic Fluid Gains without Evidence of Adverse Effect

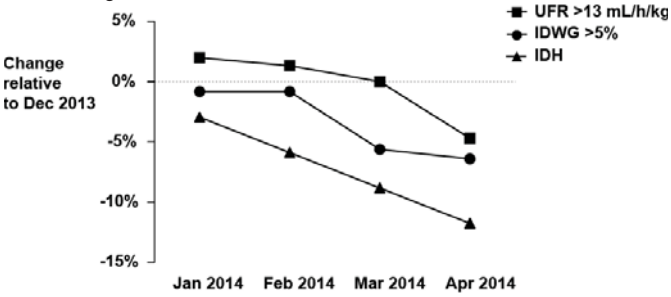
Jason Zhang, Andrew Lee, Steven M. Brunelli, Deborah A. Benner, Irina Goykhman, David B. Van Wyck, Mahesh Krishnan, Allen R. Nissenson. *DaVita HealthCare Partners Inc, Denver, CO.*

Background: Positive dialysate-serum sodium gradient results in intradialytic sodium loading. Therefore, reducing dialysate sodium ($D_{Na[sup]+[/sup]}$) is a potentially useful approach to mitigate thirst, interdialytic weight gain (IDWG), hypertension, and fluid-related morbidity in hemodialysis patients. We examined the effect of facility-level transition to lower $D_{Na[sup]+[/sup]}$ (134-138 mEq/L) on indices of peri-dialytic fluid balance: proportion of dialytic intervals with IDWG >5% of body weight, proportion of treatments with ultrafiltration rate (UFR) >13 ml/h/kg, and frequency of intradialytic hypotension (IDH); each of these indices has been previously associated with greater risk of morbidity and mortality.

Methods: We studied data from patients treated at any of 1,339 hemodialysis facilities whose governing bodies adopted default use of standard $D_{Na[sup]+[/sup]}$ between 134 and 138 mEq/L. Higher $D_{Na[sup]+[/sup]}$ was allowed by exception if deemed necessary by the treating nephrologist.

Results: By April 2014, 96% of all patients treated in these facilities had prescribed $D_{Na[sup]+[/sup]}$ ≤138 mEq/L. Between December 2013 and April 2014, the proportion of dialytic intervals with IDWG >5% of target weight declined by 6.4%, the proportion of treatments with UFR >13 ml/h/kg declined 4.7%, and frequency of IDH declined by 11.8%. Target weight, pre-dialysis serum sodium, and mortality were unchanged.

Conclusions: $D_{Na[sup]+[/sup]}$ in the range 134-138 mEq/L is effective in reducing excessive IDWG and high UFR rates without discernible adverse effects.



Funding: Pharmaceutical Company Support - DaVita HealthCare Partners Inc

FR-PO1012

Determining Blood Pressure Threshold for Intervention in Hemodialysis Patients: A Stratified Treatment Intention Analysis

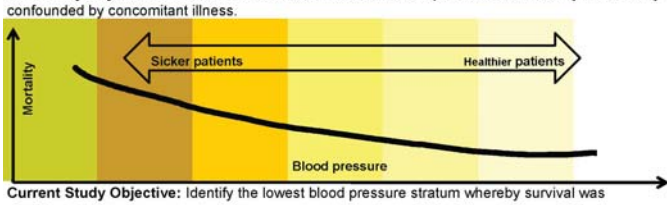
Steven M. Brunelli,[1] David B. Van Wyck,[2] Mahesh Krishnan,[2] Allen R. Nissenson.[2] *[1]DaVita Clinical Research, Minneapolis, MN; [2]DaVita HealthCare Partners, Inc., Denver, CO.*

Background: Although most hemodialysis (HD) patients have pre-dialysis blood pressure (BP) considered hypertensive in other populations, information is lacking on whether interventions to reduce higher-than-normal BP are beneficial. Given the absence of evidence from randomized controlled trials (RCTs) and targeting bias of observational studies, we conducted a stratified treatment intention analysis in order to identify the BP at which intervention versus no intervention is associated with clinical benefit.

Methods: We examined adult patients on HD as of 01-Jan-2012 (N=10,758), stratified by mean pre-dialysis systolic BP recorded during Jan-2012, and categorized patients as having had, or not had, a BP intervention (antihypertensive escalation or target weight reduction) during Feb through Mar-2012. Within each BP stratum, intervention and control patients were matched 1:1 on 18 characteristics. Mortality was considered from Apr-2012 through Mar-2013.

Results: Within BP strata, matched intervention and control patients had balanced characteristics. At BP strata ≥150-159mmHg and higher, risk of death was reduced among intervention patients versus controls, but at lower BP strata mortality risk was greater among intervention patients.

Past Study Objective: Measure the association between blood pressure and mortality that is likely confounded by concomitant illness.



Current Study Objective: Identify the lowest blood pressure stratum whereby survival was significantly better among treated versus non-treated patients. With falling stratum of blood pressure, there is reduced opportunity for confounding. Propensity score matching patients further reduces confounding.

Results: Incidence rate ratios (95% CI) by blood pressure strata

120-129 mm Hg	130-134 mm Hg	135-139 mm Hg	140-149 mm Hg	150-159 mm Hg	160-169 mm Hg	170-179 mm Hg
PS matched Treated vs non-treated (275 pairs)	PS matched Treated vs non-treated (220 pairs)	PS matched Treated vs non-treated (276 pairs)	PS matched Treated vs non-treated (930 pairs)	PS matched Treated vs non-treated (1041 pairs)	PS matched Treated vs non-treated (879 pairs)	PS matched Treated vs non-treated (570 pairs)
1.24 (0.79-1.96) p = 0.35	1.54 (0.92-2.58) p = 0.10	0.75 (0.49-1.13) p = 0.17	1.20 (0.93-1.55) p = 0.17	0.92 (0.71-1.19) p = 0.51	0.86 (0.66-1.14) p = 0.30	0.86 (0.60-1.21) p = 0.38

When considered as a continuous variable, the intervention—mortality association differed significantly according to BP (p-interaction=0.003); crossover from harm to benefit occurred at 155mmHg.

Conclusions: Among HD patients, BP-lowering interventions were associated with a lower risk of death when BP was ≥150mmHg. Our results do not support routine interventions for patients with lower BP. Stratified treatment intention analyses may prove useful when RCT evidence is lacking and robust databases are available.

Funding: Pharmaceutical Company Support - DaVita HealthCare Partners, Inc.

FR-PO1013

Early Changes in Systolic Blood Pressure and Body Weight Are Associated with Long Term Mortality in Incident Dialysis Patients

Flore Duranton,[1] Yohan Duny,[2] Ilan Szwarc,[3] Sebastien Deleuze,[4] Catherine Rouanet,[4] Isabelle Selcer,[4] Francois Maurice,[4] Jean Pierre Rivory,[4] Jean-Pierre Daures,[2] Angel Argiles.[1,3] *[1]RD - Néphrologie, Montpellier; [2]EA2415, Montpellier; [3]Nephrologie Dialyse St. Guilhem, Sète; [4]NéphroCare, Castelnau le Lez.*

Background: Systolic blood pressure (SBP) and body water are greatly altered in patients with heart and/or renal failure. We hypothesized that early changes in SBP and body weight (BW) could be associated with mortality in incident dialysis patients.

Methods: Patients incident to dialysis in 2004-2010 in 2 centers and surviving 90 days were followed-up for max 8 years. Longitudinal data were obtained from medical records (SBP and BW before dialysis) and REIN registry (outcome). Changes (linear regression slopes) and mean values in the first 15 days on dialysis (period 1) and the following 75 days (period 2: days 16-90) were associated with survival using Cox regression.

Results: 385 patients were included and 46.2% died during follow-up. In period 1 to 2, SBP and BW changed from 125±20 to 141±20mmHg and 61±14 to 70±15kg (p<0.001). Low SBP, low initial SBP slope and decreasing BW were associated with mortality (table). Low initial SBP and decreasing BW (both periods) remained significant in multivariate analysis (table). BW slopes remained significant (p<0.02) after adding heart failure (NS) in the model. Stratifying on heart failure showed that low BW slope in period 2 increased mortality in both groups, while initial BW slope was significant only in those without heart failure.

Table: Age and gender-adjusted hazard ratios of mortality

Effect	Period	Univariate		Multivariate	
		HR and 95% CI	P-value	HR and 95% CI	P-value
SBP ≤ 125 mmHg	1	1.7 [1.2; 2.3]	0.004	1.6 [1.0; 2.5]	0.047
SBP ≤ 130 mmHg	2	1.6 [1.2; 2.3]	0.003	1.2 [0.8; 1.9]	0.43
SBP slope ≤ 5 mmHg/d	1	1.7 [1.2; 2.4]	0.002	1.2 [0.8; 1.8]	0.29
BW slope ≤ -0.1 kg/d	1	1.9 [1.4; 2.7]	<0.001	1.6 [1.1; 2.3]	0.008
BW slope ≤ 0 kg/d	2	2.0 [1.5; 2.8]	<0.001	2.0 [1.4; 2.9]	<0.001

Conclusions: In the first months, patients starting dialysis have important changes in SBP and BW. Patients with BW decrease and additional low SBP have a greater risk of mortality, possibly related to heart failure.

Funding: Government Support - Non-U.S.

FR-PO1014

Ambulatory Blood Pressure (ABPM) Is Better in Identifying Hypertension (HTN) Associated with Arterial Stiffness than Casual Blood Pressure (BP) in Pediatric (ped) Hemodialysis (HD) Patients (pts) Shweta S. Shah, Jessica Geer, Fallon Campbell, Sarah J. Swartz, Poyyapakkam Srivaths. *Nephrology, Texas Children's Hospital, Houston, TX.*

Background: Few studies in ped HD pts have shown ABPM to be a better predictor of target organ damage (TOD) when compared to casual pre and post HD BP as assessed by LVMI. Pulse wave velocity (PWV) is the gold standard measure of arterial stiffness;it is shown to be elevated in adult HD pts and associated with poor outcome. However no study has assessed PWV as a marker of vascular involvement in ped pts.Aim-Compare ABPM and casual BP in assessing TOD in ped HD pts.

Methods: Casual BPs were obtained pre, intra and post dialysis on chronic HD pts during their mid-week treatment. Pulse wave tonometry to derive Augmentation index (AI), central blood pressure and PWV was done at the same time. 24 hour ABPM was started after HD treatment on same day. Echocardiogram was done to obtain LVMI. Routine monthly blood work completed as per dialysis unit protocol.

Results: 22 pts (16 male) on 3/week HD, duration 4 hrs.Mean age 17 ± 3.8 yrs, vintage 47.8 ±33.7 month, kt/v 1.6 ±0.17, 9 pts had preHD HTN but all normalized after HD. ABPM identified 6 pts with HTN. AI was elevated (>5%) in 17/22 pts but improved in 10/22 pts after HD. PWV did not change after HD. LVMI was elevated in 9/22 pts but was not associated with HTN (either casual or ABPM) or arterial stiffness. HTN identified by ABPM but not by preHD BP was associated with arterial stiffness by PWV

ABPM	Age (yrs)	BMI (%ile)	Hgb (mg/dl)	LVMI ($g/m^{2.7}$)	**PWV* (m/sec)**	AI (%)
Normal N=16	17.5 (15.5,20)	37.5 (6.5,59.5)	11.8 (10.7,12)	36 (30.6,51)	**6.15 (5.4,7.4)**	12 (-13,22)
HTN N=6	18 (12,19)	75 (18,79)	11.6 (11.4,11.8)	56.5 (38,81)	**8.45 (6.8,8.8)**	16 (12,24)

Values expressed as median (IQR), *=P value of 0.015 (Mann-Whitney test)

Conclusions: Casual BP either pre or post HD was not helpful in identifying sustained HTN in our study as most pts BP normalized post HD. ABPM identified sustained HTN which was associated with arterial stiffness. LVMI was not associated with HTN.ABPM should be part of routine monitoring in ped HD pts. Assessment of vascular stiffness in addition to routine cardiac ECHO should be considered even in ped HD pts.

FR-PO1015

Effect of Angiotensin Converting Enzyme Inhibitors on Serum Potassium Concentrations in Hemodialysis Patients. An Observational Study Ezio Movilli, Giovanni Cancarini. *UO of Nephrology Spedali Civili and Univ of Brescia, Italy, Spedali Civili, Brescia, Italy.*

Background: Angiotensin converting enzyme inhibitors (ACEi) are increasingly used in uremic patients (pts). However, their effect on serum potassium concentrations (sK) in anuric pts on chronic hemodialysis treatment (HD) is controversial. Aim of the study: To evaluate sK before and after the start of ACEi therapy.

Methods: From 1-1-2011 to 31-12-2011, 87/206 prevalent anuric HD pts on regular HD started ACEi therapy. Mean age was 67±14 years, 57/87 were men, dialytic vintage was 6-20 months. In the 2 months before and after the start of ACEi therapy, the following variables were evaluated in pre dialysis after the long interdialysis interval: sK (mean value of 8 determinations) (mmol/L), maximum sK (maximum K value observed during observations) (sKmax; mmol/L), serum sodium (Na; mmol/L), hemoglobin (Hb; g/dL), EPO dose (U/Kg/sett), pre dialysis systolic (SBP: mmHg) and diastolic (DBP: mmHg) blood pressure, body weight (BW; Kg), interdialytic weight gain (IWG; Kg), Kt/V. SBP, DBP, IWG are the mean values of the 24 HD sessions. 60 pts were on bicarbonate HD, 27 on HDF. Duration of HD, Qb and Qd were kept constant. Data are expressed as mean±SD, t test for paired data was employed to compare groups. Significant differences were defined as $p<0.05$.

Results: sK increased from 5.0±0.4 mmol/L before ACEi to 5.7±0.5 mmol/L ($p< 0.0001$). sKmax increased from 5.4±0.5 mmol/L before ACEi to 6.2±0.6 mmol/L ($p<0.0001$). 7/87 pts reduced the K in dialysate. 15/87 pts (18%) stopped ACEi therapy. sK in these pts changed from 5.2±0.3 mmol/L to 6.5±0.2 mmol/L at the moment of suspension ($p<0.0001$). sKmax changed from 5.5±mmol/L to 6.9±0.3 mmol/L at the moment of suspension ($p< 0.0001$). After the suspension of ACEi, sK and sKmax concentrations normalized within 1 month. There were no significant changes of BW, IWG, SBP, DBP, Na, Hb, EPO dose, Kt/V.

Conclusions: Treatment with ACEi causes a significant 13% increase of the sK concentrations in HD pts. This can lead, in 18% of cases, to the need to stop the drug for sK values > 6.5 mmol/L and suggests caution in the wider utilization of these drugs in HD patients.

FR-PO1016

Safety of Eplerenone in Hemodialysis Patients: A Randomized Controlled Trial Michael Walsh,[1] Braden J. Manns,[2] Amit X. Garg,[3] Joe A. Bueti,[4] Christian G. Rabbat,[1] Andrew Smyth,[1] Jessica Tyrwhitt,[1] Jackie Bosch,[1] Peggy Gao,[1] Philip J. Devereaux,[1] Ron Wald.[5] *[1]McMaster Univ/Population Health Research Inst; [2]Univ of Calgary; [3]Western Univ; [4]Univ of Manitoba; [5]Univ of Toronto.*

Background: Mineralocorticoid receptor antagonists (MRA) reduce morbidity and mortality in non-dialysis patients with heart failure but their use is limited by hyperkalemia. Whether MRAs have similar benefits and risks in in dialysis patients is unclear. We studied eplerenone in hemodialysis patients.

Methods: We randomly allocated 154 prevalent hemodialysis patients from 5 Canadian centres to 13 weeks of either eplerenone 50 mg daily or a matching placebo. The primary outcome was permanent discontinuation of the study drug for hyperkalemia or hypotension. Eplerenone was considered non-inferior if a discontinuation rate for hyperkalemia or hypotension of greater than 20% could be excluded. Secondary outcomes included rates of hyperkalemia and effects on blood pressure and cardiovascular events.

Results: Seventy-seven participants were allocated to each group and had similar baseline characteristics. The per protocol population (i.e., those taking medication for at least one week) included 74 participants in the eplerenone group and 70 in the placebo group. The primary outcome occurred in 3 in the eplerenone group and 2 (2.9%) in the placebo group for an absolute risk difference of 1.2% (95% confidence interval -4.8% to 7.1%). Eplerenone was therefore considered [non-inferior/inferior] to placebo with respect to permanent discontinuation for hyperkalemia or hypotension. In the eplerenone group, 9 (12%) developed hyperkalemia (>6.5 mmol/L) compared to 2 (2.8%) in the placebo group (relative risk 4.3, 95% CI 1.0 to 19.0). No significant differences in fatal or non-fatal cardiovascular events were seen.

Conclusions: We did not find a substantially increased risk of permanent discontinuation of eplerenone due to hyperkalemia or hypotension. Serum potassium concentrations should be carefully evaluated when aldosterone antagonism is initiated. Large studies are required to determine if aldosterone antagonism reduces cardiovascular morbidity and mortality.

Funding: Pharmaceutical Company Support - Pfizer, Government Support - Non-U.S.

FR-PO1017

Comparative Effectiveness of Dihydropyridine and Non-Dihydropyridine Calcium Channel Blockers in Chronic Dialysis Patients James B. Wetmore,[1] Jonathan D. Mahnken,[2] Milind A. Phadnis,[2] Edward F. Ellerbeck,[3] Theresa I. Shireman.[3] *[1]Nephrology, Hennepin County Medical Center, Minneapolis, MN; [2]Biostatistics, Univ of Kansas Medical Center, Kansas City, KS; [3]Preventive Medicine & Public Health, Univ of Kansas Medical Center, Kansas City, KS.*

Background: Although there is evidence of benefit for use of calcium channel blockers (CCBs) in dialysis patients, the comparative effectiveness of dihydropyridine (DHP) and non- DHP CCBs has not been well studied.

Methods: A retrospective cohort analysis of effectiveness of DHP versus non-DHP CCBs in dialysis patients was conducted. Medicaid pharmacy claims were linked with USRDS Medicare Parts A and B claims to create a cohort of hypertensive patients initiating dialysis from 2000-05. After excluding patients who were received a CCB in the first 90 days after dialysis initiation, new users were followed from their 1st day of medication exposure until they died (all-cause mortality, or ACM) or, separately, incurred a combined cardiovascular hospitalization or death (cardiovascular morbidity or mortality, or CVMM), or were censored; CCB crossover was not allowed. Cox proportional hazards models generated adjusted hazard ratios (AHRs) comparing the effect of DHPs versus non-DHPs on ACM and CVMM.

Results: There were 7307 and 6121 new users CCBs who were included in the ACM and CVMM models, respectively; approximately 14.3% received non-DHPs. Adjusted for other factors, use of DHPs, compared to non-DHPs, was associated with an AHR of 0.60 (95% CIs, 0.53 – 0.69, $P < 0.0001$) for ACM and 0.77 (0.67 – 0.89, $P < 0.0001$) for CVMM. Results were similar when only individuals who initiated therapy within 90 days of cohort entry were included, with AHRs of 0.77 (0.64 – 0.93, $P = 0.0004$) and 0.86 (0.72 – 1.02, $P = 0.024$) for ACM and CVMM, respectively. Elimination of individuals with chronic atrial fibrillation resulted in similar AHRs of 0.59 and 0.63 for ACM and CVVM, respectively.

Conclusions: DHPs are associated with a reduction in ACM and CVMM by about 40% and 23%, respectively, compared to non-DHPs. This may be related to differential effects of these classes on cardiac inotropy, cardiac chronotropy, or vascular relaxation, but more studies are needed.

Funding: NIDDK Support

FR-PO1018

Ambulatory Recording of Arterial Stiffness and Wave Reflections during Intra- and Interdialytic Periods in Hemodialysis Patients

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Background: Arterial stiffness and wave reflections are strong predictors of cardiovascular mortality in hemodialysis patients. Previous studies evaluating arterial cushioning function in this population included measurements obtained during the peridialytic period. The aim of this study was to investigate potential variations in arterial stiffness and wave reflections during the intra- and inter-dialytic intervals in hemodialysis patients.

Methods: A total of 153 hemodialysis patients underwent 48-hour ambulatory blood pressure monitoring (ABPM) with the Mobil-O-Graph device (IEM, Stolberg, Germany). ABPM included a 4-hour intradialytic period and the subsequent interdialytic interval. Mobil-O-Graph is a validated brachial cuff-based oscillometric device, which records pulse waveforms at brachial artery and assesses pulse wave velocity (PWV) and augmentation index (AIx) via mathematical transformation.

Results: Mean PWV was not changed between the intradialytic and out-of-dialysis intervals of the dialysis-on day (Day 1) (9.31±2.2 versus 9.29±2.3 m/sec, P=0.602). In contrast, heart rate-adjusted AIx (AIx (75)) was significantly lower during intradialytic than during the out-of-dialysis period of Day 1 (24.0±8.6 versus 26.4±7.7 %, P<0.001). Both parameters were increased during the dialysis-off day (Day 2) relevant to the out-of-dialysis period of Day 1 (9.39±2.3 versus 9.29±2.3 m/sec, P<0.001 and 27.5±8.2 versus 26.4±7.7 %, P<0.001 for PWV and AIx (75)).

Conclusions: This study shows a gradual increase in AIx (75) between intra- and inter-dialytic intervals in hemodialysis patients, whereas PWV exhibited only a slight and potentially BP-dependent elevation during the dialysis-off day.

Funding: Private Foundation Support

FR-PO1019

Ventriculoarterial Uncoupling in Pediatric Hemodialysis Patients

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Background: Hemodialysis (HD) patients are at high risk for cardiovascular (CV) morbidity and mortality. Intradialytic hypotension associated myocardial stun has been proposed as a potential cause. Nonivasive cardiac output measurements, validated in children, provide a dynamic bedside assessment of hemodynamic parameters. We sought to investigate intradialytic changes in hemodynamic parameters during pediatric chronic outpatient HD.

Methods: HD was performed using linear fluid removal over 4 hours. Continuous wave Doppler ultrasound was used to measure hemodynamic parameters pre HD, 120 minutes into HD, and post HD during the mid-week treatment. Pulse wave tonometry to derive augmentation index (AI), central blood pressure, and pulse wave velocity (PWV) was done at the same time.

Results: 22 pts (16 male) on 3/week HD, mean age 17 ± 3.8 yrs, vintage 47.8 ±33.7 months, spkt/v 1.6 ±0.17. No patient experienced intradialytic hypotension requiring intervention.

	Cardiac Index (L/min/m^2)	Stroke Volume (cm^3)	Systemic Vascular Resistance Index (dynes-sec/cm–5/m2)	Heart Rate (beat/min)	Pulse Wave Velocity (m/sec)
Pre	3.38 (2.9, 4.58) *	69.6 (58.04, 83.52) *	1973 (1750,2640)	81 (76,93) *	6.55 (5.8, 8.3)
Intra (120 min)	3.24 (2.5, 3.82) *	52.23 (43, 63) *	2100 (1768, 2357)	91 (84,102) *	7.1 (6.7, 8)
Post	3.33 (2.9, 3.74)	55.87 (48.13, 62.31)	1940 (1649, 2373)	91 (80,97)	6.6 (5.6, 7.5)

Values expressed median (IQR); *= p-value < 0.005

Conclusions: CI and SV decreased significantly during the HD session. Expected increase in SVRI to preserve CI was not observed. Increased PWV did not improve with HD. In children and young adults, the ventriculoarterial coupling response is disrupted in chronic HD patients, and increased arterial stiffness fails to improve with fluid removal. Noninvasive cardiovascular monitoring could identify asymptomatic patients with falling CI at risk of intradialytic myocardial stunning.

FR-PO1020

Structural Heart Disease and Outcomes in Incident Hemodialysis Patients: Correlation with Proposed Echocardiographic Criteria by the Acute Dialysis Quality Initiative (ADQI) Workgroup

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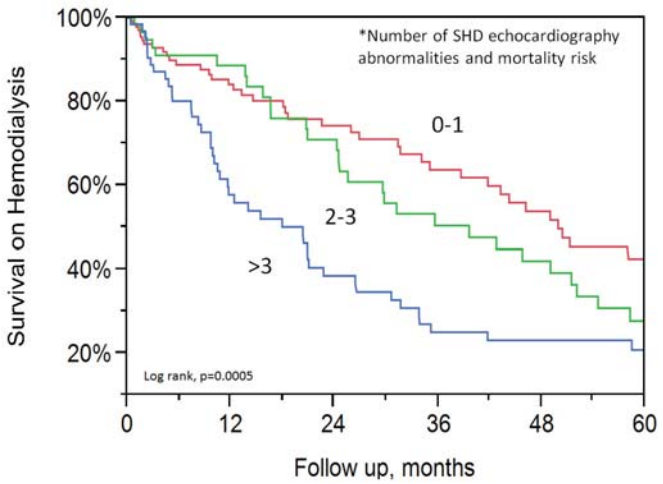
Background: Cardiovascular disease is highly prevalent among hemodialysis (HD) patients and relates to increased morbidity. The ADQI Workgroup recently proposed echocardiographic criteria for structural heart disease (SHD) in HD patients, but studies on the prevalence and clinical impact of SHD based on the new proposed classification are lacking.

Methods: AIM: To determine prevalence of SHD and its association with survival among incident HD patients. **METHODS:** A retrospective review of HD patients from a regional dialysis provider, who initiated HD from 2006-2009, and underwent echocardiography (EC) ±6 months of HD start (n=349).

Results: Mean age 65±15, 63% males, 90% Caucasian, 52% diabetes, and 45% coronary artery disease. Median time between EC and HD start was 15 days; 0-182. Accounting for missing variables (left ventricular [LV] volumes), 81% of patients had ≥1 EC finding meeting SHD criteria, and 35% had ≥3. The most common SHD abnormalities were: diastolic dysfunction (DD) (44%), left ventricular hypertrophy (LVH) (40%), aortic/mitral valvular heart disease (VHD) (33%), and LV ejection fraction (EF) ≤45% (25%). Over 1.8±2.1 years of followup, 141 (46%) died. SHD variables age- and gender-adjusted were associated with death; EF≤45% (HR 2.0, CI 1.4-2.8; p<0.01) was a strong positive predictor (DD, LVH, VHD; p>0.05). Presence of ≥2 abnormalities was associated with death (HR 1.5, CI 1.1-2.2; p=0.02) with an increased risk thereafter.



Conclusions: Based on ADQI classification, SHD is common among incident HD patients. The risk of death increases incrementally with higher prevalence of EC abnormalities. Early SHD detection may help improve management and outcomes.

FR-PO1021

Attenuated Cardiovascular Reserve Is a Major Complication of CKD That Is Only Partially Accounted for by Chronic Pressure Overload

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Background: Patients with advanced CKD experience impaired functional cardiovascular reserve with reduced oxygen consumption at peak exercise (VO_2peak). It remains unknown if this abnormality is related to impaired cardiovascular compliance resulting from chronic uremia or hypertension.

Methods: 80 kidney transplant-waitlisted patients and 80 healthy subjects with essential hypertension matched in age (53.3 versus 53.4 years, p=0.82), sex (male: 56.3 versus 51.2 %, p=0.23) and BMI (27.2 versus 27.6 kg/m^2, p=0.55) were evaluated prospectively between 2010 and 2012. Maximal cardiopulmonary exercise testing, TD-echocardiography and pulse-wave-velocity (PWV) were performed.

Results: VO_2peak was significantly lower in CKD subjects compared with the non-uremic hypertensives (18.8 versus 24.5 ml/min/kg, p<0.001). Independent cardiovascular predictors of VO_2peak for CKD included LV filling pressure (E/mean e') (standardized regression coefficient, *B*=-0.5) and PWV (*B*=-0.2); in the hypertensives, these were LV mass index (*B*=0.5), LV end-diastolic volume index (LVEDVI) (*B*=0.6), peak heart rate (HR) (*B*=0.5) and PWV (*B*=-0.2). According to the best fitted model for the CKD population (adjusted R^2=0.43), higher E/mean e' was associated with a lower VO_2peak (*b*=-3.65, p=0.001) after adjusting for demographics, hemoglobin and duration of hypertension.

In the hypertensive cohort, the final model (adjusted R^2=0.66) identified higher LVEDVI (b=0.24, p<0.001), LV mass index (b=0.09, p=0.01) and peak HR as predictors of greater VO_2peak (b=0.12, p<0.001).

Conclusions: LV compliance is significantly associated with VO_2peak in advanced CKD resulting from mechanisms beyond predominantly arterio-ventricular changes associated with hypertension. This highlights the complex kidney-heart interactions that occur in renal failure beyond the effects of pressure overload.

Funding: Private Foundation Support

FR-PO1022

The Clinical Parameters Correlating with Cardiac Dysfunction at the Start of Dialysis Therapy Yoshimichi Urahama,[1] Daijo Inaguma.[2] *[1]Dept of Nephrology, Komaki Municipal Hospital, Komaki-City, Aichi-prefecture, Japan; [2]Kidney Center, Nagoya daini Red Cross Hospital, Nagoya City, Aichi-prefecture, Japan.*

Background: It has been reported that cardiac dysfunction is an important factor of poor prognosis in dialysis patients. In addition at the start of first dialysis there are many patients who have problems with heart function already. The aim of this study was to determine which clinical parameters were associated with left ventricular ejection fraction (LVEF) in end-stage renal disease patients.

Methods: This study, named AICOPP study consisted of 1525 ESRD patients (1420 HD, 105 PD) who began dialysis therapy between October 2011 and September 2013 at 17 dialysis centers. 1255 patients were screened by ultrasonic-echocardiography (UCG). Using a cross-sectional design, the statistical analysis was performed with program JMP 10.0 (SAS Institute).

Results: Total cholesterol level, body math index, use of angiotensin receptor blocker, use of phosphate binder, use of erythropoiesis stimulating agent (positive correlation) and serum uric acid level, eGFR, history of diabetes, left ventricular hypertrophy and atrial fibrillation on electrocardiogram, use of beta receptor blocker, use of loop diuretics (negative correlation) correlated with LVEF. Multiple regression analysis of LVEF (%) demonstrated that serum BNP level (pg/ml, R=-0.00426, P<0.001), heart rate at the beginning of dialysis therapy (/min, R=-0.155, P<0.001), history of ischemic heart disease (R=-3.05, P=0.0070), age (years, R=0.167, P<0.001), systolic blood pressure at the beginning of dialysis therapy (mmHg, R=0.0518, P=0.0055), history of heart failure hospitalization (R=-1.49, P=0.00128) and female (R=1.29, P=0.0157) were significant parameters.

Conclusions: In conclusion high serum BNP level, high heart rate at the beginning of dialysis therapy, history of ischemic heart disease, advanced age, low systolic blood pressure at the beginning of dialysis therapy, history of heart failure hospitalization and male are the clinical parameters correlating with cardiac dysfunction in patients newly initiated into dialysis. ESRD patients with such clinical characteristics are necessary to receive reliable evaluation and care of cardiac function.

Funding: Private Foundation Support

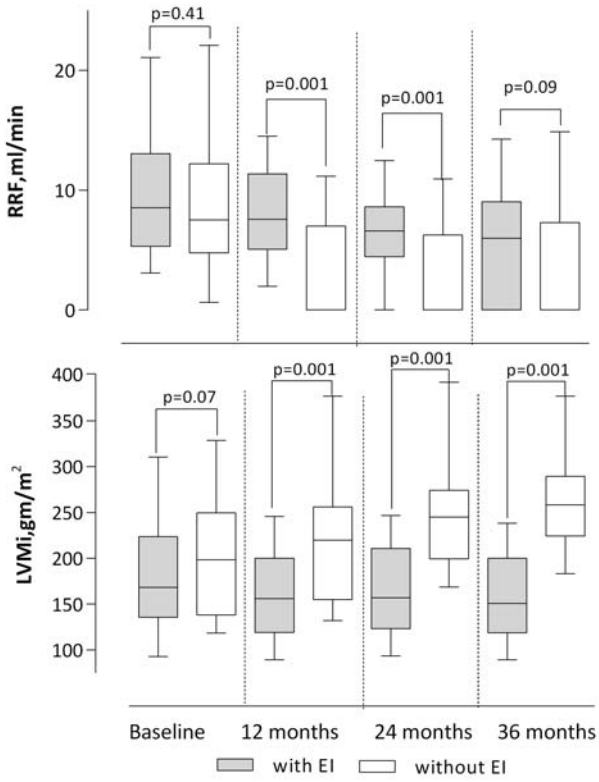
FR-PO1023

Impact of Randomized Educational Intervention on Residual Renal Function (RRF) and Left Ventricular Mass Index (LVMi) in Incident PD and HD Patients (CECODIAR Study) Gustavo Lorenzo Moretta,[1] Amilcar Vinuela,[2] Carlos Florencio Lara,[3] Eduardo Emilio Ducasse,[4] Elsa Graciela Giarrocco,[5] Carlos Alberto Bonanno,[6] Javier De Arteaga,[7] Abdul Rashid Tony Qureshi,[8,9] Jose C. Divino-Filho.[9] *[1]Centro Nefrologico Noroeste, Argentina; [2]Sanatorio de la Mujer, Argentina; [3]Inst Lanari, Argentina; [4]CIMAC, Argentina; [5]Centro de Enfermedades Renales, Argentina; [6]Hospital Militar, Argentina; [7]Dialiquen, Argentina; [8]Dialisis Berazategui, Argentina; [9]Centro Integral Nefrologico, Argentina; [10]Hospital Privado de Cordoba, Argentina; [11]Div of Baxter Novum, Karolinska Instt, Sweden; [12]Div of Renal Medicine, Karolinska Instt, Sweden.*

Background: The prevalence of traditional cardiovascular (CV) risk factors is high in dialysis patients (pts) whereas RRF preservation and LVMi reduction impact positively on survival. To evaluate the impact of educational intervention (EI) on preserving RRF and maintaining CV health status (LVMi) in HD and PD incident pts.

Methods: Prospective, multicenter, randomized EI trial in incident adult dialysis pts. The CV risk factors were evaluated after 12, 24 and 36 months of follow-up.

Results: A total of 171 pts were randomized to either EI (n=88) with median age 63 (38-70), 61% male, DM 39% and 38% initiated with PD. or without EI (n=83) with median age 60 (31-77), 53% male, DM 35% and 35% initiated with PD. Pts assigned to EI had better preservation of RRF and improved LVMi than pts without EI.



No significant survival advantage for the all-cause mortality in pts with EI was observed. The EI PD pts had a positive trend (p=0.07) to survival advantage compared to patients without EI.

Conclusions: EI, may be an important determinant of RRF preservation and LVMi improvement among incident dialysis patients. The role of EI should be further explored and applied to promote cardiac health in dialysis.

FR-PO1024

Association of Preload and Afterload with Left Ventricular Mass in Incident Hemodialysis Patients: Predictors of Arrhythmic and Cardiovascular Risk in End Stage Renal Disease (PACE) Study Esther D. Kim,[1] Stephen M. Sozio,[2] Michelle M. Estrella,[2] Bernard G. Jaar,[2] Tariq Shafi,[2] Lucy A. Meoni,[2] Wen Hong Linda Kao,[2] Joao A.C. Lima,[2] Rulan S. Parekh.[1,2] *[1]Univ of Toronto; [2]Johns Hopkins Univ.*

Background: Higher left ventricular (LV) mass strongly predicts cardiovascular (CV) mortality in hemodialysis (HD). Though several parameters of preload and afterload have been associated with higher LV mass, the relative importance of these parameters in predicting LV mass is unknown.

Methods: In a prospective cohort of 390 incident HD adults in the PACE study, we examined measures of afterload (SBP, SBP, arterial stiffness by pulse wave velocity [PWV]) and preload (volume status using estimated pulmonary pressures by echocardiogram on an interdialytic day and intradialytic weight gain [IDW]). Outcome was LV mass index (LVMI) by echocardiogram.

Results: PreHD and interdialytic SBP, DBP, and pulse pressures were associated with LVMI after adjusting for CV confounders. IDW was also associated with higher LVMI but attenuated effects after adjustment (p=0.01). PWV and pulmonary pressures were not associated with LVMI after multivariable adjustment (p=0.6 and p=0.8, respectively).

Table: Association of preload and afterload measures with LVMI by linear regression

Measurements	Unadjusted		Adjusted*	
	β (95% CI)	P	β (95% CI)	P
Vascular				
BP (per 10 mmHg)				
Predialysis[a]				
Systolic	6.2(3.3,9.1)	<0.001	7.5(4.5,10.5)	<0.001
Diastolic	10.5(6.2,14.8)	<0.001	11.6(6.4,16.8)	<0.001
Predialysis[b]				
Systolic	2.6(0.7,4.5)	0.008	2.5(0.5,4.4)	0.01
Diastolic	6.2(3.1,9.3)	<0.001	4.6(1.2,7.9)	0.008
Interdialytic[c]				
Systolic	4.8(2.9,6.7)	<0.001	5.0(3.0,6.9)	<0.001
Diastolic	7.9(4.7,11.1)	<0.001	7.4(3.8,11.0)	<0.001
Pulse pressure				
Predialysis[a]	0.3(-0.1,0.6)	0.2	0.7(0.3,1.1)	0.001
Predialysis[b]	0.1(-0.2,0.3)	0.5	0.2(-0.1,0.5)	0.1
Interdialytic[c]	0.4(0.1,0.7)	0.004	0.6(0.3,0.9)	<0.001
Arterial stiffness				
Pulse wave velocity	-0.4(-1.8,0.9)	0.5	0.4(-1.2,2.0)	0.6
Volume				
Pulmonary artery pressure	0.3(-0.3,0.9)	0.3	0.1(-0.5,0.7)	0.8
Intradialytic weight[b]	4.0(1.1,6.9)	0.007	3.7(0.8,6.6)	0.01

[a]3 month average
[b]Prior to study visit
[c]Seated
*Adjusted for demographic factors, CV risk factors, antihypertensive medications

Conclusions: Higher systolic, diastolic, or pulse pressure, regardless of timing with HD, is associated with higher LV mass. These results suggest the importance of afterload reduction in preventing increasing LV mass in HD patients.

FR-PO1025

Asymptomatic Arrhythmias on Hemodialysis by Continuous 7-Day ECG Monitoring Rulan S. Parekh,[1] Lucy A. Meoni,[2] Bernard G. Jaar,[2] Stephen M. Sozio,[2] Michelle M. Estrella,[2] Wen Hong Linda Kao,[2] Larisa Tereshchenko.[2] *[1]Univ of Toronto, Canada; [2]Johns Hopkins Univ.*

Background: Sudden cardiac death (SCD) is the most common cause of death in dialysis patients. Asymptomatic arrhythmias may lead to SCD; however, the impact of dialysis therapy on rates of arrhythmia is unknown.

Methods: One-lead surface ECG was recorded continuously for up to 7 days via an ECG patch (ZioPatch, iRhythm Technologies, Inc.) in 28 participants from the Predictors of Arrhythmia and Cardiovascular Events (PACE) Study, a prospective cohort of incident hemodialysis participants. Rates of non sustained ventricular tachycardia (VT), supraventricular tachycardia (SVT), atrial fibrillation (AF), pauses above 3 seconds (sec), and AV block were measured during 6 hours pre-dialysis, during, 6 hours post, and in between treatments.

Results: Mean age was 53.9 years, 59% were men, 14% had prevalent coronary heart disease (CHD), and had a mean left ventricular ejection fraction (LVEF) of 70.3±9%. Average heart rate was 77.3 ± 9 bpm. Nearly half had at least one arrhythmic event detected and 6 patients had at least 2 different types of arrhythmias. All events were non-sustained, lasting < 30s, and asymptomatic except one patient with paroxysmal AF. Non-sustained ventricular tacycardia (NSVT) was more frequent during or immediately postdialysis compared to pre/between dialysis treatments (63% versus 37%, P=0.01). None of the patients with NSVT had CHD. Conversely, SVT was more frequent pre/ between dialysis, as compared to during or post dialysis (84% versus 16%, P=0.01). No events of high degree AV block were detected.

Asymptomatic Arrhythmias among 28 HD Participants with Continuous 7 Day ECG Monitoring

Timing	Between dialysis	Pre-dialysis	During dialysis	Post-dialysis	Unique individuals
Events	n (%)				
Total (n=47)	18 (38)	12 (26)	8 (17)	9 (19)	13 (46)
VT (n=8)	2 (25)	1 (13)	2 (25)	3 (38)	4 (15)
SVT (n=17)	9 (53)	5 (29)	1 (6)	2 (12)	9 (32)
AF (n=21)	7 (33)	6 (29)	4 (19)	4 (19)	2 (7)
Pauses >3 s (n=1)	0	0	1 (100)	0	1 (3.5)

Conclusions: Asymptomatic supraventricular and ventricular arrhythmias are frequent in hemodialysis, and associate temporally with dialysis treatments.

Funding: NIDDK Support

FR-PO1026

Predictors of Arrhythmia Events in Incident Patients on Hemodialysis – Results from the International MONDO Initiative Viviane Calice-Silva,[1,2] Jochen G. Raimann,[1] Stephan Thijssen,[1] Aileen Grassmann,[3] Daniele Marcelli,[3] Bernard Canaud,[3] Len A. Usvyat,[4] Peter Kotanko,[1] Roberto Pecoits-Filho,[2] Mondo Consortium.[1,2,3,4] *[1]Renal Research Inst, New York; [2]Pontifical Catholic Univ of Parana, Curitiba, Brazil; [3]Fresenius Medical Care, Bad Homburg, Germany; [4]Fresenius Medical Care North America, Waltham.*

Background: Arrhythmias due to electrolytes changes, fluid overload, cardiac dysfunction, and other causes are associate to adverse outcomes in hemodialysis (HD) patients. The aim of this study was to explore predictors of arrhythmia-related events (ARR; hospitalization and mortality) in the MONDO cohort.

Methods: This analysis included incident HD patients from the MONDO database commencing treatment in clinics in the U.S. and Europe. The mean of clinical and laboratory parameters were computed for the first 12 months (baseline-BL) and clinical events (deaths and hospitalizations) were recorded in months 13 to 24 (follow-up). Hospitalizations and causes of death were classified as ARR according to ICD-9 and 10 codes. Poisson regression models were constructed to explore associations between BL parameters and the number of ARR events during follow-up.

Results: We studied 22729 patients (59.3% males, 63.5±14.9 years). Older age, higher serum phosphorus and the previous diagnosis of congestive heart failure (CHF) were associated with higher risk of ARR death and hospitalization. Higher normalized protein catabolic rate (nPCR) and hemoglobin (HGB) levels were associated with lower risk of ARR. (Figure 1; adjusted by region)

Parameter	Estimate	Wald 95% Confidence Limits		P-value
Age [per year]	**0.0228**	**0.0117**	**0.0338**	**<.0001**
Phosphate [per mg/dL]	**0.2129**	**0.0761**	**0.3497**	**0.0023**
Comorbid_CHF [Y/N]	**0.6715**	**0.3357**	**1.0073**	**<.0001**
nPCR [per g/day/kg body weight]	**-1.68**	**-2.5848**	**-0.7752**	**0.0003**
HGB [per mg/dL]	**-0.159**	**-0.3154**	**-0.0025**	**0.0465**
Male [Y/N]	-0.1581	-0.44	0.1239	0.2718
BMI [kg/m2]	0.0124	-0.0105	0.0352	0.2882
Diabetes [Y/N]	0.1967	-0.099	0.4923	0.1923
Treatment time [per hour]	-0.0018	-0.0094	0.0058	0.6466
Pre-HD SBP < 100 mmHg [Y/N]	0.7771	-0.6389	2.1932	0.2821
Pre-HD SBP > 140 mmHg [Y/N]	0.2687	-0.0343	0.5717	0.0821
IDWG [per % of post-HD weight]	0.1402	-0.0102	0.2906	0.0676
Albumin [per g/L]	-0.1611	-0.5543	0.2321	0.4221
NLR [per 1 unit]	0.0315	-0.0085	0.0715	0.1222
Potassium [per 1 mmol/L]	0.2414	-0.0271	0.51	0.0781
Calcium [per mg/dL]	-0.2246	-0.4914	0.0421	0.0988
Myocardial Infarction [Y/N]	-0.425	-1.4277	0.5777	0.4061

Conclusions: In the studied cohort higher nPCR and HGB were associated with a lower risk of ARR events while older age, higher phosphate levels and presence of previously diagnosed CHF were associated with a higher risk of ARR. These findings may assist the identification for HD patients at high risk for ARR and help to define targets for interventions.

FR-PO1027

Predictors of Sudden Death in Incident Patients on Hemodialysis – Results from the International MONDO Initiative Viviane Calice-Silva,[1,2] Jochen G. Raimann,[1] Stephan Thijssen,[1] Aileen Grassmann,[3] Daniele Marcelli,[3] Bernard Canaud,[3] Len A. Usvyat,[4] Peter Kotanko,[1] Roberto Pecoits-Filho,[2] Mondo Consortium. *[1]Renal Research Inst, New York; [2]Pontifical Catholic Univ of Parana, Curitiba, Brazil; [3]Fresenius Medical Care, Bad Homburg, Germany; [4]Fresenius Medical Care North America, Waltham.*

Background: Sudden death (SD) is defined as sudden, unexpected death within an hour of symptoms onset or without obvious non-cardiac cause. In chronic kidney disease (CKD) 5D patients SD is responsible for 25% of deaths. The aim of this study was to explore predictors of sudden death in the MONDO cohort.

Methods: This analysis included incident hemodialysis (HD) patients from the MONDO database commencing treatment in clinics in the U.S. and Europe. The mean of clinical and laboratory parameters were computed for the first 12 months (baseline) and clinical events (deaths) were recorded in months 13 to 24 (follow-up). Mortality was classified as SD-related according to ICD-10 and ICD-9 codes. Poisson regression models were constructed to explore associations between baseline parameters and the number of SD events during follow-up.

Results: We studied 22729 patients (59.3% males, 63.5±14.9 years).Older age, male gender, pre-HD SBP <100 mmHg, greater IDWG (in % of post-HD weight), higher serum calcium levels, higher neutrophil to lymphocyte ratio (NLR) and previous diagnosis of myocardial infarction (MI) were associated with higher risk of SD. Higher nPCR, albumin, hemoglobin (Hgb) levels and pre-HD SBP > 140 mmHg were associated with lower risk of SD. (Figure 1, adjusted by region).

Parameter	Estimate	Wald 95% Confidence Limits		P-value
Intercept	-2.8051	-5.4322	-0.1779	0.0364
Age [per year]	0.0344	0.0261	0.0428	<.0001
Male [Y/N]	0.2138	0.0062	0.4214	0.0435
Pre-HD SBP < 100 mmHg [Y/N]	0.9121	0.2795	1.5447	0.0047
IDWG [per % of post-HD weight]	0.295	0.1888	0.4013	<.0001
Miocardial Infarction [Y/N]	0.7048	0.2123	1.1973	0.005
Calcium [per mg/dL]	0.2191	0.0229	0.4154	0.0286
NLR [per 1 unit]	0.0655	0.044	0.087	<.0001
Pre-HD SBP > 140 mmHg [Y/N]	-0.2676	-0.4788	-0.0563	0.013
nPCR [per g/day/kg body weight]	-1.1682	-1.823	-0.5134	0.0005
Albumin [per g/dL]	-1.0077	-1.2668	-0.7486	<.0001
Hgb [per g/dL]	-0.2274	-0.3358	-0.119	<.0001
Potassium [per mEq/L]	-0.0947	-0.2848	0.0955	0.3291
Phosphate [per mg/dL]	0.045	-0.0631	0.1531	0.4145
Congestive heart failure [Y/N]	-0.1441	-0.4423	0.154	0.3433
Diabetes [Y/N]	0.1829	-0.0291	0.395	0.0909
Treatment time [per hour]	0.0048	-0.0011	0.0107	0.1105
BMI [per kg/m2]	-0.0172	-0.0371	0.0026	0.0886

Figure 1. Predictors of sudden death (Poisson regression)

Conclusions: This international study identifies predictors of sudden death in chronic HD patients, including several ones, which are accessible to interventions. Presence of predictors associated with increased risk may alert the health care providers to deploy targeted interventions to reduce the risk of sudden death.

FR-PO1028

Utilization of an Implantable Loop Recorders to Characterize Cardiac Arrhythmias in Hemodialysis Patients Christian Combe,[1] Antoine Benard,[1] Jean-Philippe Bourdenx,[2] Haddj Elmrabet Atman,[3] Keller Adrien,[4] Frederic Lavainne,[5] Julien Ott,[6] Thierry P. Hannedouche,[7] F. Sacher.[1] *[1]CHU & Univ, Bordeaux, France; [2]CTMR St. Augustin, Bordeaux, France; [3]CHU, Rennes, France; [4]CH, Libourne, France; [5]CHU, Nantes, France; [6]CH, Haguenau, France; [7]CHU, Strasbourg.*

Background: Sudden cardiac death (SCD) is the most common mode of death among hemodialysis (HD) patients (pts). However, little is known about the terminal arrhythmic events in these pts. Our objective was to identify the mechanisms which may lead to SCD in HD pts using an implantable loop recorder (ILR, Reveal XT®, Medtronic).

Methods: Pts from 9 HD centers have been included in the study. None of the pts had a cardiac pacemaker or implantable cardiac defibrillator. Continuous monitoring of the cardiac rhythm is performed using the remote monitoring capability of the ILR device (Carelink®). Clinical, biological, and technical HD parameters are recorded.

Results: 72 pts (65.8±8.7 yrs, 50M) have been included. The most common causes of ESRD are diabetes (n=31) and hypertension (n=18). 33 pts had an underlying cardiomyopathy (20 ischemic). 15 pts had Left Ventricular Ejection Franction<60%. Atrial fibrillation (AF) was noted in 9 pts prior to inclusion. 49 pts are still followed-up. 12 pts died after 11±7 months: 6 pts from a non-cardiac cause (sepsis 2, hemorrhage 1, ischemic colitis 1). SCD occurred in 6 pts; a further patient suffered an acute coronary syndrome following vascular graft surgery. In SCD patients, ILR tracings demonstrated progressive bradycardia followed by asystole. No ventricular fibrillation was recorded. With ILR monitoring, AF was diagnosed in 9 pts, a pace-maker was implanted in 2 pts. More than 700 events have been recorded in 36 pts to date.

Conclusions: In our study mortality rate in HD pts was 12%, with 4.4% SCD. In SCD pts, the terminal arrhythmic event was asystole. AF was detected in 9% of previously undiagnosed pts. ILR monitoring will be performed 2 years for each pt; at the end of the 2-yr period, links between events and recorded parameters will be analyzed. We hope to be able to identify factors predictive of SCD, whether linked to the patient or to HD technique.

Funding: Pharmaceutical Company Support - Medtronic, Government Support - Non-U.S.

FR-PO1029

Mortality following Anticoagulation for Atrial Fibrillation in End Stage Renal Disease Patients Matthew J. Diamond,[1] Jennifer L. Waller,[2] Avirup Guha,[1] Jose R. Cuellar Silva,[1] William R. Maddox,[1] Mufaddal F. Kheda,[1] Robert A. Sorrentino,[1] N. Stanley Nahman.[1] *[1]Medicine, Georgia Regents Univ, Augusta, GA; [2]Biostatistics & Epidemiology, Georgia Regents Univ, Augusta, GA.*

Background: Atrial fibrillation (AF) is a major cause of embolic stroke (CVA) and is treated with lifelong oral anticoagulation (OAT). The $CHADS_2$ and CHA_2DS_2VASc (CHAD) risk stratification tools help select anticoagulation therapy in patients with AF. We showed that CHAD scoring predicts CVA and mortality in ESRD patients (Cuellar, JACC, 63:A297 and Murphy JACC, 63:A358). It is unclear, however, what effect OAT may have on mortality in this group. To address this question we queried the USRDS for the effect of OAT on mortality in AF patients.

Methods: All incident adult hemodialysis (HD) cases from the USRDS for 2005-2008 were queried for the determinants of CHAD scores. Data were derived for comorbid diagnoses using ICD9 and CPT codes, or data from Form 2728. Descriptive statistics by CVA or mortality were calculated and simple logistic regression was used to estimate the odds ratio (OR) for each potential risk factor. The administration of anticoagulation was inferred by the presence of at least one prothrombin time per month using CPT code 85610.

Results: 443,890 incident dialysis patients were identified, of whom, 39,387 had a diagnosis of AF at incident date of dialysis (IDD).Table 1 displays the incident of stroke and death during the query period, with detail given to patients on OAT.

Table 1
Patients with AF (39,387);
with AF and OAT (5,048)

	<90d from IDD	>90d from IDD	none
Stroke (3,129)	5.3%	2.6%	92.1%
OAT with Stroke (446)	6.0%	2.9%	91.1%
Death (27,632)	50.8%	19.3%	29.8%
OAT with Death (3,837)	57.4%	18.6%	24.0%
OR for Death Controlling for OAT (95% CI)	1.31 (1.21-1.42)	1.09 (0.99-1.20)	

Conclusions: AF in incident HD patients carries a high mortality within the first 3 months of starting dialysis. OAT therapy appears to substantially increase the risk of death in these patients. The therapeutic benefit of OAT therapy in AF patients on HD cannot be determined form these studies, but suggests it may be equivocal. Additional studies are indicated to assess risk factors and outcomes in this group.

Funding: Clinical Revenue Support

FR-PO1030

Safety of Warfarin Use in Chronic Hemodialysis Patients: A Prospective Cohort Study Matsuhiko Hayashi,[1] Mieko Iwai,[1] Maho Yamashita,[1] Tadashi Yoshida,[1] Yoshihiko Kanno.[2] *[1]Apheresis and Dialysis Center, School of Medicine, Keio Univ, Shinjuku-ku, Tokyo, Japan; [2]Div of Nephrology, Tokyo Medical School, Shinjuku-ku, Tokyo, Japan.*

Background: Since there are limited data concerning the risks associated with warfarin use in hemodialysis (HD) patients, we conducted a prospective cohort study in the patients on chronic HD in Japan.

Methods: The patients on chronic HD with warfarin use and their controls were recruited from 103 HD centers in Japan. For each patient on warfarin, two dialysis-vintage matched controls were selected at each HD center. Clinical data were collected at the beginning and every 12 months after the registration for 36 months.

Results: Preliminary one-year data was presented at ASN meeting in 2012, and the final data of three-year study is presented, here. At the start, 382 cases with warfarin and 720 controls were registered. In warfarin group, the occurrence of composite incidences (40.3%), which include death, new stroke, and new cardiovascular events, were significantly higher as compared to the controls (31.0 %, $p< 0.01$). The incidence of major bleeding (gastrointestinal, intramuscular, and hemorrhagic stroke) in warfarin users (15.7%) was also significantly higher than in the controls (7.8%, $p<0.0001$). By logistic regression model analysis, however, age (OR 1.004 for each 1-year increase, 95% CI 1.003-1.005, $p<0.0001$) and the antiplatelet drug use (OR 1.58, CI 1.02-3,05, $p<0.05$) but not warfarin use were identified as the risk factors for composite incidences, while warfarin use was the sole risk factor for major bleeding (OR 2.35, CI 1.45–3.82, $p<0.001$). Patients with warfarin for preexisting Af (200 cases) and their corresponding controls (379 cases) were analyzed separately, and warfarin use was not a significant risk factor for increase in composite incidences in this subgroup, either.

Conclusions: The present study showed that occurrences of major bleeding and composite incidences were higher in warfarin group, while age and antiplatelet use but not warfarin were identified as the risk factors for composite incidences in either entire population of the patients or the patients with AF. We conclude that warfarin may be less harmful than previously reported in HD patients.

FR-PO1031

Risk of New Onset Stroke Is Increased in Incident Hemodialysis Patients and Associates with Cardiovascular Risk Factors but Not Atrial Fibrillation Mark Duncan Findlay,[1,2] Peter C. Thomson,[2] Jesse Dawson,[1] Patrick B. Mark.[1,2] *[1]Inst of Cardiovascular and Medical Sciences, Univ of Glasgow, Glasgow, United Kingdom; [2]Western Infirmary, Renal Unit, Glasgow, United Kingdom.*

Background: Hemodialysis (HD) patients are at increased risk of stroke. Risk factors for stroke are defined in the general population but are less clear in HD. We studied incidence and risk factors for new onset stroke in a single center over a six-year period.

Methods: All patients undergoing in centre HD between 1st Jan 2007 and 31st Dec 2012 were identified. Study entry was 1st January 2007 in prevalent HD patients and from HD commencement in incident patients. Baseline clinical, laboratory and demographic data were recorded. The primary endpoint was stroke defined by occurrence of fatal (using death records) or non fatal (using hospital discharge / imaging records) ischemic or hemorrhagic stroke. Follow up was to 31st Dec 2013.

Results: 1382 patients were identified (mean age 62.9 years; 58.5% male; prevalence of ever having atrial fibrillation (AF) 21.2%). 59.4% were incident patients. 164 (11.6%) experienced new stroke (94.5% ischemic) over 3471 patient-years of follow up. Stroke incidence was 39.7/1000 patient-years in prevalent patients and 54.3/1000 patient-years in incident patients. At baseline, patients who had stroke on follow up were older (mean 68.4 versus 62.1 years, $p<0.001$), more likely to have diabetes (34.8 versus 27.2%, $p<0.05$) or prior CeVD (14.0 versus 2.2%, $p<0.001$). There was no greater proportion of strokes in patients with AF compared to without (23.2 versus 20.9%) with no differences in stroke

proportion between warfarin treatment or not in AF patients (log-rank p=0.7) Significant independent risk factors for stroke were age, prior CeVD, diabetes and hypoalbuminemia. In prevalent (but not incident) patients high systolic blood pressure, phosphate and low hemoglobin associated with risk.

Conclusions: Stroke incidence is high in HD patients with high comorbidity burden, particularly when clinical, imaging and mortality data are combined to ascertain cases. Incident and hypoalbuminemic HD patients are high-risk groups. Further studies are required to identify if targeting modifiable risk factors improves outcomes.

FR-PO1032

Cerebrovascular Diseasein China Dialysis Patients Ke Zheng,[1] Xuemei Li.[1] *[1]Renal, Peking Union Medical College Hospital, Beijing, China; [2]Renal, Peking Union Medical College Hospital, Beijing, China.*

Background: Stroke is one of the most common causes of cardiovascular disease death in patients on dialysis therapy, and there will be a series of subsequent consequences of the cerebrovascular disease, especially in cognition function. But by now, there are few data about these problems in China ESRD patients.Our study is a cross-sectional study aimed to disclosure the cerebrovascular disease in our ESRD (hemodialysis and peritoneal dialysis) patients.

Methods: By the end of May 2014, We included 183 participants aged 17 to 88 years who came from our dialysis center. Among them, 116 were on hemodialysis, 63 were on peritoneal dialysis, and 4 of them were on combined dialysis modalities. Basic information, clinical evaluations, laboratory tests, and MRI images were assessed. 99 of them had cognitive tests of MMSE (Mini Mental Test Examination) and MoCA (Montreal Cognitive Assessment).

Results: By MRI, the prevalence of Cerebral hemorrhage haematoma was 6.0% (11/183), microbleeds was 40.9% (75/183), large vascular cerebral infarction was 7.1% (13/183), lacunar infarction was 39.3% (72/183) and white matter lesions 44.3% (fazekas scale≥2,81/183). Abnormal in cognitive test was 81.8% by MoCA (81/99, MOCA <26) and 18.1% by MMSE (18/99, MMSE<26). Of MMSE score, There were no statistical difference between patients with or without lacunar infarction (Z=-1.751, P=0.08); patients with or without microbleeds (Z=-1.609, P=0.11), or Fazekas scales (Z=-0.840, P=0.40). Of MoCA score, there were statistical difference between patients with or without lacunar infarction (Z=-2.011, P=0.044), and Fazekas scales (Z=-2.531, P=0.011), but no statistical difference between patients with or without microbleeds (Z=-1.543, P=0.12).

Conclusions: In our dialysis patients, there are high prevalence of lacunar infarction, microbleeds, and white matter lesions. And these patients showed higher possibility of impaired cognitive function. MOCA may be more sensitive in cognition evaluation. Further studies are needed to discover the potential influence factors on these ESRD patients' brain lesions and cognitive function.

Funding: Government Support - Non-U.S.

FR-PO1033

Cardiovascular Disease as an Independent Factor Associated with Cognitive Impairment in Hemodialysis Patients -- A Cross-Sectional Analysis of Osaka Dialysis Complication Study (ODCS) Tetsuo Shoji,[1] Katsuhito Mori,[2] Masanori Emoto,[2] Masaaki Inaba.[2] *[1]Dept of Geriatrics & Vascular Medicine, Osaka City Univ Graduate School of Medicine, Osaka, Japan; [2]Dept of Metabolism, Endocrinology and Molecular Medicine, Osaka City Univ Graduate School of Medicine, Osaka, Japan.*

Background: Dementia-cognitive impairment is common in dialysis patients. Since cardiovascular disease (CVD) is also common in this population, we examined the association of CVD with cognitive function in a large sample of hemodialysis patients.

Methods: This is a cross-sectional analysis of the baseline data of our multicenter cohort of prevalent hemodialysis patients. Global cognitive function was evaluated with modified mini-mental state (3MS). We determined CVD as composite of the clinical coronary (CAD), cerebral (CeVD), peripheral artery disease (PAD) and congestive heart failure requiring hospitalization (CHF).

Results: Among the total of 1696 patients, we analyzed data from 1229 subjects who were examined with 3MS. The median age was 67 years, median dialysis vintage was 5 years, 63% were male, 39% had diabetes, and 21% had education of college or higher degree. Pre-existing CVD was found in 36% (N=439). Median (IQR) of 3MS core was 91 (81 to 97) point, and 196 subjects (16%) had 3MS score lower than 77 point, suggesting suspicious dementia. 3MS was lower in patients with CVD, particularly those with CeVD. Other factors associated with a lower 3MS were higher age, shorter dialysis vintage, diabetes, lower BMI, lower serum albumin, lower diastolic BP, and lower education level. Multiple logistic regression analyses indicated that the pre-existing composite CVD was associated with a lower 3MS independent of the potential confounders. When composite CVD was divided into CAD, CeVD, PAD and CHF, only CeVD was a significant factor independently associated with 3MS.

Conclusions: Pre-existing CVD, especially CeVD, was independently associated with cognitive impairment in hemodialysis patients, indicating the presence of vascular cognitive impairment in this population.

FR-PO1034

Predictors of Cardiovascular Events in Incident Hemodialysis Patients – Results From the International MONDO Initiative Rakesh Malhotra,[1,2] Len A. Usvyat,[2] Michael Etter,[3] Peter Kotanko,[2] Xiaoqi Xu,[4] Bernard J. Canaud,[3] Daniele Marcelli.[3] *[1]VUMC; [2]RRI; [3]FMC Germany; [4]FMC Hongkong; [5]MONDO Initiative.*

Background: Hemodialysis (HD) patients have increased risk of cardiovascular morbidity and mortality. We explored predictors of cardiovascular events in the MONitoring Dialysis Outcomes (MONDO) initiative.

Methods: Our cohort includes 22,443 HD patients initiating in-center treatments between 1/2006 and 12/2012, who were enrolled as part of MONDO (MONitoring Dialysis Outcomes) research initiative [Usvyat, Blood Purification 2013]. We extracted data for only those patients who survived at least 12 months after the start of HD. The mean of clinical and laboratory parameters were computed for the first 12 months (baseline). We constructed a Poisson regression model to predict cardiovascular events during months 13-60 (follow-up). Cardiovascular event was defined as combination of cardiovascular death, fatal or non-fatal myocardial infarction, angina and stroke according to ICD-10 and ICD-9 codes.

Results: 22,443 HD patients were studied (Eastern Europe: 4,969, Western Europe: 228, Northern Europe: 1,940, Southern Europe: 7,492, Western Asia: 2,134, North America: 5,680). The mean (SD) age was 63.7 (15.0) years, and 60.5% were males. The model, significant predictors, and b estimates with 95% CI are presented in Figure 1.

Figure 1. Poisson regression results with adjustment by geographical region

Parameter	Estimate	Hazard Ratio	95% Confidence Limits		P-value
Age [per year]	0.0419	1.043	1.035	1.051	<.0001
BMI [kg/m2]	-0.0406	0.960	0.941	0.980	<.0001
Albumin [per g/dL]	-0.5332	0.587	0.472	0.730	<.0001
Hgb [per g/dL]	-0.2033	0.816	0.745	0.894	<.0001
Sodium [per mmol/L]	-0.0469	0.954	0.926	0.983	0.002
IDWG [per % of body weight]	0.1019	1.107	1.005	1.220	0.039
eKt/V [per 1 unit]	-0.8397	0.432	0.280	0.665	<.0001
Diabetes [Y/N]	0.2918	1.339	1.109	1.616	0.002

Conclusions: Our large international study identifies clinical relevant risk factors to predict cardiovascular events in incident HD patients. Inflammatory marker (neutrophil to lymphocyte ratio) and pre-HD SBP>140 mmHg were not associated with cardiac events. These findings may help clinicians in the assessment of cardiovascular risk providing targets for intervention in HD patients.

FR-PO1035

A 12-Year Increasing Trend of Dialysis Incidence in Those Receiving Coronary Angiography in Taiwan Yu-Kang Chang,[1] Jia-Sin Liu,[1] Shang-Jyh Hwang,[2] Chih-Cheng Hsu.[1] *[1]Div of Geriatrics and Gerontology, Inst of Population Health Sciences, National Health Research Insts, Miaoli, Taiwan; [2]Div of Nephrology, Kaohsiung Medical Univ Hospital, Kaohsiung, Taiwan.*

Background: In patients undergoing coronary angiography (CA), the risk factors and incidence trend of contrast induced dialysis (CID) are unknown.

Methods: With a retrospective analysis of the registration and reimbursement files in the National Health Insurance (NHI) Research Database (NHIRD) in Taiwan from 1997 to 2009, we determined the incidence, the independent predictors, and the trend of CID (defined as developing acute or chronic renal failure requiring at least 2 dialysis therapies within 2 weeks after the index CA).

Results: Of 507,866 patients, in acute myocardial infarction (AMI) and non-AMI patients, the overall incidences of CID were similar (0.75% versus 0.62%). After multivariate regression analysis, the independent risk factors of CID included: female, diabetes, chronic kidney disease, congestive heart failure, bypass surgery and the years after 2002 to receive CA. There was a significant time trend in the increase of CID incidence for the last 12 years: compared with risk in 1998, the risk in 2009 were 3.7 (95%CI = 3.2-4.2) and 3.0 (95%CI = 2.9-3.5) times higher for AMI and non-AMI patients. CID requiring chronic dialysis accounted for 2% of total incident chronic dialysis in 1998 but over 5% in 2009.

Conclusions: The independent predictors of CID were consistent with most traditional risk factors for CIN (contrast induced nephropathy). The persistent increase of CID has become an important factor for heavy loading of end-stage renal disease in Taiwan.

FR-PO1036

Risk Factors and Long-Term Outcome Among Young Dialysis Patients with Acute Coronary Syndrome: A Population-Based Cohort Study in Taiwan Wei-Chih Kan,[1,2] Chih-Chiang Chien.[1,2] *[1]Nephrology, Chi-Mei Medical Center, Tainan, Taiwan; [2]Chung Hwa Univ of Medical Technology, Tainan, Taiwan.*

Background: There are only little studies known about the epidemiology, risk factors, and sex differences of acute coronary syndrome (ACS) in young adult patients on dialysis. The study aims to identify the risk factors for these young dialysis patients and survey for their long-term outcome.

Methods: We conducted an observational cohort study to investigate the risk factors and long-term outcome in the young dialysis patients (18-55 years old), who started maintenance dialysis between January 1999 through December 2007, based on Taiwan's National Health Insurance claim data. 24,520 young dialysis patients were enrolled for this study. The follow-up period was from the start of dialysis to the date of ACS, death, end of dialysis, or December 31, 2008. Several risk factors possibly affecting mortality were analyzed with Cox proportional hazards models. Kaplan-Meier method was also used for their long-term survival status.

Results: ACS was diagnosed in 1,325 patients (1.28/100 person-years) during the follow-up period. Male patients had a higher incidence of ACS (1.61/100 person-years) than female patients (0.97/100 person-years). After adjustment, young male dialysis patients were still found to be at 36% increased risk of ACS (HR 1.36, 95% CI: 1.22-1.53). There was not a notable association between dialysis modality risk of ACS. Patients with baseline comorbidities (diabetes mellitus, hypertension, congestive heart failure, coronary artery disease, cerebrovascular accident, dysrhythmia, dyslipidemia, and hyperuricemia) had a higher incidence of ACS than those without. Overall in-hospital mortality was 8.3%.

Conclusions: Young male adult ESRD dialysis patients were a significantly greater risk than female patients. Having baseline comorbidities was independently associated with higher risk of ACS.

FR-PO1037

Factors Associated with First Myocardial Infarction following the Initiation of Dialysis in U.S. Patients: 1995-2010 Austin G. Stack,[1,2] Mohamed Elsayed,[1,2] Cornelius John Cronin,[1,2] Liam F. Casserly,[1,2] Hoang Thanh Nguyen.[2] *[1]Nephrology, Univ Hospital Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland.*

Background: Cardiovascular disease is the leading cause of death among patients who progress to end stage kidney disease (ESKD). We sought to explore the relationship of patient and treatment-related factors with first major myocardial infarction among new dialysis patients in a population-based cohort study.

Methods: From the U.S. Renal Data System, we constructed a national cohort of 1,097,747 incident patients (n= 86,168 on PD) who were Medicare eligible and began dialysis between 5/1995 to12/2010 and followed until 9/2011. Hospitalizations attributed to first myocardial infarction (MI) [ICD 9 codes; 410.xx] were obtained from the USRDS standard analysis files and merged with data from the medical evidence, treatment history and mortality files. Multivariable logistic regression explored the relationship of patient-related factors, pre-dialysis care and treatment modality with the likelihood [Odds Ratio (OR)] of MI occurring in the period following dialysis initiation. The analysis was repeated using Cox regression with censoring at transplantation, death, recovery of kidney function, or end of study. Approval was granted from University Hospitals Ethics Committee.

Results: The likelihood of MI was significantly higher for older patients, women, patients with diabetes, hypertension, pre-existing cardiovascular conditions, smokers, drug dependence (all P<0.001), and for patients treated with erythropoietin in the pre-dialysis period (OR=1.03*). In contrast, the likelihood was lower for patients classified as underweight (OR=0.90*) and obese (OR=0.98*) compared to overweight (OR=1.03*) or normal weight (OR=1.00); and for peritoneal dialysis versus haemodialysis (OR =0.95*). A trend analysis found that the odds of MI was significantly higher in the period 2000-2005 (OR=1.89*) and 1995-1999 (OR=1.88*) compared to 2006-2010 (OR=1.00, referent). * P< 0.001.

Conclusions: Despite adverse cardiovascular profiles of incident dialysis patients, an encouraging decline in rates of myocardial infarction has occurred from 1995-2010. This trend is associated with the use of Peritoneal dialysis.

FR-PO1038

Abstract Withdrawn

FR-PO1039

Pharmacological Stress ^{13}N-Ammonia Positron Emission Tomography Myocardial Perfusion Imaging Indicates Myocardial Microvascular Dysfunction in Hemodialysis Patients Ryo Takahashi,[1] Keiko Kimura,[1] Chieko Matsubara,[1] Kiyohito Kawashima,[1] Satoru Ohshima,[2] Yasuhiko Ito,[3] Hirotake Kasuga.[1] *[1]Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan; [2]Cardiology, Nagoya Radiology Foundation, Nagoya, Japan; [3]Nephrology, Nagoya Univ, Nagoya, Japan.*

Background: Cardiovascular disease is one of the important complications in hemodialysis (HD) patients. Cardiac dysfunction is caused by not only coronary arterial sclerosis but also by myocardial microvascular dysfunction. ^{13}N-ammonia positron emission tomography (PET) myocardial perfusion imaging (MPI) can evaluate myocardial microvascular function. However, myocardial microvascular function (by PET MPI) was not studied in detail in Japanese HD patients.

Methods: Twenty HD patients, 10 non-dialysis chronic kidney disease (CKD; eGFR<60mL/min/1.73m^2) patients and 17 non-CKD subjects without perfusion defect (Summed Stress Score<3) were enrolled. Myocardial blood flow (MBF) was evaluated at rest and underATP induced hyperemia, and coronary flow reserve (CFR) was examined by ^{13}N-ammonia PET MPI. We assessed MBF and CFR in 3 groups. Also, we compared diabetic (DMHD) and non-diabetic HD (non-DMHD) patients (n=9 and 11, respectively) in MBF and CFR.

Results: MBF at rest in HD patients was 1.13mL/g/min and significantly higher than that in non-CKD patients (0.87mL/g/min, p<0.05). We did not find significant differences in stress MBF between 3 groups. CFR was significantly impaired in HD patients compared with CKD and non-CKD patients (2.21, 2.78, 3.07, P<0.01 versus CKD and p<0.01 versus non-CKD, respectively). No significant correlations between each parameter and hemoglobin or blood pressure were observed. In MBF at rest of HD patients, there was no significant difference between DMHD and non-DMHD. However, stress MBF in DMHD was significantly lower than that in non-DMHD (2.32mg/g/mL and 2.99mg/g/mL, p=0.02). In addition, CFR in DMHD was significantly impaired compared with that in non-DMHD (2.05 and 2.76, p=0.008).

Conclusions: Our data indicate that myocardial microvascular function is impaired in Japanese HD patients, especially in DMHD.

FR-PO1040

Predictive Value of Measures of Vascular Calcification for Risk of Death in Incident Dialysis Patients Antonio Bellasi,[1] Mario Cozzolino,[2] Domenico Russo,[3] Donald A. Molony,[4] Biagio Raffaele Di Iorio.[5] *[1]Ospedale Sant'Anna-Como; [2]Univ of Milan; [3]Univ "FEDERICO II" Napoli; [4]Univ of Texas-Houston; [5]PO "A Landolfi" – Solofra.*

Background: Vascular calcifications (VC) are a useful marker of cardiovascular disease and several methods are available for presence and extension assessment. However, which one of these measures best predicts long-term survival and whether a measure of vascular calcification adds to the predictive value of traditional Framingham risk stratification, has not been determined through a concurrent comparison of these measures in a single prospective cohort.

Methods: To addresses these questions, we examined survival amongst 184 patients followed in the independent study for up to 36 months who had three measures for vascular calcification determined at baseline; coronary artery calcification (CAC) by volume or Agatston score, and abdominal aorta calcification by X-ray (Kauppila score-KS). Regression models, ROC were used.

Results: for each VC assessment separately, the most parsimonious model to predict all-cause mortality was selected starting from a model adjusted for VC measure (CAC or KS), Pulse Wave Velocity, age, Framingham score, diabetes, ASCVD, systolic blood pressure, serum levels of phosphate, calcium, PTH, use of ARBs, beta-blockers, vitamin D, calcium containing phosphate binder, calcium channel blockers and cinacalcet. The predictive value of the model with and without the measurement of VC was calculated.

	CAC-Agatston			CAC-Volume			Kauppila score		
	without	with	p-value	without	with	p-value	without	with	p-value
Overall performance (R-square)	0.12	0.35	-	0.17	0.34	-	0.13	0.27	-
Metrics of discrimination (C-statistics)	0.74	0.9	<.001	0.77	0.89	<.001	0.73	0.84	<.001
Model fit statistics (AIC)	160	225	<.001	162	214	<.001	186	222	<.001
Patients reclassification (NRI)	-	1.27	<.001	-	1.07	<.001	-	0.92	<.001

Conclusions: Overall, it seems that CAC is a better predictor of outcome than abdominal aorta VC though the difference is minimal. Of interest, in each model VC is more important than the Framingham risk score in predicting all-cause mortality.

FR-PO1041

Ankle-Brachial Index Is Associated with Coronary Artery Calcification in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease Study Bernard G. Jaar,[1] Lucy A. Meoni,[1] Stephen M. Sozio,[1] Michelle M. Estrella,[1] Wen Hong Linda Kao,[1] Joao A.C. Lima,[1] Rulan S. Parekh.[1,2] *[1]Johns Hopkins Univ, Baltimore, MD; [2]Univ of Toronto, Toronto, Canada.*

Background: Patients on hemodialysis (HD) have a high prevalence of peripheral artery disease and coronary artery disease. Ankle-Brachial Index (ABI) is an indicator of generalized atherosclerosis, but its association with coronary artery calcification (CAC) has not been well evaluated in HD patients. We aimed to assess the relationships of low (<0.9) and high (>1.4) ABI with CAC in this population.

Methods: This is a cross-sectional study of incident HD patients. At enrollment, patients had CAC assessment by multi-detector computed tomography and were stratified as: 0-99 (none/mild); 100-399 (moderate); ≥400 (severe). ABI was classified into three groups: <0.9 (low), between 0.9 and 1.4 (normal), >1.4 or non-compressible (high). Clinical predictors (age, sex, race, smoking history, hypertension, diabetes, log transformed CRP, LDL, and calcium phosphate product) were assessed at baseline. Multivariable logistic regression was used to determine the association between ABI and CAC adjusting for other risk factors.

Results: A total of 232 patients (mean age 54±13 years, 58% male, 75% African-American, 59% ever smoked, 56% diabetic) were included with a median CAC of 44 (IQR 0, 451). Low ABI was present in 6%, normal in 68% and high in 26%. The table shows the odds of CAC by ABI category. Both low and high ABI categories were significantly associated with higher CAC. Further adjustment with laboratory assays did not change the results.

	100≤ CAC <400	CAC ≥400
ABI*	Odds Ratio (95%CI)	Odds Ratio (95%CI)
Unadjusted		
<0.9	3.94 (0.82-18.95)	5.57 (1.53-20.21)
>1.4	2.14 (0.92-5.00)	2.59 (1.31-5.15)
Adjusted**		
<0.9	4.23 (0.81-22.22)	6.49 (1.57-26.77)
>1.4	2.52 (0.97-6.58)	2.55 (1.09-5.95)
* Reference group: 0.9≤ ABI ≤1.4 ** Adjusted for age, sex, race, diabetes		

Conclusions: Low and high ABI were significantly associated with the extent of CAC in this incident cohort of HD patients. ABI is a non-invasive and inexpensive tool that can be easily used to evaluate cardiovascular health in HD patients.

Funding: NIDDK Support

FR-PO1042

The Different Cut-Off Value of Ankle Brachial Index of Hemodialysis Patients, which Predict Cardiovascular Diseases, by Presence of Diabetes Mellitus Makoto Harada,[1] Koji Hashimoto,[1] Wataru Tsukada,[3] Yosuke Yamada,[1] Akinori Yamaguchi,[1] Mai Sugiyama,[1] Taro Kanno,[1] Makoto Higuchi,[2] Yuji Kamijo.[1] *[1]Dept of Nephrology, Shinshu Univ School of Medicine, Matsumoto, Nagano, Japan; [2]Dept of Nephrology, National Hospital Organization, Matsumoto Hospital, Matsumoto, Japan; [3]Ueda Kidney Clinic, Ueda, Japan.*

Background: Severe atherosclerosis and vascular calcification, causing coronary artery stenosis and/or peripheral artery diseases, are frequently detected in hemodialysis (HD) patients. Ankle brachial index (ABI) is considered to be one of the useful predictable markers of development of cardiovascular diseases (CVD); however the cut-off value of ABI in HD patients, as well as its difference by presence of diabetes mellitus (DM), has been controversial.

Methods: A retrospective cohort study using 110 maintenance HD patients was conducted. To investigate the cut-off value of ABI which predicts CVD in HD patients, we performed receiver operating curve analysis using all clinical data. To evaluate the difference of the cut-off value by presence of DM, we separated the subjects into DM group and non-DM group, and compared the cut-off value among the groups.

Results: The cut-off value of ABI which predicts CVD in all HD patients was 0.98 (area under curve 0.76, sensitivity 0.67, specificity 0.78). Among these HD patients, the cut-off value of ABI in DM group was 1.05 (area under curve 0.74, sensitivity 0.81, specificity 0.61), while that in non-DM group was 0.96 (area under curve 0.77, sensitivity 0.71, specificity 0.87). Kaplan–Meier method indicated that the patients exhibiting ABI less than the cut-off values appeared to cause CVD with high possibility in each group.

Conclusions: The current study reveals that the cut-off value of ABI in HD patients, which predicts CVD, is higher as compared to that in general population. Moreover, the cut-off value of ABI in DM group would be further elevated. Although the mechanism underlying the elevation of cut-off value of ABI is still unclear, the excess vascular calcification in HD patients, especially that in DM group, may contribute it. When we evaluate the CVD risk in HD patients, we should consider the different cut-off value of ABI by presence of DM.

FR-PO1043

Subjective Global Assessment of Nutritional Status and Mortality in Chronic Dialysis Patients: A Nationwide Prospective Observational Cohort Study in Korea Nara Shin,[1] Eunjin Bae,[1] Ji In Park,[1] Sunhwa Lee,[1] Hajeong Lee,[1] Dong Ki Kim,[1] Yong-Lim Kim,[2] Shin-Wook Kang,[3] Chul Woo Yang,[5] Yon Su Kim.[1] *[1]Internal Medicine, Seoul National Univ Hospital, Seoul, Korea; [2]Internal Medicine, School of Medicine, Kyungpook National Univ, Daegu, Korea; [3]Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [4]Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea; [5]Internal Medicine, College of Medicine, the Catholic Univ of Korea, Seoul, Korea.*

Background: Malnutrition is a common and crucial problem because it is associated with higher mortality in chronic dialysis patients. Subjective global assessment (SGA) has been proven as a clinically adequate method for assessing nutritional status in dialysis patients. We explored the relationships between initial and transitions of SGA and all-cause mortality.

Methods: A nation-wide prospective observational cohort study included adult chronic dialysis patients. We selected patients who had baseline and 12 month follow-up SGA. SGA-7 score was categorized as well-nourished (SGA 6~7) and malnourished (SGA 1~5) groups. The changes of SGA were categorized by 4 groups (well-maintained, poorly-maintained, improving, and worsening group).

Results: A total of 2,600 patients were selected and mean age was 55.7±14 years old and 57.2% of patients were male. The mean follow-up period was 28.5 months. During the follow-up period, 202 patients (7.8%) were died. The mortality rate in malnourished patients was higher in both incident (5.2% versus 13%, p<0.001) and prevalent patients (6.3% versus 20.4%, p<0.001). The survival rate was significantly higher in well-nourished patients in 12 month follow-up (p<0.001). Moreover, in terms of SGA transition, improving and well-maintained groups had better survival rate than worsening group and poor-maintained group. These findings were reproducible even afteradjustment of age, gender, smoking, BMI, comorbidity, serum albumin, cholesterol and hs-CRP (HR 2.913, 95% CI, 1.856–4.573, p<0.001).

Conclusions: Malnutrition assessed by SGA was an independent predictor for all-cause mortality. Furthermore, the transition of SGA was also correlated with mortality in chronic dialysis patients.

FR-PO1044

Dialysis or Conservative Management in Chronic Kidney Disease (Stage 5) ? Evaluation of Patients in a Tertiary Hospital Who Started Dialysis in 2012 Natacha Rodrigues,[1] Maria João Nunes da Silva,[2] André Fragoso,[5] Maria Ferin Fraga,[2] Luis Santos Pinheiro,[2] Jose António Lopes,[1] Teresa Adragao,[3] Peter G. Lawlor,[4] António Gomes da Costa.[1] *[1]Dept of Nephrology, CHLN, Portugal; [2]Dept of Internal Medicine, CHLN, Portugal; [3]Dept of Nephrology, CHLO, Portugal; [4]Faculty of Medicine, Univ of Lisbon, Portugal; [5]Dept of Nephrology, Hospital de Faro, Portugal.*

Background: Studies suggest that in elderly patients with Stage 5, Chronic Kidney Disease (CKD-5) the survival benefit with dialysis can be lost if there is high comorbidity and low Performance Status, and thus Conservative Management (CM) can be a valid option. **Aims:** Todescribe hospitalised patients who started dialysis in a tertiary hospital in 2012: to determine mortality predictors; and to identify patients who could have benefitted from CM.

Methods: We retrospectively examined data from hospitalised CKD-5 patients, who were followed over a 23-month period. Patient data included their Karnofsky Performance Status (KPS); Mental Status (MS); Charlson (CCI); and Elixauser Comorbidity Index (ECI).

Results: Of our study sample (N=185) 57% were male, 50% were diabetic, 45% were 75 years or older (75+), 51% were admitted to Internal Medicine wards and 35% to a Renal ward. Mean follow-up was 12±6 months; 35% died, 47% during their first hospitalisation. Mortality was associated with age 75+ (p<.001); KPS<50 (p<.001); confusion and dementia (CDMS) (p=.001); ECI>5 (p=.015); CCI>8 (p=.05), heart failure (HF) (p=.002); coronary artery disease (p=.027); arrhythmia (p=.01); and CKDEPI (p<.001). Mortality predictors in a Cox regression model were: 75+ (HR 3.2; p=0.003); HF (HR 1. 9; p=0.03); CCI>8 (HR 3.6; p=0.003); CDMS (HR 2.3; p=0.003); and CKDEPI (HR 1.08; p<0.001). The 75+ patients (n=11) who were referred early to Nephrology with HF and KPS<50, and met standard CM criteria benefitted less with dialysis: 6 died and 1 recovered renal function.

Conclusions: CI and KPS status were useful in predicting mortality. Dialysis use and mortality could possibly be reduced by applying CM criteria. A prospective study in an outpatient renal clinic setting could identify robust CM criteria in frail elderly patients with high comorbidity.

FR-PO1045

Depressive Symptoms and Self-Management among Chronic Dialysis Patients Mi-Kyung Song,[1] Sandra E. Ward,[2] Jessica C. Bridgman,[1] Constance A. Gilet,[3] Gerald A. Hladik.[3] *[1]School of Nursing, Univ of North Carolina-Chapel Hill, Chapel Hill, NC; [2]School of Nursing, Univ of Wisconsin-Madison, Madison, WI; [3]UNC Kidney Center, Univ of North Carolina-Chapel Hill, Chapel Hill, NC.*

Background: Having depressive symptoms as measured by self-report is an independent risk factor for mortality in patients on chronic dialysis. However, evidence about whether dialysis patients recognize such symptoms and how they manage them is largely lacking.

Methods: Data are from an ongoing longitudinal observational study of quality of life in chronic dialysis patients (NCT01530945). Baseline and monthly measurements, including CESD-10, are made for 12 months. 107 patients have completed 926 data collection sessions. If a patient's CESD-10 score was ≥ 10 or if he/she responded affirmatively to a suicidal ideation item, an event report was generated to document the patient's response to symptoms and to determine whether the symptoms required a referral for further evaluation.

Results: There were 297 depression events with an average of 2.8 per patient. Of those, 192 (64.6%) were for mild to moderate symptoms (CESD = 10-14), 104 (35.1%) were for severe depressive symptoms (CESD ≥ 15), and 29 (9.8%) involved suicidal ideation. The most frequent contributing factors were 'being on dialysis' (43 events, 14.5%), 'managing other health issues' (43, 14.5%), 'financial difficulties' (32, 10.8%), 'family/personal relationship issues' (27, 9.1%), and 'pain' (20, 6.7%). In 15 events, patients themselves had been unaware of their depressive symptoms. The most common self-management behaviors for those who recognized depressive symptoms (282 events) were 'did not act on depressive symptoms' (158, 56.0%), 'talked to primary care provider or dialysis staff' (102, 36.2%), and 'did not try to talk to healthcare providers' (99, 35.1%). The primary reason for not informing care providers of their symptoms was distrust in the staff, e.g., 'they don't care', 'they can't do anything.'

Conclusions: Despite the high prevalence of depressive symptoms, many patients on chronic dialysis lack knowledge of effective self-management strategies and do not discuss their depressive symptoms with care providers.

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FR-PO1046

Feasibility and Acceptability of Routine Palliative Care Referral in Severely Ill Hemodialysis Patients: A Pilot Study Ion D. Bucaloiu,[1] Zankhana Mehta,[2] Jamie Alton Green,[1] H. Lester Kirchner,[3] Shiloh D. Erdley,[4] Patricia Maani,[2] Sally J. Regel,[2] Evan Norfolk,[1] Neil M. Ellison.[2] *[1]Nephrology and Hypertension, Geisinger Medical Center, Danville, PA; [2]Palliative Medicine, Geisinger Medical Center, Danville, PA; [3]Center for Health Research, Geisinger Medical Center, Danville, PA; [4]Sociology, Social work and Criminal Justice, Bloomsburg Univ, Bloomsburg, PA.*

Background: ESRD patients with advanced illness have unmet end of life needs. We evaluated the feasibility of a routine in-center palliative care intervention in severely ill dialysis patients.

Methods: Eligible patients were adult prevalent ESRD patients at an academic center dialysis unit for whom their primary nephrologist answered "'No" to the question "Would I be surprised if my patient died in the next 6 months?" or had a 12-month survival probability of less than 50%. We excluded patients with cognitive deficits, deemed non-adherent to dialysis treatment, non-English speaking or already in a hospice or palliative care program. The intervention consisted of 4 monthly palliative care visits during dialysis sessions. Surveys assessing quality of life, depression, illness understanding, symptoms, functional status and satisfaction with life were obtained at baseline and after each visit. Main outcomes included recruitment, retention and palliative care acceptability to patients.

Results: Thirty four (43%) of all unit patients met eligibility criteria. Of these, 22 (65%) consented to participate in the study. Of 22 patients enrolled, 17 (77%) completed all study activities, 2 (9%) withdrew from the study after discussing with family members at home but prior to the first study visit and 1 (5%) patient withdrew after the first study visit. Two (9%) patients died before completing study. Of patients who completed the study 17 (100%) were satisfied with the overall visit experience, and 13 (77%) felt that the palliative care visits would help them in the future.

Conclusions: An in-center routine palliative care intervention in severely ill ESRD patients is feasible and acceptable. Further research evaluating the impact of such collaborative care model in this population is needed.

Funding: Private Foundation Support

FR-PO1047

Outcomes of In-Hospital Cardiopulmonary Resuscitation in Maintenance Dialysis Patients in the United States, Years 2005-2011 Fahad Saeed,[1] Jesse D. Schold,[1] Jean L. Holley.[2] *[1]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; [2]Nephrology and Hypertension, Carle Physcians Group and Univ of Illinois at Urbana-Champaign, Urbana, IL.*

Background: There is no recent study on the outcomes of cardiopulmonary resuscitation (CPR) in end-stage renal disease (ESRD) patients requiring maintenance dialysis. Our primary aim was to analyze the outcomes of in-hospital cardiopulmonary resuscitation (CPR) in these patients in comparison with the general population.

Methods: We obtained and analyzed data from the Nationwide Inpatient Sample (NIS 2005- 2011). The study population included all adults (>18 years old) from the general population and those with a history of ESRD. Baseline characteristics, in-hospital complications, and discharge outcomes were compared between the two groups. The effects of in-hospital CPR on mortality, length of stay, cost of hospitalization, and discharge destination were analyzed. Two logistic regression models were created. Model one was used to identify the association between ESRD and odds of in-hospital mortality. Model two included patients who were discharged alive and aimed at identifying the association between ESRD and likelihood of discharge to a nursing facility versus other discharge destinations.

Results: During the study period, 56,069 ESRD patients underwent in-hospital CPR as compared to 323,620 patients from the general population. In-hospital mortality rates were higher in ESRD patients (73.9 % versus 71.8%, p=<.0001) on univariate analysis. On multivariate analysis after adjusitng for age, gender, and potential confounders, ESRD patients had higher odds of mortality: odds ratio 1.2, 95 %; CI 1.1-1.3, p=<.0001. Survivors in the ESRD group were more likely to be discharged to a nursing home (odds ratio 1.1, 95 %; CI 1.0 -1.2, p=<.04).

Conclusions: Survival after in-hospital CPR remains relatively poor. CPR outcomes remain worse in comparison with the general population. ESRD patients who survive are more likely to be discharged to nursing homes. This information should be communicated to dialysis patients during CPR discussions.

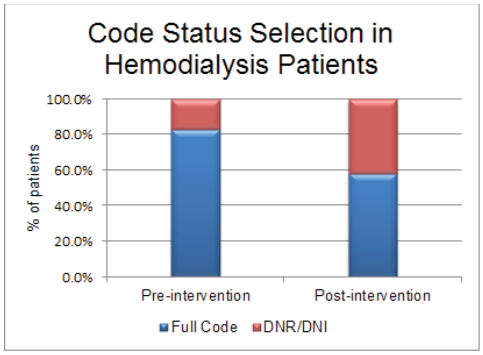
FR-PO1048

Dedicated Clinical Encounter Facilitates Advance Care Planning in Hemodialysis Patients: A Quality Improvement Project Osama W. Amro,[1] Malar Ramasamy,[1] James A. Strom,[2] Daniel E. Weiner,[1] Bertrand L. Jaber.[2] *[1]Nephrology, Tufts Medical Center; [2]St. Elizabeth Medical Center, Tufts Univ School of Medicine, Boston, MA.*

Background: The Renal Physicians Association Clinical Practice Guideline recommends that physicians address advance care planning with their patients. This quality improvement project implemented a standardized approach to educating dialysis patients with limited physician-estimated life expectancy on advance care planning and end-of-life care, with the goal of increasing autonomy and informed decision making.

Methods: Primary nephrologists identified their patients at two dialysis facilities who could most benefit from advance care planning using the 'surprise question: "Would I be surprised if this patient were to die within the next year?" Patients with a 'no' answer were approached in a dedicated clinical encounter on advance care planning. Information on the Massachusetts Medical Orders for Life-Sustaining Treatment (MOLST, a POLST analog) was provided. Family members were encouraged to participate in person or by phone.

Results: Of 201 hemodialysis patients, nephrologists answered no to the surprise question for 50 (25%). Of these, 9 (18%) had a "do not resuscitate/intubate (DNR/DNI)" order and 41 (82%) were "full-code". Following the encounter, 21 (42%) had a DNR/DNI order and 29 (58%) had a full-code order (Figure 1; P = 0.008). Encounters lasted 15-60 minutes. MOLST adoption rate increased from 10 to 90%.



Conclusions: A dedicated encounter targeting hemodialysis patients with limited life expectancy leads to significant changes in code status and identification of preferences on life-sustaining treatment limitations. This likely reflects better understanding of end-of-life care and informed decision making. Our approach was simple, practical, and effective. We will make this discussion routine for patients, repeating the encounter annually.

Funding: Other NIH Support - Dr. Osama Amro is supported by T32 NIH grant number 5T32DK007777

FR-PO1049

Validation of the Distress Thermometer in a UK Renal Population Helen Alston, Aine Burns. *UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: Older patients with advanced chronic kidney disease (CKD) and multiple co-morbidities have high symptom and depression scores in cross-sectional studies. However, long-form measurements of depression and symptom burden are not practical for regular routine screening use. We have found the Distress Thermometer (DT), a simple self-scoring visual analogue scale which records global distress (a composite of physical wellbeing, psychosocial and spiritual issues), easy to use in our multi-ethnic CKD population and have used it as a tool to enhance communication and ensure a patient-centred approach at routine CKD clinic patient visits.

Methods: Aim: To validate the Distress Thermometer (DT) in a UK renal population **Hypothesis:** The DT is a quick, acceptable and valid tool in UK renal patients, and correlates with the Beck Depression Inventory II (BDI-II), Hospital Anxiety and Depression Scale (HADS), Memorial Symptom Assessment Score (Short Form) (MSAS-SF) and SF36 scores. **Outcomes of interest:** DT, HADS, BDI-II, MSAS-SF and SF-36 scores **Study Design:** This is a method comparison study, which establishes that DT scores correlate with a variety of symptom, depression and quality of life tools. We also carried out an acceptability feedback questionnaire.

Results: 277 patients completed the study (138 haemodialysis patients, 139 low clearance clinic patients). The Distress Thermometer correlates well with depression (AUC for HADS 0.82, BDI-II 0.84) and symptoms (MSAS). There is inverse correlation with all eight SF-36 subscales, particularly energy, emotional well-being, and general health. Mean time to complete DT was <5mins, no patients found it objectionable.

Conclusions: The Distress Thermometer is correlated with depression and high symptom burden. Unlike many other widely available tools it is quick and easy to use at routine clinic visits, and is acceptable to a wide range of haemodialysis and CKD patients, including older patients and those with visual impairment. In addition, there are currently no copyright restrictions on its reproduction and use. This tool is already widely used as a screening tool in many oncology and palliative care services, and it should be adopted within the nephrology community also.

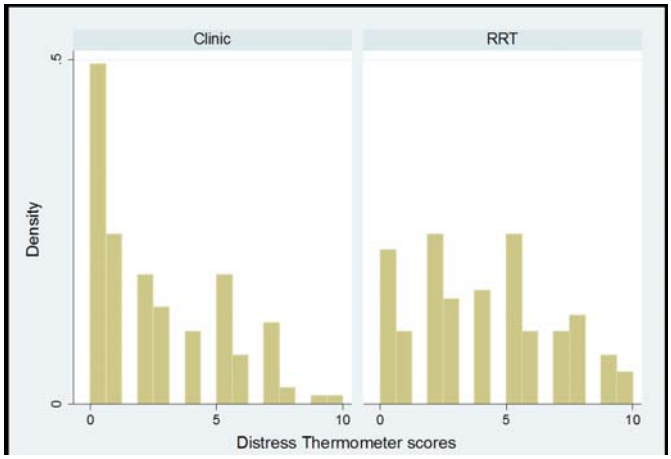
FR-PO1050

Haemodialysis Patients Experience Higher Levels of Psychosocial Distress Than Equivalent CKD Patients Helen Alston, Aine Burns. *UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.*

Background: It is known that older patients with advanced chronic kidney disease (CKD) and multiple co-morbidities have high symptom and depression scores in cross-sectional studies. However, few direct comparison studies between CKD patients and haemodialysis (HD) patients have been carried out.

Methods: Aim: To compare the Distress Thermometer (DT) scores in CKD and HD patients **Hypothesis:** Psychosocial distress is higher in HD patients than in CKD patients, even after adjusting for age and comorbidity. **Outcomes of interest:** Distress Thermometer scores **Methods:** The Distress Thermometer is a validated ultra-short screening tool used to measure psychosocial distress. We compared Distress Thermometer scores in 139 CKD patients from our pre-dialysis clinic (with eGFR <20mls/min) and 138 HD patients, and adjusted for age and comorbidity.

Results: Adjusted Distress Thermometer scores for HD patients were significantly higher than those of CKD patients [fig 1]. Median DT score for CKD patients was 2, median DT score for HD patients was 4.



Conclusions: Even after adjusting for age and co-morbidity, DT scores were higher in the HD group. Further work is needed to investigate what factors may be causing these higher levels of distress in HD patients.

FR-PO1051

A Cross Sectional Descriptive Study of Palliative Care Outcome Scale–Symptoms in End Stage Renal Disease (Pos-S-Renal) Reported by Haemodialysis Patients and Staff Thida M. Myint, Swarna Shashi Bhaskara, Michael G. Suranyi, Angela Makris. *Dept of Renal Medicine, Liverpool Hospital, Sydney, NSW, Australia.*

Background: The Palliative care Outcome Scale-Symptoms (POS-S-Renal) is a validated instrument to measure patients' physical and psychological needs in end stage renal disease. We aim to describe the symptom burden in haemodialysis patients, as self-reported by patients and as assessed by nursing staff by using this instrument.

Methods: All prevalent haemodialysis patients from five dialysis centers and their staff were invited to participate this study in March 2013. The participants simultaneously completed POS-S-Renal in the same week. Symptoms were assessed using this 18-item questionnaire, which was offered in several languages. Higher scores were associated with higher disease burden. Data were analyzed by paired t test and multivariate analysis using SPSS v21 and p considered significant if <0.05.

Results: Out of a total of 163, 150 haemodialysis patients were recruited (92% response rate), 57% were males and 28% had 3 or more comorbidities. Mean age was 63 (+/- 14) years and dialysis vintage was 69 (+/- 66) months. There was no difference in age, gender, race, religion and language spoken between the responders and non-responders. Most reported symptoms were weakness or lack of energy (70%), poor mobility (51%), difficult in sleeping (51%), pain (46%) and itchiness (45%). There was a significant difference in total scores between patients with multiple comorbidities and those with fewer comorbidities (11.6 versus 8.8, p = 0.04, 95% CI 0.83 to 5.89). Similarly, In-Centre dialysis patients had higher scores compared with Satellite patients after adjusting for age and sex (p = 0.006). In addition, there was a significant difference in total score between patients and nursing staff (9.71 versus 5.8, p = 0.001, 95% CI 2.81 to 4.99). Staff under-reported patients' symptoms in pain (t (150) = 4.72, p < 0.001), shortness of breath (t (150) = 4.45, p < 0.001), lack of energy (t (149) = 4.74, p < 0.001) and nausea (t (149) = 3.87, p < 0.001).

Conclusions: In-Centre haemodialysis patients and patients with multiple comorbidities had higher disease burden however staff tended to underestimate patient symptoms.

FR-PO1052

Program for the Attention of Terminal Renal Disease Pain in Dialysis Units: Prevalence of Pain in Chronic Hemodialysis Pedro Garcia Piqueras,[1] Daniel Herrero Rivera,[1] Alberto Martin Diaz,[1] Pablo Pagliarani Gil,[1] Roberto Martin,[2] Adriana Puente Garcia,[2] Maite Marin,[2] Ramiro Callejas,[2] Fernando Garcia Lopez,[3] Alberto Tejedor Jorge.[1] *[1]FAC-MED, Univ Complutense, Madrid, Spain; [2]Fundación Renal Iñigo Álvarez de Toledo, Spain; [3]Spanish National Inst of Health, Spain.*

Background: Pain (P) is a frequent symptom among hemodialysis (HD) patients; in some instances it is present in at least 50% of the cases with great impact in health-related quality of life. Our aims are to quantify, identify and assess control in the HD population.

Methods: Multicenter transversal study in a total of 17 dialysis units, 6 hospitals and 11 centers in Spain, with the aim of improving the management of HD P. The sample was calculated with a P prevalence of 70%, a margin of error 5,8%, and an alpha value of 0,05. The samples were stratified by center and sex in 755 patients. Exclusion criteria were the lack of knowledge of the Spanish language and severe cognitive deterioration. Participation was accepted through informed consent. The presence, type and intensity of the P were determined through the McGill-SV, Brief P inventory and CAD (coping to the chronic P) and SF-36 questionnaires. Comorbidity was determined with the Charlson index.

Results: 252 patients were examined with an average age of 65.1 years. The P prevalence found was of 101 cases; as for the intensity 47 (46,5%) quantified the P as moderate-severe, while 27 (27%) quantified it as severe. The P was weak in 10 cases (10,4%), bearable in 46 (48%), intense in 24 (25%), and terribly bothersome in 16 (17%). Among the men, 61 (59%) suffered some kind of P along with 40 (41%) women, while 24 (16%) men and 23 (23%) women suffered from moderate P, odds ratio 1,66 (IC 95% from 0,9 to 3,2).The nociceptive P was the most frequent (54%), followed by the mixed type (42%), and the neuropathic type (3,5%). There was a non-significant tendency toward greater P frequency with higher ages. For VAS<5 the average Charlson age-comorbidity index was 7,81; and for VAS>5 it was 9,38 with a difference of 1,58 (IC 95% from 0,6 to 2,5).

Conclusions: P is very frequent in HD patients and affects almost half of them. Patients who suffer from P have a higher age-comorbidity index.

Funding: Government Support - Non-U.S.

FR-PO1053

The Hemodialysis Patient Voice: Perspectives of Communication and Advance Care Planning (ACP) within Hemodialysis Units Nwamaka Denise Eneanya, Sarah L. Goff, Michael J. Germain, Mark L. Unruh, Lewis Cohen. *The Renal Supportive Care Study, Patient-Centered Outcomes Research Inst.*

Background: More than 20% of the approximately 400,000 hemodialysis patients in the U.S. die annually yet supportive care at the end of life is underutilized. We qualitatively explored dialysis patient and family experiences regarding supportive care to identify opportunities for improvement.

Methods: In-depth interviews were conducted with purposively sampled patients and family members from dialysis units at two study sites. Applying grounded theory, interviews were audio-taped, professionally transcribed, and analyzed in an iterative process by three research team members. Emergent concepts and themes were identified, discussed and then organized into major themes and sub-themes.

Results: 13 patients and 8 families participated; 12 were female. Patients identified as Black (1), Hispanic (2), Navajo (1), Pacific Islander (1), and White (8). Four major themes and numerous sub-themes were generated from analysis.

Major Themes	Sample quotes
1. Patient's prior experience with prognostic discussions and ACP	"I think a lot of people go on dialysis and they think...of that as a cure and that is not a cure..."
2. Factors that impact patients' perspectives of ACP and prognostic discussions	"...I always felt that she [a nurse] didn't really like [me]… she's never actually talked to me unless she was in charge of the unit...I feel there's a lot of favoritism..."
3. Patient recommendations for carrying out discussions about prognosis and ACP	"I would like to sit down with a physician who is compassionate but direct. Give me the fine points."
4. Family and friends' perspectives	"…I wish there was somebody that could play the role I play [advocate] on behalf [of] each patient" "I would not talk to a social worker or a nurse. I would want a physician to tell me exactly what's going on..."

Conclusions: Participants reported how poor communication and discontentment impact ACP discussions. Life experiences and personal outlook influence perspectives. These findings may be useful in designing interventional studies and protocols to improve patient management at the end of life.

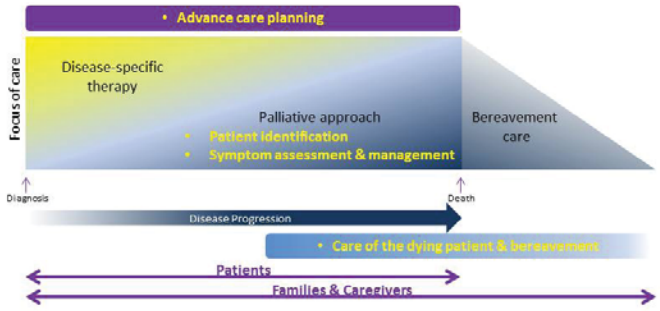
Funding: Other U.S. Government Support

FR-PO1054

Establishing an Integrated Population-Based Approach to Renal Palliative Care Helen Chiu,[1] Donna Murphy-Burke,[1] Ronald Werb,[2] John A. Duncan,[2] Gaylene M. Hargrove,[2] Adeera Levin,[2] Mohamud A. Karim.[2] *[1]BCPRA; [2]UBC, BC, Canada.*

Background: Chronic kidney disease (CKD) is characterized by high symptom burden and poor life expectancy at advanced stage. Functional and cognitive decline results in difficult end-of-life (EOL) conversations involving patients, families and care providers. An integrated approach to timely advance care planning (ACP) and EOL care spanning the CKD care continuum is needed.

Methods: Utilizing the provincial renal network in BC, Canada, an expert panel was formed to create an evidence-based renal EOL Framework that articulates 4 pillars of renal palliative care: patient identification, ACP, symptom assessment and management, and care of the dying patient and bereavement.



EOL champions from the 5 regional renal programs led the local implementation of the Framework. Education and support tools were developed provincially to facilitate uptake and capacity creation among frontline care providers. Progress over a 5 year period was verified with surveys and semi-structured interviews across the province.

Results: The 4 pillars of the EOL Framework were adapted across BC in ways that matched local needs and resources. Formal processes in ACP and symptom control with use of standardized assessment have been established in all renal programs. In a recent survey for frontline nephrologists and staff, 61% of respondents had EOL care training with 50% felt that they need more education to stay current. Enabling an organizational culture open to integrate palliative approach into routine renal care remains a challenge, and cementing relationships with palliative services is key.

Conclusions: Contextualizing the EOL Framework is crucial to integrating supportive care into daily renal care. To promote quality EOL care, there is a need to develop a person-centred evaluative rubric for continual improvement and research activities that bridge the knowledge gaps in renal palliative care.

Funding: Government Support - Non-U.S.

FR-PO1055

Self-Reported versus Measured Physical Activity in Older Adults with CKD

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Background: It is uncertain to what extent low physical activity contributes to poor physical performance in older CKD patients, and how best to estimate such activity.

Methods: The AWARD study is a randomized trial of exercise training in older adults with CKD stage 3b-4. Self-reported activity at baseline was quantified with the CHAMPS questionnaire, an instrument validated in community-dwelling older adults. Using the Step Activity Monitor over 5 days, we quantified: 1) steps/day, 2) total sedentary time, 3) total time in ≥moderate walking (≥15 strides/min). Lower extremity performance was assessed using the Short Physical Performance Battery (SPPB).

Results: Among N=46 with complete activity data, mean age was 69.5±7.9 years and eGFR was 34±10 ml/min/1.73m^2, 52% were Afr-American, 28% women, and 40% had CVD. Median steps measured were 2,702/day [interquartile range: 2,060-4,873], and median waking time spent inactive was 73.6% [61.9%-79.8%] Self-reported physical activity did not correlate with direct measures of activity (table) nor lower extremity performance. In contrast, directly measured activity and inactive time were both correlated with performance.

Correlation (ρ) of self-reported and directly measured physical activity and performance

Measured Activity/ Performance	Self-Reported Activity			Measured Activity	
	Kcals/week	Kcals/week ≥Mod. Activity	Min/week of ≥Mod. Activity	Steps/day	% Time Inactive
Steps/day	0.03 (p=0.8)	0.11 (p=0.5)	-0.01 (p=0.9)		
% Time Inactive	-0.07 (p=0.7)	-0.10 (p=0.5)	0.09 (p=0.5)	-0.74 (p<0.001)	
% Time ≥moderate pace walking	-0.01 (p=0.9)	-0.05 (p=0.8)	-0.16 (p=0.24)	0.90 (p<0.001)	-0.78 (p<0.001)
SPPB	0.17 (p=0.3)	0.17 (p=0.3)	0.13 (p=0.4)	0.41 (p=0.005)	-0.34 (p=0.02)

Conclusions: Levels of physical activity are very low among ambulatory older adults with CKD and correlate with poor lower extremity performance. However, self-reported activity is not a reliable surrogate for measured activity.

Funding: NIDDK Support, Veterans Affairs Support

FR-PO1056

Physical Function and Estimated Glomerular Filtration Rate in Advanced Chronic Kidney Disease

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Background: Older adults with reduced eGFR may have lower physical activity and poorer physical performance. Previous research focused on the relationship between eGFR and daily activity or on objective physical performance testing. This cross sectional analysis seeks to assess the association between eGFR and objective physical functioning independent of daily activity level.

Methods: The Aerobics, Weights and Renal Disease (AWARD) trial is a randomized controlled trial evaluating the effects of exercise versus health education in individuals 55 years and older with CKD stage 3b-4. Participants' physical functioning was assessed through VO_2 peak, 6-minute walk distance, 4 meter walk gait speed, Chair Rise time (CRT), and Get Up and Go time (GUGo). Daily physical activity was assessed by average daily number of steps as recorded by Step Activity Monitor worn for 5 consecutive days.

Results: Of 51 participants enrolled by May 29, 2014, 48 completed baseline testing and were included. Mean age is 69±8 years, 29% are women, 52% are African American and 40% have history of CVD. Mean eGFR is 34±10 ml/min/1.73m^2 and hemoglobin is 12.6±1.7 g/dL. Mean VO_2 is 16.9±6.2 ml/kg/min, gait speed 0.84±0.16m/s, 6 minute walk distance 396.9±114 meters, CRT 12.34±3.38 sec, and GUGo 8.36±2.2 sec. In multivariable analyses controlling for age, sex, race, CVD, Hb, and average daily steps, lower eGFR was significantly correlated with all measures of physical function. For every 10ml/min lower eGFR there was a 2.11 (95% CI: 0.71-3.51) ml/kg/min decrease in VO_2 (p=0.006), a 0.04 (95% CI: 0-0.09) m/s decreased gait speed (p=0.04), a 35.4 (95% CI: 10.4-60.4) meter decrease in 6 minute walk distance (p=0.008), a 1.33 (95% CI: 0.43-2.23) second increase in CRT (p=0.006), and a 0.77 (95% CI: 0.27-1.27) second increase in GUGo (p=0.009).

Conclusions: Lower eGFR is associated with reduced physical functioning as measured by multiple objective testing modalities. This association is not explained by habitual physical activity, demographics, anemia severity, or CVD history.

Funding: NIDDK Support

FR-PO1057

Chronic Kidney Disease Associated with an Increased Mortality Risk in Elderly Patients after Hip Fracture

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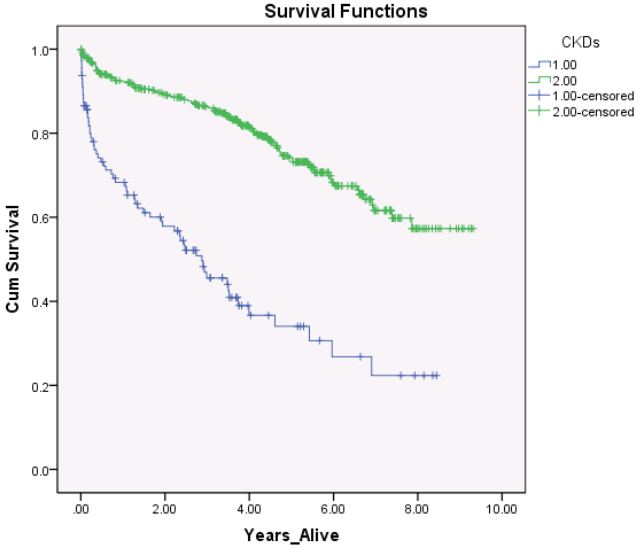
Background: Hip fractures are associated with significant morbidity and mortality. We studied if worsening renal function conferred an increased risk of mortality in patients after sustaining a hip fracture.

Methods: This was a prospective cohort study of all type 2 diabetes mellitus patients (N=850) admitted with a diagnosis of hip fracture from years 2005-2010 to a single institution. Primary end-point was all-cause mortality. Creatinine levels were taken at admission, and estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.Patients with eGFR≤30 ml/min/1.73m^2 (Group 1) were compared with patients with eGFR >60ml/min/1.73m^2 (Group 2) and their mortality risk was compared using Kaplan-Meier survival curves and multivariate Cox survival analysis.

Results: The cohort (aged 78.2 ± 8.3 years, 70.9% females) had a mean eGFR level of 62.9± 30.9 ml/min/1.73m^2. During a median follow-up of 7.4 years (range 0- 9.5 years), 295 patients died (33.5%). Patients with eGFR ≤30ml/min/1.73m^2 (N=112) had increased mortality, with median survival of 3.6years (95% CI: 2.9 – 4.3years) after a hip fracture compared to patients with eGFR >60 ml/min/1.73m^2 (N=433) who had better median survival of 7.0years (95% CI: 6.7 – 7.4years). By 5 years, only about 30% of group 1 were still alive compared to approximately 75% of patients in group 2.



Conclusions: Progressive renal impairment appears to be a predictor of poorer outcome in patients who sustained a hip fracture. This highlights the importance of chronic kidney disease (CKD) prevention and the need to look into improving the survival of CKD patients.

FR-PO1058

Only the Fittest Make It into the USRDS. Chart Abstraction versus USRDS Claims Data for Dialysis Patients over 75

Bjoerg Thorsteinsdottir, LaTonya J. Hickson, Priya Ramar, Megan Reinalda, Amy W. Williams, Nilay D. Shah. *Mayo Clinic, Rochester, MN.*

Background: The United States Renal Data System (USRDS) is designed to capture information about patients with ESRD. Most very elderly patients needing renal replacement therapy (RRT), start in the hospital after acute kidney injury. With the policy requirement of ESRD or 90-days of treatment prior to entry into the USRDS population, clinical events such as early death and renal recovery may be missed. Thus, use of this data to predict outcomes for elderly incident RRT patients may be incomplete and misleading Aim: Identify and quantify gaps in the USRDS data base determined outcomes for elderly dialysis patients.

Methods: Setting: regional not-for-profit U.S. health system. A cohort of 272 patients who started RRT in our Network in 2007-2010 and had data abstracted were matched to a USRDS cohort of 7113 patients who dialyzed at our facilities 2000-2010. Patients were grouped based on whether or not they were in the USRDS. Group characteristics and outcomes including survival to hospital discharge, 90 day and 6 month mortality and renal recovery were assessed.

Results: 43% of the cohort was not captured by USRDS. While demographics were not significantly different between the groups, there were significant differences in outcomes. Patients not in the USRDS cohort were more likely to start in the hospital and die in the first 6 months following incident RRT.

	Overall N=272	In USRDS N=155	Not in USRDS N=117
Age Median (IQR)	80.0 (77.0, 84.0)	81.0 (78.0, 84.0)	80.0 (77.0, 85.0)
Male N (%)	185 (68%)	110 (71%)	75 (64%)
Charlson Score Median (IQR)	N=269 8.0 (6.0, 10.0)	N=154 8.0 (6.0, 10.0)	N=115 7.0 (5.0, 9.0)
Hospital start* N (%)	205 (75%)	89 (57%)	116 (99%)
Total Deaths*	230 (84%)	123 (79%	107 (91%)
90 d mortality*	130 (47%)	44 (28%)	86 (73%)
6 mo mortality*	138 (50%)	49 (31%)	89 (76%)
Renal recovery*	47 (24%)	10 (12%)	37 (33%)

Conclusions: Significant differences in outcomes among elderly renal disease patients not captured by USRDS data suggest limited generalizability in drawing conclusions on survival for these patients. The USRDS cohort represents a healthier group of RRT patients significantly overestimating survival for the oldest.

FR-PO1059

Chronic Kidney Disease (CKD) Is Associated with Disorders of Balance

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Background: CKD is associated with limitations in physical activity and ADL dependence. Loss of balance can lead to falls, fear of falling, and physical inactivity. The objective of this analysis is to examine the cross-sectional association between CKD and disorders of balance among U.S. adults using the continuous National Health and Nutrition Examination Survey (NHANES).

Methods: A nationally representative sample of adults ≥40 yrs enrolled in NHANES 2001-2004 were included. Balance was tested using Modified Romberg test (MRT) consisting of four conditions (combinations of eyes open/ closed and firm/ compliant foot surfaces) designed to evaluate visual, proprioceptive, and vestibular system input in maintaining balance. Kidney function was estimated using eGFR-Creatinine. Chi-squared tests and uni/ multivariate logistic regression were used to compare prevalence of MRT failure across CKD categories.

Results: Of 5,530 subjects with results for MRT and creatinine, 49.2% were female, 17.3% black, mean age was 59.3±13.0 yrs, mean BMI 28.3±5.3, and 12.6% were diabetic. Prevalence of CKD was: Stages 1-2 (eGFR≥60), 90.6%; 3-5 (eGFR<60), 9.4%. Unadjusted OR for failing MRT was 3.7 (95% CI 3.0, 4.5) comparing those with and without CKD. More subjects with CKD failed every condition of the MRT (Table 1). The association between CKD and failure of MRT was attenuated after adjustment for age and diabetes (adjusted OR 1.53, 95% CI 1.06, 2.25).

Condition	Eyes	Surface	Sensory Input Assessed	CKD+	CKD-
1	Open	Firm	Visual, Proprioceptive, Vestibular	0.6	0.1*
2	Closed	Firm	Proprioceptive, Vestibular	7.5	2.4*
3	Open	Compliant	Visual, Vestibular	4.0	0.9*
4	Closed	Compliant	Vestibular	59.9	37.6*

Table 1. Association of CKD with Balance Disorders as Assessed by %Failing Modified Romberg Test. *p<0.05 for unadjusted comparison of CKD+/-.

Conclusions: Problems with balance, as measured by MRT, are frequent in CKD and are related to dysfunction of visual, proprioceptive, and vestibular senses. While the association between failure of Romberg test and CKD is partially explained by age and diabetes, there remains a significant association between CKD and balance even after adjustment for these factors.

Funding: Clinical Revenue Support

FR-PO1060

Predictors of In-Hospital Mortality among Elderly Patients Treated with Dialysis for Acute Kidney Injury

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Background: The factors that determine prognosis in elderly patients who are dialyzed for acute kidney injury (AKI) are uncertain. The purpose of this study was to specifically examine predictors of in-hospital mortality in this patient population.

Methods: A retrospective, single-center study of hospitalized patients ≥70 years old treated with intermittent hemodialysis for AKI. Patients with stage 5 chronic kidney disease were excluded. Clinical and demographic variables were compared between survivors and non-survivors; independent predictors of in-hospital mortality were identified by logistic regression.

Results: From March 2006 to October 2013, 137 patients, aged 70-103 met inclusion criteria. Hospital mortality was 66%; 59% of survivors were dialysis dependent at discharge. There was no significant difference in age between survivors (80.2) and non-survivors (80.5) (p=0.829). Non survivors had higher rates of hypotension (29.5% versus 13.6%, p=0.048), decreased level of consciousness (68.2% versus 22.2%, p<0.001), leukopenia/leukocytosis (62.6% versus 42.2%, p=0.024), ICU admission (59.3% versus 34.8% p=0.007), mechanical ventilation (64% versus 21.7% p<0.001), hepatic dysfunction (46.2% versus 21.7%, p=0.005), a diagnosis of sepsis (64.8% versus 26.3%, p=0.04), and vasopressors use prior to initiation of dialysis (69.8% versus 35.6%, p<0.001). Patients with ≥ 5 of these conditions had >90% mortality rate. Non survivors had lower mean serum albumin (2.48 versus 2.91 g/dL, p=0.001), and more failing systems (mean 2 versus 1.0 p<0.001) than survivors. There was no difference in chronic co-morbidities and baseline functional status. Logistic regression showed decreased level of consciousness (OR=7.4, 95% CI=3.0-18.2 p<0.0001) and mechanical ventilation (OR=6.0; 95% CI=2.5-14.6 p<0.001) independently predict in-hospital mortality.

Conclusions: Elderly patients dialyzed for AKI have a very high rate of in-hospital mortality or dialysis-dependent survival. Prognosis appears to be determined primarily by the severity of acute illness. Advanced age per-se should not be the sole criterion for decisions about dialysis in AKI.

FR-PO1061

Dialysis in the Elderly- Factors Associated with Poor Survival

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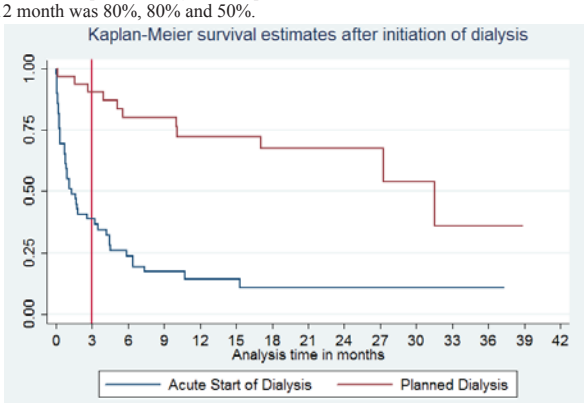
Background: A rapidly aging population worldwide presents challenges in the care of the elderly patient with ESRD. Patients and their families often choose dialysis without a clear understanding of its implications on survival and quality of life.

Methods: This is a retrospective analysis of dialysis patients 75 years and older initiated at our centre, October 1,2010 –Feb 1,2014. Outcomes were identified from our electronic database with follow up from initiation to death or May 1,2014.Univariate then multivariate Cox proportional hazards regression models were performed to assess the predictive values of demographic and clinical factors.

Results: Of the 89 patients initiated on dialysis, 55 (63%) were females, median age was 82 years (Interquartile range (IQR) 78-85), 69% had diabetes.Five patients withdrew within 30 days of initiation and were excluded from the analysis. The median follow up time was 5 months (IQR:0.8-31.5).While age at initiation did not affect survival, having 3 or more comorbid conditions increased the risk of death 3 fold.The majority, 49 patients (61%) started dialysis during an acute admission for acute medical or surgical illness. Continuous renal replacement therapy (CRRT) in the ICU was the initial modality in 32 (40%). In those initiated during an acute admission the mortality was 76% during the first 90 days. Their subsequent survival at 3 month, 6 month and 12 month was 24%,17% and 9%. However in patients dialysed in a planned manner the survival at 3 month, 6 month and 12 month was 80%, 80% and 50%.



Conclusions: This study suggests age alone as a parameter at initiation of dialysis in the elderly does not predict a worse outcome. Multiple comorbidities at initiation predicts a worse outcome. The poorest survival is seen in those with an acute hospital start to dialysis.

FR-PO1062

Predicting Survival on Variations of BMI and Serum Albumin in Elderly Hemodialysis Patients Cédric Villain,[1,2,3,4,5] Rene Ecochard,[1,2,3,4] Denis Fouque.[2,3,5,6] *[1]Service de Biostatistique, Hospices Civils de Lyon, Lyon, France; [2]Univ de Lyon, Lyon, France; [3]Univ Lyon 1, Villeurbanne, France; [4]UMR5558, Equipe Biostatistique-Santé, CNRS, Villeurbanne, France; [5]Service de Néphrologie-Nutrition-Dialyse, Centre Hospitalier Lyon Sud, Lyon, France; [6]CarMeN, U1060, INSERM, Lyon, France.*

Background: In elderly hemodialysis patients, protein-energy wasting is associated with poor outcome but the association between wasting-marker changes over time and survival has been seldom studied.

Methods: We analyzed data on patients aged ≥75 years from the French cohort ARNOS, of whom 556 had ≥2 assessments of body-mass index (BMI) and 576 had ≥2 assessments of serum albumin (SAlb) at six-month intervals. Changes in nutritional markers were estimated by individual linear regression models. Survival analyses used frailty Cox models with random effects on age and dialysis vintage categories. We considered BMI in subgroups of sex and diabetes and analyzed SAlb adjusting on sex, diabetes, and C-reactive protein.

Results: There was a great difference in BMI/survival association between men and women as between diabetic and non-diabetic patients (Table 1) but no association between survival and Baseline BMI or BMI changes in diabetic women. A 0.1 g/dL increase in Baseline SAlb was associated with a 0.95 hazard ratio of death (95% CI: 0.92-0.98, p<0.001). A 0.1 g/dL/year increase in SAlb was associated with a 0.90 hazard ratio of death (95% CI: 0.87-0.94, p<0.001).

Conclusions: Changes in BMI and SAlb over time were strongly associated with the risk of death in elderly hemodialysis patients. Monitoring and enhancing these markers should ensure better outcomes in these frail patients.

Table 1: Association between Baseline BMI, BMI annual variation, and mortality according to sex and diabetes

Sex, Diabetes	BMI criterion	Hazard Ratio	CI 95%	*p*
Men, non diabetic	Baseline BMI*	0.948	0.893-1.006	0.08
	BMI change**	0.655	0.541-0.792	<0.001
Women, non diabetic	Baseline BMI	0.932	0.861-1.008	0.08
	BMI change	0.745	0.603-0.919	0.006
Men, diabetic	Baseline BMI	0.887	0.803-0.981	0.02
	BMI change	0.628	0.515-0.765	<0.001
Women, diabetic	Baseline BMI	1.000	0.908-1.101	1.00
	BMI change	0.912	0.702-1.184	0.49

*per 1 kg/m² increment, **per 1 kg/m²/year increment.

FR-PO1063

Long-Term Clinical Outcomes of Elderly Kidney Transplant Recipients Paula Ferreira Orlandi, Marina Pontello Cristelli, Claudia Rosso Felipe, Tainá Veras de Sandes Freitas, Helio Tedesco Silva, J. Medina-Pestana. *Hospital do Rim e Hipertensão, São Paulo, SP, Brazil.*

Background: The number of elderly patients with chronic renal failure is increasing as the population ages challenging organ allocation algorithms under the current organ shortage. This study investigates the impact of age on post transplant complications, graft and patient survival.

Methods: From 1998-2010 about 7000 kidney transplants were performed in our institution. Information about all 366 patients over 60 years-old in this period were assessed (Group A). As a control group we paired 366 younger patients by sex, donor type (deceased or living donors) and year of transplantation (Group B). The data were analyzed using Kaplan-Meier survival estimates. Multivariate analysis was performed using Cox proportional hazard model.

Results: Diabetes as the cause of chronic renal disease accounted for a significantly higher proportion in the elderly (44%) compared to the younger group (12%) (p=0.002). Cardiovascular complications (12,3%), cancer (6,8%), hospital readmissions (77,3%) and delayed graft function were more common among aged recipients, even though donor characteristics were comparable in both groups. New onset diabetes (25%) and acute rejection (27%) incidences were similar between the groups. 10-year patient (54% versus 83.4%, p <0.001) and overall graft survival (39,4% versus 67,1%, p<0,001) were inferior in elderly compared to younger recipients but no significant differences were observed in death-censored graft survival (75% versus 81,1%, p=0,234). Death with functioning graft accounted for 64% and 44% of all causes of graft loss in the elderly and younger recipients groups (p=0.023). Diabetes was associated with death and graft loss in the multivariate analysis. No significant difference was found related to renal function all over the 10 years of observation.

Conclusions: This is one of the few studies comparing the outcomes of kidney transplants in elderly and younger recipients with 10 years of follow up. Higher mortality rate among the elderly population, probably related to higher prevalence of comorbidities, including diabetes, may account for the inferior allograft survival among elderly recipients.

Funding: Government Support - Non-U.S.

FR-PO1064

Prevalent Chronic Kidney Disease Is Associated with Inpatient Admission among Long-Term Care Residents with Kansas Medicaid Elizabeth W. Dehmer,[1] Theresa I. Shireman.[2] *[1]Nephrology and Hypertension; [2]Preventive Medicine and Public Health, Univ of Kansas School of Medicine, Kansas City, KS.*

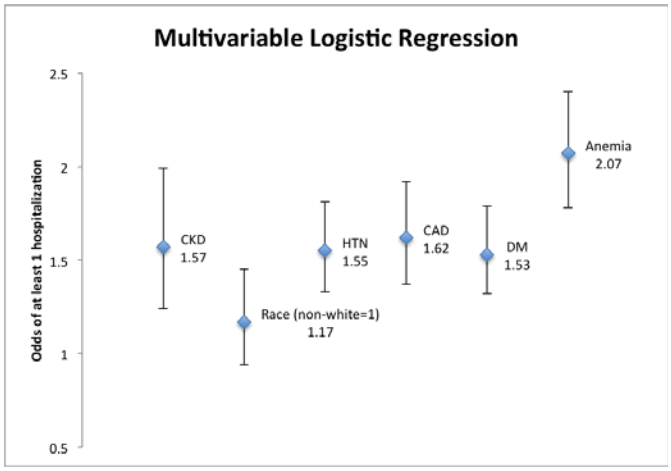
Background: The purpose of this study is to describe the prevalence of chronic kidney disease (CKD) in long-term care (LTC) residents, and to compare demographics, co-morbidities and hospital admissions in LTC residents with and without CKD.

Methods: The study population includes LTC residents (≥180 days in a facility in 2010) with continuous Kansas Medicaid during 2010-11. CKD is defined as one inpatient or two outpatient claims in 2010 containing International Classification of Diseases, Ninth Revision codes 582.xx, 583.xx, 585.xx, 586 or 587. T-tests or Fisher's exact test are used to compare measures between CKD and non-CKD residents. Logistic regression is used to examine the association between inpatient admission during 2011 and CKD.

Results: LTC residents with CKD (6.1%), compared to those without, are more likely to be of non-white race, and have comorbidities of hypertension (HTN), diabetes mellitus (DM), congestive heart failure (CHF), coronary artery disease (CAD) and anemia.

	CKD (n=410) Mean (SD) or N (%)	No CKD (n=6352) Mean (SD) or N (%)	p-value
Age (years)	73.5 (14.2)	74.1 (16.7)	0.4
Male	151 (36.8)	2073 (32.6)	0.08
White	351 (85.6)	5781 (91.0)	0.001
HTN	310 (75.6)	2676 (42.1)	<0.001
DM	244 (59.5)	1648 (25.9)	<0.001
CHF	168 (41.0)	956 (15.1)	<0.001
CAD	171 (41.7)	961 (15.1)	<0.001
Anemia	235 (57.3)	1494 (23.5)	<0.001

Prevalent CKD increases the odds (odds ratio 1.57, 95% confidence interval 1.24, 1.99) of at least one inpatient admission after adjustment for race and co-morbidities.



Conclusions: Even when controlling for co-morbidities, LTC residents with CKD have higher odds of an inpatient admission. Interventions such as review of medication lists, treatment of anemia of CKD, and routine nephrology visits may reduce risk of hospitalizations in this population.

FR-PO1065

Impact of Nursing Home Patients Undergoing Hemodialysis on Emergency Department (ED) and Hospital Admissions in the Northeast Aleef M. Rahman,[1,2] George N. Coritsidis,[1] Marie France R. DeLeon,[1] Jasjit Singh,[1] Carol Lyden,[3] Jaya Bhargava,[3] Roshan A. Patel.[1] *[1]Dept of Nephrology, Elmhurst Hospital Center, Mount Sinai's Icahn School of Medicine, Queens, NY; [2]Dept of Surgery, Elmhurst Hospital Center, Mount Sinai's Icahn School of Medicine, Queens, NY; [3]IPRO, ESRD Networks of New York & New England.*

Background: ED visit and hospitalization rates of dialysis patients are important measures of care in end stage renal disease (ESRD) patients. We are interested to see what impact nursing home patients in HDUs has on ED Visits, Hospitalizations, and LOS outcomes throughout all New England states and New York.

Methods: We retrospectively reviewed the de-identified records of all of Networks 1 and 2 (7 states: New York and New England) for 2010 regarding demographics. ED visits, hospitalization admission rates (HAD) and number of hospital days (LOS) was corrected by percent of nursing home patients (NHP) per dialysis unit.

Results: One-way ANOVAs were used to test for differences between categories. There were no differences between groups in terms of hypertension prevalence and HAD/patient. Percent diabetes, age, and LOS trends increased and differed significantly across categories. ED Visits/Patient decreased and differed significantly across categories.

% NHP/ Dialysis Unit (# of Units)	Average Patients/ Unit	Average Age	Average % of Patients w/ Diabetes	Average LOS/Per Patient	Average ED Visits/Per Patient	Average HAD/ Patient
1-10% (53)	186.7	60.14	38.01	6.81	1.248	0.92
11-20% (176)	150.3	63.6	41.28	6.68	1.37	0.95
21-30% (109)	116.6	66.32	40.46	6.97	1.469	1.0
31-40% (11)	88.18	69.13	43.85	6.72	1.48	1.02
41-50% (4)	115.75	72.53	35.70	7.0	1.09	0.91
≥ 50% (20)	69.2	69.93	51.11	7.9	0.83	0.83
P-Value	**<0.0005**	**<0.0005**	**<0.0005**	**<0.005**	**<0.0005**	**0.164**

Conclusions: As the percentage of nursing home patients rise in a given unit, there are fewer patients sent to the emergency room. This may be due to previous advanced care directives. There are no effects in the number of hospital admissions; however, the units with a higher percentage of nursing home patients have a significantly higher hospital length of stay.

FR-PO1066

Elderly Patients Starting Outpatient Dialysis in Germany Gero D. von Gersdorff,[1] Mathias Schaller,[1] Thomas Benzing.[1,2] *[1]Nephrology - QiN Group, Univ of Cologne Medical Center, Cologne - Köln, Germany; [2]KfH Kuratorium für Dialyse und Nierentransplantation, Neu-Isenburg, Germany.*

Background: Initiation of dialysis in very elderly patients is becoming more common. We evaluated recent trends in frequency and survival of incident dialysis patients (pt) aged 80 years or older in Germany.

Methods: We conducted a retrospective cohort study of pt initiating dialysis aged ≥ 65 years in the electronic health-record based registry of patients on dialysis with the largest non-profit provider in Germany. We determined rates of dialysis initiation 2007 – 2013, clinical characteristics at the start of dialysis and survival.

Results: 13872 pt ≥ 65 years initiated dialysis between 2007 and 2013. The average vintage before entry into the ambulatory setting was 119d. The fraction of pt ≥ 80y increased from 15.8 % to 19.9 % (+4.1%), i.e. an average annual increase of 0.7 % (-0.9 – +1.4). Among these older pt 3.5% were underweight (BMI < 18.5 cm/kg^2), 15.1% had initial albumin < 3.0 g/dL, and 34.3% were anemic (Hb<10 g/dL). 55.8 % had ≥ 4 comorbidities. Average 1-year survival was 74.2% and median survival was 32.3 months. At least 463 (12%) of very elderly pt survived ≥ 5y since first dialysis. These long-term survivors were more likely female and were less likely anemic, underweight, hypalbuminemic or had ≥ 4 comorbidities.

Conclusions: The fraction of very elderly patients starting dialysis continues to rise in Germany. Survival in this cohort was comparably high (cf. Kurella AnnIntMed 2007), underscoring that there is a substantial number even among the very elderly with a good prognosis on dialysis. Although early mortality events were likely not captured in this analysis, age alone is clearly not sufficient as a prognostic marker. Further efforts should be directed at better delineating prognosis for very elderly patients with renal failure.

FR-PO1067

Cognitive Impairment in Advanced Chronic Kidney Disease Simon Richard Walker, Ranveer Singh Brar, Brett M. Hiebert, Frederick Eng, Paul Komenda, Claudio Rigatto, Manish M. Sood, Clara Bohm, Leroy J. Storsley, Navdeep Tangri. *Seven Oaks General Hospital, Univ of Manitoba, Winnipeg, MB, Canada.*

Background: Chronic Kidney Disease (CKD) disproportionately affects the elderly and is associated with frailty and multiple comorbid conditions putting them at risk of developing cognitive impairment. The purpose of this study was to describe the prevalence of cognitive impairment in patients with advanced CKD (eGFR < 30ml/min).

Methods: We approached all patients with advanced CKD who attended an interprofessional non-dialysis CKD clinic at a tertiary care center for enrollment in our study. We excluded patients who did not speak English or were unable to provide informed consent. We collected demographic variables, physical examination measurements, laboratory values, and performed an assessment of cognitive function using the Montreal Cognitive Assessment (MoCA).

Results: We studied 191 patients. Their mean age was 68 years (Standard Deviation [SD]+/- 13), and 74 patients were female (39.4%). The mean eGFR was 21.2 mL/min/1.73m2 (SD +/- 9.4). Sixty seven patients (77.0%) scored < 26 on the MoCA and were thus defined as mildly cognitively impaired. In particular, the recall, visual/executive and language domains were impaired in > 60 % of the participants. Older age, higher pulse pressure, and presence of cardio/cerebrovascular disease were associated with cognitive impairment.

Conclusions: Patients with advanced CKD have a high burden of cognitive impairment. Vascular risk factors appear to be associated with worsening cognition. Further studies on the pathophysiology of cognitive decline and its impact on patient decision making and outcomes are needed.

FR-PO1068

Prevalence of Albuminuria in Older Adults Elke Schaeffner,[1] Olga Jakob,[1] Martin K. Kuhlmann,[2] Jens Gaedeke,[1] Peter Martus,[3] Natalie Ebert.[1] *[1]Nephrology, Charité, Berlin, Germany; [2]Nephrology, Vivantes Klinikum, Berlin, Germany; [3]Biostatistics, Univ of Tübingen, Tübingen, Germany.*

Background: Data on prevalence of albuminuria and associated factors in older adults are rare.

Methods: We cross-sectionally measured random albumin-creatinine-ratio (ACR) in 2054 individuals aged ≥70 (Berlin Initiative Study). ACR was categorized as <30 (no), 30-299 (micro-), or ≥300 (macroalbuminuria) mg/g. Characteristics were compared within strata of albuminuria. Odds ratios were calculated with logistic regression and refer to the second versus first row of each characteristic (Table1).

Results: Of 2054 individuals (mean age 80.4) 26% had albuminuria. Sex, age, diabetes, GFR <60 ml/min/1.73m^2, and hypertension were all statistically significantly associated with albuminuria.

	<30	30-299 (%)	≥300 (%)	Total of albuminuria	Total (%)	OR	95% CI
Whole population	1522 (73.6)	458 (22.1)	74 (3.6)	532 (25.7)	2054 (100)		
Female	851 (55.9)	208 (45.4)	22 (29.7)	230 (43.2)	1081 (52.6)	1.67	1.37-2.03
Male	672 (44.1)	250 (54.6)	52 (70.3)	302 (56.8)	973 (47.4)		
Age <80	843 (55.4)	171 (37.3)	29 (39.2)	200 (37.6)	1043 (50.8)	2.06	1.68-2.52
Age ≥80	679 (44.6)	287 (62.7)	45 (60.8)	332 (62.4)	1012 (49.2)		
Non-diabetic	1197 (78.6)	288 (62.9)	37 (50.0)	325 (61.1)	1522 (74.1)	2.35	1.90-2.90
Diabetes	325 (21.4)	170 (37.1)	37 (50.0)	207 (38.9)	532 (25.9)		
GFR $_{(BIS1)}$ ≥60 ml/min/1.73m^2	642 (42.2)	138 (30.1)	10 (13.5)	148 (27.8)	790 (38.5)	1.90	1.53-2.35
GFR $_{(BIS1)}$ <60 ml/min/1.73m^2	879 (57.8)	320 (69.9)	64 (86.5)	384 (72.2)	1263 (61.5)		
Systolic BP <140 (mmHg)	688 (45.3)	158 (34.5)	21 (28.8)	179 (33.7)	867 (42.3)	1.63	1.33-2.00
Systolic BP ≥140 (mmHg)	831 (54.7)	300 (65.5)	52 (71.2)	352 (66.3)	1183 (57.7)		
Diastolic BP <90 (mmHg)	1184 (77.9)	327 (71.4)	49 (67.1)	376 (70.8)	1560 (76.1)	1.46	1.17-1.82
Diastolic BP ≥90 (mmHg)	335 (22.1)	131 (28.6)	24 (32.9)	155 (29.2)	490 (23.9)		

70% of individuals with albuminuria were treated with an ACE inhibitor or angiotensin receptor blocker (ARB, data not shown).

Conclusions: In a huge cohort of older adults male sex, older age, diabetes, hypertension, and GFR<60 were significantly associated with albuminuria. The number of 30% albuminuric participants without ACE-Inh. or ARBs deserve attention.

Funding: Private Foundation Support

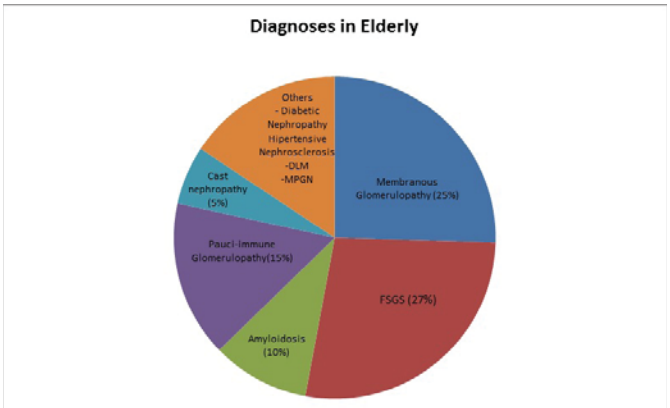
FR-PO1069

Renal Biopsy in Elderly Brazilian Patients: A Clinicopathological Correlation and Complications Alcino Pires Gama, Igor Marques, Victor Sato, Liliany P. Repizo, Simone C. Lo, Ligia Costa Battaini, Luis Yu, Cristiane B. Dias, Lectícia Jorge, Viktoria Woronik. *Div of Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil.*

Background: Elderly patients with renal disease have recently attracted increased attention. There has also been an increase in renal biopsies in these patients. The aim of this study is to report the experience of a Brazilian academic center with percutaneous biopsy in elderly subjects.

Methods: Medical and clinical records from renal biopsies registered in one single center between January 2009 and December 2013 were reviewed. Among 668 patients (female/male 394/274; age 41±16y) who underwent renal biopsies, 60 (8.9%) who were aged 65 years and older (male/female 38/22; age 72 ±6y) were selected.

Results: The elderly usually underwent percutaneous renal biopsies for renal diseases such as nephrotic syndrome (53%), acute or rapidly progressive renal failure (28%) and nephritic syndrome (8%). The main findings in pathology were FSGS (27%), membranous glomerulopathy (25%), pauci-immune glomerulopathy (15%) and renal amyloidosis (10%). In nephrotic syndrome, the most common diagnoses were membranous (44%) and FSGS (31%). In acute or rapidly progressive renal failure, the most common diagnoses were pauci-immune glomerulopathy (47%) and FSGS (18%).



The complications rates after biopsy were similar in elderly and non-elderly patients.

	≥65 y (n=60)	<65 y (n=608)	P
Delta Hb ≥ 1.5g/dL	11.7%	15%	0.2
Major complication*	10%	7.6%	0.6

*Defined as macroscopic hematuria, symptomatic hematoma or blood transfusion.

Conclusions: Renal biopsy in elderly patients is a safe and valuable diagnostic tool. This procedure should be performed without any age limitation when a renal biopsy indication emerges in elderly patients. Brazil has a very high incidence of FSGS as shown by Malafrante et al. This diagnosis remains relevant even in an unusual age.

FR-PO1070

Glomerulonephritis in the Elderly – Incidence, Treatment and Outcome
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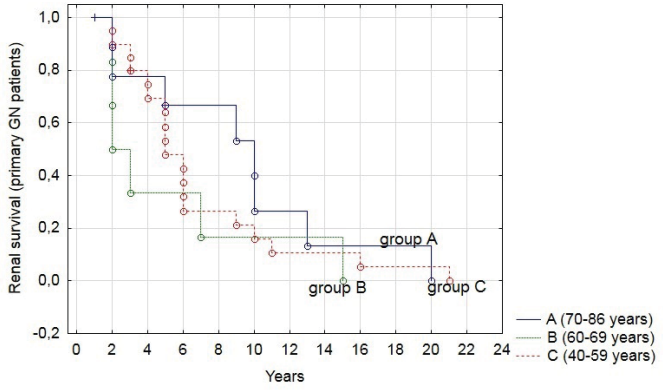
Background: The aging population and doubts about the strength of immunosuppression in elderly patients with comorbidities influence the search of appropriate approach to the glomerulonephritis (GN) treatment. The aim of this study was to evaluate the incidence, treatment and outcomes in elderly patients in the single centre.

Methods: The study group included 84 patients with GN, hospitalized between January 2011 and December 2013 and was divided into 2 subgroups according to age at the time of the study (A:≥70 years; B:60-69 years). The control group consisted of 47 patients with GN, aged 40-59 years (C).

Results: The observation period was 6.6±5.9years (range 0.5-26) for all patients, shorter in group A (4.7±5.2years, p=0.008). Kidney biopsy was performed more often in younger patients 70.2% versus 52.4% (p=0.03). The secondary GN were more common for older patients (53.6% versus 29.8%, p=0.007). In older patients there were 13 cases of primary and 5 cases of secondary membranous GN (21.4%), 16 cases of ANCA vasculitis (19%) and 11 cases of lupus nephritis (LN, 13.1%). The most common primary GN in younger patients was IgA nephropathy (23.4%) and secondary - LN (27.7%). There were no differences between groups in the total dose of intravenous steroids (p=0.12) and cyclophosphamide (p=0.57). However in elderly patients median doses were significantly lower for cyclosporine (A:137.5mg; B:200.0mg; C:237.5mg, p=0.006) and mycophenolate mofetil (A:1000mg; B:1000mg; C:2000mg, p=0.03). Despite the lower doses of immunosuppressive drugs there were no differences between groups in the renal survival defined as no need for renal replacement therapy p=0.63 for all patients and p=0.24 for patients with primary GN.



Conclusions: The study showed that older age does not affect negatively the renal survival of GN patients, regardless of some immunosuppressive regimen lowering.

FR-PO1071

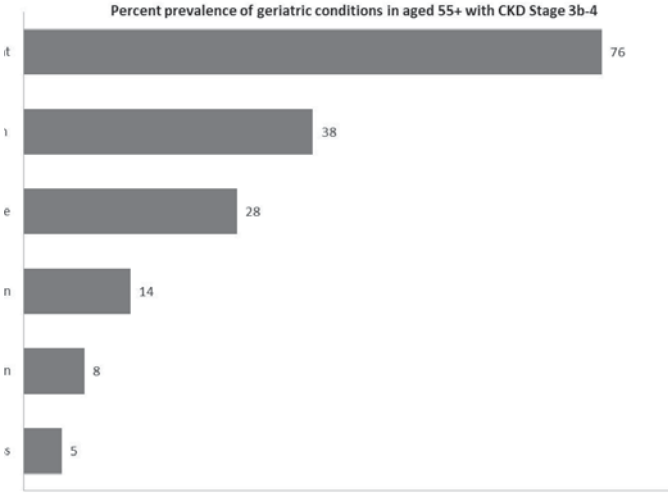
Geriatric Conditions in CKD
Christine Liu,[1] Alyson Heath,[2] Andrew M. Well,[2] Jamie Giffuni,[3] Kieran Reid,[2] Roger A. Fielding,[2] Leslie I. Katzel,[3] Stephen L. Seliger,[3] Daniel E. Weiner.[2] *[1]Medicine, Boston Univ, Boston, MA; [2]Medicine, Tufts Univ, Boston, MA; [3]Medicine, Univ of Maryland, Baltimore, MD.*

Background: Geriatric conditions are constellations of signs and symptoms not specifically linked to a disease; often they are due to deficits in multiple organ systems and impact quality of life. Examples include cognitive impairment or dizziness. Although CKD affects multiple systems, little is known about the association of CKD with geriatric conditions. We examined the prevalence of geriatric conditions and association with quality of life in older adults with CKD.

Methods: The Aerobics, Weights, and Renal Disease Study is an ongoing randomized trial of exercise in adults 55+ years with CKD stage 3b-4. Data are from baseline. Persons were defined with 1) cognitive impairment if Montreal Cognitive Assessment <26; 2) poor physical function if SPPB ≤7; 3) dizziness if answered "yes" to such symptoms on the Memorial Symptom Assessment Scale; 4) fatigue if answered ≥"good bit of time" to feeling worn out" on the SF36; 5) chronic pain if answered ≥"moderate" pain on the SF36; and 6) depression if Beck Depression Inventory ≥15. Poor health status was defined by answering ≥"mostly false" to "my health is excellent" on SF36. Logistic regression assessed the association of geriatric conditions with health status, adjusting for age, sex, race, BMI, eGFR, diabetes and hypertension.

Results: Of 50 persons enrolled (30% female, mean age 68.6±7.8 years, mean BMI 30.4±5.4 kg/m^2), 6% (3) had no conditions; 44% (22) had 1 geriatric condition while 50% (25) had ≥2 conditions (Figure). With each additional condition, odds of poor health status increased (OR 2.94, 95%CI 1.23-7.01) after covariate adjustment.

Conclusions: In older adults with CKD, geriatric conditions are common. Many have multiple geriatric conditions; these likely impact perceived quality of life in this population.



Funding: NIDDK Support

FR-PO1072

The Effect of Age on Nuclear Protein Levels in Rat Kidney Cortex and Medulla
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Background: Studies in vitro and in vivo have reported that total nuclear protein levels increase with age in mouse fibroblasts, liver cells and intestinal epithelial cells. This finding suggests the accumulation of oxidized and misfolded proteins in the nucleus with age. The present study was undertaken to investigate whether total nuclear protein levels increase with age in rat kidney cortex and medulla.

Methods: Young (3 months of age) and Old (22 months of age) female Lewis rats were used. The kidneys were harvested from anesthetized rats, separated into cortical and medullary sections and homogenized. The nuclear fractions were isolated by differential centrifugation and filtered through an 80 micron nylon mesh. The purified nuclear pellets were isolated and lysed. The protein content was isolated by dissolving the remaining pellet in 0.1 M sodium hydroxide. The protein content was measured using the Lowry Protein Assay. Differences were evaluated using a Student's t test.

Results: There was a significant decrease in nuclear protein levels in the cortex of the rat kidney with age. However, there was no change in nuclear protein levels in the medulla of the rat kidney with age. Nuclear protein levels were significantly higher in the medulla than in the cortex in kidneys from both young and old rats.

		Young (n=4)	Old (n=4)
Nuclear Protein	Cortex	11.2 ± 1.0	7.6 ± 0.7*
mg/g kidney wet wt	Medulla	14.2 ± 0.6[a]	13.8 ± 1.4[a]

All data expressed as X + SEM; * significantly different from Young; [a] significantly different from age-matched cortex.

Conclusions: Total nuclear protein levels decrease (i.e, cortex) or do not change (i.e., medulla) with age in the rat kidney.

FR-PO1073

Hemodialysis among the Elderly: Outcomes and Survival in Mexican Patients Karina Renoirte,[1,2] Guillermo Garcia-Garcia,[1,2] Xochitl Guerrero.[1,2] *[1]Nephrology, Hospital Civil, Guadalajara, Mexico; [2]Health Sciences, Univ, Guadalajara, Mexico.*

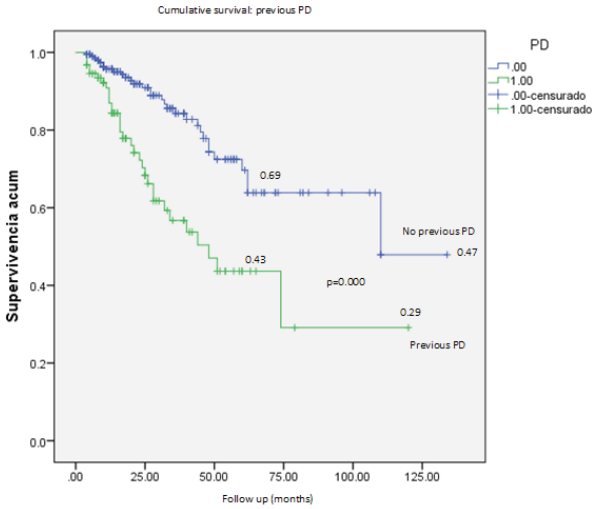
Background: The incidence and prevalence of End Stage Renal Disease (ERSD) patients (pts) in Mexico is constantly rising. In addition, the Mexican population ≥60 years of age (y/a) will duplicate by year 2050 (2.7% of total population was ≥60 y/a in 1990, 6.9% in 2000 and 9% in 2012). In 2012, the average health expectancy was 77.4 y/a, compared with 70.7 in 1990. Thus, more and more ageing pts are initiated in HD instead of PD. The average HD patient's age in the U.S. is 62 y/a compared with Mexican pts average age 46.75 ± 19.6. Nevertheless, 33% of HD pts are ≥60 y/a.

Methods: We prospectively analyzed from Jan 2010 thru Feb 2014 all incident and prevalent HD patient's ≥60 y/a, in 7 non hospital basis HD facilities. All pts were receiving 3 HD treatments per week. We used Excel and SPSS v20.0 program for statistics. We describe survival, vascular access, kt/v, Hb and socio-demographic data.

Results:

	All pts, n=1418	≥ 60y/a, n=463
Age	46.75±19.6	70.27± 6.82
Male (%)	64	63
DM (%)	34.1	64
Previous PD (%)	44	32
Average Hb, gr/dl	9.9	10.4
% pts Hb 10-12gr/dl	37	42
Average kt/v	1.16	1.16
% pts ktv ≥ 1.2	47	44
Temporary catheter (%)	32	24
Permanent catheter (%)	36	40
AV Fistula (%)	32	36

Once renal replacement therapy is initiated, the range of the expected remaining life span in the USRDS report was approx 54 months for those 60 to 64 y/a, compared with 26.3 months in our pts (range 4-110). Overall cumulative survival at 12,24, 36, 48 and 60 months was 0.93, 0.84, 0.75, 0.65, 0.61 respectively**.** Worst outcomes were identified for pts with any kind of catheter (p=0.000), previous PD (p= 0.000) and DM (p= 0.036)



Conclusions: 1/3 of HD pts in Mexico are ≥60y/a. Overall survival at 60months was 0.61. Better survival was observed for those with AV fistula, no previous PD and no DM. Hb and kt/v and were similar to those reached in younger pts.

Funding: Clinical Revenue Support

FR-PO1074

Assessment of Renal Function in Older Adults Markus Bitzer,[1] Jennifer Yi-Chun Lai,[1] Mindy Katz,[2] Richard B. Lipton.[2] *[1]Medicine, Univ of Michigan, Ann Arbor, MI; [2]Neurology, Albert Einstein College of Medicine, Bronx, NY.*

Background: Chronic kidney disease (CKD) defined by decrease in estimated glomerular filtration rate (eGFR) calculated from serum creatinine levels has the highest prevalence in adults ≥ 70 years and lower eGFR increases mortality and risk for end stage renal disease (ESRD). However, the applicability of current eGFR equations for older adults has been questioned. Here, we described differences among equations in community-based older adults and examined clinical parameters associated with lower eGFR and mortality.

Methods: Subjects ≥ 70 years of age with the first available serum creatinine measurements from 2004 to 2013 were identified from the Einstein Aging Study cohort. Kidney function was estimated using Cockcroft-Gault estimated creatinine clearance (CG CrCl) and three eGFR equations (MDRD, CKD-EPI and BIS1). Kappa statistics were applied to determine the agreement between each two equation regarding CKD staging. Linear and Cox proportional regression models were used to identify factors associated with lower eGFR and mortality.

Results: 774 subjects were included in the analysis (mean age: 79.8 (SD=5.5) years, 62% female). The average kidney function was highest using MDRD (mean=70.4, SD=19.1) and lowest using BIS1 (mean=53.2, SD=12.0). The agreement in CKD staging between MDRD and CKD-EPI was substantial, while it was low to fair between BIS1 and the other equations. Older age, lower serum creatinine levels, gender, and ethnic differences contributed to the discrepancy between equations. Higher serum homocysteine levels and presence of hypertension were associated with lower eGFR whereas presence of diabetes mellitus was associated with higher eGFR across different equations. eGFR<45 ml/min/1.73m2 was associated with increased risk of mortality (HR=2-3.3, P<0.05).

Conclusions: In this community-dwelling older population, the use of different equations to calculate eGFR results in significant differences in CKD classification and assessment of mortality risk for patients with eGFR below 45 ml/min/1.73m2. These findings support efforts to develop improved methods to determine renal function and/or modify the outcome-related CKD staging in older adults.

Funding: NIDDK Support, Other NIH Support - NIA

FR-PO1075

Knowledge and Attitudes Toward Renal Replacement Therapy Options and Decision Making in Patients with Advanced Chronic Kidney Disease Jamie Alton Green, Ion D. Bucaloiu, James E. Hartle. *Nephrology and Hypertension, Geisinger Medical Center, Danville, PA.*

Background: Little is known about the baseline knowledge and attitudes towards dialysis of patients with advanced chronic kidney disease (CKD) referred to a renal replacement therapy education class.

Methods: We administered a survey to consecutive patients referred to a dialysis education class to assess their baseline knowledge of various renal replacement therapy options, decision making preferences, and advance directive completion.

Results: Twenty-nine patients completed the survey. Mean age was 64, 17 (59%) were female, and 26 (93%) were white. Fourteen (52%) had a less than or equal to high school level of education. Nine (32%) had limited health literacy level based on a single item screen. Fourteen (48%) were unsure how close they were to dialysis start. On a 5-point Likert scale (1=lowest, 5=highest), mean level of self-reported understanding for all modalities was poor (mean 2.3). Thirteen (45%) felt unprepared to make a decision about dialysis. Twenty-three (79%) preferred to share decision making with their doctor, 5 (17%) preferred making decisions by themselves, and only one patient (3%) preferred their doctor make these decisions for them. Fourteen (48%) had no advance directive documents completed.

Conclusions: The majority of patients referred to a renal replacement education class have poor baseline knowledge, feel unprepared to make these decisions, and prefer a shared decision making model. Additional work is needed to determine the most effective educational strategy to facilitate knowledge and shared decision making about renal replacement therapy options.

Funding: Clinical Revenue Support

FR-PO1076

Predictors of Quality of Life in Patients with End Stage Renal Disease on Hemodialysis Chetana Rondla, Christine Boumitri, Magda Daoud, Marc M. Saad, Youssef El Douaihy, Suzanne E. El Sayegh. *Medicine/Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Background: Assessment of quality of life (QOL) of End Stage Renal Disease (ESRD) patients (physical, mental, and social well-being) has become an essential tool to develop better plans of care.Objective of this study is to determine which demographic and biochemical parameters correlate with the QOL scores in patients with ESRD on hemodialysis (HD) using Kidney Disease Quality of Life -36 surveys (KDQOL).

Methods: A retrospective chart review of all ESRD patients that underwent HD at an outpatient center.The 5 components of the KDQOL were the primary end points of this study (Burden of Kidney disease, Symptoms and Problems, Effects of Kidney Disease on daily life, MCS-Mental Component Survery, and PCS-Physical Component Survey). Scores grouped into 3 categories (below average, average, and above average).In addition to demographics (age, sex and race) the independent variables: weight gain, number of years on dialysis, URR, Calcium, Phosphorus, PTH, albumin, and hemoglobin in the serum were collected.Chi square analysis for dependent variables and the nominal independent variables was used; t-test analysis was used for continuous independent variables.Ordinal regression using PLUM method was used to weight out possible effects of confounders.

Results: Cohort size of 111 patients.Mean age 61.8 (±15.5), males 64.9%, mean time-on-dialysis 4.3 (4.8) years.About two thirds of responses on all 5 domains of the questionnaire ranked average when compared to national numbers.No significant relationships were found between the 5 dependent variables of interest and the independent variables.This was confirmed by regression analysis.Of note, sex carrrried the strongest statistical significance (p value 0.16) as predictor of "burden of kidney disease on daily life" in ordinal regression.

Conclusions: Prior studies have shown variables such as serum phosphate level, interdialytic weight gain and dialysis adequacy are associated with lower KDQOL scores however this was not evident in our analysis likely due to smaller sample size.Larger size studies are required to better understand the predictors of QOL in ESRD patients on Hemodialysis.

FR-PO1077

Communicating About Choices in Transplantation: Preliminary Evidence of the Efficacy of a Behavioral Communication Intervention for Kidney Transplant Candidates Heather Traino. *Social and Behavioral Health, Virginia Commonwealth Univ, Richmond, VA.*

Background: Previous research highlights the logistical and content-related difficulties patients with end-stage renal disease (ESRD) awaiting kidney transplant face while attempting to initiate and navigate discussions about kidney transplantation. In response, Communicating about Choices in Transplantation (COACH), a behavioral communication intervention, was developed combining education on living and deceased donor transplantation and the skills needed to effectively engage others in transplant-related conversations. This quasi-experimental study was conducted as a preliminary assessment of the intervention's efficacy.

Methods: ESRD patients (n=10) waitlisted for kidney transplantation at one mid-Atlantic transplant center participated in a 2-hour COACH session. Using a pre-post design, with no controls, the intervention's impact on patients' knowledge of deceased and living-donor transplantation; communication difficulties, self-efficacy, and intentions; and self-reported discussion of transplantation was evaluated.

Results: All participants reported previous conversations about transplantation prior to attending the session, however one indicated a desire for no further discussion; after program attendance all were considering, planning or going to continue to hold transplant-related conversations. Results of paired-sample t-tests revealed increases in the number of correct responses to the 12-item knowledge scale improved from 7.6 (SD=2.0; range, 5-12) pre-intervention to 9.5 (SD=1.8; range, 6-11; p=.05) post-intervention. Similarly, averaged ratings of confidence (i.e., self-efficacy) managing aspects of transplant-related conversations increased from 73.9 (SD=14.1; range, 55.9-93.5) to 89.0 (SD=6.7; range, 77.1-94.7; p=.009) post-intervention. Perceived conversational difficulties decreased from a pre-intervention average of 4.5 (SD=3.3) to 3.5 (SD=3.0) post-intervention (p=.53).

Conclusions: Results provide preliminary support for the intervention's efficacy. However, a more definitive test of the intervention is needed using a randomized controlled design and a larger, more diverse sample, with a control group.

Funding: Other NIH Support - Grant Nos. K01HS018113 and R03HS21312 from the Agency for Healthcare Research and Quality (AHRQ) and R39OT24208 from the U.S. Department of Health and Human Services, Health Resources and Services Administration's Division of Transplantation (HRSA/DoT)

FR-PO1078

Predictors and Cost of Renal Crash (Inpatient First Dialysis) Pradeep Arora,[1] John James Bono,[2] Laura L. Argauer,[2] Brian M. Murray,[1] Edwin J. Anand,[1] Rocco C. Venuto.[1] *[1]Medicine, SUNY at Buffalo, Buffallo, NY; [2]Computer Task Group, Computer Task Group, Buffalo, NY.*

Background: Mortality rate and the cost of care in dialysis patients is very high. We studied the factors associated with "crashing" into dialysis and its effect on the cost of care for the first three months after initiating chronic dialysis.

Methods: TThe population for this study was derived from a large upstate NY database spanning 6 years of health insurance claims records. Renal crash was defined if patients (1) developed end-stage renal disease (ESRD) during the observed period; (2) experienced their first instance of dialysis in an inpatient setting; (3) had at least two eGFR values indicative of CKD (less than 60 ml/min/1.73 m^2) before their first dialysis; and (4) had dialysis claims persisting for 90 days after the initial dialysis billing. Logistic regression model were built to evaluate the predictors of renal crash. Similarly MLR model was built to evaluate the impact of renal rash on cost of care in first 3 months.

Results: 332121 of 408,712 patients who had claims records with the data necessary to identify all medications and laboratory results had CKD. 664 patients were started on dialysis for ESRD: 52% had renal crash. Difference in baseline characteristics between these two groups is shown in the table.

	Renal Crash	No renal Crash	P value
Mean Age	66.6	63.4	0.03
Gender (F) %	41	38	0.5
Hypertension (%)	90	85	0.2
Diabetes	66	61	0.4
Coronary artery Disease (%)	47	33	0.01
Arthritis %	40	40	1.0
Anxiety %	6	3	0.2
other mental health	19	24	0.2
Statin use (%)	56	70	0.02
Mean number of Visit in last 12 months	5	6.4	0.001
Days between last Nephrology Visit prior to first dialysis	44	20	0.001
Last Nephrology visit between 0-4 months (%)	88	97	0.05
Last Nephrology Visit 4-12 months (%)	12	3	0.005
Mean cost of care in 3 months after First Dialysis ($)	35,376	22,367	0.00

Multivariate logistic regression revealed that coronary artery disease and less frequent visit to nephrologist were associated with increased odds of renal crash. Use of statin was protective against crash.

Conclusions: Renal crash is very common and leads to increased cost of care. More frequent nephrology visits appears to abrogate crash rates.

FR-PO1079

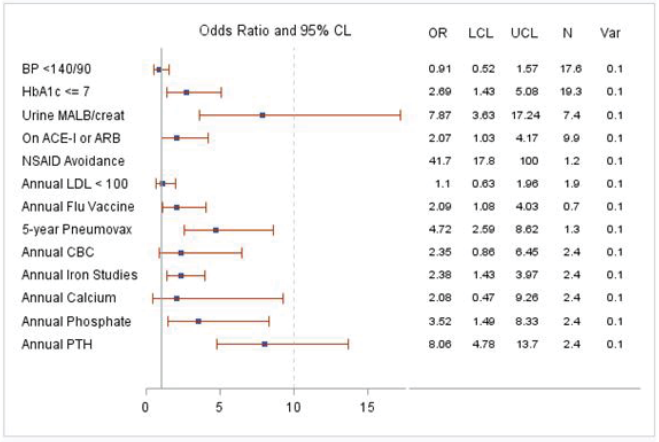
Design and Implementation of a Chronic Kidney Disease Checklist for Primary Care Providers Mallika L. Mendu, Sushrut S. Waikar. *Renal Div, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Chronic kidney disease (CKD) is associated with significant morbidity, mortality and financial burden. Guidelines outlining CKD management have been published, but there is limited knowledge and application of these guidelines among primary care providers (PCPs).

Methods: We designed a CKD checklist-a tool outlining management guidelines for CKD- and examined whether implementation in a primary care clinic improved guideline adherence. During a one year period we conducted a prospective study involving 13 PCPs, 4 of whom were assigned to utilize a CKD checklist incorporated into the electronic medical record during visits with patients with CKD stages 1-4. All PCPs received education regarding CKD guidelines. The intervention and control groups consisted of 105 and 263 patients, respectively. We examined rates of adherence to CKD management guidelines.

Results: We observed improvements in measures of CKD care in patients assigned to the checklist compared to controls. Patients in the CKD checklist group were more likely to have appropriate annual laboratory testing for albuminuria (72.4% versus 27.8 %), phosphate (65.7% versus 35.4%), and parathyroid hormone (61.0% versus 16.4%) (p <0.001 in all cases). Compared to controls, patients in the checklist group had higher rates of achieving a hemoglobin A1c target <7 (77.1% versus 53.6%), use of an angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker (67.6% versus 48.7%), documentation of non-steroidal anti-inflammatory drug avoidance (71.4% versus 6.5%), and vaccination for influenza (67.6% versus 48.3%) and pneumococcus (65.7% versus 27.8%) (p <0.001 in all cases). After adjustment for PCP, age, and CKD stage, use of a checklist remained significantly associated with increased guideline adherence.

Figure 1. Forest plot depicting adjusted impact of a CKD checklist on adherence to chronic kidney disease management guidelines'



Abbreviations: BP, blood pressure; HbA1c, hemoglobin A1c; urine MALB/creat, urine microalbumin/creatinine ratio; ACE-I, angiotensin converting enzyme-inhibitor; ARB, angiotensin receptor blocker; NSAID, Nonsteroidal anti-inflammatory drugs; LDL, Low-density lipoprotein; CBC, Complete blood count; PTH, parathyroid hormone
a. Accounting for within group correlation by PCP assignment and adjusted for Age, and CKD Stage (1 and 2, 3, 4)
b. Odds ratio greater than 1 (to right of solid vertical line) reflects increased adherence in patients assigned to the CKD checklist

Conclusions: Implementation of a CKD checklist significantly improved adherence to CKD management guidelines.

FR-PO1080

Confidence Level, Knowledge and Practices of Primary Care Physicians in Chronic Kidney Disease Management Jia Hwei Ng,[1] Robert Schoepe,[1] Chike N. Okechukwu,[2] Spencer Ng,[3] Aaroop Haridas,[1] Shaila Smith,[1] Rashmika Potdar,[1] Sonia N. Zaveri.[1] *[1]Internal Medicine, Crozer Chester Medical Center, Upland, PA; [2]Nephrology, Clinical Renal Associates Ltd, Chester, PA; [3]Widener Univ, Chester, PA.*

Background: Recent studies have shown that primary care physicians (PCP) lack confidence in management strategies for chronic kidney disease (CKD). This study aims to evaluate the confidence level, knowledge and practices of PCPs in CKD management.

Methods: A paper-based questionnaire was given to PCPs in an internal medicine residency program. In addition, physicians' actual practices were compared to survey results using a retrospective cohort analysis of 176 CKD patients.

Results: There were 31 out of 34 physicians who responded. Respondents consisted of 45% (14/31) attendings and 55% (17/31) residents. Of all respondents, 74% (23/31) were confident in diagnosing CKD, 74% (23/31) were confident in CKD staging, and 34% (10/31) were aware of CKD guidelines. Attendings were significantly more confident than residents in diagnosing CKD (p=0.0454). However, there were no significant differences between attendings and residents in CKD staging (p=1.000), and awareness of CKD guidelines (p=0.4414). In diagnosing CKD, 77% (24/31) chose eGFR, 35% (11/31) used creatinine clearance and 6% (2/31) used creatinine only. Up to 90% (28/31) of PCPs reported yearly anemia screen and 84% (26/31) urine albumin screen. But only 48% (85/176) of patients were screened for anemia, and 54% (95/176) of the patients had annual urine albumin testing. Reported yearly screening for PTH and phosphorus were 54% (16/31), and 83%

(24/31) respectively. However, bone mineral screening in actual practice was less than 40% (12/48) among patients with eGFR<45. PCPs reported use of ACE inhibitor or ARB 77% (24/31), and 13% (4/31) would use ACE inhibitor and ARB simultaneously. However 50% (75/145) of patients with hypertension were not on ACE inhibitor or ARBs.

Conclusions: Physicians were not confident at CKD management. Physicians' perception of their management did not correlate with actual practice. Making CKD a core quality measure would likely improve adherence to current guidelines.

FR-PO1081

Chronic Kidney Disease Management among Primary Care Physicians in a Residency Program Jia Hwei Ng,[1] Robert Schoepe,[1] Aaroop Haridas,[1] Shaila Smith,[1] Rashmika Potdar,[1] Sonia N. Zaveri,[1] Chike N. Okechukwu.[2] *[1]Internal Medicine, Crozer Chester Medical Center, Upland, PA; [2]Nephrology, Clinical Renal Associates Ltd, Chester, PA.*

Background: The role of primary care physicians (PCP) in managing early chronic kidney disease (CKD) is vital. However, studies have showed that management strategies for CKD by PCP were often suboptimal. This study aims to assess if the PCPs in an internal medicine residency program are managing CKD according to Kidney Disease Improving Global Outcome (KDIGO) 2012 guidelines.

Methods: This retrospective cohort study was performed on 175 patients from two group practices. Patients with two estimated glomerular filtration rates (eGFR) of < 60 for at least 3 months were included. Patients excluded were: ESRD on dialysis, HIV, pregnant, and renal transplant. Parameters measured on different aspects of CKD management included: kidney disease surveillance, hypertension, diabetes, lipids, anemia, bone mineral disease, and acid base disorder. Timely referral to nephrology was also evaluated.

Results: There were 88% (154/176) stage 3 CKD, 13% (22/176) Stage 4 CKD, and no 5 CKD. Fifty five percent (90/165) of patients with hypertension met target blood pressure (BP) of ≤ 140/90. Among diabetic patients, 26% (25/95) reached target BP ≤ 130/80. Up to 50% (75/145) of patients with hypertension were not on angiotensin converting enzyme inhibitor or angiotensin receptor blocker. HbA1C ≤ 7 was met in 51% (49/95) of diabetes patients. Hyperlipidemia screen was 82% (161/176). Yearly anemia screen for CKD Stage 3 was 53% (82/154), and twice a year anemia screen for CKD stage 4 was 32% (7/22). Phosphorus, parathyroid hormone, and vitamin D screening rates for eGFR <45 were 25% (12/48), 38% (15/48), and 52% (25/48) respectively. Only 54% (95/176) of the patients had annual urine protein testing. Appropriate nephrology referral (eGFR < 30) was done 50% (12/22) of the time.

Conclusions: By KDIGO guidelines, management of CKD by physicians in our study is suboptimal. A separate study is being done to assess barriers to physicians' non-adherence to guidelines. Larger studies involving residency programs should be done to evaluate current trends in CKD management.

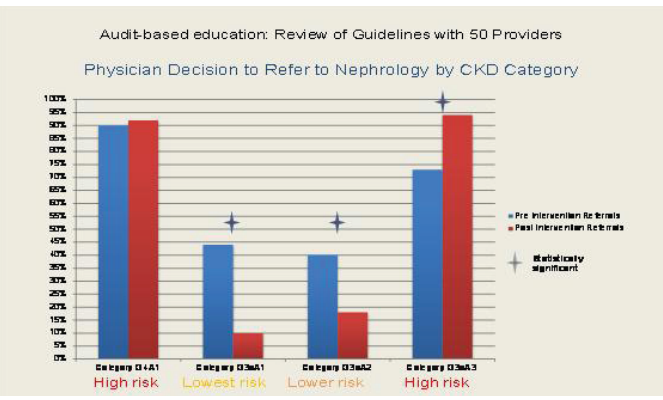
FR-PO1082

Audit-Based Education of Referring Providers Affects Albuminuria Detection and Referral Patterns of CKD Patients Konstantin Abramov, Aditi Ahlawat, Marie A. Sosa, Jayaprakash R. Dasari. *Univ of Mass.*

Background: Early referral to nephrology of low risk CKD patients is common and contributes to over-utilization of resources and prolonged wait time. This affects timely identification and referral of high risk patients. In 2013, 587 new referrals were made to our clinic, 50% for evaluation of CKD. Since 2012, the average wait time for new patient evaluation increased from 30 to 40 days. A common practice is to refer CKD patients for evaluation when eGFR is <60 ml/min. New CKD classification retains 5 GFR stages and adds 3 albuminuria stages, reflecting increased progression and complication risk in albuminuric patients. The KDIGO recommendation is to refer high risk patients to nephrology, those with eGFR <30 ml/min (category G4) or albuminuria >300 mg/day (category A3). Albuminuria is infrequently measured by referring providers.

Methods: A pre-intervention survey asked providers to identify patients for referral, based on different CKD risk categories. After an audit of current referral practices, we initiated a provider education session of the new CKD classification, guidelines and changes to the electronic medical record to facilitate ordering and tracking of urine albumin. A post-intervention survey and referral audit was obtained.

Results: Post-intervention providers indicated they would refer fewer low risk patients and more high risk patients.



Analysis of post-intervention referrals demonstrated fewer patients with low or unknown risk category (reduction from 25% to 15%) and more patients with measured albuminuria at the time of referral (increase from 25% to 85%).

Conclusions: An audit based intervention appears to be effective in changing referral patterns and albuminuria detection. A longer follow up is required to assess sustainability of this change and its effect on the referral wait time.

FR-PO1083

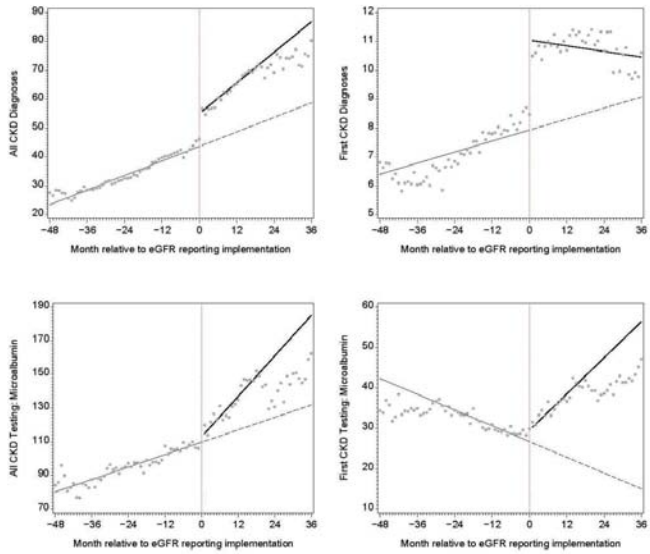
Impacts of Automated Estimated Glomerular Filtration Rate Reporting in the Veterans Health Administration (VHA) Virginia Wang,[1,2] Bradley G. Hammill,[2] Matthew L. Maciejewski,[1,2] Rasheeda K. Hall,[2] Amit X. Garg,[3] Arsh Jain,[3] Uptal D. Patel.[2] *[1]VA Med Ctr, Durham, NC; [2]Duke Univ, Durham, NC; [3]Western Univ, Ontario.*

Background: Automated lab reporting of estimated glomerular filtration rate (eGFR) has been introduced in health systems to improve recognition of chronic kidney disease (CKD), but its impact on health systems remains unclear. We examined the effects of eGFR reporting on CKD recognition and renal services in the VHA.

Methods: Time-series study examined a national cohort of 97 VA outpatient clinics or hospitals with lab data in 2000-09. Health system and patient data was drawn from VA databases. Hierarchical generalized linear models estimated short- and long-term facility-level impacts of eGFR reporting on monthly rates of overall and first outpatient CKD diagnoses, use of microalbumin and kidney ultrasound diagnostic testing, and outpatient nephrology visits.

Results: After eGFR reporting, we found increases in overall CKD diagnoses (74%; mean facility rate from 46 to 80 per 10,000 general VA outpatients per month), microalbumin testing (48%, 110 to 162), kidney ultrasounds (22%; 20 to 24), and nephrology visits (30%; 29 to 38). There was short-term growth and a long-term decline in patients' first CKD diagnoses, but immediate and sustained increases in first microalbumin testing.

Figure 1. Time series of monthly rates of outcomes (per 10,000 general VA outpatients)



In adjusted models, CKD diagnoses increased an average 11.4 per 10,000 general VA outpatients per month, with sustained long-term growth (p<.001). There was long-term growth in microalbumin testing (p<.001) and short-term growth in ultrasounds (p<.05), but no change in nephrology visits.

Conclusions: We found modest system-level effects ofeGFR reporting that may reflect the relative ease of CKD documentation and follow-up diagnostic testing. eGFR reporting had no impact on nephrology visits, suggesting opportunities to improve appropriate follow-up evaluation to confirm and effectively manage CKD.

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

FR-PO1084

Expenditures for Chronic Kidney Disease (CKD) in the United States: Results from Medical Expenditure Panel Survey (MEPS) 2011 Mukoso N. Ozieh,[1] Kinfe Gebreegziabher Bishu,[1] Leonard Egede.[1] *[1]Nephrology, MUSC, Charleston, SC; [2]Internal Medicine, MUSC, Charleston, SC.*

Background: CKD (defined as eGFR < 60ml/min/1.73m^2) is a global public health problem. According to the National Kidney Foundation, 26 million adults in the U.S. are affected by CKD. The cost of CKD has continued to rise according to the United States Renal Data System, however there are no recent national estimates of the cost of CKD in the U.S. population. This study aims to assess the cost of CKD in adults in the U.S. population.

Methods: Data on 18,945 adults in 2011 MEPS was analyzed. CKD was based on ICD-9 codes from the 2011 MEPS medical condition file which was merged with the consolidated file. Mean total expenditure in 2011 was estimated for the overall sample and by CKD status. We used a generalized linear model with gamma distribution and log

link function to estimate total overall adjusted expenditure and by CKD status. Covariates in the model include age, race, gender, marital status, education, insurance, Metropolitan Statistical Area (MSA), region, poverty status and comorbidities. STATA version 13 was used to account for the complex design of MEPS.

Results: Of the 18,945 adults analyzed, approximately 1.3percent had CKD. CKD was significantly more prevalent amongst individuals 65 years and above, widow/divorced/single individuals and publicly insured. CKD was also more prevalent in individuals with hypertension, CVD, Stroke, high cholesterol and arthritis. In the unadjusted analysis, mean total expenditure was $5,862.08 (95% CI $5,542.28-$6181.93). Mean total expenditure for those without CKD was $5,772.73 (95% CI $5,447.48-$6,097.98) and $12,739.25 (95% CI $9,762.79-$15,715.72) in those with CKD. In the adjusted analysis, mean total expenditure in those without CKD was $6,078.99 (95% CI $5,711.51-$6,446.49) and $8,366.03 (95% CI $6,301.72-$10,430.34) in those with CKD. Extrapolating to the U.S. population in 2011, the mean total expenditure for those with CKD was over $21 billion.

Conclusions: We determined that CKD had an independent effect on cost. This emphasizes the need for prevention, early diagnosis, referral, and treatment of CKD.

FR-PO1085

Barriers to Self-Care in Type 2 Diabetes and Chronic Kidney Disease: A Qualitative Approach Shayan Shirazian,[1] Natalie Crnosija,[2] Joseph Mattana,[1] Amy C. Hammock.[2] *[1]Medicine, Winthrop Univ Hospital; [2]Public Health, Stony Brook Univ.*

Background: The majority of patients with type 2 diabetes (T2DM) and pre-dialysis chronic kidney disease (CKD) are not at goal blood glucose, blood pressure or cholesterol; this has been attributed, in part, to a failure of self-care. Despite National Kidney Foundation guidelines that stress the need for self-care interventions in patients with both T2DM and CKD, few studies have evaluated barriers to self-care in this population. The objective of this qualitative study is to examine barriers to self-care in patients with T2DM and CKD.

Methods: In this IRB approved study, a doctoral level expert in qualitative methods performed two 90-minute focus groups with T2DM and CKD patients. Focus group participants were asked about barriers to self-care in managing both diabetes and CKD. Participants were asked about common barriers when discussion slowed. Focus groups were audio recorded, transcribed, and systematically analyzed to identify emergent common themes.

Results: Sixteen subjects (10 men and 6 women) of varying race and at varying stages of CKD (2-5) participated in 2 focus groups. The most commonly stated barriers to CKD self-care were (1) lack of coordinated care, (2) mechanisms and risks/benefits of medications, (3) unclear dietary recommendations, (4) inadequate information about co-morbid conditions, such as cardiac disease, (5) depressed mood and hopelessness about current health, (6) anxiety about the future possibility of dialysis, and (7) family members' unhealthy food-related behavior. Of the themes that emerged, the investigators found psychological distress regarding kidney disease and the future possibility of dialysis to be additional, unique barriers faced by patients with both T2DM and CKD.

Conclusions: Psychological distress regarding kidney disease, including the future possibility of dialysis, may be important unique barriers to the management of patients with both T2DM and CKD. Patient-centered self-care interventions that target barriers to care may improve treatment target achievement in patients with T2DM and CKD.

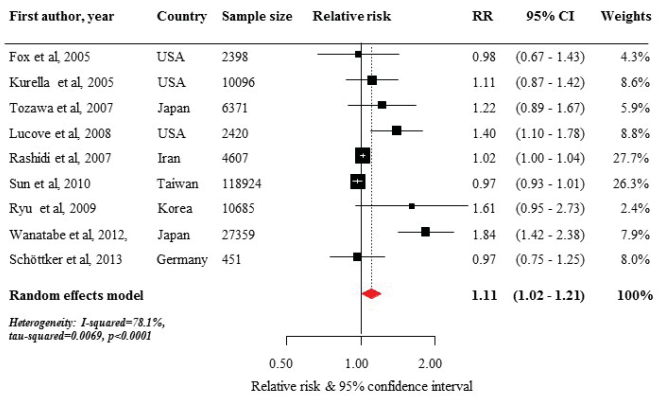
FR-PO1086

Association between Prediabetes and Risk of Chronic Kidney Disease – A Meta-Analysis Justin B. Echouffo Tcheugui,[1,3] K.M. Venkat Narayan,[1] David Weisman,[3] Sherita H. Golden,[2] Bernard G. Jaar.[2] *[1]Global Health, Emory Univ, Altanta, GA; [2]Medicine, Johns Hopkins, Baltimore, MD; [3]Medicine, MedStar Good Samaritan Hospital, Baltimore, MD.*

Background: It is unclear whether non-diabetic range hyperglycemia is a risk factor for chronic kidney disease (CKD). We assessed the prospective association of prediabetes (impaired fasting glucose [IFG] and/or impaired glucose tolerance [IGT]) and CKD.

Methods: We searched PubMed, EMBASE, and bibliographies of retrieved English-language articles pubished until March 2014. We included observational studies reporting measures of disease risk (multivariate-adjusted relativerisks [RRs], hazard ratios [HRs] or odds ratios [ORs] and corresponding 95% confidence intervals [CI] or CKD [kidney damage (microalbuminuria, albuminuria or proteinuria) and/or glomerular filtration rate < 60 cc/min/1.73m^2] to baseline prediabetes.

Results: The searches yielded nine prospective cohort studies, including 183,311 participants, mainly Asians (88%). In eight prospective studies analyzing IFG defined as fasting glucose 110-125 mg/dL, the random effects summary estimate showed an increased risk of CKD after adjustment for established risk factors including age, sex, body mass index and blood pressure (1.12; 95% CI: 1.02-1.21). When the only prospective study defining IFG as fasting glucose 100-125 mg/dL was added, the adjusted random effects summary estimate for CKD was 1.11 (95%CI: 1.02 - 1.21).



Only one study included information on IGT, and its exclusion did not change the prediabetes effect estimate (1.12, 95%CI: 1.02 - 1.21). There was no evidence of publication bias.

Conclusions: Prediabetes is associated with a modest but significantly higher risk of CKD in a diverse population. Screening for CKD among people with prediabetes, as well as aggressive management of prediabetes to prevent CKD occurrence may be warranted.

FR-PO1087

Prevalence of Microalbuminuria in U.S. Adults with Prediabetes Nishkarsh Saxena, Rukevwe Ehwarieme, Arvind R. Kunadi, Jill Gernand, Radhika Kakarala. *Internal Medicine, McLaren Regional Medical Center/ MSU, Flint, MI.*

Background: The risk of mortality with albuminuria level greater than 10 mg/g exists as a continuum. Microalbuminuria (MAU) in prediabetics (pre-DM) is associated with increased risk of diabetes, renal and cardiovascular disease. However, few studies have estimated the prevalence of MAU in the pre-DM state. The purpose of this study was to estimate and compare the prevalence of MAU in pre-DM diagnosed by either fasting plasma glucose (FPG), 2hr OGTT, HbA1c or by both FPG and HbA1c. We also determined the predictors of MAU in pre-DM patients.

Methods: We used the U.S. National Health and Nutrition Examination Survey (NHANES) 2009-2010 to identify participants with pre-DM and albumuria. Participants aged 18 years and above, with pre-DM and albuminuria were included in this study (N= 1,504). Pre-DM was defined by FPG = 100-125mg/dL, 2hr OGTT = 140-199 mg/dL or HbA1c = 5.7-6.4%. Prevalence of MAU was determined for pre-DM status. Multivariate logistic regression was used to determine the independent predictors of MAU. SPSS complex analysis was used to account for the complex sample design of the survey.

Results: Among patients with albuminuria, 5.6% had MAU. Prevalence of MAU in patients with pre-DM diagnosed by FPG only, 2 hr OGTT only, HbA1c only, and both FPG and HbA1c is 4.4%, 9.8%, 2.0% and 10.3% respectively (p-value=0.003). Age, gender, race and SBP adjusted prevalence of MAU was 2.7% and 5.5% for FPG and combined FPG and HbA1c. The odds of having MAU was 6.5 times more likely in patients diagnosed with both FPG and HbA1c compared to HbA1c only (odds ratio 6.45: [CI 2.38 to 17.50]).

Variable	OR	CI	P value
Age <60- ref ≥60	1.00 1.943	(0.777, 4.853)	0.130
Race Caucasian- ref AA Other	1.00 1.292 1.321	(0.474, 3.523) (0.443, 3.940)	0.596 0.596
Gender Male- ref Female	1.00 1.570	(0.596, 4.135)	0.339
Systolic BP <140- ref ≥140	1.00 2.238	(0.424, 11.813)	0.320
Pre DM HbA1C- ref FPG FPG + HbA1C	1.00 3.111 6.451	(1.002, 9.654) (2.378, 17.500)	0.001 0.050

Conclusions: Prevalence of microalbuminuria is higher in prediabetic patients diagnosed by both FPG and HbA1c. This group of patients may benefit from closer surveillance for progression to diabetes and/or chronic kidney disease.

FR-PO1088

Is Tertiary Prevention of Diabetic Kidney Disease with Maximum Tolerated Dose of a Renin Angiotensin System Inhibitor Achievable in Primary Care Setting? Ping Tyug Loh,[1] Shih Hui Ong,[1] Chee Kong Lim,[2] Vathsala Anantharaman.[1] *[1]Div of Nephrology, National Univ Hospital, Singapore; [2]National Healthcare Group Polyclinics, Singapore.*

Background: Singapore has the highest rate of Diabetic Kidney Disease (DKD) as the cause of End Stage Renal Disease in the world, reported at 65.5% in 2012. Despite achieved mean HbA1c of 7.7% and BP<140/90, DKD prevalence was 52.5% at one primary care cluster. A systems approach, coined Nephrology Evaluation, Management and Optimization (NEMO) program, was implemented as a collaborative effort between nephrologists and primary physicians, to augment DKD management in this primary care cohort.

Methods: This program incorporated: Information Technology (IT) to identify patients with early DKD [GFR>60 with either microalbumiuria (MI) or macroalbuminuria (MA)]. Coordinators to counsel and monitor eligible DKD patients not on maximum dose of Angiotensin Converting Enzyme Inhibitor (ACEI) /Angiotensin Receptor Blocker (ARB)

for optimization of ACEI/ARB therapy. Primary physicians to titrate ACEI/ARB dosage until maximum dose (MD), maximum tolerated dose (MTD) or normoalbuminuria (NA) was achieved.

Results: 5,620 patients had been enrolled since 2011. 2,132 have completed optimization over a mean duration of 7.6±5.1 months. Of these, 70% had MI, 30% had MA and 84% were on ACEI/ARB at baseline. Following ACEI/ARB dose titration, 87% were successfully optimized, with 24% achieving NA, 43% and 20% receiving MD and MTD of ACEI/ARB respectively. 2,066 patients with albuminuria result available at completion of program, 35.5% improved in their albuminuria status while 4.6% progressed from MI to MA.

Figure 1.Process Flow

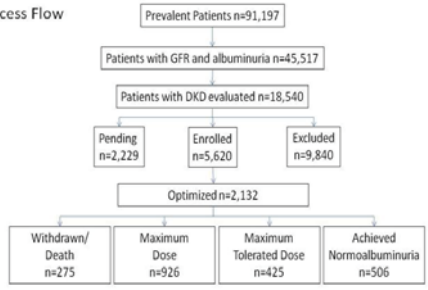
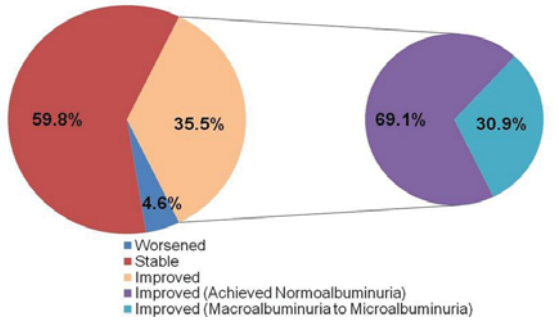


Figure 2. Albuminuria Status Post Optimization



Conclusions: Tertiary prevention of DKD is achievable in a real time, primary care setting, using IT as an enabler for patient selection and coordinators for delivery of planned care strategies, as to mitigate the high prevalence and progression of DKD.

FR-PO1089

Medication Adherence as a Predictor of Health Services Utilization in Adolescents and Young Adults with Chronic Kidney Disease Robyn Nicole Levine, Sarah Elizabeth Cohen, Jessica L. Ryan, Meredith Johnson, Karina Javalkar, Maria E. Ferris. *UNC Kidney Center, The Univ of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Adherence to treatment for CKD and ESRD is difficult to achieve as these conditions require complex medication, diet, and at times, home dialysis regimens. The relationship between medication adherence (MA) and health services utilization by adolescents/young adults (youth) needs to be elucidated.

Methods: Youth with CKD stages 2-5 from the pediatric and adult nephrology clinics at the UNC Kidney Center were invited. MA was measured using the Morisky Medication Adherence Scale, and health services utilization was measured by emergency department (ED) visits, total and preventable lifetime hospitalizations (Samuel SM, *Pediatrics* 2014), and length of in-patient admissions. Regression analyses were performed in SPSS.

Results: We enrolled 180 participants with the following characteristics: 58% female, 41% African-American, 42% White, 47% with public insurance, and 50% with CKD Stage ≥ 4. The mean age was 21 (±5.5), mean GFR was 55 (±33), and mean lifetime number of ED visits was 4.03 (±6.6). When controlling for age, gender, race, insurance, CKD stage, and glomerular filtration rate in a step-wise fashion, MA was a predictor of ED visits. Those with higher adherence had fewer total ED visits (B= -.212, p=.031). Lifetime hospitalizations (total and preventable) and length of admission were directly associated with CKD stage (p=0.000 for each metric), but ED visits were not.

Conclusions: Non-adherent youth with CKD have significantly greater ED visits compared to adherent youth. Targeted interventions to reduce health services utilization should focus on non-adherent patients and those with higher CKD stages.

Funding: Private Foundation Support

FR-PO1090

Study Design and Baseline Characteristics in the "Medication Intervention in Transitional Care to Optimize Outcomes for Chronic Kidney Disease" Clinical Trial Radica Z. Alicic,[1,3] Robert Short,[1,2] Cynthia F. Corbett,[1,2] Brian J. Gates,[2] Joshua J. Neumiller,[2] Celestina Barbosa-Leiker,[2] Sterling Mcpherson,[2] Kenn B. Daratha,[2] Katherine R. Tuttle.[1,2,3] *[1]Providence Health System, WA; [2]Washington State Univ, WA; [3]Univ of Washington Medical School, WA.*

Background: People with chronic kidney disease (CKD) are hospitalized longer and more often than people with other health conditions. Readmission rates are >20% in the 30 days after discharge. The aim of this study is to evaluate a medication information transfer (MIT) intervention in the transition from hospital-to-home for reducing acute care utilization in the CKD population.

Methods: CKD-MIT is a single-center, randomized, controlled clinical trial testing the hypothesis that improving MIT through a home-based pharmacy intervention within the first week of hospital discharge will reduce readmissions and visits to the emergency department or urgent care center for 90 days. The study is enrolling participants in 2 groups: CKD stages 3-5 (not dialyzed, target n=140) and CKD stage 5 (dialyzed, target n=70).

Results: Baseline characteristics of the CKD stages 3-5 (not dialyzed) group are reported as it is almost fully enrolled (n=115). The 3 most common reasons for the index hospitalization are: cardiovascular diseases (33%, 38/115), infections (21%, 24/115), and acute kidney injury (11%, 13/115). Participants' mean age (±SD) is 70±11 years. Nearly half, 47% (54/115) are women. Estimated glomerular filtration rate (eGFR, CKD-EPI) is 41±13 ml/min/1.73m^2. Other measures include: blood pressure 131±22/71±12 mm Hg; body mass index 33±8 kg/m^2; hemoglobin 12±2 g/dL; phosphorus 3.6±0.6 mg/dL; parathyroid hormone 72±59 pg/ml; K 4.6±0.5 mEq/L. The distribution of CKD stages includes: 7% (8/115) stage 2; 29% (33/115) stage 3a, 42% (48/115) stage 3b, 21% (24/115) stage 4; 2% (2/115) stage 5. A few participants are classified as CKD stage 2 because of eGFR shifts in the week between discharge and the baseline visit.

Conclusions: The CKD-MIT clinical trial will determine the effectiveness of an early home-based pharmacy intervention focused on reducing rates of acute care use in this high-risk group.

Funding: NIDDK Support

FR-PO1091

Increased Body Fat Rather Than Body Weight Has Harmful Effects on 4-Year Changes of Renal Function in the General Elderly Population with a Normal or Mildly Impaired Renal Function Jwa-Kyung Kim,[1] Jisuk Han,[1] Myung Jin Choi,[2] Jiwon Ryu,[2] Jung-Woo Noh,[3] Sung Gyun Kim.[4] *[1]Internal Medicine, Hallym Univ Sacred Heart Hospital, Seoul, Republic of Korea; [2]Internal Medicine, Hallym Univ Chuncheon Sacred Heart Hospital, Seoul, Republic of Korea; [3]Internal Medicine, Hallym Univ Gangnam Sacred Heart Hospital, Seoul, Republic of Korea; [4]Internal Medicine and Kidney Research Inst, Hallym Univ College of Medicine, Dongan-gu, Anyang, Korea.*

Background: With increasing age, body fat increases and muscle mass reduces. Even people with a normal weight can have a higher percentage of body fat. The aim of this study is to investigate the association between increased body fat and renal function decline (RFD) in the general elderly population with normal or mildly impaired renal function.

Methods: We conducted a study of 615 healthy individuals in the Korean general population aged ≥ 60 years who participated in two health screening check-ups separated by a 4-year period. Obesity was defined as the highest sex-specific tertiles of the percentage body fat (PBF). The main outcome was changes of estimated glomerular filtration rate (eGFR) during the 4 years. Significant RFD was defined as a decrease of eGFR over the upper quartile (≤–2.1%/yr).

Results: The mean age was 67.2±6.6 years. The median value of absolute decline in the eGFR and percent change were –3.0 mL/min/1.73m^2 and –0.87 %/yr in men and –3.1 mL/min/1.73m^2 and –0.89 %/yr in women, respectively. When stratified by sex-specific PBF tertiles, pronounced differences were observed in both sexes; those at the highest tertile of PBF showed the greatest decline in eGFR. Even after adjustments for traditional risk factors of RFD, PBF was independently associated with eGFR changes (b=-0.181, p<0.001). In addition, the harmful effect of a high PBF was consistently found in subjects with a normal weight, too (b=-0.141, p=0.006). Cases of significant RFD occurred in 181 participants (29.4%) and the risk was higher in obese participants. The odd ratios (95% CI) for significant RFD were 2.76 (1.28-7.74) in men and 2.02 (1.06-4.43) in women in a whole population and 3.15 (1,03-18.52) in men and 1.44 (1.01-3.28) in women with a normal weight, respectively.

Conclusions: Among the elderly population without comorbidites, increased body fat has harmful effect on RFD irrespective of body weight.

FR-PO1092

A Novel Approach to Improve Recognition of Geriatric Conditions among Older Adults with Chronic Kidney Disease C. Barrett Bowling,[1,2] Lynn E. Schlanger,[1,2] Steven M. Gorbatkin.[1,2] *[1]Atlanta Veterans Affairs Medical Center; [2]Emory Univ.*

Background: Nearly one in three older adults require nursing home care when starting dialysis and of these patients 45% are unable to ambulate and 60% need assistance with basic activities of daily living. Early recognition of geriatric conditions including falls,

cognitive impairment and mobility impairment may reduce the risk for functional decline as chronic kidney disease (CKD) progresses. However, assessment for geriatric conditions is not part of the routine clinical work-up for these patients.

Methods: The Comprehensive Geriatric Assessment for CKD (CGA-4-CKD) program is a partnership between Geriatrics and Renal at the Atlanta Veterans Affairs Medical Center (VAMC) designed to improve recognition of geriatric conditions and support the development of patient-centered treatment plans to prevent functional decline. The CGA-4-CKD care model includes: 1) a standardized Geriatric CKD Tool Kit in the electronic medical record, 2) a geriatrician embedded in the Renal Clinic and 3) provider-targeted education. Patients who are ≥ 70 years old with CKD seen in the Atlanta VAMC Renal Clinic had standardized screening for falls, impaired mobility, urinary incontinence and cognitive impairment. This information was reviewed by the embedded geriatrician and results were discussed with the nephrologist using an academic detailing approach.

Results: Between October 2013 and May 2014, 133 patients met inclusion criteria and 106 (80%) received geriatric screening. Eighty one patients (76%) had at least one geriatric condition and received further evaluation by the geriatrician during the clinic visit. Cognitive impairment, falls, urinary incontinence and mobility impairment were present in 24%, 27%, 31% and 46% of those screened. Of the 136 geriatric conditions identified, 92 (68%) had not been previously documented. Individualized, patient-centered care plans were developed and only 17% of patients had geriatric conditions that could not be completely addressed during the Renal Clinic visit and required further geriatric referral.

Conclusions: A CGA-4-CKD program can increase the recognition of geriatric conditions and may support patient-centered care for these patients.

Funding: Veterans Affairs Support

FR-PO1093

Physical Therapy Referral Varies Significantly Across Dialysis Facilities in the ACTIVE-ADIPOSE USRDS Special Study Nancy G. Kutner, Rebecca H. Zhang, Yijian Huang. *Emory Univ, Atlanta, GA.*

Background: Musculoskeletal problems, falling, and physical performance deficits are prominent among patients undergoing maintenance dialysis. Overall health status and collaboration of multiple organ systems to regulate energy expenditure and motor control contribute to these deficits. Physical therapists can identify impairments and activity related challenges and initiate interventions to target specific deficits. Little is known about receipt of physical therapy (PT) services by dialysis patients, however.

Methods: The USRDS special study ACTIVE-ADIPOSE evaluated 771 prevalent patients aged 20-92 undergoing maintenance hemodialysis (HD) 2009-2011 at 14 outpatient clinics located in Atlanta, GA, and the San Francisco Bay Area, CA. Follow-up evaluations were conducted at 12-months and 24-months, through September 2013. Walking disability, falls, and fractures were identified, and receipt of PT services across 24 months was determined among study participants at the 14 clinics.

Results: Almost one-fourth of the study cohort walked slower than 0.6 m/s or could not perform a walk test, indicating a high risk of physical disability, and two-thirds of ambulatory patients walked slower than 1.0 meter/second, a cut point at which individuals would benefit from fall prevention training. Over the 24-month study period, 44% of the cohort fell one or more times. Among fallers, patients who incurred a fracture were significantly more likely than those without fracture to report limitations in daily living activities but they were no more likely to receive PT. Receipt of PT across the 14 participating clinics ranged from 15% to 43% of patients; this variation was not associated with region, clinic size, or average age of patients per clinic.

Conclusions: PT services are reimbursed by third-party payers, and restorative PT is considered a critical need for chronic kidney disease patients, both non-dialysis and dialysis-dependent. Factors contributing to variation in patient referral for PT are important to understand as quality improvement initiatives focus on actionable deficits in patients' functional status.

Funding: NIDDK Support

FR-PO1094

Supplementary School Services for Children with Mild to Moderate Chronic Kidney Disease at Risk for Poor School Performance Arlene C. Gerson,[1] Allison Berryhill,[2] Matthew Matheson,[1] Stephen R. Hooper,[3] Susan R. Mendley,[4] Rica Garrison Rostad,[5] Susan L. Furth,[6] Bradley A. Warady,[7] Cynthia Wong.[2] *[1]Johns Hopkins; [2]Stanford; [3]Univ of NC; [4]Univ of MD; [5]Orting Sch Dist; [6]CHOP; [7]Childrens Mercy.*

Background: Children with poor school performance may be eligible for supplementary school services (SSS). Previously reported cross-sectional analyses from our group indicate that a sizable proportion of children with CKD receive SSS. Our aim was to longitudinally evaluate the relative significance of various risk factors for poor school performance on the likelihood of receiving SSS.

Methods: Subjects enrolled in the multicenter Chronic Kidney Disease in Children (CKiD) study with >2 yrs of data on SSS were used. Longitudinal logistic regression was done to see if risk factors were associated with concurrent receipt of SSS, and to see if gain, loss or persistence of risk factors resulted in a change in SSS. Risk factors were identified from published literature and included parent or subject report of school problems, absenteeism >20 days/yr, grade retention, below average IQ and attention problems.

Results: Of the 676 subjects, 2/3 had identifiable risk factors, yet fewer than half received SSS. Absenteeism (Odds Ratio, OR=5.0,p=.0002) and cognitive deficits (OR=4.2,p<.0001) were the strongest predictors for concurrent SSS receipt after controlling for socioeconomic status, race, GFR and duration of CKD. Poor attention (OR=2.7, p=0.002), grade retention (OR=2.4,p=.003), and report of school problems (OR=1.8,p=.03) also predicted receipt of SSS. Worsened cognitive functioning was associated with start of SSS during the observation period (OR=3.1,p=.03). Persistence of school problems (OR=2.8,p=.02), cognitive deficits (OR=3.6, p=.003), and poor attention (OR=4.1,p=.004) also predicted acquisition of SSS during the observation period. Of those not receiving SSS at the beginning of the study, 59 (23%) gained access to SSS over time. Of those receiving SSS at the beginning of the study 51 (25%) lost SSS during over time.

Conclusions: Persistent and worsening neurocognitive dysfunction was associated with SSS among CKiD subjects, yet many affected children do not receive these services.

Funding: NIDDK Support, Other NIH Support - NICHD, NHLBI, NNDS

FR-PO1095

Home Blood Pressure Telemonitoring System Among Patients with CKD: An Attempt to Achieve Optimal Blood Pressure Control with Real-Time Remote Monitoring Yoshinari Yasuda, Misao Niwa, Kanako Shibata, Mayumi Kamiya, Imai Junko, Sawako Kato, Shoichi Maruyama, Seiichi Matsuo. *CKD/Nephrol, Nagoya Univ, Nagoya, Japan.*

Background: Chronic kidney disease (CKD) is a global serious health issue. Due to high CKD prevalence in Japan, medical cooperation between general practitioners (GP) and nephrology specialists is essential. To achieve optimal blood pressure (BP) control among CKD patients treated by GP, home BP telemonitoring system was attempted and its utility was investigated.

Methods: Home BP of 79 CKD patients treated by GP were telemonitored using Medical LINK (Omron). BP data were automatically transmitted to central server by wireless-network and abnormal BP changes were reported to nephrology specialists in Nagoya Univ. Seasonal and daily variations of BP, pulse rate and room temp were analyzed.

Results: Home BP data were telemonitored well in all cases and one AKI due to hypotension was successfully detected. BP was higher in the morning, the lowest in summer and the highest in winter related to room temp. Pulse rates were comparable except winter.

	Male	Female		
Number / Age / BMI	62 / 70.0±12.2 / 24.47±3.66	17 / 73.1±10.7 / 25.39±5.78		
eGFR categories: G2 / G3a / G3b / G4 / G5	7 / 8 / 26 / 16 / 5	1 / 4 / 7 / 5 / 0		
	Spring (March-May)	Summer (June-August)	Autumn (September-November)	Winter (December-February)
Morning SBP / DBP (mmHg) / Pulse rate (/min)	137.8±16.3 / 77.8±8.4 / 67.2±8.6	129.9±12.8 / 73.8±8.4 / 67.0±8.8	134.3±11.7 / 76.6±8.5 / 67.7±8.5	139.5±13.1 / 78.8±8.1 / 69.5±9.0
Evening SBP / DBP (mmHg) / Pulse rate (/min)	130.2±15.3 / 72.6±9.2 / 70.4±9.1	125.0±15.2 / 69.0±8.2 / 69.4±9.4	128.2±12.7 / 71.7±8.6 / 71.4±9.2	131.6±13.2 / 73.6±10.0 / 72.9±8.9
Room temperature morning / evening (centigrade)	19.3±2.4 / 21.2±2.3	27.2±1.3 / 27.5±1.6	22.6±1.8 / 23.7±2.3	14.6±3.5 / 17.5±3.5

Daily BP variation correlated with age. Trend of higher seasonal BP variation was shown in association with eGFR tertiles.

Conclusions: Home BP telemonitoring system would be effective to achieve optimal BP control among CKD patients.

Funding: Government Support - Non-U.S.

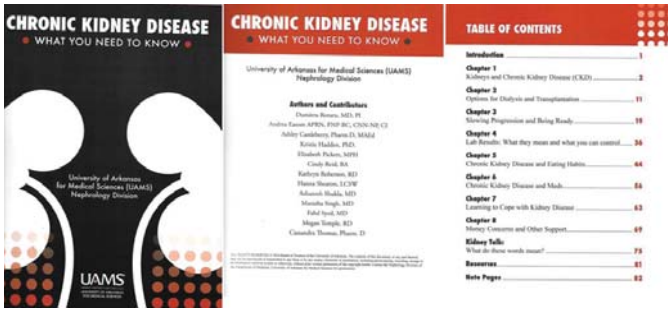
FR-PO1096

Development of Tools for a Telemedicine Based Chronic Kidney Disease Education Program Andrea K. Easom,[1] Dumitru Rotaru,[1] Fahd Syed,[1] Manisha Singh,[1] Ashutosh M. Shukla.[2] *[1]Nephrology, UAMS, Little Rock, AR; [2]Nephrology, Univ of Florida, Gainesville, FL.*

Background: Nationally, only 10% of ESRD patients are on home dialysis. We developed a multidisciplinary CKD education clinic which resulted in over 30% incident rate of home dialysis. Our hypothesis is that telemedicine is an effective tool for CKD education. The initial aim is to develop tools for CKD telemedicine education utilizing rigorous scientific methodology.

Methods: A multidisciplinary expert panel developed knowledge statements that were ranked on importance and used as the framework to create educational tools including a workbook, a questionnaire and slide sets. All materials underwent health literacy editing.

Results: The CKD Workbook is interactive and given to study subjects at the first visit. It consists of 8 chapters each based on knowledge statements.



Renal specific words are highlighted in red and defined in "Kidney Talk" sections on the same page where they first appear and at the end of the book. From a bank of 150 questions, each linked to a knowledge statement, 80 questions were tested in a small group of CKD patients to determine their difficulty. Questions that were important but either too easy or too hard were included at the end of book chapters in "Frequently Asked Questions" or "Test Your Knowledge" sections. A total of 28 questions were selected for the final questionnaire along with 2 questions on the patient's choice of modality. The CKD Questionnaire is given pre and post education to compare baseline knowledge and teaching effectiveness. The teaching slide sets and the program evaluation questions were also linked to knowledge statements.

Conclusions: The tools are now being tested at UAMS and 6 Arkansas Department of Health Telemedicine Sites. Based on this data, further validation and refinement of the tools is needed prior to statewide implementation of the program.

Funding: Pharmaceutical Company Support - Investigator Initiated Study, Baxter Renal Discoveries Extramural Grant Program

FR-PO1097

Dialysis Link Improves Clinic and Hospital Communication during Hemodialysis Patient Care Transitions in the Right TraC Program

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Background: Deficiencies in healthcare communication during care transition processes are common. Delays or inaccuracies in information transfer among health care professionals may adversely affect patient care and safety. The Right TraC™ Program surveyed standard practices of communication between dialysis services and hospitals and implemented a new method of information transfer utilizing Dialysis Link™. We sought to identify markers of performance associated with use of the Dialysis Link integrated communication system.

Methods: At 14 clinics in West Virginia, surveys were performed prior to the implementation of Dialysis Link. Baseline questions surveyed the frequency of transfer for patient information to the hospital upon admission and the availability of discharge information on return to the dialysis clinic. Dialysis Link has been implemented as of March 2014 in 9 dialysis clinics. This system is a centralized call center approach for exchanging medical information, between dialysis clinics and hospitals. First phase data was collected for utilization and satisfaction of services in the initial implementation of Dialysis Link.

Results: In the 9 clinics that implemented Dialysis Link, survey results qualitatively identified improvements in dialysis clinic and hospital communication parameters, and showed clinic satisfaction with the system (Figure 1).

Figure 1:

Baseline Survey Results in 14 Outpatient Dialysis Clinics	
Percent of clinics that do not send information to inpatient dialysis units when a patient is admitted to the hospital	54%
Percent of clinics that are not routinely notified when a patient is discharged from the hospital	50%
Percent of clinics that receive no information from inpatient dialysis when a patient is discharged from the hospital	67%
Dialysis Link Survey Results in 9 Dialysis Clinics	
Percent of clinics that used the Dialysis Link services since implementation	100%
Percent of clinics that used Dialysis Link for both sending and receiving patient admission and discharge information	67%
Percent of clinics that received more discharge records than before Dialysis Link started	44%
Average time to receive discharge documents	1-7 days
Range of clinic staff satisfaction with the Dialysis Link. Convenience and time savings were the top reasons for satisfaction	Moderate to Extremely Satisfied

Conclusions: In its early intervention stages, Dialysis Link demonstrated that improvement of medical communication and information transfer was streamlined, consistent and time saving for clinic staff. Ongoing analysis of this initiative will determine the potential impact as additional clinics implement Dialysis Link into their daily processes.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

FR-PO1098

Telephonic Case Management and Care Coordination Are Associated with Decreased Hospital Admission Rates

Sheetal Chaudhuri,[1] Fern Parlier,[1] Jane Brzozowski,[1] John W. Larkin,[1] Len A. Usvyat,[1] Chester A. Amedia,[2] Peter F. Sauer,[1] Kim L. Sonnen,[1] Franklin W. Maddux.[1] *[1]Fresenius Medical Care North America (FMCNA); [2]Fresenius Health Partners.*

Background: Fresenius Health Partners (FHP) provides care coordination to patients (Pts) with End Stage Renal Disease. As part of this, nurses' telephonically outreach to dialysis Pts based on pre-defined clinical pathways, which assess and triage signs and symptoms of hospitalized Pts related to heart failure, fluid overload, sore or open wound, diabetes, blood pressure and infections; reasons for hospitalization are documented and Pts receive targeted education to avoid readmissions.

Methods: FHP Pts treated in FMCNA clinics between Jan 1, 2013 and Mar 30, 2014 was analyzed. The first date of Pt telephone contact was captured. For every Pt, we computed mean Pt clinical and laboratory parameters 120 days before and 120 days after the first telephonic contact. Comparisons were performed using paired t-test analysis for continuous variables and Poisson regression for hospitalization rates. Only Pts with >80 days of the "before" and "after" period were included in the analysis.

Results: Overall, 386 FHP Pts were studied. Significant reductions in post-dialysis weight (0.3%) and pre-dialysis systolic blood pressure (SBP) (1.1%) were associated with implementation of telephonic FHP care coordination. Furthermore, after FHP care coordination was initiated, the hospital admissions and readmissions were identified to have significantly decreased by a notable 14.6% and 33.3% respectively.

Figure 1:	120 days before	120 days after	Percent change	p-value
Albumin (g/dL)	3.89 (SD±0.37)	3.9 (SD±0.36)	0.3%	0.232
Post-dialysis weight (kg)	76.27 (SD±19.77)	76.01 (SD±19.63)	-0.3%	0.043
Pre-dialysis systolic BP (mmHg)	154.26 (SD±19.31)	152.58 (SD±19.26)	-1.1%	0.001
Hospital admissions ppy	1.63	1.39	-14.6%	0.016
Hospital readmissions ppy	0.5	0.33	-33.3%	0.033

Conclusions: These results indicate that addition of telephonic case management is significantly associated with decreases in post-dialysis weight, pre-dialysis SBP, hospital admissions and readmissions. This study suggests that adding telephonic case management to overall patient care may be an effective intervention to decrease hospitalization rates in dialysis Pts.

Funding: Pharmaceutical Company Support - Fresenius Medical Care, North America

FR-PO1099

Development of a Smartphone-Based Kidney Care System for Patient Self-Management Support

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Background: Previously we developed smartphone-based system to support patient self-management of chronic disease. We used Bluetooth-enabled devices to remote monitor vital signs and identify real time non-acute changes in patients' health status. The systems were highly effective and well-accepted, even among older patients. We describe herein the development of a smartphone-based kidney care system for patients with advanced CKD.

Methods: Using qualitative methods, focus groups and semi-structured interviews were held with CKD patients and providers to seek their opinion about a mobile system. The information was used to form the framework and design principles to develop the first prototype. Iterative rounds of usability testing were conducted where participants provided insight on the appropriateness and ease of use of the prototype. Human factors specialists provided recommendations for the next iteration and enhancements of the prototype to improve the user experience. Validation testing helped test and refine the mobile system's features and functionalities to prepare it for the clinical trial phase.

Results: Based on interviews (n=10), we identified key CKD health behaviours and functionalities to be included in the smartphone app. The initial prototype, which included views for blood pressure (BP) monitoring, symptom assessment, CKD-related laboratory results and current medication list, underwent three rounds of usability testing (n=37) to refine the functionalities of the app. Participants commented on its design features, workflow, visual display of BP and laboratory data and ease of navigation. For the healthcare providers, we developed a dashboard which will contain detailed information on data gathered by their patients, a critical alert system and a one-page clinic visit summary report.

Conclusions: The smartphone-based kidney care system is fully developed and being piloted to assess its feasibility, acceptability and effectiveness in advanced CKD patients.

Funding: Private Foundation Support

FR-PO1100

Community-Based Disease Management Program for Patients with Chronic Kidney Disease Ingi Elsayed, Arif Khwaja. *Renal Dept, Sheffield Teaching Hospitals, United Kingdom.*

Background: CKD is common affecting 5-10% of UK, 14% of U.S. population. Cost of providing CKD care is rising; Medicare CKD costs in 1993 was 3.8%, up to 14.2 % in 2008 whereas,NHS CKD care cost in 2009-10 was $2.44 billion/year, 1.3% of NHS spending budget. More sustainable models of providing CKD care are needed. *Aim:* Evaluate impact of a remote, community-based disease management program (DMP) for patients with advanced CKD on disease progression, environmental impact and economical implications, compared to managing CKD in secondary (2ry) care.

Methods: Patients with stable CKD (Neither likely to require RRT in next 12 months, nor on immunosuppressive therapy) attending 2ry care renal clinics were offered choice of remote DMP, where an individualized care plan was generated by consultant. Their monitoring was performed at GP with results forwarded to 2ry care. The 2ry care clinic was replaced by telephone consultation with nurse specialist, detailing any change to patients' management. Written communication was also offered. Patients who declined remote DMP offer, continued to attend 2ry renal clinics. Cost to commissioners for 2ry clinics was $308/visit, while that for remote DMP consultant review was $110/review. We performed prospective analysis of lab data (eGFR, Hemoglobin level, Calcium and Phosphate levels), of all patients in both groups (age- and previous eGFR slope-matched), at time of enrolment on remote DMP and one year later.

Results: There were 75 patients in each group. There was not any statistically significant difference in the mean eGFRs, Hemoglobin, Calcium, Phopshate levels (p=0.667, 0.76, 0.915, 0.493, respectively) between either group one year later. Cost, for commissioners and distance travelled to point of care, were significantly lower in remote DMP group (p=.000, in both) than those attending 2ry care clinics, generating an annual carbon saving of 507 kg CO_2 equivalent.

Conclusions: Our data suggests that remote monitoring of CKD is deliverable, clinically safe in selected patients, whilst delivering significant carbon and cost savings. With prevalence of CKD increasing, remote monitoring of CKD may be a more sustainable model of delivery of CKD care.

FR-PO1101

Are Video Sharing Websites a Useful Source of Information on Dialysis? Neetika Garg,[1] Anand Venkatraman,[2] Ambarish Pandey,[3] Nilay Kumar.[4] *[1]Nephrology, BIDMC, Boston, MA; [2]Neurology, UAB, Birmingham, AL; [3]Medicine, UTSW, Dallas, TX; [4]Medicine, CHA/ HMS, Cambridge, MA.*

Background: End Stage Renal Disease is a growing public health problem. With increasing internet use to seek medical information, video sharing websites such as YouTube can serve as a powerful platform for dissemination of information on dialysis. Here, we analyzed the accuracy and content of videos related to dialysis posted on YouTube.

Methods: Using the search term 'dialysis,' the first 10 pages/ 200 videos on YouTube were screened for content related to dialysis. Two physicians independently categorized them as 'Useful,' 'Misleading' and 'Patient's personal experiences (PPE).' 5 point ordinal scales were used to grade reliability and quality. Information regarding source of upload, viewer interaction parameters (views per day, "likes" and comments) and content domains covered (epidemiology, etiology, signs/ symptoms, dialysis modalities, access, QOL and complications) was gathered.

Results: Of the 115 videos relating to dialysis, 67 (58.2%) were useful, 19 (16.5%) were misleading and 29 (25.2%) represented PPE (kappa statistic for interobservor agreement: 0.98, p<0.001). Useful videos were more informative, and scored highest on quality and reliability scores; however, they consistently ranked lower on viewer interaction parameters.

Variable	Useful	Misleading	PPE	p-value
Views per day Median (IQR)	3 (1–17)	11 (4–43)	14 (5–30)	0.013
Likes	4 (1–18)	43 (15–187)	19 (4–43)	<0.001
Comments	1 (0–3)	2 (0–10)	10 (2-35)	<0.001
Reliability score Mean±SD	3.39±1.03	0.32±0.48	1.93±0.75	<0.001
Quality score	3.54±0.86	1.10±0.32	2.76±0.44	<0.001
Content domains covered (total 7)	4.4± 1.7	1.6± 1.1	3.8 ± 1.6	<0.001

68% of misleading videos, all from un-credentialed sources, recommended unproven alternative therapies e.g. herbs, activated charcoal and enemas.

Conclusions: Scientifically correct information is viewed less compared to misleading videos and patients' personal stories. Authoritative sources should use this medium to provide patient specific and relevant information on dialysis. Including patients' personal stories could make this material more engaging.

FR-PO1102

Prevention Run: Opportunity for CKD Preventive Measures Attilio Di Benedetto,[1] Annalisa Ciotola,[1] Fabrizio Cerino,[1] Daniele Marcelli,[2] Bernard J. Canaud,[2] Annamaria Colao.[3] *[1]NephroCare; [2]Fresenius Medical Care; [3]Univ Federico II Naples.*

Background: Cardio vascular disease (CVD) prevalence is on the rise in industrialised countries, presenting a significant societal and economic burden. Early detection of CVD risk factors may prevent related complications, including chronic kidney disease (CKD). We report the results of a screening program focusing on a healthy population involving blood pressure, waist circumference and BMI evaluations. In addition, simple, rapid and non-invasive fluid overload screening was done by multi-frequency bioimpedance spectroscopy using the Body Composition Monitor (BCM).

Methods: During the "Napoli 2013 Prevention Race" organized by Federico II University, 191 healthy people were screened with collection of blood pressure, weight, height, waist circumference, BMI and BCM results, while CKD and CVD-related aetiology, diagnosis, and prevention were explained.

Results: 72 (37.7%) were male, mean age 52.55+13.9 yrs. Mean BPmax 128.62+19.8 and BPmin 77.57+11.8**.** 67 (35.1%) were overhydrated. 6 (3.1%) had BMI <20, 69 (36.1%) BMI 20-24, 80 (41.9%) BMI 25-29, and 36 (18.8%) BMI>30. Mean waist circumference 93.77+19.6 cm. 7 (3.7%) diabetics; 17 (8.9%) hypertensive; 6 (3.2%) with CKD. According to reference range table, 130 had LTI in a normal (N) range for their features of gender, age and race, 4 had a LTI> N, 57 had a LTI<N range. 149 had FTI in a normal (N) range, 35 had a FTI > N, and 7 had a FTI<N range.

	Overhydration (% of ideal BW)			LTI			FTI			Waist circumference (cm)		N pts
Age	<-1.0	>-1.0 < 1.0	>1.0	low	normal	high	low	normal	high	<88 (f) <102 (m)	>=88 (f) >=102 (m)	total
<20	0%	33.3%	66.7%	33.3%	66.7%	0%	0%	66.7%	33.3%	100%	0%	3
20-44	20.8%	47.9%	31.3%	27.1%	70.8%	2.1%	6.3%	77.1%	16.7%	66.0%	34.0%	48
45-64	14.6%	52.4%	33%	31.1%	66.0%	2.9%	2.9%	77.7%	19.4%	44.7%	55.3%	103
65-74	10.3%	51.7%	37.9%	34.5%	65.5%	0%	0%	82.8%	17.2%	48.3%	51.7%	29
>75	0%	50.3%	35.1%	12.5%	87.5%	0%	12.5%	75.0%	12.5%	62.5%	37.5%	8
Total Count	28	96	67	57	130	4	7	149	35	99	92	191
%	14.7%	50.3%	35.1%	29.8%	68.1%	2.1%	3.7%	78.0%	18.3%	52.1%	47.9%	100%

Figure 1. Overhydration , LTI , FTI and waist circumference results according to age ranges

Conclusions: 35% of participants had significant FO, and 18-48% had various degrees of metabolic syndrome. BCM measurements were easily incorporated into the screening for CVD and counselling. These data may constitute a useful healthy-matched reference database for advanced CKD patients. Screening is indicated for early detection of CVD risk factors in an apparent healthy population, helping to reduce public healthcare costs.

FR-PO1103

Metabolic Syndrome, Blood Pressure and Glycemic Risk amongst East Carolina University Freshman Hsiao Ling Lai,[1] Brittany S. Brown.[2] *[1]Internal Medicine and Pediatrics, East Carolina Univ, Greenville, NC; [2]College of Public Health, East Carolina Univ, Greenville, NC.*

Background: Obesity-metabolic syndrome is an increasing cause of subspecialty referral for management of hypertension and chronic kidney disease in youth.

Methods: Cardiometabolic risk in young adults of eastern North Carolina was investigated by sampling a cohort of East Carolina University (ECU) entrant undergraduates. From June to October 2010, 525 or 12% of Freshmen were screened for metabolic risk factors. Participants were classified with glycemic risk if blood glucose (BG) exceeded 140 mg/dL on random testing; or as meeting diabetic criteria if random BG exceeded 200mg/dL or previously diagnosed with diabetes. Elevated blood pressure was defined as systolic blood pressure (SBP) ≥ 140 mm Hg, diastolic blood pressure (DBP) ≥ 140 mm Hg or under treatment for hypertension.

Results: Seven participants (1.3%) met criteria for metabolic syndrome with four-fold increased risk amongst Black participants (RR 4.2, p=0.0562). Blacks had highest propensity for obesity (RR 3.9, p<0.0001). Black females (BF) had particularly high risk of obesity and inactivity (RR 3.904, p<0.0001, RR 1.59, p=0.019). Elevated BG was detected in 7% of participants. Asians and White males (WM) had highest glycemic risk (RR 4.6, p=0.007 and RR 1.7, p=0.065) and Hispanic participants most often met diabetic criteria (RR 3.5, p=0.01). Low HDL defined as HDL <50mg/dL in women and <40 mg/dL in men was the most common lipid risk factor with three-fold prevalence in BF versus Black males (BM) (35 versus 10.3%; RR 2.82, p=0.053). WM were characterized by elevated SBP (RR 2.439, p=0.003), BM by elevated DBP (RR=11.48, p<0.0001).

Conclusions: High BMI in Blacks, DBP elevation in BM and sedentary behavior in BF contribute to high incidence of cardiometabolic findings amongst Black ECU undergraduates. Glycemic risk is more prevalent in undergraduates of Asian, Hispanic and WM demographics. Improving our understanding and management of obesity-metabolic syndrome is important in impacting the long-term health of the young CKD population.

FR-PO1104

Unrecognized Chronic Kidney Disease: Effectiveness of Referral to Primary Care Units and Subsequently to Nephrologists Maria Goretti M.G. Penido,[1] Marcelo S. Tavares,[1] Celina De Faria Rezende,[2] Joao Milton Penido,[3] Mariana G. Penido de Paula,[2] Uri S. Alon.[4] *[1]Pediatric Nephrology Unit, Federal Univ of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Nephrology Center, Santa Casa de Belo Horizonte Hospital, Belo Horizonte, Minas Gerais, Brazil; [3]Internal Medicine, Federal Univ of Ouro Preto, Ouro Preto, Minas Gerais, Brazil; [4]Bone and Mineral Disorders Clinic, Children's Mercy Hospital and Clinics, Kansas City, MO.*

Background: Progression of chronic kidney disease (CKD) may be prevented by early diagnosis and referral to a nephrologist. The aim of this study was to evaluate whether primary care provider (PCP) is following individuals with CKD and refer them to a nephrologist.

Methods: During a community action on 2012 World Kidney Day in Belo Horizonte city, Brazil (2.74 million inhabitants), individuals were invited to participate. Participants underwent: blood pressure evaluation, measurement of serum creatinine and urinary protein, estimated glomerular filtration rate (MDRD/proteinuria). Individuals with grade III (A and

B) and IV, and those with grades I and II with proteinuria were referred to their PCP with a referral letter detailing our findings. Two years later the subjects were contacted by phone to find out if the PCP received our letter and referred them to a nephrologist.

Results: 298 individuals (66% female) with a mean age of 50.5±17.6 years were evaluated. We found: grade I CKD with proteinuria in 1.3%, grade II with proteinuria in 5%, grade III A and B in 20%, grade IV in 1.3%. No individual had grade V. All eighty-two individuals (grades I and II with proteinuria, grade III A and B and grade IV) were referred to their PCP. Telephone contact made two years later with 70 of these individuals showed that all provided our letter to their PCP; 45 are in regular follow-up with their PCP. Twenty-five individuals had no follow-up and only 6/45 were referred to a nephrologist.

Conclusions: A significant number of patients with CKD are not being followed and most others only by their PCP, with only a small minority referred to a nephrologist. Strategies are needed to enhance the referral rate of patients with CKD to a nephrologist.

FR-PO1105

Epidemiology and Referral Patterns of Patients with Chronic Kidney Disease in the Emirate of Abu Dhabi

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Background: According to estimates the dialysis prevalence in Abu Dhabi is around 370 ppm. The annual growth is 12-15%, and the dialysis population is likely to double in the next 5 years. Most patients present to dialysis as an emergency, only 2.7% have an arteriovenous fistula at first dialysis. The prevalence of chronic kidney disease (CKD) in the Emirate is undefined. A study of CKD epidemiology and referral patterns was undertaken.

Methods: SEHA, the Abu Dhabi Health Service delivery company, has a unified IT system containing all measurements made in its laboratories. This study considered all serum creatinine measurements performed between Sept 1, 2011 and Oct 31, 2012 from outpatient departments or emergency rooms. Estimated GFR (eGRF) was calculated using the Modification of Diet in Renal Disease formula (the Schwartz formula was used for children).

Results: We identified 331,360 samples from 212,314 individuals. Mean serum creatinine was 61+48 µMol/L in females (59+43 µMol/L Emiratis, 63+54 µMol/L expatriates) and 87+69 µMol/L in males (80+59 µMol/L Emiratis, 92+74 µMol/L expatriates). Among Emiratis, 4.6% of males and 2.8% of females had an eGFR between CKD 3-5. Among expatriates, 4.2% of males and 3.2% of females had an eGFR between CKD 3-5. On average, 8 to 3 months elapsed before a patient with CKD 3 to 5 attended a nephrology clinic respectively.

Conclusions: This study has defined the prevalence of CKD within Abu Dhabi and demonstrated the need to improve identification and referral of CKD patients. Possible solutions include campaigns to increase public and physician awareness of CKD.

FR-PO1106

Pre-Dialysis Education Program in a Tertiary Care Center of Northeast Malaysia

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Background: The prevalence of chronic kidney disease (CKD) and end-stage renal disease (ESRD) is increasing worldwide. Educational programme can slow down the progression of renal disease, improved survival rate on renal replacement therapy (RRT), enhance illness-knowledge and encourage better quality of life. Throughout this educational program, comprehensive information can be provided at an early stage to the CKD patients and their family members. This study meant to examine the distribution of RRT modalities opted by patients after pre dialysis education program.

Methods: We retrospectively reviewed the modalities chosen by patients who were attending CKD Talk from August 2011 to April 2014. Patients with CKD stage IV and V were invited to attend this program. Total patients attending CKD Talk were 571, but only 365 patients responded.

Results: Three hundred and sixty five patients, mean (SD) age was 61.46 (11.53) years. RRT modalities; HD, CAPD and CCPD were chosen by 93 (25.5%), 46 (12.6%) and 5 (1.4%) patients respectively. The remaining 221 (60.5%) patients were undecided which RRT they prefer and have been on follow-up by phone-calls after 2-3 months attending the program. Nineteen (8.6%) patients died, 124 (56.1%) alive and 78 (35.3%) were uncontactable. RRT modalities chosen by patients after follow-up were 43 (34.7%) HD; 6 (4.8%) CAPD and 75 (60.5%) were still undecided. Eight patients underwent arteriovenous fistula (AVF) creation. Possible reasons for not opting for RRT were lack of family support, in denial state and still undecided on the mode of RRT.

Conclusions: Although pre-dialysis education program is useful in assisting healthcare professionals and patients, however the results of our study revealed half of study population were unable to decide. More comprehensive measures should be taken in creating awareness of such pre-dialysis education programs.

FR-PO1107

Dialysis Patients Seeing Primary Care and Nephrologists Are More Likely to Get Preventive Services Than Those with No Primary Care

Bjoerg Thorsteinsdottir, LaTonya J. Hickson, Megan Reinalda, Priya Ramar, Amy W. Williams, Robert C. Albright, Nilay D. Shah. *Mayo Clinic, Rochester, MN.*

Background: Dialysis patients are a high risk patient population with a poor prognosis. United States Preventive Services Taskforce recommendations developed for the general population may not be appropriate for subsets in this population. With attention placed on delivering valued healthcare, one has to consider the value added of screening for patients with complex chronic disease and limited survival. We compared rates of preventive services for dialysis patients with or without primary care (PC).

Methods: Patients over 18 dialyzing in a regional dialysis network starting 2001-2010 were linked to USRDS. Analysis was limited to patients with outpatient dialysis claims, Medicare as primary payer and > 90d follow up. Family Practice, Internal Medicine, Pediatrics, Geriatrics and Preventative Medicine office visits counted as PC. Preventive services were based on CPT codes.

Results: Patients without PC were younger (56+16 versus 64+15), healthier (Charlson 5.5+3.0 versus 7.1+3.1), more likely male (66% versus 59%) and on transplant list (34.3% versus 12.1%); less likely to have DM (49% versus 62%) or CHF (39% versus 60%). Use of preventive services was low and lower in the patient group that had no PC.

Prev serv within 2 years of dialysis start			
	Primary Care		Total
	No (N=429)	Yes (N=2556)	(N=2985)
Influenza Vac*	63 (15%)	772 (30%)	835 (28%)
Pneumococcal Vac*	14 (3%)	194 (8%)	208 (7%)
Hep B Vac*	16 (4%)	256 (10%)	272 (9%)
Tetanus Vac*	5 (1%)	123 (5%)	128 (4%)
Lipids*	105 (25%)	1144 (45%)	1249 (42%)
Hgb A1C*	90 (43%)	963 (61%)	1053 (59%)
DM screening*	39 (9%)	488 (19%)	527 (19%)
Prostate ca screening*	23 (8%)	207 (14%)	230 (13%)
Colon Ca screening*	32 (8%)	405 (16%)	437 (15%)
Mammogram*	25 (17%)	379 (36%)	404 (33%)
PAP*	11 (8%)	180 (17%)	191 (16%)
Aortic aneurysm*	21 (7%)	200 (13%)	221 (12%)

Conclusions: Dialysis patients seeing PC have higher utilization of preventive services. However, in this population, specifically those ineligible for transplant with low life expectancy, services such as cancer screening may constitute overtreatment, leading to unnecessary spending and increased patient stress and burden.

FR-PO1108

Healthy Transitions: A Nursing and Informatics Program to Improve Outcomes in Late Stage Chronic Kidney Disease

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Background: Late stage chronic kidney disease (CKD stages 4-5) is a period characterized by poor outcomes and high costs. Suboptimal care is evidenced by high rates of hospitalization, inadequate patient preparation and education on ESRD modality choices, inappropriate initiation of dialysis for patients who may not be dialysis candidates, and failure to initiate hemodialysis utilizing an appropriate vascular access. The Healthy Transitions (HT) project was designed to improve processes of care and outcomes in late stage CKD while effectively controlling the rising cost of care.

Methods: All eligible patients with eGFR < 30 ml/min were enrolled into the program. Key components of the HT care delivery model include nurse care managers, disease and diet education, focused modality selection, a fistula planning protocol, daily weights, home safety evaluation, outpatient dialysis safe start program and an informatics system that drives clinical processes with daily alerts to the nurses.

Results: 162 patients were enrolled with 1024 patient-months of program follow up. Average patient age was 69.0±x years. 62% of enrolled patients were male. 71% of patients were white, 10% black or African American, 19% other. 88% of eligible patients made a modality selection within 3 months of enrollment. Of patients initiating renal replacement therapies 24% started peritoneal dialysis, 11% received preemptive transplantation and 65% hemodialysis (HD). Of HD pts 41% started without a hospitalization. Access for the first HD treatment was an AVF in 68% and only 22.7% had a catheter in place at the first HD treatment. The hospitalization rate was 1.14 per pt/year compared to 1.41 in the year prior to enrollment (RR 0.81, $p<0.05$) and 1.99 for physicians in the same practice not participating in the program (RR 0.57, $p<0.05$). The mortality rate was 0.04 per patient-year. Greater than 95% of the enrolled population reported being satisfied or extremely satisfied with program services.

Conclusions: The HT late stage kidney disease program resulted in improved patient outcomes utilizing nursing and informatics interventions.

FR-PO1109

The Rate of Change of Estimated Glomerular Filtration Rate in a Mainly Indo-Trinidadian Population: A Retrospective Database Study

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Background: Despite the global health burden of Chronic Kidney Disease (CKD) very little is known about the rate of progression in the West Indies.This study seeks to determine the rate of change in estimated glomerular filtration rate (eGFR) in patients and the factors affecting rate of decline.

Methods: A retrospective observational analysis was done on patients in renal outpatient clinic in southern Trinidad.The CKD patients were staged based on the eGFR (ml/min per1.73m^2) calculated from the Modification of Diet in Renal Disease (MDRD) equation.A linear mixed regression model was used to determine the mean annual rate of decline of the eGFR per patient and multivariate analysis was done to determine the factors affecting the rate of decline.

Results: 150 CKD patients had mean age (SD) 63.61 (9.33) years, 61.3% males, 48% were 65 or older.Indo-Trinidadians were 73.3% of the study population 24.0% were Afro-Trinidadians.There were 74.0% diabetics and 94.7% hypertensives.94% patients were stage 3 CKD or lower at presentation.Mean (SD) baseline eGFR was 34.98 (12.90) for Indo-Trinidadians and 44.10 (15.64) for Afro-Trinidadians, (p=0.001). Serum phosphate level was inversely related to baseline eGFR (B=-4.93, p=<0.001).Mean (SD) final eGFR was 28.04 (13.15) for Indo-Trinidadians and 34.91 (13.49) for Afro-Trinidadians, (p=0.006). The mean annual rate of decline in eGFR was 2.57 (95% CI 2.04 to 3.10). Decline in kidney function was seen in 84.67% patients,16 patients had progressed to stage 5 CKD, 87.3% patients had a final eGFR <45 compared with 71.3% initially.Multivariate analysis showed that the rate of decline in eGFR was slower for older patients (B = 0.007, p = 0.021) but was not related to comorbid conditions, gender or ethnicity. 37.3% patients had a rapid annual rate of decline in eGFR of >3.

Conclusions: Diabetes and hypertension were found to have no impact on rate of decline of eGFR once CKD was established.Gender and ethnicity were not associated with rate decline in this study.Older patients were associated with a slower rate of decline in renal function.

FR-PO1110

Patient's Perception Regarding Their Erythropoiesis Stimulating Agent Using a Choice Based Conjoint Analysis

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Background: Treatment adherence is essential to improve quality of care in chronic diseases. This study aimed to assess in real-life conditions patients preference and satisfaction regarding erythropoietin stimulating agent (ESA) use for the treatment of renal anemia in patients with chronic kidney disease not on dialysis (CKDnd).

Methods: This French, 6-month, non-interventional, multicentre, prospective study was conducted by 106 nephrologists. Eligible patients were on stage 2 to 5 CKD and started continuous erythropoietin receptor activator (C.E.R.A.) treatment at inclusion. They were naive or previously treated with an ESA for their anemia. The primary endpoint was the relative importance according by the patient preference to different characteristics of ESA treatments. Choice-based conjoint (CBC) analyses were performed (7 product characteristics, 2 or 3 levels per characteristic) at baseline and after 6 months under C.E.R.A. treatment.

Results: 789 patients were included, 356 ESA-naïve and 433 previously receiving an ESA. The mean age was 73±14 years, 54% men, 74% were on CKD stages 4 or 5. More than 80% of patients declare treatment efficacy as the most important expectative in ESA choice process but CBC analyses revealed that frequency of injections was more crucial, relative mean weight: ~30% versus ~20% for efficacy. Pain at injection site and hemoglobin not exceeding the recommended target were confirmed as important criteria for patients with a relative mean weights of ~15%. No new or unexplained safety signals were noted. Similar results were found for ESA naïve and non-naïve patients.

Conclusions: Using choice-based conjoint design for the first time in a non-interventional ESA study, these data showed that monthly injections, treatment efficacy and pain at the site of injection are key patients' expectations relative to ESAs use.

Funding: Pharmaceutical Company Support - Roche Pharma

FR-PO1111

Mid-Infrared Spectroscopy: Detection of Therapeutic Response in Experimental Glomerulonephritis and Application in Patients

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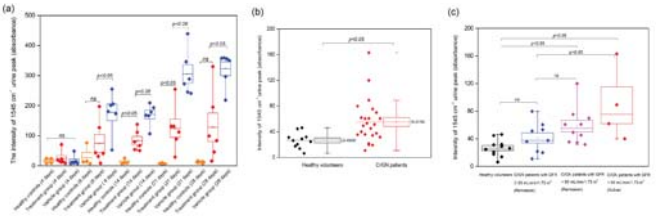
Background: We recently reported several potential markers of progressive renal injury in experimental GN using Fourier transform infrared (FTIR) spectroscopy.However, it remains uncertain whether this technique may be applicable in clinical situation.Thus, we aimed to find out whether the FTIR method was capable of detecting therapeutic response in nephritic rats treated with corticosteroid and in patients with crescentic GN (CrGN).

Methods: Experimental GN: Nephrotoxic nephritis (NTN) was induced in Wistar Kyoto (WKY) rats. The groups were **control** (n=4):normal WKY rats;**treatment** (n=6):NTN rats treated with 0.25 mg/kg of dexamethasone (DXM),*ip* every 4 days;**vehicle** (n=6):NTN rats treated with PBS,*ip*. During the course of NTN, 24 hour urine was collected at 4,8,14,21,and 28 days and plasma was taken at the time of cull. **Clinical GN**: urine was collected from 11 healthy volunteers and 24 CrGN patients. All samples were measured by FTIR spectro- scopy and analysed by the established method.

Results: In experimental GN, the intensity of the 1545 cm^{-1} urine peak was reduced in response to DXM treatment [figure1]$_a$. A similar response was also shown in three previously detected potential markers in plasma at 1705,1460 and 1240 cm^{-1}. The urinary 1545 cm^{-1} peak was significantly higher in CrGN patients compared to healthy volunteers



$_b$. Among CrGN patients,the intensity of 1545 cm^{-1} peak was higher in active CrGN patients with eGFR< 60 units [figure1]$_c$.

Conclusions: FTIR spectroscopy is a potential tool for rapid-screening and/or diagnosing patients at risk of progressive kidney disease and monitoring therapeutic response. The 1545 cm^{-1} peak of urine is the spectral marker most likely to be valuable in detecting severe CrGN.

Funding: Private Foundation Support

FR-PO1112

High Resolution MRI Renography Using Hyperpolarized [^{13}C]Urea

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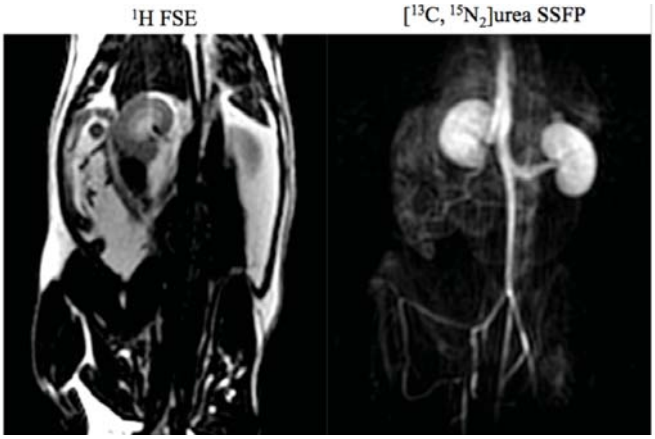
Background: Dissolution dynamic nuclear polarization (DNP) enables >50,000-fold NMR signal enhancement of ^{13}C-labeled molecules in the liquid state, introducing a new class of endogenous intravenous MRI contrast agents (Ardenkjaer-Larsen et al. *PNAS*. 2003.). A recent first clinical trial demonstrates feasibility of this approach for investigating human disease (Nelson et al. *Sci Transl Med*. 2013.). Hyperpolarized (HP) [^{13}C]urea has excellent potential as a MRI contrast agent due to high polarization and exceptional safety profile. Here we demonstrate the application of HP [^{13}C]urea for *in vivo* high resolution (<1mm) MRI renography in rats.

Methods: Preparation of HP ^{13}C media is detailed elsewhere (Ardenkjaer-Larsen et al.). Anesthetized rats placed in a clinical 3T MRI were infused with 2mL 150mM [^{13}C,^{15}N$_2$] urea over 10s via tail vein. Our group recently discovered that secondary labeling with ^{15}N extends the T_2 relaxation times of urea, resulting in greatly improved image quality (Reed et al. *IEEE Trans Med Imaging*. 2013.). Rats were imaged 30s after infusion, using a sub-mm coronal 2D projection imaging sequence with steady state free precession (SSFP).

Results: A sub-millimeter [^{13}C]urea renogram is shown in Fig. 1 (right).



The renal urea signal is primarily tubulointerstitial, according to separate experiments where chasing the HP bolus with a high MW paramagnetic complex (thereby quenching the intravascular signal) has no effect on renal urea signal. Modulating physiologic state between diuresis and anti-diuresis produces urea-specific imaging changes, evidencing differential urea transport (von Morze et al. *AJP Renal*. 2012.).

Conclusions: Sub-millimeter renography is possible using HP [^{13}C]urea MRI, with no significant potential for nephrotoxicity and no radiation exposure.

Funding: NIDDK Support, Other NIH Support - NIBIB

FR-PO1113

Predicting Clot Formation in Implanted Hemofilters Amanda Buck,[1,2] Joseph J. Groszek,[4] Shuvo Roy,[3] William Fissell.[4] *[1]Radiology, Vanderbilt Univ, Nashville, TN; [2]Vanderbilt Univ Inst of Imaging Science, Vanderbilt Univ, Nashville, TN; [3]Bioengineering and Therapeutic Sciences, Univ of California, San Francisco, CA; [4]Nephrology and Hypertension, Vanderbilt Univ, Nashville, TN.*

Background: A common failure mode of vascular devices is clotting related to the local hemodynamic environment. Computational fluid dynamics (CFD) models can be used to predict flow fields, and we have used this approach to model flow in an implantable hemofilter using a realistic flow waveform. We hypothesized that CFD simulations of blood flow would demonstrate pathophysiologically-relevant flow patterns that coincide with locations of clot formation.

Methods: We designed an implantable blood conduit to test hemofiltration membranes *in vivo*. Blood flow in the device was modeled with pulsatile flow boundary conditions based on *in vivo* ultrasound measurements. Regions of recirculation and slow near-wall flow were identified in each computational model. Hemofilter models were implanted in large animals anastamosed to iliac artery and vein for 30 days, or until thrombosis, then explanted.

Results: CFD simulations predicted two persistent recirculation regions: one along the outer wall of the proximal curve, and one zone of near-wall recirculating/slow flow along the inner wall of the distal bend (Fig. 1). Of 4 implants, 2 showed localized clot formation and two more diffuse thrombosis. Clot formation was identified in the same two areas in which CFD models predicted recirculation (Fig 1).

Conclusions: Pulsatile flow simulations predicted pathophysiologically relevant flow patterns in the same regions in which clot formation occurred. In the future, similar physiologically realistic simulations may prove useful for eliminating recirculation regions in subsequent device design.

Funding: Other NIH Support - NIBIB 1R01EB014315

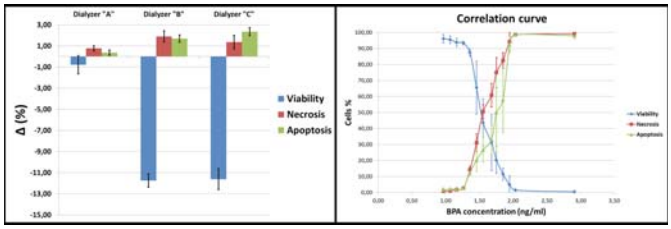
FR-PO1114

Cytotoxicity of Bisphenol A in Hemodialyzers Mauro Neri, Grazia Maria Virzì, Alessandra Brocca, Francesco Garzotto, Federico Nalesso, Monica Zanella, Claudio Ronco. *International renal Research Inst Vicenza (IRRIV).*

Background: Bisphenol A (BPA) is an environmental hormones or endocrine disrupting molecular compound and is used for the production of some plastics. In particular, in many dialyzers, polycarbonate (PC) of the housing and polysulfone (PSu) of the membranes are plastics based on polymerization of BPA. However, the release of BPA into the extracorporeal circuit may lead to cytotoxic events for blood components. Consequently, dialyzers made of different materials may avoid or reduce the BPA elution into the blood of the patients. We compared in vitro viability, necrosis and apoptosis on monocytes cell line deriving from BPA elution using 3 different dialyzers.

Methods: We compared dialyzer "A" (polypropylene housing and polynephrone membranes) versus dialyzer "B" (PC housing and PSu membranes) versus dialyzer "C" (PC housing and polynephrone membranes).We circulated in in vitro circuit 600 ml of cells medium RPMI for 240 minutes, repeating the same experiments using the 3 different dialyzers. We incubated U937 monocytes for 24 hours in medium samples taken before and after the treatments and we evaluated the differences between viability, necrosis and apoptosis by flow cytometer in the 3 dialyzers. We also tested the correlation between BPA concentrations (range between 0.97 to 2.91 ng/ml) and its cytotoxicity.

Results: Dialyzer "A" releases less BPA (46.5±53.5ng/treatment) than dialyzer "B" (232.4±32.8ng/treatment) and "C" (157.1±52.8ng/treatment). Moreover the viability of the monocytes is higher, necrosis and apoptosis are lower. The BPA citotoxicity is evident in the correlation curve with viability, necrosis and apoptosis.



Conclusions: Viability, necrosis and apoptosis of monocytes are influenced by BPA concentration. Use of alternative polymers for dialyzer's components,in particular in state of PC, may reduce this cytotoxic effect.

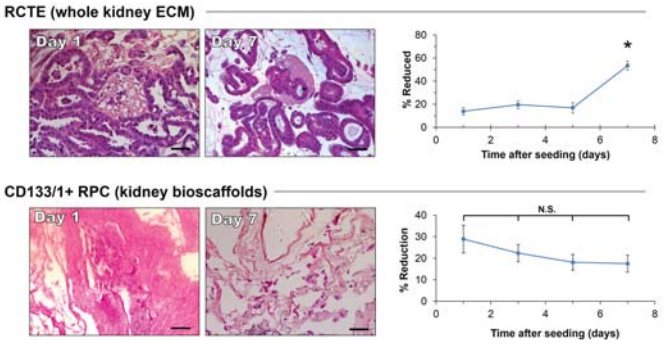
FR-PO1115

Decellularized Kidney Extracellular Matrix Supports Human Renal Parenchymal Cell Maturation in Three-Dimensional Culture Joseph S. Uzarski,[1] Heather Hilary Ward,[2] Angela Wandinger-Ness,[3] Jason Wertheim.[1] *[1]Comprehensive Transplant Center, Northwestern Univ Feinberg School of Medicine, Chicago, IL; [2]Dept of Medicine, Univ of New Mexico HSC, Albuquerque, NM; [3]Dept of Pathology, Univ of New Mexico HSC, Albuquerque, NM.*

Background: Generating a patient-specific kidney on demand would combat the donor graft shortage, extending transplantation to more patients with renal failure. Our objective was to investigate the capacity of decellularized renal extracellular matrix (ECM) to facilitate the growth and maturation of human renal parenchymal cells that could ultimately restore functionality to decellularized kidneys.

Methods: Rodent kidneys were decellularized using detergents and then repopulated with human renal cortical tubular epithelial (RCTE) cells via infusion through the renal artery. Alternatively, 1 mm kidney bioscaffold sections were seeded with primary human CD133/1+ renal progenitor cells (RPC) in multi-well dishes. We monitored cellular metabolism, dispersion, and morphological adaptation within the renal ECM over time.

Results: RCTE cells infused into whole kidney matrices dispersed in the peri-tubular space and formed patent tubular structures with recellularization of over 50% of the ECM after 24 hours. RCTE cells proliferated within the ECM over one week of perfusion culture with a significant increase in viability measured at day 7 ($p<0.001$).



RPC adhered to kidney bioscaffolds with similar efficiency, migrated into the ECM, and remained viable over one week of culture.

Conclusions: The bioactive kidney ECM supports repopulation with renal parenchymal cells and maturation into structures resembling native kidney tubules. We conclude that as a substrate the renal ECM facilitates renal cellular differentiation, and has potential as a 3D scaffold system for developing a bioartifical kidney graft.

Funding: Private Foundation Support

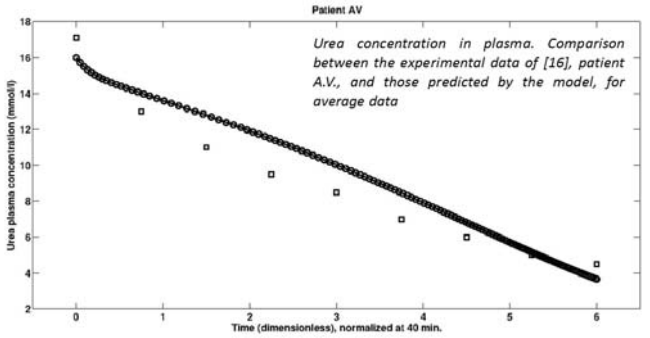
FR-PO1116

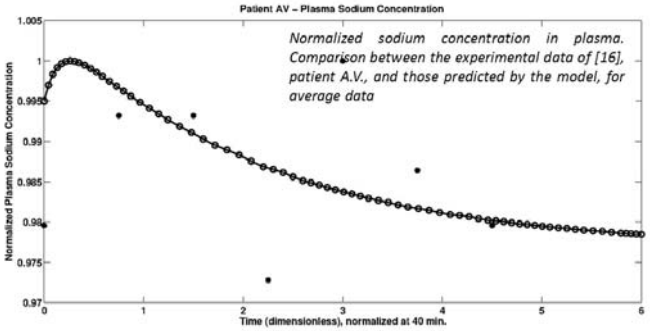
Modeling Blood Filtration in Hollow Fibers Dialyzers Matched with Patient's Dynamics Claudio Ronco,[1] Francesco Garzotto,[1] Jeong Chul Kim,[1] Antonio Fasano,[2] Angiolo Farina.[2] *[1]Nephrology-IRRIV, St. Bortolo Hospital, Italy; [2]Matematica e Informatica Dini, Univ di Firenze, Italy.*

Background: We develop a mathematical model for cross filtration in a hollow fibers dialyzer,taking into account not only the phenomena occurring within the machine,but also the redistribution of chemicals between intra-and extracellular compartments in the patient's body.

Methods: We report for brevity only steps in the derivation of the model write the full fluid dynamical equations for the blood (with some rheological model) and Darcy's law for the flow in the membrane,use a *double rescaling* of the space and velocity coordinates (e.g. $x=x^*/L^*$, $r=r^*/R^*$) and rewrite all equations in dimensionless form,select suitable time scales (one for the physics of the dialyzer,one for the dynamics of the body compartments),expand the unknowns (pressures,velocities,hematocrit,concentrations) in powers of e,match the terms of equal exponents (those with negative exponents and the zero order terms,disregarding higher order contributions).The model consists of two differential systems:one describing the three flows in the dialyzer (blood,dialyzate and cross flow),the other illustrating the dynamics of water and solutes in the organism,triggered by the modification of blood composition taking place in the device.

Results: We have performed 2 classes of numerical simulations.First we have validated the model with the experimental data reported in"A simple mathematical model applied to selection of the sodium profile during profiled haemodialysis,Nephrol Dial Transplant,Colì L et al."then we have performed some numerical experiments.In Figure we have reported the comparison between the simulation and the experimental data for a specific patient.





Conclusions: Both simulation and the experimental data for a specific patient show a good agreement,particularly in consideration of the fact that simulations refer to average data.

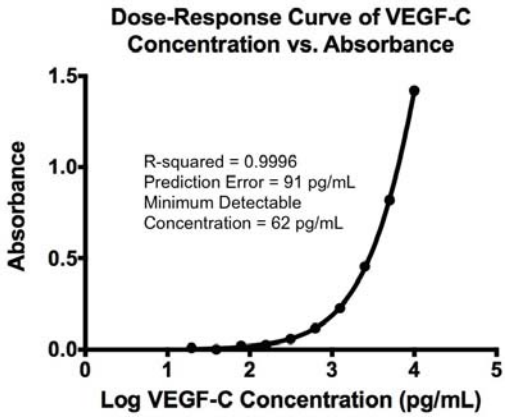
FR-PO1117

A Novel Device for the Detection of VEGF-C, a Biomarker of Chronic Allograft Rejection Demetrios S. Maxim.[1,2] *[1]Div of Nephrology, Dept of Medicine, Brigham and Women's Hospital, Boston, MA; [2]Dept of Biomedical Engineering, Tufts Univ, Medford, MA.*

Background: Long-term graft survival following renal transplantation will only become a reality when chronic allograft rejection (CAR), the leading cause of late organ loss following transplantation, can be effectively treated. If detected early enough, there can be intervention to minimize the disease. To date, however, no non-invasive test exists for routine screening. In this project, we aim to develop a simple, rapid, inexpensive, and minimally invasive device to quantitatively screen for VEGF-C, a biomarker of CAR, in human blood samples.

Methods: Test strips were prepared by coating the wells of 8-microwell strips with capture antibody directed against human VEGF-C. Biotinylated detection antibody and streptavidin-HRP conjugate were diluted in stability buffers to preserve activity and suspended in solution in the microwells. The optimal concentration for each antibody was determined by titration. Following the addition of samples, the strip was inserted into the portable device, where the wells were incubated, washed, and developed with colorimetric substrate. After stopping the reaction with sulfuric acid, the device measured the absorbance of each microwell.

Results: The completed device produced a dose-response curve with an R-squared value of 0.9996, a prediction error of 91 pg/mL, and a minimum detectable concentration of 62 pg/mL. Each test takes 25 minutes, achieves similar sensitivity to that of a commercially available ELISA, and is 12 times faster and 16 times less expensive.



Conclusions: These data show that the novel device described can serve as a simple, rapid, inexpensive, and minimally invasive screening tool for chronic allograft rejection. In addition, the device can easily be modified (simply by using different antibodies) to detect other biomarkers, and therefore other diseases.

Funding: Private Foundation Support

FR-PO1118

Point of Care Monitoring of Haemodialysis Patients with a Breath Ammonia Measurement Device Based on Printed Polyaniline Nanoparticle Sensors Frank Ward,[1] Troy Hibbard,[2] Karl Crowley,[2] Frank A. Kelly,[1] Patrick P. O'Connor,[1] John N. Holian,[1] Alan J. Watson,[1] Anthony J. Killard.[2,3] *[1]Dept of Renal Medicine, Saint Vincent's Univ Hospital, Dublin, Ireland; [2]Biomedical Diagnostics Inst, Dublin City Univ, Dublin, Ireland; [3]Centre for Research in Biosciences, Dept of Biological, Biomedical and Bioanalytical Sciences, Univ of the West of England, Bristol, United Kingdom.*

Background: Breath is an excellent candidate for diagnostic technology development. Breath ammonia levels are elevated in kidney dysfunction. Current instrumental measurements are large, heavy and expensive, thus impractical as point-of-care devices.

Methods: A device for measuring breath ammonia was developed based on a single use, disposable, inkjet-printed ammonia sensor, fabricated using polyaniline nanoparticles. The device was optimized for sampling ammonia in human breath samples and validated in normal volunteers. Breath ammonia and blood urea nitrogen (BUN) levels were sampled pre- and post-haemodialysis in patients in a single centre to assess the degree of correlation.

Results: The 20 patients recruited underwent 44 episodes of sampling. The mean pre-dialysis breath ammonia level was 930 parts per billion by volume (ppbv), (range 164 - 2243 ppbv) reduced significantly post-dialysis to a mean level of 227 ppbv (range 19 – 1138 ppbv) ($p<0.01$). The mean pre-dialysis BUN was 22 mmol/L (range 9 to 35mmol/L), reduced significantly post-dialysis to a mean level of 6 mmol/L (range 3 to 10 mmol/L) ($p<0.05$). Correlation of the combined pre- and post-dialysis breath ammonia levels with BUN yielded a Pearson coefficient of 0.61 ($p<0.01$). 11 patients underwent pre- and post-dialysis sampling on multiple occasions, with the intra-individual Pearson correlations between breath ammonia and BUN varying between 0.86 and 0.96 ($p < 0.07$ to 0.0001).

Conclusions: These findings demonstrate the potential of this system to be used in the monitoring and treatment of patients with kidney disease. Future studies should focus on similar validation in peritoneal dialysis and CKD.

FR-PO1119

Implantable Hemofilter: 32 Day Patency in a Canine Surgical Model Clark David Kensinger,[1] Seth J. Karp,[1] Joseph J. Groszek,[2] David Christopher LaNeve,[3] Phillip E. Williams,[3] Baoxia Mi,[4] Mark S. Goodin,[5] Rishi Kant,[6] Torin Yeager,[6] Shuvo Roy,[6] William Fissell.[2] *[1]Dept of General Surgery, Vanderbilt Univ Medical Center; [2]Dept of Medicine, Vanderbilt Univ Medical Center; [3]Div of Surgical Research, Vanderbilt Univ Medical Center; [4]Dept of Civil and Environmental Engineering, Univ of Maryland; [5]Dept of Bioengineering and Therapeutic Sciences, Univ of California San Francisco; [6]Dept of Bioengineering and Therapeutic Sciences, Univ of California San Francisco.*

Background: An implantable artificial kidney using silicon nanopore membranes is in development to provide the benefits of transplantation to all dialysis patients. The major challenge in implementation of chronic blood contacting devices is thrombosis. We report a successful 32-day device implant in a canine model.

Methods: A single-channel, parallel-plate hemofilter was manufactured from polycarbonate. To determine the influence of the hemofilter blood path on thrombosis and long-term patency, two silicon chips coated with biocompatible polymer (sulfobetaine methacrylate) were mounted in the hemofilter in lieu of the planned silicon nanopore membranes. The device was attached to 6 mm Polytetrafluoroethylene (PTFE) grafts anastomosed to the common iliac artery and vein. Therapeutic heparin (100 U/kg) was administered intra-operatively. The animal had no restrictions post-operatively. The dog received lovenox (0.5mg/kg) once a day at a venous thromboembolic prophylactic dose to demonstrate device patency in the absence of full anticoagulation. The grafts were serially assessed post-operatively with a pulse wave doppler ultrasound. The device was explanted at Day 32.

Results: The inflow and outflow PTFE grafts showed patent, pulsatile flow with peak flow rates between 1.7 L/min to 1.9L/min throughout the 32 day experiment. The device was patent without thrombus formation on explant. The animal had no complications.

Conclusions: Hemofilter device patency in the absence of therapeutic anticoagulation highlights successful surgical technique, nonthrombogenic blood flow conduit geometry, and manufacturing biocompatibility providing the foundation for further preclinical canine experiments.

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FR-PO1120

Mapping Murine Diabetic Nephropathy Using CEST-MRI Technique Feng Wang,[1] David J. Kopylov,[2] Keiko Takahashi,[3] Zhongliang Zu,[1] Yuna Park,[3] Christopher Chad Quarles,[1] Raymond C. Harris,[3] Takamune Takahashi.[3] *[1]Vanderbilt Univ Inst of Imaging Science, TN; [2]Drexel Univ, NJ; [3]Nephrology, Vanderbilt Univ, TN.*

Background: Diabetic nephropathy (DN) is the leading cause of renal failure. The imaging technique that enables non-invasive assessment of this disease, identifying the early stage of DN or predicting its progression, should facilitate the intensified treatment to high-risk populations. DN is associated with the changes in tissue metabolites (e.g. glucose, glycogen, glycosaminoglycan) that exhibit significant chemical exchange saturation transfer (CEST) effects in MRI. Therefore, here we evaluated the utility of CEST-MRI in this disease.

Methods: Mice were scanned on 7T MRI using CW CEST sequence and SE-EPI readout. Fat saturation was applied at RF offset (-1042 Hz). A control scan was performed with an RF offset of 100 kHz. The MTR_{asym} maps were created by asymmetric analysis. A peak fitting algorithm was used to decompose overlapped amide, amine, hydroxyl, direct saturation on free water, and aliphatic peaks around 3.5, 2.2, 1.2, 0 and -3.3 ppm RF offsets, respectively. The CEST signals, NOE, and MT effect were mapped in each kidney. Sensitivity of CEST imaging was evaluated across db/m, db/db, and db/db eNOS-/- mice at 16 wks, and longitudinally in db/db mice at week 8, 12, 16, 20 and 24 wks.

Results: Compared with db/m mice, the glucose/glycogen signals (RF offset ~1.2 ppm) were increased by 55% in outer medulla (OM) in db/db kidneys (p=0.002) at 16 wks of age, while those were more evidently increased in OM (175%) and cortex (110%) in db/db eNOS -/- kidneys (p<0.001). Glucose/glycogen signals in inner medulla (IM) and papilla (P) was increased comparably in db/db and db/db eNOS-/- kidneys (p<0.001). Longitudinally, db/db mice exhibited moderate increases (p=0.082) in glucose/glycogen levels in IM+P at 8 wks, and it is significantly and progressively increased in IM+P, OM, and cortex at 12, 16 and 24 wks.

Conclusions: The characteristic CEST features observed herein could enable the non-invasive detection of DN and differentiation between moderate (db/db) and advanced (db/db eNOS -/-) DN. This MRI technique may be effectively used for the assessment of this disease.

Funding: NIDDK Support

FR-PO1121

Athymic Rat Reduced Kidney Mass Model: A Screening Tool to Identify Human-Sourced Cell-Based Therapeutic Prototypes for Regeneration and Stabilization of Renal Function Kelly I. Guthrie, Elias Rivera, Namrata D. Sangha, Andrew T. Bruce, Tim Bertram, Deepak Jain, Joydeep Basu. *Tengion, Winston-Salem, NC.*

Background: Elucidating multi-modal mechanisms of action and efficacy of human-sourced autologous cell based therapeutic candidates is supported by availability of short-term pre-clinical models of kidney disease. The NIHRNU rat is permissive to xenogeneic transplant of human cells and Reduced Kidney Mass (RKM) models in rodents are used to identify potential CKD therapies. Selected Renal Cells (hSRC) have been identified as an autologously-sourced therapeutic for CKD patients.

Methods: NIHRNU rats underwent kidney mass reduction (KMR); removing one kidney and, approximately 1 week later, cortical tissue from remaining kidney. Proof-of-concept rats utilized for prototype testing underwent KMR in 2 pilot groups with kidney weight-based reduction averaging 59% (Grp 1) or 67% (Grp 2). Remnant kidneys were injected with hSRC formulations: Grp 1: 3D SRC aggregates (organoids) (n=4), Grp 2: SRC, 48hr hold (n=7), SRC, 72hr hold (n=8). Serum /urine chemistries, survival, terminal histology and HLA1 staining of implanted cells were employed.

Results: (1) For groups 1 and 2 respectively, 4 weeks post-KMR average sCre was 0.5 versus 1.5 mg/dL; BUN was 33 versus 108 mg/dL; 2 weeks post-KMR survival was 100% versus 64%; 18 weeks post- KMR survival was 100% versus 5%. (2) In Grp 2 rats with starting sCre>=1 at delivery, hSRC formulations provided significant survival benefit (p=0.05) and stabilization of clinically relevant biomarkers associated with independent functional niches (sCre, BUN, Hct, sPhos, sAlb, Cholesterol, UPC, p<0.05). (3) HLA1 + staining (human-origin):1-2 cells/ 20uM transverse section within tubules and interstitium at 4 months.

Conclusions: (1) Extent of KMR in NIHRNU rats significantly impacts rate of disease progression in creating short-term screening model (2) Detection of hSRC and stabilization of kidney function in this xenogeneic model (Grp 2) is consistent with that demonstrated in autologous/longer term rodent models. (3) Current POC study demonstrates application of model to SRC process development. Increased rat count in Grp2 model may assist identification of optimal process.

FR-PO1122

Effect of Blood Vessel Wall Deformation on Simulated Hemodynamics in Arteriovenous Vascular Access Models Yan-Ting E. Shiu,[1] Daniel B. Pike,[1] Michelle Fitts,[1] Christi M. Terry,[1] Alfred K. Cheung.[1,2] *[1]U of Utah, SLC, UT; [2]VASLCHCS, SLC, UT.*

Background: Previous computational fluid dynamics (CFD) simulations of blood flow in arteriovenous (AV) accesses have generally assumed a rigid vessel wall, because of computational time and power limitations as well as difficulties in measuring the deformation of thin venous wall. Here we included wall deformation in CFD, by using 2-way coupled fluid structure interaction (FSI) modeling between the fluid (blood) and solid (vessel wall) domains.

Methods: The structural boundary was an idealized AV fistula (AVF) or graft (AVG), with 300-mm thick vessel walls that were rigid or deformable (with an elastic modulus of 800 kPa). The inflow and outflow blood flow rates (Fig. 1 bottom) were actual values obtained by MRI in our porcine AVF and AVG models. FSI simulation was run in ANSYS Workbench.

Results: Fig. 1 displays color maps of velocity streamline and wall shear stress (WSS) from CFD simulations. When compared to the rigid wall models of AVF (a) and AVG (e), flow patterns in deformable AVF (b) and AVG (f) exhibit more eddy currents. The deformable AVF model has higher velocity and WSS (b, d) than the rigid model (a, c). In contrast, the deformable AVG model has similar velocity and WSS (f, h) to the rigid model (e, g).

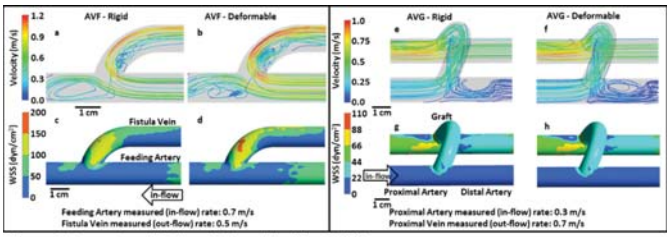

Figure 1: Comparison of Rigid and Deformable AVF and AVG.

Conclusions: Comparing the effects of rigid and deformable vessel walls on hemodynamics in AV accesses is novel. The different effects of deformable vessels on velocity and WSS between AVF and AVG may be because motion in the AVG is constrained by the presence of the rigid graft. Arterial- and venous-specific wall thickness and elastic properties will next be incorporated to further improve the accuracy of CFD models. The different influences of wall deformation on velocity and WSS in AVF versus AVG could yield further insight into the effects of blood flow on stenosis formation and maturation of AVF.

Funding: NIDDK Support

SA-PO001

Flow-Mediated Dilation and Acute Kidney Injury Prediction in Vascular Surgeries Etienne Macedo,[1] Mauricio Teixeira,[1] Daniela Calderaro.[2] *[1]Renal Div, USP, São Paulo, Brazil; [2]INCOR, USP, São Paulo, Brazil.*

Background: Among the pathophysiologic mechanisms involved in the development of Acute Kidney Injury (AKI), endothelial dysfunction (ED) and abnormal vascular reactivity may play important roles in the association of AKI and cardiovascular acute events. The endothelium is an important regulator of arterial elasticity, and assessment of Flow Mediated Dilation (FMD) is a tool to evaluate ED in coronary artery disease. We hypothesized that the Flow Mediated Dilation (FMD) could be a marker to predict AKI in patients undergoing vascular surgeries.

Methods: We analyzed data from a prospective cohort of 96 patients. All patients had been submitted to major vascular surgery and FMD had been assessed.The measurement of FMD was made with an ultrasound evaluation of the brachial artery that follows a brief compressive ischemia of the forearm. AKI was classified by KDIGO SCR criteria.

Results: All 96 patients had chronic kidney disease (19% SI, 44% SII and 37% SIII), 14% were diabetic and 95% had hypertension. 33 (35%) were submitted to endovascular procedures. The main procedure was aortic aneurysm correction (61%). During the first 7 days after the procedure, 31 patients developed AKI and 18 had cardiovascular acute events. AKI and non-AKI patients had similar rates of cardiovascular events. The median FMD was 5,7%, and was not different in patients that developed AKI or cardiovascular events.

Conclusions: Even though there is a logical pathophysiologic association between markers of endothelial function and AKI, FMD was not a predictor of AKI or cardiovascular events in this cohort of CKD patients. The high incidence of hypertension and CKD, associated with a very low mean of FMD, could be a determinant for the low discrimination. Further studies should include CKD patients in order to establish if the Endothelia Dysfunction markers could be predictors of AKI in this high-risk population.

SA-PO002

Assessing Intravascular Volume Using Inferior Vena Cava Ultrasound in ICU Patients with Renal Failure Matthew Kaptein,[1] John Kaptein,[2] Elaine Kaptein.[1] *[1]Nephrology, LAC+USC, Los Angeles; [2]Research Laboratory, Kaiser Permanente, Los Angeles.*

Background: Accurate intravascular volume assessment is essential to manage critically ill patients. Physical exam, CVP, and PCWP poorly reflect intravascular volume. IVC ultrasound (IVC US) reliably indicates right atrial pressure, and is most useful when IVC is overtly "flat" (ascending limb of Starling curve) or "fat" (descending limb). We defined IVC US parameters to accurately predict whether ≥ 1L ultrafiltration (UF) was tolerated.

Methods: 1) Population: From a convenience sample of 145 ICU patients with renal failure in 400 encounters, we analyzed a subset of 66 patients in 175 encounters who had HD/CRRT within 24h of IVC US. 2) Intravascular volume assessment: Clinical assessment recorded prior to IVC US. IVC parameters: IVCmin, IVCmax, Collapsibility Index (CI) = [IVCmax-IVCmin]/IVCmax. "Fat" IVC has large diameter and small CI = intravascular hypervolemia. "Flat" IVC has small IVCmax or large CI = intravascular hypovolemia. 3) Clinical strategy: Treatment decisions based on clinical data + IVC US ("fat or flat"). Accepted criteria of CI > 50% or collapsed to predict hypovolemia, thus pre-emptively gave volume or dialyzed without ultrafiltration. We ultrafiltrated patients predicted to be hypervolemic by small CI (~<20%), or with overriding need for volume removal and CI <50%. 4) Analysis: Pre-US intravascular volume assessment was compared to IVC US. In HD/CRRT subset, we used logistic regression to predict, using CI, the "gold standard" endpoint of whether removal of ≥ 1L UF was tolerated.

Results: Logistic regression analysis suggests CI ≤ 15% is optimal cutoff that maximizes sensitivity (90%) and specificity (90%) to predict whether ≥ 1L UF will be tolerated. Volume therapy directed by serial IVC US assessment well-tolerated. UF was possible in most with "fat" IVC, despite hypotension or vasopressors. Poor concordance of pre-US intravascular volume assessment with IVC US. Poor correlation of CVP, PCWP, CO with either IVC US or "gold standard".

Conclusions: Ours is the first study showing IVC US accurately predicts ability to rapidly remove volume in critically ill patients. Prior studies showed IVC US reliably predicts benefit from volume resuscitation.

SA-PO003

Renal Replacement Intensity and Urine Volume in Critically Ill Patients – Results from the ATN Study Finnian R. Mc Causland,[1] Josephine K. Asafuadjei,[2] Rebecca A. Betensky,[2] Paul M. Palevsky,[3] Sushrut S. Waikar.[1] *[1]Renal Div, Brigham and Women's Hospital, Boston, MA; [2]Div of Biostatistics, Harvard School of Public Health, Boston, MA; [3]Nephrology, VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: Randomized trials of higher versus lower intensity renal replacement therapy (RRT) have failed to show improvement in outcomes. We wished to test the hypothesis that higher intensity RRT may have adverse effects on kidney function by examining the differences in urine output between randomized groups of higher versus lower RRT intensity in the ATN Trial.

Methods: We performed a post-hoc analysis of the ATN Study (n=1124), a multi-center trial that randomized critically ill patients with acute kidney injury to higher (hemodialysis 6/week or CVVHDF 35ml/kg/hour) versus lower (hemodialysis 3/week or CVVHDF 20ml/kg/hr) RRT intensity. We modeled the rate of change in 24h urine output over time using mixed linear regression models with inverse probability weighting to account for dropout due to death.

Results: Mean age of participants was 59.6 ±15.3 years; 70.5% were male; 15.9% were black and 29% were diabetic. In unadjusted analyses, compared with higher intensity RRT, lower intensity was associated with an average of 72.4 mls greater urine output per day (p=0.003). Patients randomized to lower intensity RRT experienced an increase in mean urine output of 5.6 ml/day (95% CI 1.9 to 9.4); those randomized to higher intensity RRT experienced a 0.4 ml/day decrease (95% CI -3.9 to 3.2). The difference between the two groups suggested that higher intensity RRT was associated with a decrease of 6.0 ml/day in average daily urine output (p-difference=0.02).

Conclusions: Higher intensity RRT may lead to more rapid loss of residual urine output in critically ill patients with severe acute kidney injury.

Funding: NIDDK Support

SA-PO004

Administration of Allogeneic, Human Mesenchymal Stem Cells to Phase I Human Study Subjects Does Not Elicit an Immune Response, But Does in Rats with Acute Kidney Injury Anna Gooch,[1] Ping Zhang,[1] Zhuma Hu,[1] Christof Westenfelder.[1,2] *[1]Medicine, U of Utah and VAMC, Salt Lake City, UT; [2]Physiology, U of Utah, SLC, UT.*

Background: Human Mesenchymal Stem Cells (hMSC) do not express DR (HLA-II) and blood group antigens, and are negative for co-stimulatory CD40, -80 and -86. This profile predicts that the infusion of allogeneic MSC to humans should not elicit an antibody response. However, recent publications have questioned the immune privileged status of MSCs (Nat Biotech 2014). Indeed, if hMSC are cultured in fetal calf serum, as is routinely done, retained bovine antigen may induce allo-antibody production. To avoid this possibility, we cultured hMSC without animal products for our Phase I Trial in which allogeneic hMSC were infused to open heart surgery patients known to be at high risk for post-op AKI (Nat Rev Nephrol 2010). Administration of hMSC was safe overall, and none of the study subjects developed post-op AKI or CKD, while ~ 20% of closely matched historical controls experienced post-op AKI and progressively lost renal function.

Methods: To test whether administered hMSCs had elicited an immune response in the subjects, allogeneic hMSC from the stock that had been used in the Phase I Trial (dose escalating protocol) were incubated with the sera of the study subjects collected 40 days post cell infusion, then incubated with FITC-labeled anti-human IgG antibodies and FACS analyzed.

Results: Such analysis failed to detect, in all subjects, antibodies to allogeneic hMSC. Sera collected 14 days after rats with AKI had been treated with hMSC were tested in identical fashion, using FITC labeled anti-rat IgG. Although hMSC infusion had prevented development of AKI as judged by SCr, sera from all hMSC treated animals exhibited robust antibody responses to hMSC.

Conclusions: Allogeneic hMSCs cultured without animal serum infused x 1 do not elicit an antibody response in humans, while they do in rats, the latter despite MSCs' known inhibitory effects on antibody production. These data confirm the immunologic safety of clinically used allogeneic hMSC when cultured without animal serum. While effective, Xenogeneic protocol elicits an anti-body response, rendering such use unsafe.

Funding: Private Foundation Support

SA-PO005

HLA Associations with Acute Tubulointerstitial Nephritis in a Chinese Adult Population Cui Li,[1,2] Tao Su,[1] Jiawei Tang,[1] Yafang Wang,[1] Xiaomei Li,[1] Li Yang.[1] *[1]Renal Div, Renal Div, Peking Univ First Hospital, Beijing, China; [2]Renal Div, Renal Div, Beijing Shijitan Hospital, Beijing, China.*

Background: It has been reported that certain human leukocyte antigen (HLA) alleles are associated with acute tubulointerstitial nephritis (ATIN), especially tubulointerstitial nephritis with uveitis syndrome (TINU). Whereas no observation has been reported in Chinese population.

Methods: A prospective cohort of clinical-pathologically diagnosed ATIN patients, including Drug induced ATIN (DATIN) in 56 cases and TINU in 22 cases, was enrolled in the study. Two hundred healthy individuals were included as control. Typing of HLA-DRB1, DQA1, DQB1 alleles was performed by bi-directional sequencing of exon 2. Renal outcome was determined at 12m post biopsy by eGFR.

Results: A significant association was found between the HLA alleles DQA1*0104, DQA1*0105, DQA1*0303 and ATIN. These three types of HLA alleles were detected in 82% of ATIN cases but in none of the controls [risk ratio (RR) 5.6, 95% confidence interval (CI) 3.5-9.0]. Linkage disequilibrium of DRB1*1405 and DQB1*0503 was associated with ATIN (RR 70.4, 95% CI 23.7-209.1). The HLA class II haplotype DQA1*0104/ DQB1*0503/ DRB1*1405 was the most common combination in our ATIN cases (53.9%). Patients having this combination, compared with those who had not, presented more significant systemic inflammation [CRP 9.4(5.0~17.8)mg/L versus 6.7(2.0~15.2)mg/L, P=0.048], higher Peak Scr level [313.0(256.0~538.0)umol/L versus 269.3(182.8~408.8) umol/L, P=0.013], higher urinary a1-MG secretion [221.5(152.0~336.0)mg/L versus 135.0(28.0~214.0)mg/L, P<0.001], and more diffuse interstitial inflammation (P=0.035) and tubulitis (P=0.028). However, there was no association between HLA alleles and renal outcome at 12m post biopsy. No difference was found in the HLA genotypes between DATIN and TINU patients in our study cases.

Conclusions: We found a strong association between HLA-DQA1 genotypes and ATIN, whereas the alleles that were studied could not distinguish DATIN from TINU. HLA haplotype DQA1*0104/DRB1*1405/DQB1*0503 was associated with more severe renal injury but did not predict renal outcome.

SA-PO006

The Effect of Mineralocorticoid Receptor Blockade on the Incidence of Acute Kidney Injury After Cardiac Surgery

Salvador Roberto Lopez,[1] Michael Eduard Wasung,[1] Mirell Tapia,[1] Ruben Dario Barba Navarro,[1] Pamela Tella,[1] Antonio R. Villa,[2] Silvana Bazua-Valenti,[3] Norma Bobadilla,[3] Magdalena Madero,[1] Gerardo Gamba.[3] *[1]Dept of Nephrology, INCICH, Mexico City; [2]Faculty of Medicine, UNAM; [3]Molecular Physiology Unit, INCMNSZ-IIB-UNAM, Mexico City, Mexico.*

Background: Extensive experimental data from our group show that the mineralocorticoid receptor blocker spironolactone (Sp) prevents renal injury induced by ischemia-reperfusion.

Methods: We conducted a pilot, prospective, open, comparative analysis to determine the effect of Sp on the incidence of acute kidney injury (AKI) in adult patients undergoing cardiac surgery. Patients received 100 mg Sp orally 12-24 hours before surgery. Subsequently, patients received three doses of 25 mg Sp on days 1, 2 and 3 and were followed for 5 days. Patients who had not given consent served as controls. Primary and secondary outcomes were AKI incidence and length of stay in intensive care unit (ICU), respectively.

Results: The average age of the population was 54 ± 14.9 years (53.5% male). Most variables were similar between groups, except that the control group exhibited slightly higher preoperative serum potassium and lower aortic clamp time and bleeding during surgery. AKI incidence in the Sp and control groups was 36.2 versus 46.2% (p=0.12), respectively. The numbers of patients with AKIN 3 and who required renal replacement therapy were lower in the Sp group (3.1% versus 8.1%), nonetheless these results did not reach statistical significance (p= 0.097). Length of stay in the ICU was shorter in the Sp group (3.9 ± 2.2 versus 4.7 ± 3.1 days, p<0.03). Logistic multivariate analysis presented Sp as protective against AKI and length of stay in the ICU. There was no difference in the incidence of postoperative hyperkalemia (>5.5 mEq/L) between groups (14.6% versus 12.6%, Sp versus control, respectively).

Conclusions: Perioperative administration of Sp is safe and could potentially reduce the incidence of AKI in cardiac surgery patients. A randomized controlled study is going on to confirm our findings.

Funding: Government Support - Non-U.S.

SA-PO007

The Effects of Renin-Angiotensin-Aldosterone System Blockades on Acute Kidney Injury in Critically Ill Patients in Intensive Care Unit

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Background: Acute kidney injury (AKI) is a major clinical problem and predictor of outcome especially in critically ill patients in intensive care unit (ICU). Renin-angiotensin-aldosterone system (RAAS) blockades are commonly used but can cause AKI during inter-current illness. RAAS blockades on outcomes in patients undergoing AKI in hospital remain uncertain. The aim of this study was to evaluate whether the use of RAAS blockades affected major outcomes of AKI in ICU patients.

Methods: We conducted a large retrospective study of 26,287 patients who admitted to an ICU of a tertiary care academic hospital from January 2003 to December 2013. AKI was defined according to RIFLE and AKIN criteria. Main outcomes measured were AKI occurrence, all-cause mortality, and hospital and ICU length of stay.

Results: AKI developed in 3,192 (12.1%) according to RIFLE criteria and 5,238 (19.9%) according to AKIN criteria. We grouped the cohort into RAAS blockades users (n=2,157) and non-users (n=24,130). There were significant differences between RAAS blockades users and non-users in the incidence of AKI during the ICU stayby RIFLE criteria (27.1% versus 10.8%, p<0.01) and AKIN criteria (49.7% versus 17.3%, p <0.01). RAS blockades users group also significantly increased all-cause mortality within 90 days of ICU admission (Hazard ratio: 1.91, 95% Confidence interval: 1.65-2.22, p<0.01) than non-users. The hospital day (17.64 days versus 20.32 days, p=0.12) and ICU length of stay (3.61 days versus 3.76 days, p=0.63) among the survivors were not different between the two groups.

Conclusions: In this study, use of RAAS blockades was associated with increased development of AKI and mortality. Large, multi-center randomized trials are needed to confirm whether temporary withholding of these medications can affect outcomes in ICU patients.

SA-PO008

Does Adjustment of Serum Creatinine for Changes in Weight Improve the Definition of AKI in Premature Infants?

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Background: Newborn often lose up to 10% of their weight in the first days of life. Studies in children and adults suggest that correcting serum creatinine (SCr) for fluid changes may improve the ability to predict clinical outcomes.

Methods: We enrolled 125 infants (birth weight (BW) <1200 grams or gestational age < 31 weeks) between February 2012 and June 2013. We prospectively collected daily SCr, AKI status was first categorized according to the neonatal AKI definition (adapted from KDIGO definition using lowest prior SCr value as a baseline for each SCr). We then adjusted each SCr value by changes in total body water (TBW = 0.8 x weight) to define AKI. The adjusted SCr was defined as SCr x [TBW + (current wt. – BW)]/ TBW. AKI was compared between unadjusted and adjusted SCr measurements using Bowker's test of symmetry. A logistic regression was used to estimate odds ratios (ORs) for mortality and combined bronchopulmonary dysplasia (BPD)/ mortality between adjusted and unadjusted SCr definition.

Results: Using SCr, 34/122 (28%) met the neonatal KDIGO AKI definition, [(5/34 (15%) had class 2 or 3 AKI]. Using the adjusted SCr, 23/122 (18.9%) had AKI [(6/23 (26%) had class 2 or 3 AKI)]. The SCr and adjusted SCr definitions were significantly different (p=0.044), with most discordance in those classified as AKI 1 using unadjusted SCr and AKI 0 using adjusted SCr. The association between AKI and the composite of BPD/Mortality was stronger for the SCr-adjusted definition (OR 2.72, 95% CI 1.08-6.89) than unadjusted SCr definition (OR 2.14, 95% CI 0.96-4.81), with only the former being statistically significant.

Conclusions: These results suggest that not accounting for changes in fluid status may results in an overestimation of the true incidence of AKI and an underestimate of the association between AKI and BPD/mortality, which can potentially result in an increased type-II error (i.e., saying no association exists when truly one does). Evaluation of larger cohorts and performance of AKI damage markers with adjusted and unadjusted SCr definitions are greatly needed.

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SA-PO009

Polymyxin B Hemoperfusion Improves Renal Outcomes in Septic Shock

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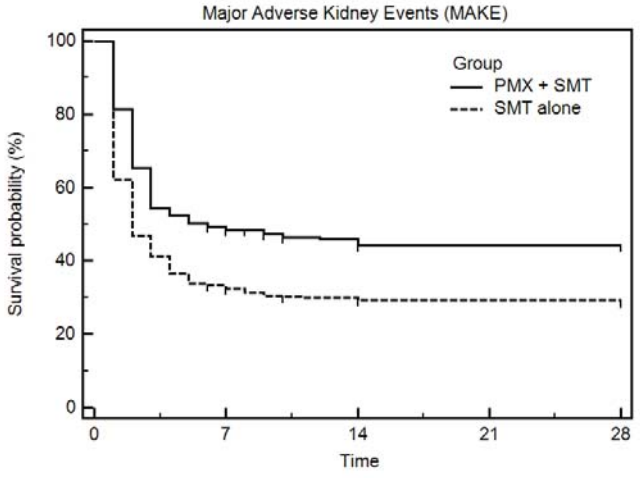
Background: Septic acute kidney injury(AKI) is a common ICU complication associated with high mortality. Hemoperfusion with Polymyxin B fiber column (PMX) reduces blood endotoxin levels and vasopressor requirement, and improves blood pressure, which may improve renal perfusion. In-vitro studies suggest PMX reduces proapoptotic activity of plasma of septic patients on cultured renal tubular cells. We hypothesized that PMX may improve renal outcomes in sepsis.

Methods: Retrospective study of septic shock patients eligible for PMX in Japan. Patients were offered PMX if they met all criteria: SIRS, documented/suspected infection, vasopressors despite adequate fluid resuscitation, ≥1 organ failure, Gram(-) organism and/or elevated endotoxin levels. If patient consented, PMX was performed twice, in addition to standard medical therapy(SMT). If no consent, they were treated with SMT alone. PMX and SMT groups were compared using survival curve analysis and multivariable Cox regression for Major Adverse Kidney Events (MAKE; composite of RRT, death, increase in renal SOFA score) at 28 days.

Results: We compared 337 PMX patients versus 360 SMT alone. PMX group was younger, more likely to be male, had higher SOFA, APACHE and endotoxin levels. ICU admission sCr was lower in PMX than SMT (2.3 versus 2.4 mg/dl, p<0.001). PMX group had shorter ICU and hospital stays. PMX group was less likely to have MAKE compared to SMT (log rank test, p<0.001). After adjustment for age, sex, SOFA score, type of organism and endotoxin levels, PMX remained associated with lower risk for MAKE.



Adjustments	OR(95%CI) for PMX	p
None (univar)	0.66(0.55-0.80)	<0.001
Age, Sex	0.66(0.55-0.80)	<0.001
Age, Sex, SOFA, sCr, endotoxin level, organism	0.67(0.55-0.82)	<0.001

Conclusions: PMX, when added to SMT, appears to improve renal outcomes in septic shock. These results warrant further exploration with prospective trials.

SA-PO010

An Acute Kidney Injury Follow-Up Clinic: Initial Experience Samuel A. Silver,[1,4] Ziv Harel,[1,4] Andrea K. Harvey,[1] Andrew Slack,[2] Neill Adhikari,[2,4] Chaim Bell,[3,4] Ron Wald.[1,4] *[1]Nephrology, St. Michael's Hosp; [2]Crit Care, Sunnybrook Hosp; [3]Internal Med, Mt. Sinai Hosp; [4]Univ of Toronto.*

Background: Survivors of acute kidney injury (AKI) are at increased risk of chronic kidney disease (CKD) and death. Despite this, the kidney care offered to AKI survivors is fragmented and inconsistent. To address this problem, we established an AKI follow-up clinic (AKIFC). Our objective was to assess 50% of KDIGO stage 2/3 AKI survivors within 30 days of hospital discharge and to characterize the interventions provided at the AKIFC.

Methods: All patients who developed KDIGO stage 2/3 AKI in a single teaching hospital were eligible. Exclusion criteria included ongoing Nephrology post-discharge care, kidney transplantation, glomerulonephritis or a pre-hospitalization eGFR≤30mL/min/1.73m^2. The Nephrology consult service patient census was audited daily to identify eligible patients, who were automatically scheduled into the AKIFC at hospital discharge. AKIFC visits consisted of a standardized assessment by a nephrologist that highlighted blood pressure and albuminuria control, cardiovascular risk, CKD complications, medication reconciliation and specialist referrals.

Results: Between September 2013-April 2014, we identified 64 eligible patients of whom 47 (73.4%) were seen within 90 days of discharge, and 25 (39.1%) within 30 days of discharge. The mean pre-hospital serum creatinine was 103(SD 36)µmol/L (eGFR 66.7(SD 24)mL/min/1.73 m^2). The mean serum creatinine was 141(SD 69)µmol/L at the initial AKIFC visit. A medical intervention was provided for 70% of AKIFC patients at their initial visit. These included medication addition (29.8%), medication discontinuation (21.3%), radiologic investigations (25.5%) and specialist referrals (17%). Added medications included renin-angiotensin inhibitors (n=4), diuretics (n=3) and statins (n=3). Discontinued medications included renin-angiotensin inhibitors (n=5), oral hypoglycemics (n=3) and NSAIDs (n=2).

Conclusions: An AKI follow-up clinic for AKI survivors after hospital discharge was feasible and led to interventions for most patients. Our ongoing research program will assess whether structured follow-up for AKI survivors reduces kidney and cardiac events compared to usual care.

SA-PO011

Renal Oxygen Supply and Demand Balance in Congenital Heart Disease Is Associated with AKI After Cardiopulmonary Bypass Catherine Morgan, Nicholas Majaesic, Sudeshna Bhattacharya, Mohamad Alaklabi, Lindsay Ryerson, Dominic Cave. *Univ of Alberta, Edmonton, AB, Canada.*

Background: Cardiac surgery associated AKI (CS-AKI) increases morbidity and mortality in children with congenital heart disease(CHD). Mechanisms of injury may include ischemia-reperfusion injury and tissue damage from glomerular filtration of free hemoglobin and labile iron generated during CPB. Ischemic preconditioning could have a protective role in CS-AKI. Regional near-infrared spectroscopy (NIRS) can provide a non-invasive measure of renal oxygen supply and demand balance before, during, and after cardiopulmonary bypass (CPB).

Methods: This is a preliminary analysis of an ongoing study of intra-operative renal oxygen saturation (rSO_2) as a predictor of AKI in children with CHD. CS-AKI was defined according to the KDIGO definition. A NIRS oximeter was placed on the flank of 26 children undergoing CPB and rSO_2 was recorded every 30 seconds preCPB, during CPB, and postCPB until skin closure. Non-parametric methods were used for testing associations.

Results: 18/26 (69%) children developed CS-AKI. Pre-CPB $rS0_2$ was higher in children with CS-AKI compared to those without (median of 74.6% (IQR 16.3) versus 66.4% (IQR 19.1)). Median $rS0_2$ during CPB was 81.6% (IQR 11.1) and 73.8% (IQR 17.7) in children with and without AKI, respectively. Total time with rSO_2 below a threshold value of 70% or 50% during CPB between those with and without AKI was the same, however, only 4 children had rSO_2 <50%. In children with CS-AKI, there was a significant increase in $rS0_2$ following CPB relative to baseline (p=0.004) but not in those without AKI.

Conclusions: Renal NIRS suggests that a relative ischemic state preCPB may be protective against CS-AKI. Also, children with a larger increase in oxygen supply relative to demand from baseline may be at increased risk of CS-AKI; reduced demand for oxygen postoperatively in the setting of injury, excessive reperfusion/ oxygenation postCPB, or failure of renal auto-regulation may play a role. Analysis of a larger dataset needs to confirm these findings and kidney injury biomarker evaluation will provide further insight.

SA-PO012

Rationale and Design of the Genetic Contribution to Drug Induced Renal Injury Study (DIRECT) Linda Awdishu,[1] Caroline M. Nievergelt,[2] Andrew Davenport,[3] Patrick T. Murray,[4] Etienne Macedo,[5] Jorge Cerda,[6] Rajasekara Chakravarthi Madarasu,[8] Arthur L. Holden,[9] Satish P. Ramachandrarao,[2] Stuart Goldstein,[7] Ravindra L. Mehta.[2] *[1]Pharmacy, UC San Diego; [2]Medicine, UC San Diego; [3]Royal Free Hospital, United Kingdom; [4]Univ College Dublin, Ireland; [5]Univ Sao Paolo, Brazil; [6]Albany Medical College; [7]Univ of Cincinnati; [8]CARE Hospitals, India; [9]International Serious Adverse Events Consortium.*

Background: Drug induced renal injury (DIRI) accounts for 18-27% of cases of acute kidney injury. DIRI is characterized as Type A (dose dependent) and B (idiosyncratic) reactions. Determining the genetic predisposition can play a role in minimizing risk. The aims of this study are to ascertain for DIRI: 1. To determine if a genetic predisposition exists, using genome-wide association and whole genome sequencing studies. 2. To describe the frequency, course, risk factors, resolution and outcomes. 3. To investigate the role of ethnic/racial variability. 4. To explore the use of different tools establishing causality.

Methods: 1000 patients will be enrolled worldwide and blood samples for DNA collected. Data on the DIRI course, drug exposure, risk factors, vital signs, and laboratory parameters will be recorded in web based database. A panel of nephrologists will adjudicate all cases. Genome wide association studies will be conducted using population controls matched on biogeographic ancestry. This study is funded by the International Serious Adverse Events Consortium.

Results: The primary endpoint is identification of specific drug-related polymorphisms associated with DIRI. Secondary endpoints include frequency of DIRI by causal drug and drug combinations; DIRI genetic variability; exploration of causality assessment tools; risk factor identification; description of the course of DIRI; and mortality and dialysis dependency at hospital discharge, 28 and 90 days post-event.

Conclusions: The DIRECT study is the first observational study investigating genetic determinants of DIRI. The findings from this study may translate into safer patient outcomes, through genotypic individualization of therapy and minimization of harm.

Funding: Private Foundation Support

SA-PO013

Phenotype of Drug Induced Renal Injury: Initial Results from the DIRECT Study Linda Awdishu,[1,2] Satish P. Ramachandrarao,[3] Marcela Zhou Huang,[3] Andrew Davenport,[4] Etienne Macedo,[5] Jorge Cerda,[6] Michael Zappitelli,[8] Dinna Cruz,[3] Rajasekara Chakravarthi Madarasu,[9] Stuart Goldstein,[7] Ravindra L. Mehta.[3] *[1]On Behalf of the DIRECT Investigators; [2]Pharmacy, UC San Diego; [3]Medicine, UC San Diego; [4]Royal Free Hospital, United Kingdom; [5]Univ of Sao Paolo, Brazil; [6]Albany Medical College; [7]Univ of Cincinnati; [8]McGill Univ, Canada; [9]CARE Hospitals, India.*

Background: Drug induced renal injury (DIRI) accounts for 18-27% of cases of acute kidney injury. However, there is a lack of prospective studies on the phenotype.

Methods: This is a prospective study enrolling 1000 patients with DIRI in 12 countries to identify drug-related polymorphisms associated with two phenotypes (AKI and glomerular injury) by genome wide association studies. Data on the DIRI course, drug exposure, risk factors, vital signs, and laboratory parameters are recorded.

Results: Preliminary results from the first 192 patients (151 adult and 41 pediatric patients) are described. The most common causal drugs were vancomycin, piperacillin/tazobactam, tacrolimus, ibuprofen, diclofenac, amphotericin and acyclovir. Multi-drug injury was common with 33% on more than one causal drug. The mean Scr increased from 1.22±0.64 to 3.77±2.38 mg/dL in adults and 0.66 + 0.37 to 2.01 + 0.92 mg/dL in pediatrics. The cases were non-oliguric and need for dialysis was low. Concomitant use of additional nephrotoxins was common. The doses of the common causal drugs appear to be within the standard ranges (adults: vancomcyin 2417.5 ± 1053.8 mg/day, tacrolimus 5.2 ± 2.9 mg/day, ibuprofen 917 ± 422 mg/day and pediatrics vancomcyin 2687 ± 1578 mg/day, ibuprofen 800 ± 283 mg/day). However, accompanying trough concentrations are above accepted targets (vancomcyin 23.7 ± 9.7 ng/mL in adults and 24.7 ± 31.1 ng/mL in pediatrics).

Conclusions: DIRECT is the first international observational study to investigate the genetic determinants of DIRI. Preliminary data suggest that certain patients may be susceptible to kidney injury from standard drug dosing. Genotyping studies will further elucidate the mechanisms for these injuries.

Funding: Private Foundation Support

SA-PO014

Frequency of Nephrotoxicity in Patients Receiving Intravenous Vancomycin Gina Marotto, Dinna Cruz, Ravindra L. Mehta, Linda Awdishu. *UC San Diego Health System.*

Background: Vancomycin nephrotoxicity is frequently suspected in hospitalized patients however the incidence and prevalence is variably described. We hypothesized that vancomycin related acute kidney injury (AKI) would be common and would correlate with vancomycin serum concentrations.

Methods: Retrospective study of adult patients who received intravenous vancomycin for at least 48 hours during June 2013 at an academic medical center. All patients had therapeutic drug monitoring and adjustment by a pharmacist. Exclusion criteria included pregnancy, history of dialysis, renal transplant, or CKD stage 5. The primary endpoint was the frequency of AKI (AKIN Scr criteria (≥ 0.3mg/dl change over 48 hrs or 50% rise over 7 days) during vancomycin treatment.

Results: AKI developed in 17 (13.5%) of 126 patients. 76% developed AKIN Stage 1; only 1 patient required dialysis. The mean Scr increased from 1.02±0.56 (pre-drug) to a peak of 1.73± 0.9 mg/dL during drug treatment. The mean Scr at hospital discharge was 1.3 ± 0.64 mg/dL and Scr returned to baseline in 6 (35%) patients. AKI patients were older than those without AKI (AKI 61.8±12.9 versus non-AKI 52.4 ±18.1 yrs, p=0.014). There was no difference in the loading doses or maximum daily dose (AKI 2550±1038 mg and non-AKI 2929 ±1099 mg, (p=0.18) for the AKI and no AKI groups. Supra-therapeutic vancomycin concentrations occurred more often in patients with AKI (53%) than controls (28%). Stepwise logistic regression revealed surgery (odds ratio [OR] and [95% CI]) (3.71 [1.05-14.28]; P = 0.04) and heart failure (OR, 8.52 [1.71-50.04]; P = 0.01) as independent risk factors for AKI. Duration of vancomycin (9.4±6.8 versus 6.6±4.8 days; P = 0.013) and hospital length of stay (19.1±18.4 versus 13.4±15.4 days, P = 0.011) were longer in the AKI group. There was no significant difference in mortality.

Conclusions: Vancomycin nephrotoxicity was common and associated with longer duration of therapy. A significant number of patients had toxicity despite therapeutic concentrations. This may indicate an underlying pre-disposition to this injury or the need for more frequent therapeutic drug monitoring.

SA-PO015

Vancomycin Associated Acute Kidney Injury Ramapriya Sinnakirouchenan, Anne M. Larosa, Hariprasad S. Trivedi. *Medicine, Div of Nephrology, Medical College of Wisconsin, Milwaukee, WI.*

Background: Recently, we have observed a high incidence of vancomycin-associated acute kidney injury (VAAKI), possibly related to higher targeted trough levels to avoid treatment failures. The present study aims to characterize the clinical features of VAAKI and we present preliminary results.

Methods: The design is a single center, retrospective study. The inclusion criteria are age ≥ 18 years and a nephrology consult indicating VAAKI. Data collected include demographics, weight, body mass index, dose, duration and level of vancomycin, course of acute kidney injury, temporal characteristics, clinical features and course, need for renal replacement therapy, renal function, and biopsy findings, if any. Acute kidney injury is defined as 0.3mg/dl or more rise in serum creatinine.

Results: Over a 6-month period, out of 272 patients with acute kidney injury 21 patients with VAAKI (8%) were identified. There was a male preponderance (62%) with 71% of them being between 40-70 years of age. No race predilection was noted. All patients were overweight or obese (mean BMI 32.1+ 9) and 33% had a body weight of >100 kg (mean body weight 95.48 + 26 kg). Mean maximum dose of vancomycin per day was 3.13 + 0.87 g/day (average 32.8 mg/kg/day); mean cumulative dose was 14.44 + 17.8 g; mean highest vancomycin level was 41.06 + 14.8 mg/L; the average duration of vancomycin therapy was 5.25 + 4 days; mean time to the development of AKI from the start of therapy was 3.8 + 3.8 days and the mean time to peak plasma creatinine from the start of therapy was 9.5 + 5.6 days; mean time to peak plasma creatinine after the development of AKI was 5 + 3.1 days. There were insufficient data to establish mean time to reach the nadir. Only one patient underwent a biopsy that showed acute interstitial nephritis and he was the only one treated with steroids. Three patients needed dialysis.

Conclusions: Our preliminary results suggest a high preponderance of VAAKI in overweight patients possibly indicating that dosing should be based on ideal body weight rather than actual body weight. More studies are necessary to further characterize this entity, to develop better dosing protocols and design interventions.

SA-PO016

Risk of Everolimus-Associated Acute Kidney Injury Was Increased in Cancer Patients with Impaired Kidney Function Seung Yeon Son, Jung Eun Lee, Hye Ryoun Jang, Wooseong Huh, Yoon-Goo Kim, Dae Joong Kim, Ha Young Oh. *Of Medicine, Samsung Medical Center, Seoul, Republic of Korea.*

Background: Everolimus has been recently introduced as second-line treatment for renal cell carcinoma (RCC) and many other cancers. A few prospective studies have shown that serum creatinine levels increase in a significant proportion of the patients receiving everolimus. However, there are sparse data on the occurrence of acute kidney injury (AKI) during everolimus treatment in clinical practice. Therefore, we conducted this retrospective study to examine incidence, risk factors and clinical significances of AKI associated with everolimus in patients with cancer.

Methods: We analyzed patients who received everolimus for more than 4 weeks in Samsung Medical Center. AKI was defined as increase of creatinine more than 1.5 fold from baseline or decrease of estimated glomerular filtration rate (eGFR) more than 25% from baseline. AKI was divided to 3 categories: Risk (AKI-R increased creatinine less than 2 times from baseline), Injury (AKI-I 2.0?3.0 times baseline), and Failure (AKI-F 3.0 times baseline). Recovery from AKI was defined as returns of creatinine within 1.2 fold of baseline.

Results: One hundred twenty patients were enrolled in this study. Majority of patients were RCC (N=93, 84.5%). AKI was developed in 21 (23%) of RCC patients and none of the patients (N=27) with other cancers showed AKI. Fourteen out of 21 were considered to be an everolimus-associated AKI in which there were no other nephrotoxic insults. The incidence of AKI was increased progressively as baseline GFR decreased (12% in subjects with eGFR 60-90 mL/min/1.73m2, 17% in eGFR 30-60, 100% in eGFR 15-30, P=0.001 for trend). Baseline eGFR was an independent risk factor for development of AKI (HR for 10 increases, 0.70; 95% CI, 0.49-0.99; p=0.044). However, most common reason of treatment withdrawal was progression of underlying malignancy for both AKI and non-AKI group.

Conclusions: The study suggested that AKI was common adverse effect of everolimus, especially in subjects with impaired renal function. However, AKI occurrence did not require discontinuation of drug and treatment decision should be made through multidisciplinary approach.

SA-PO017

First Cases of "Warfarin-Related Nephropathy" Induced By Fluindione Leonard Golbin,[1] Cecile M. Vigneau,[1] Guy Touchard,[3] Pascale Siohan,[4] Elie Zagdoun,[5] Nathan Lagoutte,[1] Nathalie Rioux-Leclercq,[2] Frouget Thierry.[1] *[1]Nephrologie, CHU Pontchaillou, Rennes, France; [2]Anatomie et Cytologie Pathologiques, CHU Pontchaillou, Rennes, France; [3]Nephrologie, CHU Miletrie, Poitiers, France; [4]Nephrologie, CH Cornouaille, Quimper, France; [5]Nephrologie, CH Memorial, Saint-Lô, France.*

Background: Acute Kidney Injury (AKI) caused by fluindione is usually due to immunoallergic reaction. AKI with tubular obstruction by Red Blood Cells (RBC) casts have been recently described with warfarin and defined as " Warfarin-related nephropathy " (WRN) (Brodsky and al., 2009). Does WRN could be extensible to other anticoagulant drugs?

Methods: All suspected cases of AKI with tubular obstruction by RBC casts in patients treated by Vitamin K antagonists (Vka) have been searched in all hospitals from the french West Society of Nephrology, between January 2006 and October 2013. Clinical and histological data have been analyzed in order to confirm cases with tubular obstruction by RBC casts on kidney biopsy.

Results: Kidney biopsies have been systematically reviewed and we found tubular RBC casts, acute tubular necrosis and an underlying kidney disease (IgA, acute post-infectious glomerulonephritis, IgG kappa glomerulonephritis) in 7 patients (5 under fluindione and 2 under warfarin). They all have developed gross hematuria during overanticoagulation (median INR was 4,2) complicated by severe AKI (median serum creatinine (SCr) 473 µmol/L). WRN was initially suspected in patients treated with warfarin while initial diagnosis was incorrect in 4 of 5 patients treated with fluindione (acute interstitial nephritis for 3 patients and acute tubular necrosis for the last patient). One patient died, 5 kept chronic kidney disease (median SCr was 162 µmol/L) and 1 fully recovered renal function after 1-year following.

Conclusions: We describe the firsts cases of AKI by obstructive tubular with RBC casts induced by fluindione. With the recent publication of WRN cases under dabigatran and acenocoumarol, " Anticoagulant-related nephropathy " appears more appropriate than WRN. Development of gross hematuria during Vka treatment, in patients with underlying kidney disease, needs rapid INR control and close monitoring of renal function because of nephropathy prognosis.

SA-PO018

Risk Factors of Intravenous Acyclovir Associated Acute Kidney Injury According to RIFLE Classification Eun Ju Lee, Kyungo Hwang, Hyun Seop Cho, Hyun-Jung Kim, Se-Ho Chang, Dong Jun Park. *Div of Nephrology, Dept of Internal Medicine, Gyeongsang National Univ Hospital, Jinju, Gyeongsangnam-do, South Korea, Korea.*

Background: Intravenous (IV) acyclovir is commonly administered medication for viral infection but is well known for its nephrotoxicity. However, there was no study for risk factors of acyclovir associated acute kidney injury (AKI).

Methods: We retrospectively reviewed the medical records of 287 patients who were medicated IV acyclovir from January 2008 to May 2013 in Gyeongsang National University Hospital. All had documented medical histories and underwent medical review. Demographic data, risk factors, concomitant drugs, laboratory findings and outcome were gathered from the medical records and analyzed.

Results: AKI occurred in 51 patients (17.8%). As per RIFLE classification, renal injury was graded as either at risk of renal dysfunction (62.7%), renal injury (15.6%), and renal failure (21.6%). There was no significant difference in age, sex, total dose, drug duration, and presence of hydration between AKI and non-AKI group. However, both occurrence and severity of AKI according to RIFLE criteria was high in diabetes (p=0.001, 0.002, respectively). Concomitant vancomycin and NSAIDs use was positively correlated with AKI occurrence (p=0.005, 0.037, respectively) whereas mannitol and renin angiotensin blockade was not. Higher mortality was observed in AKI patients (p=0.001) and this was also correlated with AKI severity (p=0.006). Multivariate analysis also presented that presence of diabetes, concomitant NSAIDs, and vancomycin use was independent risk factor of acyclovir associated with AKI (p=0.000, OR 4.284 (CI:1.945-9.434), p=0.025, OR 3.014 (CI :1.150-7.899), p=0.007, OR 4.650 (CI :1.523-14.195), respectively).

Conclusions: AKI is relatively common in patients administrating acyclovir injection. Physicians should attempt to prevent, detect, and manage acyclovir associated AKI in patients prescribing acyclovir due to possible association of poor prognosis.

SA-PO019

Factors Used by United States Nephrologists to Stop Dialysis when Acute Kidney Injury Resolves Mary C. Mallappallil,[1] Ravindra L. Mehta,[2] Ellen H. Yoshiuchi,[3] Gary R. Briefel,[1] Edgar V. Lerma,[4] Moro O. Salifu.[1] *[1]Internal Medicine, Div o f Nephrology, State Univ of New York at Downstate, Brooklyn, NY; [2]Dept of Medicine, Univ of California, San Diego, San Diego, CA; [3]National Kidney Foundation, New York, NY; [4]Dept of Medicine, Univ of Illinois Chicago School of Medicine, Advocate Christ Medical Center, Chicago, IL.*

Background: Despite attempts to define, classify and standardize Acute Kidney Injury (AKI), there is considerable variation in management and no standard approach to withdraw dialysis in the setting of AKI. We conducted a survey of of U.S. nephrologists to determine what parameters they use to wean patients with AKI off dialysis when renal function improves.

Methods: We designed an anonymous electronic survey with 16 questions and sent it to the U.S. National Kidney Foundation database of practicing nephrologists after IRB approval. The collected data had no duplicate reponses and was de-identified. Only aggregate data was analyzed with descriptive statistics. In addition to factors showing recovery of kidney function and weaning criteria, we used comparative situations to determine what was considered the most important factor to discontinue dialysis.

Results: 264 U.S. nephrologists responded to the survey, 80% of whom, were in Nephrology practice for at least 10 years. Most responders (88%) treated over 20 cases of AKI annually. The most important criteria to take a patient off dialysis with resolving AKI was resolution of oliguria (51%). In decreasing order of importance were: improvement of volume overload (48%) and respiratory parameters (37%). Of lesser importance were: correction of metabolic acidosis and hyperkalemia. Interestingly, only a minority would get a timed urine collection to assess creatinine clearance.

Conclusions: Although, the most common indicator used to discontinue dialysis in AKI was resolution of oliguria (51%) there still is considerable variation among U.S. nephrologists while discontinuing dialysis. This variation suggests that there may be a benefit in having a standardized set of criteria when withdrawing dialysis in the setting of improving AKI.

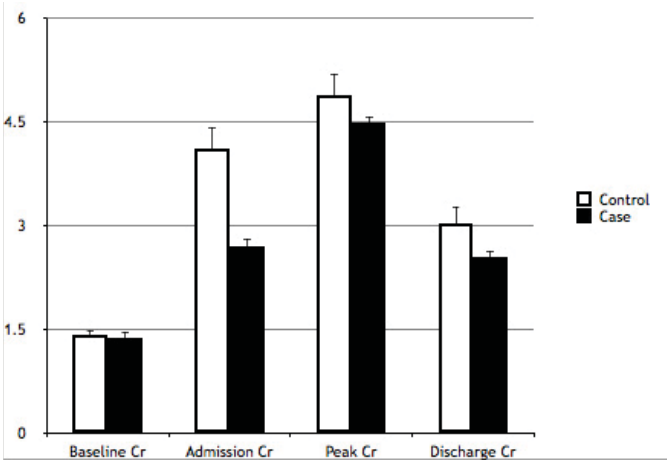
SA-PO020

Intravenous Iron in Acute Kidney Injury Jones S. John, Annie Culver, Liliana Osadchuk, Richard J. Marcus, Barbara A. Clark. *Nephrology and Hypertension, Allegheny General Hospital, Pittsburgh, PA.*

Background: Little is known about benefit versus risk in treating anemia with IV iron in patients with acute kidney injury. Concerns about adverse outcomes with infection or worsening of acute kidney injury due to exposure to free oxygen radicals may dissuade use of IV iron.

Methods: We performed a retrospective case control study of patients identified over a two-year period with a diagnosis of acute kidney injury who received IV iron (cases) compared to those with AKI without IV iron (controls).

Results: We identified 67 cases and 67 controls matched for age (66±16 versus 68±12yr), stage of CKD (1.7±1.7 versus 1.7±1.7) and severity of anemia (Hgb, 7.7±0.1 versus 7.5±0.1mg/dl). They were also similar in comorbidities including diabetes, congestive heart failure, coronary artery disease, liver disease, and hypertension. Cases tended to be sicker with longer length of stay (27±4 versus 15±1.3 d, p<0.05) and more ICU days (13±2 versus 5±1, p<0.05), diagnosis of sepsis (51% versus 39% p=0.16), number of antibiotics used (2.7±0.3 versus 1.8±0.2, p<0.05). Sepsis diagnosis and antibiotic use preceded use of IV iron. Despite this there was no difference in need for dialysis (38.8 versus 34.3%, NS), mortality (24 versus 21%, NS) or severity or recovery of AKI (sCr in mg/dl).



In most cases iron levels were checked and IV iron use was in response to resistant anemia rather than proactive. Therefore use of Procrit and transfusion tended to be higher in the cases rather than the controls. Discharge Hgb was similar, (9±0.1 versus 9.1±0.1 mg/dl, NS).

Conclusions: No adverse effect of IV iron use in AKI was seen even in patients with a diagnosis of sepsis and antibiotic use. A prospective randomized control trial of timely assessment of iron stores and preemptive use of IV iron in AKI with anemia is warranted to determine risk versus benefit including possible reduction in procrit use, transfusion and associated cost.

SA-PO021

Provider Acceptance of a Short Messaging System-Based, Electronic Alert for Acute Kidney Injury Muhammad Ubaid Ullah, Yevgeniy Gitelman, Yuliya Borovskiy, Iram Aqeel, Dan Negoianu, Barry D. Fuchs, Francis Perry Wilson. *Univ of Pennsylvania.*

Background: The implementation of electronic health records in the inpatient setting has facilitated the development of automated alert systems for acute kidney injury. Few studies have evaluated provider opinions on these systems.

Methods: We identified a convenience sample of physicians and pharmacists who had received an electronic alert for acute kidney injury as part of a randomized, controlled trial at our institution (clinicaltrials.gov #01862419). Providers were asked to provide demographic and occupational information, and to complete a brief survey regarding their opinions of the alert system within one month of receiving an alert. Alert approval was defined as the proportion of providers who indicated they would like to continue receiving alerts at the conclusion of the study. We used chi-square and Fisher exact testing to compare responses among groups of providers. We used logistic regression to evaluate the impact of survey-timing on alert approval.

Results: Of 98 individuals participating in the survey, 62 were physicians, 7 were non-physician practitioners and 27 were pharmacists. Alert approval was high at 69% of respondents. There was no significant difference in AKI alert approval by specialty, with 82% of surgeons approving of the alert compared to 62% of medical practitioners (p=0.31). There was evidence of alert fatigue as the trial progressed with the odds ratio for alert approval 0.79 (0.65 – 0.97, p=0.02) for each additional month after alerting began. Four percent of participants stated that the alert impeded their care of patients, and one percent indicated it impeded their workflow. 79% of participants stated that they were already aware of the presence of AKI "all of the time" or "most of the time" when they had received an alert.

Conclusions: An electronic alert for AKI was well-received at our institution, though alert approval declined over time. Most practitioners stated that they were already aware of the presence of AKI when the alert fired. The study is limited by the convenience nature of the sample surveyed.

Funding: NIDDK Support

SA-PO022

Bile Pigment Nephropathy in Advanced Liver Disease and Predictors of Bile Pigment Nephropathy Suman Nayak,[1] Rajendra Prasad Mathur,[1] Sivaramakrishnan Ramanarayanan,[1] Suresh Chandra Tiwari,[2] Gyan Prakash,[1] Shiv Sarin,[1] Chitranshu Vashishtha,[1] Archana Rastogi,[1] Chhagan Bihari,[1] Amar Mukund.[1] *[1]Inst of Liver and Biliary Sciences; [2]Fortis vasant kunj.*

Background: Acute kidney injury (AKI) is a major cause of mortality in patients with advanced liver disease. Majority of them have acute tubular necrosis (ATN) secondary to sepsis and ischemia. Since majority of patients are sick and coagulopathic there is paucity of literature on structural renal failure in this group.

Methods: We reviewed the post mortem kidney biopsy reports of 43 patients with severe liver dysfunction with acute kidney injury (AKI). Biopsy tissues were processed and subjected to light microscopy and immunofluorescence. In patients with pigment casts in tubules, additional special stains for iron (Pearl's stain) and bile (Fouchet's) were used to characterise the pigments.

Results: Of 43 patients bile pigment nephropathy was found in 20/43 (46.51%) and acute tubular necrosis in 23/43(53.49%) patients. Mean age of study population was 43.26±11.44 years. The Mean CTP score was higher in bile pigment nephropathy group (12.6±1.1) as compared to ATN group (11.9±1.2) (p = 0.046). The Mean MELD score was higher in bile pigment nephropathy group (39.3±7.9) as compared to ATN group (31.3±7.8) (p = 0.002). The mean urea (98.80±55.78 versus 90±44.68 mg/dl, p value = 0.294) and creatinine (4.02±2.3 versus 3.42±1.5 mg/dl, p value = 0.081) were higher in bile pigment nephropathy group compared to ATN group. The Mean total bilirubin was higher in bile pigment nephropathy group (26.1±9.3) as compared to ATN group (9.2±5.2) (p <0.001). On multivariate logistic regression analysis high bilirubin was found to be an independent predictor of bile pigment nephropathy.

Conclusions: Bile pigment nephropathy is a common pathological finding in patients of advanced liver dysfunction with AKI and high serum bilirubin was a strong predictor.

SA-PO023

Burden of Prescription Related Acute Kidney Injury in Acute Care Setting: A Quality Improvement Program Paras Dedhia,[1] Anthony C. Leonard,[1] Kotagal Shashi Kant,[1] Charuhas V. Thakar.[1,2] *[1]Nephrology, Univ of Cincinnati, Cincinnati, OH; [2]Renal Section, Cincinnati VA, Cincinnati, OH.*

Background: Acute kidney injury (AKI) is an area of focus for Medicare in reducing patient harm. Yet characterization of hospital acquired (HA) AKI and the burden of prescription related AKI (PR-AKI) is not well studied.

Methods: A 3-month clinical quality improvement (QI) program assessed 108 AKI patients out of 252 renal consults at a tertiary care hospital. We recorded clinical information and exposure to nephrotoxic drugs temporally associated with AKI. AKI, defined by KDIGO guidelines, was codified on the day of consult as: Rx related, Multifactorial, and Rx unrelated. Multifactorial AKI was scored (Rx score) based on an association with prescribed agents (1 to 10 scale). PR-AKI was defined as Rx related or with Rx score > 5. AKI was also classified as community acquired (CA-AKI) or HA-AKI, if it occurred within 2 or > 2 days after admission respectively. Primary composite outcome was death/dialysis at discharge/length of stay (LOS) of 1.5 times the median. Bivariate analysis compared patients with and without PR-AKI. Multivariable models (adjusting for age, gender and baseline creatinine) examined the associations between HA-AKI and composite outcome; PR and HA-AKI; and expressed as odds ratios (OR) and 95% confidence intervals (CI).

Results: Of the 108 AKI subjects, 65% were male. Average age was 58 years, and the median LOS was 12 days. PR-AKI occurred in 38 cases (35%). The commonest drugs associated with PR-AKI included diuretics (66%), nephrotoxic antimicrobials (63%), and contrast (45%), among others. Of the 31 cases with HA-AKI, 55% had PR-AKI compared to 27% in CA-AKI (p=0.008). Compared to CA-AKI, HA-AKI had higher odds of association with PR-AKI (OR, 2.9, CI, 1.2 – 7.4). 63% of AKI patients experienced the composite outcome; and it occurred with 3.0 times greater odds in HA- versus CA-AKI (CI 1.1 – 8.2).

Conclusions: PR-AKI occurs 1-in-3 renal consults, and this association needs to be examined in all hospitalized AKI patients. HA-AKI consults are at a higher risk of being PR-AKI, and also face poor hospital outcomes. This calls for a hospital-wide QI program to reduce the burden of nephrotoxic AKI.

Funding: Clinical Revenue Support

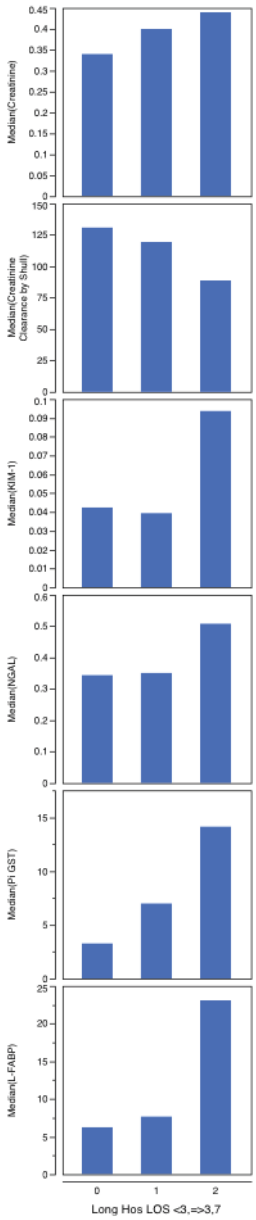
SA-PO024

The Validation of an Estimated Creatinine Clearance Formula in the Emergency Department for the Diagnosis of Acute Kidney Injury in Children: A Pilot Study Marie-Carmelle Elie-Turenne,[1] Emily Weeks,[1] Mark S. Segal.[1,2] *[1]Emergency Medicine, Medicine, Div of Neurology, Univ of Florida, Gainesville, FL; [2]Renal Service, Veterans Association Medical Center, Gainesville, FL.*

Background: The early identification of children at risk for renal failure is challenging in the acute setting, where baseline serum creatinine (SCr) and length are not readily available to perform estimates of creatinine clearance (eCrCl) by pediatric formulas. Consequently, there is no validated tool for the eCrCl among children in the emergency department (ED). We propose a modified formula, p_{ED}RIF for the assessment of acute kidney injury (AKI) by comparing its performance with renal biomarkers.

Methods: This is a pilot enrollment of 30 age-matched children <18 years with and without sepsis from the ED prior to inpatient admission. Those with prior diagnosis of chronic kidney disease or dialysis were excluded. Demographic and laboratory data was collected including SCr to calculate eCrCl and assess for AKI by p_{ED}RIF (ED modification of pRIFLE criteria using the Shull equation). Urine samples were collected in the ED to assess for urine AKI biomarkers: kidney injury molecule (KIM-1), neutrophil gelatinase associated lipocalin (NGAL), alpha and pi glutathione S-transferase (aGST, pGST), and liver-fatty acid fatty binding protein (L-FABP) by standard ELISA.

Results: Among children with AKI by p_{ED}RIF (5/30, 17%), median aGST, pGST, and NGAL were above 75% IQR of non-AKI subjects, though not statistically different. Elevation in injury biomarkers trend inversely with decreased eCrCl by p_{ED}RIF, predicting ICU admission and longer hospital stays >7 days.



Conclusions: Detection of compromised renal function using p_{ED}RIF is a relevant and simple diagnostic tool that forecasts poor outcome in the pediatric ED. Future studies are needed to validate its utility.

SA-PO025

Defining Baseline Renal Function to Improve Acute Kidney Injury Diagnosis Kerry L. Horne,[1] Rebecca A. Packington,[1] Timothy T. Reilly,[1] John Monaghan,[1] Nitin V. Kolhe,[1] Richard J. Fluck,[1] Nicholas M. Selby.[1,2] *[1]Royal Derby Hospital, United Kingdom; [2]Univ of Nottingham, United Kingdom.*

Background: Detecting acute kidney injury (AKI) in clinical practice is often hampered by lack of a baseline creatinine within the preceding 7 days. We aimed to describe the time between available baseline and AKI episode in a group of hospitalized patients and evaluate the impact on performance of AKI diagnostic criteria.

Methods: All patients sustaining AKI at our centre between 1st Jan and 31st Dec 2013 were studied.AKI was defined using KDIGO serum creatinine criteria, applied by an electronic AKI detection system.Each positive AKI result and choice of baseline was manually checked.We defined baseline creatinine as the most recent stable creatinine value in the last 12 months.In those without a baseline, a value was estimated using MDRD equation and eGFR of 75ml/min.

Results: 3578 AKI episodes occurred in 3375 patients; the cohort was representative of a generalized AKI population (mean age 76±14yrs, 30 day mortality was 27%, 54.5% AKI stage 1). 1996 patients (59.1%) had community acquired AKI (c-AKI). 579 (17.2%) patients did not have creatinine measurement within the preceding 12 months; this represented 25% c-AKI patients but only 5.7% hospital acquired AKI (h-AKI) p<0.001. 9.8% c-AKI patients had a baseline within the preceding 7 days versus 62.9% h-AKI (p<0.001). Median time between baseline and AKI episode was 58 days (IQR 121) in c-AKI and 3 days (IQR 13) in h-AKI (p<0.001). In patients with AKI stages 2/3, mortality rates were unaffected by increasing time between baseline and AKI episode. In those with AKI stage 1, mortality rates fell significantly when the time between baseline and AKI was >90days compared to those with more recent baseline values (24.3% versus 12.0%, p<0.001).

Conclusions: The majority of patients with c-AKI do not have a baseline creatinine value within 7 days. In these patients it seems reasonable to use baseline values from the preceding 12 months to compare with large increments in serum creatinine; greater caution is needed when diagnosing AKI stage 1 without baseline values within 90 days. This has relevance to clinical practice as well as research study and electronic detection system design.

Funding: Private Foundation Support

SA-PO026

N-acetylcysteine Prevents Acute Kidney Injury in Patients Undergoing Myocardial Revascularization Eduesley Santana Santos,[1] Valéria A. Costa-Hong,[2] Suely Pereira Zeferino,[1] Luiz Aparecido Bortolotto,[2] Jose Jayme Galvão De Lima.[2] *[1]Intensive Care Unit - Post Operative Care, Heart Inst (InCor) Univ of Sao Paulo Medical School, São Paulo, SP, Brazil; [2]Hypertension Unit, Heart Inst (InCor) Univ of Sao Paulo Medical School, São Paulo, SP, Brazil.*

Background: The prevention of acute kidney injury using N-acetylcysteine (NAC) in the diverse clinical settings is controversial. The objective of this study was to test efficacy of maximum IV dose of NAC on the incidence of kidney injury in 70 patients underwent elective coronary artery bypass graft (CABG).

Methods: We assessed the renoprotective effect of the highest dose of N-acetylcysteine sanctioned for clinical use. This was a prospective, double-blind placebo-controlled study which included 70 chronic kidney disease patients (stage 3 or 4) who underwent CABG and were randomized to receive either N-acetylcysteine 150 mg/kg followed by 50 mg/kg for 6 h in 0.9% saline (NAC group) or just 0.9% saline (Control group). Acute kidney injury was defined by the Acute Kidney Injury Network (AKIN) classification.

Results: The incidence of acute kidney injury was reduced in the NAC group (57.1% versus 28.6%, p=0.016; figure 1). Non-use of NAC (RR= 3.58 95% CI 1.04 to 12.33, p=0.04) and cardiopulmonary bypass (RR=4.55, 95% CI 1.28 to 16.15, p=0.02) were independent predictors of acute kidney injury.Whereas NAC reduced the incidence of AKI, the reverse was observed when patients underwent CPB.The risk of AKI developing was 20-fold higher among patients with the latter characteristic than with the former (p = 0.008). Among patients treated with CPB, the use of NAC was associated with a fall in the incidence of AKI from 63% to 41%. Oxidative stress was increased in Controls (p=0.01).

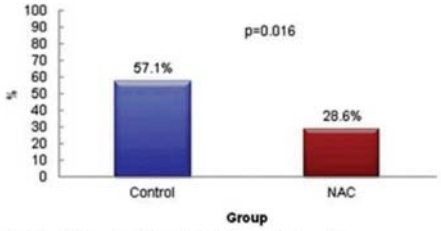


Figure 1: Effect of N-acetylcysteine (NAC) on the incidence of acute kidney injury in patients.

Conclusions: High-dosages of IV N-acetylcysteine was associated with reduction on the incidence of acute kidney injury in patients undergoing CABG.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO027

Outcomes of Albumin Use in Treatment of Hepatorenal Disorders - A Single Center Experience Krishna Pothugunta, Santhi Voora, Holly J. Kramer, Anil K. Bidani, Kavitha Vellanki. *Dept of Nephrology and Hypertension, Loyola Univ Medical Center, Maywood, IL.*

Background: Intravenous albumin along with Midodrine and Octreotide is recommended for treatment of type I hepatorenal syndrome (HRS). The dose and duration of albumin infusions along with cautions for use are not generally addressed. We reviewed patient outcomes by amount of albumin infused in patients with HRS at our institution. We hypothesized that patients receiving albumin infusions with no renal recovery have a higher risk for pulmonary edema.

Methods: A total of 93 cirrhotic patients were admitted to Loyola University Medical Center between 2011 and 2013 and received intravenous albumin for treatment of HRS. All patients had a normal CXR and were not receiving dialysis at hospital admission. Fisher's extract test was used to compare frequency of pulmonary edema stratified by renal recovery. Logistic regression was used to examine the association between renal recovery and pulmonary edema while adjusting for total albumin infused, presence of oliguria and MELD score.

Results: Among the 93 patients, 20 had complete renal recovery,17 had partial recovery and 56 had no recovery. Risk of pulmonary edema was 47%, n=44 and was significantly higher in patients with no renal recovery versus those with partial or full recovery (63% versus 24%; P = 0.003) compared to patients with renal recovery. In the logistic regression model, those with no renal recovery had 4.5 fold (95% CI 2.0-13.0) higher odds of pulmonary edema versus those with full or partial recovery.

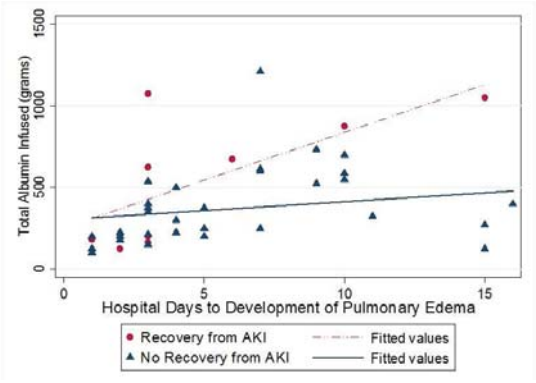


Figure 1 shows the hospital days to pulmonary edema by presence or absence of renal recovery.

Conclusions: In summary,Continued administration of intravenous albumin did not have a significant effect on renal recovery, but the risk of new onset pulmonary edema was significantly higher in patients with no renal recovery.

SA-PO028

Efficacy of Midodrine/Octreotide or Norepinephrine in the Treatment of Hepatorenal Syndrome, and Its Dependency on Rise in Mean Arterial Pressure Manish Kadian,[1] Margarita Taburyanskaya,[3] Paul Nietert,[2] Andrew Goodwin,[4] Timothy Whelan,[4] Juan Carlos Q. Velez.[1] *[1]Div of Nephrology, MUSC; [2]Div of Public Health Sciences, MUSC; [3]Dept of Clinical pharmacy, MUSC; [4]Div of Pulmonary, Critical Care, Allergy, and Sleep Medicine, MUSC.*

Background: Studies suggest that midodrine and octreotide (M/O) combination leads to improvement in kidney function in ~50% of patients with hepatorenal syndrome (HRS). We hypothesized that the success rate of M/O outside of clinical trials is lower than 25%, and that the lack of success is associated with failure to attain a substantial rise in mean arterial pressure (MAP). In addition, we explored the benefit of norepinephrine (NE) as alternative therapy in HRS.

Methods: Retrospective chart review of patients was done with a discharge diagnosis of HRS who received ≥3 doses of M/O for HRS over the last 5 years. Improvement in kidney function was defined as ≥50% decrease in serum creatinine (sCr).

Results: We identified 73 subjects treated with M/O for HRS and found a significant correlation between attained increase in MAP and improvement in sCr [mean absolute change in sCr from the value at therapy initiation: +0.45 ± 0.4, +0.04 ± 0.4, +0.08 ± 0.5 and -0.19 ± 0.5 mg/dL, for quartile I (-14.1 to +1.8 mmHg), II (+1.9 to +7.5 mmHg), III (+7.8 to +15.8 mmHg) and IV (+15.9 to +29.4 mmHg) of change in MAP from baseline, respectively; p=0.0008]. This correlation remained unchanged after adjusting for baseline MAP, sCr or MELD. Eleven patients were either transitioned from M/O to NE (n=8) or treated de novo with NE (n=3). Only 30.1% (22/73) of M/O-treated patients achieved an increase in MAP >15 mmHg, compared to 63.6% (7/11) of those treated with NE (p=0.029). Compared to M/O therapy, more patients improved kidney function when treated with NE [45.5% (5/11) versus 13.6% (10/73), p=0.011].

Conclusions: During M/O therapy for HRS, a fall in MAP is associated with worsening kidney function, whereas a rise in MAP correlates with improvement in kidney function. NE appears to be more effective than M/O in raising MAP and thereby improving kidney function in HRS.

SA-PO029

Acute Kidney Injury Risk Assessment Among VA Patients Undergoing Diagnostic Angiograms: Insights from the National VA CART Program Michael Edwin Matheny,[1,2] Thomas M. Maddox,[3,4] Jeremiah R. Brown,[5] Thomas T. Tsai,[3,4] Edward D. Siew,[1,2] James Fly,[1,2] Mary E. Plomondon,[3,4] Theodore Speroff,[1,2] John S. Rumsfeld,[3,4] Frederic S. Resnic.[6] *[1]VA Tennessee Valley Healthcare System, Nashville, TN; [2]Vanderbilt Unversity, Nashville, TN; [3]VA Eastern Colorado Health Care System, Denver, CO; [4]Univ of Colorado, Denver, CO; [5]Dartmouth Inst, Hanover, NH; [6]Lahey Clinic, Burlington, MA.*

Background: Acute kidney injury (AKI) following cardiac catheterization is a common iatrogenic complication of the procedure with strong variability in risk-adjusted AKI rates among catheterization centers. We evaluated the national catheterization registry for peri-procedural AKI risk assessment practices.

Methods: Data were from the VA Clinical Assessment Reporting and Tracking (CART) Program and the VA corporate data warehouse for all cardiac catheterizations performed in the VA between 07/01/2012 to 06/30/2013. Baseline renal function was defined as the most recent pre-procedural serum creatinine up to one year prior to the procedure. Chronic kidney disease (CKD) was stratified by KDOQI Stages IIIa-b and IV. AKI following cardiac catheterization was defined as ≥0.3 mg/dL within 48 hours of the procedure or ≥50% increase in serum creatinine from baseline at any time during the hospitalization or up to 7-days following the procedure.

Results: total of 46886 cardiac catheterizations among 40925 patients were performed in the study period. Pre-procedural serum creatinine measurement was performed within 30 days prior for 45,273 (97.2%) procedures. 24,494 (52%) of patient procedures had baseline and follow-up creatinine measurements. AKI occurred for 1426/17065 (8.4%), 688/4511 (15.3%), 550/2058 (26.7%), and 283/579 (48.9%) of patients with GFR values of >=60, 45-59, 30-44, and 15-29 mL/min/1.73 m2, respectively. A total of 2168, 607, and 79 patients with GFR values of 45-59, 30-44, and 15-29 mL/min/1.73 m2, respectively, had baseline creatinine measured but no follow-up value.

Conclusions: Post-procedural AKI ascertainment rates were low, even among CKD patients, and AKI rates among CKD patients were over double those with normal renal function.

Funding: Veterans Affairs Support

SA-PO030

Acute Kidney Injury Risk Management Practices for Veterans Affairs Patients Undergoing Diagnostic Angiograms: A Survey of Laboratory Directors Michael Edwin Matheny,[1,2] Freneka F. Minter,[1,2] Thomas M. Maddox,[3,4] Jeremiah R. Brown,[5] Thomas T. Tsai,[3,4] Edward D. Siew,[1,2] Mary E. Plomondon,[3,4] Theodore Speroff,[1,2] John S. Rumsfeld,[3,4] Frederic S. Resnic.[6] *[1]TVHS Veterans Administration, Nashville, TN; [2]Vanderbilt Univ Medical Center, Nashville, TN; [3]VA Eastern Colorado Health Care System, Denver, CO; [4]Univ of Colorado, Denver, CO; [5]Dartmouth Inst, Lebanon, NH; [6]Lahey Clinic, Burlington, MA.*

Background: Acute kidney injury (AKI) following cardiac catheterization is a common iatrogenic complication of the procedure. We conducted a national Veterans Affairs (VA) survey to ascertain patterns of care surrounding pre-procedural AKI risk assessment and management.

Methods: A survey was administered to characterize local care protocols for pre- and peri- procedural AKI risk management of patients. All VA cardiac catheterization directors were identified through the Clinical Assessment, Reporting, and Tracking Program. Surveys were administered from 07/2013 to 11/2013 by email attachment. Re-contact was performed at 1 month and 3 months for non-responders.

Results: A total of 21/77 (27%) directors responded to the survey. Nine sites used an established protocol for AKI risk assessment. All sites required a serum creatinine measurement prior to the procedure, but the measurement interval allowed varied widely: <1 day (1), <5 days (1), <7 days (7), <14 days (2), <30 days (7), and no response (2). Sites used the following GFR (mL/min/1.73 m^2) thresholds to determine increased AKI risk: <60 (13), <50 (1), <40 (1), <30 (2). One site used a risk prediction model for risk stratification. Nine sites provided pre-procedural volume expansion patient education, and 14 sites had protocols for overnight hospitalization and/or IV hydration for high risk elective procedures. 19 sites practiced strategies for minimizing contrast volume, 7 calculated a maximum contrast load, and 7 used an automated contrast injection device.

Conclusions: There is significant practice variation in AKI risk stratification and management among the surveyed VA cardiac catheterization laboratories. Opportunities exist for care protocol standardization with validated management strategies.

Funding: Veterans Affairs Support

SA-PO031

Modified Acute Kidney Injury Classification Does Not Predict Survival after Orthotopic Liver Transplantation (OLT) Thais Nemoto Matsui, Adriano Luiz Ammirati, Nadia K. Guimaraes, Maria C.C. Andreoli, Marcio Dias Almeida, Jose Ben-Hur Ferraz-Neto, Marcelo Costa Batista, Marcelino Souza Durao, Virgilio Gonçalves Pereira, Oscar Santos, Bento C. Santos. *Hospital Israelita Albert Einstein, Sao Paulo, SP, Brazil.*

Background: Recently, a modified AKIN criteria (ModAKI) incorporating the traditional definition of kidney impairment (serum creatinine [SCr]>1.5mg/dL) has been associated with lower survival rates in hospitalized cirrhotic patients. The aim of this study was to determine the impact of preoperative AKI assessed by the ModAKI classification on survival at 28 days and at 1 year after OLT.

Methods: This study included 283 patients with chronic liver disease who were consecutively admitted in a medical center to undergo OLT. AKI score was determined at the moment of admission and immediately before OLT. ModAKI classification was defined as follows: A) AKI stage 1 and SCr<=1.5mg/dL; B) AKI stage 1 and SCr>1.5mg/dL; and C) AKI stage 2/3.

Results: From the 283 included patients (52.4±11.7yrs), 54% had viral-related disease, with a MELD of 19.4±9.7, and 22% had pre-OLT diabetes. AKI at admission and on the day of OLT was observed in 83 and 73 subjects, respectively, with the following ModAKI scores: A=19%, B=29%, C=52% at admission; and A=19%, B=29%, C=52% on the day of OLT. RRT was required in 28%. Mortality rate at 28 days was 6%, with similar rates across ModAKI scores (0=7%, A=13%, B=4%, C=5% at admission, p=0.627; and 0=6%, A=14%, B=0%, C=8% on the day of OLT, p=0.967). Likewise, the mortality rate 1yr after OLT (14%) was not influenced by ModAKI score on the day of OLT (0=13%, A=21%, B=5%, C=18%, p=0.685). ModAki scores B and C at admission tended to be associated with higher mortality 1yr after OLT (0=12%, A=13%, B=21%, C=21%, p=0.061). After adjustment for MELD score and pre-OLT diabetes, RRT (OR 4.036, CI95% 1.793–9.084, p=0.001) and older age (OR 1.039, CI95% 1.002–1.078, p=0.039) were independently associated with death within 1yr of OLT.

Conclusions: The modified AKIN criteria proposed for cirrhotic patients could not accurately predict the survival rate at 28 days and within 1yr of OLT. The most important prognostic factor for survival was the need for peritransplant RRT.

SA-PO032

Erythropoietin May Ameliorate Acute Kidney Injury after Cardiac Valves Surgery Nanmei Liu. *Dept of Nephrology, Jimin Hospital of Shanghai, Shanghai, China.*

Background: A series of studies have provided evidence that Erythropoietin (EPO) administration exerts significant renal protective effects in various experimental models, but it had not been verified in clinical trial. To value if EPO can reduce Acute kidney injury (AKI) in patients undergoing cardiac valve replacement surgery.

Methods: Prospective, random, control clinical study was conducted in 18-70 years old patients need cardiac valves replacement. 1wu of EPO was injected subcutaneous before operation and 24h after operation in EPO group, other treatment was the same to the control group. AKI was defined as Serum creatinine was increased more than 0.3mg/dl within 48~72h after operation. (clinical trial registered number : ChiCTR-TRC-12002266).

Results: 105 patients was selected, 3 was excluded because of serious complications in surgery, 1 was excluded because of insufficiency data. 101 patients had been completed, 47 in EPO group, 64 in control group. There was no significant difference in Age, weight, height, baseline Serum creatinine, eGFR, operation time, CBP time and aorta block time between the two groups. The total incidence of AKI was 24.8%, 19.2% in EPO group, 29.6% in control group (P=0.09).The difference of serum TNF-α, IL-6 2h after operation was not significant. AKI patients compared with no AKI patients, 24h after operation Scr were 165±64 and 83.8±22 μmol/L, Uric acid were 398±144 and 235±97mmol/L, insulin 45.8±26.4 and 28.0±19.1(P<0.05). AKI patients were more older (62.5±6.9 versus 50.1±13.2 years), had longer operation time(301.4 ±108.0 versus 229.6±60.5 minutes) and longer CBP time (151.3±53.5 versus 114.7±39.4 minutes).

Conclusions: 1. primary results showed that EPO may ameliorate acute kidney injury after cardiac valves surgery. The study sample needs to be enlarged to confirm the result. 2. AKI patients had longer operation and CBP time, it is indicated that operation insult may play more important role in the development of AKI in cardiac valves surgery patients.

Funding: Government Support - Non-U.S.

SA-PO033

Development of an Electronic Algorithm to Identify Acute Kidney Injury Ioannis Konstantinidis, Girish N. Nadkarni, Rajiv Nadukuru, Vaneet Lotay, Stephen B. Ellis, Omri Gottesman, Erwin P. Bottinger. *Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Acute kidney injury (AKI) is a commonly occurring event that is a risk factor for morbidity and mortality and adds to healthcare cost. Epidemiological studies often rely on billing codes to classify patients; however, these were shown to have poor sensitivity and moderate negative predictive value (NPV). A tool for accurate identification of AKI from electronic medical records would have significant utility for research purposes. We aimed to assess an algorithm that uses International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes, laboratory results, and textual information from clinical notes.

Methods: We deployed the algorithm in 2,000 randomly selected Mount Sinai BioMe Biobank participants. AKI was defined by the 2012 KDIGO criteria (increase in serum creatinine of ≥0.3 mg/dl over 48 hours or ≥50% over 7 days or urine volume <0.5 ml/kg/hour for 6 hours). Two blinded physicians performed manual chart review. We calculated the sensitivity, specificity, NPV and positive predictive value (PPV) with 95% confidence intervals (CI) for both the algorithm and ICD-9-CM codes.

Results: We identified 104 cases and 346 controls. The sensitivity of algorithm-identified AKI was 100% compared to 59.6% for billing code-identified AKI. The NPV of algorithm-identified AKI was 100% compared to 89.2% for billing code-identified AKI. Specificity and PPV were 100% in both cases.

Table 1: Summary statistics for algorithm and ICD-9-CM codes

	Chart review				Chart review		
Algorithm	Case	Control	Total	**ICD-9-CM codes**	Case	Control	Total
Case	104	0	104	Case	62	0	62
Control	0	346	346	Control	42	346	388
Total	104	346	450	Total	104	346	450
Sensitivity (95% CI)	100% (95.6-100)			Sensitivity (95% CI)	59.6% (49.5-69.0)		
Specificity (95% CI)	100% (98.6-100)			Specificity (95% CI)	100% (98.6-100)		
PPV (95% CI)	100% (95.6-100)			PPV (95% CI)	100% (92.7-100)		
NPV (95% CI)	100% (98.6-100)			NPV (95% CI)	89.2% (85.5-92.0)		

Conclusions: We developed an automated algorithm that outperformed identification of AKI by billing codes. This can be deployed to identify AKI in health systems with EMR for research and quality improvement.

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SA-PO034

The Impact of a Renal Intensivist in a Surgical Trauma Intensive Care Unit (STICU) Roshan A. Patel,[1] Aleef M. Rahman,[2] Viral G. Gandhi,[1] Omar Mudallal,[1] George N. Coritsidis.[1,2] *[1]Medicine, Elmhurst Hospital Center (EHC), Icahn School of Medicine, Elmhurst, NY; [2]Surgery, EHC, Icahn School of Medicine, Elmhurst, NY.*

Background: Nephrology is one of 3 medical subspecialties that require a year of critical care fellowship for eligibility to work as an intensivist. Our program is one of few in the U.S. that offers an opportunity for both. It has been shown that early involvement by renal services in acute kidney injury (AKI) decreases morbidity and mortality. We were interested to see the impact on the STICU when primarily managed by a renal intensivist (2005) as compared to earlier management by surgical intensivists (2002).

Methods: A retrospective chart review of adult STICU admissions for the first half of 2002 and 2005.Total 418 charts were reviewed. Medical records with insufficient information, patients with terminal extubation and on palliative care were excluded. The data was further divided between trauma and nontrauma. Data was analyzed using STATA v. 12.0 (Austin, Texas). Unpaired t-tests and chi-squared analysis were utilized.

Results: 418 patients were identified, of which 218 were non-trauma. In the total population, there were no differences between groups except for significantly more trauma patients in 2005. Data was then stratified by trauma status. Significant differences were only seen in the non-trauma surgical group.

Non-trauma Surgical Patients	2005-Renal Intensivist	2002-Surgical Intensivist	P value
Number of Patients	113	103	
Age	54.03+ 1.74	55.20 + 1.48	0.5901
APACHE II score	10.02 + 0.60	11.60 + 0.80	0.1163
Mortality	9.56 + 0.03	18.44 + 0.04	0.0617
ICU LOS	6.85 + 1.16	10.19 + 1.43	0.0720
Ventilator days	2.04 + 0.40	5.42 + 1.57	0.4770
Δ Weight(lbs)	-1.38 + 0.82	1.12 + 0.72	**0.0221***
AKI	0.10 + 0.03	0.13+ 0.03	**0.0398***

Δ Weight=ICU discharge minus ICU admission weight, AKI=RIFLE criteria Injury stage. APACHE II score=Acute Physiology, Age, Chronic Health Evaluation.LOS=length of stay, *denotes significance.

Conclusions: The benefits of the renal intensivist seems to be primarily in non-trauma surgical patients. Within that cohort, there was significantly less AKI with better volume management. Benefits in mortality and ICU LOS approached significance.

SA-PO035

Calciprotein Particles Induce an Inflammatory Response in Macrophages Prakash Chandak,[1] Rakesh Kumar Bijarnia,[1] Edward Robert Smith,[2] Andreas Pasch.[1] *[1]Nephrology, Hypertension and Clinical Pharmacology, Univ Hospital Bern, Bern, Switzerland; [2]Nephrology, The Royal Melbourne Hospital, Melbourne, Australia.*

Background: Calciprotein particles (CPP) are nanoscale mineral-protein aggregates, which have been found in the blood of patients with chronic kidney disease (CKD). These particles contain amorphous (primary CPP) or crystalline (secondary CPP) calcium phosphate along with serum proteins. We investigated whether CPP might induce an inflammatory response in macrophages.

Methods: Prim. and sec. CPP were generated using phosphate- and calcium-enriched cell culture media with varying amounts of FBS. Particles were characterized morphologically by transmission electron microscopy (TEM). Murine RAW-264.7

macrophage-like cells were exposed to increasing amounts of CPP for 24 hrs. RT-PCR was performed to assess interleukin (IL)-6, IL-1β, IL-10, MCP-1, TNF-α and NLRP3. The involvement of toll-like receptor-4 (TLR-4), nuclear factor-kappa B (NF-κB) and NLRP3-dependent pathways were evaluated using selective chemical inhibitors.

Results: TEM imaging of synthetic CPP revealed populations of amorphous spherical (prim. CPP) and larger crystalline spindle-shaped particles (sec. CPP). Exposure of RAW-264.7 cells to sec. CPP resulted in a dose-dependent increase in the expression of pro-inflammatory cytokines IL-6, IL-1β, MCP-1 and TNF-α. IL-10 expression was unaffected by sec. CPP exposure. In contrast, no inflammatory response was detected upon exposure to prim. CPP. Inhibition of TLR-4, NF-κB and NLRP3 pathways reduced the sec. CPP-induced inflammatory response in RAW-264.7 cells.

Conclusions: Sec., but not prim. CPP induce pro-inflammatory cytokine expression in the macrophage and this effect may be mediated by activation of TLR-4/NF-κB and NLRP3 pathways. CPP-II might be involved in the induction and maintenance of the chronic inflammatory state commonly encountered in CKD patients.

Funding: Government Support - Non-U.S.

SA-PO036

Iron Stimulation Enhanced Calcification in Vascular Smooth Muscle Cells

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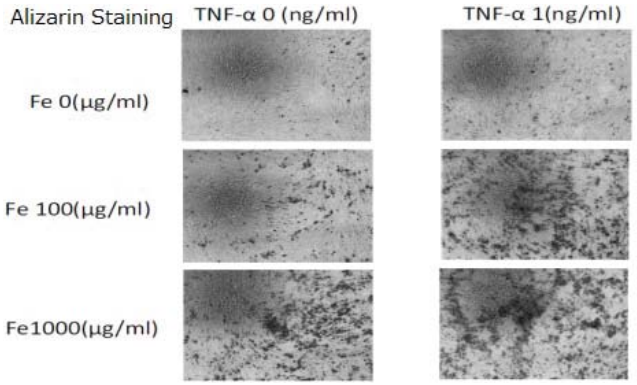
Background: In CKD patients, atherosclerosis is one of important key factors which determine their prognosis. It was reported the calcification induced by TNF-alpha was related with iron in HUVEC cells by our group (Nanami-M, et al, Atreriscler Thromb Vasc Biol, 2005). The feature of the atherosclerosis in CKD patients was called as Moenckeberg's arterisclerosis which was seen in vascular media. To reveal the relationship between calcification in vascular media and iron stimulation using vascular smooth muscle cells.

Methods: The aorta smooth muscle cells were cultured for three weeks. At day 0, we changed the usual culture medium to calcification medium, and TNF-alpha and iron were added to the calcification medium. Calcification in each condition was confirmed by Alizarin staining. And to reveal early mechanism to enhance the calcification by iron and TNF-alpha stimulation, we compared the gene expression profile between each condition in day 1 and day 3 using microarray analysis.

Results: We confirmed both iron TNF-alpha stimulation enhanced calcification by Alizarin Staining. Moreover iron TNF-alpha stimulation at the same time enhanced calcification more strongly than single stimulation (shown below).



There were several gene expressions which increases more than 45 times one day after iron stimulation, such as CABS1,OR10V1, FBP2, CRAMP1L.

Conclusions: Iron stimulation enhanced calcification in vascular smooth muscle cells along with TNF-alpha stimulation. The mechanism of this calcification seemed to begin from early phase through gene expression changes.

Funding: Government Support - Non-U.S.

SA-PO037

Adiponectin: A Novel Regulator of Calcium and Phosphate Balance?

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Background: Mineral homeostasis is a key factor in chronic cardiovascular disease and osteoporosis with the kidney at the center of a complex interplay of hormones regulating calcium and phosphate storage, excretion, and uptake. Adiponectin is an important adipose tissue-derived hormone in energy homeostasis; it has also been characterized as cardioprotective, but is potentially detrimental in osteoporosis through osteoclast activation. Several recent studies have identified adiponectin to be renoprotective in the restoration of renal function following injury.

Methods: We sought to identify how adiponectin regulates renal calcium and phosphate homeostasis in adiponectin knockout (ADN-KO), wildtype, and adiponectin overexpressing transgenic (ADN-Tg) mice.

Results: At baseline, ADN-KO mice exhibited lower serum Ca^{2+} levels, lower urinary Ca^{2+}excretion (UCaV), and lower fractional excretion of Ca^{2+} (FECa). In contrast, ADN-Tg mice showed the opposite with increased serum Ca^{2+}. A 2 month 2% phosphate enriched diet challenge resulted in many changes that diversified across adiponectin levels. While we observed increases in serum PTH, 1,25-vitamin D, and FGF23 and decreases in secreted klotho in all mice, ADN-Tg mice were "hyper-responsive" exhibiting 2-fold higher serum FGF23 levels and a concomitant increase in FEPi. Similar to their baseline state, these mice had higher UCaV and FECa with Pi-enriched diet. In contrast, ADN-KO mice exhibited a significantly smaller increase in both phosphaturic hormones; PTH levels were half those of wildtype mice; FGF23 levels were also significantly less. Chronic Pi loading also induced interstitial fibrosis with increased susceptibility in ADN-KO mice. Removal of the Pi-enriched diet for one month restored hormones and clearance to near baseline values for all mice. ADN-Tg mice exhibited the greatest decrease in histologic fibrotic area.

Conclusions: Adiponectin plays a role in hormonally regulating calcium and phosphate homeostasis and exerts protective effects from a fibrotic challenge. This highlights the importance of adipokines in kidney and cardiovascular disease and has potentially identified a new mechanism by which adiponectin regulates bone mass.

Funding: NIDDK Support, Private Foundation Support

SA-PO038

Passive Gut Phosphate Absorption Is Increased in CKD Mice

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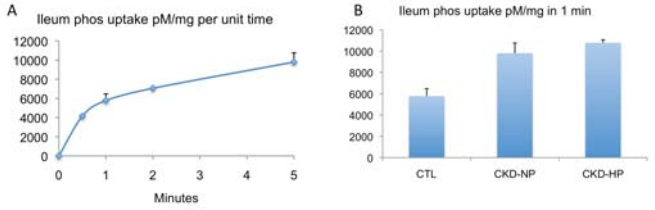
Background: Dietary phosphate absorption occurs by sodium-dependent active uptake (Npt-2b transporter) or passive diffusion (paracellular pathways). Chronic gut inflammation with breakdown of intercellular tight junctions is present in CKD and is a likely source of systemic inflammation. The potential impact of this gut inflammation on phosphate uptake has not been explored. We used an *in situ* gut loop assay to measure active and passive phosphate uptake in the ileum of CKD and control mice.

Methods: CKD was induced in adult mice via adenine diet x18 days. Mice were then placed on normal 0.5% (NP) or high 1.5% (HP) phosphate diet for 6 weeks. Mice were re-exposed to adenine diet x1 week in the midst of special phosphate diet to maintain CKD. Under isoflurane anesthesia, 2.5 cm ileum loops (with intact blood supply) were created and injected with sodium- or choline-phosphate buffer radiolabeled with P-33. Phosphonoformic acid (Npt-2b inhibitor) was added to the choline-phosphate buffer to ensure exclusion of Npt-2b active transport. Amount of absorbed phosphate, normalized to mg protein, was calculated after timed incubation. Adjacent ileum tissue was processed for histology and Western blot.

Results: Passive phosphate uptake (measured with sodium-free buffer) in the ileum of control mice plateaued after 1-minute incubation (Figure A). Active transport was less than passive uptake and sometimes measured out as a negative value, suggesting efflux transport into the gut lumen. CKD mice on NP and HP diet (n=4 per group) showed increased passive phosphate uptake compared to controls (Figure B, P<0.05). Histology showed influx of macrophages in the CKD ileum wall, and expression of the tight junction protein claudin-2 was significantly decreased.



Conclusions: Passive phosphate uptake is increased in the gut of CKD mice and may contribute to systemic phosphorus overload.

Funding: Pharmaceutical Company Support - Sanofi

SA-PO039

Small Returns for Big Investment: CKD-MBD Therapy from 2005 to 2013

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Background: Although significant resources are devoted towards the control of phosphorus (Pi), calcium (Ca) and parathyroid hormone (PTH) in dialysis patients as treatment for chronic kidney disease–mineral bone disorder (CKD-MBD), there is a deficiency of evidence supporting this practice. We aimed to describe the trends in Pi, Ca, PTH in a prevalent dialysis cohort following the introduction of non-calcium based phosphate binders (NCBPB)–sevelamer (year introduced into formulary:2002) and lanthanum (2007) – and cinacalcet (2006).

Methods: An observational cohort study was conducted on 7645 patients receiving dialysis in British Columbia (BC) from 2005–2013 entered into the PROMIS (Patient Records and Outcome Management Information System) database. Demographic, clinical, medication and laboratory data were obtained from the database. All results of laboratory investigations performed for these patients within BC were automatically uploaded into PROMIS. Target ranges of Ca, Pi and PTH were defined according to the 2009 KDIGO (Kidney Disease Improving Global Outcomes) guidelines.

Results: Phosphate binders (PB) of any kind were used by 86–91% of the population. 18–21% were prescribed NCBPB/cinacalcet +/- conventional PB. Pi was unchanged over the study period (p=0.77). There was a decrease in Ca of 0.01 mmol/L per year (95% CI=-0.02 – -0.001, p=0.03) and increase in log(PTH) of 0.04 per year (95% CI = 0.03–0.04,

p<0.001). There was no significant change in % of patients within target range of Pi (OR=1.01, 95% CI = 0.99–1.02, p=0.09) or PTH (OR=1.01, 95% CI=0.99 – 1.02, p=0.05) but patients were less likely to have low PTH (OR=0.93, 95% CI=0.92 – 0.95, p<0.001). Patients within target range of Ca decreased (OR=0.97, 95% CI=0.96–0.98, p<0.001) and patients were less likely to be hypercalaemic (OR=0.95, 95% CI=0.92–0.98, p=0.005).

Conclusions: Following prescription of new treatments for CKD-MBD there has been a reduction of patients with high Ca and low PTH. However, the proportion of patients reaching target ranges for Pi and PTH is unchanged. These data bring into question the utility of these costly therapies.

SA-PO040

Mineral Metabolism and Outcomes in Long-Term Kidney Transplantation Sinee Disthabanchong. *Div of Nephrology, Dept of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol Univ, Phayathai, Bangkok, Thailand.*

Background: In non-dialysis chronic kidney disease and chronic dialysis populations, phosphate retention, increased PTH and FGF-23 levels, and 25-hydroxyvitamin D deficiency predict future patient outcomes including ESRD and mortality. The relationships between mineral metabolism and outcomes in long-term kidney allograft recipients have not been extensively studied. This is a prospective cohort study that examined the relationship between mineral parameters and the composite outcome of allograft loss and mortality in long-term kidney allograft recipients.

Methods: Two hundred twenty nine kidney allograft recipients from Ramathibodi Hospital, Mahidol University, Bangkok, Thailand who were at least 1 year post-transplantation were enrolled between March, 2009 to January, 2010. Demographic and laboratory data including serum calcium, phosphate, intact PTH, c-terminal FGF-23 and 25-hydroxyvitamin D were collected at baseline. The composite outcome of allograft loss and mortality were followed until February 2013.

Results: The median follow-up time was 38 months. Thirteen patients died and 16 patients lost the allograft. In univariate Cox regression analyses, increased systolic blood pressure, deceased donor transplantation, increased serum phosphate, FGF-23 and PTH and decreased 25-hydroxyvitamin D were associated with the outcome. There was no association between serum calcium and the outcome. After adjustment for cardiovascular risk factors and other mineral parameters, the highest tertile of serum phosphate, FGF-23 and PTH remained significantly associated with the outcome. However, these associations were lost after further adjustment for allograft function (eGFR).

Conclusions: In conclusion, increased serum phosphate, PTH and FGF-23 predict future outcome of allograft loss and mortality in long-term kidney allograft recipients.

Funding: Private Foundation Support

SA-PO041

Serum Phosphorus and Prevalence of Cardiovascular Disease within a Large, Diverse CKD Population Dean A. Kujubu, In-Lu Amy Liu, Michael Batech, John J. Sim. *Nephrology and Hypertension, Kaiser Permanente LAMC, Los Angeles, CA.*

Background: Elevated serum phosphorus has been associated with greater morbidity and mortality among ESRD patients. We sought to determine whether incremental increases in serum phosphorus are associated with prevalent cardiovascular disease across the spectrum of eGFR from the large, diverse population of Kaiser Permanente Southern California (KPSC).

Methods: A cross sectional study of KPSC members age >/= 18yrs was conducted during 1/1/1999 - 12/31/2009. Individuals w 1yr continuous membership who had serum phosphorus and eGFR determinations were included. Dialysis and transplants excluded. The cohort was stratified by eGFR (>89ml/min, 60-89, 30-59, and < 30). Coronary artery disease (CAD), congestive heart failure (CHF), and cerebrovascular accident (CVA) were identified using inpatient and outpatient ICD9 codes. The primary outcome was risk for combined cardiovascular disease. Subsequent analysis examined each individual outcome separately stratified by eGFR. Multivariable logistic regressions used to calculate odds ratio's after adjusting for age, gender, race, and Charlson Comorbidity Index.

Results: 195,097 individuals were identified in the study cohort. There was no association between every 0.5 mg/dl increment in serum phosphorus and risk of the combined cardiovascular diseases across all eGFR strata (Table 1), though there was a higher risk for CHF. For eGFR >89 ml/min. every 0.5 mg/ml of phosphorus increased risk for combined cardiovascular disease. For all other categories of eGFR, there was no significant risk of either combined or individual cardiovascular prevalence with higher serum phosphorus.

All GFR		OR	95% CI
	Combined	0.99	0.92-1.07
	CHF	1.58	1.01- 1.10
	CAD	1.008	0.98-1.04
	CVA	0.99	0.92-4.07
GFR>=90	Combined	1.229	1.04-1.45
	CHF	0.986	0.87-1.11
	CAD	0.992	0.91-1.07
	CVA	1.229	1.04-1.45
GFR 60-89	Combined	0.897	0.79-1.02
	CHF	0.997	0.92-1.07
	CAD	0.981	0.91-1.06
	CVA	0.897	0.79-1.02
GFR 30-59	Combined	1.041	0.86-1.25
	CHF	1.055	0.96-1.16
	CAD	1.037	0.95-1.13
	CVA	0.966	0.79-1.19
GFR < 30	Combined	3.636	0.45-29.0
	CHF	1.07	0.96-1.19
	CAD	0.951	0.86-1.06
	CVA	0.874	0.67-1.14

Conclusions: In a large, diverse population, we did not observe increased risk of prevalent CAD, CHF, and CVA with higher serum phosphorus levels among individuals with eGFR<89ml/min. Whether phosphorus binder use will modify these results remains to be seen.

SA-PO042

Serum Phosphorus May Be an Incomplete Surrogate Endpoint for the Prediction of a Phosphate Binder's Impact on Mortality in Non-Dialysis-Dependent Chronic Kidney Disease Lisa Bernard,[1] Elizabeth S. Dunn,[2] Daniel Grima.[1] *[1]Cornerstone Research Group Inc., Burlington, ON, Canada; [2]Consultant to Cornerstone Research Group Inc., Brookline, MA.*

Background: Surrogate endpoints are often used to predict the effect of a therapy on final clinical outcomes such as survival, when clinical outcomes are rare and/or occur over a long time period. Serum phosphorus (SP) has been used as a surrogate endpoint in the clinical and health economic assessment of phosphate binders for treatment of hyperphosphatemia in non-dialysis dependent chronic kidney disease (NDD-CKD). The objective of this study was to assess the ability of SP to predict the difference in survival between sevelamer and calcium-based binders (CBBs).

Methods: The hazard ratio (HR) for mortality over three years associated with sevelamer compared to CBBs from the INDEPENDENT CKD Study (Di Iorio, 2012) was compared to the HR produced by a published model that predicts mortality based on SP levels. This model used previously published data on the association between SP and mortality.

Results: Modeling via the surrogate endpoint of SP underestimated the survival benefit associated with sevelamer relative to CBBs compared to empirically measured survival reported in the INDEPENDENT CKD study. The calculated HR based on SP was 0.84 compared to the observed HR of 0.36 [95% CI 0.0.15-0.83]) (Di Iorio, 2012).

Conclusions: Based on this comparison of the INDEPENDENT CKD Study and a model of SP and mortality risk, SP may be an incomplete surrogate endpoint for predicting the effect of phosphate binders on survival in NDD-CKD patients. The discrepancy could be due to several factors, including sevelamer's previously demonstrated benefits beyond SP control, such as attenuation of coronary calcification and reduction of low-density lipoprotein (LDL) cholesterol, which may positively impact clinical outcomes. Unless phosphate binder trials measure mortality directly, further work is warranted to develop a multivariate predictive model for survival in NDD-CKD.

Funding: Pharmaceutical Company Support - Sanofi

SA-PO043

Increased Infection-Related Mortality in Dialysis Patients with Low Serum Phosphorus Level: A Prospective Multicenter Cohort Study Ji-Eun Lee,[1,2] Jang-Hee Cho,[1,2] Hye Min Jang,[1,2] Yon Su Kim,[1,3] Shin-Wook Kang,[1,4] Chul Woo Yang,[1,5] Eugene Kwon,[1,2] Jeung-Min Park,[1,2] Hee-Yeon Jung,[1,2] Ji-Young Choi,[1,2] Sun-Hee Park,[1,2] Chan-Duck Kim,[1,2] Yong-Lim Kim.[1,2] *[1]Clinical Research Center for End Stage Renal Disease (CRC for ESRD) in Korea; [2]Kyungpook National Univ; [3]Seoul National Univ; [4]Yonsei Univ; [5]The Catholic Univ of Korea.*

Background: It remains unknown whether mineral metabolism parameters are associated with infection-related death. This study investigated the effect of the serum calcium and phosphorus level on the infection-related mortality in dialysis patients.

Methods: Maintenance patients on hemodialysis and peritoneal dialysis were enrolled from a multicenter prospective cohort study in Korea. The patients were divided into low, normal, and high groups according to their serum calcium or phosphorus levels. Cox proportional analysis was used to calculate hazard ratios(HRs) for the association of serum calcium and phosphorus levels with all-cause, infection-related and cardiovascular-related death. Time-dependent values of calcium and phosphorus were also evaluated to assess the effect of longitudinal change in mineral metabolism parameters on various mortalities.

Results: A total of 3,226 dialysis patients were followed up for mean 19.8 ± 8.2 months. The patients with baseline low phosphorus level showed significantly increased infection-related death compared to patients with normal phosphorus level (HR = 1.75, confidence interval [CI] = 1.08-2.83, p = 0.024). The low phosphorus level was also significantly associated with all-cause and infection-related death using time-dependent values (HR =

1.48, CI = 1.11-1.97, p = 0.008 and HR = 1.83, CI = 1.13-2.96, p = 0.014, respectively). However, the baseline and time-dependent calcium levels were not related with infection-related mortality. Serum phosphorus was correlated with nutritional factors such as serum albumin, creatinine, and body mass index.

Conclusions: Low serum phosphorus in maintenance dialysis patients was an independent risk factor for infection-related death. Persistently low serum phosphorus levels might be a nutritional biomarker to predict the susceptibility to infection and in turn worse outcomes in dialysis patients.

Funding: Government Support - Non-U.S.

SA-PO044

Inorganic Phosphate Handling in Salivary Glands Kayo Ikuta, Hiroko Segawa, Shohei Sasaki, Ichiro Kaneko, Yuji Shiozaki, Sawako Tatsumi, Ken-Ichi Miyamoto. *Molecular Nutrition, Institution of Health Bioscience, Univ of Tokushima Graduate School, Tokushima, Japan.*

Background: Hyperphosphatemia is contributes to vascular calcification in patients with chronic kidney disease and hemodialysis patients and is associated with cardiac mortality. Recently, several studies reported the relationship among salivary phosphate concentration, renal function, serum phosphate concentration, and the potential target of new therapy for hypephosphatemia. However, it is unclear the inorganic phosphate (Pi) handling mechanisms in salivary glands. In the present study, we investigated the Pi handling in salivary glands using several models mice.

Methods: Normal C57B6J mice, type II sodium-phosphate (NaPi) transporters knockout (KO) mice; NaPi-2a KO, NaPi-2c and NaPi-2a/NaPi-2c double KO (DKO) mice, and X-linked hypophosphatemia hypophosphatemic (Hyp) mice were used. Mice fed several phosphorus-modified diet, or administered fibroblast growth factor (FGF) 23 and parathyroid hormone (PTH) were used. We examined Pi concentration in saliva and urinary Pi excretion, and transporter expression.

Results: Hypophosphatemic Npt2a KO and Npt2a/Npt2c DKO mice did not show a reduction of salivary Pi concentration. Hyp mice showed hypophosphatemia, but not the reduction of salivary Pi levels. Furthermore, FGF23 and PTH lowered plasma Pi levels, but not saliva.In mice fed a high phosphorus diet, salivary Pi and urinary Pi excretion levels were significantly higher than in those fed the low Pi diet. Furthermore, high Pi diet, but not FGF23 and PTH, decreased the NaPi-2b expression levels, which localized at the apical side of duct cells.

Conclusions: The present study suggests that salivary Pi levels correlate with dietary Pi content, but not plasma Pi.

Funding: Government Support - Non-U.S.

SA-PO045

Relationship between Timed Urine and Spot Urine Collections for Measurement of Phosphate Excretion Sven-Jean Tan,[1,2] Michael Cai,[1,2] Eugenia Pedagogos,[1] Edward Robert Smith,[1,3] Stephen G. Holt,[1,2] Timothy Hewitson,[1,2] Nigel David Toussaint.[1,2] *[1]Dept of Nephrology, The Royal Melbourne Hospital, Parkville, Victoria, Australia; [2]Dept of Medicine (RMH), The Univ of Melbourne, Parkville, Victoria, Australia; [3]Monash Univ, Clayton, Victoria, Australia.*

Background: Twenty-four hour UPE reflects intestinal phosphate absorption in steady state and can be used to evaluate effects of phosphate-lowering interventions. UPE may be more informative than serum phosphate(sPi) in assessing phosphate homeostasis. However, timed urine collections are cumbersome and prone to inadequate collection. Spot uPiCr assessment may be a useful, simple surrogate for UPE, but is yet to be systematically evaluated in CKD. **Aim:**To determine the relationship between spot urine phosphate:creatinine ratio(uPiCr) and total urinary phosphate excretion(UPE) in chronic kidney disease(CKD) patients.

Methods: Blood samples, spot and 24-hour urine were collected from patients with CKD(Stages 1-5). Serum biochemistry was analysed. Urine phosphate concentration(uPi) and creatinine concentration measurements were performed on spot and 24-hour urine collections. Pearson's correlation coefficients, multiple regression analysis and Bland-Altman plots were used to assess agreement between spot uPiCr and UPE.

Results: 65 CKD patients (49 male) were studied, median age 67yrs (IQR 53-74) and mean(±SD) serum creatinine 182(±84)µmol/L. Mean(±SD) spot uPi, spot uPiCr and total UPE were 12.6(±6.2)mmol/L, 1.58(±0.55)mmol/mmol and 24.5(±11.7)mmol/d respectively. There was no significant correlation between spot uPiCr and UPE (r=0.116, p=0.336). Spot uPi correlated with 24-hour UPE significantly (r=0.306, p=0.019). Bland-Altman analysis of 24-hour versus spot uPi showed acceptable agreement with bias +0.2mmol/L(95%CI -1.2284 – 1.6508). Multiple regression analysis was undertaken to predict UPE from gender, sPi, spot uPi and eGFR. Apart from eGFR, these variables significantly predicted UPE, $F(3,51)$=5.321, p=0.003, R^2=0.238. Gender, sPi and spot uPi added significantly to the prediction, p<0.05.

Conclusions: This study suggests that normalisation of uPi to uCr on spot urine samples may not be appropriate when evaluating urinary phosphate excretion in adults with CKD.

SA-PO046

Parameters of Phosphorus Homeostasis at Normal and Reduced GFR Kenneth R. Phelps,[1,2] Darius Mason.[1,2,3] *[1]Stratton VAMC, Albany, NY; [2]Albany Medical College, Albany, NY; [3]Albany College of Pharmacy and Health Sciences, Albany, NY.*

Background: At equilibrium, influx (I_P) determines urinary excretion of phosphorus (E_P). Contributions of I_P and reabsorption (TR_P) to serum P ($[P]_s$) can be depicted by normalization to C_{cr} (E_P/C_{cr} and TR_P/C_{cr}) or by fractional excretion and reabsorption of P (FE_P and FTR_P). We analyzed $[P]_s$, E_P/C_{cr}, TR_P/C_{cr}, FE_P, and FTR_P at normal and reduced GFR.

Methods: We measured [cr] and [P] in fasting morning serum and urine in 28 controls (C) and 29 patients with stages 3-4 CKD. We calculated E_P/C_{cr} as $[cr]_s([P]_u/[cr]_u)$, TR_P/C_{cr} as $[P]_s - E_P/C_{cr}$, FE_P as $(E_P/C_{cr})/[P]_s$, and FTR_P as $1 - FE_P$. We compared means and examined pertinent linear regressions.

Results: $[P]_s$ was not different in CKD and C. $[Cr]_s$, E_P/C_{cr}, and FE_P were higher and TR_P/C_{cr} and FTR_P were lower in CKD. $[P]_u/[cr]_u$, a surrogate for I_P, was similar in both groups. In CKD, $[P]_s$ correlated with E_P/C_{cr} and TR_P/C_{cr}; E_P/C_{cr} with $[P]_u/[cr]_u$ and $[cr]_s$; and FE_P with E_P/C_{cr}, TR_P/C_{cr}, $[P]_u/[cr]_u$, and $[cr]_s$. In C, $[P]_s$ correlated with TR_P/C_{cr}; E_P/C_{cr} with $[P]_u/[cr]_u$; and FE_P with E_P/C_{cr} and $[P]_u/[cr]_u$. Despite wide ranges of TR_P/C_{cr}, FTR_P was > 80% in 26 of 28 C and < 80% in 27 of 29 CKD.

Group	CKD (n=29)	C (n= 28)	P	Group	CKD (n=29)*	C (n = 28)	
Parameter	Mean (SEM)	Mean (SEM)		Regression	R^2	R^2	P
$[P]_s$, mg/dL	3.7 (0.2)	3.4 (0.1)	0.27	$[P]_s$ on E_P/C_{cr}	0.52	0.046	0.27
$[P]_u/[cr]_u$	0.52 (0.02)	0.48 (0.03)	0.35	$[P]_s$ on TR_P/C_{cr}	0.37	0.95	<0.001
$[cr]_s$, mg/dL	2.5 (0.2)	0.9 (0.03)	<0.001	E_P/C_{cr} on $[P]_u/[cr]_u$	0.48	0.81	<0.001
E_P/C_{cr}, mg/dL	1.4 (0.1)	0.4 (0.03)	<0.001	E_P/C_{cr} on $[cr]_s$	0.74	0.03	0.38
TR_P/C_{cr}, mg/dL	2.3 (0.1)	3.1 (0.1)	<0.001	FE_P and FTR_P on E_P/C_{cr}	0.71	0.81	<0.001
FE_P, %	36.5 (2.3)	12.1 (0.9)	<0.001	FE_P and FTR_P on TR_P/C_{cr}	0.34	0.12	0.07
FTR_P, %	63.5 (2.3)	87.9 (0.9)	<0.001				

*P < 0.001 for all regressions.

Conclusions: GFR affected parameters of P homeostasis. E_P/C_{cr} varied with influx ($[P]_u/[cr]_u$) and GFR ($[cr]_s$) in CKD, but with influx only in C. $[P]_s$ varied with influx (E_P/C_{cr}) and reabsorption (TR_P/C_{cr}) in CKD, but with reabsorption only in C. FE_P varied with influx (E_P/C_{cr}) and reabsorption (TR_P/C_{cr}) in CKD, but with influx only in C. FTR_P depicted reabsorption imprecisely in CKD and C.

Funding: Veterans Affairs Support, Pharmaceutical Company Support - Genzyme Corporation

SA-PO047

Phosphorus Clearance Is Associated with Mineral Metabolic Parameters in Peritoneal Dialysis Patients Yongjin Yi, Hajeong Lee, Dong Ki Kim, Yon Su Kim, Kwon Wook Joo, Jin Suk Han, Curie Ahn, Kook-Hwan Oh. *Internal Medicine, Seoul National Univ Hospital, Seoul, Republic of Korea.*

Background: The present study was undertaken in order to explore the influence of phosphorus (P) clearance on the serum P level and other mineral metabolic parameters in the peritoneal dialysis (PD) patients.

Methods: In this cross-sectional study, measurements of dialysis adequacy and P clearance conducted between April 2013 and May 2014, were collected. The impact of P clearance on the serum P levels, and other parameters such as heart-to-femoral pulse wave velocity (hfPWV) and abdominal aortic calcification score (AAC) were analysed by using multiple linear regression analysis.

Results: Among total 187 subjects, 110 were male and the age was 48.0 ± 14.7 years. Duration of PD was 48.0 ± 45.7 months and total Kt/V were 1.90 ± 0.48, respectively. 107 subjects had residual renal function. The mean peritoneal, renal and total P clearances (PPhCl, RPhCl and TPhCl) were 35.3 ± 13.4, 11.7 ± 17.8 and 47.0 ± 18.9 L/week/1.73m^2, respectively. In multiple linear regression analysis, serum P level was associated with TPhCl (β= -0.019 ± 0.005, p<0.001), non-calcium based P binder uses (β= 1.136 ± 0.222, p<0.001) and age (β= -0.016 ± 0.007, p=0.022, r^2 = 0.240). However, neither PPhCl nor UPhCl was associated with the serum P level. Aortic stiffness, represented by hfPWV, was only associated with conventional risk factors such as age (β= 9.840 ± 1.636, p<0.001) and presence of diabetes (β= 295.988 ± 55.970, p<0.001, r^2 = 0.393). In case of vascular calcification, AAC score was also associated with conventional factors such as age (β= 0.107 ± 0.024, p<0.001), diabetes (β= 2.902 ± 0.851, p=0.001), duration of PD (months, β= 0.028 ± 0.008, p=0.001), and male gender (β= 1.645 ± 0.681, p=0.017, r^2 = 0.368). Neither hfPWV nor AAC score was associated with P clearance.

Conclusions: In PD patients, total phosphorus clearance, non-calcium based P binder and age were independently associated with the serum P level. Total P clearance accounted for the serum P level more than Kt/V, D/P creatinine ratio, and creatinine clearance. Monitoring P clearance may be warranted for the adequate control of mineral metabolic parameters in PD patients.

SA-PO048

Potential Therapeutic Target for Hyperphosphatemia: Sulforaphane Reduces the Expression of the SLC34a2 Gene through Pregnane X Receptor

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Background: NaPi-IIb sodium-dependent phosphate transporter protein, which is encoded by SLC34 gene, plays a dominant role in intestinal phosphorus absorption. The suppression of SLC34 gene expression leads to the reduction of intestinal phosphorus absorption. Therefore, SLC34a2 gene provides a potential therapeutic target for hyperphosphatemia. Previous research also indicates that pregnane X receptor (PXR) is the transcription factor of SLC34a2 gene, which can increase the expression of SLC34a2 gene. On the other hand, sulforaphane (SFN) is the antagonist of pregnane X receptor (PXR), which can suppress the activity of PXR. The objective of this paper is to demonstrate that SFN reduces the expression of SLC34a2 gene through PXR.

Methods: The human colon cancer cell LoVo was selected as its both PXR mRNA and SLC34a2 mRNA expression level were high. In the first stage, LoVo cells were incubated with SFN at a series of concentrations in DMEM (0, 10, 25μmol/ml) for 24[thinsp]h. Then the agonist of PXR, rifampin (RFP), was added into the medium for 24h before SLC34a2 mRNA extraction. Quantitative RT-PCR was conducted to evaluate the expression of SLC34a2 mRNA. In the second stage, the expression of PXR was knocked down by an anti-PXR siRNA. After that, SFN was injected into the PXR-knockdown stable LoVo cells. The SLC34a2 expression level was compared in three groups: the PXR-knock down cells with SFN, the PXR-knock down cells without SFN and the normal cells without SFN.

Results: For the first stage, it was observed that cells treated by SFN showed low expression of SLC34a2 mRNA. Moreover, the expression of SLC34a2 mRNA decreased as concentration of SFN increased. For the second stage, the results showed that PXR-knockdown cells expressed much lower level of SLC34a2 mRNA than that of normal cells. In addition, the gene expression in PXR-knockdown cells with SFN was lower than that of PXR-knockdown cells without SFN.

Conclusions: In conclusion, our study demonstrated that SFN reduced the expression of SLC34a2 gene through its suppressive effect on the activity of PXR. In addition, the effects of SFN was concentration-dependent.

SA-PO049

A Novel, Plant-Derived Natural Polymer, VS-505, Is a Non-Absorbed, Calcium- and Aluminum-Free, Highly Effective Phosphate Binder

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Background: Inadequate control of serum phosphate in chronic kidney disease can lead to pathologies of clinical importance. Effectiveness of on-market phosphate binders is limited by safety concerns and low compliance due to high pill size/burden and gastrointestinal discomfort.

Methods: VS-505, a non-absorbed, calcium- and aluminum-free, chemically-modified, plant-derived polymer, was evaluated for its efficacy and potential side effects in various studies including chronic dosing (1 month) in 5/6 nephrectomized (NX) uremic SD rats.

Results: In vitro studies show that VS-505 has a high density (density by helium pycnometer: 1.95 versus 1.27 g/cm^3 for sevelamer) and a low swell volume when exposed to simulated gastric fluid (0.4 versus 4 cm^3/0.1g for sevelamer). VS-505 binds phosphate within a wide physiologically relevant pH range, enabling it to bind phosphate along much of the GI tract. Results from drug-drug-interaction studies show that VS-505 exhibits minimal interaction with enalapril, ciprofloxacin, digoxin and warfarin. In 5/6 NX uremic SD rats on high-phosphate diet, increasing dietary phosphate led to an increase in serum phosphate, which was prevented in rats treated with VS-505 or sevelamer (0.2 - 5% in food). Urinary phosphate increased from 67 ± 40 at Week 0 to 932 ± 108 μmol/24 hr at Week 4 ($p<0.001$) in the vehicle-treated group; VS-505 or sevelamer (0.2 - 5% in food) reduced urinary phosphate in a dose-dependent manner (to 40 ± 7 and 54 ± 9 μmol/24 hr for 5% VS-505 at Week 2 and 4, respectively). High phosphate diet also increased serum FGF-23 and parathyroid hormone in 5/6 NX rats, which was prevented by VS-505 or sevelamer. VS-505 or sevelamer increased fecal phosphate in a dose-dependent manner. More aortic calcification was observed in 5/6 NX rats treated with 5% sevelamer, while VS-505 and sevelamer did not show significant effects on cardiac parameters, fibrosis, intestine histology and intestinal sodium-dependent phosphate cotransporter gene expression.

Conclusions: These results strongly support the conclusion that VS-505, a high density plant-derived polymer, effectively controls phosphate imbalance in the uremic rats by adsorbing phosphate in the GI tract with minimal swell volume.

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SA-PO050

Serum Phosphate Intervention in Renal Replacement Therapy

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Background: Although hyperphosphatemia is statistically associated with vascular calcification and decreased life span in observational studies (CKD 3-5d), no randomised controlled trials exist to prove cause and effect. The ideal target for serum phosphate (SPhos) in dialysis patients is unknown. We wished to assess the feasibility of a future large prospective multicentre RCT of 'lower range' versus 'higher range' control of SPhos in dialysis patients, to investigate clinical end-points including mortality.

Methods: Two centre, prospective RCT feasibility study, with titration to target (8 wks) then maintenance phase (10 mths). After a binder wash-out of 3 to 5 weeks, those whose SPhos rose to >1.7mmol/L at end of washout were randomized to lower range group (LRG) or upper range group (URG). LRG was titrated to SPhos 0.8-1.4mmol/L and URG to SPhos 1.8-2.4mmol/L using only non-calcium based binders. Outcomes include physician acceptance, number of patients screened and suitable, number consenting and ITT, separation of groups by SPhos, drop-out.

Results: 768 dialysis patients screened, 263 suitable, 202 approached, 131 consented (64.9%). 104/131 patients randomized at end of washout. 60 patients have completed 20 weeks follow-up

	SPhos LRG (median,IQR)	S Phos URG (median,IQR)	P
Randomisation	2.01 (1.87-2.32) N=29	2.03 (1.8-2.29) N=31	0.8
4 week	1.53 (1.17-2.01) N=29	1.96 (1.62-2.12) N=31	*0.05
8 week	1.67 (1.23-2.03) N=26, 3 withdrew consent	2.18 (1.71-2.48) N=30, 1 transplanted	*0.02
12 week	1.69 (1.39-2.25) N=25, 1 withdrawn - severe GI effects	1.95 (1.64-2.35) N=29, 1 death	0.3
16 week	1.65 (1.16-2.06) N=25	1.96 (1.34-2.22) N=27, 2 transplanted	0.39
20 week	1.46 (1.20-2.02) N=23, 1 transplanted, 1 death	1.87 (1.52- 2.1) N=26, 1 death	0.09

Table 1: Serum phosphate levels for LRG and URG. Mann-Whitney U test (data not all normally distributed)

Conclusions: A significant difference in SPhos was achieved by end of titration and maintained to week 20. It appears possible to establish and maintain 2 cohorts with separation by SPhos of 0.3 - 0.4 mmol/L. 12 month follow-up will enable feasibility assessment and power calculation for a future large multinational study.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

SA-PO051

Dietary Barley β-Glucans Markedly Attenuate Uremia- and High Phosphate-Induced Renal and Vascular Lesions in Experimental Kidney Disease

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Background: In chronic kidney disease (CKD), systemic inflammation aggravates the renal and vascular aging causing high mortality rates. Increases in renal ADAM17 and in ADAM17 release of the pro-inflammatory cytokine TNFα contribute to CKD- and inflammation-driven Klotho loss, oxidative stress, damaged-DNA accumulation, and tissue lesions. In LPS-induced sepsis, specific silencing of monocyte ADAM17 to suppress TNFα release suffices to prevent fatal organ lesions. Since yeast β-glucans reduce LPS-driven lesions in normal rats, this study examined the efficacy of dietary barley β-glucans to attenuate uremia- and high phosphate (P)-induced renal damage and vascular calcification (VC).

Methods: Nephrectomized rats were fed a high P diet (0.9% P) with or without barley flour sufficient to provide 2mg of β-glucans/g diet, for a month. Immunohistochemistry measured tissue ADAM17, Klotho and cathepsin L (CTSL) content.

Results: Dietary β-glucans had no effect on body weight, serum P or Ca levels, but markedly reduced serum BUN ($p<0.05$), proteinuria ($p<0.001$), and the prevalence of aortic calcification (None (n=13) versus 36% (n=14)) despite marginally significant reductions in serum PTH (3107 versus 2044pg/ml; $p=0.06$). β-glucans renoprotection is partially accounted for by an effective attenuation of both, increases in renal ADAM17 ($p<0.001$) and Klotho loss ($p<0.01$), two contributors to CKD progression and VC. Furthermore, direct exposure to extracts of barley β-glucans effectively prevented LPS-induced increases in ADAM17 and oxidative stress (superoxide production) in the murine monocyte-macrophage cell line Raw264.7. In addition, in rat CKD, dietary β-glucans also attenuated renal and vascular increases in CTSL, a protease that contributes to the loss of podocyte filtration barrier causing proteinuria, and the accumulation of damaged DNA causing VC.

Conclusions: Dietary barley β-glucans effectively reduce oxidative stress, ADAM17 and CTSL expression and renal klotho loss thereby attenuating CKD- and high P-driven pro-inflammatory and pro-aging signals that cause renal and vascular lesions.

SA-PO052

SNPs Associated with Serum Phosphorus Concentration in Japanese

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Background: Previous GWAS study has identified 7 SNPs associated with serum phosphorus (P) concentration in European ancestries. Here, we investigated whether the associations can be similary found in Japanese population.

Methods: We investigated 85 young healthy Japanese (45 men and 40 women, 21 to 34 years old). Blood samples were collected in the morning fasting and measured serum level of P, intact-PTH (iPTH), FGF23, and soluble alpha-klotho (s-klotho). In addition, we also investigated the effect of genotype on phosphatemic response after high P diet (1200 mg P/

meal). Subjects were genotyped by TaqMan SNP genotyping assay for 7 SNPs as previously described (rs1697421, rs17265703, rs4074995, rs9469578, rs453639, rs947583, rs2970818).

Results: In our population, subjects with AA genotype in rs1697421 trended to be higher serum P level in men (p=0.06) than those with GG genotype. Subjects with TT genotype in rs9469578 were significantly higher serum P level than those with CT genotype (p<0.05). Conversely, no significant relationship was observed between serum P concentration and other SNPs. We also found that subjects with AC genotype in rs453639 showed significantly higher increase in serum P level after the ingestion of high P diet than those with AA genotype (p<0.05).

Conclusions: Our study suggests that rs1697421 located adjacent to the tissue-nonspecific ALP gene and rs9469578 located in inositol hexakisphosphate kinase 3 genes can be possible genetic factor to determine fasting serum P level in Japanese population as well as European ancestries. In addition, rs453639 located adjacent to ectonucleotide pyrophosphatase/ phosphodiesterase 3 gene may be associated with phosphatemic response after the ingestion of high P diet.

Funding: Government Support - Non-U.S.

SA-PO053

Time-Varying Trends in Serum Calcium over 5-Years in 135,854 Incident Dialysis Patients

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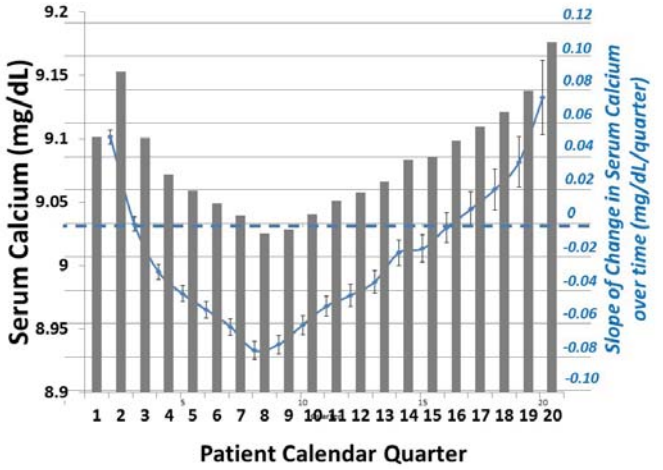
Background: Serum calcium (Ca) may change over time upon transition to renal replacement therapy given impact of dialysis therapy, dietary regimens, and medications. We hypothesized that serum Ca shows an upward tendency over time independent of secular trends.

Methods: We examined changes in serum Ca for up to 5 yrs in 135,854 incident dialysis patients (125,892 HD and 9,779 PD) who initiated therapy between 2007-2011 in one of the clinics of a large dialysis organization across the U.S.A. We examined mean serum Ca in each patient quarter (91-day increments) from the day of transition to dialysis for up to 20 quarters and calculated the rate of change in serum Ca from the first quarter to the nth quarter using time-varying mixed models.

Results: The 135,854 incident dialysis patients were 61±15 yrs old and included 43% women, 30% African Americans, 14% Hispanics, and 57% diabetics. Mean serum Ca (±SD) at the start of dialysis (Qtr 1) was 9.10±0.56 mg/dL, peaking at 9.15±0.54 mg/dL during the 2nd qtr, followed by consistent drop for the next 7 qtrs and reaching a nadir of 9.02±0.55 mg/dL in the 8th qtr. There was subsequently an upward trend reaching 9.17±0.65 mg/dL in the 16th qtr. The slope of change in rate of serum Ca change showed a similar downward trend for the first 2 yrs followed by an upward trend.



Conclusions: After an initial rise in serum Ca in the first few months of transition to dialysis, we observed a consistent drop in serum Ca for the first 2 yrs followed by a consistent rise in serum Ca from Year 3 to 5. The discovery of this complex pattern of changes in serum Ca over time and its potential impact on patient outcomes warrants additional studies.

Funding: NIDDK Support

SA-PO054

Serum Calcium Reductions among Patients on Hemodialysis Initiating Cinacalcet

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Background: Cinacalcet lowers parathyroid hormone (PTH) and serum calcium (Ca) levels as a result of its mechanism of action. The frequency and degree of, physician response to, and patient recovery from Ca reduction after initiating cinacalcet has not been described.

Methods: The cohort included all new cinacalcet users with Ca >8.4 mg/dL at cinacalcet initiation who received in-center hemodialysis at a large dialysis organization (LDO), and were enrolled in the LDO's prescription benefits service (N=13,291). Patients were categorized as those who did not experience a reduction in Ca to ≤8.4 and those in whom Ca fell to levels 8-8.4, 7.5-7.9, and <7.5 mg/dL. Baseline patient characteristics were compared between these levels. We examined the frequency of physician response and the likelihood of Ca recovery according to different levels of Ca reduction.

Results: Overall, 6763 (50.9%) patients experienced a reduction in Ca to ≤8.4 mg/dL. The majority of these (76%) had a Ca level of 8-8.4 mg/dL, while only 5% had a Ca <7.5 mg/dL. Higher baseline PTH and alkaline phosphatase were associated with lower resulting Ca levels. Among patients with Ca reductions, 48.2%-63.5% received intervention, 16.5%-28.8% discontinued cinacalcet, and the majority of patients recovered to Ca >8.4 mg/dL within 90 days. Only modest recovery differences were noted between patients who did versus did not receive intervention and patients who did versus did not discontinue cinacalcet.

	Calcium Maintained	Calcium Reduction Levels		
	> 8.4 mg	8-8.4 mg/dL	7.5-7.9 mg/dL	< 7.5 mg/dL
	n = 6528	n = 5165	n = 1253	n = 345
At cinacalcet initiation				
Age (y), mean ± SD	54.9 ± 14.6	56.0 ± 14.3	56.6 ± 14.6	56.3 ± 15.1
Caucasian, %	19.4	22.3	22.3	27.5
Diabetic etiology of ESRD, %	35.6	42.2	43.9	42.6
Vintage (m), median [p25, p75]	47 [24, 80]	44 [21, 77]	44 [22, 77]	43 [20, 74]
Weight (kg), mean ± SD	82.8 ± 23.6	83.9 ± 24.0	82.4 ± 23.2	84.6 ± 25.6
Calcium (mg/dL), mean ± SD	9.48 ± 0.54	9.19 ± 0.52	9.15 ± 0.51	9.21 ± 0.55
Phosphate (mg/dL), mean ± SD	5.56 ± 1.56	5.53 ± 1.64	5.56 ± 1.66	5.87 ± 1.88
PTH (pg/ml), median [p25, p75]	591 [389, 856]	642 [426, 903]	681 [464, 954]	767 [495, 1154]
Alkaline phosphatase (IU/L) , mean ± SD	105 ± 65	119 ± 74	129 ± 88	138 ± 111
Dialysate calcium ≤ 2mmol/L, %	37.3	37.9	38.1	44.1
Calcium acetate use, %	14.7	15.4	14.3	13
IV vitamin D use, %	91.4	91.3	91.2	87.3
Within 90 days of index low calcium				
Any intervention, %[a]	NA	48.2	53.5	63.5
Discontinued cinacalcet, %	NA	16.5	19.7	28.8
Recovered to calcium > 8.4 mg/dL, %	NA			
With intervention[a]		83.9	76.4	66.1
Without intervention[a]		82.9	69.8	62.9
Recovered to calcium > 8.4 mg/dL, %	NA			
Discontinued cinacalcet		88.3	85.4	71.1
Continued cinacalcet		82.5	70.4	62.4

a. Any of added/increased vitamin D, stopped/decreased cinacalcet, added/increased calcium binder, or increased dialysate calcium.
Abbreviations ESRD, end-stage renal disease; IV, intravenous; m, months; PTH, parathyroid hormone; y, years

Conclusions: After cinacalcet initiation, Ca reductions to ≤8.4 mg/dL were common, but Ca levels below 7.5 mg/dL were infrequent. Patients with more severe secondary hyperparathyroidism were more likely to manifest Ca reductions to ≤8.4 mg/dL following cinacalcet initiation. Recovery was common with or without directed intervention.

Funding: Pharmaceutical Company Support - Amgen Inc.

SA-PO055

Serum Magnesium (Mg) Is a Strong, Independent Predictor of Mortality in ESKD Patients and May Be Influenced By Dialysis Modality

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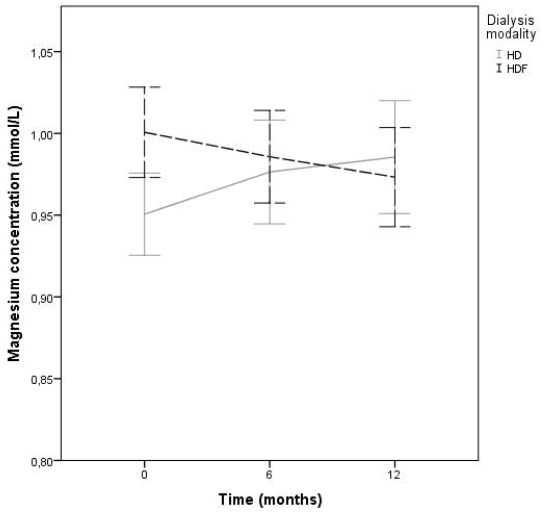
Background: Magnesium (Mg; mmol/L) may be associated with survival in hemodialysis (HD) patients. The type of dialysis treatment (HD versus hemodiafiltration [HDF]) may modulate Mg concentrations.

Methods: We performed a post-hoc analysis on the CONTRAST study (NCT 00205556), a RCT evaluating the survival effect of HDF compared to HD (mean follow-up 3.14 years). Patients were followed until death or censored if alive at the end of the study. Mg was measured at baseline and after 6 and 12 months. Cox regression model, adjusted for confounders, was used to calculate the predictive value of baseline Mg regarding mortality. A linear mixed model (LMM) was used to investigate the change of Mg over time and if this change differed between HD and HDF, using an interaction term.

Results: Mean age of 372 analyzed patients was 63.5±14.0 years and 61.6% was male. Baseline Mg was 0.98±0.18 and strongly related to mortality with an adjusted HR of 0.86 (p=0.003) for every 0.1 mmol/L increase in baseline Mg. Baseline Mg differed between HD (0.96) and HDF (1.00). Mg increased in HD and decreased in HDF over time with a significant difference in Mg slope between the 2 modalities (p=0.003 for interaction in LMM).



Conclusions: Mg is a strong, independent predictor of mortality. At M12, Mg levels were increased in HD and decreased in HDF. Whether these alterations result from differences in Mg balance deserves further study.

Funding: Pharmaceutical Company Support - Fresenius Medical Care Deutschland GmbH

SA-PO056

Proton Pump Inhibitors Increase the Risk of Hypomagnesemia in the General Population Brenda C.T Kieboom,[1] Robert Zietse,[1] Albert Hofman,[1] Bruno H. Stricker,[1] Ewout J. Hoorn.[1] *[1]Erasmus MC; [2]Erasmus MC; [3]Erasmus MC.*

Background: Proton pump inhibitors (PPIs) are one of the top selling drugs because of the wide range of indications, the high efficacy and the low risk of adverse effects. Since 2006, however, cases of severe hypomagnesemia have been reported in association with the use of PPIs. The mechanism of PPI-induced hypomagnesemia is unclear and it is unknown if this is an idiosyncratic drug reaction. Therefore, we investigated if PPI-use is associated with hypomagnesemia in the general population.

Methods: We used data from 9820 participants aged 45 and over from the population-based Rotterdam Study to construct multivariable linear and logistic regression models to analyze the association between PPI-use, serum magnesium levels, and hypomagnesemia. Hypomagnesemia was defined as ≤ 0.71mmol/L. The following potential confounders were included in the analyses: age, sex, body mass index, estimated glomerular filtration rate, diabetes mellitus, and the use of thiazide or loop diuretics.

Results: PPI-users (n = 407) had a significantly lower serum magnesium level compared to non-users (β:-0.013 [95%CI: -0.019; -0.007] P < 0.001), adjusted for confounders. This association was even stronger in loop diuretic users (β:-0.041 [95%CI: -0.068; -0.013] P= 0.004). No association was found in thiazide users. We also found no effect in H2-receptor antagonist users, making residual confounding by indication less likely. There was an increased risk of hypomagnesaemia (OR: 1.80 [95%CI: 1.03;3.15] P=0.04) in PPI-users. When stratifying by duration of PPI-use, this effect was only observed in the highest tertile (183 – 1351 days), resulting in an almost four times increased risk of hypomagnesemia (OR: 3.83 [95%CI 1.86;7.88] P<0.001).

Conclusions: PPI-use is strongly associated with lower serum magnesium levels, leading to a nearly fourfold increased risk of hypomagnesaemia in long-term users. Therefore, health care professionals should be aware of the risk of hypomagnesemia in chronic PPI-users, especially when there is concomitant use of loop diuretics.

SA-PO057

Low Serum Magnesium Is Associated with Severe Coronary Artery Calcification in Non-Dialyzed CKD Patients Yusuke Sakaguchi,[1] Takayuki Hamano,[2] Chikako Nakano,[3] Yoshitsugu Obi,[1] Isao Matsui,[1] Akihiro Shimomura,[1] Yasuo Kusunoki,[1] Daisuke Mori,[1] Hiromi Rakugi,[1] Yoshiharu Tsubakihara,[2] Yoshitaka Isaka.[1] *[1]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Japan; [2]Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Japan; [3]Internal Medicine, Kisei Hospital, Osaka, Japan.*

Background: Low serum magnesium is associated with an increased risk of cardiovascular mortality in patients with CKD. Although experimental studies have shown inhibitory effects of magnesium on calcification of vascular smooth muscle cells, clinical evidence regarding the relationship between magnesium and vascular calcification is scarce. Here we studied the relationship between serum magnesium levels and severity of coronary artery calcification (CAC) in non-dialyzed CKD patients.

Methods: Stage3-4 CKD patients with either diabetes mellitus or prior history of cardiovascular disease were enrolled. We excluded patients with atrial fibrillation or who had received percutaneous coronary intervention. Patients were classified as a lower (serum magnesium levels <2.1 mg/dL) or a higher (>=2.1 mg/dL) magnesium group. CAC scores (CACS) were calculated according to Agatston scoring using multidetector computed tomography.

Results: Seventy-nine CKD patients with mean age of 69.6 years and mean estimated glomerular filtration rate (eGFR) of 34.4 ml/min per 1.73 m^2 were examined. Median CACS was 240, and 46.8% of the patients had severe CAC (i.e., CACS >400). Those patients in the lower magnesium group were significantly younger and had a higher eGFR. After adjustment for age and eGFR, the lower magnesium group was significantly associated with severe CAC (odds ratio 3.77; the higher magnesium group as a reference, p=0.04). This association was robust after additional adjustments for traditional coronary risk factors or CKD-MBD related factors.

Conclusions: In non-dialyzed CKD patients, low serum magnesium is associated with severe CAC. Interventional studies are warranted to clarify whether magnesium supplementation can retard CAC progression.

Funding: Other NIH Support - The Kidney Foundation, Japan, Pharmaceutical Company Support - Mitsubishi Tanabe Pharma Corporation

SA-PO058

Association of Proton-Pump Inhibitor Use and Urinary Magnesium Excretion Jeffrey H. William, Kenneth J. Mukamal, John Danziger. *Dept of Medicine, Beth Israel Deaconess Medical Center, Boston, MA.*

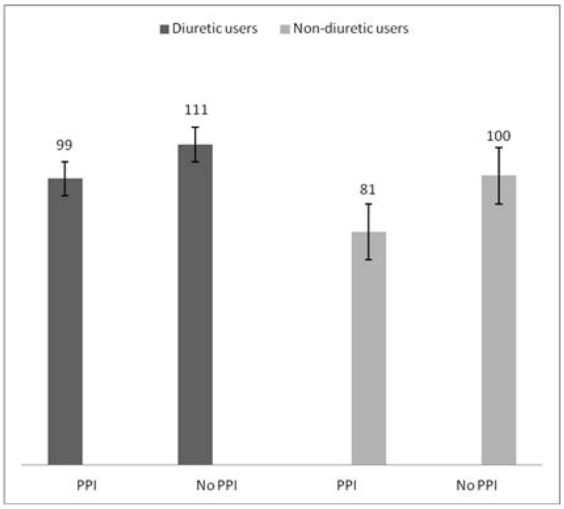
Background: Although multiple recent studies have confirmed an association between chronic proton-pump inhibitor (PPI) use and hypomagnesemia, the physiologic explanation for this association remains uncertain. To address this, we investigated the association of PPI use with urinary magnesium excretion.

Methods: We measured 24-hour urine magnesium excretion in collections performed for nephrolithiasis evaluation in 278 consecutive ambulatory patients and determined PPI use from contemporaneous medical records. A total of 18% of patients used PPIs.

Results: The mean daily urinary magnesium was 83.9±43.9 mg in PPI users, compared to 102.2±45.0 mg in non-PPI users (p=0.01). In adjusted analyses, PPI use was associated with 11.4 ± 5.7 mg/day (p=0.04) lower daily urinary magnesium excretion. Diuretic use was associated with increased magnesuria, but did not significantly modify the effect of PPI on urinary magnesium. As a control, PPI use was not associated with other urinary indicators of nutritional intake.

	Proton-pump inhibitors		*H_2-receptor antagonists*	
	Urinary Mg (β-coefficient ± SE)	**p-value**	**Urinary Mg (β-coefficient ± SE)**	**p-value**
Unadjusted model	-18.23 ± 7.04	0.01	0.55 ± 12.37	0.96
Model 1[a]	-13.38 ± 6.81	0.05	5.19± 11.81	0.66
Model 2[b]	-11.49 ± 5.71	0.04	0.50 ± 9.90	0.96

Figure 1. Urinary magnesium in PPI users, stratified by diuretic use (mg/24 hours)



Conclusions: Our findings suggest that PPI use decreases urinary magnesium excretion, reflecting decreased overall enteric absorption. When combined with diuretic-induced urinary magnesium wasting, PPI use may provide a "second hit", resulting in hypomagnesemia.

SA-PO059

The Effect of Calcium and Vitamin B6 Supplementation on Oxalate Excretion in a Gastric Bypass Model of Hyperoxaluria Benjamin Canales,[1] Christopher Anthony Monsour,[1] Saeed R. Khan,[2] Marguerite Hatch,[2] Jesse Gregory.[3] *[1]Urology, Univ of Florida, Malcom Randall VAMC, Gainesville, FL; [2]Pathology, Immunology and Laboratory Medicine, Univ of Florida, Gainesville, FL; [3]Food Science and Human Nutrition, Inst of Food and Agricultural Sciences, Univ of Florida, Gainesville, FL.*

Background: Patients who develop enteric hyperoxaluria and oxalate stones after Roux-en-Y gastric bypass (RYGB) surgery are encouraged to supplement with calcium or vitamin B6 to lower stone risk. We tested the effect of these therapies on urinary oxalate excretion in an established RYGB animal model of hyperoxaluria.

Methods: Obese male Sprague Dawley rats underwent sham (n=7) or RYGB (n=10) surgery. Animals were maintained on low oxalate/fat (LOF), normal calcium (NC) diet and completed a 2-phase crossover metabolic study. During each 2-week phase, groups were fed LOF, high calcium (HC) and LOFNC with high vitamin B6 (1600% of laboratory rodent requirement) diets with 2 week washout. Urine was collected before and after each intervention. Plasma levels of pyridoxal phosphate and associated metabolic products were determined twice before supplementation.

Results: Urinary oxalate excretion remained low and unchanged in shams on each of the 3 diets. RYGB animals on LOFHC had a 28% decrease in urine oxalate (16.9 ± 1.6 versus 12.2 ± 1.1 umol/day, p<0.001) without affecting urinary calcium. Prior to B6 supplementation, RYGB animals had similar plasma pyridoxal phosphate and biproduct levels to sham except for slightly higher plasma homocysteine (hcy) (5.25 ± 0.5 versus 3.7 ± 0.3 umol/L, p=0.001) that remained within normal range. Vitamin B6 supplementation lowered urinary oxalate excretion 15% (20.2 ± 3.0 versus 17.1 ± 1.7 umol/day; p=0.055) although not statistically significant.

Conclusions: In our obese RYGB model, increasing dietary calcium lowered urinary oxalate excretion more than B6 supplementation, and neither affected urinary calcium. RYGB animals were B6 sufficient with variations in plasma hcy likely reflective of greater protein turnover and catabolism. Both calcium and B6 appear to be reasonable therapies in hyperoxaluric RYGB patients who already maintain a low fat and low oxalate diet.

Funding: NIDDK Support, Pharmaceutical Company Support - Ethicon Endosurgery, Private Foundation Support

SA-PO060

Zn^{2+} and Zip10 Knockdown Alter In Vivo and Ex Vivo Calcium Oxalate (CaOx) Crystal Formation in Drosophila Stone Model Kari A. Strohmaier,[1,2,3] Paige Nicole Williams,[1,2] Taku Hirata,[1,2] Pablo Cabrero,[4] Julian A.T. Dow,[2,4] Eva Furrow,[2,3] Michael F. Romero.[1,2] *[1]Physiology and BME; Nephrol and Hypertens, Mayo Clinic, Rochester, MN; [2]O'Brien Urology Res Center, Mayo Clinic, MN; [3]Veterinary and Biomed Sci, U MN College Veterinary Med, St. Paul, MN; [4]Biomedical and Life Sciences, U Glasgow, Glasgow, United Kingdom.*

Background: Zn^{2+} has an implicated role in calcium oxalate (CaOx) stone formation. We have developed a model of CaOx crystallization in Drosophila (fly)[Hirata, PMID22993075]. Canine studies indicate that Slc39a10 (Zip10) may be involved. Zip10 is identifiable in human, dog and fly and all transport Zn^{2+} [Williams et al, FASEB J 28, 2014]. The crystallization effects of Zn^{2+} and Ox were assessed using Malphigian tubules (MT, renal tubule) ± Zip10-knockdown. CaOx crystal phenotypes were also analyzed after dietary alterations in flies.

Methods: Zip10 knockdown flies (dZip10 KD) were created using the GAL4-UAS system: UAS-RNAi-CG10006 x Uro-GAL4 (MT-specific). MTs were dissected from WT and dZip10 KD flies and immersed in a Na_2Ox solution. Next, other metals (Zn, Mn, Cd) were added to Ox solutions to assess the metal effect on CaOx crystal formation (*ex vivo*). These results were compared with those of feeding experiments (*in vivo*), in which Ox combined with Zn, Mn, or Cd were added to fly food, followed by MT dissection and analysis after 48 h. All crystals were visualized by DIC microscopy, photographed (Zeiss Observer), and quantified (AxioVision software).

Results: In ex vivo experiments, the average crystal volume in all solutions tested increased in Uro::dZip10 KD flies in comparison to WT flies. In feeding experiments, Ox only diets resulted in significantly larger stones in Uro::dZip10 KD flies v WT flies. However, when placed on an Ox diet plus Zn^{2+}, Uro::dZip10 KD flies formed significantly smaller crystals than WT flies. In both ex vivo and feeding experiments performed on WT flies, the presence of Zn^{2+} significantly increased mean stone volume.

Conclusions: Our results suggest that Zn^{2+} transport by Zip10 interacts with CaOx crystal formation. The changes in crystal size and dynamics could reveal important aspects of initial crystallization in oxalate nephrolithiasis. **Support**: DK92408, DK083007/DK100227.

Funding: NIDDK Support

SA-PO061

Involvement of Renin Angiotensin Aldosterone System in Calcium Oxalate Crystal Induced Activation of NADPH Oxidase and Renal Cell Injury Hidenori Tsuji,[1] Atsunori Esa,[1] Nobutaka Shimizu,[2] Masahiro Nozawa,[2] Kazuhiro Yoshimura,[2] Hirotsugu Uemura,[2] Joshi Sunil,[3] Saeed R. Khan.[3] *[1]Urology, NTT WEST Osaka Hospital, Osaka, Osaka, Japan; [2]Urology, Kinki Univ Faculty of Medicine, Osakasayama, Osaka, Japan; [3]Pathology and Laboratory Medicine, College of Medicine, Univ of Florida, Gainesville, FL.*

Background: Reactive oxygen species (ROS) are produced during the interaction between oxalate/CaOx crystals and renal epithelial cells and are responsible for the various cellular responses through the activation of NADPH oxidase (Nox). CaOx crystals also activate the renin-angiotensin aldosterone system (RAAS). Aldosterone stimulates ROS production through activation of Nox with the involvement of mineralocorticoid receptor (MR), Rac1, and mitogen-activated protein kinases (MAPK). We investigated RAAS pathways in vivo in rat model and in vitro by exposing renal eithelial cells to CaOx crystals.

Methods: Group of SD rats with hyperoxaluria was additionally given apocynin. Normal rat kidney epithelial cell line (NRK-52E) were incubated with aldosterone and CaOx crystals with or without MR inhibitor, spironolactone, a selective inhibitor of SRC family of kinases, pp2,and DPI. Rac1 expressions were measured by Western blotting analysis. Superoxide levels were determined by dihydroethidium staining.

Results: Genes encoding for angiotensinogen (AGT), Renin 1 (Ren 1), aldosterone synthase (Cyp11b), and mineralocorticoid receptor (Nr3c2) were assessed. The relative expression of these genes was upregulated in the kidneys of rats with hyperoxaluria. Treatment with apocynin negatively affected gene expressions. Both aldosterone and COM crystals activated Nox and Rac1 expression, while spironolactone inhibited Nox and Rac1 expression. Increased Rac1 expression was significantly attenuated by treatment with SRC inhibitor; PP2 and MR antagonist spironolactone.

Conclusions: The relative expressions of the genes of AGT, Ren1, Cyp11b and Nr3c2 were upregulated in the kidneys of rats with hyperoxaluria. The treatment with apocynin attenuated these gene expressions in vivo. Apparently, CaOx crystals stimulate ROS production through activation of NADPH oxidase with the involvement of MR, Rac1, and MAPK.

SA-PO062

Interleukin-1R-Deficiency Unexpectedly Accelerates Early Renal Failure in Chronic Oxalate Nephropathy Felix Knauf,[1,2] John R. Asplin,[3] Peter S. Aronson.[1] *[1]Internal Medicine/Nephrology, Yale Univ School of Medicine, New Haven, CT; [2]Internal Medicine/Nephrology, Univ Erlangen, Erlangen, Bavaria, Germany; [3]Internal Medicine/Nephrology, Litholink Corporation, Chicaco, IL.*

Background: Severe hyperoxaluria is a serious condition associated with chronic kidney disease. We have recently established a model of chronic oxalate nephropathy. Using this model we have demonstrated that progressive renal failure in oxalate nephropathy results primarily from NLRP3-mediated inflammation. Since NLRP3-activation can trigger IL-1β cytokine release, we used IL-1 receptor (IL-1R)-null mice to test the hypothesis that IL-1β drives inflammation and renal failure in chronic oxalate nephropathy.

Methods: We placed age- and gender-matched wild-type and IL-1R-null mice on an oxalate- and calcium-free diet to determine baseline BUN and creatinine. We then switched the animals to a diet high in soluble oxalate (high oxalate, no calcium). Progression of renal failure was measured longitudinally by measuring plasma creatinine and BUN via retroorbital blood collections, and animals were monitored for mortality. In addition, urine oxalate levels were measured.

Results: Renal failure was accelerated in IL-1R-null compared with wild-type mice at early time points (day 12) as indicated by increased BUN and creatinine. However, at later time points BUN and creatinine became similar between the two groups (day 25), with a subsequent trend to worsen in wild-type versus IL-1R-null mice at day 40. Wild-type mice demonstrated markedly increased mortality as compared with IL-1R-null mice, likely secondary to worsening renal function. Urinary oxalate excretion was identical between wild-type and IL-1R-null mice, excluding differences in intestinal oxalate handling to explain the observed phenotypes.

Conclusions: Taken together, these data indicate an unexpected biphasic effect of IL-1R knockout on development of oxalate nephropathy, with accelerated renal dysfunction during the first two weeks, but then protection against renal failure and death at later time points.

Funding: NIDDK Support

SA-PO063

Calcium Oxalate Monohydrate Crystals Internalized Into Renal Tubular Cells Are Degraded By Endolysosomes Visith Thongboonkerd, Sakdithep Chaiyarit, Nilubon Singhto. *Siriraj Hospital, Mahidol Univ, Bangkok, Thailand.*

Background: Interaction between calcium oxalate crystals and renal tubular cells has been recognized as one of the key mechanisms for stone formation. While crystal adhesion and internalization have been extensively investigated, subsequent phenomena (i.e. crystal degradation and dissolution) remained poorly understood.

Methods: To explore these mechanisms, we used fluorescein isothiocyanate (FITC)-labelled calcium oxalate monohydrate (COM) crystals (1,000 μg/ml of crystals/culture medium) to confirm crystal internalization into MDCK renal tubular cells after exposure to the crystals for 1 h and to trace the internalized crystals. Crystal size, intracellular and extracellular fluorescence levels were measured using a spectrofluorometer for up to 48 h after crystal internalization. Moreover, markers for early endosome (Rab5), late endosome (Rab7) and lysosome (LAMP-2) were examined by laser-scanning confocal microscopy.

Results: Fluorescence imaging and flow cytometry confirmed that FITC-labelled COM crystals were internalized into MDCK cells (14.83 ± 0.85%). The data also revealed a reduction of crystal size in a time-dependent manner. In concordance, intracellular and extracellular fluorescence levels were decreased and increased, respectively, indicating crystal degradation/dissolution inside the cells and the degraded products were eliminated extracellularly. Moreover, Rab5 and Rab7 were both up-regulated and were also associated with the up-regulated LAMP-2 to form large endolysosomes in the COM-treated cells at 16-h after crystal internalization.

Conclusions: We demonstrate herein, for the first time, thatCOM crystals could be degraded/dissolved by endolysosomes inside renal tubular cells. These findings will be helpful to better understand the crystal fate and pathogenic mechanisms of kidney stone formation.

Funding: Government Support - Non-U.S.

SA-PO064

Pathogenesis of Inflammatory Bowel Disease-Associated Hyperoxaluria

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Background: Patients with IBD have increased risk of kidney stones, with hyperoxaluria being the major risk factor. Hyperoxaluria is largely attributed to fat malabsorption, which occurs in CD but not UC; however, hyperoxaluria is also seen in UC. Therefore, additional factor(s) must be contributing to the IBD-associated hyperoxaluria (IBDAH), and that an ideal model for the IBDAH is needed.

Results: To this end, we used the SAMP1/YitFc (SAM; a model of human CD-like ileitis) mice and their AKR controls. The SAM mice were found to have significantly higher (>1.8-fold) urine oxalate compared to AKR. Intestinal oxalate absorption is predominantly passive through the paracellular pathway, while anion exchanger SLC26A6 (A6) plays a critical role in transcellular intestinal oxalate secretion. We observed significant reduction in ileal (>87%) and jejunal (>45%) A6 mRNA expression in SAM mice compared to AKR using qPCR, without a change in SLC26A1 expression. We also observed significant reduction in ileal (>90 and 86%) and colonic (>40 and 48%) occludin and ZO-1 protein expression, respectively, in SAM mice compared to AKR. We hypothesize that the observed reduction in A6 and occludin/ZO-1 expression is likely mediated by proinflammatory cytokines (PCs: e.g. TNF-α and IFN-γ, which are elevated in IBD), leading to reduced intestinal oxalate secretion and/or increased intestinal oxalate absorption, and thus contribute to the observed hyperoxaluria. Indeed, we previously found (JASN 24:277A, 2013) that PCs significantly increased ^{14}C-oxalate absorption both in vitro and ex-vivo, effects completely blocked by the peptides AMP-18 and GLP-2. We also observed that PCs significantly inhibited apical ^{14}C-oxalate uptake by Caco2 cells through mechanisms involving reduced A6 mRNA/total protein expression. Importantly, TNF-α caused significant inhibition (>55%) of mouse jejunal oxalate secretion.

Conclusions: We conclude that SAM mice have significant hyperoxaluria and that reduced A6 and occludin/ZO-1 expression, which is likely mediated by PCs, contribute to the observed hyperoxaluria by decreasing active intestinal oxalate secretion and/or increasing passive paracellular intestinal oxalate absorption.

Funding: Private Foundation Support

SA-PO065

Fat Malabsorption Contributes to Hyperoxaluria after Roux-en-Y Gastric Bypass in Rats

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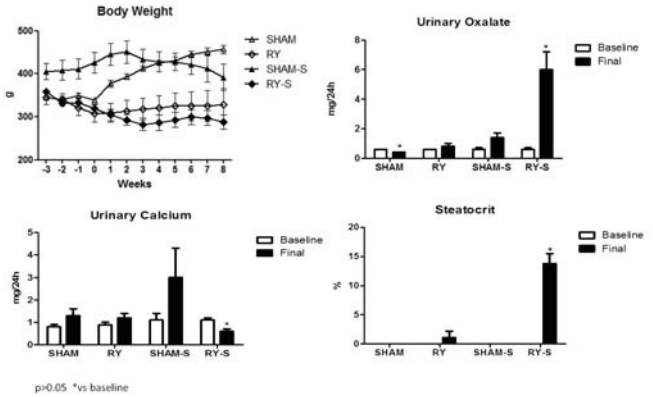
Background: It has been previously shown that increased intestinal absorption of dietary oxalate is a predisposing mechanism for enteric hyperoxaluria among Roux-en-Y (RY) gastric bypass patients (Froeder et al CJASN 7:2033, 2012). However, the contribution of fat intestinal malabsorption to hyperoxaluria after RY surgery remains controversial. Therefore, we aimed to investigate the presence of steatorrhea and its relation with urinary oxalate and other parameters in a RY gastric bypass model in rats.

Methods: Wistar rats underwent RY or Sham surgeries, and after 2 weeks, started supplementation with either 1% sodium oxalate + 18% lipids (RY-S, n=8 and Sham-S, n=5) or with a regular chow (RY, n=8 and Sham, n=8) during 8 weeks. Twenty-four urine collections (for lithogenic parameters) and stool samples (for determination of fecal fat by steatocrit) were obtained before surgery (baseline) and at the end of study (final).

Results:



At the final period, both RY groups lost weight regardless of the diet. As shown in the figures, urinary oxalate and steatocrit were markedly and significantly increased and urinary calcium significantly decreased compared to baseline only in the RY-S group. Their final urinary pH and uric acid were significantly lower, urinary sodium was significantly higher and urinary creatinine did not differ from baseline.

Conclusions: We concluded that a high fat and oxalate rich diet in this RY model, induced a significant and marked increase in urinary oxalate and fecal fat suggesting that under these dietary conditions, fat malabsorption leads to hyperoxaluria after RY gastric bypass.

SA-PO066

The Causal Role of Salt Wasting and Volume Depletion in Hypercalciuria, Nephrogenic Diabetes Insipidus and Kidney Stone Generation

Manoocher Soleimani,[1,2,3] Sharon L. Barone,[1,2,3] Jie Xu,[1,2] Marybeth Brooks,[1,2] Kamyar A. Zahedi.[1,2,3] *[1]Internal Medicine, Univ of Cincinnati, Cincinnati, OH; [2]Center on Genetics of Transport, Univ of Cincinnati, Cincinnati, OH; [3]Research Services, Veterans Affairs Medical Center, Cincinnati, OH.*

Background: The most common cause of nephrolithiasis is excess calcium in the urine, which in the context of volume depletion, high levels of oxalate, phosphate or uric acid or low levels of citrate can lead to the formation of calcium crystals in kidney tubules. The role of salt wasting and the subsequent volume depletion in the pathogenesis of hypercalciuria and its association with nephrolithiasis is not clear.

Methods: Kidney sections of NCC/pendrin double KO mice were examined and DNA microarray analysis, northern and western blot analyses, immunofluorscent microscopy and relevant enzymatic assays were performed.

Results: Salt wasting and volume depletion in NCC/pendrin dKO mice leads to the up-regulation of prostaglandin E synthase and Cyp450a12a, and increased synthesis of PGE2 and 20-HETE. The dKO animals displayed multiple defects in renal ion- or fluid-transport systems, including: the downregulation of uromodulin and NKCC2, resulting in salt wasting and impaired calcium absorption in the thick limb; aberrant regulation of Aquaporin 2, leading to nephrogenic diabetes insipidus despite severe volume depletion and elevated AVP levels, and phosphate and oxalate wasting. Collectively, these defects resulted in hypercalciuria, exacerbation of salt wasting and volume depletion, and calcium stone formation in medullary collecting ducts. Inhibition of PGE2 or 20-HETE synthesis significantly improved nephrogenic DI, hypercalciuria and salt wasting in dKO mice. in parallel, correction of volume depletion with salt replacement improved calcium wasting and inhibited PGE2 and 20-HETE synthesis.

Conclusions: Salt wasting followed by volume depletion activates PGE2 and 20-HETE, resulting in hypercalciuria, exacerbation of salt wasting and volume depletion, and nephrolithiasis. Our results suggest that patients with salt wasting and volume depletion are at increased risk of nephrolithiasis due to dysregulated production of arachidonic acid metabolites.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO067

Evidence for a Blunted Response of the 25-Hydroxyvitamin D 1-Hydroxylase to Fibroblast Growth Factor-23 in First Time Stone Formers

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Background: First time stone formers (SF) have higher serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) concentrations, reduced serum 24,25-dihydroxyvitamin D/25-hydroxyvitamin D ($24,25(OH)_2D/25(OH)D$) ratios, and increased fractional phosphate excretion (FE Pi) compared to a group of matched control subjects (C) (JASN, 24: 2013, 276A).

Methods: We correlated concentrations of full-length serum FGF-23, a peptide which reduces serum $1,25(OH)_2D$ and increases Pi excretion, in 160 SF (mean age 46 ± 14) and 201 C (age 45 ± 15) subjects with serum $1,25(OH)_2D$ and FE Pi using age and gender-adjusted multivariate analysis.

Results: The results and relationships amongst variables are shown in Table 1.

Variable	*Controls (201)*	*Stone Formers (160)*	*P value*
Serum Chemistries			
Ca, mg/dL	9.2 ± 0.7	9.4 ± 0.5	*0.01*
Pi, mg/dL	3.4 ± 0.5	3.4 ± 0.6	0.47
PTH, pg/mL	38.8 ± 17.1	42.9 ± 20.6	*0.04*
$1,25(OH)_2D$, pg/mL	38.1 ± 13.3	44.0 ± 13.8	*<0.001*
25(OH)D, ng/mL	32.1 ± 9.4	33.7 ± 11.8	0.16
$24,25(OH)_2D$, ng/mL	3.2 ± 1.5	3.1 ± 1.6	0.51
$24,25(OH)_2D/25(OH)D$	0.097 ± 0.028	0.087 ± 0.027	*0.002*
FGF-23, pg/mL	59.2 ± 22.5	61.8 ± 28.3	0.33
Urine Chemistries			
FE Pi, %	14.9 ± 5.0	16.0 ± 6.8	0.08
FE Ca, %	1.9 ± 1.0	2.0 ± 1.2	0.32
Associations between variables: input (x) and response (y) adjusted for age and gender	C Slope, p	SF Slope, p	SF vs. C slope
X=FGF-23, Y=$1,25(OH)_2D$	-0.163, *<0.001*	-0.05, 0.2	*0.04*
X=$1,25(OH)_2D$, Y=FE Pi	-0.03, 0.3	-0.10, *0.005*	0.08
X=FGF-23, Y= FE Pi	0.03, *0.01*	0.004, 0.81	0.14

Conclusions: Although there are no differences amongst FGF-23 concentrations in the C and SF groups, serum $1,25(OH)_2D$ decreases to a greater extent in association with increases in serum FGF-23 in C compared with SF, demonstrating a blunted response of the 25(OH)D 1-hydroxylase to FGF-23 in SF. Adjusting for PTH, Ca and Pi does not alter this relationship. The blunted response to circulating FGF-23 in SF could partially be responsible for the increase in serum $1,25(OH)_2D$ seen in SF.

Funding: NIDDK Support, Private Foundation Support

SA-PO068

Vitamin D3 Prophylaxis or Repletion in Children with Nephrolithiasis Does Not Increase the Risk of Hypercalciuria and Renal Stone Disease

Ismail Dursun, Rulan S. Parekh, Elizabeth A. Harvey, Jun Chuan Teh, Lisa Robinson. *Hospital Sick Children.*

Background: Hypercalciuria is the most detected metabolic abnormality in nephrolithiasis and is an important risk factor for decreased bone mass. Many pediatricians hesitate to treat these patients with vitamin D3 because of the theoretical risk of hypercalciuria and renal stone formation. Our aim is to evaluate whether vitamin D prophylaxis or repletion in children with nephrolithiasis secondary hypercalciuria adversely affects urinary calcium excretion and formation of renal stones.

Methods: We reviewed the medical records of 388 children with nephrolithiasis of which 71 children had evidence of hypercalciuria. Of these patients, 14 were treated with vitamin D3 (vitamin D group) and 57 were not (non-vitamin D group). By vitamin D3 status, baseline and follow-up measurements of serum calcium, phosphate, PTH, 25-hydroxy vitamin D, urinary calcium and creatinine were obtained. The primary end point was the urinary calcium: creatinine ratio (Uca/Ucr) and the secondary end point included new renal stones 5 years after initial presentation.

Results: 64% of children in vitamin D group and 5% of children in non-vitamin D group had various co-morbid conditions, risk factors for stone formation. The average daily vitamin D intake was 21.8±10.8 IU/kg*in vitamin D group.* We found no statistical difference for the primary and secondary end points between the groups. Median Uca/Ucr at baseline was 1.32 *mol/mol* in vitamin D group and 1.06*mol/mol* in non-vitamin D group ($p<0.05$), and on follow-up, the ratio was 0.74*mol/mol* in vitamin D group and 0.70 *mol/mol* in non-vitamin D group ($p>0.05$). There was a significant reduction in Uca/Ucr in both groups at follow up as compared to baseline ($p<0.05$). We did not find any differences between the two groups in *treatment modalities including hydration, diuretics and alkaline supplement.* The percent of the patients with hypercalciuria at last follow-up was similar in both groups ($p>0.05$).

Conclusions: Our results suggest that vitamin D3 can be given safely to children with nephrolithiasis associated with hypercalciuria, even in children with co-morbid diseases predisposing them to hypercalciuria and kidney stone formation.

SA-PO069

Hypercalciuria in Patients with Osteoporosis Is Independent of the Underlying Bone Turnover

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Background: It is well documented that patients with osteoporosis (OP) have high incidence of hypercalciuria (HC). However, the mechanism of HC in patients with OP is not well established. It is thought to be the result of high bone turnover (HBT) with excessive bone resorption. There are few reports of HC in patients with low bone turnover (LBT) OP. The purpose of this study is to compare urinary calcium (UCa) excretion in patients with HBT and LBT OP diagnosed by bone biopsy.

Methods: This is a retrospective study of patients with OP who underwent diagnostic bone biopsy and 24-hr UCa measurement at the University of Kentucky between January 2010 and December 2012. Patients were divided according to the underlying bone turnover. The following information was collected from the medical records: demographic data, history of kidney stones, serum calcium (Ca), phosphorus (P), creatinine (Cr), parathyroid hormone (PTH), 25-hydroxy vitamin D (25VD) levels as well as 24-hr UCa and creatinine (UCr).

Results: A total of 132 patients were identified. Fifty-nine had HBT and 73 LBT. Patients' age, UCa, UCa/UCr and incidence of HC are reported in Table 1 (mean ± SD; * = $p<0.05$).

	HBT	**LBT**
Age (yrs)	56 ± 15	61 ± 12*
UCa (mg/day)	236 ± 126	214 ± 107
UCa/UCr (mg/mg)	0.23 ± 0.1	0.21 ± 0.1
% HC (> 250 mg/day, male; > 200 mg/day, female)	45.5%	43.7%
% HC (> 4 mg/kg/day)	30.3%	26.4%

There was no difference in Ca, P, PTH and 25VD between the groups. Also, there was no correlation between UCa and oral Ca or VD supplementation. Presence of HC defined as >4 mg/kg/day was significantly associated with kidney stones ($n = 12$; $p=0.01$).

Conclusions: HC is very common in patients with OP regardless of underlying bone turnover. HC is not explained by oral calcium or vitamin D supplementation. Further studies are needed to understand the underlying mechanism(s), assess for possible long term effect(s), and address the potential role of the kidney in this clinical syndrome.

Funding: Other NIH Support - NCATS, UL1TR - 000117

SA-PO070

Urinary Metabolic Profile and Stone Composition among Stone Formers with and without Heart Disease

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Background: Kidney stone disease seems to be associated with increased risk of incident cardiovascular outcomes. The reasons for this association are still unknown. We analyzed differences in 24-h excretory profiles and stone composition among stone formers with and without heart disease (HD).

Methods: We used data from patients attending the UCL Centre for Nephrology's metabolic stone clinic from 1995 to 2012. We divided the sample into two groups according to the presence or absence of a history of HD (myocardial infarction, angina, coronary revascularization, or surgery for calcified heart valves) and analyzed 24-h urines and stone composition measurements for differences between groups with univariate and multivariate regression models.

Results: 1826 patients had available data for 24-h urine analysis. Among these, 108 (5.9%) had a history of HD. Those with HD were older (59±13 *versus* 46±13 years, $p<0.001$), whereas the prevalence of males was similar (73 *versus* 70%, $p=0.52$). Univariate analyses showed that patients with HD had significantly lower urinary excretions for citrate (2.4±1.5 *versus* 2.6±1.4 mmol, $p=0.04$) and magnesium (3.9±1.3 versus 4.2±1.3 mmol, $p=0.03$); adjustment for age and sex did not change these findings. A subgroup of 677 patients had available data for stone composition analysis. The proportion of calcium oxalate (57 *versus* 53%, $p=0.99$), calcium phosphate (19 *versus* 28%, $p=0.76$), and uric acid (16 *versus* 12%, $p=0.64$) in analyzed stones was similar for those with and without HD.

Conclusions: Stone formers with HD have lower urinary excretions for citrate and magnesium. Since both citrate and magnesium have been implicated in the pathogenesis of arterial plaque formation, as well as being protective factors in nephrolithiasis, this may indicate a common mechanism underlying the processes of kidney stone formation and of arterial calcification and plaque formation.

SA-PO071

Use of Lipid-Lowering Drugs and the Risk of Incident Kidney Stones

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Background: A history of kidney stones has been associated with an increased risk of cardiovascular disease; whether this association is due to common pathophysiological mechanisms is not known. Abnormal serum lipids, a known predictor of cardiovascular disease, might be related to kidney stone disease. A cross-sectional study found the use of lipid-lowering drugs (LLD) was associated with a decreased prevalence of kidney stones. However, this association has never been examined in prospective longitudinal studies.

Methods: We analyzed data from three large prospective cohorts, the Health Professionals Follow-up Study (HPFS) and Nurses' Health Studies (NHS) I and II. Information on use of LLD and other covariates was collected through biennial questionnaires. Incidence of kidney stones was confirmed through further validated questionnaires. The risk of kidney stones in participants according to use of LLD was assessed using Cox proportional hazards regression adjusted for age, BMI, race, geographic region, diabetes, high blood pressure, use of thiazides, calcium supplements and intakes of sodium, calcium, potassium, magnesium, caffeine, animal protein, vitamin C, fructose, oxalate, phytate, alcohol and total fluid.

Results: The analysis included 263,712 participants, mean age 54 (HPFS), 53 (NHS I) and 37 (NHS II) years. After up to 20 years of follow-up, 5,987 incident cases of kidney stones occurred. After multivariate adjustment, there was no statistically significant associations for risk of kidney stones among participants who used LLD compared with those who did not (HPFS: HR 0.91, 95% CI 0.74, 1.10, p=0.32; NHS I: HR 1.19, 95% CI 1.00, 1.41, p=0.05; NHS II: HR 0.98, 95% CI 0.70, 1.36, p=0.89). Analyses restricted to statins did not change the findings.

Conclusions: In three large prospective cohorts, there was no association between the use of LLD and incidence of symptomatic kidney stones.

SA-PO072

Physical Activity, Energy Intake, and the Risk of Incident Kidney Stones

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Background: It has been reported that among postmenopausal women, physical activity was inversely associated and energy intake was positively associated with risk of kidney stones. Whether these associations can be reproduced in other populations after accounting for lifestyle and dietary factors is not known.

Methods: We analyzed data from three large prospective cohorts, the Health Professionals Follow-up Study (HPFS) and Nurses' Health Studies (NHS) I and II. Information on physical activity, dietary intake, and incidence of kidney stones was collected through validated biennial questionnaires. The relative risk of incident stones among participants within different categories of physical activity (<5, 5-9.9, 10-19.9, 20-29.9, ≥30 METs/week) and energy intake (<1,800, 1,800-2,000, 2,000-2,200, 2,200-2500 and >2,500 Kcal/day) was assessed with Cox proportion hazards regression adjusted for age, BMI, race, comorbidities, medical treatments, calcium supplement use, fluid and nutrient intakes.

Results: The analysis included 215,133 participants, mean age 54 (HPFS), 53 (NHS I) and 37 (NHS II) years. After up to 20 years of follow-up, 5,355 incident cases of kidney stones occurred. In age-adjusted analyses, higher levels of physical activity were associated with a lower risk of incident kidney stones in women (NHS I and II) but not men. However, after multivariate adjustment, there was no significant association between physical activity and risk of kidney stones (HPFS: HR for highest versus lowest category 1.00, 95% CI 0.87, 1.14, p for trend=0.94; NHS I: HR 1.01, 95% CI 0.85, 1.19, p=0.88; NHS II: HR 1.03, 95% CI 0.90, 1.18, p=0.64). Analyses restricted to postmenopausal women did not change the results. Energy intake was not associated with stone risk.

Conclusions: In three large prospective cohorts, there were no independent associations between physical activity, total energy intake, and incidence of symptomatic kidney stones.

SA-PO073

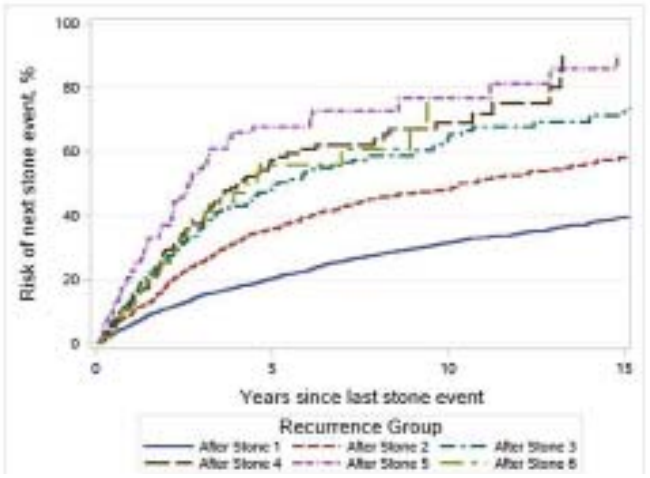
Risk of Clinical Care for a Kidney Stone Event Increases with Number of prior Stone Events: A Rochester Epidemiology Project Study

Prince Singh,[1] Eric J. Bergstralh,[1] John Knoedler,[1] Amy E. Krambeck,[1] William E. Haley,[2] John C. Lieske,[1] Andrew D. Rule.[1] *[1]Nephrology and Hypertension, Mayo Clinic; [2]Nephrology and Hypertension, Mayo Clinic, FL.*

Background: The risk of symptomatic events in kidney stone formers (SF) as predicted by the number of prior stone events is unknown. The long-term benefit of prevention interventions in SF with increasing number of past events is unclear.

Methods: We identified, validated and abstracted the comprehensive medical record of symptomatic adult first-time kidney SF in Olmsted County, Minnesota, from 1984 to 2012. We identified the number of stone events, use of stone prevention medications(potassium citrate, thiazides, allopurinol or other) and stone prevention clinic referral (included diet interventions). Cox models estimated impact of interventions at each event on risk of subsequent events.

Results: There were 3057, 845, 334, 162, 87, and 26 stone formers with at least 1 to 6 stone events. The figure shows the risk of future events increased with number of past events until the 6th stone. Stone prevention clinic referral was 15%, 39%, 57%, 69%, 81%, and 88% and medication use was 10%, 24%, 30%, 36% and 49% after stone events 1 to 6. After adjusting for number of past stone events, stone clinic referral (HR=1.4, p<0.001) but not medication use (HR=1.0, p=0.5) associated with an increased risk of future events.



Conclusions: Risk of future symptomatic events leading to clinical care progressively increases with the number of past events;this increase is limited, possibly by SF learning to manage stone events on their own. For the same number of past stone events, risk of future events is not lower among SF who have interventions compared to those who do not have interventions. Higher-risk patients selectively receiving interventions may explain this finding.

Funding: NIDDK Support, Other NIH Support - Rochester Epidemiology Project (AG034676)

SA-PO074

Comparison of Chronic Kidney Disease Markers between Incident Symptomatic Stone Formers and Controls

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Background: Retrospective studies have demonstrated an increased risk of CKD in kidney stone formers, but prospective studies are lacking.

Methods: Continuous surveillance of clinical databases at the Mayo Clinic in Rochester, MN and Jacksonville, FL were used to identify and recruit local incident symptomatic adult stone formers (SF). Controls without stones (age and sex frequency matched) were recruited through local mailings and community flyers. Participants were examined and administered a survey on co-morbidities. Blood and 24-hour urine were collected at this 1st visit and at a 2nd visit at least 90 days later. The kidney function of SF was compared to controls with and without adjusting for age, sex, hypertension, diabetes, and body mass index. CKD was defined by an estimated GFR-Cr (CKD-EPI)< 60ml/min/1.73m^2 or urine albumin > 30 mg/day at both visits.

Results: There were 374 SF (12% from FL, 54% men, 98% white, mean age 47 y) and 449 controls(13% from FL, 49% men,97% white, mean age 47y) at visit 1 and 307 SF and 402 controls at visit 2. Visit 1 was a median 81 days and visit 2 was a median 178 days after the incident stone event. SF had more obesity and a higher cystatin C, urine protein, and urine albumin at both visits compared to controls.

	SF	Control	p-value
Hypertension	25%	22%	0.29
Diabetes	11%	9%	0.38
Body mass index, kg/m^2	29.5	27.6	<0.001
First Visit			
Serum creatinine, mg/dl	0.88	0.84	0.14
Cystatin C, mg/L	0.92	0.87	<0.001
Urine Protein, mg	35	24	<0.001
Urine Alb>5 mg	28.5%	19%	0.003
Second Visit			
Serum creatinine, mg/dl	0.85	0.84	0.45
Cystatin C, mg/L	0.90	0.86	0.001
Urine Protein, mg	32	25	<0.001
Urine Alb>5 mg	31%	21%	0.006

In multivariable-adjusted analysis at visit 1, SF had a 43% higher urine albumin (p=0.002), 127% higher urine protein (p<0.001), 0.02 mg/dl higher serum creatinine (p=0.15), and a 0.04 mg/L higher cystatin C (p=0.01) than controls. Only 1.5% of SF and 1.9% of controls had CKD (p=0.39).

Conclusions: Even early after their 1st kidney stone event, stone formers had more altered CKD markers (but not overt CKD) compared to controls that was not explained by conventional risk factors.

Funding: NIDDK Support, Other NIH Support - Rochester Epidemiology Project (AG034676)

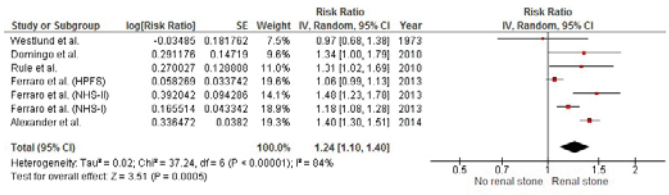
SA-PO075

The Risk of Coronary Heart Disease in Patients with Kidney Stones: A Meta-Analysis Wisit Cheungpasitporn,[1] Charat Thongprayoon,[2] Michael A. Mao,[1] Stephen B. Erickson.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Anesthesiology, Mayo Clinic, Rochester, MN.*

Background: The reported risk of coronary heart disease (CHD) in patients with a history of kidney stones is conflicting. The objective of this meta-analysis was to evaluate the association between the history of kidney stones and CHD risk.

Methods: A literature search was performed using MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews until April 04, 2014. Studies that reported odd ratios or hazard ratios comparing the risk of CHD in patients with kidney stones versus those without history of kidney stones were included. Pooled risk ratios (RR) and 95% confidence interval (CI) were calculated using a random-effect, generic inverse variance method.

Results: Seven study populations from four cohort studies and one cross-sectional study were identified and included in the data analysis. The pooled risk ratio (RR) of CHD in patients with kidney stones was 1.24 (95% CI, 1.10 - 1.40). The result remained significant (RR, 1.23 [95% CI, 1.08-1.41]) when the sensitivity analysis was restricted to only cohort studies. History of kidney stones was associated with increased CHD risk in females (RR, 1.43 [95% CI, 1.12 - 1.82]), whereas the association was not significant in males (RR, 1.14 [95% CI, 0.94-1.38]).



Conclusions: Our study demonstrates a statistically significant increased risk of CHD in female patients with prior kidney stones. This finding suggests that a history of kidney stones is a risk factor for CHD in females and may impact clinical management.

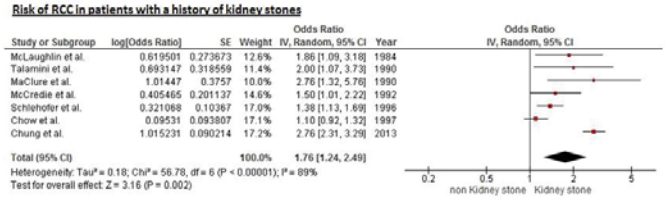
SA-PO076

The Risk of Renal Cell Carcinoma and Transitional Cell Carcinoma in Patients with Kidney Stones: A Meta-Analysis Wisit Cheungpasitporn,[1] Charat Thongprayoon,[1] Patompong Ungprasert,[2] Oisin O Corragain,[3] Stephen B. Erickson.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Internal Medicine, Bassett Medical Center, Cooperstown, NY; [3]Univ College Cork, Cork, Ireland.*

Background: The risk of renal cell carcinoma (RCC) and transitional cell carcinoma (TCC)in patients with a history of kidney stones is controversial. The objective of this meta-analysis was to evaluate the association between kidney stones and the two most common kidney cancers, RCC and TCC.

Methods: A literature search was performed using MEDLINE, EMBASE, and Cochrane Database of Systematic Reviews from inception until May 06, 2014. Studies that reported odd ratios or hazard ratios comparing the risk of RCC and TCC in patients with the history of kidney stones versus those without the history of kidney stones were included. Pooled risk ratios (RR) and 95% confidence interval (CI) were calculated using a random-effect, generic inverse variance method.

Results: Seven studies were included in our analysis to assess the association between a history of kidney stones and RCC. The pooled risk ratio of RCC in patients with kidney stones was 1.76 (95% CI, 1.24 - 2.49). The subgroup analysis found that the history of kidney stones was associated with increased RCC risk only in males (RR, 1.41 [95% CI, 1.11 - 1.80]), but not in females (RR, 1.13 [95% CI, 0.86-1.49]). Five studies were selected to assess the association between a history of kidney stones and TCC. The pooled risk ratio (RR) of TCC in patients with kidney stones was 2.14 (95% CI, 1.35 – 3.40).



Conclusions: Our study demonstrates a statistically significant increased risk of RCC and TCC in patients with prior kidney stones. However, the increased risk of RCC was noted only in male patients. This finding suggests that a history of kidney stones is associated with kidney cancer and may impact clinical management and cancer surveillance.

SA-PO077

Impact of Being Overweight (OW) on Urinary Metabolic Risk Factors for Kidney Stone Formation Linda Shavit,[1,2] Nikhil Johri,[1] William G. Robertson,[1] Stephen B. Walsh,[1] Pietro Manuel Ferraro,[3] Shabbir H. Moochhala,[1] Robert J. Unwin.[1] *[1]Centre for Nephrology, Royal Free Campus Medical School, Univ College Hospital, London, United Kingdom; [2]Adult Nephrology Unit, Shaare Zedek Medical Center, Jerusalem, Israel; [3]Div of Nephrology, Catholic Univ of the Sacred Heart, Italy.*

Background: Obese and overweight (OW) patients share most of the same risk factors for cardiovascular morbidity, while the impact of being OW, rather than obese, on urinary metabolic parameters of kidney stone formers (KSF) is less well known. In this study urinary metabolic parameters, stone composition, and probability of stone formation (Psf) in OW KSF were compared with normal weight (NW) and obese KSF.

Methods: Patients with a recorded BMI and confirmed diagnosis of kidney stone disease were divided into three categories – BMI ≤ 25.0 kg/m2 (NW group), BMI of 25 to 30 kg/m2 (OW group), and BMI >30.0 kg/m2 (obese group). 24 hour urinary volume (U.Vol), pH (U.pH), calcium (U.Ca), oxalate (U.Ox), citrate (U.Cit), uric acid (U.UA), magnesium (U.Mg), sodium (U.Na) and potassium (U.K) excretions along with stone composition and Psf were then compared among the groups.

Results: . A total of 2230 patients were studied, of whom 852 (38.2%) were NW, 902 (40.4%) were OW, and 476 (21.3%) were obese. OW and obese KSF were older, demonstrated increased female predominance and higher prevalence of diabetes, hypertension and gout. There were no statistically significant differences in U.Vol, U.Ca, U.K and U.Mg. However, significantly higher levels of U.Ox (p< 0.001 for trend), U.Cit (p= 0.005 for trend), U.UA (p<0.001 for trend), U.Na (p<0.001 for trend) and lower U.pH (p< 0.001 for trend) were demonstrated in OW and obese KSF by both crude and age- and sex-adjusted models. Stone composition data (N=677) showed significantly higher incidence of uric acid stones in OW KSF.

Conclusions: Similar to obese KSF, OW KSF demonstrate clear alterations in metabolic urinary profiles that are associated with increased overall risk of stone formation. This greater risk is primarily due to raised U.UA, U.Ox, U.Na and lower U.pH. Whether modest weight loss in OW KSF will have a favorable impact on their metabolic urinary profiles is worth exploring.

SA-PO078

Vascular Calcifications and Bone Mineral Density in Recurrent Kidney Stone Formers (KSF) Linda Shavit,[1,2] Daniela Girfoglio,[1] Vivek Vijay,[1] Pietro Manuel Ferraro,[3] Shabbir H. Moochhala,[1] Robert J. Unwin.[1] *[1]Centre for Nephrology, Royal Free Campus, Univ College London Medical School, London, United Kingdom; [2]Adult Nephrology Unit, Shaare Zedek Medical Center, Jerusalem, Israel; [3]Div of Nephrology, Catholic Univ of the Sacred Heart, Rome, Italy.*

Background: Recent epidemiological studies have provided evidence for an association between nephrolithiasis and cardiovascular disease (CVD), though the underlying mechanism is unclear. Vascular calcification (VC) is a strong predictor of CVD; the hypothesis that VC is more prominent in KSF has been investigated in the current study. The aims of this study were to: 1) determine whether recurrent KSF have more VC and osteoporosis compared with control; 2) evaluate an association between hypercalciuria and VC/osteoporosis.

Methods: We investigated 111 subjects, of whom 57 KSF and 54 age- and sex-matched controls. Abdominal aortic calcification (AAC) and vertebral body mineral density (CT BMD) were assessed using existing CT imaging. Manual calcium scoring was undertaken to calculate total aortic calcium load. The prevalence, severity and associations of AAC and CT BMD between KSF and controls were then compared.

Results: Mean age was 46.7 ± 6.4 years in KSF and 46.9 ± 5.6 in controls, 57 % of the patients in both groups were male. The prevalence of diabetes, HTN and dyslipidemia was higher in KSF, and smoking history was more prevalent in controls. AAC was found in both groups, the incidence was slightly higher in KSF (39% versus 35%). However, the severity of AAC was significantly higher in KSF by both univariate and multivariate models adjusted for age, sex, HTN, diabetes and smoking status (p-value < 0.001). Similarly, KSF had significantly lower CT BMD by both crude and adjusted analyses (p-value < 0.001). Among stone formers, the association between AAC score and hypercalciuria was not statistically significant (p-value 0.86).

Conclusions: VC might be an underlying pathogenic mechanism explaining reported associations between nephrolithiasis and CVD. Moreover, bone demineralization is much more prominent in KSF, providing preliminary evidence of possible common underlying pathways leading to increased extraosseous calcium deposition and osteoporosis.

SA-PO079

Effect of Potassium Citrate on Urine Supersaturation and Stone Formation in Genetic Hypercalciuric Stone-Forming Rats Kevin K. Frick,[1] John R. Asplin,[2] Nancy S. Krieger,[1] Ignacio Granja,[2] Christopher D. Culbertson,[1] David A. Bushinsky.[1] *[1]Medicine, Univ of Rochester, Rochester, NY; [2]Litholink, LabCorp, Chicago, IL.*

Background: Potassium citrate (K-cit) is commonly used to decrease recurrent stone formation in patients with calcium (Ca) nephrolithiasis. The increased alkali load should decrease urine (u) Ca excretion through both a direct reduction of bone resorption and an increase in renal tubular Ca reabsorption. The increased dietary citrate should bind both intestinal Ca and u Ca but will increase u pH. Citrate binding of intestinal Ca should allow greater absorption and u excretion of both phosphate (P) and oxalate (Ox). Thus, the effect of K-cit on u supersaturation (SS) with respect to CaOx and CaP and stone formation is complex and difficult to predict. To study the effects of K-cit on u SS and stone formation we utilized the inbred Genetic Hypercalciuric Stone-forming (GHS) rats which form CaP stones.

Methods: GHS rats were fed a fixed amount of a normal Ca (1.2% Ca) diet supplemented with either K-cit or KCl (each 4 meq/d) for 18 wks. Urine was collected at 6, 12 and 18 wks and mean values determined. At study end stone formation was visualized by X-ray.

Results: Urine citrate was higher with K-cit as compared to KCl (K-cit: 182±3.4 mg/d versus KCl: 110±2.7, p<0.001) and u Ca was lower (K-cit: 16.1±0.5 mg/d versus KCl: 18.6±0.3, p<0.001). Urine P (K-cit: 0.029±0.001 g/d versus KCl: 0.020±0.001, p<0.001), Ox (K-cit: 1.11±0.04 mg/d versus KCl: 0.62±0.02, p<0.001) and pH (K-cit: 7.51±0.04 versus KCl: 6.71±0.05, p<0.001) were all increased with K-cit. In rats fed K-cit, CaOx SS was higher (K-cit: 9.8±0.5 versus KCl: 5.5±0.2, p<0.001), as was CaP SS (K-cit: 8.5±0.6 versus KCl: 4.9±0.2, p<0.001); however, uric acid SS was lower with K-cit (K-cit: 0.003±0.0005 versus KCl: 0.022±0.003, p<0.001). Stone formation was present in all rats in both groups.

Conclusions: Thus K-cit effectively raises u citrate and lowers u Ca; however, the increases in u pH, Ox and P lead to an increase in u SS with respect to CaOx and CaP. K-cit did not alter stone formation. Provision of K-cit induces complex changes in u chemistries and resultant u SS which may not be beneficial in preventing CaP stone formation.

Funding: NIDDK Support

SA-PO080

Activation of Nucleotide Binding Oligomerization Domain-Like Receptor Family, Pyrin Domain Containing -3 Inflammasomes in Hyperoxaluric Rats, Is It Caused By Oxalate or Calcium Oxalate Crystals? Sunil Joshi, Wei Wang, Saeed R. Khan. *Dept of Pathology, College of Medicine, Univ of Florida, Gainesville, FL.*

Background: Hyperoxaluria is a major risk factor for calcium oxalate (CaOx) crystal deposition in the kidneys, renal injury and inflammation. Recent studies have shown activation of innate immunity through nucleotide binding oligomerization domain-like receptor family, pyrin domain containing -3 (NLRP3) inflammasomes in hyperoxaluric mice and rats. What provokes the activation, oxalate or CaOx crystals is unknown.

Methods: Male rats were divided into two groups. Rats in one group were fed normal diet and the other group diet supplemented with hydroxy proline (HP). After 28 days rats were euthanized, kidneys explanted and total RNA extracted for micro array analysis using Illumina bead array reader™. Gene ontology (GO) and KEGG pathway analyses was performed. Micro array data were verified by quantitative RT PCR and immunohistochemical staining for inflammasome associated gene products.

Results: All rats became hyperoxaluric and at day 28, 3 of 6 rats had deposition of CaOx crystals in their kidneys.Gene analysis showed that in rats with renal CaOx crystal deposits, 20 and 33 pathways were up regulated in cortex and medulla, respectively and in rats with hyperoxaluria but no renal CaOx crystals, 17 and 26 pathways were up regulated. The expression of genes involved in activation of NLRP-3 inflammasome, genes encoding for NLRP-3, TXNIP (thioredoxin interacting protein), Caspase-1, PYCARD (ASC: apoptosis –associated spec-like protein containing a CARD), IL-1β, and IL-18 were up regulated in the HP treated hyperoxaluric rats that developed renal CaOx crystal deposits. Same genes were however, down regulated in HP treated rats that developed hyperoxaluria only. Results were confirmed by RT-PCR and IHC analyses.

Conclusions: Results highlight the prominent role of CaOx crystals in activation of NLRP-3 inflammasome in hyperoxaluric kidneys. As we have suggested elsewhere, retained CaOx crystals are more injurious than hyperoxaluria alone.

Funding: Other NIH Support - Supported by National Institute of Health grant # RO1-DK078602 and the University of Florida Center for the Study of Lithiasis

SA-PO081

Comparison of Morning versus Evening Administration of Chlorthalidone (CTD) for Idiopathic Hypercalciuria Hasan Fattah,[1] Ignacio Granja,[2] Daniel A. Wollin,[1] John R. Asplin,[2] David S. Goldfarb.[1] *[1]Nephrology, New York Harbor VAMC and NYU Langone MC, New York, NY; [2]Litholink Corp, Chicago, IL.*

Background: Hypercalcuria is common in calcium stone formers. As the result of higher post-dinner urine calcium excretion (UCa) and lower urine volume (V) while sleeping, higher calcium oxalate (CaOx) supersaturation (SS) occurs in the evening. Thiazides (TZ) decrease UCa and prevent recurrent calcium stones. This prospective crossover study examined if TZ is more effective in lowering CaOx and calcium phosphate (CaP) SS if taken at night as compared to daytime.

Methods: We enrolled 5 men with history of calcium kidney stones and hypercalciuria (>200mg/24h). Participants ate self-selected diets and kept food diaries so that the diet 1 day before and on the day of the urine collections could be replicated for each of 3 experimental periods: 1) no drug; 2) 25 mg CTD in AM and 3) 25 mg CTD after dinner (PM). Two 12h urine collections were done from awakening to 12 hours later ("daytime") and then from 12 hours to awakening the next day ("nighttime"). Urine was sent to Litholink (Chicago, IL) for analysis of urinary analytes. Results were compared by non-parametric Wilcoxon paired analysis.

Results: Comparing TZ to control, 24h V was unchanged (2.9 L at baseline, 2.8L with CTD AM, 2.9L CTD PM). CTD reduced both 24h UCa and SS CaOx significantly when given in AM (UCa 136 mg, SS CaOx 10) and PM (UCa 143 mg, SS CaOx 10.2) compared with no drug (UCa 280 mg, SS CaOX 15.7); P=0.04. Nighttime 12h UCa, SS CaOx and SS CaP did not differ when CTD was administered in AM (69 mg, 4.4, 0.9) or PM (65.9 mg, 5.6, 1.4; P=NS). Daytime values were also not affected by time of taking CTD.

Conclusions: TZ significantly reduced 24h UCa excretion in men with hypercalciuria. V and UCa were the same in the control period during daytime and nighttime. TZ was equally effective in reducing both daytime and nighttime UCa excretion whether given in AM or PM. We conclude that the time of day of administration of CTD does not affect its effectiveness. CTD is a relatively long acting TZ; a different result might be seen with varying time of administration of hydrochlorothiazide, a drug with a shorter duration of action.

Funding: Veterans Affairs Support

SA-PO082

Inhibitory Effect of Febuxostat, a Non-Purine Xanthine Oxidase Inhibitor, on Renal Urate Transporters Promsuk Jutabha, Naohiko Anzai. *Pharmacology and Toxicology, Dokkyo Medical Univ, Mibu, Tochigi, Japan.*

Background: Febuxostat, a non-purine xanthine oxidase (XO) inhibitor, is an effective drug superior to allopurinol for treatment of hyperuricemia, a risk factor of hypertension and cardiovascular diseases. In addition to its XO inhibitory effect, we aim to test its action on renal urate transporters URAT1, URATv1 and OAT10.

Methods: *In vitro* XO activity assay was tested among 3 drugs; febuxostat, allopurinol and benzbromarone using commercial fluorometric determination kit. The [^{14}C]urate transport was determined in *Xenopus* oocytes expressing URAT1, URATv1 and OAT10 in the presence or absence of the test drugs, IC_{50} value of febuxostat against urate transport was also determined.

Results: Febuxostat had strong inhibitory effect on *in vitro* XO activity with an IC_{50} value of 0.22 ± 0.11 nM compare to 9.20 ± 1.14 μM of allopurinol. The urate transport via an apical urate exchanger URAT1 and a basolateral voltage-driven urate transporter URATv1 were inhibited by febuxostat with IC_{50} values of 13.41 ± 1.29 μM and 20.36 ± 1.64 μM, respectively. Febuxostat did not show any significant inhibition on urate transport by organic anion transporter 10 (OAT10).

Conclusions: The efficacy of febuxostat in reducing serum urate level might be due to its inhibition both on xanthine oxidase which subsequently reduced uric acid production and on renal urate reabsorption via URAT1 and URATv1 transporters.

Funding: Government Support - Non-U.S.

SA-PO083

Transcriptional Heterogeneity of the SLC2A9 Gene Encoding the GLUT9 Urate Transporter David B. Mount, Asim Mandal. *Renal Divs, Brigham and Women's Hospital and VABHS, Boston, MA.*

Background: There is a strong genetic basis for hyperuricemia, with almost 30 associated genes. Variation in SLC2A9, which encodes the urate transporter GLUT9, remains the major single genetic determinant; however, the causal variant(s) within SLC2A9 have not been identified. Two N-terminal isoforms, GLUT9a and GLUT9b, are generated by alternative 5' ends. We have characterized the 5' end of SLC2A9 to further understanding of how variation in this gene generates hyperuricemia.

Methods: 5' UTR exons, alternative splicing, and transcriptional start sites were identified by 5'-RACE PCR and RT-PCR. GLUT9 cDNA expression constructs were characterized by ^{14}C-urate uptakes in Xenopus oocytes. Promoter analysis utilized the dual luciferase system.

Results: Seven novel 5' UTR exons were identified, with substantial alternative splicing. Alternative splicing that deletes coding exon 3 was also identified, in both isoforms. Exon 3 encodes most of transmembrane domain 1 (TM1) and part of the first, glycosylated extracellular loop. GLUT9b constructs with deletion of exon 3 were functional (GLUT9b-delta3), generating urate uptakes that were 10-fold higher than that of water-injected control Xenopus oocytes. Western blotting indicated that GLUT9b-delta3 protein is not glycosylated, presumably due to altered topology of the first extracellular, glycosylated loop. A single GLUT9a transcriptional start site was identified, flanking exon 1a. Luciferase constructs for this promoter were highly active in multiple cell lines (66-fold > vector controls), with a minimal promoter construct of 200 bp. Four GLUT9b start sites were identified, including one ~35 kb 5' of exon 1b flanking a novel 5' UTR exon. Only 2 out of 4 GLUT9b promoters were functional, with substantially lower activity (6-fold > controls) than the GLUT9a promoter.

Conclusions: We have identified substantial transcriptional heterogeneity in SLC2A9, identifying seven new 5' UTR exons and five transcriptional initiation sites. Alternative splicing that removes TM1 generates functional GLUT9 urate transporters. The single GLUT9a promoter is strongly active in multiple cell lines, whereas only 2 out of 4 GLUT9b promoters are weakly active.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO084

Fructose Reabsorption in the Proximal Tubule Is Enhanced by a Moderate Fructose Diet Pablo D. Cabral, Agustin Gonzalez-Vicente, Jeffrey L. Garvin. *Physiology and Biophysics, Case Western Reserve Univ, Cleveland, OH.*

Background: Fructose consumption, and by inference plasma fructose, is implicated in the epidemic of diabetes, obesity and hypertension. The factors controlling plasma fructose concentrations during enhanced fructose consumption are not understood. The proximal tubule (PT) expresses several potential fructose transporters including Na-glucose linked cotransporter 5 (SGLT5) and glucose transporters (GLUT) 2 and 5. Indirect data suggest that fructose is reabsorbed in the PT; however, direct evidence of fructose reabsorption by this nephron segment has not been shown. We hypothesized that fructose is reabsorbed by isolated PTs and its reabsorption is enhanced by consumption of a moderate fructose diet.

Methods: We studied the localization of SGLT5, GLUT2 and 5 by immunofluorescence in PTs. We measured SGLT5 protein expression in PT lysates by Western blot and we directly assessed luminal fructose reabsorption using a microfluorometric fructose assay in isolated, perfused PT. Sprague Dawley rats that were fed either a control standard diet or a 20% fructose diet for one week.

Results: We found expression of SGLT5 in the luminal and GLUT2 and 5 in the basolateral membrane. PTs isolated from rats that were fed a normal diet reabsorbed fructose at a mean rate of 12.8±2.5 pmol/min/mm (n=5). In the presence of Phlorizin (100 uM), a non-selective SGLT inhibitor, fructose reabsorption decreased to 3.4 ± 1.7 pmol/min/mm ($p<0.03$;n=3). In PTs isolated from rats that were fed a 20% fructose diet, fructose reabsorption was 19.3 ± 0.5 pmol/min/mm, significantly greater than controls ($p<0.03$;n=5). Fluid reabsorption was not significantly different among all groups. SGLT5 expression increased by 59±11% in PTs from rats fed a 20% fructose diet compared to rats fed a control diet ($p<0.02$;n=6).

Conclusions: Fructose is reabsorbed by the PT. Fructose possibly enters the cell via luminal SGLT5 and exits the cell via GLUT2/5. A 20% fructose diet enhances reabsorption in part by increasing SGLT5. Fructose-enhanced fructose reabsorption likely contributes to elevated plasma fructose when dietary fructose increases.

Funding: Other NIH Support - HL28982-HL70985-HL90550, Private Foundation Support

SA-PO085

Rehydration with Fructose-Containing Beverages Produce Renal Oxidative Stress and Injury in both Passive and Active Dehydration States L. Gabriela Sanchez-Lozada,[1] Fernando E. García-Arroyo,[1] Magdalena Cristobal,[1] Edilia Tapia,[1] Abraham Said Arellano-Buendia,[1] Horacio Osorio,[1] Virgilia Soto,[2] Magdalena Madero,[1] Carlos Alberto Roncal-Jimenez,[3] Richard J. Johnson.[3] *[1]Lab. Renal Physiopathology and Nephrology, INC Ignacio Chavez, Mexico, D.F., Mexico; [2]Pathology, INC Ignacio Chavez, Mexico, D.F., Mexico; [3]Renal Diseases, U. of Colorado, Aurora, CO.*

Background: Elevated vasopressin and sugary beverages are associated with renal damage. In the present study we evaluated the effect of mild intermittent dehydration, and subsequent fluid replacement with a sweetened beverage on plasma copeptin and renal alterations.

Methods: Mild intermittent dehydration was induced either passively (daily water restriction) or actively (daily exposure to heat). After both maneuvers animals rehydrated for 2 h with water, or water sweetened with either a non-caloric edulcorant (stevia) or 11% fructose-glucose. All groups were studied for 4 weeks.

Results: The animals which received a sweetened beverage as rehydration fluid, in both active or passive mild dehydration, developed a heightened vasopressin response, noted by an increase in plasma and urine osmolality, increased plasma copeptin, decreased electrolyte-free water clearance, development of mild renal lesions with increased plasma creatinine, urinary excretion of NAG, N-GAL and nephrin, greater oxidative stress in renal cortex, tubular alterations, mild fibrosis, and overexpression of fructokinase, aldose reductase and the vasopressin AV1a receptor in the renal cortex.

Conclusions: We report that mild intermittent dehydration (active or passive), when combined with sweetened beverage ingestion, leads to a heightened vasopressin response associated with renal injury and oxidative stress. These data is also relevant to the general increased consumption in the population of sweetened beverages with reduced intake of plain water. Thus current rehydration practices might have a deleterious effect on the kidney.

Funding: Government Support - Non-U.S.

SA-PO086

A Mathematical Model of the Rat Nephron: Renal Glucose Transport Alan Mark Weinstein. *Dept of Physiology and Biophysics, Weill Medical College of Cornell, New York, NY.*

Background: Mathematical models of proximal tubule (PT), loop of Henle (LOH), and complete distal nephron have been combined to simulate transport by rat renal tubules.

Methods: The ensemble is comprised of 24000 identical superficial (SF) nephrons and 12000 juxtamedullary (JM) nephrons in 5 classes (according to LOH length); all coalesce into a single set of 7200 connecting tubules. Medullary interstitial solute concentrations are specified. The model requires iterative solution so that each nephron GFR satisfies a tubuloglomerular feedback (TGF) relation, and each initial hydrostatic pressure yields a common end-distal tubule pressure for all nephrons; that common initial connecting tubule pressure is determined from an overall distal hydraulic resistance to flow. By virtue of the greater GFR for JM nephrons, overall fluid processing by SF and JM tubules is comparable. Glucose reabsorption is restricted to proximal tubule, cotransported with one Na in convoluted tubule (SGLT2), and two Na in straight tubule (SGLT1).

Results: Increasing ambient glucose from 5 to 10 mM, increases proximal Na reabsorption, and decreases distal delivery. This is mitigated by a TGF-mediated increase in GFR, but there is still a decrease in Na and K excretion. Conversely, when distal delivery is increased by inhibition of SGLT2, the increase in distal hydrostatic pressure is transmitted back to PT, distending the tubule, and thus slowing axial fluid velocity. By virtue of glomerulotubular balance, slower flow blunts proximal Na reabsorption, amplifying renal Na and K wasting. In this case, TGF acts to reduce GFR, reduce proximal tubule distention, and thus limit natriuresis.

Conclusions: In sum, the model captures TGF-mediated diabetic hyperfiltration, and predicts glomerular protection with SGLT2 inhibition.

Funding: NIDDK Support

SA-PO087

Proximal Tubular Claudin 2 Expression Is Necessary for Paracellular Water Reabsorption and Is Sensitive to Nephrotoxin Exposure Paul Jennings, Lydia Aschauer, Alice Limonciel, Gerhard Gstraunthaler, Walter Pfaller, Anja Wilmes. *Div of Physiology, Innsbruck Medical Univ, Innsbruck, Austria.*

Background: Claudins are the major proteins of the tight junctions and the composition of claudin subtypes is critical for the selective permeability of the paracellular route and thus tissue specific function. It has been suggested that claudin 2 acts not only as paracellular cation channel but is also a paracellular water channel.

Methods: RPTEC/TERT1 cells were cultured on microporous growth supports or solid cell culture plates in serum free hormonally defined DMEM/F12. Cells were exposed to nephrotoxins and claudin 2 expression was determined at an mRNA and protein level. Claudin 2 knock down cells were generated with shRNA and puromycin selection.

Results: We have shown that several nephrotoxic compounds including Adefovir, Cyclosporine A (CsA), cadmium, chloracetaldehyde, Cidofovir, potassium bromate and Zoledronate decreased claudin 2 expression in cultured human proximal tubule cells (RPTEC/TERT1). Since the cellular changes due to these compounds were not limited to claudin-2 we investigated the transport properties of RPTEC/TERT1 cells with and without claudin 2 knock down using shRNA. Knock down of claudin 2 had no discernable effect on transepithelial electrical resistance (TEER) or dome formation, but severely attenuated apical to basolateral water reabsorption when cultured on microporous filters. Generation of an osmotic gradient in the basolateral compartment rescued water transport in claudin 2 knock down cells. Inhibition of Na^+/K^+ ATPase with ouabain inhibited dome formation in both cell types. Thus, dome formation is primarily due to transcellular water transport following a solute driven osmotic gradient. However, in RPTEC/TERT1 cells cultured on filters under iso-osmotic conditions, water transport is primarily paracellular, via pore-forming claudin 2, most likely due to local increases in osmolarity in the intercellular space.

Conclusions: In conclusion, we provides strong evidence that claudin 2 is required for paracellular water transport in the proximal tubule and that claudin 2 expression is sensitive to nephrotoxin exposure.

SA-PO088

Renal Effects of Sortilin-Deletion in Mice Kamila Bikulowa, Aljona Borschewski, Christin Dathe, Sebastian Bachmann, Kerim Mutig. *Anatomy, Charité- Universitätsmedizin Berlin, Berlin, Germany.*

Background: The family of the VPS10P-domain receptors comprises five l-type transmembrane proteins which share as a common hallmark the VPS10P-domain and are involved in intracellular sorting of diverse ligands in various cell types. Two of the family members, the sorting-protein-related receptor with A-type repeats (SORLA) and the sortilin, are abundantly expressed in the distal nephron of the kidney. We have previously described decreased NKCC2 phosphorylation and activity in SORLA-deficient mice and suggested that SORLA mediates intracellular trafficking of kinases and phosphatases responsible for the phosphoregulation of the transporter. In this study we focus on the role of the sortilin in the regulation of epithelial transporters and channels along the distal nephron.

Methods: Sortilin-deficient (Sort-/-) mice were analyzed for their physiologic kidney performance, kidney morphology, and biochemical profile of renal transporters.

Results: In wildtype (WT) mouse kidneys, sortilin was expressed along the distal nephron and in the principal cells of collecting duct. Sortilin deficiency had no major effects on kidney morphology. Physiologic evaluation of WT and Sort-/- mice showed significantly increased urinary volume and calcium excretion upon sortilin disruption. Immunoblotting evaluation of major distal epithelial salt transporters and water channels revealed decreased levels of the Na-Cl cotransporter (NCC) and of the phosphorylated, activated aquaporin 2 (AQP2) in sortilin-deficient kidneys compared to WT controls.

Conclusions: In sum, our results suggest that sortilin facilitates the reabsorptive function of renal distal epithelia by interfering with function of NCC and AQP2.

SA-PO089

TRPC3 Channel Regulates Sensitivity of Murine Distal Nephron Cells to Hypotonicity Mykola Mamenko, Oleg L. Zaika, Nabila Boukelmoune, Roger G. O'neil, Oleh Pochynyuk. *Integrative Biology and Pharmacology, The Univ of Texas Health Science Center at Houston, Houston, TX.*

Background: Mechanical stimuli, such as changes in tubular flow and fluid composition, are important regulators of transport rates of water and ions in distal nephron (DN). DN cells respond to mechanical stimuli with increase of $[Ca^{2+}]_i$. Our group has recently identified that TRPV4 channel is essential for flow-induced $[Ca^{2+}]_i$ elevations in DN cells, however little is known about molecular nature of the response to osmotic gradients.

Methods: Here, we employ Fura-2 Ca^{2+} imaging in split-opened distal nephrons of C57BL/6 and genetically modified mice to study molecular determinants of $[Ca^{2+}]_i$ response to hypotonicity in DN.

Results: We found that hypotonic stimulus elicits a uniform and reproducible increase of $[Ca^{2+}]_i$ in DN cells. $[Ca^{2+}]_i$ elevations in response to osmolality changes occur in a dose-dependent manner. Hypotonicity-induced response is mainly mediated by the entry of extracellular Ca^{2+} through the plasma membrane. However, in contrast to flow-induced $[Ca^{2+}]_i$ elevations, pharmacological inhibition or genetic ablation of TRPV4 channel fails to abrogate $[Ca^{2+}]_i$ increase elicited by hypotonic stimuli, pointing to involvement of another membrane channel. Indeed, we found that pharmacological inhibition of TRPC3 channel with pyr3 significantly attenuates elevations of $[Ca^{2+}]_i$ induced by hypotonicity. Moreover, half-maximal response to decreased osmolality requires a stronger shift in tonicity of the bath solution in TRPC3-/- mice, indicating that TRPC3 channel determines sensitivity of DN cells to hypotonic stimuli.

Conclusions: Thus, $[Ca^{2+}]_i$ elevation induced by hypotonicity is a multifactorial response. While the channel triggering $[Ca^{2+}]_i$ increase in response to low osmolality remains to be identified, our results argue that TRPC3 channel serves as a regulator of sensitivity to hypotonic stimuli in DN cells.

Funding: NIDDK Support, Private Foundation Support

SA-PO090

STIM1 Dysfunction Causes NDI-Like Symptoms in Rats Mykola Mamenko,[1] Oleg L. Zaika,[1] Nabila Boukelmoune,[1] Oleh Pochynyuk,[1] Peter A. Doris.[2] *[1]Dept of Integrative Biology and Pharmacology, The Univ of Texas Health Science Center at Houston, Houston, TX; [2]Inst of Molecular Medicine, The Univ of Texas Health Science Center at Houston, Houston, TX.*

Background: Arginine Vasopressin (AVP)-regulated water transport in the collecting duct (CD) is a critical determinant of bodily fluid homeostasis. AVP promotes water reabsorption in CD via expression and trafficking of Aquaporin 2 water channel (AQP2) to the luminal membrane of principal cells. Inability of CD cells to properly respond to AVP results in a devastating disorder – nephrogenic Diabetes insipidus. A paramount role of V2 receptor – cyclic AMP – PKA pathway in AQP2-dependent water transport has been well established. At the same time, an emerging body of evidence shows that AQP2 trafficking and AVP-induced osmotic water permeability in CD cell lines require $[Ca^{2+}]_i$. However, molecular mechanisms underlying AVP-induced $[Ca^{2+}]_i$ response remain obscure. Recent studies point to the involvement of store-operated Ca^{2+} entry (SOCE) in AVP-stimulated $[Ca^{2+}]_i$ signaling.

Methods: Here we use spontaneously hypertensive stroke-prone rats (SHR-A3) as an animal model for SOCE disruption.

Results: While being 87% genetically identical to stroke-resistant spontaneously hypertensive rats (SHR-B2), SHR-A3 have a nonsense mutation in *STIM1* gene. This results in truncation of C-terminal region of STIM1 protein – an endoplasmic reticulum Ca^{2+} sensor, responsible for interactions with plasma membrane Ca^{2+} channels. Using Fura-2 Ca^{2+} imaging in freshly isolated split-opened CDs, we demonstrate that truncation of STIM1 disrupts SOCE in SHR-A3. AVP-stimulation elicits a sustained $[Ca^{2+}]_i$ elevation in CD cells from SHR-B2, as opposed to a transient $[Ca^{2+}]_i$ response in SHR-A3. SHR-A3, but not SHR-B2, manifest increased water intake and decreased urine osmolality, while having elevated plasma AVP levels.

Conclusions: This suggests that STIM1-activated Ca^{2+} entry is critical for AVP-induced water reabsorption. Disruption of STIM1 signaling compromises ability of the kidney to concentrate water resulting in a pathological state, closely resembling nephrogenic Diabetes insipidus.

Funding: NIDDK Support, Private Foundation Support

SA-PO091

Transepithelial Sodium Permeabilities in Isolated Perfused Segments of Rat Renal Inner Medullary Thin Limbs of Henle's Loops and Vasa Recta Kristen K. Evans, William H. Dantzler, Thomas L. Pannabecker. *Physiology, Univ of Arizona, Tucson, AZ.*

Background: Sodium fluxes and resulting sodium permeabilities in specific segments of thin limbs of Henle's loops are critical to concentrating urine in rat renal inner medulla (IM) but have not yet been well defined. There are no published accounts of isolated perfused inner medullary vasa recta. To better understand the role that sodium fluxes may play in the urine concentrating mechanism, sodium permeabilities (P_{Na}) were determined in inner medullary thin limbs of Henle's loops and vasa recta. Transepithelial sodium flux in thin limbs is believed to occur by way of the paracellular pathway.

Methods: P_{Na} was determined from ^{22}Na fluxes in perfused rat descending (DTLs) and ascending (ATLs) thin limbs isolated from 1) initial 2.5 mm below the outer medullary-inner medullary border (upper IM) or 2) terminal 2.5 mm of the IM (lower IM). P_{Na} was determined in isolated perfused vasa recta from the lower IM.

Results: P_{Na} (x 10^{-5} cm/s, mean ± SE) was 71.8 ± 21.9 (n=9) in upper IM DTLs and 329.6 ± 18.5 (n=2) in lower IM DTLs. P_{Na} from the lower IM ATL was 397.8 ± 66.0 (n=5). In isolated perfused papillary vasa recta P_{Na} was 385 ± 83.2 (n=2). For all segments, temperature reduction to 16° C produced little or no change in permeability.

Conclusions: As previously shown for urea permeability, P_{Na} is similar in lower DTL and ATL, segments that lack water channel AQP1 and have no osmotic water permeability, but is significantly lower in upper DTLs, segments that express AQP1 and have high osmotic water permeabilities. The very high P_{Na} in the lower DTL was not considered in original formulations of the passive hypothesis mechanism. P_{Na} in the isolated perfused papillary vasa recta is comparable to P_{Na} determined with in vivo microperfusion. The isolated perfused tubule technique offers potential to gain new insights into inner medullary vasa recta function.

Funding: NIDDK Support

SA-PO092

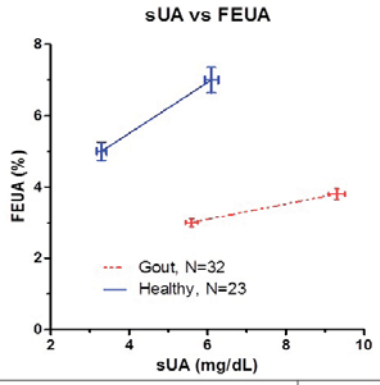
Gout Patients Differ from Healthy Subjects in Their Renal Response to Serum Uric Acid Alterations, Suggesting Less Saturated Uric Acid Reabsorption Systems in Gout Patients Sha Liu, Jeffrey N. Miner. *Ardea Biosciences, San Diego, CA.*

Background: The literature is discrepant with regard to the impact of changing sUA levels on the fractional excretion of uric acid (FEUA) in gout patients. The objectives of this study are to determine whether a change in sUA induced by xanthine oxidase inhibitors (XOI) results in a corresponding change in FEUA and to determine whether the effects are different in gout patients compared to normal healthy volunteers (NHV).

Methods: An analysis based on data from 4 Phase I/Ib studies, in which XOI were given to either NHV or gout patients, was conducted. Plots were created denoting change in FEUA versus baseline FEUA, as well as FEUA versus sUA. Change in FEUA per mg/dL change in sUA was calculated, and NHV and gout patients were compared using Student's *t*-test.

Results: A total of 62 subjects, 55 of whom had sUA and FEUA values at screening (baseline) and day 7, were pooled for analysis (NHV, n=23; gout patients, n=32). The correlation coefficient between baseline FEUA and change in FEUA was -0.83, $P<0.0001$, indicating the higher the baseline FEUA, the greater change in FEUA. When FEUA versus sUA before and after XOI treatment were plotted, the magnitude of FEUA change was greater in NHV (who have higher baseline FEUA) than gout patients (Figure). The slope of the FEUA curve in NHV was significantly steeper than that for those with gout (0.81 ± 0.114 versus 0.22 ± 0.036; $P<0.0001$).

Figure. The magnitude of changes in the FEUA are different between gout and NHV when sUA is lowered with XOIs.



	FEUA at baseline	FEUA with XO inhibitor	P value vs baseline (paired t-test)	delta FEUA per mg/dL change in sUA	P-value vs gout (t-test)
Healthy	7.0 ± 0.36	5.0 ± 0.26	<0.0001	0.81 ± 0.114	<0.0001
Gout	3.8 ± 0.15	3.0 ± 0.11	<0.0001	0.22 ± 0.036	

Conclusions: Reduction of sUA with XOI treatment results in differential effects on FEUA in gout patients versus NHV. Gout patients demonstrate a smaller change in FEUA than NHV with a similar reduction in sUA. This suggests the possibility of a less saturated uric acid reabsorption system in gout patients compared to that in NHV.

Funding: Pharmaceutical Company Support - Ardea Biosciences/AstraZeneca

SA-PO093

Integrated Collecting Duct Response of Cilia, Polycystins, ATP, NO, and ET-1 to Flow Donald E. Kohan,[1] Rajeev Rohatgi,[2] G. Luca Gusella,[2] Jing Zhou,[3] Edward W. Inscho,[4] Tsugio Seki,[4] Bellamkonda K. Kishore,[1] Meghana Pandit.[1] *[1]Univ of Utah, Salt Lake City, UT; [2]Icahn School of Medicine, NY, NY; [3]Brigham and Women's Hospital/Harvard Medical School, Boston, MA; [4]Georgia Regents Univ, Augusta, GA.*

Background: Collecting duct (CD)-derived endothelin-1 (ET-1) is an autocrine inhibitor of Na and water reabsorption. CD-specific ET-1 knockout causes marked hypertension. CD ET-1 production is increased by Na or water loading, however the responsible mechanisms remain unclear.

Methods: To address this, regulation of ET-1 synthesis by mouse IMCD3 cells was studied. Shear stress of 2 dyne/cm^2 increased ET-1 mRNA by 230% as compared to no flow; the effect was first evident after 1 hr of flow.

Results: The flow response required intracellular and extracellular Ca^{2+}, PKC, PLC, calmodulin and calcineurin. Examination of known flow-dependent Ca^{2+} channels in CD cells revealed that the ET-1 flow response required purinergic P2X receptors and polycystin-2, but not TRPC3, TRPC6 or TRPV4. Absence of polycystin-1 or cilia deletion with chloral hydrate blocked the flow response. Both $P2Y_2$ and P2X receptors were required for the flow response. IMCD3 cells lacking polycystin-2 did not increase ET-1 mRNA in response to exogenous ATP, while ATP release was reduced in PKD2-deficient cells. Western and PCR analysis revealed that IMCD3 cells express virtually all known P2X and P2Y receptors. Flow also increased IMCD3 nitric oxide (NO) production; blockade of NO synthesis reduced the ET-1 flow response by 50%.

Conclusions: These findings indicate that luminal flow, likely via ciliary bending, activates polycystins-1 and -2, releasing ATP which acts upon P2X and $P2Y_2$ receptors, increasing intracellular Ca^{2+}, which in turn activates PKC, calmodulin/calcineurin and NO synthase which augment CD ET-1 production. Since ATP and NO can independently inhibit CD transport, we propose that the CD contains a highly interactive network of factors wherein NO and ATP cause both acute (via their own direct actions) and chronic (via ET-1) inhibition of CD transport. These findings demonstrate, for the first time, how discrete autocrine factors in the CD may interact to exert an immediate and sustained response to volume loading.

Funding: Other NIH Support - NHLBI

SA-PO094

Enhanced Autophagy in the Proximal Tubule with Intracellular Vacuoles from AQP11 Null Mice Leading to the Development of Polycystic Kidneys Yasuko Tanaka,[1] Mayumi Watari,[1] Emi Takagi,[1] Yuki Sakamoto,[1] Yoshiyuki Morishita,[2] Kenichi Ishibashi.[1] *[1]Pathophysiology, Meiji School of Pharmacy, Kiyose, Tokyo, Japan; [2]Nephrology, Jich School of Medicine, Shimotuke, Tochigi, Japan.*

Background: AQP11 is an intracellular water channel, which is selectively expressed at ER of the proximal tubule in the kidney as well as other tissues. Its disruption leads to intracellular vacuole formation at 1-wk old and eventually to the development of the polycystic kidney at 3-wk old. We previously reported the upregulation of genes involved in ER stress and apoptosis in the kidneys from 1 to 4-wk old AQP11 null mice. However, some of the vacuolated cells should survive to be the cyst epithelium since all cysts are derived from the proximal tubule.

Methods: To visualize autophagy in tissues, we introduced GFP–LC3 as a marker for autophagy in AQP11 null mice by crossing with GFP-LC3 transgenic mice kindly provided by Dr. N. Mizushima. We examined the tissues from 1, 2, 4, and 5-wk old wild and AQP11 null mice.

Results: The expression of GFP-LC3 puncta was limited to the proximal tubule in the kidney, whose amount was higher in the proximal tubule of 2-wk old AQP11 null mice than wild mice. Its expression was particularly high at the deep cortex where the intracellular vacuoles were smaller with PAS-positive inclusion bodies. The GFP-LC3 puncta expression was also observed in the cyst-lining epithelial cells which still had vacuoles. The active autophagy was also documented by electron microscopy to reveal autophagosomes, mitophagy, and their fusion with lysosomes in the proximal tubule from 1 and 2-wk old AQP11 null mice. Quantitative PCR revealed the enhanced expression of LC3 and beclin-1 in the kidney of 2-wk old AQP11 null mice. On the other hand, the expression of GFP-LC3 puncta was not increased in hepatocytes and intestinal epithelial cells with intracellular vacuoles, suggesting the kidney-specific augmentation of autophagy in the absence of AQP11.

Conclusions: In conclusion, the proximal tubule of AQP11 null mice undergoes active autophagy especially at the deep cortex at 2-wk old with extensive intracellular vacuoles, which may support the survival for later development and maintenance of cysts.

Funding: Government Support - Non-U.S.

SA-PO095

Negative Transcriptional Regulation of Aquaporin-1 By Unfolded Protein Response Ayaka Kato, Hiroko Sonoda, Kanako Shigemura, Saki Takahashi, Masahiro Ikeda. *Veterinary Pharmacology, Univ of Miyazaki, Miyazaki, Japan.*

Background: The unfolded protein response (UPR) is an intracellular signaling pathway in response to the accumulation of misfolded proteins in the endoplasmic reticulum (ER). When misfolded proteins accumulate in the ER, the dissociation of GRP78 (an ER chaperon) from the ER stress sensor proteins (PERK, ATF6, and IRE1) activates downstream signaling pathways characterized by the transcriptions of several genes including CHOP mRNA. Renal ischemia-reperfusion (I/R) injury is a major cause of acute kidney injury. So far, renal UPR activation has been observed following renal I/R and this activation is suggested to protect against the injury. Aquaporin-1 (AQP1) is a water channel protein and its expression has been shown to be decreased following renal I/R.

Methods: In this study, we investigated whether AQP1 expression is directly regulated by UPR activation in rat renal proximal tubular epithelial (NRK52E) cells.

Results: When NRK52E cells were treated with chemical UPR inducers, including tunicamycin and thapsigargin, AQP1 mRNA and protein expression were decreased in association with up-regulation of CHOP mRNA. Since GRP78 knockdown is expected to activate the UPR pathway due to the increase in the free form of the sensor proteins, we examined the effect of GRP78 siRNA on the expression of AQP1 and CHOP mRNAs. As a result, the knockdown of GRP78 caused a decrease in AQP1 and an increase in CHOP mRNAs. Similarly to Aqp1 gene, the expression of Glut1, Hmox1, Ldha, Akr1b1, and Smit1 has been reported to be increased by either hypoxia or hypertonicity, and we then checked the effects of chemical UPR inducers on the expression levels of these genes. Consequently, chemical UPR inducers did not dramatically lower the expression of these genes. Finally, reporter assay with 1991 bp of 5'-flanking region of rat Aqp1 gene was performed, and the result showed that this region did not contain silencer elements in response to UPR activation.

Conclusions: In conclusion, these findings suggest that AQP1 is selectively down-regulated by UPR activation and this regulation might be involved in decreased expression of AQP1 following renal I/R.

SA-PO096

Prostanoid Receptors EP2 and EP4 Regulate Aquaporin-2 Membrane Targeting and Phosphorylation Through Multiple Signaling Pathways Emma T.B. Olesen,[1] Christian Berg,[3] Mette Marie Rosenkilde,[3] Nanna Macaulay,[2] Robert A. Fenton.[1] *[1]Dept of Biomedicine, Aarhus Univ, Aarhus C, Denmark; [2]Inst for Cellular and Molecular Medicine, Copenhagen Univ, Copenhagen, Denmark; [3]Dept of Neuroscience and Pharmacology, Molpharm, Copenhagen Univ, Copenhagen, Denmark.*

Background: Aquaporin-2 (AQP2) is the vasopressin-sensitive water channel expressed in the kidney collecting duct principal cells. Upon stimulation of the Gs coupled vasopressin type 2 receptor, intracellular vesicles containing AQP2 mobilize and fuse with the plasma membrane, causing AQP2 membrane accumulation (MA) and phosphorylation at 3 COOH-terminal sites. This process can be mediated by a Gαs induced increase in cAMP. We have previously shown that the prostanoid receptor EP4 induces AQP2 MA through a signaling pathway that does not involve an increase in cAMP in kidney epithelial cell lines, whereas the prostanoid receptor EP2 increases cAMP and AQP2 MA. EP4 is a known Gs coupled receptor, that can also signal via the pertussis toxin sensitive G-protein Gi, which decreases cAMP through the Gαi subunit and activates PI3K most likely through Gβγ.

Methods: To explore the novel EP4 signaling pathway, we stimulated Madin-Darby Canine Kidney cells, which natively express EP2 and EP4 and are stably transfected with AQP2, with the EP4 agonist CAY10580 (CAY) or EP2 agonist butaprost (BUTA) and studied AQP2 MA by cell surface biotinylation.

Results: Inactivation of Gαi with pertussis toxin did not affect 1 μM BUTA or 3 μM CAY mediated AQP2 MA. The Gβγ inhibitor gallein markedly inhibited 3 μM CAY mediated AQP2 MA, whereas gallein did not prevent 50 nM BUTA mediated AQP2 MA. Gallein enhanced 50nM BUTA induced phosphorylation of AQP2 at ser-264. Gβγ signals through pathways with a variety of second messengers, including cAMP, Ca^{2+} and inositol 1,4,5-triphosphate (IP3). However, neither 3 μM CAY nor 1μM BUTA significantly increased intracellular Ca^{2+} or IP3.

Conclusions: EP4 mediated AQP2 membrane accumulation may be instigated at least in part by the Gβγ subunit via a pathway that does not involve an increase in cAMP or Ca^{2+}/IP3, whereas Gβγ modulates the effect of the EP2 receptor on AQP2 phosphorylation at ser-264.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO097

Increased Phosphorylation of Ser-269 Is Not Sufficient for Regulated AQP2 Apical Accumulation Naofumi Yui, Sei Sasaki, Shinichi Uchida. *Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

Background: Ser-269 is one of the vasopressin (VP)-sensitive phosphorylation sites in the C-terminus of AQP2, and this AQP2 phspho-form is observed in the apical plasma membrane after VP stimulation for several tens of minutes. Therefore, it is recently supposed that S269 phosphorylation occurs in the apical plasma membrane preventing its endocytosis for the regulated AQP2 apical accumulation.

Methods: In this study, we studied its subcellular localization by using specific pS269-AQP2 polyclonal antibody in MDCK cells that express wild-type rat-AQP2 with no tag sequence.

Results: Immunoblotting analysis revealed that pS269-AQP2 was dramatically increased just after 1 min forskolin (FK, 20 μM) treatment with a faint increase of pS256-AQP2. In immunofluorescence, pS269-AQP2 was accumulated in the apical regions after 10 min FK treatment. Unexpectedly, it was mainly detected in the intracellular sites at 1 min. Next, we investigated whether or not all of pS269-AQP2 is stably inserted in the apical plasma membrane after 10 min FK stimulation. To elucidate this question, we applied cold shock (4 °C, 15 min) to AQP2-MDCK cells. Briefly, cold shock instantly arrested clathrin-mediated endocytosis, therefore, it successfully revealed the previously unrecognized AQP2's basolateral targeting that is usually undetectable due to its rapid internalization. Cells were treated with FK for 10 min, then subjected to cold shock, and fixed at 4°C.

Surprisingly, significant amount of pS269-AQP2 was detected in the basolateral plasma membrane after cold shock. Site-specific biotinylation assays also revealed basolateral targeting of pS269-AQP2 after 10 min FK treatment.

Conclusions: In summary, pS269-AQP2 was localized intracellularly at the initial phase of FK stimulation. Even after 10 min FK stimulation, pS269-AQP2 was not exclusively inserted into the apical membrane, but some were targeted to basolateral membranes. In conclusion, the increased S269 phosphorylation might be an essential trigger but not sufficient for the VP-regulated AQP2 apical accumulation. Following mechanisms induced by S269 phosphorylation for the regulated AQP2 apical accumulation have to be elucidated.

Funding: Government Support - Non-U.S.

SA-PO098

Integrin Linked Kinase Regulates the Transcription and Phosphorylation State of Aquaporin2 Jose Luis Cano-Peñalver,[1] Mercedes Griera,[1] Maria P. Ruiz-Torres,[1] Diego Rodriguez-Puyol,[2] Sergio De Frutos Garcia,[1] Manuel Rodriguez-Puyol.[1] *[1]Dept of Physiology, Univ de Alcalá, Alcalá de Henares, Madrid, Spain; [2]Research Unit, Hospital Univ Principe de Asturias, Alcalá de Henares, Madrid, Spain.*

Background: The water channel Aquaporin2 (AQP2) is regulated both transcriptionally and location-based phosphorylation stages. We recently published (FASEB J 2014) that depletion of the scaffold protein integrin-linked kinase (ILK) diminishes AQP2 abundance and membrane presence. Here we show that the lack of ILK reduce AQP2 transcription and phosphorylation in conditional ILK knock-down mice (cKD-ILK) and ILK-depleted inner medullary collecting duct cells (mIMCD3).

Methods: To decrease ILK activity, mIMCD3 were transfected with ILK siRNAs (Si-ILK) or treated with collagen I (COL I), an extracellular matrix component increased in renal interstitial fibrosis. ILK activity was determined measuring the phosphorylation levels of its substrates GSK and AKT. The AQP2 transcriptional stage was measured in mIMCD3 transfected with AQP2 promoter or NFAT transcriptional activity reporter plasmids. The AQP2 phosphorylation in ser256 was determined by immunoflorescence in kidney slides from WT and cKD-ILK.

Results: Decreased phosphorylations in GSK and AKT were observed in ILK-depleted or COL I-treated mIMCD3, compared with control. ILK-depleted cells reduced their AQP2 promoter activity basally, and it was not completely restore when cAMP-based AQP2 activation was performed by forskolin. The lack of ILK reduced the NFAT activity basally or under activation of NFATc/AP1 tandem of transcription factors by ionomycin and PMA. Finally, AQP2 phosphorylation at s256 and its plasma membrane presence were diminished in cKD-ILK slides.

Conclusions: ILK depletion reduces the phosphorylation of GSK and AKT, and this may initiate the further signaling in AQP2 regulation. ILK depletion reduced the AQP2 promoter activity. ILK mediates AQP2 transcriptional activity independently of cAMP-dependent activation, probably by NFATc/AP1. In-vivo depletion of ILK reduced the ser256-phosphorylated AQP2 quantity and apical location in the kidney, attributing ILK a new role in post-transcriptional control of the AQP2 relocation to the plasma membrane.

Funding: Government Support - Non-U.S.

SA-PO099

Estradiol Attenuates Increased Cortical AQP2 Expression and Water Retention in Rats Subjected to Ovariectomy for 7 Days Rikke Norregaard, M.Umar Cheema, William A. Miller-Little, Jorgen Frokiaer. *Inst of Clinical Medicine, Aarhus Univ, Aarhus, Denmark.*

Background: Female sex hormones are suggested to be involved in the regulation of renal water handling. We hypothesize that changes in renal water handling in response to ovariectomy (OVX) is associated with dysregulation of renal aquaporin-2 (AQP2) and can be improved with estradiol or progesterone treatment.

Methods: To examine this, rats were subjected to OVX for 7 days and received subcutaneous administration of estradiol (25 μg/kg/day) or progesterone (10 mg/kg/day) for 7 days. Expression and trafficking of AQP2 and phospho-Ser256-AQP2 (pAQP2) were examined in cortex and inner medulla. Using cortical collecting duct (mpkCCD) principal cells, the effect of estradiol exposure on AQP2 expression and cAMP production was investigated.

Results: OVX was associated with decreased serum estradiol and progesterone levels. Rat subjected to OVX had increased bodyweight (BW) and food intake which was normalized by estradiol treatment, whereas progesterone had no effect. Decreased urine output and increased urine osmolality after 7 days OVX were prevented by estradiol treatment. AQP2 and pAQP2 expression were increased in cortex from OVX rats and this was prevented by estradiol administration whereas progesterone had no effect. Furthermore, confocal microscopy analysis showed decreased apical distribution of both AQP2 and pAQP2 in cortical collecting duct principal cells in estradiol treated OVX rats compared to untreated OVX rats. In inner medulla, there was no significant change in AQP2 and pAQP2 abundance among the four groups. Transcript of estrogen receptors including the G protein-coupled estrogen receptor 1 (GPER1) as well as estrogen receptors ERα and ERβ was detected in mpkCCD cell. Moreover, estradiol attenuated the vasopressin induced AQP2 expression and cAMP production in mpkCCD cells.

Conclusions: In conclusion, estradiol attenuates dysregulation of AQP2 in cortical collecting duct principal cells and water retention in rats subjected to OVX for 7 days.

SA-PO100

The Decreased Excretion of Urinary Exosomal Aquaporin-2 During Gentamicin-Induced Urine Concentrating Defect in Rats Hiroko Sonoda, Ahmed Abdeen, Masahiro Ikeda. *Veterinary Pharmacology, Univ of Miyazaki, Miyazaki, Japan.*

Background: Urinary exosomes are nano-sized vesicles secreted into urine from various renal cells. It has been reported that urinary exosomes containmany types of renal functional proteins that are involved in renal reabsorption and secretion of water and solutes. Aquaporin-2 (AQP2), a member of water channels, is known to be excreted into the urine via exosomes. As renal water reabsorption is known to be regulated by the level of renal AQP2 expression, it is considered that urinary exosomal AQP2 excretion reflects the changes of renal urine concentrating ability. Gentamicin (GM), an aminoglycoside antibiotic, is used for treatment of many types of bacterial infection. One serious limitation to the use of GM is that it can cause acute kidney injury. Besides this injury, GM has been reported to cause urine concentrating defect. By employing this action of GM, in this study we examined whether excretion of exosomal AQP2 was related to urine concentrating ability.

Methods: GM (160 mg/kg/day) or saline was intraperitoneally administered to male rats once every day starting on day 0. Urinary exosomes were isolated by ultracentrifugation.

Results: Plasma creatinine levels were significantly increased at day 5 and beyond in the GM group. GM caused polyuria and urinary concentrating defect at day 7. GM significantly increased urinary exosomal AQP2 excretion at day 1, whereas decreased it at day 7. GM increased urinary excretion of TSG101, frequently used as an exosome marker protein at day 1, but did not have any significant effect on it at day 7. Renal AQP2 expression level in the GM group was slightly decreased at day 2 and was markedly decreased at day 8.

Conclusions: These data suggest that the urinary exosomal AQP2 excretion was decreased in GM-induced urine concentrating defect in association with the reduction of renal AQP2 expression level. Furthermore, the use of urinary exosomal AQP2 may allow early detection of GM-induced nephrotoxicity and the underlying mechanism may be related to the number of exosomes.

SA-PO101

Urinary Excretion Patterns of Exosomal AQP1 and AQP2 in a Rat Model of Renal Tubulointerstitial Fibrosis Minami Kinouchi,[1] Hiroko Sonoda,[1] Saki Takahashi,[1] Naoko Yokota-Ikeda,[2] Masahiro Ikeda.[1] *[1]Veterinary Pharmacology, Univ of Miyazaki, Miyazaki, Japan; [2]Nephrology, Miyazaki Prefectural Miyazaki Hospital, Miyazaki, Japan.*

Background: Chronic kidney disease (CKD), characterized by renal tubulointerstitial fibrosis, affects more than 10% of adult population in the U.S.A. and Japan. The reason for this high incidence is a lack of reliable non-invasive biomarkers. Urinary exosomes secreted from all nephron segments are known to contain many types of renal functional proteins such as aquaporin-1 (AQP1) and AQP2, which suggests that urinary exosomes represent a potential reservoir of biomarkers for renal diseases. Renal AQP1 and AQP2 have been reported to be down-regulated in a rat model of tubulointerstitial fibrosis, suggesting that the urinary excretion of exosomal AQP1 and AQP2 would be altered in tubulointerstitial fibrosis. In this study, we examined urinary excretion of exosomal AQP1 and AQP2 in an experimental model of renal tubulointerstitial fibrosis.

Methods: Because a strong epidemiologic linkage between episodes of acute kidney injury (AKI) and subsequent development of CKD has been reported, in this study we employed an AKI [renal ischemia/reperfusion (I/R)]-induced renal tubulointerstitial fibrosis model.

Results: Blood biochemistry and urine analyses showed that the levels of urea nitrogen and creatinine were significantly increased until 7 days and 21 days, and urinary osmolality was significantly decreased 3 days after renal I/R. Masson-Trichrome stain demonstrated progressive renal tubulointerstitial fibrosis from 7 to 35 days after renal I/R. Immunoblotting studies showed that urinary exosomal AQP1 was significantly decreased until 7 days and AQP2 was decreased only 3 days after renal I/R. Moreover, urinary exosomal Alix and TSG101, commonly used as marker proteins of exosomes, were significantly increased from 7 to 21 days after renal I/R.

Conclusions: These data suggest that urinary exosomal AQP1 and AQP2 can be used to detect early renal injury following renal I/R, and exosomal AQP2 excretion might be related to urinary osmolality. Furthermore, urinary exosomal Alix and TSG101 excretion could be useful for detection of progressive process of renal fibrosis.

SA-PO102

Eps15 Homology Domain Containing Protein 4 (EHD4) Is a Novel Player in the Control of Urine Volume Shamma Shakila Rahman, Alexandra Elizabeth Jane Moffitt, Andrew Trease, Hamid Band, Erika I. Boesen. *Univ of Nebraska Medical Center.*

Background: Eps15 Homology Domain containing protein 4 (EHD4) belongs to a family of endocytic recycling regulatory proteins. Our preliminary data show that EHD4 is expressed in the kidney, including the collecting duct, but the role of EHD4 in renal function is unknown. Accordingly, we tested whether whole body knockout of EHD4 affects baseline urinary salt and water excretion, systolic blood pressure, or acute excretory responses to diuretics or a water load.

Methods: Female wildtype (WT) and EHD4-/- mice were studied (n=4 per group). Urine was collected via metabolic cages: 24h for baseline; for 6h following 2ml of water i.p.; for 4h following i.p. injection of furosemide (40mg/kg), hydrochlorothiazide (HCTZ; 50mg/kg), amiloride (5mg/kg) or vehicle. NKCC2, αENaC and AQP2 were measured by Western blot. Systolic blood pressure was measured by tailcuff plethysmography.

Results: Spot urine osmolality was significantly lower in EHD4-/- versus WT mice (768±152 versus 1645±270mOsmol/kgH_2O; P<0.05). Baseline 24h urine flow was higher in EHD4-/- versus WT mice (1.17±0.24 versus 0.56±0.11ml/d; P=0.06), and urinary osmolality was lower (2198±121 versus 3178±155mOsmol/kgH_2O in WT; P<0.01). Basal Na^+, K^+ or Cl^- excretion did not differ between groups. EHD4-/- mice excreted significantly more of the 2ml i.p. water load in 6h than WT (1.5±0.2 versus 1.0±0.1ml; P<0.05). Urine flow but not Na^+ excretion was higher in EHD4-/- versus WT mice following vehicle injection (0.57±0.11 versus 0.30±0.03ml/4h; P=0.06). Urine flow and Na^+ excretion were significantly higher in EHD4-/- versus WT mice following amiloride (48±6 versus 23±8umolNa^+/4h; P<0.05). Responses to furosemide and HCTZ did not differ significantly between groups, nor did αENaC, NKCC2, or AQP2 protein levels in cortex, outer or inner medulla respectively. Systolic blood pressure was similar in EHD4-/- and WT mice (95±5 and 97±2mmHg). These data were from female mice, but similar trends were observed in male mice (EHD4-/- n=2).

Conclusions: Our data show that EHD4-/- mice produce higher volumes of more dilute urine than WT mice, suggesting a role for EHD4 in water homeostasis. Whether EHD4 regulates subcellular localization of AQP2 remains to be tested.

Funding: Other NIH Support - NCI, Other U.S. Government Support

SA-PO103

Renal Inner Medullary Architecture of Human, Rabbit, and Pig Guojun Wei, William H. Dantzler, Thomas L. Pannabecker. *Physiology, Univ of Arizona, Tucson, AZ.*

Background: The architecture of the simple type of renal outer medulla (human, rabbit, guinea pig) has long been known to be distinct from that of the complex type outer medulla (rat, mouse, kangaroo rat). In contrast, distinctions between architecture of the inner medullas of these two animal groups have not been reported.

Methods: Human, rabbit and pig medullas were fixed in PLP or glutaraldehyde and embedded in paraffin or Spurrs. Immunofluorescence histochemistry was conducted using antibodies against water channels AQP1 and AQP2 and urea transporters UTA and UTB. Tissue prepared for electron microscopy was stained with uranyl acetate. Architecture is compared with prior studies of rat and kangaroo rat.

Results: Expression of UTB in human descending vasa recta (DVR) declines with depth below the outer medulla. Similarly, AQP1 in human is abundantly expressed in long loop descending thin limbs (DTLs) and declines with depth below the outer medulla. In the outer region of the human inner medulla AQP1-positive long-loop DTLs are juxtaposed with DVR lying in vascular bundles. DTLs and DVR lie at margins of CD clusters. In rat and kangaroo rat inner medulla, fenestrated capillaries (ascending vasa recta, AVR) abut approximately 25-50% of CD surface area along their entire lengths. In contrast, rabbit AVR abut less than 5% of CD surface area. Interstitial nodal spaces consisting of one CD with two abutting AVR and one ascending thin limb, that may allow preferential mixing of water, NaCl and urea, are virtually absent in the simple type medulla of rabbit. UTB is abundantly expressed along the papillary epithelium.

Conclusions: Water or urea transepithelial permeabilities in human DTLs or DVR likely decline at progressively deeper levels below the outer medulla. Because of the physical separation of DTLs and DVR from CDs, fluid and solute reabsorbate from DTLs and DVR and diffusion of oxygen from DVR may have limited exchange with CDs. Absence of interstitial nodal spaces in the rabbit and their abundance in the complex type medulla of rat and kangaroo rat correlates with low and high urine concentrating capacity, respectively. UTB may provide a transcellular pathway for urea flux across the human papillary epithelium.

Funding: NIDDK Support

SA-PO104

Lithium-Induced NDI, Hypercalcemia and Urinary PGE2 Excretion Develop Independently in Mice Theun de Groot,[1,2] Lena K. Ebert,[1,2] Ruben Baumgarten,[3] Peter M.T. Deen,[2] Ron Korstanje.[1] *[1]The Jackson Laboratory, ME; [2]Radboud Univ Med. Center, Netherlands; [3]St. Exp. Lab. Med., Netherlands.*

Background: Lithium is the mainstay treatment for bipolar disorder. However, it leads to nephrogenic diabetes insipidus (NDI) and hypercalcemia in 10-20% of patients. Lithium-induced NDI is caused by the inability of the renal collecting duct to reabsorb water. In C57BL/6 mice, lithium-NDI develops in all animals and its extent correlates with the level of excreted prostaglandin E2 (PGE2). Lithium-induced hypercalcemia is ascribed to a decreased sensitivity of the parathyroid gland to blood Ca^{2+} levels, however, as hypercalcemia is known to induce NDI independently of lithium, its development may aggravate lithium-NDI. As lithium-induced NDI and hypercalcemia only occur in a subset of patients, we investigated whether these processes are linked by analyzing their development in 15 different inbred mouse strains.

Methods: Starting at 10 weeks of age, female mice had unlimited access to normal chow or lithium diet (40mmol/kg). At day 8 and 26 mice were housed for 48 hours in metabolic cages to collect urine and at day 10 and 28 blood was isolated.

Results: Lithium treatment for 10 days significantly elevated the urine production of several strains, including C57BL/6J and FVB/NJ, while other strains like A/J and NZW/LacJ were not affected. Differences in urine output highly correlated with changes in urine osmolality. Interestingly, in none of the strains lithium significantly affected urinary PGE2 levels. Furthermore, lithium did not significantly alter blood pH, while urinary proton excretion was elevated in several strains. Lithium-induced hypercalcemia was observed in 4 strains, and did not correlate with the effect of lithium on urine production. Lithium treatment for 28 days only mildly modified the results obtained at 10 days.

Conclusions: Our results demonstrate that development of lithium-induced NDI, hypercalcemia and excretion of PGE2 varies among inbred strains, indicating an independent development of these lithium-related features. Moreover, the diversity in response reveals that inclusion of additional strains may allow identification of susceptibility genes for these lithium-induced disorders.

SA-PO105

Transcriptome Changes in Rat Cortical Collecting Duct (CCD) during Vasopressin Escape Carolyn M. Ecelbarger,[1] Jae Wook Lee,[2] Mark A. Knepper.[2] *[1]Dept of Medicine, Georgetown Univ, Washington, DC; [2]Epithelial Systems Biology Laboratory, NHLBI/NIH, Bethesda, MD.*

Background: Vasopressin escape is a physiological phenomenon by which the kidney is able to overcome the antidiuretic effects of vasopressin when levels are inappropriately high (SIADH). Previously we showed escape was associated with a decrease in aquaporin-2 (AQP2) protein and mRNA. However, the mechanism remains obscure.

Methods: To identify genes that change during escape, we utilized RNA-seq in microdissected CCDs from rats infused with dDAVP (5 ng/hr) and consuming either a normal or a high-water volume (escape) for 1, 2, or 4 days. RNA-seq, a sensitive method to profile the entire transcriptome in small samples, was used to generate CCD transcriptomes (>6000 transcripts) in a total of 24 rats (n = 3-6/time point/group). The generated 50-bp reads were aligned to a rat reference genome. Individual mRNAs were quantified in terms of "reads per kilobase exon normalized to million mapped reads" (RPKM). Data were filtered to eliminate low abundance transcripts.

Results: AQP2 and several other transporters were found to change significantly ($p < 0.05$ and outside the 95% CI): AQP3 (down days 2 and 4), Slc2a1 (facilitated glucose transporter, down day 2) and Rhbg (ammonium transporter, up day 2), Slc38a3 (glutamine transporter, up days 2 and 4), Atp6v1b1 (H-ATPase subunit, up day 2). Overall, transporter transcripts expressed primarily in principal cells were decreased while those in intercalated cells were increased, suggesting reciprocal regulation. Thirteen transcription factors (TFs) with possible roles in initiating escape were significantly altered on day 1, including *Cebpg* (down), *Bmyc* (down) and 4 zinc-finger TF transcripts (2 up, 2 down). The secreted frizzled related protein 2 (*Sfrp2*), an inhibitor of Wnt signaling, was reduced on all 3 days (5-, 16-, and 100-fold on days 1, 2, and 4, respectively). The frizzled family receptor 4 (*Fzd4*) was increased on day 4. These changes might suggest a role for Wnt signaling in CD remodeling.

Conclusions: Overall, several novel transcripts were identified in vasopressin escape. Future studies will evaluate relationships between these transcripts and protein expression.

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-PO106

Regulation of Antidiuretic Hormone (ADH) Secretion in End-Stage Renal Disease (ESRD): Evidence for the Role of Urea during Acute Hemodialysis (HD) Usman Z. Bhutta,[1,2] Kai Lau.[1,2] *[1]Medicine, Univ of Oklahoma Health Sciences Center, OKC, OK; [2]Medicine, VAMC, Oklahoma City, OK.*

Background: Of the 3 normal blood osmotic species, due to intrinsic reflection coefficients, Na is the most effective, urea the least, and glucose intermediate, depending on transport and insulin. In insulin deficiency, hyperglycemia is known to exert hydro-osmotic forces to shrink cell volume and stimulate ADH. In insulin-repleted diabetics or normal, acute hyperglycemia fails to raise ADH despite increased serum osmolality (S osm), findings attributed to ready cell entry of glucose and dissipation of the osmotic gradient. Small and uncharged, urea is thought to freely diffuse across cells and osmotically ineffective. However, marked drop in BUN during the 1st HD in some uremic patients is known to precipitate the dysequilibrium syndrome, raising the hypothesis that before the elevated intracellular urea diffuses back to blood, water is abstracted into neurons to cause swelling. If true, ADH should go down with HD.

Methods: To test this hypothesis, in 13 stable ESRD patients, we measured ADH pre- and post-HD x 3.5 h, using a bath with 138 mM Na and 200 mg% glucose.

Results: Mean S creatinine fell from 15 to 6 mg%. Mean ultrafiltration was 4 L. Mean arterial blood pressure and heart rate were stable between the first and last 30 min of HD. S [Na] rose from 138 to 140 mM. S [Glucose] rose from 113 to 147 mg% as BUN fell from 126 to 47 mg%. Although the component of S osm due to Na and due to glucose rose by 3.4 and 1.9 mOsm/kg respectively, that due to urea fell by 28.3 mOsm/kg, causing a net drop in S osm of 23.1 mosm/kg (329 to 305). Mean plasma ADH fell from 4.9 pre- to 1.3 pg/ml post-HD ($p<0.001$). Plasma ADH did not correlate with S osm based on [Na] x 2 ($r=0.1$) or [Na] x 2 + [glucose]/18 ($r=0.26$), but it was linearly correlated with S osm based on Na, glucose and urea ($r=0.7$, $p<0.01$).

Conclusions: 1. These data support our hypothesis that acutely urea exerts a significant effective hydro-osmotic force to abstract water across cells and alter ADH secretion. 2. They suggest the utility of ADH as an acute cell volume marker in monitoring cerebral edema in patients with head trauma or brain tumor getting mannitol or hypertonic saline.

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SA-PO107

Urinary Arginine Vasopressin and Aquaporin-2 in Edema Patients Byoung Geun Han, Youngsub Kim, Jae Seok Kim, Jae Won Yang, Seung-Ok Choi. *Div of Nephrology, Dept of Internal Medicine, Yonsei Univ Wonju College of Medicine, Wonju, Republic of Korea.*

Background: Edema is frequently uncertain whether it really exists. Especially, idiopathic edema is difficult to diagnose because it has a cyclic feature of edema. BIS (Bioimpedance spectroscopy) is a useful method to estimate body fluid excess. However,

other useful markers for edema have not been investigated sufficiently. This study aims to investigate urinary AVP (arginine vasopressin) and AQP-2 (aquaporin-2) in edema patients.

Methods: The subjects included thirty-three patients complaining of edema. We measured body fluid excess using BIS which was presented as OH value (overhydraion, liter). We collected 24-hr urine samples, in which we measured the excretion amounts of AVP, AQP-2. We also investigated patients with idiopathic edema. We made the subjects to check their body weight at morning and evening in the nude to diagnose idiopathic edema that is characterized by cyclic swelling.

Results: The subjects had normal range of OH value (0.1 liter), while they showed decreased urinary AVP excretion (5.9 ng/day, reference value by Merkelbach U, 1975; 34-70 ng/day) and AQP-2 excretion (0.9 ug/day, reference value by Rai T, 1997; 11 ug/day). The urinary AVP excretion had a significant negative correlation with OH value (r= - 0.438, p=0.020). The subjects with body fluid excess whose OH value was more than 1 liter showed more decreased urinary AVP excretion than the one whose OH value was less than 1 liter (2.6±0.9 versus 7.4±3.7 ng/day, p=0.018). Also in the patients with idiopathic edema, the subjects showed no finding of body fluid excess (OH= - 0.28 liter), while urinary AVP excretion decreased (8.7 ng/day) and urinary AQP-2 also decreased (0.9 ug/day).

Conclusions: We believe that decrease in urinary excretion of AVP and AQP-2 suggests the status of edema. This study demonstrates that in patients who are complaining of edema but show uncertain finding of edema, the measurement of urinary AVP and AQP-2 excretion can be useful for diagnosis of edema. In particular, it is believed that in idiopathic edema which is difficult to diagnose because of the cyclic feature of swelling, it can be also helpful to diagnose.

SA-PO108

Acute and Chronic Regulation of the Osmoprotective Transcription Factor NFAT5 Yuichiro Izumi,[1] Maurice B. Burg,[2] Joan D. Ferraris.[2] *[1]Nephrology, Kumamoto Univ, Kumamoto, Japan; [2]National Heart, Lung, and Blood Inst, National Insts of Health, Bethesda, MD.*

Background: Nuclear Factor of Activated T-Cells 5 (NFAT5) is a transcription factor that increases expression of osmoprotective target genes in response to high NaCl. High NaCl activates NFAT5 by increasing its transcriptional and transactivating activities and its mRNA and protein expression. Despite multiple investigations, regulation of NFAT5 expression has not been completely elucidated.

Methods: We examined acute and chronic effects of high NaCl on the expression of NFAT5 mRNA and protein in HEK293 cells. Cells were incubated either at 300 or 500 mOsm/kg·H_2O (NaCl added) for 2, 4, 8, 24, or 48h and harvested to extract total RNA and protein. Alternatively, cells were first adapted to 300 or 500 mOsm through several passages ("chronic" high NaCl). We quantified the abundance of NFAT5 mRNA and protein by real time PCR and Western blot analysis, respectively. We used actinomycin D and cycloheximide chase to test the effect of high NaCl on NFAT5 mRNA and protein stability, respectively.

Results: High NaCl increased the abundance of NFAT5 mRNA 2.8-fold by 8h, the abundance remained elevated 1.6-fold at 48h (n=3). In cells adapted to 500 mOsm the abundance of NFAT5 mRNA was 1.7-fold greater than in cells adapted to 300 mOsm (n=3). High NaCl increased the abundance of NFAT5 protein 4.1-fold by 24h. The abundance remained elevated 2.6-fold at 48h (n=3). In 500 mOsm adapted cells, the abundance of NFAT5 protein was 1.5-fold greater than in 300 mOsm adapted cells (n=3). ActinomycinD chase showed no difference in stability of NFAT5 mRNA comparing 300 and 500 mOsm, either acutely (n=3) or chronically 500 mOsm (n=3). Cycloheximide chase showed that high NaCl stabilizes NFAT5 protein both with acute (n=3) and chronic high NaCl (n=2).

Conclusions: In HEK293 cells, acute high NaCl increases NFAT5 expression by increasing its transcription and stabilizing its protein both acutely and chronically. The chronic effect of high NaCl explains how NFAT5 activity can remain high in renal medullary cells even following prolonged adaptation to high NaCl.

Funding: Other NIH Support - The Intramural Research Program of the National Heart, Lung, and Blood Institute

SA-PO109

Erythropoietin Production by the Nephron Yuichiro Izumi,[1] Yukiko Yasuoka,[2] Takanori Nagai,[3] Yushi Nakayama,[1] Hideki Inoue,[1] Takeshi Nakanishi,[3] Masashi Mukoyama,[1] Katsumasa Kawahara,[2] Hiroshi Nonoguchi.[4] *[1]Nephrology, Kumamoto Univ, Kumamoto, Japan; [2]Physiology, Kitasato Univ School of Medicine, Sagamihara, Japan; [3]Div of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Japan; [4]Internal Medicine, Kitasato Univ Medical Center, Kitamoto, Japan.*

Background: Erythropoietin (Epo) production is known to occur in the peritubular fibroblasts in the kidney. Since the Epo production in the nephron is controversial, we examined erythropoietin production in the kidney.

Methods: We examined Epo mRNA expressions in the kidney using high-sensitive in situ hybridization (ISH). The mRNA expression of Epo (dots/10 cells) was quantified in basal and hypoxia states. To investigate the mechanism of Epo production, we examined mRNA and protein expressions of HIF PHD2 using ISH and immunohistochemistry (IHC), respectively. We further investigated the Epo production by hypoxia using rat intercalated cell line (IN-IC cells).

Results: ISH in mice showed Epo mRNA expression in PCTs, DCTs and CCDs but not in the peritubular cells (PTEC) under normal conditions. Hypoxia (7% O_2, 4hr) induced Epo mRNA expression largely in PTEC and slightly in PCTs, DCTs, and CCDs.

Epo mRNA (dots/10cells), * p<0.05 vs. control	PCT	MTAL	DCT	IC	PTEC
control	0.50±0.12	0.68±0.06	0.68±0.15	2.27±0.10	0.00±0.00
hypoxia	1.85±0.16*	0.71±0.04	1.19±0.11*	2.97±0.07*	8.90±0.58*

Double staining with AQP3 or AE1 indicated that Epo mRNA expresses mainly in β- or non α/non β-intercalated cells of the collecting ducts. ISH showed mRNA expression of HIF PHD2 in PCTs, DCTs, and CCDs in control and its increase by hypoxia in DCTs, CCDs, and peritubular cells. IHC in rats showed the expression of HIF PHD2 in CCDs and peritubular cells and its increase by anemia in peritubular cells. In IN-IC cells, hypoxia (1% O_2, 24hr) increased Epo mRNA expression, Epo concentration in the medium and protein expression of PHD2.

Conclusions: Epo is produced by the cortical nephrons mainly in the intercalated cells, but not in the peritubular cells, in normal hematopoietic condition and by mainly peritubular cells in hypoxia, suggesting the different regulation between the nephrons and peritubular cells.

Funding: Government Support - Non-U.S.

SA-PO110

Hyperaldosteronism in $K_{Ca}1.1$ Channel β_2-Subunit Knockout Mice Jens G. Leipziger, Mads Vaarby Sorensen, Helle A. Praetorius, Casper K. Larsen. *Biomedicine, Physiology, Aarhus Univ, Aarhus, Denmark.*

Background: Whole body K^+ homeostasis depends on matching K^+ excretion to the daily K^+ intake. K^+ is excreted via the colon and the kidney, the latter handling the majority of the total K^+ excretion. Aldosterone is an important regulator of both renal and colonic K^+ excretion. Renal K^+ secretion depends on 2 apical K^+ channels, ROMK and $K_{Ca}1.1$, while colonic K^+ secretion depends on apical $K_{Ca}1.1$ channels. We have acquired a novel global knockout mouse for the β_2-subunit of the $K_{Ca}1.1$ channel, which is the only $K_{Ca}1.1$ β-subunit expressed in murine colonic epithelium.

Methods: Ussing chamber, Metabolic balance studies, Q-PCR, Plasma Aldo measurement.

Results: The β_2 KO mice have increased plasma aldosterone. Primary hyperaldosteronism is characterized by low plasma K^+, low renin and hypertension. β_2 KO mice have normokalemia and low renin, indicating a secondary cause of hyperaldosteronism. The low renin in β_2 KO mice indicates that a K^+ handling deficiency, rather than hypotension, is causing the hyperaldosteronism. BK channel-dependent colonic K^+ secretion was not disturbed in β_2 KO mice. In contrast, urinary K^+ excretion was reduced in β_2 KO mice (P=0.06, n=5), suggesting that β_2 KO mice have a reduced capacity for renal K^+ secretion. mRNA expression of the β_2 subunit has previously been found in rabbit cortical collecting ducts, where expression is increased by K^+ loading and reduced by K^+ depletion.

Conclusions: These data indicate an important function of the β_2 subunit of the $K_{Ca}1.1$ in renal K^+ excretion.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO111

Novel *KCNJ5* Mutations in Sporadic Aldosterone-Producing Adenoma Reduce Membrane Abundance of Kir3.4 Channel Chih-Jen Cheng, Chih-Chien Sung, Shih-Hua P. Lin. *Dept of Medicine, Div of Nephrology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.*

Background: Aldosterone-producing adenoma (APA), the second most common cause of primary aldosteronism, has been recently linked to mutations in *KCNJ5* gene, which encodes inward rectifying potassium Kir3.4 channel. The reported prevalence of *KCNJ5* mutation in APA is 30-70%. These mutations abolished the potassium selectivity of Kir3.4, and consequently led to sodium leak current, constant depolarization of membrane potential and nonsuppressible aldosterone secretion in zona glomerulosa cells. Here we investigate the *KCNJ5* mutations in Taiwanese APA patients.

Methods: We screened *KCNJ5* gene in resected adrenal adenoma and peripheral blood of 69 Taiwanese patients with APA and functionally characterized novel Kir3.4 mutations using electrophysiological and biochemical methods.

Results: 37.6% (26/69) of Taiwanese APA patients carried heterozygous somatic mutations in KCNJ5 gene. Other than three previously reported mutations (G151R, L168R and E145Q), we identified two novel mutations (R115Q, E246G) in five patients. E145Q mutant conducted choline-sensitive but barium-insensitive sodium-leak current. In contrast, R115W and E246G mutants showed barium-sensitive Kir current, which were 30% and 10% of wild-type current, respectively, and preserved potassium selectivity. The biotinylation assays revealed markedly reduced membrane abundance of R115W and E246G mutants. All mutants exerted dominant-negative inhibition on wild-type Kir3.4 via disrupting membrane insertion (R115W and E246G) or potassium selectivity (E145Q) of wild type channel. Furthermore, tertiapin-Q, a Kir3.x antagonist, depolarized membrane potential and increased *CYP11B2* mRNA transcript in human adrenocortical H295R cells.

Conclusions: Novel Kir3.4 mutations reduce the membrane abundance of wild type Kir3.4 channel. Pharmacological inhibition of human Kir3.4 channel induced membrane depolarization and aldosterone synthesis in H295 cells. Reduced Kir3.4 membrane abundance in zona glomerulosa cells could be a new disease mechanism of APA.

Funding: Government Support - Non-U.S.

SA-PO112

KLHL2 Mediates Angiotensin II-WNK3 Signaling Involved in the Regulation of Vascular Tonus Moko Zeniya, Daiei Takahashi, Yutaro Mori, Takayasu Mori, Fumiaki Ando, Naohiro Nomura, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

Background: Recently, Kelch-like protein 3 (KLHL3)-Cullin3 complex was identified as E3 ubiquitin ligase for WNK kinases, and the impaired ubiquitination of WNK4 causes hereditary hypertensive disease, pseudohypoaldostronism type II (PHAII). However, the involvement of WNK kinase regulation by ubiquitination in the situation other than PHAII has not been identified. Previously, we identified With-no-lysine kinase 3 (WNK3)–STE20/SPS1-related proline/alanine-rich kinase (SPAK)–Na/K/Cl cotransporter isoform 1 (NKCC1) phosphorylation cascade in vascular smooth muscle cells, which constitutes an important mechanism of vascular constriction by angiotensinII (AngII). In this study, we investigated the involvement of KLHL proteins in AngII-induced WNK3 activation in vascular smooth muscle cells.

Methods: We determined which KLHL was expressed in aorta and mouse vascular smooth muscle cells (MOVAS) by RT-PCR, and investigated whether the protein levels of KLHL and WNK3 was regulated by different salt diets and AngII. Then, we performed cell culture study using MOVAS to investigate the molecular mechanisms of reregulation of WNK3 and KLHL2 by AngII.

Results: In mouse aorta and MOVAS, KLHL3 was not expressed but KLHL2, the closest homologue of KLHL3, was expressed. Salt depletion and acute AngII infusion decreased KLHL2 and increased WNK3 in mouse aorta. Surprisingly, AngII-induced KLHL2 decrease and WNK3 increase occurred in minutes in MOVAS. Overexpression and knockdown experiments of KLHL2 in MOVAS confirmed that KLHL2 is the major regulator of WNK3 protein abundance. AngII-induced KLHL2 decrease was not caused by its decreased transcription, but by its increased autophagy-mediated degradation.

Conclusions: We identified that KLHL2 is a novel component of signal transduction in AngII-induced vascular contraction, which could be a promising drug target.

Funding: Government Support - Non-U.S.

SA-PO113

Molecular Pathogenesis of PHAII in KLHL3$^{R528H/+}$ Knock-In Mice Koichiro Susa,[1] Eisei Sohara,[1] Tatemitsu Rai,[1] Moko Zeniya,[1] Yutaro Mori,[1] Takayasu Mori,[1] Motoko Chiga,[1] Daiei Takahashi,[1] Yuichi Inoue,[1] Kiyoshi Isobe,[1] Naoki Takeda,[2] Sei Sasaki,[1] Shinichi Uchida.[1] *[1]Dept of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental Univ, Tokyo, Japan; [2]Div of Transgenic Technology, Inst of Resource Development and Analysis, Kumamoto Univ, Kumamoto, Japan.*

Background: Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by salt-sensitive hypertension, hyperkalemia, and metabolic acidosis, due to activation of the WNK kinase-OSR1/SPAK kinase-NaCl cotransporter (NCC) phosphorylation cascade, and mutations in the WNK1 and WNK4 genes are known to be responsible. Recently, Kelch-like 3 (KLHL3) and Cullin3, components of KLHL3-Cullin3 E3 ligase, were newly identified as responsible for PHAII. However, it remains unclear which molecule is the target(s) of KLHL3.To clarify the pathophysiological role of KLHL3, a mouse model of PHAII caused by mutant KLHL3 was required.

Methods: We generated KLHL3$^{R528H/+}$ knock-in mice that carry the same mutation as autosomal dominant type PHAII patients. To analyze phenotypes of KLHL3$^{R528H/+}$ knock-in mice, blood pressure measurement, blood analysis, immunoblot analysis, quantitative PCR, and immunofluorescence were performed.

Results: KLHL3$^{R528H/+}$ knock-in mice exhibited salt-sensitive hypertension, hyperkalemia, and metabolic acidosis. Moreover, the phosphorylation of NCC was increased in the KLHL3$^{R528H/+}$ mouse kidney, indicating that the KLHL3$^{R528H/+}$ knock-in mouse replicates human PHAII. Interestingly, the protein expression of both WNK1 and WNK4 was significantly increased in the KLHL3$^{R528H/+}$ mouse kidney, confirming that increases in these WNK kinases activated the WNK-OSR1/SPAK-NCC phosphorylation cascade in KLHL3$^{R528H/+}$ knock-in mice.

Conclusions: The activation of OSR1/SPAK-NCC signaling by the increased protein levels of WNK1 and WNK4 caused PHAII in the KLHL3$^{R528H/+}$ knock-in mice. The impaired degradation of both WNK1 and WNK4 by the mutant KLHL3 would be the major pathogenic mechanism.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO114

Mutations in Familial Hyperkalemic Hypertension Gene KLHL3 Disrupt Binding of ROMK Endocytic Regulatory Protein ARH and Increases ARH Abundance Owen M. Woodward, Boyoung Kim, Liang Fang, Paul A. Welling. *Physiology, Univ of Maryland School of Medicine, Baltimore, MD.*

Background: Mutations in CUL3 and KLHL3, components of an E3 ligase complex, cause Familial Hyperkalemic Hypertension (FHHt). CUL3 /KLHL3 have been proposed to ubiquitinate two key kinases, WNK1 and 4, which regulate sodium reabsorption via NCC and K+ excretion via ROMK in the distal nephron. Unrestrained ROMK endocytosis contributes to urinary potassium retention and hyperkalemia in FHHt. Here we explore a new target of the CUL3/ KLHL3 complex, ARH (LDLRAP1) an endocytic protein that controls the surface expression of ROMK.

Methods: Mutagenesis, cell culture, and invitro protein-protein assays were employed.

Results: We found that KLHL3 /CUL3 directly targets ARH for ubiquitination and proteasomal degradation. KLHL3 directly bound ARH, with the KLHL3 propeller domain (aa 300-585) and the ARH PTB domain (aa 1-187) necessary and sufficient for the interaction. Presence of another known KLHL3 substrate, WNK1, did not prevent the ARH/KLHL3 interaction. Next we focused on the molecular consequences of KLHL3 FHHt mutations. Surprisingly, we found many of KLHL3 propeller mutant proteins are not efficiently expressed, revealing a mechanism for lost-of-function. One mutant, S433G, which did not disrupt stability, significantly blocked ARH interaction. And finally we found co-expression of mutant KLHL3 and ARH results in increased ARH abundance as compared to wt KLHL3.

Conclusions: FHHt mutations in KLHL3 disrupt direct ARH binding and increase ARH abundance, a finding consistent with increased ROMK endocytosis and reduced K+ excretion, contributing to the hyperkalemia in FHHt patients.

Funding: NIDDK Support

SA-PO115

Phosphorylation of KLHL3 in the Kelch-Repeat Regulates Its Binding Ability to WNK4 Yuki Yoshizaki, Eisei Sohara, Takayasu Mori, Yutaro Mori, Mai Wakabayashi, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Nephrology, Tokyo Medical and Dental Univ, Bunkyo-ku, Tokyo, Japan.*

Background: Mutations in the WNK1 and WNK4 genes result in an inherited hypertensive disease, pseudohypoaldosteronism type II (PHAII). In addition, the KLHL3 and Cullin3 genes were also identified as responsible genes for PHAII. Recently, we have reported that WNK kinases are substrates of KLHL3-Cullin3 mediated ubiquitination. However, the physiological regulation of ubiqutination of WNK kinases by KLHL3-Cullin3 complex is still unknown.

Methods: We analyzed phosphorylation site of KLHL3 using mass spectrometry. We further generated phosphor-specific antibody of KLHL3 against a specific phosphorylation site we found.

Results: Mass spectrometry analysis determined a phosphorylation site of KLHL3 at S433, located within the kelch repeats that directly bind toWNK kinases. Interestingly, a phospho-mimicking S433D KLHL3 mutant exhibited decreased binding ability to WNK4, leading to decreased ubiquitination and increased protein expression of WNK4. In addition, immunoprecipitated KLHL3 by WNK4 showed decreased phosphorylation at S433, compared to native KLHL3 protein before immunoprecipitation. These data indicated that phosphorylated KLHL3 at S433 resulted in the decreased binding ability of KLHL3 to WNK4. Since S433 is a component of PKA and Akt motif, we further examined the effect of forskolin and insulin on phosphorylation of KLHL3 at S433. As expected, both forskolin and insulin stimulation increased phosphorylation of KLHL3 at S433 in cultured cells.

Conclusions: We identified a novel phosphorylation site of KLHL3, which was regulated by insulin and forsklin. This phosphorylation of KLHL3 at S433 decreased the binding ability of KLHL3 to WNK4, suggesting that WNK ubiqutination mechanism by KLHL3 could be physiologically regulated by KLHL3 phosphorylation at S433.

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SA-PO116

p62-Mediated Selective Autophagy Is Involved in KLHL3-Dependent WNK4 Degradation Yutaro Mori, Mai Wakabayashi, Takayasu Mori, Moko Zeniya, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. *Dept of Nephrology, Tokyo Medical and Dental Univ, Tokyo, Japan.*

Background: We reported that KLHL3 binds to WNK4 and Cullin3 (CUL3) and ubiquitinate WNK4 and that the impaired WNK4 ubiquitination and degradation cause human hypertensive disease through the activation of WNK-OSR1/SPAK-NCC cascade. We also found that KLHL3-induced WNK4 degradation could not be inhibited completely by proteasome inhibitors. Recently, Kelch-like ECH-associated protein 1 (Keap1), which belongs to the same Kelch family as KLHL3, was reported to be degraded by p62/SQSTM1(p62)-mediated selective autophagy under oxidative stress. Therefore, we investigated whether WNK4 degradation induced by KLHL3 was also mediated by autophagic mechanisms.

Methods: Transient expressions, knockdown by si-RNA, and co-immunoprecipitation experiments of WNK4, KLHL3 and p62 were performed in HEK293T cells We also performed immunofluorescenct staining of p62 in mouse kidney.

Results: Co-immunoprecipitation assays revealed that KLHL3 bound to p62 by the kelch repeat domain like Keap1. WNK4 did not directly bind to p62. p62 overexpression increased WNK4 degradation, which was not inhibited by proteasome inhibitors but by autophagy inhibitors, and p62 knockdown dramatically increased the transiently expressed WNK4 protein level. In mouse kidney, p62 was confirmed to be present in distal convoluted tubules even under normal condition.

Conclusions: WNK4, which is ubiquitinated by KLHL3-CUL3 E3 ligase complex, is degraded not only by proteasomes but also by p62-mediated selective autophagy. The WNK4 degradation by autophagy may be involved in the regulations of WNK signaling under certain pathophysiological conditions.

SA-PO117

Rare Mutations in NKCC2 Gene Associated with Protection From Hypertension Differentially Regulate NKCC2 Isoforms A and F

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Background: Apical bumetanide-sensitive Na-K-2Cl co-transporter, NKCC2, is the major salt transport pathway in kidney thick ascending limb (TAL). Recent studies revealed that nine rare mutations in NKCC2 are associated with a reduction of blood pressure and protection from hypertension. However, the functional characterization of these variants addressed by two independent groups, revealed that some of these mutations do not reduce NKCC2 activity. In addition, there are some discrepancies between the findings of the two reports. Consequently, the molecular mechanisms underlying NKCC2 regulation by five of these nine mutations remain unclear. Moreover, the two groups focused on the regulation of only one NKCC2 isoform, whereas two active NKCC2 isoforms (A and F) are expressed in TAL cells. The aim of the present study was to examine the consequence of these five NKCC2 mutations on the expression and activity of NKCC2A and NKCC2F.

Methods: NKCC2 protein expression was monitored in transiently transfected OKP and HEK cells, using immunoblot and confocal imaging. NKCC2 activity was measured as bumetanide sensitive NH4 influx using BCECF fluorescence of intracellular pH.

Results: NKCC2A-P348L displayed partial loss of complex glycosylation at the cell surface, whereas NKCC2F-P348L showed only immature form and was trapped in the ER. Consequently, these mutants exhibited dramatically diminished basal transport activity. In contrast, no obvious effect on the protein expression and trafficking of NKCC2A and F was seen with the four remaining mutants. Nevertheless, these mutations were able to differentially compromise the activity of NKCC2 isoforms. Indeed, N399S reduced the activity of NKCC2F, whereas it had no effect on NKCC2A. Likewise, P250A, Y1070C and P1083A decreased NKCC2A activity, while they did not affect NKCC2F.

Conclusions: In sum, our findings indicate that all nine rare mutations in NKCC2 associated with protection from hypertension exhibit, actually, impaired transport function. Most importantly, they reveal that some of these mutations exert their effects on NKCC2 by differentially compromising the expression and/or activity of its isoforms, A and F.

Funding: Government Support - Non-U.S.

SA-PO118

Moesin, a Cytoskeletal-Associated Protein, Plays an Important Role in the Regulation of Membrane Localization of NKCC2

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Background: Electrolytes as Na^+, K^+, and Cl^- have a vital role in maintaining body fluid homeostasis. In the thick ascending limb of Henle (TALH), 20 to 40 % Na^+ filtered by glomerulus are reabsorbed by NKCC2 (Na-K-2Cl cotransporter 2), which plays essential roles in the reabsorption of electrolytes and volume balance regulation. Despite of the physiological importance of NKCC2 in the regulation of NaCl homeostasis, the molecular mechanisms for its membrane trafficking are not elucidated. Recently, it is reported that Moesin, which is a member of ERM (Ezrin-Radixin-Moesin) family, plays an important role in the apical membrane trafficking of NKCC2 by *in vitro* experiments.

Methods: We examined the physiological importance of Moesin in the regulation of renal function by using Moesin knockout (KO) mice. Wild type (WT) and Moesin KO mice were kept in metabolic cages and daily urinary volume and urinary contents of electrolytes were measured.

Results: We found that moesin deficient mice exhibited the significant increase in the fractional urinary excretion of electrolytes (Na^+, K[sup+[/sup], and Cl[sup-[/sup]), whereas total urinary contents of these electrolytes were not different between WT and Moesin KO mice. Furthermore, Moesin deficient mice showed moderate hypotensive phenotype and significantly reduced glomerular filtration rate, suggesting the possible compensation for the urinary loss of electrolytes. Immunofluorescent analysis also indicated the reduced apical surface expression of NKCC2 in Moesin deficient mice.

Conclusions: In summary, our study suggests that Moesin plays an important role in the maintaining the apical surface expression of NKCC2 in TALH and regulation of the electrolyte reabsorption *in vivo.*

Funding: Government Support - Non-U.S.

SA-PO119

Genetic Deletion of $P2Y_2$ Receptor Offers Long-Term (5 mo) Protection against Lithium-Induced Polyuria, Natriuresis and Kaliuresis

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Background: Chronic lithium(Li) administration for bipolar disorder is associated with polyuria, natriuresis and kaliuresis. Using a 2-week model, previously we reported that $P2Y_2$-R knockout (KO) mice are significantly resistant to Li-induced polyuria, natriuresis and kaliuresis as compared to the wild type (WT) mice. Here we show that this protection is long-lasting.

Methods: Groups of age-matched adult WT and KO mice were fed regular (n = 5/genotype) or Li-added diet (40 mmol Li/kg chow; n = 10/genotype) with free access to food, water and salt blocks for 5 months. Twenty-four hour water intake and urine output and osmolality were monitored every month. At the end of the experimental period mice were euthanized and serum and kidneys were analyzed.

Results: Li-fed KO mice consistently had significantly lower polyuria, natriureis and kaliureis over the 5 mo period as compared to Li-fed WT mice. Table presents data of terminal urine and AQP2 protein in the kidney. Furthermore, there were no significant differences in serum Li between the WT and KO mice, and serum BUN and Na levels were within normal limits in both groups.

Group	Water Intake[a]	Urine Output[a]	Urine Osmolality[b]	Urine Na[c]	Urine K[c]	AQP2 in Cortex[d]	AQP2 in Medulla[d]
WT-CT	4.2 ± 0.5	1.3 ± 0.2	2184 ± 168	112 ± 11	424 ± 45	100 ± 5	100 ± 24
WT-LI	8.1 ± 0.6*	3.1 ± 0.2*	1474 ± 95*	380 ± 15*	670 ± 26*	15 ± 7*	23 ± 4*
KO-CT	4.9 ± 0.6	1.7 ± 0.4	1934 ± 252	129 ± 8	442 ± 45	100 ± 10	100 ± 7
KO-LI	6.3 ± 0.7	1.4 ± 0.1	2136 ± 84**	240 ± 17**	427 ± 33**	34 ± 2**	73 ± 7**

[a]ml/day/20 g bw; [b]mOsm/Kg H_2O; [c]μEq/day/20 g bw; [d]band density as % or respective control diet; CT - control diet; LI -lithium diet: $P < 0.05$ vs. WT-CT (*) or WT-LI (**)

Conclusions: Our results demonstrate that genetic deletion of $P2Y_2$-R protects against Li-induced polyuria, natriuresis and kaliuresis on long-term basis, and thus underscore the potential utility of $P2Y_2$-R antagonism for the treatment of Li-induced nephrogenic diabetes insipidus.

Funding: Veterans Affairs Support

SA-PO120

Computational Analysis of the Effect of Aldosterone and Low pH on Gene Expression and Cell Function

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Background: Metabolic acidosis is a major problem in chronic kidney disease. Although aldosterone is produced in response to the decrease of blood pH and stimulates collecting duct to excrete proton into urine, we hypothesize that there may be urinary acidification mechanisms in aldosterone independent manner. Since low pH is accompanied by the increase of aldosterone *in vivo*, it is difficult to examine the individual effect of aldosterone and low pH. In the present study, we attempted to distinguish the effect of low pH from that of aldosterone on gene expressions and cell functions using computational analysis.

Methods: We searched publications and picked up gene transcripts that are induced in epithelial cells in the kidney either by administration of aldosterone or acid loading. We applied bioinformatic tools to analyze the genes. Using DAVID Bioinformatics Database, we examined Gene Ontology (GO) analysis for the gene transcripts. Using oPOSSUM, we analyzed promoter regions of the genes and estimated enriched consensus motifs in the promoters of the genes.

Results: Among publications, 57 and 125 genes were picked up as aldosterone- and acid-stimulated genes, respectively. 5 genes were overlapped between two stimulations. GO analysis showed 10 and 17 terms in molecular function ($p > 0.05$) for aldosterone- and acid-stimulated genes, respectively. There were 9 terms overlapped such as hydrogen ion and inorganic cation transmembrane activities between two stimulations. The promoter analysis raised 28 and 21 consensus motifs for aldosterone- and acid-induced genes, respectively (Z-score > 5.0). There were 10 motifs overlapped between two stimulations, which include the hormone response element, a target of mineralocorticoid receptor. Specific motifs for acid-induced genes include the motifs for Ets and NFAT5 TF families, and they locate on the promoter region of SLC26A7 (Cl^-/HCO_3^- exchanger) and ATP6AP2 (a component of H^+-ATPase).

Conclusions: The results suggest that there should be aldosterone independent mechanisms which are stimulated directly by low pH.

SA-PO121

Active, Sex-Dependent Fluid Secretion in the Collecting Duct

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Background: The focus of the present study was to evaluate the mechanism of collecting duct (CD) fluid transport by in vitro microperfusion of mouse cortical CD (CCD) and outer medullary CD (OMCD).

Methods: Water flux, J_V, was measured by using the radiolabeled volume marker, [methoxy-^{3}H]inulin, exhaustively dialyzed, in the perfusate during two experimental periods.

Results: Data from our laboratory show that in the absence of an osmotic gradient, the collecting duct actively secretes fluid. A lumen-to-bath osmotic gradient reversed fluid secretion to reabsorption. An unbiased analysis of the rate of fluid secretion versus the percent leak into the bath in 67 consecutive experiments demonstrated no correlation of these two measurements under symmetrical, isosmotic conditions. Interestingly, fluid secretion in the CCD of female mice (n = 8) was twice that of male mice (n = 10, P <0.02). In paired, time-control experiments, fluid secretion did not change between the two collection periods in either sex. We further tested the effect of inhibiting Na absorption with luminal 1 μM benzamil. Application of 1 μM luminal benzamil significantly reduced fluid secretion, and this inhibition occurred in both sexes. Fluid secretion persisted in the CCD of desoxycorticosterone pivalate-treated male mice (1.7 mg IM and 0.4% Na diet, 6-8 days). However, in the OMCD of male mice (n = 4) the rate of fluid secretion was approximately 2.5 times greater compared to the rate in the CCD (n = 10, P < 0.01). The greater rate of fluid secretion in the OMCD compared with the CCD of male mice indicates that this mechanism exhibits axial heterogeneity. The difference in fluid secretion between

the CCD of male and female mice implies that a primary or secondary sex characteristic influences this process, which may be inhibited by in vivo hormones or signaling factors.

Conclusions: The collecting duct possesses the capacity for active fluid secretion which is sex-dependent and exhibits axial heterogeneity between the cortex and outer medulla.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO122

Impact of Shifts in Renal Tubular Reabsorption Sites on Kidney Oxygen Consumption

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Background: The vast majority of the kidney's oxygen consumption (QO2) is attributable to the reabsorption of ~99.5% of filtered sodium. The ratio of QO2 to sodium reabsorption (TNa) has been shown to vary; e.g., QO2/TNa has been observed to be higher in spontaneously hypertensive rats than in Wistar-Kyoto rats. Changes in QO2/TNa may occur when the burden of reabsorption is shifted from the proximal tubule to the loop of Henle, which is less energy efficient in reabsorbing sodium.

Methods: To understand the impact of changes in tubular reabsorption on renal metabolism, we have developed a mathematical model of detailed epithelial solute transport and metabolism of a rat superficial nephron. We simulated the application of benzolamide, a proximal diuretic that inhibits membrane carbonic anhydrase (CA).

Results: Model simulations of benzolamide application predict a reduction in TNa and a paradoxical increase in QO2, as observed experimentally. Our results confirm the hypothesis of Deng et al. (AJP Renal 2006) that the inhibition of CA increases the active reabsorption of chloride in the proximal tubule while reducing passive NaCl transport, thereby raising QO2. Additionally, we have conducted model simulations to investigate the impact of pathophysiological changes in renal structures and functions (e.g., changes in membrane transporter density, altered tight junction permeabilities, mitochondrial uncoupling, etc., as observed in diabetes and hypertension) on renal solute transport and oxygen consumption.

Conclusions: Oxidative stress associated with conditions such as hypertension and hyperglycemia reduces tubular transport efficiency and causes metochondrial uncoupling. The resulting increase in kidney QO2, without a corresponding increase in oxygen supply, may lead to intrarenal tissue hypoxia.

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SA-PO123

Renal Tubular NHE3 Expression Is Not Required for Caffeine-Induced Diuresis and Natriuresis

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Background: The diuretic and natriuretic effects of the methylxanthine caffeine are well-known and our previous data indicated that both effects are mediated by blockade of adenosine A1 receptors (A1R). While it is assumed that the increase in cAMP levels, due to blockade of A1R, inhibits NHE3 and therefore proximal tubule-induced diuresis and natriuresis, this hypothesis has never been tested *in vivo.*

Methods: To test if tubular expression of NHE3 is required for caffeine-induced diuresis and natriuresis we generated tubulus-specific NHE3 knockout mice by crossing floxed NHE3 mice with Pax8Cre mice. Renal NHE3 knockout was determined by immunohistochemistry and semi-quantitative immunofluorescence. To test for caffeine-induced diuresis and natriuresis, $NHE3^{lox/lox}$ (Con, n=8) and littermate $NHE3^{lox/loxCre}$ (knockout, n=8) mice were randomized to acute application of caffeine (caff, 45 mg/kg) or vehicle (veh, 0.85% NaCl) via oral gavage (3% of body weight) and placed in metabolic cages for quantitative urine collection over 3 hours. Urinary flow rate and excretion of Na and Cl were determined.

Results: In $NHE3^{lox/loxCre}$ mice, cortical NHE3 expression was absent and medullary NHE3 abundance was reduced by >80%. In Con, caff significantly increased urinary excretion of fluid, Na and Cl (Con-caff versus Con-veh: 324±16 versus 203±19 nl/min/g; 35±2 versus 24±2 nmol/min/g; 25±2 versus 16±2 nmol/min/g, respectively, P<0.05 versus veh). Urinary excretion between Con and $NHE3^{lox/loxCre}$ was not different when given veh alone and in $NHE3^{lox/loxCre}$ a comparable response to caff was observed ($NHE3^{lox/loxCre}$-caff versus $NHE3^{lox/loxCre}$-veh: 331±22 versus 208±10 nl/min/g; 36±4 versus 23±1 nmol/min/g; 27±3 versus 18±1 nmol/min/g, respectively, P<0.05 versus veh).

Conclusions: In contrast to the accepted hypothesis that NHE3 mediates caffeine-induced natriuresis, our data demonstrate that tubular expression of NHE3 is not required for these effects. We conclude that different transport protein(s) are mediating these effects.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO124

The Pathogenesis of Metabolic Alkalosis in Cystic Fibrosis: Dysregulation of Pendrin and Impaired HCO_3^- Secretion in the Kidney

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Background: Cystic fibrosis (CF) is the most common life-threatening genetic disease in the United States. It is caused by mutations in CFTR, a cAMP-activated chloride channel expressed in epithelial tissues, and affects lung, pancreas and intestine. However, no apparent kidney phenotype has been identified in CF patients, despite abundant expression of CFTR in the kidney, including B-intercalated cells (AJP Renal, 1996). Patients with CF are prone to the development of metabolic alkalosis, which produces a diminution of respiratory effort that can be detrimental in CF patients, who suffer from lung injury. However, the pathogenesis of metabolic alkalosis in CF remains unknown. CFTR plays a vital role in HCO_3^- secretion in tracheal eithelial cells by activating the apical Cl^-/HCO_3^- exchangers SLC26A4 (pendrin) (JBC, 2011). We hypothesized that a synergistic interaction could occur between CFTR and pendrin in B-intercalated cells, specifically during volume depletion.

Methods: CFTR knockout (CF-KO) mice with the intestinal rescue (overexpressing wt CFTR in their intestine-Science 1994) were examined for pendrin expression and function.

Results: Baseline parameters, including acid-base status and urine pH were comparable in CF-KO and WT mice. Compared to WT animals, CF-KO mice demonstrated significant increase in blood HCO_3^- concentration (23.5 in wt versus 28.2 mEq/l in CF-KO mice, p<0.04) and pH (7.28 in wt and 7.38 in CF-KO, p<0.05) and impaired HCO_3^- excretion (urine pH 8.1 in wt and 7.2 in CF-KO mice, p<0.02) following a 3-day oral bicarbonate load. Immunofluorescence labeling in CF-KO mice demonstrated reduction in apical expression and increased cytoplasmic and basolateral localization of pendrin at baseline state and in response to bicarbonate load, consistent with impaired trafficking.

Conclusions: We propose that patients with cystic fibrosis are prone to the development of metabolic alkalsosis secondary to the impairment of pendrin, specifically during volume depletion, which is a common occurrence in CF patients.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO125

Increased Phosphorylation of the Renal NaCl Cotransporter in Transplant Patients with Hypertension

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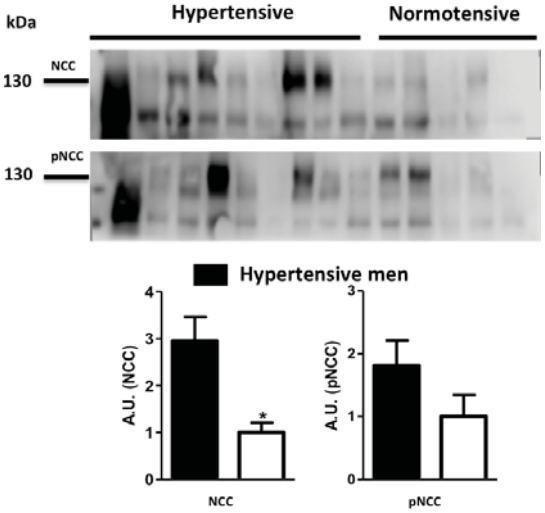
Background: In roedent models tacrolimus-induced arterial hypertension is associated with increased activity and phosphorylation of the renal NaCl cotransporter (NCC) in the distal convoluted tubule. In this study we assessed if a similar situation occurs in humans after renal transplantation.

Methods: From January 2013 all adult patients receiving a kidney allograft in our institution and consented to be included were enrolled into a prospective, longitudinal and observational study. All patients received tacrolimus as immunosuppresive therapy. At three, six, and 12 months after surgery we assessed general clinical and laboratory variables, tacrolimus blood levels, and arterial blood pressure. At six months blood pressure was determined by ambulatory blood pressure monitoring (ABPM). At three, six, and twelve months urinary exosomes were extracted to perform Western blot analysis using total and phospho-NCC antibodies. The amount of exosomes per lane was adjusted according to urinary creatinine.

Results: At this report 21 patients (66% male) have been followed for six months after surgery and completed ABPM. Mean age and serum creatinine 37.8 ± 14.3 years and 1.2 ± 0.3 mg/dl, respectively. Of seven women only one had hypertension. Of 14 males 9 were hypertensive and 5 normotensive (mean blood pressure 91.7 ± 8.8 versus 83.2 ± 6 mmHg, p<0.01), with tacrolimus blood levels of 11.7 ± 4 and 7.3 ± 1.8 ng/ml, respectively (p<0.005).Western blot analysis of urinary exosomes of these 14 male patients revealed a significant difference in NCC expression and phosphorylation between normotensive and hypertensive patients (Figure; *p<0.05).



Conclusions: Hypertension in renal transplant patients is associated with increased activity of NCC.

Funding: Government Support - Non-U.S.

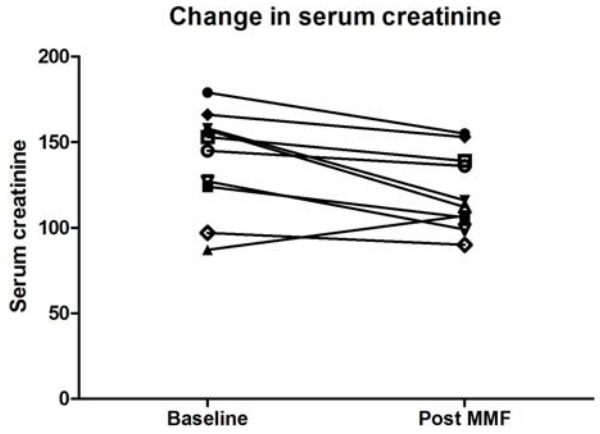
SA-PO126

Mycophenolate Improves Renal Function in Tubulointerstitial Nephritis (TIN) due to Primary Sjögren Syndrome (pSS) Rhys David Russell Evans, Maryam Khosravi, Chris Laing, Stephen B. Walsh. *UCL Centre for Nephrology, Univ College London, United Kingdom.*

Background: pSS is characterized by lymphocytic infiltration of epithelial tissue. Renal involvement occurs in up to 30%. Histologically, the predominant lesion is TIN, impairing tubular function and GFR. No treatment is of proven benefit in pSS. We report the response of 10 patients with pSS TIN to treatment with MMF.

Methods: We recorded patient demographics, baseline symptoms and organ involvement, immunology, excretory renal function, urinary acidification testing and renal histology. We reviewed the response to MMF treatment.

Results: 90% were female with a mean age of 54.7 ± 3.5 years. 70% had sicca symptoms and all were ANA and anti-Ro positive. 50% were anti-La positive; 80% had raised IgG levels. 80% of patients had a urinary acidification test. Of those, 75% had distal renal tubular acidosis (dRTA). Renal histology revealed TIN in all patients. Treatment was with MMF 500mg-2g/day. 30% of patients were also treated with hydroxychloroquine, 70% had prednisolone (weaned in 57%). Treatment was well tolerated by all bar one patient in whom it caused leucopenia. Those with dRTA were treated with potassium citrate. Mean follow up was 20 months: 80% of patients had an improvement in renal function; it was stable in 10%, and progressive in 10% (fig 1). There was a significant improvement in renal function with treatment (mean creatinine 139.5 ± 9.5 mmol/L to 121.3 ± 7.2 mmol/L; p=0.013). IgG levels improved after treatment, but not significantly (mean IgG 20.6 ± 1.3 g/L to 17.1 ± 1.5g/L, p=0.09) In patients with improved renal function, symptoms stabilized but did not resolve. Treatment had no effect on the need for supportive treatment of dRTA.



Conclusions: Immunosuppression with MMF results in an improvement of renal function in the majority of patients with pSS TIN, with a partial but incomplete effect on extrarenal symptoms.

SA-PO127

Thiazide-Induced Hyponatremia (HN): Report from the HN Registry Volker Rolf Burst,[1] Franziska Grundmann,[1] Torsten Kubacki,[1] Arthur Greenberg,[2] Despina Rudolf,[3] Joseph G. Verbalis.[4] *[1]Univ Hospital Cologne, Germany; [2]Duke Univ Medical Center, Durham, NC; [3]Otsuka Pharma GmbH, Germany; [4]Georgetown Univ Medical Center, Washington, DC.*

Background: Thiazide-induced HN (TIH) is common but insufficiently characterized. This analysis of the HN Registry (5028 pts, 225 sites, NCT01240668) compared pts with thiazide-associated HN (TAH, presumably TIH) and SIADH pts.

Methods: Demographics, choice and efficacy of treatment, uni- and multivariate analysis of biochemical parameters.

Results: Of 1946 SIADH pts classified by treating physicians, only 1556 were confirmed with SIADH; 390 were re-categorized as TAH during adjudication. TAH pts were older and more likely to be female. Serum [K^+] (3.8±0.65 mEq/L versus 4.2±0.6 mEq/L), urine osmolality (373±170 mOsm/kg versus 421±182 mOsm/kg) and urine [Na^+] (62.8±40.3 mEq/L versus 81.9±50.5 mEq/L) were significantly lower and serum creatinine (79±38 µmol/L versus 69±34 µmol/L), BUN (6.5±5.9 mmol/L versus 4.9±2.8 mmol/L), and serum uric acid (268±123 µmol/L versus 185±86 µmol/L) were significantly higher in the TAH group. However, in 12% of TAH and 11% of SIADH pts, none of the lab tests required to properly evaluate euvolemic HN were performed. Normal saline (NS) was the most common treatment applied in TAH pts (28.9%, SIADH pts 22.6%, P=0.01). With NS use efficacy endpoints including serum [Na^+] rise to >130 mEq/L or ≥135 mEq/L, and increment ≥5 mEq/L were achieved significantly more often in TAH than SIADH (32.0% versus 16.4%, 13.4% versus 3.9%, 60.8% versus 36.7%, respectively). Overly rapid correction occurred in 3.6% of TAH versus 1.7% of SIADH pts. Other treatments used in TAH pts: fluid restriction (FR, 23.1%), FR plus NS (9.7%), hypertonic saline (5.7%), NS plus loop diuretic (4.4%), and salt tablets (3.6%).

Conclusions: In the HN Registry, clinicians frequently failed to recognize thiazide use as a cause of HN. Compared to SIADH pts, the better response of TAH pts to NS administration is in line with its pathophysiology and confirms accepted treatment recommendations. Although significant differences can be detected in the biochemical profile, these parameters probably only mirror the volume depletion seen in some TAH patients and do not reliably distinguish TAH from SIADH.

Funding: Pharmaceutical Company Support - Otsuka America Pharmaceuticals Inc.

SA-PO128

Clinical Severity of Hyponatremia: Thiazides versus Psychotropics versus Combination of Both Hyunju Yoon, Jeong Gwan Kim. *Nephrology Div, Dept of Internal Medicine, Presbyterian Medical Center, Jeonju, Jeonbuk, Korea.*

Background: The aim of this study is to evaluatethe difference of clinical characteristics in patients with hyponatremia, according to the causative drugs such asthiazide diuretics and psychotropic drugs, including tricyclics, selective serotonin reuptake inhibitors, and monoamine oxidase inhibitors, phenothiazines and butyrophenones.

Methods: From 2007 to 2013, 266 patients were diagnosed with hyponatremia (P-Na < 130 mmol/L). We compared clinical characteristics among thiazide (T) group (n=93), psychotropic drug (P) group (n=83), and combination (C) group (n=90). We investigated the severity of hyponatremia based on initial level of serum sodium, initial symptom of the patients and correction time (serum sodium level ≥ 130 mmol/L).

Results: The mean age was younger in P group than in other two groups (65±8 versus 71±10 versus 74±13 year, P=0.000). There were no difference in initial urine osmolality (378±131 versus 396±154 versus 341±168 mmol/L, P=0.061) and serum osmolality (249±30 versus 244±17 versus 245±37 mmol/L, P=0.528), but serum uric acid level was significantly different among groups T, P and C(3.85±2.41 versus 2.87± 1.27 versus 3.42±2.03 mg/dL, P=0.003). Serum uric acid level was higher in group T than in group P by Scheffe's post-hoc analysis (P=0.046). All patients were divided into three categories based on the serum sodium level (mild: >125 mmol/L, moderate: 120-125 mmol/L, severe: <120 mmol/L), patient's symptom (mild: general weakness, moderate: nausea or vomiting, severe: syncope or seizure). Incidences of severe hyponatremia and severe symptoms were not different among groups T, P and C(73.1 versus 67.5 versus 71.1 %, P=0.710, 20.4 versus 30.1 versus 17.8 %, P=0.192). Correction time was significantly different among groups T, P and C (41.98±26.89 versus 34.91±23.96 versus 51.10±43.86 mg/dL, P=0.026). Correction time was longer in group C than group P by Scheffe's post-hoc analysis (P=0.010).

Conclusions: Serum sodium level and severity of symptoms were not different among groups T, P and C. Serum uric acid level was higher in group T than in group P. Correction time was longer in group C than in group P.

SA-PO129

Mild Chronic Hyponatremia and Risk of Hip Fracture in the Elderly Juan Carlos Ayus,[1] Nora Angelica Fuentes,[2] Armando Luis Negri,[3] Diego Giunta,[2] Kamyar Kalantar-Zadeh,[4] Fernan De Quiros.[2] *[1]Renal Consultants of Houston, Houston, TX; [2]Internal Medicine, Hospital Italiano, Buenos Aires, Argentina; [3]Univ del Salvador, Buenos Aires, Argentina; [4]Univ of California Irving, CA.*

Background: Bone has recently been identified as a target for hyponatremia, with fractures occurring as a result. Hip fractures are common in the elderly. We analyzed if there is an independent association between chronic hyponatremia and hip fracture in the elderly.

Methods: We designed a Cohort study of patients >60 years old who had chronic hyponatremia defined as two or more consecutive plasma sodium measurements <135 mmol/L during a period of more than 90 days. Patients were considered normonatremic if they never had a plasma sodium <135 mmol/L. The study was carried out between January 2005 and December 2012. We calculated the hip fracture incidence rate and used multivariate Cox regression analysis to determine hip fracture risk associated with chronic hyponatremia, adjusting for the hyponatremia propensity score, baseline characteristics, comorbidities, and clinical risk factors for hip fracture. Follow-up continued until the time of hip fracture or the end date of the study period.

Results: Of the 31,527 patients evaluated, 288 had chronic hyponatremia and 31,299 were normonatremic. The mean plasma sodium level was 132±5 mmol/L in hyponatremic patients, and 139±3 mmol/L in normonatremic patients (P < 0.001). The incidence rate of hip fractures in hyponatremic patients was 781 per 100,000 person-years (95% CI, 463-1320), which was higher than that of normonatremic patients (365/100,000 person-years; 95% CI, 333-400). The incidence rate ratio was 2.4 (95% CI, 1.26-3.94). The cumulative risk of hip fracture was higher in the hyponatremic group than in the normonatremic group (P < 0.001). The unadjusted and adjusted hazard ratios for hip fracture in patients with chronic hyponatremia were 6.7 (95% CI, 3.2-14.2) and 4.52 (95% CI, 2.14-9.6), respectively. Mortality among chronic hyponatremic patients was double that of normonatremics and the time from surgery to death much shorter.

Conclusions: Mild chronic hyponatremia is an independent risk factor for hip fracture in the elderly.

SA-PO130

Prevalence of and Risk Factors for Hyponatremia in Individuals with Hypertension Kausik Umanath,[1] Jamie P. Dwyer,[2] Alfred K. Cheung,[3] Raymond R. Townsend,[4] Ana C. Ricardo,[5] David Reboussin,[6] Paul L. Kimmel,[7] Daniel E. Weiner.[8] *[1]Henry Ford Hospital, Detroit, MI; [2]Vanderbilt Univ Medical Center, Nashville, TN; [3]Univ of Utah, Salt Lake City, UT; [4]Univ of Pennsylvania, Philadelphia, PA; [5]Univ of Illinois - Chicago, Chicago, IL; [6]Wake Forest Univ, Winston-Salem, NC; [7]National Insts of Health, Bethesda, MD; [8]Tufts Medical Center, Boston, MA.*

Background: The prevalence of and risk factors for hyponatremia are unclear. The Systolic Blood Pressure INTervention (SPRINT) trial randomized individuals with hypertension and CKD or cardiovascular disease risk to assess the effect of intensive blood pressure control on outcomes, providing a large sample of well characterized individuals to evaluate hyponatremia.

Methods: Outcomes in this cross-sectional study included moderate (serum Na 130-134 mEq/L) and severe hyponatremia (serum Na <130 mEq/L). Logistic regression was used to assess factors associated with hyponatremia.

Results: Among 9336 SPRINT participants, 204 (2.2%) had Na 130-134 mEq/L and 20 (0.2%) had Na <130 mEq/L. Table 1 presents characteristics and results of univariate regression. In multivariable logistic regression models, older age, higher eGFR, RAAS blocker use, higher SBP, lower BMI and female gender were associated with hyponatremia. Notably, hyponatremia prevalence among women above 65 was more than twice that of men and younger women, after adjusting for other covariates.

	Serum Na (mEq/L)				
Characteristic	≥145 (n=210)	135-144 (n=8902)	130-134 (n=204)	<130 (n=20)	Univariate p-value
Age, years	69±9	68±9	71±9	73±11	<0.01
Women	41%	35%	54%	35%	<0.01
eGFR (mL/min/1.73m^2)	65±20	72±20	76±25	74±21	<0.01
eGFR <60	41%	28%	24%	40%	0.28
Systolic BP (mm Hg)	142±18	140±15	144±16	139±18	<0.01
Diastolic BP (mm Hg)	77±13	78±12	77±12	72±13	0.02
BMI (kg/m^2)	30±6.4	30±5.7	27±5.7	27±4.3	<0.01
Loop Diuretic Use	11%	4.4%	2.4%	5%	0.18
Thiazide Diuretic Use	38%	39%	43%	55%	0.11
RAAS Blocker Use	56%	57%	66%	80%	<0.01
NSAID Use	38%	38%	40%	35%	0.80

Conclusions: The prevalence of moderate or severe hyponatremia was 2.4% in SPRINT. Hyponatremia is associated with older age, female sex, lower BMI and SBP, RAAS blockade and higher eGFR. Diuretic use was not associated with hyponatremia.

Funding: NIDDK Support

SA-PO131

Efficacy and Safety of Tolvaptan in Hyponatremic Octogenarians Cem I. Sungur. *Nephrology, Medicana International Ankara Hospital, Ankara, Turkey.*

Background: Hyponatremia is the most common electrolyte imbalance and it affects %15 of the elderly population. Approximately %50 of chronic hyponatremia is due to SIADH. V2 receptor antagonists offer a new therapeutic approach to hyponatremia and improve patient outcomes. We present the short term efficacy and safety of tolvaptan in five octogenarian patients with long-standing hyponatremia due to SIADH.

Methods: Five female patients with an average age of 83 (Range: 81 - 87) and with chronic hyponatremia due to SIADH, were admitted to the hospital because of symptomatic hyponatremia. The average serum Na level was 123 mEq/L (Range 117 - 126 mEq/L) on admission. Three patients were on low dose (12.5 mg/day) thiazide previously. Four patients were hypertensive and one had pumonary hypertension. Their left ventricular systolic functions were normal by echocardiogram. None of them had chronic kidney disease. All were euthyroid and hypocortisolism was ruled out. Simultaneous serum and urine osmolality measurements confirmed SIADH.

Results: All patients were treated with tolvaptan 15 mg/day as a single daily dose. None of them received hypertonic sodium chloride infusions. The serum Na level was monitored every 8 hours during the initial 72 hours and their body weights measured daily. ALT and GGT levels were also monitored. In two patients who were on oral furosemide 40 mg/day treatment, this treatment was maintained.The average hospitalization period was 4 days (Range 3-7 days). Average weight loss was 2.8 kg (Range 2.5 -3.8 kg). The average serum Na level at the end of 72 hours was 136 mEq/L (Range 134 - 141 mEq/L). Liver function tests were stable and no neurologic implications were encountered. Three patients were followed for 3 months of which two were on furosemide treatment. When these patients were treated with 15 mg/day, thrice weekly, the serum Na level remained within normal limits.

Conclusions: These findings provide additional information that low dose tolvaptan therapy in elderly hyponatremic patients due to SIADH, may be a safe and effective treatment. Maintenance treatment with thrice weekly 15 mg/day tolvaptan was able to provide a stable serum Na levels and prevent hyponatremic episodes in three patients during a follow-up period of three months.

SA-PO132

Desmopressin-Associated Hyponatremia in Elderly Patients with Nocturia Eun Young Choi, Joon-Sung Park, Gheun-Ho Kim. *Internal Medicine, Hanyang Univ College of Medicine, Seoul, Republic of Korea.*

Background: Desmopressin is being used for treatment of nocturia, but hyponatremia is concerned because of its further impairment of urine dilution in the elderly. This study was undertaken to characterize hyponatremia occurring in adults using desmopressin for nocturia and to examine risk factors for desmopressin-associated hyponatremia.

Methods: Data were retrospectively analyzed from patients who were prescribed desmopressin for nocturia at a urology clinic from September 2010 through February 2013. A total of 236 patients were reviewed, and 61 and 3 patients were excluded due to absence of follow-up serum sodium data and due to prior hyponatremia (< 135 mmol/L), respectively. In addition to demographic and laboratory parameters, hyponatremia-predisposing comorbidities and concurrent medications were evaluated to estimate risk factors.

Results: In 172 patients (144 men, 28 women), age was 69.5 ± 9.6 years. The basal serum sodium was 140 ± 2 mmol/L, and follow-up serum sodium measured at 21 ± 22 days after using desmopressin was 138 ± 5 mmol/L. Desmopressin-associated hyponatremia occurred in 24 patients (14%), and 7 were severe (< 126 mmol/L). In hyponatremic patients, serum sodium decreased by 11 ± 6 mmol/L. Patients with hyponatremia were older than those with normonatremia (78 ± 7 versus 68 ± 9 years, P<0.0001). Notably, none of the patients younger than 65 years had hyponatremia. Although women tended to increase the frequency of hyponatremia (7/28 versus 17/144), the statistical significance was not reached. Presence of either comorbidities or concurrent medications was associated with hyponatremia. Patients with hyponatremia had lower basal hemoglobin (11 ± 2 versus 13 ± 2 g/dL, P<0.001) and serum sodium (139 ± 2 versus 140 ± 2 mmol/L, P<0.05) than those with normonatremia. Multiple logistic regression showed that advanced age (OR, 1.15; 95% CI, 1.03-1.27) and lower hemoglobin levels (OR, 0.64; 95% CI, 0.43-0.94) were independently associated with hyponatremia.

Conclusions: Hyponatremia is not infrequently associated with desmopressin use in adult nocturia patients. Those with advanced age (≥65 years) and anemia are at risk of desmopressin-associated hyponatremia and need to be carefully monitored.

SA-PO133

Trimethoprim Associated Hyponatremia Revekka Babayev, Demetra Tsapepas, Maya K. Rao, Sumit Mohan. *Columbia Univ Medical Center.*

Background: Trimethoprim-sulfamethoxazole (TMP-SMX) is a commonly prescribed antibiotic used at high doses for treatment of pneumocystis pneumonia and other infections. Trimethoprim is structurally related to the potassium-sparing diuretic amiloride and has been associated with hyperkalemia and hyponatremia through blocking of epithelial sodium channels in the distal nephron. Objective: To determine the incidence of hyponatremia in hospitalized patients treated with high dose TMP-SMX.

Methods: We performed a single center retrospective chart review for all hospitalized patients who received high dose TMP-SMX (n=215) in 2012. Patients with congestive heart failure, cirrhosis, Stage 4 chronic kidney disease or higher, baseline hyponatremia, and those on other medications associated with hyponatremia were excluded. Hyponatremia was defined as a serum Na <136 meq/L.

Results: Analysis was restricted to 48 patients who received more than 8mg/kg/day of TMP-SMX for ≥3 days. The population had a mean age of 46.1± 16yrs, was mostly male (68.75%), with eGFR >60 ml/min/1.73m2 (95.8%). Majority of the patients were being treated for a pulmonary infection (91.6%). 77% of patients developed hyponatremia while on therapy with more than half of these patients (20/37, 54%) developing significant hyponatremia (serum Na < 130 mEq/L). Hyponatremia was noted on average 5.3 days after initiation of therapy, was more severe with higher cumulative dose of TMP-SMX and often resolved within a week of drug discontinuation (63.8%).

Nadir sodium	>135	130-135	<130	Total
N (%)	11 (22.9)	17 (35.4)	20 (41.7)	48 (100)
Baseline GFR (ml/min/1.73m^2) >60 30-59	10 1	16 1	20 0	46 (95.8) 2 (4.2)
Daily TMP dose (mean mg/day ± SD) IV PO Day of nadir Na (mean ± SD)	840 ±243 3 8 4.6 ±3	903.5 ±252 0 17 4.1±1.7	969 ± 274 5 15 6.9±5	916 ± 260
Cumulative TMP dose to nadir	3816 ± 3000	3614± 1773	6647 ± 4928	4924 ± 3870

Limitation: In 12 cases we did not have follow up serum Na off of therapy.

Conclusions: There is a high incidence (77%) of hyponatremia associated with the use of high dose of TMP-SMX among hospitalized patients. This is an overlooked and potentially reversible cause of hyponatremia.

SA-PO134

A Low Salt Diet Increases the Estimated Net Endogenous Acid Production in Non-Diabetic CKD Patients Treated with Angiotensin Receptor Blockade

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Background: High net endogenous acid production (NEAP) levels precede renal disease progression in patients with chronic kidney disease (CKD). Angiotensin receptor blockade (ARB) exacerbates metabolic acidosis by inducing a distal-tubular acidification defect. Little is known about the acid-base effects of low salt diet (LSD) on patients treated with ARB. Therefore, we evaluated the effects of LSD on the NEAP in non-diabetic patients with CKD treated with ARB.

Methods: A total of 202 adult subjects from the original trial (ESPECIAL trial: NCT01552954) were enrolled in the present study. Non-diabetic CKD patients were divided into a good LSD compliance group and a poor LSD compliance group. All patients were treated with olmesartan for 8 weeks.

Results: During the interventional 8 weeks, the NEAP in the good compliance group increased compared with the control group (12.9 ± 32.0 versus -2.0 ± 35.0, P = .002). NEAP was positively associated with the good LSD compliance after adjusting for age, gender, body mass index, levels of serum creatinine, sodium, cholesterol, and uric acid, and the NEAP baseline, with previous randomization (r = .135, P = .016). A decrement of 1 g/d of salt intake predicted an increment of 1.75 mEq/d in the NEAP (r = -.180, P = .008). The additional reduction of 2.39 g/d of protein intake with the reduction of 1 g/d of salt intake does not increase the NEAP under ARB treatment with LSD (r = .546, P < .001).

Conclusions: We found that LSD may increase the NEAP in non-diabetic CKD patients using ARB, which suggests that additional dietary protein restriction should be required for maintaining the NEAP in non-diabetic CKD patients with ARB and LSD.

SA-PO135

Serum Sodium and Physical Function in SPRINT

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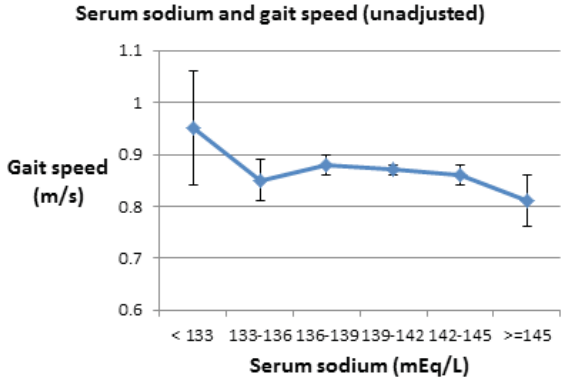
Background: Dysnatremias are common electrolyte disturbances in clinical medicine. Although limited data suggest that hyponatremia may be associated with subtle motor and functional disturbances including gait speed and falls in older adults, this has not been studied in large outpatient populations.

Methods: We analyzed data from participants in the Systolic Blood Pressure Intervention Trial (SPRINT) who underwent gait speed testing (n=2,513) or completed the Falls Efficacy Scale International (FESI) questionnaire (n=2,307) in addition to having measures of serum sodium (Na). Cross-sectional association of serum Na levels with gait speed and FESI score was assessed using linear regression.

Results: Mean (SD) age was 68 (9.4) years and 36% were women. Median (IQ) Na level was 140 (139-142) mEq/L and 387 had a Na level of <135 mEq/L. Estimated GFR decreased with increasing Na levels. Gait speed was fastest (0.95 m/s) in participants with serum Na <133 mEq/L and slowest (0.81 m/s) in those with serum Na >145mEq/L.



In univariate analyses, serum Na levels, age, female sex, CKD, diastolic BP and loop diuretic use were associated negatively with gait speed (p<0.05). Each 10 mEq higher serum Na was associated with 0.05 m/s lower gait speed after adjusting for above variables (p<0.01). Serum Na levels were not associated with FESI scores.

Conclusions: In community dwelling older adults, higher rather than lower serum Na levels are associated with reduced gait speed after adjustment. Further studies are needed to confirm our findings in specific populations such as those with hyponatremia, and in persons with other causes for mobility limitation like dementia and arthritis.

Funding: NIDDK Support

SA-PO136

Initial Serum Sodium Level Predicts Mortality in Patients Undergoing Continuous Renal Replacement Therapy

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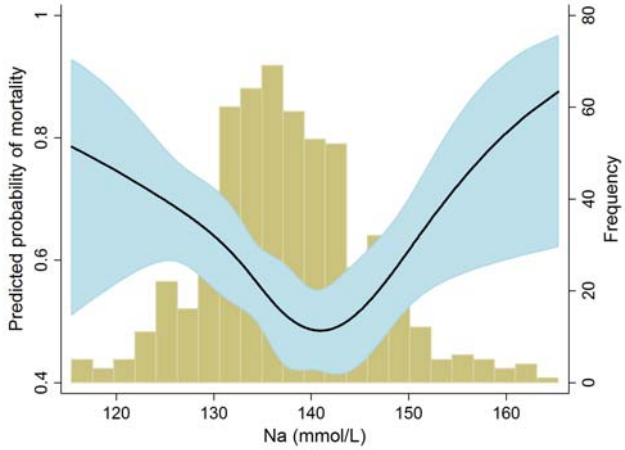
Background: Although dysnatremia is known to be related to the mortality risk, this issue remains unresolved in the patients undergoing continuous renal replacement therapy (CRRT). Furthermore, it is not determined whether the correction of dysnatremia leads to the reduction of mortality risk.

Methods: A total of 569 patients were prospectively enrolled at the time of starting CRRT from May 2010 through September 2013. The patients were divided into 5 groups: normonatremia (135–145 mmol/L), mild hyponatremia (131.1–134.9 mmol/L), severe hyponatremia (115.4–131.0 mmol/L), mild hypernatremia (145.1–148.4 mmol/L), and severe hypernatremia (148.5–166.0 mmol/L). The non-linear relationship between sodium and mortality was initially explored. Next, the odds ratios (ORs) for mortality were calculated after adjustment of multiple covariates.

Results: The relationship between baseline sodium and mortality was U-shaped.



The mild hyponatremic, severe hyponatremic, and severe hypernatremic groups had greater ORs of 30-day mortality (1.65, 1.91, and 2.32, respectively) than the normonatremic group (all Ps < 0.05). However, sodium levels at 24- and 72-hour after the start of CRRT did not predict 30-day mortality. Furthermore, the changes of sodium during 24 or 72 hours did not have any relationship or significance with 30-day mortality, irrespective of baseline sodium level.

Conclusions: Sodium level at the time of starting CRRT was a strong predictor of mortality. However, the change of sodium level or the degree of sodium correction was not associated with the mortality risk.

SA-PO137

Quantifying Vanished Sodium after Hypertonic Saline Infusion in Healthy Subjects

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Background: Sodium (Na^+) is important in maintaining volume homeostasis and blood pressure (BP). Regulation of total body Na^+ and extracellular volume is mainly attributed to the kidney. Recent animal studies have demonstrated non-osmotic Na^+ storage in skin interstitium in response to high Na^+ intake via glycosaminoglycan (GAG) binding. Studies in humans are limited, especially regarding acute salt loading.

Methods: 9 healthy male volunteers pursued a low sodium diet (<50 mmol Na^+/d) for 8 days. In order to achieve 3 mM increase of serum Na+ (according to the Adrogue-Madias formula), on day 8 hypertonic saline was administered IV in 30 minutes, adjusted for total body water (TBW=0.6 x body weight). Before infusion subjects were instructed to void. Urine was collected after 240 min. Blood samples were collected after 0, 10, 60, 240 min. BP was measured with an automatic device (Omron). Cardiac output (CO) and systemic vascular resistance (SVR) were measured with Nexfin (Edwards Lifescience).

Results: Baseline median 24h-urine Na^+ excretion was 15.5 mmol (IQR12.2-25.6) and calculated TBW was 43.9L (43.5-45.8). Ten minutes after infusion (median 220 mmol Na+ in 542 mL), baseline serum Na^+ (137.8 mM (136.5-139.0)) increased to 140.8 mM (139.0-142.5). This was in line with the expected calculated rise of 3.2 mM. Thereafter, serum Na^+ decreased with 1.0mM after 60 min and 2.0 mM after 240 min. According to the Adrogue-Madias formula, this 2.0 mM Na^+ drop at 240 min with median TBW of 44.4L, expected urinary Na^+ excretion would be 95.7 mmol. However, observed total urinary Na^+ excretion after 240 min was 20.6 mmol (median: 49.9 mM in 413 mL) after 240 min, leaving 75.1 mmol Na^+ undetected. This calculation was not corrected for potential residual bladder volume or extrarenal Na^+ loss. BP, CO and SVR remained stable after infusion. Ht (p=0.007) decreased significantly after 240 min, indicating intravascular volume expansion.

Conclusions: After acute hypertonic saline infusion in healthy subjects, 78% of Na^+ expected to be excreted via urine was missing, suggesting that Na^+ may be stored elsewhere, possibly at GAG binding sites.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO138

Association Between Extracellular Water Excess and Renal Outcomes in Patients with Chronic Kidney Disease Reibin Tai,[1] Yasushi Ohashi,[1] Toshiyuki Aoki,[1] Sonoo Mizuiri,[2] Yoshihide Tanaka,[1] Atsushi Aikawa,[1] Ken Sakai.[1] *[1]Nephrology, School of Medicine, Faculty of Medicine, Toho Univ, Tokyo, Japan; [2]Nephrology, Ichiyokai Harada Hospital, Hiroshima, Japan.*

Background: Extracellular water (ECW) excess is a major clinical problem in patients with chronic kidney disease (CKD). However, it is unclear whether ECW excess is associated with disease progression. We investigated whether ECW excess is a risk factor for adverse renal outcomes.

Methods: We performed a retrospective cohort study of 149 patients with CKD who underwent bioelectrical impedance analysis (BIA) between 2005 and 2009. Patients were categorized according to tertiles of extracellular volume status. ECW excess was assessed by examining the ratio of ECW measured by the BIA device (ECW_{BIA}) to total body water calculated using the Watson formula (TBW_{Watson}). Main outcomes were adverse renal outcomes, as defined by a decline of 50% or more from baseline glomerular filtration rate or initiation of renal replacement therapy.

Results: Patients with a higher percentage of ECW_{BIA} to TBW_{Watson} tended to: be older and male; have diabetes mellitus, resistant hypertension, lower renal function and serum albumin, higher proteinuria, and higher frequency of furosemide use. The $\%ECW_{BIA}/TBW_{Watson}$ had a weak negative correlation with body mass index (BMI) (r = -0.36 in male, P = 0.001 and r = -0.28 in female, P = 0.02). In multivariate analysis, male sex, BMI, and proteinuria remained independently associated with $\%ECW_{BIA}/TBW_{Watson}$. During a median follow-up of 4.9 years, patients in the highest tertile of $\%ECW_{BIA}/TBW_{Watson}$ were at greater risk of adverse renal outcomes (16.6 per 100 patient years) compared with those in the lowest tertile (6.0 per 100 patient years) or those in the second tertile (7.5 per 100 patient years) (P = 0.005). After adjustment for covariates, $\%ECW_{BIA}/TBW_{Watson}$ was significantly associated with adverse renal outcomes (hazard ratio 1.21, 95% confidence interval 1.09–1.33, P < 0.001).

Conclusions: ECW excess was independently associated with adverse renal outcomes during a relatively long follow-up period.Proteinuria is independently associated with ECW excess, and leaner patients with CKD may be more susceptible to volume overload.

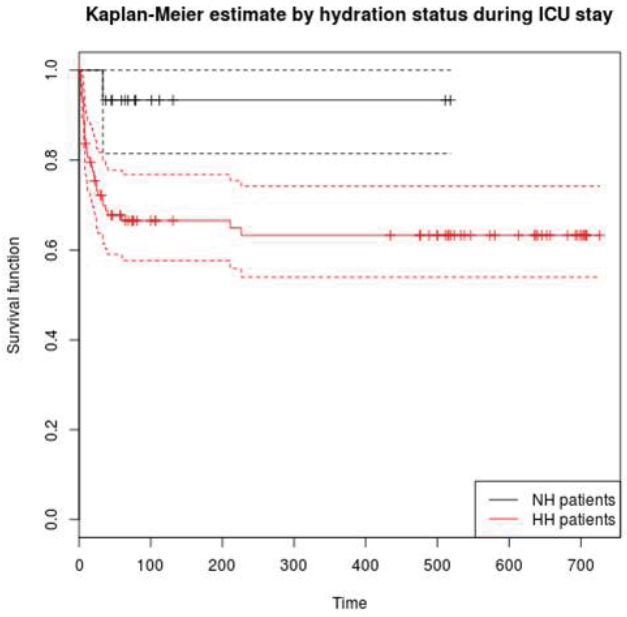
SA-PO139

Evaluation of the Impact of Persistent Fluid Overload on Outcome in Critically Ill Patients Sara Samoni,[1,2] Federico Nalesso,[1] Valentina Vigo,[2] Enrico Tonini,[1] Flavio Basso,[1] Alessandra Brendolan,[1] Monica Zanella,[1] Leonardo Claudino Ribeiro,[1] Jose Jesus Zaragoza,[1] Alessandra Spinelli,[1] Gianluca Villa,[1] Carla Estremadoyro,[1] Carlo Donadio,[2] Claudio Ronco.[1] *[1]IRRIV; [2]Univ of Pisa.*

Background: Data indicate role of fluid overload(FO) in increased mortality,non-recovery of kidney function in patients with acute kidney injury(AKI) and higher rates of complications.Also,mortality seems to be higher till the patient with AKI is exposed to FO.Aim of this study is to evaluate the impact of persistent FO on outcome in critically ill patients.

Methods: A prospective,dual-center study in critically ill patients. Anthropometric,medical history and laboratory data of patients admitted in ICU with expected stay of 72 hours or more were recorded.Assessment of body fluid status was performed using Bioelectric Impedance Vectorial Analysis(BIVA),using a single frequency analyzer,at the baseline and daily for a period of 72-120 hours.Patients were considered normo-hydrated(NH) if hydration level was 72.7%-74.3% of fat-free body mass and hyper-hydrated(HH) if hydration level was >74.3%.

Results: 483 BIVA measurements were taken in 114 patients.A Cox model studied the relation between mortality and hydration status,ceteris paribus.We found that not only the maximum level of hyper-hydration reached in observation period,but also the percentage of days in which patients had FO,was correlated with mortality(p=0.0235),either in patients with or without AKI.



Conclusions: FO is a relevant risk factor for mortality in critically ill patients. Therefore,assessment of body fluid volume in ICU is of great importance,but there are currently few methods to obtain an accurate and timely assessment of hydration status.We suggest the use of BIVA,which is simple,non-invasive and feasible to repeat measurements,in addition to clinical evaluation to estimate hydration status in critically ill patients in ICU.

SA-PO140

Classification of Acid-Base Disorders by Simultaneous Arterial and Venous Blood Gas - An Observational Study Mohamed A. Sheta,[1] Thomas H. Hostetter,[2] Paul E. Drawz.[1] *[1]Univ of Minnesota, Minneapolis, MN; [2]Case Western Reserve Univ, Cleveland, OH.*

Background: Arterial blood gas (ABG) is the gold standard for defining acid-base disorders. A high correlation between arterial and venous pH has led to the routine use of venous blood gases (VBG) to classify acid-base disorders at some institutions. However, the rate of agreement between acid-base diagnoses based on arterial and venous blood gases is unknown.

Methods: All patients at the University of Minnesota Medical Center with a VBG within 60 minutes of an ABG between November 2010 and November 2013 were included in this retrospective study. Patients' acid-base status was categorized based on pH (<7.35 or > 7.45), pCO2 (<40 or >40), and bicarbonate (<24 or >24) from both the ABG and VBG (after applying a correction factor of 0.05 for pH and 5 for pCO2 to VBG values). Primary analyses focused on patients with mild to moderate acid-base disturbances (ABG pH 7.25-7.50).

Results: 1814 patients had a VBG within 60 minutes of an ABG with an ABG pH between 7.25 and 7.50. The average age (SD) was 61.0 (15.7) years and average eGFR was 61.1 (26.4) mL/min/1.73m^2. 318 patients were unclassified based on their ABG. Concordance between acid-base diagnosis based on ABG and diagnosis based on VBG ranged from 37% for respiratory alkalosis to 64% for mixed alkalosis. Results were similar in the subset of patients with a VBG within 15 minutes of an ABG.

	Diagnostic cutoffs				VBG category same as paired ABG	
ABG category	**pH**	**pCO2**	**bicarb**	**Total N**	**N**	**%**
metabolic acidosis	< 7.35	< 40	< 24	120	66	55%
metabolic alkalosis	> 7.45	> 40	> 24	35	21	60%
mixed acidosis	< 7.35	> 40	< 24	172	78	45%
mixed alkalosis	> 7.45	< 40	> 24	56	36	64%
respiratory acidosis	< 7.35	> 40	> 24	118	66	56%
respiratory alkalosis	> 7.45	< 40	< 24	52	19	37%
Total with abnormal pH				**553**	**286**	**52%**
normal ABG pH	≥ 7.35 and ≤ 7.45			943	816	87%
Total				**1496**	**1102**	**74%**

Conclusions: In this study, concordance between acid-base diagnoses based on an ABG and a VBG was only 52% among patients with an abnormal pH on ABG. While there may be a high degree of correlation between pH measured on an ABG and a VBG, classification of patients by ABG and VBG differs significantly.

Funding: NIDDK Support

SA-PO141

Acid-Base Status in Circulatory Failure: Relationship between Arterial and Peripheral Venous Blood Gas Measurements in Hypovolemic Shock

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Background: In severe circulatory failure agreement between arterial and mixed or central venous values is poor; venous values are more reflective of tissue acid-base imbalance. No prior study has examined the relationship between peripheral VBGs and ABGs in hemodynamic compromise.

Methods: Data were obtained prospectively from adult trauma patients. Patients were excluded if the ABG and VBG were drawn > 10 minutes apart.

Results: 178 patients were included. The correlations between arterial (A)-peripheral venous (PV) pH, A-PV pCO_2, and A-PV bicarbonate and systolic blood pressure (SBP) were not statistically significant (p=0.55, 0.17, and 0.09, respectively). Although hypotensive patients had a lower mean A and PV pH and bicarbonate compared to the hemodynamically stable patients, mean A-PV differences for pH, pCO_2, and bicarbonate were similar between hypotensive and normotensive groups. Table 1: ABG and Peripheral VBG Values [Mean (SD)].

	SBP≤90	SBP > 90
n	15	163
A pH	7.26 (0.18)	7.41 (0.09)
PV pH	7.20 (0.16)	7.36 (0.10)
A-V diff pH	0.03 (0.06)	0.05 (0.06)
A pCO_2	38.86 (12.40)	37.62 (7.84)
PV pCO_2	44.37 (13.64)	46.26 (16.21)
A-V diff pCO_2	-3.52 (9.62)	-8.53 (12.36)
A Bicarb	17.51 (6.06)	22.94 (3.11)
PV Bicarb	17.65 (6.34)	24.84 (3.43)
A-V diff Bicarb	-0.22 (3.80)	-1.81 (2.41)

Conclusions: In hypovolemic shock, the peripheral VBG does not demonstrate a higher CO_2 concentration and lower pH compared with arterial blood. Therefore, the peripheral VBG is not a surrogate for the tissue acid-base status in hypovolemic shock, likely due to peripheral vasoconstriction and central shunting of blood to essential organs. This contrasts with the selective venous respiratory acidosis previously demonstrated in central and mixed venous measurements in circulatory failure, which is more reflective of acid-base imbalance at the tissue level than arterial blood. Further work needs to be done to better define the relationship between ABG and both central and peripheral VBG values in various types of shock due to ↓ cardiac output versus ↓ systemic vascular resistance.

SA-PO142

Acid-Base Disorders at Dialysis Initiation: A Multicenter Cross-Sectional Study in Japan

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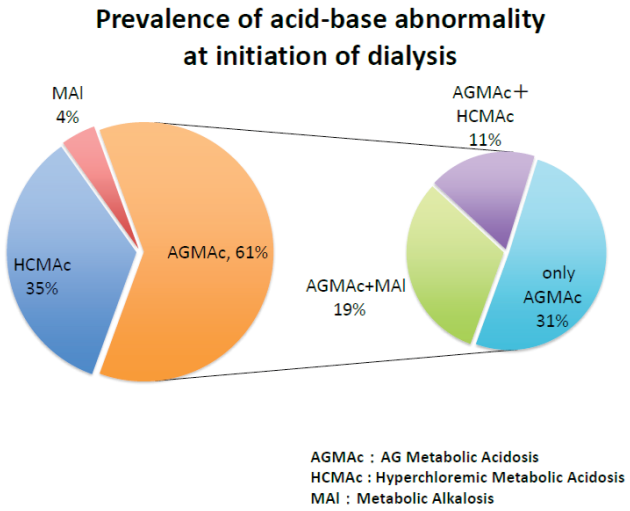
Background: Although anion gap metabolic acidosis (AGMAc) is a major acid base disorder of end stage renal disease (ESRD), only a few studies have investigated the prevalence of acid-base abnormalities of ESRD. The aim of the present study was to clarify the prevalence of acid-base abnormalities and their associated factors in patients at dialysis initiation.

Methods: This study included 1,525 patients starting maintenance dialysis between 10/1/2011, and 9/30/2013. Eighteen care centers in Japan participated in the Aichi Cohort Study of Prognosis in Patients Newly Initiated into Dialysis "AICOPP". After exclusion of 501 (32.8%) patients without acid base data, 1024 (67.1%) patients were enrolled. We defined 5 groups as 1)AGMA+Metabolic alkalosis (MAl) : AG ≥ 16 + corrected $HCO3^-$ ($cHCO3$) ≥ 28, 2)AGMAc : AG ≥ 16 + ($cHCO3^-$) 23 ≥ to < 28, 3)AGMAc + HCMAc (Hyperchloremic metabolic acidosis) : AG ≥ 16 + $cHCO_3^-$ < 23, 4)HCMA : AG8 ≥ to < 16 + HCO_3 < 23 + pH < 7.4, 5)Mal :HCO_3 > 28 + pH ≥ 7.4. Multiple logistic regression analyses were used to identify factors associated with AGMAc.

Results: Prevalence of acid-base abnormality at dyalysis initiation.



In multivariate analyses, estimated eGFR [Odds Ratio(OR) 0.81; 95% confidence interval(CI) = 0.75-0.87], and GI symptoms [OR 1.80; 95% CI = 1.45 - 2.23], not planned initiation [OR 1.98; 95% CI = 1.49 - 2.64], pulmonary edema [OR 1.57; 95% CI = 1.24 - 1.95] were independently associated with AGMAc.

Conclusions: Although AGMAc values increased as eGFR levels decreased, significant AGMA at dialysis initiation was observed only in 61.1% in this study. Mixed acid base types were up to 30% ; MA were 23%. We should not overtreat metabolic acidosis with $NaHCO_{[sup]3[/sup]}$.

SA-PO143

Prevalence of Hyperkalemia and Associated Factors in End-Stage Kidney Disease Patients at Dialysis Initiation-AICOPP Study in Japan

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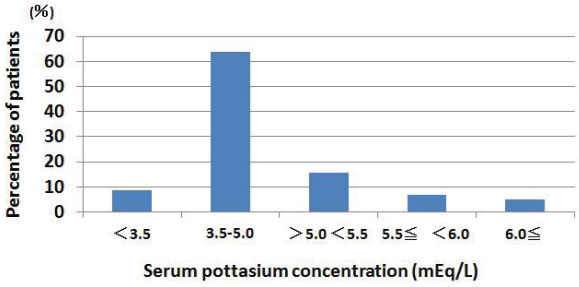
Background: CKD is an important risk of hyperkalemia, and recent studies showed prevalence and associated factors of hyperkalemia in various stages of CKD. GFR of these patients were not so low, and patients at dialysis induction were not evaluated. This is the first study which investigate prevalence and associated factors of hyperkalemia in End-Stage Kidney Disease patients at dialysis initiation.

Methods: This study is a cross-sectional study which data was collected by "Aichi Cohort study of Prognosis in Patients" (AICOPP) newly initiated into dialysis. This study enrolled 1,525 patients (mean age, 67.4 ± 13.0 years; mean eGFR level, 5.40 ± 2.2 mEq/l) who started chronic dialysis between 10/1/2011 and 11/30/2013. We evaluated prevalence of hyperkalemia in these patients, and multiple logistic regression analyses were used to identify factors associated with hyperkalemia in End-Stage Kidney Disease patients at dialysis initiation in Japan.

Results: This research showed the prevalence of hyperkalemia.



In multiple logistic regression analyses showed male sex (OR 1.637, 95%CI 1.027-2.611), eGFR (OR 0.794, 95%CI 0.697-0.904), pH<7.35 (OR 4.310, 95%CI 2.661-6.981), emergency dialysis initiation (OR 2.061, 95%CI 1.252-3.392) were independently associated with hyperkalemia. Gastrointestinal symptom, heart failure, use of angiotensin converting enzyme inhibitor, angiotensin receptor blocker, and diuretics were not associated with hyperkalemia.

Conclusions: In this study, prevalence of hyperkalemia was 27.4% in dialysis induction, and male sex, low eGFR low, pH<7.35, emergency dialysis initiation were associated with hyperkalemia.

SA-PO144

Associations of Serum Bicarbonate Concentration with Cardiovascular Outcomes in the Multi-Ethnic Study of Atherosclerosis (MESA)

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Background: Metabolic acidosis is associated with increased risks of mortality and heart failure in patients with CKD. Whether serum bicarbonate concentrations are associated with incident cardiovascular disease (CVD) in the general population is unknown.

Methods: We performed a cohort study of 6230 participants in MESA, who were free of clinical CVD at baseline. Serum bicarbonate was examined as a continuous variable and in clinically significant categories (<21, 21-22, 23-24, ≥25 mEq/L). Multivariable Cox proportional hazards models were used to test associations of serum bicarbonate with incident cardiovascular events (CVE) (composite of myocardial infarction, resuscitated cardiac arrest, stroke, coronary heart disease death and stroke death) and incident heart failure.

Results: The mean bicarbonate concentration was 23.1 ± 1.8 mEq/L. 331 (5.3%) participants had an incident CVE and 174 (2.8%) had incident heart failure during a median (IQR) follow-up of 8.5 (7.7-8.6) years. Participants with bicarbonate levels <21 mEq/L were more likely to be younger, to be Hispanic, and to have diabetes, higher body mass index, and higher estimated GFR. We found no association between serum bicarbonate and incident CVE or heart failure in unadjusted or adjusted analysis. After excluding patients on diuretics, we found that each 1 mEq/L higher serum bicarbonate concentration was associated with a 14% higher risk of incident heart failure in models adjusted for demographics and CVD risk factors (Adjusted HR 1.14, 95% CI 1.02 to 1.28). Bicarbonate concentrations were not associated with incident CVE in patients not on diuretics.

Conclusions: In a cohort of participants initially free of CVD, higher not lower serum bicarbonate concentrations were associated with an increased risk of incident heart failure in patients not on diuretics. There was no association between serum bicarbonate and incident atherosclerotic CVE.

Funding: NIDDK Support

SA-PO145

Association of Serum Bicarbonate Levels with Ankle Brachial Pressure Index in the Third National Health and Nutrition Examination Survey

Shyamal K. Palit,[1] Jessica B. Kendrick.[1,2] *[1]Univ of Colorado Denver; [2]Denver Health Medical Center.*

Background: Metabolic acidosis, as reflected by a low serum bicarbonate level, may be a modifiable risk factor for cardiovascular disease. Several studies have found that metabolic acidosis is associated with incident and prevalent hypertension. Whether metabolic acidosis is associated with arterial stiffness is unknown.

Methods: The cross-sectional association between serum bicarbonate concentration and peripheral vascular disease as measured by an ankle brachial pressure index (ABPI) >1.3, was examined among 1002 participants in the Third National Health and Nutritional Examination Survey. Serum bicarbonate was examined as a continuous variable and in clinically significant categories (<21, 21-22, 23-24 and ≥ 25 mEq/L). Multivariable logistic regression was used to examine the relationship between serum bicarbonate concentration and an ABPI >1.3.

Results: The mean ± SD age and serum bicarbonate concentration of the study population was 47 ± 21 years and 28.8 ± 4.3 mEq/L, respectively. 72 (7.2%) had an ABPI >1.3. Eighty-four percent and 1.4% had a bicarbonate level >25 mEql/L and <21 mEq/L, respectively. There were no significant differences in age, sex, race, body mass index, smoking status, diabetes, hypertension, estimated glomerular filtration rate, C-reactive protein, low density lipoprotein cholesterol or high density lipoprotein cholesterol levels between participants with higher versus lower ABPI. We found no significantly increased risk of an ABPI > 1.3 in unadjusted or adjusted analyses with serum bicarbonate levels <21, 21-22 or ≥25 mEq/L when compared to the reference group (23-24 mEq/L). When serum bicarbonate was examined as a continuous variable, we found a U-shaped relationship in that lower and higher bicarbonate levels compared to 29.6 mEq/L were increasingly more associated with an increased risk of an ABPI > 1.3 (p=0.013 each for linear and quadratic bicarbonate terms).

Conclusions: We found a U-shaped relationship between serum bicarbonate and risk of ABPI >1.3. Further studies are needed to confirm our results and to evaluate the potential mechanism by which bicarbonate may contribute to arterial stiffness.

Funding: NIDDK Support

SA-PO146

Metabolic Alkalosis in Critically Ill Patients: Learning What We think We Already Know

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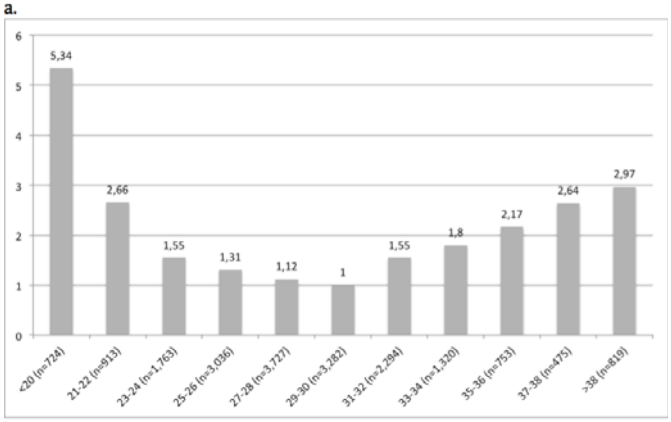
Background: Although commum in intensive care unit (ICU), m has not been studied in controlled studies regarding its associated factors and prognostic implications.

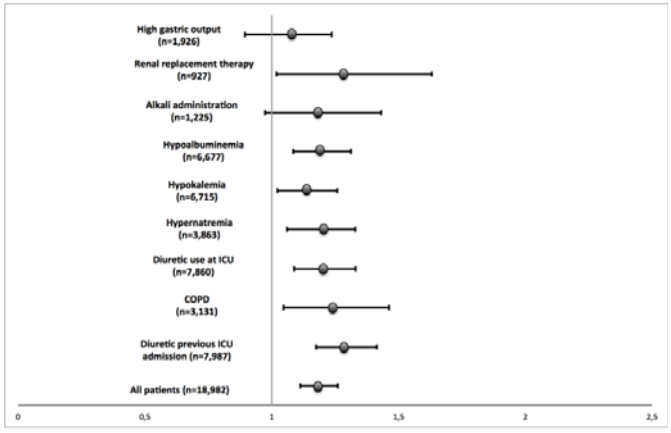
Methods: Retrospective study performed using extracted data from the Multiparameter Intelligent Monitoring in Intensive Care II (MIMIC -II) database.

Results: 18,982 patients were included. Mean age on admission was 63.8±17.5 years and 8,159 were females (43.0%). The mean SOFA and SAPS-I scores on admission were 5.9±4.0 and 13.5±5.9, respectively. Serum bicarbonate (sBIC) showed an inverted J-shaped association with mortality (fig1a). There was an increment in mortality when maximum sBIC was less than 25 or higher than 30mEq/L.

a.



b.



During ICU stay, 5,565 patients (29.3%) had at least one day of sBIC > 30mEq/L. Patients with at least one sBIC level > 30mE/L had higher severity scores at ICU admission. The majority of patients were exposed to multiple possible etiologies - mainly, diuretic use, hypernatremia, hypokalemia and high gastric output. Patients with sBIC>30mEq/L had higher ICU LOS (5.6 [2.8-12.0] versus 2.2 [1.5-3.6], p<0.001), more days on mechanical ventilation (677.4 versus 291.6 per 1,000 patient days of ICU stay, p<0.001) and higher inhospital mortality. After multivariate adjustment, each 5-mEq/L increment in sBIC above 30mE/L was associated with a 22% higher risk of inhospital death. Also, duration of such high sBIC in relation to ICU LOS was associated with mortality. Subgroup analysis showed that the association between high sBIC level and mortality occurs independently of its etiologies, except when associated with high gastric output(fig1b).

Conclusions: Elevated sBIC is common in critically-ill patients; it can be attributed to multiple factors in the majority of cases and its presence and duration has a negative impact on outcome.

SA-PO147

Effect of ZS-9 on Serum Bicarbonate and BUN in a Phase 3 Randomized, Double-Blind, Placebo-Controlled Trial

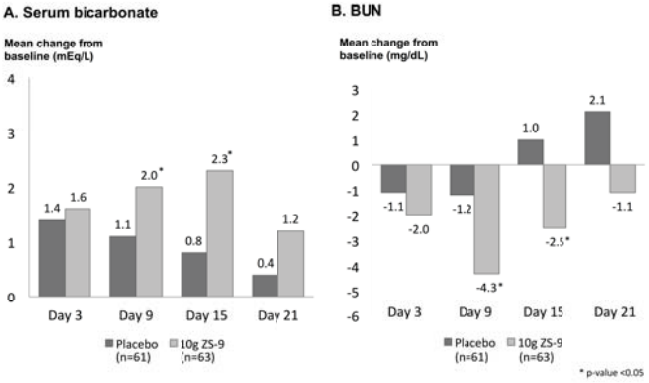
Simon D. Roger,[1] Henrik S. Rasmussen,[2] Philip T. Lavin,[3] Alex Yang,[4] Bhupinder Singh.[5] *[1]Renal Research, Gosford, Australia; [2]ZS Pharma, Inc., Coppell, TX; [3]Boston Biostatistics Research Foundation, Framingham, MA; [4]Xelay Acumen, Inc., Belmont, CA; [5]Apex Research, Riverside, CA.*

Background: Increase in serum bicarbonate (HCO_3) may benefit chronic kidney disease (CKD) patients (pts) by delaying disease progression (Kovesdy 2012, Goraya 2013). In addition, even small increases in HCO_3 can enhance insulin sensitivity, which may be important for CKD pts who are diabetic. ZS-9, a non-absorbed cation exchanger that specifically traps K^+ in the gut, was well tolerated and acutely reduced and maintained serum K^+ over 15 days in a Phase 3 study in pts with hyperkalemia (HK; Singh 2014). Here we present the effects of ZS-9 versus placebo (PBO) on serum HCO_3 and blood urea nitrogen (BUN).

Methods: Pts (N=753) with K^+ 5.0-6.5 mEq/L were randomized (1:1:1:1:1) to ZS-9 (1.25, 2.5, 5 or 10g) or PBO 3×/day for 48 hours (acute phase), after which those with K^+ ≤4.9 mEq/L were re-randomized to PBO or the same ZS-9 acute dose 1×/day on Days 3-15 (extended phase). Serum HCO_3 and BUN were measured on Days 1, 3, 9, 15 and 21. Results from the 10g dose group are presented (n=61 PBO; n=63 ZS-9).

Results: At baseline (BL), mean HCO_3 was 22.8 mEq/L and BUN was 35.8 mg/dL. HCO_3 increased in the ZS-9 10g dose group by 2.0 and 2.3 mEq/L, and BUN decreased by 4.3 and 2.5 mg/dL on Days 9 and 15, respectively ($p < 0.05$; Figure). Pts who switched to PBO exhibited no changes in HCO_3 or BUN. There was a dose-proportional effect on percentage of pts with >10% changes in HCO_3 or BUN.

Figure. Changes in serum bicarbonate and BUN through Day 21.



Conclusions: In HK pts treated with ZS-9, the increase in serum HCO_3 may be explained by removal of ammonium, as illustrated by the decrease in BUN. As prior studies have suggested that amelioration of metabolic acidosis may be kidney-protective (Loniewski 2013), longer term studies are needed to determine whether ZS-9 can delay progression of CKD.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

SA-PO148

The Association of Serum Potassium with Cardiovascular Events and Mortality in Community-Dwelling Individuals: The Multi-Ethnic Study of Atherosclerosis (MESA) Jan M. Hughes-Austin,[1] Dena E. Rifkin,[1] Tomasz Beben,[1] Ronit Katz,[2] Andrew N. Hoofnagle,[2] Rajat Deo,[3] Shunichi Homma,[4] Mark J. Sarnak,[5] David Siscovick,[6] Michael Shlipak,[7] Ian H. de Boer,[2] Bryan R. Kestenbaum,[2] Joachim H. Ix.[1] *[1]UCSD; [2]Univ Washington; [3]UPenn; [4]Columbia Univ; [5]Tufts Univ; [6]NYAM; [7]UCSF.*

Background: High serum potassium (K) is associated with death in chronic kidney disease (CKD) patients, and in acute illness. Associations in other settings are uncertain. We determined associations between K concentrations with cardiovascular (CV) events and mortality in a community-dwelling population.

Methods: Among 6484 MESA participants, we evaluated associations between serum K categories [< 4.0, 4.0-4.5, 4.5-5.0, and ≥ 5.0 mEq/L] with fatal and non-fatal CV events and all-cause mortality using Cox proportional hazards models; and whether associations differed by ACE/ARB use, diuretic use, and CKD status.

Results: Mean age was 62 years, 47% were male, 39% were White, 12% were Chinese, 28% were African, and 22% were Hispanic. Older persons, lower eGFR, higher glucose, and use of ACE/ARB were associated with higher K levels; and diuretics with lower K levels. There was no significant association between serum K and CV events; however, the association differed by ACE/ARB use ($p_{int} = 0.037$), and this interaction was qualitative. Compared to the 4.0-4.5 mEq/L reference category, those with K ≥ 5.0 mEq/L were at 55% higher risk of all-cause mortality in fully adjusted models (95% CI: 1.12-2.14). These associations did not significantly differ by diuretic or ACE/ARB use or by CKD status (p_{int} = 0.84, 0.47, and 0.55 respectively).

Hazard Ratios and 95% Confidence Intervals for Serum Potassium Categories, CV Events, and All-Cause Mortality^

	K < 4.0 mEq/L (n=960)	4.0 ≤ K < 4.5 mEq/L (n=3398)	4.5 ≤ K < 5.0 mEq/L (n=1882)	K ≥ 5 mEq/L (n=249)
CV Events				
All (n=6484)	1.15 (0.90-1.46)	1.0 (ref)	0.84 (0.33-2.14)	1.54 (0.33-7.08)
No ACE/ARB Use* (n=5338)	0.90 (0.67-1.22)	1.0 (ref)	1.09 (0.87-1.36)	**1.61 (1.07-2.43)**
ACE/ARB Use* (n=1149)	**1.81 (1.18-2.77)**	1.0 (ref)	1.35 (0.92-1.96)	0.71 (0.32-1.61)
All-Cause Mortality				
All (n=6484)	1.21 (0.96-1.52)	1.0 (ref)	**1.20 (1.00-1.43)**	**1.55 (1.12-2.14)**

*ACE/ARB use p for interaction = 0.037; bold type indicates statistical significance

^Analyses adjusted for age, sex, race eGFR, urine albumin/creatinine ratio, diabetes, systolic blood pressure, and use of any ACE/ARB, diuretics, other anti-hypertensive medications, NSAIDs, and K supplements

Conclusions: High serum K is independently associated with all-cause mortality in community-dwelling individuals. A similar association was seen for CV events, but was limited to non-ACE/ARB users.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

SA-PO149

Logistic Organ Dysfunction System Score Predicts the Prognosis in Patients with Alcoholic Ketoacidosis Kyungo Hwang, Eun Ju Lee, Hyun Seop Cho, Hyun-Jung Kim, Se-Ho Chang, Dong Jun Park. *Div of Nephrology, Dept of Internal Medicine, Gyeongsang National Univ Hospital, Jinju, Gyeongsangnamdo, Korea.*

Background: Although alcoholic ketoacidosis (AKA) is rarely diagnosed, it might be associated with various organ dysfunctions and death in chronic alcoholics. However, there have been no studies for prognostic factors in these patients. Therefore, we analyzed clinical and laboratory data to evaluate prognostic factors in patients with AKA.

Methods: We retrospectively reviewed the medical records of patients who were diagnosed as AKA from January, 2011 to December, 2013 in Gyeongsang National University Hospital. We enrolled total 34 patients diagnosed as AKA confirmed by clinical manifestations and various laboratory findings.

Results: The mean age was 50.7 years old and male patients were dominant (85.3%). Initial mean arterial pH was 6.92 and anion gap was 39.4.The mean logistic organ dysfunction system (LODS) score was 6.8. Probability of mortality based on LODS score was 38.8% and actually fourteen patients (41.2%) among them was dead. Prevalence of acute kidney injury (AKI) as per RIFLE criteria was 88.2% including risk (23.3%), injury (30.0%), and failure (46.7%). Twelve patients (32.3%) received hemodialysis. There were no differences in age, arterial pH, anion gap, bicarbonate level, lactate level, and occurrence and severity of AKI between survival and non-survival group. However, total LODS score was significantly high in non-survival group, compared with survival group (5.3 versus 9.0, p=0.014). Prothrombin activity, serum platelet number, and albumin level were significantly high in survival group (64.4% versus 42.6%, 209.9 versus 108.4 $x10^3/mm^3$, 3.6 versus 3.1 g/dL, p=0.001, p=0.004, p=0.016, respectively). There was a significant correlation between LODS score and the following parameters: arterial pH (r= .563, p=0.001), albumin level (r= .396, p=0.020), and PT activity (r= .558, p=0.001).

Conclusions: High mortality was found on patients with AKA and LODS score well reflected the prognosis of patients of AKA. Clinician might just as well to use LODS score for predicting the prognosis of AKA patients.

SA-PO150

Preoperative Potassium Disorder and Perioperative Complications Pradeep Arora,[1,2] Prateek Shukla,[4] Leili Pourafkari,[4] Rajiv Ranjan,[1,2] Poorva Bindal,[4] Nader Nader.[3,4] *[1]Medicine, SUNY at Buffalo, Buffalo, NY; [2]Medicine, VAMC, Buffalo, NY; [3]Anesthesiology, VAMC, Buffalo, NY; [4]Anesthesiology, SUNY at Buffalo, Buffalo, NY.*

Background: Potassium disorders have been linked to increased morbidity and mortality in a variety of medical conditions. However, the association of preoperative serum potassium (K) level with postoperative outcome has not been studied. We aimed to study whether preoperative hypokalemia (<4 mEq /l) or hyperkalemia (.>5.5 mEq /l) compared to normal K (4-5.5 mEq/l) were associate with increased 30 days mortality and adverse cardiac outcome (MACE).

Methods: We conducted a cohort study using a prospective database of patients undergoing surgical procedures since 2000in the VA Western New York Healthcare System which are in part reported to the National Surgical Quality Improvement Program (NSQIP). We used multivariate logistic regression to estimate the risk of MACE within 30 days of surgery.

Results: Study included total of 10,861 patients who underwent surgery,between 1998-2013. The differences in baseline characteristics of patients in 3 groups, Group 1- Normal K (4-5.5 mEq/l), group 2 Hypokalemia and group 3 hyperkalemia are shown in Table 1.

	Hypokalemia (n=6914)	Normokalemia (n= 3635)	Hyperkalemia (n=312)
Age	66.3±12.1	65.8±12.6	69.3±10.9
Body Mass Index	28.2±7.4	28.7±6.9	27.8±7.2
Serum Creatinine (mg/dl)	1.35±1.23	1.29±1.09	2.83±2.97
Hematocrit	38.1±6.4	39.5±5.8	35.4±6.6
Serum Sodium (mEq/l)	138±3.6	138.8±3.0	138.2±3.7
Anesthesia Time (min)	185±107	150±79	150±92
Duration of Surgery (Min)	140±107	105±79	105±92
Preopertaive Probability of Death	0.046±0.124	0.022±0.124	0.61±0.123
Probability of Complications	0.172±0.157	0.104±0.092	0.169±0.154
Postop Creatinine	1.65±1.65	1.37±1.20	3.38±3.23
MACE (%)	10	3.3	18.6
30 Day Mortality (%)	5	2.1	16

Multivariate logistic regression analysis revealed that compared to group 1, group 2and group 3 both had increased odds of MACE. (1.34 (1.05-1.87) for hypokalemia and 1.18 (1.12-1.36) for hyperkalemia. Adjusted linear regression analysis showed that decrease of K by 1mEq/l from 5.5 increases MACE by 7%.

Conclusions: Preoperative low and high K are associated with increased risk of MACE at 30 days.

SA-PO151

Overview of Interventions and Outcomes in Hospitalized Patients with Hyperkalemia

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Background: Hyperkalemia is a frequent problem in patients who are admitted to the emergency room and the medical floors, especially in patients with kidney disease. The evaluation for effects of hyperkalemia as well as the interventions performed to treat it are sometimes based on individual judgement rather than a standardized protocol. Some medications given to patients with hyperkalemia might not be warranted and can lead to adverse effects.

Methods: Electronic charts for patients who presented to our hospital from the period between January 1, 2012 to December 31, 2013 were reviewed. Patients with serum potassium levels above 6 were identified. The charts were reviewed for symptoms, ECG findings, interventions performed, repeat serum potassium level, repeat ECG findings, and clinical outcomes.

Results: In our population, the average serum potassium level was 6.5. An ECG was obtained in 70% of the cases; 28% had findings consistent with hyperkalemia (peaked T waves). 40% of patients received intravenous calcium gluconate and 60% received hemodialysis. There was a 20% mortality among this group of patients. 20% of patients were on an ACE-i or an ARB. In our population, there was no correlation between the degree of hyperkalemia and ECG findings, symptoms or outcomes.

Conclusions: Evaluation and management of hyperkalemia varies greatly in our institution even among patients with similar potassium values and similar underlying medical conditions. Given our inability to predict risks from symptoms or objective data, the high mortality rate and the risk of complications from the treatments provided to these patients, we plan to institute a protocol for management of hyperkalemia to try to improve patient outcomes.

SA-PO152

Once Daily ZS-9 for Treatment of Hyperkalemia: Achievement and Maintenance of Normokalemia in Black Patients in a Phase 3, Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial

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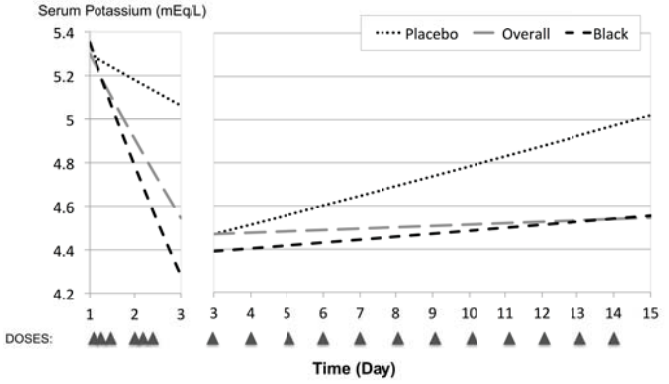
Background: Compared with other races, black patients (pts) have a lower incidence of hyperkalemia (HK) but derive less clinical benefit from RAAS therapies, which are limited by HK (Vardeny 2013). Control of HK facilitates use of cardio- and renoprotective RAASi. ZS-9 is a nonabsorbed cation exchanger that traps K^+ in the gut. A subgroup analysis was conducted in black pts from a large Phase 3 trial of ZS-9 for HK.

Methods: Pts (N=753) with K^+ 5.0-6.5 mEq/L were randomized (1:1:1:1:1) to ZS-9 (1.25, 2.5, 5 or 10g) or placebo (PBO) orally 3× daily for 48hr (acute phase). Pts with K^+ 3.5-5.0 mEq/L (n=542) were re-randomized 1:1 to the same acute dose of ZS-9 or PBO 1× daily on Days 3-15 (extended phase). RAASi was maintained. Results from black pts in the 10g ZS-9 dose group are presented.

Results: Of 753 pts, 87 (11.6%) were black; 36 were in the 10g dose group (n=17 PBO; n=19 ZS-9). Mean baseline (BL) K^+ was 5.4 mEq/L. At 48h, mean K^+ declined to 4.4 mEq/L for ZS-9, compared with 5.2 mEq/L for PBO. In the extended phase, mean BL K^+ was similar between the 10g ZS-9 black subgroup (n=8, 4.4 mEq/L) and the overall 10g ZS-9 and PBO groups (4.5 mEq/L for each; Figure). Mean K^+ was maintained through Day 15 in black pts on 10g ZS-9 (4.6 mEq/L), similar to the overall 10g group, whereas mean K^+ increased with PBO (5.0 mEq/L).

Figure. Phase 3 Mean K^+ (Acute, Extended Treatment) – Placebo (n=158) vs. 10g ZS-9 (n=143) vs. 10g ZS-9 Black subgroup (n=8)



Conclusions: Efficacy and safety of ZS-9 in black pts were similar to the overall population. Hence, by achieving and maintaining normokalemia, ZS-9 may allow use of cardio- and renoprotective drugs in black pts with HK.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

SA-PO153

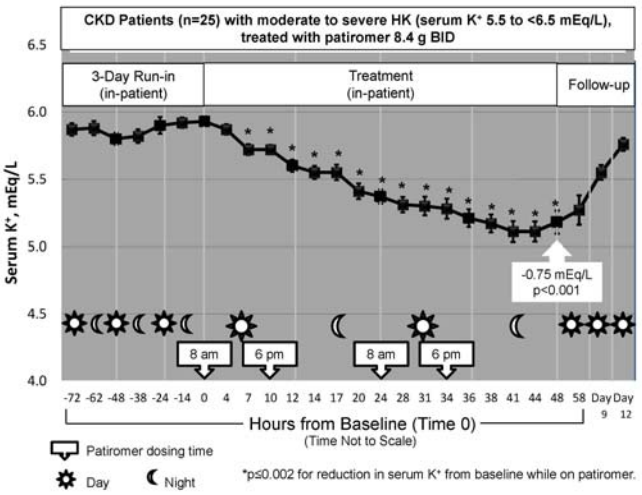
Patiromer Induced a Rapid Onset of Action and Sustained K^+ Lowering throughout the Dosing Period in CKD Patients with Hyperkalemia

David A. Bushinsky,[1] George L. Bakris,[2] Gordon Williams,[3] Bertram Pitt,[4] Martha Mayo,[5] Dahlia Garza,[5] Yuri Stasiv,[5] Elizabeth Li,[6] Lance Berman.[5] *[1]Univ of Rochester, Rochester, NY; [2]Univ of Chicago, Chicago, IL; [3]Harvard Medical School, Cambridge, MA; [4]Univ of Michigan, Ann Arbor, MI; [5]Relypsa, Redwood City, CA; [6]PharmaStat, Newark, CA.*

Background: Patients (pts) with CKD on RAAS inhibitors (RAASi) have a high risk of hyperkalemia (HK), which increases mortality and can lead to RAASi dose reduction/discontinuation. Patiromer, a novel, nonabsorbed metal-free polymer with high K^+ binding capacity and good GI tolerability, has previously been shown to normalize serum K^+ in CKD pts with HK on RAASi. In this study, we determined the onset-of-action of patiromer in CKD pts with HK taking ≥1 RAASi.

Methods: After a 3-day controlled diet (K^+ intake, 60 mEq/d) pts with serum K^+ from 5.5 to less than 6.5 mEq/L received patiromer 8.4 g/dose with AM/PM meals for a total of 4 doses. Serum K^+ was assessed: at baseline (0 hr), 4 hr post-dose, then every 2-4 hr to 48 hr, and at Day 9 and 12.

Results: From a mean baseline serum K^+ of 5.93 mEq/L, a numerical reduction in mean serum K^+ occurred 4 hr after the 1st dose, with a significant reduction (p=0.002) at the next assessment (7 hr after 1st dose). Significant reductions occurred at all subsequent assessments through 48 hr (p<0.001; Figure). Mean serum K^+ <5.5 mEq/L was achieved within 20 hr (p<0.001). At 48 hr (14 hr after last dose), the mean reduction was -0.75 mEq/L (p<0.001). There was no rebound in mean K^+ levels overnight.



Patiromer was well tolerated, with no serious AEs and none leading to withdrawal. The most common AE was mild constipation (2 pts [8%]). No hypokalemia (serum K^+ <3.5 mEq/L) was observed.

Conclusions: Patiromer induced an early and sustained reduction in serum K^+ with no nighttime rebound and was well tolerated in CKD pts with HK on RAASi.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

SA-PO154

Efficacy and Safety of Phosphate Binders for Hyperphosphatemia in Maintenance Hemodialysis Patients: A Systematic Review and Bayesian Network Meta-Analysis

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Background: Hyperphosphatemia is one of the most common complications of end-stage renal disease, which can cause secondary hyperparathyroidism, bone diseases, and cardiovascular calcification. Phosphate binders are widely used to control serum phosphorus levels in ESRD patients. Currently, calcium carbonate, calcium acetate, sevelamer, and lanthanum carbonate are four popular phosphate binders. The objective of this study is to evaluate the efficacy and safety of these four different phosphate binders for the treatment of hyperphosphatemia in ESRD patients.

Methods: Three major database, including Pubmed, ISI Web of Science and Cochrane Central Register of Controlled Trials (CENTRAL), are searched. Bayesian network meta-analysis which combines direct and indirect evidence is used to estimate the relative effects among these five treatments and the probabilities of ranking for each treatment.

Results: Twenty-five trials (3,111 patients) were included in this systemic review. Calcium carbonate, calcium acetate, sevelamer and lanthanum carbonate showed no evident difference in affecting the change of serum phosphorus, Ca×P and parathyroid hormone levels. In contrast to calcium carbonate and calcium acetate, sevelamer and lanthanum carbonate had no significant influence on the change of serum calcium. Compared with calcium carbonate and calcium acetate, the incidence of hypercalcemia is much lower

in treatments using sevelamer or lanthanum carbonate. Existing data are insufficient to conduct a network meta-analysis of the effects of these four phosphate binders on mortality or other patient-level outcomes.

Conclusions: Non-calcareous phosphate binders (lanthanum carbonate and sevelamer) are effective and riskless in treating hyperphosphatemia patients in hemodialysis. Compared with the calcareous phosphate binders, lanthanum carbonate and sevelamer can reduce the incidence of hypercalcium. Additional large randomized, double blinded clinical trials are still required to investigate the effects of different phosphate binders on mortality or other patient-level outcomes.

SA-PO155

Magnesium Lactate in the Treatment of Gitelman Syndrome: Patient Reported Outcomes Caroline M. Robinson, Fiona E. Karet. *Medical Genetics, Univ of Cambridge, Cambridge, United Kingdom.*

Background: Gitelman Syndrome (GS) is an inherited renal electrolyte wasting disorder characterised by hypomagnesemia and hypokalemic alkalosis. The need for magnesium (Mg) and potassium replacement is lifelong and dose requirements may be very high. Oral Mg preparations are often poorly tolerated due to gastro-intestinal side effects. Few studies have assessed efficacy or tolerability of different Mg compounds, either in healthy volunteers or patients with renal disorders. In an effort to improve quality of life for our GS patients, we have trialled use of slow release Mg Lactate (SRMgL).

Methods: Using a purpose-designed questionnaire, we evaluated the experiences of 26 patients with genetically proven GS, whose usual Mg formulation (Mg Glycerophosphate n=19; Mg Oxide n=1; Mg Chloride ± Glycinate or Aspartate n=3) was replaced with SRMgL or who commenced it de novo (n=3).

Results: In the majority, serum Mg levels were reported as improved. Maximal SRMgL doses ranged from 14-126 mEq (2-18 tablets)/day. Median dosage time was 15 months (range 4-40), excluding 3 patients who discontinued it immediately due to difficulty swallowing the tablets. Tablet swallowing was judged more difficult by 8 patients. Overall, 15 patients (65%, n=23) reported an improvement in symptoms since commencing SRMgL, with 7 (30%) reporting no change and 1 (4%) reporting some worsening. None perceived significant worsening. Compared with previous replacement regimens, their reported side-effect profile was lessened or had resolved in 11, similar in 7 and worse in 2. Laxative effect was the principal problem reported with all Mg formulations: for SRMgL this was mild-moderate in 8 patients (35%), more severe in 3 (13%) but absent in over half. Gastrointestinal loss of Mg is counterproductive and dose reduction was sufficient to improve tolerability in the 'severe' group, with all tolerating 2-12 tablets/day (median 6). Other reported side-effects were stomach cramp (n=3), increased thirst (n=1) and insomnia (n=1).

Conclusions: Thus in terms of patient experience, SRMgL is useful for treating hypomagnesemia and is particularly appropriate for those with GS where large doses of Mg may be required to maintain adequate serum Mg levels.

Funding: Government Support - Non-U.S.

SA-PO156

Adolescent Alcohol Use and the Development of Hypertension in Early Adulthood Sarah A. Twichell,[1] Alison Field,[1,3,4] Alan Flint,[3,4] John P. Forman.[2,4] *[1]Medicine, Boston Children's Hospital, Boston, MA; [2]Renal Div, Brigham and Women's Hospital, Boston, MA; [3]Epidemiology, Harvard School of Public Health, Boston, MA; [4]Channing Div of Network Medicine, Brigham and Women's Hospital, Boston, MA.*

Background: While there is extensive literature regarding adult alcohol use and hypertension, little is known about the associations of alcohol use during adolescence and hypertension. This study evaluated associations between pattern and quantity of adolescent and young adult alcohol use and the development of hypertension in early adulthood.

Methods: We analyzed data from the Growing Up Today Study (GUTS), a prospective cohort study of children aged 8-14 years in 1996 and followed with detailed surveys every 1-2 years. We assessed associations of pattern of alcohol use, quantity of alcohol use, and age at alcohol use initiation with the development of hypertension among the 8,605 participants who completed the 2010 survey.

Results: In young men, frequent binge drinking over the past year was associated with a significantly increased odds of hypertension after adjusting for potential confounders (OR 1.70, 95% CI 1.03-2.78). However, there was no significant association between binge drinking during adolescence or quantity of alcohol use and hypertension in early adulthood among males. In young women, binge drinking was not associated with hypertension. However, light (OR 0.55, 95% CI 0.35-0.88) and moderate (OR 0.38, 95% CI 0.19-0.78) alcohol use in young adult females was associated with a significantly reduced odds of hypertension. There was no significant association between age at alcohol use initiation and the development of hypertension in either gender.

Conclusions: Alcohol use patterns in adolescence were not associated with the development of hypertension in early adulthood. However binge drinking in early adulthood was associated with an increased odds of hypertension in males; whereas, low to moderate alcohol use in early adulthood was associated with a decreased odds of hypertension in females. Further study of alcohol use in early adulthood, particularly among boys who are frequent binge drinkers, may provide insights into the early development of hypertension.

Funding: Other NIH Support - T32 training grant

SA-PO157

Initial Screening by Ambulatory Blood Pressure Monitoring Avoids Unnecessary Testing in Children with White Coat Hypertension Peter C. Shorter,[1] M. Khurram Faizan.[2] *[1]Dept of Medicine, Rhode Island Hospital; [2]Dept of Pediatrics, Hasbro Children's Hospital, Providence, RI.*

Background: Ambulatory Blood Pressure Monitoring (ABPM) is a validated method for assessing blood pressure in pediatrics. Children with elevated clinic BP (CBP) often undergo a workup for secondary hypertension (HTN) before a diagnosis of true HTN is confirmed. This leads to unnecessary costs as well as patient and parental anxiety. Pediatric study models using ABPM in the initial evaluation of HTN suggest significant cost savings.

Methods: Retrospective cohort study on children referred by their pediatrician due to elevated CBPs who underwent an ABPM for initial screening. Prehypertension (PH) (90th percentile) and HTN (95th percentile) was defined using age and height criteria outlined in the 4th report (2004). Children with elevated CBP but normal ABPM studies were defined as having White Coat Hypertension (WCH). CBP, ABPM measurements, and diagnostic testing for secondary HTN ordered by the referrer were obtained from patients charts. Reimbursement rates of the common tests for evaluation of HTN including metabolic, lipid, and thyroid panels, EKG, renal ultrasound, pediatric echocardiogram, plasma renin activity and plasma free metanephrines were obtained from the 2013 Medicaid Fee Schedule.

Results: 391 children were referred to the Nephrology Clinic for evaluation of HTN from 2007-2013. 314 children met criteria. 259 (82.4%) were males. Mean age was 14.2 yrs. An ABPM study showed WCH, HTN and PH in 206 (65.6%), 86 (27.4%) and 22 (7%) children, respectively. Of the 206 children confirmed to have WCH, 116 (37%) had diagnostic testing to evaluate for secondary HTN prior to the ABPM study. 302 tests were ordered (2.6 tests/pt) for a total cost of nearly $11,500, with an average of nearly $100 spent per patient with WCH.

Conclusions: Our data show that about a third of children with WCH undergo unnecessary testing and procedures. Postponement of a diagnostic evaluation until after the presence of HTN is confirmed with an ABPM study, may reduce both cost and anxiety. ABPM serves as a cost-effective method not only to streamline the clinical evaluation, but also to minimize needless and expensive testing in children with WCH.

SA-PO158

Risk Factors for Hypertension in Obese Children Emine Sonmez,[1] Salim Caliskan,[2] Nur Canpolat,[2] Lale Sever.[2] *[1]Pediatrics, Istanbul Univ Cerrahpasa Medical Faculty, Istanbul, Turkey; [2]Pediatric Nephrology, Istanbul Univ Cerrahpasa Medical Faculty, Istanbul, Turkey.*

Background: In the present study, we aim to determine i) the risk factors for hypertension in obese children, and ii) its association with artherosclerosis.

Methods: A cross-sectional study was carried out in 60 obese children aged between 10-18 years and 20 age-matched healthy children. Ambulatory blood pressure monitoring was performed in the controls and obese children. Obese children were classified into two clinical groups. Twenty seven of them who had 24h mean arterial pressure >95th percentile and/or who were using antihypertensive drugs were described as hypertensive (HT-obese group), and the remaining 33 were described as normotensive (non HT-obese group). Antropometric parameters [height, weight, body mass index, waist circumference, fat mass (by multi-frequency bioelectrical impedance analysis)], biochemical markers (serum glucose, insulin, HbA1c, LDL, HDL, total cholesterol and triglyceride), inflammation markers [serum high sensitive C reactive protein (hsCRP), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and adiponectin] and the carotid artery intima media thickness (cIMT) were measured in the study population.

Results: In the HT-obese group, waist circumference–SDS, fat mass-z score and serum IL-6 levels were significantly higher as compared to non HT-obese group (p=0.007, p=0.005 and p=0.003, respectively). Logistic regression analysis showed that IL-6 was the only predictor of hypertension in the obese children. In the HT-obese group, cIMT-SDS was significantly higher than the healthy controls (p=0.021); however, there was no significant difference in cIMT-SDS between the HT-obese and the non HT-obese groups.

Conclusions: Our results suggested that both central obesity and increased fat mass seem to be the risk factors for hypertension;however, inflammation appears to play a key role in obesity-associated hypertension. In addition, hypertension itself may be a risk factor for arteriosclerosis in this population.

SA-PO159

The Relationship Between Adiposity and Left Ventricular Mass in Children with Hypertension Tammy M. Brady,[1] Barbara A. Fivush,[1] Kathryn W. Holmes,[2] Edgar R. Miller.[1] *[1]Johns Hopkins Medical Institutions, Baltimore, MD; [2]Oregon Health and Science Univ, Portland, OR.*

Background: Left ventricular hypertrophy (LVH) is highly prevalent at diagnosis of hypertension in children, yet degree of blood pressure (BP) elevation does not predict its presence. We hypothesized that obesity and obesity-related risk factors are associated with left ventricular mass index (LVMI) in hypertensive children and that the relationship between adiposity and LVMI is mediated by both BP dependent and independent pathways.

Methods: We conducted a longitudinal study of 49 treated hypertensive children 3-19 yrs for 1 yr. Student's t-tests, Fisher exact tests and Wilcoxon Rank sum were used to compare those with and without LVH as appropriate. Multivariable regression analyses were used to investigate the relationship of body mass index (BMI) z-score with ΔLVMI and to determine which cardiovascular (CV) risk factors mediated this relationship.

Results: At baseline, 51% were overweight/obese and 41% had LVH. Those with LVH had greater BMI z-score than those without LVH, but this was not associated with 24-hr

ambulatory BPs. Over time, measures of adiposity increased while BP was unchanged. Children overweight/obese at both study visits had the greatest increase in LVMI over time: mean change=6.4 g/m$^{2.7}$ [95% confidence interval (CI) 2.4, 10.5] versus 0.95 g/m$^{2.7}$ (95% CI -3.2, 5.1) among children of healthy weight at each visit (p=0.056). Overweight/obese children with and without baseline LVH had a larger increase in LVMI compared to healthy weight children. Only healthy weight children with LVH had LVMI decrease over time. Baseline BMI z-score was associated with ΔLVMI (β 4.08, 95% CI 1.54, 6.61, p=0.002) after adjusting for age, sex, race, and baseline LVMI. After sequential adjustment for postulated mediating pathways between BMI z-score and ΔLVMI, only pulse pressure and serum aldosterone partially mediated this relationship.

Conclusions: Hypertensive children demonstrate a high BMI z-score which was the greatest risk factor for LVH and increasing LVMI over time. This suggests that greater emphasis on overweight/obesity prevention and treatment should be made among hypertensive children.

Funding: Other NIH Support - NIH/Johns Hopkins Institute for Clinical and Translational Research KL2 Clinical Research Scholar Award Program (5KL2RR025006), Private Foundation Support

SA-PO160

Neonatal Hypertension and Antenatal Exposure to Non-Steroidal Anti-Inflammatory Drugs Mounira Habli,[4] Tammy M. Brady,[1] M. A. Eschenbacher,[2] Zahidee Rodriguez,[3] Emily Defranco,[3] Beena D. Kamath-Rayne.[3] *[1]Pediatric Nephrology, Johns Hopkins Univ School of Medicine, Balitmore, MD; [2]TriHealth Hatton Research Inst, Good Samaritan Hospital, Cincinnati, OH; [3]Perinatal Inst, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; [4]Maternal-Fetal Medicine, TriHealth, Cincinnati, OH.*

Background: Non-steroidal anti-inflammatory drugs (NSAIDS) are known to affect nephrogenesis, yet little is known about the association between NSAIDs used antenatally for tocolysis and neonatal hypertension (HTN).

Methods: We performed a case/control study, identifying premature infants with HTN from a single Level III NICU from 2007-2012. HTN cases were defined as neonates ≥35 weeks corrected gestational age (GA), with systolic blood pressure >100 mm Hg on 3 consecutive days. Controls were randomly chosen from a list of births with similar GA, plurality, and delivery dates. Maternal/neonatal charts of 40 cases and 134 controls were reviewed. Groups were compared for differences in NSAID exposure and maternal/neonatal factors.

Results: Maternal demographic/medical factors were similar between groups. Infants with HTN were slightly more premature (27.7 versus 28.9 weeks), had higher rates of bronchopulmonary dysplasia, umbilical artery catheter placement, and longer hospital stay (all p<0.05). Infants with HTN had higher rates of antenatal exposure to indomethacin (40% versus 24.5%) and sulindac (25% versus 17%), but neither reached statistical significance. Infants without HTN had higher rates of exposure to calcium channel blockers (CCB, 17% versus 24%, p<0.01). In multivariable regression, days of indomethacin exposure was associated with greater odds of neonatal HTN (OR 1.17 [1.00-1.38], p=0.055), after adjustment for days of sulindac exposure, doses of antenatal steroids, CCB exposure, maternal race, infant sex, and GA at birth.

Conclusions: Our data suggest an association of antenatal NSAIDs use with neonatal HTN. Our study was not powered to fully examine the effects of NSAIDs, but longer exposure time may play a role. These findings are worth further investigation in larger studies to assess short and long term neonatal effects of antenatal NSAIDs.

SA-PO161

Risk Factors for Increased Blood Pressure among Aboriginal Children and Adolescents Siah Kim,[1,2] Petra Macaskill,[2] Elisabeth M. Hodson,[1,2] Jennifer Daylight,[1] Rita Angeline Williams,[1] Nicola Vukasin,[1] Rachael Kearns,[1] David M. Lyle,[3] Jonathan C. Craig.[1,2] *[1]Centre for Kidney Research, The Children's Hospital at Westmead, Westmead, NSW, Australia; [2]School of Public Health, Univ of Sydney, Camperdown, NSW, Australia; [3]Dept of Rural Health, Univ of Sydney, Camperdown, NSW, Australia.*

Background: Hypertension is a risk factor for adult chronic kidney disease (CKD). Aboriginal Australian children have a higher prevalence of overweight/obesity, a known risk factor for hypertension in adolescence. We aimed to determine relative changes in blood pressure between Aboriginal and non-Aboriginal children as move into young adulthood.

Methods: A prospective cohort of Aboriginal and non-Aboriginal children commenced in 2002 across 213 high schools across urban, regional and remote NSW. Blood pressure and BMI were measured every 2 years. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were standardized for sex, age and height. We fitted a series of mixed linear regression models adjusting for age, sex, BMI SDS, Aboriginality, birth weight and socioeconomic status (SES).

Results: 3418 (1949 Aboriginal) participants were screened over a total of 11, 387 participant years follow up. At baseline, the prevalence of systolic hypertension was 7.2% (mean age 11 yrs) which increased to 15.4% at eight years follow up. The prevalence of diastolic hypertension remained at 3% from baseline to eight years follow up. Multivariate analysis showed mean SBP SDS increased with age (0.06 SDS per yr, P<0.01) but there was no significant difference between Aboriginal and non-Aboriginal children. Low birth weight was significantly associated with an increase in SBP (0.12 SDS, P=0.01) as was increasing BMI (0.31 SDS per BMI SDS, P<0.01). Lower SES was associated with increasing SBP (<25th quartile: 0.20 SDS, P <0.01). DBP also increased with age (0.03 SDS per yr, P<0.01) and BMI (0.14 SDS per BMI SDS, P<0.01), but lower SES was associated with a lower DBP (<25th quartile: -0.12 SDS, P<0.01).

Conclusions: Increasing BMI remains the greatest modifiable risk factor for SBP and DBP, with prevention of low birth weight and improving SES also important to reduce cardiovascular risk in children and adolescents.

Funding: Government Support - Non-U.S.

SA-PO162

Fat Free Mass, Serum Uric Acid and eGFR as Determinants of BP Levels in an Early Adulthood Lean Cohort: A Cross Sectional Survey to Plan Lifestyle Changes Interventions Antonino Sidoti,[1] Santi Nigrelli,[2] Alberto Rosati,[3] Raffaele Caprioli,[4] Luigi Tonelli,[5] Roberto Bigazzi.[6] *[1]Nephrology, Ospedale Alta Val D'Elsa, Poggibonsi, Siena, Italy; [2]Nephrology, Ospedale dell'Annunziata, Firenze, Italy; [3]Nephrology, Ospedale San Luca, Lucca, Italy; [4]Nephrology, Azienda Ospedaliera Pisana, Pisa, Italy; [5]Consiglio Sanitario Regionale, Regione Toscana, Firenze, Italy; [6]Nephrology, Spedali Riuniti, Livorno, Italy.*

Background: Actual body weight, serum uric acid(UA) are linked with subsequent hypertension(HTN). In pediatric age UA as HTN causal factor seems not related with renal function.

Methods: 18-21y.o. high school last year students, n=2092, 999 females, BP double oscillometric measurements in two different days, height, weight, waist circumference (Wc) were measured. Fat Free Mass (FFM) was calculated (Janmahasatian 2005). In a subset n=60, 29 females, UA, eGFR -via CKD-EPI- were measured. Pearson per quartiles and cluster analysis in n=2092 for FFM, BMI Wc, linear regression (LinReg) with SBP -dependent variable- were performed and in n=60 subset also for UA.

Results: Overweight 11%, obesity 1.1 %, HTN 0.7%, high-normal BP 21.9%, SBP 124.1±12 DBP 70.7±8 BMI 21.8±2.8 Wc 79.6±9cm FFM 48.5±11Kg UA 4.55±0.97mg/dl eGFR 113±13. No DBP relationships. No SBP sex clustering with BMI or Wc. SBP-FFM sex centroids were: females 116-39, males 131-57. SBP-FFM mantained a relationship 1st thru 4thquartile, respectively: 0.223, 0.135, 0.125, 0.206, SBP-BMI and SBP-Wc instead only on4thquartile: 0.162 and 0.142, p<.001. LinReg n=2092, R=0.492, partial r-β: FFM 0.373-0.478, BMI 0.095-0.132, Wc -0.077 and -0.113, p<.001 for all three. LinReg n=60: multiple R=0.655, partial r-β: FFM 0.139-0.161 NS, BMI 0.114-0.106 NS, UA 0.456-0.503 p<.001: males multiple R=0.614, partial r-β 0.530-0.544 p<.001, in females FFM and UA NS, BMI 0.316-0.492 p<0.098. eGFR-UA relationship was -0.55 females, -0.42 males.

Conclusions: In an early adulthood lean cohort, FFM best describes relationship between body weight and SBP in both sexes. UA is related with SBP independently from FFM, BMI and Wc particularly in males. Precocious dietary interventions aiming at reducing UA could be of help in preventing HTN and also in preserving renal function.

SA-PO163

Effects of Catheter-Based Renal Denervation on Ambulatory Blood Pressure, Renal Function and Albuminuria Daniel Greinert, Alexander Plehn, Matthias Girndt, Silke Markau. *Martin Luther Univ (MLU); Dep. of Internal Medicine, Nephrology, Halle (Saale), Germany; MLU; Dep. of Internal Medicine, Cardiology, Halle (Saale), Germany; MLU; Dep. of Internal Medicine, Nephrology, Halle (Saale), Germany; MLU; Dep. of Internal Medicine, Nephrology, Halle (Saale), Germany.*

Background: Catheter-based renal denervation (CBRD) is an invasive procedure, developed to treat resistant hypertension. Few studies focus on renal outcome. Patients with diabetes (DM) and impaired renal function (IRF) were neglected or excluded from the majority of the studies so far. Aim of this study was to emphasize on these patients, evaluate the impact of CBRD on ambulatory blood pressure measurement (ABPM) and renal parameters.

Methods: All adult patients with therapy resistant hypertension undergoing CBRD (Symplicity Flex Catheter, Medtronic Inc.) from Jan. 2011-Mar. 2013 and completing the 6 month follow up were analysed concerning their ABPM, renal function and albuminuria. Patients with secondary hypertension causes were excluded. Additionally evaluated were two subgroups of patients with DM and IRF (cystatin c levels >1.11 mg/l).

Results: All patients and patients with IRF showed no significant effects regarding ABPM. A significant decline of ABPM was found in patients with DM. The renal function improved in patients with IRF. Albuminuria was generally reduced, though significantly only in patients with DM.

ABPM (mmHg)	Baseline	6 month	Diff.	P
All patients (AP) n=129	153,6	149.8	3.8	0.08
Diabetes (DM) n=61	155.2	148.7	6.5	0.044
Renal impairement (RI) n=27	155.2	150.3	4,9	0.36
Creatinin (umol/l)				
AP n=134	88.0	83.0	5.0	0,31
DM n=60	88.6	82.3	6.3	0.24
RI n=29	123.1	84.7	44.4	0.035
Albuminuria (mg/d)				
AP n=91	197.5	146.6	50,9	0.44
DM n=48	307.5	128.4	179.1	0.038
RI n=20	523.9	148.6	375.3	0.11

Conclusions: Only patients with DM showed significant decline in ABPM and albuminuria. IRF seems to improve over time following CBRD. In conclusion CBRD is a controversially discussed procedure with varying published results. Further high quality studies are necessary to find out what the benefits of CBRD are, not only regarding blood pressure, but determining predictors of blood pressure response and impact on target organ damage.

SA-PO164

Effect of Renal Denervation in Patients with Multiple Renal Arteries Margreet F. Sanders,[1] Eva Vink,[1] Willemien Verloop,[2] Peter J. Blankestijn.[1] *[1]Nephrology and Hypertension, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]Cardiology, Univ Medical Center Utrecht, Utrecht.*

Background: During the diagnostic work-up of patients with treatment resistant hypertension we found in approximately one third of patients multiple renal arteries. These patients are often excluded from renal denervation (RDN) conform guidelines. The aim of this study was to investigate the relation between presence of multiple arteries and blood pressure (BP)-lowering effect of renal denervation. Moreover the role of renin angiotensin aldosterone system (RAAS) in the pathophysiology of hypertension in patients with multiple arteries is investigated.

Methods: Hundred-twenty-six patients referred for RDN who underwent non-invasive imaging of the renal arteries before treatment were included in present analysis.

Results: Thirty-four percent had multiple arteries. Sixty-nine patients underwent RDN. Office BP significantly changed from 195(±26)/ 106(±14) mmHg to 165(±24)/ 95(±14) mmHg (P<0.001). BP-reduction in patients with multiple arteries which were all treated was comparable to patients with solitary arteries. However patients with multiple arteries which were not all treated showed a trend towards a less pronounced effect of RDN (ß:12.2, P=0.09). Renal function at 6 months did not differ from baseline in all subgroups. In a multivariable model adjusting for kidney function, PAC determined in standing position was significantly higher in patients with multiple renal arteries compared to patients with single arteries (ß:0.293, 95%CI: 0.020-0.565, p=0.04).

Conclusions: Current analysis suggests that BP reduction may be less pronounced in patients with multiple renal arteries of whom not all arteries were treated with renal denervation. Patients with multiple renal arteries have higher levels of aldosterone, this can be involved in the pathogenesis of hypertension in these patients.

SA-PO165

The Effect of Renal Denervation on Kidney Oxygenation as Determined by Blood Oxygen Level Dependent MRI in Patients with Hypertension Eva Vink,[1] Anneloes De Boer,[1] Willemien Verloop,[2] Wilko Spiering,[3] Michiel Voskuil,[2] Evert-Jan Vonken,[4] Hans Hoogduin,[4] Michiel Bots,[5] Tim Leiner,[4] Peter J. Blankestijn.[1] *[1]Nephrology and Hypertension, UMC Utrecht, Netherlands; [2]Cardiology, UMC Utrecht, Netherlands; [3]Vascular Medicine, UMC Utrecht, Netherlands; [4]Radiology, UMC Utrecht, Netherlands; [5]Julius Center for Health Sciences and Primary Care, UMC Utrecht, Netherlands.*

Background: Renal denervation (RDN) is a promising therapy for resistant hypertension. RDN is assumed to decrease sympathetic activity. Consequently, RDN can potentially increase renal oxygenation. Blood oxygen level dependent MRI (BOLD-MRI) provides a non-invasive tool to determine renal oxygenation in humans. The aim of current study was to investigate the effect of RDN on renal oxygenation as determined by BOLD-MRI.

Methods: Patients with resistant hypertension or the inability to follow a stable drug regimen due to unacceptable side effects were included. BOLD-MRI was performed before and 12 months after RDN.

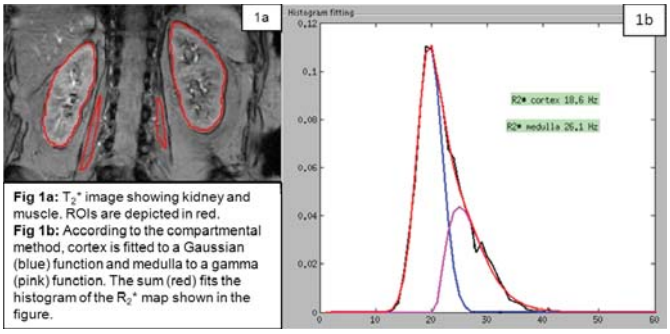


Fig 1a: T_2* image showing kidney and muscle. ROIs are depicted in red. **Fig 1b:** According to the compartmental method, cortex is fitted to a Gaussian (blue) function and medulla to a gamma (pink) function. The sum (red) fits the histogram of the R_2* map shown in the figure.

Results: 54 patients were included, 46 patients (23 males, mean age 57) completed the study. Mean 24-h BP changed from 163(±20)/98(±14) mmHg to 154(±22)/92(±13) mmHg (p=0.001 and p<0.001). eGFR did not change after RDN (77(±18) versus 79(±20) mL/min/1.73m^2 p=0.13). RDN did not affect renal oxygenation (1.5T: cortical R2*: 12.5(±0.9) versus 12.5(±0.9)p=0.94, medullary R2*: 19.6(±1.7) versus 19.3(1.4) p=0.40, 3T: cortical R2*: 18.1(±0.8) versus 17.8(±1.2) p=0.47, medullary R2*: 27.4(±1.9) versus 26.7(±1.8) p=0.19).

Conclusions: RDN does not lead to changes in renal oxygenation one year after RDN as determined by BOLD-MRI.

SA-PO166

The Effect of Renal Denervation on Left Ventricular Hpertrophy Determined by Cardiac Magnetic Resonance Imaging Eva Vink,[1] Willemien Verloop,[2] Wilko Spiering,[3] Evert-Jan Vonken,[4] Tim Leiner,[4] Michiel Bots,[5] Michiel Voskuil,[2] Peter J. Blankestijn.[1] *[1]Nephrology and Hypertension, UMC Utrecht, Netherlands; [2]Cardiology, UMC Utrecht, Netherlands; [3]Vascular Medicine, UMC Utrecht, Netherlands; [4]Radiology, UMC Utrecht, Netherlands; [5]Julius Center for Health Sciences and Primary Care, UMC Utrecht, Netherlands.*

Background: Renal denervation (RDN) is a promising treatment for resistant hypertension. One can hypothesize that as a consequence of decreased sympathetic activation and blood pressure (BP), left ventricular hypertrophy (LVH) will decrease after RDN. The aim of the current study was to investigate the effect of RDN on LVH assessed by cardiac magnetic resonance imaging (cMRI). cMRI has an high accuracy and reproducibility in the evaluation of LV-mass.

Methods: Patients with a SBP ≥160mmHg despite the use of ≥3 antihypertensive drugs or the inability to follow a stable drug regimen due to unacceptable side-effects, meeting in- and exclusion criteria for RDN were included. Patients underwent cMRI before and 12 months after RDN. Body surface area-corrected myocardial mass was quantified by end-diastolic contour tracing on short axis balanced steady state free precession. Left ventricular trabeculae were included in myocardial mass. To assess the effect of RDN on blood pressure (BP), 24-h ambulatory BP measurements at 12 months follow-up were compared to baseline values.

Results: 54 patients were included. Mean age was 58±10 years, 50% was male. One year after RDN, mean ABPM decreased by -7±18 /-5±11 mm Hg (P=0·01/P<0·01). In the patients followed-up in a standardized fashion ABPM decreased by -5±18/-4±12 mm Hg(p=0·11/p=0·09). Mean body surface area indexed left ventricular mass decreased by 3·3±11·5 g/m^2 (corresponding to a 3±11% reduction; P= 0·09).

Conclusions: The current study shows no change in LV-mass, as assessed with cMRI, 12 months after treatment with RDN. However, in patients classified as responder, LV-mass decreases after RDN.

SA-PO167

Activity of Vasocontrictor Systems Is Inversely Related to Renal Oxygenation: A Blood Oxygen Level Dependent MRI Study Eva Vink,[1] Anneloes De Boer,[1] Hans Hoogduin,[2] Michiel Voskuil,[3] Tim Leiner,[2] Michiel Bots,[4] Jaap A. Joles,[1] Peter J. Blankestijn.[1] *[1]Nephrology and Hypertension, UMC Utrecht, Netherlands; [2]Radiology, UMC Utrecht, Netherlands; [3]Cardiology, UMC Utrecht, Netherlands; [4]Julius Center for Health Sciences and Primary Care, UMC Utrecht, Netherlands.*

Background: The renin angiotensin aldosterone system (RAAS) and sympathetic nervous system (SNS) are key factors in the pathophysiology of hypertension. Renal hypoxia is the putative mechanism stimulating both systems. Blood oxygen level dependent MRI (BOLD-MRI) provides a non-invasive tool to determine renal oxygenation in humans. The aim of the current study was to investigate the relation between blood pressure (BP), kidney function and direct and indirect variables of the RAAS, and SNS with renal BOLD-MRI.

Methods: 75 hypertensive patients (38 males) were included. Antihypertensive medication was temporarily stopped. Patients collected urine during 24-hr (sodium, catecholamines), blood samples were taken (creatinine, renin, aldosterone), a captopril challenge test was performed and ambulatory BP was taken.

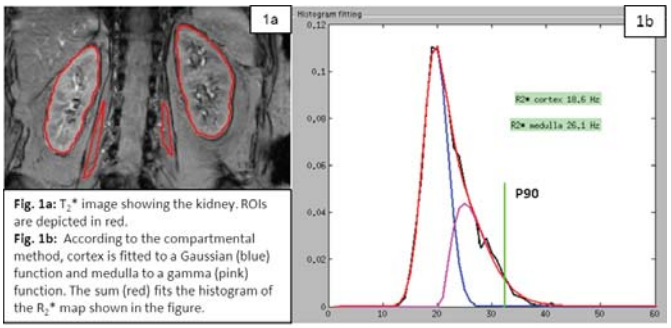


Fig. 1a: T_2* image showing the kidney. ROIs are depicted in red.
Fig. 1b: According to the compartmental method, cortex is fitted to a Gaussian (blue) function and medulla to a gamma (pink) function. The sum (red) fits the histogram of the R_2* map shown in the figure.

Results: Mean age was 58 (±11) years, day-time BP was 167(±19) /102(±16) mmHg, eGFR was 75 (±18) mL/min/1.73m^2. In multivariable regression analysis, renal medullary R2*-values inversely related to eGFR (p=0.02). Moreover the BP-lowering effect of captopril positively related to cortical- (p=0.02) and medullary (p=0.008) R2*-values as well as to P90 (p=0.02).

Conclusions: In patients with hypertension,kidney function as well as direct and indirect variables of the RAAS and SNS relate to renal R2*-values, which can be interpreted as a measure of renal oxygenation.

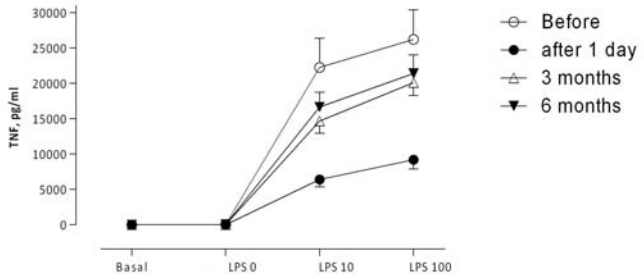
SA-PO168

The Cholinergic Anti-Inflammatory Pathway in Refractory Hypertension Treated with Renal Denervation Marie Hilderman,[1] Abdul Rashid Tony Qureshi,[1] Nils Witt,[2] Björn Anderstam,[1] Annette Bruchfeld.[1] *[1]Renal Medicine, CLINTEC, Karolinska Instt, Stockholm, Sweden; [2]Cardiology, Karolinska Insititutet, Södersjukhuset, Stockholm, Sweden.*

Background: Modulation of renal sympathetic-nerve activity through renal denervation (RDN) has recently been introduced as a treatment for resistant hypertension. By reducing sympathetic tone it may be possible to alter the sympathetic-parasympathetic balance. The autonomic nervous system is partly a regulator of innate immunity via the cholinergic anti-inflammatory pathway (CAP), also referred as the inflammatory reflex which via the vagus nerve inhibits inflammation by an α-7 nicotinic acetylcholine receptor (α7nAChR) mechanism. The aim of this study was to investigate if modulation of renal sympathetic nerve activity could affect CAP and thereby inflammation.

Methods: Ten patients with refractory hypertension (median BP 180/100 mmHg) were analysed for TNF, IL-1 and IL-10 before RDN (Medtronic Inc), 24-h after and at 3 and 6 months follow up. Whole blood samples were stimulated *ex vivo* at each time point with two concentrations of LPS (10 and 100 ng/mL) to induce an inflammatory reaction and TNF, IL-1 and IL-10 were again measured at these concentrations.

Results: Median blood pressure was significantly lower (p=0.03) at 6 months (median BP 162.5/91.5). Levels of **TNF** were significantly lower 24-h after RDN (LPS 0, 10 and 100 ng/mL; p=0.0009, p=0.0009 and p=0.001) (see fig.) **IL-1** (p=0.0001, p=0.002 and p=0.005) whereas **IL-10** levels were significantly higher (p=n.s, p=0.02 and p=0.01). These differences however declined during follow up and were at 6 months similar to baseline.



Conclusions: RDN affects the CAP and cytokine release ex vivo but the effect seems to wane over time. Modulation of parasympathetic nerve activity is an emerging anti-inflammatory treatment but current RDN techniques may not have long-lasting beneficial effects.

SA-PO169

Real Life' Experience of Renal Denervation for Resistant Hypertension From Two NHS Hospitals in Birmingham, UK Mohammed Awais Hameed,[1] Mark R. Pucci,[2] Dr Una Martin,[2] Richard Watkin,[1] Sagar N. Doshi,[2] Jonathan S. Freedman,[1] Peter Riley,[2] Jonathan N. Townend,[2] Lisa J. Tebbit,[1] Louise Beesley,[2] Paul Crowe,[1] Graham W. Lipkin,[2] Indranil Dasgupta.[1] *[1]Heart of England NHS Foundation Trust, Birmingham, West Midlands, United Kingdom; [2]Univ Hospitals Birmingham NHS Foundation Trust, Birmingham, West Midlands, United Kingdom.*

Background: Resistant hypertension is common in the hypertensive population with reported prevalence of 5-30%. Renal denervation has been a promising new treatment option for such patients, however, the recently published SIMPLICITY HTN-3 trial did not meet its primary (change in office systolic BP at 6 months) or secondary (change in mean 24-hour ambulatory systolic BP) efficacy endpoints, but has been shown to be safe.

Methods: We report our experience of renal denervation from combined data of two specialist hypertension clinics in Birmingham. Patients who were identified to have truly resistant hypertension (by careful exclusion of secondary causes and non-adherence) underwent renal denervation. Baseline demographics, clinic BP, ambulatory BP and medications were recorded. Patients were followed up for six months with repeat measurements.

Results: Thirty Three patients have completed the six months follow up. Eighteen were males. The mean age [±SD] was 53.7 [±11.3] years. On average patients were taking 4.40 [±1.79] medications at baseline and 4.30 [±1.66] medications at six months. Mean [±SD] clinic systolic BP at baseline was 184.3 mmHg [±23.6] and mean daytime ambulatory systolic BP was 163.4 mmHg [±18.3]. There was a significant reduction in clinic systolic BP of 13.5 mmHg at six months (95% CI -4.54 to -22.5; p=0.005). There was a statistically non-significant drop in daytime ambulatory systolic BP (11.4 mmHg; 95% CI +4.76 to -27.6; p=0.159). 42% of patients had a clinically significant reduction in clinic BP of 10 mmHg or more (mean 30.2 mmHg; 95% CI -19.6 to -40.9; p<0.001) and 39% had reduction in daytime ambulatory systolic BP of 5 mmHg or more (mean 19.5 mmHg; 95% CI -13.0 to -25.9; p<0.001).

Conclusions: Our 'real life' data suggest that renal denervation does appear to be an effective treatment option for some patients with resistant hypertension, whilst in others it has no effect.

SA-PO170

Estimated 24 Hour Urine Sodium Is Correlated with Blood Pressure in General Population: 2009-2011 Korean National Health and Nutritional Examination Survey Ho Jun Chin,[1] Jieun Oh,[2] Jeonghwan Lee,[3] Eun Jung Kim,[2] Jung-Woo Noh.[4] *[1]Internal Medicine, Seoul National Univ Bundang Hospital, Seongnam, Republic of Korea; [2]Internal Medicine, Hallym Univ, College of Medicine, Kangdong Sacred Heart Hospital, Seoul, Republic of Korea; [3]Internal Medicine, Hallym Univ, College of Medicine, Hangang Sacred Heart Hospital, Seoul, Republic of Korea; [4]Internal Medicine, Hallym Univ, College of Medicine, Kangnam Sacred Heart Hospital, Seoul, Republic of Korea.*

Background: We analyzed population-based data from a nationwide cross-sectional health survey to determine the association of estimated urine sodium excretion over 24-h (24HUNa) with hypertension.

Methods: To investigate the relationship of blood pressure to salt consumption, we analyzed data from 19,476 participants in the 2009-2011 Korean National Health and Nutritional Examination Survey. Urinary sodium excretion over 24-h was estimated from spot urine tests using Tanaka's equation. The study subjects were stratified into hypertensive and normotensive groups.

Results: Hypertensive participants (n=6,552, 33.6%) had higher estimated 24HUNa, 150.4 ± 38.8 mEq/day, than normotensive participants, 140.5 ± 34.6 mEq/day (P < 0.001). The association between 24HUNa and blood-pressure outcomes was not affected by adjustment for other risk factors for hypertension (odds ratio 0.001; 95% confidence interval 0.001 - 0.003; P < 0.001). Differences in 24HUNa between hypertensive and normotensive subjects were significant in the young and middle-aged populations. Although 24HUNa did not differ significantly in elderly subjects (> 65 years old), SBP and DBP of hypertensive persons were significantly higher than those of normotensive persons. Increases in 24HUNa of 100 mEq/day were associated with a 6.1 ± 0.3/2.9 ± 0.2 mmHg increase in systolic/diastolic blood pressure in all participants.This effect was stronger in hypertensive participants (increase of 8.1 ± 0.5/ 3.4 ± 0.3 mmHg) and smaller in normotensive participants (2.9 ± 0.3/ 1.3 ± 0.2 mmHg).

Conclusions: These results support recommendations for low salt intake in Korean population and suggest the importance of moderating sodium intake beginning in the early life.

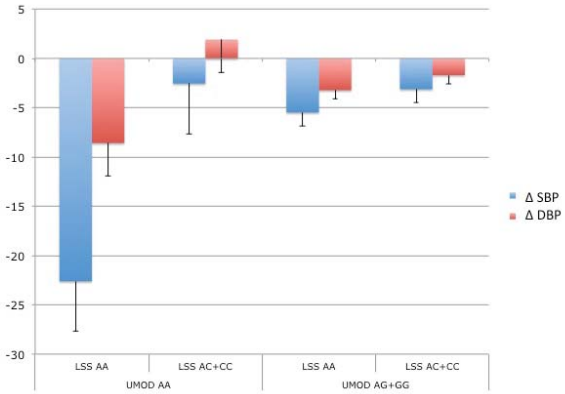
SA-PO171

Genes Involved in Blood Pressure Response to Chronic Low Salt Intake: Identification of a New Pathway Chiara Lanzani,[1] Guido Gatti,[1] Lorena Citterio,[1] Elisabetta Messaggio,[1] Simona Delli Carpini,[1] Chiara Maggioni,[1] Marco Simonini,[1] Nunzia Casamassima,[1] Laura Zagato,[1] Luca Rampoldi,[1] John Hamlyn,[2] Paolo Manunta.[1] *[1]San Raffaele Scientific Institution, Milan, Italy; [2]Univ of Maryland, Baltimore.*

Background: Due to the 'preventive paradox' and to the large inter-individual variation in blood pressure (BP) response to salt reduction, it is not always the case that every individual will ultimately benefit from a moderate reduction in salt intake. A moderate reduction in salt intake reduces BP in most but not all individuals. Recently, the uromodulin (UMOD) gene has been associated with renal damage and hypertension. The present study evaluated the response of Endogenous Ouabain (EO), candidate genes related to its synthesis sterol synthase (LSS), and UMOD polymorphisms in response to a chronic low Na diet.

Methods: 394 naïve hypertensives followed a low Na intake. In the analysis, only compliant patients who reduced urinary sodium at least of 40% of basal value or achieved a urinary sodium less than 100 mmol/day were included.

Results: In the compliant group (170 m, f ~60% of all patients), the reduction of UNa excretion was associated with significant increases in PRA ((β=-0.228, p=0.006) and aldosterone (β=-0.252, p=0.002) as expected. A direct (β=0.213) relationship (p=0.026) between the change in EO and BP was found. When LSS and UMOD gene variants were analyzed together, a significant interaction was detected: those patients homozygous for the AA alleles of both genes displayed a 5-fold greater decline in SBP than patients carrying other allele combinations.



Conclusions: In response to a low salt intake, circulating EO and BP both increased in some patients, and may be linked with adverse outcome. Among other patients, the interaction of LSS and UMOD amplifies the drop in BP evoked by reduced Na intake.

SA-PO172

Effects of Atorvastatin on Salt Sensitivity and Blood Pressure in Hypertensive Patients (DUET Study) Akira Ishii,[1] Masato Kasahara,[2] Hideki Yokoi,[1] Takashige Kuwabara,[1] Shinji Yasuno,[2] Kiyoshi Mori,[3] Akira Fujimoto,[2] Sachiko Tanaka,[2] Noriko Satoh-Asahara,[4] Takashi Sakamoto,[5] Narito Morii,[6] Kazuko Horii,[7] Takeru Sakai,[8] Masashi Mukoyama,[1] Kenji Ueshima.[2] *[1]Dept of Nephrology, Kyoto Univ Graduate School of Medicine; [2]Inst for Advancement of Clinical and Translational Science, Kyoto Univ Hospital; [3]Medical Innovation Center, Kyoto Univ; [4]National Hospital Organization, Kyoto Medical Center; [5]Fukuchiyama City Hospital; [6]Morii Clinic; [7]Horii Clinic; [8]Kyoto Ohashi General Hospital.*

Background: It is reported that statins have an anti-hypertensive effect, but precise mechanisms have not yet been revealed. In order to clarify the effects of atorvastatin on salt sensitivity and blood pressure (BP) in hypertensive patients, we conducted DUET study.

Methods: Hypertensive patients with high LDL-C levels without taking any anti-dyslipidemia drugs were registered. (1st phase) After they started taking amlodipine, they took low-salt diet (6 g/day, 3d) and collected 24-hr urine sample, measured BP. Then they took normal-salt diet (10 g/day, 3-7d), and collected 24-hr urine and measured BP. (2nd phase) Then, they started atorvastatin additionally. After one to four weeks, they repeated the same regimen. The primary endpoint was a slope change of pressure-natriuresis curve. The secondary endpoints were the changes of BP, sodium excretion, urinary and blood laboratory data.

Results: Of 32 registered patients, 30 (mean, 59.5 years) were eligible for this study (FAS). The initial systolic BP was 141.0±16.2 mm Hg, and LDL-C level was 166.8±20.6 mg/dl. There was no significant change in the slope of pressure-natriuresis curve after atorvastatin treatment. Atorvastatin significantly decreased mean BP under low-salt diet (93.3±7.1 versus 90.4±6.5 mmHg, p=0.016), but not under normal-salt diet. Serum LDL-C, TG and potassium levels decreased significantly, but urinary protein, sodium and potassium did not change after taking atorvastatin.

Conclusions: Treatment with atorvastatin did not change the slope of pressure-natriuresis curve. However, atorvastatin enhanced the BP-lowering effect under salt restriction, suggesting its additional mechanism of BP handling.

Funding: Pharmaceutical Company Support - Pfizer Company

SA-PO173

Subclinical Adrenomedullin Levels Help Distinguish between Future Aldosterone Producing Adenoma and Essential Hypertension Ryan J. Altenburg,[1] Austin Parker,[2] Dustin J. Little,[1] Stephen W. Olson.[1] *[1]Nephrology, Walter Reed National Military Medical Center; [2]Nephrology, Naval Medical Center Portsmouth, Portsmouth, VA.*

Background: Adrenomedullin levels (ADM) are elevated at diagnosis of aldosterone producing adenoma (APA) and reduced after surgical resection. APA cells have ADM receptors. ADM both stimulates APA cell proliferation and inhibits angiotensin-II driven aldosterone secretion in vitro without altering basal production. This dysregulation could manifest an autonomous adenoma. We hypothesize that ADM is a principle participant in the early pathogenesis of APA.

Methods: We performed a Department of Defense Serum Repository case-control study comparing ADM before histopathologic diagnosis of 11 APA patients to 30 age, race, sex, and age-of-serum-matched essential hypertension disease controls (HTN-DC). ADM were measured by ELISA. Data analysis was performed with Fisher's exact test and Student's t test for categorical and continuous data, respectively.

Results: APA patients had a higher average index ADM than matched HTN-DC (17.6 VS. 7.5 pg/ml; p<0.001). There was no change in ADM from index to last (mean 15 and 5.1 years before diagnosis respectively) sample (17.6 versus 17.5 pg/mL, p=0.98). A higher percent of APA patients had index ADM >9.9 pg/ml and >19.9 pg/ml compared to matched HTN-DC prior to diagnosis (82% versus 20%, p<0.001 and 46% versus 3%, p=0.003 respectively).

Conclusions: ADM are elevated at least 15 years prior to APA diagnosis compared to HTN-DC. ADM remains unchanged over time which supports an innate overproduction. ADM levels could help distinguish which hypertensive patients are at risk to develop APA. ADM may also assist in APA diagnosis because it is unaffected by Renin Angiotensin Aldosterone System (RAAS) blockade medications. Finally, ADM may represent an attractive future target for medical intervention. Future prospective evaluation is required.

Funding: Other U.S. Government Support

SA-PO174

Adrenal Vein Sampling: OHSU Experience 1995-2013 Adam Protain,[1] Frederick S. Keller,[2] Kevin C.J. Yuen,[3] Jose F. Rueda.[1] *[1]Nephrology, OHSU, Portland, OR; [2]Dotter Interventional Inst, OHSU, Portland, OR; [3]Endocrinology, OHSU, Portland, OR.*

Background: Distinguishing unilateral aldosterone-producing adenomas (APAs) from bilateral idiopathic hyperplasia is mandatory for surgical treatment of primary aldosteronism (PA). Adrenal venous sampling (AVS) is considered the gold standard for identification and localization of the lesion causing PA. However, the success rate of this procedure depends on the expertise of the angiographer. Success rates have been reported to be up to 96% in some centers in the U.S. with a complication rate of 2.5%.

Methods: A retrospective analysis of all AVS procedures performed at Oregon Health and Science University for PA between June 1995 and June 2013. A ratio of adrenal vein to IVC cortisol concentration of 5:1 or greater was used to confirm successful catheterization of the adrenal veins (SI: Sensitivity Index). Right and the left adrenal vein aldosterone concentrations were divided by their respective cortisol concentrations, and the ratio from high to low was calculated (LI: Lateralization index). A ratio of more than 4:1 was used as the cutoff for diagnosing unilateral disease. Concurrently, CT/MRI reports were reviewed, when available, and complications were noted.

Results: AVS for PA was performed successfully in 105 of 126 (83.3%) procedures and 101 of 115 patients (87.8%). AVS was successfully repeated in 10 of 11 attempts (90.9%). More than half [54/105 (51.4%)] of the successful AVS procedures had a LI ≥4:1, and 31/54 (57.4%) patients of this group underwent an adrenalectomy at OHSU. Pathology reports yielded 23 APAs, 6 glands adrenal hyperplasia, 1 normal adrenal gland, and 1 adrenal gland with hemorrhage. CT/MRI and AVS concordance was 53%, and 5.8% of AVS lateralized to opposite side of a mass seen on CT/MRI. Asymptomatic adrenal hemorrhage during adrenalectomy was the only noted complication (complication rate 0.8%).

Conclusions: Adrenal vein sampling was performed successfully in 87.8%of patients at our institution with minimal complications. The high success rate of AVS at our institution is highly dependent on the proficiency of the angiographer.

SA-PO175

Blood Pressure, Graft Survival and Renal Function in Kidney Allograft Recipients – The Effect of Intervention Christian Morath,[1] Peter Schnuelle,[1] Vedat Schwenger,[1] Martin G. Zeier,[1] Gerhard Opelz.[2] *[1]Dept of Nephrology, Univ of Heidelberg, Heidelberg, Germany; [2]Transplantation Immunology, Univ of Heidelberg, Heidelberg, Germany.*

Background: Long-term kidney allograft function is strongly correlated to the blood pressure. Systolic blood pressure (bp) above 140mmHg determines reduced graft survival. The database of the Collaborative Transplant Study (CTS) provides a high number of renal transplant recipients and their long-term follow-up.

Methods: A total of 18982 patients with continuous information on blood pressure, s-creatinine and graft outcome were followed over a time period of 10 years. Systolic blood pressure at year 1 and year 5 was categorized either <140mmHg or >140mmHg. Only patients with normal renal function s-creatinine <1.4mg/dl at year 1 post transplantation were analysed. At year 10 renal function and graft survival were analysed. Altogether four groups were followed: A: bp<140 at year 1 and year 5; B bp>140 at year 1 and <140 at year 5; C: bp <140 at year 1 and >140 at year 5 and bp >140 at year 1 and >140 at year 5.

Results: 10-year graft survival according to A-D was: A 83.3%(p<0.00001 compared to B,C and D), B 78.2% (p<.007 compared to C and p<0.0001 compared to D), C 75% and D 71.8%. Kidney function, e.g. the relative number of recipients with s-creatinine < 1.4mg/dl was: A 68.9% (p<0.0005 A compared to C and D; p n.s. for A compared to B), B 66.4% (p<0.02 B compared to D), C 63% and D 60.7%. The relative number of patients with bp >140mmHg after year 10 was 41.6%.

Conclusions: Blood pressure control significantly improves graft function and increases the number of patients with excellent graft function 10 years plus. In addition treatment intervention between year 1 and 5 (group B) has a significant impact on graft survival and renal function. In contrast inadequate blood pressure control (group C and D) resulted in an inferior graft outcome and a reduced relative number of recipients with excellent renal function. After 10 years of follow-up a significant number of patients has no adequate blood pressure control.

Funding: Government Support - Non-U.S.

SA-PO176

Nocturnal Hypertension Is the Dominant Component of the Blood Pressure Burden in Renal Transplant Patients Francesca Mallamaci, Rocco Tripepi, Daniela Leonardis, Angela Mafrica, Maria Carmela Versace, Pasquale Fabio Provenzano, Giovanni Tripepi, Carmine Zoccali. *CNR-IFC/IBIM and Nephrology and Transplantation Unit, Reggio Calabria, Italy.*

Background: 24h ambulatory monitoring (ABPM) is formally recommended for the diagnosis of hypertension at community level (BMJ. 2012 Jan 13;344:e181). Hypertension is common in renal transplant patients but the relevance of ABPM for the care of these patients is undefined.

Methods: We performed systematic ABPM recordings and carotid intima media thickness measurements (echo-color Doppler) in a series of 240 renal transplant patients representing the 90% of the whole renal transplant population at a single institution.

Results: The average 24h ABPM was 125±12/77±9 mmHg and the daytime and night time average BPs values were 126±12/78±9 mmHg and 123±13/74±10 mmHg, respectively. Ninety-six patients were classified as hypertensive (40%) by ABPM criteria (>130/80, ESH Guidelines 2013) and 44 (18%) by conventional, office criteria (>140/90 mmHg). As much as 35% of patients had a night/day systolic BP ratio >1 denoting a nocturnal rise in BP. The average night-time Systolic BP (r=0.24, P=0.001) and the night day systolic ratio (r=0.21, P=0.001) were strongly related to IMT and these associations were much more robust than that of average 24h systolic BP with the same outcome measure (r=0.16, P=0.04). In contrast average day-time and office systolic BP did associate with IMT (r=0.12, P=0.13 and r=0.11, P=0.17). In analyses adjusting for age, gender, triglycerides, hemoglobin, 24h average systolic ABPM, eGFR and past CV comorbidities both average night-time systolic BP and the night/day ratio remained independently related (β=0.32, P=0.03 and β=0.14, P=0.03, respectively) with IMT indicating that nocturnal hypertension is a relevant component of the BP burden in renal transplant patients.

Conclusions: Nocturnal hypertension is exceedingly frequent (35%) in renal transplant patients and it is the sole BP component showing an association with carotid atherosclerosis in this population. Current recommendations by transplant guidelines to diagnose and monitor hypertension exclusively by conventional BP measurements need to be reconsidered.

SA-PO177

In Preeclampsia, Early Hyperuricemia Is due to Increased Renal Reabsorption Refael Gery. *Nephrology and Hypertension Unit, Ziv Medical Center, Safed, Israel.*

Background: Hyperuricemia is frequent in preeclampsia (PE) .In these pregnancies, plasma levels of uric acid (UA) may start to rise early, long before hypertension and proteinuria develop. This is mainly due to reduced renal excretion.We postulated that since the GFR is usually preserved at these stages, the reduced excertion of UA is due to increased Urate reabsorption reflecting changes in volume status. Hence, we investigated the Fractional Excertion of UA (FEua) in mid (week23)and late(week37)pregnancy as well as other traditional parameters of clinical PE.

Methods: · 48 pregnant women were recruited. Women with chronic hypertension, renal disease, DM, morbid obesity, any other systemic diseases, and those with PE in previous pregnancies were excluded. · All participants were examined during their 23rd and 37th weeks, and during delivery. serum and urine creatinine and uric acid, and urine microalbumin were measured at each visit. Sitting BP and BMI were also recorded. · Preeclampsia was diagnosed according to the accepted criteria.

Results: · Five of the 48 women (10.4%) developed preeclampsia past the 37th week. · In the preeclampsia group,the mean serum uric acid was significantly higher,(3.8MG/dL versus 3.0mg/dL p;0.025 at week and 4.2mg/dL versus 3.0mg/dl P;0.001A at week37)and the mean FEua was significantly lower(2.0% versus 4.2% P;0.003 at week23 and2.9% versus 4.0% P:0.025 at week37) than in the normal group. At 23 weeks,Mean diastolic(69.0mmHg versus 61.3mmHg P;0.032) but not systolic (102mmHg versus 110.8mmHg P;0.293), BP was higher in the preeclampsia group. · ACR in the preeclampsia group was slightly(Mean 0.030 versus 0.023 P;0.093) but not significantly higher compared with the normal group at 23 weeks.

Conclusions: 1. In pregnant women who will develop PE, plasma levels of UA are elevated in early pregnancy before overt hypertension and proteinuria appear. 2.The findings in week 23 of reduced FE of UA that indicates enhanced tubular reabsorption, and of higher diastolic BP(althought within normal), may reflect a state of relative plasma depletion and vasoconstriction, conditions known to characterize the fully expressed PE syndrome. 3. It is suggested that these pathophysiological events start in early pregnancy, long before clinical PE appears.

SA-PO178

Serial Measurements of Proteinuria in Normal and Hypertensive Pregnancy Andrea G. Kattah,[1] Wendy White,[2] Vesna D. Garovic.[1] *[1]Div of Nephrology and Hypertension, Mayo Clinic; [2]Dept of Obstetrics and Gynecology, Mayo Clinic.*

Background: The optimal method for monitoring proteinuria in pregnancy is still debated. Urine protein to creatinine (P/Cr) ratio correlates well with 24-hr urine protein, but issues, such as false positives, remain. There is little data on albumin to creatinine (Alb/Cr) ratios in pregnancy. Our goal was to compare P/Cr and Alb/Cr ratios in women with normal pregnancy, gestational hypertension (GH) and preeclampsia at different gestational ages.

Methods: We prospectively enrolled women with normal pregnancy (n=204), GH (n=14) and preeclampsia (n=15), with diagnosis being made at time of delivery using standard criteria through 2012. Serial measurements of P/Cr and Alb/Cr ratios were collected between <13 weeks, 14-28 weeks and >28 weeks gestation and the last value in each period was used. There were 24-hr urine proteins in 18 women.

Results: The median P/Cr and Alb/Cr ratios were significantly higher in those with preeclampsia versus normal pregnancy and GH at >28 weeks (all p<0.02, Wilcoxon-rank sum test).

Proteinuria Measurements	Gestational Age (weeks)	Normal (n=204)		Gestational Hypertension (n=14)		Preeclampsia (n=15)	
		n	Median (IQR)	n	Median (IQR)	n	Median (IQR)
Albumin/Cr ratio (mg/g Cr)	<13	181	3.6 (2.1-6.5)	12	2.2 (0.15-4.1)	14	3.7 (0.5-9,4)
	14-28	110	4.1 (2.4-6.4)	10	7.5 (3.9-14.2)	9	5.3 (1.9-7.8)
	> 28	165	18.6 (6.4-65.8)	14	22.8 (5.7-35.8)	15	100.5 (17.8-703.5)
Protein/Cr ratio (mg/mg Cr)	<13	181	0.02 (0.01-0.03)	12	0.02 (0.01-0.02)	14	0.03 (0.01-0.03)
	14-28	110	0.04 (0.03-0.05)	10	0.04 (0.02-0.07)	9	0.05 (0.02-0.08)
	> 28	165	0.08 (0.04-0.17)	14	0.06 (0.04-0.11)	15	0.26 (0.07-1.20)

Table 1 - Protein measurements at various gestational ages in each pregnancy group.

The correlation between P/Cr and both Alb/Cr ratio and 24-hr urine protein was strong (Spearman ρ 0.66 and 0.75, respectively). In those with normal pregnancy, 23/456 (5%) of P/Cr ratios were >0.3 mg/mg Cr and 79/456 (17%) of Alb/Cr ratios were >25 mg/g Cr.

Conclusions: P/Cr ratio was significantly increased in those with preeclampsia only in late pregnancy, supporting its use as a measure of proteinuria. Alb/Cr ratios correlated well with P/Cr ratios, but would increase the number of false positives.

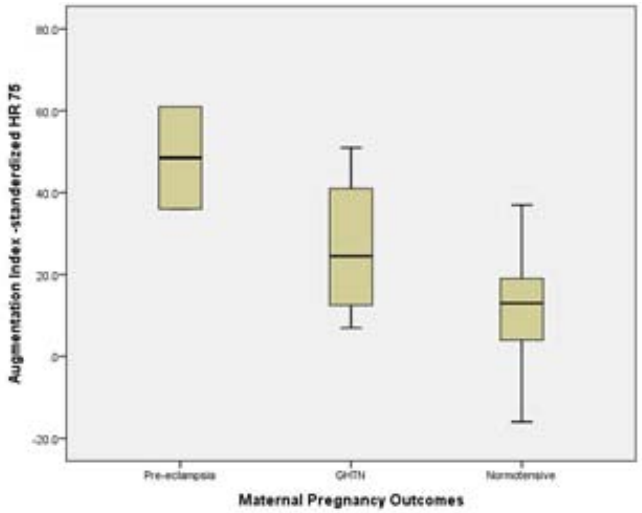
SA-PO179

Central Aortic Pressure and Arterial Stiffness as Early Predictors of Gestational Hypertension and Preeclampsia Simarta F. Brennan-Prescod, Paul G. Schmitz, Thinh Nguyen, Suwan Mehra, Ashraf M. Mohammed. *Nephrology, St. Louis Univ, St. Louis, MO.*

Background: Parameters of arterial stiffness using SphygmoCor Excel® are viewed as important markers of cardiovascular disease. This novel technique may become a useful screening tool to identify pregnant women at higher risk for developing preeclampsia and gestational hypertension during pregnancy.

Methods: A prospective study was done of 61 pregnant women in an outpatient clinic. During first half of pregnancy, noninvasive assessment of central aortic pressure and arterial stiffness was performed. The subjects were followed postpartum to determine maternal and fetal outcomes.

Results: Among the 61 women, 11.4% had central pulse pressure >50mmHg and 26% had central systolic pressure >125mmHg. In addition 26% had elevated augmentation pressures and an increase of 39% augmentation index with standardized heart rate of 75 (Ax75). There was a strong positive correlation between central pulse pressure and brachial pulse pressure (R^2=0.847, p < 0.01). Subgroup analyses of 18 women who delivered to date revealed that 2 developed preeclampsia, 4 developed gestational hypertension, and 12 were normotensive. The patients who developed preeclampsia and gestational hypertension had higher indices of arterial stiffness, such as Ax75, in the first trimester of pregnancy.



Conclusions: Results suggest that noninvasive methods to measure arterial stiffness identify patients at risk for pregnancy-induced hypertension. Patients with elevated pulse and central systolic pressures are at high risk for cardiovascular disease. The patients with higher augmentation pressure and augmentation index who later develop hypertensive disease may respond to agents that target arterial compliance (e.g. CCB). Over the next 3 months, outcomes on the remaining subjects will provide further insights into the role of central pressure monitoring during pregnancy.

SA-PO180

Independent Association of Vegetarian Diet with Vascular Endothelial Function Lea Borgi, John P. Forman. *Renal, Brigham and Women's Hospital, Boston, MA.*

Background: Vegetarian and vegan diets have been associated with a lower prevalence of hypertension when compared to animal-based diets in cross-sectional studies. Small, short-term interventional trials reported that replacing an omnivorous diet with a vegetarian diet led to a reduction in blood pressure. The mechanisms by which vegetarian diets are linked to a reduced prevalence of hypertension are not well understood, but endothelial dysfunction induced by eating meat has been proposed.

Methods: We performed a cross-sectional analysis of the NIH-funded ongoing Modifiable Effectors of Renin System Activation Treatment Evaluation (MODERATE) trial. Dietary information and endothelial-dependent vasodilation (EDV, a measure of endothelial function using brachial artery ultrasound) were ascertained at baseline, as were other factors including demographics and 24-hour blood pressure. We used multivariable linear regression to analyze the independent association of self-reported long-term dietary pattern (which we categorized as vegetarian/vegan or non-vegetarian) and EDV, while controlling for multiple potential confounders.

Results: At the time of the analysis, 174 participants had completed their baseline visits. The mean (SD) of EDV for the entire study population was 7.0±4.4%. Compared with non-vegetarians, those who were vegetarian or vegan had significantly greater endothelial function in fully-adjusted models (difference in EDV = 3.0 ± 1.4%). By comparison, the difference in EDV associated with a 1 year younger age was 0.13±0.03% in fully-adjusted models. Thus, the magnitude of the difference in EDV associated with being vegetarian or vegan was approximately equivalent to the difference observed with being 23 years younger.

Conclusions: This preliminary analysis found that a vegetarian or vegan diet was associated with substantially better endothelial function as compared with a non-vegetarian diet. Future prospective studies should examine the effects of animal-based foods on endothelial function and other potential mechanisms of hypertension and cardiovascular disease.

SA-PO181

Urinary Angiotensin-Converting Enzyme 2 Is Associated with Urinary Liver-Type Fatty Acid Binding Protein and Albuminuria in Patients with Chronic Kidney Disease Masanori Abe, Kazuyoshi Okada. *Nephrology, Hypertension and Endocrinology, Nihon Univ School of Medicine, Tokyo, Japan.*

Background: Angiotensin converting enzyme 2 (ACE2) is a recently identified member of the renin-angiotensin system (RAS), and degrades Angiotensin (Ang) II to the seven-amino acid peptide fragment Ang-(1-7). ACE2 is thought to function as a negative regulator in this respect to counterbalance the Ang II-forming activity of ACE. ACE2 is expressed in the kidney and may be a renoprotective enzyme. In addition, ACE2 has been detected in urine from patients with chronic kidney disease (CKD).

Methods: To determine the associated of ACE2 with urinary liver-type fatty acid binding protein (L-FABP) and albuminuria in CKD, we assessed 152 patients with CKD, including 72 with diabetes. Parameters were urinary ACE2, urinary albumin/creatinine ratio (UACR), urinary L-FABP, estimated glomerular filtration rate, and other factors determined to be associated with elevated urinary ACE2.

Results: Urinary ACE2 was significantly higher in patients with diabetes (P = 0.01) and in patients with CKD stage G4 compared with stages G1–G3 (P < 0.0001). Multivariable regression analysis revealed that urinary L-FABP and UACR were significantly associated with urinary ACE2 levels, indicating that urinary ACE2 is increased in patients with diabetes and advanced stage CKD.

Conclusions: ACE2 might continuously protect from both glomerular and tubulointerstitial injury during CKD progression. Taken together, urinary ACE2 could be a marker of kidney renin-angiotensin system activation in such patients.

SA-PO182

Severity of Burn Injury in Active Duty Military Personnel Is Associated with the Development of Long Term Hypertension Ian J. Stewart,[1] Brian Snow,[1] Jonathan Sosnov,[1] Mary Bollinger,[2] Kevin Chung.[3] *[1]San Antonio Military Medical Center; [2]South Texas Veterans Health Care System; [3]U. S. Army Inst of Surgical Research.*

Background: There is a paucity of data regarding long term morbidity in patients that survive a burn injury. We hypothesized that the severity of burn injury is associated with the subsequent development of hypertension (HTN) in a population of military personnel burned while supporting of combat operations in Iraq and Afghanistan.

Methods: Patients were included for analysis if they were admitted to our institution from Jan 2003 to Nov 2008, were injured in Iraq or Afghanistan, survived their hospitalization and did not have pre-existing HTN. Data on age, eGFR by CKD-EPI, race, presence of inhalation injury (IH), injury severity score (ISS), and total body surface area (TBSA) of burn injury were collected at hospital admission. To determine the diagnosis of HTN, we used ICD-9 codes from both the Department of Defense and Veteran's Affairs medical records systems. To determine factors associated with subsequent HTN, we used a cox regression model with step wise selection (p<0.1 for inclusion and output).

Results: A total of 637 patients were included in the analysis. The patients had an average age of 25.3±5.7 years. The majority (97.6%) were male and 7.5% were African American. Median ISS and TBSA were 9 (IQR 1,18) and 8% (IQR 4%, 20%), respectively. IH was present in 13.4%. Mean eGFR at admission was 112±20 ml/min/m^2. Average patient follow up was 5.5±1.7 years. On univariate analysis, ISS (HR 1.04, p<0.01), age (HR 1.05, p<0.01), absence of inhalation injury (HR 0.45, p<0.01), non-black race (HR 0.6, p=0.06), TBSA (HR 1.03, p<0.01), and eGFR (HR 0.99, p<0.01) were associated with subsequent HTN. On multivariable analysis, only age (HR 1.04, p<0.01), TBSA (HR 1.03, P<0.01), and non-black race (HR 0.56, p=0.04) were independently assocaited with HTN. eGFR trended towards significance (p=0.06).

Conclusions: Our data suggests that the size of a burn injury may impact a patients risk of developing HTN as after adjustment, TBSA was associated with subsequent HTN. Each 1% increase in burn size results in a 3% increase in the risk of developing HTN.

Funding: Other U.S. Government Support, Veterans Affairs Support

SA-PO183

Renal Prognosis in Patients with Essential Hypertension: Role of Serum Phosphorus Changes During Follow-Up Enrique Morales, Sara Santana, Julian Segura, Luis M. Ruilope, Manuel Praga. *Nephrology, Hospital 12 de Octubre, Madrid, Spain.*

Background: Our aim was to analyze whether changes in plasma phosphorus (P) were associated with renal prognosis in a cohort of patients with essential hypertension.

Methods: 1361 patients with essential hypertension, 50.2% male, age 60.5±12.3 years, usually followed at our center, were analyzed. All were followed for a minimun of three years. We classified the patients in two groups, defined by the presence of renal event (development of albuminuria ≥30 mg/g or progression of albuminuria during follow-up) or absence of renal event (maintenance of albuminuria <30 mg/g during the follow-up). Changes in plasma phosphorus (ΔP) were assessed absolutely (final P - initial P) and relatively ((final P – initial P)/(initial P) x100).

Results: At baseline the mean serum creatinine was 1.0±0.4 mg/dl, estimated glomerular filtration rate (eGFR) using the formula CKD-Epi was 78.4±25.1 ml/min/1.73m2, and mean plasma phosphorus was 3.3±0.5 mg/dl. Patients with renal event (n=363, 26.7%) were older (64.2±11.5 versus 59.2±12.3 years), higher systolic blood pressure (144±20 versus 136±18 mmHg), higher requirements of antihypertensives drugs (2.7±1.2 versus 2.1±1.2), higher serum creatinine (1.18±0.51 versus 0.93±0.27 mg/dl) and lower eGFR (71.1±32.7 versus 81.1±21.1 ml/min/1.73m2) (p<0.001 for all comparisons). Serum phosphorus showed no significant difference (3.29±0.56 versus 3.28±0.52 mg/dl). Mean absolute ΔP was 0.11±0.55 mg/dl in patients with renal event and 0.06±0.50 mg/dl in patients without renal event (p=0.083). Mean relative ΔP was 4.9 % among patients with renal event and 2.9% among patients without event (p=0.048). The logistic regression analysis showed that independent factors for the occurrence of renal event were age, female sex, baseline serum creatinine, diabetes and relative ΔP.

Conclusions: In conclusion, in a cohort of essential hypertensive patients regularly followed in our center, changes in plasma phosphorus during follow-up are an independent risk factor for the development or progression of renal disease.

SA-PO184

Relevance of Central versus Brachial Blood Pressure in Chronic Kidney Disease Patients Naman Barman,[1] Vinaya R. Soundararajan,[2] Ramesh Soundararajan.[3] *[1]Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Nephrology, Univ of Illinois College of Medicine, Chicago, IL; [3]Nephrology, Midwestern Univ College of Medicine, Downers Grove, IL.*

Background: Central blood pressure may be more relevant to end organ damage and a more appropriate target than brachial blood pressure. The purpose of this study is to evaluate the role of brachial versus central blood pressure in patients presenting to a nephrology clinic. We wanted to determine the percentage of patients in which the central systolic pressure was controlled, but the brachial systolic pressure was higher than optimal.

Methods: Central and brachial blood pressures were measured during the nephrology clinic visit by the SphygmoCor XCEL by AtCor Medical. Central blood pressure was considered controlled if systolic was less than or equal to 130 mmHg. Brachial systolic was considered controlled if it was less than or equal to 140 mmHg.

Results: Of the 109 adult patients who presented to the clinic, 23 patients (21%) had controlled central systolic blood pressure, but suboptimal control of brachial systolic blood pressure. On evaluating home blood pressure medications of each patient, 46% of patients were on an angiotensin converting enzyme inhibitor (ACE) or angiotensin receptor blocker (ARB), 50% were on calcium channel blockers, 23% were on diuretics and 16% were on beta blockers. Of the 64 chronic kidney disease (CKD) stage III patients alone, 14 (21%) had controlled central but high brachial systolic pressures. There were no CKD III patients with elevated central blood pressure and optimal brachial blood pressure. Evaluating their medications, 43% were on ACE/ARB, 35% were on calcium blockers, 23% were on diuretics and 16% were on beta blockers.

Conclusions: Results demonstrate that if therapy was solely based on brachial blood pressure, 21% of patients may be over treated, putting them at higher risk for side effects, medication intolerance, and noncompliance. Treatment based on central blood pressure may be better tolerated by the patients. Additionally, in this setting ACE/ARB and calcium blockers seemed to be more effective at controlling central blood pressure than diuretics and beta blockers.

SA-PO185

Comparison of Central Aortic and Brachial Blood Pressure during a 48-Hour Ambulatory Recording in Hemodialysis Patients Antonis Karpetas,[1] Pantelis Sarafidis,[2] Panagiotis I. Georgianos,[1] Georgios Koutroumpas,[3] Vasilios Raptis,[1] Thanasis Bikos,[1] Athanase Protogerou,[4] Dimitrios Stamatiadis,[5] Christos Syrganis,[3] Vassilios Liakopoulos,[1] George Efstratiadis,[2] Anastasios Lasaridis.[1] *[1]Div of Nephrology, 1st Dept of Medicine, AHEPA Hospital, Thessaloniki, Greece; [2]Nephrology Dept, Hippokrateion Hospital, Thessaloniki, Greece; [3]Nephrology Dept, General Hospital of Volos, Greece; [4]Hypertension Unit and Cardiovascular Research Laboratory, Laiko Hospital, Athens, Greece; [5]Hemodialysis Unit, General Hospital of Serres, Greece.*

Background: In clinical states of accelerated arterial stiffening, brachial blood pressure (BP) cannot accurately reflect BP in the aorta, due to the phenomenon of "aortic-to-brachial BP amplification". End-stage renal disease (ESRD) is characterized by elevated arterial stiffness and in these patients aortic BP was shown to be better predictor of mortality than brachial BP. This study includes comparative evaluation of aortic versus brachial BP during a 48-hour ambulatory BP monitoring (ABPM) in hemodialysis patients.

Methods: Aortic and brachial ABPM was performed with the Mobil-O-Graph device (IEM, Stolberg, Germany) for a 48-hour period covering a dialysis session and the following interdialytic interval in 153 hemodialysis patients. Mobil-O-Graph is a new brachial cuff-based device, which records oscillometric brachial BP and pulse waveforms and assesses central BP via mathematical transformation.

Results: Mean 48-hour aortic systolic BP (SBP) and pulse pressure (PP) was significantly lower than SBP and PP at brachial artery (120.1±14.6 versus 131.9±16.7 mmHg, P<0.001 for SBP and 40.4±9.4 versus 53.8±12.8 mmHg, P<0.001 for PP). In contrast, 48-hour diastolic BP was higher in the ascending aorta than in brachial artery (79.7±10.8 versus 78.1±10.6 mmHg, P<0.001). These differences were observed in both day-time and night-time periods, as well as during the hemodialysis-on and hemodialysis-off days.

Conclusions: This study shows a difference of 12 mmHg in ambulatory SBP and PP between the ascending aorta and brachial artery, consistent during the 48-hour period in hemodialysis patients. Future studies are warranted to investigate any possible effects of this difference on cardiovascular risk.

SA-PO186

Comparison of Home Blood Pressure Recordings and Office Blood Pressure Recordings with Respect to Ambulatory Blood Pressure Recordings Among Post Renal Transplant Recipients Amit Kumar,[2] Sivaramakrishnan Ramanarayanan.[1] *[1]Inst of Liver and Biliary Sciences, India; [2]All India Inst of Medical Sciences, India.*

Background: Ambulatory blood pressure monitoring (ABPM) is currently the gold standard for the diagnosis of hypertension. However, ABPM is not widely available in primary care practice and is not a feasible method to monitor blood pressure in post renal transplant patients. Office based blood pressure recordings (OBPM)may not be representative of true blood pressure recordings as it is influenced by many other factors. Home blood pressure monitoring (HBPM), may co-rrelate well with ABPM, but this has not been studied well among renal transplant patients. This study assess the agreeement between OBPM, HBPM and ABPM among renal transplant recipients.

Methods: The study enrolled 52 adult kidney transplant recipients. Apart from baseline clinical, demographic and laboratory parameters, measurements of **OBP, HBP**, **ABP** and Echocardiography were done at 3 months. ABPM was performed using automated electronic ABPM instrument(Bravo automated Ambulatory blood pressure monitor). HBPM was performed by automated electronic BP instrument. The patients were instructed to measure blood pressure at home two a day i.e. in morning and evening for three days. Each time they should take at least, 3 reading while sitting comfortably as in office BP recording and write the readings in diary. The average of all readings were then used. Office BP was recorded with automatic oscillometric device on non- fistula hand for at least 3 time over at least 5-10 minute interval and average of systolic and diastolic blood was taken.

Results: In patients at 3 months after transplant the mean difference of agreement for systolic BP with HBPM versus ABPM was 0.3±20.9 and for OBP versus ABPM was 2.6±22.3. Similarly for diastolic BP after transplant the mean difference of agreement was 2.3±13.9 for HBP versus ABPM and 4.8±24.0 for OBP versus HBPM. This suggests Both HBP and OBP has acceptable agreement with ABPM in post-transplant setting but the agreement with home BP was better.

Conclusions: Home BP is an acceptable alternative to ABPM for monitoring of blood pressure among renal transplant patients.

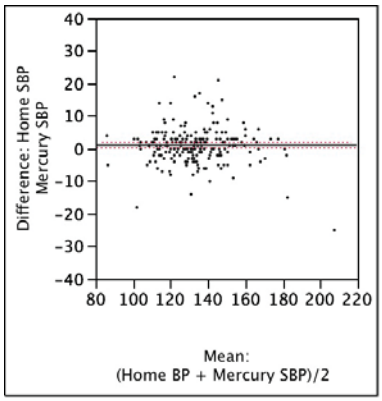
SA-PO187

Are Home Blood Pressure Monitors Accurate Compared to Validated Devices? Eva Bruketa, Ayub Akbari, Marcel Ruzicka, Swapnil Hiremath. *Div of Nephrology, Dept of Medicine, The Ottawa Hospital and Univ of Ottawa, Ottawa, ON, Canada.*

Background: Hypertension remains the most common cause of morbidity and mortality from cardiovascular disease. Major guidelines recommend home blood pressure (BP) monitoring to guide diagnosis and treatment of hypertensive patients. Notwithstanding this, little data exists on the real-world accuracy of home BP monitors used by patients. The objective of our study was to compare measurements from Home BP monitors with validated mercury sphygmomanometers.

Methods: We conducted a retrospective review and abstracted data from patient charts who had home BP monitor validated in the hypertension clinic at a tertiary care academic centre. After casual measurements, 3 resting measurements are taken with the home monitor and an office mercury sphygmomanometer using standard methods. The means of these three measurements taken with each method were compared with the t-test. In addition, we analyzed the proportion of home BP readings within 5 and 10 mm Hg of the mercury readings.

Results: We analyzed data from 210 patients (60% men, mean age 67 + 14 years). The mean body mass index was 29 kg/m^2, and the mean arm circumference was 32 + 5 cms. There was a significant difference between the resting mercury systolic BP (SBP) (132.3 + 18.5 mm Hg) compared to the home monitor resting SBP (133.4 mm Hg + 18.1 mm Hg), p = 0.011.



For diastolic BP (DBP) the difference was 71.4 + 11.9 versus 68.9 + 12.7 mm Hg (p<0.001). 63/210 of the Home Monitor SBP readings (30%) were > 5 mm Hg different and 16 (8%) were > 10 mm Hg different from the mercury SBP measurement. For DBP, these proportions were 32% (67/210) and 9% (18/210) respectively.

Conclusions: A large proportion of home BP monitors were not accurate when compared to mercury sphygmomanometers when validated in our clinical practice. Validation is a necessary step before relying on home BP monitor measurements.

Funding: Clinical Revenue Support

SA-PO188

Impact of Cuff Inflation on Ambulatory Blood Pressure in Type 2 Diabetes Emilie Hein Petersen,[1] Simone Theilade,[1] Tine Hansen,[1] Morten Lindhardt,[1] Peter Rossing.[1,2,3] *[1]Steno Diabetes Center; [2]Aarhus Univ; [3]Univ of Copenhagen.*

Background: 24-hour ambulatory blood pressure (BP) measurement is increasingly recommended in guidelines and applied in daily clinic. Discomfort related to cuff inflation might bias the measurements, especially during nighttime. We assessed the impact of cuff

inflations by measuring 24-hour BP with previously validated devices: a cuff-less wrist device based on tonometry and an upper-arm cuff device in patients with type 2 diabetes. Reproducibility of both devices was evaluated.

Methods: Clinical trial with random assignment to sequence of cuff device (Takeda, TM2431) and cuff-less device (BPro, HealthStats) in a cross-over study with 4 visits. 24-hour BP was measured with BPro at visit 1 or 2 (after randomization) 3 and 4.Takeda device was used at visit 1 or 2, and 4. Day- and nighttime was defined based on patient's diary. Antihypertensive treatment was unchanged 4 weeks prior to and during the study. Paired t-test compared inter- and intra-device differences for systolic 24-hour, nighttime BP and nocturnal BP decline.

Results: We included 53 patients, mean (±SD) age was 64 (±9.6) years, office BP was 134 (±15)/75 (±10) mmHg, 21% female, and 74% had albuminuria (≥30 mg/g). There was no significant difference in 24-hour or nighttime BP between the two BPro recordings mean difference of 2.8 (±15.3) and 2.9 (±16.4) mmHg, $p \geq 0.19$ for both; or between the two Takeda recordings 1.5 (±8.9) and 3.1 (±12.3) mmHg, $p \geq 0.09$ for both. However, 24-hour and nighttime BP was significantly higher when measured with Takeda than BPro, 141.6 (±14.6) versus 128.3 (±14.6), and 130.0 (±16.6) versus 123.1 (±15.5), $p \leq 0.01$ for both. The nocturnal BP decline was significantly lower measured with BPro than Takeda (6.7±5.3% versus 10.3±7.6%, $p<0.01$). Few patients reported sleep disturbances in general, but most patients preferred BPro when asked.

Conclusions: In type 2 diabetic patients ambulatory BP measurements with BPro or Takeda were reproducible, but revealed significantly higher values for Takeda - both for 24-hour and nighttime BP. Overall, nocturnal BP decline was higher for Takeda suggesting that cuff inflation did not increase nighttime BP.

SA-PO189

Losartan and Ambulatory Blood Pressure Variability Koichi Azuma,[1] Kouichi Tamura.[2] *[1]Naruse jin Clinic, Zenjinkai Group, Yokohama, Kanagawa, Japan; [2]Dept of Medical Science and cardiorenal Medicine, Yokohama City Univ School of Medicine, Yokohama, Kanagawa, Japan.*

Background: Previous studies have shown increases in ambulatory short-term blood pressure (BP) variability to be related to cardiovascular disease. In this study, we examined whether an angiotensin II type 1 receptor blocker losartan would improve ambulatory short-term BP variability in hypertensive patients on hemodialysis.

Methods: 40 hypertensive patients on hemodialysis therapy were randomly assigned to the losartan treatment group (n=20) or the control treatment group (n=20). At baseline and 6 and 12 months after the treatment, 24-h ambulatory BP monitoring was performed. Echocardiography and measurements of brachial-ankle pulse wave velocity (baPWV) and biochemical parameters were also performed before and after therapy.

Results: After 6- and 12-months of treatment, nighttime short-term BP variability, assessed on the basis of the coefficient of variation of ambulatory BP, was significantly decreased in the losartan group, but remained unchanged in the control group. Compared with the control group, losartan significantly decreased left ventricular mass index (LVMI), baPWV, and the plasma levels of brain natriuretic peptide and advanced glycation end products (AGE). Furthermore, multiple regression analysis showed significant correlations between changes in LVMI and changes in nighttime short-term BP variability, as well as between changes in LVMI and changes in the plasma levels of AGE.

Conclusions: These results suggest that losartan is beneficial for the suppression of pathological cardiovascular remodeling though its inhibitory effect on ambulatory short-term BP variability during nighttime.

Funding: Private Foundation Support

SA-PO190

Poor Sleep Quality Are Responsible for Nondipping Pattern in CKD with Hypertension Patients Jun Zhang. *Div of Nephrology, Dept of Medicine, 3rd Hospital of Sun Yat-sen Univ, GuangZhou, GuangDong, China.*

Background: This study aimed to evaluate the relationship between sleep quality and hypertension and to determine if there was any association between the non-dipping status and sleep quality in CKD patients.

Methods: A total of 565 pre-dialysis CKD patients (normotensive patients 224, hypertension patients 341) defined as dippers or nondippers by ambulatory blood pressure monitoring were recruited in this study. Demographics and clinical correlates, including BMI and eGFR etc, were collected. The sleep quality was measured by Pittsburgh Sleep Quality Index (PSQI).

Results: A total of 134(59.8%) CKD normaltensive and 253(74.2%) hypertensive patients had a nondipping BP pattern. Hypertension group had higher prevalence of nondipping BP, smoking, alcohol intaking, diabetes mellitus and had lower eGFR level and poorer sleep quality than normaltensive group ($P<0.05$). Patients with nondipping BP pattern had worse renal function and poorer sleep quality when compared with dipping BP pattern in hypertension CKD patients ($p<0.05$). Nondipping BP pattern group had higher bedtime BP than dipping BP pattern in all CKD patients ($p<0.05$). The rate of decline of nocturnal BP correlated significantly with PSQI in hypertension CKD patients ($p<0.05$). We failed to prove the association between poor sleep quality and hypertension in CKD patients ($p>0.05$). But poor sleep quality was an independent factor affecting BP pattern in hypertension CKD patients after multivariate logistic regression analyses ($p<0.05$).

Conclusions: Poor sleep quality, which is commonly found in pre-dialysis CKD patients, is an independent associated factor of nondipping BP pattern in hypertension CKD patients.

Funding: Private Foundation Support

SA-PO191

Long Term Effect of Deviced-Guided Breathing Exercises on Sympathetic Nervous System Activity in Primary Hypertension Silvana de Barros,[1] Giovanio Vieira da Silva,[1] Josiane Lima de Gusmão,[1,2] Tatiana Goveia de Araujo,[1] Decio Mion Junior.[1] *[1]Nephrology Div, Univ of Sao Paulo, Sao Paulo, SP, Brazil; [2]Univ Guarulhos, Guarulhos, SP, Brazil.*

Background: Deviced-guided breathing exercises reduce blood pressure and are indicated as a non-pharmacological treatment for hypertension. However, the physiological mechanism involved in the reduction of blood pressure is unknown. The reduction in sympathetic nervous system activity may be one explanation.

Methods: 19 patients with primary hypertension (systolic blood pressure (SBP) between 140 and 179 mmHg and/or diastolic blood pressure (DBP) between 90 and 109 mmHg) and without any other co-morbidities were selected. Participants were randomly allocated into two groups: Control Group (CG, n=8) or Guided Breathing Group (GBG, n=11). For 8 weeks, participants in the CG used MP3 player that reproduced loud music, daily, for 15 minutes. Participants in the GBG used RESPeRATE® device for the same period of time. The device induces reduction in breathing rate while prolonging exhalation: patients were requested to synchronize breathing with the guiding tones. Before and after the intervention period, patients underwent ambulatory blood pressure monitoring (ABPM), dosage of plasma catecholamine and measurement of sympathetic nerve activity by microneurography.

Results: There was no change in mean mean 24 hour SBP (129±8 versus 133±11, p=0,20) and DBP (89±5 versus 91±7, p=0,19) before and after intervention in GBG. However, in the CG SBP (128± 8 versus 123±8, p = 0,01) and DBP (90±5 versus 86±6, p = 0,02) showed decrease. There was no difference in plasma catecholamine concentration (pg/ml) before and after the intervention in both groups: GBG (302 (220-256) versus 234 (156-318), p = 0,35) and CG (201 (144-230) versus 221 (179-274), p = 0,97). The peripheral sympathetic nerve activity (bursts/min) measured by microneurography also showed no change: GBG (20±8 versus 18±5, p=0,36) and CG (21±4 versus 21±6, p= 0,92).

Conclusions: Device-guided breathing showed no change in blood pressure and sympathetic nervous system activity after 8 weeks. Other mechanisms should be involved in the reduction of blood pressure observed in other studies.

Funding: Government Support - Non-U.S.

SA-PO192

The Effect of the Addition of Allopurinol on Blood Pressure Control in African Americans Treated with a Thiazide-Like Diuretic Mark S. Segal,[1,4] Titte Srinivas,[2] Jogiraju V. Tantravahi,[1,4] Richard J. Johnson.[3] *[1]Medicine, Malcom Randall VA Medical Center; [2]Medical Univ of South Carolina; [3]Univ of Colorado; [4]Medicine, Univ of Florida.*

Background: Since in African-Americans there may be a strong association between hyperuricemia and hypertension, we decided to test the hypothesis that xanthine oxidase inhibition in African-Americans receiving diuretics can result in improved blood pressure (BP) control with few side effects. We tested this hypothesis in a randomized double blind, placebo controlled study of African-Americans with stage I hypertension in the absence of clinically significant renal disease.

Methods: 150 African-American men and women between the ages of 18 and 65 years of age who meet the exclusion/inclusion criteria with untreated or treated hypertension were started on chlorthalidone (25mg/day) and potassium chloride after stopping current anti-hypertensives. After 5 weeks we performed baseline testing and randomized subjects to allopurinol (300mg/dl) or placebo. After 2 weeks, if their uric acid was above 5.5mg/dl the allopurinol or placebo was doubled. After another 6 weeks they were brought in to repeat baseline testing.

Results: The baseline BP after 5 weeks on chlorthalidone was 119.9±13.6 in the allopurinol group and 116±11.2 in the placebo group. After at least 8 weeks following randomization to placebo or allopurinol, there was a change in the clinic BP between the placebo and allopurinol group that did not quite reach significance (p=0.1). The group randomized to allopurinol saw a 3.32 mm Hg decrease in systolic BP at visit 8 compared to visit 5 whereas the group randomized to placebo saw a 0.58 mm Hg increase in systolic BP at visit 8 compared to visit 5. The same trend was seen in the clinic diastolic BP.

Conclusions: Although allopurinol treated subjects had a reduced BP compared to placebo treated subjects, the decrease in BP did not quite reach significant difference. We believe the negative result was due to the excellent BP control achieved with chlorthalidone. Importantly allopurinol was well tolerated. Decreasing uric acid levels deserves to further study as a mechanism to control hypertension.

Funding: Other NIH Support - NHLBI, Veterans Affairs Support

SA-PO193

Different Effects of Febuxostat and Allopurinol on Renal Function and Blood Pressure in Patients with Hypertension Kentaro Kohagura,[1] Takeshi Tana,[3] Yusuke Ohya,[1] Kunitoshi Iseki.[2] *[1]Cardiovascular Medicine, Nephrology and Neurology, Univ of the Ryukyus, Nishihara-cho, Okinawa, Japan; [2]Dialysis Unit, Univ Hospital of the Ryukyus, Nishihara-cho, Okinawa, Japan; [3]Shuri Jokamachi Clinic, Naha, Okinawa, Japan.*

Background: Hyperuricemia was reportedly associated with elevated renal vascular resistance and developing hypertension. However, the effect of allopurinol (AP) and febuxostat (Feb), on renal function and blood pressure (BP) was unknown.

Methods: We retrospectively examined 223 hypertensive patients (152 men and 71 women) who were newly started with either AP (n=153) or Feb (n=70) without any change of other drugs in outpatient clinic. We examined the short-term changes (from the starting to next visit) in estimated glomerular filtration rate (eGFR) and BP after starting these drugs.

Results: The mean ± standard deviation values for patients' age, blood pressure, serum uric acid, eGFR were as follows: 64 ± 13 years, 127 ± 17/72 ± 12 mmHg, 8.8 ± 1.4 mg/dl and 47 ± 19 ml/min/1.73 m^2, respectively. Baseline uric acid and SBP were higher, while eGFR was lower in Feb group compared with those of AP group. Moreover, Feb decreased in serum uric acid greater than that of AP (-2.5mg/dl v.s -2.0mg/dl, p=0.03). In Feb group, eGFR (ml/min/1.73m^2) was significantly increased (43 v.s 46, p=0.01), while systolic BP (mmHg) tend to be decreased (131 v.s 128, p=0.06). Higher baseline SBP was significantly associated with higher increased in eGFR(r=0.35, p=0.003). Furthermore, the changes in SBP, but not in uric acid was negatively correlated with the changes in eGFR (r=-0.26, p=0.03). In contrast to the effects of Feb, neither systolic BP nor eGFR showed significant changes in AP group.

Conclusions: These results suggested that Feb might increase eGFR, independent of lowering effects of uric acid, by reducing vascular resistance of preglomerular vessel in the patients with hypertension.

SA-PO194

The Overlooked Effects of Sulodexide on Blood Pressure: A Systematic Review and Meta-Analysis Rik Hg Olde Engberink,[1] Nienke M.G. Rorije,[1] Bert-Jan Van den Born,[2] Dick de Zeeuw,[3] Hiddo Jan Lambers Heerspink,[3] Liffert Vogt.[1] *[1]Nephrology, Academic Medical Center, Amsterdam, Netherlands; [2]Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands; [3]Clinical Pharmacy and Pharmacology, Univ Medical Center Groningen, Groningen, Netherlands.*

Background: Sulodexide, a highly purified mixture of glycosaminoglycans, has been hypothesized to be renoprotective. Recent large randomized, placebo-controlled trials (RCT) in diabetic nephropathy failed to demonstrate significant effects on any renal endpoint, including albuminuria. Because glycosaminoglycans affect the composition of the endothelial surface layer, thereby improving endothelial function and possibly blood pressure (BP), we performed a systematic review of antihypertensive potency of sulodexide.

Methods: Medline, Embase and Cochrane were searched. RCTs that investigated sulodexide treatment of at least one month, and reported BP measurement at baseline and after sulodexide/control treatment were included. Two reviewers independently selected studies and extracted data. Primary outcome was the BP change after sulodexide treatment, corrected for the observed BP changes in parallel control groups. Meta-regression analysis was used to explore whether BP changes were associated with anti-albuminuric effects or patient characteristics.

Results: 8 studies, totalling 13 comparisons and 3,060 subjects (mean follow-up: 4.4 months) were included. Four comparisons were performed in hypertensive patients. Sulodexide treatment resulted in a significant systolic (-3.5 mmHg [95% CI -6.2,-0.9]; p=0.010) and diastolic (-2.3 mmHg [95% CI -3.9,-0.8]; p=0.004) BP reduction. Hypertensive patients displayed largest BP reductions (systolic/diastolic BP, -9.6/-4.9 mmHg; p<0.001). Higher baseline mean arterial pressure (MAP) was significantly associated with larger MAP reductions after sulodexide treatment (R^2 0.73, p<0.001). In addition, MAP reduction showed a significant association with albuminuria reduction (%) by sulodexide (R^2 0.66, p=0.019).

Conclusions: Sulodexide treatment results in a significant BP reduction with hypertensive patients showing the largest BP reduction. Greater BP reductions by sulodexide were associated with larger reductions in albuminuria.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO195

Single Pill-Based Combination Therapy with Amlodipine and Atorvastatin Improves Renal and Vascular Function and Clinic Blood Pressure Variability in Hypertension with Chronic Kidney Disease Kouichi Tamura,[1] Kengo Azushima,[1] Kazushi Uneda,[1] Hiromichi Wakui,[1] Masato Ohsawa,[1] Ryu Kobayashi,[1] Kohji Ohki,[1] Koichi Azuma,[2] Toru Dejima,[1] Tomohiko Kanaoka,[1] Yoshiyuki Toya,[1] Satoshi Umemura.[1] *[1]Dept of Medical Science and Cardiorenal Medicine, Yokohama City Univ Graduate School of Medicine, Yokohama, Kanagawa, Japan; [2]Dept of Nephrology and Hypertension, Yokohama Dai-ichi Hospital, Yokohama, Kanagawa, Japan.*

Background: The study aimed to examine the effects of single pill-based combination therapy with amlodipine and atorvastatin on clinic blood pressure (BP) profile including within-visit BP variability, a recently emerging marker of linking between kidney and vasculature, and parameters of vascular and renal function in hypertensive CKD patients who did not achieve the target BP and lipid level.

Methods: Hypertensive CKD patients who did not achieve the goal of BP (clinic systolic BP≥130 mmHg and/or diastolic BP≥80 mmHg) and lipid (LDL-C≥100 mg/dL) were enrolled. The patients were given a single pill of amlodine/atorvastatin tablet (amlodipine/atorvastatin; 2.5/5, 2.5/10, 5/5, 5/10 mg) for 16 weeks. The dose of the single amlodipine/atorvastatin tablet was titrated up as needed to achieve the BP and lipid goal.

Results: The combination therapy with amlodipine and atorvastatin for 16 weeks decreased clinic BP, and achievement of target BP control was attained in an average of 45% after the combination therapy in spite of the presence of no achievement at baseline. In addition, the combination therapy decreased the within-visit BP variability. With respect to the effects on renal damage markers, combination therapy with amlodipine and atorvastatin for 16 weeks significantly decreased albuminuria (UACR, 1034±1480 versus 733±1218 mg/g-Cr, P<0.05) without decline in eGFR. Concerning parameters of vascular function, the combination therapy significantly improved both baPWV and cSBP (baPWV, 1903±353 versus 1786±382 cm/sec, P<0.05; cSBP, 148±19 versus 129±23 mmHg, P<0.01).

Conclusions: These results suggest that the combination therapy with amlodipine and atorvastatin may exert additional beneficial effects on renal and vascular damages as well as BP profile in addition to BP lowering in hypertension with CKD.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO196

Effect of Antihypertensive Agents on the Severity of Obstructive Sleep Apnea: A Systematic Review and Meta-Analysis Kiran Khurshid,[1] Jonathan Yabes,[1] Patricia Weiss,[1] Mark L. Unruh,[2] Manisha Jhamb.[1] *[1]Nephrology, Univ of Pittsburgh, Pittsburgh, PA; [2]Nephrology, Univ of New Mexico, NM.*

Background: Adults with hypertension (HTN) have a high prevalence of obstructive sleep apnea (OSA). OSA is a known risk factor for HTN. However, the relationship may be bi-directional. The treatment of HTN to a lower BP target may improve sleep apnea both by improving upper airway tone and by reducing the nocturnal rostral fluid shifts through the use of a low-sodium diet and diuretics. The evidence on this is very limited. The aim of this study was to conduct a systematic review and meta-analysis of the effect of anti-hypertensive medications on OSA severity among hypertensive adults.

Methods: A literature search of PubMed and Embase was carried out for studies from 1980 to 2013 by a research librarian. Search concepts of sleep apnea, hypertension and drug classes used to treat HTN were used. Studies that reported objectively measured sleep apnea severity as an outcome were included. We excluded studies involving surgery or benzodiazepines as an intervention. If the sleep apnea was due to obesity-hypoventilation syndrome or high altitude sickness, it was excluded. There were no language restrictions, both published and unpublished articles were considered. Data was abstracted by 2 reviewers independently.

Results: Initial search yielded 18,577 articles, out of which 23 studies met our inclusion and exclusion criteria. These included 4 randomized controlled trials, 8 single arm studies, 5 cross-sectional studies, and 3 abstracts. Here we present results of meta-analysis of the 7 RCTs having a total of 198 participants including 28 women (6.5%). Trial quality was assessed by using Jadad scoring. Pooled estimate [95%CI] =-0.2752 [-0.4611, -0.0893] by fixed effects model. Pooled estimate [95%CI]= 0.5749 [-1.1712,0.0215] by random effects model.Test for homogeneity was rejected. (Q=84.2112, p<0.001). A funnel plot of the random effects model using REML showed asymmetry.

Conclusions: Evidence from small, single center studies with short-term follow-up suggests that use of anti-hypertensives may reduce severity of OSA in adults with hypertension.

SA-PO197

Evaluating the Impact of High-Dose Chemotherapy and Autologous Stem Cell Transplantation on Blood Pressure and Glomerular Filtration Rate in Multiple Myeloma Patients Chadi Y. Saad,[1] Joshua Fogel,[3] Leah Balsam.[1] *[1]Nephrology, NUMC, East Meadow, NY; [2]Finance and Business Management, Brooklyn College, Brooklyn, NY.*

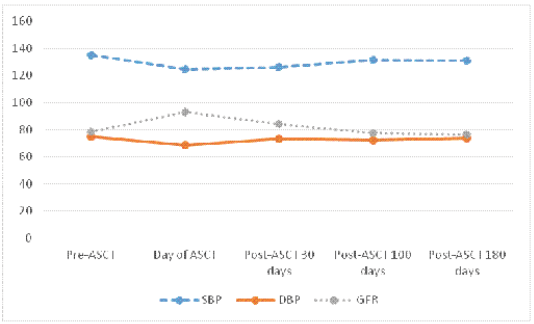
Background: Renal insufficiency is found at the time of diagnosis in almost 50% of multiple myeloma patients. Hypertension occurs in 35.8% -84.1%, of patients with CKD stages 1-5. Autologus stem cell transplantation (ASCT) may reverse kidney failure in one-third of multiple myeloma patients, which can lead to blood pressure improvement. We are not aware of any study that evaluates the impact of ASCT on improving blood pressure in multiple myeloma patients.

Methods: We studied 192 patients with an established diagnosis of multiple myeloma that underwent ASCT. We compared blood pressure readings and glomerular filtration rate GFR at 4 weeks before ASCT and then at day of ASCT and post-ASCT at 30, 100 and 180 days.

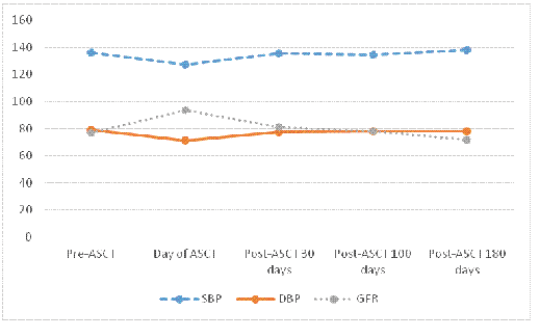
Results: For systolic blood pressure (SBP) and diastolic blood pressure (DBP), there was significantly lower mean SBP and DBP at day of ASCT and at both 30 and 100 days post-ASCT as compared to pre-ASCT SBP and DBP. For GFR, there was significantly higher mean GFR at day of ASCT and 30 days post-ASCT and significantly lower mean GFR at 180 days post-ASCT as compared to pre-ASCT. Whites had similar patterns for SBP, DBP, and GFR except for SBP which was still significantly lower and GFR which was not significantly different at 180 days. However, Blacks showed no significant reductions in the mean values of SBP and DBP and no significant increases for GFR in follow-up after day of ASCT. Furthermore, the mean value of GFR was significantly lower at 180 days post-ASCT.

Figure 1

Impact of ASCT on BP and GFR in White patients.



Impact of ASCT on BP and GFR in Black patients.



Note: SBP=systolic blood pressure, DBP=diastolic blood pressure, GFR=glomerular filtration rate.

Conclusions: ASCT in multiple myeloma patients had a positive impact on SBP and DBP and GFR but the impact was minimal for Black patients. We recommend that clinicians should consider more intense therapy for elevated blood pressure and closer follow-up of kidney function in Blacks.

SA-PO198

Immediate Blood Pressure Effect of Furosemide in Peritoneal Dialysis Patients

Caroline Lamarche, Vincent Pichette, Jean-Philippe Lafrance, Georges Ouellet, Robert Zoël Bell, Sarah Bezzaoucha, Michel Vallee. *Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.*

Background: Blood pressure (BP) lowering effect of furosemide is well documented and may be explained by a reduction in plasma volume. Nevertheless, some studies point out a possible immediate vascular effect, which could be mediated by the inhibition of the cotransporter NKCC1. Large doses of furosemide are frequently used in peritoneal dialysis (PD) patients to maintain residual diuresis. The aim of our study was to evaluate the immediate BP effect of large doses of oral and intravenous (IV) furosemide in PD patients.

Methods: The study was carried out on twelve PD patients. Each patient was given an oral dose of 500 mg of furosemide after a washout period of 7 days. BP was measured before the medication administration, at 30 minutes and one, two, four, six and twelve hours after furosemide administration. We also repeated the experiment in the same patient cohort with 250 mg of IV furosemide, after another washout period.

Results: In both oral and IV treatment there were no significant differences between systolic and diastolic BP at any time. Especially, BP was the same at baseline and 30 minutes after furosemide administration (139±15 / 76±11 to 137±20 / 78±15 mmHg, $p > 0.30$ for oral and 137±22 / 78±11 to 136±21 / 79±11 mmHg, $p > 0.44$ for IV). Thereafter the systolic BP decrease slowly, reaching a nadir at 12h which was not significant (-6.92 mmHg, $p = 0.070$ for oral and -2.58 mmHg, $p = 0.41$ for IV), whereas there was no effect on diastolic BP, even at 12h (+4.5 mmHg, $p = 0.28$ for oral and +3.08 mmHg, $p = 0.37$ for IV). In a mixed model analysis, the results was the same but the drop in systolic BP at 12h became significant (-6.92 mmHg, $p = 0.009$).

Conclusions: Large furosemide doses do not induce immediate blood pressure reduction in PD patients. There was a significant drop on systolic BP only twelve hours after furosemide administration. This may be explained by a natriuretic effet. As some authors point out, in vivo arterial response may occur only at supratherapeutic concentration, which is not achieved in a real clinical context.

Funding: Private Foundation Support

SA-PO199

Impact of 2014 Evidence Based Guidelines for Hypertension on Patients with CKD

Ankit Sakhuja, Stephen C. Textor, Sandra J. Taler. *Mayo Clinic.*

Background: The recently published 2014 Evidence-Based Hypertension Guideline (2014 EBG) revised goal blood pressure for those 60 years or older, and for those with chronic kidney disease (CKD) or diabetes. The impact of these guidelines on the prevalence of uncontrolled hypertension in these subgroups is of interest to Nephrologists, particularly in regard to control rates in CKD.

Methods: The prevalence of uncontrolled hypertension was estimated by 2014 EBG and JNC 7 guidelines using data from the National Health and Nutrition Examination Survey for the years 2011 and 2012**.** We estimated the prevalence of uncontrolled hypertension stratified by age-group, sex, race and presence of CKD or diabetes. Predictors to being reclassified to controlled hypertension by the 2014 EBG were assessed using multivariable logistic regression.

Results: Prevalence of uncontrolled hypertension was 12.8% by the 2014 EBG in comparison to 16.6% by JNC 7 ($p<0.001$). 23.0% of those with uncontrolled hypertension by JNC 7, were reclassified to controlled hypertension by the 2014 EBG. Those reclassified to controlled hypertension were more likely to be in the 60-74 year age group (OR 3.89; 95% CI: 1.58-9.59), female (OR 1.88; 95% CI: 1.30-2.73) and with diabetes (OR 2.03; 1.24-3.30). When stratified based on the presence of CKD or diabetes, those with CKD only were least likely to be reclassified (22.1% for CKD alone versus 35.8% with Diabetes alone, 34.2% with CKD and diabetes and 41.4% for those ≥60 years old without diabetes or CKD; $p=0.01$).

Conclusions: The prevalence of uncontrolled hypertension has declined from 16.6% to 12.8% using the 2014 EBG in comparison to JNC 7 guidelines. The greatest impact of reclassification from uncontrolled to controlled hypertension is on the elderly, females and those with diabetes. Those with CKD only were less often reclassified, indicating a group where greater attention to treatment is still needed.

SA-PO200

Tetrahydrobiopterin and Exercise Capacity in Patients with Chronic Kidney Disease

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Background: Patients with chronic kidney disease (CKD) have poor physical capacity and exercise intolerance. Our prior studies have shown that CKD patients have an exaggerated blood pressure (BP) response during acute exercise, which could contribute to reduced exercise capacity and adverse cardiovascular events. Nitric Oxide (NO) levels increase substantially during exercise to increase muscle blood flow and prevent excessive hypertension; therefore, reduced NO bioavailability may be one major factor contributing to the exaggerated exercise pressor response, since CKD is characterized by decreased NO bioavailability. Tetrahydrobiopterin (BH4) is an essential cofactor for endothelial nitric oxide synthase (eNOS) that has been shown to increase NO bioavailability in experimental models of CKD. Therefore, we hypothesized that long-term BH4 supplementation may ameliorate the exaggerated increase in BP during exercise, and improve exercise capacity in patients with CKD.

Methods: We conducted a randomized, double-masked, placebo-controlled trial testing the potential benefits of oral BH4 treatment on exercise capacity and exercise pressor responses in 36 patients with CKD Stages II and III. Patients were randomized to 12 weeks of: 1) 200mg BH4 twice daily + 1mg folic acid once daily; versus 2) matching placebo twice daily + 1mg folic acid once daily. The primary endpoints were change in exercise capacity measured via peak oxygen uptake (V02 max) during maximal exercise treadmill testing, and change in BP during maximal exercise. Secondary endpoints included change in exercise-induced oxidative stress.

Results: We observed no significant difference in change in exercise capacity measured as V02 max between patients treated with BH4 versus placebo. We also observed no differences in BP responses, or oxidative stress responses during exercise between the groups.

Conclusions: Chronic BH4 treatment does not improve exercise capacity or lower BP responses to maximal exercise in hypertensive patients with CKD.

Funding: Other NIH Support - NHLBI grant K23 HL098744; PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program, National Center for Research Resources, Veterans Affairs Support, Private Foundation Support

SA-PO201

Hypertension and Blood Pressure Variability in NEPTUNE

Christine B. Sethna,[1] Laura H. Mariani,[2,7] Robert J. Weyant,[7] Tammy M. Brady,[6] Kevin E.C. Meyers,[4] Keisha L. Gibson,[5] Larysa T. Wickman,[2] Crystal A. Gadegbeku.[3] *[1]Cohen Children's Medical Center of New York, NY; [2]U. of Michigan, MI; [3]Temple U., PA; [4]The Children's Hospital of Philadelphia, PA; [5]U. of North Carolina, NC; [6]Johns Hopkins U., MD; [7]Arbor Research, MI.*

Background: The prevalence, variability and treatment of hypertension (HTN) in nephrotic syndrome (NS) are not well described. Increased visit-to-visit blood pressure variability (BPV) has been associated with progression of renal disease, but has not been characterized in NS.

Methods: Data were from 376 participants in the multicenter Nephrotic Syndrome Study Network (NEPTUNE). HTN was defined as BP ≥140/90 mmHg for adults and ≥95% for children, and was the reference for BP index (BPi). Pre-HTN was defined as BP≥120/80

or ≥90% for children. BPV was measured as standard deviation (SD) and average real variability (ARV) in 246 participants with ≥3 BP readings. Regression models examined the association of BPV with eGFR at last visit.

Results: 248 adults (45.7±16.6 yr, 61% M, 21% Black) and 128 children (9.7±5.1 yr, 57% M, 40% Black) with mean eGFR of 81.7±37.8 ml/min/1.73m^2 and UPC 3.4±4.1 were evaluated. At baseline, 26% and 34% had pre-HTN and HTN, respectively. 55% were on RAAS blockade. Children were more likely hypertensive (43% versus 29%, p<0.001) and less likely on anti-hypertensives compared to adults. SD (β=-0.91, p<0.01) and ARV (β=-0.74, p<0.01) were associated with eGFR at last visit, adjusting for demographics, diagnosis, medications and baseline eGFR.

	Adults	Children	All	P
Pre-HTN	88(35%)	13(10%)	101(26%)	<0.0001
HTN	75(29%)	57(43%)	132(34%)	
SBP/DBP	125±18/77±12	112±14/70±12	121±18/75±12	<0.0001
SBP%/DBP%		73±26/77±25		
SBPi	0.90±0.13	0.94±0.11	0.91±0.13	<0.001
DBPi	0.86±0.13	0.93±0.19	0.88±0.16	<0.0001
SD	11.5±5.6	8.2±4.4	10.4±5.7	<0.0001
ARV	12.2±7.2	8.4±5.1	10.9±8.4	<0.0001
Steroid	61(24%)	78(59%)	139(36%)	<0.001

Conclusions: In NEPTUNE, HTN is common and under-treated despite significant use of anti-hypertensives. Differences in prevalence, BPV and treatment were found between adults and children. Lastly, BPV is an independent predictor of renal function in this cohort.

Funding: Other NIH Support - NIH grant to NEPTUNE: U-54-DK-083912, Private Foundation Support

SA-PO202

Practice Audit of Renal Denervation Procedure for Drug-Resistant Hypertension Praveena Sivapalan,[1] John Floras,[1,5] Douglas Jeffrey Ing,[1,5] George Dimitrios Oreopoulos,[2,3,5] Dheeraj K. Rajan,[3] Coimbatore Srinivas,[4] Duminda N. Wijeysundera,[4] Alexander G. Logan.[1] *[1]Medicine, Univ Health Network (UHN) and Mount Sinai Hospital, Toronto, ON, Canada; [2]Surgery, UHN, Toronto, ON, Canada; [3]Interventional Radiology, UHN, Toronto, ON, Canada; [4]Anesthesia, UHN, Toronto, ON, Canada; [5]Peter Munk Cardiac Center, UHN, Toronto, ON, Canada.*

Background: While two seminal but unblinded trials demonstrated substantive reductions in office blood pressure (BP) following renal denervation (RDN) in patients with drug-resistant hypertension (DRH), this was not confirmed in the large American randomized, sham-controlled Symplicity HTN-3 trial. Thus, we suspended our institution's RDN program pending audit of our "real-world" experience with the procedure, which we report herein.

Methods: In a retrospective office chart review, we assessed the effects of RDN in patients with 'true' DRH at 6- and 12-months after the procedure. Office BP was measured sitting quietly alone 5 times using an automated device.

Results: Of 105 patients referred for initial evaluation, 21 were eligible and agreed to have the procedure. BP averaged 170.0±26.3/89.6±18.8 (mean ± SD) mm Hg at baseline, 148.6 ± 23.0/81.9±19.0 at 6 months (p = 0.01 and 0.18 for systolic BP and diastolic BP, respectively) and 156.5±22.4/83.0±18.3 mm Hg at 1 year (p = 0.16 and 0.27). 63 and 42% of patients achieved a systolic BP reduction of > 10 mm Hg at 6 and 12 months, respectively. There was no significant change in the average number of antihypertensive drugs used at 6 months or 1 year (p = 0.18, 0.22). Serum creatinine, which averaged 90.4±22.9 umol/L at baseline, rose to 106.8±42.3 umol/L at 1 year (p = 0.03). There were 9 peri-procedural complications, including a renal artery dissection and acute renal failure, which resolved.

Conclusions: RDN had no effect on diastolic BP and only lowered systolic BP significantly at 6 months. Moreover, there was a wide variation in BP response and we observed several serious complications with a trend towards worsened renal function. Based on the outcomes of this audit, we are reserving RDN for patients who are adherent to antihypertensive drug treatment and suspected to have neurogenic DRH.

Funding: Private Foundation Support

SA-PO203

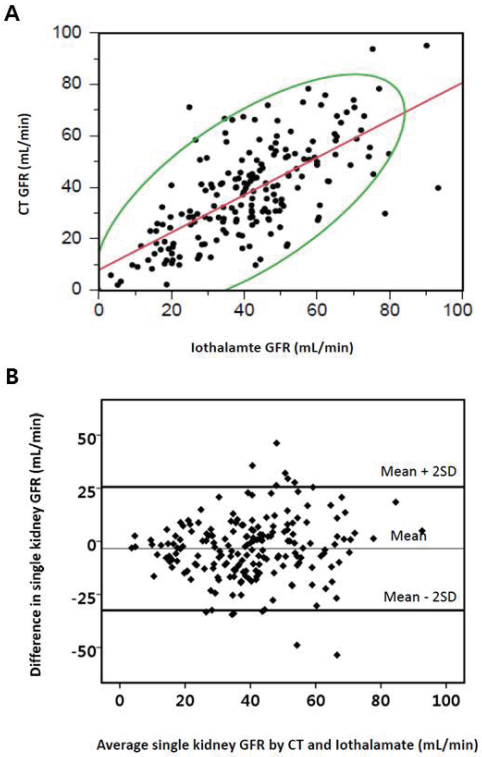
Determination of Single-Kidney Glomerular Filtration Rate in Human Subjects By Computed Tomography Soon Hyo Kwon,[1] Ahmed Saad,[1] Sandra Herrmann,[1] Cynthia H. Mccollough,[2] Stephen C. Textor,[1] Lilach O. Lerman.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Medical Physics and Biomedical Engineering, Mayo Clinic, Rochester, MN.*

Background: Determination of single-kidney glomerular filtration (SK-GFR) is important in various clinical settings, but difficult to obtain noninvasively. Multi-detector computed tomography (MDCT) provides reliable GFR measurements in animal models. This study assessed the feasibility and validated MDCT assessments of SK GFR in human subjects (n=96).

Methods: Patients with essential hypertension (EH; n=50) or renovascular hypertension (n=46) were prospectively studied under controlled conditions (sodium intake and renin-angiotensin blockade). GFR were measured using MDCT (calculated from contrast-media time density curves), and compared to GFR measured by iothalamate clearance, which was assigned to the right and left kidney by their relative volumes. The reproducibility of CT-GFR over a 3-months period was assessed in patients with renal artery stenosis (RAS) on stable medical treatment (n=21).

Results: SK CT-GFR values were similar to SK iothalamate clearance (38.2±1 versus 41.6±17 mL/min). Stenotic kidney CT-GFR was lower compared to contralateral GFR and to EH single kidney GFR (23.6±14 versus 37.2±17 and 45.2±16 mL/min, respectively, both p<0.0001), as was iothalamate clearance. CT-GFR correlated well with iothalamate-GFR (r=0.66, p<0.0001), and Bland-Altman analysis confirmed their agreement.

Figure 1.



CT-GFR also showed high reproducibility over 3 months.

Conclusions: CT assessments of SK-GFR are reproducible and agree well with a reference standard. CT can be useful to estimate noninvasively bilateral SK function in human subjects.

Funding: NIDDK Support

SA-PO204

Left Ventricular Hypertrophy (LVH) Assessment by Cardiac Magnestic Resonance Imaging (cMRI) in Relation to 24 Hour Ambulatory Blood Pressure Monitoring (ABPM) in Children Katarina Supe-Markovina, James Nielsen, Laurie E. Panesar, Robert Woroniecki. *Pediatrics, Stony Brook Children's Hospital, Stony Brook, NY.*

Background: LVH is a clinical marker of hypertensive end-organ damage. In adults cMRI is considered "gold standard" for left ventricular mass (LVM) measurement, whereas in children LVM is derived from ECHO measurements indexed to age/height norms to assess for presence of LVH. Although ECHO is widely available, there are several issues with its ability to assess LVM. ECHO has technical, observer, and patient variables which may hinder its clinical use especially in overweight children. Quantification of volumes and mass relies on geometric assumptions that do not apply to ventricles undergoing asymmetric cardiac remodeling.

Methods: Our objective is to compare LVM measurements by ECHO and cMRI in relation to severity of hypertension assessed by ABPM. We collected existing data on children evaluated in our Hypertension Center. Each subject had ECHO, cMRI, ABPM, urine microalbumin, eGFR (modified Schwartz formula). LVM by ECHO was obtained and calculated by m-mode utilizing Devereux equation. cMRI LVM was measured from standard short-axis diastolic cine-images covering the entire left ventricle.

Results: We had a total of 15 subjects, 33% females, 66% caucasian, 15±3.8 years old; BMI 28.6±6.6, eGFR=83.8 ± 19.8 ml/min/1.73m^2. 1/15 subjects had microalbuminuria, 7/15 subjects had LVH by ECHO and 6/15 had LVH by cMRI. By ECHO LVM was 172.5 ± 60.9g and by cMRI 152.8 ± 43.6g, p=0.099 (paired t-test). 10/15 patients had consistent ECHO and cMRI results, 2 subjects who had normal ECHO had abnormal cMRI. 3 subjects with abnormal ECHO had normal cMRI, p=0.31 (Fisher's exact). Pairwise correlation of abnormal LVM between ECHO and cMRI was 0.76, p=0.003. We found no statistically significant correlation among ABPM variables with ECHO and cMRI including LVM/volume.

Conclusions: In our hypertensive children cMRI and ECHO showed different LVM yet the difference was not statistically significant. Relationship of ABPM parameters and LVM were not statistically different between the 2 methods. Our study is limited by small sample size and longitudinal assessment in the future will be required.

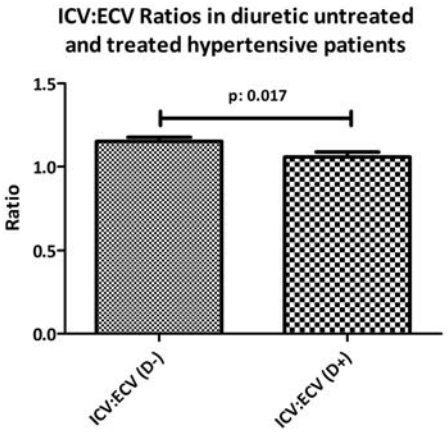
SA-PO205

Effect of Diuretics on Body Fluid Spaces in Hypertensive Patients as Measured by Bioimpedance Neville R. Dossabhoy,[1] Tibor Fulop,[2] Istvan Barna,[3] Maria Faludi,[4] Klara Berta,[4] Zsolt Lengvarszky,[5] Tibor Szarvas,[5] Mihaly B. Tapolyai.[3] *[1]Medicine, VA Medical Center, Shreveport, LA; [2]Univ Medical Center, Jackson, MS; [3]Semmelweis Univ, Budapest, Hungary; [4]Fresenius SOTE, Hungary; [5]LSUS, Shreveport, LA.*

Background: Many of the side-effects of diuretics come from a reduction in Extracellular Fluid Volume (ECV). We examined the association of diuretics and body fluid spaces in hypertensive patients.

Methods: This is a cross-sectional study of 60 hypertensive patients - with no known endocrine, urological or renal abnormalities - in a hypertension clinic who underwent a multichannel bioimpedance analysis.

Results: The use of diuretics had no correlation with achieved blood pressure (diuretics treated *versus*. untreated: 137/76 *versus*. 135/81 mmHg; P=0.66), but it did with the total number of antihypertensive medications (3.1 *versus*. 1.3; P<0.0001) and age (66.7 *versus*. 75.2 years; P<0.0001). Overhydration is greater numerically in the diuretic-use group only to a non-significant degree (5.9 *versus*. 2.9%; P=0.21). The total body water (39.8 *versus*. 40.5 L; P=0.64), ECV (18.5 *versus* 19.7 L; P=0.35) and Intracellular Fluid Volume (ICV) (21.3 *versus*. 20.8 L; P=0.75) were not significantly different in the untreated v. treated group. The ratio of ICV:ECV, however, seems to have been affected significantly; this ratio is 1.15 among patients who do not take diuretics, but 1.05 among those who do (P=0.017).



Conclusions: These results seem to challenge the notion that the ICV would be constant or inaccessible to renal function or fluid loss by diuresis. The diuretic-related distortion of ICV:ECV ratio indicates that the intracellular space is also affected or the fluid is redistributed in the body. To clarify the effect of diuretics, further studies are needed. The use of bioimpedance analysis may represent a potential clinical tool to help achieve euvolemia in hypertensive patients.

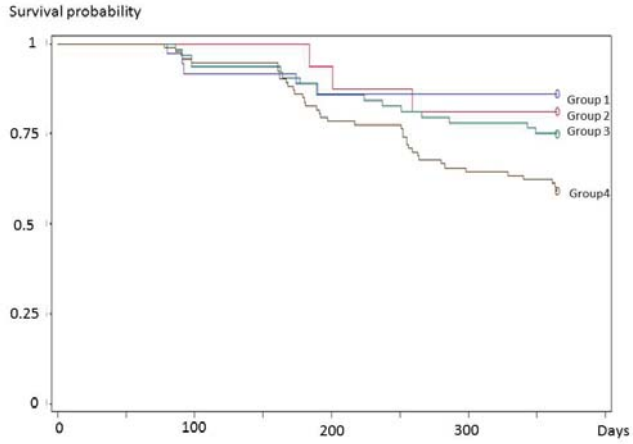
SA-PO206

Changes in Protein and Salt Intakes Are Associated with the Progression of Chronic Kidney Disease Eiichiro Kanda,[1] Masumi Ai,[2] Renjiro Kuriyama,[3] Masayuki Yoshida,[2] Tatsuo Shiigai.[4] *[1]Tokyo Kyosai Hospital, Japan; [2]Tokyo Medical and Dental Univ; [3]Kokubunji Minamiguchi Clinic; [4]Shiigai Clinic.*

Background: Although protein and salt restriction prevents the progression of chronic kidney disease (CKD), adhering to such dietary restriction is sometimes difficult. In this retrospective cohort study, we investigated the longitudinal effects of changes in protein and salt intakes on the progression of CKD.

Methods: CKD patients (249) under a low-protein (0.6 to 0.8g/kg/day) and low-salt (<6g/day) diet were enrolled in this study in Japan. Their protein intake was determined by Maroni's formula every three months for one year. The longitudinal changes in protein and salt intakes were evaluated by their coefficients of variation (CVs), on the basis of which patients were categorized into 4 groups. The primary outcome was a 25% decline in estimated glomerular filtration rate (eGFR) or starting dialysis. Dietary intakes were treated as time-dependent variables.

Results: Mean age (SD) was 70.6(7.0)years; eGFR, 22.2(14.1)ml/min; protein intake, 0.85(0.22)g/day, CV, 0.14(0.08); salt intake, 7.8(3.1)g/day, CV, 0.22(0.12); follow-up period, 322.5(82.6)days. The primary endpoint was observed in 65 patients (26.1%). Kaplan–Meier survival curves showed that group 1 (low CV) had a higher survival of kidney function than group 4 (high CV).



Time-dependent Cox proportional hazard models adjusted for patient characteristics showed that the outcome was associated with very low protein intake (<0.6g/kg/day) [aHR 7.125 (1.233, 41.160)] and high salt intake [aHR 8.446 (1.482, 48.140)]. A generalized estimating equation model showed a positive longitudinal relationship between protein and salt intakes (p=0.0001).

Conclusions: Protein and salt intakes and their changes are associated with CKD progression. It is important to maintain stable dietary intakes to prevent CKD progression.

SA-PO207

Long Term Impact of Low Protein Diet on Outcomes in Patients with Chronic Kidney Disease Elaine Ku,[1] David V. Glidden,[1] Kirsten L. Johansen,[1] Barbara A. Grimes,[1] Mark J. Sarnak,[2] Hocine Tighiouart,[2] Chi-Yuan Hsu.[1] *[1]UCSF; [2]Tufts.*

Background: We previously reported that in extended follow-up of the Modification of Diet in Renal Disease (MDRD) study (Ku SA-OR048, ASN 2013), strict blood pressure control appeared to confer lower risk of mortality post-ESRD (although it did not change risk of ESRD). In this study, we hypothesized that exposure to lower protein diet during CKD would lead to higher risk of death post-ESRD due to malnutrition.

Methods: We extended follow-up of the MDRD trial (1989-1993) via cross-linkage with U.S. Renal Data System and National Death Index to ascertain ESRD onset and vital status through 12/31/2010. MDRD Study A participants had GFR between 24.5 and 55 mL/min/1.73 m^2 and were randomized to 1.3 versus 0.58 g protein/kg/day (N=423). Study B participants had GFR ranging from 13 up to 24.5 mL/min/1.73 m^2 and were randomized to 0.58 versus 0.28 g protein/kg/day (N=417). We pooled both studies and compared the long-term effect of higher versus lower protein diet using a Cox model for risk of death with ESRD as a time-dependent covariate.

Results: Of the 840 MDRD enrollees, 627 developed ESRD by 12/31/2010. Contrary to our hypothesis, we found no difference in risk of death post-ESRD among patients assigned to lower protein diets during the study. There were 157 deaths in the lower protein diet arm and 167 deaths in the higher protein diet arm (unadjusted hazard ratio [HR] 1.13; 95% CI 0.91-1.41; p = 0.26). We also noted no difference in risk of death pre-ESRD (unadjusted HR 0.93; 95% CI 0.65-1.32; p = 0.67). For the subset of patients who started dialysis after 1995 in whom we had laboratory data per CMS-2728 form, no significant differences were noted in body mass index (p=0.25) or serum albumin (p=0.34) among those previously randomized to higher versus lower protein diet. We did note a lower risk of ESRD in the lower protein diet arm (unadjusted HR 0.84; 95% CI 0.72-0.98; p = 0.03). Results were similar when study A and B were analyzed individually.

Conclusions: Unlike strict BP control, low protein diet during CKD did not appear to impact mortality post-ESRD.

Funding: NIDDK Support

SA-PO208

Low Score of Dietary Approaches to Stop Hypertension (DASH) Diet Is Associated with Chronic Kidney Disease (CKD) Risk in U.S. Adults Tanushree Banerjee,[1] Deidra C. Crews,[2] Jennifer L. Bragg-Gresham,[3] Rajiv Saran,[3] Meda E. Pavkov,[4] Desmond Williams,[4] Neil R. Powe.[1] *[1]UCSF; [2]JHU; [3]UM; [4]CDC.*

Background: In addition to lowering blood pressure, a DASH-type diet reduced kidney function loss in women with mildly decreased estimated glomerular filtration rate (eGFR), an effect primarily associated with reduced red meat intake.We investigated whether a DASH diet, rich in fruits, vegetables, nuts and legumes, is associated with prevalent CKD.

Methods: We conducted a national cross-sectional study using the 24-hr dietary recall of 15,171 adults aged ≥20 years enrolled in the National Health and Nutrition Examination Survey III, between 1988 and 1994. We calculated a DASH-diet adherence score based on 9 target nutrients: total fat (TF), saturated fat, protein, fiber, cholesterol, calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K). Adherence was defined as score ≥4.5 out of a possible score of 9. Logistic regression was used to explore the relation of DASH

score tertile and (i) eGFR<60 ml/min/1.73 m^2 and (ii) albumin-to-creatinine ratio (ACR) ≥ 30 mg/g, adjusting for demographics, socio-economic factors, smoking, diabetes, and hypertension.

Results: The mean age of participants was 48.6 years, 46.9% were males, 26.9% were blacks, and median DASH score was low (2.5, interquartile range 2.0-4.0). 7.8% of participants had an eGFR<60 ml/min/1.73 m^2. Those in the lowest DASH tertile were more likely to be males, poor, current smokers, and have lower education level while those in the highest tertile were more likely to have diabetes and hypertension. Among those with CKD (15≤eGFR<60 ml/min/1.73 m^2 or ACR≥30 mg/g), 5.5% were adherent. Intake of fiber, Mg, Ca, K were lower while cholesterol, Na, TF, and protein were higher among the CKD versus non-CKD group (v P<0.05). The lowest DASH tertile compared to highest was associated with greater odds of eGFR<60 (OR [95% CI]:1.6[1.2-2.0]) but no statistically significant association with high ACR was found (1.1[0.9-1.3]), after full adjustment.

Conclusions: A low Dash diet score was associated with lower eGFR but not ACR levels. More studies are needed to examine the role of diet on both the prevalence and progression of CKD.

SA-PO209

Dietary Acid Load and Incident Chronic Kidney Disease: The Atherosclerosis Risk in Communities Study C. Rebholz, M. Grams, Deidra C. Crews, Lawrence J. Appel, Josef Coresh. *Johns Hopkins Univ.*

Background: Higher dietary acid load can result in metabolic acidosis and is associated with faster kidney disease progression in chronic kidney disease (CKD) patients. However, this relationship has not been evaluated in the general population.

Methods: We conducted prospective analyses of ARIC Study participants without CKD at baseline (1987-89, N=15,100). Dietary acid load was estimated by calculating potential renal acid load of usual dietary intake assessed by a food frequency questionnaire (PRAL=0.49*protein+0.037*phosphate-0.021*potassium-0.026*magnesium-0.013*calcium). Incident stage 3 CKD was assessed from baseline through 2010. Cox proportional hazards regression was used to estimate the association of dietary acid load (PRAL) with incident CKD, adjusting for age, sex, race, total caloric intake, and baseline eGFR. Analyses were repeated after stratifying by race.

Results: Median (25th, 75th percentiles) PRAL was 4.37 (-3.86, 12.79) mEq/day. At baseline, higher levels of PRAL were associated with younger age, black race, and male sex (p for all <0.001). During a median follow-up of 24 years, there were 2,351 (15.6%) incident CKD cases [765 (19.6%) in blacks, 1,586 (14.2%) in whites, p<0.001]. Those participants who developed CKD had higher dietary intake of protein, and lower intake of magnesium and calcium than those participants who did not develop CKD. Dietary intake of phosphate and potassium was similar by CKD status. In the overall study population, higher levels of PRAL were significantly associated with risk of incident CKD. The findings were significant only among blacks.

Population	Hazard Ratio	95% Confidence Interval	P for interaction
Overall	1.06	1.00, 1.11	
Blacks	1.13	1.03, 1.24	0.14
Whites	1.02	0.96, 1.09	

Conclusions: Dietary acid load is associated with incident stage 3 CKD among blacks, but not whites. Reducing dietary acid load, previously associated with slower rates of kidney disease progression in CKD patients, might also prevent CKD.

Funding: Other NIH Support - NHLBI

SA-PO210

High Protein Diet Alters the Metabolic Status of Mice Comparable to Changes in Uremic Toxin Levels Observed in Renal Failure Patients Jitske Jansen,[1,2,3] Henricus A.M. Mutsaers,[1,2,3] Lambertus P.W.J. Van den Heuvel,[2] Joost Hoenderop,[3] Rosalinde Masereeuw.[1] *[1]Pharmacology-Toxicology, Radboudumc, Netherlands; [2]Pediatrics, Radboudumc, Netherlands; [3]Physiology, Radboudumc, Nijmegen, Netherlands.*

Background: Many of the well-studied uremic toxins originate from the diet and are generated in the colon due to protein fermentation by intestinal bacteria. Here, we examined the impact of a high protein diet on the plasma concentration of a variety of uremic toxins to obtain insight in the interplay between dietary protein and uremic solutes.

Methods: Wild type (WT) Friend leukemia virus B (FVB) mice were fed control (21% crude protein; n=10) or high protein (HP; 45% crude protein; n=10) diet for 21 days, after which mice were sacrificed and uremic toxins were measured by LC-ESI-MS/MS.

Results: Mice fed HP diet developed polyuria (HP: 0.8 ± 0.2 mL/18 h versus control: 0.3 ± 0.04 mL/18 h; p=0.025), implying the presence of renal failure, and showed higher plasma levels of the phenol-derived metabolites phenylacetic acid (1.9 ± 0.3 μM, p=0.01), phenyl sulfate (3.9 ± 0.8 μM, p=0.0061), phenyl glucuronide (0.2 ± 0.05 μM, p=0.0095), p-cresyl glucuronide (1.6 ± 0.3 μM, p=0.0093) and hippuric acid (0.1 ± 0.01 μM, p=0.0002) compared to mice provided with control diet (1.1 ± 0.1 μM, 1.4 ± 0.3 μM, 0.03 ± 0.007 μM, 0.7 ± 0.1 μM and 0.04 ± 0.008 μM, respectively). In addition, a reduction in tryptophan concentration (HP: 93 ± 11 μM versus control: 127 ± 5 μM, p=0.011) was observed as well as 1.2 fold increase in indoleamine 2,3-dioxygenase (IDO) activity (P=0.0389). Furthermore, in mice fed HP diet, indoxyl sulfate levels significantly increased from 3.6 ± 0.7 μM (control) to 7.1 ± 1.7 μM (p=0.015). In contrast, p-cresyl sulfate and kynurenic acid levels remained unaltered, and kynurenine and indole-3-acetic acid concentrations diminished (p<0.05).

Conclusions: A HP diet alters the metabolic status of mice comparable to changes observed in chronic kidney disease patients. This supports the hypothesis that managing the levels of dietary protein intake in patients may be of key importance since high protein intake will augment uremic toxin levels, whereas a restriction in dietary protein might cause protein-energy wasting.

Funding: Government Support - Non-U.S.

SA-PO211

Influence of a High Protein Diet on the Human Metabolome Ruben Poesen,[1] Karen Windey,[2] Rosalinde Masereeuw,[3] Petra Van Den Broek,[3] Pieter Evenepoel,[1] Kristin Verbeke,[2] Bjorn Meijers.[1] *[1]Nephrology, Univ Hospitals Leuven, Belgium; [2]TARGID, Univ of Leuven, Belgium; [3]Pharmacology and Toxicology, Radboud Univ Medical Center, Netherlands.*

Background: There is a long held belief that protein restriction may attenuate CKD progression. Lately, there is a renewed interest in metabolites originating from protein fermentation as potential driving forces behind adverse outcome in renal disease. Indoxyl sulfate and p-cresyl sulfate, both protein fermentation metabolites, have been associated with CKD progression, supporting the so-called protein metabolite hypothesis. The influence of protein intake on these and other metabolites has not been fully elucidated.

Methods: 29 healthy volunteers were randomized to a high or a low protein diet for 2 weeks. Blood and urine were sampled before and after intervention. All samples were analyzed by liquid chromatography mass spectrometry for measurement of a panel of metabolites, focusing on tryptophan and phenol metabolites. Urinary collections were used to calculate 24h urinary excretion rates as a surrogate of intestinal generation. Differences in plasma levels and urinary excretion rates were compared with the Wilcoxon rank sum test.

Results: After randomization, 14 subjects received a high protein diet and 15 subjects were allocated to a low protein diet. There were no between-group differences in age, gender and BMI. In the high protein group, we observed a significant increase in plasma levels of indoxyl sulfate (median 19% increase in high protein group versus 18% decrease in low protein group, P 0.004). When considering urinary excretion rates, we also noted significant increases of indoxyl sulfate (+33% versus -9%, P 0.001), indoxyl glucuronide (+80% versus -6%, P 0.01), kynurenic acid (+34% versus -4%, P 0.006) and quinolinic acid (+15% versus -6%, P 0.02).

Conclusions: High protein intake is associated with increased plasma levels and generation rates of different metabolites, including indoxyl sulfate, indoxyl glucuronide, kynurenic acid and quinolinic acid. As part of these metabolites are known uremic retention solutes possibly contributing to CKD progression, these findings give additional insights in the presumed beneficial effects of protein restriction in CKD.

Funding: Government Support - Non-U.S.

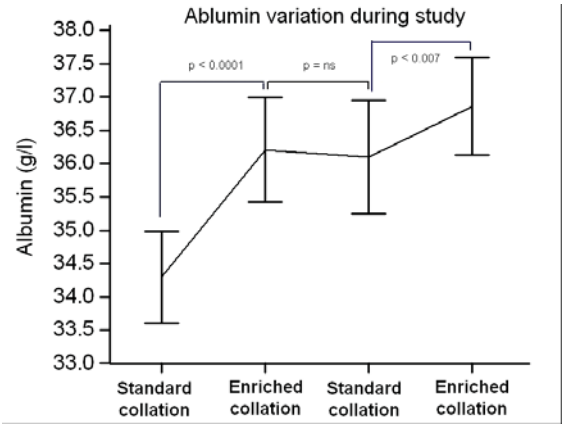
SA-PO212

Nutritional Impact of a Protein-Enriched Snack During the Hemodialysis Session: Results of the COLENDIA Study Patrik Deleaval, Anne-Lise Bernollin, Guillaume Jean, Charles Chazot. *Nephrology and dialysis, Nephrocare Tassin Charcot, Sainte-Foy-Les-Lyon, France.*

Background: Protein energy wasting (PEW) is frequent in hemodialysis (HD) patients. Therefore, it is necessary to develop simple but effective nutritional strategies to prevent this complication. The aim of our study was to assess the impact of an intradialytic protein-enriched snack on nutritional parameters.

Methods: The study design was a prospective double-cross over study during 4 periods of 4 months each. Patients were given alternatively standard (protein : 8.2 g) or protein-enriched (28.2g) snack (hot drink, DELICAL floridin® cream and 3 DELICAL Nutra'cake® biscuits). 73 patients on 3 weekly HD sessions for more than 3 months and receiving the usual snack during dialysis were included. Patients receiving immunosuppressive therapy, treated for active cancer or hospitalized for more than 4 weeks were excluded. No selection on nutritional criteria was applied.

Results: Albumin increased significantly from 34.29 ± 2.83 g/l to 36.20 ± 3.21 g / l (p <0.0001) between the first (standard snack) and second period (enriched snack). In the third period (standard snack), the albumin concentration remain stable (36.09 ± 3.52 g/l). After the 4th period (enriched collation) there was a further significant increase in serum albumin (36.86 ± 3.02 g/l) as compared to the second as third period (respectively p = 0.05 and p = 0.007).



Prealbumin, weight and NPNA did not vary significantly during the 16-month study. A bioimpedance was conducted at the end of each period. The hydration status did not change throughout the period.

Conclusions: This study demonstrates that the distribution of a protein-enriched snack during the haemodialysis session in unselected patients significantly increases their serum albumin. As markers of PEW are powerful predictors of mortality, this simple and effective strategy can be of great interest.

Funding: Pharmaceutical Company Support - Lactalis Nutrition Santé, Torcé, F-35370, France

SA-PO213

High Amylose Resistant Starch Diet Ameliorates Oxidative Stress, Inflammation, and Progression of Chronic Kidney Disease (CKD) Shuman Liu,[1] Sohrab Nazertehrani,[1] Seyed Farzaneh,[1] Wei Ling Lau,[1] Mahyar Khazaeli,[1] Sean H. Adams,[2] Dorothy Kieffer,[2] Martin Roy J,[2] Nosratola D. Vaziri.[1] *[1]Div of Nephrology, UC Irvine; [2]Dept of Nutrition, UCD.*

Background: The gut microbiome is profoundly altered in advanced CKD due, in part, to: influx of urea in the intestinal tract which leads to the dominance of urease-possessing bacteria and restriction of potassium-rich fruits and veggies which are the main source of dietary fiber. The latter leads to contraction of bacteria that convert fiber to short chain fatty acids (SCFA). Conversion of urea to NH_3 by bacterial urease damages epithelial tight junction which evokes inflammation via influx of endotoxin in the circulation. This is compounded by diminished supplies of SCFA which are essential for growth of anti-inflammatory regulatory T cells. We, therefore, hypothesized that soluble fiber supplementation may attenuate oxidative stress, inflammation and CKD progression.

Methods: SD rats were fed a chow containing 0.7% adenine for 2 weeks to induce chronic interstitial nephropathy (CKD). They were then placed on either regular or 30% resistant starch containing diet for 3 weeks. Rats fed regular diet served as controls.

Results: Compared with the controls, CKD rats exhibited impaired urinary concentrating capacity, reduced creatinine clearance, interstitial fibrosis, leukocyte infiltration, tubular damage, activation of NF_kB, upregulation of pro-inflammatory (MCP-1, COX-1, COX-2, iNOS), pro-oxidant (gp91, and NOX-4) and pro-fibrotic (PAI-1, TGF-β, and α-SM actin) molecules; impaired Nrf2 activity, and down-regulation of antioxidant/cytoprotective enzymes (catalase, superoxide dismutase, glutathione peroxidase and heme oxigenase-1). The resistant starch diet improved creatinine clearance, urine concentrating capacity, interstitial fibrosis, inflammation, and tubular damage and attenuated oxidative, inflammatory, and fibrotic pathways and partially restored Nrf2 activity.

Conclusions: Resistant starch supplementation retards CKD progression, attenuates oxidative stress and inflammation, and improves endogenous cytoprotective pathways in CKD rats. Future studies are needed to explore the impact of high fiber diet in CKD patients.

SA-PO214

Obesity Induced Chronic Inflammation in Long-Term High Fat Diet Challenged C57Bl6j Mice Is Associated with the Development of Renal and Cardiac Amyloidosis Roel A. van der Heijden,[1] Johannes Bijzet,[2] Davith De Vries,[1] Bouke P. Hazenberg,[2] Grietje Molema,[1] Peter Heeringa.[1] *[1]Dept Medical Biology, ; [2]Rheumatology, Univ Medical Center Groningen, Netherlands.*

Background: Obesity-induced chronic inflammation is considered an accelerating factor in the development of age-associated cardiovascular diseases (CVD) and is a risk factor for chronic kidney diseases (CKD). Little is known about the sequence of events involved in obesity induced CVD. The aim of the current study was to investigate the effects of obesity on the evolution of age associated CV complications in high fat diet challenged C57Bl6j mice.

Methods: C57Bl6J male mice (99 in total) were fed a low (10% lard; LFD) or high (45% lard; HFD) fat diet for a maximum of 52 weeks. Mice were monitored for development of obesity, metabolic alterations and cardiovascular complications. Blood and urine samples were taken at monthly intervals. Cohorts of mice were sacrificed after 24, 40 and 52 weeks, and organs were collected for histological and gene expression analyses.

Results: High fat feeding induced an obese phenotype characterized by increased body and organ weights, altered lipid and insulin homeostasis, low grade systemic inflammation, and increased adipokine release in time. Renal and cardiac gene expression analyses showed upregulation of pro-inflammatory and pro-fibrotic genes, along with elevated urinary albumin and NGAL levels. A progressive sclerotic phenotype in kidney and heart was observed in time in both LFD and HFD mice, but was more extensive in HFD mice. Lesions stained positive for Congo red and exhibited birefringence in polarized light consistent with the development of amyloidosis. The degree of amyloidosis correlated significantly with body weight. By immunohistochemistry, the amyloid deposits stained positive for serum amyloid A (SAA) protein. Moreover, plasma levels of SAA were chronically elevated upon HFD and significantly higher compared to LFD mice.

Conclusions: Our data suggest a causal link between obesity induced chronic inflammation and amyloidosis in C57Bl6J mice and indicate obesity-enhanced chronic secretion of SAA may lead to extravascular misfolding and accumulation in time due to SAA's amyloidogenic properties.

Funding: Government Support - Non-U.S.

SA-PO215

Protein-Energy Wasting and Quality of Life in Chronic Hemodialysis Patients Camiel L.M. de Roij van Zuijdewijn,[1] Muriel P. Grooteman,[1,2] Menso Jan Nubé,[1,2] Peter J. Blankestijn,[3] Michiel Bots,[4] Pieter M. Ter Wee.[1,2] *[1]Nephrology, VU Univ Medical Center, Amsterdam, Netherlands; [2]Inst for Cardiovascular Research, VU Univ Medical Center, Amsterdam, Netherlands; [3]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [4]Julius Center for Health Sciences and Primary Care, Univ Medical Center Utrecht, Utrecht, Canada.*

Background: Protein-energy Wasting (PEW) describes the state of decreased bodily protein and energy fuels and is associated with mortality. Despite various nutritional tests, it is currently unknown how PEW can best be measured. As this syndrome is highly prevalent among hemodialysis (HD) patients and is associated with an impaired quality of life (QoL), the nutritional test that correlates best with QoL may be the preferred test to assess PEW.

Methods: a cross-sectional study was performed using data from CONTRAST (NCT 00205556), a cohort of ESRD patients treated with HD or hemodiafiltration. Subjective Global Assessment (SGA), Malnutrition Inflammation Score (MIS), Geriatric Nutritional Risk Index (GNRI), composite Protein Energy Nutritional Score (cPENS), normalized Protein Nitrogen Appearance (nPNA), Body Mass Index (BMI), serum albumin and serum creatinine were assessed at baseline. QoL was assessed with the Kidney Disease Quality of Life Short Form 1.3, which summarizes QoL in 2 general and 12 kidney-disease specific (KDS) domains. Higher scores indicate better QoL. As none of the values is normally distributed, Spearmans rho (ρ) was calculated to determine relations between the nutritional tests and domains of QoL.

Results: 489 patients were analyzed. SGA, MIS, cPENS, GNRI, nPNA, albumin and creatinine correlated significantly with the Physical Component Score (ρ 0.222, -0.467, 0.216, 0.196, 0.103, 0.196 and 0.185, respectively; $p \leq 0.03$). SGA and MIS correlated significantly with the Mental Component Score (ρ 0.128 and -0.202, respectively; $p \leq 0.01$). SGA correlated significantly with 10 KDS QoL domains with ρ's between 0.095 and 0.213 ($p < 0.04$) and MIS with 11 KDS QoL domains with ρ's between -0.124 and -0.494 ($p < 0.01$).

Conclusions: The MIS correlates strongest with the different aspects of QoL and may therefore be the preferred test to assess the relation between PEW and QoL.

SA-PO216

To Eat or Not to Eat - International Experiences with Eating during Hemodialysis Treatment Brandon Kistler,[1] Deborah A. Benner,[2] Mary B. Burgess,[2] Maria Melts Stasios,[2] Kamyar Kalantar-Zadeh,[3] Ken Wilund.[1] *[1]Univ of Illinois, Urbana, IL; [2]DaVita Healthcare Partners, Denver, CO; [3]UC Irvine, Irvine, CA.*

Background: Poor nutritional status is common in hemodialysis (HD) patients and may lead to reduced quality of life, increased hospitalizations, and higher death risk. Providing food or nutrition supplements during HD is associated with improved nutritional status and reduced mortality, yet despite these benefits eating policies vary across countries and clinics.

Methods: To examine practices related to eating at HD treatment we surveyed clinicians about their clinic policies during the 2014 International Society of Renal Nutrition and Metabolism Conference.

Results: We received 73 responses from six continents. Respondents were primarily dietitians (71%) and nephrologists (26%) working at units housed in a hospital (63%). Sixty-one clinics (85%) allowed patients to eat during treatment, with 47 of these (65%) actively encouraging eating. Of the 53 clinics (73%) providing food during HD, 49 (93%) provided food at no cost to the patient. Generally, clinics provided meals high in carbohydrates. However, none of the nine clinics from North America (100%) provided food during treatment. The majority (47 clinics; 64%) provided supplements during treatment, with 43 (92%) providing at no cost. Among clinics that allow eating, providing energy (89%) and teaching opportunities (47%) were the primary reasons. We also asked clinicians about their experience with six commonly cited reasons to restrict eating using a four-point scale. Clinicians responded they observed the following "rarely" or "never": choking (98%), reduced Kt/V (98%), infection control issues (96%), spills or pests (85%), GI issues (72%), and hypotension (63%).

Conclusions: Our results indicate that internationally eating is common during treatment, disparities may exist in global practices, and most of the proposed negative sequelae of eating during HD are not commonly observed in clinical practice. These data describe current nutrition practices globally, provide a potential contributor to global differences in albumin, and highlight the need for more research to inform decisions regarding eating during HD.

SA-PO217

Changes in Practices and Opinions on In-Center Food Consumption in a Large Dialysis Organization in the United States Mary B. Burgess,[1] Deborah A. Benner,[1] Maria Melts Stasios,[1] Becky Brosch,[1] Rich Mutell.[2] *[1]DaVita Healthcare Partners, Denver, CO; [2]APEX Health Innovations, Simi Valley, CA.*

Background: Oral nutritional supplement (ONS) provided during dialysis is associated with improved mortality. Limitations in ONS programs include restricted patient participation due to eligibility criteria; supplement intolerance and taste fatigue. A complimentary program that encourages appropriate food consumption during dialysis may demonstrate similar clinical improvement as ONS programs in a larger population and provide opportunities for counseling on "usual food" choices. The purpose of this pilot

was to measure changes in practices, opinions and perceived barriers to consuming food on dialysis in a large dialysis organization (LDO) with an ONS program where facility specific policies related to food consumption are determined by the Medical Director and facility.

Methods: In May 2011 and June 2014, registered dietitians (RDs) within an LDO in the U.S. were surveyed on facility practices and opinions on food consumption during dialysis using an online survey. RDs provided input on facility practice and collected Medical Director responses. In 2013, optional guidelines and educational materials on Eating at Treatment (EAT) were developed and shared with RDs.

Results: Analysis of responses to these two questions show significant differences between the 2011 and the 2014 survey (X2, $p < .001$) and indicate a practice pattern change toward increased consumption of food on dialysis.

Facility – "What is the current practice in this facility regarding consuming food on dialysis?"

			Facility Practices					
			Not allowed, does not occur	Not allowed, does occur	No guidelines	Allowed, not encouraged	Allowed, encouraged	Total
Year	2011	Count	60	288	64	580	218	1210
		% within Year	5.0%	23.8%	5.3%	47.9%	18.0%	100.0%
	2014	Count	59	254	91	566	437	1407
		% within Year	4.2%	18.1%	6.5%	40.2%	31.1%	100.0%

Medical Director – "What is your opinion on allowing patients to consume food on dialysis?"

			Medical Director Opinion					
			Strongly Discourage	Discourage	No Opinion	Encourage	Strongly Encourage	Total
Year	2011	Count	255	161	160	212	49	837
		% within Year	30.5%	19.2%	19.1%	25.3%	5.9%	100.0%
	2014	Count	226	152	170	291	124	963
		% within Year	23.5%	15.8%	17.7%	30.2%	12.9%	100.0%

RDs reported top reasons some Medical Directors provided for not allowing eating at dialysis remain unchanged: potential increased hypotension, chance of choking, infection and GI problems.

Conclusions: This pilot demonstrates that food consumption patterns during dialysis are evolving. Further studies are warranted to understand the risks and benefits of consuming food on dialysis.

SA-PO218

Concordance of Sodium and Phosphate Load in Western Diet: Implications for Dietary Counseling Charlotte A. Keyzer, Jelmer K. Humalda, Arjan J. Kwakernaak, Maartje C.J. Slagman, Martin H. De Borst, Gerjan Navis. *Nephrology, Univ Medical Center Groningen, Netherlands.*

Background: Both sodium and phosphorus load are associated with cardio-renal risk and require dietary management. Sodium and phosphate are both abundantly present in processed food, but counseling dietary phosphate restriction is limited since phosphate content is uncommonly reported on food labels. We hypothesized that dietary sodium and phosphate load are associated in Western populations, both in healthy individuals and various patient cohorts.

Methods: We calculated habitual sodium and phosphate load from 24h urinary excretion of sodium and phosphate in healthy subjects, diabetic (DM) patients and renal transplant recipients (RTR) in the Netherlands and tested their association. We also tested the effect of dietary counseling to limit sodium intake on phosphate load in two independent cohorts, with non-DM and DM-CKD.

Results:

Population	Number of patients	Urinary sodium excretion (mmol/day)	Urinary phosphate excretion (mmol/day)	Correlation coefficient
Healthy volunteers	252	195±76	28±10	0.435
Renal transplant recipients	706	157±62	25±9	0.519
Diabetic patients	95	205±89	27±12	0.452
CKD regular sodium diet	46	189±56	31±10	0.494
CKD low sodium diet	48	106±48*	28±10*	0.463
DM-CKD regular sodium diet	43	224±76	26±11	0.747
DM-CKD low sodium diet	41	148±65*	23±9*	0.560

Urinary sodium and phosphate excretion were correlated in all cohorts ($P<0.001$), despite absolute differences. Moreover, successful dietary sodium restriction also reduced phosphate excretion, both in non-DM-CKD and DM-CKD (Table, * $P<0.05$ versus regular sodium diet).

Conclusions: Dietary exposure to sodium and phosphate are robustly associated across several Western populations. Successful dietary sodium restriction also reduces phosphate intake. Given this concordance, dietary phosphate load can be lowered by counseling aimed at dietary sodium restriction which is more efficient since sodium but not phosphate content is indicated on most food labels.

Funding: Government Support - Non-U.S.

SA-PO219

Sodium Reduced Meat and Poultry Products Contain a Significant Amount of Potassium from Additives Arti Sharma Parpia,[1,2] Marc B. Goldstein,[1,3] Joanne Arcand,[2] Mary R. L'abbe,[2] Pauline Darling.[1,2] *[1]St. Michael's Hospital, Toronto, ON, Canada; [2]Dept of Nutritional Sciences, Univ of Toronto, Toronto, ON, Canada; [3]Dept of Medicine, Univ of Toronto, Toronto, ON, Canada.*

Background: Sodium reduced food products are becoming increasingly available to consumers, however it is unclear if they are suitable for inclusion in a renal diet. Food manufacturers may use phosphate and potassium additives to replace the functional and flavor properties of sodium, and the amount is usually not listed on food labels. Increased intake of phosphorus and potassium in individuals with chronic kidney disease (CKD) can cause hyperkalemia and hyperphosphatemia, both of which are associated with increased risk of mortality. The objective of our study was to determine if the reduction of sodium in meat and poultry products is associated with increased amounts of phosphorus and potassium from food additives.

Methods: Grocery stores from the top 3 grocery chains in Canada were scanned for all sodium reduced meat and poultry products. Protein, sodium, phosphorus and potassium content were analyzed using the Association of Analytical Communities (AOAC) official methods 992.15 and 984.27, and values for sodium reduced products were compared with their non-sodium reduced counterparts.

Results: Sodium reduced meat and poultry products (n=19) contained 25-55% less sodium (mg/100g) than their non-sodium reduced counterpart (mean difference (95%CI): 460 (225-585)mg/100g, $p<0.001$). Potassium content of sodium reduced products ranged from 210-1500 mg/100g and was significantly higher than non-sodium reduced products by 195 (106-284)mg/100g, $p < 0.001$). Potassium containing additives were found on the ingredient list in 63% of the sodium reduced products and 25% of the non-sodium reduced products ($p = 0.02$). The amounts of phosphorus, protein and phosphorus: protein ratio did not differ significantly between the two groups.

Conclusions: Potassium additives are frequently added to sodium reduced meat and poultry products in amounts that significantly contribute to the potassium load for CKD patients. Patients requiring a potassium restriction should limit their intake of sodium reduced meat and poultry products.

Funding: Private Foundation Support

SA-PO220

Potassium Restriction and Dietary Satisfaction Are Associated with Quality of Life in Patients on Dialysis Junichi Yatabe,[1] Midori Sasaki Yatabe,[2] Kozue Takano,[2] Koichi Asahi,[1] Hiroyuki Terawaki,[1] Kohji Nomaki,[3] Kenichi Nakazawa,[3] Masaaki Nakayama,[1] Tsuyoshi Watanabe.[1] *[1]Dept of CKD Initiatives, Fukushima Med Univ, Fukushima, Japan; [2]Dept of Pharmacol, Fukushima Med Univ, Fukushima, Japan; [3]Fujitsu Home and Office Services Limited, Kawasaki, Japan.*

Background: Dietary potassium (K) restriction for dialysis patients may decrease dietary satisfaction and compromise quality of life (QOL). However, the associations between K restriction, eating satisfaction and QOL have not been examined.

Methods: Fukushima Patient Association of Kidney Disease in Japan conducted the survey on 759 subjects on dialysis (66.6±11.3 years old, male:female=399:345). Kidney disease-related QOL was assessed by KDQOL version 1.3.

Results: The percentage of subjects satisfied with their diet was lower in patients on dialysis compared to healthy subjects (68% versus 89%). Majority of patients (94.8%) know the risks of serum K elevation, and 76% work on K restriction, but 46% feel it stressful to reduce K intake. In the QOL survey, patients on dialysis had lower physical component summary score (33.5) and relationship component summary score (41.0) than the standardized Japanese average of 50. Mental component summary (MCS) score (50.3) was close to national average. Dietary dissatisfaction and the level of stress due to K restriction were significantly associated with lower MCS score independent of age, sex and number of years on dialysis (R^2=0.153, $P<0.01$). The level of K restriction-related stress positively associated with the severity of kidney disease-related symptoms, effects of kidney disease, burden of kidney disease and work status. Furthermore, the stress of K restriction was associated with less satisfaction with dialysis care. Many patients (81%) answered that they would like to include low-K produce in their diet as a method to manage dietary K.

Conclusions: There was a strong inverse relationship between the stress of K restriction and dietary satisfaction. Mental stress of dietary restriction may affect not only QOL but also the tolerability of dialysis therapy. Efforts to include a variety of low-K produce may be essential to alleviate the burden of K restriction and improve quality of life among patients on dialysis.

Funding: Government Support - Non-U.S.

SA-PO221

Low-Potassium Lettuce Grown with Novel Technology Can Be Safely Enjoyed Fresh by Patients on Dialysis

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Background: End-stage renal disease (ESRD) patients are instructed to reduce potassium (K) intake. In a separate study, 73% of patients on dialysis answered that they work on K restriction and 45% take K binders, but they still want to eat fresh vegetables and fruits. Therefore, companies in Japan developed a unique hydroponics technology to grow low-K produce.

Methods: Low-K lettuce cultivation method is licensed by Akita Prefectural University. Seedlings were grown using a special "zero K" solution to produce low-K lettuce. Eighty-two patients consumed salad prepared with 30-40 g of fresh (raw) low-K lettuce during each dialysis for 2 weeks. Participants took blood tests and completed questionnaires before and after the study, which included quality of life scores by SF-36 version 2. (UMIN000013058).

Results: Low-K lettuce contained 49.8 ± 16.9 mg of K per 100 g, close to 90% less K than regular lettuce, with similar dietary fiber and vitamin contents. Eighty participants completed the study, with no dropout due to serum K elevation. Rather, serum K decreased after the study (4.83 ± 0.09 versus 4.57 ± 0.08 mEq/L, P<0.01). Weight gain between dialyses and blood concentrations of albumin, CRP, sodium, calcium, zinc, and vitamins A, B1, B2, C and E were not affected. Many (79%) liked the taste of low-K lettuce, and 84% want to include low-K produce in their diet. SF-36 mental health subscore significantly improved after the study in subjects not satisfied with their diet (44.2 ± 23.2 versus 48.9 ± 21.0, N=13, P<0.05) and those who felt stressed by K restriction (45.3 ± 11.9 versus 49.5 ± 9.3, N=23, P<0.05).

Conclusions: Low-K lettuce is safe for patients on dialysis. Being able to eat fresh low-K lettuce may alleviate the stress of dietary restrictions and bring better eating satisfaction and mental health among ESRD patients. A growing number of advanced-stage CKD patients may benefit from a variety of low-K produce.

Funding: Government Support - Non-U.S.

SA-PO222

Vitamin C Overload Leads to Secondary Hyperoxalosis in Young Pediatric Dialysis Patients

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Background: Vitamin C supplementation is recommended in dialysis patients to prevent anemia, gingival disease and scurvy. Case reports suggest that intake of very large doses of vitamin C led to secondary hyperoxalosis in some adult dialysis patients. The impact of current enteral formulas on oxalate levels in dialyzed children is unknown.

Methods: We identified oxalate crystal deposits in bone of two 5-year-old hemodialysis patients who underwent routine bone biopsy prior to parathyroidectomy. Gene analysis of *AGXT* ruled out primary hyperoxaluria, and both had a history of high vitamin C intake from enteral feeding. Therefore, we assessed the daily recommended intake (DRI) of vitamin C from enteral feedings in 13 pediatric patients aged 8 months to 8 years fed primarily formula through a gastrostomy tube, treated with either hemodialysis (n=3) or peritoneal dialysis (n=10) in order to assess the impact on oxalate burden. Total vitamin C intake, serum oxalate levels and ascorbic acid levels were assessed in these patients.

Results: All patients were receiving 1.2-8.3 times the DRI for vitamin C from enteral feedings. Ascorbic acid levels ranged from 1.5 to 10.0 mg/dL (ref range: 0.2-1.9 mg/dL); values were elevated in 85% of patients. Oxalate levels were elevated (>30 µmol/L) in 69% of patients and ascorbic acid levels did not correlate with oxalate levels (r=0.44, p=0.13). Ascorbic acid and oxalate levels did not correlate with the amount of vitamin C received corrected by the DRI (r=0.42, p=0.15 and r=0.09, p=0.77 respectively).

Conclusions: Oxalate deposition in bone of two index cases demonstrates that oxalate is present in toxic levels in some dialyzed children; excess vitamin C ingestion contributes to secondary oxalosis in predominantly formula-fed infants and children. Plasma ascorbic acid levels vary, correlate poorly with oxalate levels, and may not reflect total body stores, suggesting that a portion of ascorbic acid is unavailable for clearance during dialysis.

SA-PO223

Acute Fructose Loading and Marked Metabolic Increases in the Circulation of Uric Acid, Triglycerides and IGFBP-1 in Chronic Kidney Disease Patients

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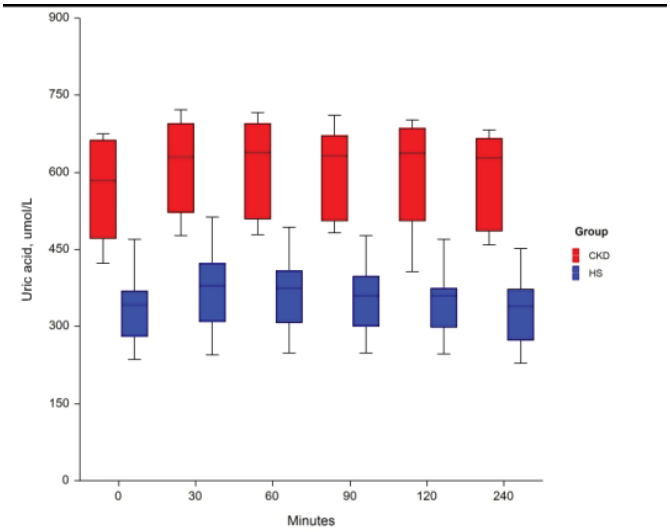
Background: The consumption of fructose has increased in recent decades and coincides with the prevalence of the metabolic syndrome and chronic kidney disease (CKD). Fructose uses fructokinase and ATP when metabolized in the liver and this can lead to that ATP degrades into uric acid. This may occur normally in healthy state, but does it occur differently in CKD? We studied the acute metabolic effects of low fructose or sucrose loading in patients (pts) with CKD (stages 4-5, GFR<30 mL/min, n=10,) and healthy subjects (HS, n=10).

Methods: All subjects underwent 6 interventions, i.e. a pure fructose drink, coca-cola or blueberry juice, containing 35 g carbohydrate and consumed with or without a pizza slice (442 Kcal). Metabolites in plasma were analyzed post-prandially up to 240 min after consumption. Factors which may statistically influence the variability were studied in a mixed model. The fixed parameters (group, intervention and time) and factors which may have different impact by fructose in CKD pts (uric acid, triglycerides (trig) and IGFBP-1).

Results: We found significant differences between CKD pts and HS of fructose intervention compared to coca-cola or blueberry juice. Fructose intervention increased circulating plasma levels of uric acid (p<0.001), trig (p=0.04) and IGFBP-1 (p=0.002), without affecting the insulin levels.



Conclusions: The hepatic degradation of fructose is quick and complete however this is unknown in CKD pts. Fructose consumption, increases amplitude and time of its metabolites which remain in circulation in CKD pts. Further analyses are in progress to study the carbohydrate metabolism in CKD pts.

SA-PO224

Phosphorus-Containing Food Additives in Processed Foods in Brazil

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Background: An increased body of evidence implicates serum phosphorus as a risk factor for cardiovascular morbidity, metabolism bone disorders and mortality in individuals with or without chronic kidney disease. The addition of phosphorus to processed foods is permitted but frequently not specified on food labels. The purpose of this study was to determine the actual phosphorus content of a number of products often consumed by hemodialysis patients and to compare the actual content with that estimated in a reference source.

Methods: Thirty-two frequently processed products consumed in the diet of the patients treated at the hemodialysis unit were analyzed. Among these processed foods were: milk in cartons, a type of cream cheese, cold cuts, instant noodles, salad dressings, sodas, and powdered juices. The concentration of phosphorus (mg P/100g of food) was determined according to the Adolfo Lutz Institute methodology, and in triplicate. This method is based on phosphorus complexation with vanadomolybdate of amonium and determined by spectrophotometry in the visible UV region. The comparison of the phosphorus obtained with the expected phosphorus was based on two national reference tables.

Results: Twenty-nine (90.6%) of the products had phosphorus-containing additives listed among their ingredients. For the category of milk in cartons, a type of cream cheese, cold cuts (salami, bologna and turkey breast), instant noodles and sodas, all containing additives, the actual phosphorus content was greater than the content expected from the reference tables. Two food categories, salad dressings and powdered juices, contained less than the expected levels. The difference between the actual and expected phosphorus content (mg/100g or mL) ranged from 22.83 to 52.87 for milk in cartons; -37.15 to 155.12 for powdered milk; 28.3 to 603.09 for a type of cream cheese; 258.47 to 632.15 for cold cuts; 41.32 to 189.15 for instant noodles, and 1.06 to 3.78 for sodas.

Conclusions: Processed foods with phosphorus additives contain higher levels of phosphorus than those listed in reference tables. Variation among similar products difficults the orientation of the patients.

SA-PO225

Creatinine Index as a Surrogate Marker of Lean Body Mass in Hemodialysis Patients: Comparison with Anthropometric and Bioimpedence Assessment

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Background: Assessment of malnutrition in HD patients is still debated. The creatinine index was proposed as a surrogate of Lean Body Mass (LBM), but its calculation was complex. Recently, a simplified formula has been proposed by Canoud et al. Aim of the study is to compare the simplified formula of creatinine index with classical anthropometric measurements and Lean Tissue Index (LTI) with bioimpedance.

Methods: 111 prevalent HD patients (mean age 69.02 ± 13.51 years; F/M 43/68, in HD for a mean of 74.49 ± 89.15 months, 36.9% of diabetics) underwent nutritional evaluation with both traditional anthropometric parameters and BCM).

Results: At anthropometric measurement, 72 (65%), 6 (6%), 17 (15%) and 16 (14%) had a normal, mild, moderate and severe decrease in LBM, respectively. Creatinine index was significantly lower in patients with severe decrease in LBM (16.8 ± 1.7 mg/kg/day) compared to the other groups (Normal: 19.5 ± 2.3; Mild 20.2 ± 2.8; Moderate: 19.4 ± 2.5). At linear regression we found a moderate relationship between creatinine index and either LBM measured with anthropometry (R = 0.47, B = 14.8, P< 0.0001; figure 1) or LTI. The capability of the formula to detect malnutrition is less effective in women and patients younger than 65 years. The highest degree of correlation with LBM (R = 0.63, B = 9.76, P =0.001) and LTI (R = 0.56, B = 13.1, P=0.035) was in patients with severe reduction of LBM. At logistic regression analysis creatinine index was the only independent variable influencing severe loss of LBM (B - 0,595, ES 0.249, P = 0.017); transferrin was and albumin, PTH, LTI were not significant.

Conclusions: Creatinine index as a moderate degree of correlation with LBM measured with anthropometric evaluation and bioimpedance. The formula may be more reliable in patients with severe malnutrition.

SA-PO226

Adiponectin, Lipids, and Body Composition in Hemodialysis Patients

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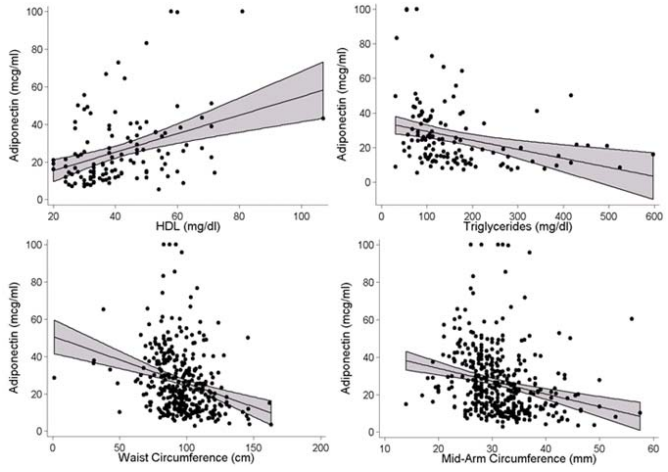
Background: In the general population, circulating adiponectin is inversely associated with triglycerides (TG), LDL, and body fat; and positively associated with HDL and lean body mass (LBM). Hemodialysis (HD) patients have higher adiponectin levels, but its association with lipids and body composition in HD is not well defined.

Methods: In cross-sectional analyses of 501 HD patients in the MADRAD study from 13 large dialysis organization centers (2011-2013), we estimated unadjusted (uR) and adjusted Pearson correlations (aR) of adiponectin with lipids and body composition surrogates: visceral fat (waist circumference, WC), subcutaneous fat (biceps/triceps skinfold, SF), LBM (mid-arm muscle circumference, MAMC, mid-arm circumference MAC), and near infrared body fat%. We examined associations between lipids and body composition with high adiponectin (>50th percentile) using multivariable logistic regression.

Results: Adiponectin was positively correlated with HDL (uR=0.37) and inversely correlated with TG, WC, biceps SF, triceps SF, MAMC, MAC, and body fat%: uR=-0.30,-0.27,-0.15,-0.11,-0.20,-0.23, and -0.12, respectively (p<0.05 for all).



In adjusted analyses, adiponectin remained positively correlated with HDL and had strongest inverse correlation with WC: aR 0.31 and -0.30, respectively (p≤0.001 for both). TG, LDL, cholesterol, WC, biceps SF, triceps SF, MAMC, MAC, and body fat% were associated with ↓ odds of high adiponectin, while HDL was associated with odds of high adiponectin.

Conclusions: In HD patients, inverse associations of adiponectin with TG, LDL, visceral and subcutaneous fat, as well as positive associations with HDL are preserved. In contrast to the general population, adiponectin is associated with ↓ LBM.

Funding: NIDDK Support

SA-PO227

Effects of Lifestyle Interventions on the Serum Concentration of Soluble (Pro)renin Receptor

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Background: The blood levels of soluble form of (pro)renin receptor (sPRR) have been reported to be affected by various pathophysiological conditions, such as diabetes mellitus, heart failure, sleep apnea and renal insufficiency. However, it is largely unknown whether lifestyle interventions such as exercise and diet affect the blood sPRR levels.

Methods: The study population consisted of 112 subjects who participated in the health promotion program conducted in Motoyoshi Town, Kesennuma, Japan. The program consisted of 2 interventions: (1) weekly 90-min instructor-led group sessions of mild aerobic exercise, and (2) monthly dietitian-led educational group sessions focused on salt restriction. The participants were free to choose to attend either exercise (EX) sessions or salt restriction (SR) sessions, or both. Before and after 5 month of the intervention period, we collected the blood and urine samples from the participants and measured biochemical parameters including serum sPRR levels.

Results: Fifty-eight persons (51.3 %) attended the SR sessions. At the end of the program, the mean estimated 24-hour sodium excretion in SR sessions-attending group (SR group) was significantly lower compared with non-SR group (166.9 ± 6.3 versus 185.3 ± 6.5 mEq/day, p<0.05), suggesting that the SR sessions were effective in the reduction of dietary sodium intake. Although the serum sPRR levels in non-SR group were not changed significantly, those in SR group significantly decreased from 34.5 ± 1.3 to 30.6 ± 1.3 ng/ml (p<0.05). Attending the SR sessions was associated with the decrease in serum sPRR levels, even after multivariable adjustment for confounding factors including age, gender, attending EX sessions and blood pressure change. In contrast, attending the EX sessions did not affect the serum sPRR levels.

Conclusions: Educational intervention to reduce dietary salt intake was associated with the decrease in serum sPRR levels.

SA-PO228

Effect of Estimated GFR on Serum Albumin Deterioration in Patients with Chronic Kidney Disease

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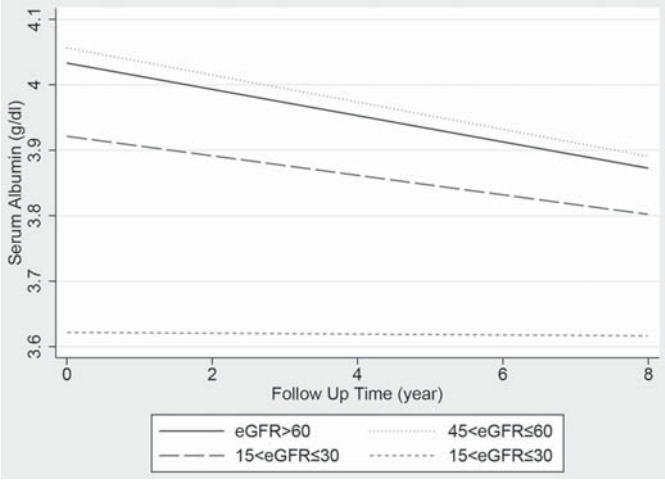
Background: Protein-energy wasting (PEW) represents a major risk factor in patients with CKD. Serum albumin (ALB) level is one of the criteria used to evaluate PEW. The independent effect of impaired kidney function on serum albumin deterioration over time is unclear.

Methods: Among 3,582,478 U.S. veterans with eGFR≥60 ml/min/1.73m^2, we identified 643,535 patients who developed incident CKD (defined as the first two eGFR values <60 at least 90 days apart). After excluding patients with pre-existing conditions that could affect ALB independent of nutritional status (liver diseases, metastatic malignancies, and chronic inflammatory conditions), our final cohort consisted of 479,491 incident CKD patients. The association of time dependent eGFR with intra-individual changes (slopes) of serum albumin was examined in mixed models stratified by estimated GFR levels.

Results: Over a mean follow-up time of 6.7 years the median (IQR) number of ALB measurements/individual were 10 (6, 15). The mean age (Standard Deviation [SD]) was 66.1(10.4) years, 12.9% were African American, 39.4% patients had diabetes and the mean baseline eGFR (SD) was 74.9 (12.8) ml/min/1.73m^2. Serum albumin levels were lower in patients with lower eGFR. However, decrease in albumin over time was not associated with lower eGFR (Figure).



Conclusions: Lower kidney function was associated with lower serum albumin, but not with worsened deterioration of ALB over time in non-dialysis dependent CKD patients.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO229

Hyperphosphatemia Is Linked to Insulin Resistance in Maintenance Hemodialysis (MHD) Patients

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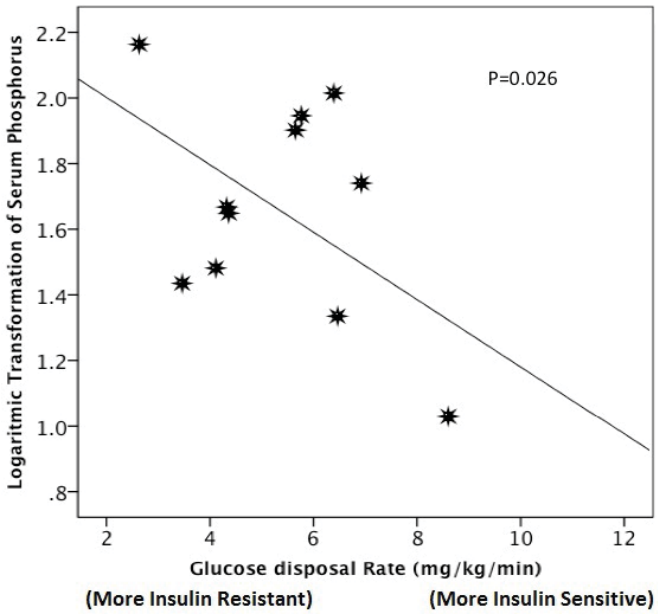
Background: Markers of mineral bone disorders (MBD), especially higher levels of serum phosphorus are associated with cardiovascular events and all-cause mortality in MHD patients. Insulin resistance (IR) is common in MHD patients and recent data suggest a potential role of phosphorus homeostasis in IR in non-dialyzed CKD patients. In this study, we examined the association between phosphorus levels and IR measured using the gold standard hyperinsulinemic euglycemic clamp (HEGC) in MHD patients.

Methods: The HEGC procedure was performed using an insulin rate of 2.0 mU/kg/min through 120 min in twelve African American MHD patients. IR was measured as the glucose disposal rate (GDR) in the last 30 minutes of the clamp. Pearson correlation and multivariate analysis were performed to evaluate the association of phosphorus and GDR. Variables were log transformed to achieve normality.

Results: Mean age was 50±9.4 years, 67% were male, 42% had diabetes, mean BMI was 33.3 +/-7.4, median dialysis vintage was 46 months. The mean GDR was 5.7 (IQR: 4.1, 6.8) mg/kg/min. The mean serum calcium, phosphorus and iPTH levels were 9.0±0.6 mg/dL, 5.2 (IQR: 3.9, 6.9) mg/dL and 285 (IQR; 228, 474) pg/mL, respectively. GDR was significantly correlated with serum phosphorus (r= -0.635, p=0.026) (Figure 1). In the multivariate analysis, serum phosphorus levels remained an independent predictor for GDR after adjusting for a) *model 1*: iPTH and Calcium (p=0.007) and b) *model 2*: age and gender (p=0.02).



Conclusions: Our results suggest that high phosphorus levels are significantly associated with IR in MHD patients. These findings represent a novel pathway for MBD-associated cardiovascular disease and all-cause mortality in ESRD patients.

Funding: Other NIH Support - CEDAR, Veterans Affairs Support

SA-PO230

Microbiome Perturbation Reduces Production of Uremic Toxin in ESRD

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Background: Indoxyl Sulfate (IS), a major protein-bound uremic toxin, is produced by the action of bacterial digestion of tryptophan to indole; indole enters the portal circulation and is hydroxylated and sulfated in the liver to produce IS. Given the evidence that high affinity protein-binding, largely to albumin, greatly limits the capacity of hemodialysis to remove this uremic solute which has been implicated in the pathogenesis of accelerated cardiovascular disease in ESRD, alternate means to reduce IS are needed. We have examined the effect of disturbing the gut microbiome on IS production in patients with ESRD.

Methods: A single dose (250 mg) of Vancomycin VANCO, an orally non-absorbable antibiotic, known to alter the gut microbiome (IKS Yap et al Proteome Res. 2008 7:3718-28) was administered to 10 subjects with ESRD undergoing hemodialysis. Plasma samples, pre- and post-dialysis, were obtained prior to and 2,4, 7, 14. 21 and 28 days following the administration of VANCO. Stool specimens for assessment of the microbiome, were collected prior to, one week following and 28 days following VANCO. IS was measured by HPLC. The gut microbiome was assessed by comparison of 16S rRNA gene sequences in stool samples collected pre- and post VANCO.

Results: Pre-dialysis IS averaged 42.7±26.4 μgm/ml prior to VANCO and decreased in all subjects. Indoxyl sulfate concentration at the nadir, usually at day 4 following VANCO, averaged 50.5 % of control. IS returned to baseline (40.2±41.6 μgm/ml) by day 28 following VANCO.

	Pre-dial	Day 2	Nadir	Nadir %	Day 28
Indoxyl sulfate (μgm/ml)	42.7 ±26.4	35.4±27.8	19.6±16.7	50.5±23.5	40.2±41.6

Conclusions: The finding that VANCO reduced the plasma concentration of IS is consistent with the evidence that bacterial flora in the gut are responsible for the production of IS. The present findings suggest that perturbing the gut microbiome, with an antibiotic or other agent such as a probiotic, by reducing the generation and plasma levels of IS, might decrease the incidence or severity of accelerated arteriosclerosis in ESRD. This would constitute a paradigm shift in the treatment of ESRD.

Funding: Private Foundation Support

SA-PO231

Association between "Obesity Paradox" and Fluid Volume Imbalance between Intra- and Extracellular Water in Patients with Chronic Kidney Disease

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Background: Body mass index (BMI) correlates inversely with mortality in patients with chronic kidney disease (CKD). We assessed the association of fluid volume status with BMI and whether fluid imbalance between intra- (ICW) and extracellular water (ECW) is a risk factor for adverse outcomes.

Methods: Body fluid composition was measured in 149 patients with CKD from 2005 to 2009, who were followed until 2013. Patients were categorized according to the ECW/ICW ratio tertile. ECW excess was assessed by examining the ratio of ECW measured by bioimpedance analysis (ECW_{BIA}) to total body water calculated using the Watson formula (TBW_{Watson}). Main outcomes were adverse renal outcomes, as defined by a decline of 50% or more from baseline glomerular filtration rate or initiation of renal replacement therapy, cardiovascular events, and all-cause mortality.

Results: The decreasing ICW slope with age was steeper than the decreasing ECW slope. The shift in the balance between ICW and ECW led to an increase in the ECW/ICW ratio. The $\%ECW_{BIA}/TBW_{Watson}$ had a negative correlation with BMI (r = -0.36 in male, P = 0.001 and r = -0.28 in female, P = 0.02). Compared with patients in the lowest tertile during a median 4.9-year follow-up, those in the highest tertile had the worst adverse renal outcomes (15.9 versus 5.1 per 100 patient-years, P < 0.001), cardiovascular events (4.1 versus 0.3 per 100 patient-years, P = 0.002), and mortality (11.2 versus 1.3 per 100 patient-years, P < 0.001) by Kaplan–Meier survival analysis. The adjusted hazard ratio (95% confidence intervals) for adverse renal outcomes, cardiovascular events, and all-cause mortality were 1.15 (1.03 – 1.26, P = 0.011), 1.12 (0.93 – 1.31, P = 0.217), and 1.29 (1.11 – 1.50, P <0.001), respectively.

Conclusions: Leaner or elderly patients with a decreased ICW were more susceptible to ECW excess. Fluid imbalance between ICW and ECW was independently associated with adverse renal outcomes and mortality, which may indicate the reserve capacity for volume overload in patients with CKD.

SA-PO232

Associations Between Body Mass Index (BMI) and Body Fat with Markers of Inflammation and Nutrition Among Patients on Hemodialysis (HD) Cynthia Delgado, Lorien S. Dalrymple, Glenn M. Chertow, George A. Kaysen, John Kornak, Barbara A. Grimes, Kirsten L. Johansen. *USRDS Nutrition Special Studies Center.*

Background: BMI does not distinguish between visceral and subcutaneous fat, which may have opposite metabolic and inflammatory effects. The purpose of this study was to determine the extent to which BMI, waist circumference (WC) and percent fat mass (%FM) are associated with markers of inflammation and nutrition in patients on HD.

Methods: We measured C-reactive protein (CRP), interleukin 6 (IL6), pre albumin (pre-alb) and albumin (alb) among 531 prevalent HD patients in the USRDS ACTIVE/ADIPOSE study. We used linear regression with BMI, WC and %FM as independent variables, along with age, sex, diabetes mellitus and race as covariates.

Results: Higher BMI was associated with higher concentrations of CRP and IL6 but not pre-alb or alb. WC associations were similar to those of BMI, but % FM was associated with higher pre-alb in addition to higher CRP. When WC* and %FM* were considered together, higher WC* was associated with higher CRP and IL6 but lower pre-alb and alb, whereas higher %FM* was associated with lower CRP and IL6 and higher pre-alb and alb.

Multivariable Association between Body Composition and Inflammatory and Nutritional Markers

Parameter	CRP, mg/L	IL6, pg/ml	Pre-alb, mg/dL	Alb,g/dL
BMI per 10kg/m2	0.59 (0.42,0.75)	0.17 (0.06,0.30)	-0.24 (-0.11,0.064)	-0.025 (-0.07,0.02)
WC, per 10cm	0.24 (0.18,0.31)	0.07 (0.02,0.12)	-0.7 (-0.43,0.30)	-0.02 (-0.04,-0.002)
%FM	3.0 (1.71,4.4)	0.05 (-0.88,0.98)	7.0 (0.05,13.9)	0.03 (-0.31,0.37)
WC*, per 10cm	0.25 (0.16,0.34)	0.12 (0.06,0.19)	-0.55 (-1.03,-0.07)	-0.037 (-0.06,-0.01)
%FM*	-0.05 (-1.76,1.65)	-1.50 (-2.7,0.27)	13.8 (4.7,23)	0.50 (0.04,0.94)

Conclusions: Visceral fat and subcutaneous fat show distinct associations with markers of inflammation in patients on dialysis with visceral fat directly associated with inflammatory markers and inversely associated with the negative acute phase/nutritional proteins. A better understanding of fat distribution may help to explain the obesity paradox in HD.

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

SA-PO233

Is There a Reverse Epidemiology of Triglyceride in Patients with Chronic Kidney Disease Stage 3-5 or Hemodialysis? Chi-Chih Hung,[1] Daw-Yang Hwang,[1] Hung-Chun Chen.[1] *[1]Dept of Nephrology, Kaohsiung Medical Univ Hospital, Kaohsiung Medical Univ, Taiwan; [2]Dept of Nephrology, Kaohsiung Medical Univ Hospital, Kaohsiung Medical Univ, Taiwan.*

Background: A reverse association is observed between cholesterol and mortality in chronic kidney disease (CKD) and hemodialysis (HD) patients. As a part of metabolic syndrome, hypertriglyceridemia causes inflammation and atherosclerosis and is prevalent in CKD and HD patients. However, whether a reverse epidemiology between triglyceride and mortality is also true in these patients is little known.

Methods: We enrolled 3300 CKD stage 3-5 patients and 2615 incident HD patients in two cohorts from southern Taiwan between 1997 and 2009. Baseline data were used for analysis.

Results: In the CKD cohort with a mean eGFR 24.7 ml/min/1.73 m^2,the mean triglyceride and cholesterol level were 155 mg/dl and 196 mg/dl, respectively. In the HD cohort, the mean triglyceride and cholesterol level was 173 mg/dl and 187 mg/dl, respectively. In the general linear regression, factors positively associated with triglyceride were HD (versus CKD), diabetes mellitus, female, cholesterol, nutritional factors and inflammation markers. In CKD patients, triglyceride <50mg/dl and 50-100 mg/dl were associated with higher risks for all-cause mortality with the hazard ratio (HR) 95% CI:2.13 (1.22-3.75)and 1.71 (1.18-2.47), respectively, compared with triglyceride 200~250 mg/dl. In contrast, in the HD patients, triglyceride 200~250 mg/dl and >250mg/dl were associated with higher risks for all-cause mortality with the HR 95% CI: 1.26 (1.11-2.27) and 1.30 (1.05-1.61), respectively, compared with triglyceride 50~100 mg/dl. The association between triglyceride and CV events showed similar results in both cohorts. Furthermore, cholesterol was not significantly associated with mortality in both CKD and HD patients.

Conclusions: Lower triglyceride in CKD stage 3-5 patients and higher triglyceride in HD patients were associated with higher mortality, while cholesterol was not associated with mortality in these patients. Treatment of hypertriglyceridemia should need further investigation in HD patients.

Funding: Government Support - Non-U.S.

SA-PO234

Association of Appendicular Lean Body Mass and Truncal Fat Mass Indexes with All-Cause Mortality in Hemodialysis Patients Akihiko Kato,[1] Yukitoshi Sakao,[1] Takayuki Tsuji,[2] Naro Ohashi,[2] Hideo Yasuda,[2] Hiromichi Kumagai.[3] *[1]Dialysis Unit, Hamamatsu Univ Hospital, Hamamatsu, Shizuoka, Japan; [2]Inter Medicine 1, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [3]School of Nutritional and Food Sciences, UNiversity of Shizuoka, Shizuoka, Japan.*

Background: Skeletal muscle wasting commonly causes frailty, thereby leading to comorbidities such as reduced activity of daily life and bone fracture in patients with chronic kidney disease (CKD). Central body fat accumulation is also associated with survival rate in CKD patients. So, we aimed this study to examine whether appendicular skeletal muscle wasting or truncal fat accumulation may be associated with clinical outcomes in regular hemodialysis (HD) patients.

Methods: We enrolled 176 male (age: 57±12 years old, time on HD: 9±7 years) and 85 female patients (age: 64±12 years old, time on HD: 9±7 years). We measured appendicular lean body mass and truncal fat mass volumes (kg) by dual-energy X-ray absorptiometry (DEXA), and calculated as appendicular lean body mass index (ALBMI) and truncal fat mass index (TFMI) dividing those by height square (m^2). We then followed all of the patients for the 5 years, and assessed the impact of changes of body composition on total mortality.

Results: Mean ALBMI was 5.8±1.0 [0.9-8.9] in male and 4.5±0.7 [3.1-7.0] kg/m^2 in female patients. There was 91% in men and 92% in women who had met the Asian criteria of sarcopenia (cut-offs: <7.0 kg/m^2 for men and <5.4 kg/m^2 for women by using DEXA). Mean TFMI was 2.2±1.3 [0.4-7.2] in male and 2.7±1.3 [0.4-6.7] kg/m^2 in female. ALBMI was correlated with age, serum creatinine and albumin in male, while with time on HD in female. TFMI was positively correlated with total cholesterol and triglyceride in both sexes. Cox proportional hazards regression analyses revealed that ALBMI was independently associated with the 5-year mortality in male patients (RR 0.71 [0.51-0.98], p<0.05). TFMI became a significant predictor of mortality in female patients (RR 0.44 [0.24-0.81], p<0.01).

Conclusions: These findings suggest that higher appendicular skeletal mass associates with better survival in male, while higher truncal adiposity affords protection in female patients.

SA-PO235

p53 Inhibition Causes a Glycolytic Shift in Renal Glucose Metabolism Takashi Hato, Timothy A. Sutton, Pierre C. Dagher. *Medicine, Indiana Univ, Indianapolis, IN.*

Background: It is increasingly recognized that glycolytic reprogramming is coupled to biosynthesis thus permitting sustained cell growth and survival under stress. Therefore, inducing renal glycolytic reprogramming could be an attractive preventative and therapeutic approach against various forms of renal injury. The tumor suppressor p53 was recently shown to be involved in the regulation of key metabolic pathways. In particular, the genetic absence of p53 was implicated in the glycolytic phenotype of tumors. Here we investigated whether the acute inhibition of p53 can also induce glycolysis in the healthy kidney *in vivo*.

Methods: To examine the effect of acute p53 inhibition on basal glucose metabolism in the kidney, wild-type mice and rats were treated with vehicle control or pifithrin-α (a pharmacologic p53 inhibitor). A combination of tools was applied including traditional biochemical analysis, PET scan and intravital imaging of the kidney in order to examine the balance between glycolysis and oxidative phosphorylation in the setting of p53 inhibition.

Results: Intravital imaging revealed increased uptake of fluorescently-labeled deoxyglucose by proximal tubules of pifithrin-α treated animals as compared to controls. Whole body ^{18}fluoro-2-deoxyglucose PET scan replicated the findings. Concomitant to increased glucose uptake, intravital imaging showed decreased mitochondrial membrane potential in proximal tubules of pifithrin-α treated animals. In kidney homogenates from pifithrin-α treated animals, pyruvate dehydrogenase activity and the ATP/AMP ratios were both significantly reduced. Finally, the expression of TIGAR (TP53-inducible glycolysis and apoptosis regulator, which downregulates glycolysis) was reduced in pifithrin-α treated kidneys. Collectively, these findings indicate that acute inhibition of p53 induces a shift towards glycolysis in otherwise normal kidney tissues.

Conclusions: We show *in vivo* evidence that renal glucose metabolism can be modulated via p53. Whether the glycolytic switch translates into renal protection in injury models remains to be determined.

Funding: NIDDK Support

SA-PO236

Genetic Deletion of Growth Differentiation Factor 15 Accelerates High Fat Diet Induced Renal Injury in Mice Sebastiaan Lambooy, Hendrik Buikema, Robert H. Henning, Leo E. Deelman. *Clinical Pharmacy and Pharmacology, Univ Medical Center Groningen, Univ of Groningen, Groningen, Netherlands.*

Background: The incidence of both Type 2 Diabetes and Metabolic syndrome (MetS) are increasing on a global scale, particularly in the Western World. In diabetes, we previously demonstrated that GDF15 plays a protective role in diabetes induced kidney damage. As high fat diet is known to increase GDF15 expression in fat, we speculate that GDF15 may also be renoprotective in a model of MetS.

Methods: The progression of kidney damage in an experimental model of MetS was compared in mice that were genetically deleted for GDF15 and in their wild type controls. Mice were given 6 weeks of high fat diet or control diet. Renal damage was assessed by the use of Real Time PCR, Western Blot and Immunohistology.

Results: Genetic deletion of GDF15 accelerated renal hypertrophy. Further, kidneys of GDF15ko mice demonstrated an increased inflammatory state and enhanced ROS production, even on normal diet. Enzymes involved in H_2S production were found to be reduced in the GDF15KO groups.

Conclusions: GDF15 exerts a protective effect against high fat diet induced kidney damage in an early model of MetS.

Funding: Government Support - Non-U.S.

SA-PO237

Does Autophagosome-Proteolysis Contribute to CKD-Induced Muscle Atrophy? Xiaonan H. Wang,[1] Zhen Su,[1,2] Janet D. Klein,[1] William E. Mitch.[3] *[1]Renal Div, Dept of Medicine, Emory Univ, Atlanta, GA; [2]Nephrology, The First Affiliated Hospital of Wenzhou Medical College, Wenzhou, China; [3]Nephrology, Dept of Medicine, Baylor College of Medicine, Houston, TX.*

Background: We have demonstrated that the ubiquitin-proteasome (UPS) system is a major pathway for the degradation of protein in CKD-induced muscle wasting. The trigger activating the UPS is a decrease in IGF-1-stimulated intracellular signaling. Since decreased IGF-1/PI3K activity can also stimulate autophagy in epithelial cells, we hypothesized that proteolysis through autophagosomes may contribute to the muscle wasting of CKD. We examined whether autophagosome activity contributes to CKD-induced muscle atrophy; and since we have shown that exercise increases the IGF-1 pathway, we asked whether exercise ameliorates CKD muscle wasting by blunting autophagosome activity.

Methods: In 25 g mice, we induced CKD by subtotal nephrectomy and examined the effect of resistant exercise (i.e., overloading of hindlimb plantaris muscles by removing gastrocnemius and soleus muscles) on muscle atrophy.

Results: mRNA expression of 4 markers of the autophagy-lysosomal proteolysis pathway in isolated plantaris muscles were measured to assess autophagosome activity. The markers were microtubule-associated protein 1A/1B-light chain 3 II (LC3 II, indicates autophagosome number), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3, indexes autophagosome formation), Vps34 (class III PI 3-kinase, a regulator of autophagic sequestration) and P62 (induction of reduced autophagosome clearance). CKD increased the mRNA expression of the 4 markers ($P<0.05$) and exercise reversed this back to levels in control mice. Protein levels of Bnip3, LC3-II and Beclin-1 (an upstream regulator of autophagic sequestration) were increased in muscle from CKD mice *versus* controls. Exercise reversed these increases in autophagosome protein markers.

Conclusions: We conclude that autophagosome-mediated proteolysis contributes to CKD-induced muscle atrophy. Exercise slows the development of muscle atrophy by up-regulating the IGF-1 signaling pathway in part by inhibiting autophagy-lysosomal proteolysis pathway in CKD.

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SA-PO238

Quantity and Quality of Nodular Sclerosis (Kimmelstiel - Wilson Lesion) Is Important in the Evaluation of Diabetic Nephropathy Fumihiko Yasuda,[1] Akiko Mii,[2] Megumi Fukui,[2] Yukinari Masuda,[1] Shuichi Tsuruoka,[2] Akira Shimizu.[1] *[1]Analytic Human Pathology, Nippon Medical School, Tokyo, Japan; [2]Nephrology, Nippon Medical School, Tokyo, Japan.*

Background: One of the most impact pathological characteristics of diabetic nephropathy (DN) is nodular sclerosis. In pathologic classification of DN (DN classification RPS 2010), nodular sclerosis is inclusion criteria of Class III. We examined clinic-pathological characterization of DN, focusing on the importance of quality and quantity of nodular sclerosis in DN, using DN classification RPS 2010.

Methods: We selected renal biopsy cases of DM (n=49) from our 5500 renal biopsy cases. We examined the clinico-pathological characterization of DN, focusing on the quality and quantity of nodular sclerosis in DN.

Results: Our DM cases (n=49) included class I (n=2), class IIA (n=6), class IIB (n=7), class III (n=24), and class IV (n=10). DN classification (RPS 2010) was significantly correlated with the duration of DM ($p<0.05$), degree of proteinuria ($p<0.05$), decreased eGFR ($p<0.001$) at the biopsy. In addition, DN classification (RPS 2010) was also significantly correlated with the degree of clinical DM stage ($r=0.451$, $p<0.01$) and CKD stage ($r=0.392$, $p<0.01$). In the cases with DN classification (RPS 2010) class III, the broad rage of proteinuria (0.2 to 11.4 g/day) was noted, however, there were correlated with the percentage of glomeruli with nodular sclerosis ($r=0.635$, $p<0.001$). If nodular sclerosis ratio (A <25%, B 25-50%, C 50-75%, D >75%) was added in the class III in DN classification (RPS 2010), more good correlation was evident between these pathologic classification and the levels of proteinuria ($r=0.444$, $p<0.01$), and degree of clinical DM stage ($r=0.506$, $p<0.001$) and CKD stage ($r=0.472$, $p<0.001$). In addition, nodular glomerular lesions that formed by predominant or only extracellular matrix (ECM) than cellular component were correlated with renal dysfunction and proteinuria.

Conclusions: DN classification (RPS 2010) is useful as pathologic DN classification that has good correlation with clinical characterization of DM. If DN classification includes quantity and quality of nodular sclerosis in class III, more increase of specificity is evident in this classification.

SA-PO239

Comparison of the Rate of Renal Function Decline in Nonproteinuric Patients with and without Diabetes Liliane Hobeika, Kelly J. Hunt, Benjamin Neely, John M. Arthur. *Internal Medicine - Nephrology, Medical Univ of South Carolina, Charleston, SC.*

Background: Patients with DM and CKD without proteinuria are often thought to have a cause of CKD other than DM. We hypothesized that if this is true, the rate of decline of renal function should be similar among non-proteinuric patients with and without DM. We compared the rate of renal function decline in patients with HTN with and without DM.

Methods: We searched the electronic medical record database for patients from the nephrology, endocrinology and general internal medicine clinics at our institution seen between 2008 and 2012, with an initial mean eGFR >35ml/min/1.73m^2 MDRD and identified 5035 patients. We used ICD9 diagnosis codes to identify patients with HTN and DM. We excluded patients with <2 measures of serum creatinine (Cr), with no urine studies and with proteinuria. We calculated the yearly eGFR for each patient from the lowest Cr in each year using CKD-EPI. 472 patients met the inclusion and exclusion criteria and had an initial eGFR between 35 and 80ml/min/1.73m^2. Annual rate of decline in eGFR was estimated by fitting a regression model with random intercept and slope.

Results: Data from 472 patients were analyzed. 38% were male, 37% African-American (AA), 65% had DM. Baseline eGFR was similar between groups (64±12 in non-diabetics versus 63±12ml/min/1.73m^2 in diabetics, $p=0.42$). In unadjusted analyses the rate of eGFR decline was greater in patients with DM than without DM (-0.74 versus -0.33ml/min/yr, $p=0.03$). After adjusting for age, race, gender, baseline eGFR and use of medications that block the RAS, the rate of decline was still greater among diabetics than non diabetics (-0.71 versus -0.39ml/min/yr, $p=0.03$). There was more decliners (defined as a decline in eGFR ≥3.3%/yr) among diabetics than non diabetics (34.43 versus 21.56%, $p=0.003$). The rate of eGFR decline in the diabetic group was much greater in those with a baseline eGFR <60 than with a baseline eGFR >60 (-2.09 versus 0.05ml/min/yr, $p<0.001$).

Conclusions: Patients with DM had more rapid decline in kidney function compared to those without DM in spite of the absence of proteinuria. These results suggest that even in the absence of proteinuria DM may be the cause of CKD.

SA-PO240

Does Pathogenesis of Diabetic Nephropathy Differ in Type 1 and Type 2 Diabetes? Katarzyna Madziarska, Slawomir C. Zmonarski, Magdalena Krajewska, Maria Magott, Hanna Bartosik, Oktawia Mazanowska, Miroslaw Banasik, Jan Penar, Katarzyna Jakuszko, Marian Klinger. *Nephrology and Transplantation Medicine, Medical Univ, Wroclaw, Poland.*

Background: Renal damage in diabetes mellitus type 1 (T1DM) and type 2 (T2DM) share similarities, but there is still an open question whether the pathogenesis of diabetic nephropathy (DN) is the same in T1DM and T2DM.We analyzed the association between proinflammatory cytokines (IL1β, IL6, IL18, TNFα) and potentially protective factors (25[OH]D3, C-peptide) with DN in T1DM and T2DM patients (pts). Data were compared with proteinuric pts with primary glomerulonephritis (GN).

Methods: In our preliminary study we assessed 68pts (18 T1DMpts, 26 T2DMpts, 24 biopsy proven GNpts) and 20 control. In all pts Hb, eGFR, lipids, glucose, albumin, CRP, C-peptide, HbA1c, insulin, Ca, P, homocysteine were examined. Using urinary albumin creatinine ratio (UACR) DMpts were divided into normo-, micro- et macroalbuminuric. DN was diagnosed in pts with micro- or macroalbuminuria and retinopathy. GNpts had proteinuria (range 0,6–2,9 g/d) and all were treated with ACEI. In all group we assessed IL1β, IL6, IL18, TNFα in blood and urine(u) and 25[OH]D3 in serum(s). All pts had stage 2 or 3 of CKD.

Results: T2DM pts were older, had higher: BMI, TG, C-peptide; lower HDL-cholesterol; shorter duration of DM thanT1DMpts. There were positive correlations of sIL6 and sTNFα with DN in T1DMpts. sIL1β, sTNFα and uIL6 directly correlated with DN in T2DM pts while 25[OH]D3 correlated inversely. In GNpts sTNFα, uIL6, uIL18 directly and 25[OH]D3 inversely correlated with proteinuria. Using univariate analysis of regression we assessed the risk of renal damage: DN in DMpts and proteinuria in GN pts. We found no relationship between analyzed factors and DN in T1DMpts while T2DMpts higher level of sIL1β was associated with DN. We revealed negative association between level of 25[OH]D3 and severity of proteinuria in GNpts.

Conclusions: The factors involved in pathogenesis of DN seem to be different in T1DM and T2DM. Higher level of sL1β can be predictor of DN in T2DMpts. 25[OH] D3 treatment may be a successful strategy in proteinuric GNpts. Our study didn't confirm nephroprotective role of C-peptide.

Funding: Clinical Revenue Support

SA-PO241

Predictors of Early Renal Function Decline in Type 1 Diabetes Trevor J. Orchard,[1] Janet Snell-Bergeon,[2] Tina Costacou,[1] Rachel G. Miller,[1] David M. Maahs,[2] Marian Rewers.[2] *[1]Dept of Epidemiology, Univ of Pittsburgh, Pittsburgh, PA; [2]Barbara Davis Center for Diabetes, Univ of Colorado Denver, Aurora, CO.*

Background: The prediction of diabetic nephropathy (DN), one of the leading type 1 diabetes complications, remains a challenge. The presence of microalbuminuria may not be an adequate marker of early renal function decline (ERFD). We thus explored additional predictors of rapid estimated glomerular filtration rate (eGFR) decline in two type 1 diabetes cohorts.

Methods: Baseline DN risk factors were assessed by the Epidemiology of Diabetes Complications (EDC, 1996-98) and Coronary Artery Calcification in Type 1 Diabetes (CACTI, 2000-02) studies. In this report, we included only participants diagnosed in 1965-80, before age 17 yrs with duration >7 yrs. Participants were followed for up to 8 yrs for development of ERFD (annual eGFR decline of >3 ml/min).

Table 1. Models predicting ERFD in the CACTI and EDC cohorts

	CACTI (n=191)		EDC (n=170)	
	OR	p-value	OR	p-value
eGFR (per 19.74 ml/min/1.73m^2)	3.9	<0.0001	1.8	0.02
Log uACR (per 1.5)	2.3	<0.0001	2.0	0.0004
Duration (per 10 yrs)	1.6	0.05	0.8	0.67
Women	1.3	0.40	1.0	0.99
Black/Hispanic/Asian vs. White	0.7	0.55	0.9	0.96
HbA1c (per 1%)	1.1	0.69	1.3	0.05
Hypertension	1.1	0.90	0.9	0.92
ACEi use	0.4	0.02	1.7	0.36

Results: Though the cohorts were similar in terms of diagnosis age, duration, sex and race/ethnicity, CACTI participants had better glycemic control: mean HbA1c 7.7% (7.5%-7.9%) versus EDC 8.5% (8.3%-8.7%) and were more likely to use hypertension medications (39.5% versus 12.3%), including ACE inhibitors (33.8% versus 16.8%). ERFD incidence was 27.5% (20.9% - 34.2%) in CACTI and 23.1% (16.8%-29.4%) in EDC. In logistic models controlling for baseline eGFR (Table), higher urinary albumin to creatinine ratio predicted ERFD in both cohorts. ACE inhibitor use was associated with less ERFD in CACTI while higher HbA1c was associated with ERFD in EDC.

Conclusions: Despite glycemic control differences, ERFD incidence did not differ between the cohorts and glycemia was only weakly related to ERFD. Interestingly, ACE use appeared to be protective only in CACTI, where the prevalence of use was twofold higher compared to the EDC.

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SA-PO242

Predictors of Progressive Renal Decline Differ among Patients with Type 1 Diabetes and Advanced Stages of Chronic Kidney Disease

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Background: It is recognized that two major factors determine the time to progression to ESRD: baseline eGFR and the speed of renal decline (eGFR slope). In this study, we search for predictors/determinants of eGFR slope in patients with diabetic nephropathy and impaired renal function.

Methods: The study group is derived from the Joslin Proteinuria Cohort that has been followed for 7 to 18 years to ascertain ESRD and estimate eGFR slopes using serial measurements of serum creatinine (using linear regression estimates). For this analysis, we focused on the 193 T1D patients in this cohort whose renal function was in CKD stage 3 (N=106, median eGFR=46.4 ml/min/1.73m^2), and in CKD stage 4 (N=86, median eGFR=24.0) at entry into the study.

Results: The medians (25th, 75th percentile) of eGFR slope are -3.9 (-8.9, -2.0) ml/min/1.73m^2/year in CKD stage 3 and -5.9 (-10.1, -3.5) in CKD stage 4, respectively. Systemic and urinary biomarkers were determined at baseline. Spearman correlation between eGFR slope and following predictors: HbA1c, serum TNFR1, ACR, urine KIM-1, urine MCP-1, and urine NGAL were significant in patients with CKD stage 3 and CKD stage 4. In multiple regression analysis, while HbA1c (β coefficient= -1.4 ml/min/1.73m^2/year for 1 % increase in HbA1c, p-value=0.001), TNFR1 (β=-1.9 ml/min/1.73m^2/year for 1000 pg/mg increase in TNFR1, p=0.040), urine KIM-1 (β=-1.3 ml/min/1.73m^2/year for 1000 pg/mg increase in KIM-1, p=0.037), urine MCP-1 (β=-3.1 ml/min/1.73m^2/year for 1000 pg/mg increase, p=0.027), and urine NGAL (β=-2.3 ml/min/1.73m^2/year for 100 ng/mg increase in NGAL, p=0.035) were significant predictors of eGFR slope in CKD stage 3, only ACR (β=-1.2 ml/min/1.73m^2/year for 1000 mg/g increase in ACR, p=0.042) was a significant predictor of decline of renal function in CKD stage 4. The R-Squared for these models were 0.522 in CKD stage 3 and 0.2794 in CKD stage 4, respectively.

Conclusions: In conclusion, our findings suggest that predictors of renal decline may vary in T1D patients according to advanced CKD stages.

SA-PO243

Predictors of Progression to Albuminuria in Patients with Type 1 Diabetes by Metabolomics

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Background: Nephropathy in type 1 diabetes (T1D) is associated with significant excess morbidity and mortality. While estimated glomerular filtration rate (eGFR) and albuminuria (AER) are routine for assessing renal impairment in clinical practice, new biomarkers could improve prediction of progression and risk stratification.

Methods: From the FinnDiane cohort we extracted 102 T1D pts with AER <30 mg/d and eGFR ≥45 ml·min^{-1}·1.73m^2 (60% male, age 34±12 yrs [mean±SD], eGFR 97±21 ml·min^{-1}·1.73m^2, AER 17±11 mg/d) who developed albuminuria over a median 3.2 years, and a sex-, age-, BMI, and eGFR-matched group of 98 T2D pts who did not develop albuminuria over a median 7.6 yrs. Non-targeted metabolomic profiling was carried out on fasting serum samples using gas chromatography or ultra-HPLC coupled with tandem mass-spec. Biomarker identification was carried out by random forest analyses using progression to albuminuria as the endpoint. Associations were tested by multivariate logistic regression and Cox proportional hazards modeling, and ROC area was calculated.

Results: Significant (p≤0.05) clinical covariates (age of onset, HbA_{1c}, and baseline AER) predicted progression with a ROC of 0.797. The top 18 identifiable metabolites most closely correlated with progression were tested for their ability to improve the clinical prediction. We selected 6 biomarkers (3-phenylpropionate, g-glutamylglutamate, sorbitol, C-glycosyltryptophan, cis-Cyclo[L-ala-L-Pro], and cortisone) which were each independently associated with the outcome. A linear combination of these biomarkers (*Pr. Index*) raised the clinical prediction ROC to 0.883 (p<0.0001). In a Cox model, pts in the top quartile of *Pr.Index* distribution had a risk ratio (RR) of progression of 2.59 [95%CI:1.66-4.02] along with RRs of 1.27 [1.05-1.54] for age of onset, 1.36 [1.10-1.67] for baseline HbA_{1c}, and 1.47 [1.22-1.75] for baseline AER.

Conclusions: A limited number of circulating intermediates mostly of amino acid metabolic pathways carry clinically significant predictivity for progression to albuminuria in pts with type 1 diabetes.

SA-PO244

The Effect of Hyperglycemia on Plasma Uric Acid Levels in Patients with Uncomplicated Type 1 Diabetes

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Background: Uric acid (UA) levels are associated with metabolic, cardiovascular and renal abnormalities in patients with type 2 diabetes. The role of UA in uncomplicated type 1 diabetes (T1D) and the physiological factors that influence UA are less well understood. The aim of the present analysis was to compare plasma UA levels in T1D patients versus healthy controls (HC), and to examine the effect of UA and clamped hyperglycemia on renal hemodynamic function in T1D.

Methods: Plasma UA levels, blood pressure (BP), glomerular filtration rate (GFR-inulin), effective renal plasma flow (ERPF - paraaminohippurate), renal blood flow (RBF), renal vascular resistance (RVR) and filtration fraction (FF) were evaluated in T1D (n=68) and HC (n=41) during clamped euglycemia (glucose 4-6 mmol/L). Studies were repeated in T1D during clamped hyperglycemia (9-11 mmol/L).

Results: T1D had lower plasma UA levels within the normal range compared to HC (232±63µmol/L versus 287±69 µmol/L, p<0.0001). During clamped hyperglycemia in T1D, UA levels further decreased to 202±62 µmol/L (p<0.0001). UA levels were positively correlated with systolic BP in T1D under euglycemic conditions (r=0.27 p=0.027), but not in HC or in T1D under hyperglycemic conditions. UA levels were negatively correlated with GFR in the HC group (r=-0.44, p=0.0042), but not in T1D under either glycemic condition. UA levels were, however, negatively correlated with ERPF in T1D during hyperglycemia (r=-0.24 p=0.048). Larger high-glucose induced declines in UA correlated with smaller increases in GFR and ERPF in response to clamped hyperglycemia in T1DM patients (r=-0.28 p=0.024 and r=-0.25 p=0.045 respectively). There were no interactions between UA and RBF, RVR, or FF in either group.

Conclusions: Plasma UA correlates with BP and ERPF in patients with T1D but not HC. Plasma UA is also lower in T1D versus HC, an effect that is amplified by clamped hyperglycemia. Studies examining blood pressure and renal effects of UA lowering are warranted in T1D.

Funding: Government Support - Non-U.S.

SA-PO245

Plasma KIM-1, a Marker of Tubular Injury, Predicts Risk of Early Progressive Renal Decline in Non-Proteinuric Patients with Type 1 Diabetes (T1D)

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Background: It has been suggested that tubular injury contributes to the development of early diabetic nephropathy. The aim of this study was to assess whether plasma and urine levels of KIM-1 are associated with risk of early progressive renal decline in patients with T1D.

Methods: The study group included 260 participants with T1D and normoalbuminuria (NA) and 204 patients with microalbuminuria (MA). At baseline all patients had normal renal function. They were followed for a median of 8 yrs with serial measurements of serum creatinine and cystatin C to determine $eGFR_{cre\text{-}cys}$ slopes. Early progressive renal decline was diagnosed if $eGFR_{cre\text{-}cys}$ slopes were <-3.3% per year. CKD≥ Stage 3 was diagnosed when $eGFR_{cre\text{-}cys}$ decreased below 60 ml/min/1.73m^2. Baseline plasma and urinary KIM1 were measured using the Kidney Biomarker Kit (R&D).

Results: During follow-up 114 patients developed early progressive renal decline, while 71 patients reached CKD Stage ≥ 3. The risk of early progressive renal decline rose with increased plasma KIM-1. It was 4.5%, 13.8%, 21.4% and 47% (p value for trend <0.001) accordingly in patients with non-detectable KIM-1, 1st, 2nd and 3rd tertiles of the distribution of KIM-1 in plasma. A similar trend was observed for incidence of CKD ≥ Stage3 (p<0.001). Similar patterns were seen in patients with NA and MA. In multiple logistic analysis, which included all relevant covariates, the association between plasma KIM-1 and risk of early progressive renal decline remained the same as in univariate analysis. Analyses between baseline urinary levels of KIM-1 and risk of early renal function decline and CKD ≥ Stage 3 showed much weaker associations.

Conclusions: In conclusion, single determination of plasma KIM-1 predicts the risk of kidney loss in non-proteinuric T1D patients. Our findings support the view that damage of proximal tubules plays a very important role in the initiation of early progressive renal decline.

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SA-PO246

Does the Combination of Two *ABCC9* Promoter SNPs Predict Microalbuminuria and Renal Function Decline in Type 1 Diabetes (T1D)? Pittsburgh Epidemiology of Diabetes Complications (EDC) Study

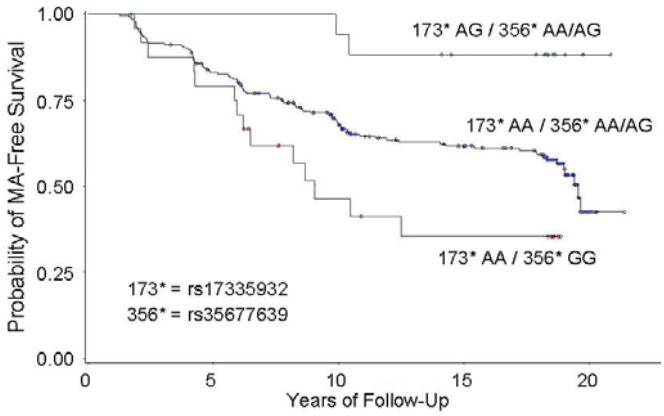
Kevin Ho, Rachel G. Miller, Trevor J. Orchard. *Univ of Pittsburgh, Pittsburgh, PA.*

Background: In prior analyses cardiovascular gene *ABCC9* promoter SNP genotypes rs17335932 (AA) and rs35677639 (GG) each predicted a higher incidence of microalbuminuria (MA) in the EDC T1D cohort, while alternate genotypes predicted lower risk. We tested if combining genotypes for both SNPs affected prediction of MA, incidence of renal function decline (RFD), low eGFR.

Methods: Data were available in 483 participants. Genotypes were combined into groups: (1) rs17335932 AG/rs35677639 AA/AG, (2) rs17335932 AA/rs35677639 AA/AG, (3) rs17335932 AA/rs35677639 GG. No rs17335932 GG genotypes were observed. At baseline 268 individuals were free of MA (AER ≥20 μg/min). Analyses were performed for incident or persistent MA (2 consecutive visits with MA or worse renal status). At baseline 438 individuals had an eGFR >60 mL/min/1.73 m^2 (CKD-EPI) and were followed to determine incidence of RFD (eGFR decrease >30 mL/min/1.73 m^2 from baseline) and low eGFR (eGFR <60 mL/min/1.73 m^2).

Results: After 22 years, incidence of MA by group was: (1) 11.1%, (2) 41.2%, (3) 58.3% (p=0.004). Group (2) combining high-/low-risk genotypes exhibited an intermediate outcome. Analyses for persistent MA showed similar but weaker results (p=0.07). With multivariate adjustment, groups (2) and (3) were at significantly higher risk of incident MA compared to group (1) (HR=4.7, p=0.03 and HR=7.6, p=0.008, respectively). Group (3) also predicted RFD incidence (HR=2.2, p=0.04). No association occurred with incidence of low eGFR.

Conclusions: Combined genotypes for 2 *ABCC9* promoter SNPs predict incidence of MA. Combining high-risk rs17335932 AA and rs35677639 GG conferred greatest risk, while combining high-(rs17335932 AA) and low-(rs35677639 AA/AG) risk genotypes conferred intermediate risk. The highest risk group further predicted RFD but not low eGFR.



Funding: Clinical Revenue Support

SA-PO247

Histopathological Review of Native Renal Biopsies in Type 2 Diabetes and Patient Outcomes

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Background: The natural course of diabetic nephropathy (DN) in type 2 diabetic patients is complex. We aimed to identify clinical parameters associated with non-diabetic kidney disease (NDKD) and correlate the histopathological findings with patient outcomes.

Methods: We conducted a retrospective study of type 2 diabetic patients with native renal biopsies performed between 2002 and 2008. Patients' clinical records and histopathology were examined independently, and categorised to histopathological diagnoses of pure DN, NDKD and Mixed (NDKD and DN). We followed this cohort until ESRD (eGFR ≤10ml/min/1.73m^2 or dialysis), death or December 2013.

Results: There were 260 patients (164(63%) men) predominantly of Maori (37%) or Pasifika (29%) ethnicity. The mean age was 57±11 years and median duration of diabetes 8(4-15) years. 84(32%) subjects had DN alone, 72(29%) NDKD and 104(40%) Mixed diagnoses. Diagnoses in the NDKD alone category included focal segmental glomerulosclerosis (35%), immune complex glomerulonephritis (10%), minimal change disease (10%) and membranous glomerulonephritis (6%). Compared to DN and Mixed groups, patients with NDKD had a shorter duration of diabetes, lower HbA_{1c}, less proteinuria, and higher eGFR at the time of biopsy (p<0.05). Maori ethnicity (OR 0.24, 95%CI 0.06 – 0.99), male gender (OR 0.18, 95%CI 0.06 – 0.60), the presence of diabetic retinopathy (OR 0.14, 95%CI 0.047 – 0.45), a lower HbA_{1c} (OR 0.96, 95%CI 0.93 – 0.99) and higher eGFR (OR 1.03, 95%CI 1.0 – 1.05) were significantly associated with NDKD diagnosis (R^2=0.47). Patients with NDKD had significantly better renal prognosis (p=0.000) and survival outcomes (p=0.0003) compared to those in the DN and Mixed groups.

Conclusions: The risk of mortality and ESRD is high in patients with pure or mixed DN. 68% of our cohort had NDKD alone or superimposed on a background of DN. Some traditional clinical parameters, in particular the absence of retinopathy, are useful at distinguishing pure NDRD from DN.

SA-PO248

Clinical and Pathological Predictors of Estimated GFR Decline in Patients with Type 2 Diabetes and Overt Proteinuric Diabetic Nephropathy

Koki Mise,[1] Junichi Hoshino,[1] Toshiharu Ueno,[1] Keiichi Sumida,[1] Tatsuya Suwabe,[1] Kenmei Takaichi,[1,2] Yoshifumi Ubara.[1,2] *[1]Nephrology Center, Toranomon Hospital Kajigaya, Kawasaki, Kanagawa, Japan; [2]Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background: Clinical predictors of the decline of the estimated glomerular filtration rate (eGFR) in patients with type 2 diabetes and relatively preserved kidney function have been investigated previously. However, the impact of various clinical and pathological parameters on the decrease of the eGFR has not been assessed in patients with type 2 diabetes and overt proteinuric biopsy-proven diabetic nephropathy (DN).

Methods: Among 223 patients with diabetes who underwent renal biopsy and were confirmed to have pure DN according to the recent classification, 128 patients with type 2 diabetes and overt proteinuria were enrolled. Receiver operating characteristic analysis was performed and the area under the curve (AUC) was calculated using models adjusted for various clinical and pathological covariates to determine the best predictors of rapid eGFR decline (defined as > 14.9 %/ year [median eGFR decline]).

Results: A model that incorported proteinuria showed a significantly larger AUC than a model with only the covariates (0.812 versus 0.661, respectively, P=0.002), and it also demonstrated the largest AUC among clinical models. Although a model incorporating interstitial fibrosis and tubular atrophy (IFTA) score did not display a significantly larger AUC than the model with proteinuria (0.843 versus 0.812, respectively, P=0.47), a model with both IFTA score and proteinuria had a significantly larger AUC than the model with proteinuria alone (0.875 versus 0.812, respectively, P=0.014).

Conclusions: Our results suggest that not only proteinuria but also tubulointerstitial lesions should be assessed to predict rapid eGFR decline in patients with type 2 diabetes who have overt proteinuria and biopsy-proven DN.

Funding: Private Foundation Support

SA-PO249

Impact of Tubulointerstitial Lesions on Anemia in Patients with Biopsy-Proven Diabetic Nephropathy

Koki Mise,[1] Junichi Hoshino,[1] Toshiharu Ueno,[1] Keiichi Sumida,[1] Tatsuya Suwabe,[1] Kenmei Takaichi,[1,2] Yoshifumi Ubara.[1,2] *[1]Nephrology Center, Toranomon Hospital Kajigaya, Kawasaki, Kanagawa, Japan; [2]Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background: An association between anemia and the renal prognosis has been reported in patients with diabetic nephropathy (DN). The aim of this study was to investigate the relationship between the progression of anemia and renal pathological findings in patients with DN.

Methods: Among 223 patients with diabetes who underwent renal biopsy from 1985 to 2010 and were confirmed to have pure DN according to the recent classification, 113 patients (baseline hemoglobin ≥ 11 g/dl) were enrolled in this study. Linear regression analysis was used to estimate the changes of hemoglobin during the follow-up period.

Results: In a multivariate model adjusted for clinical and histopathological parameters, higher interstitial fibrosis and tubular atrophy (IFTA) scores were more strongly associated with the decrease of hemoglobin than lower IFTA scores. Compared with an IFTA score of 0, the standardized coefficients for IFTA scores of 1 to 3 were -0.20 (95% CI: -0.93 – 0.31), -0.34 (-1.34 – 0.22), and -0.47 (-1.96 – -0.07), respectively. On the other hand, a higher glomerular class, a higher vascular lesion score, and the presence of exudative lesions were not strongly correlated with the decrease of hemoglobin.

Conclusions: More advanced tubulointerstitial lesions are significantly associated with the progression of anemia in patients with DN after adjustment for numerous covariates. This finding suggests that tubulointerstitial lesions may be a useful prognostic indicator for anemia in patients with DN, and that decreased erythropoietin production due to the progression of tubulointerstitial lesions is a major cause of anemia in these patients.

Funding: Private Foundation Support

SA-PO250

Insulin Resistance as a Predictor of Cardiovascular Morbidity and Chronic Kidney Disease Progression

André Fragoso, Ana Paula Silva, Pedro Neves. *Nephrology Dept, Hospital de Faro, Faro, Portugal.*

Background: Cardiovascular disease (CVD) is the main risk factor of morbidity and mortality in chronic kidney disease (CKD) patients. Insulin resistance (IR) has been reported to be a strong risk factor for CVD. The purpose of this study was to examine the usefulness of IR as a predictor of cardiovascular morbidity and CKD progression.

Methods: We followed during a period of 56 months 119 type 2 diabetic CKD patients without history of CVD at the beginning of the study. Several laboratory parameters and left ventricular mass index (LVMI) were analyzed. The degree of IR was estimated by the Homeostasis Model Assessment (HOMA-IR). Cardiovascular morbidity was assessed according to the presence of cardiovascular hospital admission during the study period and the population was divided in two groups: G-1 with cardiovascular admission (n=48) and G-2: without admission (n=71). Multivariable models were used to predict cardiovascular morbidity using HOMA-IR either a continuous or qualitative variable. CKD progression was assessed by dialysis beginning and logistic regression models were used to determine predictors for CKD progression.

Results: We found that G-1 patients showed significantly higher age, iPTH, phosphorus, IL-6, HOMA-IR (3.8 versus 0.77, p=0.0001), LVMI and lower Hg, albumin and eGFR. HOMA-IR upper tercile showed significantly higher age, eGFR, LVMI, phosphorus, iPTH and IL-6 than the other terciles. In a multivariate model HOMA-IR and IL-6 were independent risk factors of cardiovascular morbidity (HR=5.328, 95% CI, 2.884 to 32.105, p=0.048), (HR=6.239, 95% CI, 1.238 to 31.452, p=0.027), respectively. In a logistic regression model patients in the upper tercile presented significantly more cardiovascular admissions that in the lower tercile. CKD progression was observed in 24 patients and those in the upper HOMA-IR tercile showed a higher CKD progression than the rest of study patients. A multivariable logistic regression model showed that urine ACR, eGFR, IL-6 and HOMA-IR (HR=1.034, 95%CI, 1.065 to 1.650 p=0.40) were independent predictors for CKD progression.

Conclusions: In our study IR is an important risk factor for cardiovascular morbidity and CKD progression in a diabetic CKD population.

SA-PO251

Prediabetes and Its Contributing Factors in Non-Diabetic Patients with Chronic Kidney Disease Hui Peng, Qianqian Wang, Yan-Ru Chen, Jun Zhang, Xun Liu, Meirong Zhong, Yuanqing Li, Tan-Qi Lou. *Nephrology Div, Dept of Medicine, The third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Prediabetes is a condition including impaired fasting glucose (IFG) or/ and impaired glucose tolerance (IGT). Since prediabtes increases the risk of developing cardiovascular disease (CVD) or chronic kidney disease (CKD), we examined the prevalence of prediabtes in a CKD clinic in Guangzhou.

Methods: From Jan, 2013 to May 2014 all non-diabetic non-dialysis CKD patients attending the Third Affiliated Hospital of Sun Yat-sen University were analyzed in a cross-sectional observational study. CKD was defined as either an UACR≥30mg/g or eGFR <60 ml/min per 1.73m^2. Blood pressure, serum biochemistries and urine protein were collected. eGFR was calculated as described by Zhang L et al, JASN, 2006.

Results: 167 non-diabetic non-dialysis CKD patients were enrolled, 64(38.32 %) had prediabetes with age averaging 50.8±15.1yr. Prediabetes was associated principally with an increasing in IGT (90.63 %). Moreover prediabetes was associated with more hypertension (48.44% versus 21.36%, p < 0.001), higher values of serum albumin (36.70 **VS**. 34.05, p=0.019), serum creatinine (377.80 **VS**.116.7μmol/L, p<0.001), homocysteine (20.13 versus 14.81, p=0.001). Thus there were lower values of eGFR (15.75 versus 73.12 ml/min per 1.73m2, p<0.001), hemoglobin (105.50 versus 123.5g/L, p=0.002), and LDL cholesterol (2.91 versus 3.43mmol/L, p=0.029). There were more prediabetes with CKD stage 5 (50.0% versus 33.6%, p<0.001) and fewer with CKD stage 1 (12.5% versus 37.9%, p<0.001). Multivariable logistic regression analysis shows plasma creatinine (OR=1.002, 95% CI=1.001~1.003, P=0.001) was still remained significantly associated with the presence of prediabetes after multivariable adjustment.

Conclusions: Our results from a CKD clinic in China indicate that prediabetes is frequent and potentially is associated with the presence of CKD. Prediabetes in U.S. is associated with evidence of diabetic micro- and macro-vascular disease (Diabetes and vascular disease, 2014), and our results suggest that prediabetes in CKD may be linked to vascular disease.

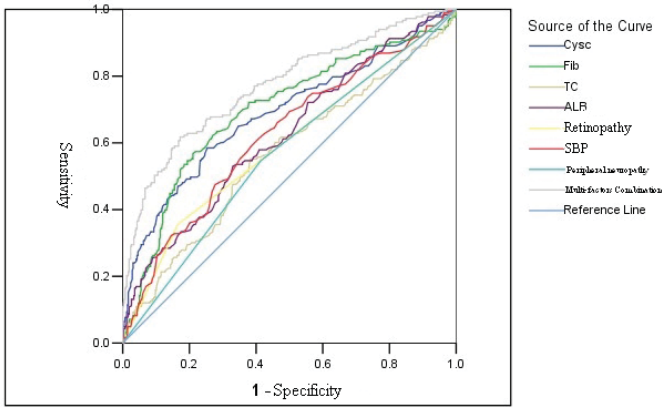
SA-PO252

A Screening Model of Correlation Factors for Type 2 Diabetes Mellitus with Microalbuminuria Wenbo Zhao, Cailian Cheng, Jun Zhang, Xun Liu, Hui Peng, Tan-Qi Lou. *Nephrology, The Third Affi liated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: To study the clinical characteristics of patients with type 2 diabetic nephropathy, analyze of the impact of the risk factors for the development of diabetic nephropathy, and establish screening model for the early stage diabetic kidney damage.

Methods: The 601 cases of the hospitalized patients with type 2 diabetes are enrolled, and recorded the patient's clinical data. Logistic regression analysis was used for finding the main risk factors for microalbuminuria Performance in type 2 diabetes. With the ROC curve principle, Multiple factors were combined to establish clinical prediction or outpatient screening equation of early diabetic kidney damage.

Results: Multivariate analysis showed that peripheral neuropathy, systolic blood pressure, diabetic retinopathy, ALB, TC, Fib, CysC, were main risk factors for the progress of the disease. The predictive value regression equation: $P = 1/[1+ e^{(-4.991+1.331X1+0.348X2+0.265X3-0.055X4+0.589X5+0.014X6+0.509X7)}]$. The under area of the ROC curve was 0.801. The maximum Value of Youden index was 0.445 and obtained 0.3518 as the diagnosis point.



Diagonal segments are produced by ties.

Conclusions: Theearly stage of diabetic kidney damage, associated with multi-factors, The screening equation of multi-factors combination can be applied to clinical risk prediction.

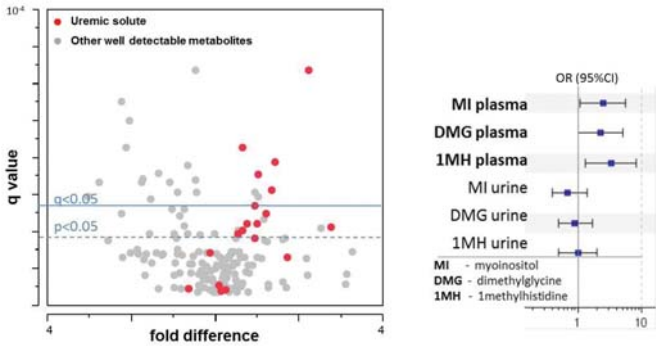
SA-PO253

Metabolomic Signature of Subjects with Type 2 Diabetes (T2D) at Higher Risk of Renal Function Decline - Replication Study Monika A. Niewczas,[1] Vladimir Tolstikov,[2] Stephanie E. Croall,[1] James R. Voelker,[2] Adam Smiles,[1] Melissa Major,[1] Kevin P. Mcdonnell,[1] Mingshang Kuo,[2] Dennis Laska,[2] Matthew D. Breyer,[2] Andrzej S. Krolewski,[1] Kevin L. Duffin.[2] [1]*Genetics and Epidemiology, Joslin Diabetes Center, Harvard Medical School, Boston, MA;* [2]*Lilly Research Laboratories, Eli Lilly, Indianapolis, IN.*

Background: In the Joslin Kidney Study of subjects with T2D we showed that plasma metabolomic signatures were associated with progression to ESRD and these included uremic solutes and branched chain (BC) derivatives. In order to test the generalizability of those findings we performed a replication study in the independent population of subjects with T2D.

Methods: Our study group consisted of 38 subjects with T2D, participants of the MBDL trial, who were followed for 3 years. At baseline mean eGFR was 80±22 ml/min and median (25th, 75th) ACR was 17 (9-74) μg/gcr. Decliners (n= 22) were defined as subjects whose eGFR loss ≥ 3.3ml/min/year. Metabolomic measurements were performed in plasma and urine of the study subjects with the usage of ABSCIEX LC/MS/MS system.

Results: 183 metabolites were detected in at least 80% of the study subjects. 26 metabolites were significantly different between decliners and non-decliners (q value <0.05). 13 uremic solutes were significantly increased (volcano plot) and certain BC derivatives were significantly decreased. Respective concentrations in urine did not correlate with plasma levels and were not different between cases and controls.



Conclusions: We confirmed associations of certain uremic solutes and BC derivatives with subsequent progression of diabetic nephropathy in an independent replication study of subjects with T2D. Further, we have shown that urinary concentrations of those uremic solutes were not altered suggesting that an increase in those solutes in plasma in subjects at risk are not a simple result of kidney filtration impairment.

Funding: NIDDK Support, Pharmaceutical Company Support - Eli Lilly

SA-PO254

Morning Hypertension and Night Non-Dipping in Patients with Diabetes and Chronic Kidney Disease Sewon Oh,[1] Kum Hyun Han,[1] Ran-Hui Cha,[2] Sejoong Kim,[3] Sunae Yoon,[4] Dong-Ryeol Ryu,[5] Jieun Oh,[6] Eun-Young Lee,[7] Dong Ki Kim,[8] Yon Su Kim,[8] Sang Youb Han.[1] *[1]Dept of Internal Medicine, Inje Univ College of Medicine, Republic of Korea; [2]National Medical Center, Republic of Korea; [3]Seoul National Univ Bundang Hospital, Republic of Korea; [4]Catholic Univ College of Medicine, Republic of Korea; [5]Ewha Womans Univ College of Medicine, Republic of Korea; [6]Hanlim Univ College of Medicine, Republic of Korea; [7]Soon Chun Hyang Univ College of Medicine, Republic of Korea; [8]Seoul National Univ College of Medicine, Republic of Korea.*

Background: Morning hypertension (MH) and non-dipping (ND) are closely associated with organ damage and cardiovascular events. The risk of MH and ND are unknown in chronic kidney disease (CKD) and diabetes (DM).

Methods: A total of 1,312 patients were prospectively recruited from 21 centers in Korea between October 2009 and May 2011. All patients had hypertension and 15-89 ml/min/1.73m^2 of estimated glomerular filtration rate (eGFR). Ambulatory 24 hour blood pressure was assessed. We divided the patients into 4 groups by status of DM and CKD (eGFR<60); no DMCKD, DM only, CKD only, and DMCKD.

Results: The rate of MH (13.6% versus 25.2%, P<0.001) and ND (48.2% versus 58.2%, P=0.002) were higher in diabetics than in non-diabetics. The level of eGFR was correlated with ND in all patients (P<0.05), and MH in only non-diabetics (P=0.005). Proteinuria (PU) was related with ND in all patients (P<0.05), and with MH in only diabetics (P=0.001). In regression analysis, the risk of MH was 2.093 (95%CI, 1.070-4.094) for the DM only group, 1.634 (95%CI, 1.044-2.557) for the CKD only group and 2.236 (95%CI, 1.401-3.570) for the DMCKD group compared with the no DMCKD group. PU was also risk factor for MH (1.591, 95% CI, 1.100-2.300). The risk of ND was not significant in the DM only group compared with the no DMCKD group. The risk of ND was significantly high in CKD: 1.581 (95%CI, 1.180-2.120) for the CKD only group; 1.872 (95%CI, 1.348-2.601) for the DMCKD.

Conclusions: Both DM and CKD are significant risk factors for MH and ND, and PU is also risk factor for MH. The risk of MH is higher in diabetics and non-diabetics with advanced CKD stages.

SA-PO255

A Urinary Peptide Classifier Predicts Albuminuria Response to Spironolactone in Patients with Type 2 Diabetes and Hypertension Morten Lindhardt,[1] Christina Stolzenburg Oxlund,[2] Harald Mischak,[3] Ib A. Jacobsen,[2] Peter Rossing,[1,4,5] Hiddo Jan Lambers Heerspink.[6] *[1]Steno Diabetes Center, Gentofte, Denmark; [2]Odense Univ Hospital, Denmark; [3]Mosaiques Diagnostics, Hannover, Germany; [4]Aarhus Univ, Denmark; [5]Univ of Copenhagen, Denmark; [6]Univ Medical Centre Groningen, Groningen, Netherlands.*

Background: We previously fund that mineralocorticoid blocker spironolactone significantly reduce albuminuria (UACR) in type 2 diabetic patients with hypertension (*Oxlund JHypertension 2014)*. However a large variability in UACR reduction was observed (5th to 95th percentile -90 to +212%) during spironolactone. We previously developed and validated a urinary proteomic classifier that predicts worsening of albuminuria (*Roscioni Diabetologia 2013)*. Here we tested whether the proteomic classifier predicts UACR response to spironolactone.

Methods: We performed a *post hoc* analysis in a randomized controlled double blind trial with allocation to either spironolactone 12.5 - 50 mg/day (n=61) or placebo (n=54) for 16 weeks. Treatment was adjuct to diuretics, calcium channel blockade and renin-angiotensin-system inhibition. UACR response was defined as change from baseline to week 16. Capillary electrophoresis mass spectrometry was used to quantify urinary peptides. A previously validated combination of 273 known urinary peptides was used as proteomic classifier (CKD273).

Results: None of the baseline clinical characteristics predicted the variability in UACR response to spironolactone at week 16. Higher values of the CKD273 classifier at baseline were associated with a larger reduction in UACR at week 16 in the spironolactone group (β=-0.70 p=0.05), but not in the placebo group (β=0.39 p=0.25). When the population was stratified in tertiles of baseline CKD273, the UACR response to spironolactone (placebo adjusted) was -13% (Cl 95%;-52 to +59) in the low, -48% (-75 to +10) in the middle, and -64% (-84 to -20) in the high CKD273 tertile (p=-0.025 for heterogeneity).

Conclusions: A urinary proteomics classifier can be used to identify individuals more likely to respond to spironolactone. These preliminary results may pave the way for more individualized therapy but need to be confirmed in a large clinical trial.

Funding: Private Foundation Support

SA-PO256

Elevated Baseline Plasma Lipoprotein(a) Levels Are Associated with Decline in Estimated Glomerular Filtration Rate Over Time Jennie Lin, Muredach Reilly, Francis Perry Wilson. *Univ of Pennsylvania, Philadelphia, PA.*

Background: Chronic kidney disease (CKD) is associated with dyslipidemia, but the role of atherogenic lipid fractions in CKD progression remains unclear. Here we assess whether baseline plasma levels of lipoprotein(a) [Lp(a)] and apolipoprotein C-III (apoC-III), causal cardiovascular (CV) risk factors being studied as therapeutic targets, are associated with decreasing glomerular filtration rate (GFR) over time.

Methods: In the Penn Diabetes Heart Study (PDHS), a single-center observational cohort of type 2 diabetes patients without clinical CV disease, we performed linear mixed effect modeling to evaluate the effects of baseline plasma Lp(a) and apoC-III on the slope of estimated GFR (eGFR) over time in subjects with longitudinal data (n=550). Adjustment for covariates was performed in the following incremental models: Model 1, demographic factors and baseline serum creatinine; Model 2 also included lipid-lowering medications, body mass index, and hypertension; Model 3 further included for hemoglobin A1c; and Model 4 additionally included urinary albumin to creatinine ratio (ACR). eGFR was calculated with the CKD-Epi equation.

Results: Each two-fold higher plasma Lp(a) level was associated with a decline in eGFR by 0.24 mL/min/year in the unadjusted model (p<0.001) and by 0.28 mL/min/year in the fully adjusted model (p<0.001). Baseline Lp(a) levels greater than the atherogenic cut-point of 30 mg/dL were associated with a decline in eGFR by 0.66 mL/min/year in the unadjusted model (p<0.001) and by 0.82 mL/min/year in the fully adjusted model (p<0.001). Although each log-transformed unit of apoC-III was associated with a decline in eGFR by 0.68 mL/min/year in the unadjusted model, the association was attenuated after adjusting for hemoglobin A1c and urinary ACR.

Conclusions: Elevated baseline plasma Lp(a) levels are associated with a decrease in eGFR over time independent of race, lipid medication use, and albuminuria. Additional longitudinal studies, including Mendelian randomization for CKD as an outcome, are needed to determine if Lp(a) is a causal risk factor for CKD progression.

Funding: NIDDK Support

SA-PO257

Clinical Predictors of Non-Diabetic Renal Disease in Patients with Type 2 Diabetes Yong Pey See, Yue-Harn Ng. *Renal Medicine, Tan Tock Seng Hospital, Singapore, Singapore.*

Background: Non diabetic renal disease (NDRD) is common amongst diabetic patients and tends to portend a better prognosis. Differentiating diabetic nephropathy (DN) from non diabetic renal disease is crucial for early diagnosis and intervention. Our study aims to identify clinical predictors that differentiate NDRD from DN.

Methods: We performed a retrospective case-control study on our diabetic patients who underwent a renal biopsy at our institution from February 2010 to May 2014. Biopsy findings were classified as DN or NDRD. Clinical parameters including age, blood pressure (BP), duration of diabetes, HbA1c, complications of diabetes including peripheral vascular disease (PVD), proteinuria, hematuria and retinopathy, were collected by chart review and compared between DN and NDRD groups.

Results: 100 diabetic patients underwent a renal biopsy during this period, 2 of whom were excluded for inconclusive biopsy findings. The mean age was 58.3 ± 12 years old and 61.2% were male. The most common indication for biopsy was for rising creatinine (43.9%), followed by sub-nephrotic range proteinuria (25.5%), nephrotic syndrome (18.4%) and hematuria (8.2%). DN was identified in 47/98 (48%) of the biopsies while 51/98 (52%) were NDRD. The most common NDRD are lupus nephritis and IgA nephropathy with 9/51 (17.6%) cases each. Male gender, presence of diabetic retinopathy, PVD, dyslipidaemia, average systolic BP, average HBA1c and HbA1c > 9% were associated with DN on univariate analysis. In the multivariate model, only diabetic retinopathy remained significantly associated with DN (OR 14.7; p <0.05) Traditional risk factors such as duration of diabetes, BP, presence of microalbuminuria, macroalbuminuria and macrovascular complications did not predict DN. Presence of diabetic retinopathy carries a positive predictive value of 78% and a negative predictive value of 87%.

Conclusions: In this study, diabetic retinopathy is strongly associated with diabetic nephropathy. The absence of diabetic retinopathy carries a strong negative predictive value for DN. Hence diabetic patients without documented retinopathy, especially in the setting of atypical features, should raise suspicion for NDRD and warrants early renal biopsy.

SA-PO258

Renal Anemia in Diabetic Patients with Nearly Normal Estimated Glomerular Filtration Rate (eGFR) Takayuki Hamano,[1] Hiromi Rakugi,[2] Yoshitaka Isaka,[2] Yoshiharu Tsubakihara.[1] *[1]Comprehensive Kidney Disease Research, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Serum erythropoietin (EPO) levels are reported to be elevated in anemic subjects with normal renal function. Renal anemia derives mainly from inadequate EPO production by the kidney relative to hemoglobin levels and is considered to emerge at chronic kidney disease (CKD) stage 3B. Diabetic patients with CKD are reported to have severer anemia, however, renal anemia has not been studied well in diabetic patients with nearly normal eGFR.

Methods: In this cross-sectional study, we enrolled 1,621 patients with eGFR>=45mL/min/1.73m^2 from 2,264 diabetic outpatients seen by diabetologists. The definition of anemia is based on WHO criteria. We performed multiple logistic regression analyses with the presence of anemia as a dependent variable. One third of the anemic patients was chosen randomly from this cohort for additional measurement of serum EPO in addition to ferritin and TSAT. We conducted a multiple linear regression analysis with serum EPO as a dependent variable.

Results: Anemia was observed in 470 patients (29%). Massive proteinuria (dipstick 3+) was significantly associated with presence of anemia after adjusting for age, sex, eGFR, triglyceride, LDL, and HbA$_1$C (Odds ratio: 2.33; 95%CI 1.02-5.29). The fact that the percentage of anemic patients increase with the increase in proteinuria suggest the presence of renal anemia even in nearly normal range of eGFR. In subgroup analysis of 166 patients, serum EPO levels were lower than upper limit of normal range despite anemia(relative EPO

deficiency)in substantial percentage of patients (61%). This percentage of EPO deficiency increases with decrease in eGFR and with the increase in urinary albumin(eGFR>90, 50%; eGFR 60-90, 58%; eGFR<60, 63%, P<0.05 for trend). The determinants of serum EPO were found to be eGFR and albuminuria in addition to BMI, serum Alb, and hemoglobin.

Conclusions: Substantial percentage of diabetic patients are relative EPO deficient even if their eGFR are normal or nearly normal, possibly due to diabetic tubulointerstitial injury.

Funding: Pharmaceutical Company Support - Cmic

SA-PO259

Glomerular MRP8 Expression Predicts Progression of Proteinuria in Obese or Type 2 Diabetic Patients Takashige Kuwabara,[1] Kiyoshi Mori,[2] Hideki Yokoi,[1] Shinji Yasuno,[3] Masato Kasahara,[3] Kenji Ueshima,[3] Kazuwa Nakao,[2] Masahito Imanishi,[4] Akira Nishiyama,[5] Motoko Yanagita,[1] Masashi Mukoyama.[1,6] *[1]Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [2]Medical Innovation Center, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [3]EBM Research, Inst for Advancement of Clinical and Translational Science, Kyoto Univ Hospital, Kyoto, Japan; [4]Nephrology and Hypertension, Osaka City General Hospital, Osaka, Osaka, Japan; [5]Pharmachology, Kagawa Univ Medical School, Kagawa, Japan; [6]Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan.*

Background: Our previous report indicated that toll-like receptor 4 and one of its endogenous ligands, myeloid-related protein 8 (MRP8), play an important role in diabetic nephropathy in mice. We performed this study to evaluate significance of renal MRP8 expression in obese or type 2 diabetic patients.

Methods: In obese, diabetic or control subjects, MRP8 mRNA and protein expressions in renal biopsy samples were determined by real-time RT-PCR and immunohistochemistry (n = 28 and 65, respectively) and their associations with baseline and prognostic parameters were analyzed.

Results: Kidney MRP8 expression was significantly higher in obese or diabetic groups than in control group both in mRNA and protein levels. At baseline, univariate analysis showed that glomerular MRP8-positive cell count and tubulointerstitial MRP8-positive area were correlated not only with various known risk factors for diabetic nephropathy (such as systolic blood pressure, proteinuria and serum creatinine) but also with extent of glomerulosclerosis and tubulointerstitial fibrosis. Multiple regression analyses were performed to identify explanatory factors predicting renal outcomes including proteinuria and renal event. Glomerular MRP8-positive cell count ($\beta = 0.59$, $P < 0.001$) was an independent predictive factor for the extent of proteinuria a year later, as well as baseline proteinuria ($\beta = 0.37$, $P = 0.002$) and systolic blood pressure ($\beta = 0.21$, $P = 0.04$) after adjustment for known risk factors.

Conclusions: Glomerular MRP8 expression appears to be associated with progression of proteinuria in obese or type 2 diabetic patients.

Funding: Government Support - Non-U.S.

SA-PO260

Zinc Alpha 2 Glycoprotein Predicts Early Renal Function Decline in Type 2 Diabetes Sharareh Saadat,[1] Kelly J. Hunt,[1,2] Nishant M. Bhensdadia,[1] Alison Bland,[1] John M. Arthur.[1,2] *[1]Dept of Medicine, Medical Univ of South Carolina, Charleston, SC; [2]Dept of Medicine, Ralph H. Johnson VA Medical Center, Charleston, SC.*

Background: Albumin to creatinine ratio (ACR) is used to predict future renal disease in patients with diabetes but it has inadequate specificity and sensitivity. We previously used proteomic analysis to identify a novel group of protein candidates to predict early renal function decline (ERFD) in patients with diabetes. One of the candidates was zinc alpha 2 glycoprotein (ZAG).

Methods: We measured ZAG concentration in 231 urine samples from type 2 diabetic patients without known kidney disease from the VADT study. Annual rate of eGFR decline (MDRD) over a median 4.4 years of follow-up was defined by fitting a regression model with random intercept and slope for each patient. We compared urinary ZAG levels between diabetic patients with and without ERFD (defined as a decrease in eGFR of ≥3.3% per year).

Results: Age, diabetes duration, baseline creatinine, blood pressure and use of ACE inhibitors were not different between subjects with ZAG concentration above and below the median. 57 patients (25%) had ERFD. Patients without ERFD had a natural log ZAG concentration of 3.03 compared to those with ERFD with a ZAG concentration of 3.40 (P=0.05). Respective natural log values for urine creatinine adjusted ZAG were -4.26 and -3.91 (P=0.09). One standard deviation change in ZAG concentration (adjusted for treatment group and ACEI use at baseline) was associated with an odds ratio of 1.38 (95% CI 1.00, 1.92, p=0.05, AUC=0.619) for ERFD. ACR had similar prognostic ability but interestingly there was a poor correlation between concentrations of the two biomarkers (0.34). The use of ZAG in combination with ACR resulted in selection of a group with higher risk for ERFD. Having high ACR and ZAG (compared to the median split) conferred a 2.7 fold risk of ERFD compared to the group with low ACR and ZAG.

Conclusions: Urinary ZAG concentrations were found to be elevated in type 2 diabetic patients who had early renal function decline. Combination of ACR with ZAG may provide better prediction of ERFD in patients with type 2 diabetes.

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SA-PO261

CNDP1 (CTG)5 Homozygosity in Type 2 Diabetic Patients: Relevance for Nephropathy and Hemodialysis Sibylle Jenny Hauske, Shiqi Zhang, Thomas Albrecht, Bernhard K. Krämer, Benito Yard. *Vth Med. Clinic, Med. Faculty Mannheim, Univ of Heidelberg, Mannheim, Germany.*

Background: Although several studies in type 2 diabetic (T2DM) patients (pat.) showed that homozygosity for the (CTG)5 allele (homCTG5) of the Carnosinase 1 (CN1) gene is associated with reduced susceptibility for diabetic nephropathy (DN),it is unclear why not all homCTG5 pat. are protected.We assume that independent factors influence CN1 concentration and activity (c+a).We performed an epidemiological study to shed light on CN1 metabolism in a T2DM cohort.

Methods: We included pat. with (n=127) and without DN (n=145).We assessed CN1 genotype and CN1 c+a.

Results: Female pat. without DN showed higher homCTG5 frequency compared to females with DN.When applying more stringent criteria for DN frequency for homCTG5 became also significant in male pat.

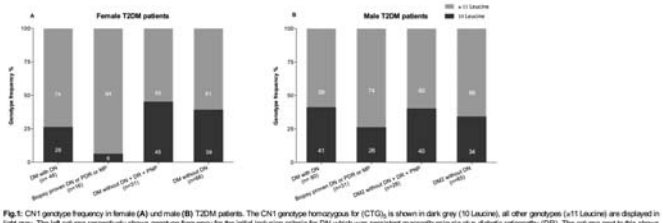


Fig.1: CN1 genotype frequency in female **(A)** und male **(B)** T2DM patients. The CN1 genotype homozygous for $(CTG)_5$ is shown in dark grey (10 Leucine), all other genotypes (≠11 Leucine) are displayed in light grey. The left column respectively shows genotype frequency for the initial inclusion criteria for DN which was persistent macroalbuminuria plus diabetic retinopathy (DR). The column next to this shows genotype frequency with more stringent inclusion criteria for DN which means only biopsy proven DN or macroalbuminuria in combination with advanced stages of DR (PDR=proliferative DR, MP=maculopathy). The right column respectively shows the initial inclusion criteria for no DN which were a diabetes duration of at least 15 years in combination with normoalbuminuria and no higher stage of DR than mild non-proliferative DR. Next to this column the genotype frequency with more stringent inclusion criteria for no DN are shown which means no microvascular complications at all were allowed (PNP=peripheral neuropathy). The comparison of CN1 genotype frequency showed significant differences in both female and male patients (χ^2 test for trend; p<0.0001 **(A)** und p=0.036 **(B)**).

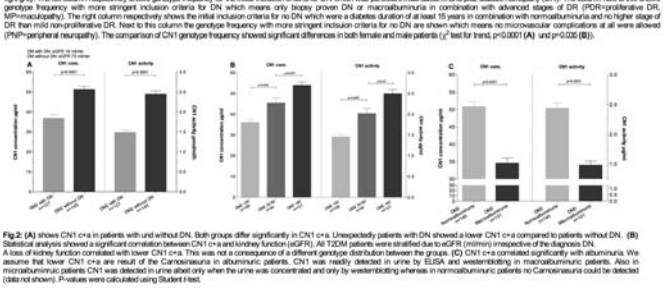


Fig.2: **(A)** shows CN1 c+a in patients with und without DN. Both groups differ significantly in CN1 c+a. Unexpectedly patients with DN showed a lower CN1 c+a compared to patients without DN. **(B)** Statistical analysis showed a significant correlation between CN1 c+a and kidney function (eGFR). All T2DM patients were stratified due to eGFR (ml/min) irrespective of the diagnosis DN. A loss of kidney function correlated with lower CN1 c+a. This was not a consequence of a different genotype distribution between the groups. **(C)** CN1 c+a correlated significantly with albuminuria. We assume that lower CN1 c+a are result of the Carnosinasuria in albuminuric patients. CN1 was readily detected in urine by ELISA and westernblotting in macroalbuminuric patients. Also in microalbuminuric patients CN1 was detected in urine albeit only when the urine was concentrated and only by westernblotting whereas in normoalbuminuric patients no Carnosinasuria could be detected (data not shown). P-values were calculated using Student *t*-test.

Pat. with DN showed lower CN1c+a as compared to pat. without DN and furthermore declining eGFR correlated with serum CN1c+a.In pat. with macroabluminuria CN1 was detected in urine by ELISA+WB. CN1c+a inversely correlated with time on hemodialysis (HD),the longer pat. were on HD the lower CN1c+a was and frequency for homCTG5 increased over time on HD (<12 versus >120 months 30 versus 67%, p<0.0001).

Conclusions: In addition to female also male pat. with homCTG5 seem to be protected if inclusion criteria for DN are chosen more stringent. Our data also indicate a correlation between loss of renal function and CN1c+a, which can be explained by the onset of canosinasuria.We speculate that CN1 might be reabsorbed in the proximal tubules where it may further deplete carnosine concentrations.Hence, not only serum CN1c+a plays an important role for progression of DN but also increased local CN1c+a in the kidney does. Finally we hypothesize that homCTG5 T2DM pat. which develop ESRD may still benefit above other genotypes as it seems to be associated with a lower mortality rate amongst these pat.

Funding: Government Support - Non-U.S.

SA-PO262

Associations of Serum Bicarbonate with Renal and Cardiovascular Outcome in Patients with Diabetes and Nephropathy Elise Schutte,[1] Hiddo Jan Lambers Heerspink,[1] Helen L. Lutgers,[1] Stephan J.L. Bakker,[1] Bruce Wolffenbuttel,[1] Kausik Umanath,[2] Dick de Zeeuw,[1] Julia Lewis,[3] Ron T. Gansevoort.[1] *[1]UMC Groningen, Groningen, Netherlands; [2]Henry Ford Hospital, Detroit; [3]Vanderbilt Univ, Nashville.*

Background: Low serum bicarbonate has been reported to be an independent predictor of renal function decline and mortality. To date, no study has investigated this association in a population with diabetes and nephropathy. We therefore aimed to investigate the association of serum bicarbonate with renal and cardiovascular (CV) outcome in this population.

Methods: Post hoc analysis of 2 multicenter randomized controlled trials (RENAAL and IDNT). Cox regression models were built to examine the longitudinal associations of baseline serum bicarbonate with endpoints. Bicarbonate was studied as continuous variable and stratified in quartiles to allow assessment of non-linear associations. Outcomes were incidence of end stage renal disease (ESRD), the combined endpoint ESRD or doubling of serum creatinine (DSCR) and CV outcome (incidence of fatal/non-fatal stroke/myocardial infarction).

Results: We included 2628 patients (mean age 60 ± 7 (SD) years, 65% male, 61% white) with a mean estimated GFR (eGFR) 44 ± 16 mL/min/1.73m2 and median UACR of 1366 mg/g (IQR 682 – 2653). Mean serum bicarbonate was 24.3 ± 3.7 mEq/L. During follow-up for 2.8 ± 1.0 years, 576 (22%) patients died, 491 (19%) developed ESRD, 731 (28%) had DSCR or ESRD, and 457 (17%) a CV event. Cox regression showed that bicarbonate had a negative association with incident ESRD (HR 0.91 (95% CI 0.90-0.93), P<0.001), and

with the incidence of the combined endpoint of ESRD or DSCR (HR 0.94 (0.92-0.96), P<0.001). These associations were independent of age, gender and CV risk factors, but lost significance when adjusting for baseline eGFR. Analysis of bicarbonate quartiles showed similar results. There were no significant associations of bicarbonate with CV events.

Conclusions: In this type 2 diabetes cohort with nephropathy, serum bicarbonate was not independently associated with renal or CV endpoints, contradicting earlier studies in non-diabetic populations.

SA-PO263

Urinary Trefoil Factor 3 Levels in Association with Albuminuria in Patients with Early Stages of Diabetic Nephropathy Naoto Terami, Daisuke Ogawa, Hitoshi Sugiyama, Toshio Yamanari, Takashi Hatanaka, Hiromi Tachibana, Naoko Nishii, Jun Wada, Hirofumi Makino. *Dept of Medicine and Clinical Science, Okayama Univ Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan.*

Background: Trefoil factor (TFF) peptides are coexpressed with mucins in the gastrointestinal tract stomach, colon and are also expressed in kidney tubules. Serum levels of TFF3 have been reported a possible biomarker of gastric cancer. Furthermore, Yu et al. reported that urinary TFF3 (uTFF3) levels were reduced, and urinary albumin levels increased in response to renal tubular injury in mice (Nat Biotechnol 2010). In this study, we determined whether uTFF3 is an efficient biomarker in patients with early staegs of diabetic nephropathy.

Methods: Spot urine samples were obtained from 79 male and 64 female type 2 diabetic patients (n=143) in Okayama University Hospital. The levels of uTFF1, uTFF2, and uTFF3 were measured quantitatively by specific ELISAs to analyze the correlation between uTFF1, uTFF2, uTFF3 and various clinical parameters. Furthermore, we also examined the correlation between Liver-type Fatty Acid-Binding Protein (L-FABP), N-acetyl-β-D-glucosaminidase (NAG), α1-microglobulin (α1-MG), β2-microglobulin (β2-MG) and uTFFs to evaluate the relation between renal tubular dysfunction and urinary levels of TFFs.

Results: The level of uTFF3 significantly increased in diabetic patients with microalbuminuria compared to those with normoalbuminuria (p=0.0139). In contrast to the level of uTFF3, the level of uTFF1 or uTFF2 did not significantly elevate in diabetic patients with microalbuminuria compared to those with normoalbuminuria.

Conclusions: These data indicate that the excretion of uTFF3 is selectively associated with microalbuminuria in patients with diabetes mellitus. Further studies are necessary to elucidate whether the selective elevation of uTFF3 in association with microalbuminuria can predict the progression of diabetic nephropathy.

SA-PO264

Two New Biomarkers in Urine (Peptides and Ceruloplasmin) for Diagnosing Early Diabetic Nephropathy Krishnamurthy P. Gudehithlu,[1] Jane Vernik,[1,2] Sandya Shivashankar,[1] Keerti K. Bhanushali,[1] Bhavi Paresh Pandya,[1] Mark A. Kraus,[1,2] Peter D. Hart,[1,2] Jose A.L. Arruda,[1,3,4] George Dunea,[1,3] Ashok K. Singh.[1,3,4] *[1]Div of Nephrology, John H. Stroger, Jr. Hospital of Cook County (JSH), Chicago, IL; [2]Dept of Internal Medicine, Rush Univ Medical College, Chicago, IL; [3]Section of Nephrology, Univ of Illinois at Chicago, Chicago, IL; [4]The Hektoen Inst of Medicine, Chicago, IL.*

Background: Early diagnosis of diabetic nephropathy (DN) is important for its optimal management. Functional change in the glomerulus leading to microalbuminuria is currently believed to be the first sign of DN. There is a growing recognition that functional renal tubular changes occur earlier than glomerular changes in DN (J.V.Bonventre, Semin Nephrol. 32:452-462, 2012). These include tubular growth, tubular hyperactivity manifested as solute hyper-absorption, oxidative stress and inflammation. We hypothesized that measuring specific tubular markers in urine may aid in the early diagnosis of DN.

Methods: Urinary peptides (a function of tubular activity and growth) and exosomal ceruloplasmin (a marker of tubular inflammation) were estimated in early DN patients with microalbuminuria (30-300 μg/g creatinine) (earlyDN, n=25) and non-diabetic controls (nonDb, n=23). Urine samples were collected from the renal and diabetes clinics at JSH. Peptide concentration in whole urine was determined by subtracting values obtained by the Lowry assay (measures peptides and proteins) from the BioRad assay (measures proteins). Ceruloplasmin level was determined in exosomes (prepared by differential ultracentrifugation) using a sandwich ELISA kit. Both, peptides and ceruloplasmin, were corrected by urinary creatinine.

Results: In earlyDN patients compared to nonDb controls, peptide levels increased by 210% (p<0.05), and ceruloplasmin levels increased by 560% (p<0.05).

Conclusions: We conclude that marked increase of peptides and ceruloplasmin in urine of earlyDN suggests a significant dysfunction of the tubular system in early diabetic kidney disease. Peptides and ceruloplasmin in urine are two potential biomarkers that need to be explored further for early diagnosis of DN.

Funding: Private Foundation Support

SA-PO265

The Association of Adiponectin with Diabetic Complications in Type 2 Diabetic Patients Sung Jin Kim, Jungyeon Ghee, Jung Eun Kim, Hye Sook Min, Jin Joo Cha, Young Sun Kang, Dae R. Cha. *Korea Univ Medical College Ansan Hospital, Ansan, Republic of Korea.*

Background: Adiponectin is a 244-amino acid collagen-like protein that is solely secreted by adipocytes and acts as a hormone with anti-inflammatory and insulin-sensitizing properties and regulates the metabolism of lipids and glucose. It plays important role in the development of insulin resistance and atherosclerosis. However, it is uncertain that the association of adiponectin with diabetic complications. We investigated the association of serum adiponectin level with diabetic complications in type 2 diabetic patients.

Methods: We conducted a prospective study of 161 patients with type 2 diabetes from 2002 to 2013. Spearman's correlation coefficients were calculated to evaluate the relationship between adipokines and biomarkers associated with diabetic complications. Linear by linear association test was performed with grouping each adiponectin, diabetic complications and biomarkers. All statistical analyses were performed using SPSS version 18.0.

Results: Age, sex, diabetes duration, cholesterol, LDL cholesterol, calcium channel blocker, thiazolidinedione, diabetic neuropathy, dialysis and 24hr protein efficiency ratio were positively correlated with serum adiponectin level(all p<0.05). eGFR was negatively correlated with serum adiponectin level(p<0.05). Quartile group of serum adiponectin was significantly linear relationship with age, sex, diabetes duration, cholesterol, LDL cholesterol, thiazolidinedione, diabetic nephropathy and chronic kidney disease stage (all p<0.05).

Conclusions: At present, medical research has shown that synthetic adiponectin may be able to raise adiponectin levels and medications could exist to prevent diabetes. Taken our research together, serum adiponectin level was significantly relationship with hyperlipidemia, diabetic neuropathy and nephropathy. Our research showed that adiponectin was potential pharmacologic treatment modality of the diabetic complication of type 2 diabetic patients.

SA-PO266

The Association of Adipokine with Diabetic Complications in Type 2 Diabetic Patients Sung Jin Kim, Jung Eun Kim, Jungyeon Ghee, Hye Sook Min, Jin Joo Cha, Dae R. Cha, Young Sun Kang. *Korea Univ Medical College Ansan Hospital, Ansan, Republic of Korea.*

Background: Glypican-4 is enhanced insulin signaling through direct interaction with the insulin receptor and promoted adipocyte differentiation. Irisin is related to glucose tolerance status and known to be lower in type 2 diabetes. RBP4(retinol binding protein 4) is known to cause insulin resistance, prediabetes, metabolic syndrome and myocardial infarction by adipose tissue inflammation. Visfatin has insulin-like activity and is able to bind to the insulin receptor. However, it is uncertain that the association of them with diabetic complications.

Methods: We conducted a prospective study of 161 patients with type 2 diabetes from 2002 to 2013. Spearman's correlation coefficients were calculated to evaluate the relationship between adipokines and biomarkers associated with diabetic complications. Linear by linear association test was performed with grouping each adipokine, diabetic complications and biomarkers.

Results: Glypican-4 was positively correlated with ACEI and beta blocker, and negatively correlated with irisin and eGFR. Quartile group of glypican-4 was linear relationship with beta blocker. Irisin was positively correlated with 2hrs post-prandial plasma glucose level, and negatively correlated with sulfonylurea, retinopathy, urine protein creatinine ratio and glypican-4. Quartile group of irisin was linear relationship with sulfonylurea, retinopathy, urine protein creatinine ratio and 2hrs post-prandial plasma glucose level. RBP4 was positively correlated with hypertension, beta blocker, calcium channel blocker, serum creatinine, nephropathy and dialysis, and negatively correlated with eGFR. Quartile group of RBP4 was linear relationship with hypertension, beta blocker, calcium channel blocker, nephropathy and CKD stage. None of them are associated with serum visfatin level.(all p<0.05).

Conclusions: Glypican4, irisin and RBP4 were correlated with hypertensive medication and renal injury. Especially, quartile group of irisin and RBP4 were linear relationship with renal injury. In type 2 diabetic patients, the measurement of serum adipokine is possible to predict the renal disease among the diabetic complications.

SA-PO267

Residual Proteinuria as a Risk Factor for Rapid Renal Function Decline During Pre-Dialysis Phase in Type 2 Diabetic Patients with Severely Impaired Renal Function Yuichiro Kitai,[1,2] Masao Koshikawa,[1] Motoko Yanagita,[2] Akira Sugawara.[1] *[1]Dept of Nephrology, Osaka Red Cross Hospital, Osaka, Japan; [2]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan.*

Background: Proteinuria is an established risk factor for progression of renal disease, including diabetic nephropathy. The predictive power of proteinuria for progressive renal function decline has been well demonstrated in diabetic patients with normal to relatively preserved renal function. However, little is known about the impact of proteinuria on renal outcome in pre-dialysis diabetic patients with severely impaired renal function.

Methods: We identified 125 incident dialysis patients with type 2 diabetes. The purpose of this study was to retrospectively analyze the relationship between nephrotic range proteinuria (urinary protein-creatinine ratio above 3.5 g/gCr) and renal function decline during the three months just prior to dialysis initiation.

Results: In total, 103 patients (82.4%) had nephrotic range proteinuria. The median rate of decline in the estimated glomerular filtration rate (eGFR) in this study population was 0.98 (interquartile range 0.51-1.46) ml/min/1.73m^2 per month. Compared to patients with non-nephrotic range proteinuria, patients with nephrotic range proteinuria showed significantly faster renal function decline (0.46 [0.24-1.25] versus 1.07 [0.64-1.54] ml/min/1.73m^2 per month; p = 0.007). A multivariate analysis after adjusting for systolic blood pressure, hemoglobin, serum albumin, total cholesterol, hemoglobin A1c, and use of renin angiotensin system inhibitors revealed that patients with nephrotic range proteinuria showed a 4.23-fold (95% confidence interval: 1.12-16.6) increased risk for rapid renal function decline defined as a decline in eGFR ≥0.5 ml/min/1.73m^2 per month.

Conclusions: Nephrotic range proteinuria is the predominant renal risk factor in type 2 diabetic patients with severely impaired renal function receiving pre-dialysis care. Reduction of residual proteinuria to the lowest achievable level might be better considered as a goal for renoprotective treatment.

SA-PO268

Urine Proteomics in Proteinuric DKD Maryam Afkarian, Leila R. Zelnick, Jonathan Himmelfarb. *Div of Nephrology, Dept of Medicine, Kidney Research Inst, Seattle, WA.*

Background: Our understanding of the mechanisms underlying various stages of diabetic kidney disease (DKD) in humans is largely derived from studies in animal models of DKD. Large-scale analysis of urine proteins may provide information on pathways dysregulated in human DKD.

Methods: We used mass spectrometry-based proteomic analysis to compare urine proteins in type 1 diabetes with (n=32) and without (n=29) kidney disease. DKD was defined as an albumin to creatinine ratio (ACR) > 300 mg/g or eGFR <60 ml/min/1.73m^2 and ACR > 30 mg/g. Absence of DKD was defined as eGFR > 90 ml/min/1.73m^2 and ACR <300 mg/g after > 30 years of type 1 diabetes.

Results: Analyzing cases and controls as groups, we identified 3065 and 3051 proteins, respectively, 1401 of which were higher in cases. Analyzing cases and controls individually, we identified 3209 proteins with 670 identified in > 50% of cases and > 50% of controls. Of these, 215 were higher in cases, 95 of which reached nominal significance (p<0.05). Cases showed an overexpression of proteins involved in cytoskeleton, response to hypoxia, immune response, cell cycle and apoptosis, as well as the endo-phagocytic machinery. The latter group included proteins involved in all steps of endo-phagoyctosis, from membrane invagination to lysosomes and lytic vacuoles, possibly reflecting the increase in proximal tubular endocytosis and intracellular proteolysis of tubular proteins in proteinuric DKD. Among overexpressed lysosomal proteins were several cathepsins, whose increase in DKD urine was confirmed using immunoassays. As cathepsins can convert angiotensin I to angioteins II and are not blocked by ACE inhibitors, their increase provides a potential mechanism of renal RAAS escape from ACE inhibition in proteinuric DKD.

Conclusions: Examination of urine proteins using mass spectrometry-based proteomics shows overexpression of proteins involved in cytoskeleton, response to hypoxia, immune response, cell cycle and apoptosis, as well as the endo-phagocytic machinery in proteinuric DKD. The latter included an increase in lysosomal cathepsins, which may provide a mechanism for renal angiotensin II generation despite ACE inhibition in proteinuric DKD.

Funding: NIDDK Support, Private Foundation Support

SA-PO269

Glycemic Markers and 1-Year Diabetic Hemodialysis Outcomes from the GIDE Study Mark E. Williams,[1] Neal Mittman,[2] Lin Ma,[3] Julia I. Brennan,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[3] Eduardo K. Lacson.[3] *[1]Joslin Diabetes Center, Boston, MA; [2]Kidney Care of Brooklyn and Queens, Brooklyn, NY; [3]Fresenius Medical Care, North America, Waltham, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

Background: Prognostic implications of glycemia in HD patients based on hemoglobin A1c (HgbA1c), albumin-adjusted and unadjusted fructosamine (AlbF; F) and glycated albumin (GA) or percent GA (%GA) have been proposed. From the multi-year prospective observational Glycemic Indices in Dialysis Evaluation (GIDE) Study, we report 1st year outcomes associated with hyperglycemic thresholds for these markers per the literature and/or manufacturer recommendations.

Methods: As of April 1, 2013, 1,392 active HD patients with DM from 26 FMCNA facilities with glycemic markers from Jan-Mar 2013 were followed until Mar 31, 2014. Poor glycemic control was based on: HgbA1c>7% (sensitivity analysis >8%), AlbF≥974 µmol/g, F>285 µmol/L, %GA>15.7%, and GA>300 µmol/L. Cox models with and without adjustment for age, sex, race, ethnicity, vintage (log), BMI, HD catheter, and baseline comorbid illnesses were utilized to determine associations between each dichotomized glycemic index with death and with hospitalization.

Results: Poor glycemic control was found in 28% (HgbA1c>7%), 13% (HgbA1c>8%), 35% (AlbF), 87% (F), 81% (%GA) and 69% (GA) of patients. AlbF was associated with 1-year hospitalization risk with HR=1.28 (1.11, 1.48) and adjusted-HR=1.33 (1.14, 1.54), both p<0.001; but not with mortality. For the other glycemic indices, Cox model analyses for both outcomes showed no significant associations.

Conclusions: Early GIDE study findings indicated an association between poor glycemic control determined by AlbF and worse 1-year hospitalization risk. No 1-year outcome associations were detected with other markers evaluated, including with HgbA1c at either 7% or 8% thresholds. Further evaluation will include use of continuous values for glycemic indices to determine other potential thresholds for risk, incorporating repeated measures of glycemic indices, adding more adjustment variables, outcomes analyses of hospital events/days and cause-specific deaths, and longer term follow-up.

SA-PO270

Predictive Factors for Non-Diabetic Nephropathy in Diabetic Patients Sheila Bermejo,[1] Maria Jose Soler,[1] Javier Gimeno,[2] Clara Barrios,[1] Eva Rodriguez,[1] Julio Pascual.[1] *[1]Nephrology, Parc de Salut Mar, Barcelona, Spain; [2]Pathology, Parc de Salut Mar, Barcelona, Spain.*

Background: Renal biopsies taken in diabetic patients are increasing in number and complexity. The objective of this study is to determine the predictability of a renal biopsy (non-diabetic nephropathy or diabetic nephropathy) by clinical and laboratory data in diabetic patients.

Methods: Retrospective descriptive study based on pathology of 110 biopsies taken at our institution in diabetic patients (DM) during 1990-2013.

Results: 110 patients, 87 men (79%), mean age 62a (50-74) were included in the study. Mean duration of DM 10.6 years, creatinine 2.6 mg/dl (0.9-4.3), estimated glomerular filtration rate MDRD 4 3.7 mL / min / 1.73 m2 (14.4-73), glycosylated hemoglobin 6.8% (5.1-8.5), basal glucose 141 mg / dl (77.2-205.8), and proteinuria of 3.5 g/24 h (0.5-6.5). The reason for renal biopsy was 25% nephrotic syndrome or rapid increase in proteinuria, 14% fast decline in GFR, 13% nephrotic range proteinuria without diabetic retinopathy and 11% proteinuria> 1 g DM and less than 5 years of DM evolution. Renal biopsy results: 38% diabetic nephropathy(DN), 5.4% DN plus-non-diabetic nephropathy(NDN). IgA nephropathy 7.3%, 5.5% nephrosclerosis, 4.5% focal and segmental glomerulosclerosis, 3.6% membranous nephropathy and chronic interstitial nephritis and others. In the multivariate binary logistic regression analysis, variables independently associated with NDN were: serum creatinine (HR: 1.48,95% CI:1,011-2, 172, p=0.044), proteinuria/24 hours (HR: 0.813,95% :0,679-0, 974, p=0.025), duration of DM (HR: 0.992, 95% CI :0, 987-0, 998, p=0.004), t age (HR: 1.068, 95% CI :1,010-1, 129, p=0.022) and diabetic retinopathy(DR) (HR 0.23, 95% CI: 0.066-0.808 p= .022). The discriminatory capacity of the model was tested using the ROC curve (95% CI): 0.805 (0.708 to 0.902).

Conclusions: 38% of diabetic patients biopsied at our center have diabetic nephropathy. Patients with serum creatinine and older have increased risk of NDN; while these patients showed shorter duration of diabetes, and less incidence of DR and proteinuria. The most frequent cause of NDN in our environment is the IgA nephropathy.

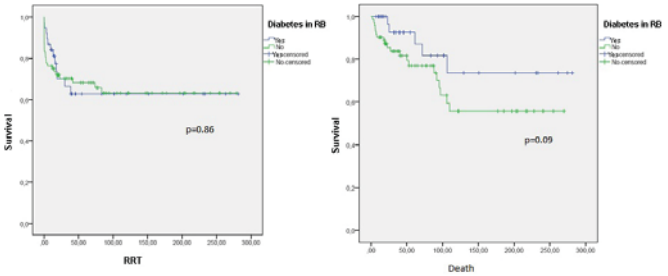
SA-PO271

Renal Prognosis and Survival of Diabetic Patients with Kidney Disease: A Comparative Analysis between Diabetic and Non Diabetic Nephropathy Sheila Bermejo,[1] Maria Jose Soler,[1] Javier Gimeno,[2] Clara Barrios,[1] Eva Rodriguez,[1] Julio Pascual.[1] *[1]Nephrology, Hospital del Mar, Spain; [2]Pathology, Hospital del Mar, Spain.*

Background: The difference of the renal and patient survival between diabetic nephropathy (DN) and non-diabetic nephropathy (NDN) in diabetic patients is not well known.

Methods: Retrospective descriptive study and analysis of renal function and survival in diabetic patients that undergoing renal biopsy at our institution between 1990-2013. We analyzed the need for renal replacement therapy and mortality between 1990 and 2013 in those diabetic patients.

Results: 110 patients were included, 87 men (79%), mean age 62 years (50-74), creatinine 2.6 mg/dl (0.9-4.3), glycosylated hemoglobin 6.8% (5.1-8.5), basal glucose 141 mg / dl (77.2-205.8) and proteinuria of 3.5 g/24 h (0.5-6.5). In renal biopsy, 35% had DN and 65% non-DM nephropathy with or without DN (NDN). 33% of patients needed renal replacement therapy.1:2 ratio between DN and NDN (11 versus 22%) at the time of renal biopsy, 1 patient in DN group and 9 patients in NDN group began dialysis. The overall mortality of the patients was 23%, 5% from the DN group and 18% from the NDN group. No differences in patient or renal survival between both patient cohorts studied by Kaplan-Meier curves were observed.



Conclusions: No differences are observed in renal and patient survival between NDN or DN in our diabetic patients. Although the crude mortality is higher in diabetic patients with NDN as compared to DN, no significant differences were observed in the actuarial survival curves.

SA-PO272

Urinary Tubular Biomarkers Predict Renal Injurious Progress in Early Diabetic Nephropathy Patients Feelan Chong,[1] Yigang Wan.[2] *[1]Oriental Academy of Chinese Medicine, Puchong, Selangor, Malaysia; [2]Dept of Traditional Chinese Medicine, The Affiliated Hospital of Nanjing Univ Medical School, Nanjing, China.*

Background: Diabetic nephropathy (DN) is a dominant cause of end-stage renal disease in China. Therefore, noninvasive detections and dynamic managements in clinic are of major importance of preventing progress. The early diagnosis of DN has focused on measurement of UAlb excretion rate, but it is not sensitive marker for DN patients with inchoate injuries in glomeruli and renal tubules. This study thus aimed to evaluate clinical significance of urinary tubular biomarkers in predicting development of DN patients at early stage.

Methods: The study was performed on 92 diabetes mellitus with different levels of UAlb and certain range of Scr (<106μmol/L). According to albumin-to-creatinine ratio (ACR) in urine, all patients were categorized into 3 groups, normoalbuminuria (A) group, microalbuminuria (B) group and macroalbuminuria (C) group. In addition to UAlb, Scr and ACR, levels of tubular biomarkers including UNAG, URBP and UCysC in urine were tested respectively before renal protective drugs intervention.

Results: Compared with A group, levels of UNAG, URBP and UCysC in B and C groups were significantly different (P<0.01). Along with UAlb, stepwise increases in levels of UNAG, URBP and UCysC were detected respectively in B and C groups. Moreover, in univariate analysis, there was immediate relevance between UAlb, ACR and tubular biomarkers including UNAG (r=0.706, P<0.01; r=0.808, P<0.001), URBP (r=0.687, P<0.01; r=0.701, P<0.001) and UCysC (r=0.727, P<0.01; r=0.790, P<0.001) in all groups. In addition, we found that UNAG was positively correlated with URBP (r=0.652, P=0) and UCysC (r=0.785, P=0). URBP was also definitely related to UCysC (r=0.673, P=0). Multivariate logistic regression showed that body mass index and fasting blood glucose were two predictive factors of increased UCysC.

Conclusions: At early stage of DN, increased levels of UNAG, URBP and UCysC are independently associated with UAlb, and these urinary tubular biomarkers similar to UAlb may be widely used as practical targets in clinic in detecting and managing DN, and predicting renal tubular damaged progression.

Funding: Government Support - Non-U.S.

SA-PO273

Diabetic Nephropathy Underdiagnosed: Results from an Autopsy Study Celine Klessens,[1] Kimberley Veraar,[1] Tess Dorine Woutman,[1] Malu Zandbergen,[1] Elisabeth J. Valk,[1] Joris I. Rotmans,[2] Jan A. Bruijn,[1] Ingeborg M. Bajema.[1] *[1]Pathology, Leiden Univ Medical Center; [2]Nephrology, Leiden Univ Medical Center.*

Background: The reported incidence of diabetic nephropathy (DN) in both type 1 and type 2 diabetes patients varies considerably. In clinical practice, a renal biopsy is often not performed to confirm a suspected DN. In this study, we report the incidence of DN on the basis of an autopsy study with 150 patients with either type 1 or type 2 diabetes.

Methods: Renal tissue specimens were collected from cases from 1984-2004 who were reported to have diabetes in the autopsy report. Tissue specimens had to be non-autolytic and at least 100 glomeruli had to be available for evaluation. Patients with renal and or pancreas transplantation were excluded. New tissue slides were cut and new stainings were performed (HE, PAS, silver). All specimens were evaluated by 2 observers and the histopathological classification for DN was scored as well as interstitial and vascular parameters. GBM width was measured by electron microscopy if no light microscopic changes were found consistent with DN. Clinical parameters included: age at death, cause of death, proteinuria and renal function (eGFR measured with MDRD formula) when available in a period of at least 6 months before death.

Results: A cohort of 150 patients was included (average age 69, sd=12.1). No light microscopic changes compatible with DN were found in 76 patients. Of these cases, we have currently measured the GBM width of 40 cases, resulting in the diagnosis of class I DN in 12. Of those patients with LM changes compatible with DN (N=74; 49.3%), 18 (12%) had class IIA, 10 (6.7%) had class IIB, 41 (27.3%) had class III and 5 (3.3%) had class IV. We found a trend for severity of DN being related to cardiovascular death (p=0.059). Also DM duration showed a trend for being related to the severity of DN (p=0.07). No significant correlation with proteinuria and class (p=0.112) was found. There was a significant difference (p<0.05) in eGFR over the classes.

Conclusions: This study shows that the incidence of DN (49.3%) in patients with diabetes is much higher than currently assumed. The clinical relevance of silent DN should be further investigated.

SA-PO274

Control of dGlucose Is Fundamental to Renal Preservation in Diabetes Anil K. Mandal,[1] Linda M. Hiebert.[2] *[1]Medicine, Univ of Florida, Gainesville, FL; [2]Veterinary Biomedical Sciences, Univ of Saskatchewan, Saskatoon, SK, Canada.*

Background: We previously reported that dglucose predicts renal function change in diabetes (Diab Res Clin Prac 2011;91:190). This study is an expansion of a previous study but with longer duration.

Methods: 85 diabetic patients (M:F,34:51;60.8±13.8[SD] yrs) were treated with a combination of glargine or detemir and regular insulin for 26.3±24.6(SD) months. Blood pressure was controlled by beta blockers, calcium channel blockers, sympathetic inhibitors or a combination and chlorthalidone in resistant cases. Treatment with angiotensin converting enzyme inhibitors (ACEIs) or receptor blockers (ARBs) was excluded. Objectives were to determine if this paradigm of treatment prevented progression of diabetic nephropathy into end stage renal disease(ESRD). Fasting(F) and 2-hour postprandial(2hPP) glucose, serum creatinine(Scr) and estimated glomerular filtration rate(eGFR); hemoglobin $A1_C$($HbA1_C$); sitting and standing systolic and diastolic blood pressures(SBP,DBP) were recorded for first and last visits. Mean blood pressure (MBP) and differences (d, 2hPP-F) were calculated for glucose, Scr and eGFR. Parameters between first and last visits were compared using a paired t-test adjusted for age, gender and duration of treatment. P<0.05 was considered significant.

Results: Differences were not significant between first and last treatments for F and 2hPPglucose (175.2±83.6[SD] versus 166.2±87.9 mg/dL, n=59, P=0.5243; 244.0±98.2 versus 217.2±94.8 mg/dL, n=57, P=0.1119, respectively), F and 2hPPScr (1.11±0.44 versus 1.11±0.45 mg/dL, n=60, P=0.9364; 1.22±0.53 versus 1.27±0.60 mg/dL, n=50, P=0.5186, respectively), F and 2hPPeGFR (68.2 ±26.3 versus 65.8±26.3 ml/min, n=60, P=0.1419; 61.0±24.3 versus 58.5± 23.0 ml/min, n=50, P=0.2475,respectively) and $HbA1_C$ (8.2±2.0 versus 8.2±1.8%, n=29, P=0.9643, respectively). Dglucose, sitting SBP and MBP were significantly reduced at the last versus first visit (36.6±65.6 versus 63.5±68.1 mg/dL, n=41, P=0.0449; 128.4±13.9 versus 133.1±17.1 mmHg, n=74, P=0.0319; 95.4±10.9 versus 98.9±11.3 mmHg, n=74, P=0.0151 respectively).

Conclusions: This study reinforces the importance of control of dglucose (2hPP-F) with insulin and exclusion of ACEI/ARB in the prevention of ESRD in diabetes.

Funding: Clinical Revenue Support

SA-PO275

Effect of Metformin Compared to Insulin in Renal Preservation of Adults with Diabetes Aswini Prasad Patnaik,[1] Anil K. Mandal,[2] Linda M. Hiebert.[3] *[1]Medicine and Nephrology, South Eastern Railway Hospital, Kolkata, West Bengal, India; [2]Medicine, Univ of Florida, Gainesville, FL; [3]Veterinary Biomedical Sciences, Univ of Saskatchewan, Saskatoon, SK, Canada.*

Background: It was reported that oral antidiabetic agents, including metformin, have a variable risk for decline in renal function including end stage renal disease or death (Kidney Int 2012; 81: 698). This prospective study compares the effect of biguanide monotherapy (metformin) versus insulin treatment, in controlling postprandial hyperglycemia and maintaining renal function.

Methods: Seventy-eight adults with diabetes (M:F,38:40; 55.7±11.1[SD] yrs [range 32–85]) were treated with metformin alone for mean 13.3±11.4 months and were compared with 64 diabetic patients (M:F,24:40, 58.8±14.4 yrs, [range 24-87]) treated with a combination of glargine or detemir and regular insulin for 23.6±30.5 months. The 2-hour postprandial (2hPP) glucose, 2hPP serum creatinine (Scr), and hemoglobin $A1_C$ ($HbA1_C$) were determined for the first and last visit. Efficacy of metformin and insulin were compared using multiple regression analyses adjusting for age, gender, duration of diabetes and treatment. P<0.05 was considered significant.

Results: In the metformin treated patients, when values for first versus last visit were compared, no difference in 2hPP glucose (231±19[SD] versus 215.5±48.1 mg/dL, P=0.0565), an increase in Scr (1.37±1.22 versus 1.47±1.22 mg/dL, P=0.0002) and no difference in $HbA1_C$ (7.2±0.9 versus 6.9±0.6 %, P=0.0555) were found. In the insulin treated patients, a significant decrease in 2hPP glucose (281.6±129.1 versus 241.0±77.8 mg/dl, P=0.0466), no change in Scr (1.15±0.38 versus 1.32±0.73 mg/dl, P=0.8503) or $HbA1_C$ (8.9±2.0 versus 9.0±1.8 %, P=0.2387) were found. When the difference in change between first and last visits in the metformin and insulin treated groups were compared a significantly greater decrease in 2hPP glucose with insulin treatment (50.6±19.5 mg/dL, P=0.0109), a significantly greater increase in Scr with metformin treatment (0.17±0.06 mg/dL, P=0.0075) but no difference in change in $HbA1_C$ (-0.4 %, P=0.3781) between the treatments were noted.

Conclusions: Thus this study indicates that insulin but not metformin preserves renal function in diabetes.

Funding: Clinical Revenue Support

SA-PO276

Personalized Podocyte Biology for the Prediction of Diabetic Kidney Disease Mayrin Correa-Medina,[1] Evadnie Rampersaud,[1] Christopher E. Pedigo,[1] Viji Nair,[2] Matthias Kretzler,[2] Robert G. Nelson,[3] Sandra M. Merscher,[1] Alessia Fornoni.[1] *[1]Univ of Miami; [2]Univ of Michigan; [3]National Inst of Diabetes and Digestive and Kidney Diseases, Phoenix, Arizona.*

Background: Diabetic kidney disease (DKD) is the leading cause of end-stage kidney disease in the U.S, and podocyte injury correlates with DKD progression in type 2 diabetes. In order to identify key signalling pathways that are induced in podocytes in early DKD, we utilized a cell-based assay where normal human podocytes are cultured for 24 hours with sera of DKD patients.

Methods: We used sera obtained from a total of 31 patients enrolled in the "*Renoprotection in Early Diabetic Nephropathy in Pima Indians trial*" and collected on average 7 years after enrolment. We studied two groups of patients with extreme phenotypes based on the rate of decline in glomerular filtration rate (GFR) between enrollment and last examination (mean time of 10±1.7 years). The two groups were defined as progressors (delta GFR of -97.39±8.2, n=15) and non progressors (delta GFR of 40.62±8.6, n=16). mRNAs obtained from podocytes were utilized to generate microarray data and for validation of gene expression by real-time PCR.

Results: We identified 397 genes differentially expressed in podocytes exposed to progressor sera. Using selection criteria (p<0.05 and 2 ≥ fold-change in expression), we found 26 down and 28 up regulated genes in progressor group. Selected genes were validated by real-time PCR where we found reduction by ~60%, p<0.05 of the ABCA1

gene in podocytes treated with progressor sera. A significant negative correlation between ABCA1 gene expression and GBM thickening was identified. BODIPY staining showed 3 fold, $p<0.05$ increases in cholesterol accumulation in podocytes treated with DKD sera when compared with cells treated with healthy control sera. Exposure of podocytes to progressor sera also induced apoptosis ($p<0.05$).

Conclusions: In summary, we propose a novel method to identify podocyte molecular fingerprint in DKD. Once validated in different cohorts, this method may serve as a non-invasive approach to predict DKD progression and as a tool to identify new podocyte-specific targets in DKD.

Funding: NIDDK Support, Pharmaceutical Company Support - University of Miami, Private Foundation Support

SA-PO277

Effects of Febuxostat Rx on Adipokines and Markers of Kidney Fibrosis in Asymptomatic Hyperuricemic Patients with Diabetic Nephropathy: A RCT Srini Beddhu,[1,2] R. Filipowicz,[2] Bin Wang,[2] G. Wei,[2] Xiaorui Chen,[2] E. Constantz,[2] Abinash C. Roy,[2] A. N. Habib,[2] Donald E. Kohan,[1,2] Mark Munger,[2] Tom Greene,[2] Yufeng Huang.[2] *[1]SLC VA; [2]Univ of UT.*

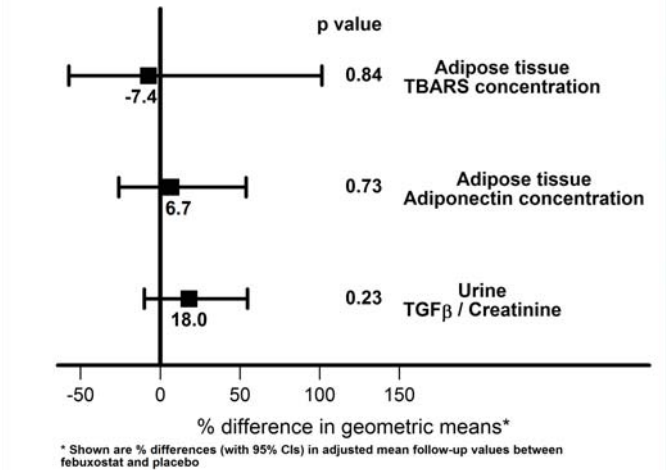
Background: Experimental data suggest that decreasing uric acid(UA) impacts adipokines and kidney disease. Therefore, we hypothesized febuxostat(FBX) Rx in humans with diabetic nephropathy(DN) will ↓ hyperuricemia and thereby, impact adipose tissue(AT) oxidative stress (OS) and adipokines; ↓ plasma markers of inflammation and OS; and ↓ urinary markers of kidney fibrosis.

Methods: Overweight or obese adults with hyperuricemia and type 2 DN were randomized to FBX(n= 40) or placebo(n=40) for 24 wks. Abdominal subcutaneous fat biopsy was performed at baseline and 24 wks. Blood and urine samples were collected at baseline, 12 and 24 wks. Mixed effects models were used to examine the impact of FBX on primary (AT thiobarbituric acid reducing substances(TBARS), AT adiponectin and urinary TGF-β) and secondary endpoints.

Results: Baseline characteristics are summarized in the table.

	FBX	Placebo
	N=40	N=40
Age(year)	67±10	68±11
Male(%)	60.0	70.0
Black(%)	2.5	5.0
CAD(%)	20.0	17.5
CHF(%)	10.0	10.0
PVD(%)	2.5	5.0
Stroke(%)	5.0	2.5
BMI(kg/m^2)	36.2±7.0	30.1±6.2
Plasma UA (mg/dl)	7.2±1.5	7.1±1.2
eGFR(ml/min/1.73m^2)	52.2±15.3	54.8±19.0
Urine ACR<30mg/g(%)	64.1	65.0

92% in FBX and 97.5% in placebo groups completed the study. FBX ↓ plasma UA by 50%($p<0.001$).



No detectable differences with FBX Rx were observed for the primary endpoints [fig) or the secondary endpoints of plasma levels of high molecular weight adiponectin(14.6%, 95% CI -3.7 to 36.3, p=0.12), TNF-α(-2.8%, 95% CI -31.8 to 38.7, p=0.87), MCP-1(4.9%, 95% CI -21.1% to 39.5%, p=0.34) or IL-6(-16.6%, 95% CI -33.0 to 5.2, p=0.13).

Conclusions: FBX Rx affected plasma UA levels in DN. However, no significant effects on the primary or secondary endpoints were observed.

Funding: Pharmaceutical Company Support - Takeda Pharmaceuticals USA, Inc.

SA-PO278

Elevated Neutrophil Gelatinase-Associated Lipocalin (NGAL) Level in Chronic Kidney Disease (CKD) Is Function of Both Reduced Glomerular Filtration Rate (GFR) and Circulating Neutrophil Count Shweta Bansal,[1,2] Chakradhar Velagapudi,[1,2] William E. Friedrichs,[1] Perla R. Zarate-Abbott,[1,2] Sue Cunningham,[1,2] Paolo Fanti.[1,2] *[1]Medicine/Renal, Univ of Texas Health Sciences Center at San Antonio, San Antonio, TX; [2]Medicine/Renal, South Texas Veterans Healthcare System, San Antonio, San Antonio, TX.*

Background: Serum neutrophil gelatinase-associated lipocalin NGAL levels increase progressively with chronic kidney disease (CKD) and predict worse outcome. However, the source of serum NGAL, whether it is injured renal tubular cells or other tissues or both, remains unclear.

Methods: NGAL and other parameters were assessed on two occasions with 3-month interval in stored blood samples of 51 diabetic nephropathy patients with stage III and IV CKD, who participated in a clinical study evaluating the effect of N-acetyl cysteine and silymarin on albuminuria.

Results: The study population was 61.6±7.5 year old, 85% male, 71% Hispanic white, 53% with stage III and 47% with stage IV CKD, and with body mass index 35.3±8.8 kg/m^2. In Pearson's correlation, $\log_{10}$-NGAL levels were correlated negatively with eGFR ($r=-0.71$, $p<0.001$), plasma hemoglobin ($r=-0.37$, $p=0.008$) and serum albumin ($r=-0.28$, $p=0.04$); and positively with total leucocyte count ($r=0.5$, $p<0.001$), absolute neutrophil count (ANC) ($r=0.58$, $p<0.001$) and serum phosphorus ($r=0.58$, $p<0.001$). In a standard multivariate regression model, the same parameters explained 75% of NGAL variation ($p<0.001$), with partial F-test analysis showing significance for only eGFR ($\beta=-0.47$, $p<0.001$) and ANC ($\beta=0.40$, $p<0.001$). Very similar results were obtained at the 3-month follow up.

Conclusions: The results of our study suggest that elevated serum NGAL is a function of both decreased kidney function and circulating neutrophils in CKD patients. Further studies are needed to evaluate the cause(s) of increased NGAL production in circulating neutrophils.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO279

Crosstalk between Hippo and EGFR Signaling Pathway in Development of Diabetic Nephropathy Jianchun Chen, Raymond C. Harris. *Nephrology/ Medcine, Vanderbilt Univ, Nashville, TN.*

Background: The Hippo signaling pathway in mammalian cells controls cell proliferation, apoptosis and differentiation. In mammalian cells, this pathway is a kinase cascade in which Mst1/2 kinases and Sav1 form a complex to phosphorylate and activate LATS1/2, which phosphorylate and inhibit two major downstream effectors YAP and TAZ. Our recent studies have demonstrated that EGFR is activated in renal proximal tubule injury during diabetic nephropathy (DN) development, and EGFR activation plays an important role in DN progression.

Methods: We induced type I diabetes in our proximal tubule EGFR deletion mice(*EGFR^{ptKO}*) and their wild type littermates (WT) by daily low dose streptozotocin injections for 5 consecutive days. Cell signaling studies were performed in a proximal tubule epithelial-like cell (LLCPKCl4).

Results: STZ injection induced similar levels of hyperglycemia in *EGFR^{ptKO}* and their *WT* mice. Both total and phosphorylated YAP (at Ser127) increased in diabetic *WT* mice, and these increases were inhibited in *EGFR^{ptKO}* mice compared with *WT* mice. In LLCPKCl4cells, 24 hour exposure to high glucose (25 mM) induced EGFR and YAP phosphorylation. These effects were inhibited by treatment with the EGFR tyrosine kinase inhibitor erlotinib or transfection with EGFR siRNA. Akt phosphorylation was also upregulated in response to high glucose treatment, and the PI3K inhibitors LY29004 or wortmannin inhibited both Akt and YAP phosphorylation. Further studies revealed that knockdown of YAP expression by siRNA transfection inhibited cell proliferation in response to EGF and also abrogated proliferative responses to high glucose.

Conclusions: This study indicated that the hippo signaling pathway activation mediated by EGFR activation may play a role in diabetic nephropathy.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO280

The Interaction Between DPP-4 and Integrin β1 Regulates the Signaling that Is Responsible for the Induction of Endothelial to Mesenchymal Transition in Diabetic Kdiney Keizo Kanasaki, Shi Sen, Megumi Kanasaki, Swayam Prakash Srivastava, Daisuke Koya. *Dept of Diabetology and Endocrinology, Kanazawa Medical Univ, Kahoku, Ishikawa, Japan.*

Background: Endothelial-to-mesenchymal transition (EndMT) is an important source of matrix-producing mesenchymal cells. Dipeptidyl peptidase-4 (DPP-4) plays a crucial role in transforming growth factor (TGF)-β2-induced EndMT. Vascular endothelial growth factor (VEGF)-A signaling antagonizes EndMT through VEGF-receptor (R)2, but VEGF-R1 has been shown to promote EndMT via reducing the bioavailability of VEGF-A.

Methods: In vivo, streptozotocin-induced diabetes in CD-1 mice were used. The functional interaction between DPP-4 and integrin β1 was analysed double labeled immunofluorescence in vivo, western blot, and siRNA knockdown in endothelial cells. The proximity of two proteins was analysed by Duolink in situ kit.

Results: Here, we found that the interaction between DPP-4 and integrin β1 plays a crucial role in regulating the intracellular signaling responsible for EndMT. In STZ-induced diabetic kidneys of CD1 mice, the endothelial DPP-4, integrin β1 and phospho-integrin β1 levels were increased compared to control kidneys; linagliptin suppressed the elevation

of these proteins in diabetic kidneys. In cultured endothelial cells, TGF-β2 increased/linagliptin suppressed proximity of DPP-4 and integrin β1. DPP-4 suppression by siRNA was associated with suppressed levels of integrin β1, and vice versa. Knockdown of either integrin β1 or DPP-4 inhibited TGF-β2-induced TGF-β receptors heterodimer formation, smad3 phosphorylation, and EndMT. When we analyzed the VEGF-A-mediated signal transduction in endothelial cells, we found that DPP-4 negatively regulates endothelial viability signaling via VEGF-R2 suppression and VEGF-R1 induction.

Conclusions: These data suggest that the interaction between DPP-4 and integrin β1 regulates key endothelial cell signal transduction in both physiological and pathological conditions, and abnormal over activation of these proteins would induce EndMT. Therefore, inhibiting DPP-4 is a reasonable therapeutic target for the vascular complications associated with EndMT, such as diabetic nephropathy.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO281

Renal Complement in Ischemia and Diabetes Katherine J. Kelly,[1] Jesus H. Dominguez.[1,2] *[1]Medicine/Nephrology, Indiana Univ School of Medicine, Indianapolis, IN; [2]Medicine/Nephrology, Roudebush VA Medical Center, Indianapolis, IN.*

Background: ZS obese-diabetic rats (D) develop diabetic nephropathy (DN) as early as 12 weeks of age. The DN is accelerated by a single episode of bilateral renal ischemia at 8 weeks of age (DI). Sham operated D rats (DS) develop a less aggressive form of DN when compared to DI. DI, and to a lesser extent DS, develop renal capillary loss, inflammation and fibrosis. We hypothesized that renal ischemia activates the renal complement system which causes chronic inflammation.

Methods: We studied primary renal tubular cells and normal SD rats subjected to 30 minutes of ischemia. Also, ZS obese/diabetic and lean (L) rats were operated at 10 weeks of age and terminated at 28 weeks of age (DI, n = 11; DS, n 7; LS; n = 7). Whole renal transcriptomes were analyzed by RNAseq.

Results: First, we tested if ischemia activated renal complement directly. As little as 8 hours of ischemia in a hypoxic chamber activated renal C5 (3.3 fold, $p< 0.05$) and C3 (2.9 fold $p< 0.05$) expression in renal tubular cells. Second, in SD rats, renal C3 and C5 were upregulated after 30 minutes of bilateral ischemia and 24 hours of reperfusion. Third, DN was accompanied by activation of the renal complement system, manifested by 5.2, 8.8, 18.1, and up to 81.2 fold increases, over lean normal controls of C3, C6, C8 and C9 mRNAs. Moreover, mRNA for the receptor for C3a is increased 2.4 fold, and mRNA for the complement receptor 2 is increased 42 fold ($p < 0.05$). Furthermore, critical regulators of the complement system were significantly attenuated in DN. These include mRNAs encoding CD55, decay accelerating factor, down 65 % and CD59, which inhibits the membrane attack complex, also down by 65 %. We found C3, C4 and C9 proteins in renal tubules and glomeruli.

Conclusions: Activation of the intrinsic renal complement system participates in renal inflammation in renal ischemia and in DN, a condition characterized in part by ischemia. The self-sustained and chronic complement activation is likely to participate in diabetic and ischemic nephropathies.

Funding: NIDDK Support, Other U.S. Government Support, Veterans Affairs Support, Private Foundation Support

SA-PO282

Increased B-Oxidation and Fatty Acid Flux Characterizes Diabetic Nephropathy Kelli M. Sas, Pradeep Kayampilly, Viji Nair, Hongyu Zhang, Jaeman Byun, Matthias Kretzler, Frank C. Brosius, Subramaniam Pennathur. *Internal Medicine, Univ of Michigan, Ann Arbor, MI.*

Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in the United States. While it is well-recognized that diabetes leads to altered fatty acid metabolism, the utilization and flux of fatty acids *in vivo* has not been systematically studied.

Methods: To obtain a global systems view of fatty acid metabolism we utilized gene expression studies in parallel with targeted metabolomics approaches. We developed a metabolomics platform for simultaneous quantitative analysis of glycolytic, tricarboxylic acid (TCA) and fatty acid oxidation intermediates by liquid chromatography tandem mass spectrometry (LC/MS/MS). Animals which exhibit characteristic pathological features of DN (BKS db/db) were used as a model system to examine the dynamic changes in fatty acid utilization at 12-weeks (early DN) and 24-weeks (established DN).

Results: Transcriptomics analysis identified significant upregulation of pathways involved in fatty acid metabolism and B-oxidation. Consistent with the murine transcriptomics data, steady state levels of acylcarnitines were statistically significantly elevated in the mouse diabetic renal cortex (1.5-5-fold) and mitochondria (2.9-6.7-fold). *In vivo* LC/MS metabolic flux analysis (MFA) was performed to study the dynamic changes in fatty acid metabolism following administration of 0.5 g/kg U-$^{13}C_{16}$ palmitate by gavage. MFA revealed statistically significant increases in cortical palmitate uptake and an elevation of labeled long-chain acylcarnitines and TCA cycle intermediates in the diabetic mouse cortex, consistent with increased fatty acid flux. Acetylation of enoyl-CoA-hydratase/3-hydroxyacyl-CoA dehydrogenase (EHHADH) in the diabetic renal cortex was increased, showing activation of a primary enzyme in B-oxidation. Diabetic renal cortical mitochondria exhibited reduced ATP production, impaired complex 2 activity and increased proton leak indicating diminished activity.

Conclusions: Taken together, these results highlight a previously unrecognized role for altered fatty acid metabolism and flux in the pathogenesis of DN and raise the possibility of a novel therapeutic target of DN.

Funding: NIDDK Support, Other NIH Support - National Center for Advancing Translational Sciences

SA-PO283

Insulin Signaling in Exfoliated Kidney Tubule Cells Mollie E. Jacobs,[1] Alan C. Pao.[1,2] *[1]Medicine, Stanford Univ, Stanford, CA; [2]Veterans Affairs Palo Alto Health Care System, Palo Alto, CA.*

Background: One significant barrier to defining insulin resistance in the kidney is obtaining sufficient kidney tissue for metabolic and molecular characterization. Current methods for detecting activation of insulin signaling proteins in clinical specimens require large quantities of tissue, which must be procured by invasive needle biopsies.

Methods: We have developed a protocol to overcome this technical barrier by using the following approaches: 1) isolation and expansion of exfoliated renal tubular epithelial cells from human urine in culture; and 2) detection of insulin signaling phospho-proteins through application of nano-immunoassay (NIA) technology. NIA technology, which utilizes isoelectric focusing of proteins followed by antibody detection, has the capability to detect changes in the phosphorylation profile of low abundance proteins in nanoliter volumes.

Results: Using gene expression studies, we found that exfoliated cells in human urine contained a mixture of renal proximal tubule and collecting duct cells, as well as fibroblasts. Preliminary NIA assays examining key components of the insulin signaling pathway demonstrated that the exfoliated cells in human urine robustly express activated/phosphorylated components of the insulin signaling network (PI3K subunits, Akt1-3, and Erk1/2).

Conclusions: The ability to isolate viable exfoliated tubule cells from urine samples overcomes a significant technical barrier to defining insulin resistance in the kidney. We next propose to identify defects in insulin signaling pathways in insulin resistant individuals who have hypertension or uric acid kidney stones. This information could be used to diagnose insulin resistance in the kidney and suggest novel strategies for the prevention and treatment of obesity-related hypertension or kidney stones.

Funding: Other NIH Support - NIH Post-Doctoral Fellowship 5T32DK076541-04 (Mollie Jacobs)

SA-PO284

Atrasentan, a Selective Endothelin A Receptor Antagonist, Treatment of Diabetic db-db Mice Prevents the Progression of Diabetic Nephropathy Xiaoxin Wang, Yuhuan Luo, Cherelle Parker, Moshe Levi. *Univ of Colorado Denver.*

Background: Diabetes is the leading cause of renal disease and new treatment modalities are needed to slow the burden and progression of diabetic nephropathy.

Methods: The purpose of the present study was to determine if treatment of hyperglycemic and proteinuric db-db mice with Atrasentan (A) can prevent the progression of diabetic nephropathy.

Results: Treatment resulted in significant decreases in urinary albumin (337±54 mg/g in db-db versus 189±23 mg/g in db-db+A, $p<0.05$), mesangial index (4±0.5 in db-db versus 2.4±0.2 in db-db+A, $p<0.05$), prevented decrease in synaptopodin immunofluorescence (podocyte marker, 3.8±0.4 in db-db versus 4.4±0.3 in db-db+A, $p<0.05$) and fibrillary collagen accumulation as determined by two photon excitation-second harmonic generation (TPE-SHG) microscopy. These beneficial effects of Atrasentan were associated with significant decrease in the expression of the profibrotic growth factor TGF-β (1.59±0.19 in db-db versus 1.08±0.03 in db-db+A, $p<0.05$) as well as CTGF and PAI-1. In addition Atrasentan induced increased expression of Nrf-2 (1.55±0.06 in db-db versus 1.88±0.04 in db-db+A, $p<0.05$) and HO-1 (2.62±0.17 in db-db versus 5.16±1.09 in db-db+A, $p<0.05$). Furthermore Atrasentan also prevented increased expression of the lipogenic enzymes SCD-1 (4.76±0.18 in db-db versus 3.05±0.16 in db-db+A, $p<0.05$) and LPK (52.0±4.73 in db-db versus 24.2±6.19 in db-db+A, $p<0.001$).

Conclusions: In summary treatment of diabetic db-db mice with the selective endothelin A receptor antagonist Atrasentan prevents the progression of diabetic nephropathy at least in part by inhibiting the increased expression of profibrotic growth factors, enhancing the Nrf2 antioxidant stress response, and inhibiting increased expression of fatty acid synthesis pathways.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO285

Human Mesenchymal Stem Cells Promote Alternative Activation of Monocytes Isolated from Healthy and Type 2 Diabetic Subjects Andrea F. Wise,[1] Timothy M. Williams,[1] Stephen Rudd,[2] Christine A. Wells,[3,4] Peter G. Kerr,[5] Sharon D. Ricardo.[1] *[1]Anatomy and Developmental Biology, Monash Univ, Melbourne, VIC, Australia; [2]QFAB Bioinformatics, Univ of Queensland, Brisbane, QLD, Australia; [3]The Australian Inst of Bioengineering and Nanotechnology, Univ of Queensland, Brisbane, QLD, Australia; [4]Inst of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, Univ of Glasgow, Scotland, United Kingdom; [5]Dept of Medicine, Monash Univ, Melbourne, VIC, Australia.*

Background: Pro-inflammatory monocytes and macrophages are the main immune cells that infiltrate the damaged kidney in type 2 diabetes, where they contribute to disease progression. Mesenchymal stem cells (MSCs) possess unique immunomodulatory properties, however, their effect on monocytes is unclear. This study investigated whether MSCs could modulate the phenotype of monocytes isolated from control subjects and type 2 diabetic patients with end-stage renal disease (ESRD).

Methods: Monocytes from control subjects (n=4) and type 2 diabetic patients with ESRD (n=5) were compared by flow cytometry for the expression of CD14, CD16 and

HLA-DR. Microarray gene expression profiling was used to analyze the gene expression profile of the monocytes following 48 hours of co-culture with MSCs using an *in vitro* transwell system.

Results: Control subjects had a greater proportion of $CD14^{++}CD16^{-}$ classical monocytes compared to diabetic patients (73.9%versus56.3%; $P<0.05$). In contrast, the diabetic patients had a higher proportion of intermediate ($CD14^{++}CD16^{+}$; 32.2%versus19.2%; $P<0.05$) and non-classical ($CD14^{+}CD16^{++}$; 7.5%versus2.8%; $P<0.05$) monocytes compared to control subjects. Following co-culture with MSCs, CD14 and CD16 expression was significantly up-regulated on diabetic blood monocytes, while HLA-DR expression was down-regulated. Principal component analysis revealed that MSC-treated monocytes clustered separately from the untreated monocytes. Genomic profiling revealed MSCs up-regulated several 'M2' macrophage associated genes such as *IL10*, *IGF1*, *MRC1*, *CD163* and *MMP9*.

Conclusions: MSC-derived factors can alter the phenotype of human monocytes isolated from healthy and diabetic subjects towards an M2 phenotype.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO286

Potential Impact of IV versus Oral Route of Iron Administration on Pancreatic Beta Cell Viability and Function in Rats with CKD

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Background: Type-2 diabetes is a common cause of ESRD and residual pancreatic b-cell s is critical for wellbeing of these patients. ESRD patients routinely receive IV iron for anemia treatment. Via divalent metal transporters beta cells which are exquisitely vulnerable to oxidative stress take up non-transferrin-bound iron. By catalyzing Fenton reaction, labile iron within the b-cell triggers oxidative stress and cell damage. We compared the effect of IV iron which bypasses body's natural safeguards for iron transport with oral iron in CKD rats and examined the effect of an IV iron product on isolated pancreatic islets in vitro.

Methods: CKD (5/6 Nx) rats were treated with either IV ferric sucrose (10 mg/kg/week) or an iron sulfate-fortified diet (400 ppm) for 2 weeks. CKD rats fed regular diet served as controls. Intra-peritoneal glucose tolerance test (IPGTT) was performed and pancreatic tissues were harvested and assessed for islet yield, islet viability, cellular composition, apoptosis, and 8'OH-guanosine (marker of DNA oxidation) content by FACS and IHC. Also normal rats' islets were studied after incubation in media containing pharmacologically-relevant concentrations of ferric sucrose or vehicle for 24 hr.

Results: Compared to CKD rats fed regular diet IV iron-treated CKD rats showed significant impairment of IPGTT. The islet isolation yield, islet viability, and b/acell ratio were lower and DNA oxidation and cell necrosis were higher in islets from IV- than oral-iron treated CKD rats. Incubation of islets from normal animals in media containing pharmacologically-relevant concentrations of iron sucrose resulted in significant concentration-dependent rise in DNA oxidation and islet cell necrosis, and a significant fall in cell viability and b/a cell ratio.

Conclusions: Use of clinically relevant doses of IV iron products adversely affected beta cell viability and function in CKD animals. Future studies are needed to examine the impact of these compounds on pancreatic endocrine function in ESRD patients.

SA-PO287

Hyperglycemia Induces Downregulation of Podocyte-Associated Proteins in the Albuminuric $GIPR^{dn}$ Mouse Model of Diabetic Nephropathy and in Cultured Glomerular Epithelial Cells

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Background: Proteinuria, as a consequence of podocyte damage, is a common clinical feature of diabetic nephropathy (DN). The exact pathogenesis of glomerular damage, however, remains unclear. We hypothesized that downregulation of key podocyte proteins is induced directly by high levels of glucose and triggers albuminuria in a murine model of DN.

Methods: Kidney samples and glomerular preparations of 21-week old transgenic $GIPR^{dn}$ mice (n=6) and wild-type littermates (n=7) were analyzed by immunohistochemistry, immunofluorescence and real-time qPCR. As a proof of concept, differentiated cultured podocytes were supplemented with low and high glucose and mannitol as osmotic control. After 48 hours mRNA expression was assessed by real-time qPCR.

Results: In the $GIPR^{dn}$ model of early-onset diabetes, transgenic mice displayed decreased number of podocytes (podocyte number: 12.8±1.6 versus 10.8±1.0; $p<0.05$) and pronounced albuminuria with markedly elevated urinary albumin/creatinine-ratio (ACR; 5.7±1.4 mg/ml versus 0.04±0.01 mg/ml, $p<0.01$). Semiquantitative glomerulosclerosis index was substantially higher in $GIPR^{dn}$ animals (GSI; 1.13±0.3 versus 0.29±0.1, $p<0.001$) and correlated significantly with ACR ($r=0.791$, $p=0.001$). mRNA levels of podocyte-associated proteins were significantly downregulated up to 60% in diabetic animals and also correlated with ACR: Synaptopodin ($r=0.670$, $p=0.012$), Podoplanin ($r=0.648$, $p=0.017$), WT-1 ($r=0.588$, $p=0.035$), Nephrin ($r=0.533$, $p=0.061$). Similarly, specific staining revealed reduction of these proteins. Stimulation of cultured podocytes with high glucose confirmed hyperglycemia induced downregulation of Synaptopodin, Podoplanin, WT-1 and Nephrin.

Conclusions: Our data suggest a direct action of high glucose condition on downregulation of proteins essential for podocyte structure and slit-diaphragm integrity. Decrease of these proteins is paralleled by a loss of podocytes and subsequent albuminuria *in vivo*.

SA-PO288

Wnt5a/SFRP5 Axis in Macrophages: Potential Role in Diabetic Nephropathy

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Background: Macrophage recruitment correlates strongly with the progression of renal dysfunction in diabetic nephropathy (DN). Wnt5a, a non-canonical Wnt protein, is expressed in macrophages and has recently been implicated in chronic inflammation. Secreted frizzled related protein 5 (SFRP5) suppresses Wnt5a signaling and is a regulator of Wnt5a resulting in reduction of chronic inflammation. We begun to test the hypothesis that Wnt5/SFRP5 axes is involved in the development of DN, and related to macrophage infiltration and polarization.

Methods: To test our hypothesis, we used db/db mice, a model of type 2 diabetes mellitus, with functional and morphologic DN, and control heterozygotes (n=6/group). At 4 months of age, when the mice are severely hyperglycemic and have mild DN, whole kidneys were harvested and macrophages were isolated by immunoseparation. Gene expression of Wnt5a and SFRP5 was analyzed by real-time PCR.

Results: Our results showed no significant difference in Wnt5a expression in control and db/db mouse kidneys, however SFRP5 expression was elevated in db/db mouse kidneys compared to controls ($p<0.05$). Wnt5a expression was elevated in the kidney macrophages of db/db mice. We also examined the macrophage phenotype by measuring the expression of macrophage inhibitory cytokine-1 (MIC-1), a marker of the M2 macrophage phenotype, and arginase II, a marker of the pro-inflammatory M1 phenotype. We found increased MIC-1 and decreased arginase II expression levels in renal macrophages of db/db mice compared to controls, indicating a predominant M2 macrophage population in db/db mouse kidneys. Wnt5a secretion/protein expression is mainly associated with the M2 phenotype in the atherosclerotic lesions. Renal M2-like macrophages have critical roles in tissue repair and fibrosis.

Conclusions: Our findings suggest that Wnt5a and SFPR5 may play a role in the pathological tissue repair during development of DN in db/db mice. Our next step is to investigate the detailed mechanisms of Wnt5a/ SFRP5 axis in macrophages related to the progression of DN.

Funding: NIDDK Support

SA-PO289

Gonadectomy Prevents Mesangial Expansion and Podocyte Loss in Angiotensin Converting Enzyme 2 Knockout Diabetic Male Mice

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Background: Whereas ACE2 deletion worsens kidney injury, its amplification ameliorates diabetic nephropathy.The effect of gonadectomy(Gdx) in diabetic ACE2KO male mice has not been previously studied.

Methods: We studied the effect of ACE2 deletion and Gdx on systolic blood pressure(SBP), glomerular filtration rate(GFR), glomerular tuft area(GTA), mesangial index(MI), podocyte number(%POD), glomerular cellularity, and serum(s) and kidney(k) ACE expression in c57bl/6 streptozotocin(STZ)-induced male mice and their controls. Mice were followed-up for 19 weeks after induction of diabetes with STZ injection. Study groups:Wild-type(WT)-CONT,ACE2KO-CONT,ACE2KO-CONT+Gdx,WT-DB,ACE2KO-DB,ACE2KO-DB+Gdx.

Results: ACE2KO-DB mice had increased SBP, GFR, and MI as well as decreased %POD compared to diabetic WT. ACE2KO-DB + Gdx group showed lower values of these measured parameters excepted for augmented %POD.sACE was increased in WT and ACE2KO DB mice.Gdx decreased sACE in both CONT and DB ACEKO mice.kACE was reduced by diabetes in terms of enzymatic activity, protein and mRNA. kACE was also decreased in ACE2KO-CONT mice as compared to WT-CONT.kACE activity positively correlated with protein($r=0.7$;$p<0.001$) and gene expression($r=0.481$, $p=0.005$).

*$P<0.05$ vs CONT; †$P<0.05$ vs WT; §$P<0.05$ vs non-Gdx	WT-CONT (n=12)	ACE2KO-CONT (n=12)	ACE2KO-CONT + Gdx (n=9)	WT-DB (n=10)	ACE2KO-DB (n=10)	ACE2KO-DB + Gdx (n=10)
Blood glucose,mg/dL	204.7±4.7	194.0±6.7	208.5±10.0	538.3±25.3*	545.6±22.2*	242.1±10.1*†§
SBP,mmHg	96.4±1.3	96.4±1.7	100.7±1.4	97.5±1.7	104.4±1.8*†	95.4±0.5*§
GFR(μL/(min·gBW))	18.4±2.2	25.3±3.1	22.5±1.8	28.9±2.8*	34.9±5.2*†	22.9±4.6§
GTA(μm²)	3161.2±88.7	3283.5±138.7	3377.7±161.2	3744.4±168.9*	3638.7±102.5*	3173.1±80.0†§
MI	0.38±0.02	0.46±0.02†	0.40±0.03	0.44±0.01*	0.52±0.02†	0.44±0.03§
WT-1 positive cells (%)	38.6±1.6	35.5±0.6	37.3±1.0	32.4±1.1*	28.7±1.0*	33.0±1.2*§
Glomerular cellularity (cells/glom)	26.2±0.7	26.4±1.3	28.5±0.7	25.1±0.8	28.2±1.0†	24.2±1.7*§
sACEactiv (RFU/(μL·min))	2143.9±133.4	2699.7±174.5†	1891.6±107.6§	2773.7±121.5*	3115.7±187.5*	2222.9±150.0†§
kACEactiv (RFU/(μg·min))	213.7±18.8	148.1±13.1†	140.6±35.8†	92.2±19.4*	73.7±11.4*	70.8±13.8*
kACEprotein	1.10±0.18	0.64±0.14†	0.57±0.18†	0.43±0.07*	0.40±0.09	0.41±0.15
kACEgene	1.03±0.09	0.78±0.07†	0.54±0.06†§	0.53±0.10*	0.30±0.05*	0.54±0.08

Conclusions: Diabetic ACE2KO showed increased blood pressure and accentuated glomerular injury as compared to diabetic WT.These alterations were prevented by gonadectomy.Diabetes and *ace2* deletion supposed increased circulating ACE but decreased renal ACE expression.Thus, the alterations observed in this model of diabetes may be related to a modulation of ACE at serum and kidney level.This regulation may be mediated,at least in part,by AngII accumulation.

SA-PO290

ACE2 Amplification by Minicircle Gene Delivery in Diabetic Mice
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Background: Angiotensin-Converting Enzyme-2 (ACE2) enhances the degradation of Ang II. We used minicircle delivery for chronic ACE2 amplification *in vivo*.

Methods: The cDNA of soluble mouse ACE2 was cloned into a circular expression cassette and the resulting ACE2 minicircle (MC) was injected to *FVB* mice in which STZ diabetes was induced.

Results: At 3-7d after MC administration, serum ACE2 activity in mice that received 10ug ACE2MC (n=9) was over 100-fold higher than in controls (n=9) (138±48 versus 0.7±0.2 RFU/uL/hr) and with a larger dose (30ug) (n=8) increased even further (480±153 RFU/uL/hr). The marked increase in serum ACE2 activity was sustained for several months but did not result in a detectable increase in either kidney ACE2 activity or immunostaining. We reasoned that ACE2MC could be used to examine if chronic amplification of circulating ACE2 affects the development of glomerular lesions in diabetic mice. Control or ACE2MC(10-30ug)-treated mice were either injected with vehicle or STZ. Urinary ACR measured over the entire 20 wks after the injection was significantly increased in STZ (n=16) and in STZ-MCACE2 mice (n=14) as compared to vehicle controls(n=9). Glomerular mesangial score, cellularity, glomerular area and GFR were all similarly increased in STZ and STZ-ACE2MC mice.

Parameter	Veh	STZ	STZ-ACE2MC
Serum ACE2 act (RFU/ul/hr)	1.4±0.3	2.4±0.3*	497±135*#
ACR (ug/mg)	19±2	118±18*	91±14*
Mesangial Score	1.0±0	2.33±0.15*	2.15±0.15*
Glom. Cellularity	1.0±0	2.14±0.17*	2.05±0.11*
Glom. Area(um^2)	4173±67	5738±197*	5427±267*
GFR (uL/min/g)	3.6±0.7	10.8±1.3*	10.3±0.5*

*p<0.05 or p<0.01 vs. veh; #p<0.01 vs. STZ

Conclusions: Mini-circle delivery of ACE2 results in a dose-dependent and sustained long-term increase in serum but not kidney ACE2 activity. A profound augmentation of ACE2 activity confined to circulation is not sufficient to prevent glomerular pathology and hyperfiltration in diabetic mice. Strategies to achieve kidney over-expression of ACE2 are needed to examine its postulated beneficial effect in diabetic kidney disease.

Funding: NIDDK Support, Private Foundation Support

SA-PO291

Calcitriol and Its Analogue BXL-628 Attenuate High Glucose-Induced Epithelial-Mesenchymal Transition of HK-2 Cells By Suppressing RhoA/ROCK Signaling Pathway
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Background: Vitamin D has been shown to be renoprotective in diabetic nephropathy recently, of which the exact mechanism still remains much unknown. In order to investigate the protective role of vitamin D in high glucose-induced epithelial-mesenchymal transition (EMT) in human renal proximal tubular cell, we detected the effect of calcitriol and its analogue BXL-628 in high glucose induced activation of RhoA in HK-2 cells.

Methods: HK-2 cells were divided into five groups: Normal control group (glucose, 5.6 mmol/L); High glucose group (glucose, 30 mmol/L); Calcitriol group (high glucose + calcitriol, the active form of vitamin D); BXL-628 group (high glucose + BXL-628, a selective vitamin D receptor agonist); Positive control group (high glucose + Y-27632, a RhoA inhibitor). Active RhoA protein were assessed by Western Blot and immunofluorescence. The expression of α-SMA, E-Cadherin were determined by Western Blot analysis and qPCR. Collagen-I and Fibronectin were observed by ELISA and qPCR.

Results: Active RhoA protein were significantly increased by high glucose treatment for 2 h. Moreover, the expression of α-SMA, Collagen-I and Fibronectin were significantly up-regulated at 48 h treatment of high glucose. Compared with high glucose group, both calcitriol and BXL-628 treatments reduced the expression of active RhoA protein thus consequently decrease the level of α-SMA Coll1 and Fibronectin while increased E-cadherin expression at both protein and mRNA levels.

Conclusions: High glucose activates RhoA/Rock pathway in HK-2 cells, leading to EMT and up-regulation of ECM. Vitamin D receptor agonist calcitriol and its analogue BXL-628 attenuate high glucose-induced EMT and ECM accumulation in HK-2 cells by suppressing the activation of RhoA/Rock pathway.

Funding: Government Support - Non-U.S.

SA-PO292

Reduced Nuclear Translocation of DNMT3a Contributes to CTGF Hypo-Methylation in Human Mesangial Cells
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Background: Connective tissue growth factor (CTGF) plays an essential role in the pathogenesis and development of diabetic nephropathy. We have previously identified that high glucose induces the expression of CTGF by decreasing DNA methylation. The aim of the present study was to investigate the underlying mechanisms that contribute to the hypo-methylation of CTGF in diabetic nephropathy.

Methods: Human mesangial cells (HMCs) were treated with normal glucose (5mM), mannitol (30mM) or high glucose (30mM). Immnofluorescence staining was used to determine the cellular distribution, and real-time PCR or western blotting were performed to measure the mRNA or protein expression levels of CTGF, DNMT1, DNMT3a, and DNMT3b, respectively. ChIP assay was applied to investigate the capacity of DNMTs to bind the putative CpG island on CTGF promoter.

Results: High glucose induced both the mRNA and protein expression levels of CTGF in human mesangial cells. Although the protein expression of total DNMT1, DNMT3a, or DNMT3b was not altered by high glucose, both immunofluorescence staining and western blotting of nuclear/cytosol fractionation showed that the nuclear translocation of DNMT3a was significantly reduced in high glucose group compared to normal glucose or mannitol group. In addition, DNMT3a was bound to the putative CpG island of CTGF promoter and high glucose promoted this binding potential.

Conclusions: High glucose attenuates the nuclear translocation of DNMT3a, which is responsible for the methylation of CTGF, and possibly contributes to CTGF hypo-methylation in diabetic nephropathy.

SA-PO293

Hydrogen Sulfide (H2S) Inhibits High Glucose-Induced NOX4 Expression By Activating Nitric Oxide (NO)-AMPK Axis in Proximal Tubular Epithelial (MCT) Cells
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Background: Renal cells constitutively generate gasotransmitters, H2S and NO; their interaction is not understood. High glucose increases NOX4 expression, ROS generation and matrix synthesis by inhibiting AMPK in renal cells. Since H2S inhibits high glucose-induced matrix increment by activating AMPK, we examined if H2S inhibits high glucose–induced NOX4 and matrix protein expression and if H2S-NO pathways are integrated.

Methods: MCT culture, immunoblotting, confocal microscopy.

Results: High glucose (30 mM) increased NOX4 expression at 1 hr and 24 hrs. that was inhibited by H2S at both time points. H2S inhibited high glucose-induced ROS generation and matrix protein laminin γ1 expression at 24 hrs. High glucose decreased activating AMPK phosphorylation on Thr172 that was restored to baseline by H2S. Compound C, an AMPK inhibitor, prevented H2S inhibition of high glucose-induced NOX4 expression at both 1 hr and 24 hrs. H2S inhibition of high glucose-induced NOX4 expression was abrogated by L-NAME, an inhibitor of NO synthase (NOS), at 24 hrs, but not at 1 hr. L-NAME tended to abolish the inhibitory effect of H2S on high glucose-induced laminin γ1 expression. H2S increased the expression of iNOS at 4 hrs; but had no effect on eNOS content. ODQ, a selective inhibitor of soluble guanylyl cyclase, did not affect H2S inhibition of high glucose-induced NOX4 expression at 24 hr.

Conclusions: H2S inhibits high glucose-induced NOX4 expression at both 1 hr and 24 hrs. by activating AMPK in MCT cells. This effect depends on NO generation at the late time point. Interestingly, iNOS, but not eNOS, appears to be involved in the ameliorative effect of H2S on high glucose-induced NOX4 expression; however, guanylyl cyclase seems not to be involved. Thus, H2S recruits NO to inhibit high glucose-induced NOX4 expression which plays a role in matrix protein accumulation; this is an example of integration of actions of two gasotransmitters in the renal cells.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO294

Ursolic Acid Attenuates HG-Induced Podocyte and Mesangial Injury By Inhibiting PI3K/Akt/mTOR and Wnt/β-Catenin Pathways
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Background: Autophagy and podocyte epithelial-mesenchymal transition (EMT) implicated with HG-induced renal injury. Ursolic acid (UA) has been identified to inhibit early lesions of diabetic nephropathy. We investigate the effects of Ursolic acid on autophagy, EMT, PI3K/AKT/mTOR and Wnt/β-catenin pathways in podocyteand mesangial cellscultured by highglucose (HG).

Methods: Podocyte andglomerularmesangial cells were cultured in normalglucose, HG and HG with Ursolic acid. The cell proliferation and intracellular ROS were detected by MTT and DCF-DA respectively. Cell cycle and apoptosis were investigated by flow cytometry.The PI3K/Akt signaling signatures,apoptosis, autophagy, EMT and fibrosis associated proteins were detected by immunofluorescence, real-time RT-PCR, western blotting andelectron microscope.

Results: Ursolic acid inhibited HG-induced mesangial cell proliferation and decreased ROS generation and TGF-β, fibronectin expression. Ursolic acid induced mesangial cell apoptosis by increased Bcl-xl, Bax and Survivin expression. The expression of podocin, ZO-1 was down-regulated and the expression of α-SMA was up-regulated in podocyte cultured by high glucose and inhibited by ursolic acid. The cells exposed to HG for 48h showed up-regulated pAkt, pmTOR, pβ-catenin and down-regulated Wnt5a, GSK3β, LC3BII expression. Ursolic acid down-regulatedpAkt, pmTOR andpβ-catenin expression and up-regulated Wnt5a, GSK3β, LC3BII expression in mesangial cell and podocyte cultured by HG. Mass abnormal mitochondrion and decreasedautophagosomeswere observed by electron microscopy in cells cultured by HG for 48h and Ursolic acid decreasedautophagosomes expression.

Conclusions: Ursolic acid can regulate autophagy and EMT and ameliorate high glucose induced podocyte and mesangial cell injury by inhibiting PI3K/AKT/mTOR and Wnt/β-catenin pathways.

SA-PO295

Paricalcitol Has the Renoprotective Effect Related to Catalase Pathway in Kidney Injury Young Sun Kang,[1] Sung Jin Kim,[1] Jung Eun Kim,[1] Jungyeon Ghee,[1] Hye Kyung Song,[1] Hye Sook Min,[1] Mi Jin Lee,[1] Jin Joo Cha,[1] Dae R. Cha,[1] Ji Eun Lee,[2] Hyunwook Kim,[2] Mihwa Lee,[1] Jee-Young Han.[3] *[1]Nephrology, Korea Univ, Ansan, Korea; [2]Nephrology, Wonkwang Univ, Gunpo, Korea; [3]Pathology, Inha Univ, Incheon, Korea.*

Background: Paricalcitol (Pari), vitamin D receptor agonist, is known to have an antioxidative effect on tissue injury, where catalase activity is involved. However, there is no data for the role of Pari on catalase pathway of kidney. The aim of this study is to investigate it in catalase knock-out (CKO) diabetic and non-diabetic mice.

Methods: 8-week-old CKO C57/BL6 and wild-type (WT) mice were treated by i.p. streptozotocin (STZ) (50mg/kg/day) injection for 7days and followed by i.p. Pari injection for two months into 7 groups; I) WT 2) CKO 3) CKO+Pari 4) WT+STZ 5) WT+STZ+Pari 6) CKO+STZ 7) CKO+STZ+Pari.

Results: CKO mice showed lower body weight and more urinary volume than WT, without any changes by Pari treatment. Blood pressure, heart rate, and organ weights were similar among groups. However, FBG level, HbA1c, glucose tolerance test, and insulin tolerance test were of significantly improved by Pari treatment in CKO mice. In addition, urinary albumin excretion was increased in CKO and reduced significantly by Pari treatment. As expected, CKO mice presented lower catalase activity in kidney. However, the catalase deficiency was recovered by Pari treatment, interestingly, only in diabetic mice, not in non-diabetic mice. Consistently, expressions of MCP-1, IL-10, TGFb and type IV collagen were inhibited by Pari treatment in diabetic CKO mice using RT-PCR, western blot analysis, and immunohistochemistry. The lipid contents and liperoxidase of kidney were not changed by Pari, although they were increased more in CKO than WT mice. In addition, urinary 8-isoprostane level did not show any difference among groups. On the other hand, Pari suppressed the PI3K/Akt pathway and urinary nephrin excretion.

Conclusions: These results suggest that the catalase deficiency might be recovered by Pari treatment in kidney injury. However, that renoprotective effect of Pari may occur through the nephrin/PI3K/Akt pathway, independent of the antioxidative effect of catalase.

SA-PO296

Calcitriol Prevents Podocyte Injury via Regulation of Macrophage M1/M2 Phenotype in STZ-Induced DN Rats Yinfeng Guo, Zhixia Song, Min Zhou, Xiaoliang Zhang. *Nephrology, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Increasing evidences suggest that the heterogeneity of macrophage phenotype and function ultimately determines the outcome of diabetic nephropathy (DN). Therefore, finding method of regulating macrophage phenotype and function is a strategic point of preventing podocyte impairment in DN. In this study, we investigated the effect of calcitriol on macrophage M1/M2 phenotype and its role in protecting podocyte impairment in DN rats.

Methods: DN model rats were established by intraperitoneal injection with streptozocin (STZ). The rats were subsequently receiving either calcitriol (0.1μg/kg/d) or vehicle by gavage. Rats were sacrificed at three time points (8, 14 and 18w) for histological and molecular analysis. In addition, we performed *in virto* study using U937 cells cultured with either high glucose or high glucose followed by 1,25-dihydroxyvitamin D3 medium to assess macrophage phenotype.

Results: Calcitriol decreased proteinuria and attenuated podocyte damage with up-regulating expressions of nephrin and podocin. DN rats showed increased $CD68^+$ macrophages infiltration in glomeruli and interstitium with enhanced protein expressions of M1 markers, including iNOS and TNF-α. Calcitriol significantly decreased the infiltrating macrophages and suppressed M1 macrophage activation. Interestingly, calcitriol improved M2 macrophage activation with elevated protein expressions of M2 markers, including CD163, Arg-1 and MR at 18w but not 8w or 14w. The ratio of CD163/CD68 considered as the proportion of M2 macrophage was about 2.9 fold highly after calcitriol treatment. Moreover, the protein expression of M1 marker iNOS was negatively correlated with expression of either nephrin or podocin, while M2 marker CD163 was positively correlated. *In vitro*, 1,25-dihydroxyvitamin D3 also switched high glucose-induced M1 macrophage toward an M2 phenotype.

Conclusions: Calcitriol regulates macrophage phenotype, via inhibiting M1 macrophage activation as well as enhancing M2 macrophage activation to prevent podocyte impairment in STZ-induced DN rats.

Funding: Government Support - Non-U.S.

SA-PO297

1,25-Dihydroxyvitamin D3 Upregulates the PI3K/p-Akt Signal Pathway via Inhabition of TRPC6 Expression to Ameliorate High Glucose Induced Podocyte Injury *In Vivo and In Vitro* Zhixia Song, Yinfeng Guo, Min Zhou, Xiaoliang Zhang. *Nephrology, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Podocytes injury plays a critical role in the development and progression of diabetic nephropathy(DN).Growing evidenceshave demonstrated PI3K/p-Akt signal pathway in the protection of podocytes. Besides, TRPC6 on the podocytes has been revealed to cause podocyte injury. Recent investigations demonstrated that vitamin D3 reduced albuminuria and prevented podocyte injury. The aim of this study is to investigate whether 1,25-dihydroxyvitamin D3 (VD) upregulates the PI3K/p-Akt signal pathway via inhabition of TRPC6 expression to ameliorate injury of podocyte *in vivo and in vitro* high glucose induced podocyte injury.

Methods: DN model rats were established by intraperitoneal injections of streptozocin. The rats were subsequently receiving either calcitriol (0.1μg/kg/d) or vehicle by gavage and then sacrificed after 18 weeks treatment. In addition, we performed in virto study using MPC5 cells (conditionally immortalized mouse podocyte clonal cells) cultured with either high glucose or high glucose followed by VDmedium orhigh glucose followed by VD and SKF-96365(a generic blocker of TRPC6 channels) medium.

Results: DN rats exhibited increased proteinuria accompanied by elevated TRPC6 expression. Treatment with calcitriol not only reduced proteinuria, but also normalized TRPC6 expression. Meanwhile, the expression of podocyte specific markers, including nephrin and podocin, together with PI3K/p-Akt were significantly decreased in DN rats, whereas calcitriol reversed these above changes. *In vitro*, podocyte injury induced by high glucose exposure in cultured MPC5 increased TRPC6 expression and decreased PI3K/p-Akt expression. Expectedly, VD reversed the increased TRPC6 as well as decreased PI3K/p-Akt expression in a does dependent manner, which were abolished by SKF-96365, a blocker of TRPC6.

Conclusions: 1,25-dihydroxyvitamin D3 upregulates the PI3K/p-Akt signaling pathway via inhabition of TRPC6 expression to ameliorate *in vivo and in vitro* high glucose induced podocyte injury.

Funding: Government Support - Non-U.S.

SA-PO298

The Circulating Inactive Form of Matrix Gla Protein (MGP) Is Associated with Diabetic Nephropathy but Not with Artery Intima-Media Thickness (cIMT) Stefanos K. Roumeliotis, Athanasios K. Roumeliotis, Anna Tavridou, Marios Theodoridis, Stelios A. Panagoutsos, Elias Dimitrios Thodis, Ploumis Stavros Passadakis. *Medical School, Democritus Univ of Thrace, Alexandroupolis, Greece.*

Background: Vitamin K-dependent MGP acts as a calcification inhibitor *in vitro* and *in vivo*and needs modification by γ-carboxylation to become biologically active. Vitamin K deficiency leads to accumulation of high levels of inactive uncarboxylated MGP (dpucMGP) in calcified vessels. The vitamin K oxidoreductase (VKOR) reduces vitamin K to support the γ-carboxylation and consequent activation of MGP. Single nucleotide polymorphism (SNP) *VKORC1 -1639 G>A*results in lower activity of VKOR complex subunit 1 in subjects with the AA genotype and probably higher levels of dpucMGP. We sought to determine the association between dpucMGP levels in the five progressive stages of diabetic nephropathy with *VKORC1 -1639 G>A* polymorphism and cIMT.

Methods: Measurements of cIMT were performed in 124 patients (mean age 68.1±8.6 years) in different stages of diabetic nephropathy, using a high resolution, real-time B-mode ultrasonograph. *VKORC1 -1639 G>A* polymorphism was determined using PCR-RFLP. Plasma dpucMGP levels were measured in a subgroup of 71 patients using ELISA.

Results: Plasma dpucMGP levels increased significantly ($p<0.001$) with disease severity (stage 1: 345±211 pM, stage 2: 513±488 pM, stage 3 and 4: 958±532 pM, stage 5: 1175±428 pM) but were not associated with cIMT. Moreover, no significant difference in dpucMGP levels was observed between *VKORC1* GG/GA and AA genotypes. Multiple linear regression analysis revealed gender (b=-0.139, p=0.001), BMI (b=0.009, p=0.032), and stage of diabetic nephropathy (b=0.063, p=0.001) as independent predictors of cIMT.

Conclusions: Plasma dpucMGP levels were significantly elevated as the stage of diabetic nephropathy progressed but not associated with *VKORC1* genotype and cIMT. Male gender, increased BMI and advanced stage of diabetic nephropathy are independent predictors of cIMT in diabetic nephropathy.

SA-PO299

Contrast-Induced Nephropathy in Hyperglycemic Kidney Cells and Elderly Diabetic Mice Altaf-M. Khan,[1] Federico Teran,[1] Kristina Angela Rathmell,[1] Madlin Alzoubi,[1] Kathleen S. Hering-Smith,[1] Eric E. Simon,[1,2] Vecihi Batuman.[1,2] *[1]Medicine, Section of Nephrology and Hypertension, Tulane Univ School of Medicine; [2]Veterans Affairs, SLVHCS, New Orleans, LA.*

Background: We studied the toxicity of contrast media (CM) on human kidney epithelial (HK-2) cells in high-glucose medium and in aged db/db (diabetic obese) mice to evaluate the effect of diabetes on the vulnerability to contrast-induced nephropathy (CIN).

Methods: HK-2 cells were exposed to nonionic (iohexol or iodixanol) CM, 25 to 100 mg iodine/ml, for 6–24 h. HK-2 cells were studied in high-glucose medium (25 mM glucose, or mannitol as osmolal control) for 6–24 h. For studies *in vivo*, 24-wk-old diabetic db/db and non-diabetic control mice (n = 5 in each group) were dehydrated for 24 h and nonionic low osmolal (iohexol) or isosmolal (iodixanol) CM at a dose of 3 g of iodine/kg bw was injected via a jugular vein catheter. All mice were sacrificed at 24 h after CM injection.

Results: Both CM caused significant cytotoxicity at 50 mg iodine/ml in HK-2 cells. Cells treated with glucose or mannitol combined with iodixanol showed a significant ($p < 0.01$) decrease in cell proliferation compared to cells treated only with iodixanol. The 24-wk old db/db mice gained significant weight, had increased urine volume, GFR, NGAL, and blood and urine glucose levels compared to non-diabetic mice. 24 h after CM (iohexol) injection, db/db mice had a significant decrease in GFR and a significant increase in serum creatinine, urine and kidney KIM-1 levels, and showed kidney damage histologically compared to control db/db mice. The mRNA level of TLR2 (Toll-like receptor 2) was significantly upregulated by iohexol in db/db mouse kidney. Iohexol also caused acidosis in diabetic mice evidenced by decreasing levels of serum pH and HCO_3 levels.

Conclusions: HK-2 cells in high-glucose medium exhibited more severe contrast-induced toxicity than normoglycemic HK-2 cells. Iohexol is more nephrotoxic than

iodixanol both *in vitro* and *in vivo*. At age ≥ 24 wk, db/db mice are prone to CIN and innate immunity mediated by TLR2 may play a role in its pathophysiology. This diabetic mouse model could be useful for in depth studies of mechanisms involved in CIN.

Funding: Private Foundation Support

SA-PO300

The Role of the Intestinal Microbiota in the Development of Pre-Diabetes and Diabetes in BTBR and BTBRob/ob Mice Kelly D. Smith, Tomasz A. Wietecha, Xiaodan Zhao, Charles E. Alpers. *Pathology, Univ of Washington, Seattle, WA.*

Background: Obesity and diabetes are associated with an altered gut microbiota that may aggravate metabolic syndrome, and promote inflammation and diabetic complications. The BTBR mouse is insulin resistant due to mutations in 3 loci and develops pre-diabetes; the BTBR$^{ob/ob}$ mouse is leptin-deficient, resulting in progressive obesity and severe type II diabetes. BTBR$^{ob/ob}$ mice also develop features that closely mimic diabetic nephropathy in humans. In this study we analyzed the role of the microbiome in modulating pre-diabetes in BTBR and type II diabetes in BTBR$^{ob/ob}$ mice.

Methods: Starting at 4 weeks, BTBR and BTBR$^{ob/ob}$ mice were fed normal mouse chow, with or without an antibiotic cocktail. Mice were analyzed for weight, blood glucose, glucose tolerance, urine protein, and histopathology. Fecal bacteria were analyzed by culture and 16S rDNA sequencing.

Results: Antibiotic treatment suppressed intestinal microbiota in BTBR mice and significantly improved glucose levels, glucose tolerance and cardiac hypertrophy. In contrast, antibiotic treatment significantly decreased survival (50% at 16 wk, log rank test P=0.0016), increased proteinuria and did not alter glomerular histopathology. Analysis of the microbiome revealed modest, non-significant changes in gut bacterial composition between BTBR and BTBR$^{ob/ob}$ mice. Antibiotic treatment dramatically altered microbiota, but did not completely suppress microbiota in BTBR$^{ob/ob}$ mice, which had an outgrowth of fungi and antibiotic resistant bacteria.

Conclusions: Antibiotic-mediated suppression of the intestinal microbiota improved pre-diabetes in non-obese BTBR mice, suggesting that gut microbiota contribute to the development of pre-diabetes in this genetic model of insulin-resistance. In contrast, antibiotics did not alter the course of severe type II diabetes in BTBR$^{ob/ob}$ mice and significantly increased mortality. Thus the contribution of gut microbiota to severe diabetes is complex and may not be adequately assessed with broad spectrum antibiotic treatment. Future studies in germ-free mice may help clarify the role of gut microbiota in diabetes and diabetic nephropathy.

Funding: NIDDK Support

SA-PO301

Moderate Exercise Training Improves Renal and Cardiac Parameters in Diabetic Rats. Is It Enough to Change the Progression of Hypertrophy? Luciana Jorge,[1] Kleiton Augusto Santos Silva,[1] Rafael Luiz,[1] Rodolfo Rosseto Rampaso,[1] Tatiana Sousa Cunha,[2] Nestor Schor.[1] *[1]Medicine/Nephrology, Federal Univ of Sao Paulo, Sao Paulo, SP, Brazil; [2]Science and Technology Inst, Federal Univ of Sao Paulo, Sao Jose dos Campos, SP, Brazil.*

Background: Diabetes mellitus (DM) causes damage in both heart and kidneys, leading to the cardiorenal syndrome that is associated with increased complications and mortality. Moreover, DM induces cardiac and renal hypertrophy. The aim was to verify if moderate exercise training (MET) improves renal and cardiac parameters and whether it is able to prevent cardiac and kidney hypertrophy.

Methods: Male Wistar rats were divided into 4 groups: control (C=8), control trained (CT=8), diabetic (D=8) and diabetic trained (DT=8). DM was induced by STZ. Trained groups were submitted to a MET protocol on a treadmill (8 wk). We measured SBP, HR, proteinuria and microalbuminuria in all groups. The cardiac and kidney hypertrophy index was assessed using weight/body weight ratio. By western blot were assessed p-Akt, p-TSC2, p-AMPK. TGF-β1 was evaluated by multiplex.

Results: MET improved renal parameters in the DT group showing a decreased urinary volume (DS:153; DT:106; CS:10 and CT:13ml), proteinuria (DS:46; DT:29; CS:18; CT:7mg/24h) and the microalbuminuria (DS:8.7; DT:4.7; CS:1.5; CT:2.3mg/24h). The SBP was similar between groups (CS:124, CT:128; DS:122; DT:130 mmHg), however, the HR decreased in the DS (323bpm), while the DT (342bpm) was increased. Diabetes resulted an increased cardiac and renal weight, surprisingly, the MET not prevented it. The cardiac expression of p-TSC2 and p-AMPK were reduced in DS group and MET normalized this expression. Moreover, the cardiac expression of p-AKT was increased in the DM and MET did not change it. The cardiac TGF-β1 was elevated in DM and the MET normalized this variable.

Conclusions: MET attenuated the progression of diabetic nephropathy and cardiac dysfunction; those improvements could be a result of the increased expression of p-TSC2, an important protein in the inhibition of the mTOR pathway, a hypertrophic signaling. Ours findings demonstrated that MET is an important approach to prevent complications caused by diabetes.

SA-PO302

High Glucose Upregulates ADAM17 through HIF-1α in Mesangial Cells Renzhong Li,[1] Lalita Uttarwar,[1] Claire M. Dubois,[2] Martine Charbonneau,[2] Bo Gao,[1] Joan C. Krepinsky.[1] *[1]Medicine, McMaster Univ, Hamilton, ON, Canada; [2]Pediatrics, Univ de Sherbrooke, Sherbrooke, PQ, Canada.*

Background: We previously showed that ADAM17 mediates high glucose (HG)-induced matrix production by mesangial cells (MC). ADAM17 expression is increased in diabetic kidneys, suggesting that its upregulation may augment the HG profibrogenic response. We thus studied the effects of HG on ADAM17 gene regulation.

Methods: Primary rat MC were treated with HG (30mM) or mannitol as osmotic control. ADAM17 regulation was assessed by real-time PCR, immunoblotting and activation of a promoter luciferase (-2304 to -1bp) construct.

Results: ADAM17 transcript and protein levels and promoter activity were dose-dependently increased by 24h of HG, but not mannitol. This correlated with increased ADAM17 activity at 24h versus 1h of HG. We tested the involvement of transcription factors shown in other settings to regulate ADAM17 transcription. Promoter activation by HG was not affected by inhibitors of NF-κB or Sp1, but was blocked by inhibition of HIF-1α which also prevented the increase in protein and transcript levels. HIF-1α siRNA also decreased HG-induced promoter activation. HIF-1α activation by HG was confirmed by its increased nuclear translocation and activation of the HIF-responsive plasmid HRE-luciferase. A series of ADAM17 promoter deletion constructs identified decreased promoter activation by HG upon deletion of -2304 to -1567 and complete abolition of activation upon deletion of -903 to -410. These contain two potential HIF-1α regulatory binding sites, termed H1 and H4 respectively. Only mutation of H4 prevented HG-induced promoter activation. HG-induced HIF-1α binding to this region was confirmed by ChIP studies. Finally, inhibitors of EGFR or ADAM17 itself prevented HG-induced ADAM17 promoter activation and protein upregulation and HIF-1α activation.

Conclusions: Thus, HG induces ADAM17 upregulation in MC which is associated with augmentation of its activity. This is mediated by HIF-1α and requires EGFR/ADAM17 signaling, demonstrating the potentiation by ADAM17 of its own upregulation. ADAM17 inhibition thus provides a potential novel therapeutic target for the treatment of diabetic nephropathy, which will be evaluated in further studies.

Funding: Government Support - Non-U.S.

SA-PO303

Mitochondrial Targeting of Superoxide by MitoQ Improves Metabolic Renal Dysfunction Micheal S. Ward,[1,2] Josephine M. Forbes.[1,2] *[1]Glycation and Diabetic Complications, Mater Research Inst - The Univ of Queensland, Brisbane, Queensland, Australia; [2]School of Medicine, The Univ of Queensland, Brisbane, Queensland, Australia.*

Background: Pathological progression of renal disease (RD) in diabetes is associated with mitochondrial dysfunction attributed in part to excess reactive oxygen species (ROS) generation. Progression of ~30% of diabetic individuals to RD highlights possible underlying susceptibility.

Methods: Groups (n=10/group; 8W of age) of randomised male db/db and db/m mice to (i) Vehicle (H_2O), (ii) MitoQ (MQ), (iii) Ramipril (Ram) or (iv) Co-therapy (MQRam) were followed for 12 weeks. Human immortalized podocytes and primary proximal tubular cells (PTC) were also studied using a Seahorse XF24 analyser to define mitochondrial respiratory function after normal (NORM; 5mM glucose and 100pM insulin), diabetic-like (DIA; 15mM and 500pM) and modulating (MOD; 2 hour alternating treatments) conditions in the presence and absence of MQ.

Results: *In vivo* pharmacological interventions each improved renal function (Sinistrin GFR and UAER) in db/db mice. Oral glucose tolerance testing suggested improvements in renal function with MQ were independent of improved glycaemic control. Analysis of freshly isolated renal mitochondria showed improved function with MQ treatments. *In vitro*, human PTCs had greater maximal respiratory capacity (MRC) and ATP content than podocytes in NORM. Under DIA, MRC and ATP content were reduced in both cell types, significantly in podocytes. However, mimicking glucose excursions, via MOD, podocytes and PTCs elevated MRC and ATP content, significantly in podocytes, compared with NORM. MQ targeting of podocytes attenuated significant decreases presenting under DIA.

Conclusions: Targeting of mitochondrial ROS by MQ has efficacy in experimental models of type 2 diabetes and mechanistically improves mitochondrial function both in vivo in renal cortical mitochondria and in cultured human cells.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO304

Metallothionein (MT)-3 Is a Novel Direct Target of Hypoxia Inducible Factor-1 Alpha (HIF-1α) Yumi Takiyama, Manami Kobayashi, Kuralay Atageldiyeva, Tsuyoshi Yanagimachi, Jun Honjo, Yukihiro Fujita, Masakazu Haneda. *Dept of Medicine, Asahikawa Medical Univ, Asahikawa, Hokkaido, Japan.*

Background: Diabetic nephropathy (DN) represents excessive production of reactive oxygen species (ROS) and renal hypoxia, leading to cellular dysfunction accompanied with subjugated antioxidant mechanisms.Metallothionein (MT) is a cysteine-rich protein with low molecular weight, and act as an antioxdant against the toxicity of metals, ischemia, and ROS. To investigate the pathophysiological role of MT in DN, we studied MT expression in human renal proximal tubular epithelial cells (HRPTECs) and in human kidney tissue derived from subjects with type 2 diabetes.

Methods: The regulation of MT expression was evaluated using HRPTECs by RT-qPCR. Transfection of HRPTECs was carried out by electroporation using the Nucleofection system. Expression of MT3 in human kidney was tested by immunohistochemistry.

Results: HRPTECs constitutively expressed the mRNAs of MT 1-3. Hypoxia (1% O_2) induced a remarkable increase in MT3 mRNA (~300-fold) and a moderate increase in MT2 mRNA (~5-fold), but not in MT1 mRNA in HRPTEC. The genetic inhibition of HIF-1α by siRNAs abolished the stimulatory effect of hypoxia on MT3 mRNA expression, indicating that hypoxia induced MT3 mRNA dependent on HIF-1α. We also found four putative hypoxia response elements (HREs) containing the consensus sequence (A/G) CGTG within the human MT3 gene. In addition, for investigation of which molecules are regulated by MT3, we analyzed microarray data using Affymetrix GeneChip (Human Gene 1.0 ST Array) with siRNA-mediated knockdown of MT3 under hypoxia in HRPTECs. A total of 28,869 transcripts were analyzed, among them, the crucial players in iron or hemoglobin metabolism, ceruloplasmin (-2.333-fold) or cytochrome b reductase 1 (-2.019-fold), were downregulated. Finally, MT3 protein expression in tubular cells in DN was increased compared with those in minimal change nephrotic syndrome (MCNS).

Conclusions: Our study, for the first time, suggests that MT3 is a novel direct target of HIF-1α-mediated signaling during hypoxia-induced renal injury in DN.

SA-PO305

Interaction of RhoA/ROCK Signaling and Renal Angiotensin System in Macrophages Infiltration of Diabetic Nephropathy Jialing Rao, Zengchun Ye, Tan-Qi Lou. *Dept of Nephrology, The Third Affiliated Hospital of Sun Yet-sen Univ, Guangzhou, Guangdong, China.*

Background: Recent studies suggests activation of RhoA/ROCK signaling and renal angiotensin system(RAS) may play an important role in the development of diabetic nephrophathy(DN). The macrophages infiltration is found in diabetic kidneys and macrophage-mediated inflammation may be critical in the early stage of DN. However, whether the interaction of RhoA/ROCK and RAS affect the infiltration of macrophages has not been determined. Therefore, a comprehensive understanding of the macrophages infiltration in the presence and absence of RhoA/ROCK signaling and RAS can contribute to provide more and safer therapies.

Methods: The effect of interaction of RhoA/ROCK signaling and RAS was investigated in the cultured rat glomerular endothelial cells(RGECs). RGECs were incubated with advanced glycation end products(AGEs). Y27632, an inhibition of ROCK and RAS inhibitor losartan were cultured with these cells. RT-PCR, immunofluorescence and Western blots were used to detected the expression of ICAM-1, MCP-1, PECAM-1, AT-1, AGT and renin in cell lysate and culture medium. Using a transwell system, we evaluated the effect of blockage of ROCK and RAS on migration of macrophages.

Results: The experiments showed that, incubated with an increasing dose and time of AGEs, the expression of ICAM-1, MCP-1, PECAM-1, AT1, AGT and rennin in RGECs was gradually enhanced. Either Y27632 or losartan could depress the increased expression of these cytokines. When AGEs-incubated cells cultured with both inhibitors, significant reducing expression of these cytokines could be observed. Using a transwell system, we demonstrated that RGECs grown with AGEs were associated with significant increase in macrophages migration. This effect was completely blocked by the addition of Y27632 and losartan.

Conclusions: These findings suggest that RhoA/ROCK and RAS play a critical role in macrophages' adhesion and migration to endothelial cells. Blocking both signalings can mitigate the expression of adhesion molecule and infiltration of macrophages. This effect can offer protection for the kidney on early stage of DN.

Funding: Government Support - Non-U.S.

SA-PO306

The Persisting Pro-Inflammatory Effects of Transient Glucose Exposure in the Kidney Are Equivalent to That Observed in Chronic Hyperglycemia with Diabetes Chris Tikellis, Raelene J. Pickering, Andrew Murphy, Merlin C. Thomas. *Diabetic Complications, Baker IDI Heart and Diabetes Inst, Melbourne, Victoria, Australia.*

Background: It is now clear that even transient hyperglycemia can have long-lasting effects on the development and progression of diabetic complications, including diabetic nephropathy. This '*metabolic karma*' may explain why many patients with pre-diabetes manifest albuminuria and other diabetic complications, and why even brief periods of poor control in patients with diabetes may have a sustained adverse legacy for the development and progression of renal damage in diabetes.

Methods: Male C57Bl6 mice (n=10/group) were randomised to receive 4 sequential injections of D-glucose (3g/kg IP) or an equivalent volume of saline or L-glucose delivered 2 hours apart. This protocol produces a sustained elevation in plasma glucose levels (15-25mM) for 8 hours, after which time no difference in plasma glucose levels is detectable between treated and control mice. Mice were then followed for 7 days at which time they were killed and glomeruli were isolated by differential sieving. To explore the long term effects mice were injected with glucose or saline weekly using the same protocol for 10 weeks.

Results: Glomeruli isolated from mice 1 week after transient glucose exposure showed persistent up-regulation of ICAM-1, VCAM-1, MCP-1 and NFKB expression when compared to mice that received saline alone. Glomerular leukocyte recruitment was also increased as indicated by the expression of CD11b and FACS analysis. Aortae taken 1 week after glucose exposure also demonstrated up-regulation of adhesion molecules and increased adhesiveness when exposed to labelled human leucocytes *ex vivo*. Repetition of the transient hyperglycaemia protocol every week for 10 weeks in complications-prone apolipoprotein E KO mice led to the development of atherosclerosis, renal damage and albuminuria comparable to that observed in streptozotocin induced diabetic mice.

Conclusions: These experimental data support the hypothesis that transient glucose excursions have adverse proinflammatory effects in the kidney and vasculature potentially contributing to the development of diabetic complications before the onset of diabetes.

SA-PO307

The Role of Renal Lipid Metabolism and Lipotoxicity in Human Obesity-Related Glomerulopathy Michal Herman-Edelstein,[1] Amalia Getsztain Bakshi,[1] Talia R. Weinstein,[1] Pnina Scherzer,[1] Uzi Gafter,[1] Vivette D. D'Agati,[3] Moshe Levi.[2] *[1]Nephrology, Rabin Medical Center-Felsenstein, Tel Aviv Univ, Petah-Tikva, Israel; [2]Nephrology, Univ of Colorado, Denver; [3]Renal Pathology, Columbia Univ.*

Background: Obesity-related glomerulopathy(ORG) is an emerging complication of the obesity epidemic. The pathophysiological mechanism ofglomerular injury in ORG is incompletely understood. There is growing evidence that ectopic lipid accumulation pathways (fatty kidney) may cause renal disease.The aim of this project was to study the mechanisms of kidney injury in ORG.

Methods: To identify metabolic pathways involved in the pathogenesis of obesity-related renal disease, we studied by qtPCR the expression of candidate genes involved in cholesterol and fatty acid metabolism, using RNA isolated from formalin-fixed, paraffin-embedded (FFPE) renal biopsies from patients with established ORG (n=33) versus normal kidneys (n=15). We also studied differential expression of these genes in glomeruli versus tubulescollected using laser-capture microdissection(LCMD) method.

Results: Glomerular surface area was markedly enlarged in ORGand was accompanied by significant decreases in the podocyte marker genes, including nephrin and podocin. There was marked lipid accumulation in both glomeruli and tubules by EM and adipophilin staining which was mediated in part by decreased expression of PPARα andfatty acid β oxidation which would favor accumulation of fatty acids and triglycerides.

Conclusions: lipotoxicity may contribute to obesity induced kidney injury.

Funding: NIDDK Support

SA-PO308

Cholecystokinin Activates AMPK and Protects against Progression of Diabetic Kidney Disease in db/db Mice Satoshi Miyamoto,[1,2] Maggie K. Diamond-Stanic,[1,2] Manjula Darshi,[1,2] Tammy Quach,[1,2] Larkin B. Slater,[1,2] Kumar Sharma.[1,2] *[1]Center for Renal Translational Medicine, Univ of California, San Diego, La Jolla, CA; [2]Veterans Administration San Diego HealthCare System, La Jolla, CA.*

Background: We have previously shown that cholecystokinin (CCK) is expressed in the rodent kidney, and that administration of CCK-octapeptide (CCK-8S) confers protection against renal inflammation in early stage of type 1 diabetic kidney disease via an unclear mechanism. We investigated whether interventional CCK arrests diabetic kidney disease (DKD) in a model of type 2 diabetic kidney disease and to determine if AMPK may play a role.

Methods: Sixteen weeks-aged male diabetic db/db (D) mice (BKS.Cg-Dock7^{m} +/+ Lepr^{db}/J strain) were randomly assigned to three groups: db/db treated with vehicle (D+V), db/db treated with low-dose CCK-8S (D+LC) and db/db treated with high-dose CCK-8S (D+HC), n=12/group. Nondiabetic db/m mice were used as control. Vehicle or CCK-8S was infused subcutaneously via osmotic pumps over a 4 weeks period. Glomerular size and mesangial matrix area were assessed by morphometric analysis. AMPK was measured via immunostaining and immunoblotting.

Results: Urinary albumin/creatinine ratio was elevated in D+V (273 ± 47, $P<0.001$) versus control db/m mice (49 ± 4), and reduced in both D+LC (135 ± 16, $p<0.05$) and D+HC (128 ± 29, $p<0.05$), whereas there were no significant differences in body weight, food intake and HbA1c among diabetic groups. There was no significant difference in glomerular size between D+V and D+LC or D+HC, but mesangial matrix was increased by 32 % in D+V and reduced to 88% and 84% of D+V levels by low and high dose CCK treatment, respectively ($p<0.001$). Interestingly, both AMPKα phosphorylation in the renal cortex and average number of pAMPKα positive cells in glomeruli was significantly increased in the D+HC group compared with D+V ($p<0.01$).

Conclusions: We found that a cholecystokinin agonist can improve features of established DKD with type 2 diabetes and may be renoprotective via activation of AMPK. Regulation of gut-kidney axis by CCK may be a novel therapeutic approach for the treatment of established DKD.

Funding: NIDDK Support, Other NIH Support - DP3DK094352-01, Veterans Affairs Support

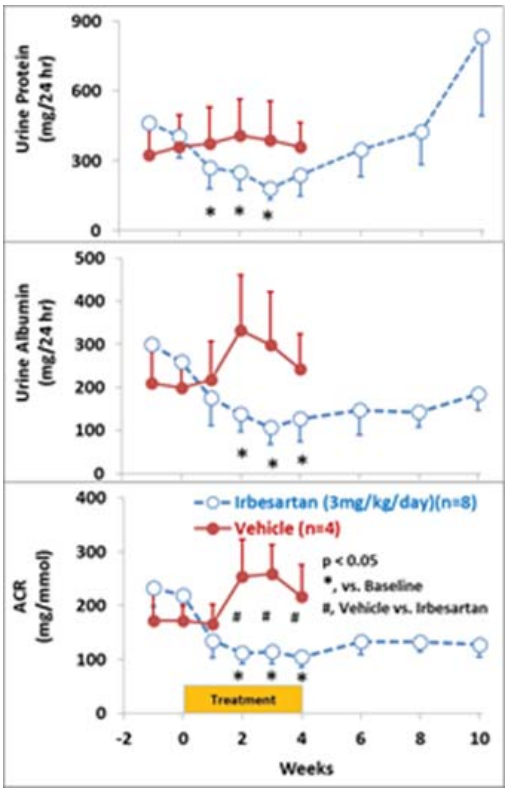
SA-PO309

Angiotensin II Receptor Blockers (ARB) Reduce Proteinuria in Diabetic Monkeys Zhonghua Qi,[2] Yixin (Jim) Wang,[1] Xiaoli Wang,[1] Yupeng Fang,[1] Yong-Fu Xiao,[1] Yongqiang Liu,[1] Jing Wu,[1] Guofeng Sun,[1] Bingdi Wang,[1] Mark Kowala,[2] Matthew D. Breyer.[2] *[1]Pharmacology, Crown Bioscience Inc, Taicang, China; [2]Lilly Research Labs, Eli Lilly & Co., Indianapolis, IN.*

Background: Diabetic Nephropathy (DN) is a grave microvascular complication of diabetes. DN initially presents as microalbuminuria, however as it progresses, glomerular damage and proteinuria increase and ultimately renal failure ensues. Efficacy of monoclonal antibody (mAb) therapies for human disease can often only be tested in monkeys but not other species due to lack of mAb cross-reactivity.

Methods: To determine whether DN in monkeys is similar to human DN this study tested whether ARBs reduce proteinuria in spontaneously diabetic cynomolgus monkeys. Two groups of diabetic monkeys were compared: Vehicle (n=4) versus Irbersartan treated (n=8).

Results: Oral administration of Irbesartan (3mg/kg/day), achieved a plasma concentration at 70±17 ng/mL at 24h and significantly reduced urinary protein and albumin excretion as well as ACR. After termination of a 4 week treatment, both gradually returned towards baseline .



There were no significant changes in urinary protein or albumin excretion, in the vehicle group, nor were there significant changes in blood pressure, serum creatinine concentrations or glomerular filtration rate (GFR) measured by creatinine clearance in either group. In a separate experiment in the same model, daily treatment with losartan (1mg/kg) resulted in a similar reduction of proteinuria.

Conclusions: These data demonstrate, to our knowledge, for the first time, that blockade of Ang-II receptors reduces proteinuria in monkeys with DN, and is consistent with the clinical observations in patients with DN. This supports the feasibility of testing therapeutics in the monkeys with DN and their use as a translational model for studying pathophysiological mechanism(s) of DN.

Funding: Pharmaceutical Company Support - Eli Lilly and company, Crown Biosciences Inc.

SA-PO310

Glomerular Parietal Epithelial Cell Activation Induces Collagen Secretion and Thickening of Bowman's Capsule in Diabetes Alexander Holderied,[2] Simone Romoli,[2] Jonathan Nicodemos Eberhard,[2] Lukas A. Konrad,[2] Satish Kumar Devarapu,[2] Julian A. Marschner,[2] Susanna Mueller,[1] Hans J. Anders.[2] *[1]Ludwig-Maximilians-Univ, Pathologisches Institut, München, Germany; [2]Klinikum der Ludwig-Maximilians-Univ, Nephrologisches Zentrum, Medizinische Klinik und Poliklinik IV, München, Germany.*

Background: The metabolic and hemodynamic alterations in diabetes activate podocytes to increase extracellular matrix production leading to thickening of the glomerular basement membrane, i.e. an early sign of diabetic nephropathy (DN). We hypothesized that diabetes would activate parietal epithelial cells (PEC) and cause thickening of Bowman's capsules.

Methods: Human kidney biopsies and in vitro experiments with human PECs were used to address this issue.

Results: Periodic Acid Schiff staining of human kidney biopsies of 30 patients with DN revealed a significantly thicker Bowman's capsule as compared to 20 non-diabetic controls. The average thickness was 4.55 +/- 0.21 µm in the group of patients with DN compared to 2.92 +/- 0.21 µm in the group of non-diabetic controls ($p<0.001$). Transmission electron microscopy confirmed this finding. In vitro, exposure of human PECs to hyperglycemic conditions, advanced glycation end-products or tumor growth factor-β1 increased the mRNA expression of collagen Iα1, collagen IV, bamcan, nidogen, laminin 1 and perlecan. Western-blot and colorimetric collagen assays confirmed these results for collagen IV on a protein level. To validate these findings in vivo, activation of the PECs was assessed by immunohistochemical staining for CD44 of twelve human biopsy cases with DN. Thickening of the Bowman's capsule showed strong association to CD44 positive PECs.

Conclusions: In summary, metabolic alterations in diabetes activate PECs to increase the expression and secretion of Bowman's capsule proteins. This process should contribute to the thickening of the Bowman's capsule similar to the thickening of the glomerular basement membrane that is driven by activated podocytes. These data may also imply that activated PECs contribute to extracellular matrix production once they migrate to the glomerular tuft, a process resulting in glomerular scaring, e.g. in diabetic glomerulosclerosis.

Funding: Government Support - Non-U.S.

SA-PO311

Paricalcitol Modulates Plasma ACE2 Activity and Renal ADAM17 Content in Type1 Diabetic Mice Marta Riera, Lidia Anguiano, Marta Rebull, Sergi Clotet-Freixas, Julio Pascual, Maria Jose Soler. *Nephrology, Hospital del Mar Medical Research Inst (IMIM), Barcelona, Spain.*

Background: We demonstrated that circulating and renal ACE2 activity is increased in the NOD DB mice. We studied the role of Paricalcitol in modulating ACE2 in these mice and the renal ADAM-17, implied in ACE2 shedding. Also, the effect of Paricalcitol on tubular epithelial cells was studied.

Methods: Diabetic NOD females age-matched with non-DB controls were studied for 21 days after DB onset. Treatments (n=10,each): Diabetic (DB) animals given vehicle **NOD_pe**; DB treated with Paricalcitol 0.4µg/kg; **NOD+PARI_L**; DB treated with Paricalcitol 0.8µg/kg; **NOD+PARI_H**; DB treated with Aliskiren **NOD+ALSK**; DB with the combination **NOD+PARI_L+ALSK**. Non-obese Resistant mice were controls **NOR**. Mouse tubular epithelial cells were incubated for 24h with Paricalcitol (0.04, 0.4, 4 ng/ml) and high concentration of glucose(25mM).

Results: PARI alone or in combination with ALSK significantly reduced cACE2 activity in DB NOD mice without variations in UAE. Serum renin activity significantly decreased in ALSK-receiving groups but no effect was found with Paricalcitol. Renal content of ADAM17 significantly decreased in NOD+PARI_H. Oxidative stress evaluated by means of renal Nitrotyrosine IHQ and H_2O_2 content in blood. Oxidative stress was reduced in NOD+PARI_H as compared to NOD_pe. In MTC cells, HG increased ACE2 protein expression as compared to cells in low glucose. Paricalcitol significanlty increased the ACE2 expression as compared to HG non-treated cells.

	Blood Glucose t=21d (mg/dL)	UAE (µgAlb/mgCrea)	Circulating ACE2 activity (RFU/µl/hr)	Renal cortex ACE2 expression (ACE2/βactin)	Serum Renin Activity (RFU/µl/hr)	Renal TACE/ADAM17 (pg/µg prot)
NOR	156.5 ± 7.1*	22.17 ± 7.50	111.4 ± 5.0*	0.41 ± 0.07*	1293.1 ± 124.9*	37.56 ± 3.53*
NOD pe	582.3 ± 11.6	482.93 ± 275.41	403.1 ± 42.6	0.66 ± 0.07	1941.5 ± 117.1	75.01 ± 7.00
NOD+PARI_L	525.3 ± 32.8	419.68 ± 209.93	316.2 ± 23.6$	0.81 ± 0.06$	1931.4 ± 80.0	67.34 ± 6.36
NOD+PARI_H	581.6 ± 17.6	311.93 ± 57.63	301.4 ± 12.4$	0.87 ± 0.09$	2034.1 ± 126.3	40.57 ± 4.42$
NOD+ALSK	582.4 ± 9.3	234.41 ± 54.04	357.7 ± 43.6	0.70 ± 0.10	1623.5 ± 130.3$	61.68 ± 6.05
NOD+PARI+ALSK	538.5 ± 23.7	433.10 ± 202.60	263.6 ± 32.5$	0.77 ± 0.09	1507.3 ± 111.0$	64.86 ± 6.56
P	*p<0.05 vs. NOD groups; $p<0.05 vs. NOD_pe					

Conclusions: In NOD diabetic mice, with type 1 diabetis, Paricalcitol modulates ACE2 activity, ADAM17 and oxidative stress renal content independently from the glycemic profile and UAE. In tubular cells, Paricalcitol may modulate ACE2 by blocking its shedding. In the early DN stage, Paricalcitol treatment counterbalances the effect of diabetes on circulating ACE2 activity.

Funding: Pharmaceutical Company Support - abbvie

SA-PO312

Meprin Alpha Modulates the Immune Response in Mice with Diabetic Nephropathy Kasheena Burris,[1] Jean-Marie V. Niyitegeka,[1] Radiah Minor,[2] Dawn Conklin,[2] Elimelda Moige Ongeri.[1] *[1]Biology; [2]Animal Sciences, North Carolina A&T State Univ, Greensboro, NC.*

Background: Pre-existing chronic kidney disease increases the mortality rate in sepsis. Meprins, metalloproteases that are abundantly expressed in the brush border membranes of kidney proximal tubules, have been implicated in the pathology of diabetic nephropathy (DN). The mechanisms by which meprins modulate kidney injury in DN are not fully understood. Recent studies have shown that meprins are also expressed in leukocytes and play a role in the inflammatory response. The objective of the current study was to determine whether meprin α modulates the immune response associated with sepsis in mice with pre-existing DN.

Methods: Streptozotocin (STZ) was used to induce type 1 diabetes in 8-week old wild-type (WT) and meprin α knockout (αKO) male mice on a C57BL/6 background. At 5 weeks post-STZ injection, the mice were subjected to cecal-ligation and puncture (CLP)-induced sepsis, sacrificed 18h later, and kidney tissue processed for proteomic analysis. Blood samples were collected pre-and post-CLP, and flow cytometry used to evaluate blood leukocyte profiles. The levels of blood urea nitrogen (BUN) and serum creatinine were also measured.

Results: The mortality rate associated with CLP was significantly lower in meprin αKO mice with DN when compared to WT counterparts. BUN levels significantly increased at 18h post-CLP, but were comparable in both genotypes. There was no significant change in the serum creatinine levels at 18h post-CLP. Flow cytometric data showed a 1.5 fold increase in B-cell numbers in WT mice with DN when compared to meprin αKO counterparts at 5 weeks post-STZ injection. However, there were no significant changes in B-cell numbers at 18h post-CLP for either genotype. The levels of neutrophils were 4 times higher in meprin αKO mice at 5-weeks post STZ-injection when compared to WT mice. There was a 3.3 fold increase in neutrophil levels in diabetic WT mice after CLP, an increase that was not observed in diabetic meprin αKO mice.

Conclusions: The data suggest that meprin α deficiency protected mice with DN from injury associated with CLP-induced sepsis. The impact appears to be in part via modulation of neutrophil levels.

Funding: Other NIH Support - NIGMS

SA-PO313

Deletion of Aminopeptidase-N Ameliorates Kidney Function in Diabetic Mice Denis Feliers, Robert T. Day, Rita de Cassia Cavaglieri, Balakuntalam S. Kasinath, Hanna E. Abboud. *Medicine/Nephrology, UTHSCSA, San Antonio, TX.*

Background: Aminopeptidase N (AP-N), which degrades angiotensin III (Ang III), is upregulated in diabetic nephropathy, and renal content of Ang III is decreased. Renal levels of Ang III show a strong inverse correlation with kidney hypertrophy, extracellular matrix expansion and albuminuria. We hypothesized that deletion of AP-N will increase renal levels of Ang III and reduce indices of kidney injury in mice with type 1 diabetes.

Methods: Type 1 diabetes was induced with streptozotocin in APN knockout mice (APN-KO) and their wildtype counterparts (APN-wt). Renal levels of Ang II and Ang III, expression of AP-N and indices of kidney injury were assessed after 2 weeks of diabetes.

Results: In Ove26 mice with type 1 diabetes, renal cortical levels of Ang III were decreased, and inversely correlated with albuminuria ($r = -0.67$, $p = 0.01$), kidney hypertrophy ($r = -0.79$, $p < 0.0001$) and fibronectin expression ($r = -0.77$, $p = 0.0001$). AP-N deletion had no effect on glycemia of control or diabetic mice, and slightly reduced blood pressure in diabetic mice. Renal cortical Ang III expression was significantly increased in control and diabetic APN-KO mice but Ang II levels were unchanged. There was a ~ 50% reduction in urinary albumin excretion and kidney hypertrophy in diabetic APN-KO mice compared to diabetic APN-wt mice. Superoxide production as well as expression of NAD(P)H-oxidase Nox4 and fibronectin were significantly reduced in diabetic APN-KO mice. Expression of pro-inflammatory molecules VCAM1 and MCP1 was significantly increased by diabetes in wild type mice but not in APN-KO mice.

Conclusions: Our data show for the first time that deletion of aminopeptidase-N increases renal levels of Ang III without affecting the levels of Ang II, reduces albumin excretion, kidney hypertrophy, oxidative stress and expression of pro-fibrotic and pro-inflammatory molecules. Our results suggest combination of blockade of Ang II synthesis and increase in renal Ang III may contribute to amelioration of kidney injury in diabetes.

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

SA-PO314

miR-21 Contributes to Proximal Tubule Cell Dysfunction in Diabetic Nephropathy Through Augmentation of TGFβ-Induced Changes in Mitochondrial Morphology and Metabolism Aaron D. McClelland, Phillip Kantharidis, Karin Jandeleit-Dahm, Mark E. Cooper. *JDRF Danielle Alberti Memorial Centre for Diabetes Complications, BakerIDI Heart and Diabetes Inst, Melbourne, Victoria, Australia.*

Background: miR-21 is strongly upregulated by TGFβ and known to directly play roles in the development of tissue fibrosis and cancer metastasis. These major roles have encompassed a small number of the predicted targets of miR-21 and therefore likely represent an equally minute understanding of the pathophysiology of miR-21. To further this understanding, miR-21 was studied in context of mitochondrial dysfunction in PTCs, a major contributor to the oxidative stress induced in the diabetic kidney.

Methods: PTCs were grown in high glucose conditions with or without 10ng/μl TGFβ. miR-21 was over-expressed and knocked-down using miRNA mimics and locked-nucleic-acids (LNAs) respectively. The effect of miR-21 on mitochondrial morphology and metabolism were analysed by an array of biochemical and imaging based techniques.

Results: Mitochondria underwent increased fission characteristic of TGFβ treatment. This was accompanied by changes in levels and localisation of mitochondrial dynamics proteins. TGFβ-induced changes in mitochondrial basal and maximal respiration in addition to spare respiratory capacity were all increased by miR-21 overexpression by ~2-fold. TGFβ-induced mitochondrial reactive oxygen species production was increased 50% above baseline. This was further increased by miR-21 over-expression to 2-fold above baseline. This was partially overcome by the addition of glutathione. Reduction of oxidative stress was accompanied by downregulation of a number of genes important to PTC DN pathophysiology including Collagen1 and Fibronectin.

Conclusions: These data provide further insight into the role of miR-21 in DN and how it mediates the pathogenic gene expression changes associated with its dysregulation. Undoubtedly, these findings add support to the notion of targeting miR-21 in a clinical setting with the aim of attenuating the progression of diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO315

Macrophage TNF-α Mediates Diabetic Renal Injury Hanning You,[1] Ting Gao,[1] Timothy K. Cooper,[2] Jean Vacher,[3] Francis Xavier Farrell,[4] William Brian Reeves,[1] Alaa S. Awad.[1] *[1]Medicine, Penn State Univ College of Medicine, Hershey, PA; [2]Comparative Medicine, Penn State Univ College of Medicine, Hershey, PA; [3]Clinical Research Inst of Montreal, Univ de Montréal, Canada; [4]Immunology Research, Centocor Research and Development.*

Background: Monocyte/macrophage recruitment correlates strongly with the progression of diabetic nephropathy (DN). Tumor necrosis factor-alpha (TNF-α) is produced by monocytes/macrophages. However, the direct role of TNF-α and/or macrophage-TNF-α in the progression of DN remains unclear. We hypothesize that deficiency or blockade of TNF-α confers kidney protection in DN via macrophage-TNF-α dependent pathway.

Methods: Experiments were conducted in TNF-α deficient mice ($TNF\text{-}\alpha^{-/-}$), macrophage-specific TNF-α deficient mice ($CD11b^{cre}/TNF\text{-}\alpha^{flox}$) and their wild type littermates following STZ induced diabetes and $Ins2^{Akita}$ mice treated with a TNF-α blocking antibody.

Results: Pharmacologic blockade of TNF-α using a TNF-α blocking antibody conferred kidney protection in $Ins2^{Akita}$ mice as indicated by reductions in albuminuria ($p<0.0001$), plasma creatinine ($p<0.05$), histopathologic changes ($p<0.05$), kidney macrophage recruitment ($p<0.001$) and plasma inflammatory cytokine levels (GM-CSF; $p<0.001$, KC; $p<0.01$, TNF-α; $p<0.05$ and MCP-1; $p<0.05$) compared to vehicle-treated $Ins2^{Akita}$ mice at 18 weeks of age. Likewise, diabetic $TNF\text{-}\alpha^{-/-}$ mice had reduced albuminuria ($p<0.05$) after 20 weeks of STZ-induced diabetes compared to diabetic $TNF\text{-}\alpha^{+/+}$ mice. To assess the direct role of macrophage-TNF-α in DN, we generated a macrophage specific TNF-α deficient mouse ($CD11b^{cre}/TNF\text{-}\alpha^{flox}$). Our results show that conditional ablation of TNF-α in macrophages significantly reduced albuminuria ($p<0.01$), the increase in plasma creatinine ($p<0.05$), histopathologic changes ($p<0.01$) and kidney macrophage recruitment ($p<0.05$) compared to diabetic $TNF\text{-}\alpha^{flox}$ control mice after 12 weeks of STZ-induced diabetes.

Conclusions: These findings indicate that production of TNF-α by macrophages plays a major role in diabetic renal injury and that blocking TNF-α could be a novel therapeutic approach for treatment of DN.

Funding: NIDDK Support

SA-PO316

Aerobic Exercise Training (AET) Improves Proteinuria in Rats with Diabetic Nephropathy Rodolfo Rosseto Rampaso, Rafael Luiz, Kleiton Augusto Santos Silva, Luciana Jorge, Edson Andrade Pessoa, Mario Luis Ribeiro Cesaretti, Nestor Schor. *Nephrology Div, UNIFESP-EPM, Sao Paulo, Brazil.*

Background: Moderate AET is an important tool for the treatment of diabetic patients. Several studies show that EXE decreases blood glucose, the incidence of CKD and contributes to a better control on hypertension and obesity among other effects. The aim of this study was to evaluate the effects of AET in controlling the progression of diabetic nephropathy, and its possible renoprotective effects.

Methods: Wistar rats divided into 4 groups: Sedentary controls (C-SED), Diabetes/Sedentary (DM-SED), Diabetes/Exercise(DM-EXE) and Exercise Controls (C-EXE). DM was induced with streptozotocin,50mg/kg i.v. The AET were done on treadmill 60 min/day, 5 days a week for 8 weeks. Weekly determined Maximal Exercise Test (set at 65-70% of MEtest). Glycemia after 24h post training (24h glycemiapt), creatinine clearance/BW (CrCl/BW), arterial pressure(AP), proteinuria(uProt) were measured. Data in Mean±ED.

Results:

	C-SED	DM-SED	DM-EXE	C-EXE
glycemiapt (mg/dl)	103 ± 2.03	551 ± 7.03 *&	491 ± 5.50 *&	83 ± 2.57
uProt (mg/24h)	17 ± 0.88	46 ± 2.05 *#&	18 ± 0.72	16 ± 0.99
CrCL/(ml/min/BW)	5.65 ± 0.66	5.02 ± 0.43	4.19 ± 0.37	4.21 ± 0.29
AP (mmHg)	122 ± 1.89	133 ± 1.79 *#&	122 ± 1.35	121 ± 2.11
Weight (g)	455 ± 6.00	236 ± 14.41 *#&	324 ± 9.34 *&	387 ± 8.71
MEtest (m/min)	23.2 ± 0.49 #&	19.5 ± 0.57 *#&	35.1 ± 0.97	37.5 ± 0.57

$p<0.05$:* VS C-SED. # VS DM EXE. & VS C- EXE

Conclusions: Reductions in glycemia and AP (~11%) were observed when comparing the groups DM-EXE versus DM-SED. The DM-EXE group showed a reduction in the

progression of weight loss (~40%) compared to DM-SED, but did not prevent alteration in the CrCl/BW with this protocol. However, the effect of the EXE was strikingly observed in the reduction of mean uProt excretion (~60%) comparing DM-SED versus DM-EXE. Therefore, preliminary data suggest that moderate aerobic exercise can reduce proteinuria in diabetic animals and consequently diminish the potential effects caused by diabetic nephropathy and could reduce the progression of renal failure.

SA-PO317

AMPK Regulates Fibronectin in Renal Cells and in Kidney of Diabetic Mouse Samy L. Habib,[1,2] Adam Kosti,[2] Sitai Liang.[2] *[1]Geriatric Research, Education, and, Clinical Center, South Texas Veterans Healthcare System, San Antonio, TX; [2]Cellular and Structural Biology, Univ of Texas Health Science Center, San Antonio, TX.*

Background: Tubular cells are a primary target of hyperglycemia contribute to the tubulointerstitial in diabetic nephropathy. The rate of deterioration of kidney function is highly correlated with the degree of tubular fibrosis and accumulation of cell matrix proteins. The mechanisms by which hyperglycemia contributes to matrix expansion and fibrosis are not known.

Methods: Mouse proximal tubular (MPT) cells were grown in normal glucose (NG) or treated with high glucose (HG) or pretreated with AICAR or compound C. MPT cells were transfected with DN-AMPK before exposure to HG to measure AMPK activity, protein expression and promoter activity of fibronectin. Db/db mice were treated with AICAR for 1 month, physiological and kidney parameters as well as p-AMPK/fibronectin expression were measured.

Results: HG significantly reduced AMPK activity and increased fibronectin expression in MPT cells while pretreatment with AICAR reversed these changes. Downregulation of AMPK by DN-AMPK or by compound C significantly increased protein expression and promoter activity of fibronectin. Further, HG significantly increased the promoter activity of fibronectin while pretreatment with AICAR reversed HG effects to NG levels. In addition, HG decreases YY1 expression and activates of mTORC1 while pretreatment the cells with AICAR reversed these changes. Moreover, kidney cortex of diabetic mice showed significant decrease in YY1 and AMPK and increase in mTOR activity that resulted in significant increase in fibornectin expression. While, mice treated with AICAR showed decrease in proteinuria and decrease in fibronectin protein expression compared to non-treated mice.

Conclusions: Our data showed that AICAR activates AMPK to block mTOR and prevent binding YY1 to mTORC1 that led to decrease accumulation of fibornectin in renal cells. In addition, AICAR treatment improves kidney parameters as well as blocks cell matrix protein accumulation in kidney of diabetic animals. These data shed the light on AMPK as potential therapeutic kinase target for treatment of renal complications in diabetes.

Funding: Veterans Affairs Support

SA-PO318

Thioredoxin Interacting Protein Mediates Dysfunction of Tubular Autophagy in Diabetic Renal Interstitial Fibrosis Chunling Huang,[1] Yuan Zhang,[2] Darren J. Kelly,[2] Christina Yan Ru Tan,[2] Xinming Chen,[1] Carol A. Pollock.[1] *[1]Renal Lab, Kolling Inst of Medical Research, Univ of Sydney, Sydney, NSW, Australia; [2]Dept of Medicine, St. Vincent's Hospital, Univ of Melbourne, Fitzroy, VIC, Australia.*

Background: Autophagy is a major pathway that delivers damaged proteins and organelles to lysosomes in order to maintain cellular homeostasis. Thioredoxin interacting protein (Txnip) expression is induced by a variety of cellular stresses including high intracellular glucose, which is associated with activation of oxidative stress and tubulointerstitial fibrosis in diabetic nephropathy. The aim of the study is to define the role of Txnip on dysfunctional tubular autophagy in the development of diabetic renal interstitial fibrosis.

Methods: Transgenic (mRen-2) 27 rats with streptozotocin-induced diabetes were randomly assigned to receive Txnip DNAzyme or control (scrambled DNAzyme) delivered by implanted minipump. Using immunofluorescence staining, the formation of autophagosomes was measured with LC3, and the effectiveness of autophagic clearance was determined by p62 expression in human kidney biopsy specimens from patients with diabetic nephropathy as well as experimental rat kidney tissues. The expression of Txnip and fibrotic marker collagen I was assessed with immunohistochemical staining. The activation of oxidative stress was measured by nitrotyrosine using immunohistochemical staining.

Results: Immunofluorescence staining results showed increased LC3 and P62 in renal tubular cells of human diabetic kidneys compared to non-diabetic kidneys, which indicated tubular accumulated autophagosomes and suppressed autophagic clearance. Similarly, increased LC3 and P62 were also found in renal tubular cells of experimental rat model of diabetic nephropathy compared to non-diabetic kidneys, which were reversed by Txnip DNAzyme treatment. Diabetic induced upregulation of Txnip, collagen I and nitrotyrosine were dramatically attenuated in the animals treated with Txnip DNAzyme.

Conclusions: Hyperglycemia induced overexpression of Txnip may contribute to the dysfunction of tubular autophagy in diabetic renal interstitial fibrosis through oxidative stress.

SA-PO319

Blockade of Smad3 Signalling Reduces Podocyte Injury in High Fat Diet-Induced Obesity Jinhua Li, Yu Bo Yang Sun, Xinli Qu. *Anatomy and Developmental Biology, Monash Univ, Melbourne, Vic, Australia.*

Background: Central obesity poses an increased risk for the development of renal-cardiovascular diseases and type 2 diabetes. TGF-β/Smad signalling plays a key role in renal fibrosis with the majority of the biological effects mediated via Smad3. Smad3-null mice are protected from high fat diet (HFD)-inducedobesity and exhibit reduced adiposity. We hypothesize that Smad3 deficiency may reduce obesity-related kidney injury.

Methods: Smad3 wild type and knockout mice were given HFD or normal diet (ND). Mice were killed 1, 4, 8 or 16 weeks after HFD or ND treatment.

Results: In response to a HFD, we have observed activation of Smad3, a significant increase of albuminuria and the incipience of insulin resistance occur at 1, 4 and 8 week(s) respectively. This suggests a temporal pattern of Smad3 signalling activation leading to kidney injury and subsequent insulin resistance in the aetiology of obesity-related kidney disease. With prolonged HFD exposure (16 weeks), there is a progressive increase of renal fibrosis with associated with loss of synaptopodin expression. Smad3 deficiency attenuated HFD-induced proteinuria and glomerulosclerosis, reduced alternatively activated M2 macrophages, and prevented down-regulation of synaptopodin. Confocal microscopy also demonstrated that mitochondrial marker significantly decreased in podocytes in HFD-treated group compared with that in ND-treated group. Smad3 deficiency rescued the loss of mitochondrial markers in podocytes. In a vitro model, palmitate acid addition to cultured podocytes induced a similar rapid activation of Smad3 and loss of synaptopodin after 5 days treatment. Addition of the specific Smad3 inhibitor, SIS3, prevented palmitate-induced down-regulation of synaptopodin expression and loss of mitochondrial marker.

Conclusions: Our studies provide the first evidence that high fat diet may directly cause kidney injury and Smad3 plays essential roles in HFD-induced podocyte damage by down-regulation of synaptopodin. Smad3 may be a novel therapeutic target in obesity-related kidney disease.

Funding: Government Support - Non-U.S.

SA-PO320

Effect of Bilirubin on Triglyceride Synthesis in Streptozotocin-Induced Diabetic Nephropathy Ho Jun Chin, Seong Woo Lee, Seon Ha Baek, Ki Young Na, Youn-Su Park. *Internal Medicine, Seoul National Univ Bundang Hospital, Seong nam, Kyeongkido, Republic of Korea.*

Background: Recent studies have indicated that bilirubin, in modest levels, acts as a major physiologic cytoprotectant for diabetes, diabetic complications, cardiovascular diseases, and renal diseases, mainly through anti-oxidative and anti-inflammatory properties. We investigated the other effects of bilirubin on diabetic nephropathy and probable mechanisms of them in rats with type I diabetes induced by streptozotocin (STZ).

Methods: Sprague–Dawleyrats were grouped into control, DM, and DM with bilirubin treatment (Bil) groups.Bil group was treated with bilirubin (60 mg/kg IP x 3/week). Hepatoma cells were also cultured withbilirubin(0.3 mg/dL).

Results: In SD rats of Bil group, serum creatinine level was preserved at 5 weeks after diabetes and decreased mesangial matrix with lower expression of renal collagen VI and TGF-β1, lower level of apoptosis determined by TUNEL staining in the kidney compared to DM group. That finding accompanied by decreased tissue level of hydrogen superoxide and protein expressions of subunits of NADPH oxidase. Bilirubin decreased serum total cholesterol, HDL cholesterol, free fatty acid, and triglyceride, and the content of triglyceride in liver tissue, also. The protein expression of genes involved in triglyceride synthesis, LXRα, SREBP-1, SCD-1, and FAS,was suppressed by bilirubin, which was enhanced in liver of DM rats or hepatoma cells in HG condition.

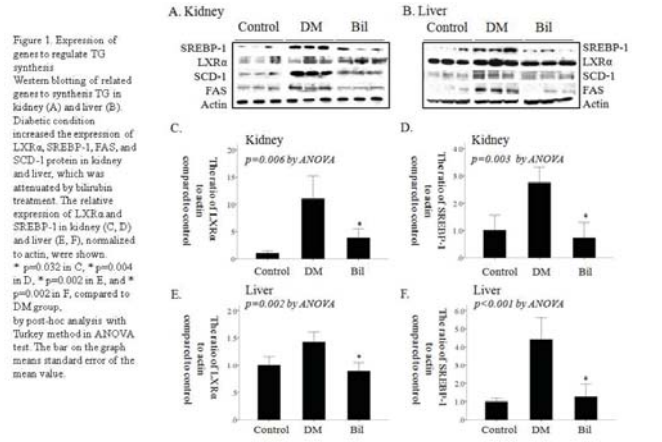


Figure 1. Expression of genes to regulate TG synthesis
Western blotting of related genes to synthesis TG in kidney (A) and liver (B). Diabetic condition increased the expression of LXRα, SREBP-1, FAS, and SCD-1 protein in kidney and liver, which was attenuated by bilirubin treatment. The relative expression of LXRα and SREBP-1 in kidney (C, D) and liver (E, F), normalized to actin, were shown. * p=0.032 in C, * p=0.004 in D, * p=0.002 in E, and * p=0.002 in F, compared to DM group, by post-hoc analysis with Turkey method in ANOVA test. The bar on the graph means standard error of the mean value.

Bilirubin suppressed the SREBP-1 gene, indirectly, via downregulation of LXRα, and through direct pathway, also.

Conclusions: Bilirubin attenuated diabetic renal dysfunction, and dyslipidemia in diabtes through suppression of LXRα and SREBP-1 genes.

Funding: Private Foundation Support

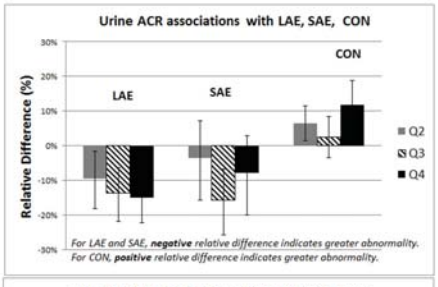
SA-PO321

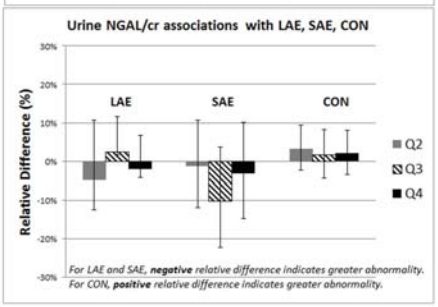
Urine ACR, NGAL, and KIM-1 and Subclinical Cardiovascular Disease: The Multi-Ethnic Study of Atherosclerosis (MESA) Meyeon Park,[1] Michael Shlipak,[1] Ronit Katz,[2] David Siscovick,[2] Mark J. Sarnak,[3] Chi-Yuan Hsu,[1] Carmen A. Peralta.[1] *[1]UCSF; [2]UW; [3]Tufts.*

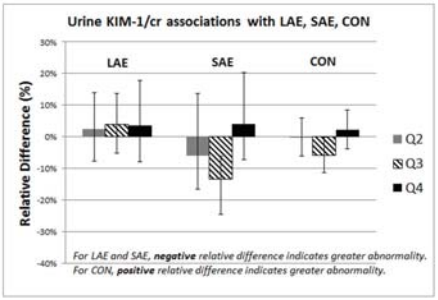
Background: Elevated levels of urine markers of glomerular and tubular injury are associated with heart failure and death. Whether or not these biomarkers are associated with subclinical abnormalities in vascular function or cardiac structure is not established.

Methods: In a nested case-control study of MESA, we investigated cross-sectional associations of urine markers and 3 subclinical measures predictive of later cardiac events in MESA: large (LAE) and small artery elasticity indices (SAE), measured by arterial tonometry; and left ventricular mass to end-diastolic volume ratio (concentricity (CON)), measured by cardiac MRI. Urine albumin (ACR), neutrophil gelatinase-associated lipocalin (NGAL/cr), and kidney injury molecule-1 (KIM/cr), normalized to creatinine, were measured at baseline. We used inverse probability weighting to account for the study design. Using linear regression, we evaluated associations of quartiles of urine markers with log-transformed outcomes of LAE, SAE, and CON, adjusting for age, race/ethnicity, sex, height, pulse, blood pressure, smoking, medications.

Results: All 686 participants (37% white, 30% black, 26% Hispanic, 6% Asian) were free of cardiovascular disease at baseline. Mean (SD) age was 67 (8.7) years; median (IQR) baseline eGFR was 75.7 (69.8-82.4) ml/min/1.73m². Compared to the lowest quartile, higher quartiles of ACR were associated with worse LAE (Q4 v. Q1 relative difference 15%, 95% CI 7.7-21.8) and more severe CON (11.7%, 95% CI 4.5-19.3), but not with SAE. NGAL/cr and KIM/cr were not significantly associated with LAE, SAE, or CON.







Conclusions: Higher levels of urine ACR, but not NGAL or KIM-1, are associated with subclinical vascular and cardiac abnormalities. Arterial elasticity and cardiac remodeling do not readily explain the association between kidney injury and elevated cardiac risk.

Funding: NIDDK Support

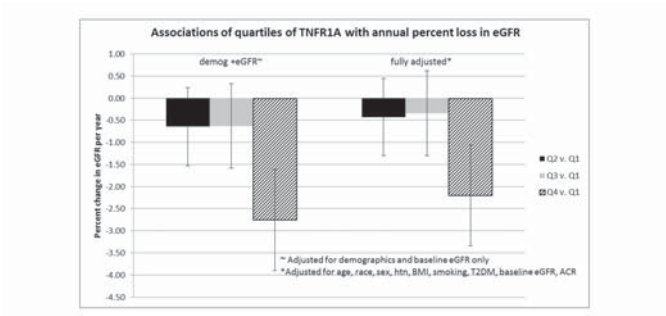
SA-PO322

TNF-Alpha Receptor 1 Is Associated with Kidney Function Decline in Persons with Coronary Artery Disease Meyeon Park, Eric Vittinghoff, Mary Whooley, Michael Shlipak. *UCSF.*

Background: Inflammation is a possible mechanism to explain the association between atherosclerosis and kidney disease. This study evaluated circulating tumor necrosis factor alpha receptor type 1 (TNFR1A), a marker of inflammation, as a risk factor for kidney function decline and albuminuria.

Methods: In the Heart and Soul Study, a cohort with established coronary artery disease (CAD), we measured TNFR1A from baseline serum samples and defined elevated levels of TNFR1A by the highest quartile (Q4, > 3.3 ng/ml). We calculated estimated glomerular filtration rate (eGFR) at baseline and after 5 years of follow-up using the CKD-EPI equation. We evaluated the associations of high TNFR1A with annual percent loss in eGFR, rise in urine albumin:creatinine ratio, rapid eGFR loss (>3% per year), and incident CKD (change from eGFR>60 to <60). We adjusted for age, race, sex, hypertension, smoking, diabetes, HDL, and medications.

Results: Among 629 participants who had TNFR1A measurements at baseline and follow-up measures of kidney function, median TNFR1A was 2.33 ng/ml (1.8-3.1). Higher levels of TNFR1A (Q4 v. Q1) were associated with a 1.94% (95% CI 1.03-2.86%) annual percent loss in eGFR (Figure) and rise in ACR (β=41.45, 95% CI 0.28-82.62, p=0.0016) in adjusted analyses. The highest TNFR1A quartile had similar associations with rapid kidney function loss (OR 3.66, 95% CI 2.03-6.6, p<0.001) and incident CKD (OR 1.89, 95% CI 0.89-4.02), although the latter outcome was non-significant after adjustment.



Conclusions: TNFR1A is independently associated with increased albuminuria and greater loss of kidney function longitudinally. These findings implicate inflammation as a potential contributor to the elevated kidney disease risk in persons with CAD.

Funding: NIDDK Support, Other NIH Support - NHLBI

SA-PO323

Validation of Molecular Signatures Linked to Asymmetric Dimethylarginine Dysregulation in Chronic Kidney Disease Varsha Pathak,[1] Wenjun Ju,[2] Viji Nair,[2] Matthias Kretzler,[2] Crystal A. Gadegbeku.[1] *[1]Temple Univ School of Medicine; [2]Univ of Michigan.*

Background: Asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of nitric oxide synthase, is implicated in endothelial dysfunction and accelerated atherosclerosis in patients with chronic kidney disease (CKD). Here, we validate and extend data on gene expression profiles from renal biopsy samples in a large CKD cohort, European Renal c-DNA Bank (ERCB).

Methods: Gene expression analysis was performed using Affymetrix 133 plus GeneChips on renal biopsy tissue obtained from consented individuals in ERCB. Steady state gene expression levels of PRMT 1, DDAH and oxidative stress markers (gp91-phox, p67-phox, mitochondrial [Mn] superoxide dismutase) were measured from the microdissected tubulo-interstitial (n=162) and glomerular (n= 143) compartments from renal cortical samples. Pearson correlations were used to compare enzyme profiles and eGFR (CKD-Epi equation) with adjustment for multiple comparisons.

Results: In the tubulo-interstitial compartment, PRMT1 was significantly inversely correlated with DDAH1 and positively correlated with the oxidative stress markers gp91-phox, p67-phox and Mn-SOD. These oxidative stress markers were inversely correlated with DDAH1. Further, eGFR correlated positively with DDAH1 and negatively with PRMT1 and oxidative stress markers. In the glomerular tissue, only the oxidative stress markers demonstrated significant associations and were inversely correlated with eGFR. Also, we observed a negative co-relation with DDAH1 and not DDAH2 (known to be expressed predominantly in vascular endothelium).

Compartment	Enzyme	gp91-phox	p67-phox	Mn-SOD	eGFR
Interstitium	PRMT1	0.40**	0.45**	0.44**	-0.37**
	DDAH1	-0.43**	-0.50**	-0.30**	0.29**
	eGFR	-0.43**	-0.45**	-0.43**	--
Glomerulus	PRMT1	0.4**	-0.42**	-0.39**	0.07
	DDAH1	-0.25**	-0.29**	-0.01	0.02
	DDAH2	-0.03	0.13	-0.16	-0.06
	eGFR	-0.4**	-0.42**	-0.4**	--
*p≤0.05, **p≤0.001					

Conclusions: Here, we confirm the relationships between renal tissue gene expression profiles of ADMA-related enzymes and renal function and provide further molecular evidence of the kidney's role in ADMA dysregulation.

Funding: NIDDK Support

SA-PO324

Endocan as a Biomarker of Endothelial Function in End Stage Renal Disease Vinod K. Bansal,[1] Debra Hoppensteadt,[2] Daneyal Syed,[2] Jawed Fareed.[2] *[1]Nephrology, Loyola Univ Medical Center; [2]Pathology, Loyola Univ Medical Center.*

Background: Endocan also described as endothelial cell specific molecule (ESM1) is a proteoglycan of 50kda which is highly upregulated in various vascular diseases. Endocan binds CD11a / CD18 integrin on human leukocyte. Pro-inflammatory mediators such as TNFalpha and proantiogenic molecules such as VEGF or FGF 2 stimulate the formation of this mediator. Elevated blood levels of endocan have been reported in patients with cancer (lung, kidney, blood), severe sepsis, and post-transplantation veno-occlusive disease. The purpose of this study is to measure endocan in ESRD patients to demonstrate its relevance to this disease.

Methods: Citrated plasma samples were collected from 85 patients who were on maintenance hemodialysis in the dialysis clinic at Loyola University Chicago Hospital. The control group represents 50 normal, drug free individuals. Endocan levels were measured in these plasma samples using a commercially available ELISA method (Lunginnov,Paris, France). VEGF and FGF 2 levels were also measured using commercially available ELISA methods (R&D, Minneapolis, Minnesota).

Results: Endocan levels were found to be significantly higher (p=<0.05) in the ESRD patients (2.6 + 1.3ng/ml) with a wide range (0.9 + 14.7 ng/ml) in contrast to normal (1.8 + 0.6ng/ml) with a narrower range of (1.3 + 3.4ng/ml). Of the 85 patients, 10 showed greater than 5 ng/ml of this biomarker. Both the VEGF level and FGF 2 levels were also elevated (2-5 folds) in the ESRD group in comparison to the normal. There was a poor correlation (r=<0.3) between the elevation of endocan with either VEGF and FGF 2.

Conclusions: Consistent with earlier reports endocan is elevated in various diseases with endothelial dysfunction. These studies suggest that this biomarker may be a useful prognostic indicator for ESRD. Contrary to the earlier reports the observed poor correlation between endocan and VEGF / FGF 2 may suggest independent regulation of endocan through other mechanisms. This data warrants additional studies to validate the relevance of this marker with the pathogenesis of ESRD.

SA-PO325

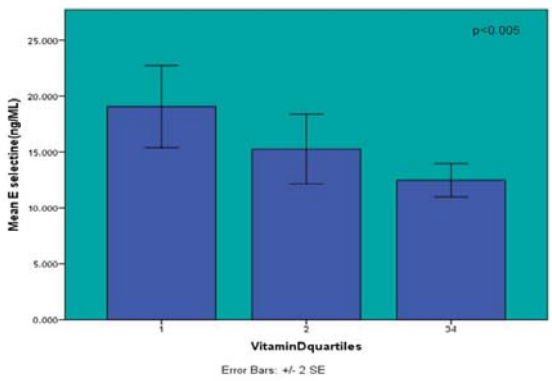
Vitamin D Deficiency and Biomarkers of Endothelial Function in Kidney Transplant Recipients Ashok Kumar Yadav,[1] Matthew Steel,[2] Raja Ramachandran,[1] Juan Carlos Kaski,[2] Vivekanand Jha,[1] Debasish Banerjee.[2] *[1]PGIMER, Chandigarh; [2]St. Georges, Univ of London.*

Background: Vitamin D deficiency is common in kidney transplant (KTx) recipients and associated with adverse outcomes. We have previously demonstrated abnormal endothelial function in KTx patients and worse endothelial cell biomarker profile in vitamin D deficient CKD patients, which improves with vitamin D supplementation without a change in FGF23 concentration. However the relationship between vitamin D deficiency and biomarkers of endothelial dysfunction in KTx recipients is unknown which the present study investigated.

Methods: We assessed 93 non-diabetic, stable KTx patients from Chandigarh, India, none on vitamin D therapy. Data on demographics, anthropometrics, and treatment were collected. Blood samples were stored at -70^{0}C until analysis for bone and endothelial cell biomarkers including FGF 23, iPTH and E-Selectin using standard ELISA techniques.

Results: Clinical characteristics were: age 37±10 years, 80% men, 23% past smokers, SBP 125±15 mmHg, DBP 78±10 mmHg, cholesterol 172±47 mg/dL, haemoglobin 13±2 g/dL, calcium 9.4±0.6 mg/d and iPTH 58±33 ng/mL and Vitamin D 36±39 nmol/L. Patients with Vitamin D <37.5 nmol/L (66%) had similar age, creatinine, phosphate, iPTH, BP but lower calcium (9.3±0.7 versus 9.6±0.5 mg/dL, p<0.05), lower FGF23 (59±73 versus 107±97 pg/mL, p<0.05) and higher E Selectin (16±8 versus 13±5 ng/mL, p<0.05). With increasing quartiles of Vitamin D concentration the levels of E Selectin decreased (p<0.005) and this was associated with rising levels of FGF23 (p<0.05).

Figure 1: Showing relationship of quartiles of Vitamin D and concentration of E Selectin



Legend: The concentration of E Selectin rises with decreasing Vitamin D, quartiles 3 and 4 are combined

Conclusions: This study demonstrates that Vitamin D deficiency is common in KTx recipients in North India, associated with low calcium, low FGF23 and high E Selectin. These findings set up the basis for further studies to assess whether vitamin D deficiency associated endothelial dysfunction play a role in CV complications in KTx recipients.

SA-PO326

Vitamin D Supplementation Improves Central Arterial Stiffness and Inflammatory Biomarkers in Patient with Chronic Kidney Disease Ashok Kumar Yadav,[1] Vivek Kumar,[1] Anupam Lal,[2] Manphool Singhal,[2] Vivekanand Jha.[1] *[1]Nephrology, Post Graduate Inst of Medical Education and Research, Chandigarh, India; [2]Radio-Diagnosis and imaging, Post Graduate Inst of Medical Education and Research, Chandigarh, India.*

Background: Cardiovascular abnormality is a significant cause of morbidity and mortality in patients with Chronic Kidney Disease (CKD). Vitamin D deficiency is common and associated with mortality in CKD patients. Vitamin D supplementation might mitigate the cardiovascular risk of CKD by favourably influencing endothelial and vascular function.We investigated the effect of vitamin D supplementation on arterial stiffness, and inflammatory and endothelial dysfunction biomarkers in patients with stage 3 and 4 CKD.

Methods: In this prospective randomized, placebo-controlled trial, 38 vitamin D deficient (<20ng/ml) CKD patients were randomized to placebo (n=16) and vitamin D (n=22) group. Subjects received 2 directly observed oral doses of 300,000 IU cholecalciferol at 0 and 8 weeks or matching placebo. Circulating vitamin D3, interleukin-6 (IL-6), highly sensitive C-reactive protein (hsCRP), Von Willebrand factor (VWF), e-selectin, fibroblast growth factor-23(FGF-23), pulse wave velocity (PWV) and augmentation index (AI) were analysed at baseline and week 16.

Results: Baseline characteristics of the two groups were similar. Two patients (one from each group) dropped out. Serum level of vitamin D3 increased in vitamin D group (17.04±5.4 versus 46.22±14.91, p<0.0001) whereas in placebo group no difference was noted (17.66±5.03 versus 17.47±10.37, p=0.95). There was significant decrease in IL-6 (p=0.05), FGF-23 (p<0.0001) and PWV (p=0.005) in vitamin D group. No difference in these parameters was noted in placebo group except FGF23 which showed increased level at follow-up (p=0.03).

Conclusions: Achievement of vitamin D sufficiency (>30ng/ml) by supplementation of oral vitamin D leads to improvement in markers of inflammation, FGF-23 and arterial stiffness.

Funding: Government Support - Non-U.S.

SA-PO327

Calcitriol Pretreatment Blunts the Permeability Inducing Effect of Vascular Endothelial Growth Factor Through Reducing the Expression and Protein Level of Caveolae- and Fenestrae Associated PV-1 Protein Adrienn Németh,[1] Csaba Bodor,[1] Sharokh Mirza Hosseini,[1] Laszlo Kohidai,[2] Laszlo Rosivall.[1] *[1]Dept of Pathophysiology, MTA-SE Pediatrics and Nephrology Research Group, Semmelweis Univ, Budapest, European Union, Hungary; [2]Dept of Genetics Cell and Immunobiology, Semmelweis Univ, Budapest, European Union, Hungary.*

Background: Vascular endothelium provides a semipermeable barrier that plays a pivotal role in both physiological and pathophysiological processes involving several endothelial structures including fenestrae and caveolae. VEGF was initially described as a potent endothelial permeability-increasing factor involved in tumor development, hypertension and diabetic nephropathy. Hence VEGF is known to induce endothelial fenestration and the expression of plasmalemma vesicle associated protein (PV-1), which is the key structural component in fenestrae and caveolae formation. While calcitriol, the most active form of vitamin D, is an anti-angiogenic and anti-inflammatory agent, it has beneficial effects on glomerulosclerosis and hypertension. We hypothesized that pretreatment with calcitriol may modulate the VEGF-induced increase in permeability of human umbilical vein-derived endothelial cells(HUVEC).

Methods: Cells were pretreated with 0,1 nM calcitriol for 30 min and/or with 100 ng/ml VEGF for 48hours. Permeability of endothelial monolayers was determined by using 40 kDa FITC-dextrane. The mRNA expression and protein levels of PV-1 were measured by real-time PCR and western blot. The activation (phosphorylation) of VEGFR-2 at tyrosine 1175 was detected by western blot.

Results: Pretreatment with calcitriol reduced the VEGF-induced increase in permeability, moreover, calcitriol lowered PV-1 protein and mRNA levels. Calcitriol prevented the VEGF-induced activation of VEGFR-2 by inhibiting the phosphorylation of tyrosine 1175.

Conclusions: We assume that the VEGF-induced increase in permeability of HUVEC is the consequence of increased PV-1 expression. Reduction in PV-1 protein expression and endothelial permeability by calcitriol pretreatment may ameliorate endothelial function in hypertension and glomerulosclerosis.

SA-PO328

VDR Deficiency Aggravates Atherosclerosis: A Possible Role for Endothelial NF-kB Activation Governing the Onset of an Early Leukocyte-Endothelium Interplay Milica Bozic,[1] Carmen de Pablo Bernal,[2] Angeles Alvarez Ribelles,[2] Maria D. Sanchez-Niño,[3] Alberto Ortiz,[3] Elvira Fernandez,[1] Jose M. Valdivielso.[1] *[1]IRBLleida; [2]Univ de Valencia; [3]Fundacion Jimenez-Diaz.*

Background: Cardiovascular mortality is very high among CKD patients. Endothelial cell activation plays an essential role in atherosclerosis. Vitamin D has been proposed to have an important role in cardioprotection. The aim of this study was to evaluate the effect of vitamin D receptor (VDR) deficiency on atherogenesis and the possible mechanisms.

Methods: ApoE-VDRdouble-knockout (DKO) mice were generated and fed an atherogenic diet. Atherosclerotic burden was quantified. Cellular composition of the atherosclerotic lesions was assessed. Expression of VCAM-1, ICAM-1 and IL-6 mRNA in the aorta was analyzed. Knockdown of VDR in human endothelial cells was performed by shRNA. Interactions between peripheral blood mononuclear (PBMC) cells and endothelial cells *in vitro* were evaluated.

Results: Serum total cholesterol, HDL, LDL and triglycerides were significantly lower in DKO than in apoE-/-. However, DKOs showed larger plaques. DKO plaques had fewer smooth muscle cells and more macrophages, as well as higher immunoreactivity for MCP-1 and F4/80. Serum levels of IL-6 were significantly higher in DKOs compared with apoE-/-. Furthermore, DKOs showed significantly higher expressions of VCAM-1 and ICAM-1 and IL-6 in the arteries. Knockdown of VDR in endothelial cells led to a decrease in PBMC rolling velocity and an increase in PBMC rolling flux and adhesion to the endothelium, together with an upregulation of VCAM-1 and ICAM-1. The levels of κB inhibitor (IκB) α were decreased in sh-VDR cells compared with controls. Level of p65 in the nuclear extracts was higher in sh-VDR cells than in controls. The IκB kinase inhibitor, PS-1145, prevented upregulation of VCAM-1 and ICAM-1 in endothelial cells carrying shRNA targeting VDR.

Conclusions: Endothelial NF-kB activation in cells lacking VDR leads to endothelial dysfunction with pronounced leukocyte-endothelial interactions, which may lead to bigger plaques in DKOs. Data reveal an important role for basal levels of endothelial VDR in limiting endothelial cell dysfunction and atherosclerosis.

Funding: Government Support - Non-U.S.

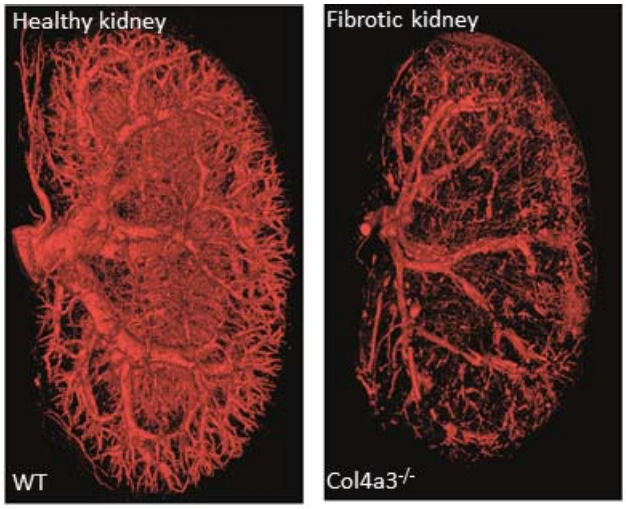
SA-PO329

Imaging of Renal Vasculature in Murine Kidney Fibrosis Peter Boor, Josef Ehling, Janka Babickova, Barbara Mara Klinkhammer, Felix Gremse, Fabian Kiessling, Jürgen Floege, Twan Lammers. *RWTH Univ, Aachen, Germany.*

Background: Renal fibrosis is associated with rarefaction of the renal vasculature and this might contribute to fibrosis progression. The aim of this study was to establish anatomical, functional and molecular imaging of renal vessels in fibrosis.

Methods: We analyzed three murine fibrosis models: unilateral ureteral obstruction (days 1-10), unilateral ischemia-reperfusion (days 1-56) and Alport mice (6 and 8 weeks old). We assessed renal blood volume using *in vivo* contrast-enhanced micro-computed tomography (μCT), analyzed vasculature using *ex vivo* high-resolution μCT after perfusion with a polymerizing cast agent, and used an elastin-specific contrast agent and magnetic resonance imaging (MRI) for molecular imaging of vessels and fibrosis.

Results: Functional *in vivo* μCT showed an early, significant, and progressive reduction of renal blood volume in all models. These findings correlated with peritubular capillary rarefaction. In 3D rendered renal vasculature obtained by high-resolution μCT we found in all three models a significant reduction in the number and diameter of renal vessels, vascular branching, and a significantly increased vessel tortuosity (fig. 1). *In vivo* a stronger MRI signal was found in fibrotic kidneys of mice with UUO compared to healthy kidneys using the elastin-specific probe. The up-regulation of elastin in fibrotic kidneys was confirmed on mRNA and protein level.



Conclusions: Using new methods to assess the murine renal vasculature, we observed a significant vascular rarefaction and pathological alteration in different models of fibrosis. Increased MRI signals using an elastin-specific probe suggests this to be a potential molecular imaging approach in renal fibrosis. These methods lay the basis for better understanding of renal fibrosis and may become novel outcome parameters in intervention studies targeting progressive renal disease.

Funding: Government Support - Non-U.S.

SA-PO330

Alterations of Peritubular Capillaries in Experimental Renal Fibrosis Peter Boor, Janka Babickova, Eva Miriam Buhl, Sonja Djudjaj, Barbara Mara Klinkhammer, Jürgen Floege. *RWTH Univ, Aachen, Germany.*

Background: Renal fibrosis is associated with rarefaction of peritubular capillaries (PTCs). However, functional and ultrastructural alterations of the renal microvasculature in renal fibrosis are not well described.

Methods: We studied three murine models of fibrosis with distinct mechanisms of injury, i.e. unilateral ureteral obstruction (UUO, day 1, 3 and 5), unilateral ischemia-reperfusion injury (IR, day 14 and 21) and Col4A3 deficient (Alport) mice. In all models we quantified PTC to tubule ratios using immunohistochemistry, analyzed vascular leakage using Evans Blue dye and fibrinogen extravasation and ultrastructure by electron microscopy.

Results: Compared to healthy kidneys, we found significantly lower numbers of PTC in UUO day 3 (-11%) and day 5 (-16%), but not day 1. Capillary rarefaction was also observed after IR injury on both days 14 (-12%) and 21 (-17%). Compared to healthy kidneys, we observed significantly higher extravasation of Evans blue in UUO day 3 (+250%) and day 5 (+167%), but not day 1, as well as in IR injury day 14 (+460%) and day 21 (+157%) and in Alport mice (+100%). Compared to healthy kidneys, we found significantly more interstitial deposition of fibrinogen in the fibrotic kidneys in all three models (+109 to +459%). In fibrotic kidneys, ultrastructural studies revealed loss of fenestrations, increased thickness of endothelial cell soma and of lamina densa of the PTC basal membrane.

Conclusions: Independent of the underlying mechanism, all fibrosis models were characterized by progressive loss of renal microvasculature, a significant increase in vascular leakage and substantial alterations of the endothelial ultrastructure. These data show that renal fibrosis not only involves loss of PTC but also significant functional alterations of remaining capillaries.

Funding: Government Support - Non-U.S.

SA-PO331

Platelet-Derived Growth Factor CC and Capillary Rarefaction in Experimental Renal Fibrosis Peter Boor,[1] Janka Babickova,[1] Ina V. Martin,[1] Claudia R.C. van Roeyen,[1] Frank Eitner,[3] Jürgen Floege,[1] Carine Peutz-Kootstra,[2] Tammo Ostendorf.[1] *[1]RWTH Univ, Aachen, Germany; [2]Maastricht Univ, Netherlands; [3]Bayer Pharma AG, Germany.*

Background: Renal fibrosis is associated with progressive capillary rarefaction. Neutralization of platelet-derived growth factor (PDGF)-CC potently reduced kidney fibrosis. However, PDGF-CC has also been shown to be a potent proangiogenic factor in experimental glomerular diseases. Here we asked, whether PDGF-CC neutralization might have potentially deleterious anti-angiogenic effects during renal fibrosis, i.e. promote capillary rarefaction.

Methods: We analyzed capillary rarefaction in a model of renal fibrosis, unilateral ureteral obstruction (UUO) in genetically PDGF-C-deficient mice or by using neutralizing PDGF-CC antibodies.

Results: Lack or antagonism of PDGF-CC significantly reduced renal fibrosis. In contrast, extensive quantification yielded in a similar number of peritubular capillaries in control mice compared to mice with PDGF-CC-deficiency or -neutralization in both healthy and fibrotic kidneys (apart from significantly preserved peritubular capillaries in PDGF-CC knock-out mice in UUO day 5). Similarly, no difference in microvascular leakage (assessed by extravasation of Evans Blue) was found between the WT and PDGF-CC knock-out mice in UUO day 5. Expression of angiogenic molecules, such as VEGF, VEGF receptors 1 and 2 and angiopoietins 1 and 2 was similar in these mice, whereas FGF-2 was significantly reduced in mice with PDGF-CC deficiency or neutralization. In healthy mice or the contralateral non-obstructed kidneys the antagonism or deficiency of PDGF-CC had no effect on the number or functionality of the tubulointerstitial microvasculature.

Conclusions: Despite a significant reduction of renal fibrosis, PDGF-CC deficiency was not associated with an accelerated loss of peritubular capillaries, suggesting that in the fibrotic renal tubulointerstitium, proangiogenic effects of PDGF-CC are much less potent than its pro-fibrotic properties. Thus PDGF-CC's proangiogenic effects do not limit its therapeutic antagonism in renal fibrosis.

Funding: Government Support - Non-U.S.

SA-PO332

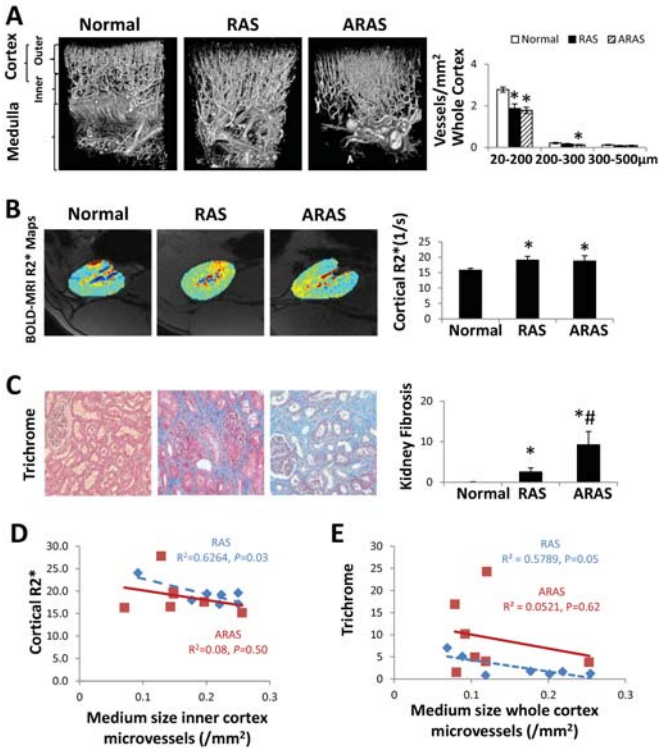
Atherosclerosis Aggravates Renal Microvascular Loss and Fibrosis in Swine Renal Artery Stenosis Dong Sun, Behzad Ebrahimi, Alfonso Eirin, Stephen C. Textor, Amir Lerman, Lilach O. Lerman. *Mayo Clinic.*

Background: Renal function in patients with combined atherosclerosis and renal artery stenosis (RAS) deteriorates more than in non-atherosclerotic RAS. Whether microvascular loss is a comparable determinant of renal dysfunction in RAS and atherosclerotic RAS (ARAS) remains unclear. We hypothesized that ARAS aggravates stenotic kidney loss of microvessels and fibrosis compared to RAS.

Methods: Domestic pigs were randomized to Normal, RAS, and ARAS groups (n=7 each). Stenotic kidney oxygenation was determined 10 wks later using blood oxygen-level dependent (BOLD) magnetic resonance imaging (MRI). Single-kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were evaluated using multidetector CT (MDCT), and microvascular density by Micro-CT.

Results: Mean blood pressure in RAS and ARAS groups was elevated compared with normal (117.5±10.7 and 127.4±10.9 versus 98.4±1.6 mmHg, respectively, $P<0.05$), and RBF and GFR were similarly decreased in their stenotic kidneys. RAS decreased the

density of small-size cortical microvessels (diameter <200 μm), while ARAS extended the decrease to medium-sized microvessels (200-300 μm) (Fig. A). Cortical R2* (BOLD-MRI hypoxia index) increased in both RAS and ARAS (Fig. B), but correlated inversely with microvascular density only in RAS (Fig. D). Interstitial fibrosis was higher in ARAS than in both Normal and RAS cortex (Fig. C), but correlated inversely with microvascular density only in RAS (Fig. E).



A-Micro-CT images of Microvascular Architecture; **B**-Measurement of Kidney Oxygenation by MRI; **C**-Trichrome staining for renal fibrosis; **D** and **E**-Correlation analysis.
*P<0.05 compared with Normal; #P<0.05 compared with RAS.

Conclusions: Atherosclerosis aggravates loss of stenotic-kidney cortical microvessels, yet unlike RAS, additional determinants may contribute to cortical hypoxia and fibrosis in swine ARAS.

Funding: NIDDK Support

SA-PO333

A Novel Role for Renal NMDA Receptors: Regulation of the Renal Microcirculation Kadeshia N. Dunn,[1] Edward W. Inscho,[2] Scott S.P. Wildman,[1] Claire M. Peppiatt-Wildman.[1] *[1]Medway School of Pharmacy, The Univs of Kent and Greenwich at Medway, United Kingdom; [2]Physiology, Georgia Regents Univ.*

Background: Glutamate and GABA receptors have been detected throughout the kidney, although the precise role(s) these play in the kidney remain unclear. Previously we have reported that glutamate and its substrate, GABA, are involved in the regulation of the renal microcirculation [1]. Specifically, we observed that GABA and glutamate induce vasoconstriction of afferent arterioles in the renal cortex, whereas in the medulla, glutamate evokes vasodilation and GABA evokes vasoconstriction of vasa recta capillaries via their actions at contractile pericytes.

Methods: It is well established that pericytes regulate vasa recta diameter and thus medullary blood flow. We have utilised a rat live kidney slice model to investigate glutamate-evoked vasodilation of vasa recta capillaries [2].

Results: We tested a panel of glutamate receptor antagonists (MK-801, GYKI, MSOP, LY341495, UBP302) and found that only the NMDA receptor antagonist, MK-801 (300 uM, n=11), significantly attenuated glutamate-evoked vasodilation mediated via pericytes. Accordingly, NMDA alone evoked a pericyte-mediated vasodilation of vasa recta via pericytes, similar to that seen with glutamate. In addition, glycine (1 mM), a co-agonist for glutamate NMDA receptors, evoked a pericyte-mediated vasodilation of vasa recta (16.5 ± 1.1%, n=6). Interestingly, MK-801 (300 uM, n=6) significantly attenuated glycine-evoked vasodilation of vasa recta via pericytes. In parallel experiments in the cortex, glycine (100 nM – 1 mM) evoked vasodilation of afferent arterioles, which was disparate to the glutamate mediated-constriction previously reported.

Conclusions: In conclusion, we provide evidence to suggest that glutamate-mediated vasodilation of vasa recta occurs via NMDA receptors, presumably expressed on pericytes. Moreover, we provide novel observations regarding the differential roles of glutamate and glycine in the regulation of afferent arteriole diameter. The mechanisms underlying these observations require further delineation. References [1] Dunn et al, 2013 FASEB J. 27, 1110.13. [2] Crawford et al, 2012 Nephron Physiol. 120, 17-31.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO334

The Effect of L-Arginine on Cutaneous Microvascular Function Is Inversely Related to Blood Urea Nitrogen in Patients with Stage 3-4 Chronic Kidney Disease Meghan G. Ramick,[1] Jennifer DuPont,[1] William B. Farquhar,[1] Raymond R. Townsend,[2] David G. Edwards.[1] *[1]Dept of Kinesiology and Applied Physiology, Univ of Delaware, Newark, DE; [2]Clinical and Translational Research Center, Univ of Pennsylvania, Philadelphia, PA.*

Background: Either a relative or absolute deficit of the nitric oxide (NO) precursor L-arginine likely contributes to reduced NO synthesis in chronic kidney disease (CKD) however the effectiveness of L-arginine in studies of endothelial dysfunction in CKD has been mixed. This may be due to inhibition of L-arginine uptake by urea and other uremic toxins as demonstrated in cell studies. The purpose of this study was to determine the effect of local L-arginine delivery on cutaneous microvascular function in patients with CKD. Further, we investigated whether the effect of L-arginine in CKD was related to blood urea nitrogen (BUN) levels.

Methods: Ten patients with stage 3-4 CKD (62±4 years; 4M/6F; eGFR: 38±5 ml·min^{-1}·1.73m^{-2}) and 10 healthy controls (HC; 55±2 years; 4M/6F; eGFR: 90±6 ml·min^{-1}·1.73m^{-2}) were instrumented with 2 intradermal microdialysis fibers for the local delivery of Ringer's solution and 10 mM L-arginine. Red blood cell (RBC) flux was measured via laser Doppler flowmetry. A standardized non-painful local heating protocol (42°C) was used. Cutaneous vascular conductance (CVC) was calculated as RBC flux/MAP and all data were expressed as a percentage of the maximum CVC at each site (28 mM sodium nitroprusside, 43°C).

Results: The plateau %CVCmax, which is largely mediated by NO, was attenuated in CKD (CKD: 80±3 versus HC: 89±1 %CVCmax; $p<0.05$) and augmented with local L-arginine delivery (L-arg: 88±2 versus Ringer's: 80±3 %CVCmax; $p<0.05$) such that it did not differ from HC ($p>0.05$). The difference in plateau %CVCmax between L-arginine and Ringer's sites was variable in CKD (range: -8.5 to 20.3) and was related to eGFR ($r=0.46$, $p<0.05$) and inversely related to BUN ($r=0.63$, $p<0.01$).

Conclusions: These data suggest that the effectiveness of L-arginine in restoring vascular function in patients with CKD may be dependent on the level of BUN and other uremic toxins, which may explain why L-arginine is ineffective in some patients with CKD.

Funding: Other NIH Support - R01 HL104106

SA-PO335

Nicorandil Prevents Endothelial Dysfunction, Renal Dysfunction, and Anemia in Dahl Salt-Sensitive Hypertensive Rats Ken-Ichi Serizawa, Kenji Yogo, Yoshihito Tashiro, Ken Aizawa, Koichi Endo. *Product Research Dept, Chugai Pharmaceutical Co., Ltd., Gotemba, Japan.*

Background: Cardiorenal anemia syndrome (CRAS) is closely associated with extremely poor outcomes in patients with heart failure, and chronic kidney disease is an important factor in its development. Dahl salt-sensitive (DS) hypertensive rats incurs heart failure, renal failure, and anemia by salt loading. Nicorandil, used to treat angina and acute heart failure, is reported to improve the prognosis of patients with ischemic heart disease. This study examined the beneficial effects of nicorandil on multiple disorders in salt-loaded DS rats.

Methods: Male DS rats (6 wks old) were divided into 3 groups: DS-NS (fed normal-salt diet), DS-HS (fed high-salt diet containing 8% NaCl), and DS-HS+nicorandil. Nicorandil (15 mg/kg/day) was administered concurrently with salt loading. After 7 wks of treatment, urine was collected from individual metabolic cages and jugular vein blood was sampled to evaluate renal function and anemia. Then, as a marker of cardiovascular risk, endothelial function was assessed by ultrasound measurement of flow-mediated dilation (FMD) in femoral arteries.

Results: FMD was lower in DS-HS rats than in DS-NS rats (DS-NS, 24.0±2.7%; DS-HS, 9.4±1.6%; n=6–7). Renal function as evaluated by urinary protein excretion (DS-NS, 16.0±1.9 mg/day; DS-HS, 342.0±38.1 mg/day; n=8–10) and creatinine clearance (46.5±5.7%, % of DS-NS, n=8) was also reduced in DS-HS rats. Furthermore, DS-HS rats developed anemia (hemoglobin: DS-NS, 15.4±0.1 g/dL; DS-HS, 11.1±1.4 g/dL; n=9–10). Nicorandil prevented the reduced FMD (16.8±1.6%, n=9), the increased urinary protein excretion (165.6±20.7 mg/day, n=10), the decreased creatinine clearance (80.8±7.9%, % of DS-NS, n=10), and anemia (hemoglobin: 15.2±0.4 g/dL, n=8). Systolic blood pressure was increased in DS-HS rats, and nicorandil showed no influence on systolic blood pressure (DS-NS, 133.4±2.8 mmHg; DS-HS, 215.9±8.3mmHg; DS-HS+nicorandil, 218.5±4.8 mmHg; n=6–9).

Conclusions: These results suggest that nicorandil prevented endothelial dysfunction, renal dysfunction, and anemia in salt-loaded DS rats, and are expected to clarify how nicorandil will affect the prognoses of patients with CRAS.

SA-PO336

3D Modeling and Analysis of the Human Glomerular Capillary Network
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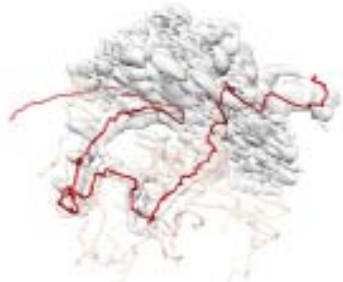
Background: 3D modeling of the glomerulus can provide information about the physiology of glomerular filtration and perfusion and reveal deviations from the normal renal vasculature that occur with glomerular diseases. We developed a technique that enables 3D reconstruction of the glomerular capillary lumen structure. The models generated using this technique allow analysis of network topology and provide detailed renderings of the interior of the glomerulus, which is information that cannot be obtained by scanning electron microscopy.

Methods: Digital photomicrographs of serial one-micron thick tissue sections that were stained with Toluidine blue of an entire normal human glomerulus were obtained by light microscopy. The serial images were registered using MIDAS (Boulder, CO) and segmented semi-automatically using TrakEM2 and ImageJ (Bethesda, MD) using a Fast-Marching algorithm and binary thresholding. 3D renderings of the model were created using UCSF Chimera.

Results: Topological analysis shows that there are numerous parallel paths that red blood cells can travel from the afferent to the efferent arteriole and one possible path (red pipe) is shown.



The transparent surface models glomerular capillary lumina. The orange pipes represent the skeleton of the capillary network. There are also numerous anastomoses along these parallel paths. We found no path that bypassed the glomerular filtration system.

Conclusions: Modeling of additional normal glomeruli will help establish the conserved and variable architecture of the capillary structure. 3D modeling of diseased glomeruli can provide important insights into the pathophysiologic alterations that may occur in a variety of glomerular diseases.

Funding: NIDDK Support

SA-PO337

FIR-Induced Autophagy in Vascular Endothelia Cells Protects Vascular Endothelium From Advanced Glycation End Products-Induced Injury
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The accumulation of advanced glycation end products (AGEs) in diabetic patients can trigger apoptotic changes in vascular endothelial cells and contribute to the pathogenesis of diabetes mellitus and atherosclerosis. Far-infrared radiation (FIR) therapy has been proved to be effective in increasing patency of dialysis vascular access. In this study, we investigate the protective effects of FIR on AGEs-induced injury in vein endothelial cells in vitro and in vivo. We found that FIR irradiation induced autophagy in human umbilical vein endothelial cells (HUVECs). FIR irradiation induced the expression of autophagic markers, such as Beclin-1 and LC3-II. FIR irradiation also induced nuclear translocation of promyelocytic leukaemia zinc finger (PLZF) and the expression of phosphatidylinositol-3 kinase (PI3K) class III, an important kinase in autophagic signaling pathway. PLZF siRNA transfection inhibited FIR-induced autophagy. Immunofluorescence staining showed that AGE-bovine serum albumin (BSA) was engulfed by HUVECs and presented at lysosomes. FIR irradiation promoted AGE-BSA degradation in HUVECs, which was inhibited by autophagy inhibitor 3-methyladenine. In streptozotocin-induced type 2 diabetic mice, blood AGEs and MCP-1 significantly increased. FIR therapy dose-dependently reduced blood AGEs and MCP-1. FIR therapy also reduced AGE deposition, inducible nitric oxide synthase expression and leukocyte infiltration in the intestinal vascular endothelium. Aorta ring relaxation analysis revealed that FIR therapy improved vascular endothelia function in diabetic mice. However, we can't found these protective effects of FIR therapy in PLZF-knockout mice. Our data suggest that FIR-induced autophagy in vascular endothelia cells protects vascular endothelium from AGE-induced inflammation in diabetic mice.

Funding: Government Support - Non-U.S.

SA-PO338

PPAR-β Agonist Protects the Tight Juntions of Glomerular Endothelial Cells Through RhoA/ROCK1 Pathway
Zengchun Ye, Wei-Yan Lai, Canming Li, Tan-Qi Lou. *Dept of Nephrology, The Third Affiliated Hospital of Sun Yat-sun Univ, Guangzhou, Guangdong, China.*

Background: The destruction of junctions between adjacent glomerular endothelial cells (GEnCs) can lead to increased glomerular permeability, which may be one of the most important mechanisms of early proteinuria in diabetic kidney disease. In our previous study, we found that treatment of GEnCs with advanced glycation end products(AGEs) can reduce the expression of occludin and JAM-A, while increased rGEnCs permeability. The present study is to investigate whether the agonist of peroxisome proliferator activated receptor β(PPAR-β) L165041 can reverse the damage of tight junctions of rGEnCs caused by AGEs, and the role of RhoA/ROCK1 pathway.

Methods: The rGEnCs were pretreated with inhibitor of ROCK and PPAR-β agonist before AGEs treanment. RNAi of PPAR-β or ROCK-1 was also used to study the role of PPAR-β and ROCK-1. JAM-A, Occludin, ROCK1, PPAR-β and phosphorylation of MYPT1 were detected by western blotting. The endothelial permeability was investigated by transendothelial electrical resistance and the flux of FITC-conjugated bovine serum albumin. The activity of RhoA was evaluated by pull-down assay. Immunofluorescence was used to demonstrate the distribution of the tight junction proteins.

Results: AGEs reduced the expression of occludin and JAM-A, and increased the rGEnC monolayer permeability. Inhibition of PPAR-β by siRNA can promote the destruction of the tight junctions cause by AGEs. Treatment of GEnCs with AGEs activated RhoA/ROCK1 pathway and increased the phosphorylation of MYPT1 in rGEnCs, while PPAR-β agonist L165041 pre-treatment can block these effects and protect the tight junction and endothelial permeability.

Conclusions: The agonist of PPAR-β can protect the tight juntions of glomerular endothelial cells from the damage of AGEs partly through RhoA/ROCK1 pathway. * Zengchun Ye and Weiyan Lai contributed equally to this work.

Funding: Government Support - Non-U.S.

SA-PO339

Targeting CKD-Associated High-Density Lipoproteins-microRNA Intercellular Communication to Treat Atherosclerosis
Valentina Kon, Carrie B. Wiese, Jianyong Zhong, T. Alp Ikizler, Kasey C. Vickers. *Vanderbilt.*

Background: In addition to cholesterol, high density lipoproteins (HDL) transport other cargo, including proteins and nucleic acids. Recently, we found that HDL transport and deliver functional microRNAs (miRNAs) to recipient cells where they regulate gene expression through post-transcriptional regulatory modules. We also showed that HDL's anti-inflammatory capacity is mediated by HDL-miRNA suppression of adhesion molecules. We now compare HDL-miRNA signatures and transfer ability in patients with ESRD on hemodialysis (ESRD) and matched controls (C). We also use a subtotal nephrectomy mouse model (5/6Nx) to assess HDL delivery to endothelial cells and the impact of blocking miRNAs on atherosclerosis.

Methods: Real-time PCR-based OpenArrays were used to profile HDL-miRNAs of ESRD (n=12) and C (n=12). Human coronary artery endothelial cells (HCAEC) were used as recipient cells in HDL-miRNA transfer studies. Apolipoprotein E-deficient mice (n=14) underwent 5/6Nx and 7 wks later were injected with HDL alone or complexed with locked-nucleic acid (LNA) miR-92a inhibitors (20/mg/kg) followed by gene expression studies and phenotypic characterizations of plaques 1 wk later.

Results: Compared to C, HDL of ESRD have 8.5-fold increase in miR-92a levels ($p=0.001$), a pro-atherogenic miRNA in endothelial cells. Further, uremic HDL increased HCAEC miR-92a ($p<0.05$) with concomitant down-regulation of 18 putative miR-92a targets. Likewise, HDL of 5/6Nx have >20-fold increase in miR-92a ($p<0.05$), versus controls. *In vivo*, HDL complexed with LNA-92a, but not HDL alone, did not affect blood pressure but decreased miR-92a levels in aortic endothelium and decreased Oil Red O staining for atherosclerosis by 21% ($p<0.05$) along with greater macrophage (MOMA) and collagen content (Masson).

Conclusions: HDL intercellular communication is significantly altered in ESRD, which results in increased pro-atherogenic miR-92a levels in arterial endothelial cells. Inhibition of HDL-miR-92a transfer and activity in CKD-induced atherosclerosis decreased atherosclerotic burden. Thus, nucleic-acid based therapies may be a viable approach to attenuate cardiovascular disease in ESRD.

Funding: Other NIH Support - Heart, Lung, and Blood

SA-PO340

The Renal Microvasculature Is a Primary Target for Blood Pressure Regulation by Inorganic Nitrate and Nitrite
Xiang Gao,[1] Ting Yang,[2] Eddie Weitzberg,[2] Jon Lundberg,[2] Erik G. Persson,[1] Mattias Carlstrom.[2] *[1]Dept Medical Cell Biology, Uppsala Univ, Uppsala, Sweden; [2]Dept Physiology and Pharmacology, Karolinska Instt, Stockholm, Sweden.*

Background: Oxidative stress and nitric oxide (NO) deficiency in kidney are key events in development of hypertension. Stimulation of a nitrate-nitrite-NO pathway reduces blood pressure, but the mechanism(s) are not clear. We investigated the hypothesis that inorganic nitrate and nitrite attenuates reactivity of renal microcirculation and blood pressure responses to angiotensin II (ANG II) by modulating NADPH oxidase activity and NO bioavailability.

Methods: Isolated and perfused mice renal afferent arterioles were performed, then arteriolar diameters changes were measured to estimate the effect of vasoactive substances.

Fluorescent detection of NO production in afferent arterioles, renal NADPH oxidase activity analysis, renal NADPH oxidase expression analysis, plasma analysis, telemetric study for blood pressure response to long-term Ang II infusion were also taken.

Results: Nitrite dilated afferent arterioles in the physiological concentration range (0.1-10 μM), and this dilatation was associated with increased NO formation. Contractions to ANG II alone and during inhibition of NO synthase were attenuated by nitrite. Abnormal ANG II responses in arterioles from SOD-1 deficient mice were normalized by simultaneous nitrite administration. The effect of nitrite was abolished by NO scavenger (cPTIO) and by xanthine oxidase inhibitor (oxypurinol). In the presence of NADPH oxidase inhibitor apocynin, no effect of nitrite on ANG II-induced contractility was observed, suggesting this enzyme as a possible target. In preglomerular vascular smooth muscle cells and in kidney cortex, nitrite dose-dependently reduced both basal and ANG II-induced NADPH oxidase activity. Supplementation with dietary nitrate (10 mM, 7 days) reduced renal NADPH oxidase activity and attenuated ANG II-mediated arteriolar contractions. Finally, dietary nitrate markedly attenuated gradual hypertension induced by chronic ANG II infusion in rats.

Conclusions: All findings position NADPH oxidase in renal microvasculature as a prime target for blood pressure-lowering effects of inorganic nitrate and nitrite.

Funding: Government Support - Non-U.S.

SA-PO341

Metabolomics of Renal Venous Plasma from Individuals with Unilateral Renal Artery Stenosis and Essential Hypertension Eugene P. Rhee,[1,2] Clary B. Clish,[2] Kerry A. Pierce,[2] Ahmed Saad,[3] Lilach O. Lerman,[3] Stephen C. Textor.[3] *[1]Nephrology Div, Massachusetts General Hospital, Boston, MA; [2]Metabolite Profiling, Broad Inst, Cambridge, MA; [3]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Comparing venous effluent from both kidneys of individuals with unilateral atherosclerotic renal artery stenosis (ARAS) provides an opportunity to directly examine how impaired renal blood flow impacts renal metabolic function in humans.

Methods: We applied liquid chromatography-mass spectrometry based metabolite profiling to venous plasma obtained from the stenotic (STK) and contralateral (CLK) kidneys of ARAS patients (n=16) and both kidneys of essential hypertensive (EH) controls. Study samples were acquired during a 3-day protocol that included radiographic kidney phenotyping (Duplex ultrasound, multidetector CT, and blood oxygen-level dependent MRI) and controlled sodium and caloric intake and anti-hypertensive treatment.

Results: Partial least squares-discriminant analysis demonstrated clear separation of EH kidney metabolite profiles versus STK and CLK metabolite profiles, but no separation between metabolite profiles of STK and CLK samples. All of the discriminating metabolites were similarly elevated in the STK and CLK samples, likely reflecting the lower GFR in the ARAS versus EH subjects (mean 66.1versus 89.2 mL/min/1.73m^2). In a paired analysis within the ARAS group, no metabolite was significantly altered in STK compared to CLK samples; notably, creatinine was the same in STK and CLK samples (STK/CLK ratio = 1.0, P=0.9). Results were unchanged in an examination of ARAS subjects in the bottom half of renal tissue perfusion or oxygenation.

Conclusions: Metabolite profiling does not differentiate venous effluent from STKs or CLKs in individuals with unilateral ARAS, despite measurable loss of kidney volume and blood flow on the affected side. These findings are consistent with the kidney's ability to adapt to ARAS to maintain a range of metabolic functions.

Funding: NIDDK Support, Pharmaceutical Company Support - Satellite Healthcare (Extramural Grant Program)

SA-PO342

Albuminuria Is Associated with the Loss of Endothelial Glycocalyx in Diabetic Mice – A Quantitative Electron Microscopy Analysis Using Cationic Hydrous Thorium Dioxide Colloids ($cThO_2$) Jan Hegermann,[1] Putri Andina Agustian,[2] Anna Bertram,[2] Nelly Shushakova,[2] Joon-Keun Park,[2] Torsten Kirsch,[2] Jan Menne,[2] Hermann G. Haller.[2] *[1]Hannover Medical School, Dept of Anatomy, Hannover, Germany; [2]Hannover Medical School, Clinic of Nephrology and Hypertension, Hannover, Germany.*

Background: The endothelium is covered by the glycocalyx. Damage of the glycocalyx enhances inflammation and permeability in diabetes. The analysis of the glycocalyx is difficult because of (1) its complex structure and (2) its instability ex-vivo. We have therefore established novel electron microscopy techniques to test the hypothesis that hyperglycemia damages the endothelial glycocalyx and that this damage is associated with albuminuria.

Methods: StSTZ-treated mice and control animals were analyzed after 4 and 8 weeks of hyperglycemia. Urinary albumin was measured by ELISA. Immunohistochemistry was performed on cryostat or on paraffin sections. Glycocalyx was assessed by (1) quantitative electron microscopy using cationic hydrous thorium dioxide colloids ($cThO_2$), (2) staining for alcian-blue, and (3)perfusion-fixation with lectins.

Results: Lectin-staining and alcian-blue showed a continuous linear lining of the capillary endothelium. In contrast, the EM analysis revealed a bushel-like structure of the glycocalyx with a spacing varying between 30 and 100 nm. These structures were regularly aligned. Hyperglycemia induced a rapid loss (4 weeks) of lectin staining. Both, the size and the number of glycocalyx "bushels" were significantly reduced by hyperglycemia.

Conclusions: We show here for the first time the use of $cThO_2$ to visualise the endothelial glycocalyx in glomerular capillaries in the mouse. Within weeks a reduction of glycocalyx structures both in size and number were observed. We suggest that the loss of glycocalyx molecules changes the functional properties of the endothelium with enhanced permeability, inflammation and coagulation.

SA-PO343

Inhibition of Endothelial TGF-β Signaling Reduces Fibrosis and Endothelial-Mesenchymal Transition (EndoMT) in CKD Sandhya Xavier, Radovan Vasko, Kei Matsumoto, Joseph A. Zullo, Robert Chen, Julien Maizel, Praveen N. Chander, Michael S. Goligorsky. *New York Medical College, NY.*

Background: Recently, endothelial cells undergoing EndoMT have been shown to contribute significantly to fibrotic process. While several studies focused on the ablation of epithelial or fibroblast TGF-β signaling on development of fibrosis, there is lack of information on its ablation in the endothelium due to embryonic lethality. We previously used TbRII$^{endo+/-}$ mice and reported that curtailed TGFb signaling in the endothelium modifies nephrosclerosis. Here we extend our findings to the UUO model and also study tissue hypoxia.

Methods: Unilateral ureteral obstruction (UUO) surgery was performed on TbRII$^{endo+/+}$ and TbRII$^{endo+/-}$ mice. Renal blood flow was evaluated 14 days post UUO. To analyze the degree of renal hypoxia in UUO mice, before sacrifice mice were i.p. injected with pimonidazole HCL and kidneys collected.

Results: TbRII$^{endo+/-}$ mice exhibited less tubulointerstitial fibrosis and less alpha-smooth muscle actin staining as compared to TbRII$^{endo+/+}$ mice. While renal blood flow was impaired in TbRII$^{endo+/+}$ mice, flow was significantly preserved in TbRII$^{endo+/-}$ mice, associated with lesser tissue hypoxia and much better preserved the patency (intravital lectin labeling), than its TβRII$^{endo+/+}$ counterpart. These findings shed light on the mechanisms underlying loss of patency of microvessels observed in injured kidneys. Partial deletion of TbRII in the endothelium also significantly reduced endothelial-to-mesenchymal transition (EndoMT). In vitro, TGF-β-induced canonical Smad2 signaling was reduced in TbRII$^{+/-}$ EC's, however, ALK1 mediated Smad1/5 phosphorylation in TbRII$^{+/-}$ EC's remained unaffected. Endoglin S/L mRNA expression ratio was significantly lower in TbRII$^{+/-}$ EC's as compared to TbRII$^{+/+}$ EC's.

Conclusions: Our data collectively document that partial ablation of TbRII in the endothelium reduces fibrosis during chronic kidney injury. Our results indicate the critical role of excessive endothelial TGF-β signaling in enhanced EndoMT and development of fibrosis. EndoMT may represent a proximal cause of the loss of vessels' patency, even when microvascular density is minimally impaired.

Funding: NIDDK Support

SA-PO344

Estrogen Augments while Progesterone Inhibits Arginine Transport in Human Endothelial Cells, through Modulation of Cationic Amino Acid Transporter-1 Ohad S. Bentur, Doron Schwartz, Gil Chernin, Idit F. Schwartz. *Nephrology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel.*

Background: Decreased generation of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS) has emerged as a primary factor provoking endothelial dysfunction (ECD). Delivery of L- arginine to membrane bound eNOS, by the cationic amino acid transporter-1 (CAT-1) has been shown to modulate eNOS activity. We have found in female rats, as oppose to males, that CAT-1 activity is preserved in aging and in chronic renal failure, two established experimental models of ECD. In contrast, during pregnancy CAT-1 is inhibited. The current experiments were designed to explore the role of female sex hormones on arginine transport.

Methods: Using radio-labeled arginine {[^{3}H] L-arginine} uptake was determined in HUVEC following incubation with progesterone, 17β estradiol, and both. Subsequently, western blotting for CAT-1, PKCα, ERK 1/2, JNK, and their phosphorylated forms were performed.

Results: Exposing HUVEC to 17ß estradiol (50 and 100nM) for 30 minutes resulted in a significant increase in arginine transport and in diminished phosphorylated CAT-1(the inactive form) protein content. The aforementioned findings were associated with a decrease in phosphorylated ERK1/2 abundance while PKCα and JNK proteins were unchanged. Incubating HUVEC with UO 126 (ERK 1/2inhibitor) or SP600125 (JNK inhibitor) augmented arginine uptake while tocopherol (PKC inhibitor) had no effect. Progesterone (1 and 100 pM) for 30 minutes attenuated arginine uptake, increased phosphorylated CAT-1 and phosphorylated PKCα protein content while JNK and ERK protein expression remained unchanged. Tocopherol prevented the decrease in arginine transport by progesterone. Co-incubation with both progesterone and estrogen for 30 minutes resulted in attenuated arginine transport.

Conclusions: While estrogen increases arginine transport and CAT-1 activity through modulation of constitutive cellular signaling transduction pathways involving ERK 1/2, progesterone inhibits arginine transport and CAT-1 via PKC phosphrolyation, an effect which predominates over that of estrogen.

Funding: Government Support - Non-U.S.

SA-PO345

Astragaloside IV Synergizes with Ferulic Acid to Reduce Endothelial Cell Microparticles Release in 5/6 Nephrectomized Rats Liqiang Meng, Jiawei Tang, Lei Qu, Xiaomei Li. *Renal Div, Dept of Medicine, Peking Univ First Hospital, Beijing, China.*

Background: Astragaloside IV (AS-IV) and ferulic acid (FA) were demonstrated to possess a renoprotective effect partly via endothelial cell protection. Here, the mechanisms of AS-IV and FA in improvement of endothelial cell function were studied.

Methods: Male SD rats were divided randomly into 5 groups: sham, 5/6 nphrectomy (Nx), Nx+AS-IV-treated, Nx+FA-treated and Nx+AS-IV+FA (AF)-treated group. Blood endothelial microparticle (EMP), platelet microparticle (PMP) were evaluated using flow

cytometry. Cultured human umbilical vein endothelial cells (HUVEC) were divided into 5 groups: CON, TNF-α stimulated, TNF-α+FA-treated, TNF-α+AS-IV treated, TNF-α+AF treated (AF). Flow cytometry was used to analysis the type and concentration of EMP release from endothelial cells. Amino phospholipid translocase (APLT) activity, Caspase 3, Caspase 9, calpain, ROCK-I, II expression in the supernatant fluid were detected.

Results: Compared with sham group, EMP was increased significantly in Nx group. In AS-IV and AF group EMP was lower than that in Nx group. PMP was increased significantly in Nx group than that of sham group, but it was significantly decreased in the treatment groups. Compared with CON group, EMP release from endothelial cells was increased significantly in TNF-α group. While in AF group, the concentration of EMP was lower than that of TNF-α group, but there were no difference in FA or AS-IV group. Compared with CON group, endothelial cell APLT activity was decreased, whereas the expression of Caspase 3, Caspase 9, ROCK-II increased in TNF-α group. In AF group, the activity of APLT was significantly decreased and the expression of Caspase 3, Caspase 9 increased than that of TNF-α group, but no significant difference in FA and AS-IV group. The expression of calpain, ROCK-I was shown no change between each group.

Conclusions: In chronic renal failure, the apoptosis of endothelial cell is increased and release EMP that might be associated with reducing APLT activity. AF could protect endothelial cell and reduce EMP release, which might relate to APLT activity enhancement by inhibiting the expression of Caspase 3 and Caspase 9.

Funding: Government Support - Non-U.S.

SA-PO346

Atherosclerotic Plaque Characterization in Chronic Kidney Disease (CKD)

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Background: CKD is characterized by accelerated atherosclerosis as compared to the general population. MCP1, ICAM, VCAM, CD36 and MMP2 are pro-atherogenic enzymes involved in plaque progression and plaque (in)stability, characterized by inflammation and Neoangiogenesis (nAng). Heme oxygenase-1 (HO-1) is a well-known anti-atherogenic enzyme.

Methods: Peripheral-artery biopsies retrieved during vascular surgery of 27 CKD stages 3-5D patients were compared with those of 43 nonCKD patients. Atherosclerotic plaques were scored blindly for lesion type, nAng, cap characteristics, inflammation and/or plaque complications. Immunostainings for HO-1, CD36, MMP2, MCP1, ICAM1, VCAM and Activated Caspase-3 (aCasp3) were judged semi-quantatively (0-3) and means were calculated. Patients were further phenotyped by clinical and lab evaluation.

Results: Atherosclerotic plaques of CKD patients showed more inflammatory activity (foam cells 2.2 versus 1.5 (p=0.004) and MCP1 (2.2 versus 1.6 p=0.04)), nAng (1.5 versus 1.0 p=0.04) and complicated plaques (68 versus 27 % p=0.002) than clinically well-matched nonCKD patients. In univariate analyses nAng correlated positively with CD36, aCasp3, ICAM1, HO-1, BMI and plaque inflammation, and negatively with eGFR and HDL-cholesterol level (HDL). Main predictors for nAng in multivariate linear regression analysis were plaque inflammation, ICAM, CD36, MMP2, HDL and eGFR ($R^2 = 0.5791$). Univariate correlation analyses in CKD versus nonCKD including HDL, HO-1, MCP1, CD36, ICAM, VCAM and nAng reveal the loss of the classical protective associations for HDL and HO-1 in atherosclerosis in CKD.

Conclusions: Atherosclerosis in CKD is characterized by a vulnerable plaque phenotype, with more inflammation, nAng and plaque complications. Besides the classical pathological cardiovascular markers and plaque inflammation, lower eGFR is associated with more nAng. The loss of the correlations for HO-1 and HDL with atherosclerosis-specific markers such as MCP1, ICAM, VCAM, CD36 and nAng in CKD requires further elucidation.

SA-PO347

Higher Plasma Level of Carnitine Contributes to Preventing Arteriosclerosis via the Reduction of Advanced Glycation End Products in Hemodialysis Patients

Yumi Kamada,[1,2] Takashi Masuda,[2] Yoko Itoh,[1] Ibuki Moriguchi,[1] Naoyuki Kobayashi,[1] Michihito Okubo,[1] Yasuo Takeuchi,[3] Kouju Kamata.[3] *[1]Sohbudai Nieren Clinic, Zama, Japan; [2]Kitasato Univ Graduate School of Medical Sciences, Sagamihara, Japan; [3]Kitasato Univ School of Medicine, Sagamihara, Japan.*

Background: Hyperglycemia or aggravated oxidative stress is well known to promote production of advanced glycation end products (AGEs). There is accumulating evidence that AGEs play an important role in cardiovascular disease in patients with maintenance hemodialysis (HD). It has been known that carnitine, derived from diet or synthesized in liver or kidney, improves insulin resistance and reduces oxidative stress. We hypothesized that higher plasma carnitine leads to prevention of arteriosclerosis via reduction of AGEs. The aim of this study was to investigate the relationships among carnitine, AGEs and arteriosclerosis in HD patients.

Methods: We recruited 79 patients (43 men and 36 women, 60.9 ± 13.2 years) undergoing HD thrice a week. Patients with diabetes or those regularly taking carnitine drug were excluded. We measured pre-HD plasma free carnitine and pentosidine as a parameter of plasma AGEs at the first weekly session. Skin AGEs level was quantitatively evaluated by measuring skin autofluorescence with an AGE-reader. Arteriosclerosis was evaluated by measuring post-HD cardio-ankle vascular index (CAVI). Correlations among carnitine, AGEs and arteriosclerosis were analyzed using Pearson's correlation coefficient.

Results: Free carnitine ranged from 14.9 to 53.3 μmol/L, and was negatively correlated with pentosidine (r=-0.32, P<0.01), skin AGEs level (r=-0.26, P<0.05) and CAVI (r=-0.30, P<0.05). Skin AGEs level was positively correlated with CAVI (r=0.42, P<0.01).

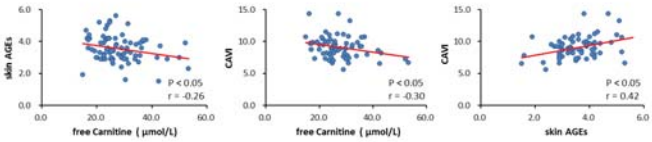

Figure1 Relationships among carnitine, AGEs and arteriosclerosis

Conclusions: Higher plasma level of carnitine contributed to preventing arteriosclerosis via reduction of AGEs in HD patients.

SA-PO348

Carotid Intimal Medial Thickness and Kidney Dysfunction in Rheumatoid Arthritis

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Background: Both glomerular and tubular damage contribute to kidney damage(KD) in Rheumatoid arthritis(RA) patients. Cardiovascular disease(CVD) reported to play a pivotal role in the increased KD in RA patients. With significant increase inKD with duration and severity of RA. KD in RA however is usually asymptomatic and is detected only on laboratory investigations. Despite the presence of number of parameters that reflect KD, these cannot be applied to day to day practice and still remain research tools.

Methods: KD was assessed in 37 RA patients(mean age 44.7) by estimated Glomerular Filtration Rate(GFR) using MDRD equation. Risk factor for KD and CVD were recorded or measured in all participants. Linear regression used to test the independence of the association between GFR and each of KD and CVD risk factors.

Results: Univariable analysis revealed significant associations between GFR and age of the participants (p<0.001), age at RA onset(p=0.001), number of cigarettes consumed per day(p=0.036), smoking duration(p=0.031), pack year history of smoking(p=0.024), systolic blood pressure(p=0.002), cIMT(p=0.026), uric acid(UA) level(p<0.001), urine microalbumin level(p=0.029) and urine microalbumin creatinine ratio(0.035). Of traditional CVD risk factors, age had the most powerful association with KD(r=0.42), followed by age at RA onset(r=0.265), and systolic blood pressure(r=0.241). Pack year of smoking and cIMT had almost a same power(r=0.137 and 0.134, respectively). A baseline model was created, incorporating all of the above parameters along with ESR and CRP values. Factors that maintain a significant association with GFR were age, uric acid and cIMT.

Conclusions: This is the first study to report that cIMT is a correlate of KD in RA, in addition to age and UA. CVD-associated factors appear to play a role in reduced KD development. The presence of RA disease with reduced KD may lead to an increase of CVD risk as manifested by a progression of cIMT. Based on these findings: kidney function monitoring; by uric acid and cIMT may be used to pick up early KD and minimize the risk of KD deterioration and subsequent CVD complications in RA population.

Funding: Government Support - Non-U.S.

SA-PO349

Improved Arterial Stiffness Is Associated with Blood Pressure But Not Inflammation, Dialysis Modality or Bioimpedance in Incident Dialysis Patients

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Background: A recent ANZDATA study suggests commencing PD has superior survival at 90-days compared to HD in ESKD patients. This could be explained by preserved residual renal function and induction of a lesser inflammatory response with PD.

Methods: We assessed the effects of conventional haemodialysis (HD) or continuous ambulatory peritoneal dialysis (PD) on surrogate markers of cardiovascular disease in incident end-stage kidney disease (ESKD) patients. We conducted a prospective study of 75 ESKD patients commencing HD or PD between January 2009 to December 2012. We measured inflammatory markers (IL-18, hs-CRP), SBP, bioimpedance and arterial stiffness (PWV) 0-3 months prior to dialysis initiation and 3-6months following commencement of HD or PD. Dialysis modality was chosen independent of this study.

Results: Baseline patient characteristics, inflammatory markers, bioimpedance and arterial stiffness were similar between HD and PD patients. Compared with pre-dialysis values, commencing any dialysis significantly lowered PWV (10.2±1.3 versus 8.9±1.0m/s;p<0.001), SBP (156±2.9 versus 136±3.0mmHg;p<0.001), and IL-18 [568(439-711) versus 494(343-578); p=0.004) but not ECW:ICW ratio (0.91±0.02 versus 0.88±0.02;p=0.08). While hs-CRP increased in HD compared with PD [rCRP 0.5±1.6 versus -1.2±1.9 g/L, p=0.02], changes in PWV, SBP, ECW:ICW ratio or IL18 were not significantly different between HD or PD. In multivariate analysis, after adjusting for age, and time on dialysis, only ΔSBP, but not ΔIL-18, ΔCRP, ΔECW:ICW ratio or dialysis modality, was an independent predictor of improved PWV (B=0.03;p=0.005).

Conclusions: Commencement of conventional dialysis in the short-term improves SBP and arterial stiffness, and lowers IL-18 levels. Improvement in arterial stiffness is mediated by changes in SBP rather than pro-inflammatory cytokines, volume status or specific dialysis modality per se. The beneficial effect of PD over HD on 90-day mortality is not supported by our data.

Funding: Government Support - Non-U.S.

SA-PO350

Abstract Withdrawn

SA-PO351

Peripheral Monocytes Are Constitutively Primed for Scavenger Receptor in Hemodialysis Patients, which Likely Facilitates the Development of Atherosclerosis Miki Nishida,[1] Minoru Ando,[2] Yusuke Iwamoto,[3] Ken Tsuchiya,[1] Kosaku Nitta.[1] *[1]Dept of Medicine kidney Center, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Renal Div, Dept of Medicine, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [3]Dialysis Center, Saito Memorial Hospital, Tokyo, Japan.*

Background: Atherosclerosis is closely associated with morbidity and mortality in hemodialysis (HD) patients. Scavenger receptor (SR) plays a pivotal role in the initial stage of atherosclerosis by ingesting oxidized low-density lipoprotein (ox-LDL) in monocyte lineage cells.

Methods: Subjects included 61 HD patients and 29 healthy controls. Peripheral monocytes were isolated using magnetically labeled Whole Blood $CD14^+$ micro-beads. Transcriptional levels of SR class A and class B (CD36) were simultaneously measured in peripheral monocytes by quantitative real-time RT-PCR, using the comparative threshold (Ct) method. The gene expressions were compared between HD patients and controls, and between HD patients who had cardiovascular disease (CVD) and those who did not. Additionally,CD36 protein expression was analyzed by a flow cytometry.

Results: Both SR-A and CD36 gene expressions were significantly higher in monocytes from HD patients than in those from controls (mean [95% CI of the mean]: 2.35 [1.97-2.74] versus 1.24 [0.91-1.56], P=0.0001; and 1.52 [1.30-1.74] versus 1.30 [0.92-1.69], P=0.0351). Both SR receptor expressions were significantly higher in HD patients with CVD than in those without (SR-A, 2.78 [2.28-3.29] versus 1.64 [1.16-2.13], P=0.0023; and CD36, 1.72 [1.43-2.00] versus 1.20 [0.89-1.50], P=0.0056).

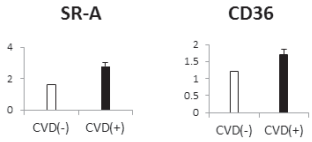

Figure1. Gene expression of SR-A and CD36 in HD patients with and without CVD

The proportion of $CD14^+CD36^+$ cells was 1.3-fold higher in the HD patients than in the controls (35.65% versus 27.65%).

Conclusions: Peripheral monocytes in HD patients may be primed for SR expressions, suggesting that they are predisposed to uptake of atherogenic ox-LDL.

SA-PO352

The Interaction between C5a and Sphingosine-1-Phosphate in Neutrophils for ANCA-Mediated Activation Min Chen,[1,3,4,5] Jian Hao,[1,2] Yimin Huang,[1,3,4,5] Minghui Zhao.[1,3,4,5] *[1]Renal Div, Dept of Medicine, Peking Univ First Hospital, Beijing, China; [2]Renal Div, Dept of Medicine, The Affiliated Hospital of Inner Mongolia Medical Univ, Huhehot, Inner Mongolia, China; [3]Key Laboratory of Renal Disease, Ministry of Health of China, Beijing, China; [4]Key Laboratory of Chronic Kidney Disease Prevention and Treatment (Peking Univ), Ministry of Education, Beijing, China; [5]Peking-Tsinghua Center for Life Sciences, Beijing, China.*

Background: C5a plays an crucial role in antineutrophil cytoplasmic antibody (ANCA)-mediated neutrophil recruitment and activation. The current study further investigated the interaction between C5a and sphingosine-1-phosphate (S1P) in neutrophils for ANCA-mediated activation.

Methods: The plasma levels of S1P from 29 patients with ANCA-associated vasculitis (AAV) in active stage and in remission were tested by ELISA. The generation of S1P was tested in C5a-triggered neutrophils. The effect S1P receptor antagonistwas tested on respiratory burst and degranulation of C5a-primed neutrophils activated with ANCA.

Results: The plasma level of circulating S1P was significantly higher in patients with AAV with active disease comparedwith patients in remission (2034.2±438.5 versus 1489.3±547.4 nmol/L, *P*<0.001). S1P can prime neutrophils for ANCA-induced respiratory burst and degranulation. Compared with non-triggered neutrophils, the MFI value for CD88 expression up-regulated significantly in S1P-triggered neutrophils. S1P receptor antagonist decreased oxygen radical production in C5a primed neutrophils induced by ANCA-positive IgG from patients.Blocking S1P inhibited C5a-primed neutrophils migration.

Conclusions: S1P triggered by C5a-primed neutrophils could further activate neutrophils. Blocking S1P could attenuate C5a-induced activation of neutrophils by ANCA. The interaction between S1P and C5a plays an important role in neutrophils for ANCA-mediated activation.

Funding: Government Support - Non-U.S.

SA-PO353

Analysis of Mitochondrial DAMPs in ANCA Vasculitis Lovisa Odén,[1,2] Eóin O'Brien,[1] Alice M. Coughlan,[1] Paul O'Hara,[1] Mark Alan Little,[1] Fionnuala B. Hickey.[1] *[1]Trinity Health Kidney Centre, Trinity College Dublin, Dublin, Ireland; [2]Karolinska Instt, Solna, Sweden.*

Background: Anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis is an autoimmune condition characterised by inflammation and necrosis of blood vessels. In its most severe form it manifests with rapidly progressive glomerulonephritis and vasculitis of the respiratory tract. Danger-associated molecular patterns (DAMPs) are cellular components released from injured tissues that activate the innate immune system. As mitochondria are considered endosymbionts that evolved from bacteria, components of these organelles including mitochondrial DNA (mtDNA) can act as DAMPs. This study examined mtDNA copy number in serum of patients with ANCA vasculitis compared to healthy controls, and analysed the ability of mtDNA to prime neutrophils and monocytes for activation by ANCA, thus exacerbating the course of the disease.

Methods: Samples derived from the Irish Rare Kidney Disease Registry and Biobank. DNA was extracted from the serum of 173 patients and 53 controls. Mitochondrial and nuclear genes (MTND3 and UCP2) were quantified by real time-PCR. Neutrophils and monocytes isolated from whole blood were stimulated with mtDNA (extracted from HeLa cells) and/or ANCA. Cell activation was measured by DHR123 assay and secretion of IL-1β.

Results: Serum mtDNA levels were significantly higher in patients with active disease (n=74) versus those in remission (n=99) (p<0.05). Analysis of UCP2 indicated that nuclear DNA was significantly elevated in patients versus healthy controls (p<0.0001) and also significantly higher in patients with active disease versus remission (p<0.0001). Treatment of neutrophils with mtDNA primes them for activation by ANCA, as measured by oxidative burst. Similarly, treatment of monocytes with mtDNA results in enhanced activation and IL-1β secretion in response to ANCA.

Conclusions: Increased levels of serum mtDNA are seen in patients with active disease compared to remission. mtDNA enhances the activation of both neutrophils and monocytes by ANCA and as such may propagate the autoimmune response in ANCA vasculitis, although it remains unclear whether the elevated level is a driver of inflammation or a consequence of tissue damage.

Funding: Government Support - Non-U.S.

SA-PO354

Cyclosporine Enhances Macrophage Lipid Accumulation via the TLR Pathway Joseph Mattana, Nobuyuki (Bill) Miyawaki, Laura Kaplan, Iryna Voloshyna, Allison B. Reiss. *Dept of Medicine, Winthrop Univ Hospital, Mineola, NY.*

Background: Cyclosporine A (CsA), an immunosuppressive used in renal transplantation, is associated with nephrotoxicity, atherosclerosis and dyslipidemia. Chronic CsA use may be pro-atherosclerotic and nephrotoxic due to mechanisms involving endothelial injury and macrophage infiltration. Modified low density lipoproteins (LDL), particularly carbamylated (c)LDL, are elevated in renal disease and can promote atherosclerosis through vascular endothelial dysfunction or foam cell formation. Toll-like receptors (TLRs) play a key role in inflammation and atherosclerosis. TLR2 is activated by CsA. Here we determined that CsA enhances lipid accumulation in cultured THP-1 human macrophages through the TLR pathway.

Methods: THP-1 macrophages (10^6/ml) were incubated 24h and 48h in media alone (control), oxidized (ox)LDL or cLDL (50 μg/ml) ± CsA (1 μg/ml). Cholesterol influx analysis and foam cell detection were performed under these conditions ± 5μg/ml 1,1'-dioctadecyl 3,3,3',3'-tetramethylin docarbocyaninet (Dil)-oxLDL. Message levels of the scavenger receptors CD36, LOX1, SRA1 and CXCL16 were evaluated by real-time PCR. Inflammatory response was analyzed by expression of TLR2 and TLR4. The TLR pathway was inhibited with BAY 11-7082. Protein levels were confirmed by Western blot. Cellular dil-oxLDL was quantified by fluorescent intensity using confocal microscopy.

Results: CsA doubled expression of the scavenger receptors CD36, SRA1 and CXCL16 in THP-1 macrophages exposed to oxLDL or cLDL, but not in control cells (set at 100%) (n=3, P<0.05). CsA doubled TLR2 under all 3 conditions (control, oxLDL, cLDL) (n=3, P<0.05), while TLR4 was unchanged. In THP-1 macrophages exposed to oxLDL or cLDL + CsA, enhanced cholesterol influx was accompanied by acceleration of oxLDL uptake (by 46% and 56%, respectively versus no CsA). Blockade of the TLR-2 signaling pathway reduced lipid influx and accumulation to the level of control cells.

Conclusions: CsA acts synergistically with modified LDL to induce an atherogenic macrophage phenotype. CsA-induced activation of innate immunity provides a link to accelerated lipid accumulation and pro-atherogenic changes in renal disease states.

Funding: Other NIH Support - NCCAM

SA-PO355

Serum YKL-40 Level Is Associated with Vascular Injury and Predicts Proteinuria in Nephrotic Syndrome Patients Ismail Kocyigit,[1] Ozkan Gungor,[2] Ender Dogan,[3] Serhat Karadavut,[4] Cigdem Karakukcu,[5] Eray Eroglu,[3] Ozcan Orscelik,[4] Aydin Unal,[1] Murat H. Sipahioglu,[1] Bulent Tokgoz,[1] Oktay Oymak.[1] *[1]Dept of Nephrology, Erciyes Univ Medical Faculty, Kayseri, Turkey; [2]Nephrology Clinic, Kahramanmaras Education and Research Hospital, Kahramanmaras, Turkey; [3]Dept of Internal Medicine, Erciyes Univ Medical Faculty, Kayseri, Turkey; [4]Dept of Cardiology, Erciyes Univ Medical Faculty, Kayseri, Turkey; [5]Biochemistry Laboratory, Kayseri Education and Research Hospital, Kayseri, Turkey.*

Background: Nephrotic syndrome (NS) is associated with increases in cardiovascular events. YKL-40 levels are associated with atherosclerosis, endothelial dysfunction and proteinuria in renal and non-renal populations. The aim of this study was to investigate the relationship between YKL-40 levels with vascular injury and proteinuria in NS patients.

Methods: Sixty-nine NS patients and 20 healthy subjects were enrolled in the study. Endothelial function was measured by flow mediated dilatation (FMD) and arterial stiffness by pulse wave velocity (PWV). Serum YKL-40 levels were measured by ELISA.

Results: YKL-40 level and PWV were higher and FMD was lower in NS patients compared with healthy controls. However, CA-IMT and LVEF levels were not statistically significant between the two groups. Patients were divided into three groups in terms of proteinuria levels: the normoproteinuria group (n:18), non-nephrotic proteinuria group (n:33) and nephrotic proteinuria group (n:18). YKL-40 levels and PWV were significantly increased and FMD was decreased in the nephrotic proteinuria group compared to both the non-nephrotic proteinuria and normoproteinuria groups. The YKL-40 level correlated with FMD and PWV in NS patients. In patients with NS, proteinuria correlated with YKL-40, FMD, PWV, eGFR and fasting LDL cholesterol. Multivariate linear regression analyses showed that YKL-40 and eGFR were effective in predicting proteinuria in NS patients.

Conclusions: Serum YKL-40 level in NS patients is associated with endothelial dysfunction and increased arterial stiffness, and YKL-40 level is an indicator of the amount of proteinuria seen in these patients.

SA-PO356

Hyperuricemia Is a Risk for Coronary Artery Calcification in Asymptomatic Subjects Undergoing General Health Examination Ah Ran Choi, Miok Cho, Tae Hoon Kim, Sung Chang Bae, Hoon Young Choi, Sung-Kyu Ha, Hyeong Cheon Park. *Yonsei Univ College of Medicine, Gangnam Severance Hospital, Seoul, Republic of Korea.*

Background: Recent studies suggest that hyperuricemia may be a potential risk factor for atherosclerosis even in healthy subjects. Quantity of coronary artery calcium (CAC) correlates with atherosclerotic plaque burden and increased coronary artery calcium score (CACS) predict future coronary heart disease (CHD) events. We aimed to assess the relationship between CACS and serum uric acid (sUA) as well as other cardiovascular risk factors in asymptomatic subjects undergoing general health examination.

Methods: We consecutively enrolled 5,491 asymptomatic subjects without history of CHD who underwent coronary CT angiography as part of a general health examination. Patients with gout orthose taking uric acid lowering medications were excluded.

Results: Mean age of enrolled subject was 52.97 ± 9.5 years, 61 % of the study participants were male, mean CACS was 36.13 ± 148.9. Overall, 8.5% of subjects had an Agatston score greater than 100. The proportion of subject with serum uric acid of greater than 6.8 mg/dL in male were 24.6 %, and sUA greater than 6.0 mg/dL in female were 6.2 %. Compared to subjects with CACS less than 100, subjects with CACS > 100 had significantly higher sUA and higher fasting blood sugar (FBS), old age, and hypertension. CACS was positively associated with age, male gender, diabetes, body mass index, FBS, calcium-phosphate product (CPP), and sUA in CACS > 100 group according to multivariate logistic regression. Odds ratios (OR) of CACS for CPP and sUA was 1.021 (P <0.001) and 1.126 (P <0.012), respectively, after adjusting for other variables. In multivariate linear regression analysis, male, old age, hypertension, diabetes, smoking, increased CPP, serum uric acid, and fasting blood glucose were significantly associated with log transformed CACS.

Conclusions: Our data suggest that in addition to other known traditional risk factors, high sUA and CPP are independent risk factors for high CACS in asymptomatic subjects undergoing general health examination.

Funding: Clinical Revenue Support

SA-PO357

Endothelial RIG-I Signaling: A Crucial Pathway for Endotoxin-Induced Inflammatory Activation and Leukocyte Recruitment Jill Moser,[1,2] Peter Heeringa,[2] Rianne Jongman,[2] Jan G. Zijlstra,[1] Grietje Molema,[2] Matijs Van Meurs.[1,2] *[1]Dept Critical Care, UMCG, Groningen, Netherlands; [2]Dept Medical Biology, UMCG, Groningen, Netherlands.*

Background: Sepsis is a well-known risk factor for the development of AKI. RIG-I-like receptors have been suggested to play a role in the pathophysiology of sepsis yet very few studies have investigated this in detail. The aim of the current study was to investigate the role of RIG-I signaling in the development of endothelial dysfunction contributing to sepsis-induced kidney failure.

Methods: In this study, we investigated the mRNA levels of RIG-I and endothelial inflammatory markers in sepsis mouse models, LPS administration and CLP. Moreover, using laser microdissection/qRT-PCR analysis we investigated RIG-I expression in the renal microvascular compartments. In vitro studies using siRNA technology were adopted to ascertain whether there was a functional role for RIG-I in the regulation of endothelial cell (EC) responses to LPS.

Results: EC responses to LPS lead to the upregulation of RIG-I mRNA levels which followed the expression kinetics of the endothelial adhesion and inflammatory cytokine genes. We found that NFκB-mediated endothelial adhesion molecule and inflammatory cytokine expression was markedly inhibited in response to LPS in RIG-I and MAVS deficient ECs. Accordingly, less leukocyte adherence to the endothelium was observed in RIG-I and MAVS deficient ECs treated with LPS. We also identified RIG-I to regulate LPS-induced IRF1 transcriptional responses in ECs which we show specifically regulates VCAM-1 expression. In our mouse sepsis models we found renal RIG-I mRNA was upregulated which was paralleled by endothelial inflammatory activation. Moreover, endothelial-specific RIG-I was upregulated by LPS in all microvascular beds of the kidney despite variations in their basal levels.

Conclusions: Here we describe the identification of two unanticipated factors, RIG-I and MAVS, as essential core components required for LPS-induced activation and inflammatory responses which promote leukocyte adhesion to the endothelium. In vivo models of sepsis-induced AKI furthermore provide evidence of RIG-I involvement in the microvascular pathogenesis associated with septic shock.

SA-PO358

Mechanisms and Prevention of Low Birth Weight Associated Acute and Chronic Renal and Vascular Impairment May M. Rabadi, Joseph A. Zullo, Wasan Abdulmahdi, Ashvary Parth Dwivedi, Michael S. Shen, Kavneet Kaur, Brian B. Ratliff. *New York Medical College, NY.*

Background: In the United States, 8.1% of annual live births result in low birth weight (LBW) infants, contributing up to 80% of all neonatal deaths, and is the major cause of childhood morbidity. These babies are also predisposed to chronic illness later in life.

Methods: We used a maternal malnourished mouse model to examine the effects of LBW on kidney and vascular development and short/long-term function. Circulating cyto-/chemokines, nephron development, renal blood flow, renal developmental gene expression, autophagy, renal function and systemic blood pressure were examined in the LBW offspring. The therapeutic potential of glutamine, methionine and calendula treatment was examined in these animals.

Results: LBW male offspring birth weight and kidney size were reduced by 50%. During the first week after birth of the LBW neonate, BUN levels were elevated by 66%, renal blood perfusion was attenuated by 50%, and circulating proinflammatory cyto-/chemokines including IL-1β, IL-5, IL-12, IFNγ and GM-CSF were elevated. During mid to late nephrogenesis, the LBW fetus/neonate demonstrated a 25-50% reduction of pre-glomerular structures (renal vesicles, comma and s-shaped bodies) and glomeruli, a blunting of autophagy, and altered expression of critical developmental genes including elevated Wnt9b, Bmp7 and FoxD1, but reduced Eya1 and Sall1. Supplementation of methionine and glutamine during nephrogenesis normalized kidney development and function in the LBW neonate. At 2 months post-birth, LBW mice had 30% fewer glomeruli, showed early tubular damage and glomerular abnormalities. At 5 months post-birth, LBW animals had 10% elevated blood pressure, elevated serum creatinine and proteinuria, and enhanced circulating proinflammatory cyto-/chemokines. Treatment with calendula improved blood pressure and renal function in the older animals.

Conclusions: LBW compromises the kidney and vascular system, enhances circulating proinflammatory cyto-/chemokines, interferes with normal nephron/glomeruli development and predisposes mice to acute and chronic illness. Effects are improved upon treatment with glutamine, methionine or calendula.

Funding: Private Foundation Support

SA-PO359

Loss of Endothelial Surface Glycocalyx (ESG) in Early Sepsis (S) Jie Fan,[1] Wan-Yi Yen,[1] Min Zeng,[1] Jun Chen,[2] Brian B. Ratliff,[2] Jungol Song,[2,3] John Tarbell,[1] Bingmei M. Fu,[1] Michael S. Goligorsky.[2] *[1]City College, CUNY; [2]NY Medical College, NY; [3]Ulsan Medical College, Seoul, Korea.*

Background: ESG is a surface layer consisting of glycoproteins, proteoglycans and glycosaminoglycans; heparan sulfate (HS) is the most abundant. ESG provides a barrier to water and solute transport, sensing of mechanical forces, and shielding receptors from hyper-activation. S disorganizes ESG, but the mechanism is nebulous. Among the earliest responses of activated endothelial cells to LPS are exocytosis of Weibel-Palade bodies (WPB) and lysosomes. We hypothesized that those responses result in the focal degradation of ESG unleashing the cascade of events culminating in multiorgan failure.

Methods: bEnd3 endothelial cells and HUVEC were treated with LPS (5ug/ml) and stained with mAb against HS or vWF; intravital microscopy of lysosomal motility was performed using LysoTracker. C57BL/6 mice were injected with LPS IP. After 1h or 6h treatment, the cremaster microvessels were in vivo stained with FITC-anti-HS; in another group, the aorta was similarly stained en face. Zeiss LSM 710 confocal microscopy and 3-D multi-color ultra-high resolution Nikon-STORM (Stochastic Optical Reconstruction Microscopy) were used to detect ESG, vWF/WPB and lysosomes.

Results: At 10 min after LPS treatment of bEnd3, we observed a significant loss of ESG and exocytosis of vWF/WPB (Fig. 1). At 30 min, ESG was degraded by ~90%, 30% more lysosomes and 4-fold vWF/WPB externalized compared to the control. 1h and 6h LPS treatment removed ESG by ~50% and more than 90%, respectively, in microvessels; by ~60% and ~90%, respectively, in aorta of mice.

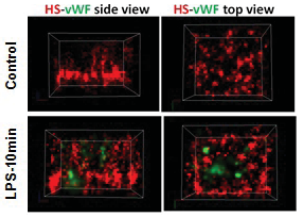

Fig.1.Representative STORM-acquired images of anti-HS labeled ESG (red) and vWF (green) distribution in control and 10min post-LPS (side and top views) in bEnd3 cells. In control ESG is richly represented, whereas it is sparse and vWF density hightened in LPS group. HS-vWF-*side view* images depict the relative distribution of ESG and vWF (note that ESG is impregnated with vWF and is fragmented in LPS series); *top view* illustrates the formation of ESG "halo" around vWF-labeled WPB. Bar = 1 um.

Conclusions: Patchy loss of ESG is detectable after 10-30 min of LPS treatment, coinciding with exocytosis of WPB and lysosomes in cultured cells. Loss of ESG is a very early event in S. and may serve as a therapeutic target in preventing the complications of S.

Funding: NIDDK Support, Private Foundation Support

SA-PO360

The Kidney Enriched Proteome Defined by Transcriptomics and Antibody Based Profiling Masato Habuka,[1,2] Ichiei Narita,[3] Tadashi Yamamoto.[1] *[1]Dept of Structural Pathology, Inst of Nephrology, Medical and Dental School, Niigata Univ, Niigata City, Japan; [2]Affinity Proteomics, Science for Life Laboratory, KTH - Royal Inst of Technology, Stockholm, Sweden; [3]Clinical Nephrology and Rheumatology, Niigata Univ Graduate School of Medical and Dental School, Niigata City, Japan.*

Background: The kidney is a specialized tissue and plays a vital role in maintaining body homeostasis.To understand renal biology and disease, it is important to define the molecular constituents of the various subcompartments of the kidney, including glomerulus and tubule.

Methods: we have used an integrated omics approach involving genome wide transcriptomics analysis using deep RNA sequencing across 27 different human tissue types combined with antibody-based immunohistochemistry in 48 different tissues to identify the genes overrepresented in the kidney and to further map the localization of the corresponding protein. All human genes were classified into categories of expression patterns with regards to expression pattern in the kidney as compared to the other anlyzed tissues.

Results: Altogether 387 genes were found to be elevated in kidney as compared to the other analyzed tissues and 64 genes were found to have a significant higher expression in kidney and these genes are designated as highly and moderately enriched. In-depth analysis of all the elevated genes using antibody-based allowed us to create a nephron and collecting duct segment-specific map, including 149 proteins with a unique localization, including 12 proteins in glomeruli, 120 in proximal tubule, 9 in distal tubule and 8 in collecting duct. An analysis of the identified genes supports their role in the function of each segment. Among the gene products identified as kidney-enriched, we found several proteins not previously described in the context of the kidney.

Conclusions: In summary, we have identified and mapped proteins with elevated expression in kidney through an integrated omics approach and these proteins are important starting points for further functional studies. The identified protein targets can also serve as the vantage point for identification of early kidney specific biomarkers of disease and injury before clinical symptoms arise.

SA-PO361

Tracking the Fate of Renal Mesenchymal Stem Cells with In Vivo Multiphoton Imaging James L. Burford, Janos Peti-Peterdi. *Physiology and Biophysics and Medicine, Univ of Southern California, Los Angeles, CA.*

Background: Currently there is no cure for chronic kidney disease (CKD) other than non-specific drugs that only slow down progression. The unmet medical need and inadequacy of current treatments have led to great interest in regenerative stem cell approaches. We aimed to track the localization, migration, and fate of resident mesenchymal stem/progenitor cells in the renal cortex in intact mouse kidneys.

Methods: Tamoxifen-inducible chondroitin sulfate proteoglycan NG2-Tomato reporter mice were subjected to multiple surviving surgeries to exteriorize and image the same region and glomeruli of the left kidney by in vivo serial multiphoton microscopy (MPM). Blood vessels were labeled green by 500 kDa dextran. Specific renal cortical regions and glomeruli were z-scanned to visualize changes in tissue morphology and cell dynamics.

Results: Compared to baseline, the density of cells that belong to the NG2 lineage (red) was increased 3-fold and observed in a scattered pattern throughout the renal interstitium, along the afferent arteriole towards the MD tubular segment, within the glomerulus (including mesangial and parietal epithelial cells, and podocytes), and in the early proximal tubule 1-2 weeks after UUO (a common model of CKD and tubulointerstitial fibrosis) or low salt diet+captopril treatment. Spherical or stellate-shaped cells of the NG2 lineage were observed in the Bowman's space and in the proximal tubule lumen suggesting cell migration.

Conclusions: In summary, these in vivo MPM imaging data suggest the dynamic cellular remodeling of the renal interstitium, juxtaglomerular vasculature, mesangium, the Bowman's capsule, and the proximal tubule by renal progenitor cells in these conditions. The more precise mechanistic understanding of this novel NG2 cell function needs further study.

Funding: NIDDK Support

SA-PO362

Analysis of Renal Tubular Function by ^{99m}Tc-MAG3 Secretion Requires MRP2 Transporter with Interference from Morphine as a Co-Ligand Samriti Dogra,[1] Kang Cheng,[2] Pravin C. Singhal,[2] Chris Palestro,[2] Sanjeev Gupta,[3] Kuldeep Bhargava.[2] *[1]Pediatric Nephrology, Montefiore Medical Center, Bronx, NY; [2]North Shore-Long Island Jewish Health System, Manhasset and New Hyde Park, NY; [3]Pathology and Medicine, Albert Einstein College of Medicine, Bronx, NY.*

Background: The widespread use of ^{99m}Tc-MAG3 complex in evaluation of renal proximal tubule function requires molecular details of tracer biology. Recent studies established that ^{99m}Tc is a ligand for the bile canalicular apical MRP2 transporter, which is also expressed in renal tubules, with specificity for ^{99m}Tc-MAG3 due to the small size of MAG3. As glucuronidated metabolites of morphine are also transported by MRP2 and individuals undergoing renal imaging may have received this drug, we investigated the effect of morphine on renal ^{99m}Tc-MAG3 excretion.

Methods: The study used 9 mice in 3 groups: untreated controls, morphine given continuously for 72 h via s.c. 75 mg pellet, and morphine for 72 h via s.c. pellet of 75 mg followed by morphine withdrawal for 72 h. Administration of 3.7 MBq ^{99m}Tc-MAG3 via tail vein was followed by acquisition of 60 1-second images and 29 1-minute images by gamma camera. Regions of interest were drawn around kidneys for time–activity curves to illustrate split renal function and time-to-peak activity (Tpeak). Immunostaining and westerns were used for MRP2 expression.

Results: We observed differences in Tpeak for ^{99m}Tc-MAG3 activity, which was 6, 27 and 10 min in untreated controls, morphine-treated mice and morphine-treatment-withdrawal mice, respectively, $p<0.05$, ANOVA. No urinary excretion of activity was noted during imaging study in morphine-treated mice. MRP2 was expressed equally by westerns in kidneys of animals in all groups. Similar localization by immunostaining of MRP2 in tubular cells of controls and morphine-treatment excluded dysregulation of transporter expression.

Conclusions: Morphine blocked renal ^{99m}Tc-MAG3 excretion, which was in agreement with preferential MRP2-binding of morphine. These findings have clinical implications because presence of morphine may confound results of ^{99m}Tc-MAG3 imaging.

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SA-PO363

CD36: A New Therapeutic Target for Chronic Kidney Disease Progression Ana C. Souza,[1] Alexander V. Bocharov,[2] Irina Baranova,[2] Tatyana Vishnyakova,[2] Yuning George Huang,[1] Kenneth J. Wilkins,[1] Xuzhen Hu,[1] Adam E. Mullick,[4] Alan Remaley,[3] Thomas Eggerman,[1] Peter S.T. Yuen,[1] Robert A. Star.[1] *[1]NIDDK; [2]CC; [3]NIHLBI, NIH; [4]ISIS.*

Background: CD36 is a scavenger receptor important for lipid metabolism and inflammation. To test for a direct role of CD36 in CKD, we compared wild type (WT) versus CD36 knockout (KO) versus WT mice treated with 5A-37pA (5A), a CD36 antagonist peptide, in a CKD model.

Methods: 16wk old male C57BL6 WT and KO mice were subjected to 5/6 nephrectomy (5/6Nx) with AngII infusion (0.75 µg/Kg/min) through osmotic minipump. Some mice got 5A (5mg/kg/day) in a second minipump. WT mice with 5/6Nx without AngII served as controls. All groups (WT5/6Nx, WT 5/6Nx+AngII, KO 5/6Nx+AngII, WT 5/6Nx+AngII+5A, N=11-19/group) were followed for 4wk, and telemetry blood pressure monitored in WT and KO 5/6Nx+AngII (N=6/group). Serum creatinine was measured by HPLC; albuminuria, serum amyloid A (SAA), and FGF-23 by ELISA. Kidney sections were stained for histology. We measured renal cytokines and NLRP3 by RT-PCR (N=4-6/group). Fluorescent 5A was injected IV and kidneys analyzed after 3h by 2 photon microscopy. Statistical analyses primarily involved ANOVA; repeated measures data were analyzed by mixed models.

Results: Non-treated WT 5/6Nx+AngII mice developed albuminuria, substantial decline in kidney function, histological damage, and a metabolic profile typical of CKD with increased FGF-23 and SAA. Kidney function was improved by ~ 40% in the KO 5/6Nx+AngII and 5A-treated WT 5/6Nx+AngII groups, with decreased albuminuria (~ 50%), improved histology and metabolic profile. There was no difference in blood pressure between WT 5/6Nx+AngII and KO 5/6Nx+AngII. 5A decreased mRNA expression of renal cytokines and inflammasome NLRP3 and IL-1ß genes. Fluorescent 5A localized in proximal convoluted tubules (PCT) after IV injection, coinciding with the most prominent CD36 staining by IHC.

Conclusions: CD36KO and 5A-treated WT mice are protected from CKD progression. This protection occurs without changes in blood pressure. 5A decreases inflammation and inflammasome activation in the kidney. Both 5A and CD36 localized in PCT. CD36 is a new therapeutic target for CKD, and 5A is a promising protective agent.

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SA-PO364

5A-37pA, a CD36 Antagonistic, Decreases Kidney Interstitial Fibrosis in Mice Subjected to Unilateral Uretheral Obstruction Ana C. Souza,[1] Alexander V. Bocharov,[2] Irina Baranova,[2] Xuzhen Hu,[1] Jonathan Street,[1] Alan Remaley,[3] Thomas Eggerman,[1] Peter S.T. Yuen,[1] Robert A. Star.[1] *[1]NIDDK; [2]CC; [3]NIHLBI, NIH.*

Background: Inhibiting CD36 receptor with 5A-37pA (5A) slows CKD progression after 5/6 nephrectomy with Ang II infusion in C57BL6 mice (ASN 2014). To test the efficacy of 5A in a outbred strain in a fibrosis model that is not Ang II- dependent, we evaluated 5A in CD-1 mice subjected to unilateral uretheral obstruction (UUO).

Methods: CD-1 mice (male, 9wk, n=8/group) were divided as follows: 1) Sham operated; 2) UUO; 3) UUO + 5A, 5mg/kg/day (5A5); 4) UUO + 5A, 15mg/kg/day (5A15). 5A or vehicle (water) was infused through subcutaneous osmotic minipump that was implanted 24h before sham surgery or UUO. After 10 days, mice were euthanized. A biochemistry serum panel was analyzed by automated analyzer. Kidney sections were stained with Masson's trichrome for interstitial fibrosis and morphometric analysis. IHC for F4/80$^+$ cells and RT-PCR analysis of cytokines and NLRP3 expression in the kidney was performed (n=4/group). Statistical analyses were performed by ANOVA.

Results: Mice subjected to UUO had significant kidney histological damage with substantial interstitial fibrosis, which was less prominent in mice receiving 5A15: (a) interstitial fibrosis scores- sham 1.1; UUO 4.8 $p<0.0001$ versus sham; 5A5 4.3; 5A15 3.9 $p<0.05$ versus UUO (b) cortex thickness- sham 1000 pixels; UUO 469.7 $p<0.0001$ versus sham; 5A5 664.9; 5A15 771.4 $p<0.001$ versus UUO). F4/80$^+$ cells infiltration was significantly elevated after UUO ($p<0.001$ versus sham), and decreased in both 5A-treated groups to levels similar to sham. mRNA expression of TGF-ß and 1L-1ß were decreased in the kidney in the 5A15 group in comparison to untreated UUO ($p<0.05$ and $p<0.01$, respectively). mRNA expression of other inflammatory cytokines trended toward decreased levels in the 5A15 group. Biochemistry panel did not show any drug toxicity at either dose.

Conclusions: 5A treatment protected mice subjected to UUO from kidney interstitial fibrosis in a dose-dependent manner, potentially through anti-inflammatory actions. 5A did not cause toxicity at the doses and route of administration used.

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SA-PO365

Blockade of Mitochondrial Complex-1 Remarkably Attenuates Fibrosis via Inhibition of Oxidative Stress and Inflammation in Chronic Obstructive Nephropathy Ying Sun,[2] Yue Zhang,[1] Guixia Ding,[1] Aihua Zhang,[1] Songming Huang,[1] Zhanjun Jia.[2] *[1]Nephrology, Nanjing Children Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China; [2]Nanjing Key Laboratory of Pediatrics, Nanjing, Jiangsu, China.*

Background: Mitochondrial abnormality has been shown in many kidney disease models. However, its role in the pathogenesis of chronic kidney diseases (CKDs) is still uncertain. In present study, a mitochondrial complex-I inhibitor rotenone was applied to the mice subjected to unilateral ureteral obstruction (UUO) to evaluate its effect on the tubular injury, fibrosis, as well as the oxidative stress and inflammation.

Methods: Following the UUO surgery, mice were treated with rotenone at a dose of 500 ppm in diet for 7 days. Then the mice were sacrificed and the kidney tissues were harvested for analysis.

Results: By PAS staining, a remarkable attenuation of tubular injury was observed after rotenone administration. In line with the improvement of kidney morphology, we found that rotenone markedly suppressed expressions of FN (-80%), PAI-1(-71%), collegen-1(-66%), collagen-3 (-72%), and α-SMA (-70%) at protein and mRNA levels as determined by Western Blotting and qRT-PCR. Meanwhile, the protein and mRNA expressions of TGF-β1 were reduced by 71% and 62%, respectively. To investigate the potential mechanisms, we examined the oxidative stress and inflammatory response. Importantly, the oxidative stress level in obstructed kidney was robustly inhibited by rotenone as evidenced by the significant blockade of renal TBARs content (-62%)and HO-1 protein expression (-75%). Similarly, the inflammatory markers of TNF-α, IL-1β, and ICAM-1 were also markedly ameliorated by 60-80% as determined by ELISA and qRT-PCR.

Conclusions: Mitochondria complex I inhibitor rotenone effectively attenuated obstructive kidney injury and fibrosis, possibly via the inhibition of mitochondrial oxidative stress and the subsequent inflammatory response, suggesting an important role of mitochondrial dysfunction in mediating the pathogenesis of obstructive kidney disease. These results also suggested that mitochondria might be a potential target for the treatment of chronic kidney disease.

SA-PO366

Pro-Inflammatory and Pro-Fibrotic Action of the Protease-Activated Receptor-2 (PAR-2) in the Rodent Unilateral Ureter-Obstraction-Model (UUO) Christoph Daniel,[1] Iris Scheitacker,[1] Marko Bertog,[2] Christoph Korbmacher,[2] Kerstin U. Amann.[1] *[1]FAU Erlangen-Nuremberg; Nephropathology, Germany; [2]FAU Erlangen-Nuremberg; Physiology, Germany.*

Background: Protease activated receptor-2 (PAR-2) is a G-protein coupled receptor, that can be activated by a panel of serine proteases being secreted in response to injury. In different disease models it has been shown that pro-inflammatory as well as pro-fibrotic signal transducing cascades were activated by PAR-2. At present it is unknown whether PAR-2 mediated processes also play a role in kidney injury following unilateral ureter obstruction (UUO).

Methods: To investigate this, the UUO model was induced in wildtype (wt, n=16) and PAR-2-/- mice (n=26). Seven days later non-treated and ureter ligated kidneys were collected and analyzed for morphologic changes, fibrosis and inflammatory response using immunohistochemistry (n=9/20); real-time PCR (n=6/6) and flow cytometry analysis (n=6/6).

Results: UUO kidneys from wt mice showed pronounced tubular injury including tubular dilatation and atrophy. These changes were significantly lower in PAR-2-/- mice (38.8%±7.2 versus 23.4%±8.9 of tubular damage, p<0,001). The milder tubular damage was accompanied with a slightly lower degree of fibrosis (Sirius red semiquantitative score 2.9±0.3 versus 2.2±0.5, $p<0.001$) and lower CTGF mRNA expression (rel. expr.: 1.04±0.5 versus 0.48±0.2, p<0,05) in the obstructed kidney of PAR-2-/- mice. In addition, a non-significant trend was observed that in PAR-2-/- mice the expression of TGF-ß and of the myofibroblast markers α-smooth muscle actin and collagen-I was lower than in wt mice. Furthermore, inflammatory response in PAR-2-/- kidneys after UUO was also attenuated compared to injured wt controls. In PAR-2-/- mice MCP-1 expression was significantly lower (rel. expr.: 31.2±12.7 versus 15.7±7.5; $p<0.05$) with a non-signficant trend to lower TNF-α und IL-6 mRNA expression. Influx of F4/80 positive macrophages and CD3 positive T-cells in diseased kidneys from PAR-2-/- mice also showed a tendency to lower numbers as assessed by immunohistochemistry and flow cytometry compared to wt animals.

Conclusions: Thus, PAR-2 might be a potential new target for the therapy of inflammatory and fibrotic kidney disease.

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SA-PO367

The Fate of Nephrons in Congenital Obstructive Nephropathy: Adult Recovery Is Limited by Nephron Number Maria Sergio, Carolina I. Galarreta, Barbara A. Thornhill, Michael S. Forbes, Robert L. Chevalier. *Dept of Pediatrics, Univ of Virginia, Charlottesville, VA.*

Background: Pediatric chronic kidney disease (CKD) is most often due to congenital anomalies of the kidneys and urinary tract (CAKUT), and obstructive nephropathy is the leading cause. Progression to renal failure, however, is more likely to develop in adulthood than childhood (Wuhl, CJASN 8: 67–74, 2013). Frequently associated with CAKUT, reduced nephron number (NN) at birth is an independent risk factor for adult CKD.

Methods: To determine the role of NN in progression of congenital obstructive nephropathy, wild-type (WT) and reduced NN mice (Os/+) were subjected to sham operation or partial unilateral ureteral obstruction (UUO) in the first 2 days of life (prior to completion of nephrogenesis). To assess the impact of NN on recovery, additional WT and Os/+ mice underwent release of UUO at 7 days. All kidneys were harvested at 3 weeks (weaning) or 6 weeks (adulthood). Glomerular number and area, glomerulotubular junction integrity, proximal tubular volume fraction, and interstitial fibrosis were measured by histomorphometry.

Results: *In the obstructed kidney*, UUO caused additional nephron loss in Os/+ but not WT mice. Glomerular growth from 3 to 6 weeks was impaired by ipsilateral UUO and was not preserved by release in WT or Os/+. Proximal tubular growth was impaired and interstitial collagen was increased by ipsilateral UUO in all mice. These were attenuated by release of UUO in WT mice but were not restored in Os/+ mice. *In the contralateral kidney* of Os/+ mice, UUO decreased proximal tubular growth and increased interstitial collagen which were not prevented by release of obstruction.

Conclusions: Congenital partial UUO impairs glomerular and proximal tubular growth, and causes additional nephron loss in mice with reduced NN. Release of UUO does not restore glomerular growth and does not preserve tubular mass when NN is reduced. Because tubular injury and fibrosis develop in *both* kidneys of adult Os/+ mice with UUO, the concept of "renal reserve" should include tubular as well as glomerular components. Children with CAKUT should be followed throughout adulthood regardless of earlier surgical intervention.

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SA-PO368

In Unilateral Ureteral Obstruction Rat Model Mesenchymal Stromal Cells Downregulate Renin-Angiotensin System Inhibiting HuR Chiara Rocca,[1] Marilena Gregorini,[1] Samantha Milanesi,[1] Valeria Corradetti,[1] Eleonora Francesca Pattonieri,[1] Teresa Valsania,[1] Manuela Cannone,[1] Pasquale Esposito,[1] Carolina Bianco,[2] Ilaria Benzoni,[2] Marcello Maestri,[2] Maria Antonietta Avanzini,[3] Ingo Daniela,[3] Teresa Rampino,[1] Antonio Dal Canton.[1] *[1]Nephrology, IRCCS Policlinico San Matteo, Italy; [2]Chirurgia Generale, IRCCS Policlinico San Matteo, Italy; [3]Oncoematologia Pediatrica, IRCCS Policlinico San Matteo, Italy.*

Background: Mesenchymal Stromal Cells(MSC) prevent inflammation and renal scarring in Unilateral Ureteral Obstruction model in rats(UUO) suppressing renin-angiotensin system(RAS),but mechanisms by which MSC exert their effect is unknown. Renin synthesis is critically dependent on renin mRNA(RENmRNA) stability.HuR is a protein that target a cis-element 3'UTR of RENmRNA and regulate renin production.We investigated the mechanisms by which MSC suppress RAS.

Methods: *In vivo*:We studied 3 groups of SpragueDawley rats.MSC were isolated by bone marrow.A:5 sham operated.B:8 UUO received saline at day 0.C:8 UUO received MSC at day 0 via tail vein.Rats were sacrified at days 1 and 7.*In vitro*:HK-2 cell line were cultured in high glucose medium(HK2HG) to induce upregulation of RENmRNA and incubated in presence and absence of MSC,in presence of IL10,and MSC+anti IL10 antibody.Macrophages(M) derived from circulating monocytes(Mo) were isolated by Ficoll density gradient centrifugation and adhesion for 24h.Mo to M shifting phenotype was assessed by FACS flow cytometer.M and MSC were cocultured for 4 days.RENmRNA and HuRmRNA expression were evaluated on renal tissue and on cell lysate by RT PCR.

Results: MSC reduced RENmRNA in C,compared with A and B.At day 1 HuRmRNA expression was significantly higher in rats MSC untreated compared to sham operated rats($p<=0.05$).MSC treatment in C suppressed HuRmRNA expression compared with B($p<0.005$).*In vitro* MSC suppressed RENmRNA expression in HK2HG($p< 0.005$). RENmRNA expression was lower in presence than in absence of IL10($p<0.001$) and increased whether in MSC HK2HG cocultures was added antibody blocking IL10($p<0.001$). MSC suppressed RENmRNA in M.

Conclusions: MSC in UUO model suppress HuR transcription via IL-10.The inhibition of HuR transcription may decrease RENmRNA stability and suppress RAS in UUO.

SA-PO369

Phosphodieterase-4 Inhibitor Roflumilast Attenuates Renal Fibrosis in a Rat Model of Chronic Kidney Disease Xianzhong Lau,[1] Yuan Zhang,[1] David Stapleton,[2] Robyn G. Langham,[1] Darren J. Kelly.[1] *[1]Dept of Medicine (St Vincent's), Univ of Melbourne, Fitzroy, Victoria, Australia; [2]Howard Florey Inst, Parkville, Victoria, Australia.*

Background: The prevalence of chronic kidney disease (CKD) is increasing globally. There has been recent interest in the role of Phosphodiesterase (PDE) enzymes in mediating inflammation and fibrosis in acute kidney injury. Given the predominance of cAMP-signaling regulation by PDE4 isoforms in the kidney, we sought to examine the potential efficacy of a selective PDE4 inhibitor, roflumilast (RFL) in abrogating progressive fibrosis of the remnant kidney. Having demonstrated *in vitro* efficacy of RFL, in reducing inflammatory and fibrotic responses to injury in renal cells, the question of potential efficacy of RFL as an effective therapy in *in vivo* renal fibrosis was raised.

Methods: Sprague-Dawley rats underwent either STNx or sham surgery, further randomised after 2 weeks to receive an oral dose of either RFL (1 mg/kg/day) or vehicle, and sacrificed at 12 weeks. Renal function was assessed via glomerular filtration rate (GFR) and urinary protein. Glomerulosclerosis and tubulointerstitial fibrosis were evaluated histologically. Macrophage infiltration was measured with CD68 immunohistochemistry. Gene expression for MCP-1 was evaluated via PCR.

Results: RFL significantly attenuated GFR decline (treated:2.32±0.20 VS untreated:0.96±0.34 ml/min/kg, P<0.05) and proteinuria (treated:199±21 VS untreated:277±27 mg/day, P<0.05) in STNx rats. Histologically, RFL treatment significantly reduced glomerulosclerosis (treated:0.97±0.11 VS untreated:1.59±0.22 AU, P<0.05) and macrophage infiltration (treated:123±9 VS untreated:214±12 count/field, P<0.001), and though a trend towards reduced tubulointerstitial fibrosis was seen, this did not reach statistical significance. MCP-1 gene expression was also significantly lower in the treated group (treated:2.19±0.28 VS untreated:3.10±0.38 AU, P<0.05).

Conclusions: RFL attenuated the functional and structural deterioration in a rat CKD model, at least in part, by reducing inflammation. Therefore, PDE4 inhibition may represent a potential therapeutic target for the treatment of CKD.

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SA-PO370

Erythropoietin Protects Integrity of Tubular Basement Membrane by Up-Regulating miR-144 Level in Serum Microvesicles Yang Zhou, Jing Niu, Junwei Yang. *Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

Background: Chronic kidney disease is characterized by tubulointerstitial fibrosis. Disruption of the integrity of tubular basement membrane (TBM) contributes to renal fibrosis. Tissue plasminogen activator (tPA) is one of the major components in the matrix proteolytic network by activating matrix metalloproteinase (MMP)-9. tPA is the predicted target of miRNA (miR)-144. It was reported by anti-doping researchers that serum miR-144 was up-regulated by erythropoietin (EPO), which was reported to attenuate renal fibrosis. Serum miRNAs are probably encapsulated in microvesicles. Here, we investigated whether EPO protected the integrity of TBM to attenuate renal fibrosis by up-regulating miR-144 in serum microvesicles, which then targeted fibroblasts' tPA expression and inhibited MMP-9 activation.

Methods: Renal tubulointerstitial fibrosis mouse model was induced by unilateral ureteral obstruction (UUO). EPO was administered intraperitoneally at a dose of 1000U/kg body weight every other day. Serum microvesicles were isolated by ultracentrifugation and administered through tail vein injection. Q-PCR, western blot and zymography were applied to evaluate expression of miR-144 and tPA and activity of MMP-9, respectively.

Results: In obstructed kidney, EPO attenuated disruption of TBM, relieved up-regulation of tPA and activation of MMP-9. MiR-144 level in serum microvesicles was up-regulated by EPO. The miR-144-containing microvesicles attenuated TGF-β1-induced up-regulation of tPA and activation of MMP-9 in renal fibroblasts. Moreover, administration of miR-144-containing microvesicles attenuated disruption of TBM and relieved up-regulation of tPA and activation of MMP-9 in obstructed kidney. MiR-144 negatively regulated tPA expression and therefore interfered MMP-9 activation of fibroblasts. Ectopic expression of miR-144 alleviated, while inhibition of miR-144 facilitated TGF-β1-induced up-regulation of tPA and activation of MMP-9 of renal fibroblasts.

Conclusions: These results suggest that EPO protects the integrity of TBM in obstructed kidney by up-regulating miR-144 level in serum microvesicles, which then inhibits the expression of tPA and activity of MMP-9 of fibroblast.

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SA-PO371

Autophagy Is the Predominant Mediator of TGF-β1-Induced Profibrotic Dedifferentiation in Tubular Epithelial Cells Hailong Wang,[1,2] Min Pang,[1,3] Ye Zhao,[1] Yun Zhang,[1,3] Qi Cao,[1] Yiping Wang,[1] Yuan Min Wang,[4] Stephen I. Alexander,[4] Guoping Zheng,[1] David C. Harris.[1] *[1]Centre for Transplant and Renal Research, Westmead Millennium Inst, Univ of Sydney, Sydney, NSW, Australia; [2]Biochem and Mol Biol, Shanxi Medical Univ, Taiyuan, Shanxi, China; [3]1st Hospital of Shanxi Medical Univ, Shanxi Medical Univ, Taiyuan, Shanxi, China; [4]Centre for Kidney Research, Children's Hospital at Westmead, Sydney, NSW, Australia.*

Background: Autophagy and tubular cell profibrotic dedifferentiation are both increased in kidney fibrosis, but the role of autophagy in kidney fibrosis is controversial, and details of cross-talk between autophagy and profibrotic dedifferentiation are unclear.

Methods: Mouse tubular epithelial C1.1 cells were treated with TGF-β1 in presence or absence of Rapamycin or 3-methyladenine (3-MA) to augment or inhibit autophagy respectively, and to examine the role of autophagy in tubular cell profibrotic dedifferentiation. MG132 and chloroquine or NH_4Cl were used to inhibit proteosomal or lysosomal protein degradation respectively. Smad7 and β-catenin degradation chimera F-TrCP-Ecad plasmids were used to inhibit TGF-β1/Smad and β-catenin signalling.

Results: TGF-β1-induced both autophagy and profibrotic dedifferentiation in C1.1 cells, as demonstrated by increased levels of autophagy markers (beclin 1 and LC3) and vimentin and integrin linked kinase (ILK) with reduction of E-cadherin. Serum rescue or inhibition of autophagy reduced (by more than 90%) while augmentation increased (about 2 fold) TGF-β1-induced profibrotic dedifferentiation. Inhibition of lysosomal but not proteosomal degradation prevented reduction of E-cadherin. Autophagy increased Src kinase, Src kinase-mediatedphosphorylation of Y654-β-catenin (pY654-β-catenin) and pY654-β-catenin/pSmad2 complex formation, while pY654-β-catenin/pSmad2 mediated upregulation of ILK, a known inducer of tubular cell dedifferentiation and fibrosis.

Conclusions: Autophagy accounts for the majority of TGF-β1-induced tubular cell profibrotic dedifferentiation, through pSmad2/pY654-β-catenin-mediated upregulation of ILK. Autophagy is a key target to limit fibrosis.

Funding: Government Support - Non-U.S.

SA-PO372

Targeting Tubulointerstitial Remodeling By Lymphangiogenesis Blockage and Macrophage Depletion in Rat Proteinuric Nephropathy Saleh Yazdani,[1] Ryanne S. Hijmans,[1] Gerjan Navis,[1] Harry Van Goor,[2] Jacob van den Born.[1] *[1]Nephrology, UMCG, Groningen, Netherlands; [2]Pathology, UMCG, Groningen, Netherlands.*

Background: Proteinuria is an important cause of progressive tubulointerstitial damage. We previously showed that proteinuria can trigger renal lymphangiogenesis (LA) before the onset of interstitial inflammation and fibrosis. However, the interrelationship of these tubulointerstitial events is not clear yet. Also, macrophages (MΦs) have shown to be involved in tubulointerstitial remodelling and promoting LA. Therefore, in a proteinuria model we targeted LA (anti-VEGFR3 antibody (IMC-3C5)) and inflammation (deplete monocytes/ MΦs by Clodronate liposomes (CL)) and evaluated interstitial pathology and renal function.

Methods: Proteinuria was induced in 3 month-old male Wistar rats by adriamycin injection (1.8 mg/kg). After 6 weeks, when proteinuria was developed (~200 mg/ml /24hrs), the treatment by IMC-3C5 (Imclone, U.S.A., i.p. 40mg/kg, 3 times/week) and CL (i.p. twice weekly 1 ml) started for another 6 consecutive weeks. At week 12, rats were sacrificed and blood, urine and organs were collected.

Results: : In proteinuric rats, LA (podoplanin+ vessels), inflammation (ED1+ MΦs), and fibrosis (Collagen III, and α-SMA for myofibroblasts) significantly increased at week 12 versus week 6 (all p<0.05). IMC-3C5 completely blocked LA (p<0.0001), diminished interstitialMΦs, and improved plasma creatinine (p<0.05) and urea levels in proteinuric rats. However, LA blocking did not prevent α-SMA expression, collagen III deposition and progression of proteinuria. Additionally, CL significantly reduced MΦ influx into kidney (p<0.05), partly reduced α-SMA expression, however, neither prevented LA, fibrosis and proteinuria, nor improved renal function.

Conclusions: This study showed the dissociation of renal LA from inflammation (MΦ), and to some extent from profibrotic/fibrotic response (α-SMA and Collagen III), at least in this proteinuric model. It seems that, opposite to several earlier reports both in renal diseases and other organs, MΦs are not the main player in inducing LA in proteinuric kidneys. Moreover, blocking LA evidently reduced plasma creatinine/urea which might be therapeutically relevant in proteinuric conditions.

SA-PO373

Macrophage Phenotypes in Acute and Chronic Kidney Disease Magnus Soderberg,[1] Alan Sabirsh,[1] Johan C. Molne.[2] *[1]AstraZeneca R&D, Sweden; [2]AstraZeneca R&D, Sweden; [3]Sahlgrenska Univ Hospital, Sweden.*

Background: Macrophages (MØ) play an important role in the progress of kidney disease. In animal models, modifications of MØ phenotype (M1/M2) have been described that retard disease progression, leading to the assumption that steering MØ response from a "pro-inflammatory" M1 type towards a "pro-reparative" M2 type is a target for therapeutic intervention. However, there is currently a lack of scientific data on the phenotypic profiles of MØ subsets in human kidney disease.

Methods: Renal biopsies from healthy renal transplant donors and from patients with renal disorders have been used, including 5 patients each with diabetic nephropathy (DN), IgA nephropathy (IgAN), acute transplant rejection and chronic transplant rejection. Immunohistochemistry was done using CD68 as a pan-MØ marker. MØ subtype phenotyping was performed using: M1: CD80; M2a: CD206; M2b: IL10; M2c: CD163. Dendritic cells were identified using CD1a and CD83. Positive cells were counted manually using light microscopy or automated using image analysis.

Results: A limited number of MØ were found in healthy controls. All disease groups showed increased MØ infiltration revealed by CD68 stain; the increase was 4-fold in IgAN, 5-fold in DN and 6-fold in rejection. M1 MØ were few (<2% of total MØ in controls), with no clear increase in any disease. M2a was the dominating MØ subtype (>90% of all MØ in all groups). M2a increased 1.5-fold in IgAN and 2.5-fold in DN and rejection. M2b and M2c were few (<1 % each of total MØ in controls). M2b increased 10-fold in IgAN, 20-fold in rejection and 25-fold in DN. M2c doubled in rejection, but did not change in DN or IgAN. M2b and M2c varied considerable between individuals within disease groups. Dendritic cell presence was minimal, except in chronic disease when positive cells appeared in intrarenal lymphoid follicles.

Conclusions: Proper interpretation of MØ response in human kidney must take both type of disorder and stage of disease development into account. The observations of M2a domination both in acute and chronic renal disease and the limited contribution and response of the M1 phenotype in disease challenge the current paradigm of the M2 response being the more benign.

Funding: Pharmaceutical Company Support - AstraZeneca

SA-PO374

RNA-seq Profiling of S1 Proximal Tubules Microdissected from the Uninephrectomized Rats

Jae Wook Lee, Mark A. Knepper. *Systems Biology Center, NHLBI, NIH, Bethesda, MD.*

Background: Nephron hypertrophy occurs as a compensatory response to reduction in nephron mass. To identify early gene expression changes in nephron hypertrophy, we measured global gene expression in microdissected S1 proximal tubules of the contralateral kidney of uninephrectomized rats using next-generation sequencing of RNAs (RNA-seq).

Methods: 5-week-old male Sprague-Dawley rats received uninephrectomy (U, n=4) or sham surgery (S, n=4). 48 hours after surgery, S1 proximal tubules (8 mm per each animal) were microdissected under a stereomicroscope. Poly(A)'-tailed mRNAs were reverse-transcribed and the adapter-ligated cDNA libraries were sequenced using an Illumina HiSeq2000 platform. FASTQ sequences obtained were mapped to rat reference genome. Expression levels are calculated for RefSeq transcripts in terms of reads per kilobase exons per million mapped reads (RPKMs).

Results: The contralateral kidneys in uninephrectomized rats were heavier than their sham counterparts (0.87±0.11 g/100 g BW in U versus 0.76±0.09 in S). Each library had 35-42 million reads and more than 80% of reads were uniquely aligned to the reference genome. There were 6093-7995 RefSeq transcripts detected in individual samples with RPKM > 1. Using dual statistical criteria (U/S ratios outside of 95% confidence interval for S/S ratios and $p<0.05$ for t-test, "volcano plot") giving a conservative criterion for changes, more transcripts were downregulated (41) than upregulated (8). Among these were *Zfp36*, an immediate early gene induced by growth factors as well as a secreted growth factor (*Ctgf*) and several modulators of growth-factor signaling (*Errfi1*, *Igfbp1*, and *Igfbp3*). Also downregulated were three regulators of cell cycle (*Pim3*, *Plk3* and *Rgcc*), a scaffold/ubiquitin E3 ligase (*Sh3rf1*, also known as 'plenty of SH3 (POSH)'), and two glucocorticoid-regulated genes (*Sgk1* and *Tsc22d3*). Upregulated transcripts included a transcription factor (*Hnf1b*).

Conclusions: The RNA-seq method is applicable at the level of single microdissected tubules and that the overall method is sufficiently facile to allow us to map transcriptional responses over a full set of time points after uninephrectomy in S1 proximal tubules and other relevant structures.

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SA-PO375

Kidney Angiotensin and Bradykinin Levels Are Decreased in Captopril-Treated Uninephrectomized Fawn-Hooded Hypertensive Rats

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Background: The intrarenal renin-angiotensin system is a therapeutic target of glomerular disease. We previously reported the effect of chronic intravenous infusion of angiotensin (Ang) 1-7 and Ang2-10 in comparison to captopril on severity of kidney damage in uninephrectomized fawn-hooded hypertensive rats (Unx-FHH). Here we report on the intrarenal concentrations of Ang peptides, bradykinin (BK) 1-9, and their relationship with hemodynamic/renal outcomes.

Methods: AngI, AngII, Ang2-10, Ang1-7 and BK1-9 levels were determined using liquid chromatography tandem mass spectrometry.

Results: Vehicle-treated rats developed hypertension and lesions of FSGS. Both hypertension and renal disease were markedly attenuated by captopril treatment. Tissue AngII levels were 1.4 fold lower in the captopril- versus vehicle-treated groups (3.3+/-0.8 versus 5.5+/-1.6 fmol/mg, respectively, $p<0.05$), which is consistent with Ang converting enzyme inhibition. Paradoxically, although we predicted a rise in BK1-9 in captopril–treated rats due to inhibition of BK degradation, tissue BK1-9 levels were 2.2 fold lower in animals treated with captopril versus control (12.8+/-4.3 versus 28.4+/-11.1 fmol/mg, respectively, $p=0.006$). Correlation analysis of all Ang and BK peptide levels at 30 weeks showed positive correlations between BK1-9 levels and urine volume ($R=0.46$, $p<0.01$), blood pressure ($R=0.63$, $p<0.001$), and Ang1-7 ($R=0.48$, $p<0.01$). In contrast, AngII levels were only significantly correlated with kidney weight normalized to body weight ($R=0.38$, $p<0.05$), Ang2-10 ($R=0.55$, $p<0.001$), and Ang1-7 ($R=0.45$, $P<0.001$). Ang1-7 levels were correlated to urine volume ($R=0.46$, $p<0.01$). No peptide correlated to proteinuria.

Conclusions: The counterintuitive finding that BK1-9 levels are reduced in response to captopril suggests downregulation of the renal bradykinin system under conditions of reduced levels of AngII. Further, intra-renal BK1-9 may contribute to the regulation of renal function in Unx-FHH.

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SA-PO376

Combination Treatment with ACE Inhibitor and Nicorandil in the Rat Remnant Kidney Model

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Background: An inhibition in the renin-angiotensin system is one of the most widely used therapies to treat chronic kidney disease. However, its effect is not sufficient and in such cases, additional treatment is required. Recently, we reported that nicorandil exhibited renoprotective effects in a mouse model of diabetic nephropathy. Here we examined if nicorandil can provide an additive protection on enalapril in chronic kidney disease.

Methods: RK rats were divided into five groups: Sham operated rats (n = 6), remnant kidney (RK) rats without treatment (n = 6), RK rats treated with enalapril 2 mg/kg/day (Ena, n = 6), RK rats treated with nicorandil 15 mg/kg/day (Nico, n = 6), RK rats treated with Ena + Nico (Ena + Nico, n = 6). Twelve weeks later, the rats were sacrificed.

Results: Single treatment with either enalapril or nicorandil significantly ameliorated Ccr, proteinuria and histological injury in the kidney of RK model whereas the combination of these two compounds provided additive effects (Table). In addiiton, an increase in oxidative stress was also blocked by either enalapril or nicorandil while the combination of the drugs was more potent. A mechanism was likely due for nicorandil to preventing MnSOD and sirtuin-3 from being reduced in injured kidneys.

	Ccr	Proteinuria	Glomeruli	Tubulointerstitium
	ml/min/100g	mg/day	CollagenIV (+) area (%)	CollagenIII (+) area (%)
Sham	0.72±0.08**††	4.2±2.3**	11.9±1.8**	5.4±1.1**
RK	0.13±0.07††	98.5±42.0††	34.5±8.9††	24.7±0.3††
Ena	0.23±0.02*†	55.3±38.0**††	23.2±2.7**††	14.6±0.8**††
Nico	0.30±0.07**	69.6±26.0*††	22.1±2.7**†	14.5±1.4**††
Ena+NIco	0.34±0.04**	19.0±6.8**	13.8±2.2**	7.1±1.5**

**p<0.01, *p<0.05 vs RK, ††p<0.01, †p<0.05 vs Ena+Nico

Conclusions: Nicorandil can be added on enalapril to obtain further protection in the patients with chronic kidney disease.

SA-PO377

Chronic Angiotensin II Infusion Causes Kidney Angiotensin II Accumulation and Aggravated Glomerular Injury in Aminopeptidase A-Deficient Mice

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Background: The intrarenal renin-angiotensin system is a therapeutic target in progressive proteinuric glomerular diseases. Aminopeptidase A (APA) is abundantly expressed in the kidney and metabolizes angiotensin (Ang) peptides at the amino terminus. However, it is not known whether APA is renoprotective in Ang II-mediated hypertensive kidney disease.

Methods: We tested the effect of a 3-week subcutaneous administration of Ang II (400 ng/kg/min) via osmotic mini-pumps in BALB/c APA-deficient (APA-KO) and wild-type (WT) mice. Systolic blood pressure (SBP) was measured by tail-cuff, urine albumin-to-creatinine ratio (UACR) was determined by ELISA, and intrarenal Ang peptide content was measured by liquid chromatography/tandem mass spectrometry.

Results: Baseline SBP was not different between APA-KO and WT mice (104 ± 3 versus 103 ± 2 mmHg). Although no significant difference was found in SBP after chronic infusion of Ang II between the strains (220 ± 4 versus 216 ± 6 mmHg), APA-KO mice developed a significant rise in UACR that was not observed in WT controls (245 ± 106 versus 28 ± 10 μg/mg, for APA-KO and WT, respectively; $p=0.009$). Under baseline conditions, the intrarenal concentration of Ang II was similar between groups. Following chronic Ang II infusion, the intrarenal concentration of Ang II in APA-KO mouse kidneys was significantly greater than that observed in WT controls (16 ± 4 versus 7 ± 3 fmol/mg, for APA-KO and WT, respectively; $p=0.005$). On histology, arterial wall thickening was noted in renal vessels in both groups. Furthermore, focal areas of glomeruli with near total collapse and markedly dilated tubules filled with proteinaceous material were found in kidneys of APA-KO mice, while those abnormalities were absent in WT mouse kidneys.

Conclusions: These data demonstrate that deficiency of APA increases Ang II-mediated hypertensive kidney injury, supporting a protective role for APA in Ang II-mediated kidney disease, likely explained by APA-mediated degradation of Ang II to Ang III and diversion of Ang I conversion to Ang 2-10.

SA-PO378

Renin Angiotensin System (RAS) Blockade Attenuates Growth and Metastasis Formation of Renal Cell Carcinoma in Mice Wedson Araujo,[1] Juliana N. Ravanini,[2] Josne Carla Paterno,[1] Marcelo Andery Naves,[1] Nestor Schor,[1] Vicente De Paulo Castro Teixeira.[1] *[1]Medicine / Nephrology Div, Univ Federal de Sao Paulo/EPM, Sao Paulo, Brazil; [2]Pathology, USP, Sao Paulo, Brazil.*

Background: Renal cell carcinoma (RCC) is the most frequent cancer among renal neoplasms in adults and poor responsive to radio-, chemo- and immunotherapy There is increasing evidence that blockade of RAS has antineoplasic effects. Objective: Investigate the effects of RAS blockade on renal cell carcinoma (RCC) in a murine model.

Methods: Murine renal cancer cells (RENCA) were cultivated and injected ($1x10^5$) into subcapsular space of the left kidney of BALB/c mice (8 weeks). The animals were divided into 4 groups : control group (no treatment), Losartan (100 mg / kg / day), Captopril group (10 mg / kg / day) and Losartan + Captopril group (100 mg / kg / day + 10 mg / kg / day). The animals received the drugs by gavage 2 days before tumor induction and continued until euthanasia (21 days after inoculation). After sacrifice, kidneys and lungs were removed, weighed and processed for histopathological. Angiogenesis and vascular microvessel were determined by immunohistochemical evaluation for VEGF and CD34. The statistical significance of the results was evaluated by ANOVA followed by Tukey test.

Results: All inoculated animals developed renal tumor. Treated animals presented smaller tumors, regardless the therapeutic regimen and far fewer lung metastases in both quantity and dimension in relation to controls. The expressions of VEGF and CD34 were significantly decreased in renal tumors of animals treated when compared with controls.

Conclusions: Our findings suggest that blockade of RAS decrease the tumor proliferation capacity and metastatic potential of renal cell carcinoma in this experimental model.

SA-PO379

Blockade of Wnt/β-Catenin Signaling Ameliorates Hypertension and Kidney Injury By Targeting Renin-Angiotensin System Liangxiang Xiao,[1] Lili Zhou,[1] Fan Fan Hou,[1] Youhua Liu.[2] *[1]State Key Laboratory of Organ Failure Research, Nanfang Hospital, Southern Medical Univ, Guangzhou, Guangdong, China; [2]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA.*

Background: Local activation of the intra-renal renin-angiotensin system (RAS) plays a critical role in mediating hypertension and chronic kidney diseases. As Wnt/β-catenin regulates multiple RAS genes in the kidneys, we speculated that this developmental signaling might be linked to blood pressure regulation and kidney pathology after injury.

Methods: To test this hypothesis, we investigated the role of β-catenin in regulating RAS activation, hypertension and kidney injury in rat remnant kidney model induced by 5/6 nephroectomy (5/6NX).

Results: At 12 weeks after 5/6NX, renal β-catenin was markedly up-regulated predominantly in renal tubular epithelium. Delayed administration of a small molecule β-catenin inhibitor (ICG-001), starting at 6 weeks after 5/6NX, substantially reduced albuminuira, serum creatinine and blood urea nitrogen. Interestingly, ICG-001 blunted blood pressure elevation in 5/6NX rats, and markedly alleviated renal fibrotic lesions. Renal expression of fibronectin, collagen I, collagen III and plasminogen activator inhibitor 1 was reduced. ICG-001 also inhibited renal infiltration of $CD3^+$ T cells and $CD68^+$ monocytes/macrophages. Subtotal renal ablation by 5/6NX induced multiple RAS components such as agiotensinogen, renin and angiotensin type I receptor in the kidneys. However, inhibition of β-catenin signaling by ICG-001 dramatically blocked their induction. In vitro, incubation with losartan, an angiotensin type I receptor blocker, prevented Wnt/β-catenin-mediated fibronectin, α-smooth muscle actin and Snail1 expression, suggesting that the fibrogenic action of Wnt/β-catenin is dependent on RAS activation.

Conclusions: These results illustrate that hyperactive Wnt/β-catenin signaling drives hypertension and kidney damage via RAS activation after subtotal renal ablation.

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SA-PO380

Sustained Activation of Wnt/β-Catenin Signaling Drives AKI to CKD Progression Liangxiang Xiao,[1] Dong Zhou,[1] Roderick J. Tan,[2] Haiyan Fu,[1] Lili Zhou,[3] Fan Fan Hou,[3] Youhua Liu.[1] *[1]Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA; [2]Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA; [3]State Key Laboratory of Organ Failure Research, Nanfang Hospital, Southern Medical Univ, Guangzhou, Guangdong, China.*

Background: Acute kidney injury (AKI) is increasingly recognized as a major risk factor leading to subsequent progression to chronic kidney disease (CKD). However, what factors govern AKI to CKD progression remains poorly understood.

Methods: In this study, we investigated this issue by using both moderate (20 min) and severe (30 min) ischemia/reperfusion injury (IRI) in mice.

Results: Moderate IRI led to acute kidney failure and transient Wnt/β-catenin activation, which was followed by the restoration of kidney morphology and function. However, severe IRI resulted in sustained Wnt/β-catenin activation, which was accompanied by development of renal fibrotic lesions characterized by interstitial myofibroblast activation and excessive extracellular matrix deposition. To ascertain the role of sustained Wnt/β-catenin in AKI to CKD progression, we manipulated this signaling by either over-expression of Wnt ligand or pharmacologic inhibition of β-catenin. At 5 days after IRI, over-expression of Wnt1 in vivo induced β-catenin activation and accelerated AKI to CKD progression. Conversely, blockade of Wnt/β-catenin by small molecule ICG-001 prevented AKI to CKD progression. In vitro, Wnt ligands induced myofibroblastic activation of fibroblasts. However, activated myofibroblasts readily returned to quiescent fibroblast phenotype after Wnts were removed.

Conclusions: These results indicate that sustained, but not transient, activation of Wnt/β-catenin signaling plays a decisive role in AKI to CKD progression.

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

SA-PO381

Lubiprostone Ameliorates the Progression of Chronic Kidney Disease By Altering the Intestinal Environment Eikan Mishima,[1] Shinji Fukuda,[2] Yasutoshi Akiyama,[1] Hisato Shima,[1] Sadayoshi Ito,[1] Takaaki Abe.[1] *[1]Tohoku Univ, Sendai, Japan; [2]Keio Univ, Japan.*

Background: The accumulation of uremic toxins is involved in CKD progression. It is well known that various uremic toxins are derived from gut microbiota and that an imbalance of gut microbiota is related to renal failure. In CKD, the intestinal environment is reported to be adversely altered. However, the pathophysiological mechanisms underlying the relationship between the gut microbiota and renal failure are still obscure.

Methods: Using an adenine-induced renal failure mouse model, we evaluated the potentially beneficial effects of the ClC-2 chloride channel activator lubiprostone, commonly used for constipation treatment, on CKD. Intestinal and fecal properties were evaluated. Renal fibrosis and inflammation were evaluated with histology, IHC and quantitative PCR. Gut microbiome was analyzed at the genus level by pyrosequencing of microbial 16S rRNA genes with the next generation sequencer.The plasma concentration of various uremic solutes was measured by capillary electreophoresis-masss pectrometry (CE-MS)-based metabolome analysis.

Results: Oral administration of lubiprostone (500 μg/kg/day) changed the fecal properties and reduced the collagen deposition in the colon of the renal failure mice. Lubiprostone treatment also reduced the elevated blood urea nitrogen and protected against tubulointerstitial damage, renal fibrosis and inflammation. Gut microbiome analysis in the renal failure mice showed that lubiprostone treatment altered their microbial composition, especially the recovery of the level of the *Lactobacillaceae* family and *Prevotella* genus, which were significantly reduced in the renal failure mice. Furthermore, CE-MS-based metabolome analysis showed that lubiprostone treatment decreased the plasma level of uremic toxins, such as 3-indoxyl sulfate and hippurate, which are derived from gut microbiota as well as newly identified uremic toxin, trans-aconitate.

Conclusions: This suggests that lubiprostone ameliorates CKD progression and uremic toxin accumulation through an improvement of the gut microbiota and intestinal environment.These findings provide supporting evidence to recent reports on the gut-kidney axis.

Funding: Government Support - Non-U.S.

SA-PO382

Differential Roles of C5a Receptors C5aR and C5L2 in Kidney and Liver Fibrosis Ina V. Martin,[1] Annika Bohner,[2] Peter Boor,[1] Frank Lammert,[2] Jürgen Floege,[1] Tammo Ostendorf,[1] Susanne N. Weber.[2] *[1]Nephrology, Univ Hospital, RWTH, Aachen, Germany; [2]Gastroenterology, Saarland Univ Hospital, Homburg, Germany.*

Background: Complement factor C5a has two known receptors, C5aR and C5L2. C5aR is involved in transduction of the proinflammatory effects of C5a, whereas C5L2 has been suggested as a decoy receptor. We previously demonstrated a pro-fibrotic role of C5a and C5aR in kidney and liver, but nothing is known concerning the potential involvement of C5L2 in organ fibrosis.

Methods: $C5L2^{-/-}$-, $C5aR^{-/-}$- and wildtype (WT) control mice were subjected to 5 days of unilateral ureteral obstruction (UUO), a model of renal fibrosis, or liver fibrosis by CCl_4-treatment for 6 weeks. Extracellular matrix (ECM) deposition, the inflammatory status and injury markers were analyzed by gene expression analyses and immunohistochemistry.

Results: During progression of kidney fibrosis both C5a receptors are overexpressed. Compared to WT, C5aR deficiency led to a significant reduction of renal collagen I and IV, fibronectin, αSMA and vimentin mRNA. At the protein level similar changes were observed. Renal infiltration of macrophages and CCL2, CCL5 and TNFα expression did not change in $C5aR^{-/-}$-mice versus WT. In contrast, C5L2 deficiency in renal fibrosis resulted in only mild reduction of ECM compared to WT, whereas CCL2, CCL5, IL6 and TNFα expression increased. Accordingly, there was a trend towards increased renal infiltration of macrophages in $C5L2^{-/-}$-mice. Transcripts of TGFβ and KIM1 were markedly reduced in both, $C5aR^{-/-}$- and $C5L2^{-/-}$-mice versus WT. In liver fibrosis, collagen contents were significantly lower in $C5aR^{-/-}$- but unchanged in $C5L2^{-/-}$-mice. Transcript analyses of cytokines in the chronic fibrosis model (TGFβ, TNFα, IL6, IL10, IL12, IL23, IL27) showed a prominent reduction in both receptor-deficient mice versus WT. One day after an acute single CCl_4-challenge, these cytokines were highly upregulated specifically in $C5L2^{-/-}$-mice versus WT and $C5aR^{-/-}$-mice.

Conclusions: In conclusion, C5aR is involved in chronic fibrogenesis in both liver and, albeit less potently, also in the kidney. In the liver, C5L2 provides protection from early inflammatory insults, and in kidney fibrosis C5L2 might also play an anti-inflammatory role.

Funding: Government Support - Non-U.S.

SA-PO383

Loss of Expression of the SMAD2/3 Phosphatase, PPM1a during Kidney Fibrosis Promotes a Maladaptive Repair Response Rohan Samarakoon,[1] Nidah S. Khakoo,[1] Amy D. Dobberfuhl,[2] Sevann Helo,[2] Lucas Falke,[3] Jessica Overstreet,[1] Roel Goldschmeding,[3] Paul J. Higgins.[1] *[1]Center for Cell Biology and Cancer Research, Albany Medical Center, Albany, NY; [2]Div of Urology, Albany Medical Center, Albany, NY; [3]Dept of Pathology, Univ Medical Center Utrecht, Utrecht, Netherlands.*

Background: Deregulation of negative regulators of TGF-βeta1 pathway (e.g., BMP-6/7, Ski, SMAD7) are documented in the progression of kidney disease. PPM1a is a recently identified c-terminal SMAD2/3 phosphatase that inhibits TGF-βeta signaling. The role of PPM1a in renal fibrosis, however, is unknown.

Methods: Ureteral unilateral obstruction (UUO) and Aristololic acid nephropathy (AAN) mouse models and genetic manipulation in HK-2 tubular epithelial cells and NRK-49F renal fibroblasts were utilized to investigate role of PPM1a in fibrosis.

Results: PPM1a expression is dramatically decreased in UUO and AAN-injured kidneys both in the tubular and interstitial regions, correlating with increased pSMAD3 and fibrosis marker expression. Stable gene silencing of PPM1a in HK-2 cells further enhanced TGF-βeta1 induced pSMAD3 activity and PAI-1, fibronectin and CTGF target gene expression. Conversely, transient PPM1a overexpression suppressed this response. Prolonged loss of PPM1a by stable RNA interference initiates pSMAD3 activation as well as PAI-1, CTGF and fibronectin, vimentin and alpha-SMA induction while transient PPM1a gene depletion in NRK-49Fs promotes a fibro-proliferative response. Nuclear levels of PPM1a are dramatically reduced by PTEN knockdown, enhancing SMAD3 activation and PAI-1 induction. These responses are reversed by ectopic PPM1a expression in PTEN depleted HK-2 cells.

Conclusions: A negative regulator of the SMAD2/3 pathway, prolonged PPM1a gene silencing initiated renal mesenchymal gene induction and proliferative arrest in tubular epithelial cells. PTEN is an upstream regulator of PPM1a as loss of PTEN promotes PPM1a mislocalization and a SMAD3-dependent renal fibrotic phenotype.

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SA-PO384

Increased Expression of Par1a/1b in Proximal Tubular Injury in Murine Kidneys Natalie S. Uy,[1] Samriti Dogra,[1] Jennifer R. Charlton,[2] Michael S. Forbes,[2] Robert L. Chevalier,[2] Sanjeev Gupta,[3] Kimberly J. Reidy.[1] *[1]Pediatric Nephrology, Children's Hospital at Montefiore, Bronx, NY; [2]Pediatric Nephrology, Univ of Virginia, Chralottsesville, VA; [3]Dept of Gastroenterology, Albert Einstein College of Medicine, Bronx, NY.*

Background: Apico-basal polarity is required for directional transport by renal epithelial cells. Defects in apico-basal polarity occur in kidney diseases such as acute kidney injury (AKI) and obstructive nephropathy. Partitioning defective Par1 is a serine-threonine kinase that localizes to the basolateral aspect of cells and establish apico-basal polarity in vitro. Mammlian homologues Par1a and 1b are functionally redundant on kinase assays and appear to compensate for one another in vivo. We have recently examined the role of Par1a/b in vivo and identified a role for Par1a/b in establishing proximal tubular polarity in developing mouse kidneys. Par1a/b proteins are highly expressed in developing kidneys and down-regulated in the adult. We hypothesized that Par1a/b would be re-expressed in the setting of kidney injury and sought to define Par1a/b expression in the setting of tubular injury.

Methods: We examined expression of Par1a and 1b using immunofluorescence with homologue specific antibodies in two models of renal tubular injury as compared to adult controls. Adult mice underwent unilateral ureteral obstruction (UUO) by complete ligation of the left kidney ureter. Kidneys were examined 14 days after obstruction. To examine the effect of proximal tubular toxin, we examined Par1a/b expression in kidneys from cisplatin treated mice (20 mg/kg).

Results: In controls, Par1a/b expression was almost completely absent in adult proximal tubules. Increased expression of both Par1a and 1b was identified in both UUO injury and cisplatin-induced AKI. Par1a/1b expression localized predominantly at the basolateral aspect of proximal tubules.

Conclusions: Par-1a are 1b are required for normal kidney development, but are downregulated in the adult. We identified up-regulation of Par1a/b in the setting of two mouse models of tubular injury, suggesting the possibility that re-expression of Par1a/1b and apico-basal polarity signaling may contribute to tubular repair.

SA-PO385

L-Carnitine Reverses the Effect of Maternal Smoking on Renal Oxidative Stress and Mitochondrial Dysfunction Stefanie Stangenberg,[1] Long The Nguyen,[2] Ibrahim Al-Odat,[2] Hui Chen,[2] Martin Edward Gosnell,[3] Carol A. Pollock,[1] Sonia Saad.[1] *[1]Kolling Inst of Medical Research, Univ of Sydney, Australia; [2]School of Medical and Molecular Sciences, Univ of Technology, Sydney, Australia; [3]Macquarie Univ, Sydney, Australia.*

Background: Maternal smoking has been associated with underdevelopment of fetal/neonatal organs and low birth weight, which is closely linked to a susceptibility to chronic kidney disease (CKD) in later life. Evidence suggests that oxidative stress and mitochondrial dysfunction may play a role. L-carnitine is a nutritional supplement that was shown to benefit both antioxidant defense and mitochondrial performance in different disorders, however its role in the kidney is not known.

Methods: Balb/c mice were sham exposed to air or exposed to cigarette smoke from 2 cigarettes twice daily for 6 weeks before mating, throughout gestation and lactation. A subgroup of smoke exposed (SE) mice received L-carnitine in drinking water. Offspring's kidneys were harvested at birth, weaning and adulthood. Mitochondrial function was examined by levels of oxidative phosphorylation (OXPHOS) enzymes, mitochondrial protein (TOM20) and antioxidant levels of Manganese Superoxide Dismutase (MnSOD) and Glutathione Peroxidase (GPX-1).Oxidative stress was evaluated by determining total and mitochondrial reactive oxygen species (ROS). Body and kidney weight and and glucose tolerance were also measured.

Results: L-carnitine reversed low birth weight, glucose intolerance and high levels of ROS in the SE offspring. Maternal smoke exposure caused a significant reduction in most of the OXPHOS complexes, TOM20, MnSOD and GPX-1 at birth and adulthood, which were normalized under L-carnitine supplementation.

Conclusions: L-carnitine significantly reduces renal oxidative stress and mitochondrial dysfunction in offspring of smoke exposed mothers. This suggests a potential role for L-carnitine in preventing CKD.

Funding: Government Support - Non-U.S.

SA-PO386

The Effect of Maternal Smoking on Mitochondrial Structure and Function in Offspring Kidneys Stefanie Stangenberg,[1] Long The Nguyen,[2] Ibrahim Al-Odat,[2] Hui Chen,[2] Martin Edward Gosnell,[3] Carol A. Pollock,[1] Sonia Saad.[1] *[1]Kolling Inst of Medical Research, Univ of Sydney, Sydney, Australia; [2]School of Medical and Molecular Sciences, Univ of Technology, Sydney, Australia; [3]Macquarie Univ, Sydney, Australia.*

Background: The role of an adverse in-utero environment on programming of chronic adulthood diseases is emerging. Maternal smoking during gestation is associated with low birth weight and increased oxidative stress in mothers and newborns. The association between low birth weight and chronic kidney disease in later life is well described, however the mechanisms underlying such susceptibility remain unknown.

Methods: Female Balb/c mice were sham or cigarette smoke exposed (SE) for 6 weeks before mating, throughout gestation and lactation. Male offspring were sacrificed at day 1, day 20 (weaning) and week 13 (adulthood). Blood and urine were collected, kidneys were harvested to determine oxidative stress and mitochondrial function. Oxidative stress was assessed by CellROX® and localized by Mitotracker® co-stain. Mitochondrial structure was examined by electron microscopy. Mitochondrial copy number was determined by real-time PCR. The activity of mitochondrial superoxide dismutase (MnSOD) and levels of the oxidative phosphorylation (OXPHOS) complexes I-V and translocase of outer mitochondrial membrane (TOM20) protein were determined.

Results: Offspring from SE mothers had low birth weight and increased albumin/creatinine ratio at adulthood. Kidneys of SE offspring had fewer and enlarged mitochondria at adulthood while oxidative stress was increased and localized to mitochondria. OXPHOS enzyme levels, TOM20 and MnSOD level and activity were significantly reduced at birth and adult age but not at weaning.

Conclusions: Maternal smoking leads to oxidative stress, structural mitochondrial changes and mitochondrial dysfunction as well as adult onset albuminuria in the offspring kidney. The mitochondrial dysfunction and reduced antioxidant capacity may predispose to further renal damage in the setting of other insults.

Funding: Government Support - Non-U.S.

SA-PO387

Mitigation of Radiation Injury by Low Anti-Oxidant Diet Eric P. Cohen,[1] Brian L. Fish,[1] Meetha Medhora,[1] Jessica Flowers,[2] John E. Moulder.[1] *[1]Radiation Oncology, Medical College of Wisconsin, Milwaukee, WI; [2]Nutrition, Harlan Laboratories, Madison, WI.*

Background: Oxidative stress may be a significant mechanism of renal injuries. Radiation causes prompt oxidative stress (OS) and has been hypothesized to cause chronic OS, with fibrosis and organ failure. We tested the chronic OS hypothesis by using low anti-oxidant diets.

Methods: Radiation nephropathy (rad np) was created by 11 Gy single fraction total body irradiation (rads) with one leg shielded to avoid early hematologic death. Rad np and renal failure determine survival in this model. Low antioxidant diets (modified AIN-76A) were started 2 weeks before or 1 week after rads to test the effect of low antioxidant status on development and progression of rad np. Starting one week after injury is mitigation; the modifying agent starts after the initiating injury but before its expression. The standard diet was Teklad 8904, which has complex ingredients: corn, wheat, and soybean meal. The AIN-76A diets (casein or soy versions) have refined ingredients and reduced antioxidants compared to 8904. All 3 diets have typical levels of protein (18-24%), sodium (0.1-0.3%), and polyunsaturated fat (2-2.9%). Body weight, survival and renal function were monitored. Results are medians for weight, BUN and survival. ANOVA was used for statistical comparisons, * is $p < 0.05$ versus standard diet.

Results:

Diet	8904	AIN pre	AIN mitig-casein	AIN mitig-soy
weight, grams, 17 weeks	200	214	228*	233*
BUN, mg%, 21 weeks	180	300	40*	60*
Survival, days	152	126	228*	192*

The low anti-oxidant diet started before rads does not protect but instead exacerbates injury as expected, but when started 1 week after irradiation it mitigates rad np. The protein source did not change this unexpected mitigation benefit.

Conclusions: 1) These data do not support a major role for chronic oxidative stress in rad np, consistent with genomic and biochemical evidence. 2) The low anti-oxidant diet mitigation benefit compares favorably to past studies of captopril in this model. 3) Low anti-oxidant diets could be used to test the role of chronic OS in non-radiation models of renal injury. 4) Diets may exert unsuspected effects: diet must be taken into account in any renal injury model.

Funding: Other NIH Support - NIAID, Veterans Affairs Support

SA-PO388

Impact of Mild Therapeutic Hypothermia on Cell-Free DNA

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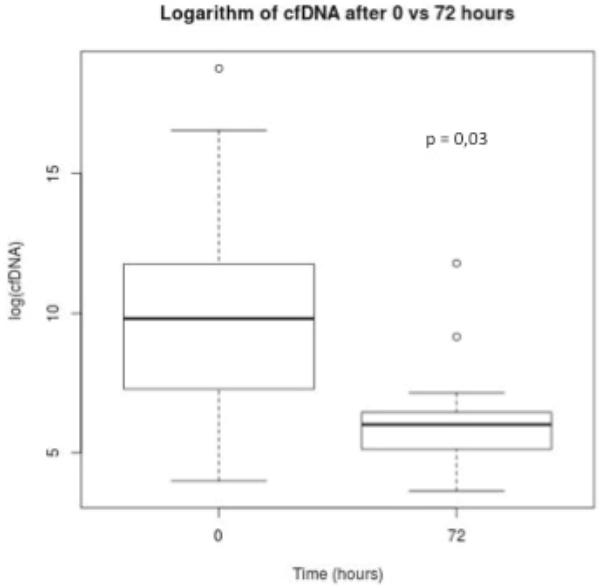
Background: There is growing interest in using hypothermia to prevent hypoxic damage in experimental models, although the mechanisms regulated by hypothermia are still unclear.Cell-free DNA (cfDNA)is a circulating extracellularDNA fragment that originates from necrotic and apoptotic cells derived from infiammation and tissue damage.The study examined the effect of Mild Therapeutic Hypothermia (MTH) on plasma cfDNA levels to evaluate the antiapoptotic effects of MTH.

Methods: We performed a observational study of 19 patients resuscitated from out-of-hospital cardiac arrest.On Admission time and at 72 hours after the start of MTH, we collected EDTA blood samples.The cfDNA was extracted from plasma and quantified by Real time PCR in Genome Equivalent (GE)/ml for β-globin gene.We applied a linear trasformation of cfDNA in log cfDNA to manage the data better.

Results: Mean age was 66 ± 11, 16(84%)males, 14(80%)experienced ventricular dysrhythmia as the primary rhythm of arrest,14(74%) survived, and 12(63%)GCS 15 at discharge.The concentration range of cfDNA in plasma at time 0 was 15566 GE/ml(IQR 15686 – 132985).The median concentration of cfDNA at 72 hours was 410 GE/ml(IQR166 - 630);We observed an significantly descease in cfDNA levels after 72 hrs of MTH(p<.05)



Conclusions: In conclusion,we observed from our data that the MHT induced a decrease of cfDNA levels.Thus,it could be associated with an antiapoptotic and antinecrotic effect of this treatment.Our results provide new evidence concerning the protective mechanism of hypothermia in vivo.

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NEFA Induces Tubular Damage via CPT-1 Inhibition and Energy Depletion and Could Be Reversed By Malonyl-CoA Modulation

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Background: Diabetic nephropathy has become the major single cause of end-stage renal disease and its prevalence is dramatically increasing. In DN tubulointerstitial damage has a better correlation with impaired renal function than the degree of glomerular damage. Among the most relevant mediators involved are the circulating non-esterified fatty acids (NEFA), a characteristic feature of type 2 diabetes and obesity, that are able to induce tubular damage.

Methods: We use functional assays (apoptosis, CPT-1 activity, ATP) and protein quantitation to assess NEFA lipotoxicity in proximal tubular epithelial call (PTEC). A NEFA lipotoxicity model, by intraperitoneal injection in mouse, was employed to confirm tubular damage and test therapeutical intervention by AICAR/carnitine or metformin.

Results: Palmitic and linoleic acids are capable to inhibit carnitine-palmitoyl transferase 1 (CPT-1) in PTEC. CPT-1 inhibition, depletes ATP and depolarizes mitochondrial membranes, causing a mitochondrial dysfunction that leads to cytochrome c release and triggers apoptotic intrinsic mechanisms. NEFA inhibition of CPT-1 is mediated by an increase on malonyl-CoA level. The inhibition of its synthesis, reverted the observed effects. We additionally examined apoptotic damage and mitochondrial dysfunction in a protein-overload nephropathy in mice, and tested if the modulation of CPT-1 inhibition, using AICAR or Metformin, could attenuate tubulointerstitial lesions in proteinuric kidney diseases.

Conclusions: High NEFA-content albumin elicits CPT-1 inhibition and mitochondrial dysfunction both in vitro and in vivo. Mechanisms capable to overcome CPT-1 inhibition may improve cell energy status and ameliorate tubular damage in DN.

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Apoptotic Pathways in Cardiorenal Syndrome Type 5

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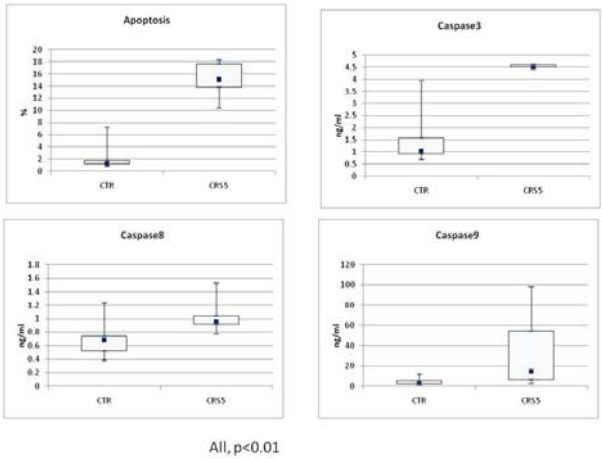
Background: Cardiorenal Syndrome Type 5 (CRS5) is characterized by the presence of combined cardiac and renal dysfunction and damage due to systemic disorder. CRS5 mechanism is not well-known, although specific cellular and molecular changes with time-specific pattern are reported as a potential mechanisms involved in CRS5. We examined the effect of plasma from patients with CRS5 on Renal Tubular Epithelial Cells (RTCs) apoptosis as a pathogenic mechanism in this syndrome. In particular, we investigated the Caspase pathway involved in this induced apoptosis.

Methods: We enrolled 11 patients with CRS5 (68.4±10.8yrs), and 16 controls (CTR) (52.0±7.7yrs). Plasma from different groups were incubated with RTCs for 24h and, subsequently, cell apoptosis was evaluated by Flow Cytometer. Renal Tubular cells were assayed for activation of Caspase3, 8 and 9.

Results: Quantitative analysis of apoptosis by Annexin-V/Propidium Iodide (PI) showed significantly higher apoptosis rates in CRS5 compare to CTR (p<.01).This increasing apoptosis was confirmed by Caspase3 levels with a strong correlation (rho=.71). Caspase8 and 9 were significantly higher in CRS5 compare CTR (p<.01)



Furthermore, Caspase3 levels showed a significantly positive correlation with Caspas8 (rho=.72) and 9(rho=.49).

Conclusions: This pilot study demonstrates the significantly heightened presence of dual apoptotic disequilibrium with high upregulation of Caspase8 and with mild activation of Caspase9 in RTCs treated in vitro with CRS5 plasma.We observed the activation of 2 distinct apoptotic pathways in CRS5.These apoptotic pathways could be proved an important contribution of the development of CRS5 and may play a role in the mechanism of this syndromeand may be essential for the damage of distant organs. Our findings indicate that apoptosis could be a potential therapeutic target.

Funding: Private Foundation Support

SA-PO391

Congestive Heart Failure in the Rat Induces Subtle Renal Damage via Neurogenic Pathways Tilmann Ditting,[1] Peter Linz,[1] Martin Hindermann,[1] Kristina Rodionova,[1] Christian Ott,[1] Sonja Heinlein,[1] Roland E. Schmieder,[1] Karl F. Hilgers,[1] Kerstin U. Amann,[2] Roland Veelken.[1] *[1]Medical Clinic 4, Nephrology and Hypertension, Friedrich-Alexander Univ Erlangen Nürnberg, Erlangen, Germany; [2]Pathology, Friedrich-Alexander Univ Erlangen Nürnberg, Erlangen, Germany.*

Background: Cardiomyopathy in experimental renal insufficiency is putatively influenced by neurogenic pathways of renal origin. We wondered if neurogenic effects in congestive heart failure could likewise harm the kidney. We hypothesized that increased renal sympathetic nerve activity (RSNA) in rats after myocardial infarction (CHF) induces renal structural damage.

Methods: 21 day after induction of CHF renal morphology was evaluated by immunohistology (interstitial and glomerular mononuclear cell infiltration (ED1), cell proliferation (PCNA), collagen I,III,IV,V,VI, laminin und fibronectin). RSNA was assessed by volume challenge (VE) to decrease RSNA. CHF and control rats were investigated with and without renal denervation (DNX). Blood pressure (BP), heart rate (HR) and RSNA were recorded. Nodose ganglion neurons (NGN) with vagal cardiac afferents were cultured for 1 day. Whole cell recordings were obtained and current-voltage relationships established. Cells were characterized by osmomechanical stress with a mannitol solution.

Results: In CHF rats with intact renal nerves (nonDNX) formation of collagen I occurred, that was reduced after DNX (12.2+0.7 %area versus 9.1+1.1 %area*, n=6, * p<0.05).VE-induced RSNA decreases were impaired in CHF versus controls suggesting increased RSNA (-D 34+8% versus -D 54+6% *, n=6, * p<0.05). NGN from CHF exhibited altered conductance in response to mechanical stress as compared to controls (change in holding current at -80 mV: control_normoosmotic: -144±30 pA; control_hypoos.: -282±34 pA versus CHF_normosmotic: - 230±55 pA; CHF_hypos.: -540±100* pA; *p<0.05 CHF versus control).

Conclusions: CHF induced subtle renal structural damage due to increased renal sympathetic tone which was likely due to altered NGN mechanosensitivity.

Funding: Pharmaceutical Company Support - Medtronic

SA-PO392

Human Kidneys with Diabetes and Stage 3 CKD Are Ischemic and Hypoxic as Evaluated by MRI Jon Thacker,[1] Huan Tan,[2] Luping Li,[3] Stuart M. Sprague,[4] Orly F. Kohn,[5] Ivana Lazich,[5] Pottumarthi V. Prasad.[1,3,5] *[1]Biomedical Engineering, Northwestern Univ; [2]Dept of Surgery, Univ of Chicago; [3]Dept of Radiology, Northshore Univ HealthSystem; [4]Div of Nephrology, Northshore Univ HealthSystem; [5]Pritzer School of Medicine, Univ of Chicago.*

Background: Renal ischemia and hypoxia are key contributors to CKD progression according to chronic hypoxia theory [PMID: 9551436]. Identification of suitable markers to predict risk of progression remains as an unmet need. In this study, we evaluated both renal cortical oxygenation and perfusion using blood oxygenation level dependent (BOLD) and arterial spin labeling (ASL) MRI in subjects with diabetes and moderate CKD, known to be risk of progression.

Methods: Coronal acquisitions of ASL [PMID: 23447145] and BOLD MRI were acquired in healthy and CKD (N=10 per group) on a 3T scanner. *Parameters* BOLD: mGRE, TR 62ms, TE 3.09 - 30.53ms, 8 echoes; ASL: FAIR, post labeling delay=1.5s (healthy) or 2.0s (CKD); other: resolution 1.48x1.48mm, slice 8mm (ASL) and 5mm (BOLD). Regions of interest were defined in the cortex and medulla.

Results: Both perfusion and oxygenation were reduced (i.e. R2*) in CKD compared to healthy (see table). BOLD MRI data is consistent with previous report [PMID: 21757771]. However, other studies reported no difference between groups [e.g. PMID: 24760031]. We report for the first time a combined study of ASL and BOLD MRI, and show consistent trends with both measurements. An inverse correlation between R2* and perfusion was observed, suggesting that decreased perfusion may be responsible for increased hypoxia (r=-0.55; p<0.05; CI 95% [-0.71,-0.27]; n=19).

Cortical Measurements	Stage 3 CKD with diabetes	Healthy	T-Test
Perfusion (ml/100g/min)	111±28	207±65	p<0.01; t=4.20
R2* (s-1)	26.7±3.4	20.2±2.1	p<0.01; t=-5.13

Conclusions: The reductions in renal perfusion and oxygenation in subjects with diabetes and moderate CKD are consistent with chronic hypoxia theory. It is important to note that the reported differences may actually be lower compared to those in progressors because only 1 in 3 with CKD stage 3 are estimated to progress toward ESRD [PMID: 19176795].

Funding: NIDDK Support

SA-PO393

Evidence for Thalidomide Treatment Blocking the Development of Diabetic Nephropathy in Mice Xiaojie Shen,[1] Zhaoyong Hu,[2] Jing Xu.[1] *[1]Nephrology Div, Changhai Hospital, Shanghai, China; [2]Nephrology Div, Baylor College of Medicine, Houston, TX.*

Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD), however, the effective treatments for DN is lack. Down-regulation of Phosphatase and tensin homolog (**PTEN**) had linked to mesangium expedition and renal interstitial fibrosis. While thalidomide, an anti-inflammatory and anti-angiogenesis drug, reportedly up-regulates PTEN by inhibiting its degradation. Therefore, we investigated if thalidomide administration can block the progression of DN.

Methods: In human mesangial cells, we examined if thalidomide addition blocks TGF-β1 stimulated the expressions of fibronectin (FN), Collagen IV (Col IV), alpha-smooth muscle actin (α-SMA). In C57/BL6 wild-type mice (32, male), and db/db mice (32 male, C57BL / 6 background, 6-month age), we assessed whether thalidomide administration (100mg/kg, three days/week by gavage) suppresses proteinuria and the expression of α-SMA, FN and Col IV. Furthermore, we examined PTEN expression and pathologic changes in kidneys of Wt and db/db mice treated with or without thalidomide.

Results: Thalidomide addition blocked the expressions of α-SMA, FN, ColIV in mesangial cell cultures. Without thalidomide treatment, db/db mice developed DN characterized by a marked increase in proteinuria and histological changes including mesangial cell proliferation and mesangium expansion. Electron microscopy revealed that the GBM thickness and foot processes enfacement. In contrast, thalidomide treatment significantly reduced proteinuria and suppressed the expressions of fibrotic proteins followed by improved pathological changes in glomeruli of db/db mice. Western blot also indicated that PTEN expression was decreased in glomeruli of db/db mice, but thalidomide treatment effectively blocked this response.

Conclusions: our results demonstrated that thalidomide blocks or slows-down the progression of DN, this benefic effect might be due to the up-regulation of PTEN in glomeruli of db/db mice. These findings could provide a therapeutical strategy to combat the development of DN.

Funding: Government Support - Non-U.S.

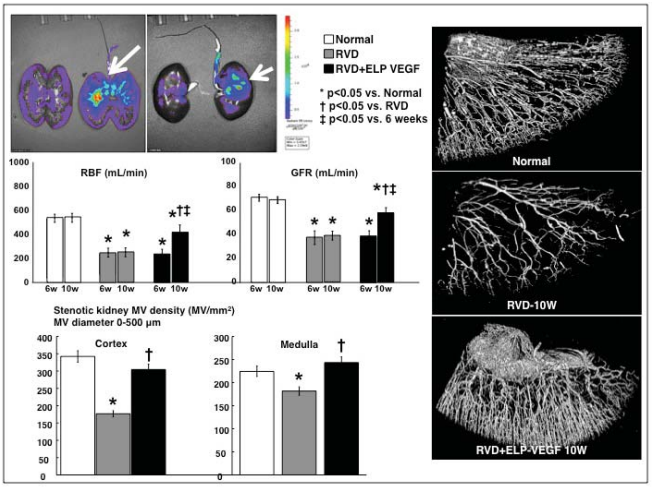
SA-PO394

Administration of Elastin-Like Polypeptide-VEGF in the Stenotic Kidney to Recover Renal Function Alejandro Chade,[1] Gene Lee Bidwell.[2] *[1]Physiology and Biophysics, Medicine, Radiology, Univ of Mississippi Medical Center, Jackson, MS; [2]Neurology, Biochemistry, Univ of Mississippi Medical Center, Jackson, MS.*

Background: Elastin-like polypeptides (ELP) are drug delivery vectors with high affinity for kidney tissue. ELPs can be fused to peptides to increase their tissue binding and half-life. We designed a novel fusion of ELP and vascular endothelial growth factor (VEGF). We showed that intra-renal administration of *free* VEGF in renovascular disease (RVD) improved but not fully recovered the kidney, possibly due to its short half-life and/or rapid wash-out by the renal circulation. We tested the therapeutic feasibility of ELP-VEGF fusion and hypothesized that by augmenting binding of VEGF to renal tissue, intra-renal infusion of ELP-VEGF will recover renal function.

Methods: Unilateral renovascular disease (RVD) was induced in 8 pigs by renal artery stenosis. After 6 weeks, stenotic kidney blood flow (RBF) and filtration (GFR) was quantified *in vivo* using multi-detector computed tomography. Then, pigs were randomized in placebo (RVD) or treated with a single stenotic kidney infusion of ELP-VEGF (RVD+ELP-VEGF, 100 ug/kg, n=4 each). Pigs were observed for 4 weeks, *in vivo* studies repeated and then euthanized for *ex vivo* studies.

Results: Placebo and ELP-VEGF treated pigs showed similar stenosis. Notably, ELP-VEGF fusion significantly bound to stenotic kidney parenchyma and improved RBF, GFR, and cortical and medullary microvascular (MV) density compared to placebo.



Conclusions: Our results support the feasibility of a novel therapeutic intervention. A single intra-renal administration of an ELP-VEGF fusion largely recovered the function and expanded the MV architecture of the stenotic kidney. Our data constitutes a solid first step for a novel therapeutic application of an existing compound never tested before to protect the kidney.

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SA-PO395

Nrf2 Is Activated and Has a Protective Role in Cyclosporine Nephrotoxicity

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Background: Oxidative stress-causing agents interact with cytosolic Keap1 complex, leading to stabilization of Nrf2, an active form of a transcriptional factor, which readily induces the expression of antioxidant genes including heme oxygenase-1 (HO-1). This study aimed to clarify the role of Keap1-Nrf2 system in the pathogenesis of cyclosporine A (CyA) nephrotoxicity.

Methods: Nrf2 knockout (NKO) and wild type (WT) mice were injected with CyA (50mg/kg/day, i.p.)(n=9 and 6, respectively) or vehicle (both n=7) for 3 weeks. Separately, similar experiment was done using Keap1 knockdown (KKD) (n=10 and 8, respectively) and WT mice (both n= 6). Renal mRNA expression of Nrf2-regulated genes (HO-1, GSTM1, and NQO1) and those related to kidney damage (TGFß1 and Kim1), and malondialdehyde(MDA) content in the kidney reflecting oxidative stress were quantified.

Results: CyA upregulated HO-1 (6.71±5.20 versus 1.00±0.27, p=0.007) in WT mice. This was blunted in NKO+CyA (2.44±1.19). GSTM1 and NQO1 were downregulated in NKO, but not upregulated in response to CyA. Renal MDA content was higher in NKO+CyA than WT+CyA (0.14±0.01 versus 0.07±0.02 nmol/mg•prot, p=0.041). Renal TGFß1 mRNA was more abundant in NKO+CyA than WT+CyA (1.77±0.62 versus 1.00±0.47). In addition, Kim1 was significantly upregulated by CyA in WT (24.8±16.7 versus 1.00±0.88), and further exaggerated in NKO+CyA (103.2±133.9). As expected from the Nrf2-regulatory role of Keap1, HO-1 mRNA was more abundant in KKD+CyA than WT+CyA (7.16±3.58 versus 2.14±1.57, p=0.016). However, renal TGFß1 and Kim1 were not suppressed in KKD+CyA compared to WT+CyA.

Conclusions: CyA induces oxidative stress in the kidney, and also induces HO-1 expression mediated by Nrf2, leading to amelioration of the oxidative stress and renal injury. However, constitutive Nrf2 activation does not contribute to further prevention of CyA nephrotoxicity, suggesting that CyA itself sufficiently activates Nrf2 to maximally ameliorate oxidative stress-related renal injury induced by CyA.

Funding: Government Support - Non-U.S.

SA-PO396

Correlation of Urinary β2-Microglobulin with Kidney Injury Molecule-1 Staining in Renal Biopsy

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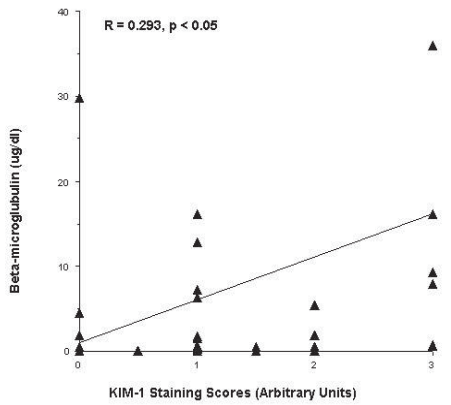
Background: After filtration through glomeruli, β2-microglobulin (β2-MG) is reabsorbed by proximal tubules. Increase in urine β2-MG indicates proximal tubular injury and measurement of β2-MG in urine is useful to determine the source of kidney injury. Kidney injury molecule-1 (KIM-1) has been characterized as a injury marker for proximal tubules. This study was designed to evaluate the correlation of urinary β2-MG with KIM-1 staining, i.e. proximal tubular injury.

Methods: During 2009-2012, 46 patients had urine β2-MG (RenalVysion™) and renal biopsies with various diagnoses including minimal change disease, IgA nephropathy, lupus nephritis and crescentic glomerulonephritis. Immunohistochemical staining for KIM-1 was performed. The intensity was graded from 0 to 3+ (0, no staining; 0.5 to 1+, focal to diffuse weak fine granular staining; 2 to 3+, moderate to strong granular staining). Linear regression was used to correlate urinary β2-MG and KIM-1 staining.

Results: 30 patients had elevated urinary β2-MG.KIM-1 staining. KIM-1 was positive in 36 cases. There was a significant correlation between urinary β2-MG and KIM-1 staining (R value = 0.293, p < 0.05). Odds ratio was 7.229, so for every unit increase in KIM-1 staining level, β2-MG increased by 7.229. Confidence interval at the 95% range was 2.868 to 12.01.



Conclusions: Increase in urine level of β2-MG is significantly correlated with KIM-1 up-regulation in injured proximal tubules. Measurement of urine β2-MG is a reliable assay for proximal tubular injury.

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DNA Hypermethylation Contributes to Mouse Kidney Fibrosis and the Hypermethylation of Homeobox A5 Promotes the Expression of Smad1

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Background: Renal fibrosis is a common, pathogenic process in chronic kidney diseases. Emerging evidence has implicated epigenetic regulation in renal fibrosis. However, the underlying epigenetic mechanisms, including DNA methylation, remain largely unclear.

Methods: In this study, we analyzed the genome-wide DNA methylation in mouse kidney tissues following unilateral urethral obstruction (UUO) by using the Next Generation Sequencing (NGS). We also examined the effect of 5-aza-2'-deoxycytidine(5-aza), a DNA methylation inhibitor, on UUO-induced renal fibrosis. Further studies verified the methylation changes of genes such as HoxA5 and suggested the regulation of important renal fibrosis regulators, including Smad1.

Results: The global methylation analysis demonstrated an overall DNA hypermethylation following UUO, which was attenuated by 5-aza. 5-aza also suppressed the expression of fibrosis markers (eg. a-SMA, collagens, fibronectin) as well as DNA methylation enzymes including DNMT1, DNMT3A, and DNMT3B in UUO. To identify the key hypermethylated genes involved in renal fibrosis, we analyzed the global sequencing data and showed hypermethylation in the promoter CpG islands of Homeobox A5 (HoxA5) gene. The hypermethylation was associated by a marked decrease of HoxA5 expression in UUO kidney tissues. In cultured mouse proximal tubule cells, overexpression of HoxA5 inhibited the expression of Smad1.

Conclusions: The results reveal DNA hypermethylation in UUO and support a critical role of DNA methylation in renal fibrosis. Moreover, the results highlight the involvement of HoxA5 methylation in the regulation of fibrosis-related signaling pathways.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO398

Mechanisms of Gremlin1 Signalling and BMP Antagonism In Vivo and In Vitro

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Background: Gremlin (Grem1) is a secreted glycoprotein antagonist of bone morphogenetic proteins (BMPs) that plays a critical role in development and disease. Grem1 is implicated in fibrotic diseases of the kidney, lung and other tissues. Increased levels of Grem1 are detected in the kidney tubules of diabetic nephropathy patients, and correlate with progressive renal disease. The specific BMP targets of Grem1 in development and disease have not yet been identified.

Results: Levels of Grem1 were highest in colon, brain and spleen with lower levels detected in kidney, liver, lung, stomach and skin. In contrast, levels of Grem2 were highest in kidney, followed by colon, brain and liver. Mating *grem1*+/- mice on a C57Bl/6:FVB background produced viable *grem1*-/- offspring that displayed the previously described limb deformities. Approximately 95 % of the *grem*-/- offspring died at P2 due to the absence of kidneys with less than 5 % surviving into adulthood. The surviving *grem1*-/- mice were, on an average, 33 % smaller in size and weight compared to controls. Consistently, the right kidney was absent in *grem1*-/- mice of both genders, with an enlarged left kidney evident. Average kidney:body weight ratios were 6.07 g/kg for wild-type versus 8.52 g/kg for grem1-/- at 4 wk and 6.54 g/kg versus 13.96 g/kg at 4 months of age. The glomerular density of *grem1*-/- mice was significantly lower than wild-type (5.41 glomeruli/mm^2 versus 9.26 glomeruli/mm^2, p<0.001). However, the total number of glomeruli was comparable between wild-type and *grem1*-/- mice when normalised to body weight, suggesting that the increase in kidney size was due to expansion of the tubular area. Interestingly, levels of Grem2 mRNA were increased 4-fold in *grem1*-/- kidney compared to wild-type, suggesting compensatory changes may be occurring here.

Conclusions: We are currently using these mice to identify exact mechanisms of BMP signalling in kidney and Grem1-mediated pathophysiology in acute and chronic fibrosis models.

SA-PO399

A Module IV-Defective CCN2 Mutant and Module IV-Derived Decoy Peptides Attenuate Renal Fibrogenesis

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Background: CCN2 is a TGF-β-induced profibrogenic mediator. It consists of four different functional modules, although the most profibrogenic module remains to be clarified.

Methods: Using mutant cDNAs, each of which lacked one of Exon 2~5 corresponding to module I~IV, we found that a module IV-defective CCN2 expressed in tubular epithelial cells didn't induce fibronectin (FN) synthesis in fibroblasts in the co-culture system. We next generated Exon 5-deleted CCN2 gene knock-in mice (Ex5-/-). By using unilateral ureter obstruction (UUO) model of these mice, we evaluated the levels of mRNAs encoding the

profibrogenic molecules and the area of interstitial fibrosis. Western blot analysis was also used to measure intracellular signaling molecules. Decoy peptides corresponding to partial amino acid sequences of module IV were administered to Ex5+/+ with UUO to examine the profibrogenic roles of module IV. To confirm our results in another model, a subtotal nephrectomy (SNx) model was also tested.

Results: The kidneys in the UUO showed a significant reduction in the levels of mRNAs encoding FN and collagen type I (COLI) in Ex5-/- compared to those in Ex5+/+ (FN; 8.63 ± 1.87 versus 4.06 ± 0.47, COLI; 33.77 ± 11.75 versus 8.37 ± 0.59). Fibrotic area was also significantly reduced in Ex5-/- (13.2 ± 5.1 versus 9.6 ± 6.6). Additionally, the administration of some of decoy peptides significantly attenuated the expansion of the interstitial fibrotic area in the UUO model of Ex5+/+. Levels of pAkt and pGSK-3β, but not pLRP6, were significantly lower in the fibrous kidneys of Ex5-/- than in those of Ex5+/+. We also confirmed that, in the SNx model, the fibrotic area in the kidneys was significantly reduced in Ex5-/- in comparison with that in Ex5+/+.

Conclusions: The module IV-defective CCN2 mutant and module IV-derived decoy peptides attenuated renal fibrogenesis. This suggests that CCN2 promotes renal fibrogenesis via module IV, probably through the integrin/integrin-linked kinase/Akt/GSK-3β pathway, which appears to be an appropriate, anti-fibrotic therapeutic target.

SA-PO400

Total Flavone Glycosides of Flos *Abelmoschus manihot*, Ameliorates Renal Fibrosis in Diabetic Nephropathy Rats via Inhibiting Oxidative Stress and p38MAPK Signaling Activity, Compared with Alpha-Lipoic Acid

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Background: Total flavone glycosides of flos *Abelmoschus manihot* (TFA)has been widely used for treating renal fibrosis in patients with diabetic nephropathy (DN) in China. However, therapeutic mechanisms remain unclear. Oxidative stress (OS) is a determinant during renal fibrotic progress under hyperglycemia. As one of regulative approaches of OS, p38MAPK signaling pathway plays a pivotal role. This study thereby aimed to investigate effects and mechanisms in vivo of TFA on renal fibrosis, compared with alpha-lipoic acid (ALA) as an antioxidant in clinic, through attenuating OS-related injury and p38MAPK signaling activity.

Methods: Rats were randomly divided into 5 groups, sham-operated group, vehicle-given group, low dose of TFA-treated group, high dose of TFA-treated group and ALA-treated group. TFA, ALA and saline were daily administrated for 8 weeks after induction of DN by streptozotocin with unilateral nephrectomy. DN rats' general state, biochemical indicators, renal pathological changes, OS-related markers, as well as key protein expressions in p38MAPK signaling pathway, fibrogenic cytokines and inflammatory factors were examined, respectively.

Results: DN model rats exhibited typical renal fibrosis, OS-related features and increases in expressions of p-p38MAPK, TGF-β1 and TNF-α. UAlb, BUN, UA, Alb, TG, TC and OS-related markers including MDA, T-SOD, GSH-Px, 8-OHdG and NOX4 in serum or kidneys were ameliorated in treated groups, especially in high dose of TFA-treated group. Of note, TFA synchronously inhibited p38MAPK signaling activity and TGF-β1 and TNF-α protein overexpressions, whereas, ALA only suppressed TNF-α protein overexpression in kidneys.

Conclusions: By means of DN models, we demonstrated that OS promotes renal fibrosis and p38MAPK signaling activity. TFA, as a natural regulator in vivo, can improve OS-related renal damage via regulating protein overexpressions of p-p38MAPK, TGF-β1 and TNF-α, which is different from ALA.

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SA-PO401

Alpha2A-Adrenoceptors Deficiency Protects From Renal Fibrosis and Inflammation

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Background: Chronic kidney disease (CKD) is one of the major health issues. Inflammatory processes play a pivotal role in the pathogenesis of CKD. Alpha2A-adrenoceptors (α2A-AR) regulate sympathetic nerve activity by controlling presynpatic norepinephrine (NE) release. Activation of SNS leads to the progression of CKD. On the other side, there is increasing evidence that α2A-ARs on non-adrenergic cells, like immune cells, modulate pro-inflammatory processes during tissue damage. Here we tested the impact of α2A-AR on the progression of renal fibrosis.

Methods: Unilateral ureteral obstruction (UUO) – a model of renal fibrosis - was performed in α2A-AR-KO mice (KO) and compared to its wild-type (WT). Renal NE tissue content was measured by HPLC. 7d after UUO immunohistochemistry and gene expression analysis by real-time PCR analyses were carried out. Murine macrophages were isolated from the peritoneal cavity, subsequently cultured and stimulated.

Results: Renal sympathetic neurotransmission and renal NE tissue content was significantly exaggerated in KO compared to WT. Despite an increased sympathetic activity, renal fibrosis, assessed by sirius red/ fast green collagen staining (p=0.0428) and renal collagen-1 expression (p=0.001), was significantly attenuated in KO compared to WT 7 days after UUO. Moreover, the expression of the pro-inflammatory and pro-fibrotic cytokines TNF-α (p<0.05) and TGF- β (p<0.05) as well as the chemokines CCL2 (p<0.05) and CCL5 (p<0.05) were significantly reduced in KO compared to WT indicating a pro-inflammatory role of α2A-AR on immune cells in the progression of renal fibrosis. To test that, we isolated macrophages from WT and stimulated them with the α2-AR-agonist UK14.304 (0.1 μM). Stimulation of α2A-AR, expressed on macrophages, induced a 1.5-fold expression of TNF-α (p<0.05).

Conclusions: Our results show, α2A-ARs appear not only to be key players in regulating renal sympathetic activity, but also promote inflammation and the progression of renal fibrosis in response to kidney injury. Activation of non-adrenergic α2A-ARs on immune cells seems to be at least partly responsible for this effect.

SA-PO402

Rho Kinase Mediates Renal Fibrosis via Nox4-Derived Reactive Oxygen Species

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Background: We previously showed that TGF-β1-induced renal myofibroblast differentiation *in vitro* is regulated via a Rho-GTP/Rho kinase (ROCK) signaling mechanism involving NAD(P)H oxidase (Nox4)-derived reactive oxygen species (ROS) and a Nox4 enhancing protein, polymerase delta interacting protein 2 (Poldip2). Here we link the ROCK and Poldip2/Nox4 axis *in vivo* during renal fibrosis.

Methods: The effect of a ROCK inhibitor, Fasudil (2.0 mg/kg/day, i.p.) on Poldip2 and Nox4-generated ROS during renal fibrosis was examined 7 days after unilateral ureteral obstruction (UUO) and compared to vehicle treated UUO and unobstructed contralateral kidneys. Protein and RNA expression levels of ROCK substrate pMYPT1, Poldip2, Nox4, and markers of fibrosis (transforming growth factor-beta 1 (TGF-β1), alpha-smooth muscle actin (α-SMA), cellular fibronectin (Fn-EIIIA) and collagen type 1 were examined by immunoblot, RT-PCR and immunohistochemistry.

Results: Expression of pMYPT1 (ROCK activation) and NADPH oxidase activity (ROS generation) was significantly elevated in kidneys of vehicle-treated UUO rats relative to unobstructed controls. Similarly, renal expression of Poldip2, Nox4, TGF-β1, α-SMA, Fn-EIIIA and collagen type 1 were significantly increased after UUO-induced renal fibrosis. Fasudil treatment significantly inhibited renal pMYPT1 expression and NADPH oxidase activity relative to vehicle-treated UUO rats. Likewise, Fasudil reduced protein and mRNA expression of Poldip2, Nox4, TGF-β1, α-SMA and Fn-EIIIA relative to kidneys from vehicle-treated UUO rats. Moreover, Fasudil treatment reduced interstitial expression of α-SMA, Fn-EIIIA, and collagen type 1 relative to vehicle-treated UUO rats assessed by immunohistochemistry.

Conclusions: Fasudil interrupts ROCK activation and reduces Poldip2, Nox4 expression, NADPH oxidase activity, and myofibroblast activation after UUO, indicating that a Rho/ROCK-Poldip2/Nox4 axis plays an important role in ROS-mediated renal interstitial fibrosis.

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SA-PO403

Sirt1 in Kidney Fibrosis: Human, Cell Culture, and Animal Intervention Studies

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Background: Sirt1, the mammalian ortholog of silent information regulator 2 (Sir2) has been implicated in the pathogenesis of fibrosis, in addition to its role in longevity. To explore this further, we conducted human, transgenic and interventional animal and cell culture studies.

Methods: We extracted RNA from formalin-fixed archival kidney biopsies from patients with focal and segmental glomerulosclerosis (FSGS, n=6) and thin basement membrane disease (TMN, n=10). Sirt1 mRNA levels were determined by real-time QPCR. We also investigated the effects of Sirt1 activation in the subtotal nephrectomised (SNX) rat, a rodent model of FSGS. One week after 5/6 nephrectomy, rats were randomized to receive either regular chow or chow containing the Sirt1 activator, SRT3025 (Glaxo Smith-Kline), then followed for a further 12 weeks. We next examined kidney function in aged (2 year old) sirt1-/- (129/CD1) mice and their age-matched wild-type littermates to further investigate the role of Sirt1 activity in age-associated decline in kidney function. Finally, the effects of Sirt1 activation on TGF-ß induced collagen production in renal fibroblasts was quantified by 3H-proline incorporation.

Results: When compared with TMN, Sirt1 mRNA was reduced by >70% in biopsies from patients with FSGS (p<0.05). When compared with untreated SNX rats, those that received the Sirt1 activator had less glomerulosclerosis (GSI: 1.3±0.17 v 1.9±0.26), higher GFRs (2.9±0.25 v 1.7±0.20 μl/min/g) and less proteinuria (224±41 v 363±47 mg/d), p<0.05 for all. Blood pressure was unaffected. When compared with wild-type mice, aged Sirt1-/- mice had substantially lower GFRs (7.0±0.58 v 4.2±0.22 μl/min/g). TGF-ß induced a 5-fold increase in fibroblast 3H-proline incorporation that was abrogated by Sirt1 activation (p<0.001) in association with a reduction in acetylation of the downstream TGF-ß signalling molecule, Smad2.

Conclusions: Together, these findings implicate Sirt1 in the pathogenesis of kidney fibrosis and suggest that its activation may provide a new therapeutic strategy in fibrosis-associated kidney disease.

SA-PO404

Loss of Tumor Suppressor PTEN Expression in Renal Injury Initiates SMAD3 and p53 Dependent Fibrotic Responses Rohan Samarakoon,[1] Sevann Helo,[2] Amy D. Dobberfuhl,[2] Nidah S. Khakoo,[1] Lucas Falke,[3] Jessica Overstreet,[1] Roel Goldschmeding,[3] Paul J. Higgins.[1] *[1]Center for Cell Biology and Cancer Research, Albany Medical Center, Albany, NY; [2]Div of Urology, Albany Medical Center, Albany, NY; [3]Dept of Pathology, Univ Medical Center Utrecht, Utrecht, Netherlands.*

Background: Deregulation of the tumor suppressor PTEN occurs in lung and skin fibrosis, diabetic and ischemic renal injury. However, the potential role of PTEN and the associated mechanisms in the progression of kidney fibrosis remains unclear.

Methods: We utilized Ureteral unilateral obstruction (UUO) and Aristocolic acid nephropathy (AAN) mouse models and in vitro molecular approaches to investigate PTEN function in renal tubular cells and fibroblasts.

Results: Tubular and interstitial PTEN expression is dramatically decreased in the UUO and AAN-injured kidney, correlating with Akt, p53 and SMAD3 activation and fibrosis. Stable gene silencing of PTEN in HK-2 tubular epithelial cells induces CTGF, PAI-1 and fibronectin expression. Prolonged inhibition of PTEN with OHpic trihydrate in NRK-49F cells similarly initiated a profibrotic gene cascade. RNA interference or inhibition of PTEN stimulated Akt, SMAD3 and 53^{Ser15} phosphorylation with accompanying proliferative arrest in HK-2 cells. Gene depletion or pharmacological blockade of either SMAD3 or p53 partially suppressed fibrotic gene expression and relieved growth inhibition associated with either loss or inhibition of PTEN. Similarly shRNA suppression of PAI-1 induction rescued growth inhibition orchestrated by PTEN loss. Moreover, TGF-βeta1-initiated fibronectin, PAI-1 and alpha-SMA expression is further enhanced by PTEN deletion. Furthermore, combined TGF-βeta1 treatment and PTEN silencing potentiates epithelial cell death.

Conclusions: PTEN loss initiates tubular dysfunction via SMAD3 and p53 mediated PAI-1, CTGF and fibronectin induction with accompanying PAI-1 dependent proliferative arrest. Moreover, PTEN loss cooperates with TGF-βeta1 in inducing profibrotic responses and tubular cell apoptosis.

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SA-PO405

Impaired Cyclooxygenase-2 Expression Leads to Aggravated Renal Fibrosis in Response to Unilateral Ureteral Obstruction in Mice Kirsten Madsen,[1,2] Signe Skou Tofteng,[1] Line Nilsson,[3] Boye Jensen,[1] Rikke Norregaard.[3] *[1]Dept of Cardiovascular and Renal Research, Inst of Molecular Medicine, Univ of Southern Denmark, Odense, Denmark; [2]Dept of Pathology, Odense Univ Hospital, Odense, Denmark; [3]Dept of Clinical Medicine, Aarhus Univ, Aarhus, Denmark.*

Background: Renal fibrosis is the final common pathway in chronic kidney diseases and is the most consistent predictor of irreversible loss of renal function. Prostaglandins have anti-fibrotic effects in several organ systems and have also been suggested to diminish the development of renal fibrosis. We tested the hypothesis that renal cyclooxygenase-2 (COX-2) expression is increased in an experimental model of renal fibrosis, and that renal COX-2 activity impairs renal fibrogenesis.

Methods: Seven days unilateral ureteral obstruction (7dUUO) was performed in adult COX-2 knock-out (KO) mice and in wild-type (WT) littermate controls on a C57BL6 genetic background.

Results: Both the obstructed and the contralateral kidney increased in size in both genotypes in response to 7dUUO with no changes in total body weight. Quantitative PCR analysis of WT kidney tissue showed significantly increased renal COX-2 expression after 7dUUO compared to baseline. Sirius red staining showed no renal fibrosis at baseline in either genotype. After 7dUUO, development of fibrosis was seen with accumulation of extracellular matrix (ECM) in the renal interstitium and tubular atrophy with kidneys from COX-2 KO mice being more severely affected than WT controls. Messenger RNA expression and protein abundance of ECM proteins fibronectin, collagen I and III increased significantly in both genotypes after 7dUUO and were significantly higher in COX-2 KO mice compared to WT controls. Expression of fibroblast markers alpha-smooth muscle actin (αSMA) and fibroblast specific protein-1 (FSP-1) increased significantly in both genotypes after 7dUUO with the level of expression being significantly higher in COX-2 KO mice compared to WT controls.

Conclusions: In conclusion, renal COX-2 expression increases in response to 7dUUO, and impairment of COX-2 activity aggravates renal fibrosis suggesting an anti-fibrotic effect of COX-2 in renal fibrogenesis.

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SA-PO406

Astragaloside IV Ameliorates Renal Fibrosis via the Inhibition of MAPKs and Anti-Apoptosis *In Vivo* and *In Vitro* Weijia Xu,[1] Xinghua Shao,[1] Lei Tian,[1] Qin Wang,[1] Minfang Zhang,[1] Ling Wang,[1] Jufang Yao,[2] Xiaoping Xu,[3] Shan Mou,[1] Zhaohui Ni.[1] *[1]Dept of Nephrology, Molecular Cell Laboratory for Kidney Disease, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong Univ, Shanghai, China; [2]Animal Centre, Ren Ji Hospital, Shanghai, China; [3]Dept of the Biochemical Laboratory, Ren Ji Hospital, Shanghai, China.*

Background: Apoptosis of renal tubular cells plays a crucial role in renal fibrosis. Astragaloside IV (AS-IV) has been shown to inhibit renal tubular cell apoptosis induced by high glucose, but its role in preventing renal fibrosis and the underlying molecular mechanisms is still unknown.

Methods: As an in vivo model, mice subjected to unilateral ureteral obstruction (UUO) were administered AS-IV (20 mg/kg) by intraperitoneal injection for 7 days after operation. Bilateral kidneys were collected in postoperation days 7, 14. In vitro, human HK2 cells induced by TGF-β1 (10ng/ml) were utilised to investigate the protective role of AS-IV in anti-fibrosis. TUNEL, Western Blotting and real-time PCR were respectively used to detect apoptosis, protein and mRNA levels.

Results: AS-IV significantly alleviated renal mass loss, decreased the expression of TGF-β1 induced by UUO, and reduced the expression of α-smooth muscle actin (α-SMA), fibronectin, and collagen IV both in vitro and in vivo, suggesting that this compound functions in the inhibition of renal tubulointerstitial fibrosis. Furthermore, TUNEL assay results both in vivo and in vitro showed that AS-IV significantly attenuated both UUO and TGF-β1-induced cell apoptosis and prevented renal tubular epithelial cell injury in a dose-dependent manner. Western blotting results also revealed that the anti-apoptotic effect of AS-IV was reflected in the inhibition of caspase-3 activation, which might be mediated primarily by the downregulation of MAPK effectors phospho-p38 and phospho-JNK. SB203580 was given to inhibit p38 phosphorylation, which decreased the expression of fibronectin, α-SMA.

Conclusions: These data infer that AS-IV is effectively attenuates the progression of renal fibrosis after UUO injury and may have a promising clinical role as a potential anti-fibrosis treatment in patients with chronic kidney disease.

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SA-PO407

EZH2 Blockade Inhibits Renal Interstitial Fibroblast Activation and Attenuate Renal Interstitial Fibrosis in Obstructive Nephropathy Xiaoxu Zhou,[1] Shougang Zhuang.[2] *[1]Dept of Kidney Disease and Hypertension, Alpert Medical School and Rhode Island Hospital of Brown Univ, Providence, RI; [2]Dept of Medicine, Alpert Medical School and Rhode Island Hospital of Brown Univ, Providence, RI.*

Background: Interstitial fibroblast activation and proliferation is central to the development and progression of renal fibrosis after various insults, however, the signaling mechanism regulating this process is not fully understood.

Methods: In this study, we examined the role of EZH2, the enzymatic component of the polycomb repressor complex 2 (PRC2) that is responsible for methylation at H3K27 in renal interstitial fibroblast activation and proliferation in cultured renal interstitial fibroblasts (NRK-49F) and development of renal fibrosis in a murine model of obstructive nephropathy induced by unilateral ureteral obstruction.

Results: Exposure of cultured NRK-49F cells to 3-DZNeP (3-Deazaneplanocin A) and GSK126, two EZH2 inhibitors, respectively, resulted in decreased expression of alpha-smooth muscle actin and fibronectin, two hallmarkers of fibroblast activation and type 1 collagen, a key extracellular matrix protein, in a dose and time dependent manner. Silencing EZH2 with siRNA also significantly inhibited the expression of these proteins. Moreover, inhibition of EZH2 activity with 3-DNZeP reduced cell proliferation and expression of cyclin D1, PCNA. Finally, blocking EZH2 with 3-DZNep, GSK126 or siRNA also inhibited expression of transforming growth factor-beta receptor I and phospholation of Smad2/3 and STAT3. In a murine model of obstructive nephropathy, administeration of 3-DZNep also attenuated expression of fibronectin, α-SMA and type I collagen, reduced Di/Tri-Methyl-Histone H3 expresion, and suppressed phosphorylation of Smad-3 and STAT3.

Conclusions: Collectively, our results reveal an important role of EZH2 in mediating activation and proliferation of renal interstitial fibroblasts and renal fibrogenesis, and suggest that EZH2 inhibition might be a potential therapeutic approach for treatment of chronic fibrotic kidney disease.

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SA-PO408

Proteomic Analysis of Secretome of Cultured Pericytes Isolated from Unilateral Ureteral Obstruction (UUO) Mice Didier Portilla,[1,2] Shenyang Li,[1,2] Srinivas Ayyadevara,[2] Judit Megyesi,[1,2] Peter M. Price,[1,2] Jeremy Stuart Duffield.[3] *[1]Nephrology, Medicine, Univ of Arkansas for Medical Sciences, Little Rock, AR; [2]Nephrology, Medicine, Central Arkansas Veterans Healthcare Systems, Little Rock, AR; [3]Biogen Idec, Cambridge, MA.*

Background: Interstitial pericytes/fibroblasts transform into myofibroblasts in kidney tissue in *in vivo* models of renal fibrosis. To understand cellular mechanisms responsible for this transformation we performed proteomic analysis of supernatants obtained from cultured pericytes isolated from sham and UUO mice.

Methods: Pericytes were isolated using magnetic beads containing anti-PDGFβ Receptor ab and grown in DMEM media. Confluent cells were incubated for 24 hrs in serum-free medium, and both cell lysates and supernatants were collected. Supernatants were concentrated and resolved in one dimension on 1% SDS acrylamide gels, stained with Coomassie, and 1-mm slices were digested in trypsin prior to LC-MS analysis of peptides using an LTQ Orbitrap-FT mass spectrometer. Proteins were identified directly with MASCOT software, matching known peptide patterns of secondary fragmentation.

Results: Pericytes isolated from 3 and 7-day UUO mice have increased cellular proliferation when compared to sham pericytes. Collagen 1 alpha (VI, VIII, XIV and XV chains) increased by 45-fold in the pericyte secretome at 3-day UUO. Collagen up regulation correlated with increased mRNA and protein expression of TGFβ-1 and αSMA in cell lysates. Thrombospondins-1 and 2 were upregulated by 47-fold at 3 day UUO. There was increased expression of phosphorylated osteopontin with increased expression of cysteine protease cathepsin S (7-fold day 3) and ADAMTS-2 (6-fold at day 3) and increased expression of ECM ligands fibrillin-1 and fibulin 2 (5-fold) both at day 3 and 7. Complement C1q subunits and Class 2 detoxification enzymes like SOD and GSTs were up regulated. DAVID analysis showed increased secretion of several glycoproteins with increased acetylation and phosphorylation.

Conclusions: Proteomic analysis of the secretome of cultured pericytes isolated from sham and UUO mice represents a novel tool to examine cellular mechanisms of kidney fibrosis.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO409

Reversal and Prevention of the Myofibroblast Phenotype through Hyaluronic Acid Internalisation and Breakdown by BMP7 Adam Midgley, Aled O. Phillips, Soma Meran. *Inst of Nephrology, Cardiff Univ School of Medicine, Cardiff, United Kingdom.*

Background: Fibrosis is mediated through myofibroblasts activity. The glycosaminoglycans hyaluronan (HA) is a key mediator of myofibroblast phenotype, with its accumulation promoting TGFβ1 driven myofibroblast differentiation. However HA can have both antifibrotic and profibrotic properties depending on its composition, size and localisation. BMP7 is an antifibrotic cytokine known to antagonise the effects of TGFβ1, however the mechanistic processes for this are not fully determined. Here we investigate whether BMP7 drives alterations in HA that then mediate prevention/reversal of TGFβ1-driven myofibroblast differentiation.

Methods: Fibroblasts were used to test two models of BMP7-driven antagonism of differentiation - a reversal model, where cells were exposed to TGFβ1 followed by BMP7, and a prevention model where cells were exposed first to BMP7, then TGFβ1. RT-QPCR, immunofluorescence, ELISA, and siRNA transfection techniques were used to delineate associated alterations in HA matrix and cell phenotype.

Results: BMP7 both prevented and reversed myofibroblast differentiation, and this was associated with removal and internalisation of cell surface HA into perinuclear endosomes. HA co-localised with the HA-degrading enzyme, Hyaluronidase-1 (Hyal1) in endosomes, and Hyaluronidase-2 (Hyal2) at the nuclear margin. This process was also associated with increased expression of the cell-surface HA receptor, CD44, and its redistribution to the perinuclear region. In addition, BMP7 markedly increased expression of the variant CD44 isoform CD44v7/8. Using siRNA techniques against Hyal2 and CD44v7/8 we identified these proteins to be essential for HA internalisation, as well as BMP7 driven prevention and reversal of myofibroblast phenotype.

Conclusions: A novel mechanism of TGFβ1 antagonism by BMP7 was identified. BMP7 prevented TGFβ1-driven pericellular HA accumulation by internalisation of HA into catalytic, perinuclear endosomes. CD44v7/8 and membrane-bound Hyal2 were critical to this process; and to the prevention and reversal of myofibroblast phenotype by BMP7, thus identifying new potential therapeutic targets for intervention in fibrosis.

SA-PO410

Role of Bone Marrow-Derived Fibroblasts in Renal Fibrosis Jun Zhou, Xiaogao Jin, Yanlin Wang. *Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Renal fibrosis is the final common manifestation of chronic kidney disease resulting in progressive loss of kidney function. Although activated fibroblasts are responsible for the production and deposition of the extracellular matrix, the origin of activated fibroblasts mediating renal fibrosis has been controversial. Recent evidence indicates that bone marrow-derived fibroblasts contribute significantly to the pathogenesis of renal fibrosis. In this study, we characterized and examined the functional role of bone marrow-derived fibroblasts in the development of renal fibrosis.

Methods: In a well-established mouse model of renal fibrosis induced by unilateral ureteral obstruction, we characterized bone marrow-derived fibroblasts in the kidney using Col-GFP mice that express GFP driven by collagen I promoter and examined the functional role of bone marrow-derived fibroblasts in the development of renal fibrosis using Col-DTR mice that have a diphtheria toxin (DT)-induced depletion of collagen I-producing cells in the bone marrow.

Results: Bone marrow-derived fibroblasts constitute 45% of total collagen-producing fibroblasts in the obstructed kidney. The bone marrow-derived fibroblasts are of hematopoietic origin, can proliferate and differentiate into myofibroblasts in the obstructed kidney. Treatment of Col-DTR mice with DT resulted in a marked reduction of circulating fibroblasts in the peripheral blood. Col-DTR mice treated with DT accumulated significant fewer bone marrow-derived fibroblasts in the kidney in response to obstructive injury. Furthermore, Col-DTR mice treated with DT exhibited fewer myofibroblasts and expressed substantial less fibronectin, type I collagen, and a-smooth muscle actin in the obstructed kidney. Finally, Col-DTR mice treated with DT displayed significant less collagen deposition in the obstructed kidney.

Conclusions: Our results demonstrate that hematopoietic fibroblasts migrate into the kidney, proliferate and differentiate into myofibroblasts and contribute functionally to the pathogenesis of renal fibrosis.

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SA-PO411

Quercetin Inhibits Fibroblast Activation and Kidney Fibrosis Involving the Inhibition of mTORC1 and β-catenin Signaling Pathways Jiafa Ren, Weichun He, Junwei Yang, Chunsun Dai. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

Background: Previous studies demonstrated that both mTORC1 and β-catenin signaling activation contribute to fibroblast activation and kidney fibrosis. Although it has been reported that Quercetin, a dietary flavonoid, is able to inhibit mTORC1 and β-catenin signaling pathways in other cell types, the role and mechanisms for it in fibroblast activation and kidney fibrosis are not fully understood.

Methods: Rat kidney interstitial fibroblasts (NRK-49F) were stimulated with TGFβ1 and kidney fibrosis was induced by unilateral ureter obstruction (UUO) in CD1 mice.

Results: Here, we show that in cultured rat renal fibroblast cells (NRK-49F), Quercetin treatment inhibited TGF-β1-induced mTORC1 and β-catenin signaling activation but not for smad3 phosphorylation. a-SMA and fibronectin expression induced by TGFb1 treatment were also remarkably inhibited by Quercetin treatment at a dose-dependent manner. In mice with UUO nephropathy, administration of Quercetin significantly diminished total collagen deposition, fibronectin and a-SMA expression in the kidneys compared to vehicle control. Macrophages infiltration and proinflammatory cytokines expression such as TNF-α and MCP-1 were also remarkably inhibited in the UUO mice treated with Quercetin. In addition, the abundance of p-4EBP1 and β-catenin nuclear translocation in kidney interstitial cells was also reduced after Quercetin treatment.

Conclusions: Take together, these results suggest that Quercetin may act as a novel therapeutic strategy for treatment of kidney fibrosis through inhibiting both mTORC1 and β-catenin signaling pathways.

Funding: Government Support - Non-U.S.

SA-PO412

Tenascin-C Is a Major Component of Fibrogenic Niche That Promotes Fibroblast Proliferation Haiyan Fu,[1] Dong Zhou,[1] Liangxiang Xiao,[1] Roderick J. Tan,[2] Youhua Liu.[1] [1]*Dept of Pathology, Univ of Pittsburgh, Pittsburgh, PA;* [2]*Dept of Medicine, Univ of Pittsburgh, Pittsburgh, PA.*

Background: It is thought that after chronic kidney injury, fibrogenic niches are formed at various focal sites, which provide a microenvironment that favors fibroblast proliferation and activation. However, the molecular identities and compositions of such a fibrogenic niche are poorly understood. Here we report that tenascin-C (TNC), an extracellular matrix glycoprotein with both structural and cell-signaling properties, is a major component of kidney fibrogenic niche.

Results: In vivo, TNC was markedly induced in animal models of chronic kidney disease (CKD) induced by ischemia/reperfusion injury (IRI) and unilateral ureteral obstruction (UUO). Immunostaining revealed that TNC was predominantly localized at the foci rich in fibroblasts in renal interstitium. In vitro, treatment of normal rat kidney fibroblast (NRK-49F) cells with TGF-β or sonic hedgehog (Shh) dramatically induced TNC expression in a time dependent manner. TNC was also induced in human proximal tubular epithelial cells (HKC-8) after sustained incubation with TGF-β1. To investigate the potential role of increased TNC, we studied its effect on cultured interstitial fibroblasts. We found that incubation with TNC promoted NRK-49F cell proliferation, which was assessed by cell counting, MTT assay, and BrdU incorporation. Furthermore, TNC activated MEK/ERK signaling pathway and stimulated the expression of numerous proliferation-related genes in fibroblasts.

Conclusions: Taken together, these results suggest that after chronic kidney injury, TNC is induced with a unique spatial and temporal expression pattern, and forms a fibrogenic niche that ensures fibroblast proliferation and activation.

Funding: NIDDK Support

SA-PO413

IL-4 Receptor α Deficiency Suppresses Bone Marrow-Derived Fibroblast Activation and Renal Fibrosis Jingyin Yan, William E. Mitch, Yanlin Wang. *Medicine-Nephrology, Baylor College of Medicine, Houston, TX.*

Background: Renal fibrosis is the final common pathway for chronic kidney diseases of different causes. Recent evidence indicates that bone marrow-derived fibroblast precursors contribute significantly to the development of renal fibrosis. These cells express hematopoietic marker such as CD45 and mesenchymal marker such as platelet derived growth factor receptor-β (PDGFR-β). However, the signaling mechanisms underlying the activation of bone marrow-derived fibroblast precursors in the kidney are incompletely understood. We have recently found that Th2 cytokines - IL-4 and IL-13 are induced in the kidney during the development of renal fibrosis. In this study, we investigated the role of IL-4 receptor α in the activation of bone marrow-derived fibroblasts and the development of renal fibrosis.

Methods: IL-4 receptor α knockout (IL4Ra-KO) mice and wild-type (WT) mice were subjected to unilateral ureteral obstruction (UUO).

Results: Our results showed that the number of CD45 and PDGFR-β double positive fibroblasts was significantly increased in the injured kidneys of WT mice, whereas the number of double positive fibroblasts was significantly reduced in the injured kidneys of IL4Ra-KO mice. Furthermore, IL4Ra deficiency resulted in a significant reduction in the number of α-smooth muscle actin (α-SMA) positive myofibroblasts in the obstructed kidneys. Consistent with these findings, IL4Ra deficiency significantly reduced mRNA and protein expression of α–SMA in the injured kidneys compared with WT mice. There was also a marked increase in the mRNA and protein levels of collagen I and fibronectin in the obstructed kidneys of WT mice, while these fibrotic changes were significantly attenuated in the obstructed kidneys of IL4Ra-KO mice.

Conclusions: Our results demonstrate that IL4Ra plays an important role in the activation of bone marrow-derived fibroblast precursors in the kidney and the development of renal fibrosis. These results indicate that IL4Ra signaling may represent a novel therapeutic target for fibrotic kidney disease.

Funding: NIDDK Support, Other NIH Support - NHLBI, Private Foundation Support

SA-PO414

Thrombin Induces Epithelial-Mesenchymal Transition Through the Activation of PAR-1 in Tubular Epithelial Cells Wai Han Yiu, Dickson W.L. Wong, Joseph C.K. Leung, Loretta Y.Y. Chan, Kar Neng Lai, Sydney C.W. Tang. *Dept of Medicine, The Univ of Hong Kong, Queen Mary Hospital, Hong Kong.*

Background: Fibrin deposition is commonly observed in tubulointerstitial injury of chronic kidney diseases. Recent studies have suggested that local activation of thrombin contributes to renal fibrosis. However, the effect of thrombin on tubular cells is not fully understood. Since epithelial-mesenchymal transition (EMT) is an important mechanism of renal tubulointerstitial fibrosis, we investigated whether thrombin plays a role in tubular EMT and dissected the underlying mechanism.

Methods: Rat kidney tubular epithelial cells (NRK52E) were treated with thrombin (1-4U/ml) for 72 h and were examined for the expression of EMT and profibrotic markers. To determine the potential role of protease activated receptor (PAR) in tubular EMT, selective PAR antagonists, SCH7979 for PAR-1 and tcY-NH2 for PAR-4 were co-incubated with thrombin.

Results: After 72-h treatment, cells underwent a transition from an epithelial to a mesenchymal phenotype as evidenced by reduced expression of E-cadherin, and increased expression of α-smooth muscle actin in a dose-dependent manner. Expression of snail, the known inducer of EMT, was also upregulated by thrombin. Blockade of PAR-1, but not PAR-4 partially restored the altered expression of thrombin-induced EMT markers. Besides, thrombin also stimulated the expression of profibrotic growth factors (TGFβ1 and CTGF) and extracellular matrix (ECM) proteins (fibronectin and collagen IV). The induction of TGFβ1 and collagen IV were suppressed by PAR-1 antagonist; while the induction of fibronectin was inhibited by both PAR-1 and PAR-4 antagonists.

Conclusions: Our data demonstrated that thrombin promoted EMT and increased the production of profibrotic factors and ECM proteins in tubular epithelial cells. These effects were partially mediated by PAR-1 and, to a lesser extent, PAR-4. These results suggest that modulation of PAR signaling may provide a potential therapeutic strategy for the treatment of renal fibrosis. Fund support: Research Grants Council of Hong Kong (GRF grant number 7796/11M) and Small Project Funding (project code 201309176032) from the University of Hong Kong.

SA-PO415

Signaling of a Receptor for Leukotriene B_4, BLT1 Plays Roles in Tubulointerstitial Fibrosis Mariko Kamata,[1,2] Kanako Hosono,[1] Tomoe Fujita,[1] Kouju Kamata,[2] Masataka Majima.[1] *[1]Pharmacology, Kitasato Univ School of Medicine, Japan; [2]Nephrology, Kitasato Univ School of Medicine, Japan.*

Background: Kidney fibrosis develops on the interaction of chemokines and cytokines secreted from migrated cells and kidney cells, and accumulates type I collagen and type III collagen in the tubulointerstitium. We investigated a role of the signaling pathway of LTB_4-BLT1 on the tubulointerstitial fibrosis of the kidney using BLT1 knockout mice ($BLT1^{-/-}$).

Methods: Ninety male C57BL/6 wild type mice (WT) and 80 male $BLT1^{-/-}$ were used. All the mice had the unilateral ureteral obstruction (UUO) at 8-wk-old. Collagen type I, III and IV, F4/80 (a macrophage marker), S100A4 (a fibroblast marker) and Gr-1 (a neutrophil marker) were stained on frozen or paraffin sections of isolated kidneys. COL1A1, COL3A1, COL4A1, S100A4, alpha-smooth muscle actin (αSMA), TGF-β, FGF-2, MCP-1, F4/80, 5-lipoxygenase (5-LOX) and BLT1 were quantitated by RT-PCR in the kidney specimens with UUO.

Results: The mRNA of BLT1 increased in UUO kidney in WT, but not in $BLT1^{-/-}$. The 5-LOX mRNA increased in both of WT and $BLT1^{-/-}$ UUO kidneys, but the increase in $BLT1^{-/-}$ was significantly blunted compared with WT UUO kidneys from day-3 to day-7. The immunoreactive type I, III and IV collagens in UUO kidneys were less deposited in $BLT1^{-/-}$ mice from day-1 to day-7 compared with those in WT. mRNA levels of COL1a1, COL3a1, and COL4a1 confirmed the above immunostaining results. The expressions of TGF-β, and FGF-2 also decreased in $BLT1^{-/-}$ from day-1 to day-7. The mRNA levels of αSMA, a marker of myofibroblast, significantly decreased in $BLT1^{-/-}$ UUO kidney from day-2 to day-7. The numbers of S100A4-positive cells also decreased in $BLT1^{-/-}$ UUO kidneys.

Conclusions: LTB_4-BLT1 signaling plays roles for tublointerstitial fibrosis of the kidney, possibly via upregulation of TGF-β, and FGF-2 together with enhanced recruitments of myofibroblasts and fibroblasts.

SA-PO416

Low-Dose Colchicine Treatment Attenuates Renal Fibrosis in Mice Unilateral Ureteral Obstruction Seiji Itano, Minoru Satoh, Atsushi Uchida, Hiroyuki Kadoya, Chieko Ihoriya, Tamaki Sasaki, Naoki Kashihara. *Dept of Nephrology and Hypertension, Kurashiki, Okayama, Japan.*

Background: Colchicine is known to exert anti-inflammatory action through inhibition of migration and chemetaxis of neutrophils by blockade of microtubule polymerization. Recently, it has been reported that colchicine treatment could prevent recurrence of pericarditis and development of atrial fibrillation after the cardiac surgery. Meanwhile, colchicine has been demonstrated to possess anti-fibrotic properties through suppression of collagen synthesis and secretion. We investigated the effects of colchicine on renal fibrosis using unilateral ureteral obstruction (UUO) model.

Methods: (1) Eight week-old male C57BL/6 mice were divided to two groups: Control group and Colchicine group. Colchicine was administrated by osmotic pump (0.5mg/kg/day). UUO operation was induced at 7 days after insertion of osmotic pump. Mice were sacrificed at 14days from UUO. (2) In vitro scratch assay were performed to evaluate the effect of colchicine on cellular migration. NRK-49F cell (rat fibroblast) were cultured with or without colchicine under TGF-β1 stimulation.

Results: (1) Any significant renal nor liver dysfunction was not observed, but slight degree of body weight loss was noted in Colchicine group. The UUO-Colchicine group showed suppression of kidney interstitial fibrosis comparing to UUO-Control group. CTGF and FN-1 mRNA expressions were elevated in UUO-Control group and decreased by colchicine administration. (2) In vitro, colchicine inhibits fibroblast migration on scratch assay. Fibrotic gene expression were ameliorated with addition of colchicine.

Conclusions: Colchicine directly affects fibroblast activity, exerts anti-fibrotic effects, and attenuates renal fibrosis in UUO mice.

SA-PO417

Combination TGF-β and CTGF In Vivo Transfection via Minicircles: A Novel Renal Fibrosis Model Peng Sun, Scott MacDonnell, Hu Sheng Qian, Tammy Bigwarfe, Danielle M. Fowler, Chung-Wein Lee, Mark Mchugh, James W. Tanner, John Miglietta, Glenn Gibson, Glenn A. Reinhart, Steven M. Weldon. *Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT.*

Background: Transforming growth factor beta (TGF-β) and connective tissue growth factor (CTGF) have been identified as mediators of kidney fibrosis. Minicircles (MC) are DNA vectors devoid of any bacterial backbone that exhibit enhanced expression and duration compared to classic plasmids vectors. In the present study, we used MC technology to express TGF-β and CTGF transgenes to develop a novel, non-surgical model for of renal fibrosis in the mouse.

Methods: MC-DNA constructs for human TGF-β1 and CTGF were delivered by hydrodynamic injection (HI). Mice (n=6/group) received TGF-β1+CTGF transfection and antibodies, 1D11 or FG3019, to neutralize TGF-β and CTGF. Hepatocyte transfection was evaluated via MC-GFP florescence. Plasma TGF-β and CTGF levels were measured by ELISA. Renal tubulointerstitial fibrosis (TIF; cortical Sirius Red staining) and fibrotic gene transcription (Col1a1, etc.) were evaluated 10 days post-HI.

Results: GFP labeled MC transfection produced a robust GFP florescence signal in hepatocytes (10 days post HI) of a control MC injection group compared to naïve mice (523±145 GFP^+ units/area versus 0). Mice receiving TGF-β+CTGF MCs exhibited elevated plasma levels of TGF-β and CTGF by Day 4 and remained increased 10 days post-HI. TIF and col1a1 mRNA were significantly increased (54% and 40%, respectively) compared to sham MC-control. Both 1D11 and FG3019 markedly reduced TIF (~40%) in the MCs transfection groups compared to control.

Conclusions: We show for the first time in mice that co-transfection with TGF-β1+CTGF via MCs produces high levels of circulating TGF-β and CTGF and induces a significant renal fibrosis that can be attenuated by administration of neutralizing antibodies. These data support the importance of TGF-β1 and CTGF as drivers of organ fibrosis. Finally, this novel, non-surgical model of kidney fibrosis has utility for evaluation of novel anti-fibrotic agents and may be extended to organ systems.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim Pharmaceuticals, Inc.

SA-PO418

Inhibition of EGFR Alleviates the Development and Progression of Hyperuricemia Associated Nephropathy Na Liu,[1] Shougang Zhuang.[2] *[1]Dept of Nephrology, Shanghai East Hospital, Tongji Univ School of Medicine, Shanghai, China; [2]Dept of Nephrology, Shanghai East Hospital, Tongji Univ School of Medicine, Shanghai, China.*

Background: Although mechanisms and therapeutic approaches of uric acid-associated nephropathy (UAN) have been widely studied, so far there is no any effective treatment for this disorder.

Methods: In this study, we examined the effect of epidermal growth factor factor receptor (EGFR) inhibition on the progression of UAN and the mechanism involved.

Results: In a rat model of UAN induced by feeding a mixture of adenine and potassium oxonate, severe glomerular sclerosis and renal interstitial fibrosis were observed, which was accompanied by increased expression of EGFR phosphorylation in the kidney. In paralell, serum uric acid, BUN and creatinine levels and urine micro albumin levels were increased. Adminstration of gefitinib, a specific EGFR inhibitor, prevented progressive renal injury as demonstrated by blunting the rise of serum uric acid, BUN and creatinine levels, reducing increased urine micro albumin and inhibiting expression of α-smooth muscle

actin (α-SMA), collagen I and fibronectin. Hyperuricemia resulted in downregulation of major urate transporters, OAT1 and OAT3. Gefitinib treatment preserved their expression. EGFR blockade also inhibited expression of multiple profibrogenic cytokines/chemokines including transforming growth factor-β1 (TGF-β1), MCP-1, RANTES, TNF-α, IL-1β. Furthermore, gefitinib markedly abrogated hyperuricemia-induced phosphorylation of Smad3, ERK1/2, and NF-κB(p65).

Conclusions: Taken together, blocking EGFR can alleviate the development and progression of UAN through enhancing expression of uric acid transporters, inhibit profibrotic signaling pathways and suppress proinflammatory responses. Thus, inactivation of EGFR activity may provide a novel therapeutic approach for treatment of UAN.

Funding: Government Support - Non-U.S.

SA-PO419

Podocyte Proliferation Is Associated with Loss of Contact to the Glomerular Basement Membrane (GBM) Kevin Schulte,[1] Antonio Sechi,[2] Katja Berger,[1] Bart Smeets,[1] Jürgen Floege,[1] Marcus J. Moeller.[1] *[1]Div of Nephrology and Clinical Immunology, Univ Hospital of the Aachen Univ (RWTH), Aachen, Aachen, Germany; [2]Inst of Biomedical Engineering, Div of Cell Biology, Univ Hospital of the Aachen Univ (RWTH), Aachen, Aachen, Germany.*

Background: Podocytes are thought to be postmitotic cells. Nevertheless, under specific circumstances podocytes may undergo cellular divisions. Here we investigated the role of the extracellular matrix on podocyte proliferation.

Methods: Podocytes were labeled in quadruple transgenic Pod-rtTA/LC1/R26R/eGFP-histone mice and traced in culture, as well as *in vivo* during development and in disease models. Cellular outgrowths from isolated glomeruli and podocyte proliferation were monitored in primary culture. Podocyte expression of the proliferation marker Ki67 was analysed in different models in vivo, which are characterised by (parietal) podocytes on the parietal basement membrane (PBM).

Results: *In vitro*, podocytes on isolated glomeruli did not proliferate as observed by 72 hours time-lapse video microscopy. However, outgrowing podocytes started to proliferate within 12 - 24 hours as soon as they lost contact to the GBM. In kidneys of newborn mice, expression of proliferation marker Ki67 was observed on a small fraction of "committed" podocytes on Bowman's capsule (the recently described "podocyte reserve"), but not on visceral podocytes. In a model of atubular glomeruli in adult mice, cellular proliferation was observed again exclusively in parietal podocytes located on the PBM. Visceral podocytes on the GBM did not proliferate regardless if they were filtering or not. Finally, podocytes within cellular crescents expressed Ki67 in mouse tissue.

Conclusions: Contact to the GBM is associated with an antiproliferative signal. This is consistent with histological patterns observed in glomerular diseases, where proliferation occurs in general along Bowman's capsule (e.g. cellular crescents).

Funding: Government Support - Non-U.S.

SA-PO420

Precision-Cut Kidney Slices (PCKS): A Novel Model to Study Development and Treatment of Renal Fibrosis *Ex Vivo* Fariba Poosti,[1] Bao Tung Pham,[2] Klaas Poelstra,[3] Harry Van Goor,[1] Jan-Luuk Hillebrands.[1] *[1]Pathology, Univ Medical Center Groningen, Groningen, Netherlands; [2]Pharmaceutical Technology and Biopharmacy, Univ of Groningen, Groningen, Netherlands; [3]Pharmacokinetics, Toxicology and Targeting, Univ of Groningen, Groningen, Netherlands.*

Background: Renal fibrosis is a serious clinical problem forming the utmost cause of need for renal replacement therapy. As yet, no adequate preventive or curative therapy is available for clinical use to specifically target the development of renal fibrosis. The search for new efficacious treatment strategies is therefore warranted. *In vitro* models using homogeneous cell populations have contributed to the understanding of the mechanisms involved in fibrosis. However, models as such mimic the complex *in vivo* multicellular architecture poorly. Aim of this study was to evaluate the value of the precision-cut kidney slice (PCKS) model as an *ex vivo* model to study development of fibrosis, and to test the anti-fibrotic effects of IFNγ.

Methods: Precision-cut slices (250 μm thickness) were prepared from healthy mouse kidneys using a Krumdieck tissue slicer. To induce fibrosis, slices were incubated with TGFβ1 (5 ng/ml) for 48 hrs in the presence or absence of the anti-fibrotic cytokine IFNγ or a modified derivative thereof (PPB-PEG-IFNγ; *i.e.* IFNγ targeted to the PDGFR). After culture, tissue viability (ATP content) and expression of fibrotic markers (α-SMA, fibronectin, and collagen 1) using real-time PCR and immunohistochemistry were determined.

Results: Kidney slices remained viable up to 48 hrs of incubation and no significant effects of TGFβ1 and/or IFNγ on viability were observed. TGFβ1 significantly increased α-SMA, fibronectin, and collagen 1 mRNA and protein expression levels. IFNγ and PPB-PEG-IFNγ significantly reduced TGFβ1-induced fibronectin and collagen I mRNA and protein expression.

Conclusions: Fibrotic kidney slices are a promising tool to test anti-fibrotic drugs *in vitro* in a multicellular and profibrotic milieu, which cannot be achieved *in vitro* using other models. Importantly, this method provides the opportunity to study anti-fibrotic compounds not only in animal but also in human renal tissue.

SA-PO421

Nephronectin Promotes Normal Glomerular Structure and Function Denise K. Marciano,[1] Susan E. Zimmerman,[1] Zhufeng Yang.[1] *[1]Dept of Medicine, Div of Nephrology, Univ of Texas Southwestern Medical Center, Dallas, TX; [2]Dept of, Univ of California San Francisco, San Francisco, CA.*

Background: Previous studies have demonstrated that Nephronectin production by the ureteric bud is critical for the early inductive events of nephrogenesis and that Nephronectin null mice often have unilateral or bilateral renal agenesis.

Methods: In the current study, we use control and Nephronectin null mice to examine the localization and function of Nephronectin in later kidney development.

Results: We find that Nephronectin is highly produced by tubules and glomeruli. Specifically we find that Nephronectin is most highly produced by podocytes and localizes to the glomerular basement membrane. We examined glomerular structure in Nephronectin null mice that had escaped the early phenotype and found a slight expansion of the glomerular mesangium in adult mice. We subsequently conditionally deleted Nephronectin from nephron progenitors and observed significant mesangial expansion with collapse of glomerular capillary loops. The mesangial expansion was accompanied by increased mesangial matrix deposition. Furthermore, we find that in the absence of nephronectin, alpha 8 integrin produced by mesangial cells was mislocalized.

Conclusions: These results suggest that the nephronectin-alpha8 interaction is critical for normal glomerular structure.

Funding: NIDDK Support, Pharmaceutical Company Support - Satellite Healthcare, Private Foundation Support

SA-PO422

Podocalyxin Mediates Polarized Lumenogenesis of Human Pluripotent Stem Cells in Three-Dimensional Culture Benjamin S. Freedman, Albert Q. Lam, Ryuji Morizane, Craig R. Brooks, Jing Zhou, Joseph V. Bonventre. *Renal Div, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: Epithelial polarization and lumenogenesis are critical to development and disease, but epithelial cell lines used to study these processes lack genetic diversity. Podocalyxin is an apical/luminal sialomucin expressed by glomerular podocytes and vascular endothelia, whose functional and mechanistic roles remain controversial and poorly understood. We investigated lumenogenesis in human pluripotent stem cells (hPSCs), which are genetically diverse and express endogenous podocalyxin.

Methods: hPSCs were cultured in three dimensions in extracellular matrix or suspension. Podocalyxin expression was disrupted using siRNA or CRISPR/Cas9 genome editing. Lumenogenesis was assayed by confocal microscopy, and tight junctions were assessed by ZO-1 immunohistochemistry and dextran dye exclusion.

Results: In 3D culture, epiblast-stage hPSCs formed expanding spheroidal cysts, comprising simple columnar epithelia surrounding hollow lumens lined with size-selective tight junctions and podocalyxin. Cysts were composed of pluripotent cells, which expressed pluripotency markers and gave rise to new cysts in serial 3D cultures. Lumenogenesis occurred via apicobasal polarization of ZO-1, ß-catenin, and podocalyxin, without requiring apoptosis, CFTR, or extracellular matrix. More primitive "naïve" hPSCs did not express podocalyxin and did not form cysts. CRISPR/Cas9 knockout of podocalyxin reduced lumen formation seven-fold, without disrupting tight junctions or pluripotency. Protamine sulfate caused podocalyxin mislocalization and collapse of cystic structures, indicating a charge-based mechanism.

Conclusions: Polarized lumenogenesis is a fundamental property of epiblast-stage human pluripotent stem cells in 3D microenvironments. Polarized, charge-repulsive interactions by podocalyxin at the apical plasma membrane are essential for expansion of the cyst lumen, independent of tight junction organization and function. hPSCs establish a new, genetically diverse model system in which to investigate the role of kidney proteins in epithelial polarity and lumenogenesis.

Funding: NIDDK Support, Private Foundation Support

SA-PO423

Anti-Inflammatory Role of miR-146a in the Pathogenesis of Diabetic Nephropathy Kirti Bhatt,[1] Linda L. Lanting,[1] Ye Jia,[1] Marpadga A. Reddy,[1] Mark Boldin,[2] Rama Natarajan.[1] *[1]Diabetes and Metabolic Diseases Research, Beckman Research Inst at City of Hope, Duarte, CA; [2]Molecular and Cellular Biology, Beckman Research Inst at City of Hope, Duarte, CA.*

Inflammation plays a pivotal role in the pathogenesis of diabetic complications including diabetic nephropathy (DN). MicroRNAs have recently emerged as critical regulators of DN. However, the role of microRNAs in the regulation of inflammation and development of DN is poorly understood. Here we show that miR-146a, a known anti-inflammatory microRNA, is up regulated in mice models of DN suggesting the involvement of inflammation/immune modulatory microRNA components in disease progression. miR-146a up regulation was consistently observed in the macrophages and kidneys of type 1 and type 2 diabetic mice by RT-PCR and in situ hybridization. Notably, miR-146a deficient mice exhibited increased diabetic kidney injury and severity in the pathogenesis of DN as compared to wild type mice. miR-146a null mice displayed a significant increase in proteinuria, macrophage infiltration, glomerular hypertrophy and fibrosis as compared to wild type mice after STZ induced diabetes. Mechanistically, the expression of pro-inflammatory mediators like MCP-1, TNF-α and pro-fibrotic genes like PAI-1, TGF-β were significantly elevated in the renal cortices of diabetic miR-146a null mice relative to wild type. Interestingly, the expression of key genes associated with anti-inflammatory M2 phenotype was significantly suppressed whereas expression of those associated with pro-inflammatory M1 phenotype

was up-regulated in macrophages from diabetic miR-146a-/- mice, suggesting a shift to more pro-inflammatory states. Furthermore, miR-146a target genes *Traf6* and *Irak1* related to NF-kB activation and inflammation were found to be up-regulated in the renal cortical tissues derived from diabetic miR-146a null mice as compared to the diabetic WT mice. These observations suggest that miR-146a plays an anti-inflammatory and anti-fibrotic role in the diabetic kidney. Approaches to overexpress miR-146a can be evaluated to confer protection from the development of DN.

Funding: NIDDK Support

SA-PO424

CXCL10 Expression Induced By Mxi1 Inactiviation Causes Mesangial Cell Apoptosis in Mice Habu Nephritis Xiang-Mei Chen, Lingling Wu, Yang Lu, Guangyan Cai. *Dept of Nephrology, Chinese PLA General Hospital, Chinese PLA Inst of Nephrology, State Key Laboratory of Kidney Diseases, National Clinical Research Center of Kidney Diseases, Beijing, China.*

Background: The Mxi1 proteins are antagonists of c-myc. It has been pointed out that Mxi1 expression decreased significantly during the proliferative period in anti-Thy1 nephritis model rats and then gradually rised as proliferation declined, thereby suggesting a possible role for Mxi1 in mesangial proliferation, while the specific mechanisms that how Mxi1 affects mesangial proliferative glomerulonephritis need to be studied further.

Methods: *In vivo*, we used Mxi1 gene knockout mice ($Mxi1^{-/-}$ mice) and wild-type mice ($Mxi1^{+/+}$ mice) to establish mesangial proliferative glomerulonephritis mice model. In vit*ro* we down-regulated mouse mesangial cell Mxi1 expression by RNAi technology.

Results: We found that $Mxi1^{-/-}$ mice exhibited more typically and more severe pathological phenotypes compared with wild-type mice, which mainly manifested as the more obvious dissolution phenotype, the higher proportion of apoptotic cells and chemokine CXCL10 expression in the early days of the model, and the higher levels of cell proliferation in late days. In vitro, we found increased expression of CXCL10 and activiation of caspase 3, and increased proportion of apoptotic cells when the expression of the Mxi1 was inhibited in mouse mesangial; while the proportion of apoptotic cells decreased after we interfered CXCL10.

Conclusions: These *in vivo* and in vit*ro* experiments demonstrate that the mouse mesangial cell apoptosis in the early age of mesangial proliferative glomerulonephritis model was related with the expression of CXCL10 induced by Mxi1 inactiviation, and this finding will provide a possible intervention target for the treatment of the mesangial proliferative glomerulonephritis.

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SA-PO425

Concomitant Inhibition of Renin Angiotensin System and Endothelin-1 Reduce Renal Fibrosis Induced By Unilateral Ureteral Obstruction in Mice Dae Eun Choi,[1] Jin Young Jeong,[1] Hyunsu Choi,[2] Ye Jin Kim,[1] Sarah Chung,[1] Yoon-Kyung Chang,[3] Ki Ryang Na,[1] Kang Wook Lee,[1] Young Tai Shin.[1] *[1]Internal Medicine, ChungNam National Univ Hospital, Daejeon, Korea; [2]Inst of Clinical Medicine, Saint Mary Hospital, Catholic Univ, Daejeon, Korea; [3]Internal Medicine, Saint Mary Hospital, Catholic Univ, Daejeon, Korea.*

Background: Both Endothelin-1(ET-1) and Renin angiotensin system (RAS) may play an important role in renal fibrosis in obstructed kidney. However, there was few study for the relationship between RAS and renal ET-1 activation in experimental unilateral ureteral obstruction (UUO). We investigated the role and relationship of renal RAS and ET-1 in UUO.

Methods: 8 weeks old male C57BL/6 mice were divided into the 6 groups; 1)Sham, 2) bosentan+Sham, 3) valsartan+Sham, 4)vehicle+UUO, 5)bosentan+UUO 6)valsartan+UUO, and 7)Valsartan + bosentan+UUO. Valsartan and bosentan were administrated via oral using NG tube (valsartan 10mg/kg/day, bosentan 100mg/kg/day for 8days). We performed realtime RT PCR and immunohistochemistry for molecular study and H&E stain and Masson trichrome (MT) stain for histologic examination of kidneys.

Results: Although angiotensinogen and angiotensin II receptor are increased in bosentan + UUO kidney compared to vehicle + UUO kidney, bosentan treatment reduced significantly renal expression of PKC-β, TGF-β, and α-SMA in UUO kidney. Also, it reduced renal tubular injury in H&E stain and blue stained area in MT stain of UUO kidney. Valsartan treatment in UUO mice showed similar results to Valsartan treated UUO mice. Co-treatment of valsartan and bosentan decrease significantly renal expression of PKC-β,TGF-β, and α-SMA compared to both valsartan+UUO and bosentan+UUO respectively. Also, they reduced significantly renal tubular injury in H&E stain and blue stained area in MT stain of UUO kidney compaired to both valsartan+UUO and bosentan+UUO respectively.

Conclusions: Concomitant inhibition of Renin Angiotensin System and Endothelin-1 reduces renal fibrosis induced by UUO in mice. It may have additive effects compare to single inhibition of RAS or ET-1.

SA-PO426

G-Protein Coupled Chemokine Receptor Cxcr4 Contributes to Kidney Fibrosis By Inducing Multiple Effectors Amy Yuan,[1] Yashang Lee,[1] Gilbert W. Moeckel,[1] Anil K. Karihaloo.[1] *[1]Medicine, Yale School of Medicine, New Haven, CT; [2]Medicine, Yale School of Medicine, New Haven, CT; [3]Pathology, Yale School of Medicine, New Haven, CT.*

Background: Kidney fibrosis is the common endpoint for every type of chronic kidney disease (CKD). G-protein coupled chemokine receptor Cxcr4 is ubiquitously expressed. The expression in an adult kidney is very low. In the present study, we hypothesized that chronically increased Cxcr4 expression will augment fibrosis.

Methods: Tubule and macrophage-specific conditional mouse knockouts of Cxcr4 were generated. Fibrosis was induced via UUO.

Results: Following UUO Cxcr4 expression in tubular cells increased by several-fold. This increased Cxcr4 expression correlated with their increased mRNA expression of PDGFα, TGFβ1 with concurrent loss of BMP7. Artificially over-expressing Cxcr4 in a human proximal tubular cell line led to almost complete loss of BMP7 mRNA. Conditional knockout of tubular Cxcr4 in-vivo was partially protective against UUO-mediated fibrotic response leading to 50% less fibrosis. This correlated with a significant reduction in PDGFα, TGFβ1 levels and the preservation of BMP7 expression after UUO. Furthermore, $Cxcr4^{+}$ immune cells infiltrated into the interstitium of the obstructed kidneys that further contributed to the increase in total Cxcr4 expression in injured kidneys. Increased Cxcr4 expression in macrophages partly correlated with increase in their pro-fibrotic markers. Selectively ablating Cxcr4 from macrophages significantly reduced UUO-mediated fibrotic response with concomitant reduction in the expression of total kidney TGFβ1 mRNA that correlated with reduced SMAD activation and αSMA levels. Finally, administering a Cxcr4 inhibitor, AMD3100, subcutaneously once every 12 hours from the time of UUO, resulted in a dose dependent significant protection from UUO-induced fibrotic response.

Conclusions: Our data demonstrate that chronic high Cxcr4 expression in multiple effector cell types contributes to the pathogenesis of renal fibrosis by altering their biological profile. We present a novel cross-talk between Cxcr4-TGFb1 and BMP7 pathways that may provide novel avenues for interrupting the progression of fibrosis and hence CKD.

Funding: Other NIH Support - Yale O'Brien Center for Kidney Research

SA-PO427

Latent Transforming Growth Factor Beta Binding Protein 4 Enances Renal Fibrosis in Obstructive Nephropathy Chi-Ting Su,[1,2] Jenq-Wen Huang,[2] Chih-Kang Chiang,[2] Branka Dabovic,[3] Zsolt Urban.[1] *[1]Human Genetics, Univ of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; [2]Nephrology, Internal Medicine, National Taiwan Univ Meical College and Hospital, Taipei, Taiwan; [3]Cell Biology, New York Univ Langone School of Medicine.*

Background: Although transforming growth factor-β (TGFβ) signaling is known to play an essential role in renal fibrosis, the role of latent TGFβ binding proteins (LTBPs) remains largely unknown. LTBP4 has been recognized to regulate the secretion and deposition and activation of latent TGFβ molecules in the extracellular matrix.

Methods: To investigate the impact of Ltbp4 on renal fibrosis, we subjected *Ltbp4S*-/- mice and wild type littermates to unilateral ureteral obstruction (UUO) at 4 weeks of age. Two weeks later, the mice were euthanized. Expression of mRNA and protein markers of fibrosis and inflammation were studied by quantitative real-time PCR, immunoblotting and immunofluorescent staining of kidney samples.

Results: LTBP4 was up-regulated by UUO both at the protein and at the mRNA levels in wild type mice, and was mainly expressed around peri-glomerular cells. As expected, UUO increased the expression of both profibrotic [TGFβ1, plasminogen activator inhibitor-1 (PAI1)] and proinflammatory [interleukin1 (IL1), IL6] molecules, and increased immunoreactivity for α-smooth muscle actin (SMA) in wild type mice. *Ltbp4S* deficiency significantly attenuated TGFβ1 and PAI1 expression and SMA immunoreactivity, but did not change the expression of either IL1 or IL6 in UUO mice.

Conclusions: Ltbp4 acts downstream of inflammatory molecules to enhance fibrosis in UUO-induced renal injury. Therefore, Ltbp4 antagonism may be considered for therapeutic suppression of renal fibrosis.

SA-PO428

Dnmt1 as a Therapy Target for *Attenuating* Diabetic Nephropathy and Podocyte Injury Li Zhang, Wei Shi, Shuangxin Liu, Xinling Liang, Ting Lin, Chunping Yu, Yuanhan Chen, Zongshun Huang, Ruizhao Li. *Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Podocyte injury plays a key role in the development of diabetic nephropathy(DN). Recent studies have suggest a potential role for epigeneticmechanisms apart from genetic predisposition in the etiology of DN. In this study, experiments were undertaken to assess the role of DNA methylation on DN, especially on podocyte injury.

Methods: Type 2 diabetic *db/db* mice received i.p. injections of DNA methylation inhibitor 5-azacytidine three times a week and were killed after 8 weeks. Immortalized mouse podocytes were cultured under different experimental conditions.

Results: Our findings indicate that the albuminuria in diabetic *db/db* mice was markedly attenuated after 5-azacytidine treatment, accompanied with alleviated glumerular hypertrophy, mesangial matrix expansion and podocyte injury. However, body weight, food and water consumption, and glucose levels remained unaffected. In addition, we also found that themethyltransferase Dnmt1, nuclear factor Sp1and NFκB-p65 expression were

markedly increased in vivo and vitro podocytes under diabetic state, and increasedDnmt1 expression was also attenuated after treatment with 5-azacytidine or 5-Aza- 2'-deoxycytidine or Dnmt1 knockdown, accompanied with improved podocyte motility and the filtration barrier function of podocyte monolayer. Further study found that increased Sp1 and NFκB-p65 are interact in the nucleus of podocytes incubated with high glucose, and Sp1 binds to Dnmt1 promoter region(-119~+102). The involvement of Sp1/NFκB-p65 in Dnmt1regulation is further demonstrated by the observation that Sp1 knockdown using mithramycin A or siRNA decreases Dnmt1 protein levels. Our results provide the first in vivo and vitro evidence that inhibition of DNA methylation ameliorate glomerular pathologies and podocyte injury in diabetic state, and thus suggest that aberrant DNA methylation is involved in DN and podocyte injury.

Conclusions: DNA methylation inhibitor might be a new therapeutic avenue for the treatment of DN. Sp1/ NFκB-p65-Dnmt1 pathway may be exploited as a therapeutic target for protecting against podocyte injury of DN.

Funding: Government Support - Non-U.S.

SA-PO429

Podocyte-Specific NFAT Activation in Mice Causes Glomerular Injury That Progresses from MCD- to FSGS-Like Features Alexis J. Sloan, Saurav Singh, Karla J. Schramm, Alexander Grabner, Ansel P. Amaral, Alessia Fornoni, Christian Faul. *Medicine, Miller School of Medicine, Miami, FL.*

Background: Aberrant elevation in cytoplasmic calcium levels in podocytes causes changes in podocyte morphology and glomerular permeability. The phosphatase calcineurin responds to elevated calcium by dephosphorylating the nuclear factor of activated T-cells (NFAT). We have shown that in podocytes NFAT is activated by proteinuric stimuli, like angiotension II or adriamycin, and that podocyte-specific activation of calcineurin or NFAT in transgenic mice is sufficient to cause proteinuria within days. We investigate the precise glomerular phenotype of NFAT transgenic mice.

Methods: Transgenic mice with the podocyte-specific, doxycyclin (Dox)-inducible expression of constitutively active NFAT (NFATc1nuc) were fed Dox chow, and urine albumin levels were monitored over time. Mice were sacrificed at different time points for histological studies of the kidney, including H&E, TEM analysis of the podocyte foot process (FP) structure and labeling for WT1 to determine podocyte numbers. In subgroups Dox was removed at different time points to study a potential reversibility of the phenotype.

Results: NFATc1nuc mice are proteinuric within three days of Dox exposure with podocyte FP effacement. NFATc1nuc induction for four months leads to sustained proteinuria with an increase in FP effacement, but without other histological changes within the kidney. Animals that are left on constant Dox treatment for 1 year develop glomerular sclerosis, extraglomerular lesions and impaired renal function. Proteinuria in mice with short-term NFATc1 activation in podocytes is reversible, whereas mice with NFATc1nuc expression for two months are unable to revert to normal podocyte morphology and kidney function.

Conclusions: Podocyte-specific NFAT activation in mice causes a two-phase renal phenotype, first MCD-like alterations that are reversible, followed irreversible FSGS-like changes. We are currently determining the precise "point of no return", and postulate that the phenotypic switch from a MCD- to an FSGS-like phenotype occurs in concert with changes in the responsiveness to pharmacological interventions like steroid resistance.

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SA-PO430

Thrombin Causes Human Podocyte Apoptosis in a Protease Activated Receptor (PAR)-3/-4 Dependent Manner Ruchika Sharma,[1] Amanda P. Waller,[1] Adam J. Guess,[1] Shipra Agrawal,[1] Berend Isermann,[3] William E. Smoyer,[1] Marvin T. Nieman,[2] Bryce Kerlin.[1] *[1]Center for Clinical and Translational Research, Nationwide Children's Research Inst, Columbus, OH; [2]Pharmacology, Case Western Reserve Univ, Cleveland, OH; [3]Clinical Chemistry and Pathobiochemistry, Otto-von-Guericke Univ, Magdeburg, Germany.*

Background: Nephrotic Syndrome (NS), one of the most common forms of glomerular disease, is associated with increased endogenous thrombin activity. *In vitro* evidence suggests that thrombin may injure podocytes and thus contribute to progressive glomerular injury. The molecular mechanisms by which thrombin induces podocyte injury are not yet known. Thrombin is known to signal through Protease Activated Receptors (PARs) in other cell types. Thus, we hypothesized that thrombin exacerbates glomerular injury by enhancing podocyte apoptosis in a PAR dependent manner.

Methods: Experiments were performed with differentiated, conditionally immortalized human podocytes. Podocyte apoptosis was determined after 36 hours of thrombin (20nM) exposure by TUNEL assay. Specific PAR antibodies and activating peptides were utilized to determine which PARs mediate thrombin-induced podocyte apoptosis.

Results: Thrombin exposure induces a significant increase in apoptosis ($p<0.05$). Blockade of PAR-3 or PAR-4 resulted in a significant decrease in apoptosis ($p<0.05$) as did inhibition of thrombin enzymatic activity with hirudin, a direct thrombin inhibitor ($p<0.05$). In comparison to a control peptide, PAR-4 activation peptide significantly increased apoptosis ($p<0.05$), while PAR-3 activation peptide did not.

Conclusions: Thrombin-induced human podocyte injury is mediated in a PAR-dependent fashion. Specifically, in this *in vitro* model, PAR-3 and PAR-4 appear to mediate thrombin induced podocyte injury. Furthermore, these data suggest that thrombin induced podocyte injury may be mediated in a manner dependent on PAR-3/-4 heterodimerization. Co-immunoprecipitation experiments are underway to evaluate for the presence of PAR-3/-4 heterodimers in human podocytes. Interrupting thrombin-mediated podocyte injury may provide a novel therapeutic approach for NS.

Funding: NIDDK Support

SA-PO431

Disrupted ABIN1 Function Results in a Pro-Inflammatory Phenotype in Human Glomerular Cells David W. Powell, Rachel Therese G'Sell, Ryan M. Sheehan, Dawn J. Caster, Michael Merchant, Kenneth R. McLeish, Erik Korte. *Medicine/Nephrology, Univ of Louisville School of Medicine.*

Background: NF-κB activation is implicated in various types of glomerulonephritis. ABIN1 is a ubiquitin binding protein that functions as a physiological NF-κB inhibitor. We reported that mice expressing an ABIN1[D485N] mutation lacking ubiquitin binding activity develop lupus-like autoimmunity and proliferative lupus nephritis (LN) and ABIN1 polymorphisms are associated with LN. The present study tested the hypothesis that enhanced NF-kB activation in human podocytes and mesangial cells containing a loss of function ABIN1[D472N] mutation leads to local production of cytokines that amplify glomerular injury.

Methods: Expression and secretion of NF-κB target proteins were assessed in response to TNF-α and IL-1β in wild-type and ABIN1[D472N] expressing human-derived podocyte and mesangial cell lines using gene and protein arrays, RT-PCR, ELISA, and mass spectrometry. Primary human lymphocyte and leucocyte chemotaxis was measured using transwell permeable supports in response to TNF-α stimulated wild-type and ABIN1[D472N] mesangial cell supernatants.

Results: Array analysis showed that expression and secretion of NF-κB targets including interleukins (IL-6, IL-8) interferons (INF-β1, INF-γ) chemokines (CXCL1, MCP1), complement factors (CFB, C3), and colony stimulating factors (G-CSF, CSF3) were elevated in pro-inflammatory stimulated ABIN1[D472N] human glomerular cells as compared with wild-type control cells. This effect was confirmed for MCP-1, CSF3, IL-8, IL-6, and C3. Primary B/T cell and neutrophil chemotaxis was stimulated by incubation with supernatant from stimulated ABIN1[D472N] glomerular cells as compared to that of control cells.

Conclusions: Our findings suggest that dysregulation of NF-κB activation in glomerular cells containing ABIN1 polymorphisms acts to enhance glomerular inflammation in LN through production of pro-inflammatory cytokines and recruitment of immune cells, providing novel mechanistic insight for development of improved therapeutics. Our studies have focused on LN, but these finding also provide promising directions for events controlling inflammation in other types of kidney disease.

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SA-PO432

Podocyte-Specific Loss of Krüppel-Like Factor 6 Accelerates Kidney Fibrosis Under Diabetic Conditions Sandeep K. Mallipattu,[1] Sylvia Horne,[1] Victoria Ly,[1] Timothy W. Miller,[1] John C. He.[2] *[1]Stony Brook Univ; [2]Icahn School of Medicine at Mount Sinai.*

Background: Krüppel-like factor 6 (KLF6) is a zinc-finger DNA-binding transcription factor, shown to play a critical role in preventing mitochondrial injury in the podocyte. Although mitochondrial injury and podocyte loss are features of diabetic kidney disease (DKD), mechanism(s) by which podocyte injury contributes to eventual interstitial fibrosis remain elusive.

Methods: Immunostaining was used to assess KLF6 expression in kidney biopsies from patients with DKD. *In vivo*: mice with podocyte-specific loss of *Klf6*, *Cre*$^{+}$ *podocin Klf6*$^{flox/flox}$, were generated and made diabetic with streptozotocin (STZ) at age 12 weeks. *In vitro*: stable knockdown (shRNA-*KLF6*) and overexpression (lentiORF-*KLF6*) of *KLF6* in human podocytes was performed under high (HG-30mM), normal glucose (NG-5mM), and mannitol (30mM) conditions for 24 hours.

Results: We observed that the glomerular expression of KLF6 was reduced in kidney biopsies from patients with DKD as compared to healthy donors. These findings were confirmed by two independent tissue arrays from Nephromine, publicly available renal gene expression database. Diabetic *Cre*$^{+}$ *podocin Klf6*$^{flox/flox}$ mice exhibited a 20-fold increase in albuminuria, kidney hypertrophy, mesangial expansion, and thickened BM with increased glomerular and interstitial collagen deposition as compared to all other groups. Immunostaining showed a significant increase in glomerular and interstitial cytochrome c, TGF-β1, and vimentin levels in the diabetic *Cre*$^{+}$ *podocin Klf6*$^{flox/flox}$ mice. Under HG conditions, shRNA-*KLF6* increased cleaved caspase 3 and 9 expression and reduced cytochrome c oxidase levels as compared to all other groups. In contrast, lentiORF-*KLF6* reduced cleaved caspase 3 expression and rescued cytochrome c oxidase levels under HG conditions, as compared to all other groups.

Conclusions: We showed that under diabetic conditions, podocyte-specific loss of *Klf6* resulted in glomerular injury, earlier activation of the intrinsic apoptotic pathway, and acceleration of interstitial fibrosis. Combined, this suggests that KLF6 may serve as the link between podocyte injury and interstitial fibrosis in DKD.

Funding: NIDDK Support

SA-PO433

Identification of Neuropilin-1 as a Target for Glomerular Disease Christina S. Bartlett,[1] Monika Lucyna Wnuk,[2] Vera Eremina,[3] Chengjin Li,[3] Yashpal S. Kanwar,[1] Jeffrey H. Miner,[4] Maria Pia Rastaldi,[5] Susan E. Quaggin.[1] *[1]Feinberg Cardiovascular Research Inst and the Div of Nephrology, Northwestern Univ, Chicago, IL; [2]Univ of Bern, Switzerland; [3]Mount Sinai Hospital, Toronto, Canada; [4]Washington Univ, St. Louis, MO; [5]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy.*

Background: Neuropilin-1 is a co-receptor for a variety of growth factors and is critical for development and patterning of the vascular and nervous systems. In the kidney NP-1 is expressed in the specialized perivascular mesangial cells, yet its role in glomerular function and disease is unknown. In biopsies from human diabetic patients, we show increased expression of NP-1. Therefore, we hypothesize NP-1 on mesangial cells, and in perivascular cells in other tissues, is required to modulate receptor tyrosine kinase activity in adjacent endothelium to maintain a quiescent vasculature.

Methods: NP-1 expression was observed in biopsy samples from diabetic and IgA nephropathy patients using IF. To further investigate the function of NP-1, mice with perivascular cell specific deletion of NP-1 were utilized.

Results: Patients with diabetic nephropathy and IgM nephropathy showed substantially increased expression of NP-1 protein within the glomerular mesangium, suggesting NP-1 may play an important role in pathogenesis of these diseases. In mice, deletion of NP-1 from mesangial cells during glomerular development results in dramatic mesangial expansion, thickening of the GBM, and tubular dilation. These mice show 10-fold higher proteinuria and an over 60% reduction in GFR at 4 weeks of age, and die within 5 weeks of age due to renal failure. Profound collagen accumulation and increased matrix protein expression was observed in mutant glomeruli, suggesting NP-1 plays a critical role in matrix production. Additionally, enhanced VEGFR2 activation and signaling was observed in mutant mice, consistent with our hypothesis.

Conclusions: These studies suggest a protective role of NP-1 in renal diseases such as diabetic nephropathy or IgA nephropathy, via regulation of matrix production and stabilization of the glomerular endothelium, and present NP-1 as an attractive target for the treatment of renal diseases.

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SA-PO434

T Cells Infiltration in the Kidneys of Patients with Both Anti-Glomerular Basement Membrane Antibodies and Anti-Neutrophil Cytoplasmic Antibodies Shuiyi Hu, Zhao Cui, Ming-Hui Zhao. *Renal Div, Dept of Medicine, Peking Univ First Hospital, Inst of Nephrology, Peking Univ, Beijing, China.*

Background: Patients with anti-glomerular basement membrane (GBM) antibodies and anti-neutrophil cytoplasmic antibodies (ANCA) present severe kidney injury and poor outcomes. Cellular immunity plays important role in the pathogenesis of anti-GBM disease and ANCA associated vasculitis. In double positive patients, the participation of T cells in kidney injury is unclear.

Methods: T cells (CD3, CD4, CD8, IL-17 and foxp3) and macrophages (CD68) were examined by immunohistochemistry on renal biopsy tissues from 22 patients with anti-GBM disease, including nine patients with positive ANCA. The correlation between cell infiltration and clinical data was further analyzed.

Results: T cells and macrophages were shown in the kidneys of all patients. The distribution of cell infiltration was predominant in the peri-glomerular and interstitial areas ($p<0.001$). T cells infiltration in the kidneys of double positive patients was much severer than that of patients with anti-GBM antibodies alone (3527.6±1561.6 versus 1776.4±1209.0 cells/mm^2, $p=0.024$). This difference was much notable on CD8+ T cells and IL-17 producing cells in the peri-glomerular and interstitial areas. In double positive patients, the number of CD8+ T cell around glomeruli was positively correlated with the crescent percentage in glomeruli ($r=0.707$, $p=0.033$), and CD8+ T cells aggregated highly around the glomeruli with ruptured Bowman's capsule (13.9±6.7 versus 4.4±4.0 cells/gcs, $p=0.006$). In patients with anti-GBM antibodies alone, IL-17 producing cells showed a positive correlation with cellular crescents percentage ($r=0.968$, $p=0.007$).

Conclusions: T cell infiltration might play a crucial role in the inflammatory kidney injury of double positive patients, especially the CD8+ cytotoxic T cells around glomeruli.

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SA-PO435

Zonulin, a Circulating Factor That Regulates Podocyte Function and Glomerular Permeability Karla J. Schramm,[1] Britta Sylvia Walter,[1] Alexis J. Sloan,[1] Saurav Singh,[1] Sandra M. Merscher,[1] Alessia Fornoni,[1] Cristina Zennaro,[2] Mary Artero,[2] Michele Carraro,[2] Alessio Fasano,[3] Christian Faul.[1] *[1]Dept of Medicine, Univ of Miami Miller School of Medicine, Miami, FL; [2]Dept of Medicine, Surgical and Health Sciences, Univ of Trieste, Trieste, Italy; [3]Dept of Pediatric Gastroenterology, Mass General Hospital for Children, Boston, MA.*

Background: Zonulin, a 47kDa secreted protein, stimulates protease-activated receptor (PAR) 2 on enterocytes, modifies the phosphorylation and localization of zonula occludens (ZO)-1 and alters actin dynamics. Thereby zonulin transiently disassembles tight junctions (TJ) and reversibly regulates small intestine permeability. Zonulin expression is elevated in patients with Celiac Disease (CD) and causes abnormal paracellular transport and diarrhea. It is also increased in the serum of patients with CD, indicating that zonulin could function as a circulating modifier of TJs in other tissues. Since podocytes express PAR2 and TJ proteins, zonulin might act as a regulator of the slit diaphragm (SD), and prolonged PAR2 activation may lead to irreversible podocyte injury and proteinuria.

Methods: We treated cultured podocytes (wild type and PAR2 knockdown) with AT1002, a zonulin peptide analogue, and examined its effects on the cytoskeleton and SD. We measured albumin permeability of isolated rat glomeruli treated with zonulin, and we determined urine albumin levels of zonulin knockin mice.

Results: AT1002 induced changes in the podocyte actin cytoskeleton and cell migration via PAR2, and altered ZO-1 phosphorylation and paracellular flux. Zonulin and serum from FSGS patients increased albumin permeability of isolated rat glomeruli, which was blocked in the presence of a PAR2 inhibitor. Zonulin knockin mice developed proteinuria.

Conclusions: Zonulin might act as a circulating factor that regulates the podocyte SD and paracellular permeability. It is possible that zonulin is involved in a variety of glomerular diseases and that FSGS patients would benefit from pharmacological PAR2 inhibition. Anecdotal reports suggest that a subgroup of FSGS patients show a favorable response to a gluten-free diet, which in CD has been shown to reduce serum zonulin.

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SA-PO436

Rostafuroxin Protects From Podocyte Injury and Proteinuria Induced By Adducin Genetic Variants and Ouabain Mara Ferrandi,[1] Isabella Molinari,[1] Patrizia Ferrari,[3] Maria Pia Rastaldi,[2] Laura Zagato,[1] Giuseppe Bianchi,[1,3] Paolo Manunta.[1] *[1]San Raffaele Scientific Inst, Milan, Italy; [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore, Italy; [3]CVie Therapeutics, Hong Kong, China.*

Background: Glomerulopathies are important causes of morbidity and mortality. Selective therapies addressed to the underlying mechanisms are still lacking. Recently, our group identified two mechanisms, mutant β-adducin and ouabain, involved in glomerular podocytopathies and proteinuria through nephrin down-regulation. Here, we proposed that rostafuroxin, a novel antihypertensive agent developed as a selective inhibitor of Src-SH2 interaction with mutant adducin and ouabain-activated Na-K ATPase, may protect podocytes from adducin and ouabain effects, thus representing a novel pharmacological approach for the therapy of podocytopathies and proteinuria caused by the above mentioned mechanisms.

Methods: To study rostafuroxin effect on podocyte protein changes and proteinuria, mice carrying mutant β-adducin and ouabain hypertensive rats, OHR, were orally treated with 100 μg/kg/day rostafuroxin. Primary podocytes from congenic rats carrying mutant α- (NA) or β-adducin (NB) from the Milan hypertensive strain, MHS, and normal rat podocytes incubated with 10^{-9} M ouabain, were cultured with 10^{-9} M rostafuroxin.

Results: We showed that mutant β-adducin and ouabain caused podocyte nephrin loss (range from -28 to -38%, $p<0.05$) and proteinuria (range from +50 to +60%, $p<0.05$) in these animal models. Such nephrin alteration was reproduced in primary podocyte cultures from NB rats (-60%, $p<0.05$ versus NA) and from normal rat incubated with ouabain (-34%, $p<0.05$ versus control). Treatment of animals, or incubation of cultured podocytes with rostafuroxin, reverted mutant β-adducin and ouabain-induced effects on nephrin protein expression (range from +25 to +35%, $p<0.05$) and proteinuria (range from -55% to -65%, $p<0.05$).

Conclusions: These findings indicate that rostafuroxin prevented podocyte lesions and proteinuria due to mutant β-adducin and ouabain in animal models. This suggests a potential therapeutic effect of rostafuroxin also in patients affected by glomerular disease progression associated with these two mechanisms.

SA-PO437

Mammalian Target of Rapamycin Complex 1 Activation in Podocytes Promotes Cellular Crescent Formation Junhua Mao, Weichun He, Junwei Yang, Chunsun Dai. *Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical Univ, Nanjing, China.*

Background: Podocytes play a key role in the formation of cellular crescents in experimental and human diseases. However, the underlying mechanisms for podocytes in promoting crescent formation need further investigation.

Results: Here we demonstrated that mammalian target of rapamycin complex 1 (mTORC1) signaling was remarkably activated and HIF1alpha expression was largely induced in cellular crescents from patients with crescentic glomerular diseases. Specific deletion of Tsc1 in podocytes led to podocyte mTORC1 activation and kidney dysfunction in mice. Interestingly, about 15% of the glomeruli developed cellular or mixed cellular and fibrous crescents in the knockouts at 7 weeks of age and the percentage was increased to about 30% at 12 weeks of age. Most notably, bridging cells between the glomerular tuft and the parietal basement membrane and cellular crescents were WT1, p-S6, HIF1alpha or Cxcr4 immunostaining positive. Furthermore, many cells within the cellular crescents were also immune-staining positive for anti-claudin 1, suggesting an involvement of parietal cells for the crescent formation in the knockouts. In addition, administration of rapamycin started at 7 weeks of age for 5 weeks abolished crescent formation as well as the induction for p-S6, HIF1alpha and Cxcr4 in the glomeruli from the knockouts.

Conclusions: It is concluded that mTORC1 activation in podocytes promotes cellular crescent formation and targeting this signaling may shed a new light for the treatment of patients with crescentic glomerular diseases.

SA-PO438

Essential Role of iqgap2 for Maintenance of Podocyte Structure and Function in Zebrafish Yuya Sugano,[1,2,4] Ines Auberger,[1,3,4] Urs Ziegler,[5] Clemens D. Cohen,[3,4] Stephan C. Neuhauss,[2,4] Johannes Loffing.[1,4] *[1]Inst of Anatomy, Univ of Zurich, Zurich, Switzerland; [2]Inst of Molecular Life Sciences, Univ of Zurich, Zurich, Switzerland; [3]Inst of Physiology, Univ of Zurich, Zurich, Switzerland; [4]Zurich Center for Integrative Human Physiology, Univ of Zurich, Zurich, Switzerland; [5]Center for Microscopy and Image Analysis, Univ of Zurich, Zurich, Switzerland.*

Background: Podocytes are essential for the maintenance of the glomerular filtration barrier. In nephrotic syndrome (NS), podocyte dysfunction impairs the glomerular size selectivity, leading to proteinuria. In this study, we screened human and mouse mRNA datasets for genes possibly relevant to NS and tested their functional relevance in the zebrafish pronephros.

Methods: The human European renal cDNA bank, biopsies from kidneys of NS patients and isolated mouse nephron segment were screened for genes that are dysregulated in patients with NS and are also enriched in mouse glomeruli. Subsequently, the orthologues of the mammalian candidate genes were analyzed by *in situ* hybridization for expression in the pronephros and subjected to functional analyses by morpholino-based gene knockdown.

Results: One of the identified candidate genes was the IQ motif containing GTPase activating protein 2 (iqgap2), of which mRNA was found to be expressed in the zebrafish pronephros with highest expression levels in the glomerulus. Immunohistochemistry revealed that iqgap2 protein is mainly abundant in the zebrafish podocytes. Morpholino-based knockdown of *iqgap2* caused a dilation of Bowman's space that became apparent with the onset of glomerular filtration. Electron microscopy showed intact glomerular endothelial and mesangial cells. Podocytes were well developed but showed some degree of progressive foot process effacement. Consistent with an impaired glomerular filtration barrier, *iqgap2* morphants showed glomerular filtration of large fluorescent dextrans (~500 kDa), which had been i.v. injected.

Conclusions: IQGAP2 is a glomerulus-enriched gene product that is dysregulated in patients with NS, which appears to be required for normal podocyte structure and function in zebrafish larvae.

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SA-PO439

Knockdown of Podocyte Foot Process Protein Klhl35 Results in Proteinuria in Zebrafish Pronephros Sonia Zambrano Sevilla, Patricia Rodriguez, Jaakko Patrakka. *Dept of Medical Biochemistry and Biophysics, Karolinska Instt, Stockholm, Sweden.*

Background: In our previous microarray study, a transcript encoding for Kelch-like family member 35 (Klhl35) was enriched in the glomerulus. As the protein is very poorly characterized, we aimed in this study to analyze the expression and function of Klhl35 in the kidney.

Methods: Expression was studied in adult and developing kidneys, as well as in cultured podocytes using RT-PCR, immunofluorescence and immune-electron microscopy. Functional studies were performed in zebrafish by inactivating the gene expression using morpholinos.

Results: Klhl35 was expressed by podocytes and it localized to foot processes. During glomerulogenesis Klhl35 was detected first at S-shaped stage glomeruli in where its expression seemed to preceed nephrin expression. In cultured podocytes, no significant expression was detected in undifferentiated cells. In differentiated podocytes the expression was induced and Klhl35 was detected in stress fibers. In zebrafish, the knockdown of Klhl35 orthologue resulted in pronephros abnormalities including foot process effacement. Functionally, pronephros was leaking as detected by the presence of of 500kD fluorescent dye in the tubular compartment.

Conclusions: Klhl35 is a novel podocyte foot process protein. It is important for the integrity of zebrafish pronephros. Based on its sequence, we speculate that Klhl35 is an actin-associated protein that regulates the organization of foot process cytoskeleton.

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SA-PO440

Studying the Function of ApoL1 in the Pronephros of Zebrafish Larvae Ahmed Kotb, Elisabeth Rumpel, Ole Simon, Karlhans Endlich, Nicole Endlich. *Anatomy and Cell Biology, Univ Medicine Greifswald, Greifswald, MV, Germany.*

Background: APOL1 which encodes for a secreted high density lipoprotein (HDL) is expressed in a number of human tissues, including the kidney. Recently it was shown that genetic variants of APOL1 are associated with non-diabetic kidney diseases, kidney diseases attributed to hypertension and focal segmental glomerulosclerosis (FSGS) in African Americans. In human, ApoL1 is expressed mainly in podocytes, in extraglomerular endothelial cells and somes tubules. Since an ortholog of ApoL1 does not exist in mice, the zebrafish is an ideal model to study the function of this protein.

Results: We found that the expression of zApoL1 in zebrafish larvae is similar to the expression in human tissue. After the knockdown (KD) of zApoL1 in zebrafish larvae by the injection of specific morpholinos (zApoL1 MO) into fertilized eggs, the larvae developed severe pericardial edema accompanied by a lower number of glomerular capillaries. To determine whether the KD of zApoL1 affects also glomerular filtration we injected two different fluorescence-labeled dextran molecules (10 kDa and 500 kDa) into the vein of zebrafish larvae and studied the filtration process by measuring the fluorescence intensity in vessels and in the pronephric tubules. With this method we found that the glomerular filtration barrier was leaky for 500 kDa dextran after zApoL1 MO injection. After the injection of in vivo morpholinos into zebrafish larvae we observed a reorganization of the glomerulus directly in living zebrafish larvae by the use of two-photon micoscopy. Further, we found by immunohistochemical staining of zebrafish sections that the expression of nephrin, a slit diaphragm protein, was significantly reduced after zApoL1 KD. Interestingly, we also found a correlation between the ApoL1 and nephrin expression in biopsies of patients suffering from FSGS and membranous glomerulonephritis.

Conclusions: In summary, our data demonstrate that zApoL1 is important for the proper expression of nephrin in podocytes and necessary for an intact glomerular filtration barrier in zebrafish larvae.

Funding: Government Support - Non-U.S.

SA-PO441

Misexpression of Mouse Nephrin in the Drosophila Developing Eye Leads to Irregular Cell Sorting Resulting in a Rough Eye Phenotype Britta George,[1] Hermann Pavenstaedt,[1] Christian Klämbt.[2] *[1]Nephrology Dept, Univ Hospital Münster, Münster, Germany; [2]Inst for Neurobiology, Westfälische-Wilhelms-Univ Münster, Münster, Germany.*

Background: Mutations in the murine NPHS1 gene which encodes the slit diaphragm protein Nephrin result in incomplete formation of podocyte cell processes and the development of proteinuria. Nephrin binds to the cell adhesion molecule Neph1 at the slit diaphragm and upon activation mediates signals to the actin cytoskeleton in a phosphotyrosine-dependent fashion. In the *D. melanogaster* developing eye Nephrin orthologs *hibris* and *sns* are expressed by ommatidial cells while Neph1 orthologs *kirre* and *roughest* are expressed by interommatidial cells and mediate cell sorting. This work aims at analyzing molecular mechanisms of Nephrin family signaling to the actin cytoskeleton.

Methods: An *in vivo* model was established which allows analyzing the signal transduction of murine Nephrin in the *Drosophila* developing eye. Wild-type as well as several mutant murine UAS Nephrin transgenes were manufactured and integrated into the genome of *Drosophila* by ΦC31-based transformation to assure equal expression strength.

Results: We expressed wild-type or one of several different mutant murine UAS Nephrin transgenes in cells of the developing eye using the GMR-Gal4 driver. Flies that express wild-type murine Nephrin in the developing eye show a characteristic rough eye phenotype which arises from defective cell sorting during the development of the compound eye. To analyze the functional role of tyrosine residues within the cytoplasmic domain of murine Nephrin, we generated a murine Nephrin mutant in which all tyrosine residues within the cytoplasmic domain were mutated to phenylalanine. Expression of this mutant Nephrin protein in the developing eye results in a weaker rough eye phenotype, compared to flies expressing the wild-type murine Nephrin.

Conclusions: SH2 domain protein-dependent Nephrin signaling as well as phosphotyrosine-independent Nephrin signaling appears to play a role in this *in vivo* model. The *Drosophila* model is a valid system to decipher Nephrin signaling.

Funding: Government Support - Non-U.S.

SA-PO442

Podocyte MicroRNA-92a Controls Glomerular Damage in Extracapillary Glomerulonephritis Carole Hénique,[1] Guillaume Bollee,[1,4] Patrick Bruneval,[1,2] Eric Thervet,[1,3] Dominique Nochy,[2] Laurent Mesnard,[5] Pierre-Louis Tharaux.[1,3] *[1]Paris Cardiovascular Centre - PARCC, INSERM, Paris, France; [2]Pathology; Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France; [3]Nephrology; Hôpital Européen Georges Pompidou, AP-HP, Paris, France; [4]Nephrology, Centre Hospitalier de l'Univ de Montréal, Montréal, Canada; [5]UMR 702, INSERM, Paris, France.*

Background: Mature podocytes are growth-arrested because of the expression of cyclin-dependent kinase inhibitors. Under pathological conditions, podocytes may undergo mitosis, but not cell division. Exceptions to this rule is necrotizing crescentic rapidly progressive glomerulonephritis (RPGN) where podocytes differentiate and proliferate, achieving "extracapillary glomerulopathies", the most severe forms of kidney diseases. We hypothesized that break on disease tolerance with pathological dedifferentiation of glomerular cells may be elicited by microRNA (miRNA) deregulation.

Methods: RPGN was induced by injection of anti–glomerular basement membrane serum in mice with modified EGFR or STAT3 pathways. miR-92a expression was studied by in Situ Hybridization and RT-qPCR in kidney biopsies from patients diagnosed with crescentic RPGN and non-crescentic GN. We manipulated the HB-EGF/EGFR, IL-6, STAT3 and miR92a pathways in primary podocytes and in vivo in mice and evaluated functional and structural impact on RPGN.

Results: We found that microRNA-92a (miR-92a) expression in diseased glomeruli is up-regulated in experimental and clinical RPGN. An EGFR and IL-6 - STAT3 cascade induced expression of miR-92a in podocytes. The cyclin-dependent kinase inhibitor 1C/p57^{Kip2} was found to be a direct target of miR-92a. Inhibition of miR-92a upregulated expression of p57^{Kip2} and decreased podocyte proliferation and dedifferentiation. In vivo silencing of miR-92a de-repressed p57^{Kip2} expression in podocytes and prevented extracapillary proliferation, glomerular demolition and renal failure.

Conclusions: mIR-92a is the first miRNA that is found to modulate podocyte quiescence *in vivo*. A specific common miR-92a extracapillary expression pattern in murine and human RPGNs is likely to have significant pathogenic, diagnostic, and/or therapeutic implications.

SA-PO443

Differential Expressions of miRNAS in Kidney in Puromycin Aminonucleoside Nephropathy Model and Intervened Effects of Triptolide

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Background: *Triptolide* (TP), a major active ingredient from Chinese herbal medicines, *Tripterygium wilfordii* Hook. f. (TWHF) and its extract named multi-glycoside of TWHF (GTW), has been proved effective in reducing proteinuria in China. The aim of this study is to demonstrate the mechanism in vivo of TP for reducing proteinuria and protecting podocyte in puromycin aminonucleoside (PAN) nephropathy rat.

Methods: Fifty male Wistar rats were randomly subdivided into five groups, Control (A), Model (B), TP(C), GTW (D), and Valsartan(E). Except the rats in A group, PAN nephropathy model was induced byinjection of PAN 100 mg/kg in B, C, D, and E groups. At the 2nd day after PAN nephropathy model was established, daily oral administration of the fluid of TP in C, GTW in D, valsartan in E, and physiological saline in A and B groups as a control was started respectively and lasted until sacrifice at the 10th day. Proteinuria, glomerular ultrastructure, and glomerular immunofluorescence staining of dicer enayme, nephrin, podocin and synaptopodin were examined. The differential expressional characteristics of miRNAsin renal cortex were analyzed through biochip assay. Moreover, the differential expressional volumes of no-miR-23a, rno-miR-300-3p, rno-miR-24, and rno-miR-30c were measured by real-time PCR respectively.

Results: In PAN-induced rats, proteinuria and podocyte foot processes effacement were investigated, as well as the expressions of nephrin, podocin, and synodopotodin were affected by dicer enayme. The differential expressionalmiRNAs in kidney included rno-miR-24, rno-miR-30c, rno-miR-23a, and rno-miR-300-3p. TP could improve proteinuria, alleviate podocyte foot processes effacement, reduce the expression of dicer enayme, increase the expressions of nephrin, podocin, and synodopotodin, and regulate the differential expressionalmiRNAs as well in PAN nephropathy model.

Conclusions: In PAN nephropathy rat, with the treatment of TP, proteinuria can be reduced and the expressions of nephrin, podocin, and that, synaptopodin can be regulated. The mechanism of which is probably due to intervening dicer enayme and differential expressions of miRNAs in kidney.

Funding: Government Support - Non-U.S.

SA-PO444

Apolipoprotein L1 Has Diverse Isoforms and APOL1-B3 Activates the NF-kB Pathway

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Background: Apolipoprotein L1 (APOL1), a trypanosomal lytic factor, is a component of the innate immune system. *APOL1* gene variants, present in individuals of recent African descent, are strongly associated with glomerular disease risk. APOL1 has RNA splice variants, summarized as A, B and C isoforms. The A isoform is encoded by exons 1 and 3-7 and has been studied most extensively. Little is known about the B isoform, encoded by exon 1-7, and the C isoform, encoded by exons 1, 3, and 5-7. The roles of distinct APOL1 isoforms in mediating renal injury are unknown.

Methods: We examined mRNA expression by TA cloning and RT-PCR and protein localization using immunofluorescence (IF) of FLAG-tagged APOL1 in stably transfected HeLa cells. Since only APOL1-B isoforms contain exon 2, we raised rabbit antiserum specific against APOL1-B peptide by immunizing rabbits with an exon 2 peptide. Mitochondrial fractions were isolated using a detergent-based method.

Results: We cloned 7 APOL1 splicing variants from cultured human podocytes. Three variants contained exon 2, which we termed APOL1-B1, B2, and B3. APOL1-B1 and B2 RNA contained exon 1-7, with B2 having an alternate spliced exon 3. APOL1 B3 lacked exon 4, which contains the signal sequence of APOL1; this isoform has not been previously reported. Using RT-PCR, we found that normal human kidney expressed four APOL1 splicing variants: APOL1-A, B1, B3 and C. In cultured human podocytes, transfected FLAG-tagged APOL1-B3 was detected by both APOL1-B and anti-FLAG antibodies (Ab). APOL1-B isoforms, detected by IF using the exon 2 Ab, localized to mitochondria in serum-starved human podocytes and were detected in mitochondrial fractions of HeLa cells by WB; further, transfected APOL1-B3 localized to mitochondria in HeLa cells. Impairment of autophagy causes NF-κB activation; when autophagy was inhibited with chloroquine, the NF-κB pathway was activated in APOL1-B3 transfected HeLa cells, compared to empty-vector transfected cells.

Conclusions: APOL1 mRNA splicing produces multiple distinct protein isoforms. APOL1-B3 over-expression activated the NF-κB pathway in HeLa cells; if this occurred in immune cells, this could promote immune response.

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SA-PO445

***APOL1* mRNA Renal Risk Variants Activate Protein Kinase R and Reduce Cell Protein Synthesis In Vitro and Increase Susceptibility to Proteinuria In Vivo**

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Background: Coding variants in APOL1 (apolipoprotein L1), S342G (G1) and NYK388K (G2), compared to the ancestral allele (G0) are strongly associated with renal disease among individuals with sub-Saharan ancestry. APOL1 circulates as component of HDL and is also expressed in podocytes and vascular cells. Wiggins and colleagues have shown that in transgenic rats, suppression of protein translation via expression of a dominant-negative 4E-BP1 in podocytes leads to focal segmental glomerulosclerosis (FSGS). We asked whether *APOL1* variants might affect global protein synthesis.

Methods: For *in vitro* assay, we generated stable HEK293 cell lines bearing recombinant *APOL1* variants. For the cell free protein kinase R (PKR) activation assay, we used a synthetic mRNA T7 transcript system to generate APOL1 transcripts and we made recombinant PKR protein using E.coli. For *in vivo* studies, we generated nephrin transgenic mice in which the nephrin promoter driven drives expression of the G1 stem-loop RNA.

Results: In APOL1-overexpressing HEK293 cells, phosphorylated PKR (the active form) and inactive (phosphorylated) elongation initiation factor-2α were both increased with the G1 and G2 variants compared to G0 cells. PKR is activated by long dsRNA, which is absent in normal eukaryotic cells but is present with certain viral infections including HIV. As shown in the figure, in a cell free PKR activation assay, G1 and G2, but not G0 mRNA, generated phoshorylated PKR; poly-IC served as the positive control. Predictive software suggested that the regions near G1 and G2 have long dsRNA sequence, forming a stem-loop. When we disrupted this stem-loop with synonymous mutations, PKR activation by the risk alleles was lost. Finally, in an FSGS model involving basic fibroblast growth factor and puromycin (also a protein synthesis inhibitor), the G1 stem-loop transgenic mice had higher levels of proteinuria compared to wild-type mice.

Conclusions: These results suggest that the risk *APOL1* variant mRNAs activate PKR and thereby reduce protein synthesis. These effects appear to be mediated by dsRNA structure. Reduced protein synthesis may act as susceptibility factor which promotes podocyte injury.

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SA-PO446

Structure and Function of the Podocyte Slit Diaphragm

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Background: The slit diaphragm is a multiprotein complex and constitutes the final component of the glomerular filtration barrier. Despite intense research its molecular ultrastructure and function of individual components have remained elusive.

Methods: We established a complimentary set of *Neph1*, *Neph2*, *Neph3* and *Nphs1* -/- mice. To compare SDs across the animal kingdom, chicken and mice kidneys were used. Detailed analysis was performed with functional assays, WB, light microscopy, STORM, TEM, Immuno-EM, HIM, as well as Cryo-Electrontomography.

Results: NEPHRIN and NEPH1 are essential to build a proper slit diaphragm. Yet in both types of constitutive -/- animals rudimentary SDs based on the remaining Super-IgG molecule could be detected. Interestingly, Neph2 and Neph3 -/- mice did not show any obvious phenotype over a 2 year observational period. Within the native cryopreserved SD ultrastructural analysis revealed that the SD is a multi-layered cell-cell contact that does not overlap in the midline, but rather forms multi-layered junctions. Individual components arch from cell membrane to cell membrane. Immuno-EM localized NEPH1 to the basal and narrower aspects of this junction, while NEPHRIN molecules form the top layer facing Bowman's space. Despite NEPHRIN's fundamental role in mammals, reptiles and birds live healthily without NEPHRIN and present with narrower and leakier NEPH1 based slit diaphragms.

Conclusions: This unique comparative approach revealed fundamentally new insights into the composition of the slit diaphragm. While NEPHRIN is essential for the formation of the slit diaphragm in mammals, it is dispensable in both reptiles and birds. NEPH1 seems to form the basal aspects of this junction in mammals and is the main component in reptiles and birds. This ultrastructural analysis challenges old views from the SD as a thin grey line linking two adjacent footprocesses but rather points towards a multi-layered cell-cell contact.

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SA-PO447

***In Vivo* Disruption of Podocyte Cytoskeletal Organization as a Result of Podocyte-Specific Deletion of NDST1**

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Background: Previous work from our laboratory has shown that heparan sulfate (HS) plays a key role in mediating podocyte (POD)-glomerular basement membrane (GBM) interactions by intracellular signaling mediated via the core protein of the cell surface proteoglycan, syndecan-4 (Sdc4). Recent work has shown that (Kid. Int. 85: 307-318) N-sulfation of HS is important for the proper organization of POD on the GBM. Podocytes from the Pod-Cre Ndst1$^{-/-}$ mice were shown to develop foot process effacement with an age-related increase in albuminuria. The focus of this current study was to explore the pattern of organization of Sdc4 along with known downstream components of the POD cytoskeleton.

Methods: Frozen tissue sections from 12 month and 15 month old age-matched control and mutant mice (Pod-Cre Ndst1$^{-/-}$) were double-label immunostained using antibodies directed against Sdc4 and α-actinin-4, nephrin, or synaptopodin (Synapt). The sections were examined using either wide-field fluorescence microscopy (WFM) or laser scanning confocal microscopy (LSCM).

Results: In control animals, Sdc4 immunoreactivity was present in a punctate pattern along the length of the GBM, and was colocalized with α-actinin-4 along the GBM. Synapt and Sdc4 showed a different pattern of immunostaining in WFM, having an evenly spaced, alternating punctate appearance along the GBM. In LSM sections showing the outside of the capillary, Synapt stained the 1° and 2° processes. Sdc4 staining was found on the lateral borders of the pedicels along their length. The relationship of nephrin to Sdc4 staining resembled that for Synapt. In the mutant mice, there was a complete disruption of the Sdc4/α-actinin-4 colocalization, with α-actinin-4 staining now located within the podocyte cell body. The Sdc-4 and Synapt relationship was also disrupted, with a distinct loss of

Sdc4 staining along the length of the pedicel. The pattern of nephrin staining appeared to remain the same, but its relationship to Sdc4 staining was altered, due to the change in the Sdc4 staining pattern.

Conclusions: The loss of N-sulfation on Sdc4 HS in POD causes disengagement of α-actinin-4 and Synapt but not nephrin, leading to pedicel disorganization.

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SA-PO448

Clathrin Mediated Endocytosis Regulates Podocyte Migration

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Background: The cytoplasmic tail of human nephrin contains the Y-R-S-L sequence (Y^{1139}RSL) that conforms to the canonical endocytic motifs of the YxxØ type where x is any amino acid and Ø is an amino acid with a hydrophobic side chain. This sequence is conserved among several IgCAMs, including the neuronal CAM L1 where the Y-R-S-L motif directs clathrin-dependent endocytosis of L1 critical for motility of the nerve growth cone. The role of Y^{1139}RSL in human nephrin is unknown.

Methods: Phosphorylation of tyrosine residue in the YxxØ motif determines endocytic activity and comprises the information whether a protein should remain at or be removed from the cell surface. De-phosphorylation activates YxxØ as an endocytic motif and can be mimicked by substitution with phenylalanine (Y/F-Nephrin). By contrast, tyrosine substitution by alanine disables the endocytic signal (Y/A-Nephrin). The T-SV40 immortalized non-differentiated human podocytes were stably transduced with the wild type (WT)-Nephrin, Y/F-Nephrin, or Y/A-Nephrin using the lentiviral vector pLOC.

Results: The Y/F-Nephrin was less abundant at steady-state and had decreased cell surface stability compared to WT-Nephrin. By contrast, Y/A-Nephrin was more abundant at steady-state and had increased cell surface stability compared to WT-Nephrin. The difference in the cell surface abundance between the nephrin mutants further increased by stimulating nephrin endocytosis. The Y/F-Nephrin was more abundant in clathrin-coated vesicles (CCV) compared to Y/A-Nephrin, confirming the role of the Y^{1139}RSL motif in clathrin-dependent nephrin endocytosis. Podocyte migration was reduced in cells expressing WT- or Y/A-nephrin but not the Y/F-Nephrin. Finally, there was no difference in the paracellular flux of 40kDa dextran between cells expressing the Y/F- or Y/A-Nephrin.

Conclusions: The Y^{1139}RSL motif regulates cell surface abundance and stability of human nephrin by adjusting nephrin endocytosis. Clathrin-dependent endocytosis mediated by the Y^{1139}RSL motif may play a role in podocyte migration rather that the glomerular filtration barrier. The Y^{1139}RSL in human nephrin plays a similar role to the the Y-R-S-L motif in CAM L1.

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SA-PO449

Acute Effects of Angiotensin II on TRPC6 Channels and Calcium Influx in the Podocytes of the Murine Glomeruli

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Background: A key role for glomerular epithelial cells (podocytes) in the pathogenesis of proteinuric renal diseases has been established since podocyte injury leads to proteinuria and foot process effacement. It was previously shown that angiotensin II (Ang II) caused depolarization and increased intracellular Ca^{2+} in podocytes. Members of the TRPC channels family, and particularly TRPC6, are proposed as proteins responsible for this calcium flux; gain-of-function mutation in the gene encoding TRPC6 channel results in the development of focal segmental glomerulosclerosis.

Methods: Changes in intracellular Ca^{2+} stimulated by Ang II in presence of various pharmacological agents were measured with live confocal microscopy in Fluo4/FuraRed loaded podocytes of the freshly isolated glomeruli; electrophysiological experiments (single channel patch clamp) were performed to assess the activity of TRPC6 channels in this preparation, as well as in transfected cells.

Results: First, we examined the effects of Ang II on intracellular Ca^{2+} in intact podocytes of freshly isolated rat glomeruli. Depletion of internal Ca^{2+} stores with thapsigargin did not affect calcium influx activated by Ang II, but SKF 96365 decreased Ang II - triggered increase in intracellular calcium concentration. Single channel patch-clamp analysis demonstrated that Ang II acutely activates native TRPC6 channels in the podocytes of rat and mouse glomeruli and in CHO cells transiently overexpressed with TRPC6 and AT_1 receptor; the effect is mediated by changes in the channel open probability. Importantly, Ang II did not activate any currents in TRPC6$^{-/-}$ mice. Further we used losartan and PD 123319, inhibitors of AT_1 and AT_2 receptors, in order to discriminate between the effects of Ang II on its specific receptors; these experiments indicated that both AT_1 and AT_2 might be involved into this signal transduction pathway.

Conclusions: Our data have provided the critical evidence of Ang II-dependent activation of TRPC6 channels and calcium influx in podocytes, which might play a significant role in the pathogenesis of kidney diseases.

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SA-PO450

Up-Regulation of TRPC6 in Podocyte By Glomerular Hyperfiltration and Effect of Calcineurin Inhibition

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Background: TRPC6 in podocytes functions as a sensor of pressure load at the time of glomerular hyperfiltration (GHF). In this study, we aimed at examining the following two points: (1) in vivo changes in the expression of TRPC6 in 5/6 nephrectomized rats, which are a GHF model, and (2) effects of calcineurin inhibition, which is a downstream signal of TRPC6, on podocyte damage in GHF.

Methods: We removed 5/6 of the kidney from male SD rats and conducted histological/molecular biological examinations between a Sham group (S) and a nephrectomy group (N) six weeks later. We set up a group to which tacrolimus (T: 0.08 mg/kg) was administered subcutaneously every day in each group.

Results: The glomerular volume, urinary albumin excretion (UAE) and desmin expression score all increased in the N group. No changes were observed in the glomerular volume by the administration of T, but the UAE and desmin expression score showed a tendency for improvement. The electron microscopic images showed morphological changes of podocytes in the N group, such as loss of the foot processes and improvement in the T groups. The FGS score also increased in the N group and improved in the T groups. The mRNA expression of TRPC6 increased in the N group, and it was suppressed in the T groups. The double staining of TRPC6 with WT-1 showed the up-regulation of TRPC6 in WT-1 positive podocytes in the N group and attenuation in the T groups. The double staining of TRPC6 with synaptopodin showed a decreased staining of synaptopodin along the loop wall in the N group and improvement in the T groups. Evaluation of changes in TRPC6 mRNA by in situ hybridization showed an increased expression in the N group and suppression in the T groups.

Conclusions: The present study demonstrated the functional relationship between podocyte damage and increased expression of TRPC6 in podocytes in vivo. In addition, a direct inhibition of podocyte damage by calcineurin inhibition without suppression of GHF was evoked.

SA-PO451

TRPC5-Mediated Podocyte Calcium Toxicity Drives Progressive Glomerular Disease

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Background: Podocyte injury, with proteinuria as the earliest sign, is the critical step in various forms of kidney disease. Podocyte-specific expression of the human angiotensin receptor type 1 (AT1R) in rats (AT1R TG rats) causes progressive podocyte damage and loss, leading finally to kidney failure and death after 40-50 weeks. Our laboratory established that deletion or pharmacological blockade of TRPC5 channels is protective in acute models of filter barrier damage, but the role of TRPC5 in disease progression remains unknown. Previously we have also shown that AT1R activation promotes TRPC5-mediated cytoskeletal disruption in podocytes.

Methods: We investigated the influence of TRPC5 on disease progression in AT1R TG rats by 2-week treatment with the TRPC5 inhibitor ML204, starting after proteinuria had developed. For mechanistic studies, we activated TRPC5 pharmacologically in conditionally immortalized mouse podocytes after 11 days of differentiation. Annexin V/PI labeling was used as cell death assay, and mitochondrial permeability transition (MPT) was measured with Calcein/Co^{2+}. Analysis was done by flow cytometry.

Results: Treatment with the TRPC5 blocker ML204 decelerated disease progression in the AT1R TG rats. Specifically, albuminuria remitted under ML204 treatment. In vivo, podocytes showed signs of recovery following treatment. Mechanistically, we show that sustained TRPC5 activation leads to podocyte calcium toxicity via MPT and finally cell death, in a dose and time dependent manner. These effects can be blocked effectively by ML204.

Conclusions: This study reveals sustained TRPC5 activation as a driving force in progressive glomerular disease. Mitigated disease progression by ML204 treatment in AT1R TG rats is an important proof of concept study for the potential of TRPC5 blockade as a therapeutic strategy in progressive proteinuric kidney diseases.

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SA-PO452

Genetic Interactions between crb2b and moe in Podocytes

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Background: In podocytes, cell polarity is manifested in the presence of molecularly and functionally different apical and basal membrane domains, slit diaphragm cellular junctions, and well defined cellular structures like actin rich foot processes. Genetically induced derangement of apical basal polarity through gene specific mutations in polarity protein components results in loss of foot process structure and ensuing proteinuria suggesting that cell polarity is intimately coupled to podocyte function. We have used the *crb2b* gene as an entrance point into exploring the wider roles of polarity complexes

within zebrafish podocytes. *Crb* proteins directly bind to the FERM domain protein Moesin/Radixin/Ezrin/Band 4.1 through a FERM binding domain located in the Crb intracellular tail. In zebrafish, the Moesin/Radixin/Ezrin/Band 4.1 protein is mutated in the mosaic eyes (moe) mutant and moe mutants have a podocyte structural defects (Dev Biol. 2005 Sep 15;285(2):316-29). Based on studies in zebrafish and fruit flies, we hypothesize that both crb2b and moe may function antagonistically in a common protein trafficking pathway during podocyte differentiation.

Methods: The zebrafish has become a popular genetic model system for studying gene expression, gene function, and modelling kidney disases. In this study, we perform molecular genetics in the zebrafish pronphros.

Results: We present data showing the Nephrin targeting is affected in zebrafish moe mutants. We further analyze genetic interactions between crb2b and moe, through crb2b; moe double mutant using a panel of apical basal polarity markers.

Conclusions: We conclude that both crb2b and moe participate in a common pathway required for correct Nephrin localization in podocytes.

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SA-PO453

Podocyte-Specific Deletion of the Planar Cell Polarity Gene Vangl2 Leads to Glomerular Abnormalities Eugenia Papakrivopoulou,[1] Sabrina Pacheco,[1] Deborah Henderson,[2] Hortensja Lucja Brzoska,[1] Adrian S. Woolf,[3] David A. Long.[1] *[1]Developmental Biology and Cancer, UCL Inst of Child Health, London, United Kingdom; [2]Inst of Genetic Medicine, Newcastle Univ, Newcastle, United Kingdom; [3]Inst of Human Development, Univ of Manchester, Manchester, United Kingdom.*

Background: The development and maintenance of podocyte architecture involves cytoskeletal re-organisation, but little is known about the pathways regulating this process. In this study, we hypothesised that the planar cell polarity (PCP) pathway, which controls cytoskeletal organisation in a variety of cell systems, may be critical in maintaining podocyte structure and the integrity of the glomerular filtration barrier.

Methods: Podocyte-specific knock-down mice of *Vangl2*, a core component of the PCP pathway, (*PodCre*$^{+/}$*Vangl2*$^{fl/fl}$) were generated by combining floxed *Vangl2* animals with *Podocin-Cre* mice (both on C57Bl/6 background); littermates without Cre were used as controls (*Vangl2*$^{flox/flox}$). Glomerular morphology, proliferation and overnight albumin excretion were assessed at 12 weeks of age. RNA was extracted from glomeruli isolated by Dynabead perfusion.

Results: *PodCre*$^{+/}$*Vangl2*$^{fl/fl}$ mice had a 40% reduction in *Vangl2* glomerular mRNA levels compared with *Vangl2*$^{flox/flox}$. Expression of other PCP genes in isolated glomeruli was unaltered. Abnormal glomerular morphology, characterised by loss of capillary loops and tuft collapse, was seen in 16.6±4.8% of glomeruli of *Vangl2*^{Dpodocyte} mice compared with 3±1.5% in control littermates. This was accompanied by increased glomerular proliferation (0.17±0.03 *versus* 0.07±0.02 cells/glomerulus, $p<0.05$) and nephrin phosphorylation. Expression of the extracellular matrix protein collagen (α_3) IV was also significantly reduced in *PodCre*$^{+/}$*Vangl2*$^{fl/fl}$mice. Despite these findings, we did not observe any changes in albuminuria or haematuria at this time-point.

Conclusions: Our data indicates that loss of *Vangl2* in the podocyte leads to subtle glomerular abnormalities including altered proliferation, matrix turnover and nephrin phosphorylation. However, this does not manifest as altered integrity of the filtration barrier.

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SA-PO454

Podocyte Number in Humans: Associations with Glomerular Hypertrophy and CKD Risk Factors Victor G. Puelles,[1] Luise A. Cullen-McEwen,[1] Wendy E. Hoy,[2] John F. Bertram.[1] *[1]Anatomy and Developmental Biology, Monash Univ, Melbourne, Victoria, Australia; [2]Centre for Chronic Disease, The Univ of Queensland, Brisbane, Queensland, Australia.*

Background: Podocyte depletion can be defined as absolute (reduction in podocyte number) or relative (reduction in podocyte density), and it has been directly associated with the development of glomerulosclerosis. The aim of this study was to determine if podocyte depletion is observed in subjects without renal disease in the context of glomerular hypertrophy and in association with multiple CKD risk factors.

Methods: Tissue from 32 autopsied Caucasian American males (Jackson, Mississippi, U.S.A.), comprised of four young children (≤3 years) and 28 adults (≥18 years) without overt renal disease was used for stereological quantification of podocyte number and density by the disector/fractionator method. A total of 192 glomeruli (6 glomeruli per subject) were analyzed. Demographic variables such as age, hypertension and body mass index (BMI) were obtained from medical records and autopsy reports. Nephron number was estimated using design-based stereology.

Results: Podocyte number (podocytes per glomerulus) varied 2.1-fold in children (302-634) and 7-fold in adults (140-983). Podocyte density (podocytes per $10^6\mu m^3$ of glomerular tuft) showed a similar trend: a 2.8-fold range in children (565-1590) and an 11.4-fold range in adults (46-526). While there was a strong direct correlation between glomerular volume and podocyte number in children ($R=0.81$, $P<0.0001$), this correlation was weaker in adults ($R=0.37$, $P<0.0001$). Among adults, podocyte number was lower in subjects with older age (≥40 years; $P<0.001$), hypertension ($P<0.05$) and low nephron number (≤0.6 million nephrons; $P<0.01$), but not in subjects with overweight (BMI ≥25Kg/m^2). Podocyte density was reduced in subjects with older age, hypertension, low nephron number and overweight ($P<0.0001$).

Conclusions: The wide variation in podocyte number and density in adults may be related to a combination of podocyte loss (associated with older age, hypertension and low nephron number) and possible podocyte gain associated with glomerular hypertrophy. Further studies in controlled animal models are urgently needed.

SA-PO455

Nephrin Phosphorylation in Minimal Change Disease Gabriel M. Cara-Fuentes, Eduardo H. Garin. *Univ of Florida.*

Background: Minimal change disease (MCD) is considered a podocytopathy. Proteinuria in MCD is thought to be due to an increased CD80 podocyte expression. Experimental models of nephrotic syndrome showed that CD80 podocyte expression leads to foot processes effacement and proteinuria. Nephrin, a slit diaphragm protein, displays a structural and functional role that is critical to maintain the integrity of the filtration barrier. **Objective:** To define the pattern of nephrin phosphorylation in MCD patients during relapse and remission. This is a necessary step to our hypothesis that increased podocyte CD80 expression leads to a decrease in nephrin phosphorylation in MCD patients during relapse resulting in proteinuria.

Methods: Human cultured podocytes were incubated with serum from 11 MCD patients in relapse and 5 in remission. CD80, CTLA-4 and phosphorylated nephrin expression were measured by quantitative PCR and Western-Blot analysis. Immunofluorescence staining of CD80 and phosphorylated nephrin were performed in biopsies from MCD patients in relapse and MCD in remission.

Results: Human cultured podocytes exposed to serum from MCD patients in relapse showed a significantly increased in CD80 and decreased phosphorylation of nephrin compared to those exposed to serum from MCD patients in remission ($p=0.02$ and $p=0.03$ respectively).Expression of CD80 was decreased when podocytes were cultured with CTLA-4 ($p=0.04$). In addition, kidney biopsies of MCD patients in relapse showed a weak signal for phosphorylated nephrin by Immunofluorescence in contrast to the strong signal obtained in kidney biopsies of MCD patients in remission.

Conclusions: (1) Decreased phosphorylation of nephrin was found in podocytes exposed to serum from MCD patients in relapse along with increased CD80 expression. (2) CTLA-4 prevented the increase CD80 expression in podocytes exposed to serum from MCD patients in relapse. (3) Weak signal for phosphorylated nephrin was observed in kidney biopsies of MCD patients in relapse in contrast with those in remission. (4) We postulate that CD80 decreases nephrin phosphorylation resulting in reorganization of podocyte cytoskeleton and proteinuria.

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SA-PO456

Nephrin Preserves Podocyte Viability and Glomerular Structure and Function in Adult Mice Xuezhu Li,[1] Peter Y. Chuang,[1] Vivette D. D'Agati,[2] Lawrence B. Holzman,[3] John C. He.[1] *[1]Dept of Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Dept of Pathology, Columbia Univ, New York, NY; [3]Renal Electrolyte and Hypertension Div, Univ of Pennsylvania, Philadelphia, PA.*

Background: Nephrin is required during kidney development for the maturation of podocytes and formation of slit diaphragm. As nephrin is downregulated in various acquired glomerular diseases, nephrin deficiency is considered a pathologic feature of glomerular injury. Nephrin's role in the adult kidney and in acquired glomerular diseases, however, has not been confirmed experimentally.

Methods: Mice with doxycycline inducible RNA-interference-mediated nephrin knockdown were generated. Unilateral nephrectomy (UNPX) was performed to generate a model of glomerular hyperfiltration. Adriamycin (ADR) nephropathy model was used to characterize the response of mice with nephrin knockdown to podocyte injury and glomerulosclerosis. Urinary albumin excretion, serum creatinine, renal histology, glomerular ultrastructure, glomerular gene expression, AKT phosphorylation, and podocyte apoptosis were performed.

Results: Short-term nephrin knockdown (6 weeks) has no impact on glomerular structure and function. In contrast, chronic nephrin knockdown (20 weeks) causes proteinuria, foot process fusion and effacement, filtration slit narrowing, mesangial proliferation, glomerular basement membrane thickening, sub-endothelial zone widening, and podocyte apoptosis. When subjected to UNPX and ADR, mice with short-term nephrin knockdown developed more severe glomerular injury compared to mice without knockdown. In addition, nephrin knockdown mice developed more exaggerated glomerular hypertrophy when subjected to UNPX, and more podocyte apoptosis and depletion after ADR challenge. AKT phosphorylation was markedly reduced in mice with chronic nephrin knockdown as well as short-term nephrin knockdown mice challenged with UNPX and ADR.

Conclusions: Our data established that under basal conditions as well as in acquired glomerular diseases, nephrin is required to maintain slit diaphragm-mediated signaling and podocyte viability to preserve glomerular function and podocyte viability in adult mice.

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SA-PO457

MAP-Kinase p38 – Pivotal Mediator of Slit Diaphragm Integrity

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Background: Albuminuria is an early symptom of diabetic damage of the glomerular filter. Diabetic albuminuria has been shown to be attenuated by inhibition of MAPK p38. We could recently demonstrate that diabetic mice with albuminuria show increased nephrin endocytosis. Several other groups now confirmed that the endocytosis of slit diaphragm proteins is paramount for podocyte function. In our project we further unravel the molecular mechanism of nephrin endocytosis and identify p38 as pivotal mediator of proteinuric signaling and slit diaphragm integrity.

Methods: Diabetes was induced in C57BL/6 mice by streptozotocin. For inhibition of p38, the mice were treated with SB202190. Albuminuria was quantified as albumin/creatinin ratio. Nephrin endocytosis was analyzed by immunofluorescence staining. To quantify endocytosis in murine kidneys in vivo a special biotin-based endocytosis assay was established. Western blotting and kinase assays were performed to analyze the phosphorylation of nephrin mediated by p38 and PKCα.

Results: Hyperglycemic mice developed a significant albuminuria already four days after induction of diabetes. In vivo analysis showed a significant increase in podocytic p38 activity and nephrin endocytosis only in albuminuric animals. Pharmacologic inhibition of p38 nearly completely prevented nephrin endocytosis and albuminuria. P38 mediated phosphorylation of the nephrin c-terminus facilitates the coupling of nephrin to the endocytotic machinery via PKCα – β-arrestin2 – signaling.

Conclusions: Here we show that p38 is a pivotal mediator of hyperglycemia-induced nephrin endocytosis and albuminuria. Our in vivo endocytosis assay allowed for the first time a quantification of nephrin endocytosis in murine kidneys under diabetic conditions. Inhibition of p38 decreases nephrin phosphorylation and endocytosis, supporting the hypothesis that stabilization of the slit diaphragm prevents albuminuria. These findings suggest p38 as pivotal mediator of early diabetic damage and thus as a promising therapeutic target.

SA-PO458

Early Proteinuria in Nephrotoxic Nephritis Is Associated with p38 Activation and Nephrin Endocytosis

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Background: Nephrotoxic nephritis is an established animal model of rapid progressive glomerulonephritis. The application of a polyvalent sheep anti-GBM serum induces two peeks of an inflammatory response and proteinuria. The first one can be detected after 24 hours, called heterologous phase. In contrast to the autologous phase which appears after 7 days and is caused by an invasion of inflammatory cells the pathomechanism of the heterologous phase is not well understood. In the following project we examined whether p38 mediated nephrin endocytosis is involved in this marked dysfunction of the glomerular filter caused by the anti-GBM serum.

Methods: C57BL/6 mice were administered intraperitoneal with 5 µl/g body weight of sheep anti-GBM serum. The animals were housed in metabolic cages for 12 h after anti-GBM serum injection for urine collection and sacrificed 24 h after injection.Albuminuria was quantified as albumin/creatinine ratio. To quantify the surface expression of nephrin murine kidneys were perfused with biotin. Afterwards glomeruli were instantly harvested and analyzed with streptavidin via Western blot and analyzed by immunofluorescence staining.

Results: Twenty-four hours after treatment with the anti-GBM-Serum the mice developed severe albuminuria. Western blot analysis of murine glomeruli revealed a strong activation of p38. Immunofluorescence studies demonstrated a translocation of nephrin from the cell surface to the cytoplasm. Quantification of nephrin endocytosis with our in vivo biotinylation assay showed a decrease of surface nephrin of about 73%.

Conclusions: The present study shows for the first time that the severe proteinuria in the heterologous phase of nephrotoxic nephritis is associated with significant nephrin endocytosis mediated by activation of p38.

SA-PO459

Combined Treatment with Pioglitazone and Glucocorticoids Enhances Protection against Nephrotic Syndrome

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Background: Oral glucocorticoids (GCs) are the primary therapy for nephrotic syndrome (NS), although GCs have serious side effects and are clinically ineffective in ~20% of patients. We previously reported that like GCs, pioglitazone (Pio) also directly protects podocytes and modulates the GC receptor pathway. We thus hypothesized that Pio could enhance reduction in proteinuria seen with GCs during NS.

Methods: Proteinuria was induced in Wistar rats (N=13/group) by single PAN injections (50 mg/kg). Treatment groups received PAN+low-dose GCs (5 mg/kg), PAN+high-dose GCs (15 mg/kg), PAN+Pio (10 mg/kg), PAN+Pio+low-dose GCs, and PAN+Pio+high-dose GCs. Analyses included proteinuria, glomerular gene and protein expression 11 days after PAN injection. Translation to a childwith refractory NS included the addition of Pio to GCs and other immunosuppressive drugs at 15 mg/day for 4 weeks, followed by 30 mg/day for 20 weeks.

Results: PAN induced severe proteinuria, which was significantly reduced by high-dose (79%;P=0.005) but not low-dose (25%;P=NS) GCs. Pio alone reduced proteinuria moderately (61%;P=NS), but low-dose GCs+Pio reduced proteinuria significantly (63%;P=0.025), similar to high-dose GC alone. High-dose GCs+Pio reduced proteinuria to almost control levels (97%;P=0.001). PAN increased glomerular cycloxygenase-2 and decreased podocyte synaptopodin expression, while Pio+GCs treatment restored expression levels to control values. Analogous addition of Pio to GCs in a child with refractory NS correlated with a ~80% decrease in proteinuria.

Conclusions: Repurposing Pio as a combination therapy with GCs has the potential to notably increase the clinical efficacy of GCs in reducing proteinuria during NS, and potentially enable reduced GCs dosing and toxicity. This efficacy likely results in part from the restoration of podocyte/glomerular expression of synaptopodin and cycloxygenase-2.

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SA-PO460

The Superiority of Urinary Angiotensinogen to Proteinuria as a Marker of Podocyte Injury

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Background: Proteinuria (PU) is widely used as a marker for renal prognosis in clinical setting, as well as urinary angiotensinogen (U-AGT). Our preliminary data confirmed that patients with focal segmental glomerulosclerosis (FSGS) represented higher U-AGT/urinary protein (UP) ratio than patients with minimal change disease (MCD) (3.1 ± 1.3 versus 0.7 ± 0.3 µg/10mg UP) and showed AGT deposition in segmental lesions of glomeruli, suggesting U-AGT is a more specific marker of podocyte injury than PU.

Methods: We tested the impacts of U-AGT and PU on two different nephrotic models induced by puromycin aminonucleoside (PAN) in Wistar rats; 15 mg/100 g·body weight (BW) of PAN administration at week 0 similar to MCD, and 5 mg/100 g·BW of PAN administration for three times at weeks 0, 1 and 2 similar to FSGS. Next, we observed renal tissue and urinary metabolism of exogenous injected human recombinant AGT (hAGT; little affinity for enzymatic cleavage by rodent renin) in FSGS and control rats. Furthermore, we tested AGT production levels in cultured mouse podocytes injured by PAN.

Results: PU levels were comparable between MCD and FSGS rats. U-AGT levels from FSGS rats were higher than MCD rats, and podocin and nephrin expressions were reduced in FSGS, but not in MCD rats. Intravenous injection of hAGT showed about 100-fold increases in urinary hAGT excretion from FSGS rats compared to undetectable levels of those from control rats. Immunostaining for rat-AGT and hAGT identified that rat-AGT was detected in injured podocytes as well as proximal tubules, but filtrated hAGT was detected only in superficial proximal tubules. Finally, we confirmed that PAN induced increases in AGT production in cultured podocytes in dose dependent manner and these were negatively associated with podocin and synaptopodin expression in podocytes.

Conclusions: U-AGT, which was affected by AGT produced from injured podocytes at least in part, in addition to exogenous filtrated AGT from glomeruli, is a reliable marker of podocyte injury.

SA-PO461

Sulfatase 2, Identified By Genome Association Analysis, Contributes to Protection against Nephrotic Syndrome

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Background: Heparan sulfate proteoglycans (HSPGs) is major components of glomerular basement membrane (GBM) and regulating podocyte adhesion to GBM. Although Sulfatase 2 (SULF2) modulates intracellular signaling through controlling sulfation pattern on HSPGs, it is unclear whether SULF2 activity contributes to podocyte injury in nephrotic syndrome. In our previous genome-wide association study, a single nucleotide polymorphism (SNP) rs11086243 upstream of the *SULF2* gene showed a significant association with nephritic syndrome [Nat Genet. 2011;43:459-63].

Methods: We conducted a genetic association study by using 300 control samples and 201 nephrotic syndrome samples from Japanese population. High density SNP mapping was performed on the *SULF2* gene and its upstream region. As a functional study, renal Sulf2 expression was evaluated with mouse and human kidney sections. The effects of Sulf2 knockdown were examined in nephrotic syndrome model by injecting puromycin aminonucleoside and basic fibroblast growth factor.

Results: Seventeen candidate SNPs were obtained by expression-QTL analysis, transcript factor-related analysis, and imputation analysis and one haplotype was demonstrated to be significantly associated with nephrotic syndrome. Immunofluorescence staining showed intraglomerular SULF2 expression in mouse and human kidney. SULF2 was dominantly expressed in nucleus and cell surface of glomerular epithelial cells. In mouse nehprotic syndrome model, proteinuria that peaked at day 7 was significantly higher in SULF2 knockdown mice than wild-type. Blood urea nitrogen was also higher

at day 10 in knockdown mice. Segmental and global glomerular sclerosis accompanied with tubulointerstitial damage were present at day 28 only in SULF2 knockdown mice.

Conclusions: We identified a significant haplotype near SULF2 gene that was associated with nephritic syndrome. Functional analysis using knockdown mice suggested that SULF2 expressed in podocytes may have protective role in nephritic syndrome.

SA-PO462

Podocyte-Specific Deletion of Yes-Associated Protein Leads to Progressive Glomerulosclerosis Monica H. Schwartzman,[1] Antoine Reginensi,[2] Jenny Wong,[1] Vivette D. D'Agati,[3] Helen McNeill,[2] Kirk N. Campbell.[1] *[1]Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Molecular Genetics, Samuel Lunenfeld Cancer Center, Mount Sinai Hospital, Toronto, Canada; [3]Pathology, Columbia Univ, New York, NY.*

Background: Podocytes and the interposed slit diaphragm form a critical component of the glomerular filtration barrier. This explains why podocyte injury is typically associated with proteinuric kidney disease. We previously identified Yes-associated protein (YAP) as a pro-survival signaling molecule whose silencing in vitro increases podocyte susceptibility to apoptotic stimuli. YAP is the key downstream effector of the Hippo signaling pathway whose role in podocyte homeostasis has not been explored. In the present study we tested the hypothesis that podocyte specific deletion of YAP would lead to proteinuric kidney disease through increased podocyte apoptosis.

Methods: A YAP flox allele was generated by inserting LoxP sites for Cre-mediated excision flanking exon 2. YAP was selectively silenced in podocytes using Cre-mediated recombination controlled by the podocin promoter.

Results: YAP loss in podocytes resulted in proteinuria at 6 weeks with subsequent development of podocyte-derived apoptotic bodies and extensive apoptosis with TUNEL staining. At 12 weeks glomeruli from podocyte-targeted YAP knockout mice displayed histologic features characteristic of focal segmental glomerulosclerosis including podocyte depletion, tubular atrophy, interstitial fibrosis and the development of tubular casts. These changes progressed in severity through 32 weeks. These animals also developed progressive renal failure as determined by an increase in serum creatinine.

Conclusions: These findings, taken together, suggest a role for YAP as a physiological antagonist of podocyte apoptosis. This could have future implications in the quest for targeted therapeutics. The results also highlight the importance of podocyte survival in maintaining the integrity of the glomerular filtration barrier.

Funding: Private Foundation Support

SA-PO463

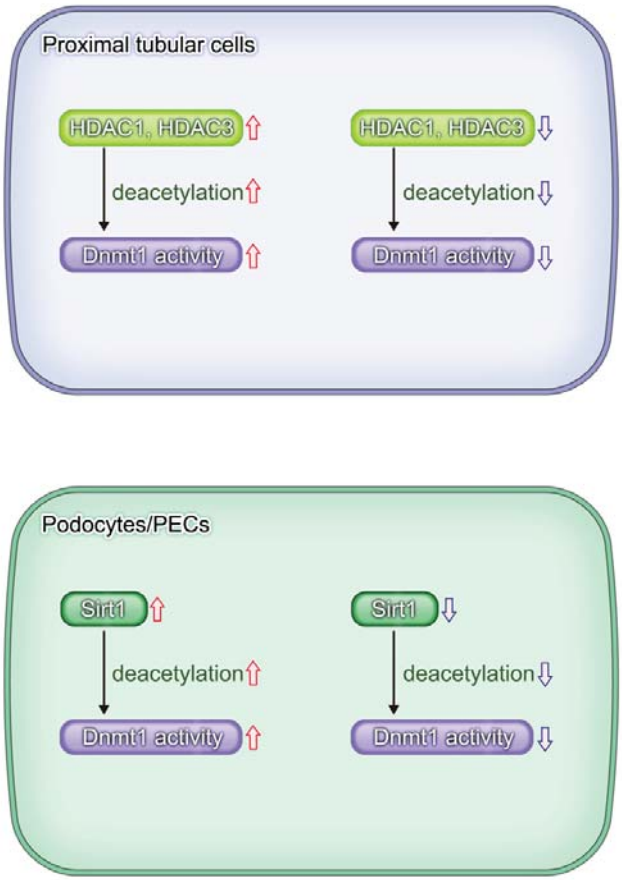
Divergent Usage of Histone Deacetylases for the Activation of DNA Methyltransferase1 in the Kidney; HDAC1, 3 (Class I HDAC) in Proximal Tubules and Sirt1 (Class III HDAC) in Podocytes De-Acetylate and Activate Dnmt1 Activity Hirokazu Muraoka, Shu Wakino, Kazuhiro Hasegawa, Koichi Hayashi, Hiroshi Itoh. *Keio Univ.*

Background: DNA Methyl Transferase 1 (Dnmt1) plays an important role in renal fibrosis and progression of CKD. We recently reported that Dnmt1 was deacetylated and activated by Class III histone deacetylase (HDAC), **Sirt1** in **podocytes (Pods)** leading to the epigenetic regulation of tight junction protein, Claudin-1. (Hasegawa K, Nature Medicine 2013). However, the regulatory mechanisms of Dnmt1 in other segments of the kidney, such as **proximal tubules (PTs)** or **parietal epithelial cells (PECs)** remains to be elucidated.

Methods: We used cultured **PT**s (HK2 and LLCPK1), **Pod**s (provided by Prof. Mundel, University of Washington), and **PEC**s (provided by Prof. Shankland, University of Washington). To determine which HDACs deacetylate Dnmt1, we transfected these cells with HA-Dnmt1, c-myc-PCAF (PCAF; P300/CBP-associated factor, which has histone acetyltransferase activity), Flag-HDAC1, V5-HDAC3 or 6×His-Sirt1.

Results: First, we confirmed that HDACs 1, 2, and 3 and Sirt1, 6, and 7 were expressed in mostly nucleus, which are more likely to colocalize with and regulate Dnmt1 in the nucleus. Since in other type of cells, HDAC1, HDAC3, and Sirt1 were reported to deacetylate Dnmt1, whereas HDAC2, SIRT6, and SIRT7 did not, we examined the effects of Sirt1, HDAC1, and HDAC3 on the activation of Dnmt1. Among the three HDACs studied, only **Sirt1** deacetylated Dnmt1 in cultured **Pod**s and **PEC**s. Conversely, in **PT**s, **HDAC1** and **HDAC3** deacetylated Dnmt1 whereas Sirt1 did not.

Conclusions: These findings demonstrated that the different types of HDACs were responsible for the activation of Dnmt1 in PTs, PECs, and Pods. Our data suggested that the divergent regulatory systems of epigenetic factor **Dnmt1** provide a novel mechanism for the functional disparity of each kidney cells or segments.



SA-PO464

FSGS-Associated Mutations in MYO1E Disrupt Intracellular Localization of Myo1e and Result in Podocyte Junctional Defects Jing Bi, Christopher D. Pellenz, Mira Krendel. *Cell Developmental Biology, SUNY Upstate Medical Univ, Syracuse, NY.*

Background: Focal segmental glomerulosclerosis (FSGS) is one of the most common primary glomerular disorders that may lead to end-stage kidney disease. The pathological features of FSGS include formation of scar tissue that obliterates glomerular capillary loops and the presence of massive proteinuria. Pathogenesis of FSGS involves impaired function of glomerular podocytes, specialized epithelial cells in renal glomeruli, which cover the surface of the capillaries. Podocytes play an essential role in maintaining proper renal filtration function. Mutations in the *MYO1E* gene (A159P and T119I) were found to be associated with childhood familial FSGS (Mele et al., NEJM, 2011; Al-Hamed et al., J. Hum. Gen., 2013). Myosin 1e (Myo1e) is a non-muscle myosin, which is expressed in kidney podocytes in humans and mice. It has been found to be a component of the slit diaphragm (specialized junction between podocytes). However, the molecular mechanism that links Myo1e and FSGS still remains unclear.

Methods: Using immortalized Myo1e-null cultured podocytes that were isolated from knockout mouse kidneys, we were able to mimic cell-cell junction assembly using calcium-switch experiments. We introduced GFP-tagged Myo1e constructs into cultured podocytes using adenoviral vectors. We utilized live-cell imaging to visualize the recruitment of junctional components during cell-cell contact assembly.

Results: We found that without functional Myo1e, cultured podocytes were unable to assemble proper junctional structures. Furthermore, FSGS-associated Myo1e mutants did not colocalize in the nascent junctions with other junctional components, such as actin and ZO-1.

Conclusions: FSGS-associated mutations in *MYO1E* result in defects in cell-cell junction assembly, which may lead to formation of "leaky" slit diaphragm complexes *in vivo*.

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SA-PO465

The Role of the Scaffolding Protein Shank2 in Cytoskeletal Organization in Podocytes Evgenia Dobrinskikh,[1] Patricia M. Zerfas,[2] R. Brian Doctor,[3] Linda Lewis,[1] Kayo Okamura,[1] Jeffrey B. Kopp,[3] Judith Blaine.[1] *[1]Medicine, Univ of Colorado Denver, Aurora, CO; [2]Office of Research Services, Office of the Director, NIH, Bethesda, MD; [3]NIDDK, NIH, Bethesda, MD; [4]Dept of Biology, Univ of Mississippi, Oxford, MS.*

Background: Shank2 is a scaffolding protein that interacts with a number of cytoskeleton-associated proteins. We have found that Shank2 is expressed in podocytes both *in vitro* and *in vivo*. Shank2 knockout (KO) mice develop proteinuria by 18 weeks of age suggesting that Shank2 is involved in cytoskeletal organization in podocytes.

Methods: The present study utilized immunofluorescence and functional assays in podocytes isolated from Shank2 knockout mice to assess the impact of Shank2 on the actin and microtubule cytoskeleton. In addition, scanning electron microscopy (SEM) was used to evaluate podocyte morphology in wild type and KO mice.

Results: Podocytes isolated from wild type and Shank2 KO mice express the podocyte markers podocin and WT-1. Immunofluorescence analysis shows that in Shank2 KO podocytes actin stress-fibers are short, broken and do not run axially. In addition, some Shank2 KO cells lack actin stress-fibers entirely. In Shank2 KO podocytes, the microtubule meshwork is disorganized and in some podocytes microtubules are completely depolymerized. To examine the functional consequences of cytoskeletal disruption in Shank2 KO podocytes, *in vitro* wound closure assays were performed. The percent of the wound closed is significantly less at both 6 hrs and 24 hrs in Shank2 KO podocytes compared to wild type (WT: 31±16% and 96±3%; KO: 10±7% and 53±18% of wound area closed after 6 hrs and 24 hrs respectively) suggesting defects in podocyte migration in the KO podocytes. Scanning electron microscopy (SEM) was performed to evaluate podocyte architecture in Shank2 KO versus wild type mice. SEM demonstrates a loss of foot process complexity and foot process disorganization in the KO mouse podocytes.

Conclusions: Taken together these data suggest that Shank2 plays an important role in the cytoskeletal organization and motility of podocytes.

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SA-PO466

Sphingomyelin-Phosphodiesterase-Acid-Like-3b Affects Insulin Signaling in Podocytes Alla Mitrofanova,[1,3] Ximena A. Morales,[1] Christopher E. Pedigo,[1] Rodrigo Villarreal,[1,2] Mayrin Correa-Medina,[1] George William Burke,[3] Sandra M. Merscher,[1,2] Alessia Fornoni.[1,2] *[1]Dept of Molecular and Cellular Pharmacology Peggy and Harold Katz Family Drug Discovery Center, Univ of Miami, Miami, FL; [2]Diabetes Research Inst, Univ of Miami, Miami, FL; [3]Dept of Surgery, Univ of Miami, Miami, FL.*

Background: Disruption of physiological insulin signaling in podocytes may contribute to the pathogenesis of diabetic kidney disease (DKD). Sphingomyelin-phosphodiesterase-acid-like-3b (SMPDL3b) is upregulated in DKD and may facilitate to podocyte injury. Lipid rafts and the associated protein caveolin-1 (Cav-1) modulate insulin receptor (IR) dependent signaling. We tested the hypothesis that SMPDL3b expression modulates insulin signaling in human podocytes.

Methods: Podocytes were treated with insulin (1nM/ml, 1h) or methyl-beta-cyclodextrin (5mM/ml, 1h). Plasma membranes were separated using the ultracentrifugation (100,000xg, 1h) protocol. Proteins were separated in 4-20% SDS-PAGE gels (BioRad) and transferred to nitrocellulose membranes for the Western blot analysis. All antibodies were obtained from Cell Signaling.

Results: Insulin treatment of SMPDL3b overexpressing (OE) podocytes did not result in a significant protein kinase B phosphorylation when compared to wild type (WT) podocytes. Plasma membrane localization of IR was significantly ($p<0.05$) reduced in OE podocytes (19.00±0.04%) compared to WT (99.00±0.11%). The amount of total and phosphorylated Cav-1 at the plasma membrane was not affected in OE podocytes. However, OE podocytes were more sensitive to cholesterol depletion (via cyclodextrin) causing reduced total (17.88±0.03% in OE versus 62.10±0.10% in WT cells, $p<0.01$) and phosphorylated (26.55±0.04% in OE versus 62.93±0.04% in WT cells, $p<0.01$) Cav-1. Reduction of Cav-1 was associated with increased IR plasma membrane localization in OE podocytes.

Conclusions: SMPDL3b interferes with the insulin receptor and caveolin-1 interaction. SMPDL3b overexpression could cause insulin resistance in podocytes by altering the subcellular distribution of the insulin receptor. Upregulation of SMPDL3b in podocytes may contribute to insulin sensitivity and cause podocyte damage in DKD.

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SA-PO467

Comparative Phosphoproteomic Analysis of Mammalian Glomeruli Reveals Podocin Carboxyl Terminus Phosphorylation as Determinant of Slit Diaphragm Complex Architecture Markus M. Rinschen,[1] Trairak Pisitkun,[2] Bernhard Schermer,[1] Thomas Benzing,[1] Paul T. Brinkkoetter.[1] *[1]Internal Medicine, Univ Hospital Cologne, Cologne, Germany; [2]Faculty of Medicine, Chulalongkorn Univ.*

Background: Signaling in podocytes in health and disease largely depends on phosphorylation, but technologies to understand its signaling have not been available. Recently, we performed in-depth analysis of the mouse glomerular phosphoproteome by tandem mass spectrometry and confidently identified more than 4000 phosphorylation sites. Comparisons of phosphoproteomic evidence across species is a powerful mean to functionally prioritize phosphorylation sites (Beltrao et al. Cell 2012). The purpose of this study was to use comparative phosphoproteomic analysis to prioritize phosphorylation sites for reductionist studies.

Methods: We performed an extensive phosphoproteomic analysis of glomerular fractions of bovine and rat kidneys. We performed bioinformatics analysis to determine conserved phosphorylation on homologous proteins and protein residues across mammalian species.

Results: We discovered several phosphorylation sites with potentially high biological relevance, e.g. tyrosine phosphorylation of the cytoskeletal regulator synaptopodin and the slit diaphragm protein neph-1 (Kirrel). Moreover, cross-species comparisons revealed conserved phosphorylation of the slit diaphragm protein nephrin on an acidic cluster at the intracellular terminus and conserved podocin phosphorylation on the very carboxyl terminus of the protein. Comparison of the dataset partly confirms significant cross-species conservation of signaling events in mouse, rat and cow. Given the pivotal role of podocin for glomerular biology we studied a highly conserved podocin phosphorylation site in greater detail and show that phosphorylation regulates affinity of the interaction with nephrin and CD2AP.

Conclusions: Taken together, these results suggest that species comparisons of phosphoproteomic data may reveal regulatory principles in glomerular biology.

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SA-PO468

Myosin 1e Is Required for the Maintenance of the Glomerular Filtration Barrier in the Adult Kidney Mira Krendel, Sharon E. Chase. *Cell Developmental Biology, SUNY Upstate Medical Univ, Syracuse, NY.*

Background: Glomerular visceral epithelial cells (podocytes) rely on the actin cytoskeleton and actin-associated proteins in order to develop the complex system of interdigitating foot processes and to maintain their structural and functional integrity. Myosin 1e (Myo1e), an actin-dependent molecular motor protein, is one of the key components of the podocyte cytoskeleton. Mutations in the MYO1E gene are associated with childhood FSGS (Mele et al., NEJM, 2011), and mice lacking Myo1e in podocytes develop proteinuria, foot process effacement, and glomerular basement membrane defects (Chase et al., AJP Renal Physiology, 2012). These observations indicate that Myo1e is required for the normal development of the glomerular filtration barrier. We have set out to determine whether Myo1e is necessary to maintain selective glomerular filtration in the adult kidney.

Methods: To address this question, we used a transgenic mouse model with doxycycline-inducible, podocyte-specific Cre recombinase expression to remove Myo1e from the adult podocytes. Doxycycline treatment was administered to eight week old mice for two weeks.

Results: Within one to two weeks following the treatment, experimental mice developed persistent proteinuria. Control mice, not carrying the transgenes necessary for Cre expression, did not exhibit proteinuria following doxycycline administration. Transmission electron microscopy revealed foot process effacement and glomerular basement membrane abnormalities following the induction of Myo1e knockout.

Conclusions: Our findings demonstrate that Myo1e is required not only for glomerular development but also for maintaining normal glomerular filtration in the adult. Thus, changes in Myo1e expression and activity in adults could lead to podocyte dysfunction, loss of selective filtration, and progressive kidney disease.

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SA-PO469

Cyclosporine versus Tacrolimus Therapy in Steroid plus Cyclophosphamide Resistant Nephrotic Syndrome: A Prospective Long Term Follow-Up Study Narayan Prasad, Manjunath Revanasiddappa, Amit Gupta, Akhilesh Jaiswal, Raj K. Sharma. *Nephrology, SGPGIMS, Lucknow, India.*

Background: The treatment of both steroid and cyclophosphamide resistant nephrotic syndrome(CYC-SRNS) is challenging.Both calcineurin inhibitors (Cyclosporine and Tacrolimus) vary in potency and side effects. The comparative outcomes data are lacking.

Methods: Forty five CYC-SRNS patients were categorized into 2 groups to receive either CSA or TAC on1:1 distribution. Patients were followed for complete remission (CR)/ partial remission(PR), adverse effects and progression. At 6 months, patients were declared responsive/resistant to first CNIs and resistant patients were switched to another CNI. The same CNI was continued in responsive patients for 12 months and steroid was tapered.CSA and TAC dose were kept at trough level of 120-180ng/ml and 6-10 ng/ml,respectively. CNI non responsive patient at 12 months from either group were treated with MMF.

Results: At 6 months, of 23 patients on CSA,16(69.5%) achieved remission(CR13/ PR3) and of 22 patients on TAC,18 (81.8%) achieved remission(CR11/PR7)($p=0.35$). Patients who are resistant to 1st CNI, 7/23were switched to TAC and of them 4 achieved remission (3PR, 1CR), and 3 remained resistant. Of the 4 patients who were switched to CSA,1 had PR and 3 remained resistant.Mean decline in GFR from baseline in CSA and TAC was similar at 6 months.The decline at 12 months (-24.5±10.2 versus -19.4±5.6, $P=0.04$) and end of follow up (-32.7±15 versus -24.3±10,$p=0.03$) was high in CSA group as compared to TAC. Ten patients in CSA and 4 in TAC group had doubling of creatinine/ decline in GFR by 50% ($p=0.06$). Hypertrichosis, and gum hyperplasia was high in CSA group as compared to TAC($p<0.05$). On KM survival analysis with event doubling of serum creatinine, the 1,2,3,4, and 5 years renal survival in CSA group was 96%,91%,85%,54%, and 33%, and TAC group was 96%,95%,90%,89%, and 79% respectively($p=0.02$). Of the 15 patients who did not achieve CR, 14 received MMF for 12 months, and 2 had CR, 5 had PR,and7 remained resistant.

Conclusions: Though TAC and CSA have similar efficacy in inducing and maintaining remission in CYC-SRNS patients. The side effect profile and long term outcome favour use of Tacrolimus over Cyclosporine.

SA-PO470

Elimination of AT1 Receptors from Podocytes Does Not Reduce Albuminuria Stacy Alana Johnson,[1] Natalie Mattocks,[1] Momoe Maeda,[2] Susan B. Gurley,[1,3] Thomas M. Coffman.[1,3] *[1]Duke Univ; [2]Duke-NUS; [3]Durham VA Medical Center.*

Background: The renin-angiotensin system (RAS) is a key regulator of blood pressure and fluid balance. RAS blockade is the mainstay of therapy for proteinuric renal diseases and the anti-proteinuric actions of RAS inhibitors are believed to be critical to their efficacy. This suggests that angiotensin II (AngII) acting via AT_1 receptors promotes proteinuria and this may be an important pathway in the pathogenesis of glomerular diseases. The mechanism by which AngII causes proteinuria has never been clearly demonstrated. Proposed mechanisms include glomerular hypertension, mesangial cell contraction and effects on podocytes to modify the glomerular filtration barrier. To test this last possibility, we generated mice lacking expression of all AT_1 receptors in podocytes. We hypothesized that loss of AT_1 signaling in the podocyte would reduce albuminuria.

Methods: We generated mice homozygous for a conditional *Agtr1a* allele on the *Agtr1b* null background and crossed these mice with *Pod-Cre* mice expressing Cre recombinase under the control of the podocin promoter, thus eliminating all AT_1 receptors from podocytes (*PodKO*s). The resulting offspring were then crossed with mice expressing a renin transgene (*RenTg*), which expresses renin under the control of the albumin promoter. *RenTg* mice develop hypertension with albuminuria.

Results: AT_{1A} mRNA expression was significantly reduced in glomeruli isolated from *PodKO* mice compared to controls. *PodKO* and *PodKO-RenTg* mice develop normally and there were no differences in body or kidney weight compared to controls. By 12 weeks of age, *RenTg* mice developed albuminuria compared to wild-type controls (85±14 versus 18±1 µg/24h; $p<0.05$) and the extent of albuminuria is increased at 24 weeks (210±71 versus 29±7 µg/24h, $p = 0.02$). This increased level of albuminuria was similar in the *PodKO-RenTg* and *RenTg* mice at both 12 (146±36 versus 85±14 µg/24h) and 24 weeks (229±38 versus 210±71 µg /24h).

Conclusions: Elimination of AT_1 receptors from podocytes did not attenuate albuminuria, suggesting that AT_1 receptors in other cell lineages are responsible for promoting proteinuria.

SA-PO471

Day Case Renal Biopsy Is Safe Irrespective of Renal Function: Results from a Single-Center Experience Vasantha M. Muthuppalaniappan, Sheela Anpalakhan, Conor J. Byrne, Michael Sheaff, Ravindra Rajakariar, Mark Blunden. *Renal Medicine, The Royal London Hospital, United Kingdom.*

Background: Renal biopsy is a useful diagnostic test to investigate renal disease. Day case biopsies are increasing to improve efficiency in patient and hospital time. However, concerns remain about the safety of day case biopsies especially in patients with eGFR<60ml/min.

Methods: Data was collected retrospectively on 778 consecutive day-case renal biopsies performed in our short stay facility. Patients were deemed fit if; Hb >8g/dl, platelet >100 and INR and APTT <1.2 less than 1 week prior to biopsy. Patients with a creatinine >300umol/L received 20mcg of intravenous DDAVP. Anti-platelet agents were stopped 1 week prior and the blood pressure cut-off was 160/90. Patients were observed for 6 hours post biopsy and discharged if haemodinamically stable and no visible haematuria. Complications and diagnostic adequacy were identified from hospital records.

Results: Between 01.01.11-31.05.14, 778 biopsies (460 male) were performed, comprising 429 native and 349 transplant, of which 112 were 3-month protocol biopsies. The amount of biopsies performed in patients with an eGFR of; 0-19ml/min, 20-39ml/min, 40-59ml/min, were 97, 304, 181 respectively. Biopsies were diagnostic in 762 cases (97%). 6 native, 4 transplant and 6 protocol biopsies were not diagnostic due to inadequate sampling. 21 patients required overnight admission for extended observation; 12 native (1 required blood transfusion) and 5 transplant due to visible haematuria, 2 patients for pain at the biopsy site and 2 patients had non-biopsy complications. Visible haematuria occurred in 6 patients with an eGFR >60, 4 with an eGFR 0-19, 5 with a 20-39 and 2 with an eGFR 40-59 (all ml/min). The remaining admissions were haemodinamically stable with no drop in Hb. 1 patient had an accidental biopsy of spleen and kidney causing splenic haematoma and hospital admission 4 days later.

Conclusions: Day case biopsy is a safe procedure with high diagnostic yield (97%). It can be performed as an outpatient even when significant renal impairment is present. The cancellation rates were reduced when the procedure was performed as an outpatient day case as opposed to an admission for inpatient biopsy.

SA-PO472

Association of Glomerular Macrophage Phenotypes and Urinary Soluble CD163 with Disease Activity in Human Lupus Nephritis Nobuhide Endo,[1] Naotake Tsuboi,[1] Kazuhiro Furuhashi,[2] Shoichi Maruyama,[1] Seiichi Matsuo.[1] *[1]Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan; [2]Brigham and Women's Hospital and Harvard Medical School.*

Background: In addition to the effector roles of classically activated macrophages for tissue injury, recent studies have shown that alternatively activated (M2) macrophages are involved in resolution of inflammation in animal models of kidney disease. But, clinical relevance of M2 macrophage in human disease is largely unknown. The current study aimed to evaluate renal accumulation of macrophage phenotypes in human lupus nephritis (LN) and significance of soluble form of CD163 (sCD163), a representative marker for M2 cells, for LN disease activity.

Methods: Plasma, urine and kidney biopsy samples were obtained from 74 patients with LN. Histological features were classified according to the ISN/RPS LN criteria. Immunohistochemical analyses using anti-human CD68, CD163 or CD204 antibodies were performed for identification of macrophage phenotypes. Concentrations sCD163and MCP-1in plasma and urine were measured by ELISA.

Results: Immunohistological analysis in LN glomeluli revealed more than 70% of $CD68^+$ macrophages was merged with $CD163^+$ cells and more than 90% of $CD163^+$ cells was merged with $CD68^+$ cells. However, $CD163^+$ cells appeared to be more than $CD68^+$ cells in interstitum, indicating the different origin of glomerular and interstitial $CD163^+$ macophages. The cell counts of glomerular $CD68^+$, $CD163^+$ or $CD204^+$ macrophages were increased in association with severity of biopsy active index (BAI) score in LN. Interstitial $CD68^+$, $CD163^+$ or $CD204^+$ macrophage infiltration correlated with eGFR. Urine sCD163 level showed stronger correlation with the number of glomerular CD163 positive cell counts (r=0.501) and BAI score (r=0.644) than plasma sCD163 levels with both of the above (r=0.289 and r=0.295, respectively). Correlation of urine sCD163 with BAI was comparable to that of urine MCP-1 levels (r=0.592) in LN.

Conclusions: These results suggest that $CD163^+$ or $CD204^+$ macrophage is the dominant phenotype in kidneys of LN patients, and urine sCD163 level has a potential significance for estimation of disease activity in human LN.

SA-PO473

Urine Monocyte Chemoattractant Protein-1 (MCP-1) Associates with Interstitial Fibrosis on Implantation Biopsies of Living Kidney Donors Hisham Elsherbiny, Aleksandar Denic, Vidhu Kaushik, Prince Singh, Muthuvel Jayachandran, Mariam P. Alexander, John C. Lieske, Andrew D. Rule. *Div of Nephrology and Hypertension, Mayo Clinic, Rochester, NY.*

Background: MCP-1 plays a role in renal inflammation that leads to fibrosis and loss of kidney function in a variety of kidney diseases. We hypothesized that urinary MCP-1 associates with occult renal fibrosis in healthy adults with normal kidney function.

Methods: From 2005 to 2011, 517 living kidney donors had a pre-donation spot urine sample collected and a core needle biopsy of their donated kidney during surgery. All patients had preoperative GFR by iothalamate clearance and 24-h urine collection for albumin measurement. Mean non-sclerotic glomerular volume of periodic acid-Schiff stained biopsy sections was estimated using the Weibel and Gomez stereological method and the percentage of globally sclerosed glomeruli and interstitial fibrosis were calculated. Urinary MCP-1 was assayed by ELISA.

Results: Mean+SD age was 45+12 years, 43% were men, 14% had hypertension, 24-h urine albumin was 4+6 mg, GFR was 102+17 ml/min/1.73 m^2, MCP-1 was 187+194 pg/ml and MCP-1/ urine creatinine (UCr) was 1.6+1.3 pg/mg. Fibrosis was present in 22% with 4% having more than 5%, GSG >5% was present in 27%, and the mean glomerular volume was 0.003+0.001 mm^3. Associations of MCP-1 and MCP-1/UCr with clinical and biopsy features after adjustment for age, sex, urine albumin, and GFR are shown in the Table.

Characteristic	MCP-1	MCP-1/UCr
	% difference, p value	**% difference, p value**
Age, 10y	-1.4%, 0.79	**12%, <0.001**
Male	**58%, <0.001**	-3%, 0.67
24-h urine albumin excretion, SD	**20%, <0.001**	**9%, 0.009**
GFR, SD	1.5%, 0.80	5%, 0.16
Hypertension	-3.4%, 0.84	11%, 0.32
Any interstitial fibrosis	**44%, 0.007**	**26%, 0.005**
Interstitial fibrosis >5%	44%, 0.21	**49%, 0.028**
Percent GSG >5	23%, 0.097	12%, 0.14
NSG Volume, SD	4%, 0.44	5%, 0.20

Conclusions: Urinary MCP-1 associates with interstitial fibrosis in ostensibly healthy adults, independent of age, gender, GFR, and urine albumin excretion. Thus Urinary MCP-1 may be useful for identifying occult interstitial fibrosis that is not detected by current kidney function markers.

Funding: NIDDK Support

SA-PO474

Pathologic Findings in Tenofovir Toxicity and Correlation with Severity of Acute Kidney Injury

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Background: Tenofovir disopril fumarate (TDF) is a nucleotide analog reverse-transcriptase inhibitor used for treatment of HIV and Hepatitis B virus infections. TDF nephrotoxicity is characterized by acute tubular necrosis (ATN) with distinctive clinical, pathological, and mitochondrial (MC) abnormalities. The pathology of TDF toxicity was first described in 2010 among a cohort of 13 patients at Columbia University Medical Center (CUMC). This study aimed to confirm the pathologic findings of TDF toxicity in a larger biopsy cohort.

Methods: Patients with biopsy proven TDF nephrotoxicity were identified from the CUMC Renal Pathology Lab database and their charts reviewed retrospectively.

Results: We identified 30 additional cases of TDF toxicity. Mean age was 55.4±8.2 years, 47% female and 44% White, 56% diabetic. The median duration of TDF therapy was 9 months (IQR 4-56mo), the earliest case was identified 2 weeks after starting therapy. The mean baseline serum creatinine (SCr) was 1.23 ±0.53 mg/dL, the mean SCr at biopsy was 4.05 mg/dL (IQR 2.2-8.3mg/dL). Biopsy indications were acute kidney injury (AKI) in 70% of patients and 30% were biopsied for chronic kidney disease (CKD) and proteinuria (1071 ±530 mg/gm). ATN was evident in 20/21 cases with AKI. Of those biopsied for CKD and proteinuria, 8 had evidence of mild, patchy tubular damage, 1 had diffuse tubular damage. A median of 8% (IQR 0-20%) of glomeruli were globally sclerotic, and mean tubular atrophy/interstitial fibrosis was 24% ±17%. Diabetic glomerular changes were seen in 11 cases, 5 had other unrelated glomerular lesions. All 30 cases showed dysmorphic MC on electron microscopy. Enlarged MC were seen in 21 cases and 12 had MC depletion. 5 showed patchy areas of both MC depletion and enlargement. MC changes were visible by light microscopy in 70% of the cases. There was minimal podocyte effacement 20%±10%.

Conclusions: Our findings add to previous series of TDF nephrotoxicity, confirming the potential for severe ATN and the presence of characteristic MC abnormalities. All cases had some element of MC damage.

SA-PO475

Renal Expression of the Proximal Tubular Markers Megalin and Cubulin and Mitochondrial Enzyme Activity in HIV-infected Patients with Tenofovir Nephrotoxicity

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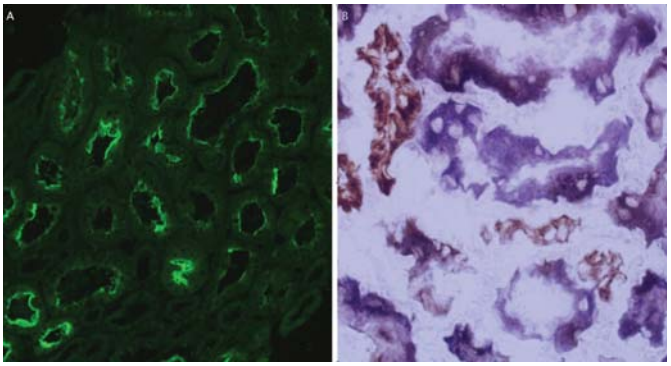
Background: Tenofovir disoproxil fumarate (TDF) is an effective and widely used molecule for the treatment of human immunodeficiency virus (HIV) infection. TDF is infrequently associated with proximal tubular dysfunction and acute tubular necrosis (ATN).

Methods: We reviewed kidney biopsy specimen from patients presenting with TDF nephrotoxicity. Proximal tubular expression of megalin and cubulin was assessed by immunostaining. Kidney frozen sections were histochemically stained for COX and succinate dehydrogenase enzyme activity. Results were compared to kidney biopsies from control groups.

Results: Twenty HIV-infected patients with TDF nephrotoxicity were identified. Median TDF exposition duration was 36 months. At the time of kidney biopsy, median estimated glomerular filtration rate was 27,5 ml/min/1,73m^2. Histologic findings usually showed toxic ATN of various severity. Loss of megalin and cubulin expression affected more than 50% of the proximal tubules (PT) in 9 cases and 15 to 50% of PT in 10 cases, compared to less than 10% PT in control groups, that included HIV-infected patients naïve for TDF and HIV uninfected patients with ATN. Giant mitochondria were observed in only 25% of cases by light microscopy and ultrastructural mitochondrial dysmorphic changes were obvious in 4 out the 5 cases analyzed. Functional histochemistry of COX and SDH showed patchy loss of COX enzyme activity in PT.



Conclusions: Mild to severe alterations of megalin and cubulin expression characterize TDF-related proximal tubular toxicity, and may differentiate ATN unrelated to TDF exposition. Additionally, specific loss of COX activity in PT could be a strong marker of TDF nephrotoxicity.

SA-PO476

Dynamics of Renal Human L-Type Fatty Acid Binding Protein Reflects the Degree of Tubular Loss which Develops to Chronic Kidney Disease After Acute Kidney Injury

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Background: Tubular loss after acute kidney injury (AKI) is an important risk factor for onset of chronic kidney disease (CKD) and, therefore, notable detector of tubular loss is needed for prediction of renal prognosis after AKI. The aim of this study is to elucidate that dynamics of renal human L-type fatty acid binding protein (hL-FABP) is connected with the degree of tubular loss on chronic phase after AKI.

Methods: hL-FABP chromosomal transgenic (Tg) mice were subjected to ischemic reperfusion (I/R) model. The Tg mice were divided into 4 groups: short duration ischemia group (short-I/R) received procedure of renal ischemia induced by left renal artery clamping for 20 min; twice short-I/R group (2-short-I/R) received ischemia for 20 min twice with interval of 20 days; long duration ischemia group (long-I/R) received ischemia for 60 min; and the sham operated group. Kidneys were obtained 20 days after last I/R.

Results: The correlation between dynamics of renal hL-FABP and the degree of tubular loss after AKI with different frequency was revealed by comparison between short-I/R and 2-short-I/R mice. Tubulointerstitial injury and fibrosis were significantly greater in 2-short-I/R than in short-I/R mice. Under these conditions, renal gene and protein expressions of hL-FABP in 2-short-I/R increased significantly compared to short-I/R mice. Urinary hL-FABP levels were significantly higher in 2-short-I/R than in short-I/R mice. In regards to the dynamics of renal hL-FABP in tubular loss after AKI with different ischemic term, tubulointerstitial injury in long-I/R were significantly more severe than in short-I/R. Up-regulation of renal hL-FABP expression and urinary hL-FABP levels increased significantly in long-I/R more than in short-I/R.

Conclusions: In conclusion, dynamics of renal hL-FABP reflects the degree of tubular loss on chronic phase after AKI and urinary L-FABP may be useful for detection of tubular loss in clinical practice.

SA-PO477

Prognostic Utility of Cell Free DNA in Critically Ill Patients

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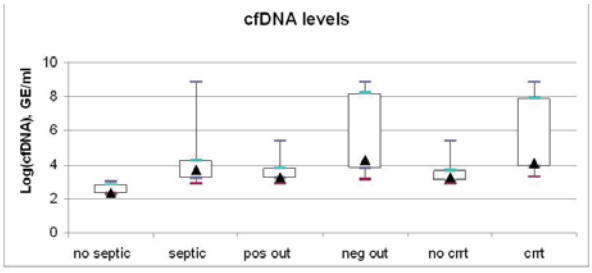
Background: Sepsis, a devastating condition characterized by systemic activation of inflammatory and coagulation pathways in response to infection, is a primary cause of morbidity and mortality in critically ill patients (pts). Recent researches suggested that cell free DNA (cfDNA), released as a result of cell necrosis and apoptosis, increases in many clinical conditions. The aim of this study was examined cfDNA levels in critically ill pts.

Methods: We enrolled 33 patients (pts) admitted in intensive care unit (ICU). 27 were septic (19 males, mean age 62±18yrs) and 7 were non septic (6 males, mean age 58±19yrs). 12/27septic pts developed acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT); 11/27 had a negative outcome during ICU stay. CfDNA was extracted from plasma and was quantified in Genome Equivalent GE/ml by Real time PCR for β-globin gene. Nonparametric variables were expressed as median and interquartile range (IQR). We applied a linear transformation of cfDNA, log(cfDNA), to manage the data better.

Results: We observed significantly higher levels of cfDNA in pts with sepsis (5331GE/ml; IQR: 1751-16113 versus 238GE/ml; IQR: 208-1001 in no septic pts) (p<.01), with no difference in its levels among pts with Gram$^{+/}$Gram$^-$ infections. Moreover, cfDNA levels resulted to be higher in septic pts who developed AKI requiring CRRT (CRRT pts: 13060; IQR: 8692-79367316 versus no CRRT: 1891; IQR: 1203-4324); and in patients with a negative outcome(negative: 18678; IQR:6175-157747 versus positive: 1891; IQR: 1497-6319)(all, p<.01).



Conclusions: cfDNA could be a potential biomarker in critically ill pts. In particular, cfDNA seems to have prognostic utility in critically ill patients with sepsis, especially if they develop AKI requiring CRRT. Moreover, it appears to be associated with negative outcomes.

Funding: Private Foundation Support

SA-PO478

Urinary NGAL Levels in Maintenance Hemodialysis Patients
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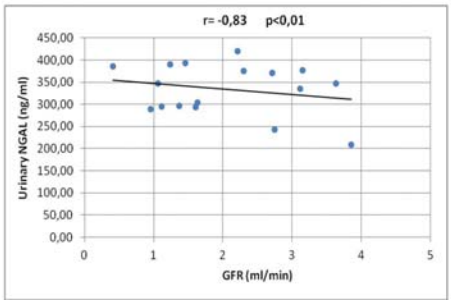
Background: Urinary concentration of Neutrophil gelatinase-associated lipocalin(uNGAL) is a sensitive biomarker for acute kidney injury.Recent studies investigated its role in chronic kidney disease(CKD) and showed that uNGAL progressively increased with the reduction of Glomerular Filtration Rate(GFR).Few data are available about its behavior in maintenance hemodialysis(MHD) patients(pts).

Methods: We enrolled 17 MHD pts with residual diuresis(>200ml/die).24h diuresis(collected before the first weekly dialysis session),uNGAL,GFR(as the arithmetic mean between Creatinine and Urea Clearances) were measured.According to diuretic dose,enrolled pts were divided in a diuretic group(DG, furosemide>50mg/die) and a non-diuretic one(NDG,furosemide≤50mg/die).Results are expressed as median[I-III interquartile].The correlation between uNGAL and residual GFR was analyzed by Spearman Test.The Mann–Whitney U test was used for comparison of two groups;a p value<.05 was considered for statistical significance.

Results: We reported GFR,diuresis and uNGAL for all pts in figure1.We observed a negative correlation between GFR and uNGAL(rho=-.83,p<.01).We compared 11 DG pts versus 6 NDG pts. DG pts compared to NDG pts.However,GFR and diuresis did not differ significantly between groups.

	All subjects		
GFR (ml/min)	1.6[1.2-2.7]		
Diuresis (ml/die)	600[400-800]		
uNGAL (ng/ml)	346.8[294.5-377.0]		
	DG (11pts)	NDG (6pts)	p
Diuretic dose (mg/die)	500[250-500]	0[0-25]	
GFR (ml/min)	1.5[1.1-2.3]	1.6[1.5-2.9]	NS
Diuresis (ml/die)	500[375-650]	650[375-750]	NS
uNGAL (ng/ml)	347.4[341.2-372.98]	296.3[294-320.1]	<.01



Conclusions: In conclusion,our data have demonstrated that MHD pts have chronically increased uNGAL levels,which progressively increase with GFR reduction.uNGAL could be a new potential biomarker to evaluate renal damage severity in MHD.In particular,at similar GFR level,pts with higher levels of uNGAL need more diuretic doses to maintain the same level of diuresis.Further trials to assess the role of uNGAL in the evaluation of residual renal function and clinical outcome are necessary.

SA-PO479

The Renal Pathological Findings in Diabetic Patients with Acute Renal Failure
Steven Salvatore, Surya V. Seshan. *Weill Cornell Medical College.*

Background: Diabetic nephropathy (DN) is the most common cause of end stage renal disease in the U.S. Typically, presenting with proteinuria and progressive renal insufficiency, the diabetic patient may be managed clinically without a diagnostic kidney biopsy. However, acute renal failure in the diabetic patient tends to have many etiologies requiring a kidney biopsy for definitive diagnosis.

Methods: Of 7485 native kidney biopsies from 2000-2014, 924 (12.3%) had diabetic nephropathy and 502 of those (54.3%) presented with acute renal failure (ARF) +/- significant proteinuria and hematuria. ARF was defined by KDIGO criteria (>0.3 mg/dL increase in serum creatinine within 48 hrs or 50% in 7 days). Clinicopathologic analysis of this cohort was performed.

Results: Renal biopsy findings of DN are increasing in recent years in our cohort, found in 8.4% of cases from 2000-2004 and 16.4% from 2011-2013. The 502 patients with ARF ranged from 14-88 years old and were 63% male. The average presenting creatinine was 4.6mg/dL (1.5-22), with 9 patients on dialysis, and proteinuria was 6.6g/d (0-34). A second diagnosis in addition to DN was rendered in 343 cases (68%), most commonly patchy or diffuse active interstitial nephritis (AIN) (27%), post-infectious glomerulonephritis (PIGN) (10.5%), acute tubular injury (12%), podocytopathy (6%) [29 collapsing glomerulopathy,10 minimal change, 6 FSGS], crescentic GN (2.6%), thrombotic microangiopathy (2.2%), and occasional membranous GN (5 cases), IgA nephropathy (3), Bence Jones cast nephropathy (3), lupus nephritis (4), atheroembolic disease (3), amyloidosis (2), cryoglobulinemia (1), and membranoproliferative GN (5). The cases without a superimposed disease (32%) showed advanced diabetic disease, class III (nodular diabetic glomerulosclerosis) 38% to class IV (having >50% global GS) 37%, interstitial fibrosis (65%), and severe vascular disease (42%).

Conclusions: DN is increasingly found in the renal biopsy population. Acute renal failure, a common but important indication for kidney biopsy in diabetic patients, is frequently associated with superimposed disease but may also disclose advanced DN alone. In these patients, renal biopsy yields diagnostic and prognostic information for appropriate management.

SA-PO480

Reproducibility of the NEPTUNE Digital Pathology Morphologic Profiling of NS
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Background: Reproducibility of conventional light microscopy analysis remains challenging. The NEPTUNE digital pathology protocol (NDPP) is a novel approach that applies a descriptor-based assessment (60 histologic (H), 10 immunofluorescence (IF) and 16 electron microscopy (EM) descriptors) to whole slide images (WSI), IF and EM digital images.

Methods: To test reproducibility, 6 pathologists and 1 trainee (P1-7) scored H descriptors in 131 glomeruli, EM podocyte descriptors (PD) (P1-5) in 178 cases, and % of interstitial fibrosis and tubular atrophy (IFTA) (P1=54, P2=38 and P4=188 cases). % IFTA was compared to IFTA morphometric measurement (64 cases). Kendal's coefficient of concordance was used to measure inter-reader reliability for glomerular H descriptors, PD and IFTA.

Results: Coefficient of concordance was *excellent* (>0.70) for PD effacement, global collapse, cellular and sclerosing tip lesion, foam cells, global spikes; *good* (<0.70 and >0.60) for PD microvillous transformation, normal glomeruli, global sclerosis with hyalinosis, global deflation, obsolescence, mid-glomerular sclerosis, hyaline droplets, global epithelial hypertrophy; *moderate* (<0.60 and >0.50) for segmental sclerosis and hyalinosis cannot determine location, perihilar hyalinosis, adhesions, segmental epithelial hypertrophy, halo, global mesangial hypercellularity. Evaluation of WSI-IFTA had excellent concordance with morphometric analysis.

Kendall's Coeff. of Concordance							
global collapse	cellular tip lesion	sclerosing tip lesion	glomerular foam cells	global spikes	IFTA %	% IFTA vs morphometric analysis	PD effacement (0-4+)
0.85	0.72	0.70	0.80	1.00	0.87-0.99	0.82-0.85	0.92

Conclusions: The NDPP has good reproducibility, independent from years of experience, enabling standardization of renal biopsy interpretation. The standardized collection of permanently recordable observational data is also suitable for correlation with clinical and molecular profiling of NS across international collaborative consortia employing the NDPP.

Funding: NIDDK Support

SA-PO481

Morphometric Analysis of Podocyte Density and Glomerular Volume Adapted for Routine Diagnostic Biopsy Evaluation
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Background: Strong evidence from model systems and human studies support the hypothesis that podocyte density (podocyte depletion relative to glomerular volume) is the critical process driving glomerulosclerosis. We have previously reported a method estimating podocyte number and density in a single histological section (Venkatareddy et al., JASN 2014) using immunofluorescence and immunohistochemistry. Here we compare three simplified methods using immunohistochemistry for WT1, Glepp1, or routine PAS.

Methods: Human kidney tissue was obtained from 19 nephrectomies under IRB approved protocols. Slides were morphometrically analyzed according to Venkatareddy et al. Additional slides from the same cases were immunostained with WT1, Glepp1, or PAS, then scanned and analyzed morphometrically using ImageJ software for podocyte number, podocyte area, and glomerular tuft area.

Results: Podocyte density, %podocyte area, and glomerular volume were calculated (Table). All methods correlated well with Venkatareddy et al., with podocyte density from PAS sections surprisingly best. WT1 performed worst due to some nonspecific staining.

	Morphometric method	Mean (SD)	Range	R-squared*	Correlation	Relative Bias,% (SD)
Podocyte Density ($x10^6$ um^3)	**Venkatareddy et al.**	**125.2 (34.3)**	**82.0-214.0**			
	WT1 only	152.0 (49.2)	90.5-285.1	0.56	P < 0.001	20.2 (24.1)
	Glepp1 only	123.9 (22.1)	94.3-155.9	0.76	P < 0.0001	1.7 (13.9)
	PAS only	125.9 (29.3)	84.0-207.9	0.81	P < 0.0001	2.5 (14.9)
Podocyte Area (% of tuft)	**Venkatareddy et al.**	**49.5 (4.6)**	**39.5-54.5**			
	Glepp1 only	51.3 (4.8)	41.0-57.8	0.49	P < 0.001	3.9 (7.6)
Glomerular Volume ($x10^6$ um^3)	**Venkatareddy et al.**	**3.7 (1.2)**	**2.4-6.7**			
	Mean of WT1, Glepp1, PAS	3.6 (1.1)	2.3-6.7	0.87	P < 0.0001	-4.2 (11.6)
				*compared to Venkatareddy et al.		

Conclusions: Building on previous work, we demonstrate the feasibility of reporting podocyte density in standard renal pathology practice with either a single immunostain, or routine PAS, using freely available analysis software and standard imaging tools.

Funding: NIDDK Support, Private Foundation Support

SA-PO482

Glycomics for Urinary Biomarker Discovery in IgA Nephropathy (IgAN) Robert H. Weiss, Renee Ruhaak, Billy T. Hour, Angela M. Zivkovic. *UC Davis, Davis, CA.*

Background: IgAN is the most common primary glomerulonephritis in the developed world, yet there are no specific biomarkers for this disease except those available on invasively obtained on biopsy tissue. Given the glycation abnormalities which are now known to be a part of this disease, we undertook a pilot study using an unbiased glycomics approach in IgAN patient urine to discover potential urinary biomarkers which, when validated, could be brought to the nephrology clinic.

Methods: After appropriate IRB approval, 11 IgAN patients and 11 age, sex, and race matched non-renal disease controls were recruited from the UCD Nephrology and Urology clinics. Unbiased glycomics analysis was undertaken after ethanol precipitation of 200 ug of urinary protein. Glycopeptide and Ig plus subtypes were analyzed using QQQ-MS and IgAN patients were compared to controls.

Results: IgAN patients had increased total urinary IgA, IgA1 and IgA2 as compared to matched controls. Total IgG as well as all the IgG subclasses were higher in the urine of IgAN patients as compared to controls. In addition, IgAN patients had a higher probability of having higher relative amounts of IgA2 in the urine. Several monofucosylated or fucosylated+sialylated IgG1 and IgG2 glycans were more likely to be found in the urine of IgAN patients than controls. IgAN patients were more likely to have several monofucosylated or fucosylated+sialylated glycans on site 205 and sialylated glycans on site 144 of IgA.

Conclusions: A glycomics analysis of IgAN urine shows promise for specific biomarkers for this disease which have the potential to obviate the need for an invasive biopsy. Further validation, including the analysis of a blinded test set, is currently underway in our laboratories and will likely lead to new and specific biofluid markers for IgAN.

Funding: NIDDK Support, Other NIH Support - NCI, Veterans Affairs Support

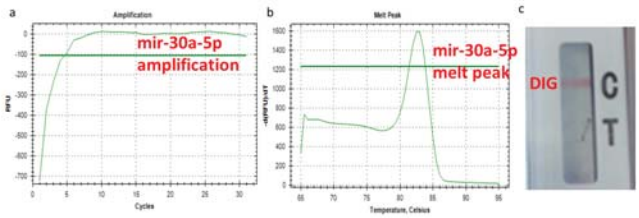
SA-PO483

CHAMP: Novel Isothermal Amplification Assay for the Detection of microRNAs in Urine as a Biomarkers for Kidney Diseases Kathrin Gassei, Ishwad Chandra, Andrew J. Bodnar, Jacqueline Ho, Abhay N. Vats. *Pediatrics, Children's Hospital of UPMC, Pittsburgh, PA.*

Background: MicroRNAs are being explored as biomarkers for several diseases including nephrotic syndrome (NS). Lately miRNA-30a-5p has been shown to be upregulated in urine of NS patients. However, miRNA detection assays can be expensive and time consuming. We aimed to develop a fast and reliable assay for microRNAs in urine and report a novel isothermal amplification method for miR-30a-5p detection called cross hybridization amplification (CHAMP).

Methods: We designed 20-25 nt isothermal primers (F-Chip, B-Chip) and a probe specific for miR-30a-5p. The primers had a binding site for mature miR-30a-5p via a 6 nt sequence at the 5'-end, and were labeled with either Biotin or Digoxigenin on the 3'-end. Amplification required the binding of the primers to the probe, Bst DNA polymerase enzyme, and incubation at 65°C for 30-60 min. Assay optimization was performed on fresh urine samples spiked with synthetic miR-30a-5p target.

Results:



Amplification and melting curves for miR-30a were obtained within 1 hr (<20 min in Fig. 1a and b). Amplicons were also detected by UV transillumination and on agarose gels. The incorporation of Biotin and Digoxigenin in primers allowed the detection of amplicons on lateral flow strips also (Fig. 1c). The presence of urine did not inhibit the reaction, and unprocessed urine spiked with synthetic miR-30a could be used in the reactions, without the need for RNA isolation.

Conclusions: CHAMP allowed a rapid, sensitive, highly specific and cost efficient alternative to current miRNA detection methods like qPCR or microarray. CHAMP could detect miR-30a-5p, with sensitivity approaching qPCR, in unprocessed urine without the need for RNA isolation or any other sample prep. Further comparative evaluation of this method will prove its utility in developing diagnostic miRNA assays.

Funding: NIDDK Support, Other NIH Support - NIAID

SA-PO484

Urinary Exosomal mRNA Analysis for Non-Invasive Diagnostics of Kidney Diseases and Damages Mizue Kawai,[1] Hideki Mochida,[1] Masashi Nishio,[1] Keisuke Fujitaka,[1] Rikako Yamauchi-Kohno,[1] Taku Murakami,[2] Masato Mitsuhashi.[2] *[1]Mitsubishi Tanabe Pharma Corporation, Saitama, Japan; [2]Hitachi Chemical Research Center, Inc., Irvine, CA.*

Background: Urinary exosomes and microvesicles (EMV) are a potential biomarker source for kidney diseases. EMV are released into the urinary space from all the areas of the nephrons by encapsulating cytoplasmic molecules of the cell of origin. Therefore, we hypothesized that mRNA profile of urinary EMV may reflect status of kidney, and be useful for non-invasive diagnosis of kidney diseases and damages. To address our hypothesis, various kidney disease models were prepared and mRNA profile of urinary EMV was examined.

Methods: Three different rat kidney disease models (drug-induced glomerulus damage (N=8), type 2 diabetes (N=10), and metabolic syndrome (N=10)) and corresponding healthy controls (N=4-5 in each group) were prepared (N=42 in total). Kidney damages were confirmed by urinalysis and kidney pathology scores. Urinary EMV was isolated from 24-hour urine by a differential centrifugation protocol. The mRNA isolation, cDNA synthesis, and quantification from the EMV fraction were conducted using oligo(dT)-immobilized microplate (Hitachi Chemical Research Center, Inc.) and real time polymerase chain reaction (PCR). The raw PCR data were normalized by the values of reference genes, and compared with the disease model types and pathology scores.

Results: Among 27 kidney specific and reference mRNA we tested, glomerulus specific Synpo mRNA was down-regulated in all the disease models compared with corresponding control groups, indicating that Synpo may be used as a marker of general kidney damages. Other kidney-specific mRNA such as Nphs1, Nphs2, Cubn, Lrp2, Aqp2, Spp1, Ppargc1, were also differentially expressed but their expression patterns were different among the disease models and pathology scores. Logistic regression analysis demonstrated that the combinations of 2 to 5 genes were capable of identifying 3 disease models as well as pathology scores with more than 95% sensitivity and specificity.

Conclusions: Urinary EMV mRNA analysis is a promising diagnostic tool for kidney diseases and damages.

SA-PO485

Improving Urine Biomarker Discovery Using Filter Assisted Sample Preparation- U-FASP James A. Hribar, Daniel Wade Wilkey, Walter Blake Kusiak, Michael Merchant. *Medicine, Univ of Louisville, Louisville, KY.*

Background: Urine can be collected using non-invasive techniques, in large quantities, and is a rich source of biomarkers of human diseases. Quantitative LCMS (qLCMS) methods are being developed to enable simultaneous quantification of multiple urine disease biomarkers. Unfortunately qLCMS analysis of urine is problematic. Our hypothesis is the irreproducible chromatographic retention times and loss of sensitivity stem from the effects of peptidoglycans found in urine tryptic digests. To address this hypothesis we adapted the Filter-Aided Sample Preparation method to the analysis of urine samples (U-FASP) with the goal of depleting high molecular weight proteoglycans to ensure a consistent urine matrix injected onto the nanoLC column.

Methods: Spot urine samples (n=3, healthy normal males) were pooled, trypsinized and then processed with or without filtration by the FASP protocol to fractionate HMW tryptic peptides from LMW peptides (<10K). A portion of the HMW tryptic peptides were deglycoslyated using PNGase F and analyzed by nanoflow-UHPLC-MS analysis using a Thermo EZ-nLC-TSQ Quantum to develop MRM methods for urinary biomarkers. UHPLC-MS performance comparing stable isotope labeled peptides (IS) retention time, sensitivity for detection and calibration curve linearity was determined. Confirmation of peptide deglycoslyation experiments was achieved using an EZ-nLC-LTQ-Orbitrap Elite and PD1.4.

Results: Application of U-FASP improved UHPLC performance by (a) extending column lifetime by approx. 50-100-fold, (b) reduced retention time drift by 25-fold, and (c) a gain of detection sensitivity for the IS of 6-10 fold. U-FASP method identified 625 proteins in 100ng urine protein; including 47 proteins only identified with the U-FASP/ PNGaseF protocol.

Conclusions: Our data shows the U-FASP method improves the reproducibility of peptide chromatography, increases the sensitivity for analyte detection by improved peak characteristics, and additionally isolates a unique urinary proteomglyome such as suPAR and SPARCL1, both markers implicated in podocentric diseases such as FSGS.

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SA-PO486

Development of Assays for Quantification of Angiotensin II Signature Proteins in Human Urine

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Background: Angiotensin II (AngII), the main effector of the renin-angiotensin system (RAS), mediates kidney disease progression. However, there are no specific markers of renal AngII activity. We previously identified 83 AngII-regulated proteins in proximal tubular cells *in vitro*, which reflected renal AngII activity in animal models of kidney disease *in vivo*. We now examine whether these proteins measured in urine represent markers of renal AngII activity in patients with CKD.

Methods: Mass spectrometry-based selected reaction monitoring (SRM) assays were developed for 37 peptides corresponding to 10 AngII-regulated proteins previously detected in urine, and 8 AngII-regulated proteins previously quantified in human kidney cells. Methods were developed in normal urine samples from 100ug of total protein. To assess reproducibility, we spiked in bovine serum albumin (BSA) and chicken ovalbumin (OVA) at known concentrations. Urine was concentrated utilizing 3kDa filter or precipitated with acetonitrile. We compared protein digestion with lys-C/trypsin to trypsin alone. After digestion, peptide fractions were analyzed on triple quadrupole mass spectrometer. We then measured peptides in 10 urine samples from patients with CKD.

Results: We repeated the optimization procedure 4 times with 2 biological and 2 technical replicates. Biological replicate analysis demonstrated excellent coefficients of variation <11% for both BSA and OVA peptides. In addition to control peptides, we detected the most peptides (16 of 37) in urine samples precipitated with acetonitrile, and digested with lys-C/trypsin. CKD urine samples contained 14 of 37 AngII-regulated peptides.

Conclusions: We optimized the procedure for SRM monitoring of peptides in urine. Acetonitrile precipitation and lys-C/trypsin digestion appeared most effective at detecting AngII-regulated peptides. We will next quantify these peptides in urine of 40 CKD patients before and after RAS blockade, and determine whether AngII-regulated proteins reflect renal AngII activity.

SA-PO487

A New Player in the Evaluation of Oncotic Pressure – Oxidative Stress

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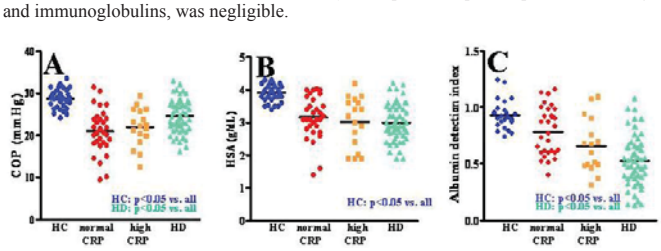
Background: Hypoalbuminemia, oxidative stress (OS) and inflammation are common in patients with chronic kidney disease (CKD). Oxidative modifications of serum albumin impair its quantification by clinical assays such as bromocresol-green (BCG), affecting albumin read-out in CKD patients. As maintenance of oncotic pressure/ colloid osmotic pressure (COP) is the main albumin function, the study aim was to examine the impact of albumin oxidation on the oncotic pressure in CKD patients.

Methods: Blood samples were collected from 134 subjects including healthy controls (HC, n=32), CKD patients with (n=17) or without (n=31) systemic inflammation (evaluated by CRP levels) and patients on chronic hemodialysis (HD, n=54).

Results: COP values in HD were significantly higher than in CKD (fig.A), although the albumin levels, measured by BCG, were statistically similar (fig.B). The decreased quantification of modified albumin by BCG was assessed using the "albumin detection index", that represents the ratio between BCG read-out (albumin-specific) to total albumin (assessed by an oxidation-insensitive assay, OD at 280nm). This index was significantly lower in the HD group compared with all other groups (fig.C). In-vitro oxidized albumin and HD-albumin showed significantly higher COP values than HC-albumin and non-modified albumin. The contribution to COP by other prevalent plasma proteins, fibrinogen and immunoglobulins, was negligible.



Conclusions: Altogether, these data demonstrate that actual albumin concentration in plasma of CKD patients, and especially in HD patients, is higher than measured and contributes to the relatively higher oncotic pressure. The physiological and clinical implications of these findings, especially in edema associated states accompanied by OS and inflammation, should be addressed.

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SA-PO488

Biological Variation and Analytical Stability of Serum Soluble α-Klotho in Healthy Volunteers

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Background: Recent evidence suggests that the cleaved extracellular domain of the α-klotho receptor, soluble α-klotho (sKl), has effects on phosphate homeostasis, ion channel regulation and anti-fibrotic/anti-oxidant pathways. However, measurements of serum sKl in healthy individuals and in cohorts of patients with renal disease have yielded inconsistent results when related to renal function, other markers of mineral metabolism and patient outcome. Pre-analytical factors such as biological variation and analyte stability may affect the interpretation of sKl results but have yet to be formally assessed.

Methods: For assessment of biological variability,serum samples were collected from 10 healthy volunteers at 3 time-points during the day (morning, midday and afternoon). For assessment of analytical stability, separate aliquots from morning samples were allowed to stand at room temperature for 30, 60 and 120 minutes, prior to centrifugation and processing. All samples were stored at -80°C until batched analysis. sKl was measured using a commercial ELISA kit (Immuno-Biological Laboratories Co., Gunma, Japan) according to the manufacturer's protocol. Differences between the groups were analyzed using non-parametric tests.

Results: Delayed separation of samples yielded median (IQR) sKl levels of 271 (204-359)pg/mL, 271 (155-358)pg/mL and 253 (152-328) g/mL, at 30, 60 and 120 minutes, respectively. A trend towards analyte degradation over time was statistically significant between 30 minutes and 60 minutes (p=0.038) as well as between 30 minutes and 120 minutes (p=0.008). Median (IQR) sKl levels were 271 (204-359) pg/mL, 276 (196-503) pg/mL and 263 (221-461) pg/mL at morning, midday and afternoon time-points respectively, showing no evidence of significant diurnal change.

Conclusions: Prompt processing of serum samples for sKlmeasurement is advisable, although the magnitude of analyte degradation over 2 hours is small. In health, sKl displays minimal variation throughout the day.

SA-PO489

Profiling the Renal Transcriptome in the North Dublin Renal Biobank: Preliminary Results

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Background: Chronic Kidney Disease (CKD) is a progressive disorder with poor outcomes. Progressive tubulointerstital fibrosis (TIF) represents the final common pathway of CKD and is a major predictor of disease progression. Elucidation of the initiation and progression of TIF will aid identification of novel targets for therapeutic intervention in CKD.

Methods: The North Dublin Renal Biobank (NDRBB) recruited patients undergoing a clinically indicated renal biopsy. Blood, urine, clinical and demographic data were collected. A dedicated sample of renal biopsy tissue was retained, and the ultimate histological diagnosis recorded. RNA was extracted from renal biopsies of individuals with CKD of diverse aetiologies and TIF severity. cDNA libraries were generated. Ultimately,44 CKD biopsies, together with 3 controls,were selected for transcriptome profiling by RNAseq analysis (IlluminaTruSeq). The sequencing reads were aligned to the Genome (TopHat) and gene expression quantified using HTseq (Ensembl70).

Results: RNAseq analysis revealed ~98% reads aligned to the reference genome GrCh37.75% genome aligned reads mapped uniquely and unambiguously to exons. Gene expression clustering analysis for the 200 most variable expressed genes shows two distinct clusters. Analysis is ongoing to determine if correlates with clinical parameters exist. Unbiased clustering of mitochondrial matrix gene expression also indicated two clusters,one of which was clearly enriched for high fibrosis cases ($p < 0.01$). Correlation analysis confirmed that the degree of fibrosis negatively correlated with the expression of transcripts associated with the human mitochondrial matrix.

Conclusions: This expression dataset shows intriguing preliminary results. Work is ongoing (GO and GSEA analysis) to determine if the observed variability in gene expression can be ascribed to sub-categories of mitochondrial function.

SA-PO490

Effect of Gender when Estimating Glomerular Filtration Rate with Beta-Trace Protein in Children Samantha Witzel,[1] Shih-Han S. Huang,[1,2] Branko Braam,[4] Guido Filler.[1,2,3] *[1]Dept of Pediatrics, Div of Pediatric Nephrology, Children's Hospital, London Health Sciences Centre, Schulich School of Medicine and Dentistry, Univ of Western Ontario, London, ON, Canada; [2]Dept of Medicine, Div of Nephrology, London Health Sciences Centre, Schulich School of Medicine and Dentistry, Univ of Western Ontario, London, ON, Canada; [3]Dept of Pathology and Laboratory Medicine, London Health Sciences Centre, Schulich School of Medicine and Dentistry, Univ of Western Ontario, London, ON, Canada; [4]Dept of Medicine, Div of Nephroloy, Univ of Alberta, Edmonton, AB, Canada.*

Background: Gender may affect the performance of small molecular weight proteins as markers of glomerular filtration rate (GFR) because of differences in fat mass between the two sexes. We hypothesized that the diagnostic performance of beta-trace protein (BTP), a marker of GFR, would be significantly better in boys than in girls.

Methods: GFR, height, serum creatinine, and BTP were measured in 755 children and adolescents undergoing ^{99}technetium diethylenetriamine penta-acetic acid (^{99}Tc DTPA) renal scans. Boys and girls were separated into formula-generation cohorts (284 boys and 220 girls) and formula-validation cohorts (140 boys and 111 girls). GFR-estimating formulae were derived using stepwise linear regression analysis of log-transformed data. Bland-Altman analysis was used for testing agreement between ^{99}Tc DTPA GFR and calculated GFR.

Results: In the stepwise regression analysis, BTP (R^2 of 0.734 for boys and 0.651 for girls) was more important than creatinine (which increased R^2 to 0.814 for boys and 0.752 for girls) and height (which increased R^2 to 0.876 for boys and 0.800 for girls). GFR can be calculated using the following formulae: GFR_{boys}=10^(2.824-0.461*log(BTP[mg/L])-0.679*log(creat[umol/L])+0.00259*height[cm]) GFR_{girls}=10^(2.772-0.433*log(BTP[mg/L])-0.661*log(creat[umol/L])+0.00256*height[cm]) Bland-Altman analysis showed better performance in boys. The new formulae performed significantly better than the previous Benlamri and White formulae.

Conclusions: We present improved and gender-specific formulae for the estimation of GFR in children based on BTP, serum creatinine, and height.

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SA-PO491

Urine-Derived Human Renal Progenitors for Modeling of Genetic Renal Diseases Elena Lazzeri, Elisa Ronconi, Maria Lucia Angelotti, Anna Julie Peired, Francesca Becherucci, Paola Romagnani. *Excellence Centre DENOTHE, Univ of Florence, Florence, Italy.*

Background: Up to 20 % of adults progressing to end stage renal disease suffer from genetic kidney diseases. The current diagnostic and therapeutic management of these diseases is in most cases still insufficient. The identification of suitable disease models, that recapitulate human kidney disorders in a personalized manner, could improve diagnosis and treatment of genetic kidney disorders. Renal progenitor cells (RPC) obtained from the affected patient may represent an ideal alternative for personalized disease modeling. Since loss of renal cells in urine naturally occurs in patients, urine may represent a potential RPC source for in vitro studies of disease.

Methods: Urine samples were centrifuged and cells were plated. Cultures were analyzed by FACS and TaqMan low density array and differentiated in tubular and in podocyte after exposure to specific media. Electronic microscopy, RT-PCR, confocal microscopy and western blot were performed.

Results: In this study we describe a cheap and reliable method to amplify u-RPC from patients with renal disorders, that exhibit identical phenotype and functional properties to those purified from kidney tissue, including the capacity to differentiate into tubular cells as well as podocytes. To evaluate the possibility to use these cells for modeling of genetic kidney disorders, u-RPC were obtained from patients with genetic forms of steroid-resistant nephrotic syndrome, carrying putative pathogenic mutations in genes encoding for podocyte cytoskeleton proteins, as well as from patients without genetic alterations. U-RPC obtained from patients carrying pathogenic mutations generated podocytes that exhibited altered synthesis of mutated proteins, abnormal cytoskeleton structure and functional abnormalities. By contrast, podocytes obtained from u-RPC of patients without genetic mutations showed normal phenotype, structure and function.

Conclusions: This study demonstrate that u-RPC represent an innovative personalized tool for modeling of genetic kidney disorders providing a rapid test of putative pathogenic mutations and an essential support for the clinical diagnosis.

SA-PO492

Circulating miR-148b and let-7b in Serum as Potential Markers for Detecting Primary IgA Nephropathy: A Multicenter Study Grazia Serino,[1,2] Francesco Pesce,[2,3] Fabio Sallustio,[1] Giuseppe De Palma,[1] Sharon N. Cox,[1,2] Kar Neng Lai,[4] Joseph C.K. Leung,[4] Aikaterini A. Papagianni,[5] Maria Stangou,[5] Dimitrios S. Goumenos,[6] Miltiadis Gerolymos,[6] Kazuo Takahashi,[7] Yukio Yuzawa,[7] Shoichi Maruyama,[8] Enyu Imai,[8] Francesco Paolo Schena.[1,2] *[1]C.A.R.S.O. Consortium, Univ of Bari, Italy; [2]Dept of Emergency and Organ Transplantation, Univ of Bari, Italy; [3]Imperial College, United Kingdom; [4]Dept of Medicine, Queen Mary Hospital, Hong Kong; [5]Dept of Nephrology, Univ of Thessaloniki, Greece; [6]Dept of Nephrology, Univ Hospital Patras, Greece; [7]Dept of Nephrology, Fujita Health Univ School of Medicine, Japan; [8]Dept of Nephrology, Nagoya Univ Graduate School of Medicine, Japan.*

Background: IgA Nephropathy (IgAN) is a worldwide disease characterized by the presence of galactose-deficient IgA1 deposits in the glomeruli. A kidney biopsy for the diagnosis is required. Aim of our study was to develop and validate a new biomarker for IgAN.

Methods: We measured two miRNAs (let-7b and miR-148b), previously identified by our group as regulators of the *O*–glycosylation process of IgA1, by Real–Time PCR in serum samples of biopsy–proven IgAN patients and healthy blood donors (HBD) recruited from the international multicenter study. Logistic regression was performed to develop a predictive model based on these two miRNAs. Diagnostic accuracy of the combined biomarker was assessed by the area under the receiver-operating-characteristic curve (AUC).

Results: Training study. The combined miRNAs biomarker was able to discriminate between 88 Caucasian IgAN patients and 102 HBD from Italy and Greece (AUC, 0.849; 95% confidence interval [CI], 0.794-0.905; p <0.0001). Validation study. The biomarker was validated in another cohort of 88 Asian IgAN patients and 97 HBD from Hong–Kong and Japan. The AUC was 0.852; 95% CI, 0.793-0.910. (p<0.0001) Test study. The diagnostic biomarker discriminated each cohort of IgAN patients from 58 Caucasian and Asian individuals affected by other primary glomerulonephritides, supporting its specificity.

Conclusions: A combined miRNA biomarker, based on let-7b and miR-148b serum levels, appears to be a novel biomarker to predict the probability of IgAN and support the clinicians in the diagnosis.

Funding: Government Support - Non-U.S.

SA-PO493

Identification of Tissue Biomarkers Differentiating Primary IgA Nephropathy from Staphylococcus Infection Related Glomerulonephritis Anjali A. Satoskar,[1] Samir Parikh,[1] Paul L. Kimmel,[2] John W. Kusek,[2] Jianying Zhang,[1] Lianbo Yu,[1] Tibor Nadasdy,[1] Michael Merchant,[3] Jon B. Klein,[3] Brad H. Rovin.[1] *[1]Ohio State Univ Wexner Medical Center, Columbus, OH; [2]NIDDK, Bethesda, MD; [3]Univ of Louisville, Louisville, KY.*

Background: Staphylococcus infection-associated glomerulonephritis (SAAGN) can manifest as proliferative GN with IgA and C3-containing immune complexes, thus mimicking idiopathic IgA Nephropathy (IgAN). We addressed the hypothesis that SAAGN and IgAN could be differentiated by characterizing the glomerular and interstitial proteomes of their kidney biopsies.

Methods: Laser capture micro-dissection was used to isolate the glomerular and tubulointerstitial (TI) compartments from SAAGN (n=4), IgAN (n=4), and normal renal tissue (n=5). Proteins were extracted, digested, and analyzed by tandem mass spectrometry. Spectral counts were modeled as negative binomial distribution and compared using Wald test.

Results: 798 proteins were identified in the glomerular compartment and 1177 proteins in the TI compartment. Normal, IgAN, and SAAGN clustered separately. Glomerular proteomes of SAAGN and IgAN showed many similarities. Coagulation and complement systems were the top canonical pathways activated in both SAAGN and IgAN relative to normal. Gluconeogenesis and glycolytic pathways were down-regulated in both. TI proteomes of IgAN and normals were similar, but SAAGN differed considerably. SAAGN showed upregulation of TGFβ, RhoGDI, inflammatory pathways (TNF, IL6, and NF-κB) and lipopolysaccharide signaling. Mitochondrial functions such as fatty acid oxidation, oxidative ethanol degradation, putrescine degradation, and lipid metabolism were downregulated.

Conclusions: The most striking feature of this proteomic analysis was the perturbation of tubular metabolism in SAAGN relative to IgAN. These data suggest that proteomic and metabolomic analysis of TI may be useful to identify specific biomarkers of SAAGN and IgAN.

Funding: NIDDK Support

SA-PO494

Pro-Inflammatory CD11c+CD68+ Mononuclear Phagocytes Promote Glomerular Injury in Lupus Nephritis Yohei Ikezumi,[1] Utako Kaneko,[1] Takeshi Yamada,[1] Hiroya Hasegawa,[1] David J. Nikolic-Paterson,[2] Akihiko Saitoh.[1] *[1]Dept of Pediatrics, Niigata Univ Medical and Dental Hospital, Niigata, Japan; [2]Monash Univ Dept of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.*

Background: Recent studies have shown a pro-inflammatory phenotype of mononuclear phagocytic cells (MPC) in the mouse kidney which co-express high levels of macrophage (CD11b) and dendritic cell (CD11c) markers. In this study, we examined the presence and potential role of CD68+CD11c+ MPC in biopsies of childhood lupus nephritis (LN).

Methods: Biopsies taken from 16 cases of childhood LN (12.8±1.8 years, M:F=4:12) were examined for histologic changes and by immunofluorescence staining for pan macrophages (MQ) (CD68+ cells), CD11c+ cells, M2-type MQ (CD163+ cells), CD3+ T lymphocytes, and glomerular expression of TNF-α. Nine biopsies from children with thin basement membrane disease (TBMD) were used as control.

Results: Significant accumulation of CD68+ MQ and CD3+ T lymphocytes was evident in LN. Most glomerular CD68+ MQ co-expressed CD11c (75%) but few co-expressed CD163. By contrast, most interstitial CD68+ MQ co-expressed CD163 (91%) but few expressed CD11c. Glomerular CD11c+ cells correlated with endocapillary proliferation ($p=0.002$), extracapillary change ($p=0.013$), the degree of hematuria ($p<0.01$) and proteinuria ($p<0.001$). Increased CD11c+ cells were seen in glomeruli with severe damage (ISN/RPS grade IV; n=5) compared to mild damage (grades II and III) (9.4 versus 5.4 CD11c+ cells/gcs, $p<0.01$). In addition, some glomerular CD11c+ cells expressed TNF-α. In contrast, glomerular CD163+ MQ failed to correlate with glomerular damage. However, interstitial CD163+ MQ correlated with interstitial fibrosis ($p<0.001$).

Conclusions: We have identified a pro-inflammatory population of CD68+CD11c+ MPC in childhood LN which contrasts with the pro-fibrotic CD163+ MQ population. CD68+CD11c+ MPC appear to be involved in the development of acute lesions (endocapillary proliferation and cellular crescents), which are important factors in evaluating disease severity in LN. Thus, CD68+CD11c+ MPC may represent an important therapeutic target in LN.

Funding: Government Support - Non-U.S.

SA-PO495

CNV Analysis of the CFHR Region by MLPA in aHUS Patients Michael Jones, Nicole Meyer, Yuzhou Zhang, Dingwu Shao, Erika Takanami, Carla M. Nester, Richard J. Smith. *MORL, Univ of Iowa, Iowa City, IA.*

Background: Atypical hemolytic uremic syndrome (aHUS) is an ultra-rare renal disease caused by uncontrolled activation of the alternative complement pathway at the cell surface level. Mutations in several complement genes have been implicated in its pathogenesis. Copy number variation (CNV) and complex genomic rearrangements of the complement factor H related (CFHR) region have also been causally linked to aHUS. Duplications, deletions and novel hybrid gene formation in the CFHR region reflect the frequent occurrence of non-allelic homologous recombination (NAHR). In this study, we sought to characterize the frequency and type of CFHR rearrangements.

Methods: Multiplex ligation-dependent probe amplification (MLPA) was used to characterize the genomic architecture of the CFHR region in 355 patients diagnosed with aHUS and 315 controls. Forty-three probes were used to cover this genomic region. Additional probes were used to delineate complex rearrangements.

Results: The most common finding was a common CNV, the deletion of *CFHR3-CFHR1*, del(*CFHR3-CFHR1*). Present in homozygosity in ~3.2% of a control European-American population, we found homozygosity for del*(CFHR3-CFHR1*) in 8.7% of aHUS patients. Of patients found to be homozygous del(*CFHR3-CFHR1*), 25.8% were positive for CFH autoantibody and another 25.8% were found to have variants in other complement genes. 28.7% of aHUS patients were heterozygous for del(*CFHR3-CFHR1*). 1.9% of aHUS patients had complex rearrangements of the CFHR region, which included rearrangements between homologous blocks containing CFH and CFHR1 as well as those containing CFHR3 and CFHR4. These rearrangements result in putative fusion proteins composed of SCRs from the two corresponding genes. Complex rearrangements were occasionally associated with familial aHUS but also represented *de novo* NAHR events.

Conclusions: The genetic evaluation of aHUS patients must include defining the genomic architecture of the CFHR region. The most frequent finding will be homozygous deletion of *CFHR3-CFHR1*. Complex rearrangements resulting in the formation of novel fusion genes are also quite common. Most of these fusion events appear to involve a portion of *CFHR1*.

Funding: Clinical Revenue Support

SA-PO496

The Clinical Application of aPLA2R Antibodies Measured By Commercial ELISA Kit in Korean Patients with Idiopathic Membranous Nephropathy (iMN) Yang Gyun Kim,[1] Jiyun Park,[1] Ju-Young Moon,[1] Kyung-Hwan Jeong,[1] Tae Won Lee,[1] Chun-Gyoo Ihm,[1] Yon Su Kim,[2] Sang Ho Lee.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Kyung Hee Univ College of Medicine, Seoul, Korea; [2]Dept of Internal Medicine, Seoul National Univ Hospital, Seoul, Korea.*

Background: aPLA2R antibody has not been measured widely in clinical field because immunofluorescence test as an only commercially available assay could be inaccurate and western blot(WB) is too complex to be applied. Though a few studies presented aPLA2R antibodies using home-made ELISA could be useful in western population, it has never been tried against Asian patients. Therefore, we investigated the suitability of commercial aPLA2R antibody ELISA test in Korean patients with iMN through comparison with the results of WB test.

Methods: We measured aPLA2R antibodies with ELISA kit(Euroimmun, Lübeck) in 124 serum samples from patients with iMN(n=93), secondary MN(n=6), IgAN(n=5), MCD(n=8) and healthy controls(n=12) in three hospitals using serums gathering at time of renal biopsy. Serums of several patients(n=43) with iMN were also analyzed with WB method. ELISA titers of aPLA2R antibody from 8 patients were followed after immunosuppressant therapies.

Results: aPLA2R antibodies were positive in 44.1% and 69.8% of patients with iMN using ELISA and WB test, respectively, in contrast the patients with other diseases and healthy controls had negative results. The concordance rate between two tests was 69.8% and the results from both tests showed positive correlation. aPLA2R antibody-positive patients presented severe disease activity and low remission rate. The titer of aPLA2R antibody was positively correlated with proteinuria and negatively associated with eGFR and serum albumin. The patients with high titer(>80U/mL) of ELISA revealed significantly low remission rate(30% versus 71%). Following reactivity of aPLA2R antibody after treatment was reversely paralleled with the existence of remission.

Conclusions: Therefore, the assessment of aPLA2R antibody with commercial EILSA kit is thought an easy and reliable method for not only diagnosis but also guidance of therapeutic plans for Korean patients with iMN.

SA-PO497

Idiopathic Membraonous Glomerulonephritis versus Class V Lupus Nephritis: Clinical-Pathologic Features and Outcomes Alcino Pires Gama, Simone C. Lo, Cristiane B. Dias, Luis Yu, Lectícia Jorge, Viktoria Woronik. *Nephrology, Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Overall renal prognosis in both Idiopathic Membranous Glomerulonephritis (IMGN) and Class V Lupus Nephritis (LNV) is worse than general population, but there is no data comparing prognosis between them. The aim of this study was to compare clinical-pathologic profile and outcomes between the two entities.

Methods: A retrospective analysis was carried out on all patients with biopsy-proven IMGN (n=195) or LNV (n=164) between 1999-2011. 86 patients with IMGN and 83 patients with LNV met inclusion criteria (age>18y and follow-up longer than 3y). The clinical endpoint was rate of loss of glomerular filtration rate(eGFR) per year(ΔeGFR/y = difference between final and initial eGFR adjusted by follow-up time).

Results:

	LNV(n=83)	IMGN(n=86)	p
Follow-up(y)	8.0±2.8	6.9±3.5	ns
Age(y)	33±10	42±13	<0.0001
%male	10	60	<0.0001
eGFR(ml/min)	87.7±41	85±44	0.67
Hb(mg/dl)	11.8±1.8	12.8±1.9	0.0001
Ptn(g/day)	4.4±4.2	7.9±7.8	0.0005
Ptn_1y(g/day)	1.4±2.4	3.3±3.8	0.0003
% Hematuria	38	24	0.06
Progression rate(ml/min/y)	4.2±4.5	5.6±5.6	0.2
Final eGFR(ml/min)	81±38	71±39	0.08
Pathologic features			
%Crescents	10	5	0.13
% IgG deposit predominance on IF	15	91	<0.0001*
% Mesangial Ig deposits on IF	57	17	<0.0001**
% Interstitial fibrosis (>10%)	26	24	0.7

Table 1. Clinical and pathological features on LNV and IMGN groups. *OR 56 CI 21-149. **OR 6.6 CI 3.2-13.7

There was no statistically difference between renal prognoses in both diseases, although CKD progression tends to occur faster in IMGN group (4.2 versus 5.6, $p=0.2$).

Conclusions: The rate of loss of renal function did not differ between the groups, but both are higher than general population (about 1ml/min/y). The persistent proteinuria, a known prognosis factor for both diseases, is more evident in IMGN. On the other hand, LNV occurs in the scenario of systemic inflammation (lower levels of hemoglobin). These factors may confer for both diseases worse renal prognosis than general population (even though the rate of progression to chronic kidney disease did not differ between the two groups).

Funding: Private Foundation Support

SA-PO498

Using Urine Adiponectin as a Biomarker of Inflammatory Kidney Lesions in Lupus Nephritis (LN) Xiaolan Zhang,[1] Anthony Alvarado,[1] Hermine Brunner,[2] Brad H. Rovin.[1] *[1]Nephrology, Ohio State Univ, Columbus, OH; [2]Rheumatology, Univ of Cincinnati, Cincinnati, OH.*

Background: Adiponectin (AD) is an adipokine that regulates metabolism, but also has pro- and anti-inflammatory properties. We previously showed that urine AD levels increase significantly at LN flare. The relationship of AD to renal histology in LN has never been studied, and forms the basis for this report.

Methods: Urine was collected at the time of kidney biopsy from 78 LN patients. Urine high molecular weight (HMW) AD was measured by ELISA, normalized to urine creatinine, log-transformed, and tested as a biomarker of specific histologic lesions. A threshold AD level for each lesion was identified by visual inspection of the cohort data. Based on the threshold level, sensitivity (sens), specificity (spec) and likelihood ratios (LR) for each lesion were calculated. Post-test probabilities of having a specific lesion were determined using Bayes Theorem plus the probability of the lesion in this biopsy cohort.

Results: The characteristics of AD as a diagnostic test for cellular crescents in >25% of glomeruli, the presence or absence of glomerular necrosis, and interstitial inflammation or fibrosis in >25% of the cortex are shown in the Table. AD did not discriminate between levels of global glomerulosclerosis. There was no association between AD and serum creatinine, proteinuria, or complement levels at biopsy.

	>25% Cellular Crescents	Presence of Glomerular Necrosis	>25% Interstitial Inflammation	>25% Interstitial Inflammation
Threshold AD (ng/mg Cr)	>20	>7.4	>7.4	>7.4
Sens	1	0.96	0.89	0.93
Spec	0.5	0.34	0.27	0.19
Positive LR	2	1.45	1.21	1.14
Probability of Lesion if AD > Threshold (%)	20	41	27	36
Negative LR	0	0.12	0.42	0.40
Probability of Lesion if AD < Threshold (%)	0	5	11	17

Conclusions: A urine HMW-AD level < 20 ng/mg Cr excludes the presence of more than 25% cellular crescents in LN biopsies. Similarly, a urine AD level < 7.4 ng/mg Cr excludes the presence of glomerular necrosis and moderate-severe interstitial inflammation and fibrosis. It is conceivable that urine AD levels could be followed post-biopsy in LN patients to non-invasively assess the resolution of active inflammatory lesions.

Funding: Other NIH Support - NIAMS

SA-PO499

Serum Anti-PLA2R Antibodies May Be Initially Absent in Idiopathic Membranous Nephropathy: Seroconversion after Prolonged Follow-Up Anne-Els van de Logt, Julia M. Hofstra, Jack F. Wetzels. *Nephrology, Radboud Univ Medical Center, Nijmegen, Netherlands.*

Background: Antibodies against PLA2R (aPLA2R) are present in 70% of patients with idiopathic membranous nephropathy (iMN). In seronegative patients, iMN may be caused by other antibodies. However, in some seronegative patients PLA2R was detected in the glomerular immune deposits, suggesting that also in these patients iMN was related to PLA2R (*NEJM 2011, Ronco and Debiec).* It was suggested that the discrepancy occurred in patients who had remitting disease. Alternatively, the kidney could function as a "sink", antibodies becoming detectable only after the buffer capacity of the kidney is exceeded. We present data to support the latter hypothesis. **Case-description:** A 47-year-old patient was diagnosed with idiopathic membranous nephropathy in 2004. Treatment with prednisone and cyclophosphamide resulted in a complete remission. In June 2013 he developed a relapse. Initially aPLA2R was not detected in the immunofluorescence test (IIFT), however they became clearly positive during follow-up.

Date	IIFT	creatinine (mg/dl)	albumin (g/dl)	Protein creatinine ratio (g/g)
05-06-2013	inconclusive	0.92	2.4	2.40
25-09-2013	negative	0.96	2.4	5.82
09-10-2013	inconclusive	0.90	2.3	4.41
20-11-2013	positive	1.00	2.1	4.84
19-02-2014	positive	0.95	1.8	4.04
19-03-2014	positive	0.94	2.2	7.28

Methods: We next evaluated if seroconversion could occur more often. We identified 21 patients with a negative IIFT at first presentation or relapse and a serum sample stored during follow-up.

Results: Three patients with an initial negative IIFT became seropositive during follow-up. Mean proteinuria at presentation was 6.8 g/g creatinine and at follow-up 6.0 g/g creatinine and the interval between the baseline sample and the follow-up sample was 4.3 months.

Conclusions: Our data indicate that " seroconversion" can occur in patients with iMN and initially absent aPLA2R in serum. Our findings support the "kidney functions as a sink" hypothesis. We suggest that in aPLA2R negative patients PLA2R related disease must be excluded by either analysis of PLA2R in kidney biopsy or renewed evaluation of serum after 6 months follow-up.

SA-PO500

Incidence of Thromboembolism and Use of Antithrombotic Primary Prophylaxis in Nephrotic Syndrome Patients Leslie N. Smith,[1] Heather Snyder,[1,2] Joanna Hudson,[1,2] Jennifer Twilla.[1,2] *[1]Pharmacy, Methodist Univ Hospital, Memphis, TN; [2]Clinical Pharmacy, Univ of Tennessee, Memphis, TN.*

Background: Compared to the general population, patients with nephrotic syndrome (NS) have a higher incidence of venous thromboembolism (VTE) ranging from 3% to 19%. Established risk factors include proteinuria, hypoalbuminemia, disease etiology, and time from diagnosis. However, there are no randomized trials to guide optimal management of hypercoagulability. The purpose of this study was to evaluate the incidence of VTE relative to bleeding events in NS patients receiving prophylactic antithrombotic therapy.

Methods: A retrospective chart review of patients diagnosed with NS between 10/2007 and 12/2012 was conducted. Patient admissions were classified as those with new-onset VTE (VTE group) or without VTE (no VTE group). Other pertinent data was collected including risk factors for VTE and bleeding events.

Results: A total of 532 patient admissions were reviewed with 500 that met inclusion. There were 30 admissions in the VTE group with 34 VTE events (6.8%) overall. Of these, 16 events (47%) occurred within 6 months of diagnosis. Compared to the no VTE group, patients in the VTE group had a lower serum albumin (2.2 +/- 0.7 versus 2.5 +/- 0.8 g/dl; p=0.03), higher prevalence of membranous nephropathy (23.3% versus 6.8%; p=0.005), and lower use of anticoagulant (0% versus 1%; p=NS) and antiplatelet (38% versus 43%; p=NS) primary prophylaxis. In the patient subset without prior VTE (n=449), statin use was significantly higher compared to the VTE group (45% versus 23%; p=0.04). Bleeding was reported in 12 of 500 patients (2.4%) with two patients on warfarin and four patients on antiplatelet therapy at the time of event.

Conclusions: In NS patients with VTE risk factors and a low risk of bleeding, clinicians should consider use of prophylactic anticoagulant or antiplatelet therapy. For primary prophylaxis, use of statins may be beneficial for VTE prevention. However, randomized clinical trials are needed to make a strong recommendation for statin therapy in all NS patients.

SA-PO501

The Clinical Features of PLA_2R-Related Idiopathic Membranous Nephropathy Ningxin Xu, Qionghong Xie, Yan Li, Chuanming Hao. *Div of Nephrology, Huashan Hospital, Fudan Univ, Shanghai, China.*

Background: Idiopathic membranous nephropathy (iMN) could be categorized into PLA_2R-associated and non-PLA_2R-associated iMN, based on the presumption that different insulting auto-immune mechanisms are involved in iMN. This study aimed to examine whether the non-PLA_2R-associated iMN has any difference in clinical features compared with PLA_2R-associated iMN.

Methods: A total of 231 adult patients diagnosed as iMN during the past 5 years were recruited to this retrospective study. Renal PLA_2R was detected by immunofluorescence using a commercial available anti-PLA_2R antibody(produced in rabbit, Sigma). Among these patients, 186 (80.5%) with complete baseline clinical data were used for further study. For those patients with follow-up duration longer than 90 days, the relationship between renal PLA_2R and sensitivity to immunosuppresants were analyzed.

Results: Renal PLA_2R positive rate in the iMN patients was 81.8% (189/231).Among the 186 patients with complete baseline data, the baseline serum creatinine levels, serum albumin and 24 hours urine protein excretion were not significantly different between PLA_2R-associated (n=145) and non-PLA_2R-associated iMN patients (n=41). However, about 1/3 of the non-PLA_2R associated iMN had abnormal serological tests, significantly more common than PLA_2R-associated iMN (31.7% versus 8.3%, p=0.000). PLA_2R-associated iMN patients had higher levels of uric acid (p=0.001) and more severe hyperlipidemia (p<0.05 for total cholesterol and triglycerides), while the non-PLA_2R-associated iMN had lower C4 levels (p=0.004) and higher conjugated bilirubin (p=0.020) in serum when compared. The non-PLA_2R-associated iMN patients also showed a better response to immunosupressants (CR 37.5%; PR 12.5%) compared with PLA_2R-associated iMN (CR 2.9%; PR 48.6%, p=0.004) at 3rd month.

Conclusions: There were no significant differences in serum creatine, albumin and 24 hours urine protein excretion between PLA_2R-associated and non-PLA_2R-associated iMN, while the non-PLA_2R-associated iMN patients showed more abnormal serological tests. The non-PLA_2R-related iMN seemed to respond more quick to the immunosuppressive therapy compared with PLA_2R-associated iMN.

SA-PO502

Low Prevalence of Anti-PLA2R Antibody in Patients with Idiopathic Membranous Nephropathy in Japan Shin'ichi Akiyama,[1] Enyu Imai,[2] Seiichi Matsuo,[1] Shoichi Maruyama.[1] *[1]Nephrology, Nagoya Univ Graduate School of Medicine, Japan; [2]Nakayamadera Imai Clinic, Japan.*

Background: We previously first reported that a prevalence of circulating anti-phospholipase A2 receptor antibody (aPLA2R) in Japanese patients with idiopathic membranous nephropathy (iMN), which was measured by standard Western blot (WB) techniques, was 49.5% (n=109). Here, we validated the low prevalence in Japanese patients with iMN by enhanced WB techniques and commercial kit.

Methods: We measured autoantibodies in plasma samples obtained at the time of biopsy from a total of 115 patients with iMN who had not yet received immunosuppressive treatment. Circulating aPLA2R was analyzed by three different techniques: WB with human glomerular extract (HGE), WB with recombinant phospholipase A2 receptor (rPLA2R), commercial indirect immunofluorescence cell-based assay (IIF-CBA) from Euroimmun AG. The WB performed with diluted plasma at x1, x0.1 and x0.04. The CBA were performed according to the manufacture's instructions.

Results: The prevalence of aPLA2R in patients with iMN which was measured by WB with HGE, WB with rPLA2R and IIF-CBA were 54% (62/115), 54% (62/115) and 50% (57/115), respectively. When the analysis was limited to nephrotic patients (Urinary protein ≥3.5g/day), the prevalence were 58% (43/74), 58% (43/74) and 54% (40/74), respectively.

Conclusions: The prevalence of aPLA2R in Japanese patients with iMN varied 50% (IIF-CBA, All patients) to 58% (WB, Nephrotic patients). Our study demonstrated once again that the prevalence of iMN caused by aPLA2R is lower in Japanese iMN compared with those in Caucasian and Chinese.

Funding: Government Support - Non-U.S.

SA-PO503

Association of PLA2R and HLA-DQA1 Genetic Variants with Serum Anti PLA2R Antibody and Clinical Outcome in Indian Patients with Idiopathic Membranous Nephropathy

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Background: M-type phospholipase A2 receptor (PLA2R), is the first autoantigen identified in adult patients with idiopathic membranous nephropathy (IMN). Risk alleles in PLA2R1 and HLA-DQA1 genes are associated with IMN. Whether these alleles are associated with the anti- PLA2R antibody serum level is not clear. To find the association of 5 SNPs in the PLA2R1 (1 in 5' UTR, 3-coding and 1-noncoding region) and 1 in the HLA-DQA1 gene with development of IMGN and their association with anti-PLA2R antibody levels and clinical outcome.

Methods: A total of 101 adult patients IMGN patients requiring treatment were enrolled in the study. PLA2R antibody (ELISA, EUROIMMUN) was estimated in serum samples collected before treatment, and after 6 and 12 months of therapy. Genotype analysis was done on DNA isolated from PBMCs of recruited patients and 95 healthy controls TaqMan SNP genotyping assays (ABI, Foster city, CA) for 6 SNPs (PLA2R: rs3749119, rs3749117, rs4664308 and rs 3828323; HLA-DQA1: rs2187668). Patients were followed up monthly for a period of 12 months to gauge response.

Results: Out of 101 nephrotic patients, 65 (64%) showed elevated serum anti-PLA2R antibody levels. The distribution of PLA2R rs3749119, rs3749117, rs4664308 and HLA-DQA1 SNPs was significantly associated with IMN. rs3749119 (TT+CT genotype (p=0.04) and T-allele), rs2187668 (AA+AG genotype (p=0.0025) and A-allele), rs4664308 (AA genotype (p=0.048) and A-allele) were associated with elevated PLA2R antibody levels. Of the 62 patients who have completed 12 months of follow-up, 71% achieved remission. Reduction in serum PLA2R (86%) correlated significantly with remission rate (p=0.0003). There were no significant association of the six genetic variants with clinical response to immunosuppressive therapy.

Conclusions: Presence of certain PLA2R1 and HLA-DQA1 genotypes confers risk for IMN. rs3749119, rs2187668 and rs4664308 correlate with elevated PLA2R antibody levels. However, these genotypes do not contribute in predicting response to immunosuppressive in Indian IMGN patients.

Funding: Government Support - Non-U.S.

SA-PO504

Urine Podocyte mRNA Excretion Is Predictive Biomarker of Therapeutic Efficacy in Patients with Idiopathic Membranous Nephropathy

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Background: Podocyte depletion in various glomerular diseases is considered as a major mechanism driving glomerulosclerosis. Podocyte cell lineage specific mRNAs can be recovered from urine pellets, and urine podocyte (u-pod) mRNA excretion could serve as useful glomerular disease biomarker in model system (Fukuda A et al. KI 2012). The purpose of this study was to test whether u-pod mRNA excretion is a useful biomarker in nephrotic patients with idiopathic membranous nephropathy (IMN).

Methods: From January 2009 to December 2013, early morning voided urine samples were obtained from 28 healthy volunteer and 17 patients with histology-proven IMN. We examined u-pod mRNA excretion and urine protein/creatinine ratio (u-PCR) at pre-treatment (renal biopsy), and evaluated at 6 months and a median follow-up of 38 months after treatment. We also evaluated the histological findings of podocyte depletion which was estimated by TLE4 immunoperoxidase staining of podocyte density counting methods (Venkatareddy M et al, JASN 2014).

Results: Compared with controls, u-pod mRNA excretion was significantly increased in patients with IMN at renal biopsy (P<0.01). U-pod mRNA excretion did not correlate with histological findings of podocyte depletion in patients with IMN. U-pod mRNA excretion before treatment in non-remission (NR) groups (u-PCR≥0.3) (n=11) tended to be increased compared with complete remission (CR) group (u-PCR<0.3) (n=6) at 6 months after treatment (p=0.49). This urinary biomarker before treatment in NR group (n=9) was significantly increased compared with CR group (n=8) at final follow-up (median, 38 months) (p=0.03). However, the histological findings of podocyte depletion before treatment were not different between these two groups.

Conclusions: These results suggest that u-pod mRNA excretion could be useful non-invasive biomarkers of on-going podocyte damage. On the other hand, the histological findings of podocyte depletion could indicate the accumulation of podocyte loss in patients with IMN.

SA-PO505

Presence of M-Type Phospholipase A2 Receptor Antibody in Membranous Nephropathy

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Background: Investigating the frequency of M-Type phospholipase A2 receptor antibody (Anti-PLAR2) seropositivity in patients with IgA and membranous nephropathy (MN).

Methods: Presence of Anti-PLA2R was investigated by using indirect immunofluorescent method in the current serum samples of the patients. Seropositivity at 1/10 dilution was accepted as significant. 108 patients with MN (97 primary, 11 secondary), 44 with IgA nephropathy and 30 healthy volunteers were included in the study irrespective of renal function.

Results: Anti-PLA2R was found to be negative in the healthy volunteers, patients with secondary MN and patients with IgA nephropathy. Anti-PLA2R was positive in 56% (n: 54) of the patients with primary MN, in 92.6% (n: 50) of the patients who were not in remission and only in 7.4% (n: 4) of the patients who were in remission. Among the Anti-PLA2R positive patients, proteinuria was >3 gr/day for 79% (n: 43) and <1 gr/day for 5.6% (n: 3). Among the Anti-PLA2R negative patients, proteinuria was <1 gr/day in 47.5% (n: 19) and >3 gr/day 30% (n: 12). (p<0,001). A positive correlation was noted between the Anti-PLA2R seropositivity and proteinuria (R=0.363, p<0.007). In a multivariable analysis, which included the patients who did not respond to the treatment, Anti-PLA2R positive patients had higher levels of proteinuria (7.2±3.7 versus 5.3±4.1 gr/day; p=0.034) and lower GFR (69.1±31.6 versus 90.8±53.1 ml/min/1.73m²; p=0.026) as compared to Anti-PLA2R negative patients.

Conclusions: In patients with primary MN, there is a correlation between Anti-PLA2R positivity and quantitative proteinuria, which can be a surrogate marker for disease progression.

Funding: Government Support - Non-U.S.

SA-PO506

Eculizumab Reduces Terminal Complement, Complement Alternative Pathway Activation, Inflammation, Endothelial Damage, Thrombosis and Renal Injury Markers in Patients with Atypical Hemolytic Uremic Syndrome

Roxanne Cofiell, Anjli Kukreja, Krystin A. Bedard, Yan Yan, Angela P. Mickle, Masayo Ogawa, Camille L. Bedrosian, Susan Faas. *Alexion Pharmaceuticals, Inc., Cheshire, CT.*

Background: To understand how eculizumab (ECU) acts in patients (pts) with atypical hemolytic uremic syndrome (aHUS), we studied markers of complement and endothelial (EC) activation, inflammation, EC damage, thrombosis and renal injury (RI) prior to and during ECU treatment (tx).

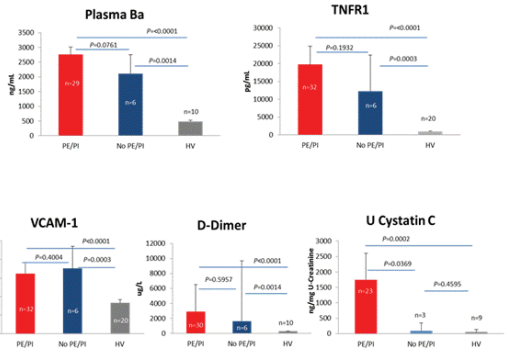
Methods: Markers were evaluated in healthy volunteers (HV; n=9–20) and adult aHUS pts (n=26–38) at baseline and >1 year of ECU tx in a prospective clinical trial.

Results: Prior to ECU, markers were significantly elevated (Table), regardless of mutational status, in pts on plasma exchange/infusion (Figure), or with normal platelets, LDH or haptoglobin values. On ECU, C5a, sC5b-9 and RI markers rapidly fell to the HV range; sTNFR1, thrombomodulin, F1+2 and D-dimer levels decreased to 77–99%, and Ba and sVCAM-1 (complement alternative pathway [CAP] and EC activation) levels decreased (mean of 30 and 60%, respectively) but remained elevated.

Conclusions: aHUS pts not receiving ECU showed CAP and terminal complement (TC) activation and elevated markers of inflammation, coagulation, EC activation and damage, thrombosis and RI. ECU normalized TC markers, reduced EC damage markers to near-normal levels, reduced inflammatory and thrombosis markers and eliminated signs of RI; ongoing ECU sustained improvement. Elevated sVCAM-1 and plasma Ba show chronic EC and CAP activation, but tissue damage markers are decreased with ongoing ECU. These data highlight the chronic complement dysregulation and risk of TMA and organ damage in aHUS pts, and the need for continued TC blockade, even when clinical presentation and lab values have improved. An analysis correlating decreases in marker levels with clinical outcomes is under way.

Disease Process	Marker (HV range, units)	BL Median Levels (*P* Value vs HV*)	n/N (%) Elevated at BL	BL Fold Increase over HV	Median Levels, During ECU (*P* Value vs HV)
CAP activation	Plasma Ba (388.0–588.0 ng/mL)	2676.4 (<0.0001)	35/35 (100)	5.53	1451.0 (<0.0001)
TC activation	U-C5a (0.0–0.7 ng[a])	9.0 (0.0007)	26/29 (89.7)	45	0.3 (0.3694)
	U-sC5b-9 (0.0–0.6 ng[a])	30.5 (0.0025)	23/27 (85.2)	305	0.15 (0.2552)
Inflammation	sTNFR1 (407.3–1391.3 pg/mL)	17616.9 (<0.0001)	38/38 (100)	18.71	4138.2 (<0.0001)
EC activation	sVCAM-1 (159.2–444.7 ng/mL)	659.8 (<0.0001)	36/38 (94.7)	1.99	569.1 (<0.0001)
EC damage	Thrombomodulin (2.0–3.6 ng/mL)	10.0 (<0.0001)	33/34 (97.1)	3.64	3.30 (0.0220)
Coagulation	F1+2 (82.9–305.5 pmol/L)	1017.6 (<0.0001)	36/38 (94.7)	5.46	494.7 (<0.0001)
	D-dimer (157.0–395.9 µg/L)	2735.0 (0.0002)	34/36 (94.4)	9.84	510.0 (0.0070)
Renal injury	U-clusterin (5.7–437.1 ng[a])	1232.3 (<0.0001)	24/29 (82.8)	8.62	200.0 (0.305)
	U-L-FABP-1 (0.0–16.9 ng[a])	58.0 (0.0130)	22/29 (75.9)	48.33	5.55 (0.1309)
	U-β2m (0.0–2.7 µg[a])	18.4 (<0.0001)	20/28 (71.4)	46	0.25 (1.0)
	U-cystatin-C (0.3–301.3 ng[a])	1256.9 (0.0001)	18/26 (69.2)	23.85	40.5 (0.4834)

*P values were calculated from the Wilcoxon rank sum test, testing for a difference between groups and measured at weeks 49–54, with the exception of urine markers, which were measured at weeks 17–30. [a]Normalized to urinary creatinine (units per mg U-creatinine).
β2m, β2 microglobulin; BL, baseline; CAP, complement alternative pathway; EC, endothelial; ECU, eculizumab; HV, healthy volunteers; L-FABP-1, liver fatty acid binding protein-1; sTNFR1, soluble tumor necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TC, terminal complement; U, urine.



Funding: Pharmaceutical Company Support - Alexion Pharmaceuticals, Inc.

SA-PO507

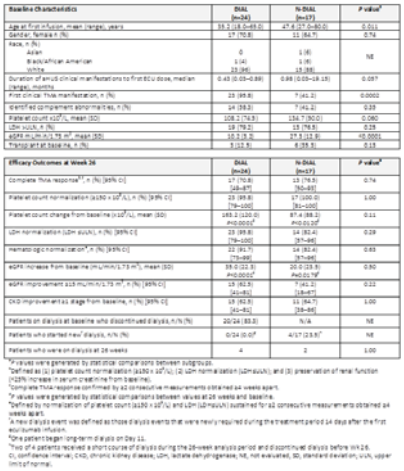
Eculizumab Safety and Efficacy in Adult Patients with aHUS, with or without Baseline Dialysis Fadi Fakhouri,[1] Camille L. Bedrosian,[2] Masayo Ogawa,[2] John Kincaid,[2] Chantal Loirat.[3] *[1]Chu Nantes, Nantes, France; [2]Alexion Pharmaceuticals, Cheshire, CT; [3]Assistance Publique-Hopitaux de Paris, Hopital Robert Debre, Paris, France.*

Background: Eculizumab (ECU) has been proven safe and effective at inhibiting terminal complement-mediated thrombotic microangiopathy (TMA), improving renal function, and eliminating the need for dialysis in many patients (pts) with atypical hemolytic uremic syndrome (aHUS). The objective of this analysis was to characterize the safety and efficacy of ECU in pts with dialysis (DIAL) and without dialysis (N-DIAL) at baseline (BL) in the largest prospective trial of adults with aHUS.

Methods: A subanalysis of an open-label, single-arm trial to evaluate safety and efficacy of ECU in pts with aHUS aged ≥18 years was performed. The primary study outcome was complete TMA response during 26 weeks (wks) of ECU therapy.

Results: BL characteristics and efficacy outcomes at Wk 26 appear in Table. ECU significantly improved plt count (x10^9/L) in both DIAL (163.2; *P*<0.0001) and N-DIAL (87.4; *P*=0.0120) pts. eGFR (mL/min/1.73 m^2) improved in DIAL (35.0; *P*<0.0001) and N-DIAL (20.0; *P*=0.0179) pts. Of BL DIAL pts, 20/24 (83%) discontinued dialysis, and all remained dialysis-free. Of N-DIAL pts, 4/17 (24%) started new dialysis and 15/17 (88%) were dialysis-free at Wk 26.

Conclusions: ECU use resulted in significant and clinically meaningful improvements in hematologic and renal parameters in BL DIAL and N-DIAL pts with aHUS at Wk 26. More than 80% of pts on dialysis at BL were able to discontinue dialysis, consistent with a prior ECU trial in adolescents and adults whereby 80% of BL DIAL pts also discontinued dialysis by Wk 26. A similar proportion of BL DIAL and N-DIAL pts were dialysis-free at Wk 26. Collectively, these data provide additional support that ECU is proven, effective, and safe in adult pts regardless of dialysis history.



Funding: Pharmaceutical Company Support - Alexion Pharmaceuticals, Inc.

SA-PO508

Eculizumab Inhibits Thrombotic Microangiopathy, and Improves Renal Function in Adult Atypical Hemolytic Uremic Syndrome Patients: 1-Year Update Fadi Fakhouri,[1] Maryvonne Hourmant,[2] Josep Maria Campistol Plana,[3] Spero R. Cataland,[4] Mario Espinosa,[5] A. Osama Gaber,[6] Jan Menne,[7] Enrico E. Minetti,[8] Francois Provot,[9] Eric Rondeau,[10] Piero Luigi Ruggenenti,[11] Laurent E. Weekers,[12] Masayo Ogawa,[13] Camille L. Bedrosian,[13] Christophe M. Legendre.[14] *[1]Chu Nantes, Nantes, France; [2]Univ Hospital, Nantes, France; [3]Hospital Clinic, Barcelona, Spain; [4]Ohio State Univ, Columbus, OH; [5]Hospital Reina Sofia, Cordoba, Spain; [6]Methodist Hosp, Houston, TX; [7]Med School Hannover, Hannover, Germany; [8]Careggi Univ Hosp, Florence, Italy; [9]CHRU Lille, Lille, France; [10]Assistance Publique des Hôpitaux, Paris, France; [11]AO Ospedali Riuniti, Bergamo, Italy; [12]CHU ULg, Esneux, Belgium; [13]Alexion Pharmaceuticals, Inc., Cheshire, CT; [14]Hôpital Necker, Paris, France.*

Background: In the largest prospective study of adults with atypical hemolytic uremic syndrome (aHUS), eculizumab (ECU) prevented thrombotic microangiopathy (TMA), and improved renal function and hematological parameters by 26 weeks (wks). Here, we report a 1-yr update.

Methods: Single-arm, Phase 2 trial of ECU in adult aHUS patients (pts); platelets <LLN at screening; prior plasma exchange/infusion and genetic screening were not required. The primary endpoint was complete TMA response at 26 wks.

Results: 41 pts treated, 38 (93%) completed 26 wks. At 1 yr, median treatment duration was 11.9 months. At Wk 26, 30 pts (73%) achieved the primary endpoint and at 1 yr, 33 pts (81%). Respective mean eGFR increases from baseline (BL) were 29.3 and 30.0 mL/min/1.73 m^2 at Wk 26 and 1 yr. 20 of 24 pts (83%) with BL dialysis discontinued by Wk 26 and remained dialysis-free at 1 yr. 4 pts with no BL dialysis began dialysis. 2 pts had meningococcal infections in the first 26 wks–both recovered; 1 continued ECU.

Conclusions: The update confirms that ECU continues to inhibit TMA in adult aHUS pts, maintaining or improving upon clinical gains seen at 26 wks.

Funding: Pharmaceutical Company Support - Alexion Pharmaceuticals, Inc.

SA-PO509

Time to End-Stage Renal Disease in Patients with Atypical Hemolytic Uremic Syndrome Receiving Supportive Care and Eculizumab

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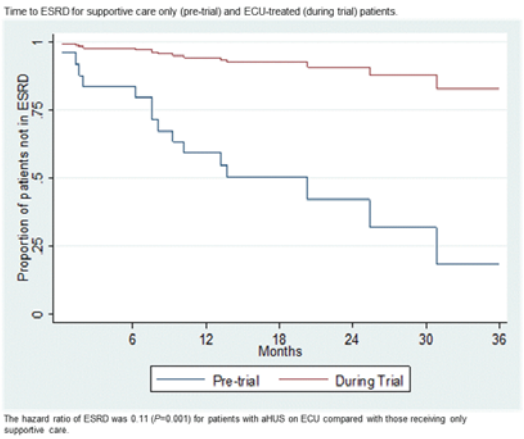
Background: Pre-eculizumab (ECU),up to 40% of aHUS patients (pts) died or progressed to end-stage renal disease (ESRD) within 1 year following a clinical manifestation. The overall 5-year mortality rate for all pts on chronic dialysis is 65%.[1] In clinical trials, ECU resulted in stabilization of renal function or reversal of renal damage. Thus, our objective was to compare time to ESRD in pts with aHUS receiving supportive care only (SCO) with those receiving ECU in 2 aHUS clinical trials.

Methods: We modeled time to ESRD (eGFR <15mL/min/1.73 m^2) using pts' initial eGFR with Kaplan-Meier and Cox proportional hazards analyses to determine the relationship of initial CKD stage and time to ESRD in pts with aHUS.

Results: 23 and 26 pts were included from pre-treatment and on-treatment data. In the pre-treatment period, 17%, 48%, and 35% were CKD stage 2, 3, and 4, respectively. Median time to ESRD among pts receiving SCO was 618 days, and the 25th percentile time to ESRD was 231 days. A Cox proportional hazards model showed that the risk of ESRD progression for pts on ECU was 0.11 (95% CI [0.03–0.42]; *P*=0.001), or an 89% reduction in the progression rate to ESRD compared with SCO (Figure). When stratifying by initial CKD stages 2, 3, and 4 at first eGFR measure, median time to ESRD for pts receiving SCO was 939 (*P*=0.134), 618 (*P*<0.01), and 231 (*P*<0.050) days, respectively. No pt initiating ECU in CKD stage 2 or 3 progressed to ESRD in 3 years; only 3 of 11 pts in CKD stage 4 reached ESRD over 3 years. The analysis did not assess if renal failure was acute or chronic.

Conclusions: The results suggest progression of renal impairment to ESRD is rapid in aHUS pts on SCO, and ECU eliminates or significantly reduces the rate of progression to ESRD. **Reference:** 1.U.S. Renal Data System 2011.



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SA-PO510

Characteristics of 406 Adult and Pediatric Patients in the Global aHUS Registry

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare, genetic, life-threatening disease of chronic complement activation leading to systemic thrombotic microangiopathy and end-organ damage. The global aHUS Registry, initiated in April 2012, prospectively collects information on patients (pts) with aHUS. Here, we report baseline demographics from pts enrolled in the aHUS Registry.

Methods: aHUS pts (regardless of treatment) are eligible for enrollment. Demographic, medical and disease histories, treatments, efficacy and safety outcomes data are collected initially and every 6 months.

Results: By March 31, 2014, 406 pts were enrolled from 13 countries. Family history of aHUS, prior kidney graft, dialysis, and PE/PI were recorded (Table). In the 6 months prior to enrollment, 52.5% reported renal symptoms, and 11% to 22% had extrarenal symptoms (GI, cardiovascular, CNS, and pulmonary). Overall, 235 pts (57.9%) received eculizumab (ECU) for a mean duration of 0.4 yrs. 46 pts discontinued ECU, with 5 of 46 pts restarting.

Conclusions: The Registry will improve understanding of aHUS and may help optimize pt care. The Registry is a successful model of partnership between industry and academia, and will provide data for additional research on multiple levels of collaboration.

Characteristic	Pediatric Patients[a] (n=161)	Adult Patients[a] (n=245)	Total (N=406)
Mean age at baseline, years (SD)	7.5 (5.2)	39.0 (15.1)	26.5 (19.6)
Race, n (%)			
Caucasian	140 (87.0)	222 (90.6)	362 (89.2)
Black/African American	4 (2.5)	12 (4.9)	16 (3.9)
Asian	4 (2.5)	3 (1.2)	7 (1.7)
Other	13 (8.1)	8 (3.3)	21 (5.2)
Country, n (%)			
United States	27 (16.8)	64 (26.1)	91 (22.4)
Germany	31 (19.3)	37 (15.1)	68 (16.7)
Italy	15 (9.3)	39 (15.9)	54 (13.3)
United Kingdom	29 (18.0)	22 (9.0)	51 (12.6)
Spain	19 (11.8)	10 (4.1)	29 (7.1)
Australia	2 (1.2)	24 (9.8)	26 (6.4)
Belgium	4 (2.5)	15 (6.1)	19 (4.7)
Russia	13 (8.1)	6 (2.4)	19 (4.7)
France	2 (1.2)	16 (6.5)	18 (4.4)
Israel	13 (8.1)	2 (0.8)	15 (3.7)
Austria	1 (0.6)	9 (3.7)	10 (2.5)
Sweden	3 (1.9)	1 (0.4)	4 (1.0)
Canada	2 (1.2)	0 (0.0)	2 (0.5)
Mean age at initial symptoms, years (SD)	3.6 (4.1)	33.1 (17.5)	21.3 (20.0)
Mean age at diagnosis, years (SD)	3.9 (4.2)	34.1 (17.2)	22.1 (20.1)
Mean disease duration from diagnosis to enrollment, years (SD)	4.3 (4.2)	5.3 (8.0)[b]	4.9 (6.7)[c]
Deceased, n (%)	0 (0)	10 (4.1)	10 (2.5)
Family history of aHUS, n (%)	33 (20.5)	48 (19.6)	81 (20.0)
History of kidney transplantation, n (%)	20 (12.4)	58 (23.7)	78 (19.2)
History of PE/PI, n (%)	86 (53.4)	166 (67.8)	252 (62.1)
History of dialysis, n (%)	84 (52.2)	153 (62.4)	237 (58.4)
Treated with eculizumab, n (%)	92 (57.1)	143 (58.4)	235 (57.9)

aHUS, atypical hemolytic uremic syndrome; PE/PI, plasma exchange/plasma infusion; SD, standard deviation.
[a]Categorized by age at enrollment in the registry. Pediatric patients were ≤17 years of age, and adult patients were ≥18 years.
[b]n=244.
[c]n=405.

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SA-PO511

Safety and Efficacy of Eculizumab in Adult aHUS Patients, with or without a History of Renal Transplant

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Background: Atypical hemolytic–uremic syndrome (aHUS) is a life-threatening, chronic disease of complement-mediated thrombotic microangiopathy (TMA). In aHUS, renal transplantation (RT) has been associated with high rates of post-RT aHUS manifestations and graft loss (~50–70% 5 yrs post-RT). Eculizumab (ECU) inhibits terminal complement-mediated TMA and is safe and efficacious in aHUS regardless of RT history (hx). This analysis reports safety and efficacy of ECU in transplant (TP) and nontransplant (NTP) pts.

Methods: A retrospective subanalysis of an open-label, single-arm trial of ECU in TP and NTP pts ≥18 yrs was performed. Pts were vaccinated against *N. meningitidis*. Inclusion criteria included platelet (plt) count <150x10^9/L, LDH ≥1.5xULN, and SCr ≥ULN. Primary outcomes were evaluated at Wk 26.

Results: There were 9 (22%) TP pts (8 pts with 1 RT; 1 pt with 2 RT). ECU improved plt count (x10^9/L) in both TP (146.2; *P*<0.0810) and NTP (132.6; *P*<0.0001) pts. eGFR (mL/min/1.73 m^2) improved in TP (19.0; *P*=0.1940) and NTP (31.5; *P*<0.0001) pts. 56% and 66% of respective TP and NTP pts achieved CKD improvement ≥1 stage. Of TP and NTP pts who had BL dialysis, 2/3 (66.7%) and 18/21 (85.7%) discontinued dialysis. Treatment-emergent adverse events were similar among groups.

Conclusions: ECU produced clinically meaningful improvements in plt count and renal function in TP and NTP pts. No pt progressed to ESRD requiring RT, and most remained dialysis-free, regardless of RT hx. These results are consistent with 2 previous subanalyses in which ECU prevented progression to graft loss, and improved renal function in both TP and NTP aHUS pts.

Baseline Characteristics	TP (n=9)	NTP (n=32)	P value[a]
Age at first infusion, mean (range), years	31.1 (18–49)	37.9 (18–63)	0.03
Gender, female n (%)	7 (77.8)	21 (65.6)	0.69
Race, n (%)			
Asian	0	1 (3.1)	NE
Black/African American	0	2 (6.3)	
White	9 (100)	29 (90.6)	
Duration of aHUS clinical manifestations to first ECU dose, median (range), months	0.38 (0.03–9.87)	0.70 (0.03–18.18)	0.63
First clinical TMA manifestation, n (%)	3 (33.3)	27 (84.4)	0.008
Identified complement mutation or autoantibody, n (%)	3 (33.3)	18 (56.3)	0.28
Platelet count (x10^9/L), mean (SD)	130.9 (59.9)	113.9 (68.6)	0.33
LDH (U/L), mean (SD)	367.6 (126.6)	928.1 (960.1)	0.76
eGFR mL/min/1.73 m^2, mean (SD)	20.0 (10.8)	18.3 (12.4)	0.04
CKD Stage, n (%)			0.14
3a/b	2 (22.2)	6 (18.8)	
4	3 (33.3)	3 (9.4)	
5	4 (44.4)	23 (71.9)	
Dialysis at baseline, n (%)	3 (33.3)	21 (65.6)	

Efficacy Outcomes at Week 26	TP (n=9)	NTP (n=32)	P value[a]
Complete TMA response[b,c], n (%)	9 (100)	19 (79.1)	0.22
Platelet count normalization (≥150 x10^9/L), n (%)	9 (100)	31 (96.9)	1.00
Platelet count change[d] from baseline (x10^9/L), mean (SD)	149.2 (140.8) P=0.0810[e]	132.6 (110.6) P<0.0001[e]	0.77
LDH normalization (LDH ≤ULN), n (%)	7 (77.8)	30 (93.8)	0.20
eGFR increase from baseline (mL/min/1.73 m^2), mean (SD)	19.0 (27.5) P=0.1940[e]	31.5 (22.9) P<0.0001[e]	0.66
eGFR improvement ≥15 mL/min/1.73 m^2, n (%)	4 (44.4)	18 (56.3)	0.71
CKD improvement ≥1 stage from baseline, n (%)	3 (33.3)	21 (65.6)	0.70
Patients on dialysis at baseline who discontinued dialysis, n/N (%)	2/3 (66.7)	18/21 (85.7)	0.44
Patients who started new[f] dialysis	1[g]	1	0.10
Patients who were on dialysis at 26 weeks	0	3	0.11

[a]P values were generated by statistical comparisons between subgroups.
[b]Defined as (1) platelet count normalization (≥150 x 10^9/L), (2) LDH normalization (LDH≤ULN), and (3) preservation of renal function (<25% increase in SCr from baseline).
[c]Complete TMA response confirmed by ≥2 consecutive measurements obtained ≥4 weeks apart.
[d]P values were generated by statistical comparisons between values at 26 weeks and values at baseline.
[e]A new dialysis event was defined as those dialysis events that were newly required during the treatment period ≥14 days after the first ECU infusion.
[f]Two of 3 patients received a short course of dialysis during the 26-week analysis period.
[g]One additional patient began long-term dialysis on Day 11.
CKD, chronic kidney disease; LDH, lactate dehydrogenase; NE, not evaluated; SCr, serum creatinine; SD, standard deviation; ULN, upper limit of normal.

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SA-PO512

Complement Biomarker Activity in Eculizumab Treated Patients Gilbert Van Schaeffer, Nicole Meyer, Dingwu Shao, Yuzhou Zhang, Richard J. Smith, Carla M. Nester. *Molecular Otolaryngology and Renal Research Laboratory, Univ of Iowa, Iowa City, IA.*

Background: Eculizumab is a monoclonal antibody directed against complement component C5. It has proven efficacy in aHUS. Isolated reports suggest effectiveness in C3G. The therapeutic level of >99mcg/mL is rarely used clinically. The compliment biomarker correlates of this level are sparse. No specific clinical test exists to directly assess the efficacy of eculizumab in patients.

Methods: We studied 11 patients treated with eculizumab. aHUS patients were in remission by hemolytic parameters. No C3G patient was in clinical remission. Usual dosing was the primary regimen. Two patients were on alternative dosing strategies. Eculizumab levels were compared with multiple assays: C5 (Binding Site Radial Immunodiffusion), C5a (Quidel EIA), C5 Functional (C5F Mayo Lab), Eculizumab level (Cambridge Biomedical), Alternative Pathway Functional Activity (APFA Wieslab) and a modification of the latter. The modified APFA consisted of patient sera mixed with normal human sera and then tested by standard APFA.

Results: Complement activity was reduced at 2 weeks in all patients. There was no correlation between the standard APFA or C5F with the eculizumab level. The eculizumab level was moderately correlated with the modified APFA (R = 0.600).

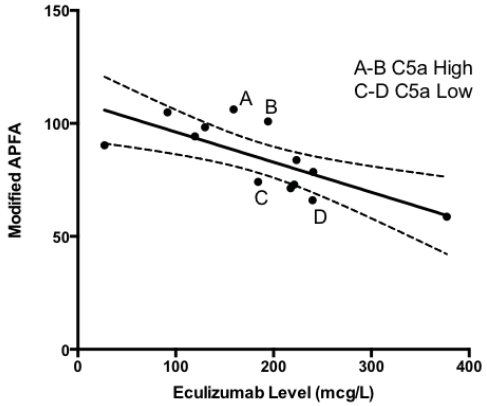

Figure 1 and values outside the 95% CI (dotted lines) correlated with the C5a level. Total C5 was significantly elevated in all patients as compared to controls and was not correlated with the eculizumab level.

Conclusions: The modified APFA had the highest correlation with eculizumab levels. C5a levels predicted non-correlation (points A-D). Assessments are ongoing to confirm the usefulness of the modified APFA as a marker of complement activity in patients treated with eculizumab. We propose this as the first step toward confirming complement blockade regardless of patient disease or dosing regimen.

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SA-PO513

Morbidity and Mortality of Shiga Toxin Associated Hemolytic Uremic Syndrome in a Contemporary Group of American Children Carla M. Nester, Larry A. Greenbaum, Myda Khalid, Stefan Kiessling, Kera E. Luckritz, John D. Mahan, Yosuke Miyashita, Michelle N. Rheault, Tarak Srivastava, Donald J. Weaver, Sharon M. Bartosh. *Midwest Pediatric Nephrology Consortium.*

Background: Shiga toxin-producing *Escherichia coli* (STEC) is the leading cause of hemolytic uremic syndrome (STEC-HUS) in the United States. There are no disease specific treatment modalities and current therapy is supportive, including fluid management, blood transfusions, and dialysis when indicated. The short-term and long-term morbidity and mortality of STEC HUS in children remains poorly defined.

Methods: A retrospective chart review was conducted within centers of the Midwest Pediatric Nephrology Consortium. Medical charts were identified using diagnosis and billing codes. Eligible patients were <18 years old. Patients with non toxin related HUS were excluded from the review. Each patient chart was quired for 145 separate data points. We present here a summary of the morbidity and mortality data.

Results: 311 patients with a clinical diagnosis of typical HUS were identified across 11 centers during the 5 year time period. The accompanying table depicts (Figure 1) the distribution of disease related events. The mortality was 1%. 44% of children required ICU care, 92% required blood transfusion and 43% required dialysis during their acute episode. Symptomatic pancreatitis was the most frequent extrarenal complication of disease with neurologic impairment being the second most likely. Many children had urine abnormalities at discharge and the majority of those patients that were followed after discharge continued to show renal dysfunction.

Morbidity and Mortality of ST-HUS in a Pediatric Population

Diagnosis	
Median of 6 days after diarrhea start	
<4 years (Median age 3.9 years)	50%
Older than 15 years	5%
Required ICU Care (Median Stay 6 Days - range 1-82)	44%
Mortality: (3 patients: Death at 6 days, 4 days and 3 days after diagnosis)	1%
Required Blood Transfusions	92%
one 17%, two 20%, three 18%, four 12%, five 8%, > 6 16% | (Median 3)	
Anuria (Median duration 5 days)	37%
Dialysis requirement during acute hospital admission (Medan duration 9 days)	43%
Renal Replacement greater than 1 week	57%
Renal Replacement greater than 2 weeks	38%
Indication: Uremia (72%), volume overload (56%), electrolyte disturbance (39%)	
CRRT 16%, HD 50%, 50% PD and 14% with more than one modality	
Complications	
Peritonitis	6%
Catheter related thrombus	5%
Blood catheter related infection	10%
Dialysis requirement at discharge	5%
Hypertension	71%
No meds 56%, 1 med 25%, 2 meds 11%, 3 meds 5%, 4 meds 3%	
Hypertension treatment significantly more likely if dialysis is required	
BP meds required at discharge	
At 1 mo 19%, at 3 mo 13%, at 6 mo 11% and 12% at both 9 & 12 months	
Urinalyses at Discharge (N= 155)	
Proteinuria of 1+ or above	82%
Hematuria	88%
Estimated GFR (intervals after discharge)	
1 month - (N = 199): < 15 in 3%, 15-30 in 2%, 30-60 in 8 %, 60-90 in 28%, > 90 in 59%	
3 months – (N = 102): < 15 in 1%, 15-30 in 5%, 30-60 in 5%, 60-90 in 16%, > 90 in 73%	
6 months – (N = 96): < 15 in 1%, 15-30 in 3%, 30-60 in 4%, 60-90 in 29%, > 90 in 63%	
12 months – (N = 90): < 15 in 1%, 15-30 in 0%, 30-60 in 7%, 60-90 in 26%, > 90 in 64%	
Complications	
ARDS	2%
Cardiac Dysfunction	1%
Pericardial Effusion	2%
Diabetes Mellitus	2%
CVA	1%
Seizure	5%
Coma	1%
Bowel Perforation	1%
Symptomatic Pancreatitis	16%
Neurologic impairment	6%

Conclusions: Of those children admitted with STEC HUS, mortality was low however morbidity was high with almost 50% requiring ICU care and/or dialysis. Thirty six percent of children were left with CKD and 12% required antihypertensive care twelve months after their episode.

Funding: Pharmaceutical Company Support - Alexion

SA-PO514

Differential Alteration in Peripheral T-Regulatory and T-Effector Cells with Change in P-Glycoprotein Expression in Childhood Nephrotic Syndrome: A Longitudinal Study Narayan Prasad, Akhilesh Jaiswal, Vikas Agarwal, Brijesh Yadav, Amit Gupta, Raj K. Sharma. *Nephrology, SGPGI, Lucknow, UP, India.*

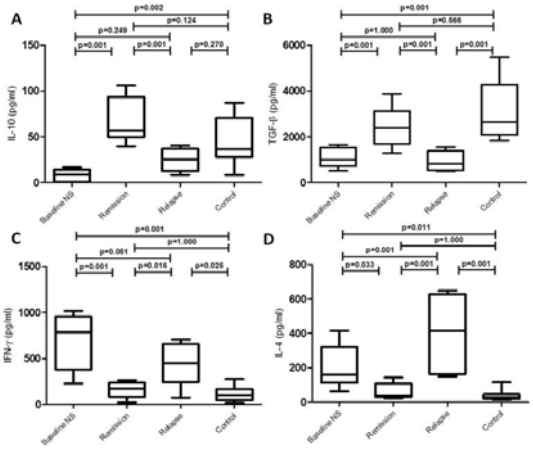
Background: We hypothesize that relapses in INS may occur due to imbalance in T-regulatory and T-effector cell and their respective cytokines and overexpression of P-gp on lymphocytes.

Methods: The frequency of peripheral blood $CD4^+CD25^+FoxP3^+$ Treg(Tregs), $CD4^+IFN\text{-}\gamma^+$ Th1(Th1) and $CD4^+IL\text{-}4^+$ Th2 (Th2) lymphocytes and their respective cytokines and P-gp expression on peripheral blood lymphocytes (PBLs) were analyzed in INS patients at baseline (n=26), during remission (n=24) and at relapse (n=15).P-gp expression was correlated with cytokine level of cultured stimulated PBMCs.

Results: Compared to baseline values(1.83±0.84),Tregs frequency was significantly increased at remission (6.82±4.12) and became similar to control(5.69±2.34) and decreased at relapse(3.03±1.18). In contrast,Th1(9.9±4.65) and Th2(4.81±1.42)frequency was significantly decreased during remission from baseline (Th116.18±7.19; Th210.5±4.66) and increased at the time of relapse(Th119.83±3.47;Th2 9.89±5.18). Similarly, expression of P-gp was high at baseline, decreased during remission and increased at relapse.

Parameters	Baseline	Remission	Relapse	Control
% P-gp positive cells	8.69±3.62	5.46±3.14	10.02±5.2	4.89±1.73
RFI	8.22±1.82	6.72±1.88	8.65±2.96	7.13±5.26
RFI×% P-gp positive cells	66.59±21.13	35.84±22.26	80.22±28.24	30.41±16.15

Cytokines Il-10 and TGF-β in supernatant of stimulated PBMCs was increased during remission and decreased at relapse. In contrast, levels of IFN-γ and IL-4 were decreased during remission and increased at relapse.



Conclusions: An increase in P-gp expression and decreased Tregs in blood at baseline and during relapse and vice versa after remission is suggestive of crucial role of T lymphocyte phenotype imbalance and P-gp overexpression in NS.

SA-PO515

Urinary Messenger RNA of Receptor Activator of NF-kappaB Could Be Useful to Differentiate Between Minimal Change Disease and Membranous Nephropathy Shuangxin Liu, Wei Shi, Li Zhang, Sijia Li, Xinling Liang, Zhiming Ye, Bin Zhang, Lixia Xu, Jianchao Ma, Zhilian Li, Wenjian Wang, Ruizhao Li, Zhonglin Feng, Wei Dong, Yiming Tao, Yuanhan Chen, Yongzhen Liang. *Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Podocyte damage and loss play an important role in the pathogenesis and progression of glomerulonephritis. Quantification of messenger RNA (mRNA) in urine by real-time PCR is emerging as a noninvasive method of screening podocyte injury and response-associated molecule. We previously demonstratedthat RANK was upregulated in podocyte of human glomerular disease, and was marker of podocyte injury response-associated molecule. We hypothesize that expression of receptor activator of NF-kappaB (RANK) in urinary sediment may provide important clinical insight into the different type of glomerulonephritis.

Methods: Glomerulonephritis patients and healthy controls were enrolled in this study. Biochemical, clinical and experimental procedures included measurement of total urinary protein, renal biopsy, and gene expression analysis of RANK. Correlations between RANK mRNA and clinical parameters were analyzed.

Results: Between July 2012 and August 2013, 145 patients with glomerulonephritis and 22 healthy controls were included. The urinary mRNA levels of RANK were significantly higher in the glomerulonephritis group compared with controls. The urinary RANK levels of glomerular subtypes was significantly correlated with proteinuria ($r = 0.72$, $p < 0.001$ for membranous nephropathy (MN); $r = 0.63$, $p < 0.001$ for IgA nephropathy (IgAN); $r = 0.58$, $p = 0.014$ for LN; and $r = 0.80$, $p < 0.001$ for focal segmental glomerulosclerosis (FSGS)). The calculated area of RANK mRNA levels under the curve was 0.61 for MCD, 0.97 for MN, 0.65 for IgAN, 0.70 for lupus nephritis (LN), and 0.70 for FSGS.

Conclusions: The urinary mRNA of RANK may be useful to differentiate histologic subtypes of glomerulonephritis, particularly between MCD and MN.

Funding: Government Support - Non-U.S.

SA-PO516

Podocyte-Associated mRNA Profile in Lupus Nephritis and Its Relation with Immunopathology Cristina Karohl,[1] Mariane Dos Santos,[1] Rafael N. Bringhenti,[2] Patrícia Garcia Rodrigues,[1] Jonathan Fraportti Do Nascimento,[1] Odirlei Andre Monticielo,[3] Andrese A. Gasparin,[3] Waldir Pedro Castro,[2] Francisco Veríssimo Veronese,[1] Rafael Zancan. *[1]Div of Nephrology, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; [2]Div of Pathology, Univ Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil; [3]Div of Rheumatology, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.*

Background: This study aimed to quantify the expression of podocyte-associated mRNAs in patients with active lupus nephritis (LN), in correlation with the immunopathology.

Methods: Thirty-three patients with LN were included, and grouped according to the presence of mild (classes I and II) or moderate-to-severe (classes III, IV and V) immune deposits, proliferation and/or inflammation. Messenger RNA of podocyte-specific proteins, and growth (VEGF-A, TGF-β1) and regulatory (FOXP3) factors were quantified by real time PCR in the kidney tissue and urine. These mRNAs were correlated with histopathology and the intra-renal cell inflammatory infiltrate.

Results: Overall, podocyte-associated mRNAs were inhibited in the renal tissue, and substantially higher levels of podocyte products were found in the urine of the patients. Urine mRNA levels were significantly higher in patients with more severe lesions, but mRNAs in kidney tissue did not correlate with class severity. In the proliferative forms, significant alterations were found to podocin, podocalyxin, TRPC-6, and TGF-β1mRNA levels, reflecting more intense podocyte injury. Urinary nephrin, podocin, podocalyxin, TGF-β1and FOXP3 mRNA levels correlated positively with proteinuria, but the podocyte mRNAs did not correlate with renal function or anti-dsDNA titers. The density of $CD8^+$ T cells, B cells and macrophages in kidney biopsy were positively correlated with the urinary podocyte mRNAs; T cells also correlated with TGF-β1and FOXP3 in biopsy.

Conclusions: Podocyte-associated mRNAs in LN were reduced in kidney tissue, irrespective of the histological class, and substantially increased in the urine, especially in biopsies with more severe lesions. Inflammatory cells were positively associated with urine podocyte mRNAs, reflecting the ongoing immune-mediated podocyte injury in LN.

SA-PO517

Morbid Obesity, Hyperinsulinemia, and Inflammation Are Associated with Podocyte Injury in Obese Individuals Cristina Karohl,[1] Mariane Dos Santos,[1] Sane Pereira,[1] Patrícia Garcia Rodrigues,[1] Jonathan Fraportti Do Nascimento,[1] João Rodolfo Telo Timm,[1] Rafael Zancan,[1] Rogerio Friedman,[2] Francisco Veríssimo Veronese.[1] *[1]Div of Nephrology, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; [2]Div of Endocrinology, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil.*

Background: Obesity is associated with glomerular lesions and podocyte injury. This study correlated the urinary expression of podocyte-associated messenger RNAs (mRNAs) with different degrees of obesity, metabolic syndrome and systemic inflammation.

Methods: Eighty-three obese or overweight patients and 18 controls were included. Expression of nephrin, podocin, podocalyxin, alpha-actinin-4, α3β1-integrin, vascular endothelial growth factor (VEGF-A), and transforming growth factor-beta ($TGF\beta_1$) mRNAs in urine was quantified by real-time PCR. mRNA was correlated with body mass index (BMI), metabolic syndrome, albuminuria, and inflammation.

Results: Patients had overweight (20.8%), obesity class I (11.9%), II (9.9%), or III (39.6%). Class III patients had higher serum lipids, plasma glucose, HbA1C, insulin resistance, and C-reactive protein levels ($p<0.05$), and 70% met criteria for the metabolic syndrome ($p=0.003$ versus other groups). Albuminuria >30 mg/g creatinine was observed in 14%, 8%, 10%, and 23% of patients with overweight, class I, class II, and class III obesity respectively ($p=0.548$). Expression of all podocyte-associated mRNAs varied with BMI, being significantly higher in the obese class III compared with controls and the other patients ($p<0.05$). Patients with overweight, class I or II obesity also had higher levels of mRNAs compared with controls: nephrin ($p=0.021$), alpha-actinin-4 ($p=0.014$), α3β1-integrin ($p=0.036$), and $TGF\beta_1$ ($p=0.005$). A higher degree of podocyturia was also found in hyperinsulinemic patients and in those with systemic inflammation.

Conclusions: In this study morbid obesity was associated with a higher degree of podocyturia even at normal urinary albumin excretion rates, suggesting that the presence of podocyte products in urine may reflect early podocyte injury in severe obesity. Hyperinsulinemia and systemic inflammation also correlated with higher levels of urinary podocyte-associated mRNAs.

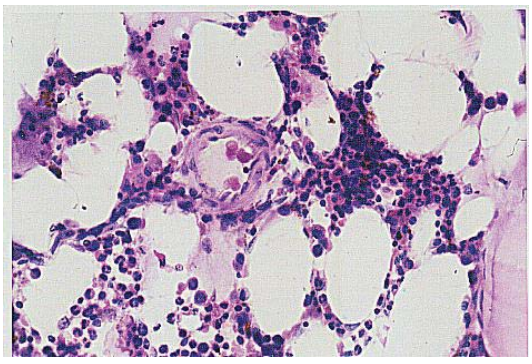
SA-PO518

Clinical and Pathological Features of Aristolochic Acid – Nephropathy Induced By Fukefenqing Pills Yanqiu Li,[1] Hua Zhou.[2] *[1]Dept of Nephrology, The First Affiliated Hospital of China Medical Univ, Shenyang, Liaoning, China; [2]Dept of Nephrology, The First Affiliated Hospital of China Medical Univ, Shenyang, Liaoning, China.*

Background: To dicuss the toxicity of the aristolochic acid in fukefenqing pills.

Methods: 14 patients who had eaten the pills for more than half a month were enrolled in the study in 2008-2009 at the department of nephrology of Chinese medical university.Collecting their clinical data and using prostaglandin E1(10ug/d)intravenous drop for 10-14 days.

Results: 7 patients underwent renal biopsy,all cases results with light microscopy examination showed renal interstitial infiltration of inflammatory cells, renal interstitial fibrosis, renal tubular atrophy or disappear, glomerular changed slightly, often accompanied by mild mesangial proliferation. Arteriole intima is not smooth, with inflammatory cell infiltration, visible stenosis or rigid wall, advanced muscle layer fracture and delamination. In a bone marrow biopsy case, part of the regional proliferation is extremely low, fat cells proliferate abnormal, interstitial edema, with micro vascular wall thickening and luminal stenosis.



Fukefenqing pills have guanmutong, it can cause Chronic interstitial nephritis and multiple organs lesion especially Anemia, the symptom is severity than others.According to the conventional dose, the daily amount of AA content up to 394.2mg, the equivalent of 50 grain of Guanxin Suhe pills.

Conclusions: The serum creatinine and hematoglobin before and after therapy were statistically different.

Table 1 treatment effect of AAN patients using prostaglandin E1

	Cr(umol/L)	Hb (mg/dl)
before	520.25±114.62	68.21±10.13
after	405.76±102.43	79.45±10.49
t value	2.8	2.9
p value	<0.05	<0.05

Aristolochic acid possibly causes abnormal of the system of prostaglandin or other blood vessel cytokines,so improving of microcirculation will be effect in therapy.

Funding: Clinical Revenue Support

SA-PO519

Decreased Tamm-Horsfall Protein Levels in Human Immunodeficiency Virus-Infected Subjects Associate with Higher Levels of Serum Tumor Necrosis Factor Alpha Receptor

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Background: Tamm-Horsfall Protein (THP), the most abundant protein in the urine of healthy humans, plays a regulatory role in renal function and was recently shown to influence systemic cytokine clearance. However, THP has not yet been investigated in HIV.

Methods: We performed a cross-sectional study of HIV-infected subjects on stable antiretroviral therapy (ART) with HIV-1 RNA of <1000 copies/mL and $eGFR_{cr}$ >50 mL/min per 1.73 m^2 to explore THP relationship with HIV-related factors and inflammation and immune activation markers. Creatinine (Cr) was measured by Jaffe's reaction. THP was quantified by Western blotting and normalized per unit urine protein. Univariable followed by multivariable linear regression was performed.

Results: 121 subjects were included; 82% were men and 65% African American. Median (Q1-Q3) age was 46 (40-52) years and $eGFR_{cr}$ 98.37 (84.92-115.79) ml/min per 1.73 m^2. Median known HIV duration was 141 (75-211) months, cumulative ART 65 (38-199) months, $CD4^+$ count 607 (425-823) cells/mm^3 and 76% had HIV-1 RNA ≤48 copies/ml. Median THP level was 116.16 (83.26-146.98) arbitrary absorbance units. THP levels were positively associated with $eGFR_{cr}$ inversely associated with the vascular inflammation marker Lp-PLA2 and the systemic inflammation marker sTNFR-I, but not with other parameters tested. Adjusting for $eGFR_{cr}$ and total urine protein, higher sTNFR-I were independently associated with lower THP level.

Conclusions: In HIV-infected patients on ART, lower urinary THP levels were associated with higher sTNFR-I even after adjustment for eGFRcr and urine total protein levels. This suggests that higher inflammation in HIV-infected subjects on ART may partially be due to lower THP. Cytokine clearance may be slower in individuals with lower levels of THF protein and this lower clearance rate might account, in part, for the inverse relationship between the two. A longitudinal study is required to confirm this novel observation.

Funding: Other NIH Support - O'Brien Center for AKI Research

SA-PO520

Association of HIV Suppression with Progression of Non-HIVAN FSGS

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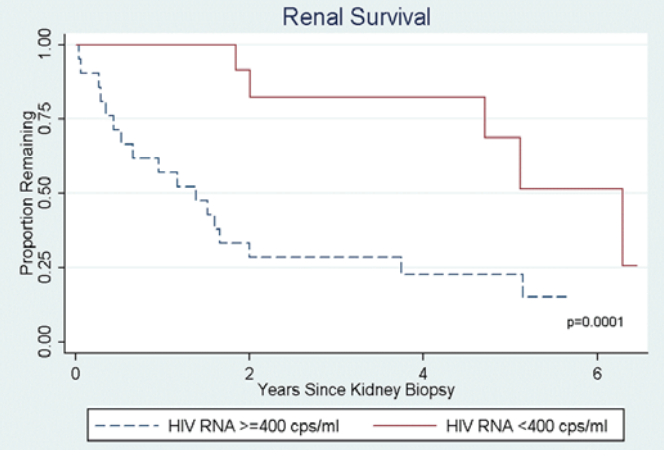
Background: In the era of potent antiretroviral therapy (ART), non-HIV associated nephropathy (non-HIVAN) FSGS is one of the most common histopathological findings in HIV+ African Americans. We aimed to examine whether HIV suppression is associated with lower ESRD risk among HIV+ African Americans with biopsy-proven non-HIVAN FSGS.

Methods: HIV+ African Americans aged ≥21 years who underwent a native kidney biopsy between 1/1996 and 6/2011 were confirmed as having non-HIVAN FSGS by the presence of FSGS without collapsing glomerulosclerosis, microcystic tubular dilatation, tubulointerstitial inflammation and tubuloreticular inclusions. Sociodemographic and clinical data were abstracted from medical records. ESRD was defined as an eGFR <15 ml/min/1.73 m^2 or dialysis initiation. Survival analyses from the time of biopsy to 1/2013 censoring for death or loss to follow-up were used to evaluate the association between viral suppression (HIV RNA<400 cps/ml at biopsy) and progression to ESRD.

Results: Among 43 HIV+ African Americans with non-HIVAN FSGS (mean age 48y, 11% women), the median CD4 count was 230 cells/μl, and 19 had suppressed viral loads at baseline. The median baseline eGFR and urine protein:creatinine ratio were 39 ml/min/1.73 m^2 and 1.3, respectively. Over a mean follow-up of 3±3 years, 22 progressed to ESRD. Those who had suppressed viral loads were less likely to progress to ESRD (Figure). Adjusting for sex and baseline CD4 count, eGFR and proteinuria, those with baseline HIV RNA levels <400 cps/ml had a 75% lower risk of progressing to ESRD (HR 0.25; 95% CI: 0.07, 0.88).



Conclusions: HIV suppression is associated with significantly lower risk of progression to ESRD among HIV+ African Americans with non-HIVAN FSGS, supporting the potential role of ART for the treatment of non-HIVAN FSGS among HIV+ African Americans.

Funding: NIDDK Support

SA-PO521

Non-Albumin Proteinuria Predominates in Biopsy-Proven Tenofovir Toxicity

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Background: Tenofovir disoproxil fumarate (TDF) is a nucleotide analog reverse-transcriptase inhibitor that can cause mitochondrial dysfunction. TDF nephrotoxicity is characterized by proximal tubular injury and dysmorphic mitochondria resulting in proteinuria, orthoglycemic glycosuria and other markers of proximal tubular dysfunction.

Methods: Patients with biopsy-proven TDF nephrotoxicity were identified from the Columbia University Medical Center Renal Pathology Lab database and their charts reviewed retrospectively.

Results: We identified 43 cases of biopsy proven TDF toxicity; mean age 54.7± 8.4 years, 53% male, 42% white. 37 cases reported dipstick proteinuria results: (0-trace: 3, 1+: 12, 2+: 13, and ≥3+: 9). 71% had dipstick hematuria. 25 patients had total protein quantified. The median proteinuria was 1800mg per 24 hours or 1667mg/gram creatinine (IQR 900mg – 2071mg); despite this only 56% had evidence of ≥2+ proteinuria on dipstick. There was a positive correlation with serum creatinine at biopsy and proteinuria (R = 0.44 P=0.028). Urinary albuminuria was quantified simultaneously (median 236 mg/gm (IQR 136.7 -343 mg/gm) for 10 patients. When both protein and albumin excretion rates were available, only 17% (IQR 13-18%) of the total protein excreted was albumin; confirming that TDF toxicity is primarily associated with non-albumin proteinuria. Among these 10 patients, urinalysis revealed orthoglycemic glycosuria in 6, and 5 had ≥2+ proteinuria by dipstick.

Conclusions: Our results suggest that only 60% (22/37) of patients with TDF nephrotoxicity have significant proteinuria by dipstick, making this is an insensitive screening tool for TDF nephrotoxicity. The simultaneous measurement of urinary albumin and protein excretion provides clinicians an inexpensive and noninvasive means of distinguishing TDF toxicity from causes of glomerular proteinuria. A low ratio of urine albumin to protein appears to be a reliable clinical feature of tenofovir toxicity, and it's diagnostic test characteristics should be elucidated by examining a large cohort of patients taking tenofovir who undergo kidney biopsy.

SA-PO522

Circulating and Urinary MicroRNA Profile in Focal Segmental Glomerulosclerosis: A Pilot Study

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Background: MicroRNAs (miRNAs) are non-coding RNA molecules that play important roles in the pathogenesis of various kidney diseases. We investigated whether patients with minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) have distinct circulating and urinary miRNA expression profiles that could lead to potential development of noninvasive biomarkers of the disease. To this end, we examined the urinary and serum miRNA profiles in patients with MCD and FSGS and compared them with subjects with no kidney disease.

Methods: Exosomal miRNAs were extracted from serum and urine samples of patients with FSGS (n=19) or MCD (n=5) and healthy controls (n=5). Differences in miRNA abundance were examined using Affymetrix GeneChip miRNA 3.0 arrays. QRT-PCR was used to validate the findings from the array.

Results: Comparison analysis of FSGS versus MCD revealed 72 and 81 differentially expressed miRNAs in serum and in urine, respectively. Only 38 of these miRNAs were

previously cited, whereas the remaining 115 miRNAs have not been described. Comparison analysis showed that a significant number of miRNAs were down-regulated in both serum and urine samples of FSGS patients compared to those with MCD. Serum levels of miR-30b, miR-30c, miR-34b, miR-34c, and miR-342, and urine levels of mir-1225-5p were up-regulated in MCD patients compared to FSGS patients and normal controls ($p<0.001$). Urinary levels of mir-1915 and miR-663 were down-regulated in FSGS patients compared to MCD and normal controls ($p<0.001$), whereas the urinary levels of miR-155 were up-regulated in FSGS patients when compared to MCD patients and normal controls ($p<0.005$).

Conclusions: Patients with FSGS and MCD have a unique circulating and urinary miRNA profile. The diagnostic and prognostic potential of miRNAs in FSGS and MCD warrants further studies.

Funding: NIDDK Support, Other NIH Support - This work is supported in part by grants R01 DK073665-01A1, 1U01DK099924-01 and 1U01DK099914-01 from the National Institute of Diabetes and Digestive and Kidney Diseases, and grant UL1TR000075 from the NIH National Center for Advancing Translational Sciences, awarded to Dominic Raj. The work was also supported by the NIDDK Intramural Research Program

SA-PO523

Focal and Segmental Glomerulosclerosis: Clinical and Kidney Biopsy Correlations

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Background: Primary focal segmental glomerulosclerosis (FSGS) can be difficult to distinguish from secondary FSGS. which can result in a number of patients with secondary FSGS undergoing unnecessary immunosuppressive therapy. We aimed to idetify clinical and histological findings that can help distinguish the two.

Methods: We reviewed the Mayo Clinic Database for patients with a diagnosis of FSGS on native renal biopsy and divided them into nephrotic syndrome(NS)-associated and non-nephrotic syndrome (NNS)-associated FSGS as a first approximation followed by dividing the lesion according to the degree of foot process effacement (FPE) on electron microscopy (EM).

Results: A total of 41 patients with an FSGS with complete evaluation were identified and divided into NS (18) and NNS (23). Baseline characteristics (age, gender, body mass index, serum creatinine) were not different between the groups. All patients with NS had diffuse FPE ranging from 80-100% (mean 96%). Conversly, of the 23 in the NNS group, 22 had segmental FPE, with all cases showing 20-70% FPE (mean of 48%).

	Nephrotic syndrome	Non Nephrotic Syndrome	P Value
n	18	23	
FPE	96 ± 5.9	48.3± 16.9	< 0.0001
FSGS Lesions (n)			
Collapsing	1	2	0.4
Tip	3	1	
Perihilar	2	7	
Cellular	2	1	
NOS	10	12	
Glomerulomegaly (Y/N)	6/12	16/7	0.02
Global glomerulosclerosis (%)	11.4 ± 13.5	30.5 ± 24.1	0.005
Tubular atrophy and interstitial fibrosis (≤25%/>25%)	18/0	18/5	0.04
Arteriosclerosis and arteriolar hylainosis (<2+/≥ 2+)	13/4	10/10	0.09

Table 1: Biopsy Characteristics

Conclusions: Adult patients presenting with NS, a FSGS lesion on LM, extensive FPE (>80%) on EM and no risk factors associated with secondary FSGS are likely to have primary FSGS. Conversely, the absence of NS, in a patient with segmental FPE on EM strongly suggests a secondary FSGS. Dividing FSGS into the presence or absence of NS together with the degree of FPE on EM examination is more helpful as it provides a more practical way to separate patients into cases of primary versus secondary FSGS.

SA-PO524

Urinary Soluble Urokinase Receptor and Alpha-1-Microglobulin Excretion in Patients with Focal Segmental Glomerulosclerosis and Chronic Kidney Disease Controls

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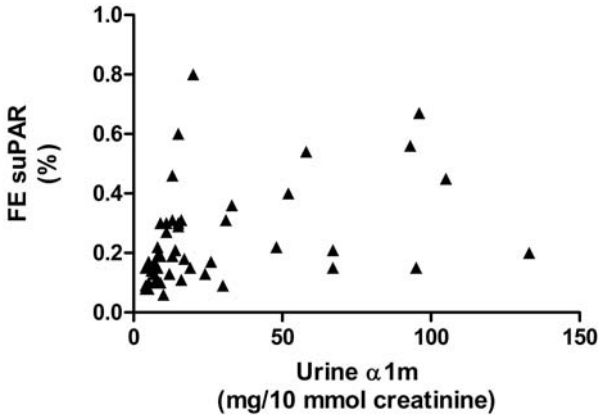
Background: Urinary excretion of suPAR has been proposed as a marker for FSGS but little is known about factors that influence suPAR excretion. We have previously demonstrated a correlation between fractional excretion of suPAR (FEsuPAR) and other low molecular weight proteins in nephrotic patients. In the present study we compared FEsuPAR with excretion of proximal tubular marker alpha-1-microglobulin (a1m) in patients with FSGS and non-FSGS CKD.

Methods: We included 27 patients with FSGS and nephrotic syndrome and 54 controls with non-FSGS CKD. Patients were matched for eGFR, age and gender. Serum and urinary suPAR were measured by ELISA (R&D Systems, Minneapolis, MN), and urinary a1m was measured by immunonephelometry in the same samples.

Results: Patients with FSGS had higher total proteinuria and lower serum albumin compared to patients with CKD. Median urinary suPAR was 281 (range 46 - 906) pmol/μmol creatinine in patients with FSGS and 236 (range 63 - 889) pmol/μmol creatinine in CKD controls ($P = 0.37$). There was a significant correlation between FEsuPAR and urinary a1m excretion in patients with FSGS (Spearman's rho 0.41; $P=0.03$), as well as in non-FSGS CKD patients (Spearman's rho 0.56; $P<0.01$, Figure 1).

Conclusions: We did not find significant differences between urinary suPAR in patients with FSGS and non FSGS CKD. There was a significant correlation between FE suPAR and urinary a1m excretion, suggesting that there is tubular reabsorption of suPAR.



Funding: Private Foundation Support

SA-PO525

Hypercholesterolemia in Nephrotic Syndrome Is Related with Enhancing Cholesterol Absorption Dependent with Niemann-Pick C1-Like 1

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Background: Although hypercholesterolemia in nephrotic syndrome is generally considered due to increased production of lipoprotein by the mechanism of compensation for hypoproteinemia, there is still no convincing evidence for this hypothesis, especially in human. We previously reported at ASN annual meeting that the serum cholestanol, a marker of cholesterol absorption, is elevated in patients with nephrotic syndrome compared to those in complete remission. Furthermore, the serum cholestanol is correlated with the severity of nephrotic syndrome. But, the relationship between nephrotic syndrome and choresterol absorption has been not fully investigated yet.

Methods: We made a comparison of lipid profile between nephrotic patients (NS) and 210 healthy subjects (CON) who were not treated with a cholesterol lowering drug and were not suffering from proteinuria. We investigated the serum cholestanol, campesterol and sitosterol, as markers of cholesterol absorption, and lathosterol as a marker of cholesterol synthesis in addition to total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride. Moreover, we examined mRNA expression of Niemann-Pick C1-like 1 (NPC1L1), that plays a pivotal role in cholesterol absorption, by using rats with puromycin aminonucleoside (PAN)-induced nephrosis.

Results: The all lipid parameters excluding high-density lipoprotein cholesterol in NS were high relative to CON. The ratio of cholesterol absorption markers (cholestanol, campesterol and sitosterol) to total cholesterol in NS were elevated compared to CON, but the ratio of cholesterol synthesis marker (lathosterol) to total cholesterol was not significantly different between NS and CON. NPC1L1 mRNA expression in the PAN-induced nephrosis approximately doubled compared to pair-fed control ($p=0.003$, $n=9$).

Conclusions: Cholesterol absorption should contribute to the mechanism of nephrotic syndrome-associated hypercholesterolemia.

SA-PO526

Effect of Metreleptin Therapy on Proteinuria in Different Forms of Human Lipodystrophy

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Background: Acquired and congenital lipodystrophies (LD) are characterized by partial or complete absence of adipose tissue, low levels of leptin with metabolic consequences including severe insulin resistance with diabetes, severe dyslipidemia and ectopic fat accumulation. Treatment with recombinant human leptin (metreleptin) has been shown to improve metabolic disease in LD. Here we report patterns of renal pathology in LD and effects of metreleptin on renal parameters.

Methods: 64 patients (pts) with LD received metreleptin for ≥12m in an open-label trial at the NIH Clinical Center from 2000-2013. Serum creatinine (Cr), Cr clearance (CrCL), and urinary protein excretion(Prot) (24 hr collection and Prot/Cr ratio) were analyzed at baseline and follow-up. Renal biopsies (bx) were performed as clinically indicated.

Results: Median age at baseline was 17y. 60% were Caucasian, 15% African American, 25% other. Median duration of therapy was 35m (range 12-149m). At baseline, pts with

congenital generalized LD (CGL) had greater Prot (P=0.017). There were no differences in baseline A1c or lipid levels between groups. Metreleptin led to decline in proteinuria in all groups but was only significant in the CGL group.

	Baseline	Last Available	Paired Test
Acquired generalized (N=13)	1831±3192	1215±2826	0.20
Acquired partial (N=5)	1099±558	579 ±754	0.25
Congential generalized (N=30)	1875±2464	759±1194	<0.0001
Familial partial (N=16)	383±528	264±246	0.92

CrCL did not significantly change with therapy (mean baseline CrCL 180 ml/min). Of 12 renal bx performed at baseline, focal segmental glomerulosclerosis (FSGS) was seen in 7, diabetic nephropathy (DN) in 3 and membranoproliferative GN in 2.

Conclusions: Proteinuria is common in patients with different forms of LD. FSGS and DN are the predominate histologies. CGL patients tended to have greater proteinuria at baseline, and better improvement in proteinuria with metreleptin. The cause of Prot and mechanisms by which metreleptin leads to improvement is unclear, but may be related to improvements in metabolic parameters and glomerular lipotoxicity.

Funding: NIDDK Support

SA-PO527

Serum 25-Hydroxy-Vitamin-D Correlation with Serum Albumin and 24-Hour Proteinuria in Glomerulopathies Epitácio Rafael da Luz Neto, Simone C. Lo, Alcino Pires Gama, Cristiane B. Dias, Lectícia Jorge, Luis Yu, Viktoria Woronik. *Div of Nephrology, Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

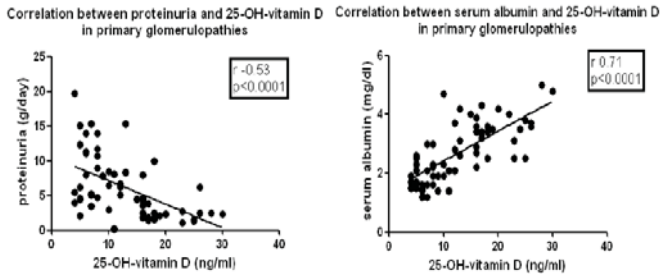
Background: Nephrotic syndrome is associated with vitamin D deficiency. Urinary losses of protein-bound intermediate metabolites of this vitamin are thought to contribute to the deficiency state as well as other factors, such as inflammatory states. The aim of this study is to evaluate possible correlations between serum 25-hydroxy-vitamin D (s25OHD) levels, 24-hour proteinuria (24PTN) and serum albumin considering nephrotic and subnephrotic states as well as inflammatory background of primary glomerulopathies (PG) in contrast to lupus nephritis (LN).

Methods: PG (n=57), LN (n=38) and others (n=22) patients medical records with proteinuria ≥ 1.5 g were reviewed. PG group was composed of FSGS (n=30), MN (n=14), IgAn (n=5), MPGN (n=4) and MCD (n=4).

Results: Subnephrotic group compared to nephrotic showed higher s25OHD levels (table 1). Considering primary glomerulopathies there was correlation between s25OHD levels and 24PTN as well as with serum albumin (fig. 1). Correlations were absent in LN group (not shown).

	Subnephrotic (n=54)	Neprotic (n=63)
Age (y)	40.9 ± 17.9	42.6 ± 16.7
Female (%)	68.5	46
24PTN (g/d)*	1.9 ± 0.8	7.5 ± 3.5
Albumin (g/dl)*	3.2 ± 0.8	2.4 ± 0.7
s25OHD (ng/ml)*	17.6 ± 8.8	12.1 ± 7.3
eGFR (ml/min/1.73 m^2)	52 ± 44	51 ± 44
Calcium (mg/dl)	4.9 ± 0.3	4.7 ± 0.3
Phosphorus (mg/dl)*	3.9 ± 1.1	4.3 ± 0.9
PTHi (pg/ml)	98 ± 85	79 ± 84

*p<0.05



Conclusions: Serum 25OHD levels in primary glomerulopathies showed negative correlation with 24PTN and positive correlation with serum albumin while in LN there was no correlation. So far, we could speculate that inflammatory state of LN is an important factor for maintaining s25OHD levels besides glomerular permeability and urinary protein loss.

SA-PO528

Role of Pituitary Adenylate Cyclase-Activating Polypeptide in the Nephrotic Kidney Benedicte Eneman,[1] Kathleen Freson,[2] Lambertus P.W.J. Van den Heuvel,[1] Chris Van Geet,[2] Henry Dijkman,[3] Elena N. Levtchenko.[1] *[1]Pediatric Nephrology, Dept of Growth and ReGeneration, Catholic Univ of Leuven, Leuven, Belgium; [2]Dept of Molecular and Vascular Biology, Catholic Univ of Leuven, Leuven, Belgium; [3]Dept of Pathology, Radboud Univ Nijmegen Medical Centre, Nijmegen, Netherlands.*

Background: Recent studies have reported a nephroprotective effect of PACAP in a variety of renal disease models. Recently, we discovered plasma PACAP deficiency in nephrotic syndrome (NS) due to urinary loss. However, renal PACAP expression in NS has never been studied. We now explored the expression of PACAP and its receptors PAC1, VPAC1 and VPAC2 in healthy and nephrotic kidney tissue and studied a potential nephroprotective role of PACAP in NS.

Methods: RT-PCR, qPCR, werstern blot and IF stainings were performed for PAC1, VPAC1 and VPAC2 in 3 renal cell lines: conditionnally immortalized (ci)proximal tubular epithelial cells (PTEC), podocytes and glomerular microvascular endothelial cells (GMVEC). IH and IF stainings for PACAP, PAC1, VPAC1 and VPAC2 were performed on kidney tissue from 4 healthy children and 2 children with congenital and 5 with acquired NS. ciPTECs were exposed to an albumin concentration range, mimicking proteinuria related damage, with and without addition of recombinant PACAP. WST-1 cell viability tests were performed.

Results: ciPTEC showed VPAC1 expression. All other cell lines did not express the 3 receptors. PACAP and VPAC1 were found in the tubular epithelial cells and VPAC1 and VPAC2 were found in the glomeruli on renal tissue of both NS patients and controls. PACAP expression was more pronounced in tubular epithelial cells of NS patients than of controls. Cell viability in ciPTECs decreased with increasing albumin concentrations. Addition of recombinant PACAP had a protective effect on cell viability.

Conclusions: Our observations provide new insights in the expression of PACAP and its receptors in the healthy and nephrotic kidney. PACAP prevents albumin induced damage of ciPTECs. The enhanced presence of PACAP in tubular epithelial cells in nephrotic kidneys is probably due to re-uptake of filtered PACAP and possibly plays a protective role in proteinuria related damage.

Funding: Private Foundation Support

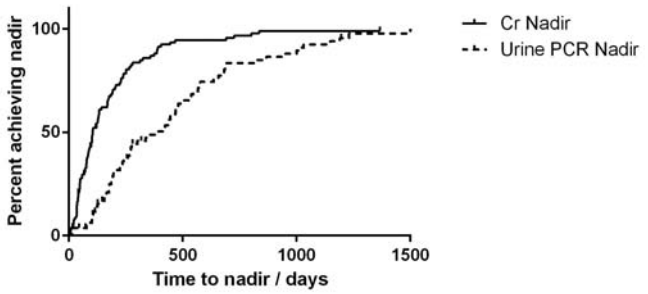
SA-PO529

Time Taken to Achieve Stable Renal Function in ANCA Vasculitis: Implications for Management and Disease Scoring Amin Oomatia,[1] Sarah Margaret Moran,[2] Claire Kennedy,[3] Sally Hamour,[1] Aine Burns,[1] Mark Alan Little,[3] Alan D. Salama.[1] *[1]Centre for Nephrology, UCL, United Kingdom; [2]Nephrology, Univ College Cork; [3]Trinity Health Kidney Centre, Ireland.*

Background: Renal involvement in ANCA associated vasculitis (AAV) is common and found in 50% of patients at presentation. Serum creatinine and proteinuria provide important information regarding renal disease activity. However, no accurate data about the time taken to achieve maximal improvement in creatinine and UPCR exist. This has implications for management and disease scoring with BVAS.

Methods: We identified the time to nadir serum creatinine and UPCR after treatment of ANCA associated glomerulonephritis (AAGN) in three seperate cohorts from UK and Ireland. Nadir was defined as a difference of < 5% between two consecutive percentage change differences in creatinine or UPCR compared to baseline. We included all patients with AAGN and renal impairment defined by eGFR<90, within 2 weeks of diagnosis. We excluded those with <3 measurements and those remaining dialysis dependent >3 months.

Results: We identified 103 patients with AAV and renal involvement, median age 64 (range 13-94) years, 50.4% male and 79.6% white British or Irish. Median presenting creatinine was 320(range 56-431) mcmol/l. MPO-ANCA was present in 55 patients, PR3-ANCA in 47 and one patient was ANCA negative. When calculating nadir for creatinine and PCR, 95(92%) and 69(67%) patients had sufficient data, respectively. Nadir creatinine was achieved at median 101 days (range 6-836), nadir PCR at 337 days (range 14-1733).



Conclusions: There is a significant delay in achieving nadirs in creatinine and proteinuria, which occur at later time points than conventionally defined clinical remission. This suggests that ongoing renal recovery is still possible for almost one year from diagnosis which should be taken into account by contemporary scoring systems.

SA-PO530

Analysis of Long-Term Morbidity in Patients with ANCA-Associated Renal Vasculitides - Data from a National Registry Vladimir Tesar, Zdenka Hruskova, Eva Jancova. *Dept of Nephrology, Charles Univ and General Univ Hospital, Prague, Czech Republic.*

Background: While long-term survival of ANCA-associated vasculitides (AAV) has been improved in the last decades, patients accumulate significant long-term disease- and treatment-related damage. The aim of this study was to describe damage in AAV patients with renal involvement included in a nationwide registry.

Methods: Clinical data from a total of 686 patients (M/F 329/357, median age at diagnosis 58 years, 89% with renal involvement) recruited from sixteen vasculitis centres participating in the Registry since 2009 were available for analysis. The damage was assessed using the Vasculitis Damage Index (VDI).

Results: VDI was available for 485/613 patients with renal involvement (biopsy-proven in 74%; 156 required dialysis anytime during the disease course and 85 were on RRT at the last visit; median S-creatinine in non-dialysed patients at the last visit 1.35 mg/dL) with median time from diagnosis 71 (range 0-480) months. The last VDI ranged between 0 and 16, with median 4; and only 42 patients (8.6%) had no items of damage. Even though the accumulation of damage increases during the long-term follow-up, it is significant in a number of patients even after the first year and seems to slow down in the subsequent years (median VDI=2.5 at 1 year, median VDI=3 at both 2 and 3 years). The most frequently scored items included those related to renal involvement: hypertension (61.2%), proteinuria (50.9%) and impaired glomerular filtration rate (48.5%). Among the other items, diabetes (17.9%), osteoporosis (17.7%), peripheral neuropathy (15.5%), cataract (14.2%) and nasal blockage/crusting (14%) were the most commonly represented.

Conclusions: In the studied population, both treatment- and disease-related damage was present, and was significant in a number of AAV patients with renal involvement. Earlier diagnosis and less toxic therapies are needed to decrease the damage burden in patients with AAV in the future.

Funding: Private Foundation Support

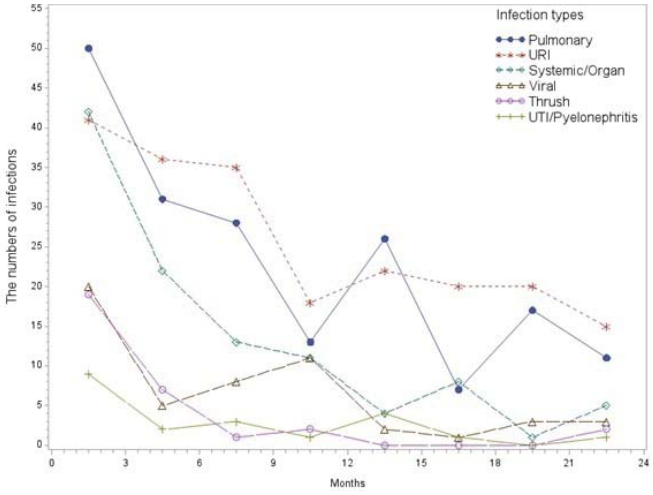
SA-PO531

Timing and Types of Infections After Diagnosis of Antineutrophil Cytoplasmic Antibody Associated Vasculitis JulieAnne G. McGregor, Caroline J. Poulton, Jason M. Kidd, Suzanne L. Katsanos, Lindsey R. Goetz, Yichun Hu, Patrick H. Nachman, Ronald J. Falk, Susan L. Hogan. *UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC.*

Background: Disease control in AAV with immunosuppression is effective but leads to serious burden of infections. Study goal was to evaluate timing and type of infections in an inception cohort with biopsy-proven AAV (1992-2011).

Methods: All patients were immunosuppressed. Infections were assessed from medical records. Severe infections were those requiring hospitalization or causing death. Induction therapy included cyclophosphamide and corticosteroids and maintenance therapy was with azathioprine, mycophenolate mofetil or rituximab. Positive cultures from all available sources were reviewed.

Results: 489 patients (median age 57 yrs; 53% male, 55% MPO-ANCA) were followed for a median of 2.8 yrs. Cumulative incidence of any infection at 1, 2 and 5 years was 51%, 58% and 65%, and of severe infections was 22%, 23% and 26%. Pulmonary and upper respiratory infections were the most common, highest in the first 3 months.



Beyond one year, there were 116 severe infections in 52 patients with 28% during relapse and 72% during maintenance therapy. In 128 patients who had cultures within 24 months, 112 patients had 192 positive cultures. Staphylococcus aureus was the most frequent organism (41%) followed by gram negatives (23%) and yeast (15%). There was one Pneumocystis carinii pneumonia 6 weeks following treatment initiation.

Conclusions: Prophylactic therapy targeted to reduce pulmonary and upper respiratory as well as S. aureus infections could be critical to decrease infectious morbidity in AAV, especially in the first 3 to 12 months. Burden of severe infections beyond 1 year is more commonly associated with maintenance therapy than relapse treatment.

Funding: NIDDK Support

SA-PO532

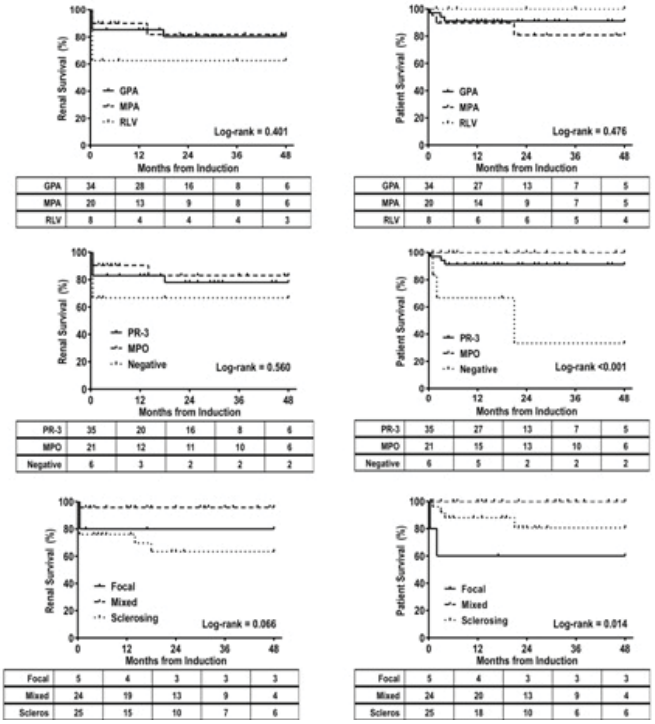
Clinical Presentation and Outcome Prediction of Clinical, Serological and Histopathological Classification Schemes in ANCA-Associated Vasculitides Juan M. Mejia-Vilet, Bertha Manuela Cordova Sanchez, Norma O. Uribe-Uribe, Luis E. Morales-Buenrostro, Ricardo Correa-Rotter. *Nefrologia y Metabolismo Mineral, Inst Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico.*

Background: Various classification schemes have been developed for ANCA associated vasculitis (AAV), with actual debate focusing on their clinical and prognostic performance.

Methods: 62 renal biopsy proven AAV were retrospectively analyzed and classified in clinical (granulomatosis with polyangiitis [GPA], microscopic polyangiitis [MPA], renal limited vasculitis [RLV]), serological (PR3-ANCA, MPO-ANCA, ANCA-Negative) and histopathological (focal, crescenteric, mixed, sclerosing) groups and followed up until death, loss to follow-up or october 2013. Outcomes included remission, relapse, renal and px survival.

Results: In this mexican-mestizo population, GPA was the most frequent AAV while RLV and ANCA-negative subgroups presented with the worst renal disfunction. PR3-ANCA patients had a higher probability of relapse (HR 2.06, p=0.043). Clinical and serological classification did not predicted remission, renal or patient survival. Histopathological classification showed poorer remission and renal prognosis for sclerosing group and those with <25% normal glomeruli, and adequately separated 24 mo clinical evolution, but it did not predicted patient survival.

Figure 1. Renal survival (a,b,c) and Patient survival (d,e,f) at 48 months according to clinical (a,d), serological (b,e) and histopathological (c,f) classification.



Renal replacement therapy (RRT) requirement (HR 35.4, CI 3.84-326, p=0.003) and proteinuria (HR 1.7, CI 1.13-2.56, p=0.011) at diagnosis predicted renal survival, while age (HR 1.08, CI 1.01-1.15, p=0.044) predicted patient survival.

Conclusions: ANCA serological classification may predict AAV relapses, but neither clinical or serological categories predict renal nor patient survival. Renal function and proteinuria at presentation, age and histopathology constitute the main outcome predictors and should be considered for individualized management.

SA-PO533

Monoclonal Gammopathy Detection in ANCA-Associated Vasculitis – Attack of the Clone? Sarah Margaret Moran,[1] Mark Alan Little,[2] Michael Clarkson.[1] *[1]Renal Medicine, Cork Univ Hospital, Cork, Ireland; [2]Trinity Kidney Health, Trinity College Dublin, Ireland.*

Background: Immunoglobulin (Ig) levels are routinely used as surrogate markers of the degree of immunosuppression in patients with anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV). Incidental detection of a monoclonal immunoglobulin (MIg) band may raise concern about the presence of an underlying plasma cell dyscrasia. MIg are detected in 1 - 3.2% of the general population. While ANCA are most commonly of the IgG subtype concomitant IgM ANCA may also occur in up to 1/3 of patients with pulmonary haemorrhage.

Methods: Laboratory records of all patients attending a Regional Vasculitis Clinic were searched for MIg reported as part of their immunoglobulin profile. Detailed clinical and laboratory parameters were also recorded.

Results: 90 patients were identified. 21 (23%) had MIg bands, most commonly IgG. 17 (81%) of the patients with MIg were ANCA positive. There was an equal distribution of MPO (8) and PR3 (9) specificity.

	Monoclonal IgG	Monoclonal IgM	Total Monoclonal Ig
ANCA positive	16	1	17
ANCA negative	2	2	4
Total	18	3	21

MIgG levels in the ANCA positive patients: Range 3.04 -18.07g/L; Mean 10.42g/L; Only 3/17 had an absolute IgG value above the normal range. MIgM levels in both ANCA positive and negative patients:Range 0.93 - 5.5g/L; 2/3 patients had absolute IgM values above the normal range. ANCA titres in MIg band patients: Range 3.8-214 IU/mL; Mean 59.7IU/mL. ANCA titres in non-MIg band patients: Range 4.6-191 IU/mL; Mean 59IU/mL. No patients were developed plasma cell dyscrasias during follow up.

Conclusions: The incidence of MIg in this cohort is at least 8 times higher than the general population (23% versus 1-3%). MIg may reflect a circulating monoclonal ANCA. Further investigation needed to ascertain whether column bead extraction of ANCA leads to resolution of the MIg band.

Funding: Clinical Revenue Support

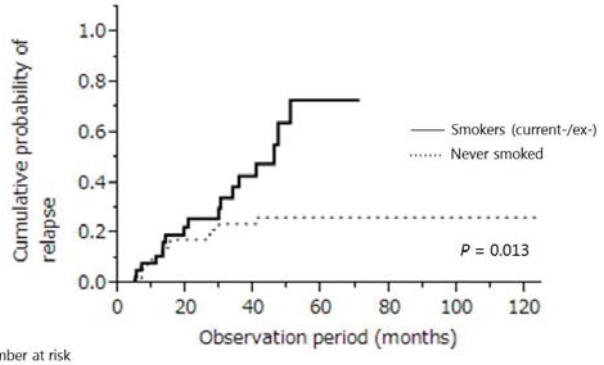
SA-PO534

Smoking Is a Risk Factor for the Relapse of Antineutrophil Cytoplasmic Antibody (ANCA)–Associated Vasculitis Makoto Yamaguchi, Sawako Kato, Takuji Ishimoto, Tomoki Kosugi, Waichi Sato, Naotake Tsuboi, Masashi Mizuno, Yasuhiko Ito, Seiichi Matsuo, Shoichi Maruyama. *Dept of Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan.*

Background: Several studies have shown predictors of relapse in antineutrophil cytoplasmic antibody (ANCA)–associated small-vessel vasculitis. However, whether smoking increases the risk for the relapse is unknown.

Methods: This is a multicenter retrospective cohort study, including 123 patients with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) from 7 nephrology centers in Japan. The dose-response relationships between cigarette smoking and the outcomes were assessed by using multivariate Cox proportional hazards models adjusted for clinically relevant factors. The primary and secondary outcomes were first relapse and first remission, respectively. A relapse was defined as clinical signs of vasculitic activity in any organ system. Remission was defined as the absence of clinical signs and symptoms of active vasculitis (BVAS.0).

Results: Throughout the observation period (median, 40 months; interquartile range, (20–67 months), 114 (92.7%) and 33 (28.9%) patients developed at least 1 remission and 1 relapse, respectively. Log-rank test showed that smokers were significantly associated with relapse ($P = 0.013$).



Multivariate Cox proportional hazards models revealed current smoking (adjusted hazard ratio [HR], 6.60 [95% confidence interval (CI), 2.39–18.4]) and cumulative smoking of ≥50 pack-years (adjusted HR, 5.05 [95% CI, 1.70–14.7]) to be associated with relapse. However, smoking was not associated with remission.

Conclusions: Smoking is a significant and dose-dependent risk factor for the relapse of ANCA–associated vasculitis. All patients with ANCA–associated vasculitis who smoke should be encouraged to quit.

SA-PO535

Long Term Damage in Patients with ANCA Associated Vasculitides Marilina Antonelou, Suceena Alexander, Anne Frances Doyle, Massimiliano Morreale, Stephen Paul McAdoo, Anisha Tanna, Charles D. Pusey, Ruth M. Tarzi. *Imperial College Kidney and Transplant Inst, London, United Kingdom.*

Background: ANCA-associated vasculitis (AAV) is associated with significant long term damage due to the disease itself and its treatments.

Methods: We collected retrospective clinical data from 265 patients with AAV between 1976-2012, and assessed the burden of disease and treatment-associated damage in our cohort over this time period.

Results: 124 patients (47%) were male. The median age at presentation was 55±16.8. Twenty six percent of patients were MPO-ANCA positive, 44% PR3-ANCA, 12% MPO- and PR3-ANCA and 20% ANCA negative. The mean follow up was 9.6±0.8 years. Forty three percent of MPO-ANCA patients and 54% PR3-ANCA patients experienced one or more relapses during follow up. During the time period management has evolved with a reduction in cyclophosphamide (CYP) exposure and increasing use of rituximab along with corticosteroids for induction. Azathioprine (AZA) or mycophenolate mofetil (MMF) were used for maintenance. The median cumulative dose of oral CYP was 5.0±50.9g, pulse CYP 3.5±4.4g and corticosteroids 12.1±47.6g. Median duration of treatment with AZA was 3.6±55.5 years and MMF 2.7±2.9 years. Long term complications occurring after the diagnosis of AAV included hypertension (31.3%), cardiovascular disease (20%), diabetes (12.9%), cataract (10.9%), venous thromboembolism (9.1%) and end-stage renal failure (14%). An association between exposure to steroids and osteoporosis was noted ($p= 0.001$). Thirty three cancers developed in 29 patients, including non-melanoma skin cancers (10), lymphoma (3), leukaemia (1), bladder cancer (1). Nine per cent of patients died during active follow up. Causes of death included cardiovascular disease (7), infection (5), active vasculitis (3). There was a higher incidence of cancer (all cause) among patients treated with oral (19/84, 22.6%) versus pulse CYP (4/70, 5.7%) ($p<0.05$). There was an association between duration of AZA use and cancer (all cause) ($p<0.05$).

Conclusions: Management of cardiovascular risk factors and bone health is an important aspect to the chronic management of AAV. There is a need to reduce the toxicity of therapies in current use.

SA-PO536

Urinary and Serum Soluble Urokinase Receptor Levels Predicts the Therapeutic Responsiveness of Nephrotic Syndrome and ANCA-Glomerulonephritis Keiji Fujimoto, Hiroki Adachi, Hiroshi Okuyama, Hideki Yamaya, Hitoshi Yokoyama. *Div of Nephrology, Kanazawa Medical Univ, Uchinada, Ishikawa, Japan.*

Background: The relationship between the therapeutic responsiveness of nephrotic syndrome (NS) and the changes of urinary (u-) and serum (s-) soluble urokinase receptor (suPAR) levels after therapy and the activation of β3 integrin is still unclear.

Methods: We investigated s- and u-suPAR levels in NS (37 cases) and ANCA-glomerulonephritis (ANCA-GN) (13 cases) and 20 healthy control subjects. We also examined the renal expression of activated β3 integrin (AP-5) by immuno-staining in primary NS, crescentic GN and normal tissues.

Results: The pretreatment s- and u-suPAR levels in NS or ANCA-GN were higher than those in the normal controls, but no differences were noted among the pathological types of NS and ANCA-GN. The pretreatment s-suPAR levels were correlated inversely with eGFR in NS and ANCA-GN, and positively with CRP in ANCA-GN. In addition, a positive correlation between pretreatment u-suPAR and proteinuria was noted in NS. By the time-course changes in the s- and u-suPAR levels over 2 months after therapy (Δ2M s- and u-suPAR), we could differentiate intractable NS from non-intractable NS (Δ2M s-/u-suPAR, $p=0.005$, $p=0.007$, respectively), and MCNS from FSGS (Δ2M s-/u-suPAR, $p=0.007$, $p=0.048$, respectively). The s-suPAR levels at 2 months were 3,373 pg/ml or higher in intractable NS (n=27, AUC=0.913, $p=0.002$). In a multiple regression analysis ($R^2=0.41$, $p=0.007$), therapeutic response was a significant predictor of Δ2M s-suPAR, whereas CRP and eGFR were not. In ANCA-GN, a positive correlation was noted between s-suPAR level before therapy and clinical severity, especially requiring hemodialysis. Activated β3 integrin was mainly detected on proximal tubular epithelium, but not glomerular capillaries in NS.

Conclusions: These findings suggested the change of s- and u-suPAR levels after therapy may serve as a clinical marker to judge the treatment response of NS and the differentiation of MCNS from FSGS. S-suPAR predicts the severity and prognosis of ANCA-GN. In addition, suPAR may activate β3 integrin on proximal tubules in human.

SA-PO537

Characteristics and Outcome of MPO-ANCA-Associated Glomerulonephritis in Patients Discharged with Seronegative for MPO-ANCA Tomohiro Saito,[1] Masayuki Iyoda,[1] Yukihiro Wada,[1] Makoto Watanabe,[2] Taihei Suzuki,[2] Ysutaka Yamamoto,[1] Ken Iseri,[1] Kei Hihara,[1] Takanori Shibata.[1] *[1]Div of Nephrology, Dept of Medicine, Showa Univ School of Medicine, Tokyo, Japan; [2]Div of Nephrology, Makita General Hospital, Tokyo.*

Background: A role for antineutrophil cytoplasmic autoantibody (ANCA) in the management of ANCA-associated glomerulonephritis (GN) is controversial.We attempted to clarify the clinical significance of seronegative for myeloperoxidase (MPO)-ANCA at the time of discharge in patients with ANCA-GN.

Methods: We retrospectively reviewed 48 patients (29 female; average age, 69.1 years) who were diagnosed as having MPO-ANCA-GN at Showa University hospital from January 1999 to December 2012. Subjects were divided into two groups: patients discharged with seronegative for MPO-ANCA (N-group, n=18) and patients discharged with seropositive for MPO-ANCA (P-group, n=30). The study outcome was relapse, defined as the condition of strengthening immunosuppressive therapy owing to relapse from clinical remission.

Results: Baseline characteristics, including clinical findings, histological features and immunosuppressive therapy, were comparable between the two groups except for the length of hospitalization. The duration of hospitalization was significantly longer in the N-group compared to the P-group (75 ± 29 versus 58 ± 22 days, Mann–Whitney U test, $P<0.01$). During the median follow-up period of 49.0 months (range 13-131 months) in the 48 patients, we observed relapse in 2 cases of N-group (11.1%) and 16 cases (53.3%) of P-group. The cumulative incidence of relapses during the follow-up period was significantly higher in the P-group compared to the N-group (log rank test, $P = 0.010$). Cox's regression model showed that seropositive for MPO-ANCA at the time of discharge increased the hazard ratio for relapse (hazard ratio 4.98; 95 % confidenceinterval 1.84-14.77, $P = 0.001$).

Conclusions: Our results showed that seropositive for MPO-ANCA at the time of discharge is a risk factor for relapse. We suggest that the achievement of MPO-ANCA seronegativity is desirable for the initial treatment of ANCA-GN, even with extending the hospitalization or performing more intensive clinical management.

SA-PO538

CD14 Expression Is Increased on Monocytes of Patients with ANCA Vasculitis, and Correlates with the Surface Expression of ANCA Auto-Antigens Ruth M. Tarzi, Joanne Tsz Ki Liu, Nicola R. Hill, Theresa H. Page, H. Terence Cook, Charles D. Pusey, Kevin Woollard. *Dept of Renal and Vascular Inflammation, Imperial College, London, United Kingdom.*

Background: Monocyte subsets with differing functional properties have been defined by their expression of CD14 and CD16. We set out to assess these subsets in AAV compared with healthy controls, and to determine their surface expression of ANCA autoantigens.

Methods: Flow cytometry for HLA-DR, CD16 and CD16 was performed on blood from 16 patients with active AAV (7 MPO-ANCA positive, 7 PR3-ANCA positive and 2 ANCA negative), 49 patients with AAV in remission (21 MPO-ANCA, 25 PR3-ANCA and 3 ANCA negative at diagnosis), and 21 controls. The proportion of classical ($CD14^{high}CD16^{neg/low}$), intermediate ($CD14^{high}CD16^{high}$) and non-classical ($CD14^{low}CD16^{high}$) monocytes, and surface expression of PR3 and MPO on monocyte subsets was determined.

Results: There was no difference in the proportion of monocytes in each subset in patients with AAV compared with healthy controls, although the absolute number of monocytes was higher in patients with active disease compared with remission patients. However, the expression of CD14 on monocytes was increased in patients with active AAV, compared with patients in remission and healthy controls ($p<0.001$ versus controls, $p<0.01$ versus remission). Patients with PR3-ANCA disease in remission also had increased monocyte expression of CD14 compared with controls ($p<0.01$), whilst the levels were normal in MPO-ANCA disease in remission. PR3 was expressed mainly on classical and intermediate monocytes, whilst MPO was expressed mainly on intermediate and non classical monocytes. There was a correlation between CD14 and both PR3 and MPO expression on classical monocytes in AAV patients ($r=0.79$, $p<0.0001$ and $r=0.47$, $p<0.001$ respectively), but this correlation was not seen in control monocytes.

Conclusions: There were variations in the expression of PR3 and MPO on different monocyte subsets, which may impact on the ability of ANCA of different specificities to interact with these monocyte subsets. The correlation between monocyte CD14 and ANCA autoantigen expression in AAV may contribute to the pathophysiology of bacterial infection in AAV.

Funding: Private Foundation Support

SA-PO539

Ficolin-1 Is Upregulated in Leukocytes and Glomeruli from Microscopic Polyangiitis Patients Eri Muso. *Div of Nephrology and Dialysis, Center for Nephrology and Urology, Kitano Hospital, Tazuke Kofukai Medical Research Inst, Osaka, Japan.*

Background: Microscopic polyangiitis (MPA) is a systemic autoimmune disease that often has a fatal outcome. Although delineating the molecular pathogenesis is essential for its remedy, an understanding of its molecular mechanism has remained elusive.

Methods: To search for new markers of active lesions that might help better understand the molecular basis of MPA and aid in its diagnosis, DNA microarray analysis with peripheral blood mononuclear cells (PBMCs)was performed for the patients with active MPA and normal control. Ficolin 1,disease specifically upregulated mRNA was detected by immunohistochemistry in glomeruli of pateints and control, Costaining of ficolin-1 dot and CD68 was performed to investigate the cellular origin of ficolin-1 and compared those of local and peripheral cell number.

Results: Compared to normal control, several genes were up- or down-regulated in MPA patients, including up-regulation of the mRNA level of ficolin-1, an innate pattern recognition complement molecule. The amount of ficolin-1, as detected by immunohistochemistry, was higher in the glomeruli of another group of MPA patients than in the glomeruli of control patients who harbored almost normal glomeruli. Many of the ficolin-1 dots were also positive for CD68, suggesting that the ficolin-1–positive cells were monocytes, such as macrophages or dendritic cells. This is not due to the difference in the number of neutrophil or monocytes in the blood samples of MPA and control patients.

Conclusions: Increased ficolin-1 expression could serve as a new marker for the characterization of MPA, especially when it is associated with local active lesions.

Funding: Government Support - Non-U.S.

SA-PO540

Incidence of Anti-Moesin Autoantibody in Patients with Small-Vessel Vasculitis and Kidney Disease Kouju Kamata, Shokichi Naito, Tomoko Okamoto, Mariko Kamata, Chikako Okina, Togo Aoyama, Junya Murano. *Nephrology, Kitasato Univ School of Medicine, Sagamihara, Kanagawa, Japan.*

Background: Newly discovered anti-moesin autoantibody has been proposed as a novel biomarker for anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV). While, the incidence of anti-moesin autoantibody in patients with kidney disease before treatment has not been reported. This study investigated the incidence of the anti-moesin autoantibody in patients with kidney disease just before treatment.

Methods: Patients aged ≥18 years who underwent initial kidney biopsy for the diagnosis and treatment of kidney disease from 2002 to 2013 were enrolled in this study. Factors analyzed included patient age, gender, serum creatinine level, estimated glomerular filtration ratio (eGFR),urinary protein excretion, serum albumin level, MPO-ANCA level by ELISA (normal value <10 EU) and Birmingham's vasculitis activity score (BVAS) at the time of the kidney biopsy. All the sera were obtained at initial kidney biopsy. The serum anti-moesin autoantibody was semi-quantitatively analyzed by western blotting using recombinant human moesin protein generated in *Escherichia coli* BL21 as an antigen.

Results: Anti-moesin autoantibody was present in the sera of 42% (14/33) of patients with microscopic polyangiitis (MPA), 63% (5/8) of patients with anti-glomerular basement membrane (GBM) disease, 18% (2/11) of patients with IgA vasculitis and 43% (3/7) of patients with lupus nephritis, but was not detected in any of the three patients with granulomatosis with polyangiitis or the 10 with minimal change nephritic syndrome. Birmingham's vasculitis activity score was significantly higher in MPA patients with than without the anti-moesin autoantibody ($p<0.05$), and was significantly higher in MPA patients with both myeloperoxidase (MPO)-ANCA and anti-moesin autoantibody than in MPA patients with MPO-ANCA alone ($p<0.02$). The estimated glomerular filtration rate was significantly lower in MPA patients with both MPO-ANCA and anti-moesin autoantibody than in MPA patients with MPO-ANCA only ($p<0.02$).

Conclusions: Anti-moesin autoantibody is a biomarker of anti-GBM disease, lupus nephritis and IgA vasculitis as well as of AAV and MPA.

SA-PO541

Expression of Calcineurin in Podocytes Associated with Remission of Primary Nephrotic Syndrome Treated by Cyclosporine Ke Wang, Haiyun Wang, Yubing Wen, Xuemei Li, Xuewang Lee, Limeng Chen. *Dept of Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China.*

Background: The expression of calcineurin in podocyte was observed in MN and FSGS patients, and associated with the injury of glomerular filtration barrier permeability.The purpose of this study is to observe the association between the expression of calcineurin in podocyte and the efficacy of NS patients treated by calcineurin inhibitor cyclosporine(CsA).

Methods: The clinical records of IMN(n=20)or FSGS(n=10) patients, who treated by CsA for at least 6ms at PUMCH, were reviewed. The expression of WT1,Calcineurin and Synaptopodin in podocytes were tested by immunohistochemical or immunofluorescence staining.

Results: The mean age of these patients was 41.8±20 years. Mean proteinuria,serum albumin and serum creatinine at baseline were (7.65±4.04) g/d, (23.9±5.9) g/L and (77.4±30.3) μmol/L. CsA was given at a dose of (3.08±0.66) mg/kg/d, combination with prednisone[(0.43±0.26) mg/kg/d] in part of patients. Complete remission and partial remission were observed in 6 and 18 patients after 6 m of treatment. Three patients with no expression of calcineurin showed no response to CsA. Compared with no remission patients, the expression of calcineurin in remission groups was significantly higher (10.96 ± 3.69% versus 3.93 ± 2.38%, $P<0.05$). But no significant difference were observed in age, gender, pathology type, proteinuria, renal function, dose and blood drug concentration of CsA. There was no correlation between calcineurin and proteinuria level. The expression of calcineurin accompanied with loss of synaptopodin was confirmed by serial section cutting.The loss of WT1 of podocyte was more obvious in FSGS patients than MN patiens.

Conclusions: The expression of calcineurin may related with the remission of NS patients treated by Cyclosporine.

Funding: Government Support - Non-U.S.

SA-PO542

Clinical Value of Podocyte and Urinary CD80 in Differentiating Treatment Considerations for Minimal Change Disease and Focal Segmental Glomerulosclerosis Eduardo H. Garin,[1] Jochen Reiser,[2] Nada Alachkar,[3] Changli Wei,[2] Gabriel M. Cara-Fuentes,[1] Richard J. Johnson.[4] *[1]Univ of Florida; [2]Rush Univ Medical Center; [3]The Johns Hopkins Univ; [4]Univ of Colorado.*

Background: Minimal Change Disease (MCD) is associated with increased CD80 expression in podocytes and elevated urinary CD80 excretion during active disease, whereas focal segmental glomerulosclerosis (FSGS) is characterized by mild or absent CD80 podocyte expression and normal urinary CD80 concentration.

Methods: One patient with biopsy proven MCD, one patient with biopsy proven primary FSGS and three patients with FSGS recurrence after transplantation received CD80 blocking antibodies (Abatacept or Belatacept). Urinary CD80 and CTLA-4 were measured by ELISA. Kidney tissue samples were stained for CD80. Random urinary protein/creatinine ratio was measured.

Results: The administration of Abatacept in the MCD patient was associated with a fall in urinary CD80 excretion to an undetectable level followed by transient resolution of proteinuria. CD80-blockers (Abatacept, Belatacept) therapy did not result in any improvement in proteinuria in one patient with primary FSGS and two patients with FSGS recurrence after transplantation despite of the presence of mild CD80 glomerular expression but normal urinary CD80 excretion. One patient with recurrent FSGS after transplantation had elevated urinary CD80 excretion immediately after surgery which fell spontaneously before receiving Abatacept. His proteinuria remained unchanged for 5 days despite of a normal urinary CD80 excretion.

Conclusions: These observations provide clinical evidence that CD80 expressed in podocytes and shed into urine may play a critical role in the development of proteinuria in MCD. In contrast, it is unlikely that CD80 plays a role in FSGS recurrence since urinary CD80 is only increased transiently after surgery and normalization of urinary CD80 does not result in resolution of proteinuria.

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SA-PO543

Th1 and Th2 Cytokines in Histology and Progression of Idiopathic Nephrotic Syndrome due to Focal Segmental Glomerulosclerosis and Minimal Change Nephropathy

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Background: Aim of the present study was to evaluate the role of Th1 and Th2 cytokine levels in distinguishing FocalSegmentalGlomerulosclerosis (FSGS) and MinimalChangeNephropathy (MCN)and their correlation with renal histology and disease outcome.

Methods: Thirty-six patients with FSGS (Age: 41.9±17yrs, Scr=1.7±0.8mg/dl, Upr=4.7±5.5g/24hr), and 21 with MCN (Age: 41.4±15χρ, Scr=1±0.4mg/dl, Upr=7.9±9.3g/24hr) were included in the study. Th1 (IL-2, IL-12, GM-CSF, INF-γ, TNF-α) andTh2 cytokines (IL-4, IL-5, IL-10, IL-13) were measured by multiple cytokine assay, in first morning urinary sample collected at the day of renal biopsy. Levels of cytokines were correlated with renal function and degree of proteinuria.

Results: No significant differences in urinary excretion of all cytokines measured were found between FSGS and MCN patients. In FSGS however, percentage of global sclerosis had significant positive correlation with urinary levels of bothTh1, IL-2, IL-12, GM-CSF, INF-γ, TNF-α (p=0.005, p=0.01, p=0.01, p=0.005, p=0.007 respectively), andTh2 cytokines, IL-4, IL-5, IL-10, IL-13 (p=0.002, p=0.04, p=0.03, p=0.04 respectively). Degree of interstitial fibrosis was correlated with IL-12, INF-γ, TNF-α andIL-4 (p=0.04, p=0.03, p=0.04 and p=0.02 respectively). IL-12 urinary excretion was independent factor correlated with both global sclerosis (R^2=0.3, p=0.009) and interstitial fibrosis (R^2=0.3, p=0.02). Th1 cytokines (IL-2 and GM-CSF) were significantly increased in FSGS patients who did not respond to treatment (p=0.03 and p=0.007, respectively). Th2 cytokines (IL-4, IL-5, IL-10, IL-13) were significantly increased in MCN patients with frequent relapses (p=0.05, p=0.001, p=0.01, p=0.03).

Conclusions: UrinaryexcretionofTh1/Th2 cytokinescannot discriminate FSGS from MCN. Th1 may be involved in pathology and progression of FSGS, while Th2 cytokines are implicated in frequent relapses of nephrotic syndrome in MCN.

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SA-PO544

Glomerular Collapse -When It Hits, Where It Hits -Does It Matter

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Background: Collapsing focal segmental glomerulosclerosis (cFSGS) and other glomerulonephritis with collapsing pattern (GNcp) presents with massive proteinuria and rapid progression to end stage renal disease (ESRD). Present observational study was undertaken to study the clinicopathological spectrum of cFSGS and compare its clinical behavior to other GNcp and poorly therapy responsive non-collapsing FSGS.

Methods: Patients with a diagnosis of cFSGS and GNcp were prospectively followed. All the patients of cFSGS and GNcp were treated with oral prednisolone (1 mg/kg/day). Patients of lupus nephritis and membranous glomerulonephritis with collapse were managed with cyclophosphamide and oral prednisolone. Patients of cFSGS were compared with a cohort of prospectively followed steroid and tacrolimus (TAC) resistant non-collapsing FSGS. Loss of differentiated podocyte marker molecule (WT-1), gain of immature podocyte marker (Pax 2) and proliferation marker (Ki 67) and Parvo B19 nested PCR were performed in all the three groups. The clinical outcome and histological parameters of cFSGS, GNcp and steroid and TAC resistant FSGS were compared.

Results: The study included 32 patients (22 cFSGS and 10 GNcp). Of the 22 with cFSGS, 11 (50%) patients each presented with nephrotic syndrome and rapidly progressive renal failure. Complete remission, partial remission, steroid resistant nephrotic syndrome, progression to ESRD and death was observed in 3 (9.3%), 1 (3.1%), 7 (21.8%), 17 (53.2%) and 4 (12.5%) patients respectively. There were no differences in the clinical outcome and the histological parameters of patients with cFSGS and GNcp. Patients of cFSGS had higher serum creatinine and advanced tubulointerstitial changes compared to steroid and TAC resistant FSGS.

Conclusions: Glomerular collapse due to either cFSGS or other GNcp is poorly treatment responsive and has a high rate of progression to ESRD. cFSGS has a poorer prognosis than therapy resistant non-collapsing FSGS.

SA-PO545

Genomic Analysis Identifies Renal Cell Carcinoma as a New Tumor Type Linked to Aristolochic Acid Exposure

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Background: Dietary intake of nephrotoxic and carcinogenic aristolochic acid (AA) leads to endemic nephropathy (EN) marked by chronic tubulointerstitial nephropathy (CTN) and urinary tract transitional cell carcinomas (TCC). The role of AA in malignancies other than TCC is unexplored. We aimed to investigate a role of AA in the etiology of renal cell carcinomas (RCC), usually not related to EN, by conducting whole exome sequencing (WES) of tumor-enriched DNAs to detect the presence of the AA mutational signature.

Methods: Four clear cell RCC patients from EN area linked to AA exposure and 1 non-EN RCC case were studied. Of the EN patients, 3 were farmers baking bread from grain from locally grown wheat; 1 patient was diagnosed with CTN and 2 with TCC. All patients were in CKD stages≥3b.Tumor DNA was isolated by macrodissection from FFPE sections, processed for WES libraries, exome capture and sequencing on Illumina HiSeq2500 (multiplexed paired-end 50 bp run, >10x coverage/sample). Reads were aligned by BWA, variants called by GATK, annotated by ANNOVAR and genetic variants observed in normal population removed. The detection of the AA signature was performed by customized R functions.

Results: High A:T>T:A transversion rates (0.9 to 1.8 /Mb) were found in all EN RCC samples. Three cases had A:T>T:A (48.1%,38.2% and 21.6%) and 1 tumor was borderline positive (15.2% A:T>T:A). The non-EN RCC control was negative (6.4% A:T>T:A). In the positive samples the mutations occurred mainly in the C**A**G context and on the coding strand, meeting all definition criteria for the AA mutational signature.

Conclusions: Our study finds AA mutational signature in 3 EN patients with RCC, suggesting a possible causal involvement of AA in the RCC etiology, with new implications for the worldwide RCC incidence due to widespread unregulated use of AA-containing herbs. Extension of this study is underway to validate the preliminary results in a larger cohort of cases. Supported by grant 04-38 Cro Nat Foundation and IARC regular budget.

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SA-PO546

Safety and Efficacy of Eculizumab in Pediatric Patients with aHUS, with or without Baseline Dialysis

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Background: Atypical hemolytic uremic syndrome (aHUS) is characterized by chronic, uncontrolled complement activation and thrombotic microangiopathy (TMA). The objective of this analysis was to characterize the safety and efficacy of ECU in aHUS pediatric pts with dialysis (DIAL) and without dialysis (N-DIAL) at baseline (BL).

Methods: Subanalysis of an open-label, single-arm trial in pts with aHUS aged 1 month to <18 y and wt ≥5 kg was performed. Primary outcome was complete TMA response during 26 weeks (wks) of ECU treatment (tx). Pts were vaccinated against *N. meningitidis*. Inclusion criteria included platelet (plt) count <150x10^9/L, LDH ≥1.5xULN, and SCr ≥97th percentile for age at screening. Exclusion criteria included ADAMTS-13 activity <5% and Shiga-toxin–producing *E. coli* infection.

Results: 12 of 22 pts (55%) received ECU without first receiving plasma exchange/infusion. ECU improved plt count (x10^9/L) in both DIAL (149.8; *P*=0.0150) and N-DIAL (180.2; *P*=0.0003) pts. eGFR (mL/min/1.73 m^2) improved in DIAL (57.7; *P*=0.0568) and N-DIAL (70.3; *P*=0.0056) pts. Of BL DIAL pts, 9/11 (82%) discontinued dialysis by 26 wks. No pt began new dialysis. At Wk 26, 2 pts were on dialysis. Adverse events were similar among groups.

Conclusions: ECU led to improvements in hematologic and renal parameters in BL DIAL and N-DIAL pediatric pts with aHUS. 82% of BL DIAL pts discontinued dialysis by Wk 26, and no pt started new dialysis. Increases in eGFR were observed for both DIAL and N-DIAL pts at Wk 26. These results are consistent with data reported from a retrospective ECU trial in 19 pediatric pts (aged 2 months to 17 y), where no pt required new dialysis during ECU tx.

Baseline Characteristics	DIAL (n=11)	N-DIAL (n=11)	P-value[a]
Age at first infusion, mean (range), years	5.5 (0.0–17.0)	7.7 (1.0–17.0)	0.25
Gender, Female, n (%)	4 (36.4)	6 (54.5)	0.67
Race, n (%) Asian White Other	0 10 (91) 1 (9)	2 (18) 8 (73) 1 (9)	NE
Duration of aHUS clinical manifestation to first ECU dose, median (range), months	0.20 (0.03–1.84)	0.18 (0.03–4.26)	0.50
First clinical TMA manifestation, n (%)	10 (90.9)	6 (54.5)	0.15
Identified complement abnormalities, n (%)	5 (45.5)	5 (45.5)	1.00
Platelet count x10^9/L, mean (SD)	326.6 (94.2)	69.4 (43.3)	0.068
LDH >ULN, n (%)	8 (72.7)	11 (100)	0.45
eGFR[b] mL/min/1.73 m^2, mean (SD)	11.2 (3.9)	94.2 (30.0)	<0.0001
Transplant at baseline, n (%)	1 (9.1)	1 (9.1)	1.00

Efficacy Outcomes at Week 26	DIAL (n=11)	N-DIAL (n=11)	P-value[a]
Complete TMA response[c,d], n (%) [95% CI]	6 (54.5) [23–83]	8 (72.7) [39–94]	0.66
Platelet count normalization (≥150 x 10^9/L), n (%) [95% CI]	11 (100) [72–100]	10 (90.9) [59–100]	1.00
Platelet count change from baseline (x10^9/L)[e], mean (SD)	149.8 (101.0) P=0.0150[f]	180.2 (34.9) P=0.0003[f]	0.94
LDH normalization, n (%) [95% CI]	9 (81.8) [48–98]	9 (81.8) [48–98]	1.00
Hematologic normalization[g], n (%) [95% CI]	9 (81.8) [48–98]	9 (81.8) [48–98]	1.00
eGFR increase from baseline (mL/min/1.73 m^2)[e], mean (SD)	57.7 (57.3) P=0.0568[f]	70.3 (37.1) P=0.0056[f]	0.076
eGFR improvement ≥25 mL/min/1.73 m^2, n (%) [95% CI]	9 (81.8) [48–98]	10 (90.9) [59–100]	1.00
Patients on dialysis at baseline who discontinued dialysis, n/N (%)	9/11 (81.8)	N/A	NE
Patients on dialysis at 26 weeks	2	0	0.48

[a]P-values were generated by statistical comparisons between subgroups.
[b]eGFR calculated using the Schwartz formula: eGFR (mL/min/1.73 m^2) = 0.413 x height (cm) / SCr (mg/dL).
[c]Defined as (1) platelet count normalization (≥150 x 10^9/L); (2) LDH normalization (LDH≤ULN); and (3) improvement of renal function (≥25% increase in serum creatinine from baseline).
[d]Complete TMA response confirmed by 2 consecutive measurements obtained 24 weeks apart.
[e]Assessment at Week 27.
[f]P-values generated by statistical comparisons between values at 27 weeks and values at baseline.
[g]Defined by normalization of platelet count (≥ 150 x 10^9/L) and LDH (LDH≤ULN) sustained for ≥2 consecutive measurements obtained ≥4 weeks apart.
CI, confidence interval; LDH, lactate dehydrogenase; NE, not evaluated; SCr, serum creatinine; SD, standard deviation; ULN, upper limit of normal.

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SA-PO547

Management of Hepatitis-Associated Renal Autoimmunity Anke Schwarz,[1] Heiner Wedemeyer,[2] Hermann G. Haller.[1] *[1]Nephrology, Hannover Medical School, Hannover, Germany; [2]Hepatology, Hannover Medical School, Hannover, Germany.*

Background: Hepatitis-related autoimmunity with renal involvement can result in life-threatening hydropic and cachectic symptoms.

Methods: We report on 10 patients with hepatitis C (n=9) and B (1) who presented between 2010 and 2014 either with severe nephrotic syndrome caused by membrano-proliferative glomerulonephritis (MPGN; hepatitis C, 3 women, 4 men; hepatitis B, 1 man) or cachexia caused by polyarteritis of mediumsized renal vessels (hepatitis C, 2 men).

Results: Mixed cryoglobulenemia was detected in 5 and rheumatoid factor in 7 patients, hypocomplementemia in all 8 patients with MPGN (not in the 2 patients with polyarteritis). All patients had progressive renal insufficiency, and 8 had hypertension. Other organs involved were hemorrhagic alveolitis (n=1), heart involvement (1), skin vasculitis (3), and peripheral neuropathy (2). Successful antiviral treatment had been performed in 5 patients with hepatitis C and the patient with hepatitis B; four patients with hepatitis C had either not tolerated therapy (1) or had no treatment because of severe concomitant diseases (3). In spite of being virus-free after treatment, active nephrotic syndrome persisted in 3 cases with MPGN up to 24 months and relapsed after 3, 5 and 6 years after symptom-free and virus-free interval in 3 cases with MPGN. All patients were treated with rituximab (375mg/m^2) twice with one week interval; further doses were given dependent on B-cell recovery or relapsing symptoms. One patient did not lower B cells even after 4 doses of rituximab and reached end-stage renal disease after ruptured sigma diverticulitis. One other patient had remission of nephrotic syndrome but developed renal failure. Six other patients responded to treatment with reduction of proteinuria and improvement of renal function; but only one is off immunosuppressive treatment. Two other patients have just started treatment. Two patients had pneumonia, one other patient relapse of hepatitis C under rituximab (successfully retreated).

Conclusions: Rituximab is the treatment of choice in hepatitis-related autoimmunity, even in cases with continuous viral replication. Active MPGN may persist or relapse years after successful antiviral treatment.

SA-PO548

Higher Serum Uric Acid Levels Increase Resistance of Afferent Arteriole, Inducing Decreased Renal Plasma Flow (RPF) and Glomerular Filtration Rate (GFR) in Human – Inulin and Para-Aminohippurate Clearance Study Hideki Uedono, Akihiro Tsuda, Eiji Ishimura, Mari Yasumoto, Mitsuru Ichii, Katsuhito Mori, Masaaki Inaba. *Osaka City Univ Graduate School of Medicine, Osaka, Japan.*

Background: Hyperuricemia has been reported to induce decrease in GFR through endothelial cells dysfunction (Kidney Int 67:1739, 2005). Increase in resistance of renal artery and decrease in RPF is caused by hyperuricemia (Curr Hypertens Res 8:120, 2006). There are, however, no data that examined the relationship between serum uric acid levels and intrarenal hemodynamic parameters in human. The aim of the present study was to evaluate this mechanism in human, utilizing clearance of inulin (C_{in}) and paraaminohippurate (C_{PAH}).

Methods: Renal and glomerular hemodynamics were assessed by simultaneous measurements ofC_{in} (GFR) and C_{PAH} (RPF) in 50 in-patients with normal renal function (C_{in} ≥60 ml/min/1.73m^2) and without hyperuricemia (56.0±13.7 years, 23 males and 27 females, 26 diabetics and 24 non-diabetics). Renal and glomerular hemodynamics, such as resistance of afferent arteriole (R_a) and efferent arteriole (R_e), were calculated using Gomez's formulae, recently examined and reported by us (Diabetes Res Clin Pract 104:234, 2014).

Results: Serum uric acid levels in all patients were within the normal range (3.2-7.5 mg/dl). Serum uric acid levels correlated negatively with C_{in} (r=-0.381, p<0.01). Serum uric acid levels correlated positively with R_a (r = 0.308, p = 0.00293), but not significantly with R_e. In multiple regression analyses, serum uric acid levels were significantly and independently associated with C_{in} and C_{PAH} (β= -0.410, p = 0.0122; and β= -0.321, p = 0.0396, respectively) after adjustment for age, gender, body mass index, hemoglobinA1c and systolic blood pressure (R^2=0.317, p<0.01; R^2=0.365, p<0.01, respectively). Serum uric acid levels (β=0.558, p=0.0005) were significantly and independently associated with R_a after adjustment for the above confounders (R^2=0.382, p<0.01).

Conclusions: These findings suggest, for the first time in human study, that higher serum uric acid levels are significantly associated with reduced GFR and RPF. This reduction may be probably caused by increase in R_a.

SA-PO549

Plasma CD147/Basigin as a Promising Biomarker of the Kidney Diseases Kayaho Maeda, Tomoki Kosugi, Hiroshi Kojima, Tomohiro Masuda, Noritoshi Kato, Waichi Sato, Seiichi Matsuo, Shoichi Maruyama. *Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan.*

Background: CD147/Basigin (Bsg), a glycosylated transmembrane protein, contributes to cell survival, migration and cancer invasion. We so far demonstrated *in vivo* that its pathophysiological roles in the kidney diseases are diverse, ranging from the occurrence of acute kidney injury frequently accompanied by ischemia, inflammation and a loss of tolerance to progression of renal fibrosis. Particularly, the biological activity of Bsg on leukocytes is indispensable for T lymphocytes differentiation and migration of leukocytes into the tubulointerstitium. Disruption a vicious chain reaction involving Bsg and its interacted molecules would be required for overcoming the kidney diseases. We therefore investigated whether Bsg could serve as a novel biomarker for disease activity and prognosis in the kidney diseases.

Methods: Plasma and spot urine samples were collected from 365 patients, who underwent renal biopsy in our affiliated hospitals between 2008 and 2012. They included healthy individuals as a pathological control (Control; n=16), rapid progressive glomerulonephritis (RPGN; n=101), lupus nephritis (LN; n=64), minimal change nephrotic syndrome (MCNS; n=45), diabetic nephropathy (DM; n=24), IgA nephropathy (IgAN; n=43), focal segmental glomerulosclerosis (FSGS; n=32), and membranous nephropathy (MN; n=40).

Results: In the normal kidneys, Bsg mainly expresses in tubules, but not glomeruli. While this expression was extremely lower in atrophic tubules of injured kidney, infiltrating leukocytes and fibroblasts, and injured glomeruli such as crescent formation showed the marked induction of Bsg. Interestingly, the close relationship between plasma Bsg and serum creatinine was found. Plasma Bsg levels were strikingly higher in RPGN, LN and DM patients with leukocytes recruitment, Furthermore, this values accurately reflected the degrees of tissue damages, leukocytes infiltration and renal dysfunction in respective diseases. Bsg on leukocytes and fibroblasts might be thus involved in inflammation in the kidney diseases.

Conclusions: Plasma Bsg may accurately reflect the disease activity in the kidney diseases.

SA-PO550

Development of a Urine Mass Spectrometry Based Test for Detection of a Mutant MUC1 Peptide in Medullary Cystic Kidney Disease Type 1 Wendy Heywood,[1] Fujun Lin,[2] Duriye Deren Oygar,[3] Thomas Michael Connor,[5] Guy H. Neild,[2] Kevin Mills,[1] Daniel P. Gale.[2] *[1]Inst of Child Health, Univ College London, London, United Kingdom; [2]Centre for Nephrology, Univ College London, London, United Kingdom; [3]Nephrology Dept, Nicosia State Hospital, Nicosia, Cyprus; [4]MRC Inst of Genetics and Molecular Medicine, Univ of Edinburgh, Edinburgh, United Kingdom; [5]West London Renal and Transplant Centre, Imperial College, London, United Kingdom.*

Background: Medullary Cystic Kidney Disease Type 1 (MCKD1) is an autosomal dominant condition that causes kidney failure in adults. The disease is clinically silent with no hypertension, haematuria, proteinuria or imaging abnormalities prior to the onset of renal impairment. Kidney biopsy in MCKD1 typically shows only tubular atrophy/fibrosis with no diagnostic histological or ultrastructural features. Only treatment is kidney transplantation. MCKD1 is caused by a single cytosine insertion mutation in an extremely (>80%) GC-rich 60-bp tandem repeat in the *MUC1* gene. The C-insertion introduces a shift in the reading frame of the protein resulting in expression of a unique peptide (MUC1fs). Reliable assays to diagnose this disease are in great demand but are not widely available.

Methods: Genome-wide SNP-based linkage, Western Blotting, MRM LC-MS.

Results: We investigated 4 families with clinical features and linkage consistent with MCKD1 with a C-insertion mutation in MUC1. Western blotting detected a protein with the MUC1fs neo-peptide protein in urine from affected individuals from each family. Using a targeted proteomic approach we have developed a 5 min peptide based multiple reaction monitoring LC-MS assay. The MUC1fs neopeptide was detected in the urine of 6 affected family members (including renal transplant recipients) and not in the urine from 10 control individuals.

Conclusions: The assay is inexpensive, requires only 2ml urine, can be automated and multiplexed with other tests and is absolutely specific for MCKD1. The assay has great potential for high throughput population screening and will make predictive and diagnostic testing available – allowing living kidney donation from unaffected relatives to patients with MCKD1 to be performed safely.

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SA-PO551

Urinary Extracellular Vesicles as Potential Biomarkers for Polycystic Kidney Disease Mahdi Salih,[1] Jeroen A.A. Demmers,[2] Karel Bezstarosti,[2] Ewout J. Hoorn,[1] Robert Zietse.[1] *[1]Nephrology and Transplantation, Erasmus Medical Center Rotterdam, Rotterdam, Netherlands; [2]Proteomics Center Rotterdam, Erasmus Medical Center Rotterdam, Rotterdam, Netherlands; [3]In Collaboration with the DIPAK Consortium.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive cyst growth and often leads to renal failure. There is no good biomarker to predict disease progression. Urinary extracellular vesicles (uEVs) contain many disease-related proteins from all renal epithelial cells and form a source of biomarkers.

Methods: We collected spot urine samples of 6 ADPDK patients (confirmed PKD1 mutation) and 6 age- and sex-matched healthy volunteers. Samples were normalized for urinary creatinine, pooled and precipitated using acetone or ultracentrifuged to isolate uEVs. Proteins were trypsinized and subsequently analyzed using LC-MS/MS. uEVs were labeled with isotopomeric dimethyl labels, allowing quantitative analysis using bioinformatics. A different set of ADPKD (n = 6), CKD (n =6) and healthy control (n = 6) subjects were selected for validation of our proteomics results with immunoblotting.

Results: A total of 1048 proteins in acetone precipitated urine and 1245 proteins in uEVs were identified, of which 718 were unique for uEVs. Quantitative analysis of the uEVs revealed a ≥ 2-fold upregulation of 227 proteins and downregulation of 116 proteins. Many proteins previously implicated in the pathogenesis of ADPKD were identified. Interestingly, most of the up- or downregulated disease-related proteins were not found in acetone precipitated urine, supporting the notion that the isolation of uEVs enriches the

urinary proteome. Immunoblotting showed that several of these uEV proteins were only increased in urine from ADPKD patients, including periplakin (10-fold) and desmoplakin (3-fold), both junctional proteins known to be mislocated in ADPKD.

Conclusions: Isolation of uEVs enriched the urinary proteome and identified potential biomarkers uniquely upregulated in ADPKD, including periplakin and desmoplakin.

SA-PO552

α-Adducin Gene Polymorphism Affects eGFR Decay in ADPKD Maria Teresa Sciarrone Alibrandi,[1] Giancarlo Joli,[1] Marina Nuzzo,[1] Elena Brioni,[1] Marco Simonini,[1] L. Merlino,[1] Francesco Trevisani,[1] Rodolfo Rivera,[2] Simona Delli Carpini,[1] Lorena Citterio,[1] Donatella Spotti,[1] Paolo Manunta.[1] *[1]San Raffaele Scientific Inst, Milan, Italy; [2]San Gerardo Hospital, Monza, Italy.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic nephropathy and an important cause of ESRD, End Stage Renal Disease, accounting for about 5% of patients requiring dialysis. The number of cysts and their complications are variable among single patients as well as the renal outcome. Adducin is a heterodimeric cytoskeleton protein consisting of an alpha-subunit and either a beta- or gamma-subunit. It is involved in cohesion of renal cells. In rats and humans, mutation of the a-adducin subunit leads to the stimulation of the sodium (Na(+)), potassium (K(+))-adenosine triphosphate (ATP)-ase activity in renal tubular cells, increased renal Na(+) reabsorption, and, subsequently, hypertension.

Methods: Patients and methods: We analyzed the genetic background of a sample of 154 patients affected by ADPKD, in order to evaluate the influence of α-adducin gene polymorphism in renal outcome. ΔGFR (Glomerular Filtration Rate loss) was evaluated at the follow-up (every 6 months). Statistical analysis has been performed by Cox Regression corrected for sex, age, blood pressure, comorbid conditions, therapy and previous renal function.

Results: ADPKD patients carrying at least a mutated allele (GW and WW) present a significant loss of renal function versus wild type ones. We observed a GFR loss of 30% versus basal value in 77 months in mutated pts and in 90 months in wild type ones (p=0.03). Mean of follow up 45.5 months in the entire sample (min 6 max 169.73).

Conclusions: α-adducin genotype is associated with loss of renal function in ADPK. As mutated genotype is known to be also associated with hypertension and renal handling of sodium it could contribute to our understanding of the pathogenetic mechanisms that lead in renal failure progression for ADPKD patients.

SA-PO553

A Urine Peptidome-Based Biomarker Score Accurately Predicts the Risk of Reaching ESRD in ADPKD Patients Andreas D. Kistler,[3] Harald Mischak,[1] Arlene B. Chapman.[2] *[1]Mosaiques Diagnostiques GmbH, Hannover, Germany; [2]Renal Div, Emory Univ School of Medicine, Atlanta, GA; [3]Div of Nephrology, Univ Hospital Zurich, Zurich, Switzerland.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) accounts for about 5% of patients with end stage renal disease (ESRD). Now that specific treatment options likely become available, prediction of disease course would be of utmost importance to select high risk patients for treatment. We have previously identified ADPKD-specific patterns of urine peptide excretion that differentiate ADPKD from control patients and correlate with disease severity. Here, based on extended follow up time, we were able to identify a set of urinary peptides that predict progression to ESRD and thus allow early detection of patients at high risk for progression.

Methods: Baseline urine samples from all patients in the CRISP cohort were analyzed by capillary electrophoresis - mass spectrometry (CE-MS). All patients were followed for >7 years and the urine peptidome of patients reaching ESRD was compared to control patients with relatively slow progression (defined as an annual GFR loss of <4 ml/min/1.73m^2 during follow up). Since patients reaching ESRD had lower baseline GFR, we matched the control group by including only patients with baseline GFR <95 ml/min/1.73m^2. Two thirds of both cases and controls were used to identify a prognostic biomarker score, the remaining patients served as validation cohort.

Results: During follow up, 22 patients reached ESRD, and 46 patients matched for baseline GFR had a low progression rate. A prognostic biomarker score based on 52 urinary peptides, applied to the validation cohort, reached an AUC of 0.94 in the training cohort upon cross validation and an AUC of 0.81 in the validation cohort to identify patients reaching ESRD during follow up. This predictive value was higher than the AUC of total kidney volume (TKV) at baseline.

Conclusions: We identified a biomarker score based on the urine peptidome at a single timepoint that allows to identify ADPKD patients with high risk for progression to ESRD. This score outperforms TKV, which has by now been the best prognostic marker in ADPKD.

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SA-PO554

Increased Expression of Semaphorin 7A in ADPKD Neera K. Dahl,[1] Yashang Lee,[1] Anil K. Karihaloo.[3] *[1]Section of Nephrology, Yale Univ School of Medicine, New Haven, CT; [2]Astra-Zenica, Södertälje, Sweden.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic disorders in humans. It is characterized by progressive cyst growth and corresponding loss of renal function. Inflammatory cells are known to be present in the cystic kidneys of ADPKD patients. It is further validated by the presence of pro-inflammatory cytokines in cyst fluid. In ADPKD patients, high urinary monocyte chemoattractant protein-1 (MCP-1) levels correlate with high rate of cyst growth. Semaphorin 7A (Sema7A), an axonal guidance molecule, plays a critical role in lung fibrosis. Interestingly, Sema7A levels on circulating mononuclear cells are significantly higher in idiopathic pulmonary fibrosis as well as in scleroderma patient population. The Sema7A levels separate slow and fast progressing IPF patient populations. We hypothesized that the circulating mononuclear cells in ADPKD patient population will have higher Sema7A expression and will inversely correlate with renal function.

Methods: Blood samples were obtained from 80 ADPKD patients and from 20 controls with no known kidney disease. PBMCs were separated from normal (control) and ADPKD patient blood using a Ficoll gradient. RNA was isolated and analyzed for Sema7A expression. Data were analyzed using Welch's t-test (Student t-test with unequal variance).

Results: We first demonstrated that in a mouse model of unilateral ureteral obstruction there is increased level of Sema7A mRNA in peripheral blood mononuclear cells (PBMCs) derived from the buffy coat. Next, we analyzed Sema7A expression on peripheral blood CD45+, F4/80+ and CD4+ PBMCs from 80 ADPKD patients (CKD stage 1-5) and 20 healthy volunteers, using FACS analysis. Our data conclusively demonstrate that as compared to healthy volunteers Sema7a expression is highly upregulated in the circulating monocytes and CD4$^+$ T cells of ADPKD patients.

Conclusions: These data suggest a potential for Sema7A as a marker of kidney injury in ADPKD patient population.

Funding: NIDDK Support

SA-PO555

Autosomal Dominant Polycystic Kidney Disease Progression: Measuring Chronic Kidney Disease Stage Transitions Christopher M. Blanchette,[1,2] Caihua Liang,[3] Deborah Lubeck,[4] Britt B. Newsome,[5] Ben Gutierrez,[1] Nancy D. Lin.[3] *[1]Otsuka America Pharmaceuticals, Inc., Princeton, NJ; [2]Univ of North Carolina, Charlotte, NC; [3]Optum, Waltham, MA; [4]Outcomes Insights, Westlake Village, CA; [5]Denver Nephrologists, Thornton, CO.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a progressive genetic disorder characterized by bilateral cysts, leading to kidney failure. Most cases are due to a mutation in the *PKD1* gene and are associated with more rapidly progressing disease. Few studies have assessed chronic kidney disease (CKD) stage transitions of ADPKD patients.

Methods: This retrospective cohort study included patients with a claim indicating ADPKD from 1/1/2000–2/28/2013 and ≥6 months of prior continuous enrollment (baseline) within a large U.S. administrative claims database. A random sample of CKD patients served as comparators. In a subgroup of patients with linked electronic laboratory results data, the estimated glomerular filtration rate was calculated via serum creatinine values (Scr) to determine CKD stage at baseline and during follow-up. Proportions of patients transitioning to another stage and the mean age at transition were calculated. Approval from the institutional review board was obtained.

Results: Identified patients included 7617 patients with ADPKD and 22,851 with CKD. At first observation, 68% of ADPKD patients were ≥40 y (91% CKD) and 53% were female (40% CKD). Baseline Scr values were available for 1962 (26%) ADPKD patients and 7305 (32%) CKD patients. Staging at baseline for ADPKD and CKD patients, respectively, were: stage I, 627 and 934; stage II, 343 and 1658; stage III, 328 and 1937; stage IV, 166 and 577; stage V, 498 and 2199. Of ADPKD patients in stage I at baseline (n=627), 99 transitioned to stage 2 (mean age 47 y), 19 to stage III (mean age 47 y), 4 to stage IV (mean age 46 y), and 7 to stage V (mean age 45 y) during follow-up. Patterns were similar for patients in initial staging of II, III, and IV.

Conclusions: The results suggest that distributions of patients by age at transition help to identify rapid progressors. Future research should explore genetic, clinical, economic, and humanistic characteristics of ADPKD patients who rapidly progress through CKD stages.

Funding: Pharmaceutical Company Support - Otsuka America Pharmaceutical, Inc., Princeton, NJ, USA

SA-PO556

Renal Transcatheter Arterial Embolization in ADPKD Patients: Increasing Its Effectiveness Tatsuya Suwabe, Yoshifumi Ubara, Koki Mise, Keiichi Sumida, Junichi Hoshino, Masahiro Kawada, Kenmei Takaichi. *Nephrology Center, Toranomon Hospital, Tokyo, Japan.*

Background: In patients with autosomal dominant polycystic kidney disease (ADPKD), massive renal enlargement is related to prominent abdominal distension. Renal transcatheter arterial embolization (TAE) is safe and effective for reducing renal volume, and has been performed for volume reduction before renal transplantation. The average renal volume reduction ratio (RVRR) after renal TAE was reported to be about 50%, but there is large variation and the reason is unknown.

Methods: All ADPKD patients who received renal TAE at Toranomon Hospital from 2006 to 2013 were enrolled. We calculated the RVRR as follows: (1 - renal volume 1 year after renal TAE / renal volume before renal TAE)x100. Factors affecting the RVRR were analyzed.

Results: A total of 372 patients (183 males, 189 females; 56.8±9.4 years old [mean±SD]) were enrolled. Average RVRR was 45.3±14.6% (range: 10~84%). Stepwise multiple linear regression analysis revealed that age, body mass index (BMI), blood pressure, number of microcoils used for renal TAE, renal calcification score (RCS), and cardiovascular disease significantly affected the RVRR.

Dependent variable	Explanatory variable	β	t	p value	Adjusted R^2
RVRR					0.346
	Age	-0.372	-6.18	<0.0001	
	BMI	-0.232	-4.06	<0.0001	
	BP	0.215	3.98	<0.0001	
	No. of microcoils	0.170	2.73	<0.01	
	Cardiovascular disease	-0.137	-2.52	<0.05	
	RCS	-0.138	-2.34	<0.05	

Analysis in a single age group (227 patients aged 50 to 65 years) revealed that RCS was correlated with dialysis duration (r=0.717, p<0.0001) and inversely correlated with RVRR (r=-0.206, p<0.05), while the aortic calcification index (ACI) was not. Average RVRR was significantly lower in patients with a dialysis duration > 5 years when compared among groups with different dialysis times (p<0.005).

Conclusions: Age had the greatest influence on RVRR, while calcification did not, suggesting that age-related changes like fibrosis might be the most important determinant of RVRR. RCS was correlated with dialysis duration and affected RVRR in patients from the same age group. Renal TAE might be more effective in patients with less renal calcification or patients within 5 years of starting dialysis.

SA-PO557

Phosphate Metabolism in Children with Autosomal Dominant Polycystic Kidney Disease Stéphanie De Rechter,[1,2] Justine Bacchetta,[3] Laurence Dubourg,[3] Pierre Cochat,[3] Maria Van Dyck,[1] Jean De Schepper,[4] Pieter Evenepoel,[5] Elena N. Levtchenko,[1,2] Djalila Mekahli.[1,2] *[1]Dept of Pediatric Nephrology, Univ Hospital of Leuven, Leuven, Belgium; [2]Laboratory of Pediatrics, KU Leuven, Leuven, Belgium; [3]Centre de Référence des Maladies Rénales Rares, Hôpital Femme Mère Enfant, Hospices Civils de Lyon, Bron, France; [4]Dept of Pediatric Endocrinology, Univ Hospital of Brussels, Brussels, Belgium; [5]Dept of Internal Medicine, Div of Nephrology, Univ Hospital of Leuven, Leuven, Belgium.*

Background: Clinical data have revealed in an ADPKD adult population that Fibroblast Growth Factor 23 (FGF23) increases while circulating Klotho levels decrease, with a low TmP/GFR even in patients with normal renal function. The underlying mechanisms are largely unknown. Moreover, it has been demonstrated in animal ADPKD models that the polycystic kidney produces FGF23 but is resistant to its actions. No data are available in a pediatric ADPKD population. To fill this gap, we prospectively assessed renal phosphate handling in children with ADPKD in a dual-center study.

Methods: Children with ADPKD and normal renal function were eligible for inclusion. Blood and urine samples were collected and analysed for parameters of bone mineral metabolism. Based on normal values according to age, we made percentile (P) charts for serum phosphate and TmP/GFR. Hypophosphatemia and urinary phosphate wasting were defined as values ≤ P5.

Results: We included 69 ADPKD patients (median (range) age 10.7 (2.3 – 17.5) years). Parameters of mineral metabolism (Vit D and PTH) were in the normal range. Hypophosphatemia and urinary phosphate wasting were observed in 10%, and 22% of the children respectively. Serum phosphate levels and TmP/GFR were in P5-P50 range in 70% and 77% of the patients respectively.

Conclusions: This is the first report highlighting hypophosphatemia and renal phosphate leak in ADPKD children with normal eGFR. These results confirm recent data in adult ADPKD patients and point to an abnormal FGF23 metabolism. Further studies are required to elucidate the underlying pathophysiology and to investigate potential clinical consequences.

Funding: Government Support - Non-U.S.

SA-PO558

Adult Polycystic Kidney Disease Patients Show a Very Similar Cardiovascular Comorbidity Profile to CKD Patients with Other Etiologies. Most Cardiovascular and Renal Risk Factors Are Not Controlled in the Majority of Patients Jose L. Gorriz, Marco Montomoli, Cristina Castro, Belen Vizcaino, Daniel A. Molina, Angela Maria Serrato, Alberto M. Martinez-Castelao, Sandra Beltrán, Carlos Del Pozo Fernandez, Ramon Lopez-Menchero Martinez, Luis M. Pallardo. *On Behalf of the MERENA and PECERA Study Investigators, Spain.*

Background: The younger age of autosomal dominant polycystic kidney disease (ADPKD) patients compared to patients with other etiologies may cause underestimation of their cardiovascular risk. The aim of this study was to analyze renal and cardiovascular risk factors in patients with ADPKD, analyzing whether there is a difference between ADPKD and other CKD patients with different etiologies.

Methods: Cross-sectional study that included 153 patients with a diagnosis of ADPKD from two observational Spanish studies (MERENA and PECERA) and a group of ADPKD patients followed up in the Hospital Dr Peset, compared to 2,010 patients with different etiologies from these studies. CKD stages were: 16%, 66% and 17% in stages 3, 4 and 5 not on dialysis in ADPKD patients, and 23%, 61% and 16% in other etiologies respectively (p = 0.25).

Results: ADPKD patients were of a younger age (53±14 versus 68±13 years, p<0.001), male sex was 48% versus 64% (p=0.001), a lower heart failure rate (3.2% versus 19%, p=0.003) a lower prevalence of peripheral vascular disease (3.5% versus 20%, P<0.001). We did not find significant differences in prevalence of coronary heart disease (7.3% versus 11.4%, p=0.07), cerebrovascular disease (3.5% versus 12%, p=0.082) or hypertension (84% versus 92%, p=0.19). Patients with ADPKD were more likely to be smokers (14% versus 9%, p=0.03), had better, but not optimal, blood pressure control (>140 sys BP: 33% versus 59%, p=0.03) and a lower degree of proteinuria (>1g/day:18% versus 38%, p=0.008). There was no difference in the excess weight (64% versus 79%, p=0.7) nor in the degree of control of dyslipidemia (LDL>100: 56% versus 64%, p=0.18) (low HDL: 48% versus 44%, p=0.67).

Conclusions: in this epidemiological analysis, ADPKD patients have a similar comorbidity profile to other CKD patients with other etiologies. Added to this, is the fact that most of renal and cardiovascular risk factors in these patients are not controlled.

SA-PO559

Clinical Significance of Endothelin-1 in the Pathogenesis of Autosomal Dominant Polycystic Kidney Disease: A Translational Study Rupesh Raina,[1] Linda Lou,[1] Bruce E. Berger,[2] Beth A. Vogt,[1] Angelique Sao-Mai Sy Do,[1] Karin Anna Herrmann,[4] Katherine M. Dell,[3] Michael S. Simonson.[2] *[1]Dept of Pediatrics, Div of Pediatric Nephrology, Rainbow Babies and Children's Hospital, Cleveland, OH; [2]Dept of Medicine, Div of Nephrology and Hypertension, Univ Hospitals Case Medical Center, Cleveland, OH; [3]Div of Pediatric Nephrology, Cleveland Clinic Foundation, Cleveland, OH; [4]Dept of Radiology, Univ Hospitals Case Medical Center, Cleveland, OH.*

Background: The pathogenesis of progressive renal insufficiency in autosomal dominant polycystic kidney disease (ADPKD) is unclear. Evidence from experimental models of ADPKD demonstrates that elevated endothelin-1 (ET-1) drives cyst growth, renal fibrosis and loss of renal function, but whether ET-1 is elevated in humans with ADPKD is unknown.

Methods: We conducted a cross-sectional analysis of patients with ADPKD recruited from the University Hospitals Case Medical Center outpatient clinics. Urine ET-1, a well-validated surrogate for kidney ET-1, was measured in spot collections and corrected for creatinine. The volume of each kidney was measured from magnetic resonance images using stereology. The relationship of log-transformed urine ET-1 with MDRD eGFR and kidney volume was modeled by multiple linear regression with adjustment for clinical covariates in Stata 13.

Results: We recruited 21 patients with ADPKD ages 18 to 53. eGFR (mean ± SD) was 78.0 ± 47.7 ml/min/1.73m^2 and albumin/creatinine ratio (ACR) was 26.0 ± 32.3 mg/g. Urine ET-1 was negatively associated with eGFR (r = -0.395, P= 0.026) independent of age and female sex (P<0.01). In contrast, urine ET-1 was positively associated with total kidney volume (r = 0.425, P < 0.01) after adjustment for age and sex (P< 0.05).

Conclusions: In a pilot study of patients with ADPKD, urinary ET-1 was positively associated with kidney volume, a surrogate for cyst growth, and negatively with eGFR. Taken together with results from experimental models, these findings suggest that the role of ET-1 in ADPKD warrants further investigation.

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SA-PO560

Arterial Stiffness in Young Non-Uremic Autosomal Dominant Polycystic Kidney Disease Patients Compared to Young Healthy Subjects Timur Selcuk Akpinar,[1] Abdullah Ozkok,[1] Fatih Tufan,[1] Murat Kose,[1] Oguz Kagan Bakkaloglu,[1] Burak Ince,[1] Elif Irem Sarihan,[1] Duygu D. Uzun,[1] Erol Bozbora,[1] Halil Yazici,[2] Yasar Caliskan,[2] Tevfik Ecder,[2] Kamil Nas,[4] Miklos Illyes.[3] *[1]Internal Medicine, Istanbul Univ, Istanbul, Turkey; [2]Nephrology, Istanbul Univ, Istanbul, Turkey; [3]Heart Inst Clinic, Univ of Pecs, Pecs, Hungary; [4]Dept of Radiology Budapest, Szent Janos Hospital, Budapest, Hungary.*

Background: Cardiovascular disease is the most important cause of mortality in autosomal-dominant polycystic kidney disease (ADPKD) patients. The ambulatory arterial stiffness index (AASI) can be used to predict cardiovascular risk in hypertensive patients. However, to our knowledge, the role of ASSI in patients with ADPKD has not been evaluated.

Methods: Age, blood pressure (BP) and GFR matched 24 normotensive and non-uremic ADPKD patients (mean age: 28±8 years, M/F: 12/12) and 18 healthy controls (31± 11 years, M/F: 12/6) were enrolled into the study. Twenty-four hour blood pressure and arterial stiffness measurements were performed non-invasively with the device Tensiomed Arteriograph24. AASI was defined as one minus the respective regression slope of diastolic BP on systolic BP.

Results: Age (p=0.36), sex (p=0.48), systolic BP (123±7 versus 124±12mmHg, p=0.85), diastolic BP (75±6 versus 76±12 mmHg, p=0.93) and GFR (104±19 versus 103±20 mL/min, p=0.72) were similar between healthy controls and ADPKD patients. However, microalbuminuria was significantly higher in ADPKD patients (106±139 versus 2.81±1.88 mg/day, p<0.001). AASI was significantly lower in patients with ADPKD (0.33±0.10 versus 0.37±0.05, p=0.03). Left ventriculer mass index was higher in ADPKD patients compared to healthy controls (93±18 versus 74±16, p=0.03). AASI was significantly correlated with microalbuminuria in patients with ADPKD (r= -0.60, p=0.03). In the linear regression analysis for predicting AASI; among age, GFR, mean BP and microalbuminuria, only microalbuminuria retained as the significant factor in the model.

Conclusions: Arterial stiffness was not found to be higher in young, normotensive and non-uremic ADPKD patients compared to young, healthy individuals. Ambulatory arterial stiffness index was significantly related to microalbuminuria in ADPKD patients.

SA-PO561

Pericardial Effusion (PE) and Autosomic Dominant Polycystic Kidney Disease (PKD) with Different Staged of Chronic Kidney Disease (CKD)

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Background: PKD is a genetic disorder characterized by CKD a progression and onset of cardiovascular (CV) complications. Greater occurrence of PE has been observed in PKD. Aim: to test the effects of PE and PKD on CV and renal outcome in an Italian Cohort.

Methods: 349 CKD patients (stage I-V) were divided into two groups: A= 83 with PKD (follow up: 53.7 months) and B= 266 without PKD (50.5 months). PE was detected by echocardiography and left ventricular mass index, and systolic and diastolic volume were used to predict a latent variable score for cardiac function (CF). Primary endpoint: time to firstCV event; secondary endpoint: dialysis treatment onset. LogRank and Cox frailty random (subjects' pedigree) models were used to test central effect of PE and PKD, adjusting for age, sex, Charlson comorbidity index, renal function, hypertensive drugs and CF score. PE was more frequent in group A (34.9%) than in B (13.5%) ($p<0.001$). There were 63 CV events and 58 onset of dialysis. LogRank did not show significant evidences of PKD on both outcomes ($p>0.05$), while significant evidences of PE were observed ($p<0.001$).Cox models returned not significant for PKD effects ($p>0.05$) on both outcomes, while PE effect resulted significant for CV outcome (Hazard Ratio= 2.8, p=0.01), but not for renal outcome (HR=1.13, p= 0.8).

Results: PE was more frequent in group A (44.6%) than in B (19.0%) ($p<0.001$). There were 63 CV events and 58 onset of dialysis. LogRank did not show significant evidences of PKD on both outcomes ($p>0.05$), while significant evidences of PE were observed ($p<0.001$). Cox models returned not significant PKD effects ($p>0.05$) on both outcomes, while PE effect resulted significant for CV outcome (Hazard Ratio= 2.8, p=0.01), but not for renal outcome (HR=1.13, p= 0.8).

Conclusions: PE is more common in PKD patients. Despite of 2.8 times higher hazard for CV events in PE patients, PKD does not increase CV events and it does not anticipate dialysis therapy. PE increases the hazard for CV events but not affecting renal outcome independently from the PKD group.

SA-PO562

Patients and Kidney Graft Outcomes in Polycystic Kidney Disease (ADPKD) Transplant Recipients following Native Nephrectomy

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Background: Native nephrectomy(NNx) is frequently needed in ADPKD patients. Timing of NNx (pre, concurrent or post) in relation to kidney transplantation (KTx) offers different challenges. We report our experience of post-transplant NNx.

Methods: Between 1977-2014, 450 ADPKD patients were transplanted in our center. 111(24.7%) underwent NNx with 80(72.1%) post-transplant and subject of this study. We reviewed the perioperative complications and compared patient and graft survival with ADPKD recipients (n=435) who did not undergo nephrectomies (NoNx).

Results: Mean age at KTx and NNx was 52.7±11.6 and 54.3±10.5 respectively, 53.3% male, 93% White, 5.4% had pretransplant diabetes, 8.3% CAD and40% were on dialysis prior to KTx for a median time of 16.8 months. NNx was done at a median of 19.9 months (IQR 8.2-71.5) post-transplant. Median follow up time was 6.4 years (IQR 3-10.3) during which 74 patients died and 117 graft failed (17.5% in NNx and 29.1% in NoNx; p=.035). Median hospital stay was 4 days after NNx. 5 patients required blood transfusions, 5 patients required admission to ICU and 2 had reversible acute graft injury. Patient survival at 1, 5 and 10 years was: 100%, 96.8% and 85.1% in the NNx group and 98.5%, 93.4% and 78.4% in the NoNx group respectively (p=.039). Graft survival at 1, 5 and 10 years was: 100%; 95.5%, and 83.9% in NNx and 95.7%, 87.9% and 67.9% in NoNx respectively (p=.022) On multivariate cox regression analysis, nephrectomy did not relate to patient or graft survival and only age at transplantation (HR= 1.1; [95% CI 1.05-1.14], p<.001) and male gender (HR=2.47[1.17-5.7]) were predictors of patient survival.

	HR	95% CI	p-value
NNx(Yes/No)	0.72	0.26-1.66	0.45
Age at Tx	1.1	1.05-1.14	<0.001
Male	2.47	1.17-5.7	0.017
Deceased donor	0.94	0.43-2.04	0.88
Diabetes	1.83	0.49-5.58	0.34
Dialysis pre-KTx	1.57	0.71-3.5	0.26

Conclusions: Native nephrectomy after kidney transplantation has low perioperative complications. Patients and grafts survival are comparable between ADPKD recipients who undergo nephrectomy and those who do not.

SA-PO563

Renal Cell Carcinoma (RCC) in Polycystic Kidney Disease (ADPKD) Transplant Recipients

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Background: The incidence of malignancy post kidney transplantation (KTx) is higher than that in the general population. Whether the risk for development of RCC in native kidneys following KTx is higher in ADPKD patients than in patients with other renal diseases is controversial.

Methods: Between 1977-2014, 450 ADPKD patients received KTx in our center. Mean age at KTx was 50.7 years (23-70). The mean follow-up time was 6.4 years (IQR 3-10.3).111 (24.7%) underwent native nephrectomies (NNx) with 80 (72.1%) post-KTx and subject of this study. We reviewed the indications and NNx renal pathology. Univariate logistic regressions were performed to identify variables associated with RCC. We included age at NNx, gender, race, smoking status, kidney volumes, symptoms, dialysis before KTx and number of years between KTx and NNx.

Results: NNx was performed at a median of 20.6 months after KTx (IQR 8.3-73.7). All were white, 58.2% male, 40% current/previous smokers and 35% on dialysis pre KTx for a median of 20.7 months. Indications for NNx were: pain 66%, hematuria 19%, cyst infection 15%, abdominal/incisional hernias 14%, renal mass 7.5 %, GI 5%,and respiratory symptoms 5%. RCC was present in 11 patients (3 had bilateral RCC) and only 6 had a suspicious mass prior to NNx.Pathology included: 8 papillary, 4 clear, 1 chromophobe and 1 unidentified RCC. Median size of RCC on pathology was 2.4cm (IQR 1.8-3.1) for clinically suspected RCC and 1.3cm (IQR 0.95-2.4) for incidental RCC(p=.128).One patient had pancreatic metastasis.Significant variables associated with RCC were age at NNx (OR 1.98 per 10 years,p .038),and number of years between KTx and NNx (OR 1.19,p .005). The incidence of clinically suspected RCC was 1.57 per 1000 person-years. The incidence of RCC including those incidentally diagnosed at NNx was 3.7 per 1000 person-years.

Conclusions: The incidence of RCC in ADPKD transplant recipients is relatively low. Most RCCs are small and papillary type. Age and time post-transplant were associated with RCC. Further studies are needed to determine whether screening is warranted.

SA-PO564

Volume Regression of Native Polycystic Kidneys after Renal Transplantation

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Background: The natural course of native kidneys after renal transplantation or dialysis in patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) is not well known.

Methods: We measured the volume of native kidneys (TKV) in 77 ADPKD patients who had pre (within two years) and at least one post-transplant renal CT or MRI, in 35 patients who had at least two post-transplant but no pre-transplant CT or MRI, and in 10 patients on chronic hemodialysis who had at least two CT or MRI. The last imaging was used in patients with multiple studies. Gender; age at transplantation; time on dialysis prior to transplantation; time from transplantation to post-transplant imaging; interval between pre-transplant imaging and transplantation; eGFR and diagnosis of hypertension or diabetes mellitus at last imaging; and immunosuppressive program (calcineurin, n=69, versus mTOR, n=8, inhibitors) were used in a multivariable model to identify factors associated with changes in TKV.

Results: TKV (±SD) prior to transplantation was 3218±1770 ml in the 77 patients who had imaging before and after transplantation and decreased by 20.2, 28.6, 36.5 and 45.8% after 0.5-1 (mean 0.7), 1-3 (1.8), 3-10 (5.7), and >10 (12.6) years, respectively. TKV was 3218 at baseline and 1348±1097 ml after the longest mean follow-up of 12.6 years. In the multivariable analysis, time on dialysis prior to renal transplantation (negatively) and time from transplantation (positively) associated with percent reduction in TKV. TKV decreased by 5.7±23.5% between 8.0±5.8 and 12.2±6.9 years in the 35 patients who only had imaging after transplantation. TKV increased by 15.9±32.3% in the 10 ADPKD patients on maintenance hemodialysis after a mean follow-up of 0.5±3.3 years.

Conclusions: TKV of native polycystic kidneys decreases substantially after renal transplantation, but not during chronic dialysis. The reduction occurs mainly during the first year and more slowly afterwards. Duration of dialysis prior to transplantation is associated with less reduction.

Funding: NIDDK Support

SA-PO565

Systematic and Standardized Employment of Laparoscopic Decortication to Control Pain Related to Enlarging Renal Cysts in Polycystic Kidney Disease

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Background: Severe pain that affects quality of life is reported in up to 60% of patients (pts) with Polycystic Kidney Disease (PKD). Enlarging cysts stretching the renal capsule are thought to be the cause of pain in the majority of PKD pts. This pain is most often experienced in the anterior abdomen, with only a minority having a posterior presentation. Laparoscopic cyst decortication (LCD) is reported to provide lasting pain relief in only

half the pts undergoing this procedure, but there are no uniform criteria for employing this treatment option. We describe and illustrate a systematic and uniform approach to addressing pain related to kidney cysts in PKD pts.

Methods: 1) The PKD pt must be able to provide a single finger pinpoint location of the chronic reproducible pain present for at least 6 months, especially located in the anterior abdomen. The chronic pain must be reported at least 5/10 on a Visual Analog Scale (VAS). 2) A skin marker is placed over the point of maximum tenderness prior to ultrasonography. 3) Kidney cyst(s) measuring at least 4 cm in biggest dimension must be identified directly below the marked area. 4) If the above criteria are met, our team urologist will employ the LCD technique, never unroofing more than 3 cysts.

Results: Using this detailed approach we have done 4 procedures, achieving uniform success in each pt. With a follow up of up to 32 months, pts have achieved uniform, complete and sustained improvement in their pain patterns. The VAS has fallen from an average 7/10 to 1/10. No change in serum creatinine or estimated GFR occurred in any pt.

Conclusions: Our case series suggests that targeting LCD towards cysts most likely to be responsible for pt symptoms, identified through careful history taking, physical examination and directed use of imaging, can increase the success rate of this procedure while minimizing damage to adjacent renal parenchyma. It also highlights the importance of a multi-disciplinary approach, involving nephrology, urology and radiology for management of pain attributable to enlarging cysts in PKD.

SA-PO566

Urinary Excretion of EGF Receptor Ligands in Autosomal Dominant Polycystic Kidney Disease Patients and Response to Tolvaptan Treatment

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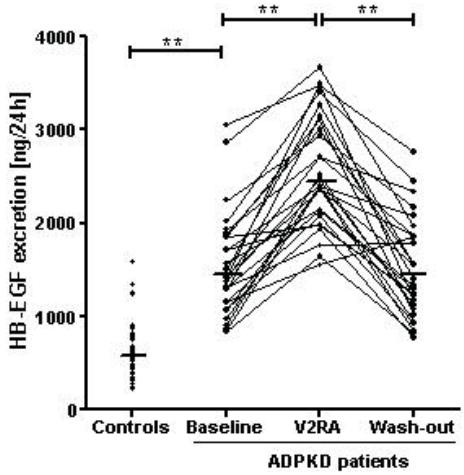
Background: Recent animal research suggests that dysregulation of the epidermal growth factor (EGF) receptor pathway plays a role in the pathophysiology of Autosomal Dominant Polycystic Kidney Disease (ADPKD). Research on EGF receptor ligands in human ADPKD is lacking. We measured EGF receptor ligands in ADPKD patients at baseline and after treatment with the vasopressin V2 receptor antagonist (V2RA) tolvaptan, because this information could provide a rationale for future combination therapy.

Methods: Blood and urine concentrations of HB-EGF, EGF and TGF-α were measured by ELISAs in 27 ADPKD patients, 27 controls and in cyst fluid. In ADPKD patients, ligands were measured at baseline, after 3 week treatment with tolvaptan and after a 3 week wash-out period. Glomerular filtration rate (mGFR) was measured by io-thalamate infusion and total kidney volume by MRI.

Results: Urinary HB-EGF excretion as well as plasma concentration were higher in ADPKD patients compared with controls (P<0.001 and p=0.04). In contrast, both urinary EGF excretion and plasma EGF concentration were lower in ADPKD patients (both p<0.001). In cyst fluid HB-EGF was present, whereas TGF-α and EGF were not measurable. Higher HB-EGF excretion was associated with more severe disease, assessed as lower mGFR or higher TKV (both p=0.05), whereas higher EGF excretion and TGF-α excretion were negatively associated with disease severity. During tolvaptan treatment, HB-EGF excretion increased (p<0.001) and returned towards baseline after drug withdrawal.



Conclusions: Our data suggest that of the EGF receptor ligands HB-EGF may play a detrimental role in the pathophysiology of ADPKD. The V2RA induced increase in HB-EGF suggests that targeting the EGF receptor pathway during V2RA treatment may hold promise.

SA-PO567

The Feasibility of Using Urine or Plasma Osmolality as Indicator of Vasopressin Concentration and Prognosis in Patients with Autosomal Dominant Polycystic Kidney Disease

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Background: Vasopressin plays an essential role in osmoregulation, but has deleterious effects in patients with ADPKD. Increasing water intake to suppress vasopressin activity has been suggested as potential renoprotective strategy. This study investigated whether urine and plasma osmolality (Uosm; Posm) can be used as reflection of vasopressin activity in ADPKD patients in early and later stage of the disease.

Methods: We measured Uosm, Posm, plasma copeptin concentration (as surrogate for plasma vasopressin concentration), GFR using constant infusion with 125 I-iothalamate (mGFR) and total kidney volume by MR imaging (TKV). In addition, change in estimated GFR (eGFR) during follow-up was assessed.

Results: 94 patients with ADPKD were included (56 males, age 40±10 year, mGFR 77±32 ml/min*1.73m^2, TKV 1.55 (0.99 – 2.40) L. Uosm, Posm Uosm/Posm ratio and copeptin concentration were 420±195 mosmol/l, 289±7 mosmol/l, 1.4 (1.1-1.8) and 7.3 (3.2 – 14.6) pmol/l, respectively. Posm was associated with copeptin concentration, whereas Uosm and Uosm/Posm ratio were not.

Multivariate linear regression analyses investigating the cross-sectional association of baseline urine osmolality, urine to plasma osmolality ratio and plasma osmolality with baseline copeptin concentration (as dependent variable) in 94 ADPKD patients.

	Model 1		Model 2		Model 3	
	St. β	p-value	St. β	p-value	St. β	p-value
Uosm	-0.10	0.35	-0.02	0.86	+0.22	0.006
Age			+0.26	0.01	-0.12	0.14
Male sex			-0.32	0.001	-0.07	0.33
mGFR					-0.66	<0.001
TKV					+0.33	<0.001
Uosm/Posm ratio	-0.09	0.42	-0.01	0.98	+0.21	0.006
Age			-0.27	0.002	-0.13	0.13
Male sex			-0.31	0.01	-0.08	0.30
mGFR					-0.66	<0.001
TKV					+0.34	<0.001
Posm	+0.54	<0.001	+0.44	<0.001	+0.18	0.07
Age			+0.09	0.36	-0.21	0.03
Male sex			-0.15	0.13	-0.10	0.26
mGFR					-0.46	<0.001
TKV					+0.30	0.004

Abbreviations: Uosm, urine osmolality; mGFR, measured glomerular filtration rate; TKV, total kidney volume; Uosm/Posm ratio, Urine to plasma osmolality ratio; Posm, plasma osmolality.

Fifty-five patients were followed for 2.8±0.8 years. Baseline Posm and Uosm were not associated with rate of kidney function decline after adjustment for age, gender and TKV (St. β = -0.11, p= 0.6 and St. β = 0.14, p= 0.3, respectively), whereas baseline copeptin concentration did show an association with change in eGFR, in both a crude analysis (St. β= -0.41, p=0.003) and after adjustment for the aforementioned variables (St. β= -0.23, p=0.05).

Conclusions: These data suggest neither Uosm nor Posm are valid measures to identify ADPKD patients that may benefit from increasing water intake. Copeptin appears a better alternative for this purpose.

SA-PO568

Decreased Urine Concentration Ability Precede Renal Function Decline in Patients with Autosomal Dominant Polycystic Kidney Diseasewith *PKD1* Mutation

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Background: It has been shown that patients with *PKD1* mutations progressed faster than those with *PKD2* mutations in the European and Japanese patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD). However, little is known about factors or predictors for faster progression of the disease in ADPKD patients with *PKD1* mutations. To know the factors contribute to a faster decline of renal function, we evaluated clinical characteristics for 106 Japanese ADPKD patients with *PKD1* or *PKD2* mutation.

Methods: We examined the correlation between a decline of estimated glomerular filtration rate (eGFR) and clinical parameters, including urinary osmolality (Uosm), serum osmolality, plasma arginine vasopressin (AVP), using a linear regression analysis in 106 Japanese patients with ADPKD in a longitudinal study design (2.00-11.92 yrs follow-up). We further performed 15-hours fasting urinary concentration test in 41 ADPKD patients with preserved renal function (eGFR ≥60 ml/min/1.73m^2, 27 *PKD1* mutated and 14 *PKD2* mutated patients).

Results: Uosm, plasma AVP and *PKD1* mutation were independently associated with eGFR decline in 106 patients with ADPKD. In a urinary concentration test, maximum values of Uosm between 13 and 15 hours fasting were significantly lower in patients with *PKD1* mutation than in those with *PKD2* mutation (674.6 ± 119.9 versus 759.9 ± 89.4 mOsm/

kg H_2O, p = 0.007). A multiple linear regression analysis revealed that *PKD1* mutation (β = -0.460, p = 0.005), total kidney volume (β = -0.426, p = 0.004) and plasma AVP (β = -0.342, p = 0.011) were independently associated with the maximum Uosm.

Conclusions: Urinary concentration ability was decreased in ADPKD patients with *PKD1* mutation prior to decline of eGFR, and may be a good indicator for predicting eGFR decline.

SA-PO569

Accuracy of Creatinine-Based Estimating Equations for GFR in ADPKD

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Background: The accuracy of creatinine-based estimating equations for GFR in ADPKD is uncertain. Alterations in tubular creatinine secretion early in disease progression may affect the accuracy of these equations.

Methods: Among 37 adults with ADPKD and eGFR >20 cc/min/1.73m^2,we measured GFR using iohexol plasma clearance (iGFR) and serum creatinine with an IDMS-traceable assay. We estimated GFR (eGFR) using 2 validated creatinine-based equations (CKD-Epi and MDRD). We compared the bias, precision, overall accuracy, and classification (for GFR<60 cc/min/1.73m^2) of each estimating equation.

Results: Mean age was 42.5±11.1years, 70%female, 84% Caucasian, 73% were treated with anti-hypertensives, mean iGFR was 77.6±38.7 cc/min/1.73m^2 and 41.0% had an iGFR<60 cc/min/1.73 m^2. There were strong linear correlations of each eGFR with iGFR (CKD-Epi: r=0.884; MDRD: r=0.882). The performance of $eGFR_{CKD\text{-}Epi}$ in terms of bias, precision, and accuracy was superior to that of $eGFR_{MDRD}$ (Table). However, both equations had comparable classification of patients with iGFR<60 cc/min/1.73m^2.

Performance Characteristic	Statistic	CKD-Epi Equation	MDRD Equation
Bias	Mean Difference (cc/min/1.73m^2)	-5.0 (-11.0, 1.0)	-11.7 (-18.0, -5.5)
Precision	Interquartile Range of Difference (cc/min/1.73m^2)	-10.2, +1.5	-20.8, -0.9
Accuracy	P30*	86.5%	73.0%
	Root Mean Square Error	18.34	18.52
Classification of iGFR<60 cc/min/1.73m^2	Sensitivity	93.3%	93.3%
	Specificity	90.9%	86.4%

*P30 represents proportion of GFR estimates that are within 30% of measured GFR. Negative values indicate under-estimation of true GFR (iGFR).

Conclusions: GFR estimated from CKD-Epi is superior to the MDRD estimating equation in ADPKD, but both equations underestimate iGFR and may have greater bias than when applied to the general CKD population.

Funding: NIDDK Support

SA-PO570

Glomerular Hyperfiltration Is a Common Risk Factor for Accelerated GFR Decline in Young Adults with Autosomal Polycystic Kidney Disease (ADPKD)

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Background: Glomerular hyperfiltration is an independent risk factor for accelerated GFR decline in ADPKD children. Here we aimed to assess whether and to what extent this finding can be extended to young (<35 years; NDT.2009.24:304-8) ADPKD adults.

Methods: Among 900 ADPKD outpatients of the "Papa Giovanni XXIII" Hospital (Bergamo, Italy), we identified all consecutive young adults with a baseline creatinine clearance (CrCl) >140 mL/min/1.73m^2, followed for at least 1 year with a minimum of 3 CrCl measurements.

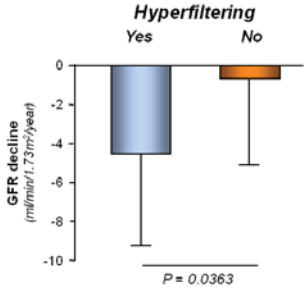
Results: We identified 91 patients (45 males, 49.5%) aged<35 years with a baseline CrCl of 110 (IQR: 86-129) mL/min/1.73 m^2. Eleven patients (12.1%) were hyperfiltering.

	Hyperfiltering (n=11)	Non-hyperfiltering (n= 80)	P
Males (%)	9 (81.1)	36 (45.0)	0.02
Age	27.2 (20.2-30.4)	29.2 (25.0-31.4)	0.17
Creatinine clearance (mL/min/1.73 m^2)	169.0 (162.0-209.0)	102.5 (84.5-122.0)	< 0.0001
Systolic BP (mmHg)	120.0 (111.0-127.0)	125.0 (112.5-136.5)	0.15
Diastolic BP (mmHg)	78.0 (63.0-81.0)	75.5 (67.5-82.0)	0.84

Over 6 (IQR:3.5-9) years, CrCl declined by 4.42 (9.35 to 0.97) and 0.50 (5.1 to -2.78) mL/min/1.73 m^2/year in hyper- and normofiltering patients at baseline, respectively (p=0.0363).

GLOMERULAR HYPERFILTRATION* AND GFR DECLINE IN 91 YOUNG ADULTS WITH ADPKD

** GFR > 140 ml/min/1.73m^2*



At final visit, median CrCl decreased by 1.5 and 57 mL/min/1.73 m^2 in normo- and hyperfiltering patients, respectively(p<0.0001). Higher baseline CrCl significantly correlated (p<0.0001,r=0.44) with faster CrCl decline on follow-up.

Conclusions: In ADPKD young adults, glomerular hyperfiltration is relatively common and associates with accelerated renal function loss. Whether hyperfiltration may be a specific target for interventions aimed at preventing disease progression merits further investigation.

SA-PO571

Serum Gamma-Glutamyl Transferase (GGT), Conjugated Bilirubin, and ^{18}F-FDG PET/CT as Potential Diagnostic Markers in Patients with Renal or Hepatic Cyst Infection

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Background: Cyst infection is a severe complication in autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (PCLD). Reliable, non-invasive diagnostic markers are required to reduce the need for invasive interventions. The aim was to investigate potential markers of cyst infection.

Methods: We identified ADPKD and PCLD patients with a suspected cyst infection from clinical and renal transplant registries that were monitored in four Dutch tertiary referral centers in the period 2001-2013. Patients were included if cyst aspirate culture led to pathogen isolation or diagnostic criteria indicating cyst infection (abdominal pain, fever, and serum CRP >50mg/L) were met. We retrospectively derived clinical, biochemical, and imaging data from medical records.

Results: We included 81 patients (94% ADPKD, 53% renal transplant, 11% dialysis, median age 56 years, male 51%) with renal cyst infection (RCI, 64%) or hepatic cyst infection (HCI, 36%). In patients without renal transplantation or dialysis, median eGFR (CKD-EPI) was 52ml/min/1.73m^2 [IQR 23-81] in RCI (n=19) and 74ml/min/1.73m^2 [IQR 28-89]in HCI (n=11). CKD-stage ≥4 (eGFR <30) was seen in 32% (RCI) and 27% (HCI) of patients without renal transplantation or dialysis. In patients with HCI, serum GGT and conjugated bilirubin were abnormal in 22/23 (median 94U/L, IQR 56-169) and 8/13 (median 0.5mg/dl, IQR 0.2-0.7). In contrast to computed tomography,^{18}F-FDG PET/CT was predominantly indicative for cyst infection in both RCI (12/14 versus 5/17) and HCI patients (15/16 versus 5/13).

Conclusions: Hepatic cyst infection is associated with elevated serum GGT and conjugated bilirubin. ^{18}F-FDG PET/CT is highly sensitive in detecting renal- and hepatic cyst infection.

Funding: Clinical Revenue Support

SA-PO572

Height-Adjusted Total Liver Volume Affects Hepatic Complications and Symptoms in the Patients with Autosomal Dominant Polycystic Kidney Disease

Hyunsuk Kim,[1] Hayne C. Park,[1] Sun Ae Han,[2] Joon Young Jang,[2] Hee Jung Jeon,[2] Kook-Hwan Oh,[1] Young-Hwan Hwang,[3] Curie Ahn.[1,3] *[1]Internal Medicine, Seoul National Univ Hospital, Seoul, Korea; [2]Transplantation Research Inst, Seoul National Univ, Seoul, Korea; [3]Internal Medicine, Eulji General Hospital, Seoul, Korea.*

Background: Polycystic liver disease (PLD) is the most frequent extra-renal manifestation of ADPKD. We investigated the relationship between hepatic complications/symptoms and the height-adjusted total liver volume (htTLV, mL/m) in a cross-sectional study with 488 Korean ADPKD patients.

Methods: PLD was defined as the presence of at least 4 liver cysts in a CT scan. Medical records were reviewed and physical examinations were undertaken to identify hepatic symptoms and complications. A questionnaire was also used to evaluate hepatic symptoms. The TLV was measured by stereotactic method and then adjusted by the height of the subject.

Results: PLD was more common among women (96.2% *versus* .86.9%, P <0.001). The common complications were ascites (16.6%), bilateral leg edema (5%), hernias (3.6%) and cyst infections (3.1%). Presence of pressure-related complications including ascites, hernia, bilateral leg edema, biliary dilatation, and IVC stenosis were associated with htTLV > 2,100 mL/m. Hepatic cyst infection or cholangitis were also related to htTLV > 2,100 mL/m (P < 0.001). The common symptoms were back pain (59.4%), flank pain (53.1%), abdominal fullness (46.5%), dyspnea or chest discomfort (44.3%). Presence of pressure-related symptoms including early satiety, dyspnea or chest discomfort, palpable mass and abdominal fullness, pain, and gastrointestinal symptoms were associated with htTLV > 1,600 mL/m (P < 0.001). Additionally, the case of pressure-related complications, hepatic cyst infection or cholangitis were also related to htTLV > 1,600 mL/m (P < 0.001).

Conclusions: Clinicians should pay more attention to symptom in case of htTLV >1,600 mL/m and complication in subjects with htTLV of 2,100 mL/m in Korea.

SA-PO573

Development and Validation of a Polycystic Liver Disease Specific Questionnaire Marie C. Hogan,[1] Tom J.G. Gevers,[2] Patrick S. Kamath,[3] Jeff Sloan,[4] Joost P.H. Drenth.[2] *[1]Nephrology, Mayo Clinic; [2]Hepatology, Radboud UMC, Nijmegen, Netherlands; [3]Hepatology, Mayo Clinic; [4]Health Sciences Research, Mayo Clinic, MN.*

Background: Symptomatic polycystic liver disease (PLD) is common in ADPKD and ADPLD and treatment focuses on improvement of symptoms. However, generic questionnaires lack sensitivity to capture PLD-related symptoms, a prerequisite to determine efficacy of therapy. The aim of this study is to develop and validate a disease specific questionnaire assessing symptoms in PLD (PLD-Q).

Methods: We identified PLD-related symptoms impacting quality of life by literature review and qualitative interviews with Dutch patients and clinicians. Subsequently, we developed questions on frequency and severity of each symptom. Individual questions scores were summarized into a total score ranging from 0-100 points. We undertook a validation study in 200 patients with mild-severe PLD from the Dutch PLD-registry. We assessed convergent validity (Spearman's correlation coefficient) of the PLD-Q score with EORTC QLQ-C30 symptoms subscale and the EQ5D-VAS score. Furthermore, we compared PLD patient scores with controls and 183 PLD; symptomatic versus asymptomatic patients and ADPKD versus ADPLD (Mann-Whitney U test) for discriminative validity. Intraclass correlation coefficient (ICC) of test-retest was calculated to assess reproducibility.

Results: In the validation survey, 167 patients (85% female, mean 55.6y, 45% ADPKD) completed the 14 item questionnaire (response rate 84%; mean score 40.1 ± 18.3, range 0-86.5 points). PLD-Q correlated well with the EORTC30 (r= 0.788,p<0.001) and EQ5D (r= -0.666,p<0.001). PLD-Q discriminated between patients and controls (40.1 ± 18.3 versus 19.3 ± 10.5, P<0.001) and between symptomatic and asymptomatic patients (44.5 ± 16.7 versus 24.9 ± 16.4, p<0.001). ADPKD patients scored higher than ADPLD patients (43.8 ± 16.6 versus 37.0 ± 19.0, p=0.016). The PLD-Q showed excellent reproducibility (ICC 0.914).

Conclusions: The PLD-specific questionnaire (PLD-Q) has good convergent and discriminative validity and reproducibility and captures symptoms in PLD patients. We have commenced validation of the translated PLD-Q in a U.S. PLD cohort, including correlation with liver/kidney volumes and comparison with ADPKD patients without PLD.

Funding: NIDDK Support

SA-PO574

Elevated Alkaline Phosphatase Predicts Response in Polycystic Liver Disease during Somatostatin Analogue Therapy: A Multi-Center Pooled Analysis on Individual Patient Data Marie C. Hogan,[1] Tom J.G. Gevers,[2] Frederik Nevens,[3] Vicente E. Torres,[1] Joost P.H. Drenth.[1] *[1]Nephrology Div, Mayo Clinic, Rochester; [2]Dept of Gastroenterology and Hepatology, Radboud UMC, Nijmegen, Netherlands; [3]Dept of Hepatology, KU Leuven, Leuven, Belgium.*

Background: Somatostatin analogues (SA) reduce liver volumes (LVs) in patients with polycystic liver disease (PLD). However, these patients show a considerable variability in treatment responses, making it difficult to predict response to SA therapy.

Methods: Our aim was to identify specific patient, disease or treatment characteristics that predict response in PLD during SA therapy. We pooled the individual patient data of 4 trials (NCT00771888,NCT00426153, NCT01157858, NCT01354405) of long-acting SAs in PLD (120 mg lanreotide or 40 mg octreotide) for 6 or 12 months that included LV as the primary outcome. We performed uni- and multivariate linear regression analysis with 9 preselected patient, disease and drug variables to identify independent predictors of response, defined as percent change in LV. Secondary outcome was percent change in kidney volume in the ADPKD subgroup. All analyses were adjusted for baseline LV and center (random) effect.

Results: 153 PLD patients (86% female, mean age 50 years, median LV 4974 ml, 69% ADPKD) from 3 international centers were treated with octreotide (n=70) or lanreotide (n=83). Mean reduction in LV was 4.2% (range -31.7% to +9.7%). Uni- and multivariate linear regression revealed that elevated baseline alkaline phosphatase (ALP) was associated with increased response during SA therapy (-2.7%, 95% CI -5.1% to -0.2%, p = 0.037), independently of baseline LV. Duration of therapy (6 versus 12 months), SA type and eGFR did not affect response. Elevated ALP remained associated with LV response (-3.2%, 95% CI -6.0 to -0.3%, p=0.029) in ADPKD patients (n=100), but did not predict response in kidney volumes (0.1%, 95% CI -3.1 to 3.3%, p = 0.97).

Conclusions: Elevated ALP is associated with response in polycystic liver disease during SA therapy, and could possibly serve as a prognostic biomarker in this disease.

SA-PO575

Outcomes of Liver Transplantation, Partial Hepatectomy, and Transcatheter Arterial Embolization for Massive Polycystic Liver in Autosomal Dominant Polycystic Kidney Disease Patients Hyunjin Ryu,[1] Miyeun Han,[1] Hee Jung Jeon,[2] Jong Cheol Jeong,[2] Hayne C. Park,[1] Young-Hwan Hwang,[3] Jaeseok Yang,[2] Curie Ahn.[1,2] *[1]Internal Medicine, Seoul National Univ; [2]Transplantation Center, Seoul National Univ Hospital; [3]Internal Medicine, Eulji Univ College of Medicine.*

Background: Polycystic liver disease(PLD) is the most common extra-renal manifestation in autosomal dominant polycystic kidney disease(ADPKD). Patients with PLD suffer from hepatomegaly symptoms. Interventions for symptomatic PLD include transcatheter arterial embolization(TAE), partial hepatic resection and liver transplantation(LT). We compared outcomes of each treatment modality.

Methods: We retrospectively analyzed 29 patients, undergone TAE, partial hepatectomy, or LT in ADPKD patients in a single center.

Results: Six and seven patients underwent LT and partial hepatectomy, respectively, while sixteen patients underwent TAE. Living donor LT was done for intractable hepatomegaly symptoms in 2 TAE patients, and for uncontrolled ascites in a patient. Deceased donor(DD) LT was performed in 2 patients for hepatic failure or uncontrolled ascites after TAE. Another patient underwent DDLT for hepatic failure after partial hepatectomy. The most common complications were ascites and pleural effusion. Two patients were under hemodialysis at LT, and acute kidney injury resulted in kidney failure in 1 DDLT patient. All patients had maintained good liver function at a median follow-up of 15.5 months. Causes of partial hepatectomy were recurrent cyst infection and hepatomegaly symptoms. One patient underwent partial hepatectomy, because multiple TAE had failed to control symptoms. Preoperative CTP score was lower in the partial hepatectomy group than in the LT group. A few serious infectious occurred, and antibiotic treatment cured them without mortality. Eleven patients received single TAE, and five patients underwent TAE twice. Two patients died of obstructive jaundice and cyst infection. Five patients (31.3%) in the TAE group needed LT or partial hepatectomy to relieve symptoms or rescue hepatic failure.

Conclusions: LT is more effective and tolerable treatment option for symptomatic PLD in ADPKD compared to partial hepatectomy and TAE.

SA-PO576

SIrolimus for ADPKD Patients with Massive Polycystic LiVER (SILVER): An Interim Analysis Hayne C. Park,[1] Hyunsuk Kim,[1] Hyunjin Ryu,[1] Sun Ae Han,[2] Joon Young Jang,[2] Hee Jung Jeon,[3] Kook-Hwan Oh,[1] Young-Hwan Hwang,[4] Curie Ahn.[1,2,3] *[1]Dept of Internal Medicine, Seoul National Univ Hospital, Seoul, Republic of Korea; [2]Transplantation Research Inst, Seoul National Univ, Seoul, Republic of Korea; [3]Transplantation Center, Seoul National Univ Hospital, Seoul, Republic of Korea; [4]Dept of Internal Medicine, Eulji General Hospital, Seoul, Republic of Korea.*

Background: Polycystic liver is the most common and often fatal extrarenal manifestation to autosomal dominant polycystic kidney disease (ADPKD) patients. We aimed to assess the efficacy and safety of mTOR inhibitor, sirolimus on liver cyst growth in patients with severe polycystic liver.

Methods: Adult (18-65 years) ADPKD patients with massive (total liver volume (TLV) > 2500 mL) or symptomatic polycystic liver received sirolimus starting from 2 mg/day for 1 year and followed up with conventional treatment for another 1 year. The drug dose was titrated to the trough level of 4-10 ng/dL. The primary endpoint was TLV change in 1 year measured by CT. This study was registered with ClinicalTrials.gov, NCT01680250.

Results: Twelve patients (mean age 49, female 83%) were enrolled between Sept 2011 and Feb 2013. The TLV, TKV, and eGFR at baseline were 5641 mL, 1387 mL, and 59 mL/min/1.73m^2. Five patients (42%) dropped out due to adverse events, and therefore, 7 patients were included in the efficacy analysis. TLV was significantly increased from 6713 to 7494 mL (+11.6%) after 1 year of sirolimus treatment (p = 0.02). TKV was not significantly changed after treatment (2450 versus 2537 mL, p = 0.47), whereas eGFR was significantly decreased in spite of sirolimus treatment (59.1 versus 50.8 mL/min/1.73m^2, p = 0.02). Serious adverse events occurred in 2 subjects (1 death from biliary sepsis, 1 admission from cyst infection). Two patients intermittently reduced or stopped study medication because of adverse events (cyst infection and increased proteinuria with renal progression). The high rate of adverse events prompted us to stop further enrollment for safety reason.

Conclusions: Sirolimus did not effectively reduce TLV in ADPKD patients with massive or symptomatic polycystic liver.

SA-PO577

Identifying Genetic Modifiers for Severe Polycystic Liver Disease (PLD) by Targeted Exome Sequencing Amirreza Haghighi,[1] Young-Hwan Hwang,[2] Ning He,[1] Kairong Wang,[1] Winnie Y. Chan,[1] Xuewen Song,[1] York P. Pei.[1] *[1]Div of Nephrology, Univ Health Network and Univ of Toronto, Toronto, ON, Canada; [2]Div of Nephrology, Eulji General Hospital, Eulji Univ College of Medicine, Seoul.*

Background: Severe PLD is a rare and poorly understood phenotype associated with *PKD1* and *PKD2* mutations in ADPKD and *PRKCSK* and *SEC63* mutations in ADPLD. Mutations of *PRKCSH* or *SEC63* reduces the functional polycystin-1 (PC-1) dosage by

decreasing endoplasmic reticulum protein-processing (ER-PP) and aggrevates cystic disease severity in-vivo (Nat Genet 43: 639-647, 2011). We hypothesize that mutations of genes in the ER-PP pathway may aggrevate PLD in patients with ADPKD/ADPLD.

Methods: To identify genetic modifiers of severe PLD, we performed WES using Illumina HiSeq2000/2500 with SSV4/5 capture in 30 unrelated patients with and 3 affected relatives without severe PLD (including 4 affected sib-pairs discordant of PLD and two affected sib-pairs concordant of severe PKD) and focused analysis on 167 genes involved in ER-PP. All patients with severe PLD had a liver span >25 cm at mid-clavicular line by CT/MRI or >3x normal total liver volume. Standard algorithms for sequence alignment, base calling, and QC filtering were applied to identify rare deleterious variants of high and moderate impact as predicted by PolyPhen-2, Sift, and Provean.

Results: Overall, 99.8% of all exomes sequenced were covered with a mean depth of 108X. We identified a total of 45 rare deleterious variants from 21 ER-PP candidate genes that affected 2-4 patients. Among them, the followings are functionally linked: SEC31B, SEC24A, SEC24D, SEC23B, MOGS, UGGT1, RPN1, OS9, EDEM3, WFS1, BAX, PARK2, ATXN3L, UBXN6, and PRKCSH.

Conclusions: If our hypothesis is correct, our preliminary results suggest extensive hetergeneity with no one single ER-PP gene accounting for a large proportion of severe PLD cases. We are currently completing exome sequencing in 50 cases of severe PKD to narrow a short list of most promising candidate ER-PP genes (i.e. each involved in >5 unrelated patients) for follow-up functional studies. Identification of genetic modifiers of PLD has the potential to improve risk prediction and treatment of this unusual complication.

SA-PO578

Genotype-Phenotype Correlations in Extra-Renal Manifestations of Autosomal Dominant Polycystic Kidney Disease (ADPKD): Insights from a Cohort of 1425 Patients Emilie Cornec-Le Gall,[1,2] Maryvonne Hourmant,[3] Cecile M. Vigneau,[4] Eric Renaudineau,[5] Christophe Charasse,[7] Philippe Gatault,[6] Marie-Pierre Audrezet,[1,2] Claude Ferec,[1,2] Yannick Le Meur.[1] *[1]CHRU Brest; [2]Inserm 1078; [3]CHU Nantes; [4]CHU Rennes; [5]CH Saint Malo; [6]CHU Tours; [7]CH Saint Brieuc.*

Background: ADPKD is marked by a high clinical variability, both in regards of the renal outcome and the occurrence of extra-renal manifestations. The aim of our study is to describe extra-renal manifestations in ADPKD and to investigate if the gene and the mutation involved predispose to specific extra-renal phenotypes such as aneurysms, diverticular disease of the colon and liver cysts.

Methods: Genkyst is an ongoing cross-sectional cohort study involving over 70 nephrologists in the western part of France. All the consenting ADPKD patients were included in 19 centers of Nephrology between 2010 and May 2014. Comprehensive clinical data were collected and a molecular analysis of *PKD1* and *PKD2* genes was undertaken.

Results: 1425 patients were included (median age 55.6 y, 659 men). The absence of liver cysts after age 45 was unusual (12% of the women and 22% of the men), irrespective of the gene and the mutation identified. 109 patients from 105 pedigrees (7.6%) had presented at least one vascular complication (86 intracranial aneurysms and 23 aortic aneurysms) at a median age of 47 y [19-71], only 26% had a familial history of vascular complication at diagnosis. Neither the gene involved, *PKD1* or *PKD2*, nor the mutation type or position (in contrast with a former study) were associated with the development of these vascular complications. Diverticular disease of the colon was reported in 10% of the patient, at a median age of 58.5 y, without any influence of the gender, or of the gene or the mutation involved. Conversely to previous publications, we did not find any evidence for infertility in ADPKD male patients.

Conclusions: Based on this large cohort, we provide insights on the exact prevalence of extra-renal manifestations in ADPKD. Despite several cases of familial clustering, there was no genic or allelic influence on the occurrence of these extra-renal features, which suggests the involvement of modifier genes.

Funding: Government Support - Non-U.S.

SA-PO579

Complex Genotypes Identified Through Genetic Characterization of the HALT PKD ADPKD Cohort Christina M. Heyer,[1] Jamie L. Sundsbak,[1] Arlene B. Chapman,[2] Ronald D. Perrone,[3] Robert W. Schrier,[4] Theodore I. Steinman,[5] Vicente E. Torres,[1] Franz Winklhofer,[6] Kyongtae Ty Bae,[7] Michael F. Flessner,[8] Katharina Hopp,[1] Peter C. Harris.[1] *[1]Mayo Clinic, Rochester, MN; [2]Emory, Atlanta, GA; [3]Tufts, Boston, MA; [4]U of Colorado, Denver, CO; [5]BIDMC, Boston, MA; [6]KUMC, Kansas City, KS; [7]U of Pittsburgh, Pittsburgh, PA; [8]NIH, Bethesda, MD.*

Background: The HALT PKD study is a phase three clinical trial investigating the affects of ACE inhibitors and ARB blockers on a cohort of 1043 hypertensive ADPKD patients. We have completed genetic analysis of 970 patients who provided DNA samples from 833 families, for the *PKD1* and *PKD2* genes.

Methods: *PKD1* and *PKD2* were sequenced in all probands by direct Sanger sequencing (5 long range and 63 nested/single copy reactions), followed by bioinformatic evaluation of potential mutations. MLPA was performed in mutation negative patients to identify large deletions/duplications.

Results: Of the resolved families, 626 (75.2%) were PKD1, 121 (14.5%) PKD2, with no mutation detected (NMD) in 66 families (7.9%). Interestingly, 20 families (2.4%) exhibited evidence of complex genotypes. These included three families with one strongly predicted change along with a second significant variant in the same gene, and two families with likely significant *PKD1* and *PKD2* variants. Four other families had strong evidence of mosaicism. For example, in family 690020 the daughter had a clear frameshifting mutation, c.12442_12443insAGGA, seen as heterozygous, but the mother's sequence had a very low level of the mutant allele, detectable only by manual inspection. Disease due to the combined effect of two (or three) likely hypomorphs, in cis or trans, was suspected in nine families, include two with the previously described p.R3277C hypomorph. Finally, two families were suspected of having gene conversion events due to the large number of variants identified matching the *PKD1* pseudogenes in *PKD1* exon 23.

Conclusions: Complex haplotypes manifesting largely as typical ADPKD presentations were found in approximately 2% of the HALT cohort. These families are a particular diagnostic challenge and show the need to completely screen both genes for full genetic characterization.

Funding: NIDDK Support, Pharmaceutical Company Support - Boehringer Ingelheim company donated drugs for clinical trail

SA-PO580

New Genetic Test for Prognosis and Diagnosis of All (Common, Rare and Ultra-Rare) Cystic Kidney Diseases M. Lara Besada-Cerecedo,[1] Lisbeth Silva,[1] Beatriz Sobrino,[2] Jorge Amigo,[2] Olaya Lamas-Gonzalez,[1] Helena Covelo-Molares,[1] Marina García,[1] Patricia Regueiro Casuso,[1] Ana Barcia de la Iglesia,[1] Francisco Barros Angueira,[2] Angel Carracedo,[2] Candido Diaz Rodriguez,[1] Miguel A. Garcia-Gonzalez.[1] *[1]Group of Genetics and Developmental Biology of Renal Diseases, Health Research Inst of Santiago de Compostela (IDIS). Clinical Univ Hospital (CHUS), Santiago de Compostela, Spain; [2]Galician Public Foundation of Genomic Medicine, Clinical Univ Hospital (CHUS), Santiago de Compostela, Spain.*

Background: Our group designed the first genetic and functional strategy for diagnostic and prognosis of all cystic kidney diseases using Next Generation Sequencing (NGS).

Methods: Three panels of genes were created: one of them includes the eight genes associated with PKD that are more common in the population, a second panel includes the eight genes with the replicated portion of PKD1 (exon 1-34) and finally, the other includes all the genes associated with PKD (72 genes).

Results: Here we show the validation of this three panels aiming to be use as a diagnostic tool in PKD. We have applied the test to 51 patients with PKD and we could detect 35 mutations in 25 patients.

Conclusions: Our results show that our genetic test detects mutations in all known genes associated with cystic kidney disease in a rapid, precise and cost-effective way.

Funding: Government Support - Non-U.S.

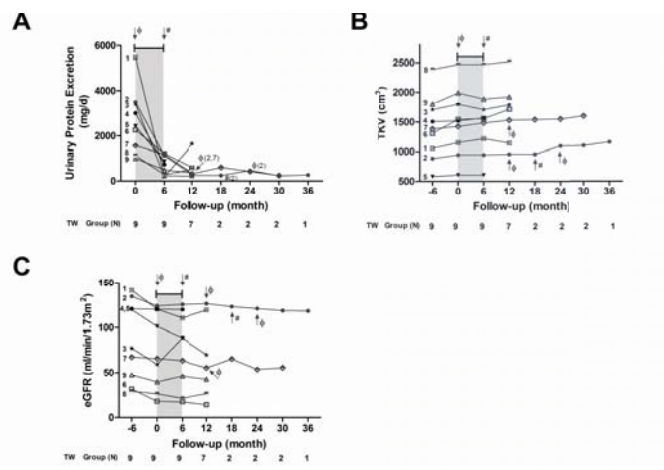
SA-PO581

Triptolide Containing Formulation in Patients with Autosomal Dominant Polycystic Kidney Disease and Proteinuria-An Uncontrolled Trial Changlin Mei, Shengqiang Yu, Dongping Chen, Yiyi Ma. *Dept of Nephrology, Shanghai Changzheng Hospital, Shanghai, China.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited cystic renal disease. Proteinuria is a risk factor for disease progression in ADPKD patients. The traditional Chinese medicine Tripterygium *wilfordii* (TW) contains triptolide and has been widely used as an effective antiproteinuric agent. Recently, triptolide was shown in experimental studies to inhibit cyst growth. The effects of TW in ADPKD patients with proteinuria are unknown.

Methods: In an uncontrolled trial, proteinuric ADPKD patients resistant to 6 months' treatment of losartan were treated with TW (1 mg/kg/d, divided into three doses per day) for 6 months. Effect of TW on 24-hour urinary protein excretion (UPE), eGFR and total kidney volume (TKV) were analyzed.

Results: Proteinuric ADPKD patients had a mean 24-hour UPE of 2,645±1,408 mg/d (range 1,004 to 5,483 mg/d) with 6-month TKV enlargement of 93±74 cm^3 and TKV growth rate of 6.8±5.1%. After 6 months of treatment with TW, the UPE decreased to 702±418 mg/d (range 189 to 1,215 mg/d) with one patient relapsed. An inhibition of the 6-month TKV enlargement (-1±61 cm^3, 0.4±3.8%) was noted. The 6-month eGFR decline was inhibited from -10±8 mL/min/1.73 m^2 to 1±12 mL/min/1.73 m^2 and the 6-month eGFR decrease rate was relieved from -14±12% to 2±20% in all the treated patients (figure1).



During treatment, 2 female patients developed menoxenia, and another 4 developed amenorrhea. The menstrual cycles normalized within 6 months after withdrawal of TW.

Conclusions: TW treatment was associated with an impressive reduction of proteinuria in ADPKD patient. It remains to be shown whether the effect of TW on proteinuria occurs independently of its possible effect on TKV enlargement and renal function protection.

Funding: Government Support - Non-U.S.

SA-PO582

Feasibility of Measuring Renal Blood Flow by Magnetic Resonance Imaging without Contrast in Patients with Autosomal Dominant Polycystic Kidney Disease

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Background: Renal blood flow (RBF) has been shown to predict disease progression in autosomal dominant polycystic kidney disease (ADPKD). The value of RBF measurement was, however, investigated in a single study, that included only subjects with a creatinine clearance >70ml/min. Furthermore, contrast enhanced MRI was used, which precludes RBF assessment in patients with impaired GFR. We investigated therefore the feasibility and accuracy of RBF measurement by non-contrast MRI (RBF_{MRI}) in ADPKD patients with a wide range of eGFR.

Methods: Accuracy of RBF_{MRI} measurement was assessed by gold standard for measuring flow using a phantom. In a test-set of 21 ADPKD patients repeated RBF_{MRI} measurements were performed to assess reproducibility. In addition, in a cohort of 91 patients RBF_{MRI} was compared with RBF estimated by continuous infusion of hippuran (RBF_{Hip}). Variability in RBF_{MRI} and RBF_{Hip} explained by disease characteristics in ADPKD severity was investigated using multivariate regression analyses.

Results: Concordance correlation coefficient between flow measured by fluid collection in a phantom (gold standard) and phase contrast MRI assessed flow was 0.97. Intra- and interobserver coefficients of variation (CV) in the test-set were 2.3% and 3.5%, resp. In the patient cohort technical success rate of RBF_{MRI} measurement was 76%. Technical problems that precluded RBF_{MRI} measurement occurred predominantly in ADPKD patients with lower eGFR (34 versus 16% of subjects with eGFR ≤70 and >70ml/min*1.73m^2, resp., p=0.02). In patients with lower eGFR, intra-CVs were higher compared to patients with higher eGFR (3.2 versus 1.5%) and inter-CV was 3.5%. RBF_{MRI} correlated with RBF_{Hip} (r=0.81, p<0.001). In subjects with higher eGFR variability explained by disease characteristics was similar for RBF_{MRI} and RBF_{Hip} (R^2 0.36 versus 0.51 resp., p=0.2) whereas in subjects with lower eGFR this was less for RBF_{MRI} (0.17 versus 0.72 resp., p<0.001).

Conclusions: Our study shows that RBF can be measured accurately in ADPKD patients by non-contrast MRI, but this technique may be less feasible in subjects with lower eGFR.

SA-PO583

Diagnostic Accuracy of Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography with Computed Tomography for Cyst Infections in Patients with Polycystic Kidney Disease

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Background: Renal/hepatic cyst infections are a common complication of polycystic kidney disease (PKD). CT and MRI are not always contributive for the diagnosis, which relies on an index of clinical suspicion. Objective: diagnostic accuracy of F18FDG PET-CT in cyst infection of PKD patients, and compare it with other imaging techniques.

Methods: Monocentric retrospective study on patients with PKD hospitalized in 2006-2013 for a suspicion of cyst infection, who underwent a PET-CT. Control group of patients with PKD undergoing PET-CT for other motives. Diagnosis of cyst infection retained a posteriori on index of clinical suspicion, absence of differential diagnosis and favorable response to treatment. PET-CT considered positive if focal hypermetabolism of the wall or one/several cysts. CT or MRI considered positive if thickened cyst walls (contrast enhanced if injection performed) and infiltration of adjacent fat.

Results: 32 PET-CT were performed in 24 patients with PKD for suspected cyst infection. The diagnosis was retained in 18/32. PET-CT was positive in 14/18 (true positive), negative in 4/18 (false negative). There was no false positive, even in the 9 control patients. PET-CT had a sensitivity of 77%, a specificity of 100% and a negative predictive value of 77%. PET-CT revealed a differential diagnosis in 3 patients (1 diverticulitis and 2 pulmonary lesions). In parallel, 21 CT (18 with contrast media injection) were performed in PKD patients with a suspicion of cyst infection. Diagnostic accuracy of CT was poor : sensibility 7%, negative predictive value 35% (p < 0,001 versus PET-CT). MRI was performed in 8 patients only (1 true positive, 1 false negative, 6 true negative).

Conclusions: Diagnostic accuracy of PET-CT is superior to that of CT in cyst infections of PKD, for comparable radiation exposure and without nephrotoxic contrast media injection. Prospective studies are needed to evaluate the cost-effectivness of PET-CT in this indication.

SA-PO584

Novel Approach to Estimate Kidney Volumes Using Computer-Assisted Segmentation Tools in ADPKD

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Background: Some recent data indicate that kidney growth is a critical predictor of progression to renal failure in autosomal dominant polycystic kidney disease (ADPKD) patients. Consequently, kidney volume growth is considered the best surrogate marker predicting the decline of renal function in ADPKD. It is, however, hard to measure kidney volume, easily, precisely and reproducibly. To evaluate whether kidney volumes can be accurately estimated using semi-automatic computer-assisted segmentation tools images of patients with ADPKD.

Methods: CT images of 23 ADPKD patients (9 men and 14 women, median age 48 years, age range 36–72 years), 46 kidneys were analyzed. For each transverse CT image slice, we measured kidney areas using stereology methods. The kidney volumes were calculated by summing up the area measurements of all the slices covering the kidney by using computer-assisted segmentation tools in Synapse VINCENT (FUJIFILM Corporation, Tokyo, Japan). We then compared the kidney volumes predicted from the conventional ellipsoid method and some clinical parameters.

Results: The median eGFR and cystatin C were 54.8 ml/min/1.73 m^2 and 0.97 mg/l, respectively. The median kidney volumes predicted by conventional ellipsoid method and VINCENT method are 715.4 ml (range; 369.2-8854.3), 621.4 ml (range; 283.4-2837.8), respectively. The kidney volume predicted from VINCENT method correlated extremely well with the kidney volume from ellipsoid method (R(2) = 0.812, p=0.002). In addition, the kidney volume predicted from VINCENT method correlated extremely well with s-Cr (R(2) = 0.806, p=0.003) and cystatin C (R(2) = 0.767, p=0.011), respectively.

Conclusions: Synapse VINCENT is an interactive segmentation tool for efficient, accurate, and reproducible boundary extraction. These tools helped us to reduce the time to get accurate kidney volume.

SA-PO585

Mutations in TRAF3IP1/IFT54 in Patients with Syndromic Nephronophtisis Lead to Cilia and Cytoskeleton Defects

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Background: Nephronophthisis (NPH), a major cause of ESRD in children, is a heterogeneous autosomal recessive renal ciliopathy, the causative genes encoding proteins being involved in ciliary function.

Results: Using NGS, we identified pathogenic recessive mutations in *TRAF3IP1* encoding the intraflagellar transport IFT-B component IFT54 in 5 families with renal and retinal involvement. As expected, IFT54 mutationslead to cilia defects in patients' fibroblasts and truncating mutations prevent interaction with IFT20, its partner within the IFT-B complex.We further demonstrate, using 3D structure modelling and mass spectrometry approaches, that missense mutations impair proper folding of the N-terminal domain and reduce the affinity of IFT54 for tubulin and microtubule-associated proteins. In addition,live-cell imaging in patient's fibroblasts and in knockdown mIMCD3 revealed that beside its known functions at cilia, IFT54 acts as a key regulator of cytoplasmic microtubules organization and dynamics. Consequently, epithelialization defects were observed in both 3D cultured knockdown renal cells and in the pronephros of zebrafish embryos invalidated for *TRAF3IP1*.

Conclusions: Altogether, our findings demonstrate that NPH causing mutations of *TRAF3IP1*, affect both ciliary and non-ciliary functions of IFT54, which can provide an explanation for kidney tubules morphogenesis defects.

Funding: Pharmaceutical Company Support - Novartis, Government Support - Non-U.S.

SA-PO586

Nephronophthisis 13: Implication of Association with Caroil Disease and Altered WDR19 Expression in the Kidney

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Background: Nephronophthisis 13 (NPHP13) is a rare disease with mutations in *WDR19*. We describe 6 additional cases with Caroli syndrome or disease, characterized by focal intrahepatic ductal dilatation with or without congenital hepatic fibrosis, respectively.

Methods: We performed targeted exome sequencing covering 96 ciliopathy-related-genes to 48 patients with a clinical suspicion of NPHP, and detected 3 unrelated index cases (6.3%) with *WDR19* mutations. One case had 2 siblings with the same mutation. We further detected 1 additional case by Sanger sequencing. We evaluated the expression of the WDR19 in the kidney for two patients and two controls by immunohistochemistry.

Results: All six patients progressed to chronic kidney disease and notably, all had Caroli syndrome/disease. Immunohistochemistry for WDR19 showed localized expression along the luminal borders of renal tubular epithelium in controls, while it showed diffuse cytoplasmic staining in *WDR19*-mutated patients.

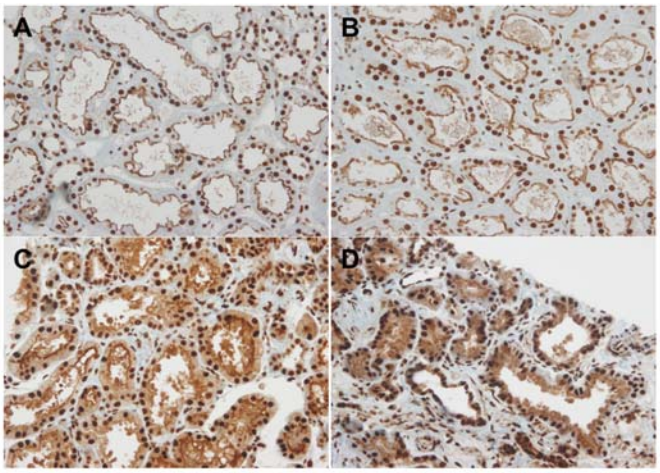

Fig.1 **Immunohistochemistry of WDR19 in kidney tissues of controls and patients.** W DR19 is expressed along the luminal border of the renal tubular epithelium without cytoplas mic staining in both normal (A) and disease controls (B). On the contrary, cytoplasmic staini ng of WDR19 is noted for Patients I-3 (C) and II-1 (D) (x400).

Conclusions: Hepatic involvement, especially Caroli syndrome/disease, may be another major extrarenal phenotype of NPHP13. We have visually validated the mutant WDR19 in NPHP13, in the human kidney using immunohistochemistry.

Funding: Government Support - Non-U.S.

SA-PO587

Studying Mechanisms Underlying NPHP6 in an Ex Vivo Murine Collecting Duct Cell Line

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Background: Nephronophthisis (NPHP) is a childhood cystic kidney disease, which invariably leads to end stage renal disease. NPHP genes encode proteins which localise to the primary cilia, basal body and centrosome. NPHP is a therefore a ciliopathy, and may be a part of a broad spectrum of clinical disease. We present an ex vivo cellular model of NPHP using Cep290 deficient collecting duct cells.

Methods: We have established immortalised wild-type and *CEP290*$^{LacZ/LacZ}$ murine cell lines. The origin of these cells was confirmed by RT-PCR using collecting duct specific molecular markers. Cells were studied to determine proliferation rates and mitotic spindle defects. Mitotic spindle defects were quantified by counting the total number of cells showing abnormal mitosis (>2 spindle poles) in the wild-type and *CEP290*$^{LacZ/LacZ}$ cells. Cells were cultured in matrigel allowing them to form 3D spheroids, develop a lumen and express primary cilia. In order to test specific disease mechanisms cells were treated with drug compounds (including purmorphamine and HPI-4) to determine effect on spheroid formation.

Results: Wild-type and *CEP290*$^{LacZ/LacZ}$ cells express the collecting duct markers epithelial sodium channel alpha subunit (E-NaC), mineralocorticoid receptor (MR), AVPR and Aquaporin-3. Proliferation rates are altered in *CEP290*$^{LacZ/LacZ}$ cells. *CEP290*$^{LacZ/LacZ}$ cells exhibit more abnormal mitotic figures as compared to wild-type cells (40% versus 8%). In 3D culture mutant cells have abnormal primary cilia and are unable to form well defined spheroid structures which is partially rescued by a hedgehog agonist purmorphamine. Wild-type cells treated with Hedghog antagonist HPI-4 mimic *CEP290*$^{LacZ/LacZ}$.

Conclusions: We have characterised a useful ex vivo model of NPHP using a *CEP290*$^{LacZ/LacZ}$ cell line. Abnormalities in cell proliferation, mitosis and hedgehog signalling have been observed. The 3D spheroid read-out has allowed us to develop a platform for drug testing and to explore pathophysiology mechanisms underlying cystogenesis.

SA-PO588

Comparison of Age of Onset of End-Stage Kidney Disease in Medullary Cystic Kidney Disease Types 1 and 2

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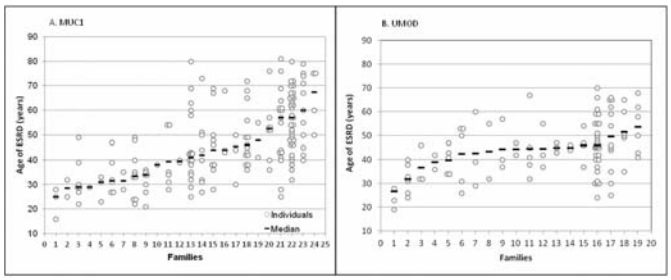
Background: Medullary cystic kidney disease (MCKD) 1 is caused by mutations in the *MUC1* gene encoding mucin-1, and MCKD2 is caused by mutations in the *UMOD* gene encoding uromodulin (Tamm Horsfall protein). Both MCKD 1 and 2 cause a similar type of autosomal dominant interstitial kidney disease. The age of onset of end-stage renal disease (ESRD) has not been compared between the two diseases. We therefore determined the age of onset of ESRD for families with each condition as a potential clue to their pathogenesis.

Methods: Families were identified with mutations in the *MUC1* and *UMOD* genes. As many family members as possible with ESRD were identified, and their ages of ESRD were compared within and between families.

Results: There were 24 families with *MUC1* mutations and 186 individuals in whom the age of onset of ESRD could be determined. The mean age of ESRD was 46.84 years ± 15.22 sd. For *UMOD* mutations, there were 114 family members with known age of ESRD in 20 families. The mean age of ESRD was 43.30 ± 12.40 sd ($p = 0.037$ for comparison between the two diseases). Figure 1 A and B show the ages of onset of ESRD for each family. There was a wide range of onset of ESRD for both types of disease.



Conclusions: While mutations in *MUC1* and *UMOD* are responsible for very distinct forms of MCKD, they have remarkably similar age ranges of onset of ESRD and variation between family members with the same mutation for the age of onset of ESRD. Modifying genes or the environment may be similar between the two disease. Identifying these factors may help slow progression of the disease.

Funding: Private Foundation Support

SA-PO589

Suppressing Angiotensinogen Synthesis Attenuates Kidney Cyst Formation in a *Pkd1* Mouse Model

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Background: Hypertension in Autosomal Dominant Polycystic Kidney Disease (ADPKD) is common and activation of the renin angiotensin system (RAS) is believed to play a role. However, results from clinical studies testing RAS inhibitors in slowing the progression of ADPKD are inconclusive. There is evidence that intrarenal RAS is elevated in ADPKD and therefore we hypothesize that current RAS inhibitors may not adequately block intrarenal RAS. For this study we compared a novel Gen 2 antisense oligonucleotide (ASO) that inhibits angiotensinogen (AGT) synthesis to lisinopril in adult conditional *Pkd1* systemic knockout mice, a model of ADPKD.

Methods: Adult *Pkd1*conditional floxed allele mice expressing cre were administered tamoxifen resulting in global knockout of *Pkd1*. Six weeks after tamoxifen injection, mice were treated with Agt ASO (50-100mg/kg/week), lisinopril (100mg/kg/day), PBS (control) or control ASO (50-100mg/kg/wk) for a total of 10 weeks. Tail cuff was used to measure blood pressure (BP). Kidneys, liver, heart, plasma and urine were collected at 16 weeks after tamoxifen and examined by histology and RT-PCR to assess AGT, renin and markers of fibrosis and tissue injury.

Results: Agt ASO resulted in significant reduction in liver and kidney AGT and increased kidney renin compared to control or lisinopril treatments. Additionally, kidneys from Agt ASO treated mice were not as enlarged and showed reduced cystic volume compared to lisinopril or control treatments. Although both Agt ASO and lisinopril treatments reduced BP, lisinopril was more effective. Markers of fibrosis and tissue injury in kidneys treated with Agt ASO were reduced relative to lisinopril treatment.

Conclusions: These data indicate that Agt ASO treatment is more efficacious in reducing cystogenesis and related sequelae in conditional *Pkd1* knockout mice. This

decrease in cystogenesis was not a result of effective BP lowering. Agt ASO may effectively attenuate intrarenal RAS and therefore be a novel and effective agent for treating ADPKD.

SA-PO590

Antisense Inhibition of Kirsten-Ras Slows Progression of Murine Polycystic Kidney Disease Ayesha Irtiza-Ali,[1] Richard N. Sandford,[2] Dorien J.M. Peters,[3] Adam E. Mullick,[4] Bruce M. Hendry.[1] *[1]Renal Medicine, King's College London, United Kingdom; [2]Medical Genetics, Univ of Cambridge, United Kingdom; [3]Leiden Univ Medical Center, Netherlands; [4]Isis Pharmaceuticals.*

Background: Ras-GTPase-MAPK signalling plays a crucial role in the control of cell proliferation, differentiation and survival. It is consistently activated in different PKD models but the role of the 3 Ras isoforms, Kirsten (Ki)-, Neural- and Harvey- Ras, in PKD are unknown. Our previous work has shown Ki-Ras upregulation as disease progresses across the proliferative to fibrotic phases in an ADPKD orthologous PKD1$^{nl/nl}$hypomorph and recessive Pcy mouse models. This work investigates the effects of Ki-Ras gene silencing using a generation 2 gapmer antisense oligonucleotide (ASO) in mouse models of PKD.

Methods: Study A evaluated the long-term effects of Ki-Ras inhibition in CD1-pcy mice with weekly subcutaneous injection of a Ki-Ras ASO, a control non-targeting ASO or vehicle (n=16/treatment group) from age 3 to 20 weeks. In a second smaller study(B), B6.CD1-PKD1$^{nl/nl}$mice were similarly treated with the Ki-Ras ASO or vehicle from age P21 to P49 (n=5/treatment group). Serum biochemistry assays, qPCR and immunohistochemistry (IHC) were used to assess the effects of treatment on renal function, inflammation and fibrosis.

Results: The targeting ASO specifically knocked down renal Ki-Ras mRNA expression by 65% (p=<0.0001) in Study A, and 85% (p=0.0263) in Study B compared to vehicle. In CD1-pcy mice, Ki-Ras ASO significantly improved serum creatinine levels (p=0.0298), and decreased renal αSMA IHC expression by 3 fold (p=<0.01), as well as expression of inflammatory markers MCP1 (p=0.029) and IL1β (p=0.0032), compared to vehicle. These effects were not seen in the control ASO treated group. As we have previously reported, B6.CD1- PKD1$^{nl/nl}$ develop progressive, severe fibrosis from P21 to P49, but treatment with Ki-Ras ASO over this period reduced αSMA positive area by 2.5 fold (p=0.029).

Conclusions: These results demonstrate specific antisense inhibition of Ki-Ras protects renal function with both anti-fibrotic and anti-inflammatory effects, and slows disease progression in mice with polycystic kidney disease.

Funding: Private Foundation Support

SA-PO591

Inhibition of Aerobic Glycolysis with 2-Deoxyglucose Retards Polycystic Kidney Disease Progression in Han:SPRD Rats Meliana Riwanto,[1] Sarika Kapoor,[1] Daniel Rodriguez,[1] Ilka Edenhofer,[1] Stephan Segerer,[2] Rudolf P. Wuthrich.[2] *[1]Univ of Zurich, Switzerland; [2]Univ Hospital Zurich, Switzerland.*

Background: Autosomal dominant polycystic kidney disease is a common genetic disorder characterized by development of multiple renal cysts. Using microarray analysis and qPCR, we identified altered glucose metabolism in Han:SPRD rat model i.e. upregulation of genes involved in aerobic glycolysis (*Hk1, Hk2, Ldha*) and downregulation of gluconeogenesis genes (*G6pc, Lbp1*), indicating a Warburg effect.

Methods: We examined the effect of 2-deoxyglucose (2DG), a glycolytic inhibitor, on renal function loss and cyst progression in Han:SPRD rats, a PKD model with a phenotype closely resemble human ADPKD. Male heterozygous cystic (Cy/+) were administered 2DG (500 mg/kg/day) for 5 weeks.

Results: Cy/+ rats treated with 2DG (n=10) displayed significantly reduced kidney weights and kidneys/total body weight ratio and decreased renal cyst index, as compared to vehicle treatment (27%, 21% and 48% reduction, respectively). Treatment with 2DG also improved creatinine clearance (1.98±0.67 versus 1.41±0.37 ml/min, p<0.05), BUN clearance (0.69±0.26 versus 0.40±0.10 ml/min, p<0.01) and uric acid clearance (0.38±0.20 versus 0.21±0.10 ml/min, p<0.05). Interestingly, administration of 2DG led to sustained increase of urine output, suggesting renal resistance to vasopressin. Western blot analysis of kidney tissues from 2DG treated Cy/+ rats showed increased AMPK phosphorylation, a negative regulator of mTOR, and restoration of ERK signaling. Notably, 2DG treatment limited cellular proliferation as assessed by Ki67 staining, while no marked increased in apoptosis was observed with caspase-3 measurement. Moreover, in cultured epithelial cells from Cy/+ rats, 2DG dose-dependently inhibited cell growth, reduced lactate and ATP production.

Conclusions: Taken together, our results show that the kidneys of Han:SPRD rats display enhanced aerobic glycolysis which may play important role in the pathogenesis of PKD. Administration of 2DG markedly delayed the loss of renal function and retarded cyst development in Han:SPRD rats with PKD. Targeting the glycolytic pathway may therefore present a novel therapeutic strategy to control cyst growth in PKD.

SA-PO592

B-type Natriuretic Peptide Gene Therapy Improves Cardiac and Renal Functions in Autosomal Recessive Polycystic Kidney Disease Sara J. Holditch,[1] Vicente E. Torres,[2] Yasuhiro Ikedo.[1] *[1]Molecular Medicine, Mayo Graduate School, Mayo Clinic, Rochester, MN; [2]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Polycystic Kidney Disease (PKD) presents with cystic manifestations, renal enlargement, progressive renal dysfunction, and hypertension; ultimately leading to end stage renal failure. B-type natriuretic peptide (BNP) is central in controlling intravascular volume. Additionally, BNP inhibits fibroblast proliferation, prevents RAAS activation, and blocks AVP release. These properties, among others, make BNP a promising therapy for PKD.

Methods: We investigated the effects of long-term BNP overexpression in the rat model of ARPKD (PCK). 3 day old PCK littermates were administered a single intraperitoneal injection of PBS, or adeno-associated virus 9 (AAV9) vectors, expressing BNP under the control of an internal CMV promoter, at 1.0E+12 gc/kg.

Results: At three months, diastolic blood pressure was significantly (*=p<0.05) reduced (DBP: 71.2±10* versus 87.6±5 mmHg), and cardiac function was preserved, in BNP treated rats (EF tech: 74±3* versus 68±3%). Urinalysis showed reduced 24 hour urine output in treated, compared to untreated PCK (UV: 8.8±3* versus 13.2±1mL/24-hr). Creatinine clearance was also significantly higher in BNP-treatment (eGFR: 0.51±0.1* versus 0.26±0.2mL/min). BNP treated rats had significantly reduced (4.71*, and 4.01* fold) renal expression of collagen and fibronectin, respectively. Histologically, treatment preserved glomerular architecture and retarded basement membrane thickening. However, no significant effects were observed on kidney size (TKW/BW 1.27±0.14 versus 1.44±0.18) or renal cystogenesis.

Conclusions: Together, these observations indicate that long-term BNP overexpression can protect renal function and architecture, preserve renal urine-concentrating ability, and provide cardiac protection. Importantly these effects occurred despite the concurrence of renal cystogenesis. This study demonstrates the efficacy of AAV-BNP gene therapy in protecting cardio-renal organ integrity and provides a novel treatment of ARPKD-associated renal damage and hypertension, secondary to progressive renal cystogenesis.

Funding: NIDDK Support

SA-PO593

Soluble Activin Type IIB Receptor Treatment Effectively Blocks Cyst Formation in a Mouse Model for ADPKD Wouter N. Leonhard,[1] Steven J. Kunnen,[1] Fatiha Bouazzaoui,[1] Kimberley Veraar,[2] Martijn H. Breuning,[1] Emile De Heer,[2] Olli Ritvos,[3] Dorien J.M. Peters.[1] *[1]Human Genetics; [2]Pathology, Leiden Univ Medical Center, Netherlands; [3]Bacteriology and Immunology, Haartman Inst, Finland.*

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by *PKD1* or *PKD2* mutations and is characterized by the formation of thousands of kidney cysts. TGFß and Activin signaling are known to play important roles in tissue regeneration, and this process is suggested to have substantial overlap with the process of cyst formation. Nuclear accumulation of pSMAD2/3 in cyst-lining cells suggests the involvement of these pathways in PKD. However, conditional inactivation of the TGFß receptor I (*Alk5*) in renal epithelial cells did not affect the progression of PKD. In this study, we therefore tested the therapeutic potential of an Activin type IIB receptor fusion protein (sActRIIB-Fc), which can be used as a soluble trap to sequester Activin ligands.

Methods: Activin signaling was studied in kidney samples of mice at various stages of adult PKD and in *Pkd1*-wt and *Pkd1*-KO cells by Western-blot and qPCR. Following *Pkd1*-deletion at post-natal day 10 (P10), the tamoxifen-inducible kidney-specific *Pkd1*-deletion mice were treated from P14 to P33 with bi-weekly intra-peritoneal injections with 0, 3 or 10 mg/kg sActRIIB-Fc in PBS. These kidneys were analyzed by qPCR and IHC analysis.

Results: In-vitro: Activin A and B stimulated SMAD2 and ERK1/2 phosphorylation, which was completely blocked by sActRIIB-Fc. In-vivo: The expression of both Activins increased at early and advanced stage PKD in an adult mouse model for ADPKD. Treatment with 3 or 10 mg/kg sActRIIB-Fc effectively blocked cyst formation.

Conclusions: The major findings of this study are 1) Activin signaling is increased at an early stage of PKD, 2) Activin not only stimulates phosphorylation of SMAD2 but also of ERK1/2, which is known to be involved in PKD, and 3) Activin signaling can be therapeutically targeted by sActRIIB-Fc, which effectively inhibited cyst formation in mice. Since soluble Activin receptors are currently being tested in various clinical trials for other purposes, our findings may become clinically relevant to ADPKD patients.

Funding: Government Support - Non-U.S.

SA-PO594

Beneficial Effect of Combined Treatment of Two Gi Protein Activators in PCK Rats, an Orthologous Model of Human Autosomal Recessive Polycystic Kidney Disease Masanori Kugita,[1] Daisuke Yoshihara,[1] Mai Sasaki,[1] Kazuhiro Nishii,[2] Atsushi Suzuki,[3] Yukio Yuzawa,[4] Tamio Yamaguchi,[5] Shigeo Horie,[6] Eiji Higashihara,[7] Shizuko Nagao.[1] *[1]Education and Research Center, Fujita Health Univ, Aichi, Japan; [2]Rehabilitation, Fujita Health Univ, Aichi, Japan; [3]Endocrinology and Metabolism, Fujita Health Univ, Aichi, Japan; [4]Nephrology, Fujita Health Univ, Aichi, Japan; [5]Human Nutritional Sciences, Univ of Manitoba, Winnipeg, MB, Canada; [6]Urology, Juntendo Univ, Tokyo, Japan; [7]Urology, Kyorin Univ, Tokyo, Japan.*

Background: A strategy of decreasing cAMP level by adenylyl cyclase inhibitory G protein (Gi) activators, pasireotide (PAS) and octreotide (OCT), was effective in animal models of polycystic kidney disease (PKD) (Masyuk, Hepatology, 2013). However, hyperglycemia caused by PAS treatment is concerned as an adverse effect in the clinical use (Shenouda, Am J Ther, 2012). Whereas, OCT and co-application of OCT and PAS did not increase serum glucose in rats (Schmid, J Endocrinol, 2012). Therefore, in the current study, we examined the efficacy of combined treatment of OCT and PAS in PCK rats, an autosomal recessive PKD model.

Methods: 4-week-old PCK male were treated with 8mg/kg OCT and/or PAS for additional 12 weeks. After termination, serum and renal tissue were used for analyses.

Results: In PCK rats, kidney weight, serum urea nitrogen, cyst area, Ki67 expression, and blood pressure were decreased either by PAS or combination of OCT and PAS (OCT/PAS), whereas no effect of OCT was shown. The components of mTOR pathway, serum IGF-1 and renal S6 kinase activity were significantly decreased in OCT/PAS group compared with vehicle-, OCT-, or PAS-treated groups. Serum glucose was significantly increased by PAS, whereas no difference was shown between normal rats and PCK rats with OCT/PAS, possibly because insulin/glucagon ratio was decreased by PAS, but not by OCT/PAS combination.

Conclusions: Present results show that co-administration of OCT and PAS has stronger effect on renal disease progression in PCK rats, rather than OCT or PAS alone. Further, since increased serum glucose level is concerned by the use of PAS, a co-application with OCT may reduce the risk of its adverse effect.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO595

An mTOR Kinase Inhibitor Slows Disease Progression in a Rat Model of Polycystic Kidney Disease (PKD)

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Background: Sirolimus and everolimus indirectly bind and inhibit mTORC1. A novel group of drugs, the mTOR kinase inhibitors, directly bind to mTOR kinase, thus inhibiting both mTORC1 and 2. The aim of the study was to determine the therapeutic effect of an mTOR kinase inhibitor, PP242, in the Han:SPRD rat (Cy/+) model of PKD.

Methods: Male rats were treated with PP242 5 mg/kg/d IP or vehicle for 5 weeks.

Results:

	+/+ Veh (n=8)	+/+ PP242 (n=6)	Cy/+ Veh (n=7)	Cy/+ PP242 (n=13)
TBW(g)	277	247	267	281
2KW	2.3	2.1	6.8*	4.7+
2K/TBW (%)	0.8	0.9	2.2*	1.6+
CVD	0.8	0.8	32*	25+
BUN (mg/dL)	23	18	56*	30+
IL-1 (pg/mg)	5.4		9.7*	10+
IL-6 (pg/mg)	2.6		5.7*	4.1
CXCL1 (pg/mg)	1.1		3.7*	3.3
TNF-α (pg/mg)	0.3		1.3 *	0.8
PCNA+ per cyst			0.9*	0.5+
PCNA + per HPF	0.58		5.6*	1.5+

***P< 0.01 vs. +/+,+P< 0.05 vs. Cy/+ Veh. BW=body weight, CVD=cyst volume density. 2K/TBW (%)=two kidney weight to total body weight ratio.**

PP242 significantly reduced the kidney enlargement, the cyst density and the BUN in Cy/+ rats (Table). On immunoblot of kidneys, PP242 resulted in a decrease in pS6, a marker of mTORC1 signaling and $pAkt^{Ser473}$, a marker of mTORC2 signaling. mTORC plays an important role in regulating cytokine production. On Meso Scale analysis, there was an increase in IL-1, IL-6, CXCL1 and TNF-α in Cy/+ rat kidneys that was unaffected by PP242. Apoptosis or proliferation are known to play a causal role in cyst growth. PP242 had no effect on caspase-3 activity, TUNEL positive or active caspase-3-positive tubular cells in Cy/+ kidneys. PP242 reduced the number of proliferating cells per cyst and per non-cystic tubule in Cy/+ rats.

Conclusions: In a rat model of ADPKD, PP242 treatment 1) decreases proliferation in cystic and non-cystic tubules; 2) inhibits renal enlargement and cystogenesis and 3) significantly reduces the loss of kidney function. Blockade of both mTORC1 and 2 has therapeutic potential in PKD.

SA-PO596

mTOR Inhibitor Rapamycin Reduces Cytogenesis in a New *Pkd2*-Mutant Mouse Model

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Background: ADPKD is caused by mutations of *Pkd1* and *Pkd2*. Recent studies showed that rapamycin treatment can improved the cystic phenotypes in mouse models of ADPKD, but clinical trials have not shown clearly benefit. Although studies have showed that rapamycin treatment could achieve therapeutic effects in both *Pkd1*- and *Pkd2*-mutant mouse models, the challenge has been arisen by a recent report that PC1 but not PC2 involves regulation of the mTOR pathway.

Methods: To validate whether rapamycin can indeed inhibit cystic phenotypes and improve renal function of a *Pkd2*-mutant mouse model, *Pkd2* conditional KO mice with *Villin*-Cre transgene (*Vil*-Cre:$Pkd2^{f3/f3}$) have been produced. *Vil*-Cre:$Pkd2^{f3/f3}$ mice exhibit full spectrum of cystic phenotypes which are closely similar to human ADPKD patients. Two groups were designed in this study: i.e. rapamycin-treated group and its solvent DMSO control group (n≥5/group). *Vil*-Cre:$Pkd2^{f3/f3}$ mice for rapamycin group received a single intraperitoneal injection of rapamycin (5 mg/kg/d) and the control group were administrated with the same amount of DMSO from postnatal days 10 (P10) to 55 (P55). The animals were sacrificed at P60.

Results: Mice with rapamycin treatment exhibit significantly prolonged lifespan (27 weeks *versus*. 17 weeks, $P<0.0001$), decreased cyst index (24.93%±4.07% *versus*. 32.25%±5.33%, $P<0.001$), kidney/body ratio (0.0162±0.003 *versus*. 0.0221±0.003, $P<0.0001$), liver/body ratio (0.0566±0.005 *versus*. 0.0605±0.003, $P<0.05$) and improved renal function (BUN) (6.11±0.98 mmol/L *versus*. 9.39±2.02 mmol/L, $P<0.0001$). *Vil*-Cre:$Pkd2^{f3/f3}$ mice with rapamycin treatment also showed significantly decreased proliferation in renal cyst-lining epithelial cells. In rapamycin group, decreased expression of phosphorylation of S6 ribosomal protein (S6Rp) which is a downstream target of mTORC1 can be seen.

Conclusions: Our results indicate that rapamycin treatment can significantly improve cystic phenotypes and prognosis in a new *Pkd2*-mutant mouse model, further validating therapeutic effects of mTOR inhibitor for *Pkd2*-mutant mice.

Funding: Government Support - Non-U.S.

SA-PO597

Reduction in Cyst Volume Requires Inhibition of mTORC2 in Polycystic Kidney Disease

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Background: Mammalian target of rapamycin (mTOR) is found in two multi-protein complexes, mTORC1 and mTORC2, through which it exerts a broad range of physiological effects. Rapamycin (Rapa) primarily inhibits mTORC1; however at high doses it also inhibits mTORC2. In animal models of polycystic kidney disease (PKD), Rapa administered at very high concentrations inhibited cyst progression. In contrast, in human studies, Rapa was given at much lower doses and its effects were disappointing. Here, we examined the hypothesis that mTORC2 inhibition is necessary to blunt cyst progression in PKD.

Methods: $Pkd1^{V/V}$ mice, which develop severe renal cystic disease in early postnatal period, were treated from P5 to P11 with either a potent competitive antagonist of mTOR (mTORcomp), which inhibits mTORC1 and mTORC2 equally, or with Rapa at doses that inhibit only mTORC1. At P11, the mice were sacrificed and the kidneys were harvested and subjected to computer-assisted morphometric analysis to calculate renal cyst area, which was used as a surrogate of cyst volume. The contralateral kidneys were used for Western blot analysis for markers of mTOR activity.

Results: Mice treated with mTORcomp had a 33.2% increase in cyst area during the treatment period, while mice receiving vehicle had a 39.5% increase ($p<0.01$). This difference represents 16% reduction in cyst enlargement for treated mice compared with controls. In contrast, Rapa-treated mice showed an increase in cyst area that was indistinguishable from vehicle treated controls. Western blots showed that Rapa inhibited only mTORC1 (reduced phosphorylation of S6K but not Akt), while mTORcomp inhibited both mTORC1 and mTORC2 (reduced phosphorylation of SK6 and Akt).

Conclusions: Inhibition of mTORC2 is required to blunt cyst growth in this animal model of PKD, suggesting that aberrant mTORC2 signaling is implicated in cystogenesis. Effects of Rapa or its analogues to blunt cyst growth in earlier animal studies are likely due to the high doses used, which inhibited both mTORC1 and mTORC2. These data suggest possible avenues toward more effective treatment of human PKD.

SA-PO598

Novel Means of Gene Therapy in Polycystic Kidney Disease

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Background: Polycystic kidney diseases (PKD) are some of the most common life-threatening monogenic abnormalities. PKD can cause end-stage renal disease (ESRD) with immeasurable suffering, mortality and cost. While mutations for many types of PKD are known and tremendous advances in understanding the pathophysiology have been made, there is no specific therapy to repair or replace the single mutated gene. Autosomal recessive PKD (ARPKD) is truly catastrophic, causing ESRD and death in neonates and children.

Methods: The mechanisms by which renal cell transplant improves structure and function in a model of PKD were examined. Adult tubular cells from normal Sprague Dawley (SD) rats that express wild type genes were given non-invasively to PCK rats, an orthologous model of ARPKD harboring a mutation in *Pkhd1*. The effects of SD renal cells and microvesicles harvested from cultured SD renal cells and the normal rat kidney (NRK) cell line were also evaluated.

Results: Renal cell therapy resulted in expression of both mutant and wild-type Pkhd1 in treated, but not control, PCK kidneys. Marked and sustained improvements in total cyst volume, renal fibrosis, albuminuria, blood urea nitrogen and kidney weight were found in treated PCK rats. We were struck by the large effects of relatively few transplanted normal cells. In examining the mechanisms of the beneficial effects, we found that microvesicles (exosomes) shed by normal primary kidney cells and NRK cells carry *pkhd1* mRNA. PCK cells treated with exosomes from normal cells express mutant and wild type *pkhd1* mRNA. Kidneys from PCK rats treated with exosomes also express both mutant and wild type *pkhd1* mRNA. We also found that primary tubular cells from PCK rats, but not those from Sprague Dawley (SD) rats, form cysts in collagen matrices. When PCK cells were incubated with exosomes harvested from SD cells, they did not form cysts and, in some cases, formed more elongate tubular structures.

Conclusions: Renal microvesicles from normal SD rats and NRK cells can be used to transfer genetic material to mutant PKD cells. The ultimate goal is the future translation of this therapy to the clinical arena.

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SA-PO599

Heavy Chain Deposition Disease Rafia I. Chaudhry,[1] Paisit Paueksakon,[2] Rachel B. Fissell,[1] Neil S. Sanghani.[1] *[1]Nephrology, Vanderbilt Univ Medical Center; [2]Renal Pathology, Vanderbilt Univ Medical Center.*

Introduction: Heavy chain deposition disease (HCDD) is a rare cause of nephrotic range proteinurea. We report a case of HCDD associated with acquired C1q esterase inhibitor deficiency and angioedema.

Case Description: A 45 yo male presented with severe hypertension (HTN), volume overload and acute renal failure. Past history included recently diagnosed HTN, and angioedema while on lisinopril. On exam, BP was 200/129, lungs had coarse crackles bilaterally, and jugular venous distension was 10cm. Workup was notable for creatinine 1.9 mg/dL (baseline 1.5 mg/dL), BNP 5,800pg/mL, albumin 2.5 mg/dL and 24 hour urine protein of 3.6 gm. Urinalysis had 63 RBCs/hpf and 6 hyaline casts on microscopic exam. Serologies resulted HepC Ab pos, hypocomplentemia with C3 60mg/dl, C4 11.7 mg/dl, and a low C1q level at 7.2mg/dL. Transthoracic ECHO showed EF 41%, with enlarged left atrium and heavily trabeculated left ventricle suggestive of protein deposits. Renal biopsy revealed diffuse matrix expansion in a nodular pattern with mesangial and endocapillary hypercellularity with extensive eosinophilic strongly PAS positive material. There was diffuse splitting of the GBM, with 2 crescents. IF positive for IgG3 staining along glomerular and tubular BMs, without kappa or lambda staining. Congo red staining was negative. EM showed extensive punctate electron dense deposits in the GBM, Bowman's capsule, and tubular basement membrane. Final diagnosis was IgG3 heavy chain deposition disease. Interestingly he had 3 episodes of angioedema during this time, despite being off lisinopril for months, suggestive of acquired C1q esterase deficiency. He was started on aminocaproic acid and has had no angioedema since. His HCDD was treated with dexamethasone plus bortezomib, and creatinine improved to 1.5 mg/dL at 3 months.

Discussion: This patient's course was consistent with acquired C1q esterase inhibitor deficiency from hypocomplementemia caused by the HCDD. Extra renal deposition of heavy chains, also seen in this case, occurs less commonly in HCDD than in the other immunoglobulin deposition diseases, and although not proven by a cardiac biopsy, we suspect our patient also had heavy chain deposition in the left ventricle.

SA-PO600

C4 Dense Deposit Disease Associated with Monoclonal Gammopathy Radhika Vemuri,[1] Girish Singhania,[1] Sanjeev Sethi,[2] Rajesh Mohandas.[3] *[1]Nephrology, Hypertension and Transplantation, Univ of Florida, Gainesville, FL; [2]Dept of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [3]Nephrology and Hypertension Section, Dept of Veterans Affairs Medical Center, North Florida/South Georgia Veterans Health System, Gainesville, FL.*

Introduction: C4 dense deposit disease is a recently described glomerulonephritis characterized by proliferative glomerulonephritis on light microscopy, negative Ig and C3 staining on immunofluorescence microscopy (IF) and dense capillary wall deposits on electron microscopy. The hallmark is staining of deposits for C4d. Monoclonal gammopathy has been associated with both Ig- mediated glomerulonephritis and C3 glomerulopathy, but has not been described in C4 dense deposit disease. Here we report a case of C4 dense deposit disease associated with a monoclonal gammopathy.

Case Description: A 53 year old veteran with substance abuse, HTN and CKD with a baseline Cr of 1.4-1.7 was admitted with AKI (Cr 3.0 mg/dL) and uncontrolled hypertension (BP 185/117). Urinalysis showed 50-100 RBC/HPF. Immunofixation studies showed IgG kappa. A bone marrow biopsy showed 5 % plasma cells with distinct population of immunophenotypically aberrant kappa restricted plasma cells. Patient's renal function worsened requiring dialysis. The kidney biopsy showed a proliferative glomerulonephritis with extensive focal segmental and global glomerulosclerosis, with negative staining for C3 and Ig, including light chains. Electron microscopy showed highly electron dense sausage –like deposits along the glomerular basement membranes. Complement assays including CH50, C3 nephritic factor and functional assays of alternate pathway were normal. Given dense deposits on EM and lack of C3 a stain for C4d was done which revealed dense (2-3+) staining for C4d along the glomerular capillary walls. A diagnosis of C4 dense deposit disease was made.

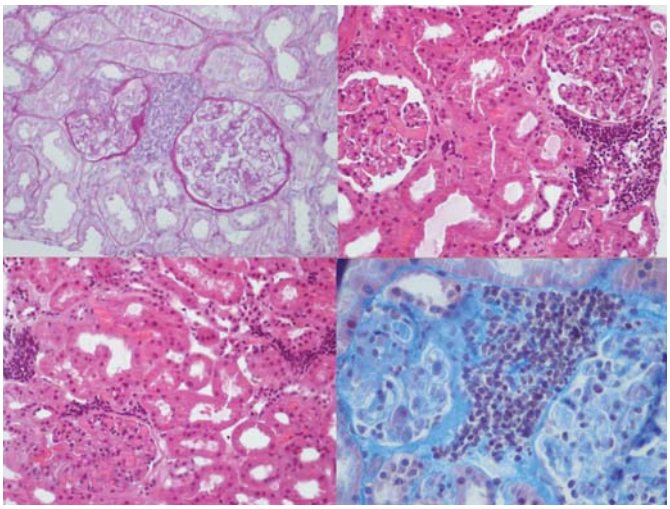
Discussion: This case highlights the association of a monoclonal gammopathy with the recently reported complement glomerulopathy that appears to be driven by over activation of the lectin pathway and deposition of C4d. Given the age of presentation we postulate that monoclonal gammopathy had a role in development of C4 dense deposit disease.

SA-PO601

Waldenström Macroglobulinemia and Minimal Change Disease: Treatment with R-CVP for a Rare Association Manuel Alfredo Podesta, David Cucchiari, Silvia Finazzi, Simona Verdesca, Rossella Valentino, Albania Calvetta, Claudio Angelini, Salvatore Badalamenti. *Nephrology and Dialysis Unit, Humanitas Clinical and Research Center, Rozzano, Milano, Italy.*

Introduction: Waldenström macroglobulinemia (WM) is defined as a lymphoplasmacytic lymphoma associated with a monoclonal IgM protein. Paraneoplastic renal manifestations of the disease are extremely rare: among them, minimal-change disease (MCD) has been reported in only 4 previous cases.

Case Description: A 54 year-old woman presented with facial and dependent edema. Physical examination was otherwise unremarkable. Laboratory investigations revealed normal renal function, microhematuria (30 RBC/HPF) and nephrotic-range proteinuria (7.8 g/day). An immune panel was negative. S-PEP showed a monoclonal peak in the gamma region, which resulted to be IgM-κ at immunofixation. Serum IgM were 1200 mg/dL, while Bence-Jones proteinuria was negative. A renal biopsy showed only focal interstitial infiltrates and 20 normal glomeruli.



IF was negative, thus confirming the diagnosis of MCD. In addition, a bone marrow biopsy revealed a $CD20^{+}/CD103^{-}$ lymphoplasmacytic infiltrate with IgM-κ monoclonal restriction, consistent with WM. The patient was treated with 6 cycles of R-CVP (Rituximab, Cyclophosphamide, Vincristine and Prednisone). After the fourth cycle, the patient underwent complete remission, with a rapid decrease of the proteinuria (0.3 g/day).

Discussion: Anecdotal reports showed that standard therapy with corticosteroids is ineffective in WM-associated MCD, probably due to specific pathogenic mechanisms underlying this condition, which may differ from idiopathic MCD. Indeed, several MCD cases have been described as paraneoplastic manifestations of Hodgkin's lymphoma. However, we report one of the few WM-associated MCD cases that required specific WM chemotherapy to obtain a remission of the nephrotic syndrome.

SA-PO602

IgG4-Related Renal Disease Presenting with Tumor-Like Masses Ghayyath Sultan, Hema Manickam, Chandandeep Takkar. *Dept of Nephrology, UTHSCSA, San Antonio, TX.*

Introduction: IgG4-related disease (IgG-4 RD) is a rare emerging entity characterized by IgG-4 - positive lymphoplasmacytic infiltration that might form tumor- like nodules and masses. We report a case of renal mass-like lesions in a patient with excellent response to steroid therapy and recurrence of the lesions when treatment was discontinued.

Case Description: A 48 year old man with no past medical history presented with weight loss, jaundice and abdominal pain for three months. He was found to have autoimmune pancreatitis, autoimmune hepatitis, sclerosing chlangitis and retroperitoneal fibrosis. Laboratories investigations revealed elevated IgG-4 level at 382 mg/dl (7-89 mg/dl). Low complement C3 level at 76 (98-162 mg/dl), normal complement C4 and negative screen for hepatitis B, hepatitis C, HIV, ANA and ANCA. CT scan of abdomen showed bilateral renal lesions concerning for malignancy. MRI of abdomen showed hypoenhancing nodular lesions (1.2–1.5 cm) in the bilateral kidneys suggesting renal involvement associated with IgG-4 related disease. He was started on prednisone 40 mg daily; azathioprine was added later on due to recurrent sclerosing cholangitis requiring biliary stenting. Renal lesions and retroperitoneal fibrosis disappeared on CT scan after steroid treatment. Numerous bilateral kidney rounded lesions (1.7 - 2.2 cm) were seen again on ultrasound, CT scan and MRI of the abdomen six months after he was taken off prednisone. Patient was restarted on prednisone and these lesions showed interval decrease in size and number on MRI six months after restarting the treatment. IgG-4 level also decreased to normal with treatment. Over three years follow up; kidney function remained normal with minimal proteinuria 200 mg/24 hour urine.

Discussion: Tubulointerstitial nephritis is the most common finding in patients with IgG4-RD; Nodular or mass lesions mimicking renal carcinoma can also bee seen. Corticosteroid is the treatment of choice and usually leads to a rapid resolution of most IgG4-RD lesions, although recurrence and relapse are frequent when corticosteroid is reduced or withdrawn.

SA-PO603

HIV – Associated Non-Hodgkin's Lymphoma Presenting with Primary Renal Involvement Seyyar A. Khan,[1] Anna T. Levy,[2] Anna Mathew.[1] *[1]Nephrology, Hofstra North Shore LIJ School of Medicine; [2]Hematology, Hofstra North Shore LIJ School of Medicine.*

Introduction: 25-40% of patients with HIV develop malignancy; 10% are Non-Hodgkin's lymphoma(NHL), commonly diffuse large B cell lymphoma (DLBLC). Common extranodal involvement includes GI tract, lung, and bone marrow. Kidney involvement is seen in 30-60%, but primary renal lymphoma is rare. Described here is an unusual case of DLBCL due to lymphomatous infiltration of the kidney, presenting as AKI.

Case Description: A 48 y/o female with HIV presented with b/l flank pain, fevers, night sweats and weightloss x 1 week. She discontinued ART, but restarted 2 weeks earlier. On exam, a 1.5 cm left axillary lymph node was palpable. On admission, Cr was 2.95, and peaked at 3.34. Pro/Cr ratio was 1.2g. CD4 count was 117 cells/uL and viral load was 1097 copies/mL. CT scan showed enlarged kidneys (right 15cm, left 13 cm). Numerous retroperitoneal and paraaortic lymph nodes measuring less than 1cm were present. A kidney biopsy was performed. Light microscopy showed <1% intact tubules and near total infiltration by a monomorphic atypical lymphoid infiltrate. Glomeruli were normal. Electron microscopy showed tubuloreticular inclusions in endothelial cell cytoplasm. Features were consistent with DLBCL. PET/CT showed uptake in b/l axillary lymph nodes and symmetric tracer activity in b/l kidneys. Bone marrow biopsy showed normal trilineage hematopoiesis. Left axillary lymph node biopsy was consistent with DLBCL. After 8 weeks of chemotherapy(DA R EPOCH) Cr decreased to 1.0 mg/dL. Six months post treatment, patient is in remission with normal renal function.

Discussion: Primary renal lymphoma accounts for 0.7% of extranodal lymphomas and < 1% of renal lesions. The low CD4 count, systemic symptoms, flank pain and kidney enlargement suggest an HIV-related lymphoma. Renal biopsy with immunohistochemical staining confirmed the diagnosis. AKI resolved and lymphoma is in remission with ART and chemotherapy. Patients with HIV-related lymphoma have numerous kidney manifestations. Hemodynamic and drug-induced mechanisms must be separated from AKI caused directly by malignancy, as therapy relies on treatment of the underlying disease.

SA-PO604

An Unusual Kidney Presentation of Multiple Myeloma in a 45-Year-Old Man Antioco Fois,[1] Daniele Derudas,[2] Emanuele Angelucci,[2] Riccardo Cao,[1] Maura Conti,[1] Alice Atzeni,[1] Antonello Pani.[1] *[1]Nephrology, Azienda Ospedaliera Brotzu, Cagliari, Italy; [2]Hematology, Ospedale Oncologico Businco, Cagliari, Italy.*

Introduction: Multiple myeloma is a plasma cell dyscrasia caused by the proliferation of a single cellular clone producing a monoclonal immunoglobulin, usually presenting with renal failure, osteolytic lesions, anemia and hypercalcemia. The most common kidney pathology is determined by light chain cast nephropathy, light chain deposition disease (LCDD or AL amyloidosis. In a small percent age of cases, renal lesions are atypical, such as interstitial nephritis and light chain proximal tubulopathy.

Case Description: Eight months before coming to our attention, a 45-year-old male had been admitted to a different hospital due to acute renal failure. Renal biopsy showed acute interstitial nephritis with chronic lesions related to NSAIDS adminstration for lower back pain. He was treated with prednisone for 7 months after which he was admitted to our unit because of fatigue, nausea and back pain. The laboratory workup showed renal insufficiency, proteinuria, hypercalcemia, hypereosinophilia and a significant increase in serum kappa free light chains with a kappa to lambda ratio of 909:1. Bone marrow biopsy revealed 70% plasma cell infiltration, and skeleton xray highlighted multiple osteolytic lesions. Kidney biopsy showed chronic interstitial nephritis with aspects of cast nephropathy and a k-restricted light chain proximal tubulopathy with crystals. Light chain multiple myeloma was diagnosed and the patient was treated with steroids, bortezomib and thalidomide. He showed no response to the therapy, and four months later he died of multi organ failure.

Discussion: Interstitial nephritis and light chain proximal tubulopathy represent an uncommon onset of multiple myeloma.We believe that the interstitial damage revealed by the first renal biopsy was due to multiple myeloma, perhaps unrecognized because of the lack of clinical suspicion. Multiple myeloma may be masked and it is fundamental to search for it depending on the clinical presentation, even if the pathologic diagnosis is atypical.

SA-PO605

T-Cell Lymphoma Presenting with Type B Lactic Acidosis Laith Farah Al-Rabadi,[1] Roberto Leon Ferre,[2] Christopher D. Blosser,[3] Mony Fraer.[4] *[1]Nephrology, Boston Univ Medical Center; [2]Internal Medicine, Univ of Iowa Hospital and Clinics; [3]Nephrology, Univ of Washington; [4]Nephrology, Univ of Iowa Hospital and Clinics.*

Introduction: Type B lactic acidosis is a rare complication of hematologic malignancies that tends to portend a poor prognosis. Although several hypotheses have been proposed, the underlying mechanisms that lead to lactic acidosis in cancer patients, mostly with hematologic malignancies, remain elusive. We report the case of a 65-year-old female who presented with lactic acidosis of unknown cause. After extensive investigations, she was diagnosed with T-Cell Lymphoma.

Case Description: Patient was in severe respiratory distress and required support with a ventilator. She was started on continuous renal replacement therapy (CVVHDF) in an attempt to correct the metabolic acidosis while definitive treatment with chemotherapy was pursued. She was started on CHOP chemotherapy regimen (cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone). lactic acid continued to rise. She had a very rapid decline with demise after 6 days.

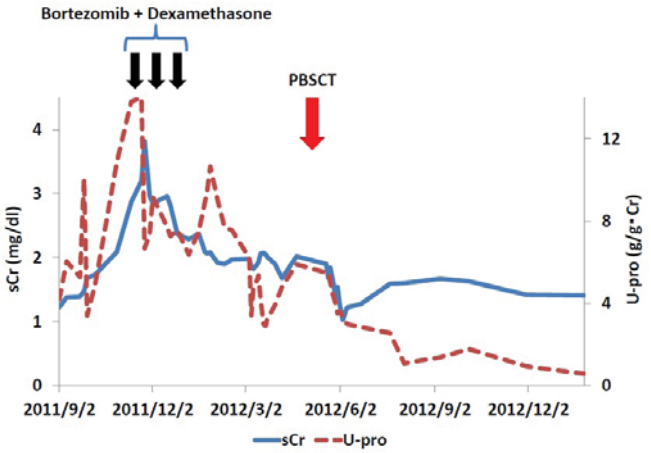
Discussion: The literature pertaining to malignancy-associated lactic acidosis and its potential treatments is scarce. Management strategies are controversial, and favorable outcomes in these patients are more often the exception than the rule. The very few patients, who survived,had received chemotherapy. Lactate clearance through continuous renal replacement therapies in order to decrease the degree of metabolic acidosis, as a temporizing measure until chemotherapy takes effect has been used but with limited results. As described by Levraut et al., the degree of lactate elimination is comparable as that of urea with an estimated excretion fraction of 25%. However, the total amount removed by dialysis is small when compared to total plasma clearance. Hence, lactate levels continue to be good reflection of the primary process without being affected much by these supportive therapies. The beneficial effect, reported in some studies, with continuous venovenous hemofiltration with dialysis on lactic acidosis likely reflects an improvement in acid-base and potentially enhanced lactate metabolism, rather than the direct removal of lactate.

SA-PO606

Light Chain Deposition Disease Successfully Treated By a Combination of Bortezomib-Based Chemotherapy and Autologous Stem Cell Transplantation Saeko Uehara, Takuya Isegawa, Masahiro Koizumi, Masafumi Fukagawa. *Nephrology and Metabolism, Tokai Univ School of Medicine, Isehara, Kanagawa, Japan.*

Introduction: Light chain deposition disease (LCDD) is a clonal plasma cell proliferative disorder characterized by systemic tissue deposits of light chain fragments (usually kappa chain), leading to organ dysfunction including kidney. Its prognosis is very poor, especially in those with kidney involvement, and no standard therapy has been established. However, recent data indicate that bortezomib, a proteasome inhibitor, is a promising agent.

Case Description: A previously healthy 39 year-old female was referred to our department for evaluation of nephrotic-range proteinuria. In the initial test, serum creatinine was 1.2 mg/dl and urine protein was 4.2 g/g Cr. The renal biopsy showed that nodular glomerulosclerosis, linear deposits of kappa chains along tubular basement membrane by immunofluorescence. Urine immunofixation detected kappa chains, and serum kappa: lambda ratio was markedly elevated. The bone marrow exam showed slightly increase of plasma cells (6.6%). As an induction therapy, this patient received three cycles of chemotherapy composed of bortezomib and dexamethasone, and showed response in three months. After the chemotherapy, peripheral blood stem cell transplantation (PBSCT) was performed. The renal function was significantly improved, and the kappa: lambda ratio fell to within normal range.



Discussion: Bortezomib could become a preferred initial therapy for patients with LCDD, and may help improving the outcomes in patients eligible for autologous stem cell transplantation.

SA-PO607

A Case Report: Immunoglobulin D (IgD) Multiple Myeloma with Rapidly Progressing Renal Failure Jwalant R. Modi, Ahmad Eter, Suzanne E. El Sayegh, Elie El-Charabaty. *Medicine/ Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Introduction: Immunoglobulin D multiple myeloma (IgD MM) is very rare form of myeloma affecting less than 2% of all myeloma patients. It has a multiorgan involvement with renal failure being the key feature.

Case Description: We present here a case of IgD MM in a 62 year white male, smoker with past medical history of hypertension, who presented to ED with complaints of lower abdominal pain, constipation and decreased urination. Physical exam was unremarkable. Laboratory investigation showed S. Cr 5.99 mg/dL, hemoglobin 8.7 g/dL and corrected S.Ca 10.6 mg/dL. Urine dipstick showed 100 protein and TP/Cr ratio was 23. Serology was positive for Serum free lambda chain level of 8947.6 mg/L as well with free κ/λ ratio <0.01. The results of serum and urine electrophoresis and immunofixation were also supportive of diagnosis of IgD MM. IgD level was remarkably elevated (27300 mg/L) too. CT scan abd/pelvis was negative for obstructive uropathy. Skelatal survey showed a solitary lytic lesion in the iliac crest. His kidney function deteriorated next day requiring hemodialysis. The bone marrow biopsy was positive for plasma cell hypercellualrity (70-80%) and flow cytometry showed 8% monoclonal IgD lambda plasma cells. The patient was started on bortezomib and dexamethasone and he underwent bone marrow transplant 6 months later. He is doing well hematologically now but he remains dialysis dependent.

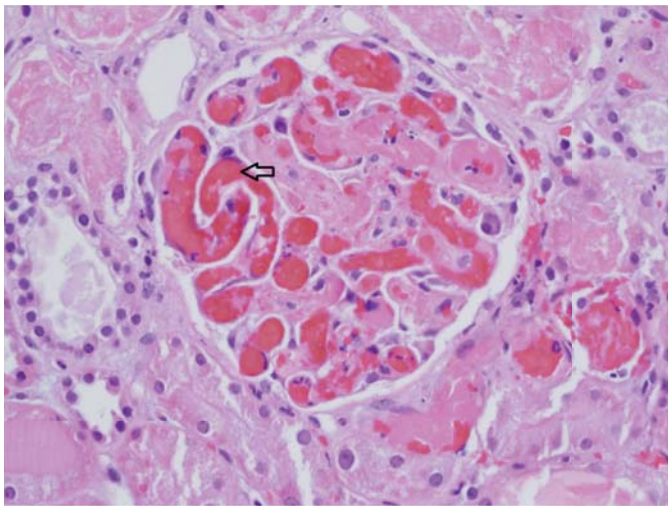
Discussion: IgD MM is very rare disease affecting younger population with poor prognosis, patients often ending up on hemodialysis despite better control of the hematological component.

SA-PO608

Renal Thrombotic Microangiopathy Associated with a Novel Chemotherapy Agent Carfilzomib Owolabi Ogunneye, Jo Abraham, Alfred K. Cheung. *Nephrology, Univ of Utah.*

Introduction: Thrombotic microangiopathy (TMA) constitutes a group of microvascular occlusive disorders characterized by systemic or intrarenal platelet aggregation, thrombocytopenia and end organ damage. Carfilzomib is a novel tyrosine-kinase inhibitor, used as a single agent for multiple myeloma. We report herein a case of TMA secondary to carfilzomib therapy, the first to our knowledge.

Case Description: A 51 year-old Caucasian male presented to the hematology clinic of our institution 12 years after he was diagnosed with smoldering myeloma. Seven years prior to his presentation, he had a recurrence of his myeloma, diagnosed as lambda-light-chain myeloma and was treated with combination chemotherapy, followed by autologous stem-cell transplantation with induction of remission. Unfortunately, he had another relapse one year before this visit. He was therefore enrolled in the Endeavour study and started on the study medication carfilzomib, a tyrosine-kinase inhibitor. On the day of presentation, while on cycle 8, the patient reported reduced urine output. He was noted to have a platelet count of 10 k/mL, a rapid elevation of serum creatinine from 1.2 mg/dl to 4.5 mg/dl in 1 week, bicarbonate of 15 mmol/l, LDH 1038 U/l, haptoglobin <10 mg/dl, ADAMSTS 13 activity >95%, and urinary FeNa 0.3%. His peripheral blood smear revealed schistocytes. Urine microscopy showed multiple dysmorphic erythrocytes and granular casts. Kidney biopsy showed TMA.



Carfilzomib was discontinued promptly and he was treated supportively with hemodialysis.

Discussion: The mainstay in the management of drug-induced TMA involves cessation of the offending agent and supportive therapies. To our knowledge, this is the first reported case of TMA that appeared to be related to carfilzomib. Clinicians should be vigilant for early signs and symptoms of TMA in patients treated with novel therapeutic agents, especially those with a prolonged course.

SA-PO609

Successful Eculizimab Withdrawal in a Patient with Pregnancy-Associated Atypical Hemolytic Uremic Syndrome Cintia Germana Mergulhão da Costa, Hugo Pinheiro, Ederson Vidal Moura, Gisele Vajgel Fernandes, Luis H.B.C. Sette, Têg Marcos Veiga, Carla Tenório Barros Cisne Pessoa, Maria Alina G.M. Cavalcante, Lucila Maria Valente. *UFPE.*

Introduction: Atypical hemolytic uremic syndrome (aHUS) is a rare and systemic disease related to dysregulation of the alternative complement system. The sporadic aHUS is related to various triggers, such as pregnancy. Eculizumab therapy was associated with a significant inhibition of complement-mediated thrombotic microangiopathy and the early infusion was associated with improvement in glomerular filtration rate and also with favorable outcome after drug withdrawal 62-64 weeks later.

Case Description: A 36y woman, in the course of 33 weeks pregnancy, was admitted with hematuria, edema and hypertension in the past 24 hours. She received magnesium sulfate and pregnancy was interrupted. After birth she developed oligoanuric AKI, hemolytic anemia (Hb 5.8g/dL), thrombocytopenia (63.000) and increased LDH (1076U/dL) with mildly increased transaminases. Schizocytes were found in peripheral blood; rate reticulocytes was 5.4%;direct Coombs was negative; haptoglobin was low (33mg/dL); anticardiolipin antibody IgG and IgM, ANA and anti-DNAds were negatives; normal C3 and C4; negative sorology for HBV, HCV and HIV; normal ADAMTS 13 (65%). She undergone daily HD after birth and one session of plasmapheresis; treatment with eculizumab was infused 72h after first session of HD. She increased diuresis (100ml/h) after drug infusion. Patient recovered renal function, not requiring HD 24h later. She underwent a 1-month induction therapy with eculizumab and maintenance up to 24 weeks. Renal biopsy was done 8 weeks after birth and was unremarkable except for mild fibrous intimal hyperplasia. After patient consent we successfully withdraw the drug and 12 weeks later she was asymptomatic with a normal Scr and controlled arterial pressure with 2 anti-hypertensives.

Discussion: Patient had an excellent response to early infusion of eculizumab which was maintained up to 24 weeks. The patient remains off dialysis and with normal renal function during a 3 months follow-up after drug withdrawal.

SA-PO610

A Rare Case of Renal Thrombotic Microangiopathy Associated with Castleman's Disease Anubha Mutneja,[1] Larry N. Cossey,[2] Helen Liapis,[1,2] Ying Maggie Chen.[1] *[1]Renal Div, Washington Univ, St. Louis, MO; [2]Nephropath, Little Rock, AR.*

Introduction: Castleman's disease (CD) is an uncommon lymphoproliferative disorder leading to high circulating levels of IL-6 and VEGF. Renal involvement in CD has only been described in single-case reports. Herein, we report a rare case of renal thrombotic microangiopathy (TMA) associated with CD.

Case Description: A 19 yo male presented with diarrhea and low-grade fever for 3 weeks with a rise of creatinine (Cr) to 1.5 mg/dl. His physical exam was notable for diffuse lymphadenopathy, splenomegaly and moderate ascites. Laboratory findings included hemoglobin 8.2, platelets 73 and normal LDH. Urinalysis showed 2+ blood and 1+ protein. Peripheral smear did not reveal schistocytes. Complement levels were normal, and ADAMTS 13 activity was decreased (10%). Shiga toxin was negative, and stool culture was negative for E coli O157:H7. Plasmapheresis was promptly initiated due to concerns for hemolytic uremic syndrome. However, his renal function kept declining. A kidney biopsy showed diffuse endothelial swelling, mesangiolysis and focal glomerular thrombi, consistent with TMA. Subsequent laboratory findings were significant for positive ANA, ENA, SS-A, SS-B and anticardiolipin antibodies. ESR 59, CRP 99.4, and serum VEGF 343 pg/ml (reference: 31-86) were high. An excisional lymph node biopsy revealed follicular hyperplasia and a diffuse intrafollicular plasma cell infiltrate. HHV-8 staining was negative. The final diagnosis was CD, plasma cell variant. After treatment with rituximab, etoposide and prednisone, his renal function, platelet count and anemia were normalized.

Discussion: Our case illustrates a rare renal histological feature of CD, although CD is known to cause endothelial injury. Other reported renal involvements include amyloidosis, acute interstitial nephritis and focal segmental glomerulosclerosis. IL-6 and VEGF are postulated to suppress glomerular VEGF expression, thereby causing renal TMA. Therapy directed against these inflammatory mediators may have important therapeutic implications.

Funding: Other NIH Support - NIH/NIDDK, Halpin Foundation/ASN Research Grant, and the Children's Discovery Institute of Washington University and St. Louis Children's Hospita

SA-PO611

Simultaneous Infusion of Eculizumab and Intravenous Immunoglobulin Does Not Decrease the Efficacy of Eculizumab Yvonne El Kassis, Juan C. Calle. *Dept of Nephrology and Hypertension, Cleveland Clinic Foundation, Cleveland, OH.*

Introduction: Eculizumab is approved for treatment of atypical hemolytic uremic syndrome(aHUS). Its efficacy with concomitant intravenous immunoglobulin(IVIG) infusion has not been determined yet. We describe the case of a patient with aHUS and toxic epidermal necrolysis(TEN) who got Eculizumab and IVIG simultaneously without subsequent decrease in Eculizumab activity.

Case Description: 39 year-old AAF who presented with hypertensive emergency and seizures and was found to have microangiopathic anemia, thrombocytopenia, acute kidney injury, and thrombotic microangiopathy on kidney biopsy. She was diagnosed with aHUS and underwent plasmapheresis for 1 week without improvement in her thrombocytopenia or kidney function. She was thus started on Eculizumab. She became febrile after the placement of a chest port and developed a generalized skin rash with biopsy proven TEN when she was started on Piperacillin-Tazobactam. She received IVIG for 3 days. She was due for her 2nd dose of Eculizumab but it was postponed for 24 hours after her last IVIG dose. As a surrogate marker of Eculizumab activity, we measured its level and the serum C5 functional activity when she received both infusions a day apart and, a week later, when she only got Eculizumab. In both cases, Eculizumab level was in the therapeutic range and C5 functional level was suppressed.

Discussion: This is the 1st case report of a simultaneous use of IVIG and Eculizumab in a patient with aHUS and TEN. There is only 1 study that looked at the concomitant infusion of IVIG and Eculizumab in 10 patients with motor neuropathy. In this study, Eculizumab was given before IVIG, unlike our patient who got IVIG first. This study showed no difference in complement activity between patients receiving Eculizumab only and those receiving Eculizumab and IVIG on a same day. Our results are consistent with this finding, suggesting a lack of inhibitory effect of IVIG on Eculizumab. This would be relevant in instances where IVIG and Eculizumab are needed together, particularly in the transplant field where Eculizumab appears to be an emerging drug for antibody mediated rejection and where IVIG is a cornerstone of the treatment.

SA-PO612

Vitamin B 12 Deficiency Mimicking Thrombotic Thrombocytopenic Purpura Manish Goyal, Farhanah Yousaf, Bruce S. Spinowitz, Chaim Charytan, Marilyn Galler. *New York Hospital Queens.*

Introduction: Thrombotic Thrombocytopenic Purpura (TTP) typically presents with hemolytic anemia, schistocytosis, and thrombocytopenia. We report a case of vitamin B 12 deficiency that was initially diagnosed and treated as TTP.

Case Description: A 43-year-old Hispanic male with no previous medical history was brought in to emergency department for syncope following a blood draw to investigate a 40

lbs weight loss during the past 6 months associated with decreased appetite and progressive fatigue. The patient also reported a 1 month history of jaundice. He denied any chest pain, dyspnea, fever, cough, tingling, numbness, headache, visual changes, focal weakness, abdominal pain, bleeding, leg swelling, rash, changes in urinary or bowel habits, sick contacts, or any previous history of anemia. On examination, he was hemodynamically stable, afebrile, with pallor and diffuse jaundice but without skin rash or palpable purpura. Neurological examination showed normal sensations and power in all extremities. The presence of hemolytic anemia, schistocytosis, thrombocytopenia, and elevated LDH was suggestive of TTP. Three units of packed red blood cells and one unit of platelets were transfused on Days 1-3. The response to 2 sessions of plasmapheresis on Days 2-3 was inadequate. The low WBC and low reticulocyte count were atypical for TTP and therefore an alternative etiology was sought. Testing revealed a low vitamin B 12 level and positive intrinsic factor blocking antibodies supporting a diagnosis of pernicious anemia with severe vitamin B 12 deficiency. Vitamin B12 replenishing was begun on Day 3. Additional plasmapheresis sessions were performed on Days 4-5 until a negative ADAMTS 13 test result was available. Hematological improvement was observed following vitamin B 12 supplementation. The patient was discharged markedly improved on Day 9.

Discussion: Pseudo-TTP is rare but should be considered in all adult patients especially when the laboratory data has inconsistent features of TTP. In a recent retrospective review of 7 patients with pseudo-TTP and 6 patients with TTP, the most prominent distinguishing features suggestive of pseudo-TTP versus TTP included very high LDH levels and lower reticulocyte counts.

SA-PO613

Postpartum Atypical Hemolytic Uremic Syndrome Successfully Treated with Eculizumab Ginius Pradhan, Rohini Prashar, Sandeep Vetteth. *Nephrology, Univ of toledo Medical Center, Toledo, OH.*

Introduction: Pregnancy associated atypical HUS (aHUS) is increasingly being recognized as a cause of thrombotic microangiopathy in postpartum females. We describe a case of postpartum thrombotic microangiopathy, resistant to plasmapheresis, successfully treated with Eculizumab.

Case Description: A 29-year-old female, postpartum day 3, was transferred to our center for thrombocytopenia and oliguric renal failure. Her blood pressure was 163/99 mm Hg. Her laboratory work showed a creatinine increase from 0.8mg/dl to 3 mg/dl in 3 days, hemoglobin of 8.2g/dl, with schistocytes and a platelet count of 29,000/mm3. She had elevated LDH and indirect bilirubin and low haptoglobin. There was no history of recent diarrhea. Hemodialysis had to be initiated because of worsening hyperkalemia and renal function. Immediate plasmapheresis was initiated for a presumptive diagnosis of Post partum TTP without much improvement in patient's clinical condition, hemogram or renal parameters over the next 10 days. The ADAMTS13 activity was resulted normal. With poor response to plasmapheresis and a normal ADAMTS13 activity, we had a high clinical suspicion for aHUS.

Discussion: aHUS is a disease of uncontrolled alternative complement pathway activation from mutations in complement activation inhibitors. Pregnancy associated aHUS (P- aHUS) is increasingly being recognized now and in a recent study P-aHUS occurred in 21 of the 100 adult female patients with aHUS. Eculizumab is a monoclonal antibody that targets C5 to prevent activation of the terminal membrane attack complex and thus slow down complement-mediated damage. To our knowledge, this is only the third description of successful use of eculizumab in a patient with P-aHUS. In a disease that carries a risk of significant morbidity and mortality, our case emphasizes the importance of considering this novel therapy early if there is a high index of suspicion.

SA-PO614

Hemophagocytic Lymphohistiocytosis Associated with Cytomegalovirus Infection in the Setting of Refractory Thrombotic Microangiopathy Imtiaz M. Ather, Taha Ayach, Hem P. Chataut, Yuvaraj Thangaraj, A. Ahsan Ejaz. *Medicine, Div of Nephrology, Univ of Florida, Gainesville, FL.*

Introduction: We herein report a rare case of hemophagocytic lymphohistiocytosis (HLH) associated with cytomegalovirus (CMV) infection during a prolonged course of treatment for thrombotic microangiopathy (TMA).

Case Description: A 46 year-old female with no past medical history was admitted with diarrhea, thrombocytopenia, and hemolytic anemia. Peripheral blood smear was notable for schistocytes, ADAMTS13 levels were normal, and there was no history of Shiga toxin positivity. Renal biopsy was consistent with thrombotic microangiopathy, for which the diagnosis of atypical hemolytic uremic syndrome (HUS) was made. Treatment was initiated with plasma exchange, eculizumab, and high-dose steroids, however the patient did not improve despite 8 weeks of treatment. Two weeks later she developed fevers and progressive lethargy, followed by acute liver failure. Ferritin was noted to be significantly elevated at 97,473 ng/mL, and triglycerides were elevated at 468 mg/dl. Bone marrow biopsy revealed evidence of hemophagocytosis. Infectious workup was notable for CMV infection with viral load of 28,971 copies/mL. The patient began treatment for HLH and CMV infection with etoposide and ganciclovir, respectively, but ultimately developed worsening multi-organ failure and passed away within a matter of days.

Discussion: Hemophagocytic lymphohistiocytosis (HLH) is a rare and aggressive disorder due to severe immune system activation leading to life-threatening cytokine storm. It can be triggered by viral illnesses including CMV and EBV. Our patient met diagnostic criteria consisting of fever, pancytopenia, elevated serum ferritin greater than 500 ng/ml, hypertriglyceridemia (greater than 265 mg/dl), and evidence of hemophagocytosis in bone marrow biopsy. We believe that HLH was triggered by her CMV infection, which likely arose from aggressive prolonged immunosuppressive therapy for atypical HUS. In these patients, early recognition of signs of HLH followed by rapid evaluation for possible infectious etiologies and directed treatment may prove helpful in preventing subsequent complications of this devastating disorder.

SA-PO615

Thrombotic Microangiopathy from Cannabinoids Rajat Lamba, Shantheri S. Shenoy, Savneek S. Chugh, Praveen N. Chander, David Selzer, Anita Kaul. *Nephrology, Westchester Medical Center, Valhalla, NY.*

Introduction: AKI (acute kidney injury) secondary to Synthetic Cannabinoid(SC) use was first reported to health department of Wyoming in March 2012. 16 cases have been reported since then in United States, of which 6 showed acute tubular injury and 3 showed acute interstitial nephritis on biopsy. We report a case of AKI and TMA(thrombotic microangiopathy) likely associated with synthetic cannabinoid use.

Case Description: A 35 year old female with history of smoking marijuana, Hepatitis C virus infection 10 years prior treated with peg interferon, type 2 diabetes, bipolar disorder, presented with complaints of fatigue and weight loss. On admission BP was 170/100. Labs showed hemoglobin of 11.3g/dl and platelet count of 86,000 with an elevated reticulocyte count of 10.1, LDH of 794, haptoglobin less than 8 mg/dl and uric acid of 8.5 mg/dl She was non oliguric with BUN of 46 and creatinine of 5.0. Urine had trace blood and 2 grams of protein. Ultrasound showed both kidneys were 12cm and isoechoeic. Peripheral blood smear showed 1-2 schistocytes per high power field. TTP/HUS was suspected. She denied any bloody diarrhea. Therapeutic plasma exchange was performed daily for 3 days. Further workup revealed normal ADAMTS 13 activity. ANA, anti-ds DNA, ANCA, HIV and Hepatitis B surface antigen were negative. Hepatitis C antibody was positive with negative HCV RNA, normal serum complements and negative Cryoglobulins. Urine toxicology was positive for cannabinoids. She underwent a CT guided renal biopsy that was consistent with TMA. She was started on Eculizumab, following which her serum creatinine stabilized. 3 months later, she had acute kidney injury from gram negative sepsis and eventually started on dialysis.

Discussion: Cannabinoids, particularly SCs abuse is becoming popular with young adults due to affordability and inability to be detected on routine urine toxicology screens. Although the exact mechanism of TMA in our patient is not known, we suspect that cannabinoids might have evoked an endotheliolitis and triggered a microangiopathic event. TMA from SCs should be included in the differential diagnosis of unexplained AKI, especially in young adults with marijuana use.

SA-PO616

An Unusual Case of Severe Bleeding in a Dialysis Patient Rajat Lamba, Savneek S. Chugh, Karim B. Solangi, Anita Kaul. *Nephrology, Westchester Medical Center, Valhalla, NY.*

Introduction: Bleeding in dialysis patients is multifactorial and is usually associated with vascular stenosis, anticoagulation and rarely from platelet dysfunction due to inadequate dialysis. We are reporting a rare case of life threatening bleeding in a patient on hemodialysis secondary to acquired hemophilia A due to factor VIII inhibitor.

Case Description: A 63 year old man on hemodialysis for 6 years presented to the emergency room for uncontrolled bleeding from the vascular access site, resulting in formation of a large hematoma requiring immediate surgical intervention. Surgery was complicated by severe intraoperative bleeding requiring transfusion. Admission labs showed hemoglobin of 10.5, platelet count of 88,000, BUN of 64, Cr of 11.7, significantly prolonged PTT of 69.7 with normal INR (1.04) and PT (11.4). Surprisingly, patient was not receiving any heparin on dialysis. Repeat PTT was again prolonged (63.5) and antiphospholipid antibodies were negative. Mixing study showed PTT of 33.8 after mixing with normal plasma and further investigation revealed the presence of an inhibitor against Factor VIII (low Bethesda ASSAY titer of 2.8 BU). Patient had another episode of severe bleeding which required administration of Factor VIII, DDAVP, IVIG and Steroids. Within few weeks of starting steroids, factor VIII inhibitor titer was undetectable and PTT improved (33.8). Further investigations were done to rule out any underlying malignancy and autoimmune disorders, which were all found to be negative.

Discussion: Acquired hemophilia A is a disorder characterized by the formation of an inhibitor against Factor VIII. Although about 50 percent of cases are idiopathic, associations have been made with pregnancy, malignancy, autoimmune disorder and medications. The formation of factor 8 inhibitor in this patient could be related to exposure to the dialysis membrane. Any patient receiving adequate dialysis who presents with severe bleeding warrants workup for coagulation disorders.

SA-PO617

Plasmapheresis in *Streptococcus pneumoniae* Associated TTP Kevin C. Roe, Sreedhar Devathi. *Nephrology, Penn State College of Medicine, Hershey, PA.*

Introduction: *Streptococcus pneumoniae* infection is a rare cause of Thrombotic Thrombocytopenic Purpura (TTP) via the action of circulating neuraminidase. However, within the heterogeneous disease of TTP it is unclear if plasmapheresis is an appropriate treatment for *S pneumoniae*-associated TTP. We report a case of TTP with *S pneumoniae* bacteremia effectively managed with plasmapheresis.

Case Description: A 23 year-old Puerto Rican female was admitted for ventilator dependent respiratory failure with *S pneumoniae* bacteremia and pneumonia after starting on belimumab for SLE associated arthritis. She had no previous history of renal disease or nephritis. Her hospital course was complicated by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure requiring renal replacement therapy. Renal biopsy was unrevealing. ADAMTS 13 levels were normal and testing for lupus anticoagulant was

negative. After appropriate antibiotic therapy and clearance of her blood cultures, it was not until starting on plasmapheresis that she had complete clinical recovery from thrombotic microangiopathy and associated acute renal failure.

Discussion: It has not been previously well reported but we believe that plasmapheresis should be considered in the treatment of *S pneumonia*e induced TTP.

SA-PO618

Acute Renal Artery Aneurysm Rupture with Bilateral Renal Infarction: An Unusual Case of Fibromuscular Dysplasia in a Young Male Rio Noto,[1] Hideki Yokoi,[2] Hiroyuki Ueda,[3] Makoto Kinoshita,[4] Nozomu Kamiura,[1] Motoko Yanagita,[2] Akihiro Yoshimoto.[1] *[1]Dept of Clinical Nephrology, Kobe City Medical Center General Hospital, Kobe, Hyogo, Japan; [2]Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan; [3]Dept of Diagnostic and Interventional Radiology, Kobe City Medical Center General Hospital, Kobe, Hyogo, Japan; [4]Dept of Cardiovascular Medicine, Kobe City Medical Center General Hospital, Kobe, Hyogo, Japan.*

Introduction: Fibromuscular dysplasia (FMD) is a nonatherosclerotic and noninflammatory vascular disease that causes arterial stenosis, occlusion, dissection, and aneurysm of medium-sized arteries. Renal artery aneurysms (RAA) occur in 5.6% of patients with FMD, and rupture of RAA is uncommon. Renal failure (1.6%) and infarction (0.9%) are both rare events.

Case Description: A 26 year old male without any underlying diseases presented with progressive left flank pain. His blood pressure was 153/101 mmHg, and his heart rate was 113 bpm. Blood tests showed an elevation in white blood cell count of 19,800/μL and other laboratory findings were within normal limit. A computed tomography angiography (CTA) demonstrated a typical "string of beads" appearance of FMD with dissection of renal arteries and infarction of bilateral kidneys. He was treated with calcium blocker to reduce blood pressure. However, on day 8, he suddenly felt severe, left-sided abdominal pain. His hemoglobin decreased from 15.9 g/dl to 11.0 g/dl, and serum creatinine increased from 0.69 mg/dl to 1.52 mg/dl. A CTA showed large retroperitoneal hemorrhage from rupture of a left RAA, arising from the above-mentioned artery dissection. Receiving blood transfusion, he was treated with a coil embolization of a left RAA. Two days later, a right RAA, found by chance, was embolized with endovascular stent emplacement in the right renal artery dissection. His renal function has recovered and a subsequent CTA showed complete resolution of dissection and no appearance of new lesions.

Discussion: FMD often affects renal arteries but renal infarction and rupture are rare complications, and we will discuss the differential diagnosis of RAA. We first demonstrated rupture of RAA with dissection in a young healthy male with FMD.

SA-PO619

Reninoma a Rare Cause of Hypertension Kerri A. McGreal, Alan S.L. Yu. *Nephrology, Univ of Kansas Medical Center, Kansas City, KS.*

Introduction: Reninoma is a juxtaglomerular cell apparatus tumor that secretes renin. It is a rare cause of secondary hypertension and more specifically secondary hyperaldosteronism, with less than 100 cases ever reported.

Case Description: A 19 year old female was referred for refractory hypertension and hypokalemia. She was healthy until 4 months ago, when her blood pressure was incidentally found to be 260/150 mmHg and potassium was 2.4 mmol/L. Her blood pressure was controlled on 5 anti-hypertensive medications. She received a work-up for secondary hypertension that showed normal urine cortisol, catecholamines, and metanephrines. Her urine drug screen was negative. Ultrasound showed normal size kidneys. Plasma aldosterone was elevated at 52 ng/dL (<21). Plasma renin activity was 10 ng/mL/hr (9-24 upright is normal). 24 hr urine aldosterone was elevated at 30 mcg (<19). She had computer tomography (CT) with contrast that showed no evidence of renal artery stenosis, but an isodense mass versus cyst in the superior pole of the right kidney. Renal vein sampling showed renin level of 25 ng/ml/hr in the right upper pole vein and 49 ng/ml/hr in the right upper pole superior medial vein. The inferior vena cava, left renal, and femoral vein were all <9 ng/ml/hr. She had resection of the mass and the pathology was consistent with juxtaglomerular tumor. Her renin and aldosterone levels normalized on post-op day one. One month later she was controlled on one blood pressure medication.

Discussion: This patient had hyperaldosteronism with unsuppressed renin causing her hypertension. Non-invasive imaging showed no evidence of renal vascular hypertension, a far more common cause of secondary hyperaldosteronism, though CTA is known to have poor sensitivity for fibromuscular dysplasia. When a common cause is not found one has to go searching for the rare causes. Reninoma lesions can be difficult to identify on imaging, as they are usually small and do not enhance on CT. Renal vein renin ratios assist in localizing a renin-secreting mass. In conclusion, reninoma is a rare cause of secondary hypertension but should always be considered when a patient has refractory hypertension with hypokalemia and no other cause of hyperaldosteronism can be found.

SA-PO620

A Curious Case of Abdominal Pain: Still Related to Kidney Pramod Kumar Guru,[1] Abbasali Akhoundi,[2] Kianoush Banaei-Kashani.[3] *[1]Pulmonary and Critical Care, Mayo Clinic; [2]Nephrology, Mayo Clinic; [3]Nephrology, Mayo Clinic.*

Introduction: Spontaneous renal artery dissection is an uncommon cause of renovascular hypertension, but renal artery is the most common site for peripheral dissection. With advent of radiologic procedures renal artery dissections are increasingly recognized. This is a unique case with unexplained abdominal pain.

Case Description: 64 year old male admitted to the intensive care unit for worsening abdominal pain of the prior 12 hours associated with nausea and diarrhea. Patient had history of hypertension, atrial fibrillation and cardiomyopathy. On arrival his blood pressure was 156/96 mmHg. Review of system was negative for dysuria, fever, renal colic or prior surgery. Laboratory studies showed elevated creatinine, leukocytosis, and hematuria. CT scan of the abdomen showed bilateral renal and right external iliac artery dissection with kidney infarcts. Given the history of ablation five days prior, possibility of intervention related dissection was entertained. Work up for associated vasculitides and infections were unremarkable. The exact etiology, spontaneous or iatrogenic, of the dissection was not ascertained during hospital stay. Following IV opioids and antihypertensives his pain subsided and hypertension improved. His subsequent hospital course was uneventful, and his discharge serum creatinine was 1.4 mg/dl, higher than the baseline of 0.9-1.1 mg/dl.

Discussion: Spontaneous bilateral renal artery dissection is a rare entity and reported cases are sparse. Abnormalities of vaso-vasorum and arterial dysplasia leading to spontaneous bleeding have been proposed as the cause of dissection. However, the exact pathogenesis is unknown. Association without proof of causality has been described with fibromuscular dysplasia. Abdominal pain, hematuria and kidney injury are common presenting features. Angiography remains the diagnostic modalities of choice for most patients. Medical therapies directed to control the pain and BP is the preferred treatment. Surgical interventions (radical or endovascular) are reserved for failed medical therapy. Anticoagulation as therapeutic options remains a controversial issue. High index of suspicion is needed to diagnose renal artery dissection.

SA-PO621

Renovascular Hypertension due to an Unusual Cause of Renal Artery External Compression Prince Singh,[1] Sherry-Ann Brown,[2] Roger Shepherd,[1,2] Thomas C. Bower,[3] Suzanne M. Norby.[1,2] *[1]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Dept of Internal Medicine, Mayo Clinic, Rochester, MN; [3]Div of Vascular and Endovascular Surgery, Mayo Clinic, Rochester, MN.*

Introduction: Renal artery (RA) stenosis is a common cause of arterial hypertension (HTN). While atherosclerosis and fibromuscular dysplasia comprise the majority of cases, any structural disorder reducing renal perfusion pressure may produce renovascular HTN.

Case Description: A 20-year-old man presented for evaluation of dyspnea and was noted to have HTN. CT angiogram was negative for pulmonary embolism and incidentally noted upward displacement of the left kidney with kinking of the left RA. Blood pressure (BP) was elevated, averaging 160/110 mmHg. Lisinopril/HCTZ 20mg/12.5 mg twice daily and amlodipine 10 mg daily were initiated. He was referred for further evaluation. 6-hour ambulatory BP averaged 143/87 mmHg with pulse 69/minute. Physical examination was unremarkable; no abdominal bruit was noted. Laboratory tests included serum creatinine (SCr) 1.7 mg/dl, negative spot urinary protein, and normal urine microscopy. Duplex ultrasound (US) noted the left kidney was smaller, 9.1 cm compared to 13.2 cm for the right kidney. Before referral, selective left RA angiogram had revealed high-grade left RA stenosis; however, attempt at angioplasty and stent was unsuccessful. Surgical exploration of the left RA was performed. The left kidney parenchyma was dusky, and the RA was found to be compressed by the left crus of the diaphragm. After wide resection of the crural muscle and fibrous tissue, the RA dilated to a normal diameter. Marked improvement of RA intraoperative Doppler signals was seen. Antihypertensive medications were held after surgery. BP on postoperative day 6 was 125/72 mmHg, and SCr was 1.2 mg/dl. After 3.5 months, Doppler US revealed left RA peak systolic velocities of approximately 140 cm/sec with aortic velocity 102 cm/sec. BP remained normal on no medications.

Discussion: External compression of the left RA by the diaphragmatic crus is an atypical cause of renovascular HTN. Appropriate surgical management can result in normalization of BP.

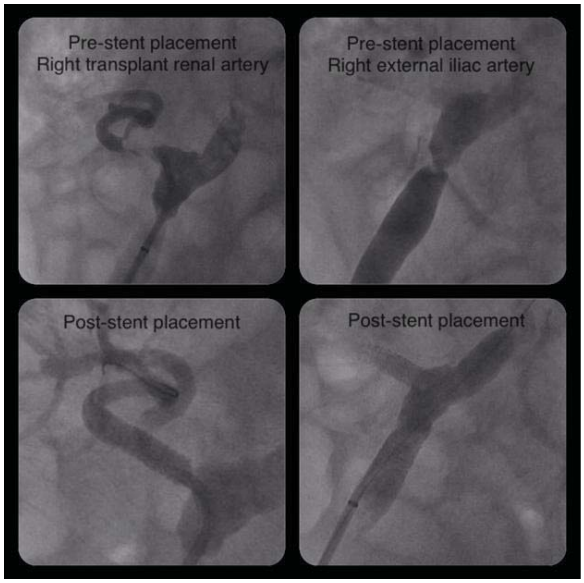
SA-PO622

A Late Presentation of Transplant Renal Artery Stenosis with a Fulminant Course Siddiq Anwar, Derek Larson, Richa A. Pandey, Daniel C. Brennan. *Renal Div, Washington Univ School of Medicine, St. Louis, MO.*

Introduction: Transplant renal artery stenosis (TRAS) is a recognized cause of renal allograft loss, hypertension (HTN) and volume overload. We report an unusual case of microangiopathic hemolytic anemia (MAHA) and malignant HTN caused by TRAS 12 years after kidney transplant (KT).

Case Description: 44yr white male with IgA nephropathy status post deceased-donor KT 12 years ago with baseline creatinine(SC) of 1.8 mg/dL was admitted with malignant HTN, MAHA (hemoglobin 6.7 g/dL platelet count, PC- 4 K/cumm),encephalopathy and anuric AKI(SC- 6.4 mg/dL). We initiated dialysis plus plasma exchange (PEX) for concern of thrombotic thrombocytopenia purpura. ADAMTS 13 activity level was subsequently 63% hence received a dose of Eculizumab for possible atypical HUS with no improvement. Donor specific antibodies,CMV, BK and HIV were negative. With HTN control PC

gradually increased and PEX was discontinued. Doppler suggested TRAS;subsequent KT renal angiogram demonstrated a 99% occlusion of the proximal KT renal artery and 80% stenosis in the external iliac artery (EIA). TRAS was treated with a bare metal Herculink-Elite stents and the EIA stenosis with an Express LD stent. He produced 7 liters of urine in the 12 hours post-stent with a marked improvement in his BP allowing the discontinuation of 4 out of 6 anti-hypertensive agents.SC remains stable at 1.6 mg/dL 18 months post-stent



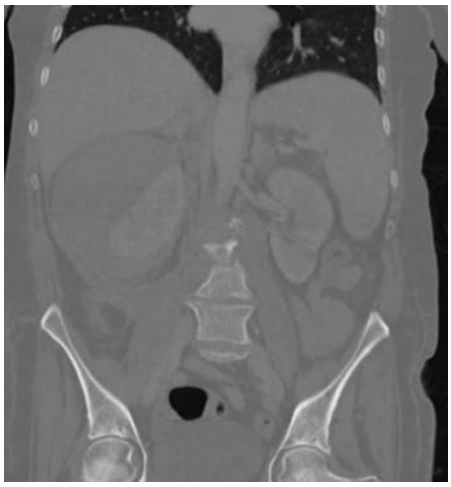
Discussion: Risk factors for development of TRAS include surgical complications,CMV,DGF and the use of older donors. This patient with anuric renal failure, thrombotic microangiopathy and malignant hypertension caused by TRAS had a complete clinical recovery after percutaneous intervention. This case highlights the need to consider TRAS in the differential diagnosis for AKI occurring late post KT and the success of percutaneous intervention if failing optimal medical therapy.

SA-PO623

Extensive Peri-Nephric Hematoma due to Ruptured Renal Artery Mycotic Psuedoaneurysm in a Patient with Bacteremia Ardavan Mashhadian,[1] Seyed-Ali Sadjadi.[2] *[1]Nephrology, Loma Linda Medical Center, Loma Linda, CA; [2]Nephrology, Loma Linda VA Medical Center, Loma Linda, CA.*

Introduction: Septic embolic from the heart can occlude the vasa vasorum of the vessel or the vessel lumen, leading to vascular wall infection and mycotic aneurysm formation. Infective endocarditis is a well-known cause of septic emboli. These emboli are commonly clinically silent and may be medically managed.

Case Description: 55-year-old female with past medical history of heroin abuse, endocarditis, osteomyelitis, and deep vein thrombosis on chronic anticoagulation with Coumadin presented with sever right-sided abdominal pain. Urine-analysis showed mild hematuria and CT scan of abdomen and pelvis showed right retroperitoneal peri-renal hemorrhage (10x8 cm), completely compressing the right kidney.



She had renal artery embolization where upper and mid portion of right renal artery was embolized. This unusual presentation of a septic embolus and its management are discussed.

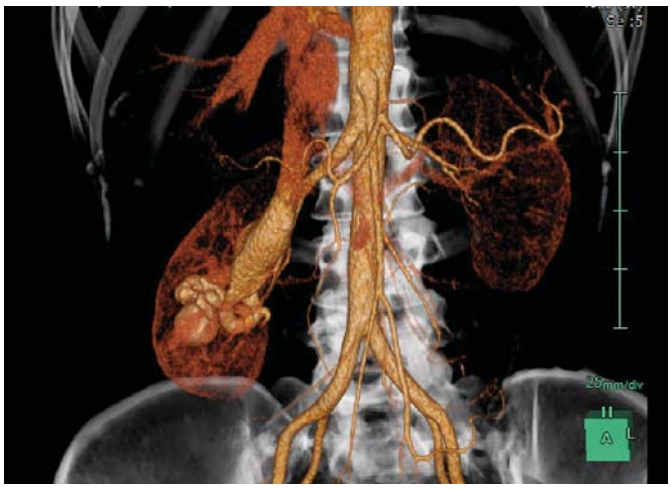
Discussion: Because of their embolic nature, mycotic aneurysms tend to be multiple. Spontaneous resolution with antibiotic therapy for endocarditis has been reported. Smaller renal mycotic pseudo-aneurysms may spontaneously thrombose and require no further management. However, larger pseudoaneurysms may rupture and result in sepsis. These septic sites usually require direct surgical intervention. Hemorrhage may also occur, presenting as gross hematuria, intra-abdominal bleeding, or retroperitoneal bleeding. Renal arterial embolization is a well-accepted procedure and the treatment of choice for these lesions.

SA-PO624

Case of Renal Arteriovenous Fistula after Renal Biopsy, Accompanied with Brugada Syndrome Rie Suzuki, Asako Gondo, Kanako Jimi, Ryuji Tsujimoto, Yoshitaka Miyaoka, Toshikazu Wada, Yume Nagaoka, Yoshihiko Kanno. *Dept of Nephrology, Tokyo Medical Univ, Tokyo, Japan.*

Introduction: Renal biopsy is one of the most common causes of acquired renal arteriovenous fistula (AVF), but its incidence is rare. Also apparently very rare is the comorbidity of Brugada syndrome and chronic kidney disease (CKD) and their relationship is unclear. Here, we report the case of a CKD patient with renal AVF after renal biopsy, who also has Brugada syndrome.

Case Description: A 48-year-old Japanese woman was referred to a nephrologist for proteinuria and an elevated serum creatinine level. She had undergone 2 renal biopsies when she was a teenager. However, she had not received any special treatment. She came to our hospital because of her concern regarding her proteinuria and increased serum creatinine level, although she did not have any symptoms. Upon examination, her blood pressure was normal, but an abdominal bruit was auscultated. Her serum creatinine level was 1.02 mg/dL. Urine analysis showed no hematuria or proteinuria. Upon abdominal ultrasonography, a right renal arteriovenous malformation (AVM) was detected.



During her hospitalization for treatment of the AVM, her electrocardiogram revealed Brugada syndrome. She was diagnosed as having type 2 Brugada syndrome by a positive response in the pilsicainide test. Her renal AVF was successfully treated with coil embolization.

Discussion: Renal AVF and Brugada syndrome were found incidentally in an asymptomatic CKD patient. Our findings suggest that the results of routine examinations should be carefully followed up, even in asymptomatic cases, for the possible detection of abnormalities.

SA-PO625

Digestive Tract Bleeding due to Chronic Kidney Disease-Related Vascular Ectasia Found in Both the Upper and Lower Gastrointestinal Tract Tomomi Itoh,[1] Junichiro J. Kazama,[1] Ryohei Kaseda,[1] Michihiro Hosojima,[1] Suguru Yamamoto,[1] Ichiei Narita,[1] Sakumi Kazama.[2] *[1]Div of Clinical Nephrology and Rheumatology, Niigata Univ, Niigata, Japan; [2]Gastroenterology, Niigata Rinko Hospital, Niigata, Japan.*

Introduction: Gastric antral vascular ectasia (GAVE) is a relatively rare cause of recurrent upper gastrointestinal tract bleeding, while its etiology and pathophysiology remain obscure. Chronic kidney disease (CKD) is known as one of its background diseases, and an elevated circulating gastrin level has been assumed to be the likely cause of CKD-related GAVE.

Case Description: A 78- year- old female was transferred to our emergency room because of faintness and abdominal discomfort. She had received aortic valve replacement therapy, and anti-coagulation therapy with oral warfarin administration had been performed subsequently. She also had CKD with unproven etiology. She had no episodes of pathological bleeding or vasodilatation. She also had no past history of liver or collagen diseases. In the emergency room, she presented with pallor face and obviously anemic palpebral conjunctiva. Atypical honeycomb- like vasodilation was observed in her oral mucosa. Laboratory examinations demonstrated the followings: TP, 6.2 g/dl; Alb, 3.2 g/dl; BUN, 82 mg/dl; Cre, 3.01 mg/dl; Hb, 4.8 g/dl; Ht, 15.6 %; MCV, 94.5 fl; MCH, 29.1 pg; Fe, 142 mg/dl; UIBC, 129 mg/dl; Ferritin, 22 ng/ml and PT-INR, 1.26. A gastroduodenal endoscopic examination detected a typical watermelon-like stomach- type vasodilation in the gastric antrum. Many clots were found around the vasodilated lesion. A capsule

endoscopic and a colon fiberscopic examinations also detected vasodilatation images similar to the watermelon stomach finding along the intestinal tract, and hemodiapedesis from the vasodilated lesion was confirmed at the transverse colon. The patient's condition was improved immediately after transfusion therapy.

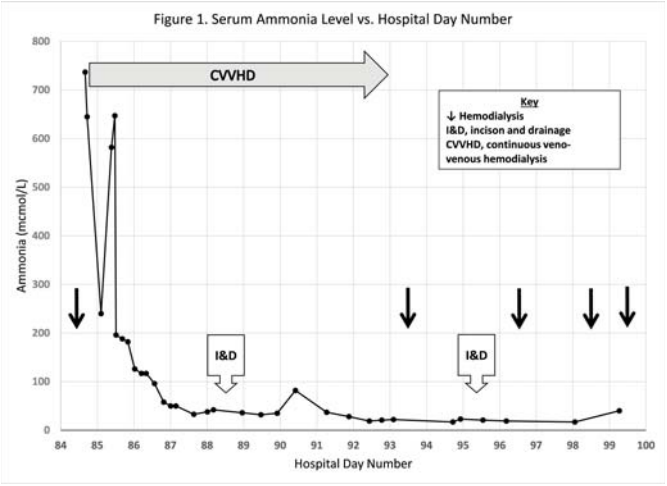
Discussion: GAVE-like vasodilation is not a stomach- specific disease, but can appear anywhere in the gastrointestinal tract in CKD patients. The role of gastrin in this disease condition must be reconsidered, because the gastrin receptors are localized predominantly in the stomach.

SA-PO626

Severe Hyperammonemia due to a *Proteus Mirabilis* Abscess in a Burn Patient without Liver Disease Treated with CRRT Sook Hyeon Park, John Doran. *Nephrology, Emory, Decatur, GA.*

Introduction: Hyperammonemia typically occurs in patients with liver disease, but can occur in normal liver function. We describe a patient with a deep tissue abscess from a urea-splitting organism that caused systemic hyperammonemia and was treated with continuous renal replacement therapy (CRRT).

Case Description: A 60 year old male with history of developmental delay and diabetes was admitted to the Grady Memorial Hospital Burn ICU for a 24% TBSA burn. Hospital course was complicated by peritonitis requiring multiple abdominal washouts, prolonged ventilatory support, atrial fibrillation and acute kidney injury from ATN due to septic shock. On hospital day 53 he began dialysis and required CRRT or intermittent hemodialysis (iHD) throughout the hospitalization to control azotemia. On hospital day 84 iHD was stopped early due to seizure-like activity. Lab tests did not indicate liver disease nor did he have cirrhosis or hepatitis, but the serum ammonia was very elevated at 737 mcmol/L (normal 11-35 mcmol/L). Urgent treatment for acute hyperammonemia was begun with high dose CVVHD (therapy fluid rate 5L/hr), hypothermia protocol, carnitine, 3% saline and holding feeds. On day 88 his thigh was noted to have fluctuance and emergent surgical exploration revealed 700 mL of foul smelling hematoma that grew *Proteus Mirabilis*. His ammonia level decreased rapidly with CVVHD and remained < 100 mcmol/L after incision and drainage.



He died of sepsis complications 2 weeks later.

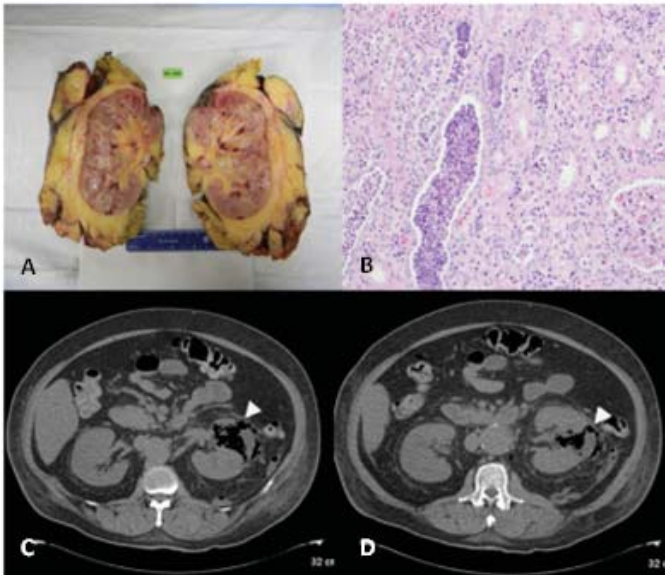
Abscesses containing urea splitting bacteria are a rare cause of systemic hyperammonemia so unexplained hyperammonemia in patients without liver disease should prompt a search for occult fluid collections. Our patient had a very large abscess that produced severe hyperammonemia that was rapidly corrected with high dose CRRT until surgical washout could be performed.

SA-PO627

Renal Emphysema – Indispensable Diagnostic Tools Isha Gupta, Eduardo J. Zouain, Germaine Z. Chan, Steven D. Smith, Ira S. Meisels. *Nephrology, Mt. Sinai St. Luke's Hospital, Icahn School of Medicine at Mt. Sinai, New York, NY.*

Introduction: Renal emphysema, popularly known as emphysematous pyelonephritis, continues to be a highly morbid condition even two centuries after Kelly and MacCullum first described it in 1898. Diabetes, especially when poorly-controlled, is a common risk factor.

Case Description: A 62-year old man presented with altered mental status. He had a history of diabetes type 2, hypertension and gout. He was hypothermic, hypotensive, tachycardic, tachypneic and hypoxemic on room air. He was started on vasopressor support and admitted to intensive care unit. On exam, he had a clouded sensorium, abdominal and left flank tenderness. Initially, blood urea nitrogen was 131 milligram per deciliter (mg/dl), creatinine level was 9.3 mg/dl and white cell count was 12.5 K/microliter. An abdominal and renal ultrasound were unremarkable but the left kidney was poorly visualized. Urine culture and blood cultures grew Escherichia Coli. Non-contrast computed tomographic (CT) scan of the abdomen and pelvis revealed intra-parenchymal air bubbles extending into the nephric space, along with infiltrative changes and small collections within the peri-nephric space diagnostic of class 3A emphysematous pyelonephritis involving the left kidney. He underwent emergent left sided nephrectomy.



A depicts the gross features of the left kidney post nephrectomy, B microscopic findings, C and D (arrowheads) depict the CT scan findings. He required hemodialysis support briefly. Over the next four months, his renal function improved to blood urea nitrogen of 20 mg/dl and creatinine of 1.8 mg/dl.

Discussion: Our case emphasizes the need for early diagnosis of this potentially fulminant disease. CT scan is the preferred imaging method for diagnosing renal emphysema. Once the diagnosis was made via CT scan, our patient underwent life-saving emergent nephrectomy.

SA-PO628

Immunoglobulin G4 Disease in Disguise Thida M. Myint, Michael G. Suranyi, Michael Lin, Ananthakrishnapuram N. Aravindan. *Dept of Renal Medicine, Nuclear Medicine, Liverpool Hospital, Sydney, NSW, Australia.*

Introduction: Immunoglobulin G4 related disease (IgG4 RD) is a rare systemic inflammatory condition of unknown aetiology characterized by lymphoplasmacytic tissue infiltration and fibrosis predominantly by IgG4-positive plasma cells and elevated serum IgG4 level. We report one such case affecting lungs and kidneys mimicking malignancy.

Case Description: A 60-year-old man was admitted for evaluation of unexplained acute kidney injury. He had been experiencing increasing thirst, polyuria, nocturia, leg cramps and 3 kg weight loss in few weeks prior to admission. He had no significant medical history except hypertension for 3 years, which was controlled well. He denied use of any analgesia, antibiotics, herbs or over-the-counter medications. There was no family history of any renal disease. Physical examination was unremarkable. Serum creatinine was 500 mmol/L. Urinalysis did not show active sediment. Chest XR showed right lower zone nodule. Non-contrast CT scan revealed multiple lung lesions, largest in right middle lobe with spiculated margins and pleural tethering suggestive of metastasis. Kidneys appeared normal on CT scan. PET scan showed intensely metabolically active lesions in the lungs, lymph nodes above and below the diaphragm, kidneys, stomach and tail of pancreas. Further investigations had ruled out lymphoma. Renal biopsy showed severe tubulointerstitial nephritis with diffuse inflammatory cells, primarily of plasma cells with positive IgG4 stain (>30/HPF). Core biopsy of the lung showed IgG4 positive plasma cells infiltration with no evidence of neoplasia. Stains for fungi and mycobacteria were negative. Serum ACE level was within range. His plasma IgG4 was high at 15.60 (0.03–2.01 g/L). A final diagnosis of IgG4 RD was made and he was started on steroids (1mg/kg/day). His renal function significantly improved to creatinine of 200 umol/L within 4 weeks. His PET scan 3 months after treatment indicated improvement of lung lesions.

Discussion: IgG4 RD is a rare systemic disease. Differentiation from malignancy changes the direction of management. Early recognition and treatment is essential to prevent extensive irreversible fibrosis. Patients usually have a good response to steroids.

SA-PO629

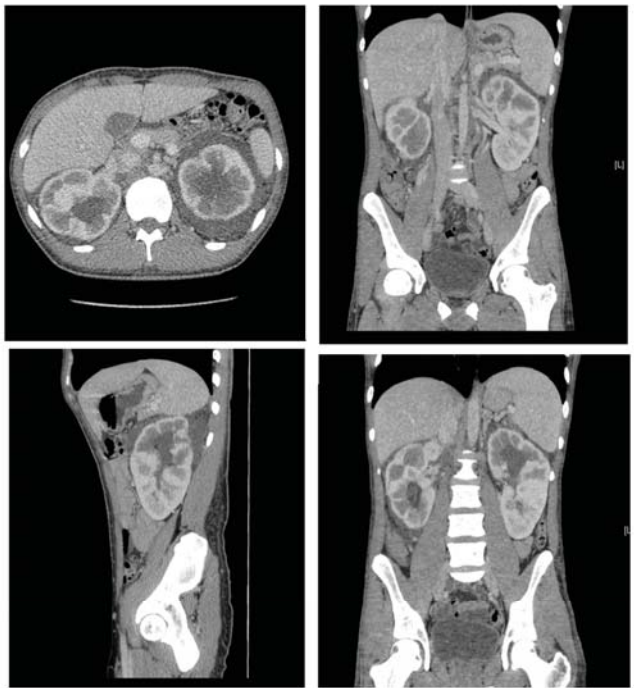
Renal Lymphangiomatosis: An Uncommon Presentation Roopkiranjot K. Kahlon. *Nephrology, Boston Univ, Boston, MA.*

Introduction: Renal lymphangiomatosis is abnormal formation of perirenal,intrarenal and parapelvic lymphatic structures.I present a case of 21 yr old male presented with nausea,vomiting and abdominal pain and found to have enlarged kidneys and renal lymphangiomatosis.

Case Description: 21 yr old male presented with epigastric abdominal pain, nausea and vomiting for 6 days.He had asthma as a child.Family history of ESRD in brother and sister and were on hemodialysis. On examination he was volume depleted and had epigastric tenderness.Lab results showed S.Cr 1.16 mg/dL(baseline unknown),BUN 15 mg/dL,Na 138 mmol/L,K 3 mmol/L,HCO3 23 mmol/L,Ca 9.2 mmol/L,albumin 3.8 gm/dL, 2+ blood and 3-5RBCs in urine and sediment showed few nondysmorphic RBCs.

Ultrasound showed bilateral enlarged kidneys with heterogenous hyperechoic cortex.CT scan showed bilaterally enlarged kidneys R 16cm,L 16.3 cm,with small amount of bilateral fluid at upper poles around collecting system,no hydronephrosis seen. Upon further review with Radiology they were typical images of renal lymphangiomatosis.Patient's symptoms resolved with intravenous hydration.His sister had FSGS(biopsy proven) and cause for his brothers ESRD was unknown.

Discussion: Renal lymphangiomatosis is a rare benign disorder of renal lymphatics and can occur at any age .It occurs due to a failure of renal lymphatic drainage into the retroperitoneal lymphatics, causing dilatation of the ducts and formation of unilocular or multifocal cystic spaces in the perirenal and renal sinuses.May be asymptomatic or present with flank pain, hypertension, proteinuria and hematuria.Differentials include PKD or hydronephrosis. Diagnosis is made by the radiologic appearance.In some case aspiration of the lymphatic fluid can be done which shows chyle rich in lymphocytes.There is no definitive treament but percutaneous drainage and sclerotherapy have proven to be effective. Longterm complications include chronic pyelonephritis and hypertension.



SA-PO630

Spontaneous Forniceal Rupture in a Pregnant Female Presenting with Acute Flank Pain Roshni Upputalla, Belinda Bun Jim. *Nephrology, Jacobi Medical Center, Bronx, Ny.*

Introduction: Spontaneous forniceal rupture usually occurs in setting of obstruction. In pregnancy, forniceal rupture is rare and may be due to increased intra-pelvic pressures.

Case Description: We present a case of a 26 year old pregnant female (G1P0) who presents at 23 wks of gestation with acute right sided flank pain for 1 day with no other associated symptoms such as fever or dysuria.Her past medical and obstetric histories were unremarkable.Her physical exam revealed right CVA tenderness.She was admitted with impression of pyelonephritis and started on antibiotics. Imaging included renal ultrasound, CT and MRI which revealed right forniceal rupture and fluid in the retroperitoneum with no evidence of nephrolithiasis.Initial aspiration of the fluid returned a sterile culture.She improved symptomatically and was discharged home.Four days later, the patient returned again with right flank pain.Repeat imaging revealed an urinoma measuring 17.5 cm.A nephrostomy tube and surgical drain were placed which decreased the size of the urinoma. She improved symptomatically and discharged home with a nephrostomy tube.Throughout this period,her renal function was preserved.She is currently being followed as an outpatient with planned nephrostomy tube changes every 4-6 weeks until her delivery.

Discussion: Pyeloureteral dilatation is a physiological process occurring in pregnancy. The right side is more affected due to relationship of right ureter to the less distensible right iliac artery and right ovarian blood vessels.This begins at 20 weeks of gestation and is continued until delivery; dilatation resolves in almost half of them within 2 days of delivery. Due to these changes, patients in rare instances have increased intrarenal pressures that lead to pelvic rupture.Risk factors include:nephrolithiasis, pyelonephritis, hamartomas and instrumentation.Few improve with symptomatic management;others require intervention with nephrostomy tube and drainage of urinoma.Monitoring and changing of nephrostomy every 4-6 weeks is recommended. Outcomes are favorable if there are no associated features of infection or tumors in pregnancy.A multidisciplinary approach involving urology,interventional radiology,obstetrics and nephrology is recommended.

SA-PO631

Successful Chemotherapy against Invasive Hydatidiform Mole in a Patient with ESRD Tomoaki Onoue, Hideki Inoue, Yuichiro Izumi, Yushi Nakayama, Kenichiro Kitamura, Masashi Mukoyama. *Nephrology, Kumamoto Univ Graduate School of Medical Sciences, Kumamoto, Japan.*

Introduction: Invasive hydatidiform mole (IHM) is a rare disease and standard chemotherapy has not been established for the patients with ESRD. This is the first report of successful chemotherapy against IHM in a patient undergoing peritoneal dialysis (PD).

Case Description: A 42-year-old female patient with ESRD undergoing PD for 4 years was diagnosed as invasive hydatidiform mole. Methotrexate and actinomycin D, which are widely used for chemotherapy against IHM, are not recommended to treat patients with ESRD. Since there are several case reports of etoposide chemotherapy against small-cell lung cancer for the patients with ESRD, we chose etoposide as single-agent chemotherapy. One course of therapy was monthly intravenous administration of etoposide with 50mg/m^2 on days 1, 3 and 5. On the days of chemotherapy, the patient received 4 hr hemodialysis after the administration of the agent. Between each chemotherapy course, PD was followed as usual. Serum human chorionic gonadotropin (hCG) was monitored as a marker of IHM. We observed three consecutive negative levels of serum hCG by 10 courses of treatment and completed the therapy after 14 courses (figure1, 2). No severe side effect and recurrence occurred during the observation period.

Discussion: We succeeded in the treatment of IHM in a patient with ESRD using our regimen, which could contribute to the advancement of chemotherapy for the patient with ESRD.

Figure 1

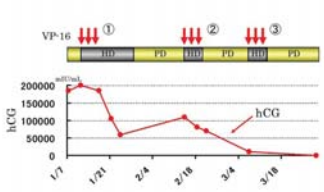
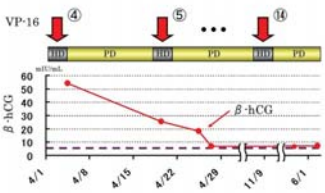


Figure 2



SA-PO632

Recurrent Pyroglutamic Acidosis Related to Therapeutic Acetominophen Hazem M. Alhourani,[1] Lekha K. George,[1] Barry M. Wall,[1,2] Aneel Kumar.[1] *[1]UTHSC, Memphis, TN; [2]VAMC, Memphis, TN.*

Introduction: Pyroglutamic acid (PGA), also known as 5-oxoproline, is an intermediate in glutathione metabolism. Elevated anion gap metabolic acidosis due to PGA has been described as a rare complication of the use of therapeutic doses of acetaminophen in adult patients.

Case Description: 36-year-old African-American female with past history of chronic pancreatitis and alcohol abuse presented with nausea, vomiting, and poor oral intake for 3 days. She took 3-4 tablets of oxycodone/acetaminophen daily for months. She had normal vital signs and general examination was unremarkable, except for cachexia and mild respiratory distress. Laboratory: sodium 133 meq/L, potassium 3.5 meq/L, HCO3 3.0 meq/L, chloride 100 meq/L, albumin 3.9 g/dL, anion gap 30 meq/L, glucose 100 mg/dL, BUN 25mg/dL, creatinine 1.0 mg/dL. ABG revealed pH 7.1, PCO2 16 mmHg, PO2 85 mmHg. Serum osmolarity 280 mosm/kg and calculated osmolar gap was 2.0. Serum ketones, L-lactic acid, D-lactic acid, ethylene glycol, methanol, salicylate, liver function tests and acetaminophen came back negative or normal. Urinary organic acid screen was positive for PGA with value of 20 mmol/mmol creatinine Patient was treated with isotonic sodium bicarbonate intravenously, and the anion gap acidosis resolved over 2 days- serum HCO3 normalized to 25 meq/L, and plasma anion gap to 5 meq/L. On further questioning, she reported two additional hospitalizations in the past month with similar presentations of high anion gap metabolic acidosis of unknown etiology. After changing pain medications to oxycodone, acidosis did not recur during 6 months of follow up.

Discussion: In adults, acquired causes of PGA from acetaminophen use are typically associated with conditions leading to acquired glutathione deficiency. Risk factors include female gender, chronic acetaminophen use, alcohol abuse, chronic liver disease, and malnutrition. PGA should be considered in at risk adult patients with unexplained anion gap metabolic acidosis despite normal acetaminophen levels.

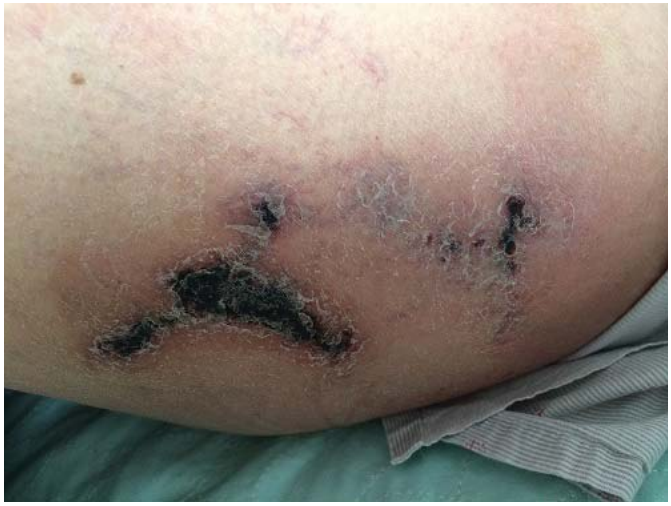
SA-PO633

Non Uremic Calciphylaxis with Pseudoxanthoma Elasticum Maria Saleem Khan, Basel Taha, Robenson Jean Marie, Salem Almaani, Alejandro Diez, Udayan Y. Bhatt. *The Ohio State Univ Medical Center.*

Introduction: Non-uremic calciphylaxis(NUC) is an uncommon condition associated with high mortality. Pseudoxanthoma elasticum(PXE) is a rare systemic genetic disorder caused by mutation in ABCC6 gene leading to abnormal elastic tissue structure in multiple systems. We describe a case exhibiting NUC with coexisting histologic changes of PXE. To our knowledge this is the second reported case of NUC with overlying histologic features of PXE.

Case Description: A 33 y/o white female with PMH of alcoholic cirrhosis, hypothyroidism and heparin induced thrombocytopenia on warfarin presented with

worsening jaundice, abdominal distention, lower extremity edema and skin lesions. Physical Exam was remarkable for jaundice, ascites, peripheral edema and tender purpuric plaques on bilateral thighs and right calf.



Labs: WBC:11.0K/uL, Hgb:9.7g/dL, BUN 6 mg/dl, Cr 0.59mg/dL, ALT 8U/L, AST 41U/L, Alk Phos 188U/L, T.Bili 9.8mg/dL, Alb 2.5g/dL, INR 3.8, Ca 7.7mg/dL, Phos 2.4mg/dL and PTH 24.8pg/mL. MELD:30. EGD showed non–bleeding varices and gastritis. Imaging of the liver was consistent with cirrhosis without evidence for hepatocellular carcinoma. Biopsy of the skin lesions were compatible with NUC with histologic changes of PXE. Treatment with cinacalcet or sodium thiosulfate is equivocal hence she was treated conservatively with local wound care. We believe the cause of NUC in this patient is alcoholic liver disease. The patient is being considered for orthoptopic liver transplantation.

Discussion: Both NUC and PXE are rare diseases and require multidisciplinary approach for management. Given the paucity of cases, treatment guidelines are lacking. Nephrologists are often consulted in the management of the disease given the recognized experience with treating uremic calciphylaxis. The main intervention remains education to keep lesions clean and dry to prevent life threatening infections.

SA-PO634

Thyrotoxic Periodic Paralysis in a Hispanic Male Rakesh Kilari, Anuradha Wadhwa, Shirin Amlani Poonja. *Nephrology, Loyola Univ Medical Center, Maywood, IL.*

Introduction: Periodic paralysis (PP), as manifested by episodes of painless muscle weakness can occur in association with hypokalemia. While most cases of PP are hereditary(autosomal dominant), sporadic cases of hypokalemic PP have been described in association with hyperthyroidism, mostly in Asian population. Here, we present a rare case of thyrotoxic hypokalemic PP in a Hispanic male.

Case Description: A 23 year old Hispanic male with no known medical conditions presented with severe muscle weakness for one day. Weakness initially started in his legs and then progressed to his arms to a point where he was unable to move. Prior to admission, he had been training for army boot camp with rigorous exercise for several weeks and lost 30 lbs during this time. Patient denied any over the counter medication use. Family history was significant for hyperthyroidism in mother. Exam revealed decreased motor strength (proximal>distal), fine tremors and diminished deep tendon reflexes. Admission labwork was significant for hypokalemia (1.4 mmol/L), mild hypophosphatemia/hypomagnesemia and mildly elevated CPK. EKG showed sinus tachycardia, with prolonged QTc and U waves. Urine potassium was low. Treatment with 80 mEq of potassium chloride resulted in rapid resolution of the hypokalemia and weakness. Further work up revealed an undetectable TSH, elevated free T4 at 4.7ng/dL(normal 0.8-1.7) and elevated free T3 at 1315 pg/dL(normal 230-420).

Discussion: Thyrotoxic PP is an often overlooked diagnosis, especially in the western countries. The diagnosis must be considered in any individual who presents with relatively quick onset (usually ascending) paralysis, hypokalemia and hyperthyroid state. These attacks are usually precipitated by strenuous exercise, high carbohydrate meals or stress. Patients may also present with life threatening arrhythmias secondary to hypokalemia. Almost all patients show prompt response to potassium replacement and/or non selective beta blockers such as propranolol. This case highlights that even though most cases have been described in Asians, this rare diagnosis must be considered in Hispanic population with similar clinical presentation and that hypokalemic PP may be the initial presentation of hyperthyroidism.

SA-PO635

Pregnant Filipino with Thyrotoxic Periodic Paralysis: A Case Report Floravil M. Mabras, Brian Michael I. Cabral. *Dept of Medicine, Philippine General Hospital, Manila, Philippines.*

Introduction: Thyrotoxic periodic paralysis (TPP) is a rare complication of hyperthyroidism characterized by acute onset of severe hypokalemia and profound proximal muscle weakness. Ninety percent of all cases reported in the literature were constituted by men of Oriental Asian descent, but rarely among women. It is also not well known that hypophosphatemia, hypomagnesemia and hypocalcemia are also present in TTP.

Case Description: This 35-year-old pregnant Filipino on her 2nd trimester (G7P6, 6006) was admitted for acute lower leg weakness. She had six similar episodes in the past two months which resolved spontaneously over 24 hours. On admission, blood pressure was 130/90, tachycardic with irregular heart rate. Physical examination revealed icteric sclera, diffuse thyromegaly, 3/5 motor strength in lower extremities. In the ER, her initial labs showed thyroid stimulating hormone of <0.005 IU/ml, free thyroxine of 32 pM, hypokalemia at 2.2 mEqs/L, hypomagnesemia at 0.36 mmol/L, hypocalcemia at 1.9 mmol/L and hypophosphatemia of 0.61 mmol/L, urine potassium of 18 mEq/L, urine sodium of 77 mEq/L and normal acid-base status. She was diagnosed as having TTP associated with diffuse toxic goiter in storm. She received propylthiouracil (600mg/day), propranolol (80mg/day), potassium iodide, 80 mEqs of intravenous potassium chloride (KCl) during the first twelve hours, then oral KCl (120mEqs/day). Serial measurements of serum potassium showed a steady return to normal values (3.6 mEqs) within four hours.

Discussion: TTP is predominantly a disease of males, the male to female ratio ranges from 17:1 to 70:1 despite the fact that hyperthyroidism is more common in females. It is ten times more frequent in the Asian population because of presence of HLA-DRw8. Patients with TTP have significantly higher Na-K-ATPase pump number and activity leading to a shift of potassium into the cells. Hyperthyroidism increases renal excretion of magnesium, increased shift of phosphate into cells and hypercalciuria. This case demonstrates a classical presentation of TTP in a female which resolved quickly with a course of non-selective beta blocker, anti-thyroid drugs and supportive potassium chloride correction.

SA-PO636

Thrombotic Microangiopathy and Acute Renal Injury as Complication of Guillain-Barré Syndrome: A Case Report Mariana Pin Andrade, Jonatas Dantas, Beatriz Seves Holanda, Cristiane B. Dias, Luis Yu, Lectícia Jorge, Viktoria Woronik. *Div of Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil.*

Introduction: Thrombotic microangiopathy and acute renal injure after malignant hypertension due to autonomic dysfunction can be a rare complication of Guillain-Barré syndrome. This syndrome is believed to result from an aberrant immune response that attacks nerve tissue. This response may be triggered by surgery, immunizations or infections. The most common form of the disease, acute inflammatory demyelinating polyradiculoneuropathy, presents as progressive motor weakness.

Case Description: We describe a case of female patient, 38-years-old, who was admitted to the hospital with status epilepticus, hypertension (BP 266/194 mmHg) and fundus alterations (cotton-wool spots). In recovery, she had been areflexic.Nerve conduction studies confirmed the clinical diagnosis of Guillain-Barré syndrome and laboratory findings showed a severe acute kidney injury and hemolytic anemia pattern of thrombotic microangiopathy: serum creatinine was 7,2mg/dL, BUN 77mg/dL (eGFR 8mL/min/1,73m2), hemoglobin 6g/dL, haptoglobin < 10mg/dL (30-200), lactate dehydrogenase 2964U/L, schizocytes 12,6%, reticulocyte 0,86% (0,5-2,7%), indirect bilirubin 1,3 mg/dL (0,1-0,6) and platelets 9000; Urinalysis: leucocytes :+100, erytrocites +100, protein >1g/dL. Serology and rheumatologic tests were negative.She was treated with antihypertensive and antiseizure medications and intravenous immune globulin with complete resolution of her autonomic symptoms and improvement in her weakness. She remained on dialysis for 1 month.In the end of the treatment, the patient recovered renal function and the anemia: serum creatinine 1,0mg/dL; hemoglobin 12,5g/dL, without evidence of haemolysis, and urinalysis was normalized.

Discussion: Guillain-Barré syndrome may result in autonomic dysfunction about two thirds of cases, and usually presenting with hypotension. In the literature, there are few cases described with malignant hypertension. We present a rare case of Guillam-barre syndrome with malignant hypertension and hypertensive encephalopathy as the first manifestation.

SA-PO637

Hypertension with Bevacizumab and Regorafenib: Treat, but with Caution Akshita Narra, Gaurav Alreja, Ruchir D. Trivedi. *Div of Nephrology, Univ of Connecticut, CT.*

Introduction: Angiogenesis inhibitors, which are increasingly used in advanced stage solid tumors primarily target vascular endothelial growth factor (VEGF) or its receptors. Hypertension is a well recognized dose-dependent side effect attributable to VEGF inhibition. The knowledge of common side effects of various VEGF inhibitors, particularly the underlying mechanisms of VEGF inhibition is essential for appropriate interventions.

Case Description: A 61-year-old Caucasian male with hypertension was diagnosed with stage II colon adenocarcinoma. He underwent partial colectomy and three years later had recurrence of metastatic disease. Several chemotherapy regimens: FOLFOX, capecitabine, FOLFIRI, and cetuximab were attempted with incomplete response. Subsequently, bevacizumab was initiated for advanced disease. His blood pressure(BP) at that time was well controlled(124/78) on metoprolol succinate. As the disease continued to progress, he was switched to regorafenib. After 1.5 cycles of treatment, his BP was persistently elevated above 160/90mmHg. His creatinine remained at baseline and urine studies revealed mild proteinuria. Amlodipine 10mg and losartan 50mg were sequentially added for effective BP control. His cycle-4 of regorafenib was interrupted due to vascular access complication; he then presented with pre-syncope and had BP of 90/60 mmHg. Lab data revealed acute kidney injury necessitating de-escalation of his antihypertensive regimen. After resuming regorafenib, his BP was again elevated warranting initiation of antihypertensive regimen. His BP was monitored closely with cautious use of reduced doses of antihypertensives during off-cycle of regorafenib.

Discussion: Multikinase inhibitors cause broad-spectrum VEGF inhibition as opposed to bevacizumab, which only binds to circulating VEGF which may explain the difference in

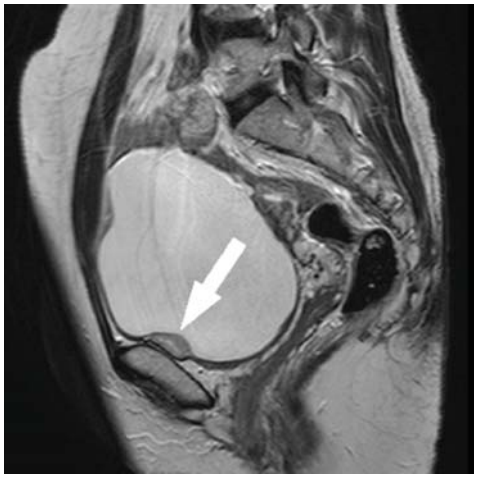
magnitude of BP elevation. Regorafenib through VEGF receptor-3 inhibition, also effects pressure natriuresis potentially contributing to higher BP elevation. Hypertension is on-target side effect hence improves with cessation of treatment. This report underscores the importance of close follow up and cautious use of antihypertensives for VEGF induced hypertension during on and off-cycles.

SA-PO638

"Avoiding" Hypertension Kainat Shahid,[1] Jordan Weinstein,[2] Catherine J. Streutker,[3] Errol Colak,[4] Jeffrey Perl,[2] Kenneth Pace,[5] Hyangsoon Shin,[2] Marc B. Goldstein.[2] *[1]Dept of Nephrology, Univ Health Network, Toronto, ON, Canada; [2]Dept of Nephrology, St. Michael's Hospital, Toronto, ON, Canada; [3]Dept of Pathology, St. Michael's Hospital, Toronto, ON, Canada; [4]Dept of Medical Imaging, St. Michael's Hospital, Toronto, ON, Canada; [5]Dept of Urology, St. Michael's Hospital, Toronto, ON, Canada.*

Introduction: Paragangliomas of the bladder are rare causes of bladder tumors. We present a case of a bladder paraganglioma highlighting the diagnostic and management challenges of these neuroendocrine tumors.

Case Description: A 53 year old Korean female presented with isolated episodes of hypertension, palpitations and diaphoresis post voiding. Urine metanephrines and catelchoamines collected immediately after the episodes were normal on two occasions. Meta-Iodobenzylguanidine (MIBG) scan did not localize for any pheochromocytoma. Pelvic ultrasound revealed no bladder pathology. MRI revealed a brightly enhancing bladder wall lesion on T2-weighted images.



An extramucosal bladder mass was confirmed on cystoscopy. The patient was started on phenoxybenzamine in advance of an uneventful laparoscopic excision of the perivesical mass. Pathology revealed positive chromogranin staining, confirming a paraganglioma. Post-operative course was challenged with prolonged hypotension managed by high sodium intake. Given an association between false-negative MIBG scan and *succinate dehydrogenase* mutations, which carry higher malignant risk, a referral is being made for genetic testing. As malignancy risk is difficult to determine on a histologic basis, she is subject to yearly symptomatic monitoring and imaging as indicated.

Discussion: The case is unique due to the clinical presentation of a functionally active tumor with negative functional testing. It highlights the diagnostic challenges in these rare tumors, and underscores the adage that the key to a diagnosis is in the history.

SA-PO639

The Pickering Syndrome; a Reason to Stent Renal Artery Stenosis Vikyath Prakash, Vijay Lapsia. *Nephrology, Ichan School of Medicine at Mount Sinai, New York, NY.*

Introduction: In 1988 Pickering reported a series of 11 hypertensive patients with bilateral renal artery stenosis (RAS) presenting with acute pulmonary edema that resolved with renal artery revascularization. The recently published CORAL study, a randomized controlled trial (NEJM 2014), showed no benefit of stenting RAS over medical therapy, thereby questioning the use of revascularization in general. However, a large proportion of patients in CORAL had low risk disease and there may still be clinical situations when revascularization is appropriate.

Case Description: A 90 year old woman was admitted with 4 weeks of increasing lower extremity edema and dyspnea on exertion. She was hypertensive and had bibasilar crackles with elevated jugular venous pressure. The chest x-ray revealed central pulmonary congestion and a cardiac echocardiogram showed preserved biventricular systolic function. Her creatinine had increased from 0.9 to 3.1mg/dl in the span of 5 weeks with decreasing urine output. The urinary sediment was bland with no red/white blood cells, casts, or proteinuria to suggest intrinsic renal disease. Shortly after admission the patient became anuric requiring emergent hemodialysis for hyperkalemia and shortness of breath. A renal doppler ultrasound revealed bilateral RAS 80-99%. Within 24 hours of stenting the right renal artery (left was completely occluded) the urine output increased to over 2.5L/day, shortness of breath resolved, dialysis was discontinued and the patient was discharged dialysis free.

Discussion: This case illustrates the benefit to renal artery stenting in selected patients with Pickering Syndrome. Although multiple case reports have demonstrated similar results in the past, with the publication of CORAL it becomes even more critical for nephrologists to identify the limitations of the study and differentiate those patients who may benefit from revascrualization. Prompt recognition and intervention with stenting of high risk presentations like Pickering Syndrome, refractory heart failure, and otherwise unexplained progressive renal insufficiency may lead to tangible outcomes like the discontinuation of hemodialysis in this patient.

SA-PO640

Interest of Diffusion-Weighted Magnetic Resonance Imaging in IgG4-Related Renal Disease Detection and Follow-Up Joelle L. Nortier,[1] Anwar A. Hamade,[1] Agnieszka Anna Pozdzik.[1] *[1]Nephrology, Erasme Hospital, ULB, Brussels, Belgium; [2]Nephrology, Tenon Hospital, Paris, France.*

Introduction: Tubulointerstitial nephritis (TIN) is a manifestation of IgG4-related diseases, a condition characterized by infiltration of target organs by IgG4+ plasma cells and severe fibrosis. Cortico-sensitivity is one of the diagnostic criteria but the treatment of steroid-resistant and-dependent forms is not well defined.

Case Description: We present one case of 47-years-old patient with IgG4-related NTI followed for 72 months. He complained of fatigue and recurrent postprandial abdominal pain. With the exception of elevated levels of gamma-glutamyl transferase (GGT), transaminases and IgG4, kidney function remained normal (serum creatinine ≤ 0,9 mg/dL). After 2 cures of methylprednisolone (2010-2011) azathioprine was associated in 2012. Due to the cortico-dependence and persistence of bilateral focal renal lesions detected by diffusion-weighted magnetic resonance imaging (DW-MRI), Rituximab (RTX) was given (2x376 mg/m^2/15 days) in 2013. Before the first injection, positron emission tomography (PET) showed metabolic hyperactivity corresponding to axillary and abdominal aorta lymph nodes but not in the kidney. After 4 months of RTX, the patient became asymptomatic. All biological alterations disappeared. PET showed a decrease in metabolic activity at extrarenal lesions described above. A dramatic regression of bilateral renal lesions was noted by DW-MRI: the apparent diffusion coefficient has almost doubled (0,776 versus 1,111x10^{-3} mm^2/sec) and the volume of renal lesions was reduced by 50%, never observed under other treatments.

Discussion: Our observations demonstrate: (1) clinical, biological and radiological efficacy of rituximab in steroid-dependent form of IgG4- related TIN and (2) the interest of DW-MRI as non-nephrotoxic radiological and PET complementary approach not only in monitoring the effectiveness of immunosuppression but also in the early detection of renal involvement during IgG4-related disease.

SA-PO641

A Tale of Two Rare Renal Diseases in the Same Patient Krishna K.R. Manda,[1] Steven P. Lamontagne,[2] Curtis Brasseur,[2] Konstantin Abramov.[1] *[1]Univ of Massachusetts, Worcester, MA; [2]Berkshire Medical Center, Pittsfield, MA.*

Introduction: We report probably the first case of Renal Replacement Lipomatosis (RRL) and Nephrogenic Systemic Fibrosis (NSF) occurring in the same patient.

Case Description: Our patient is a 68 y/o man with significant history of diabetes, CKD stage 3-4, hypertension and peripheral vascular disease. For evaluation of PVD, he had multiple MRA studies in 2007 which led to development of extensive fibrotic skin plaques- gadolinium induced NSF. Around the same time his renal function worsened to end stage and was started on hemodialysis. CT abdomen/pelvis in 2008 showed evidence of RRL in the left kidney which progressed to involve both the kidneys on a CT done in 2012.

Discussion: NSF is a systemic fibrotic disorder which occurs exclusively in patients with kidney failure. The pathogenesis of NSF is not fully understood. Exaggerated tissue fibrosis is thought to be due to activation of the transforming growth factor beta-1 pathway and an increase in circulating fibrocytes. Inciting event may be the tissue deposition of gadolinium which is poorly soluble, highly toxic, and can form precipitates with anions. RRL is a rare disorder in which a massive fatty tissue proliferation occurs within the renal sinus, hilum and perirenal region. It is an advanced form of renal sinus lipomatosis and accompanied by renal stone disease in about 70% of cases. Pathophysiology is not fully understood and it is suggested that an inciting event triggers a process of hydronephrosis and chronic inflammation that results in parenchymal atrophy and fibrofatty replacement. RRL can simulate renal malignancies like angiomyolipoma, liposarcoma. It is interesting to note that our patient never had renal calculi. We highlight the strengthening association between gadolinium exposure in patients with advanced kidney dysfunction and NSF, and the importance of awareness for the potential toxicity of gadolinium in this patient population. Both NSF and RRL are rare conditions, but the similar proposed pathogenesis of chronic inflammation and their coexistence in this one patient raises the question as to whether the two processes are linked. Further research is required to delineate this.

SA-PO642

Unexplained Pain Does Not Equal Drug Seeking Oksana I. Nimkevych, Rose Paccione, Zohreh S. Soltani, Kimberly Cox Fremin. *Section of Nephrology and Hypertension, LSUHSC, New Orleans, LA.*

Introduction: Loin pain hematuria syndrome is a rare diagnosis that occurs amongst young adults. For this reason their pain often dismissed and patients are regarded as drug seeking without the appropriate diagnosis being made.

Case Description: A 22 year old female presents with hematuria and flank pain. She has recurrent bouts of sharp bilateral lower back and flank pain every 3 days. It extends

around to the front of her abdomen. She notes gross blood in her urine daily. Her past medical history is significant for frequent urinary tract infections. Her works up included kidney ultrasound, numerous CT scans, cystoscopies, pyelograms and cystourethrograms, a nuclear renal perfusion scan with normal results. There is no contributory family history. Physical exam is unremarkable. Urinalysis reveals numerous dysmorphic RBCs and no proteinuria. Rheumatoid factor, C3, C4, C-ANCA, P-ANCA, ANA, anti-GBM were negative. Renal biopsy shows thin basement membrane, preserved foot processes, tubules containing numerous red blood cells, negative immunofluorescent stain and no electron dense deposits which are consistent with thin glomerular basement membrane (GBM). Constellation of GBM, hematuria and unexplained chronic flank pain directs us to the diagnosis of loin pain hematuria syndrome.

Discussion: Loin pain hematuria syndrome was first discovered in 1967 and there are now approximately two hundred cases documented in the literature. It is most often seen in young women in their twenties. It is characterized by combination of hematuria with recurrent flank pain that may be constant or intermittent. The etiology of the pain is poorly understood. There is some evidence to suggest that it may be associated with glomerular basement membrane defects. Kidney biopsy in these patients has shown red blood cells in the tubules, suggesting glomerular damage. Once the tubules become blocked this can increase pressure inside the kidney and result in capsular stretch which may cause the pain in these patients. Treatment consists primarily of pain management. There is some evidence to suggest that ACE inhibitor or ARB therapy may reduce the frequency or severity of the pain.

SA-PO643

A Falsely High Blood Creatinine Caused by a Model Aviation Fuel Intoxication Philippe Lachance, Fabrice Mac-Way. *Nephrology, Univ Laval, Québec, QC, Canada.*

Introduction: In many centres, determination of serum creatinine still relies on the Jaffe reaction. However, there are many products that are known to interfere with this reaction and could lead to erroneous level of serum creatinine. We report the case of a man who accidentally ingested nitromethane and presented with a significantly raised serum creatinine when measured by the Jaffe reaction.

Case Description: A 59-year-old man was admitted to the ICU after an accidental ingestion of 250 mL of "Nitrogaz", a product containing 80 % of methanol and 20 % of nitromethane. In the hour after ingestion, the patient felt dizzy, but did not vomit. He did not take any relevant medications and was not known to have a renal disease. On presentation, the serum sent for analysis by the Jaffe reaction showed a creatinine of 940 µmol/L (10,6 mg/dL) but corresponded to 70 µmol/L (0,79 mg/dL) when measured by the enzymatic method. The rest of blood tests were unremarkable except for methanolemia of 12,6 mmol/L. Hemodialysis was performed until methanolemia was undetectable. The patient was discharged from hospital 6 days after admission.

Discussion: Nitromethane is an industrial solvent. Evidence from monkey studies suggest a peak serum concentration at 24 hours and near complete elimination after 3 days. Pharmacokinetic of nitromethane in human is poorly known but it seems to be mainly eliminated by the kidneys. Acute poisoning in humans rarely results in serious toxicity and comes mostly from model fuel ingestion which also contains methanol. Nitromethane intoxication has rarely been reported in the literature and the majority of these cases describe a falsely elevated level of serum creatinine. In these few reports, it has been shown that the nitromethane was indeed the interference product in the Jaffe reaction. Nitromethane is a rare cause of intoxication but a must known product for all nephrologists because of its interaction with measurement of creatinine level. The right recognition of this interaction will allow the avoidance of additional unnecessary tests for high creatinine level.

SA-PO644

Assessment of Knowledge and Perception about Peritoneal Dialysis by in Center Hemodialysis Health Care Workers Ann Mancini,[1] Catherine Firanek,[1] Mary Gellens,[1] Lucy Barker Todd,[1] Ann Robar,[2] Sara L. Garza,[3] Karen Lattrel,[4] James A. Sloand.[1] *[1]Baxter Healthcare, Deerfield, IL; [2]Satellite Healthcare, San Jose, CA; [3]US Renal Care, Plano, TX; [4]Greenfield Health Systems, Detroit, MI.*

Background: Home dialysis use is <10% in the U.S. Most patients begin dialysis in in-center hemodialysis (ICHD) facilities. Medicare mandates all patients must receive modality education. The knowledge and attitudes of ICHD staff may influence modality education.

Methods: A validated online survey was used to assess knowledge of literature-based clinical outcomes and attitudes of responders about home dialysis. Nursing directors of 4 dialysis chains sent a 10 minute survey to 580 dialysis health care workers. Survey dates were 2/24/24 - 4/18/14.

Results: 275 surveys were returned (response rate 47%). In-center hemodialysis (ICHD) nurses, acute hemodialysis (HD) nurses and technicians provided 35% of the responses; peritoneal dialysis (PD) nurses, dieticians, social workers, administrators, managers, and others the remaining 65%. Below are statistically different responses between the groups.

	ICHD RNs, Acute HD RNs and Technicians (35%)	Other Dialysis Staff (65%)
Perceived benefits: PD vs. HD		
Avoiding temporary vascular access w/PD	49%	74%
Preservation of Residual renal function (RRF)	45%	74%
Less risk of infection	13%	26%
Better survival with PD	32%	53%
Perceptions re: Infectious Complications		
Greater risk of infection: PD vs. HD	55%	24%
Patients with peritonitis are likely to be septic	55%	30%
Perceptions of Obstacles to start PD		
Home set up/environment	57%	36%
Automated PD is a complex therapy to learn and self-administer	43%	13%
Lack of physician belief in PD	19%	41%

Conclusions: ICHD staff have misperceptions of PD that contrast with evidence-based findings and those of home-based dialysis staff. Key misperceptions include: avoiding temporary vascular access, preservation of RRF and risk of sepsis. ICHD staff also thinks PD is complex and requires extensive home set up. These misperceptions/beliefs may impact ICHD patient modality education and selection.

Funding: Pharmaceutical Company Support - Baxter Healthcare

SA-PO645

Learning Preferences in Renal Physiology: Results of a Q-Sort Survey Needs Assessment John K. Roberts, Alisa Nagler, Ruediger W. Lehrich. *Dept of Medicine, Duke Univ Medical Center, Durham, NC.*

Background: Interest in nephrology has reached an all-time low. A better understanding of medical students' opinions and preferences could reverse this trend. While planning for the re-design of a renal physiology course for first-year medical students, the authors used a Q-Sort survey to assess the students' attitudes and learning preferences to guide curricular change.

Methods: We invited first-year medical students to take a Q-Sort survey at the start of renal physiology. Students ranked statements on a scale from -4 (strongly disagree) to +4 (strongly agree) according to their understanding of renal physiology, interest in nephrology, learning styles, course characteristics, and perceived clinical relevance of renal physiology. By-person factor analysis was then performed to identify statistically different viewpoints among the students.

Results: Factor analysis revealed four statistically different viewpoints. In three of the viewpoints, the students valued learning about the kidney, but differed in how they preferred to learn: the Readers avoid lecture and prefer using a textbook or notes; the Social Auditory learners prefer attending lectures and working with peers; and the Visual learners prefer diagrams and online videos. The fourth factor represented a small group of predestined students with a strong bias against renal physiology and nephrology. (see Figure 1)

Conclusions: The major viewpoints among first-year medical students are defined by preferences for different learning styles rather than attitudes towards renal physiology or interest in nephrology. However, there are some predestined students who have a strong predisposition against nephrology. A curriculum that caters to a diversity of learning styles may impact learning and nephrology interest in students taking renal physiology.

Funding: Other NIH Support - Duke Training Grant in Nephrology

SA-PO646

Simulation-Based-Mastery-Learning for Temporary Hemodialysis Catheter Insertion at the Canadian Society of Nephrology Annual Meeting: A Before-After Study James J. Paparello,[1] Edward G. Clark,[2] Cedric A.W. Edwards,[2] Michael Schachter,[3] Rory F. McQuillan,[4] Diane Wayne,[1] Jeffrey H. Barsuk.[1] *[1]Dept of Medicine/Nephrology, Northwestern Univ Feinberg School of Medicine, Chicago, IL; [2]Dept of Medicine/Nephrology, The Ottawa Hospital - Riverside Campus, Ottawa, ON, Canada; [3]Dept of Medicine/Nephrology, Univ of British Columbia, Vancouver, BC, Canada; [4]Dept of Medicine/Nephrology, Univ of Toronto, Toronto, ON, Canada.*

Background: Simulation-based-mastery-learning (SBML) is an effective technique for teaching the insertion of temporary, non-tunneled hemodialysis catheters (NTHDCs). We sought to determine if SBML training for NTHDC insertion could be effective in training nephrology trainees at the annual meeting of the Canadian Society of Nephrology.

Methods: Trainees were surveyed regarding demographics, prior experience and procedural self-confidence. They underwent a baseline assessment of their NTHDC insertion skills using a patient simulator and ultrasound machine. Iinsertion skills were assessed using a 28-item checklist. Participants then had a training session that included a didactic presentation and 2 hours of deliberate practice. On the following day, they underwent repeat assessment using the same 28-item checklist (post-testing). All participants were required to meet or exceed a minimum passing score (MPS) of 79% checklist items correct.

Results: Twenty-two individuals participated in the training. None of the 22 met or exceeded the MPS at baseline assessment (mean checklist score of 15.5/28 [SD=7.2]). Seventeen of 22 participants took part in post-testing and improved their scores (mean of 26.9/28 [SD=1.1; $p < 0.001$]). All 17 met or exceeded the MPS on their first attempt. Participants strongly agreed that SBML-training for NTHDC insertion was beneficial and should be incorporated into nephrology fellowship training.

Conclusions: Although most participants reported having previously inserted NTHDCs in clinical practice, they did not perform well on a simulated procedure. This study establishes that SBML-training for NTHDC insertion in the setting of a medical conference is feasible and effective.

SA-PO647

Validating the Self-Audit Chart Review Method through Verification of Data Accuracy in a Nephrology Fellowship Program Will M. Schouten,[1] Suzanne M. Norby.[1,2] *[1]Dept of Internal Medicine, Mayo Clinic, Rochester, MN; [2]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Self-audit of continuity clinic (CC) patient charts is a method used to assess the Practice-Based Learning and Improvement competency in our nephrology training program and to evaluate how nephrology fellows manage patients with chronic kidney disease (CKD). However, the accuracy of data collection had not been systematically evaluated to determine the validity of this assessment tool. The purpose of this study was to determine if self-audits yield accurate data in a nephrology fellow CC practice setting.

Methods: Each of 11 nephrology fellows was assigned to review medical records of 10 consecutive non-dialysis patients with CKD stage 4 or 5 whom they had evaluated in CC. Data were abstracted from laboratory results and visit notes: blood pressure measurement (BP) recorded in the designated field; diagnosis of diabetes mellitus and, if diabetic, whether the patient was taking an ACEI or ARB; serum bicarbonate level and, if low, whether the patient was on bicarbonate supplementation; and documentation of end-stage renal disease (ESRD) treatment options discussion. Abstraction of the same data was then performed by the program director (PD).

Results: Of 106 patient records reviewed, 8 did not meet criteria and were excluded. 453 data points were collected from 98 records. Overall, 95% of fellow data points agreed with review by the PD. Individual fellow accuracy ranged from 83-100%. Accuracy did not differ by level of training: 94% for 1st year fellows, 98% for 2nd year fellows, and 95% for 3rd year fellows. Overall accuracy by category was 91% for recorded BP, 96% for diabetes diagnosis, 99% for bicarbonate level, and 97% for discussion of ESRD treatment options. For data points requiring an if-then decision, abstraction was 89% accurate for administration of supplemental bicarbonate if the level was low and 93% for ACEI or ARB use in patients with diabetes.

Conclusions: The self-audit chart review method appears to be a valid assessment tool, yielding reasonably accurate results unaffected by year of training.

SA-PO648

Comfort with Computer Technology in Patients with Chronic Kidney Disease Gordana Obradovic,[1] Andrea Lynn Berger,[2] Christina Yule,[2] Steven D. Weisbord,[3] Jamie Alton Green.[1,2] *[1]Nephrology, Geisinger Medical Center, Danville, PA; [2]Center for Health Research, Geisinger Medical Center, Danville, PA; [3]Renal Section, VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: Computer-based tools have the capacity to engage patients in their care and improve self-management, however their use depends upon adequate computer literacy by patients. We sought to assess comfort with and use of computer technology among patients with chronic kidney disease (CKD).

Methods: We administered an investigator-designed survey to adult patients with non-dialysis dependent CKD stages 3-5 in rural Pennsylvania. The survey included questions about internet usage, comfort with computer based tools, and use of electronic health portals.

Results: There were 84 participants. Average age was 71, 57% were male, 96% were white, 53% had a less than or equal to high school level of education, 68% were retired, and 33% had an income less than $25,000. Almost half (47%) reported that they had not used the internet in the preceding 12 months. Only 58% were comfortable using a traditional computer with a mouse, 10% using a cell phone with internet access (smart phone), and 17% using an iPad or touchscreen computer. Nearly one-third (32%) stated that they were not comfortable using any of the above. Forty-two percent were active users of an electronic health portal. Reasons for non-use of a health portal included: lack of internet access (39%), not interested (32%), not sure how it works (11%), and lack of a computer (7%). Forty-three percent allowed someone else to access their health information through a health portal.

Conclusions: Despite the increased reliance on computer technology in health care, many patients with CKD are not comfortable with computer-based tools. These findings may help inform the development of 'technology sensitive' self-management interventions in this patient population.

SA-PO649

Comprehensive Assessment of Health Literacy in Patients with Chronic Kidney Disease Using the Health Literacy Skills Instrument Short Form Gordana Obradovic,[1] Andrea Lynn Berger,[2] Christina Yule,[2] Steven D. Weisbord,[3] Jamie Alton Green.[1,2] *[1]Nephrology, Geisinger Medical Center, Danville, PA; [2]Center for Health Research, Geisinger Medical Center, Danville, PA; [3]Renal Section, VA Pittsburgh Healthcare System, Pittsburgh, PA.*

Background: Many health literacy screening tools use reading skills as a proxy measure of overall health literacy. However, adequate health literacy requires additional skills including the ability to interpret numbers, listen effectively, and navigate the health care system. We sought to comprehensively assess health literacy in CKD patients using a short, skills-based instrument that measures multiple health literacy domains.

Methods: We assessed health literacy in patients with CKD stages 3-5 not on dialysis using the Health Literacy Skills Instrument-Short Form (HLSI-SF), a 10-item screening tool that measures four domains of health literacy: print literacy (reading and writing), numeracy skills, oral literacy (listening), and information seeking (navigation of internet and facilities). Results are categorized as adequate (7-10 correct) and inadequate (0-6 correct) health literacy.

Results: There were 84 participants. Mean age was 71, 57% were male, 96% were white, and 53% had a high school or lower level of education. Fifty-one patients (61%) had inadequate health literacy. Only 11 (13%) were able to calculate the percent daily value of saturated fat from a nutrition label (numeracy), 43 (51%) were able to identify the appropriate timing of a medication from a chart (print literacy), 43 (51%) were able to select the correct number to press after listening to a telephone recording (oral literacy), and 43 (51%) were able to calculate the amount of calories burned during activity using an interactive web calculator (internet navigation).

Conclusions: When using a skills-based instrument, the prevalence of inadequate health literacy in patients with CKD is high. These findings should inform the development and assessment of targeted interventions to alleviate the challenges faced by patients with CKD who have inadequate health literacy.

SA-PO650

Structured Quality Improvement Curriculum Leads to Increased Productivity of Fellows Laura J. Maursetter. *Div of Nephrology, Dept of Medicine, Univ of Wisconsin School of Medicine and Public Health, Madison, WI.*

Background: Quality improvement (QI) looks at a problem through a lens that is different from that of basic science or clinical research. Its mission is not scientific discovery but, rather, improvements in the efficiency, safety or effectiveness of healthcare delivery. As organizations and individuals are more commonly being measured on quality, it is critical for graduating fellows to have a working understanding of the QI process and the contribution it can have on practice. The aim of this project was to create and deliver a QI curriculum to multiple fellowship programs over the course of the year to increase the number and quality of QI projects that these fellows created.

Methods: The curriculum was developed around the FADE model (Focus, Analyze, Develop, Execute, Evaluate). Five meetings were established among 6 fellowship groups. Fellows met for 20 minutes of didactics and 40 minutes of project discussion at each of the five sessions to develop and implement a QI project. The projects were vetted across the groups for improvement suggestions and then presented at a department-wide event.

Results: 35 fellows and 12 faculty mentors participated in at least one of the sessions. Projects were created by groups ranging from 1-6 fellows. There were 16 projects across the 6 participating divisions with at least 1 project in each division. In the Division of Nephrology, the number of projects created increased from 0 to 3 projects per year over the 2 years the curriculum as been in place. Projects ranged from mechanisms to avoid PICC line placement in CKD stage 4-5 to measuring the efficiency of care impact pre-clinic preparation had on the osteoporosis clinic. This year 10 posters were presented at the end of the year with 1 project, thus far, submitted for publication.

Conclusions: A structured QI curriculum increased the number of QI projects generated. Including fellows from various divisions in the process and discussions allows for common problems to be identified and improved with further-reaching impact. Analysis of participant survey data will help to determine how to improve the process to enhance learning and productivity for the future.

SA-PO651

Medical Trainees' Knowledge of Contrast Use in Chronic Dialysis Patients Rahmat Balogun, Chinmay P. Patel, Shital Gandhi, Hitesh H. Shah, Kenar D. Jhaveri. *Nephrology and Radiology, Hofstra North Shore LIJ School of Medicine, Great Neck, NY.*

Background: The knowledge related to IV radiocontrast use in ESRD patients among residents at our two large tertiary hospitals is not known. Hence, to gain a better insight in medical trainees' knowledge on this subject, we conducted an online survey at our institution.

Methods: We first reviewed the literature to gain knowledge related to the use of contrast agents in ESRD patients. An anonymous online survey was subsequently created. Trainees were presented with 4 clinical case scenarios to determine their knowledge on this subject. The survey was internally validated by 3 independent nephrologists that were not a part of this study. The survey was subsequently distributed to all internal medicine, emergency medicine, surgery and radiology residents at two large tertiary centers.

Results: 94 medical trainees responded to the survey (33% response rate). 95% of the residents were trained in U.S. medical schools. Nearly half (47.9%) of the respondents were internal medicine residents, while the remaining respondents were emergency medicine (21.3%), radiology (18.1%), and surgical (12.7%) residents. Over one-third of the respondents were PGY1. Question related to the timing of dialysis following IV contrast use was incorrectly answered by nearly 24.1% of the respondents. 10.1% of the residents incorrectly suggested that N-acetylcysteine should be given to ESRD patients to prevent radiocontrast nephropathy. Over one-fifths (22.7%) of the respondents were unaware of the risks of developing NSF following gadolinium based contrast use in ESRD patients. As compared to all responding trainees, radiology residents performed better on the survey.

Conclusions: While most responding trainees were knowledgeable regarding the appropriate use and risks of IV iodinated radiocontrast material in ESRD patients, a significant percentage were not aware of the risk of developing NSF following gadolinium based contrast material use in this patient group. A significant number of trainees were also not aware of the timing of dialysis following IV contrast use. Measures to enhance medical trainees' knowledge related to iodinated and gadolinium contrast use in ESRD patients are needed.

SA-PO652

Quality Improvement Process and Fellow Education Improves Referral Rates for Transplant and Dialysis Access Anuj Regmi,[1,2] N. Stanley Nahman,[1,2] Usman Afzal,[1,2] Noble Iwuagwu,[1,2] John Jason White.[1,2] *[1]Nephrology, Charlie Norwood VAMC, Augusta, GA; [2]Nephrology, Georgia Regents Univ, Augusta, GA.*

Background: Important functions of any chronic kidney disease (CKD) clinic are timely referrals for renal transplantation and dialysis access. However, the optimal timing of referral is debatable, and the reasons for low referral rates are not well understood.

Methods: As part of an on-going quality assurance initiative (QAI) in our CKD Clinic at the Charlie Norwood VA Medical Center, we evaluated all patients seen by nephrology fellows between January 1, 2013 and April 30, 2013 (4 months) (Phase 1) and assessed referral patterns for renal transplantation and dialysis. We focused on CKD 4 and CKD 5. At the conclusion of Phase I, the results were reviewed in a combined session with fellows and faculty. The need for early referral was emphasized and plans for second audit defined. We subsequently evaluated referral patterns for all patients seen between August 1, 2013 and November 30, 2013 (4 months) (Phase 2). Lastly, we assessed referral rates for eGFR < 20 ml/min/1.73m2 and documented reasons for non referral.

Results: The results are summarized in the table. Following the intervention, there was a general increase in referral rates for access, but there was a limited effect on transplant referral. Reasons for non referral in 24 patients included: advanced age/dementia (n = 5); acute malignancy (n = 4); patient refusal (n = 3); unstable psychiatric disorder (n = 1); and unknown (n = 10).

Percent of patients referred for transplant or access (N)		
Referral	Phase 1 (349)	Phase 2 (361)
Transplant		
CKD 4	9% (67)	25% (49)
eGFR < 20	32%(37)	50%(26)
CKD 5	50% (18)	67% (15)
Access		
CKD 4	21%	11%
eGFR < 20	59%	55%
CKD 5	72%	73%

Conclusions: Rates of referral for transplantation and dialysis access placement may be improved by chart audits. Furthermore, the reasons for non-referral may be due to complicating medical problems but patient refusal is also an issue. Addressing issues of non referral may help increase the rate of referral for transplant and vascular access in advanced CKD.

SA-PO653

Preparing Nephrology Fellows for Difficult Conversations in the Critically Ill Patient with Acute Kidney Injury: The Use of Simulation-Based Communication Skills Training Yusra R. Cheema,[1] Kathy Johnson Neely,[2] Paul J. Hutchison,[3] Cybele Ghossein.[1] *[1]Nephrology, Northwestern Univ, Chicago, IL; [2]Hospital Medicine, Northwestern Univ, Chicago, IL; [3]Pulmonary, Northwestern Univ, Chicago, IL.*

Background: Nephrology fellows spend a significant amount of time caring for the critically-ill that suffer acute kidney injury (AKI), many of whom are started on continuous renal replacement therapy (CRRT) despite a low likelihood of surviving to discharge from the intensive-care unit (ICU). Surveys demonstrate that many nephrology fellows feel unprepared to have conversations regarding withholding or withdrawal of renal replacement therapy in these situations and yet, few programs have addressed communication skills training. Here we describe our experience using didactic sessions followed by simulated patient encounters to prepare nephrology fellows for difficult discussions in critically-ill patients with AKI.

Methods: All 7 nephrology fellows at Northwestern University during the 2013-14 academic year underwent this training as part of a quality improvement initiative. All fellows attended a 1-hour didactic session on useful communication strategies in end-of-life situations. Each fellow then practiced these skills with a standardized patient and received feedback. All fellows completed both pre- and post-encounter questionnaires using a 5-point Likert scale to assess the experience.

Results: All fellows reported an improvement in both knowledge and skills required to lead a successful discussion with a surrogate decision-maker regarding initiation of CRRT. Additionally, 100% of fellows would recommend this type of simulation-based palliative care education.

Statements	% Responding as Good or Excellent (score of 4 or 5)	
	Pre	Post
I would assess my knowledge base to successfully lead such a discussion as..	0	85.7
I would assess my skill set to successfully lead such a discussion as..	14.2	100

Conclusions: At our institution, simulation training with standardized patient encounters served as an effective tool to prepare nephrology fellows to lead difficult end-of-life conversations regarding CRRT initiation in critically-ill patients with AKI.

SA-PO654

Journal Publications of U.S. Adult Nephrology Fellowship Training Program Directors Hitesh H. Shah, Aditya Kadiyala. *Nephrology, Hofstra North Shore-LIJ School of Medicine, Great Neck, NY.*

Background: Publication in peer-reviewed journals can be considered as one measure of scholarly activity among academicians. Peer-reviewed journal publications (PR-JPs) of U.S. adult nephrology fellowship training program directors (N-TPDs) are not known.

Methods: A PubMed search for journal publications was conducted for a period of 3 years (7/2011 to 6/2014) for N-TPDs serving in academic year 2013-14. Publications were categorized as follows: basic science research (BSR), prospective clinical study (PCS), retrospective or cross sectional clinical study (R/CCS), clinical outcomes research (COR), randomized clinical trial (RCT), meta-analysis (MA), educational research (ER), case report/series (CR/S), review article (RA), editorial (E) and letter to editor (LT-E). Data was analyzed in June 2014 for 145 N-TPDs.

Results: 33 (22.8%) N-TPDs had no PR-JP in the last 3 years. While 35.8% had ≤3 publications, 15.2% had ≥10 publications. We identified 664 PR-JPs by N-TPDs over the past 3 years, out of which 19.1% publications had an N-TPD as the primary author (PA), 29.2% had an N-TPD as a corresponding author (CA) and 15.1% had an N-TPD as both the primary and CA. 39% were clinical research (PCS, R/CCS, COR, RCT, MA) related publications. The remaining publications were as follows: RA-22.7%, CR/S-16%, BSR-9.3%, E-7.4%, LT-E-2.9%, and ER-2.7%. While 37.1% of N-TPDs had at least one PR-JP as a PA over the past 3 years, 46.2% had at least one publication as CA and 32.4% had at least one as primary and CA. In the last 3 years, 49.7% of the N-TPDs had authored at least one clinical research publication, 17.2% had a BSR publication, while 7.5% had an ER publication. When analyzed by gender, 41 female N-TPDs had a mean of 4.9 publications in past 3 years as compared to 104 male N-TPDs who had a mean of 4.4 publications with no statistically significant difference between means ($p = 0.63$).

Conclusions: Nearly one in four N-TPDs had no PR-JP over the last 3 years. Nearly half N-TPDs were CA on at least one publication over the last 3 years. Half of the PR-JPs were either clinical research, BSR or ER related publications. The ER category had the least publications. Male N-TPDs were as likely to publish as female N-TPDs.

SA-PO655

Telenephrology May Improve Rural Primary Care Provider (PCP) Satisfaction and Medical Care within Veterans Affairs Raimund H. Pichler,[1] Lauren Beste,[2] Maureen Germani,[1] Michael F. Chang,[3] Bessie A. Young.[1] *[1]Div of Nephrology, Renal Dialysis Unit S-1111, Veterans Administration Puget Sound Health Care System, Seattle, WA; [2]Div of Gastroenterology, S-111 GI, Veterans Administration Puget Sound Health Care System, Seattle, WA; [3]Div of Gastroenterology, Portland VA Medical Center, Portland, OR.*

Background: PCPs practicing in rural areas have more difficulty accessing specialty care for their patients. Rural PCPs also have been shown to report professional isolation, lack of access to professional development and decreased educational opportunities. As part of the Veterans Affairs (VA) Specialty Care Access Network-Extension for Community Health Outcomes (SCAN-ECHO) we launched a provider-to-provider Nephrology telemedicine consultation service to provide mentoring for PCPs to treat mostly rural Veterans with renal disease.

Methods: We conducted an e-mail survey of VA-based PCPs (n=13) in the rural Pacific Northwest, who attended a longitudinal Nephrology telemedicine program. We used a validated survey to assess provider measures.

Results: Most PCPs felt that the program increased their knowledge and competencies (100%), improved the quality of care for their patients (95%) and was useful in treating patients not discussed in the program (85%). The vast majority of providers felt that their participation was rewarding (85%). Almost half of the PCPs (46%) stated that they were using the Nephrology knowledge gained from the program in their daily practice. 62% of PCPs felt more integrated into a clinical team. The majority of PCPs felt that Renal SCAN ECHO increased their overall job satisfaction (70%) and 92% stated that they would recommend the program to a colleague. Almost half (46%) of the providers felt that their participation decreased traditional Nephrology consults.

Conclusions: A renal provider-to-provider Nephrology telemedicine service can be a rewarding experience that improves provider self reported knowledge, job satisfaction and quality of nephrology care in more rural areas. Future research is needed to study the effect of such an intervention on patient outcomes.

Funding: Veterans Affairs Support

SA-PO656

Nephropathology Education during Fellowship: A U.S. Nephrology Fellowship Training Program Directors' Survey Kenar D. Jhaveri,[1] Surya V. Seshan,[2] Rimda Wanchoo,[1] Hitesh H. Shah.[1] *[1]Nephrology, Hofstra North Shore LIJ School of Medicine, Great Neck, NY; [2]Pathology, Weill Cornell Medical Center, New York Presbyterian Hospital, New York, NY.*

Background: Nephropathology (NephPath) education is an important component of nephrology fellowship curriculum. To gain a greater insight into NephPath education offered to fellows during fellowship training in the U.S., we conducted this study.

Methods: An anonymous online survey was created and subsequently distributed to all U.S. adult nephrology fellowship training program directors (N-TPDs) in May 2014.

Results: So far, 36 N-TPDs have responded to our survey (25% response rate). Over half (56%) of the respondents' institution had a renal pathology division at their institution. Nearly one-fifth programs did not have an "in-house" nephropathologist (N-Path) and performed <50 kidney biopsies/year. Kidney biopsies in these programs were read by either an affiliated academic center N-Path or by N-Path working in a private setting. Over half (52%) of the programs with "in-house" N-Path had weekly NephPath conferences. Majority (80%) of the programs with no "in-house" N-Path had monthly conferences. 11% sent their fellows to other institutions to attend NephPath conferences. Measures to enhance NephPath education included: dedicated renal pathology elective (46%), send fellows to attend NephPath conferences at national meetings (46%) or dedicated NephPath courses (20%), web-based teaching (20%), and didactic lectures from invited N-Path(s) (14%). While 63% programs felt that their fellows were receiving adequate NephPath education at their institution, another 34% felt that they could do better. "No in-house N-Path" (11%), "not enough kidney biopsies" and "lack of resources" were some of the reasons for inadequate NephPath education for fellows.

Conclusions: Programs with N-Path within their institution were more likely to have frequent NephPath conferences as compared to the programs that did not. While majority of N-TPDs felt that they provided adequate NephPath experience to fellows, nearly one-third felt that they could do better. Novel methods to enhance NephPath experience such as web-based learning or videoconferencing can be considered by training programs.

SA-PO657

Trends of Education Research Abstract Presentations and Publication Rates at ASN Kidney Week 2008-2013 Kenar D. Jhaveri,[1] Rimda Wanchoo,[1] Laura J. Maursetter,[2] Hitesh H. Shah.[1] *[1]Nephrology, Hofstra NSLIJ School of Medicine, Great Neck, NY; [2]Univ of Wisconsin School of Medicine, Madison, WI.*

Background: Since the inception of the educational research category at the ASN Kidney Week (KW) in 2008, the number and types of research abstracts accepted for presentation at ASN KW from 2008 to 2013 is not known. Peer-reviewed journal publication of educational research abstracts at ASN KW is also not known.

Methods: All accepted educational research abstracts from 2008-2013 were reviewed and further categorized into one of the 4 sub-categories: medical trainee (MTE) related educational research, patient education (PE) related research, social media (SoM) and faculty development (FD). To determine the peer-reviewed journal publication rate of these abstracts, a literature (PubMed) search was performed.

Results: A total of 145 abstracts were presented in the educational research category in this time period. ASN KW 2013 had the highest number of abstracts in this category with a total of 35. Over the previous six ASN KW meetings, 48% were MTE related. The remaining abstracts were PE (33%), FD (13%) and SoM (5.5%) related. Of the 145 abstracts, 29% were survey-based studies. So far, 30 (21%) out of the 145 abstracts have been published as papers in peer-reviewed journal. Of those that were published, 13 (45%) were survey based, 2 were RCTs, 2 propensity matched pair analysis, 1 historical control comparison and 1 time controlled trial. While 47% of the published articles were MTE related papers, 23% were PE, 17% were FD and the rest were SoM related educational research papers. Overall, abstracts were more likely to be published if they were SoM related(50%), followed by FD (27%) and then MTE related(20%).

Conclusions: Based on our study, interest in conducting nephrology educational research is increasing among the training community. However, so far, only one-fifth of the educational research abstracts that have been presented at ASN KW have been published as peer-reviewed journals papers. Of those that were published, majority were survey based and MTE related. Reasons for this low publication rate is not known. Training in medical education research might increase quality of abstracts and publication rates.

SA-PO658

Narrative Medicine Reflective Writing in the Dialysis Unit Kenar D. Jhaveri, Elizabeth J. Berger, Hitesh H. Shah, Tomoko Ouchi, Heidi Mandel, Barbara R. Hirsch, Nancy Farber, Mindy B. Nelkin, Michael T. Murn, Alice Fornari. *North Shore LIJ Health System, Hofstra North Shore LIJ School of Medicine, Great Neck, NY.*

Background: Narrative medicine(NM) is being used to enhance professionalism, humanism and ultimately patient care in other areas of medicine. To assess NM utility, a pilot intervention using narrative reflection was developed and implemented with staff members in a single center dialysis unit.

Methods: A NM program was developed for our dialysis staff. Nephrologists, fellows, dietitians, social workers, nurses, technicians, and unit receptionists participated in the study. The 60 minute session began with 5 minutes of silent writing in response to a prompt (visual or words). The expressive writing was subsequently shared among the small group of participants. The skilled NM facilitators guided discussion and where appropriate, connected the writings to their role in patient care. Sessions were conducted twice a week for 3 months. Curriculum development and monitoring of the project's progress took place via monthly teleconferences and emails among the facilitators, with a documented "dialogue" for evaluation purposes.

Results: A total of 24 sessions were conducted. Each session included discussions that were around reflected thoughts of fatigue, death, and work life balance. The overarching goal to bring NM to a patient care team within a dialysis unit was achieved. A wordle image produced themes such as "appreciation, mindfulness, fathers, mothers, love, staff and protection" as examples. The participants self-reported that these sessions were enjoyable and seemed to benefit from the opportunity to reflect. They identified the need for continued support of this kind as desirable, as this is largely unfulfilled in the course of traditional medical rounds in dialysis units.

Conclusions: A pilot program using NM demonstrated preliminary promise as a tool for staff to discuss the humanistic aspect of patient care, work-life balance in a safe manner. Using the knowledge gained from this pilot in organizing such a unit based program, a future larger study with dialysis staff can include pre and post interventions to measure impact of NM sessions specific to humanism and burnout.

SA-PO659

A Latin American Online Continuing Medical Education Course for the Nephrology Community Francisco Gonzalez-Martinez,[1] Oscar A. Noboa,[1] Gustavo Cristian Greloni,[2] Irene L. Noronha,[3] Almerinda Ribeiro,[4] Alvaro Margolis,[5] Leticia Lorier,[5] Ricardo Silvariño,[1] Sofia Garcia,[5] Antonio Lopez,[5] Juan M. Fernández-Cean.[6] *[1]Nephrology, Univ de la República, Uruguay; [2]Nephrology, Hospital Italiano, Argentina; [3]Nephrology, Univ de San Paulo, Brazil; [4]Nephrology, Univ Estadual de Campinas, Brazil; [5]Evimed, Uruguay; [6]SARI, Uruguay.*

Background: In 2013, the Latin American Society for Nephrology and Hypertension (SLANH) and the Latin American and Caribbean Transplantation Society (STALYC), with support from Evimed, implemented a CME course for Latin American nephrologists. We summarize the design, implementation and results of this course.

Methods: The topic was Immunopathology in native and transplanted kidneys. Evimed provided for the educational, technological and logistics support. The course was given in Spanish and Portuguese. The activities included a distance education seven-week asynchronous on-line modality with multiple educational strategies. Thirty hours of study workload was estimated to complete the course.

Results: There were 612 participants (about 7% of the Latin American nephrology community): 498 physicians coming from 18 countries who registered for the course, 59 experts and tutors and 55 observers; 85% of attendees were nephrologists, 11% were residents. Ninety-six percent of registrants (n=479) accessed the online campus, and 92% of them (n=442) participated in the course. Of this last figure, 51% received a certificate of completion (n=226) and 29% a certificate of participation (n=128). Sixty-five percent of registrants participated in the case discussions. Eighty-six percent were very satisfied, 13% were satisfied, 1% was neutral, and none were dissatisfied or very dissatisfied. Lack of time to devote to the course was the main limitation expressed (62% of participants who answered this question), while Internet access or difficulties in the use of technology were

considered by 12 and 6% of participants, respectively. There was a significant increase in knowledge between before and after the course: The grade increased from 64 to 83%.

Conclusions: Technology-enabled education demonstrated good potential to become an instrument for Latin American nephrologists.

SA-PO660

An Educational Intervention on Communication of Prognosis by Renal Fellows Jennifer S. Scherer, Evgenia Litrivis. *Dept of Geriatrics and Palliative Medicine, Icahn School of Medicine at Mount Sinai, NYC, NY.*

Background: Nephrology trainees report being poorly mentored and unprepared to discuss end-of-life issues. We conducted a study to assess renal fellows' preparedness for communicating prognosis and to examine the effects of an educational intervention.

Methods: Fellows from 3 institutions were provided surveys that inquired about communication skills, mentorship, barriers, and knowledge of prognostic tools. Answers were on scale of 1-10. Fellows attended a 15 minute didactic on prognostication and were resurveyed 6 weeks later. Results were compared with a t-test. Study was IRB exempt.

Results: Pre-survey: n=12; Average age: 31.2 years; 66.7% male, 58.3% 2nd year fellows. Post-survey: n=5

	Fellows Pre-Intervention (n=12)	Fellows Post-Intervention (n=5)	Change	t-test p-value
Rank of frequency of discussions of prognosis with patients (average)	5.67	6.60	+0.93	0.52
Rank of frequency of incorporation of prognosis into treatment decision-making (average)	5.67	6.50	+0.83	0.56
Level of Preparedness (average)	5.09	5.29	+0.20	0.91
Frequency of observation by Attendings (average)	4.08	3.25	-0.83	0.58
Frequency of fellows asking for observation (average)	2.42	1.50	-0.92	0.52

Baseline scores were low. There was a non-significant increase in comfort and use of prognosis, with a downward trend in mentorship. 16.7% knew of prognostic tools before the intervention, 100% after. 66.7% found the didactic helpful. 100% felt there should be an increase in communication training during fellowship. Barriers identified were time constraints, inability to explore patients' values, lack of training, and clinical uncertainty.

Conclusions: This study identifies barriers to trainees' use and learning of communication skills. Communication training is taught most effectively through observed feedback, yet our data shows that fellows are not receiving this education. Limitations include the low return of surveys and the short time for the didactic. Strengths are the multi-institution participation and the identification that fellows would like more training in these skills. Future directions include partnership with palliative care departments to provide effective communication education and mentorship.

SA-PO661

NephMadness 2014, a Social Media Campaign to Promote Nephrology Education and Interest Joel Topf,[1] Matthew A. Sparks,[2] Edgar V. Lerma,[3] Warren L. Kupin,[4] Kenar D. Jhaveri.[5] *[1]Medicine, St. John Hospital/Providence, Detroit, MI; [2]Medicine, Duke, Durham, NC; [3]Medicine, Univ of Illinois, Chicago, IL; [4]Medicine, Univ of Miami, Miami, FL; [5]Medicine, Hofstra North Shore, New Hyde Park, NY.*

Background: We created an online homage to the Men's College Basketball Tournament to teach about 8 areas of nephrology. The teaching was integrated into a game where participants tried to predict winners of head-to-head match-ups between nephrology concepts. The authors provided editorial content to provide context and background to guide participants as well as rationale to explain the winners of each match-up.

Methods: The tournament was announced and hosted on the blog eAJKD.com. Signing up for the contest and predicting the winners was done via a third-party site designed to host similar contests. Twitter, blog posts, and e-mails were used to publicize the contest and educational material. We registered the hashtag #NephMadness in order to track Twitter discussions about the contest. We also tracked web site traffic.

Results: The contest was composed of 64 nephrology concepts, distributed across 8 subjects. The 8 core subjects were: toxins, hypertension, dialysis, regeneration, AKI, electrolytes, kidney stones and biologics. Detailed, evidence-based, referenced descriptions, for each concept were published to the blog by select content experts. Concurrent with the Men's College Basketball Tournament, the field of 64 concepts was narrowed in a progressive elimination pattern until a single winner remained. The contest attracted 273 entries. 160 were from doctors or medical students (93 from U.S. medical schools, 68 from international schools). Participants came from 23 countries however 79% were from the U.S. We tracked 1,448 tweets from 160 unique accounts, providing 1.9 million impressions on Twitter using the hashtag #NephMadness.

Conclusions: The social media campaign was successful in attracting people to interact and learn about nephrology. Traffic to the website, the blog of the AJKD, was the highest in its history by a wide margin. Increased employement of social media and educational games could provide a unique portal for improving medical education.

SA-PO662

Inter-Rater Reliability of the Inter-Professional Collaborator Assessment Rubric (ICAR) during Haemodialysis Simulation Maury N. Pinsk,[1,2] Andrew Reid.[3] *[1]Pedaitrics, Univ of Alberta, Edmonton, AB, Canada; [2]Pediatric Nephrology, Stollery Children's Hospital, Edmonton, AB, Canada; [3]ESIM Provincial Simulation Centre, Alberta Health Services, Edmonton, AB, Canada.*

Background: Haemodialysis is a multidisciplinary therapy that involves interaction between physicians and nursing staff. Postgraduate programs excel at teaching the medical expert roles of dialysis, but the interaction on multidisciplinary teams is less effectively assessed, and even less frequently taught. To meet the objectives of competency based learning and assessment, we built a high fidelity haemodialysis simulator for the purpose of training physicians on rare and life threatening dialysis complications, noting that the simulation offers an opportunity to assess multidisciplinary communication. The ICAR is a validated instrument for assessing team dynamics. We piloted this instrument during haemodialysis simulation to assess inter-rater reliability (IRR) and content validity of each instrument item.

Methods: Fifteen consenting adult and paediatric trainees participated in five simulations using a nursing confederate. A physician and a nurse observing the simulation exercises scored the 31-item ICAR independently. Scores for each item during each of the simulations were compared using kappa statistics for IRR, and items were identified with moderate agreement ($0.4 \leq k < 0.7$), weak agreement ($0 \leq k < 0.4$), and no agreement ($k < 0$).

Results: The 31-item ICAR instrument was assessed as cumbersome to use due to its length. Three items pertaining to patient interactions were deemed not applicable in the context of the scenarios where the patient is in crisis and non-communicative. Six items had moderate IRR and three items had low IRR. Twenty-two items did not perform reliably in the simulation environment.

Conclusions: We developed a high fidelity simulator for haemodialysis, and devised an abbreviated ICAR assessment tool that is applicable and reliable in assessment of inter-professional interactions. Scoring by a multidisciplinary team supports content validity to the use of ICAR in haemodialysis simulation. The shortened form is less cumbersome to use than the full length instrument, but requires prospective assessment of usability.

Funding: Clinical Revenue Support

SA-PO663

Are We Losing Important Patient-Doctor Interaction with Increasingly Online Clinics? Rauri A. Clark,[1] Anna Murray,[1] Mary McHugh,[1] John Andrew Sayer,[2] Iain Moore.[1] *[1]Renal Unit, City Hospitals Sunderland, United Kingdom; [2]Inst of Human Genetics, Newcastle Univ, United Kingdom.*

Background: Our study was prompted by patient reaction to increased computer use in clinic for documentation, blood work and investigations. After consultation nursing colleagues received comments from patients such as, "The doctor spent more time looking at the computer screen than me". We investigated whether this was accurate.

Methods: We asked: 1. Do we spend more time looking at the computer than the patient? 2. Do online clinic records have a detrimental effect on patient time? 3. Does computer screen position affect contact time? We studied nephrology clinics performed by the same nephrologist in 1 week, splitting clinics into 2 groups based on whether online records were part of the consultation. Two timers were used, 1 recording patient contact time, the other computer contact time. Patient contact time was defined as writing notes, examining and talking to the patient. Computer contact time was defined as time spent looking at the screen, searching for information and order entry. The angle between the patient, doctor and computer was calculated.

Results: Online record use increased computer time to the detriment of patient time by a mean 1.4 minutes per consultation. 1. At no point in consultation did the doctor spend longer looking at the computer than the patient. In online clinics, 30.2% of time was spent looking at the screen. 2. Patient contact time for clinics with online records was reduced by 20.6%. 3. There was no common positioning of the computer screen at clinic; screen position did not affect patient contact time. Angle of computer from patient ranged 54 to 135 degrees.

Conclusions: We highlight a problem with increasing online records and patient satisfaction in healthcare. Perceptions of how long the doctor spends on the computer did not correlate with actual time spent. However, perceptions need investigation. We are in danger of alienating patients by 'ignoring' them at clinic in favour of a screen. Research needs to be done into the best positioning of the computer for optimum patient contact and satisfaction. We must maintain a patient-centred approach and adjust practice to electronic records.

SA-PO664

The Association of Health Literacy with Patient Understanding of Kidney Test Results Through an Online Health Portal Stefanie L. Puher,[1] Andrea Lynn Berger,[2] Maria C. Bermudez,[1] Alex R. Chang,[1,2] William DiFilippo,[1] Jamie Alton Green.[1,2] *[1]Nephrology, Geisinger Medical Center, Danville, PA; [2]Center for Health Research, Geisinger Medical Center, Danville, PA.*

Background: The use of online health portals has the potential to increase patient engagement in care; however, little is known about the use of online health portals by patients with low health literacy and how well they understand electronic health information.

Methods: We administered an investigator-designed survey about patient understanding of kidney test results through an online health portal to patients with chronic kidney disease (CKD) stages 3-5 who attended at least 1 nephrology clinic, were active portal users, and had viewed test results online. The survey consisted of 29 knowledge questions based on published educational content. Health literacy was assessed using the Brief Health Literacy Screen (BHLS), a validated 3-item self-reported screening instrument.

Results: Out of 1,957 patients sent the survey, 512 completed the survey. The mean age was 66 ±11, 50% were male, 97% were white, 43% had a high school or lower level of education, and 17% had low health literacy. Patients with low health literacy had poorer understanding of their kidney test results (average test score 57.4% versus 64.3%, p<0.001), even when adjusting for age, gender, educational level, number of nephrology visits, CKD stage, use of educational resources, and number of portal logons in past 30 days. Compared with patients with adequate health literacy, patients with low health literacy who viewed their test results online were more likely to report that they need help understanding their test results (p<0.001) and are not sure what to do with the information (p<0.001).

Conclusions: A significant number of kidney patients with low health literacy are seeking use of online health portals to better manage their health. Despite this, patients with low health literacy may have difficulty understanding their test results online and may need tailored educational content and tools to maximize their involvement in care.

SA-PO665

Intensive Education to Chronic Kidney Disease Patients: Benefits on Glomerular Filtration Rate Decline Mario Prieto Velasco1, Benjamin De Leon Gomez, Jose Edgardo Gonzalez Arregoces, Igor Romaniouk Jakovler, Elena Astudillo, Jorge Estifan Kasabji, Aranzazu Sastre, Cristina Lucas. *Nephrology, Complejo Asistencial Univ León, León, Spain.*

Background: We implemented a program for intensive CKD patients education to educate and motivate the patients (MDRD < 20 ml/m) to take part in the therapeutic decisions and treatment election.

Methods: Patients with a MDRD < 20 ml/min are offered to be included in the program, voluntarily, from a pre-dialysis clinic. Educational methodology: multidisciplinary pedagogical group education. The multidisciplinary team is formed by nephrologist, nurses, pharmacist, nutritionist, psychologist, physiotherapist, social service, mentor patients and others, who teaches in 90 minutes sessions every two weeks, after hours, 3 topics related to the disease in every session. The group is composed by 10 CKD patients and one relative/patient. Throughout 8 sessions, twice a year.

Results: > 60 patients enrolled. Evaluation using a satisfaction survey was made (9,6 over 10). Initial-final knowledge survey (initial score of 6,9± 1,18 point and final 9,3 ± 0,23 points; p=0,004). Anxiety state survey at the beginning and at the end of the course (40% of the patients with less anxiety, 36% same as at the beginning and 24% more anxious toward the end). The mean loss of GFR in comparison with the control group, without intervention was 0,28 ± 0,27 ml/month versus 0,62 ± 0,6 ml/month; p=0,009). Renal Replacement Therapies (RRT) elected by patients were HD 45,7%, PD 40% and 13,4% transplant.

Conclusions: The "CKD School" is highly satisfactory for the patient, it helps to improve the knowledge about the disease, it might reduce the anxiety and it reduces the ratio of GFR loss. It also helps to improve transplantation as well as home therapies as first RRT. And might help, to the medical and non-medical staff to have a wider view of the problem, and a more satisfactory work.

SA-PO666

Parent-Child Dyads Preferred Platforms to Learn about Chronic Kidney Disease Ali Annaim,[1] Sarah Elizabeth Cohen,[1] Jessica L. Ryan,[1] Anthony Viera,[2] Rebecca M. Ferris,[3] Sofia Ocegueda,[1] Maria E. Ferris.[1] *[1]UNC Kidney Center, Univ of North Carolina Chapel Hill, Chapel Hill, NC; [2]Dept of Family Medicine, UNC School of Medicine, Chapel Hill, NC; [3]Student, Amercian Univ, Washington DC.*

Background: Chronic Kidney Disease (CKD) is a condition that places a large degree of responsibility on patients and parents/caregivers because of the need to manage several medications, diet and complex medical procedures.

Methods: We performed focused interviews with children and adolescents who have CKD and their parents/caregivers at the University of North Carolina Kidney Center Pediatric Nephrology Clinic. We asked these parent-child dyads to identify their preferred sources of information to learn about CKD. Informed consent or assent was obtained, and the twice reviewed recording transcriptions were analyzed qualitatively using the Atlas T.i software.

Results: We enrolled 22 parents/caregivers (9 fathers and 13 mothers) and their children. The children's characteristics were: mean age 14 (±3) years; 23% Hispanic; 27% Caucasian; 50% African-American; 36% had private insurance; 45% were males. Their disease characteristics were: mean CKD stage 3 (range 2-5); 23% transplant; mean age at diagnosis was 9 (± 5 years); mean percentage life with disease was 38% (±32%); and the mean number of medications was 8 (±4). The most common themes cited by parents and their children are depicted in Table 1.

Themes	Parent	Child
Preferred source of information	Provider (96%), Internet (68%)	Provider (91%), Internet (36%), Parents (41%)
Response to diagnosis	Shock, feeling overwhelmed	Shock and Fear
Strategies to manage condition	Habit and Instinct	Pillboxes, alarms, Schedules

Conclusions: Providers are the preferred source of information about CKD for parents of children with CKD, followed by the internet. For children with CKD, their providers, followed by their parents are the preferred sources of information on their disease. While the parents found themselves in a sense of crisis when their child was diagnosed with CKD, the children found themselves relying on the parents for information and understanding of their situation.

Funding: Private Foundation Support

SA-PO667

Shared Decision-Making and Dialysis Modality Selection in Adult Patients Living with Chronic Kidney Disease Ana Paula Rossi,[1] Fahima Nasreen,[1] Anna C. Cloutier,[2] Paul K. Han,[2] Robert Zimmerman.[1] *[1]Nephrology, Maine Medical Center, Portland, ME; [2]Center for Outcomes Research and Evaluation, Portland, ME.*

Background: For most patients with chronic kidney disease (CKD), the mode of dialysis is a preference-sensitive decision. Decision aids (DA) have been created to facilitate informed preference-sensitive decisions. The goal of this study was to determine the efficacy of a DA in improving informed decision making about dialysis modality in CKD patients.

Methods: Single center randomized controlled trial of patients with CKD stage IV/V randomized to DA or standard care (SC). At baseline and after receiving the allocated intervention in the DA arm, or six months later in the SC arm, participants completed surveys assessing several primary outcomes: CKD and dialysis-specific knowledge (14-item questionnaire), decisional conflict, and preparation for decision-making (PDM). Pre-post differences in outcome variables were compared using x^2 test and t-tests.

Results: Of 55 randomized patients, 28 completed the study (14 subjects per arm). Participants were 52% male, had a mean age of 67.2 years, eGFR of 18.4 mls/min/1.73m^2, and duration of nephrology care of 7.4 years. At baseline the percent of correct answers in the knowledge questionnaire was 64.3% for the SC and 61.4% for the DA arm (p=.7). At follow-up knowledge score improved by 20.7% in the DA arm but only by 7.9% in the UC arm (p=.05). There were no differences in the overall decision conflict scale (DCS); however, scores in the informed subscale of the DCS were marginally better in the DA arm (mean score 9.5 ±19.3 for DA versus 25.8 ±34.5 for UC; p=.1; [0–100 scale, 0 = feels extremely informed, 100 = feels extremely uninformed]). Patients in the DA arm also demonstrated high scores on the PDM scale, and reported that the DA was quite effective in preparing them for dialysis selection (mean score 77.7 ±20.9; 0–100 scale, higher scores indicate higher perceived level of PDM).

Conclusions: A dialysis-specific DA was efficacious in improving CKD patients' knowledge about their disease and dialysis options. Further testing is needed to determine if this intervention will help CKD patients make better informed decisions about dialysis modality.

Funding: Private Foundation Support

SA-PO668

An Educational Project to Improve Living Kidney Donor Transplant Rates Marie A. Sosa, Paula Ann Bigwood, Jahan Montague, Ellen Wells. *Nephrology, UMass Memorial Health Care, Worcester, MA.*

Background: Living kidney donor transplant (LDKT) is the optimal treatment for individuals with End Stage Renal disease (ESRD). Despite the benefits of LDKT, living donor transplant rates remain stagnant. Prior research points toward inadequate education of potential recipients as an obstacle to undergoing LDKT. We developed an educational program to help patients with ESRD improve their knowledge and identify barriers to LDKT and learn strategies to communicate their need.

Methods: The UMass Memorial Health Center (UMMHC) transplant department's database was used to identify 30 ESRD patients on the waiting list for Kidney transplant. Using a previously validated survey, we measured barriers to living kidney donation in our patient population. We then conducted a 90 minute 1:1 educational session with each patient which included information about the benefits of living kidney donation as well as communication tools that can assist in finding a living kidney donor. We recorded the number of living donor referrals, evaluations, and transplants pre and post intervention.

Results: The pre education survey identified several barriers to LKDT: knowledge deficit, fear, guilt, and lack of opportunity of having family and friends who could potentially donate a kidney. The educational curriculum was effective in improving knowledge regarding risks of donation and how to ask someone to donate a kidney. Post education, 73% of the participants knew how to ask someone to donate a kidney compared to pre education 40%. Donor referrals improved in the six months of the project; 158 donor referrals compared to the previous six months 117. Seven study subjects were successful at recruiting donors. Of the 7 subjects who recruited a donor, 3 have potential donors being evaluated, and 1 subject has undergone LDKT. LKDTs improved from 8 in FY 2013 compared to YTD 2014 (17).

Conclusions: An educational program that addresses risks of donation and how to communicate the need for a LDKT is effective in improving the ability of ESRD patients to recruit potenital donors. This project's results demonstrate an improvement in living donor referrals. A longer term follow up is needed to assess an impact in LDKT Rates.

SA-PO669

A Medical Student Web-Based Curriculum for Renal Pathology; Development, Optimization, and Assessment of the Teaching Tool

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Background: The concept of the "flipped classroom" has gained increasing popularity in recent years. The need for new and exciting curricula is particularly important for renal pathophysiology, as nearly 80% of surveyed medical students report that renal pathophysiology courses are too complex, lack relevance, or are simply uninteresting. This early discouragement may contribute to the dramatic decline in career interest for nephrology that has become evident in recent years. Our goal was to promote active learning by adding web-based learning tools to the already existing renal pathology curriculum in our renal pathophysiology course.

Methods: The online renal pathology curriculum consisted of an open access online textbook, short web-based concept videos, and an online quiz system. At the end of the course, we assessed students' performance on the final examination and administered general course evaluation and an additional survey through SurveyMonkey™ to assess utilization and satisfaction with the curriculum. Quantitative analysis of the tool use was available for the quiz system.

Results: 82.6% of the students used the quiz system. The greatest usage frequency was on the day before the final exam. Students repeated interactive and more challenging quizzes more often. Although the pathophysiology portion was not represented in the quiz, students who used the pathology quiz had comparable results in both pathology and pathophysiology portions of the final exam. Students that did not use the pathology quiz did equally worse in both pathology and pathophysiology on the final exam. The quizzes and concept videos were evaluated as very useful learning tools by 82.5% and 51.6% of student survey responders, respectively. 89.0%, thought the quiz improved their experience with kidney pathology in this course.

Conclusions: Online renal pathology learning tools with interactive features, especially quizzes, are very well received by students and show beneficial impact on the learning experience.

SA-PO670

The Nephrology Fellows Clinical Experience Project: A Pilot Study

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Background: Despite the emphasis on using clinical experience as a basis for graduate medical education, little is known regarding the frequency with which specific diagnostic entities are seen by nephrology fellows nor the special cognitive challenges they pose. We carried out this pilot study to begin to assess nephrology fellow clinical experience, with the aim of eventually using this information to facilitate patient-centered education and experiential learning.

Methods: Patients from the inpatient and outpatient setting who had a nephrology consult done were included in the study. Data included demographics, admission diagnosis (for inpatients), comorbid conditions, the reason for the renal consult, and the presumed/confirmed renal diagnosis by the fellow and supervising attending nephrologist.

Results: For the inpatient sample (n=300), acute kidney injury (AKI), fluid and electrolyte abnormalities, chronic kidney disease (CKD), ESRD on dialysis, renal transplant status and hypertension (HTN) were common entities seen in contrast to pregnancy-related disorders and vasculitis. Sixty cases were determined to be especially challenging from a diagnostic and or therapeutic perspective. For the outpatient setting (n=50), conditions seen included stage 3 and 4 CKD, glomerulonephritis and nephrotic syndrome, fluid electrolyte disorders, kidney transplant status and hypertensive disorders among others. Our fellowship program has an intervention to promote post-hospital discharge follow up of patients who had AKI by the same fellow who saw them in the hospital. Of note, this accounted for 11% of patients seen.

Conclusions: These pilot results from our ongoing study reveal a rich spectrum of fellow clinical experience and suggest that it is amenable to quantitative assessment. By developing a more comprehensive portfolio of nephrology fellow clinical experience this may help program directors restructure their curriculum, especially to incorporate new experiential learning approaches as well as to develop supplemental learning venues for those clinical experiences which occur at a low frequency and/or pose unique challenges.

SA-PO671

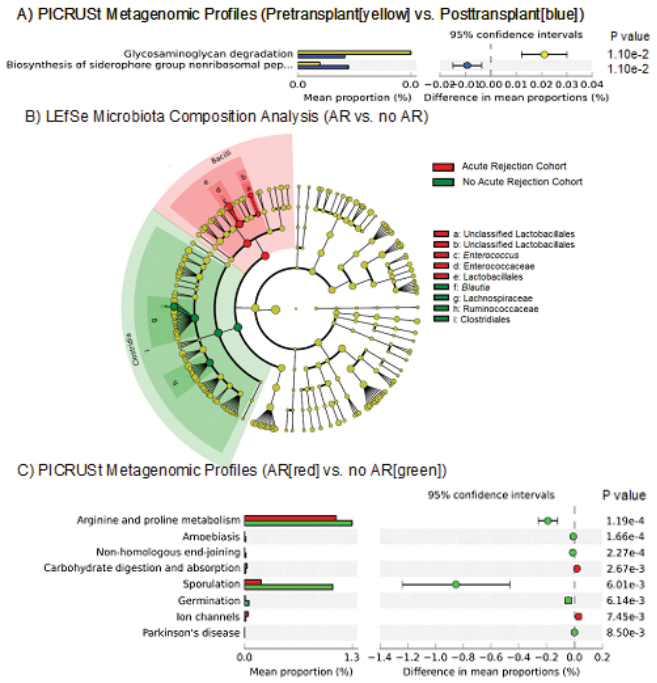
Characterization of the Gut Microbiota and Its Predicted Metagenomics in Kidney Graft Recipients

John R. Lee,[1] Thangamani Muthukumar,[1] Darshana Dadhania,[1] Lilan Ling,[2] Eric Pamer,[2] Manikkam Suthanthiran.[1] *[1]Medicine, Weill Cornell Medical College, New York, NY; [2]Medicine, Memorial Sloan Kettering Cancer Center, New York, NY.*

Background: Immunosuppressive therapy, in a similar fashion to antibiotics, has the potential to perturb gut microbial community structure and composition. Such alterations, however, have not been well characterized in recipients of renal allografts.

Methods: We prospectively enrolled 26 kidney transplant recipients and collected 85 sequential fecal specimens during the first 3 months of transplantation. We characterized the microbiota in the 85 fecal specimens by PCR amplification of the 16S rRNA V4-V5 variable region and deep sequencing of the amplified PCR product using the Illumina MiSeq platform. We utilized the PICRUSt tool to predict the metagenomic profiles and we investigated whether the fecal microbiota or metagenomic profiles change after kidney transplantation and whether these were associated with a biopsy confirmed acute rejection.

Results: The relative density of Proteobacteria was higher in the 2 week post-transplant fecal specimens compared to matched pre-transplant specimens (N=5, P=0.04, Wilcoxon Signed Rank test). PICRUSt revealed distinct metagenomic KEGG pathways that were different from pre-transplant and post-transplant fecal specimens (Figure 1A, Paired t test). The patients who developed acute rejection (AR, n=3) had significantly different microbial composition than those who did not (No AR, n=23) by linear discriminant effect size (LEfSe) method (red indicating higher taxon abundance in AR patients; green indicating higher taxon abundance in patients without AR) (Figure 1B). Several distinct metagenomic KEGG 3 pathways were associated with AR (Figure 1C, Welch's t test).



Conclusions: We identified significant alterations in the gut microbiota and the predicted metagenomes after kidney transplantation.

Funding: Other NIH Support - CTSC KL2 TR 00458

SA-PO672

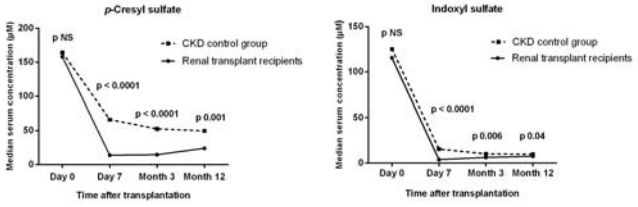
The Impact of Renal Transplantation on Microbiota Derived Uremic Retention Solutes

Ruben Poesen, Katrien De Vusser, Pieter Evenepoel, Dirk R. Kuypers, Maarten Naesens, Bjorn Meijers. *Nephrology, Univ Hospitals Leuven, Belgium.*

Background: The gut microbiota contributes substantially to uremic retention solutes accumulating in CKD. p-Cresyl sulfate and indoxyl sulfate are representatives of this group of solutes and associate with adverse outcome. Although it can be expected that serum levels of these metabolites decrease after renal transplantation, this has not been studied to date. In addition, whether serum levels of p-cresyl sulfate and indoxyl sulfate in renal transplant patients are quantitatively different than in regular CKD patients is unknown.

Methods: A cohort of 51 CKD patients was prospectively followed from time of transplantation to 12 months post transplantation. Serum levels of p-cresyl sulfate and indoxyl sulfate were determined at time of transplantation, day 7, month 3 and month 12 post transplantation. At each time point, serum levels were compared with CKD patients matched for age, gender, BMI, diabetes, dialysis modality/vintage at time of transplantation or renal function at other time points, and biochemistry.

Results: Serum levels of p-cresyl sulfate and indoxyl sulfate substantially decreased after renal transplantation ($P < 0.0001$). When compared to CKD control patients, serum levels of both solutes were still significantly lower in renal allograft recipients at each time point (Figure 1). Additional analyses demonstrated lower urinary excretion rates of microbial metabolites in renal transplant patients ($P < 0.0001$).



Conclusions: Microbiota derived uremic retention solutes substantially decrease after renal transplantation. In addition, serum levels of these solutes are lower when compared to regular CKD patients, suggesting an influence of renal transplantation or

immunosuppressive drugs on gut microbial metabolism. Whether these metabolites are also associated with adverse outcome in renal transplant patients needs further investigation.

Funding: Government Support - Non-U.S.

SA-PO673

Molecular Features of Primary and Secondary Focal Segmental Glomerulosclerosis After Renal Transplantation Michelle L. Lubetzky,[1] Valeria Mas,[2] Enver Akalin.[1] *[1]Nephrology, Montefiore Medical Center, Bronx, NY; [2]Translational Genomics Transplant Laboratory, U of Virginia.*

Background: The intragraft gene expression profiles of focal segmental glomerulosclerosis (FSGS) after renal transplant (KTx) have not been described.

Methods: All for-cause renal allograft biopsies with a diagnosis of FSGS were studied using Affymetrix HuGene 1.0 ST expression arrays and compared to normal KTx biopsies. Patients were divided into 3 groups: Stable allograft function (n=11), primary/recurrent FSGS (n=6) who either had a diagnosis of FSGS in the first 3 months after KTx and/or collapsing FSGS on biopsy and secondary FSGS (n=11) who had a diagnosis of FSGS more than 1 year after KTx.

Results: A total of 272 probe sets were differentially expressed in primary/recurrent FSGS biopsies (FDR ≤ 0.05). Top canonical pathways included dendritic cell maturation, granulocyte adhesion and diapedesis, TREM1 signaling and role of macrophages, fibroblasts and endothelial cells ($p<0.01$). Analysis of upstream regulators showed activation of lypopolysacharide, interferon gamma (IFN), tumor necrosis factor (TNF), interleukin1B, toll-like receptor (TLR)-4, CSF2 ($p<0.01$). A total of 467 probe sets were differentially expressed in secondary FSGS biopsies (FDR≤ 0.05). Top canonical pathways included dendritic cell maturation, altered T and B cell signaling, CD28, and iCOS-iCOSL signaling in helper T cells, and T cell receptor and PKCθ signaling in T lymphocytes, and PI3K signaling in B lymphocytes. Analysis of upstream regulators showed TNF activation, including lymphocyte activation, cell movement of lymphocytes and mononuclear cells. When comparing overlapping probe sets between the two groups, 82 were common to both analyses, 190 were unique to secondary FSGS, and 384 were unique to primary FSGS. In context of the overall analysis, it appears that these unique genes demonstrate innate immune response is critical for primary/collapsing FSGS while cellular/adaptive immune response is more involved in secondary/late FSGS.

Conclusions: Molecular analysis of biopsies with FSGS revealed significant immune activation, more specifically an innate immune response in primary FSGS as compared to an adaptive response in secondary FSGS.

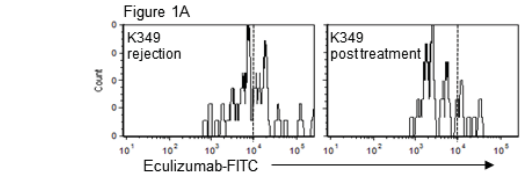
SA-PO674

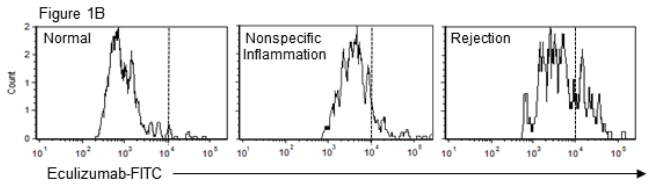
Eculizumab Binding to Human Renal Microvascular Endothelial Cells: Identifying New Therapeutic Targets in Kidney Biopsies Kimberly A. Muczynski, Nicolae Leca, Niamh Kieran, Arthur E. Anderson, Susan K. Anderson. *Univ of Washington, Seattle, WA.*

Background: Complement inhibition agents, such as eculizumab, offer a new approach to treating inflammatory kidney disease. The contribution of complement to different renal pathologic conditions and the therapeutic applications of complement inhibition remain unclear. We investigated eculizumab epitope binding in human kidney biopsies using our cytometry-cytokine detection protocol.

Methods: A direct FITC conjugate of eculizumab was made for use in multicolor flow cytometry. Human Ig-FITC was used as an isotype control. Renal biopsy samples were prepared for cytometry according to our previously reported protocol.

Results: Renal microvascular endothelial cells (RMEC), defined as HLA-DR+, CD34+, CD45-, bound eculizumab under conditions of acute cellular rejection and the level of binding was less following treatment (Figure 1A). RMEC from transplanted kidneys with non-specific inflammation showed a slight shift in eculizumab binding histograms toward the positive direction, but not to the extent of rejecting kidneys (Figure 1B, normal versus nonspecific, p = 0.04; normal versus rejection, p = 0.03).





RMEC from a patient treated with eculizumab for C3 glomerulopathy had minimal eculizumab binding detected with the assay. Tubular cells (defined as CD324+, CD326+, CD45-, HLA-DR-) from native and transplanted kidneys under a variety of conditions consistently showed 8-10% of the population expressed the eculizumab epitope. Eculizumab binding to tubular cells did not differ with pathology.

Conclusions: Our preliminary results of eculizumab binding to RMEC show correlation with renal pathology and may provide a surrogate marker for therapeutic applications of complement inhibition.

Funding: Private Foundation Support

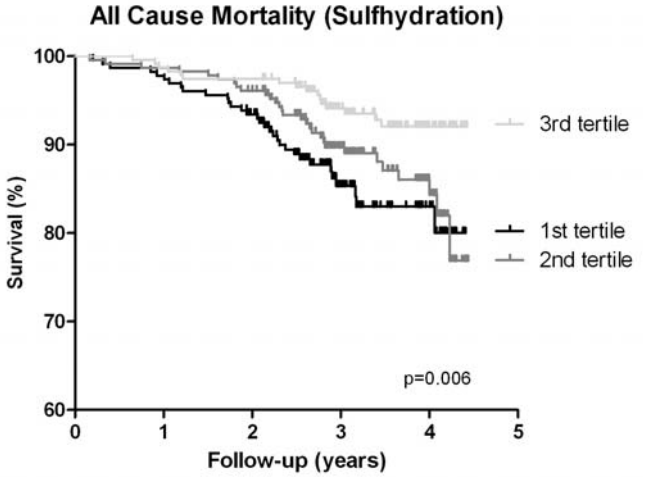
SA-PO675

Sulfhydration Is Associated with Patient and Graft Survival in Renal Transplant Recipients Anne-Roos S. Frenay,[1] Martin H. De Borst,[2] Matthias Bachtler,[3] Charlotte A. Keyzer,[2] Else Van den Berg,[2] Stephan J.L. Bakker,[2] Harry Van Goor,[1] Andreas Pasch.[3] *[1]Pathology and Medical Biology, Univ Medical Center Groningen, Netherlands; [2]Nephrology, Univ Medical Center Groningen, Netherlands; [3]Nephrology, Hypertension and Clinical Pharmacology, Univ Hospital Bern, Inselspital, Switzerland.*

Background: Urinary H_2S metabolites beneficially associate with survival in renal transplant recipients (RTR; van den Berg et al. JASN 2014). Protective effects of H_2S can be mediated through sulfhydration (SH): post-translational modification of reactive cysteine residues of targeted proteins, which modulates protein function. We prospectively investigated whether SH is favorably associated with patient and graft survival in RTR.

Methods: Serum SH (corrected for total protein [g]) was quantified in 697 stable outpatient RTR (57% male, 53±13 yr), with a functioning graft for ≥1 yr; transplant vintage 5.4 [1.9-12.1] (median[IQR]) yr. Determinants of SH were evaluated with multivariate linear regression models. Associations between SH and mortality or graft failure risk were assessed with multivariabele Cox regression analyses.

Results: SH (mean 1.83±0.67 μM/g) correlated with the serum and urinary H_2S metabolite thiosulfate ($p<0.01$). In multivariate models, eGFR and serum albumin were positively associated with SH. Male gender, serum cholesterol and calcineurin inhibitor or sirolimus use were inversely associated with SH (model $R^2=0.26$). During follow-up (3.1 [2.7-3.9] yr) 79 (11%) patients died and 45 (7%) patients developed graft failure. SH was inversely associated with all-cause mortality (HR 0.63 [95% CI 0.49-0.82]) and graft failure (HR 0.45 [0.32-0.63]), both $p<0.001$ per SD increase and independent of Framingham risk factors and eGFR.



Conclusions: Increased SH is associated with an improved patient and graft survival in RTR. SH modification may improve long-term outcomes post KTx.

SA-PO676

Comparable Renal Function in De Novo Kidney Transplantation with Prolonged-Release Tacrolimus + MMF/Sirolimus: Initial Results from the ADHERE Study Oleg Rummo,[1] Mario Carmellini,[2] Lionel Rostaing,[3] Rainer Oberbauer,[4] Maarten H.L. Christiaans,[5] Frank Lehner.[6] *[1]RSPC for Organ and Tissue Transplantation, Belarus; [2]Univ of Siena, Italy; [3]Toulouse Univ Hospital, France; [4]Medical Univ of Vienna, Austria; [5]Maastricht Univ Medical Centre, Netherlands; [6]Hannover Medical School, Germany.*

Background: ADHERE: 52-week, Phase IV, randomized, open-label study to investigate renal function with once-daily, prolonged-release tacrolimus (QD) + MMF/sirolimus.

Methods: All patients received tacrolimus QD (initial dose: 0.2mg/kg/day) + MMF (Days 0–27). Patients were randomized on Day 28: tacrolimus QD + MMF (Arm 1) or tacrolimus QD (reduced dose from Day 42) + sirolimus (1mg/day) (Arm 2). Steroids were administered throughout. Target trough levels: Day 0–14: 10–15ng/mL; Day 15–41: 8–12ng/mL; Day 42–365 (Arm 1): 6–10ng/mL; Day 42–365 (Arm 2): 4–5ng/mL. Primary endpoint (full-analysis set; FAS): measured GFR (mGFR; iohexol clearance) at Week 52. Secondary endpoints (intent to treat; ITT) included: Kaplan–Meier analyses of composite efficacy failure (defined as graft loss/early patient withdrawal), patient death, and biopsy-confirmed acute rejection (BCAR).

Results: ITT: 362, 368 in Arms 1–2; 730 randomized. FAS: 287, 282 in Arms 1–2. Baseline characteristics were comparable. Tacrolimus trough levels were comparable until Day 42 and remained lower in Arm 2 after dose reduction. At Week 52, mean (SD) trough levels were: 7.11ng/mL (2.27) and 5.17ng/mL (1.78) in Arms 1–2. mGFR at Week 52 was 40.7 versus 41.8mL/min/1.73m² for Arm 1 versus 2 (1.02; –1.39 to 3.44; p=0.405). Secondary endpoints are presented in the table. A higher incidence of patients in Arm 2 discontinued due to adverse events (AEs).

%	Arm 1 (n=362)	Arm 2 (n=368)	P value Wilcoxon–Gehan
Composite efficacy failure	11.5	18.2	0.0024
- Graft loss	2.9	2.2	0.6757
- Early patient withdrawal	5.2	14.4	<0.001
Patient death	1.1	0.3	0.1769
BCAR (post-randomization)	4.3	3.6	0.8916

Conclusions: Comparable mGFR, incidence of graft loss, mortality and BCAR were reported with tacrolimus QD + MMF/sirolimus at Week 52. A higher incidence of patients discontinued from the tacrolimus QD + sirolimus arm due to AEs.

Funding: Pharmaceutical Company Support - The ADHERE study was sponsored by Astellas Pharma Europe Ltd. Editorial assistance was provided by iS Health and funded by Astellas Pharma Europe Ltd.

SA-PO677

Serum Levels of Soluble Klotho Are a Promising Marker of Graft Function After Kidney Transplantation Makoto Tsujita,[1] Tomoki Kosugi,[2] Waichi Sato,[2] Shoichi Maruyama,[2] Yoshihiko Watarai.[1] *[1]Transplant Surgery, Nagoya Daini Red Cross Hospital, Japan; [2]Nephrology, Nagoya Univ School of Medicine, Japan.*

Background: Fibroblast growth factor 23 (FGF23) is known to predict graft function after kidney transplantation, but the roles of Klotho and vitamin D still remains unknown. The aim of this study is to identify whether serum Klotho and vitamin D are predictive markers of graft function.

Methods: Consecutive 46 patients were enrolled in this retrospective obsevational study at our hospital. Serum FGF23, soluble Klotho levels and 25-hydroxyvitamin D (25-OHD) levels, estimated glomerular filtration (eGFR), and other clinical parameters at 1 year and eGFR at 3 year after kidney transplantation were measured.

Results: Patients' characteristics and clinical data were shown in Table 1.

Age (years)	51.8±11.6
Gender (male/female)	31 / 15
DMN / non DMN	9 / 39
Systoric blood pressure (mmHg)	127.0±11.5
Diastoric blood pressure (mmHg)	77.5±7.7
Corrected Calcium (mg/dl)	9.6±0.7
Phosphorus (mg/dl)	3.4±0.4
iPTH (pg/ml)	110.0±133.3
T-cho (mg/dl)	189.7±28.98
TG (mg/dl)	122.5±65.6
HDL (mg/ml)	62.8±19.4
LDL-C (mg/ml)	96.0±17.3
Klotho (pg/ml)	535.9±171.6
FGF23 (pg/ml)	60.1±21.1
25-OHD (ng/ml)	7.7±4.4
eGFR at 1 year (ml/min/1.73m2)	43.0±12.8
eGFR at 3 year (ml/min/1.73m2)	43.2±14.3
Use of Cycrosporine / tacrolimus (n)	27 / 19
Use of mycophenolate mofetil (n)	45
Use of ARB (n)	33
Use of statin (n)	25

Serum soluble Klotho (pg/ml), FGF23 (pg/ml) and 25-OHD (ng/ml) levels were 535.9±171.6, 60.1±21.1, 7.7±4.4 respectively. ΔeGFR (eGFR at 3 year - eGFR at 1 year) (ml/min/1.73m²) values were 0.1±5.6. Serum soluble Klotho levels were associated with ΔeGFR (r = 0.38 *P* =0.008). Serum soluble Klotho / FGF23 ratio values were also associated withΔeGFR (r = 0.32 *P* =0.03), but serum FGF23 and 25-OHD levels were not associated (r = 0.04 *P* =0.80, r = 0.01 *P* =0.94 respectively).

Conclusions: Serum levels of soluble Klotho might be a promising marker of graft function after kidney transplantation, compared to FGF-23 and 25-OHD.

SA-PO678

Prediction of Calcium and Phosphorus Disorders in the Early Stage After Kidney Transplantationon on the Basis of Pretransplant Fibroblast Growth Factor 23 Levels Makoto Tsujita, Yoshihiko Watarai. *Transplant Surgery, Nagoya Daini Red Cross Hospital, Aichi, Japan.*

Background: Hypercalcemia and hypophosphatemia are common complications of kidney transplantation. Previous studies have shown that pretransplant full-length fibroblast growth factor (FGF) 23 levels predict phosphorus (Pi) metabolism after kidney transplantation; however, it remains unknown whether they are predictive of post-transplant calcium (Ca) metabolism.

Methods: Seventy-one consecutive patients were enrolled in this study at Nagoya Daini Red Cross Hospital, Japan, from 2012. Parameters of corrected Ca and Pi metabolism, including FGF23 and intact parathyroid hormone (iPTH) levels, were measured before (pre) and 6 months (6m) after kidney transplantation.

Results: Patients' characteristics and clinical parameters of this study are shown in Table 1.

Age (years)	50.6±12.7
Gender (male/female)	35 / 36
DMN (diabetic nephropathy) / non DMN	33 / 38
PEKT (preemptive kidney transplantation) / non PEKT	35 / 36
cCa pre (mg/dL)	9.1±0.8
Pi pre (mg/dL)	5.5±1.0
iPTH pre (pg/dL)	260.9±19.2
cCa 6m (mg/dL)	9.8±0.6
Pi 6m (mg/dL)	3.4±0.6
iPTH 6m (pg/dL)	102.9±51.7
FGF23 pre (pg/dL)	7350.9 (793.7, 7411.5)
Ln FGF23 pre	3.4±0.7
Duration of dialysis (months)	26.5
Use of VDRA (oral)	28
Use of VDRA (iv)	5
Use of Pi binder	18
Use of Ca-containing Pi binders	24
Use of cinacalcet	6

Table 2 shows the results of this study.

Table 2 (a)
Pretransplant risk factors associated with serum Ca levels at 6 months after kidney transplantation

Ca	Coefficient	SE	t	R^2	P-value
Univariate					
age	0.01	0.006	1.81	0.05	0.08
gender (female)	0.01	0.006	1.81	0.05	0.08
DMN	−0.3	0.14	−2.2	0.07	<0.05
PEKT	0.54	0.13	4.22	0.31	<0.001
cCapre	0.43	0.07	5.76	0.32	<0.001
Pipre	0.12	0.07	1.75	0.21	0.09
iPTHpre	−0.0003	0.0003	−0.89	0.01	0.38
LnFGF23	0.49	0.09	5.41	0.3	<0.001
multivariate				0.44	
age	−0.002	0.005	−0.36		0.72
gender (female)	0.08	0.11	0.71		0.48
DMN	−0.19	0.12	−1.54		0.13

Table 2 (b)
Pretransplant risk factors associated with serum Pi levels at 6 months after kidney transplantation

P	Coefficient	SE	t	R^2	P-value
Univariate					
age	−0.003	0.006	−0.41	0.003	0.68
gender (female)	0.25	0.15	1.7	0.04	0.1
DMN	0.112	0.15	0.8	0.009	0.43
PEKT	−0.2	0.15	−1.36	0.03	0.18
cCapre	−0.23	0.09	−2.73	0.1	<0.05
Pipre	−0.14	0.07	−1.99	0.05	0.05
iPTHpre	−0.0001	0.0003	−0.23	0.0008	0.81
LnFGF23	−0.43	0.11	−4.24	0.21	<0.001
multivariate				0.26	
age	0.005	0.006	0.8		0.43
gender (female)	0.25	0.13	1.87		0.07
cCapre	−0.1	0.1	−0.99		0.33

Multivariate linear regression analysis revealed that higher FGF23 levels before transplantation, not iPTH levels, were related to higher Ca levels and lower Pi levels at 6 months after kidney transplantation.

Conclusions: This study suggests that pretransplant FGF23 levels, not iPTH levels, are a strong predictive marker of Ca and Pi disorders after kidney transplantation.

SA-PO679

Urinary Biomarkers of Cell Cycle Arrest and Inflammation for Prediction of Dialysis or Recovery After Kidney Transplantation Timothy J. Pianta,[1] Philip Peake,[1] John W. Pickering,[2] Nicholas Buckley,[1] Zoltan H. Endre.[1] *[1]Prince of Wales Clinical School, Univ of New South Wales, Australia; [2]Medicine, Univ of Otago, New Zealand.*

Background: Prediction of allograft recovery within hours of kidney transplantation is difficult and limits the development of therapeutic strategies. Novel urinary acute kidney injury biomarkers, tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) predict AKI but performance in renal transplantation is unknown.

Methods: We studied 81 patients immediately after kidney transplantation. Serial urine concentrations of TIMP-2 and IGFBP7 were measured along with putative inflammatory biomarkers vascular endothelial growth factor (VEGF), macrophage migration inhibitory factor (MIF), monocyte chemotactic protein-1 (MCP-1), trefoil factor 3 (TFF3), and chemokine (C-X-C) ligand-16 (CXCL16). The utility of these biomarkers for prediction of delayed graft function (DGF, dialysis within 7 days) within 1 day of transplantation was assessed.

Results: 23 patients (28%) had DGF. At 4h after kidney reperfusion the areas under the receiver operator characteristic curve (AUC) for VEGF and [TIMP-2]*[IGFBP7] were good (AUCs > 0.80), TIMP-2, IGFBP7, and MIF fair (AUCs 0.70 – 0.79), and for MCP-1, TFF3, and CXCL-16 poor (AUC < 0.60). After adjusting for pre-operative clinical variables, anuria and the creatinine reduction ratio, integrated discrimination improvement analysis showed that only VEGF, TIMP-2, and [TIMP-2]*[IGFBP7] separately enhanced a clinical model for prediction of DGF at 4h. TIMP-2 and [TIMP-2]*[IGFBP7] also enhanced the model at 12h.

Conclusions: The cell cycle arrest marker urinary TIMP2 and the combination biomarker [TIMP-2]*[IGFBP7] predict DGF after kidney transplantation. VEGF appears to provide best discrimination very soon after transplantation. Other putative inflammatory biomarkers, MIF, MCP-1 CXCL16, and TFF3 performed poorly.

Funding: Government Support - Non-U.S.

SA-PO680

Urinary Soluble Urokinase Receptor (suPAR) Is Not Elevated in Membranous Nephropathy (MN) and Does Not Correlate with Proteinuria

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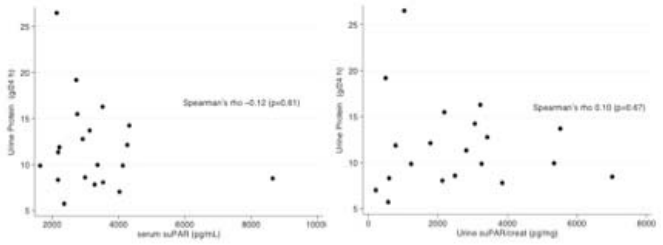
Background: Serum suPAR, a proposed marker of FSGS, is elevated in a variety of renal diseases, possibly due to reduced clearance with low GFR. Conversely, elevated urinary suPAR appears more specific to recurrent FSGS. We evaluated the relationships of proteinuria to urine and serum suPAR in MN patients with preserved renal function.

Methods: Urine and serum suPAR were measured in patients with MN and preserved renal function by ELISA. suPAR levels were correlated with proteinuria and compared to levels in pretransplant specimens from patients who experienced post-transplant recurrence FSGS. Data are expressed as mean ± SD, or mean (min-max) or median (25%, 75%).

Results: Age of MN cases (N=20) was 49 ± 13 years; 17 (85%) were male. Serum Cr was 1.49 (0.8-2.3) mg/dL and CrCl 72 (30-156) ml/min. Recurrent FSGS cases (N=5) were similar in age 48±19 years and gender 4 (80%) were male. Serum Cr was higher at 5.8 (3.1-9.9) mg/dl, p <0.01 in FSGS cases.MN compared to FSGS patients had similar proteinuria (10.6 [8.4, 13.9] versus 16.2 [14.8, 20.5] g/24hrs, p=0.12), lower urinary suPAR (2338 [910, 3337] versus 5239 [3141, 5331,] p 0.04), and lower serum suPAR (3058 [2289, 3780] versus 6195 [6137, 7009], p=0.002). In MN patients, serum and urinary suPAR did not correlate with urinary protein see figure 1.



Conclusions: Urine suPAR appears to be uniquely elevated in FSGS patients compared to MN patients despite comparable proteinuria. Thus elevated urinary suPAR could potentially serve as a marker of FSGS recurrence following transplant.

Funding: Clinical Revenue Support

SA-PO681

CD57 in CD8 Populations Is a Predictor of Cutaneous Squamous Cell Carcinoma Development in Renal Transplant Recipients

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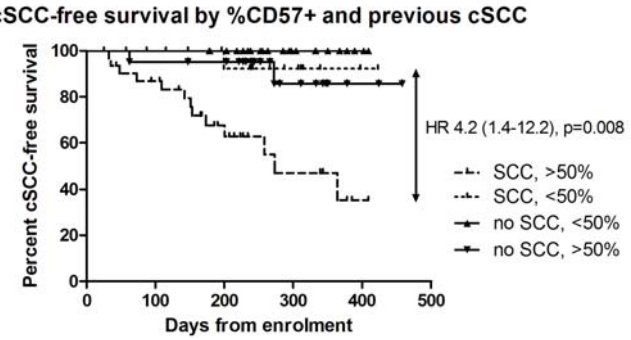
Background: Cutaneous squamous cell carcinoma (SCC), the commonest malignancy in renal transplant recipients (RTR), is more aggressive and recurrent than in non-RTR. Identification of high risk RTR may allow targeted screening and intervention. In liver transplantation and non-RTR, higher numbers of CD57-positive cells are associated with increased risk of malignancy.

Methods: We conducted a case-cohort study to identify predictive immunological markers in SCC. 51 RTR with previous SCC were recruited and matched by age, sex and immunosuppression type/duration to 50 RTR without SCC. Follow-up was (median(IQR)) 252 days (190-332));isolated peripheral blood mononuclear cells were analysed using flow cytometry.

Results: During follow-up, 21 further and 2 first SCC occurred in 16 RTR. RTR with >50% CD57-positive CD8+cells were 5.9 times (95% CI: 2.3-15.5) more likely to develop SCC during follow-up than those with <50%. In those with previous SCC, >50% CD57+ was associated with a 4-fold increase in risk (fig 1). Increasing age and clinical risk score (Harwood et al, AJT, 2013) were also associated with SCC development, but on multivariate analysis an increased proportion of CD57+ cells remained independently predictive of malignancy. RTR developing SCC were universally taking azathioprine, which markedly depleted NK cells, in contrast to mycophenolate.



Conclusions: The proportion of CD8+CD57+ cells may augment the power of clinical factors to predict SCC development. Azathioprine may deplete innate anti-tumour responses, compounding this effect. Analysis of CD8 populations may allow identification of a high-risk group for SCC, who would benefit from more intensive dermatological screening and immunosuppression reduction.

Funding: Private Foundation Support

SA-PO682

Role of Circulating Fibrocytes in Chronic Allograft Dysfunction

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Background: Interstitial fibrosis and tubular atrophy (IF/TA) is often described on renal allograft biopsy and the extent of this condition correlates with transplant outcome. Fibrocytes are circulating peripheral blood cells identified by surface progenitor markers (ie CD34) and markers of mesenchymal phenotype (intercellular pro-collagen). The role of fibrocytes in chronic allograft dysfunction has not been previously evaluated.

Methods: This is a single center observational study of patients undergoing a first kidney transplant. Baseline blood was drawn before transplant, 1 month, and 3 months after transplant. Circulating fibrocyte numbers were assessed by flow cytometry. Peripheral blood mononuclear cells were labeled for cell surface CD45 and intercellular collagen I.

Results: Eleven patients have been recruited to this study and followed to 3 months post transplant. There was a significant decline in the circulating fibrocyte count from baseline (pre-transplant) to 3 months post transplant ($5.7x10^9$/L to $1.5x10^9$/L, p=0.039). Patients whose fibrocyte counts decreased between 1 and 3 months post transplant had significantly better renal function (measured by serum creatinine) compared to those whose fibrocytes increased post transplant (3 months creatinine 111 umol/L versus 136 umol/L, p=0.047).

Conclusions: This preliminary study is the first to evaluate circulating fibrocytes post renal transplant. Fibrocytes decreased significantly after transplantation. Increasing fibrocyte counts may predict worse renal function post transplant.

Funding: Pharmaceutical Company Support - Pfizer Canada

SA-PO683

Renal Transplantation Does Not Result in Complete Restoration of the Endothelial Glycocalyx

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Background: Dialysis patients have impaired glycocalyx barrier properties and increased plasma levels of glycocalyx constituents. It is not known whether renal transplantation leads to restoration of the endothelial glycocalyx (EG). Here, we investigated the state of the EG in renal transplant recipients (RTR) treated with two different immunosuppressive therapies, compared to healthy individuals (HI).

Methods: Investigations were performed in 30 stable RTR (14 receiving cyclosporine (CsA) and 16, everolimus (EVL)) and 19 HI. Plasma levels of the glycocalyx constituents syndecan-1 and hyaluronic acid (HA), and the hyaluronidase activity were measured using ELISA. The state of EG in individual blood vessels was assessed by measuring the perfused boundary region (PBR), which reflects the erythrocyte permeable part of the endothelial surface layer, by Sidestream darkfield (SDF) imaging of the sublingual microvasculature.

Results: GFR was 52.9±19ml/min/1.73m2 in RTR, and similar in CsA and EVL group(p=0.8). Plasma levels of Syndecan-1 and HA were higher in RTR compared to HI (34±20 versus 20±11ng/ml, p=0.02; 51±35 versus 21±11ng/ml, p=0.03), but hyaluronidase activity was not different in the two groups (p=0.1). Patients treated with EVL had higher levels of HA, but not of syndecan-1, and increased hyaluronidase activity compared to the CsA group (75±37 versus 25±10ng/ml, p=0.004; 36±24 versus 31±15ng/ml, p=0.8 and 31±3 versus 27±3U/ml, p=0.03). HA levels in CsA group did not differ from the HI(p=0.2). The PBR was higher in RTR compared to HI (3.3±0.3 versus 2.9±0.5µm, p=0.02) but did not differ between the two therapies (3.1±0.3 versus 3.3±0.4µm, p=0.3).

Conclusions: The high plasma levels of glycocalyx constituents and the increased PBR in the sublingual microvasculature, indicate that RTR have alterations of the endothelial glycocalyx. Since the type of immunosuppressive therapy may influence the state of the endothelial glycocalyx, these data may guide the selection of tailor made immunosuppression in the future.

SA-PO684

Effects of Structured Physical Activity Program on Serum Adipokines and Markers of Inflammation and Heart Disease in Kidney Transplant Recipients Katarzyna Muras, Anna Masajtis Zagajewska, Michal P. Nowicki. *Dept Nephrology, Hypertension and Kidney Transplantation, Medical Univ of Lodz, Lodz, Poland.*

Background: Sedentary behavior typical for patients with chronic kidney disease (CKD) usually persists after kidney transplantation (KTx). Altered adipokine profile and inflammation has been linked to physical inactivity and increased cardiovascular mortality in CKD. We postulated that increased physical activity achieved through individually-tailored program may reverse these changes and improve cardiac function.

Methods: 25 KTx recipients at least 12 months after KTx and 15 age- and eGFR-matched CKD patients were included. Baseline nutritional condition, anthropometry, body composition (impedance spectroscopy), pattern of daily physical activity (SenseWear Armband Pro accelerometer) and serum leptin, adiponectin, NT-proBNP and hsCRP were assessed. Structured program of physical activity was scheduled for each patient. Repeated training and short message service with reminders were provided to participants. All measurements were repeated after 3 months.

Results: Active energy expenditure increased during 3 months of physical activity program in both KTx and CKD patients (from 0.30±0.21 to 0.44±0.30 cal/min, p<0.001 and from 0.30±0.17 to 0.36±0.25 cal/min, p=0.01, respectively). Time spent daily on activities changed similarly (126±87 versus 200±132 and 79±78 versus 129±114 min, respectively, p<0.001). Adipose mass decreased in KTx recipients only (from 40.8±1.6 to 38.5±10.3 kg, p=0.01). Serum leptin decreased in both groups (from 11.5±7.0 to 10.0±5.6, p=0.03 and from 14.1±8.3 to 12.2±6.1 ng/mL, p=0.01, respectively). Serum adiponectin increased in KTx (from 1666±863 to 2015±1133 ng/L, p=0.004) but not in CKD patients (1896±817 and 1905±646 ng/L, respectively). Serum CRP decreased in both groups (in KTx patients from 15.1±5.2 to 14.0±5.6 mg/L, p=0.01 and in CKD patients from 16.5±3.9 to 15.4±4,3 mg/L, p=0.05). NT-proBNP was unchanged in both groups (544±294 versus 537±317 and 514±170 pg/mL versus 512±161 pg/mL, respectively).

Conclusions: Increased physical activity induces beneficial effects on adipokine profile and inflammation but does not seem to affect heart function in kidney transplant recipients.

Funding: Government Support - Non-U.S.

SA-PO685

Left Ventricular Hypertrophy and Left Ventricular Systolic Dysfunction Affect Long-Term Outcome in Recipients of Kidney Transplantation Jung Nam An,[1] Hyosang Kim,[2] Jaeseok Yang,[3] Curie Ahn,[3] Yun Kyu Oh,[1] Chun Soo Lim,[1] Su-Kil Park,[2] Yon Su Kim,[4] Jung Pyo Lee.[1] *[1]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Asan Medical Center and Univ of Ulsan College of Medicine, Seoul, Republic of Korea; [3]Transplantation Center, Seoul National Univ Hospital, Seoul, Republic of Korea; [4]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Republic of Korea.*

Background: Left ventricular hypertrophy (LVH) and left ventricular systolic dysfunction (LVSD) are independent risk factors for cardiac death in both the general population and patients with end-stage renal disease. However, only limited data on echocardiographic prognostic parameters in recipients of kidney transplantation (KT) are available.

Methods: This study was a retrospective review of patients assessed for KT from Jan. 1997 to Jan. 2012. The LVSD was defined by ejection fraction below 50%. The primary and secondary end points were all-cause mortality and graft failure, respectively.

Results: Of 4,650 patients assessed for transplantation, 1,870 had an echocardiography. A total of 231 (12.4%) patients experienced graft failure and 116 (6.2%) died during a mean follow-up of 4.5 years. The recipients with LVH were associated with all-cause mortality (P=0.007) and showed higher occurrence of graft failure (P<0.001). The recipients with LVSD also showed higher all-cause mortality (P=0.045) and graft failure (P=0.004) by Kaplan-Meier method. In a multivariate analysis, increased age (P=0.014), previous history of CV event (P=0.015), post-transplant diabetes (P=0.013) and LVSD (hazard ratio: 2.987, 95% confidence interval: 1.061-8.412; P=0.038) were associated with all-cause mortality. However, none of LVH and LVSD was significantly associated with graft failure in a multivariate analysis.

Conclusions: In patients of KT candidate, easily determined echocardiographic finding of LVSD before KT was independently associated with all-cause mortality after transplantation.

SA-PO686

Kidney Transplantation in Patients with Adenine Phosphoribosyltransferase Deficiency Hrafnhildur L. Runolfsdottir,[1] Runolfur Palsson,[1,2] Inger Maria Agustsdottir,[2] Vidar O. Edvardsson.[1,2] *[1]Univ of Iceland, Reykjavik, Iceland; [2]Landspitali – The National Univ Hospital of Iceland, Reykjavik, Iceland.*

Background: Adenine phosphoribosyltransferase (APRT) deficiency is a rare, hereditary cause of chronic kidney disease, characterized by deposition of 2,8-dihydroxyadenine (DHA) crystals in the renal tubules and interstitium. Treatment with a xanthine dehydrogenase (XDH) inhibitor (allopurinol or febuxostat) prevents progression to end-stage renal disease (ESRD) but limited data exist on the effectiveness of pharmacotherapy in preserving renal allograft function. The aim of this study was to examine the outcome of kidney transplantation in patients with APRT deficiency.

Methods: Included in the study were patients in the APRT Deficiency Registry of the Rare Kidney Stone Consortium who had undergone kidney transplantation. Estimates of glomerular filtration rate (eGFR) were calculated using the MDRD equation. Data are presented as median and range.

Results: Among 51 patients in the Registry, 10 (5 males) of 12 with ESRD underwent 14 kidney transplantation procedures. Eight received one allograft and two patients 2 and 4 grafts, respectively. Age at first kidney transplantation was 44 (15-67) years. Six patients were taking allopurinol in a daily dose of 300 (150-400) mg when they received their first renal allograft, while 4 patients were not prescribed such therapy at the time of first kidney transplantation. At latest follow-up, 1.7 (0.2-8.8) years following kidney transplantation, 6 allografts were still functioning with eGFR of 34 (21-65) mL/min/1.73 m^2 despite a biopsy-proven recurrence of DHA nephropathy in five of these transplants. Four allografts were lost due to disease recurrence 1.3 (0.1-3.4) years post-transplant in untreated patients. Four patients died with a functioning graft.

Conclusions: Long-term renal allograft survival in patients with APRT deficiency is favourable in those receiving XDH inhibitor treatment. Lack of awareness of the disorder and the resultant late institution of appropriate pharmacotherapy is a major contributor to premature graft loss.

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SA-PO687

Blood Pressure, Graft Survival and Renal Function in Heart Allograft Recipients – The Effect of Intervention Christian Morath,[1] Vedat Schwenger,[1] Peter Schnuelle,[1] Gerhard Opelz,[2] Martin G. Zeier.[1] *[1]Dept of Nephrology, Univ of Heidelberg, Heidelberg, Germany; [2]Transplantation Immunology, Univ of Heidelberg, Heidelberg, Germany.*

Background: Hypertension in non-renal organ transplantant recipients is common. The effect of high blood pressure on the graft survival in heart allograft recpients and on long-term kidney function is unknown. The database of the Collaborative Transplant Study (CTS) provides a high number of heart transplant recipients and their long-term follow-up, including blood pressure and s-creatinine.

Methods: A total of 2853 heart transplant recipients with continuous information on blood pressure, s-creatinine and graft outcome were followed over a time period of 10 years. Systolic blood pressure at year 1 and year 5 was categorized either <140mmHg or >140mmHg. Only patients with normal renal function s-creatinine <1.4mg/dl at year 1 post transplantation were analysed. At year 10 renal function and graft survival were analysed. Altogether four groups were followed: A: bp<140 at year 1 and year 5; B bp>140 at year 1 and <140 at year 5; C: bp <140 at year 1 and >140 at year 5 and bp >140 at year 1 and >140 at year 5.

Results: 10-year graft survival according to group A-D was: A 81.2% (p<0.02 A compared to D), B 80.2% (p n.s. B compared with C), C 76% and D 77%. Normal kidney function, e.g. the relative number of recipients with s-creatinine < 1.4mg/dl was: A 65.2% (p<0.004 A compared to C and D; p n.s. A compared to B), B 58%, C 52.5% and D 42.2%.

Conclusions: Blood pressure control significantly improves heart allograft survival 10 years plus. In addition blood pressure treatment intervention (<140mmHg) between year 1 and 5 (group B) has a significant impact on heart allograft survival and on renal function. More than 60% of patients in group A had normal renal function at year 10. In contrast inadequate blood pressure control (group C and D) resulted in an inferior heart allograft outcome and a reduced relative number of recipients with excellent renal function. Blood pressure and renal functions significanty impact heart allograft survival.

Funding: Government Support - Non-U.S.

SA-PO688

Relationship of Leptin with Metabolic Syndrome and Hypophosphatemia in Pediatric Kidney Transplant Recipients Kristen Sgambat,[1] Ines Armando,[2] Christy Petyak,[1] Asha Moudgil.[1] *[1]Children's National, Washington DC; [2]Univ of Maryland.*

Background: Leptin, a hormone produced by adiopse tissue, plays a key role in regulation of body weight; recent animal studies suggest it may also influence bone mineral metabolism. A prospective study was conducted to investigate associations of leptin with risk factors for metabolic syndrome (MS) and hypophosphatemia in pediatric kidney transplant (tx) recipients.

Methods: The following parameters were measured at 6 month intervals in children 0-36 months post tx: fasting morning plasma leptin, glucose, HDL, triglycerides (TG), HbA1c%, BMI z score, waist circumference percentile (WC), blood pressure (BP), serum phosphorus and parathyroid hormone (PTH). Children with MS were identified as those meeting ≥ 3 of the following criteria: HbA1c%>5.6 or fasting glucose>100, BP>90th percentile, central obesity (WC≥95th percentile), HDL <5th percentile, TG > 95th percentile. Correlation of leptin with continuous variables was assessed by Spearman's rank correlation. Wilcoxon ranksum was used for comparisons between groups.

Results: Study population consisted of 25 kidney tx recipients age 12.9±0.8 years. One third of the population had central obesity (30%) and MS (33.3%). Leptin levels of children with obesity (39.1±3.4) and MS (28.7±4.7) were higher than those without obesity (8.1±1.8) and MS (11.8±2.4 ng/ml), p=0.0000 and p=0.002 respectively. Leptin positively correlated with BMI z score (rho 0.62, p=0.0000), WC (rho 0.77, p=0.0000), and age (rho 0.4, p=0.001). Leptin negatively correlated with HDL (rho -2.8, p=0.02) and phosphorus (rho -0.45, p=0.001). Leptin did not correlate with time post-tx or PTH. Of those with elevated leptin for sex and tanner stage, 42.1% were hypophosphatemic, while only 10.7% with normal leptin were hypophosphatemic (p=0.01). Mean phosphorus of obese (3.1±0.13) was lower than non-obese (4.02±0.14 mg/dl), p=0.001.

Conclusions: Leptin is a biomarker for obesity and MS in pediatric kidney tx recipients. Elevated leptin levels are associated with hyposphosphatemia but not with PTH levels in this population. Further studies are needed to determine if the stimulation of phosphaturic hormone FGF23 by leptin may play a role in this phenomenon.

SA-PO689

Long Term Outcomes of Drug Eluting and Bare Metal Stents as Primary Intervention in Transplant Renal Artery Stenosis Chelsea Estrada, Mersema Abate, Anil John Mani, Frank Darras, Edward P. Nord. *Nephrology, Transplantation, Stony Brook Medicine, Stony Book, NY.*

Background: Transplant renal artery stenosis (TRAS) is a common vascular complication of renal transplantation whose optimal management is uncertain. Little data exists regarding the use of drug eluting stents (DES) in this population.

Methods: This is a prospective study of the targeted deployment of DES for renal artery diameter < 5 mm and bare metal stent (BMS) for renal artery diameter >5 mm as primary treatment of TRAS. The use of DES in small renal arteries is based on prior reports showing restenosis rates up to 50% when BMS are used. Doppler Ultrasound was initially obtained based suspicion for TRAS (rising creatinine or poorly-controlled BP). Positive studies were confirmed by angiogram, and if stenotic, DES and BMS placed accordingly. Patients were started on clopidogrel post-procedure (6 weeks for BMS, 1 year for DES) and lifelong aspirin.

Results: From 3/2008 to 11/2013, 47 patients underwent endovascular intervention (EVI) for TRAS; 23 received DES, 22 received BMS and 2 received both stent types. The primary outcome, ie: resolution of stenosis was achieved in all cases. Secondary outcomes, ie: improvement in BP control and serum creatinine, were evaluated immediately and over time. Patients were followed for 32 ± 21 months, range 3.1-78. At time of EVI, mean serum creatinine was 2.9 ± 1.5 mg/dL, increased from post-transplant nadir of 1.9 ± 0.7 mg/dL, which declined to 2.1 ± 0.9 mg/dL 1 month post procedure and remains 2.1 ± 0.9 mg/dL at last follow up. Mean SBP decreased from 157 ± 19.5 mmHg at EVI to 139 ± 16 mmHg currently, on the same or fewer medications. Eight patients progressed to ESRD and 2 lost to follow-up. Complications were inability to retrieve stent (1), distal edge dissection (1), groin hematoma (2), and transient contrast nephropathy (6). In-stent restenosis occurred in 3 of the DES and in 2 of the BMS groups.

Conclusions: Targeted EVI as primary intervention for TRAS, with DES for vessel < 5mm and BMS for vessel > 5mm, is an effective strategy for blood pressure management and renal function preservation. Use of a DES may offer a lower incidence of in-stent restenosis in renal arteries <5mm.

SA-PO690

Clinical Significance of Pre-Existing Microcalcification in the Iliac Artery in Renal Transplant Recipients Hyeon Seok Hwang,[1] Hye Eun Yoon,[2] Se Young Kim,[1] Suk Young Kim,[1] Chul Woo Yang.[2] *[1]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Daejeon, Korea; [2]Div of Nephrology, Dept of Internal Medicine, The Catholic Univ of Korea, Seoul, Korea; [3]Convergent Research Consortium for Immunologic Disease, Seoul St. Mary's Hospital, Seoul, Korea.*

Background: The clinical significance of pre-existing microcalcificationin the iliac artery is undetermined in renal transplant recipients.

Methods: We obtained iliac artery segments from 90 transplant recipients at the time of renal transplantation and performed von Kossa staining for microcalcification. The clinical significance of intimal microcalcification was evaluated with allograft survival rate, rate of graft function decline and composite of any cardiovascular event or patient death. Expression of fetuin-A and C-reactive protein (CRP), key regulators of calcification,was also investigated in the iliac artery.

Results: Intimal microcalcification was positive in 48 (53.3%) patients, and its intensity was correlated positively with intimal CRP intensity (P = 0.019). Allograft survival in patients positive for intimal microcalcification was lower than patients who werenegative (P = 0.017). The patients with positivity for both intimal microcalcification and fetuin-A showed lower allograft survival rate than patients with intimal microalcification positivity alone (P = 0.012). The rate of renal graft function decline was significantly steeper in patients positive for intimal microcalcification than in patients who were negative (P = 0.036). In multivariate analysis, positivity for both intimal microcalcification and fetuin-A was an independent predictor for renal graft function decline (β = -10.21; P = 0.011). The intimal microcalcification was not associated with composite-event free survival.

Conclusions: Pre-existing intimal microcalcification in the iliac artery predicts a lower allograft survival rate and rapid decline of allograft function. Positivity of fetuin-A with intimal microcalcificationfurther reduces allograft survival rate and an independent predictor for renal graft function decline.

SA-PO691

Effectiveness of Trunk Fat as a Predictor of Renal Graft Function Using Artificial Neural Networks Catherine Kelley Pantik,[1] Ravi Behara,[2] Donna K. Hathaway,[1] Ann Cashion,[3] Vinaya Rao.[1,4] *[1]College of Nursing, Univ of Tennessee Health Science Center, Memphis, TN; [2]College of Business, Florida Atlantic Univ, Boca Raton, FL; [3]National Inst of Nursing Research, National Insts of Health, Bethesda, MD; [4]Renal Transplant, Methodist Univ Transplant Inst, Memphis, TN.*

Background: There are conflicting reports on the effect of obesity on graft survival. Body Mass Index (BMI) is the most common method used to assess obesity in clinical settings and BMI > 35 is used as exclusion criteria to transplantation. Abdominal fat is highly correlated with worsening renal function. The purpose of this pilot study was to evaluate the usefulness of BMI and percentage of trunk fat in predicting renal graft function at 1year post transplant using artificial neural network (ANN) modeling.

Methods: A hidden layer feed-forward ANN with unsupervised training was developed. The structure was used to develop two models to represent obesity: one model used BMI (N=81) and the other trunk fat percentage at baseline (N=65). Both models were trained with 2/3 of the sample and tested with 1/3 of the sample. The output node represented worsening graft function (serum creatinine (Scr) ≥ 1.5) at 1year post transplant.

Results: When BMI was used as the predictor variable, overall accuracy of prediction was 64%, predicting SCr < 1.5 at 1 year was 57% and predicting SCr ≥ 1.5 was 71%. Trunk fat percentage as a predictor variable resulted in 73% overall accuracy of prediction, predicting SCr < 1.5 was 67%, while predicting SCr ≥ 1.5 at was 80%.

Conclusions: Abdominal fat mass at time of transplant is an important clinical concern. There is a paucity of clinical research on the use of trunk fat v BMI in predicting renal graft function. This pilot study demonstrates trunk fat percentage may be a better predictor of graft function at 1year than BMI. Additional analysis with larger data sets is needed to build model fidelity. The study provides the foundation for additional work using ANN to examine the importance of body mass distribution in predicting long-term renal graft outcomes.

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SA-PO692

Does Intragraft Gene Expression Vary with the Type of Induction Therapy? Maria Ajaimy,[1] Pilib O Broin,[2] Michelle L. Lubetzky,[1] Yi Bao,[1] Aaron Golden,[2] Enver Akalin.[1] *[1]Einstein/Montefiore Transplant Center; [2]Computational Genomics Facility, Albert/Einstein College of Medicine, Bronx, NY.*

Background: Molecular evidence of allograft injury might precede histopathological findings. We aimed to investigate if the type of induction therapy has an effect on intragraft gene expression profiles of early transplant kidney biopsies.

Methods: We identified 34 near normal clinically indicated biopsy samples taken within 6 months of transplant for gene expression profiling. All patients received induction treatment with either anti-thymocyte globulin, if class I or II panel reactive antibody (PRA) levels > 20% (n=17), or basiliximab, (n=17). The gene expression profiles were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: When comparing the two groups, there was no difference in patients demographics between the 2 groups. Although, the mean cold ischemia time was longer (24.6 ±14 versus 18.8 ±11.5 hours, p=0.26) and the delayed graft function was more prevalent (76% versus 50%, p=0.08) in the basiliximab group, the difference was not statistically significant. As expected, median class I 70% (18,100) and II 35% (2,100) PRA levels were higher in anti-thymocyte group compared to basiliximab group class I 0%(0,13) and class II 0%(0,34), respectively. There were no differences in Banff acute allograft injury scores including microvascular inflammation (g, ptc),interstitial inflammation, and chronic injury scores (cg, ct, ci, cv and mm) between the 2 groups. There were no differentially expressed genes between the two groups (Both False discovery rate p<0.05 and fold change >2). Pathogenesis based transcripts showed no difference in the expression of intragraft gene transcripts associated with Cytotoxic T-cells, Regulatory T cells, B cells, Endothelial cells and Interferon-gamma and rejection induced transcripts between the 2 groups. There was also no difference in 12 months serum creatinine levels between the 2 groups.

Conclusions: Although, anti-thymocyte globulin treated patients are immunologically higher risk compared to basiliximab group, there was no difference in intragraft gene expression profiles or Banff allograft injury scores of early kidney biopsies.

SA-PO693

Superior Patient and Graft Survival after Kidney Transplantation in Patients with IgA Nephropathy Aditya Kadiyala, Anna Mathew, Cristina P. Sison, Mala Sachdeva, Steven Fishbane, Kenar D. Jhaveri. *Nephrology, Hofstra-NSLIJ School of Medicine, Great Neck, NY.*

Background: IgA Nephropathy (IgAN) is a common glomerular disease leading to End Stage Renal Disease requiring kidney transplantation. To date, there have been no large studies in the U.S. looking at post-transplant outcomes in patients with IgAN who receive a Kidney transplant.

Methods: Using UNOS/OPTN data, patients > 18 years with first transplant (1/1/1999 to 12/31/2008), and BMI >15 or < 45 were analyzed. 5-year patient survival (PS) and death censored graft survival (DCGS) were compared between patients with IgAN (IgA) and other kidney transplant recipients (non-IgA). Survival curves for PS and DCGS were plotted using Kaplan Meier method. Unadjusted hazard ratios (UHR) and adjusted Hazard ratios (AHR) were calculated using Cox regression analysis. Subgroups of diabetes (DM) and donor type (DT) were also analyzed.

Results: The IgA group (n= 4005) was younger, had more males and Asians compared to the non-IgA group (n=90845). Live donation was more frequent in IgA (47.1% versus 30.2%); DM was more prevalent in non-IgA (31.9% versus 3.9%). Hypertension and BMI were comparable in both groups. IgA had higher unadjusted 5-year DCGS (96.3% versus 87.3%, p<0.001) and PS (88% versus 83.3%, p<0.001). UHR for DCGS (0.77, 95% CI 0.72, 0.83) and PS (0.30, 95% CI 0.27-0.34) were significantly lower in the IgA group (p<0.0001). In the adjusted model for PS there was effect modification by DT. AHR for PS stratified by DT showed significantly lower risk of death in deceased donor IgA (0.54, 95% CI 0.47 to 0.62) and living donor IgA (0.39, 95% CI 0.31 to 0.49) (p<0.05) compared to the non-IgA group. In the adjusted model for DCGS, there was no effect modification by DM or DT. AHR for DCGS was also significantly lower (0.90, 95% CI 0.83 to 0.97) in IgA (p< 0.0075) compared to non-IgA.

Conclusions: This is the first UNOS-based U.S. study of post-transplant outcomes in IgAN. Our study shows that patients with IgAN who receive their first kidney transplant have superior adjusted and unadjusted patient and graft survival compared to other first-time kidney transplant recipients.

SA-PO694

Identification of Risk Factors for BK Infection – A Paired Kidney Analysis

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Background: BK nephropathy remains an important cause of premature renal allograft failure. It is thought to be primarily related to the intensity of immunosuppression, while the importance of donor and recipient factors remains uncertain.

Methods: Using data from Scientific Registry of Transplant Recipients (SRTR) between 2004 – 2010, we performed a paired kidney analysis in which both kidneys were transplanted from the same deceased donor (n = 21,575) to identify 1) concordance of BK infection – cases where both kidneys were infected, 2) recipient factors for BK infection – using discordant pairs where only one kidney was infected.

Results: Among the 21,575 pairs, 1975 pairs (9%) had discordant infection, while 174 (1%) had concordant infection. Concordant infection was 5-fold higher than would be expected at random. In a multivariate conditional logistic regression model including discordant pairs, the following factors were associated with BK infection: Age < 18 (OR 1.31; 95% CI 1.01-1.54), male gender (OR 1.53; 95% CI 1.32-1.77), HLA MM ≥ 4 (OR 1.80; 95% CI 1.28-2.53), acute rejection (OR 2.75; 95% CI 2.23-3.38), use of T cell depleting antibody (OR 1.22; 95% CI 1.02-1.47). Among recipient pairs, concordant pairs were more likely to have both recipients treated with lymphocyte-depleting antibodies (p<0.001) or tacrolimus (p<0.001), have acute rejection (p<0.001), HLA MM ≥ 4 (p<0.001) and to be from the same center (p=0.001).

Conclusions: We conclude that concordant infection among mate kidneys is higher than would be expected by random chance, suggesting the importance of donor factors. However, similar recipient and treatment factors, as well as the finding that many co-infected kidneys were transplanted at the same center suggest that risk of BK infection may be related primarily to treatment factors, rather than donor factors.

SA-PO695

The Impact of Post-Transplant Urinary Tract Infections on Graft Outcomes in Kidney Transplant Recipients

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Background: Urinary tract infections (UTI) are the most common form of infectious complication in kidney transplant recipients (KTR). However, the impact of UTI on graft outcomes remains poorly understood.

Methods: This retrospective cohort study examined 1,111 KTR from 1 Jan 2000 to 31 Dec 2010 (followed until 31 Dec 2011). A UTI was defined by a positive urine culture (>10^5 CFU/mL) and a urine dipstick positive for leukocytes dated within two days of the positive culture date. Multiple linear regression models were fitted to assess estimated glomerular filtration rate (eGFR) at 1- and 5-years post-transplant. Multivariable Cox proportional hazards models were fitted to examine the association of UTI with first acute rejection, total graft failure, death-censored graft failure, and death with graft function.

Results: There were a total of 196 first acute rejections, 99 graft losses, 90 deaths with graft function, and 189 total graft failures accrued over 5951.8 patient-years of follow-up in this study cohort. In a multiple linear regression model, having at least one UTI episode in the first year post-transplant was significantly associated with a decreased eGFR at 1-year (*b*-2.96 [95% CI: -5.66, -0.26] ml/min) and 5 years (*b* -4.76 [95% CI: -8.47, -1.05]) of follow-up. In multivariable Cox proportional hazards models, the occurrence of a UTI was significantly associated with an elevated relative hazard of total graft failure (HR 1.85 [95% CI: 1.32, 2.60]), death-censored graft failure (HR 1.64 [95% CI: 1.01, 2.67]) and death with graft function (HR 2.14 [95% CI: 1.33, 3.45]). There was not significant association of UTI with time to first acute rejection (HR 1.13 [95% CI: 0.78, 1.64]).

Conclusions: Our results suggest that post-transplant UTI increase the risk of developing adverse outcomes in kidney transplant recipients. The mechanisms by which UTI negatively impact graft and patient longevity, and the prognostic implications of recurrent UTI events, require further study.

SA-PO696

Risk Factors for Urosepsis After Renal Transplantation and Its Impact on the Renal Allograft Function

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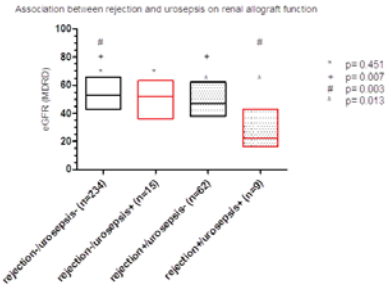
Background: Along with urinary tract infection (UTI), bacteraemia may occur. This is known as urosepsis. The aim of this study is to evaluate the risk factors of urosepsis and its impact on the renal allograft function.

Methods: Retrospective cohort study with adult renal allograft recipient, who had 1 year follow-up after transplantation. Bacteriuria was defined as positive urine culture. Urosepsis was defined as bacteriuria and positive blood culture with the same pathogen. Asymptomatic bacteriuria (ASB) was defined as bacteriuria without symptoms of the urinary tract, fever/malaise. Bacteriuria with these symptoms was defined as UTI. We compared the group with urosepsis to the group without any bacteriuria at all.

Results: In total 431 recipients were analysed. Within 1 year after transplantation; 24 (5.6%) had urosepsis and 298 (69.1%) did not have any bacteriuria at all. The rest had UTI 61(14.2%) or only ASB 48(11.1%). Multivariable analysis showed that only an indwelling urological catheter was associated with the development of urosepsis with OR 13.40 (95%CI=4.43-40.50,p<0.001). Multivariable linear analysis of the renal allograft function at 1 year after transplantation showed significant interaction between urosepsis and acute rejection. In the group without rejection, urosepsis did not impair renal allograft function 1 year after transplantation. However, having experienced both, urosepsis and acute rejection significantly resulted in even greater impairment of the renal allograft function than rejection alone.



Conclusions: The presence of a urological catheter is the greatest risk factor for urosepsis. Having experienced both, acute rejection and urosepsis, is strongly associated with impaired renal allograft function, but urosepsis alone is not.

SA-PO697

Herculink Elite® a Cobalt Chromium Alloy Bare Metal Stent Appears to Be Effective for the Treatment of Transplant Renal Artery Stenosis

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Background: Management of transplant renal artery stenosis (TRAS) remains controversial. There is paucity of data regarding timing of intervention, surgical versus endovascular repair (EVR), use of bare metal stent (BMS) versus drug eluting stent (DES), and anticoagulation use post repair.

Methods: Retrospective analysis of 13 consecutive renal transplant recipients(RTR) with a diagnosis of TRAS from 2005-13 in our center

Variables	N=13
Demographic	
Mean age (range) — yr	61 (34-79)
Male sex — no./total no. (%)	10/13 (76.9)
Black — no./total no. (%)	4/13(30.7)
Clinical	
Smoking status — no./total no. (%)	
Current smoker	2/13 (15.3)
Ex smokers	5/13 (38.5)
Coexisting conditions — no./total no. (%)	
Hypertension	13/13 (100)
On ACEI/ARB before diagnosis of RAS	6/13 (46.2)
Diabetes	4/13 (30.7)
Coronary heart disease	5/13 (38.5)
PVD	1/13 (7.7)
Renal	
Baseline serum creatinine	
Mean (range) — mg/dl	1.5 (1.1-2.7)
Level — no./total no. (%)	
< 1.5 mg/dl	8/13 (61.5)
1.5-2.5 mg/dl	4/13 (30.7)
> 2.5 mg/dl	1/13 (7.7)
Cause of ESRD — no./total no. (%)	
Hypertension	4/13 (30.7)
Diabetes Mellitus	3/13 (23.1)
PKD	2/13 (15.4)
Combined HTN/DM	1 /13(7.7)
Alport syndrome	1/13 (7.7)
Chronic Pyelonephritis	1/13 (7.7)
Reflux Nephropathy	1/13 (7.7)
Transplant	
Type — no./total no. (%)	
DDKT	12/13 (92.3)
LKT	1/13 (7.7)
CMV Serostatus Donor/Recepient	
Type — no./total no. (%)	
Positive/Positive	7/13 (53.8)
Positive/Negative	3/13 (23.1)
Negative/Positive	3/13 (23.1)
Negative/Negative	0
Post Transplant Renal Artery Stenosis(TRAS)	
Median time to diagnosis — months	4.5
Mean serum creatinine at diagnosis (range)	3.9 (1.2-12)
Level — no./total no. (%)	
<1.5 mg/dl	3/13 (23.1)
1.5-2.5 mg/dl	4/13 (30.7)
>2.5 mg/dl	6/13 (46.2)
Endovascular Repair(EVR) of TRAS	
Type — no./total no. (%)	
Bare Metal Stent (Herculink-Elite stent)	11/13 (84.6)
Drug Eluting Stent	2/13 (15.4)
Mean patient follow up post EVR (range) — in months	15.6 (1-27)
Incidence of Herculink-Elite stent restenosis	2/11 (18.2)
Mean time observed for developing HES restenosis (range) — months	6 (3-9)
Post Endovascular Repair	
No. of cases returning to baseline creatinine — no./total no. (%)	12/13 (92.3)
Mean no of months taken for serum creatinine returning to baseline value (range)	4.7 (1-6)
Success post EVR — no./total no. (%)	12/13 (92.3)

Results: Of 13 RTR, 69% where white and 92% received a deceased donor transplant. Mean nadir serum creatinine (SCr) was 1.5mg/dl. Mean time to presentation was 4.5 months post transplantation, with a mean SCr of 3.9mg/dl (Figure 1). 11 RTR received a BMS and 2 a DES. Endovascular repair was immediately successful in 12/13 RTR and there were no procedure related complications. 2 patients with a Herculink Elite® stent required repeat EVR with a DES due to in-stent restenosis (18.2%). All RTR received clopidogrel for at least one month if BMS was used, and nine months for DES. Lastly, they were all placed on lifelong anticoagulation with aspirin (12) or clopidogrel (1).

Conclusions: Herculink Elite® (Abbott Vascular, Santa Clara, CA) BMS are FDA approved for use in TRAS. The cobalt chromium composition allows for thinner stent struts to cause less in-stent restenosis. One advantage of using a BMS is the need for clopidogrel for one month only, which was helpful in 2 of our patients who subsequently required a renal biopsy for unrelated reasons. Endovascular repair is a safe and effective treatment option for TRAS. In our series BMS appear to be an effective option albeit with a risk of in-stent restenosis. Larger controlled studies are required to establish the standard of care for management of transplant renal artery stenosis.

SA-PO698

Evaluation of Long Term Cardiovascular Outcomes in Renal Transplant (RT) Patients Correlated with the Use of Cardioprotective Medications

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Background: Cardiovascular (CV) disease is a leading cause of mortality among RT recipients. Despite that, the use of cardioprotective drugs remains suboptimal. Dawson et al. (2010) evaluated the use of optimal cardioprotective regimen consisting of aspirin (ASA), ACE-I or ARB, and statin in 130 patients. Only 18% of high risk RT patients as defined by prior cardiovascular disease, diabetes or a Framingham risk score exceeding 20%, received optimal treatment. This study evaluates long term CV outcomes of high risk and standard risk RT patients and the correlation of events to the use of cardioprotective drugs.

Methods: 202 renal transplant recipients who transplanted at Houston Methodist Hospital between 9/2005 and 9/2008 were included. Baseline characteristics were recorded, and medication data was obtained at one year after transplant. A retrospective chart review was conducted. Primary end point was a composite of CV death, PCI or CABG, PVD requiring stent or amputation, MI, CHF exacerbation, and TIA/stroke. Chi Square and Students T-Test were used for statistical analysis.

Results: Of the 202 RT patients evaluated, 110 (54.5%) were categorized to standard risk (SR) and 92 (45.5%) to high risk (HR). Optimal treatment was rendered to 14.46% of HR patients and 9.9% of SR patients (p=0.48). Over the course of follow up (average 6.3 years) 7 patients (6%) in SR group reached the primary endpoint (1.1% risk per patient year) compared to 33 patients (35.9%) in the HR group (9.8% risk per patient year) (p<0.000). In the HR group, 41.67% of optimally treated patients had events versus 35% receiving suboptimal treatment (p=0.27). Further analysis of individual drugs also showed no treatment benefit; statin: 34.8% versus 36.1% (p=0.9), ASA: 40.5% versus 28.9% (p=0.28), ACE-I/ARB: 33% versus 37.5% (p=0.7).

Conclusions: Treatment of high risk RT patients with statin, ASA, ACE-I or ARB or the combination of all three did not alter outcomes. At the present time empiric treatment of standard risk patients with cardioprotective drugs is probably not warranted given the low event rate and the lack of effectiveness in the high risk group.

SA-PO699

Renal Transplantation May Improve Baroreflex Function in Patients with Diabetes Mellitus

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Background: This study was performed to assess the effect of renal transplantation (TX) and of diabetes mellitus (DM) duration on baroreflex function in DM patients with end-stage renal disease.

Methods: Continuous beat-to-beat intervals (IBI) and systolic blood pressure (SBP) were monitored in age-matched controls (C, n=34)), in DM patients on chronic hemodialysis (HD-DM, n=28)) and after renal transplantation (TX-DM), with DM of shorter (≤20 y, n=9) or longer (>20y, n=12) duration.

Results: Mean SBP, IBI variability and baroreflex indices in the low (LF) and high (HF) frequency ranges and slope (median and interquartile ranges) were:

	C	HD-DM	p (vs. C)	TX-DM (≤20y	p (vs. HD-DM)	TX-DM (>20y	p (vs. HD-DM)
Mean SBP (mmHg)	126 (20)	143(37)	0.040	139(22)	NS	120 (36)	NS
sd IBI (ms)	33 (15)	15(11)	0.001	20(16)	NS	11(5)[c]	0.049
%BEI	11.8(14.7)	0.8(3.6)	0.001	1.9 (5.4)	NS	0.2 (0.6)[a]	0.003
Slope (ms/mmHg)	6.53 (4.06)	4.58 (2.68)	0.001	7.07 (6.28)	0.022	3.12 (2.75)[b]	NS
LF IBI (ms^2/Hz)	2327 (3010)	177(756)	0.001	579 (1102)	0.030	93 (211)[a]	NS
HF IBI (ms^2/Hz)	222(243)	84(74)	0.001	78(104)	NS	33(15)[c]	0.001
LF IBI/HF IBI	5.85(3.80)	1.20(2.68)	0.001	4.00 (4.30)	0.004	1.45 (2.93)[c]	NS
LFα (ms/mmHg)	5.05(3.30)	2.53 (2.22)	0.001	4.09 (2.71)	0.028	1.82 (2.33)[b]	NS
HFα (ms/mmHg)	6.12 (5.83)	3.57 (2.25)	0.001	4.98(4.52)	NS	2.40 (1.66)	NS

sdIBI: standard deviation of normal IBI; %BEI: the ratio of SBP ramps followed by ramps in IBI ; Slope: the slope of regression line between IBI and SBP for at least 3 beats; α: the square root of the ratio of average power spectral density of IBI and SBP. DM≤20y vs.>20y,p:[a]0.001;[b]<0.008;[c]<0.05.

In a multiple regression model, baroreflex and variability indices were inversely correlated with DM duration and age and directly correlated with the TX vintage.

Conclusions: 1.Baroreflex indices were significantly impaired in HD-DM; 2.In patients with DM of shorter duration TX was associated with improved heart rate variability and baroreflex function; 3.In contrast, these measurements further deteriorated in those with DM of longer duration. The prognostic value of these findings remains to be determined.

SA-PO700

Predictors for Renal Function Recovery after Orthotopic Liver Transplantation Maria C.C. Andreoli, Nadia K. Guimaraes, Adriano Luiz Ammirati, Thais Nemoto Matsui, Fabiana Dias Carneiro, Ana C.M.S. Ramos, Jose Ben-Hur Ferraz-Neto, Marcio Dias Almeida, Marcelino Souza Durao, Marcelo Costa Batista, Júlio Martins Monte, Virgilio Gonçalves Pereira, Oscar Santos, Bento C. Santos. *Centro de Diálise Einstein, Hospital Israelita Albert Einstein, Sao Paulo - SP, Brazil.*

Background: Renal dysfunction frequently occurs in pre and perioperative period in OLT and in many cases it requires renal replacement therapy (RRT). The rate and timing of renal function recovery (RFR) after OLT are crucial information for transplant program management. We evaluated a sample of stable post-intensive care hemodialysis (HD) patients from a group of 908 adults who were submitted to OLT. We studied the average time of RFR and risk factors for RRT after 90 days post-OLT.

Methods: The Cox proportional hazards model was used to identify factors associated to the relative risk of remaining or not in HD ≥90 days post-OLT.

Results: We analyzed data of 155 patients, 53 (45–60) yo, 64% male, 28% pre-OLT diabetes mellitus, 21% pre-OLT hypertension, 40% virus-related disease, MELD 27 (22-35), 32% creatinine (SCr) at time of listing >1.5mg/dL or on HD, 50% SCr at time of OLT >1.5mg/dL or on HD, 17% acute re-OLT, 18% hepatocellular carcinoma, 14% pre-OLT CVVHDF, 25% pre-OLT intermittent HD, 41% post-OLT CVVHDF; intraoperative transfusion: 2 (0-3)U packet red blood cells, 0 (0-5)U, fresh-frozen plasma (FFP), 22% cryoprecipitate, 30% platelet; 7 (4-14) days in intensive care unit. In a period <90d post-OLT, 118 (76%) patients were removed from RRT and 16 (10%) patients died on HD. During a 1-year follow-up period, a total of 129 (83%) patients were removed from RRT, 19 (12%) patients died on HD, 2 (1%) patients subsequently received a kidney transplant and only 5 (3%) patients were on HD. The median of RFR time was 33 (95%CI 27-39) days. In the multivariate analysis, fulminant hepatic failure (p<0.001), no pre-OLT hypertension (p=0.016), less intraoperative FFP transfusion (p=0.019) and no pre-OLT intermittent HD (p=0.032) were predictors of out of need RRT ≥90d after OLT.

Conclusions: Therefore, a high proportion of OLT patients improves renal function after OLT and pre and intraoperative factors have impact on the probability of this recovery.

SA-PO701

Severe Neutropenia in Children after Renal Transplantation: Incidence, Course and Treatment with Granulocyte Colony Stimulating Factor Rachel Becker-Cohen, Efrat Ben Shalom, Choni Rinat, Sofia Feinstein, Michael Geylis, Yaacov Frishberg. *Shaare Zedek Medical Center, Jerusalem, Israel.*

Background: Infections are an important cause of morbidity and mortality in solid organ transplant recipients. Neutrophils play a crucial role in the initial host defense against bacterial pathogens. Neutropenia is not uncommon after renal transplantation in adults; however, there are scarce published data in children. We conducted a historical cohort study to evaluate the incidence, clinical course and management of severe neutropenia after renal transplantation in children.

Methods: In a single center study we collected clinical and laboratory data on all children (<19 years) who underwent renal transplantation in 2005-2013. All post transplantation blood counts were reviewed, the lowest absolute neutrophil count recorded and correlated with clinical information and laboratory findings.

Results: Of 68 children, 31 (45.6%) had neutropenia <1000/microliter. Fifteen (22%) had severe neutropenia (< 500/ microliter), 2-12 months (mean 4.4) after renal transplantation. Work up for viral infection or malignancy was performed. Initial management included dose decrease/ discontinuation of mycophenolate/ azathioprine, co-trimoxazole and valgancyclovir. Bone marrow aspiration in 4 children revealed normal marrow cellularity in all cases, with myelocyte maturational arrest in 2. Seven children (10.4%) were treated with G-CSF (5 mcg/kg/day), with excellent response in all and no adverse effects. Six children had fever during neutropenia, and were treated with antibiotics. Anti-metabolite was resumed in all patients unless contraindicated, recurrence of neutropenia was seen in 4 patients. Graft function was preserved during and after resolution of neutropenia.

Conclusions: Post-transplant neutropenia in children is common, and mostly occurs in the first few months. Its etiology is probably primarily a result of the combination of immunosuppressive agents and prophylactic treatment of infections in the early post-transplant period. Decreasing immunosuppressive or antimicrobial medications carries the risk of acute rejection or infection. Off –label treatment with G-CSF may present a safe and effective alternative.

Funding: Clinical Revenue Support

SA-PO702

Thrombotic Microangiopathy after Kidney Transplantation: A Case Series Shuang Ying Bao, Surya V. Seshan, Steven Salvatore, Manikkam Suthanthiran, Thangamani Muthukumar. *Weill Cornell Medical College, New York, NY.*

Background: Thrombotic microangiopathy (TMA) in the allograft is a rare but devastating complication after kidney transplantation.

Methods: We reviewed 25 consecutive cases of TMA in the kidney allograft diagnosed at our institution between 2000-2013. We collected their demographic, laboratory and histopathological characteristics as well as outcome. Allograft failure was defined as return to dialysis or a repeat transplant. Follow up time was censored as of 12/31/2013.

Results: The mean (±SD) age was 50±12 years. There were 18 (72%) women, 12 (48%) African-American recipients (48%), and 14 (56%) recipients of deceased donor grafts. The cause of end stage kidney disease was diabetes (20%), chronic glomerulonephritis (16%), hypertension (12%), lupus nephritis (8%), hemolytic uremic syndrome (4%), and others/unknown (40%). Except one, all patients received Thymoglobulin induction and tacrolimus. Patients were diagnosed with TMA at a median of 11 days (range: 4-2030 days) from transplant (Figure).

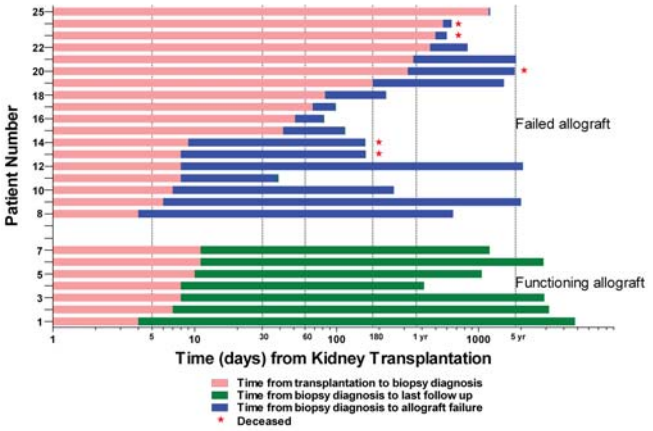


Figure: Time course for the development and outcome of TMA

TMA was renal-limited in 9 of the 16 (56%) patients in whom laboratory data related to hemolysis were available. Concomitant acute rejection was present 16 (64%). Acute antibody mediated rejection was the common (75%) type of acute rejection. Following biopsy diagnosis of TMA, 19 (76%) received pulse steroids, 17 (68%) received plasmapheresis, 13 (52%) received intravenous immunoglobulin and 8 (32%) received rituximab. Tacrolimus was stopped in 13; 5 of these 13 (39%) were started on rapamycin and 6 (46%) were started on cyclosporine. Eighteen patients (72%) had allograft failure during the study period. The median follow up was 8.3 year. The median allograft survival after the diagnosis of TMA was 94 weeks.

Conclusions: Although TMA after kidney transplant portends a poor prognosis, early diagnosis and aggressive therapy appear to improve outcome.

SA-PO703

The Usefulness of Tacrolimus without Basiliximab in Well Matched Living Renal Transplantation in Korea Chung Hee Baek, Joon-Seok Kim, Hyosang Kim, Su-Kil Park. *Dept of Internal Medicine, Div of Nephrology, Asan Medical Center, Seoul, Republic of Korea.*

Background: At the initial investigation, basiliximab was the very effective induction agent with combination of cyclosporine based immunosuppression. From those experiences, basiliximab has been a routine induction therapeutic agent even for well-matched living renal transplantation with tacrolimus based immunosuppression in Korea. As tacrolimus is a different drug from cyclosporine, we studied the usefulness of tacrolimus based immunosuppression without basiliximab in well matched living renal transplantation.

Methods: We prospectively evaluated 22 patients who underwent 1-3 HLA mismatched living donor renal transplants without basiliximab induction therapy between April 2012 and March 2014(group1). They were ABO compatible and T-flow negative transplants and we followed them until April 2014. We used tacrolimus-based triple therapy(tacrolimus, mycophenolate mofetil and methylprednisolone) as maintenance immunosuppressants. Age and sex matched 44 patients who underwent 1-3 HLA mismatched living donor renal transplants with basiliximab induction therapy in the same periods served as a control group(group2).

Results: There was 1 case(4.5%) of infection in group 1 and 9 cases(20.5%) of infection in group 2. Especially, CMV and BKV virus infection was only occurred in group2. Urinary tract infection occurred in 1 patient in group 1, and 2 patients in group2. In addition, 2 cases of pneumonia and 1 case of sepsis were reported only in group2. There was no PCP infection in both groups. Total incidence of infection was not significantly different(p=0.146), but there was a trend of lower incidence of infection in group1. Kidney biopsy was not performed in group1. However, 10 kidney biopsies were performed in group 2, and 1 acute T-cell mediated rejection was reported(p=0.549). Other biopsy results were acute tubular injuries. No mortality or malignancy was reported.

Conclusions: A tacrolimus-based triple drug maintenance immunosuppression without basiliximab might be optimal in well matched living renal transplantation in Korea.

SA-PO704

Correlation of Serum Visfatin and Clinical Outcome in Renal Transplant Recipients Kuo-Hsiung Shu,[1,2] Ming-Ju Wu,[1,2] Cheng-Hsu Chen,[1,3] Chi-Hung Cheng,[1,2] Tung-Min Yu.[1,3] *[1]Dept of Medicine, Div. of Nephrology, Taichung Veterans General Hospital, Taichung, Taiwan; [2]School of Medicine, Chung-Shan Medical Univ, Taichung, Taiwan; [3]School of Medicine, China Medical Univ, Taichung, Taiwan.*

Background: Most of the late graft loss in renal transplant (RTx) recipients can be attributed to chronic antibody-mediated allograft injury elicited by B cells directly against donor-specific antigens. Visfatin is a pre-B cell colony enhancing factor. We hypothesize that visfatin may play a role in the augmentation of B cell colony and facilitate antibody-mediated rejection. Therefore, the aim of the study was to elucidate if serum levels of visfatin might be of clinical relevance in predicting renal allograft outcome.

Methods: RTx recipients were randomly selected for the study. Fasting blood samples were obtained for the assay of visfatin. The participants were prospectively followed up for 3 years. Graft function was assessed by serial change of serum creatinine. Rejection was diagnosed based on clinical judgment and supported by a graft biopsy. Because the serum visfatin levels varied widely, patients were stratified into 3 groups (tertiles). A Chi-square test or Fisher's exact test were used to compare categorical data, while one-way analysis of variance or a Kruskal-Wallis H test were used to compare numeric data.

Results: A total of 158 patients were recruited for the study. There was no statistically significant difference in terms of demographic data or baseline laboratory data in the 3 groups. At the end of follow-up, 7 cases (4.4%) had graft loss and there was no patient mortality. Two (3.8%) graft loss occurred in tertile 1, 3 (5.6%) in tertile 2 and 2 (3.8%) in tertile 3 (p=1.000). Fifteen cases (9.5%) experienced at least one episode of acute rejection while 22 cases (13.9%) were diagnosed as having chronic rejection. The distribution of acute rejection was 7.7% in tertile 1, 16.7% in tertile 2 and 3.8% in tertile 3 (p=0.083). Chronic rejection occurred in 13.5% of tertile 1, 13.0% of tertile 2 and 15.0% of tertile 3 patients (p=0.931).

Conclusions: We conclude that serum visfatin level was not correlated with either graft failure or patient mortality.

Funding: Government Support - Non-U.S.

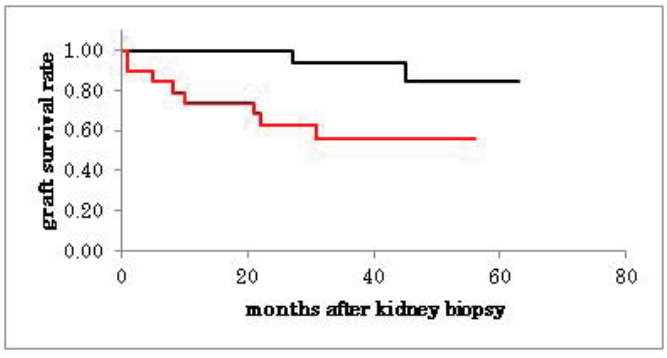
SA-PO705

The Diagnostic and Prognostic Value of Caveolin-1 Immunoreactivity in Peritubular Capirallies in Patients with Chronic Antibody-Mediated Rejection Yasuyuki Nakada, Izumi Yamamoto, Yudo Tanno, Ichiro Ohkido, Keitaro Yokoyama, Hiroyasu Yamamoto, Takashi Yokoo. *Div of Nephrology and Hypertension, Dept of Internal Medicine, The Jikei Univ of Medicine, Tokyo, Japan.*

Background: The issue of the C4d immunoreactivity in peritubular capillaries (PTCs) was its low sensitivity for the diagnosis of Antibody-Mediated Rejection (ABMR). C4d-negative ABMR was defined in the new version of Banff classification(AJT2014;14:272) and they focused on the expression of endothelial-associated transcripts (ENDATs) (AJT2009;9:2312), which is not available in any center. Our previous report showed that Caveolin-1(CAV-1), which is one of the ENDATs, is a distinct feature of chronic ABMR (AJT2008;8:2627). We investigated the diagnostic and prognostic value of CAV-1 immunoreactivity in PTCs in patients with chronic ABMR.

Methods: To examine the diagnostic and prognostic value of CAV-1 immunoreactivity in PTCs, biopsy samples from cases of chronic ABMRwere double-immunostained for CAV-1 and PAL-E; a marker of peritubular capillary. Twenty-one cases of chronic AMR were compared with 20 cases of interstitial fibrosis and tubular atrophy(IF/TA). The receiver operating characteristic curves (ROC) analysis and kaplan–meier method/log-rank test were applied for the diagnostic and prognostic value of CAV-1 immunoreactivity in PTCs, respectively.

Results: C4d immunoreactivity was positive for 61.9% while CAV-1 for 100% in chronic ABMR. Further, the %CAV-1/PAL-E in PTCs were significantly higher in chronic ABMR compared with IF/TA (71±16% versus 36±14% p<0.001). Area under the ROC curves was 0.93 when we used 55% as a cutoff value (sensitivity, 90.5%, specificity, 95%). Kaplan–meier method showed high %CAV-1/PAL-E (> 55%) was associated with high kidney graft failure (log-rank test, p=0.01. Fig).



Conclusions: The CAV-1 immunoreactivity in PTCs could be a diagnostic and prognostic marker for chronic ABMR.

SA-PO706

Omega-3 Fatty Acids and Risk of Graft Failure and Mortality in Renal Transplant Recipients Ilse Pranger,[1] Michel M. Joosten,[1] Sabita Soedamah-Muthu,[3] Gerjan Navis,[1] Rijk O.B. Gans,[1] Frits A.J. Muskiet,[2] Ido Peter Kema,[2] Stephan J.L. Bakker.[1] *[1]Internal Medicine, UMCG; [2]Laboratory Medicine, UMCG; [3]Human Nutrition, Wageningen Univ.*

Background: Omega-3 fatty acids (O3FA) have been shown to protect against acute rejection in renal transplant recipients (RTR). In the general population (GP), intake of O3FA is associated with survival benefit. Whether O3FA are associated with protection against chronic rejection or survival benefit in RTR is unknown. We examined the association of omega-3 fatty acids with graft failure (GF)and mortality in RTR.

Methods: Intake of O3FA (i.e. marine eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) and plant α-linolenic acid (ALA) were assessed by validated food-frequency questionnaire. GF was defined as return to dialysis or re-transplantation. Statistical analyses were performed using Cox-regression.

Results: We included 636 RTR (mean age 53 yrs, 57% male). Median intakes of EPA, DHA and ALA were 40, 51 and 128mg/d resp. During 3 yrs of follow-up, 41 developed GF and 67 died. Intake of O3FA was not associated with GF. In age- and sex adjusted analyses, both EPA and DHA tended to be inversely associated with mortality (HR[95%CI]=0.77[0.52-1.14] and 0.80[0.59-1.10] resp. per 100mg difference). ALA was significantly associated with increased mortality (HR=1.06[1.01-1.11], p=0.02). This association remained after adjustment for potential confounders. The highest tertile of ALA intake (>145mg/d) was at ~2-fold mortality risk (1.90[1.07-3.37]) compared to the lowest tertile (<114mg/d).

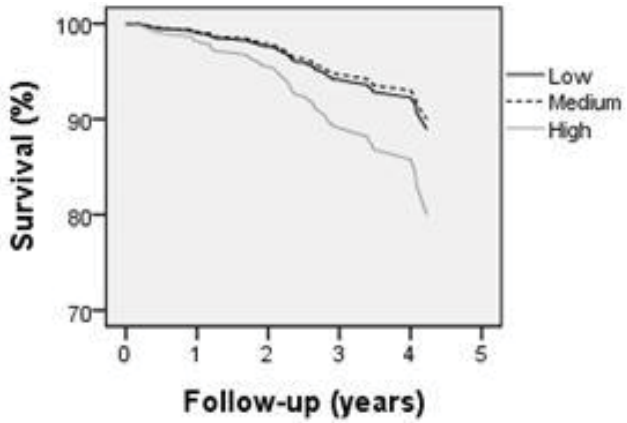


Figure 1: Survival curve for tertiles of ALA intake in RTR.

Conclusions: Intake of EPA and DHA showed trends towards decreased mortality. ALA was associated with increased mortality. The mechanisms of this llatter association might relate to differences in risk factor profile and pathophysiology between RTR and the GP. Further investigation on the long-term effects of O3FA, particularly ALA, in RTR is warranted.

Funding: Government Support - Non-U.S.

SA-PO707

Contrast Induced Nephropathy in Renal Allograft Recipients Yuvraj Sharma, Swapna Katipally, Anthony C. Leonard, Rita R. Alloway, E. Steve Woodle, Charuhas V. Thakar, Bassam G. Abu Jawdeh. *Nephrology, Univ of Cincinnati, Cincinnati, OH.*

Background: Contrast induced nephropathy (CIN) is associated with significant increase in mortality and morbidity in patients with native kidneys; however data is limited regarding its impact in renal transplants (txp). We retrospectively studied the incidence of CIN in renal txp, its risk factors and effect on long term outcome including allograft loss or death.

Methods: 135 renal txp recipients undergoing CT scan or cardiac catheterization (Cath) with iso or low osmolar contrast during 2000-2013 at a single institution were identified. CIN was defined as rise in serum creatinine (SCr) by > 0.3 mg/dl or 25% from baseline within 4 days of contrast exposure. After excluding 85 patients who had no SCr within 4 days of contrast; 76 procedures (Cath: n=31; CT: n=45) in 50 patients were analyzed. Risk factors assessed included demographics, comorbid conditions, type/volume of contrast and calcineurin inhibitor use. Bivariate and multivariate analyses were used to assess the risk of CIN. 53% were pre-treated with fluids and 36% with N-acetylcysteine (NAC).

Results: The sample included 50% males, mean age of 53.3 +/- 15.3 years, and mean SCr of 1.46 +/- 0.88 mg/dl (mean +/- sd). Characteristics included DM (32%), HTN (78%), CHF (7%), Live donor recipients (78%), Deceased donor recipients (22%), on Calcineurin inhibitor (83%). CIN was identified in 10 out of 76 procedures (incidence 13.16%). Incidence of CIN following Cath was 12.9% (4 out of 31) and CT was 13.3% (6 out 45). Significant bivariate predictors of CIN were fluids (P=0.05), lower hemoglobin (P=0.03) and lower albumin (P=0.02). In a multivariable model, CIN was predicted by NAC (P=0.03) and lower hemoglobin (P=0.01). CIN was not associated with Calcineurin inhibitors. During long term follow up CIN did not affect renal function, loss of allograft or death.

Conclusions: CIN is common in transplant recipients; and there is room for quality improvement with regards to careful renal function monitoring post contrast. In our study NAC exposure and lower hemoglobin were associated with CIN, although the sample size is small. Randomized studies of CIN in transplant setting are needed.

SA-PO708

Myocardial Perfusion (MIBI) Scanning Has Significant False Positive Results in Detecting Coronary Artery Disease in Patients with Stage 5 Chronic Kidney Disease Freya Baird, Sumith C. Abeygunasekara, Abdelgalil Abdelrahman Ali. *Broomfield Hospital, Essex, United Kingdom.*

Background: Cardiovascular disease is the leading cause of mortality following renal transplantation in patients with chronic kidney disease (CKD), accounting for 40-55% of all deaths (*Briggs, 2001*). This is attributable to both an ageing cohort, other co-morbidities (e.g. diabetes) and a reduction in other post-transplantation complications, such as infection. MIBI scans have been shown to be highly accurate for the detection of coronary artery disease (CAD), with one study [Narin et al, 2012] calculating a high sensitivity (85%), specificity (71%) and positive predicted value (90%) in non-renal patients. However, it has also been shown (*Ammann et al, 2003*) that hypertension and left ventricular hypertrophy, which are both prevalent complications of CKD, significantly increase the incidence of falsely positive MIBI scans. In this study we aim to evaluate the effectiveness of the current screening technique in identifying CAD in patients with stage 5 CKD.

Methods: From January to December 2012 at Broomfield Hospital, all prospective renal transplant patients (stage 5 CKD) who had a positive MIBI scan and subsequently went on to have a coronary angiogram, were reviewed. The outcomes were compared to that of a cohort of cardiac patients (eGFR >60) with positive MIBI scans who also went on to have coronary angiograms.

Results: Of the 11 CKD patients with a positive MIBI scan, only 2 went on to have a significant coronary angiogram requiring intervention (18.8%). This was proportionally fewer compared to the cardiac patient cohort, in which 11 of the 46 patients with a positive MIBI scan had significant coronary stenosis on angiogram (23.9%). (Chi-square 0.166, p = 0.684).

Conclusions: Despite the small patient numbers, our initial findings suggest that there may be a high false positive rate for MIBI scans in CKD patients. A larger cohort of patients across multiple centers would be needed to investigate this further and determine whether use of this scan represents the most efficient and cost-effective pre-operative work-up, or whether these patients should receive a coronary angiogram as a first line investigation.

SA-PO709

A Prospective Study of Renal Transplant Recipients with Acute Clinical and Subclinical Rejection Shan Shan Chen, Rajil B. Mehta, Riyaj A. Kasekar, Puneet Sood, Christine Wu, Nirav A. Shah, Sundaram Hariharan. *Renal-Electrolyte Div, UPMC, Pittsburgh, PA.*

Background: To study the impact of clinical rejection (CR) and subclinical rejection(SCR) on 1 year renal allograft function and to prospectively study acute and chronic histological changes in renal transplant recipients.

Methods: We prospectively followed all patients (n=81) undergoing kidney transplantation at our center from 1/1/2013 to 5/31/2013 with at least 12 months follow up. Protocol biopsies were performed at 3 and 12months post-transplant. The patients were divided into 4 groups based on 3 month protocol biopsy: those with SCR (group1,n=9), without rejection (group2,n=36), with CR (group3,n=10), and without kidney biopsy (group4,n=26). All rejection patients (n=19) were treated per our center's protocol. Serum creatinine was measured at 3,6 and 12 months. Acute and chronic (Banff) composite scores were calculated for the 3 and 12 month biopsies. Data was analyzed using repeat measures ANOVA, T-test and chi square.

Results: Baseline demographics and other variables were not significantly different in the four groups (all p>0.05). Serum creatinine at 3, 6 and 12 months within patients and between four groups was not statistically different (p=0.07 and 0.10). There was no difference in acute and chronic composite scores between 3 and 12 month biopsies among the four groups (all p>0.05).

			Group 1	Group 2	Group 3	Group 4	p value
Demographics		Mean Age (range, year)	27 (23-81)	51 (24-80)	49.6 (31-72)	53.6 (23-78)	>0.05
		Gender (M/F)	7/2	27/9	4/6	16/10	>0.05
		Race (W/AA/ Other)	9/0/0	27/8/1	6/4/0	24/2/0	>0.05
Renal Function	Serum Creatinine (mg/dl)	3month	1.49	1.39	1.71	1.41	0.3
		6month	1.49	1.40	1.66	1.28	0.11
		12month	1.62	1.39	1.84	1.32	0.01
Banff Classification Histological Parameters	Mean Acute Composite Score (i+t+v+g+c4d)	3month	5.44 (n=9)	1.32 (n=34)	3.2 (n=10)	n=0	
		12month	2.75 (n=5)	2.40 (n=22)	3.66 (n=6)	3 (n=7)	
		p value	0.25	0.06	0.52		
	Mean Chronic Composite Score (ct+cv+ci+cg)	3month	2.77 (n=9)	1.41 (n=34)	2.3 (n=10)	n=0	
		12month	3.2 (n=5)	2.52 (n=22)	2.40 (n=6)	1.71 (n=7)	
		p value	0.78	0.08	0.77		

Conclusions: Renal function and histology score (acute and chronic) did not change from 3 months to 12 months post-transplant in patients with CR and SCR.

SA-PO710

CD45, Vimentin and Periostin as Novel Markers of Graft Outcome in a Cohort of Kidney Transplanted Patients Carlo M. Alfieri,[1] Paola Simonini,[1] Anna Regalia,[1] Masami Ikehata,[2] Deborah Mattinzoli,[2] Francesca Zanoni,[1] Christos Chatziantoniou,[3] Maria Pia Rastaldi,[2] Gabriella Moroni,[1] Piergiorgio Messa.[1] *[1]Nephrology, Dialysis and Kidney Transplantation, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Milan, Italy; [2]Renal Research Laboratory, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Milan, Italy; [3]INSERM Joint Research Unit S_702, Tenon Hospital, Paris, France.*

Background: Renal biopsy gives several informations about the prognosis of Kidney Transplantation(KTx). The aim of our study was to evaluate in KTx biopsies: 1)the prevalence of histologic anomalies; 2)the prognostic role of markers of inflammation(CD45), epithelial-mesenchymal transition(Vimentin-VIM) and fibrosis(Periostin (POST)).

Methods: 149 KBx performed on clinical indication in 149 KTx patients (M=83; age 44±14 yr) between 2009 and 2012 were processed for general histology. A mean of 15±4 glomeruli/patient were evaluated. Tubular atrophy (TA), interstitial infiltration (I-Inf) and fibrosis (IF) were defined as absent,mild,moderate, and severe, whereas glomerulosclerosis (GS) as %of glomeruli affected. CD45,VIM and POST tissutal positivity, was quantified as %of positive area. Clinical and biochemical data were collected at the time (T0),12 mths before and after the KBx, whereas FGF-23, osteoprotegerin, fetuin and 25-OH-VITD (VIT D) only at T0.

Results: GS was present in 25±22% glomeruli/biopsy. I-Inf, TA and IF were slight in 18%,21% and 25%, moderate in29%,23% and 27% and severe in 18%,29% and 27% of patients respectively. CD45, VIM and POST were correlated each other. CD45 and VIM were both directly correlated with creatinine and inversely with eGFR and VITD, while POST was inversely related with VitD only. Both VIM and POST were directly related to GS. The 32pts that restarted dialysis(HD+) in the year after KBx, had lower eGFR at T0 and higher positivity for CD45,VIM and POST. CD45 and POST significantly predicted HD+, independently of renal function. POST by ROC-CURVE resulted the best marker inHD+ prediction:(AUC: CD45=0,678;VIM=0,673;POST=0,760).

Conclusions: CD45,VIM and POST,obtained in KTx biopsies, were correlated each other and seem to predict graft outcome. POST and CD45, have prognostic role independently of the renal function.

SA-PO711

Membranous Glomerulopathy After Renal Transplantation Artur Quintiliano Silva,[1] Tainá Veras de Sandes Freitas.[1,2] *[1]Div of Nephrology, Federal Univ of São Paulo – UNIFESP, São Paulo, Brazil; [2]Div of Nephrology, Hospital do Rim e Hipertensao, São Paulo, Brazil.*

Background: Membranous nephropathy (MN) is one of the more common causes of nephrotic syndrome in the adult population, accounting for about 20% of cases. It can be idiopathic (70%-80%) or secondary to various clinical conditions.The incidence of post transplant (pTx) glomerulonephritis in the renal allograft varies widely depending on the criteria of recurrence (pathologic or clinicopathologic) and of the type of glomerular disease in native kidneys.

Methods: We performed a retrospective study of pTx MN in a single transplant center, identifying 36 cases until this moment among 11525 transplants already performed.

Results: Most patients were males (54.5%), 96.7% had hypertension and 36.3% delayed graft function, 21.1% acute cellular rejection, 9.1% diabetes and 51.5% dyslipidemia. The median overall graft survival was 103 months(m) and 106m among those with graft loss (9/33). The median time for detection of the first proteinuria was 48m and of pTx

MN diagnosis was 58m Eleven patients had a secondary cause of MN (HCV, HBV, SLE, neoplasia). Primary recurrence, secondary recurrence, primary "de novo" and secondary "de novo" MN corresponded to 30.3%; 36.36%; 6.1% and 27.3%, respectively. The most common *human leukocyte antigen* (*HLA*) were A2 (54%), B35(37.5%) and DR11 (37.5%). Development of pTX MN was not associated to any immunosuppressive regimen.

Conclusions: In the present study, graft loss was not common throughout follow-up; the onset of proteinuria occurred later than in previous reports (48m versus 10m) and nephrotic syndrome did not correspond to the initial main manifestation (as usually described). Recurrence was diagnosed in 36.4% (a frequency higher than in previous studies), regardless of the immunosuppressive regimen used. Additional analyzes are underway to better define the profile of this group of patients and peculiarities of the of the disease.

Funding: Private Foundation Support

SA-PO712

Long Term Outcomes of Kidney Transplant Recipients Converting From a Calcineurin-Inhibitor to a mTOR Inhibitor in a Prednisone-Free Regimen

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Background: Our center previously described similar clinical outcomes(3 years of follow up) between kidney transplantation recipients converted from Tacrolimus(Tac) to Sirolimus(SRL) in a prednisone free regimen. The current analysis focuses on longer-term outcomes in this population of patients.

Methods: Prospective, randomized single center trial. Patients(N=200) were induced with alemtuzumab and maintained on a prednisone-free regimen with Tac and MMF. At 1 year post transplantation, patients were randomized 2:1 to be converted to SRL(n=135) or continue Tac(n=65). Recipient and donor demographics were similar in both groups. Patients with acute rejection, proteinuria, or death before randomization were excluded from analysis (n=7). Patients lost to follow up or who withdrew consent were also excluded from analysis(n=7). Patient randomized to SRL group but did not undergo conversion were included in the Tac group(n=11).

Results: Of 186 patients (Tac=71, SRL=115) analyzed, 26 experienced death or graft loss (Tac=10, SRL=16). Of the remaining 160 patients(Tac=61,SRL=99), 144 (90%) had greater than 4 years of follow up after transplant. The rates of death, graft loss, and acute rejection were similar between the 2 groups(Table 1). At a mean follow up of 5.41 years in the Tac group and 5.47 years in the SRL group, allograft function (eGFR by MDRD) was not statistically different between the two groups(figure 1).

Table 1

	Tacrolimus (Tac) N= 71		Rapamune (SRL) N=115		
Outcome	n	%	n	%	P Value*
Acute Rejection	5	7.0	13	11.3	0.44
Death	5	7.0	7**	6.1	0.77
Graft loss	5	7.0	10	8.7	0.79

*Fisher's exact test

**One patient with graft loss prior to death

Figure 1

	Prograft(FK) N=61	Rapamune(SRL) N=99	P value*
Unadjusted Mean (SD) GFR in abbreviated MDRD(ml/min per 1.73m2)	61.44(18.54)	66.12(21.19)	0.157
Mean (SD) follow up in months post-randomization	65.20(14.63)	65.69(16.02)	0.846

*Two-tailed, unpaired t-test

Conclusions: After alemtuzumab induction and rapid steroid elimination, conversion from Tac to SRL after transplantation is safe. In addition, renal allograft function is equally maintained in both groups.

SA-PO713

Vitamin D Deficiency Is an Independent Risk Factor of Urinary Tract Infection after Kidney Transplantation

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Background: Vitamin D deficiency is frequently found in patients with kidney transplantation (KT). Since vitamin D plays indispensable roles in the immune system, there may be an association between vitamin D deficiency and infection in these patients. However, this has not been fully elucidated. This study, therefore, was aimed to investigate the impact of pre-KT vitamin D deficiency on the development of urinary tract infection (UTI) after KT.

Methods: 25-hydroxyvitamin D [25(OH)D] levels were measured in 410 patients within two weeks before KT. Vitamin D deficiency was defined as 25(OH)D < 10 ng/mL. The primary outcome was the occurrence of UTI after KT. Cox proportional hazard analysis was performed to determine the independent association of vitamin D deficiency with UTI.

Results: The mean 25(OH)D levels were 12.8±6.9 ng/mL, and 171 patients (34.4%) were deficient of vitamin D. Vitamin D deficiency was significantly associated with female gender [β = 2.30, 95% confidence interval (CI) = 1.41 to 3.77, P = 0.001], hemoglobin (β = 0.83, 95% CI = 0.72 to 0.95, P = 0.006) and serum albumin concentrations (β = 0.17, 95% CI = 0.10 to 0.30, P <0.001), and the season of KT operation (summer, β = 0.22, 95% CI = 0.11 to 0.42, P <0.001; autumn, β = 0.32, 95% CI = 0.17 to 0.63, P = 0.001, spring as a reference). During a median follow-up duration of 7.1 years, the incidence rates of UTI were significantly higher in patients with vitamin D deficiency compared to those without deficiency [52 (30.4%) versus 40 patients (16.7%), P = 0.001). Moreover, multivariate Cox analysis showed that vitamin D deficiency was a significant independent predictor of UTI after KT (hazard ratio = 1.81, 95% CI = 1.11 to 2.97, P = 0.018).

Conclusions: Vitamin D deficiency was an independent risk factor for UTI after KT, suggesting that determining 25(OH)D levels might be helpful to predict infectious complications after KT.

SA-PO714

Routine Employment of Intraoperative Continuous Renal Replacement Therapy for Liver Transplantation was Safe and Effective

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Background: Liver transplantation is a lengthy and complex procedure during which there are often large shifts in volume, disturbance in sodium - potassium homeostasis, and acid/base physiology that can be difficult to manage. Intraoperative management is more challenging when the patient has severely compromised kidney function. In order to better manage these physiologic variables in the operating room, we instituted a standard policy whereby all patients on dialysis awaiting liver transplant are placed on continuous renal replacement therapy (CRRT) throughout the liver transplant procedure and for at least 24 hours post-operatively. Here in we report our experience with this transplant dialysis protocol.

Methods: We retrospectively examined 8 liver or combined liver and kidney transplant patients from 2010 - 2014, who also received dialysis support in the week prior to receiving their transplant organ(s). Data included electrolytes, volume status, daily input/output, the day of extubation, total blood products received, and blood pressure, from 2 days pre-op to 3 days post-op period.

Results: None of the patients developed hyperkalemia or acidosis. Hyponatremia resolved post operatively which is a common challenge in end stage liver disease patients. Blood pressure was well controlled perioperatively. Patients' volume status better controlled despite receiving on average 40-100 blood products intra-operatively. Four patients were extubated on post op day 1 and two patients on post op day 2. No anticoagulation was required during CRRT to maintain filter and circuit patency.

Conclusions: We found that a protocol that utilized intraoperative CRRT during the liver transplant surgery was safe and effective at maintaining tight sodium, potassium, fluid and acid/base balance despite large peri and intra-operative transfusion and crystalloid fluid requirements. Although we are not able to comment on long term outcomes and its impact on ICU stay or hospitalization length, we submit that this type of CRRT protocol could be utilized by other transplant centers and is worthy of larger, prospective studies.

SA-PO715

Urinary Alpha$_1$-Microglobulin Excretion and Renal Outcome after Kidney Transplantation

Anneke Bech, Judith M. Hoogendijk-van den Akker, Andries Jan Hoitsma, Jack F. Wetzels. *Nephrology, Radboud Univ Medical Center, Nijmegen, Gelderland, Netherlands.*

Background: Although one year survival after kidney transplantation is excellent, renal function deteriorates in many patients within the first decade. Urinary excretion of alpha$_1$-microglobulin (Ua$_1$m) reflects tubulo-interstitial damage and predicts outcome in patients with glomerular diseases. The predictive value of Ua$_1$m in the transplantation setting is unknown.

Methods: In patients who received a renal transplant between 2006 and 2010, Ua$_1$m was measured at 3 and 12 months after kidney transplantation. Ua$_1$m was expressed as mg per 10 mmol creatinine (normal values < 15).

Results: 139 patients were included with a median age of 48 years (IQR 36-58). Median follow-up time after kidney transplantation was 60 months (IQR 48-72). During follow-up 3 patients developed graft failure and 10 patients died. Ua$_1$m-ratio at 12 months was increased in 78% of patients. Increased Ua$_1$m was associated with the type of donor, gender of the recipient, eGFR, albuminuria and rejections (Table1). Ua$_1$m did not predict deterioration of renal function during follow up. Table 1: patient characteristics per quartile of Ua$_1$m at 12 months.

	Q1	Q2	Q3	Q4	P value
Postmortal donor (N,%)	12 (34)	15 (43)	8 (24)	20 (57)	0.03
Gender (M/F)	17/18	18/17	24/10	26/9	0.06
Ua_1m 3 months	17 (12-38)	28 (23-45)	46 (34-63)	79 (53-135)	<0.01
Ua_1m 12 months	10 (6-14)	22 (21-28)	38 (33-43)	75 (55-113)	<0.01
ACR 12 months	15 (5-41)	25 (9-89)	34 (17-117)	60 (17-177)	<0.01
Rejection <12 months (N,%)	6 (17)	7 (20)	8 (24)	15 (43)	0.06
eGFR 12 months	60±15	50±15	47±12	44±12	<0.01
eGFR difference last FU-12 months	2 (-4-7)	0 (-6-9)	1 (-6-5)	1 (-2-9)	0.73

Median values with interquartile ranges for skewed data and mean values with standard deviation for normally distributed data
ACR = albumin/creatinine ratio (mg/10 mmol)
eGFR = MDRD4 (ml/min/1.73m^2)

Conclusions: The majority of kidney transplant patients have elevated Ua_1m at 12 months after transplantation. Ua_1m was associated with factors contributing to kidney injury during or shortly after transplantation but did not predict renal outcome. Our data suggest that an elevated Ua_1m reflects fibrosis and not active tubular cell injury.

SA-PO716

Using Cinacalcet to Treat the Hypophosphatemia of Early Kidney Transplant Maria Coco, Enver Akalin, Daniel G. Glicklich. *Medicine, Montefiore Medical Center, Albert Einstein College of Medicine, Bx, NY.*

Background: Hypophosphatemia is common early after kidney transplantation due to residual hyperparathyroidism. We hypothesized that cinacalcet will normalize phosphorus in early renal transplantation by reducing the effect of PTH on the proximal convoluted tubules, thereby reducing urinary phosphate wasting.

Methods: This was a randomized, double-blinded, placebo controlled trial of cinacalcet start within 3 months of renal transplant in patients with serum phosphorus less than 2 mg/dl and creatinine less than 2 mg/dl and continued for 3 months. Baseline and 3 month creatinine, calcium, phosphorus, Vitamin D, FGF23, Urinary calcium and phosphorus excretion were obtained. Calcium, phosphorus and creatinine were compared 6 months after the drug was stopped. Repletion with phosphorus was allowed if phosphorus remained low.

Results: 36 subjects were randomized blindly by pharmacist. There was no difference in baseline demographics and biochemical parameters between study (CINA) and control (CON) groups. At the end of the 3 month study period, absolute values and percent change in calcium, phosphorus, creatinine, urinary excretion of phosphate and calcium were not different between the groups. Similarly, Calcium and Phosphorus were not different 6 months after the study drug was stopped. Phosphate supplementation tended to be more in control group.

drug	Ca3m(mg/dl)	Ca9m(mg/dl)	phos3m(mg/dl)	phos9m(mg/dl)	UPhos3m(mg/dl)
Con	10.2	10.2	2.6	2.9	0.78
Cina	9.8	10.1	2.9	2.9	1.5

Conclusions: Cinacalcet, given in a placebo-controlled, blinded manner, did not appreciably affect phosphate control in kidney transplant patients with adequate renal function, although phosphate supplementation may have been lower. FGF23 assay is pending: while results will be interesting, it may not have an important role in phosphate control early after kidney transplantation.

Funding: Clinical Revenue Support

SA-PO717

Post Renal Transplant Hypercalcemia Effect on Graft Function and Cinacalcet Use Gaurav Tandon, Sravan Jasti, Rasib Raja. *Nephrology, Albert Einstein Medical Center, Philadelphia, PA.*

Background: Post renal transplant hypercalcemia is common and is usually due to pre transplant hyperparathyroidism. We perviously reported a high incidence of 70% of post renal transplant hypercalcemia in our study population, mostly because of significantly high pre-transplant parathyroid hormone. In our mean follow up of 9months on the same study population, we attempt to see if post renal transplant hypercalcemia has any effect on allograft function and incidence of Cinacalcet usage.

Methods: 47 patient charts were reviewed for allograft function and Cinacalcet usage for a mean follow up period of 9 months. The patient were divided into two arms. First arm was with patients who had normal post transplant calcium (<10.2 mg/dl) and the second arm consisted of patients with post transplant hypercalcemia. Patient's allograft function and Cinacalcet usage after a mean follow up period of 9 months, in the both the arms were compared.

Results: In our study we found a trend towards worsening graft function but it was not statistically significant with our sample size. In normocalcemic group graft function improved by 20% and in hypercalcemia group graft function worsened by 5%. We observed 125% (pvalue 0.08) increased dosage of Cinacalcet after a mean follow up of 9 months when compared to 1 month post transplant. Pre transplant hyperparathyroidism usually resolves in 6-12 months post transplant. In our study population we found that 45% were still requiring Cinacalcet at 6-12 months at a 125% higher dose.

Conclusions: As it is known hypercalcemia leads to afferent arteriolar vasoconstriction, polyuria, interstitial fibrosis and nephrocalcinosis. Hence it can lead to worsening graft function. Better control of Pre transplant hyperparathyroidism may decrease the incidence of post transplant hypercalcemia and the need for Cinacalcet usage. This will lead to better transplant outcomes and cost. Further large studies with long term follow up are required to validate our findings.

SA-PO718

25OH vitD Supplementation in Kidney Transplantation: A 24 Month Follow Up Ines Aires, Isabel Mesquita, Ana Azevedo, Manuel A. Ferreira, Fernando Barbosa Nolasco. *Nephrology and Transplantation, HCC-CHLC, Lisbon, Portugal.*

Background: Native vitamin D serum levels (25-OH) are reduced in kidney transplant (KTx) patients (pts) and have been associated with chronic kidney disease-mineral and bone disorder (CKD-MBD), neoplasic, immunological and cardiovascular effects.

Methods: We evaluated the effects of 24 months cholecalciferol supplementation (median dose 2664 UI), in 121 KTx pts, 60,4% men, mean age 54.4±13 years, 25% diabetic, mean post KTx follow up:71.5±8.5 months. All pts were naïve to 25-OH vitD therapy but 29.7% were on VDRA. 25% were on ACE/ARB inhibitors, that were unchanged.Pts were supplemented accordingly to basal (T0) calcidiol serum levels (ng/mL): deficiency (<15), insufficiency (15 to 30) and normal (>30). Wilcoxon and Anova tests were used.

Results: At T0, 25-OH levels were 15.9±8.2 ng/mL and 93.4 % pts had lower than normal levels. At T24, 25OH levels increased to 31.2±13.3 with 44.6% pts attaining normal levels(versus 6.6% at T0). Proteinuria and PTH levels were significantly reduced: 0.83±1.2 g/d (T0) to 0.56±1 g/d (T24),p<0.001 and 133.9±85.3 (T0) to 110±102 pg/mL (T24), p<0.001, although phosphorus increased (3,3±0,7 mg/dl versus 3,6±0,8, p<0,005) and calcium decreased (9,8±0,9 mg/dl versus 9,7±1 mg/dl; p>0,05). As expected Pcr increased at T24 from 1.35±0.5 mg/dL to 1.48±0.8 mg/dL, p<0,05, but that did not seem to account for the reduction in proteinuria (r=0,31; p<0,005). Supplementation was well tolerated and no adverse events or acute rejection episodes were reported.There were no differences in the number of hospital admissions, cardiovascular events or neoplasia between pts with normal or insufficient 25OH levels.

Conclusions: Accordingly to our results, vitamin D deficiency is highly prevalent among KTx pts. Oral supplementation with cholecalciferol is efficient and safe in the correction of calcidiol serum levels, leading to reduction in proteinuria and CKD-MBD. In this period it was not associated with CV, infectious or neoplasic risk reduction but the short follow up and the late supplementation may have contributed to these results. Larger and longer randomized controlled studies are needed to address different results.

SA-PO719

Overweight and Fat Mass as an Aggravating for Reduced Bone Mineral Density in Renal Transplant Recipients Ita Pfeferman Heilberg, Alessandra Calábria Baxmann, Aluizio B. Carvalho. *Nephrology Div, Federal Univ of São Paulo, São Paulo, Brazil.*

Background: A high prevalence of overweight has been frequently found after renal transplant. Although a higher body mass index (BMI) has been traditionally associated with higher bone mineral density (BMD), it has been suggested more recently that fat mass may exert a detrimental effect on BMD, possibly mediated through leptin. We aimed to evaluate the impact of obesity and fat mass in non-diabetic renal transplant recipients (RTR).

Methods: A hundred nondiabetic RTR (62M/38F,42±10 years) with serum creatinine <2.0mg/dL and at least 6 months of transplantation were subjected to an anthropometric evaluation and body composition assessment through bioelectrical impedance. A blood sample was drawn for serum biochemical and hormonal determinations, and BMD was assessed by dual-energy x-ray absorptiometry.

Results: We observed overweight (BMI>25kg/m2) in 59% of RTR evaluated up to 165 months after transplant and a significant median weight gain of 5.1 kg. An inadequate distribution of body fat was evidenced in 50% of males and in 58% of females. Sixty (60%) of the patients presented low BMD. Hypovitaminosis D was observed in 65% of them, with levels indicating Insufficiency [25(OH)D< 30 ng/mL] in 53% and Deficiency [25(OH) D<15 ng/mL] in 12%. When we analyzed all factors contributing to both lumbar spine and femoral neck BMD, the univariate linear regression showed significant associations (p=0.001) with female gender, levels of 25(OH)D, weight gain, BMI, body fat, lean mass and serum leptin levels. Finally, the multivariate linear regression analysis showed that serum leptin levels and BMI were the only significant (p=0.001) variables remaining in the model predictive of low BMD, adjusting for immnunosupression and factors related to persistence or de novo CKD-MBD.

Conclusions: The present study showed a high percentage of overweight, body fat and weight gain after Tx combined with a high prevalence of low BMD and hypovitaminosis D. Serum leptin levels and BMI were considered the only independent risk factors for low BMD in these patients, suggesting that excessive fat mass may present an unfavorable impact on bone mass in RTR.

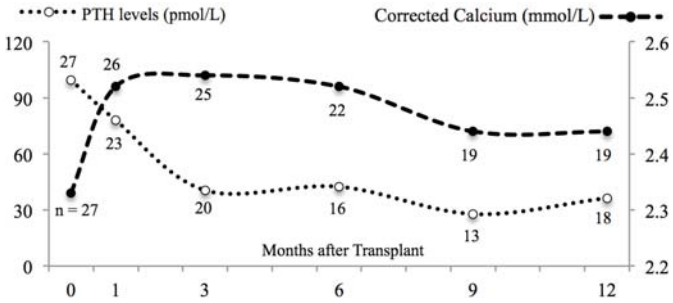
SA-PO720

Cinacalcet in the Management of Persistent Hyperparathyroidism after Renal Transplantation Habib Mawad, Robert Zoël Bell, Sarah Bezzaoucha, Hugues Bouchard, Anne Boucher, Suzon Collette, Jean-Philippe Lafrance, Denis Ouimet, Vincent Pichette, Lynne Senecal, Duy Tran, Michel Vallee. *Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.*

Background: Successful renal transplantation (RTX) corrects many abnormalities of ESRD. Nevertheless, hyperparathyroidism (HPT) persists in up to 50% of recipients 1 year after RTX. In such patients, hypercalcemia may occur thereby limiting the use of vitamin D analogues. Parathyroidectomy is hence often required. The aim of our study is to evaluate the use of cinacalcet in the management of persistent HPT.

Methods: In this retrospective study, we conducted a chart review of all patients having been transplanted from 2003 to 2012 and having received cinacalcet up to RTX and/or thereafter. Baseline characteristics as well as evolution of calcemia and PTH levels were collected.

Results: A total of 27 patients were included consisting mainly of middle-aged men. Time spent on dialysis prior to RTX was 5.8±4.3 years. Data was collected up to 3.2±2.5 years after RTX. 6 patients initiated cinacalcet only after RTX. The remaining 21 patients already had cinacalcet at the time of RTX. Cinacalcet was stopped within the first month after RTX in 12 of these patients of which 7 had to restart the treatment. The main reason for restarting cinacalcet was hypercalcemia. At the end of the study only 8 patients had stopped cinacalcet. Length of treatment post-RTX was 26±24 months. Dosage requirements decreased with time although not statistically significant. There were only 3 cases of mild hypocalcemia. PTH decreased during the first 3 months whereas calcium increased.



Conclusions: HPT is a common problem in renal transplant patients and may persist for years. Cinacalcet appears to be a safe and effective treatment. It may serve as a relay to parathyroidectomy or as an alternative. In our study, spontaneous resolution of HPT was uncommon.

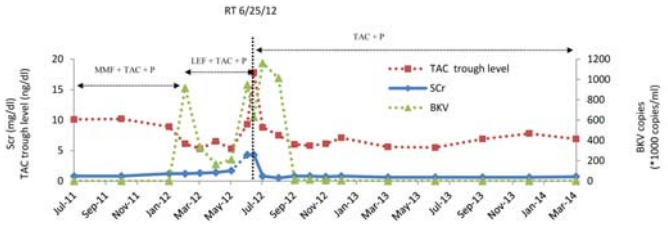
SA-PO721

Kidney Retransplantation for BK Virus Nephropathy Despite Active Viremia without Allograft Nephrectomy Jingbo Huang, Gabriel M. Danovitch, P.T. T. Pham, Suphamai Bunnapradist, Edmund Huang. *Nephrology, UCLA David Geffen School of Medicine, Los Angeles, CA.*

Introduction: Kidney retransplantation (KRT) may be done for patients with previous BK nephropathy (BKN). Whether transplant (TP) nephrectomy and viral clearance are required prior to KRT is unclear.

Case Description: A 65 year-old female with ESRD secondary to polycystic kidney disease received a living unrelated kidney TP with basiliximab induction and tacrolimus (TAC; trough 8-10 ng/ml months 0-3; 6-8 ng/ml thereafter), mycophenolate mofetil (MMF) and prednisone maintenance. She had a baseline creatinine (Scr) of 0.7 mg/dl until 11m post-TP when Scr increased to 1.2 mg/dl. Plasma BK PCR was noted to be 854K copies. Kidney biopsy confirmed BKN by SV40 staining. Leflunomide (LEF) 20 mg twice daily was initiated, MMF was stopped, and TAC trough was targeted at 4-6 ng/ml. 15m post-TP, she developed fulminant hepatic failure, with liver biopsy suggesting drug-induced injury. LEF was stopped. She was thought to have hepatorenal syndrome and initiated hemodialysis (HD). BK PCR increased to 946K copies and IVIG 2 g/kg was given. Due to a clinically deteriorating status, she was listed for liver/kidney TP (SLK). Allograft nephrectomy was considered, but she was deemed too unstable for surgery. 3 days after HD initiation and IVIG, she underwent SLK with active BK viremia. She did not receive antibody induction and was maintained on TAC (trough 4-6 ng/ml) and prednisone alone. She had immediate graft function. She received a 2nd course of IVIG 5 days post-SLK and started on ciprofloxacin. BK PCR was undetectable 3m post-SLK. At 15m follow-up, Scr was 0.6 mg/dl and BK PCR remained undetectable. The trend of BK PCR and TAC level is shown in Figure 1.

Figure 1



Discussion: To our knowledge, this is the first reported case of successful KRT for BKVN in the setting of severe BK viremia without concomitant allograft nephrectomy.

SA-PO722

BK Virus Nephropathy in the Native Kidney Anjali Narain Masand, Jordan Gabriela Nestor, Jeffrey I. Silberzweig, Steven Salvatore. *Dept of Nephrology and Hypertension, New York-Presbyterian/Weill Cornell, New York, NY.*

Introduction: BK virus associated nephropathy (BKVN) is known as an important cause of renal allograft dysfunction after renal transplantation.Case reports suggest that BKVN is not confined to allograft kidneys,and can be seen in the native kidneys of immune-compromised hosts.We present a case of a patient with refractory chronic lymphocytic leukemia and renal dysfunction who had BK virus detected in the serum and histologically confirmed on renal biopsy.

Case Description: The patient was a 72 year-old male with a history of refractory chronic lymphocytic leukemia (CLL) which was diagnosed initially in 1997 when he presented with diffuse lymphadenopathy.He had been treated with numerous chemotherapy protocols and experimental regimens since 2001 when he was treated with Fludarabine and Rituximab.His creatinine level had been mildly elevated since at least 2007, in the range of 1.1-1.5 mg/dL.It reached a peak level of 2.4 mg/dL in June 2013.He had a renal ultrasound at this time which showed no evidence of hydronephrosis and normal echogenicity.He had several urinalyses,none of which showed glycosuria,microscopic hematuria,proteinuria,or pyuria. He had none of the usual risk factors for chronic kidney disease but the various chemotherapy regimens placed him at risk of renal injury.His creatinine remained stable in the range of 1.8-2.4 mg/dL over the course of the next several months.In August 2013 a BK virus level was checked and was found to be elevated at 45,000 cpy/mL.He subsequently underwent a kidney biopsy which showed BK virus associated nephropathy with no direct renal involvement by CLL.He was started on treatment with Ciprofloxacin 400 mg twice daily and Leflunomide 20 mg once daily.Despite this, the patient's renal function did not recover, and hemodialysis was initiated in March 2014.

Discussion: BK virus associated nephropathy of the native kidneys may be under-recognized. Quantitative BK virus DNA is not routinely measured in immune-compromised patients with worsening renal function. A high level of suspicion in immune-compromised patients is required in order to facilitate early diagnosis of BKVN and optimize initiation of antiviral therapy.

SA-PO723

Poor Outcome of Occult Hepatitis C Infection in Kidney Transplant Recipient Alejandra Mena- Gutierrez, Fahd Syed, Sameh R. Abul-Ezz. *Div of Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

Introduction: Identification of Hepatitis C infection (HCV) in kidney transplant candidates relies on the detection of anti-HCV antibody and evaluation of liver function. Patients with End Stage Renal Disease may have an ineffective immune response resulting in negative anti-HCV antibody while HCV-RNA can be detected by polymerase chain reaction (PCR). If not detected before kidney transplantation, further immunosuppression may result in fatal outcome.

Case Description: A 65 years old male with ESRD secondary to IgA nephropathy received a living related kidney transplant from his son. He had no indication of liver disease or high risk behavior. Both the recipient and donor had negative HCV serology and liver function before transplantation. 9 months after transplantation, the recipient had deranged liver function with hyperbilirubinemia. Ultrasound showed cholelithiasis and he underwent laparoscopic cholecystectomy with marked improvement in liver function. He presented few weeks later with worsening liver enzymes, and all serologies for infectious and autoimmune causes were unrevealing. Immunosuppression was decreased and liver function improved again. He returned with severe jaundice and in addition to repeat viral serologies, HCV-RNA by PCR was checked. Levels came back extremely high despite negative anti-HCV antibody. Liver biopsy showed fibrosing cholestatic hepatitis. He progressed rapidly to fulminant hepatitis and was not considered candidate for HCV treatment or transplantation. He shortly died of sepsis. Blood samples from the donor were obtained and did not show evidence of HCV infection by PCR or by antibody testing. The recipient's stored blood samples dated before transplantation were retested for anti-HCV antibody and were confirmed negative.

Discussion: Occult hepatitis C infection with negative anti-HCV antibody can only be detected by HCV-RNA PCR. The devastating outcome of this occult infection indicates the need to test all kidney transplant candidates for HCV-RNA regardless of their HCV antibody status. Patients with negative anti-HCV antibody and positive HCV-RNA PCR should undergo liver biopsy and treatment before considering kidney transplant.

SA-PO724

Abatacept for Rheumatoid Arthritis in a Renal Transplant Recipient with Six-Year Follow-Up Mohamed A. Sheta, Hassan N. Ibrahim, Arthur J. Matas, Aleksandra Kukla. *Renal Diseases and Hypertension, Univ of Minnesota, Minneapolis, MN.*

Introduction: Treatment of autoimmune diseases is challenging in renal transplant patients. Inhibition of T cell co-stimulation pathway has been gaining widespread interest in the treatment of autoimmune diseases and as an Induction/maintenance therapy for renal transplantation. We are presenting a case of de novo membranous glomerulonephritis in kidney transplant recipient secondary to rheumatoid arthritis which responded well to Abatacept, a co-stimulatory inhibitor, while graft function remained stable for a follow up period of Six years.

Case Description: A 69 year-old white male patient,with a history of end stage kidney of unknown etiology received a living donor kidney transplant in 1998 on Cyclosporine,Mycophenolate mofetil(MMF) and low dose Prednisone with serum creatinine (Scr) of 1.5 to1.7 mg/dl.Past medical history includes hypertension, coronary artery disease and gout.in 2008 patient was diagnosed with rheumatoid arthritis (RA).BP 120/70. Positive examination finding were symmetrical arthritis of the PIPs,MCPs and wrists,boutonniere and swan neck deformities, and bilateral leg edema. UA showed 300 mg/dl with no RBCs, Scr of 1.6 mg/dl, and protein to creatinine ratio of 6.8 g/g. Renal graft biopsy showed membranous glomerulopathy (MGN). Methotrexate (MTX) was started, while MMF was discontinued. Scr increased to 3.6 mg/dl. A Repeated renal graft biopsy did not show new findings. MTX was discontinued because of worsening Scr and inadequate clinical response. Patient was started on monthly Abatacept, together with discontinuation of cyclosporine and re-starting MMF and continuation of low dose prednisone. This led to both clinical and laboratory response. Curently Scr is 1.6 and protein creatinine ratio is 0.76 g/g. Abatcept was tolerated well.

Discussion: Abatacept,FDA approved for treatment of RA, was chosen in our patient based on the understanding of the central role of T-cell in both normal immune response to transplanted organ and autoimmune diseases. Adopting this concept in renal transplant patients with autoimmune diseases will lead to less number of immunosuppressive use and, hence, less toxicity.

SA-PO725

Glomerular Disorders in Two Patients with Intestinal Transplant Arwa Nada,[1] Brian Becknell,[1] Peter Baker,[2] Hiren P. Patel.[1] *[1]Div of Nephrology, Nationwide Children's Hospital, Columbus, OH; [2]Div of Pathology, Nationwide Children's Hospital, Columbus, OH.*

Introduction: Prior studies of end stage kidney disease (ESKD) after intestinal transplant (IT) found the incidence of ESKD is up to 3% of children and 21% of adults. ESKD is often attributed to calcineurin inhibitors in children and diabetes and hypertension in adults, but reports of glomerular diseases in this population is rare. To our knowledge this is the first report of the pathological findings of glomerular disease in patients who received IT as children.

Case Description: We report 2 patients with IT for Hirschsprung disease who had kidney biopsy for proteinuria. The age at first IT was 2 and 16 years. Neither patient had diabetes mellitus at the time of the kidney biopsy. Both patients had progressive increase in proteinuria with urine protein/creatinine ratio of 15 mg/mg in the first patient and 30 mg/mg in the second patient at the time of biopsy. The estimated glomerular filtration rate at the time of the biopsy was 55 and 33 ml/min/1.73m^2. Both kidney biopsies showed focal segmental glomerulosclerosis (FSGS) with hyalinosis on light microscopy. Both had positive IgG, IgM, IgA, C3, C1q, C4, and fibrinogen on immunofluorescence. Electron microscopy showed primarily subendothelial deposits in the first patient and a small amount of subepithelial and intramembranous deposits in the second. Both patients had normal serum C3, and C4 complement.

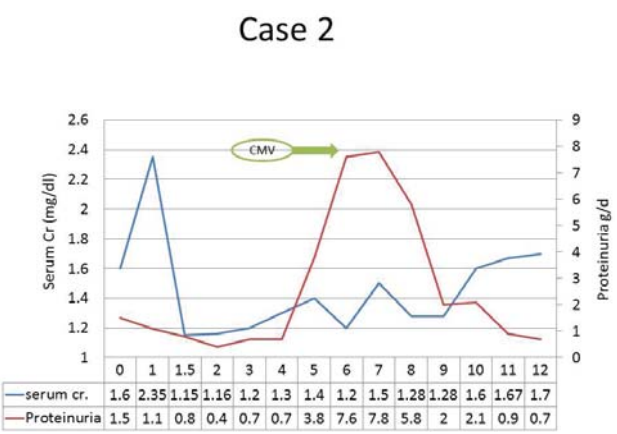
Discussion: We found FSGS with immune deposits in 2 patients with IT who developed proteinuria. This report underscores the importance of obtaining kidney biopsy in patients with IT who develop proteinuria, especially when the proteinuria is out of proportion to what one may expect from drug toxicity. Early screening and identification of underlying kidney disease may allow intervention to prevent or slow the development of ESKD in these children. Studying larger number of patients to further investigate our preliminary findings and develop a better understanding of the cause of ESKD in these patients is warranted.

SA-PO726

CMV Induced Proteinuria in Renal Transplant Recipients Syed J. Sher, Teena Tandon, Muhammad Ahmad Mujtaba, Muhammad S. Yaqub, Tim E. Taber, Asif A. Sharfuddin. *Nephrology/Medicine, Indiana Univ.*

Introduction: CMV is a common infection in kidney transplant recipients in the first year after transplantation. It is unclear if CMV infection in renal transplantation can cause allograft dysfunction with glomerulopathy.

Case Description: Case1. 54 year old CMV seronegative male with ESRD who received a CMV seropositive living kidney transplant and received 6 months of valgancyclovir for CMV prophylaxis. Ten months after his transplant, he developed acute new- onset proteinuria and rising serum creatinine from 1.1 to 1.7mg/dl. He was found to have CMV viremia of 15,000 copies while his proteinuria increased from a baseline of 0.4gm/day tot 2.5g/day. Transplant biopsy showed minimal change disease and was negative for rejection or chronic transplant changes. Treatment was initiated with valgancyclovir and CMV PCR decreased to less than 250 while proteinuria also improved back to 0.5 gms, with the addition of lisinopril. Case 2. 56 year old CMV seropositive male with ESRD who received a CMV seropositive living unrelated kidney transplant with 3 months of valgancyclovir for CMV prophylaxis. Five months after transplant the patient developed CMV disease with viremia of > 500,000 copies along with simultaneous new onset proteinuria rising from baseline 0.7g to 7.6g/day.



Transplant biopsy showed mild lymphocytic tubulitis and patchy foot process effacement of the podocytes. His proteinuria improved to 0.7 gm after his CMV was treated with valgancyclovir, initiation of lisinopril as well as reduction in mycophenolate and temporary use of high dose prednisone therapy. CMV inclusions or positive viral staining was not found in either of the biopsy cases.

Discussion: These 2 cases represent a very rare complication of CMV and clearly show that even in the presence of immunosuppression, and absence of direct visualization of virus in the kidney, glomerular changes can occur in CMV disease.

SA-PO727

Living Donor Transmitted Histoplasmosis in a Kidney Transplant Recipient Nader S. Bahri,[1] Martin L. Mai.[2] *[1]Nephrology, UF, Jacksonville, FL; [2]Transplant, Mayo Clinic, Jacksoville, FL.*

Introduction: Donor transmitted histoplasmosis (histo) post solid organ transplant is rare even in endemic areas. Most cases occur in deceased donor transplant recipients with median time from transplantation to diagnosis of 17 months. We report a case 78 days post living donor kidney transplant.

Case Description: Data was abstracted from an electronic medical record of recipient and donor data with review of medical history,exam,lab, microbiology and diagnostic studies.

A 73 year-old female native of Florida with ESRD due to hypertensive and T2DM nephropathy underwent kidney transplant from a 21 year-old donor. Donor and recipient fungal serologies by EIA were negative preop. Immunosuppression included rabbit antithymoglobin (rATG) induction (high PRA pretransplant) with maintenance tacrolimus, mycophenolate and corticosteroids with excellent kidney function. 14 days postop the recipient developed acute cellular and antibody mediated rejection managed with steroids, rATG, and IVIG and plasma exchange. 64 days later, she presented with high fever for 3 days. Travel history was unremarkable. There was no exposure to pets/birds, sick contacts, or other risk factors for fungal infection.CT scan of chest and abdomen were unremarkable. Kidney biopsy showed no rejection. Blood and urine cultures were negative for 5 days. She subsequently developed altered mental status, respiratory failure and hypotension requiring intensive care unit admission, intubation and blood pressure support. Fungal serologies by EIA were negative as were CMV and EBV PCR of serum. Bronchoalveolar lavage was remarkable for yeast. Peripheral blood smear showed intracellular yeast forms in RBC, prompting bone marrow biopsy and histo urine antigen confirming infection with histo. The donor remained asymptomatic and fungal EIA were negative postop but histo urine antigen was found to be positive.

Discussion: We believe this is the first reported case of living donor transmitted histo in kidney transplant.Donor and recipient fungal screening with EIA pre and post transplant did not identify fungal infection. Screening for histo is only recommended for high risk donors. Early use of urine histoplasma antigen is valuable in identifying acute infection.

SA-PO728

A Rare Case of Ocular Toxoplasmosis That Developed Four Years After Kidney Transplantation Toshinari Fujimoto, Izumi Yamamoto, Masatsugu Nakao, Yudo Tanno, Ichiro Ohkido, Hiroyasu Yamamoto, Keitaro Yokoyama, Takashi Yokoo. *Dept of Internal Medicine, Div of Nephrology and Hypertension, The Jikei Univ School of Medicine, Tokyo, Japan.*

Introduction: Toxoplasmosis is a zoonotic infection caused by Toxoplasma gondii, a ubiquitous obligate intracellular parasite in both humans and warm-blooded animals. Approximately one-third of humans worldwide are estimated to be chronically infected with Toxoplasma gondii. Toxoplasmosis after solid organ transplantation is associated with high morbidity post-transplantation. Here, we report a rare case of ocular toxoplasmosis that had developed 4 years after kidney transplantation.

Case Description: A 39-year-old man had undergone living-related kidney transplantation because of IgA nephropathy. Four years post-transplantation, he revisited our

hospital because of altered visual acuity. We detected significantly high serum toxoplasma IgG antibodies (×40960) and toxoplasma antigen (×2048) using a serum latex agglutination method. Furthermore, using a primer to target the '18S rDNA' area of Toxoplasma gondii DNA (GenBank no. L37415), nested-polymerase chain reaction (PCR) test results of the anterior chamber aqueous humour were positive for Toxoplasma gondii. Consequently, ocular toxoplasmosis was diagnosed and was successfully treated with clindamycin together with pyrimethamine and folinate. After initiation of therapy, his intraocular findings markedly improved, and visual acuity recovered.

Discussion: The diagnosis of ocular toxoplasmosis is difficult when patients are in an immunosuppressive state since IgM antibodies for Toxoplasma gondii do not appear in the blood. The nested-PCR test for the anterior chamber aqueous humour is useful definitive diagnostic method for ocular toxoplasmosis. In addition, ocular toxoplasmosis should be considered in the differential diagnosis of patients with a history of kidney transplantation who present with the altered visual acuity.

SA-PO729

Ileal Tuberculosis Presenting as Fever of Unknown Origin in a Post Renal Transplant Patient Paul L. Cespedes. *Dept of Nephrology, Univ of California at Davis, Sacramento, CA.*

Introduction: Mycobacterium Tuberculosis is a leading infection following renal transplant throughout the world, with reactivation being the most common mode of infection. Prevalence of post transplant TB ranges from 1.5% in the U.S. to up to 15% in Asia. Up to 1/2 of all active TB cases after transplant are disseminated or extra pulmonary. Fever is present in 91% of patients with disseminated TB. The typical time course for TB to develop in post transplant patient is 26 months in patient treated with azathioprine and prednisilone and 11 months if the patient received cyclosporin.

Case Description: Patient is a 71 year old woman with a history of hypertension, Type II diabetes, CVA, CKD stage 5 of unknown etiology who underwent a living unrelated kidney transplant in the Philippines in 2008. Patient was treated with tacrolimus, azathioprine and prednisone initially, with azathioprine slowly tapered off. Patient presented to an outside hospital 2 month prior due to declining health with intermittent fevers, decreased PO intake, abdominal pain, nausea, diarrhea, and weight loss. Patient underwent a workup at the outside facility on 2 separate occasions with no source of fever identified. Patient's family brought the patient to UC Davis for further evaluation.

On presentation, patient had low WBC count and was spiking daily fevers to 38 degrees and 39 degrees. Infectious disease evaluation was unremarkable. A CT abdomen and pelvis showed cecal and terminal ileal wall thickening with aneurysmal dilation of the terminal ileum, consistent with lymphoma or tuberculosis. A CT chest showed numerous peripheral sub centimeter ground glass nodules and right middle lobe 12mm well-circumscribed nodule. A colonoscopy with biopsy was positive for AFB and subsequent sputum samples were positive for AFB as well. Patient was started on 4 drug anti TB regimen of rifabutin, isoniazid, pyrazinamide and ethambutol with resolution of fevers.

Discussion: While post renal transplant TB is not as common an infection in the U.S. as in other parts of the world, TB should be considered in all transplant recipients with fever of unknown origin.

SA-PO730

JC Virus Associated Transplant Nephropathy Ashraf M. Mohammed, Jiries S. Dahu, Bahar Bastani, Alexandra Ileana Voinescu. *Nephrology, St. Louis Univ, St. Louis, MO.*

Introduction: Polyomavirus-associated nephropathy is a known cause of renal graft dysfunction. While the majority of cases are caused by BK virus, very few are linked to JC virus. We report the 9th case of JC-associated nephropathy, 6 yrs post renal transplant.

Case Description: 65 years old male recipient of a deceased donor kidney induced with alemtuzumab and maintained on tacrolimus and sirolimus, presented with asymptomatic allograft dysfunction with no clear cause. Laboratory and radiology evaluation were unremarkable in the setting of therapeutic immunosuppressant levels and absence of BK viremia and viruria. Renal allograft biopsy was unremarkable for any type of rejection; however it revealed mononuclear tubulointerstitial inflammation and nuclear enlargement with granular inclusions of epithelial cells suggestive of PyVAN. Immunohistochemical stain for SV40 was strongly positive. In the absence of BK viruria and viremia, JC virus became the suspected pathogen which was confirmed by serum JC PCR and urine decoy cells. Our patient was treated with reduction of tacrolimus and sirolimus. Due to suboptimal response, tacrolimus was discontinued, leflunomide was added and sirolimus was switched to cyclosporine along with 3 doses of IVIG. JC viremia and renal function has gradually improved and remained stable to date.

Discussion: Our case illustrates the possibility of PyVAN several years post transplant, contrary to the projected time for its occurence within the first 2 years of transplant. It also demonstrates the importance of immunohistochemical staining for polyomavirus in allograft biopsies even in the absence of BK viremia or viruira to avoid diagnostic and therapeutic delays of JC nephropathy. Like previously reported cases, our case shows that JC-associated nephropathy is a unique clinical entity and we suggest testing for JC PCR when BK PCR is negative in a setting of histological evidence for PyVAN and positive SV40 stain. Despite lack of clear guidelines, our patient responded favorably to similar therapeutic protocols currently in place for treating BK nephropathy. Prospective studies are needed to better understand the risk, clinical presentation, diagnostic and therapeutic approaches of JC nephropathy.

SA-PO731

Refractory BK-Virus Induced Nephropathy in the Native Kidneys of a Pediatric Bone Marrow Transplant Recipient Nirupama Gupta, Kiran K. Upadhyay. *Pediatric Nephrology, Univ of Florida, Gainesville, FL.*

Introduction: BK virus-induced nephropathy (BKVN) is a serious complication after renal transplantation; however, BKVN in the native kidneys of immunocompromised patients is not well known. In this report, we describe a child with multi-drug resistant BKVN. To our knowledge, this is the youngest surviving child with refractory BKVN in the native kidneys following complications of bone marrow transplantation(BMT) that led to end stage renal disease.

Case Description: A 10 y.o. African American male was diagnosed with severe aplastic anemia in April 2008. He received an allogeneic BMT in March 2012 with pre-and post-chemo and radiation therapy. By January 2013, he developed severe warm-autoimmune hemolytic anemia and became blood transfusion-dependent. He was treated with multiple courses of IVIG, Rituximab, Eculizumab, Bortezomib and Prednisone for several months along with 11 courses of plasmapheresis without any clinical improvement. His renal function started deteriorating at the same time from a baseline serum creatinine of 0.4mg/dL to 1.4mg/dL. The acute kidney injury (AKI) was initially attributed to pigment nephropathy and chronic renal damage from multiple nephrotoxic medications. His renal function declined (serum creatinine peaked at 4mg/dL) despite aggressive supportive therapy. His serum CMV and EBV titers were negative. The serum and CSF BK viral DNA PCRs were 5 million and 587copies/ml, respectively. The renal biopsy in July 2013 showed intranuclear viral inclusions and positive staining for SV40 T antigen in the tubular epithelial cells. There was extensive interstitial fibrosis and tubular atrophy. Therapy included reduction and discontinuation of immunosuppressive agents along with multiple courses of Leflunomide and Cidofovir; however, this did not reduce the BK viral load. He remains hemodialysis-dependent since September 2013.

Discussion: Although BKVN is more common in renal transplant patients, the use of newer and stronger immunosuppressive medications is increasing the risk of BKVN in non-renal transplant patients. Thus, BKVN should be included in the broad differential of AKI when taking care of such vulnerable patients for a prompt diagnosis and treatment.

SA-PO732

Cytomegalovirus-Induced Thrombotic Microangiopathy after Renal Transplant Treated with Eculizumab Anuja Java, Angelina Edwards, Ana Paula Rossi, Rowena B. Delos Santos, Christina L. Klein, Daniel C. Brennan. *Nephrology, Washington Univ in St. Louis, St. Louis, MO.*

Introduction: De novo thrombotic microangiopathy (TMA) after renal transplant is rare but life-threatening. The insult from ischemia-reperfusion is enhanced by viral infections, immunosuppressive drugs or dysregulated complement activation. Our case provides insight into the pathogenesis and novel treatment of de novo TMA.

Case Description: 75 yo Caucasian woman with ESRD from diabetes received a 4-antigen mismatched, CMV D-R+, DDTx with thymoglobulin induction (5 mg/kg), followed by tacrolimus, azathioprine, and prednisone. Creatinine (Cr) was 1.6 mg/dL but 5 months later 9.2 mg/dL with anuria. Allograft biopsy showed acute TMA without systemic evidence (Hgb 11 g/dL, LDH 214 U/L, Plt 137K/cumm, no schistocytes, ADAMTS13 75%, STEC negative). C4d and DSAs were negative and a diagnosis of aHUS was made. Eculizumab (1200 mg) was given with improvement in urine output. Infectious workup showed CMV viremia (2,582copies/mL) which was treated with valganciclovir (VGCV). Belatacept replaced tacrolimus for possible CNI-induced TMA. Cr improved to 1.8 mg/dL but rose to 6.6 mg/dL 4 months later. Repeat biopsy showed recurrent renal-limited TMA. C4d and DSAs were negative. CMV viremia (unable to quantify) was again noted. VGCV was resumed with clearance of viremia. Belatacept was stopped and eculizumab (1200 mg weekly x 4 doses, then 1200mg q2weeks) restarted. Cr improved to 2.0 mg/dL. 3 months later, testing for complement gene mutations was negative and eculizumab was discontinued. 14 months later, patient has remained stable (Cr 2.0 mg/dL, UP/C 0.1) on azathioprine, prednisone and low-dose VGCV.

Discussion: CMV-related posttransplant TMA has only been reported in 6 cases. Resolution of acute TMA with CMV treatment, recurrence with CMV viremia and the lack of correlation with a CNI in our case supports CMV as cause of the TMA. Eculizumab without plasmapheresis led to prompt improvement in renal function but was discontinued when no genetic cause was identified. This case highlights the beneficial effects of complement inhibitors in acute aHUS and shows that they can be safely discontinued once the inciting etiology is addressed.

SA-PO733

Advanced HIV Infection Leading to Graft Tolerance in a Kidney Transplant Patient Neha Das, Christine M. Durand, Hamid Rabb. *Johns Hopkins Univ.*

Introduction: With effective antiretroviral therapy (ART), patients with ESRD and well controlled HIV infection have excellent survival outcomes with renal transplantation; however, the higher rate of rejection in these patients is not well understood. We present an unusual case of renal allograft tolerance in the absence of immunosuppression in a patient with untreated advanced HIV infection.

Case Description: A 33-year-old male with ESRD due to congenital renal dysplasia underwent live donor kidney transplantation in September 2006. He had immediate graft function. Immunosupressive (IS) therapy included antithymocyte globulin for induction followed by tacrolimus, mycophenolate mofetil, and prednisone. He developed acute tacrolimus toxicity 8 months post-transplant. Serum creatinine (Scr) stabilized at 1.8-2 mg/dl. In May 2008, he was diagnosed with HIV infection. Both recipient and donor had tested

negative for HIV prior to transplant. After the HIV diagnosis, he was lost to follow-up and later self-discontinued IS therapy in September 2009. In November 2013, he presented with focal neurologic deficits. Brain MRI showed multifocal white matter lesions. CSF JC virus PCR was positive. He was diagnosed with progressive multifocal leukoencephalopathy. CD4 count was 232 cells/mm≥ and HIV RNA was 166,832 copies/ml. Scr was 1.7-1.9 mg/dl despite being off IS therapy for over 4 years. He was started on ART. IS therapy was not resumed given his active infection. A week later, he presented with a rise in Scr to 4.2 mg/dl. A transplant renal biopsy showed immune complex-mediated glomerulonephritis and moderate tubular injury without evidence of cellular or humoral rejection. Two months later, Scr improved to 1.9 mg/dl.

Discussion: The lack of allograft rejection despite absence of immunosuppression in this HIV-infected patient was unexpected. We speculate that dysfunction of helper T cell response due to advanced HIV infection resulted in allograft tolerance seen in our patient. Additionally, there is growing evidence suggesting a role of regulatory T and B cells in both allograft tolerance and HIV pathogenesis. A better understanding of the effect of HIV infection on alloimmunity is needed for effective IS therapy for HIV-positive patients with transplants.

SA-PO734

An Unusual Case of a Parasite in a Transplanted Kidney Billy T. Hour, Piangwarin Phaosawasdi, Angelo M. De Mattos. *Div of Nephrology, Univ of California, Davis, Sacramento, CA.*

Introduction: Microsporidia are obligate, spore-forming intracellular organisms that parasitize immunocompromised hosts, such as those who have undergone kidney transplantation. It can infect a variety of organ systems, including respiratory, ocular, GI, GU, and CNS. We describe here a case of microsporidia infection in a kidney transplant patient causing AKI requiring brief hemodialysis (HD) which subsequently improved after albendazole treatment.

Case Description: A 36 year old male s/p kidney transplant was noted to have worsening kidney function about 4 months after transplantation. He presented with fever, diarrhea, and AKI (creat 2.89) with workup negative. Biopsy revealed ATN. One month later he again presented with intermittent fever and AKI (creat 6.3) and biopsy revealed mixed interstitial inflammation with renal tubular abscesses. He was treated for urosepsis and discharged. Several weeks later, patient again presents with intermittent fever with AKI (creat 16.1), biopsy revealed fungus elements with intracellular parasites consistent with microsporidia despite a negative stool stain. He was subsequently started on albendazole 400mg bid but required HD for support and was discharged on HD. After a couple of weeks HD was discontinued due to improved kidney function and repeat biopsy 3 months later revealed only residual microsporidia species. Patient did not require removal of his transplanted kidney and maintains a stable creatinine of around 3.0.

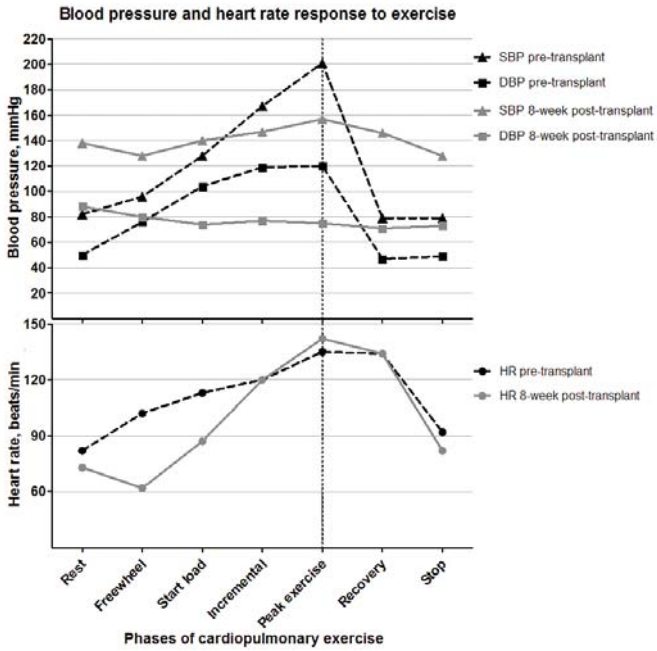
Discussion: Few cases have been described regarding microsporidia in renal transplant recipients. Common symptoms include fever and diarrhea. Diagnosis is made histologically with electron microscopy as confirmation. Treatment is typically with albendazole until stool stain is negative. This case illustrates that microsporidia should be among the differential in kidney transplant recipients with unexplained fever and diarrhea despite a negative stool stain as it can be localized to the transplanted kidney. Therefore, serial biopsies may be required for monitoring and albendazole therapy continued until it is cleared.

SA-PO735

Cardiopulmonary Exercise Triggered an Exaggerated Blood Pressure Response in a Patient with 'Refractory Hypotension' prior to Kidney Transplantation Stephen M.S. Ting,[1] Gordon Mcgregor,[1] Alice Rogan,[1] Nicolas Aldridge,[1] Susan Hewins,[1] Charles E. Weston,[2] Robert Higgins,[1] Daniel Zehnder.[1] *[1]Univ Hospitals Coventry and Warwickshire NHS Trust; [2]Dorset County Hospital NHS Foundation Trust.*

Introduction: Chronic persistent hypotension carries a significant risk of death in patients with ESRD. Such hemodynamic instability would preclude patient from major surgery.

Case Description: A 40-year old female hemodialysis-dependent patient underwent assessment in our center for antibody-incompatible kidney transplantation. A 24 months history of severe hypotension during inter- and intra-dialytic period (<80/50 mmHg) had precluded her from another transplant center. Myocardial perfusion scan and echocardiography were normal. LV ejection fraction was 62%. Aortic pulse wave velocity was preserved at 5.9m/sec. On maximal cardiopulmonary exercise testing (CPET), anaerobic threshold was 10.6ml/min/kg and oxygen consumption at peak exercise was at 73% predicted. Exaggerated rise in systolic (SBP) and diastolic blood pressure (DBP) at maximal load (ΔSBP/ΔDBP=119/70mmHg) was followed by a rapid decline during the recovery phase. Post-operatively, she was vasopressor reliant for 14 days. Eight weeks following transplantation, her resting BP was 124/82. On repeat CPET, SBP rose uniformly but DBP did not rise from baseline (ΔSBP/ΔDBP=19/-13mmHg). The heightened heart rate (HR) in the early phase of exercise was only observed prior to transplant. Cortisol, renin and aldosterone levels at rest were (pre- versus post-transplant) 433 versus 356nmol/l, <9 versus 9mU/l and 515 versus 372pmol/l, respectively.



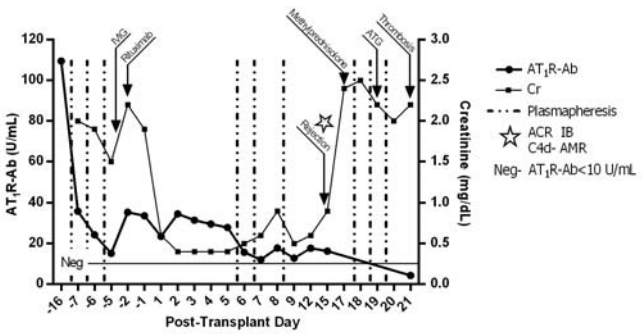
Discussion: Exercise BP profiling and measures of functional cardiovascular reserve can facilitate the management of such complex case. Severe exercise hypertension could be the result of simultaneous augmentation of cardiac contractility, HR and vascular tone induced by dysregulated catecholamine concentration.

SA-PO736

Accelerated Transplant Rejection, Allograft Thrombosis, and Failure with Angiotensin II Type 1 Receptor Antibodies Meghan Pearl, Richard K. Leuchter, Elaine F. Reed, Robert B. Ettenger, Eileen W. Tsai. *Pediatrics, UCLA, Los Angeles, CA.*

Introduction: Angiotensin II type 1 receptor antibodies (AT_1R-Ab) have been associated with vascular injury, antibody mediated rejection (AMR), and allograft (AG) failure. This case illustrates the pro-coagulant effects, accelerated rejection, and early AG loss that can occur with AT_1R-Ab despite desensitization.

Case Description: A 7yo M with ESRD secondary to posterior urethral valves lost his first renal transplant (tx) from chronic C4d+ AMR with HLA donor specific antibodies (DSA). To avoid preformed HLA antibodies (Ab) (cPRA 71%), his re-tx was a paired exchange 3/6 match. Endothelial cell crossmatch and MHC class I related chain A Ab were screened and negative, but, his AT_1R-Ab were 110U/mL (normal <10U/ml). A hypercoagulablity work-up was otherwise negative. Pre-tx desensitization with 3 days (d) of plasmapheresis (pp), IVIG, and rituximab reduced his titer to 34U/mL. He was induced with thymoglobulin (ATG), and maintained on a steroid based regimen with FK and MMF. Mild HTN (130/90) post-tx day (PTD) 3 resolved with losartan. Due to mild decline in AG function and persistent AT_1R-Ab 28U/mL, pp was performed PTD 6-9 with improvement in function and titer. PTD 15 he developed AG tenderness, fever, Cr to 2.5mg/dL, and AT_1R-Ab to 17U/ml. DSA were negative and biopsy revealed ACR IB and C4d- AMR. Cr and oliguria improved with treatment; however, he acutely developed increased pain and anuria. MR angiogram revealed renal artery thrombosis with AG loss PTD 21.



Discussion: We report the first pediatric case of AT_1R-Ab with accelerated rejection and renal artery thrombosis. We avoided tx through DSA, as DSA and AT_1R-Ab have been linked to accelerated AMR.[1] Adjunctive therapies to desensitization, such as anticoagulation, for AT_1R-Ab require further investigation. 1.Kelsch, R.et al. 'Accelerated Kidney Transplant Rejection' *Transplantation, 2011.*

Funding: Private Foundation Support

SA-PO737

IgG-Kappa Light Chain Crystalline Plasma Cell Inclusions in a Transplant Kidney Biopsy Vanessa Moreno,[1] Laura R. Kidd,[1] Rupinder Chatha,[2] William F. Glass.[1] *[1]Pathology and Laboratory Medicine, The Univ of Texas - Health Science Center, Houston, TX; [2]Nephrology, Houston Nephrology Group, Cypress, TX.*

Introduction: Intracellular immunoglobulin crystals within lymphoid cells and plasma cells are uncommon but well-documented in B-cell lymphoproliferative disorders (plasmacytoma, multiple myeloma, chronic lymphocytic leukemia, lymphoplasmacytic lymphoma, mucosa-associated lymphoid tissue lymphomas and high-grade lymphomas); but rarely occur in reactive plasmacytic infiltrates.

Case Description: A 65 year-old male with hepatitis C and status post-renal transplantation (2007) underwent kidney biopsy due to acute renal failure with elevated BUN (52 mg/dl) and Cr (2.6 mg/dl). He also presented with anemia (Hgb 7.9 g/dl), thrombocytopenia (26,000/uL), hypoalbuminemia (2.7 g/dl), and increased beta-2 microglobulin (14.1 mg/dl). SPEP and UPEP showed elevated free kappa and lambda-light chains, but kappa/lambda ratio was normal. Calcium levels were normal. The kidney biopsy showed no evidence of T-cell or antibody mediated rejection. However, focal medullary interstitial infiltrates rich in lymphocytes and polyclonal plasma cells were present. In addition, numerous eosinophilic, rectangular to rhomboid-shaped crystals were seen in the cytoplasm of plasma cells and in the interstitium which were accentuated in H&E-sections by fluorescence microcopy with the Texas red filter. The crystals were positive for IgG and kappa-light chain, but negative for IgA, IgM, and lambda-light chain. By electron microscopy, plasma cells contained intracytoplasmic rectangular to rhomboid-shaped crystals with parallel linear fine-structure (14nm repeat) within rough endoplasmic reticulum.

Discussion: The presence within a polyclonal lymphoplasmacytic infiltrate of a plasma cell subset containing monoclonal, IgG-kappa crystals has not been previously reported in native or renal allograft biopsies. While early plasma cell dyscrasia is not entirely ruled out, the overall polyclonal plasma cell population and mixed inflammatory infiltrate favors a reactive process. This conclusion is supported by reports of similar findings in *Helicobacter*-associated gastritis, osteoarthritis and bilateral papillary conjunctivitis.

SA-PO738

Takotsubo Cardiomyopathy after Simultaneous Liver–Kidney Transplantation Ekamol Tantisattamo, Sundus A. Lodhi. *Renal Div, Emory Univ.*

Introduction: Takotsubo cardiomyopathy (TC) is a rare cardiac complication of severe stress situation. Postoperative period especially after transplantation involving several immunosuppressive medications may increases risk of TC. We report a case of simultaneous liver–kidney (SLT) transplant recipient with new onset acute decompensated heart failure (ADHF) from TC.

Case Description: A 56 year-old woman with end stage liver disease secondary to non-alcoholic steatohepatitis and α-1 antitrypsin deficiency and end stage renal disease from hepatorenal syndrome requiring hemodialysis underwent SLK with basiliximab induction. Maintenance immunosuppression was with tacrolimus, mycophenolate mofetil, and prednisone. Postoperative course was complicated by delayed graft function requiring continuous renal replacement therapy (CRRT). On postoperative day 3, she developed cardiogenic shock. EKG showed sinus tachycardia without ST segment change. Troponin-I rose to 1.42 ng/mL. Transthoracic echocardiogram (TTE) showed decreased ejection fraction (EF) of 25% with basal hypokinesis and mid and apical akinesis (Figure1). Preoperative cardiac catheterization revealed normal coronary arteries and a normal EF. Her clinical condition was improved with inotropes and careful fluid management by CRRRT, which were subsequently discontinued once her renal function improved. Followup TTE one month late showed normalized EF (Figure1). She was discharged with normal liver function and serum creatinine of 1.53 mg/dL on postoperative day 15.

Discussion: TC is a rare but reversible cardiac complication after stress situation such posttransplantation. It should be considered as part of the differential diagnoses of new onset cardiomyopathy in post-organ transplantation, particulary if there is documented absence of significant coronary artery disease. Early diagnosis and treatment can normalize cardiac function and avoid morbidity and mortality.

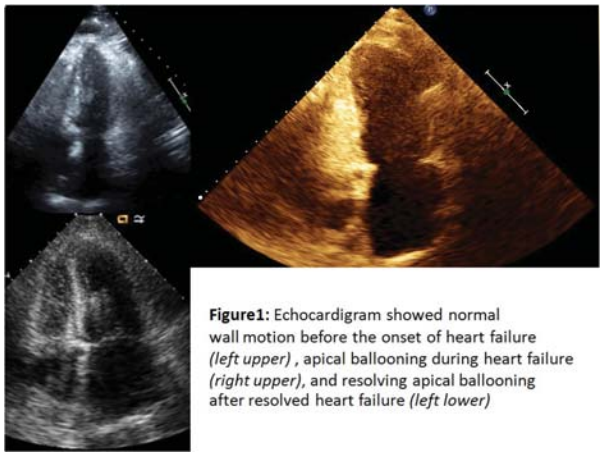


Figure1: Echocardigram showed normal wall motion before the onset of heart failure *(left upper)* , apical ballooning during heart failure *(right upper)*, and resolving apical ballooning after resolved heart failure *(left lower)*

SA-PO739

Late Onset Cerebral Toxoplasmosis in a Kidney Transplant Recipient Ekamol Tantisattamo,[1] Michael J. Connor,[2] John Doran.[1] *[1]Renal Div; [2]Div of Pulmonary, Allergy, and Critical Care Medicine, Emory Univ.*

Introduction: Opportunistic infection usually occurs during the first 6 months post-kidney transplantation when the immunity is intensely suppressed. *Toxoplama gondii* infection commonly presents in the first 3 months. We report a case of cerebral toxoplasmosis 8 years post-kidney transplantation.

Case Description: A 63 year-old woman with history of ESRD underwent deceased donor kidney transplantation with thymoglobulin induction 8 years ago. Immunosuppressive medications were tacrolimus 3 mg twice a day, mycophenolate mofetil (MMF) 500 mg twice a day, and prednisone 7.5 mg daily. She presented with left-sided weakness for 2 days. Head CT scan showed a large area of edema in the right frontal lobe with right to left shift. Brain MRI revealed a ring-enhancing mass in the right frontal lobe (Figure1). She underwent right frontal craniotomy for resection of the mass. Pathology revealed necrotizing cerebritis. Focal bradyzoite cysts and tachyzoite forms of toxoplasma were present and immunostain confirmed toxoplasmosis. She received 6-weeks of pyrimethamine, leucovorin and sulfadiazine (switched to clindamycin due to hyperkalemia), followed by lifelong prophylaxis. MMF was discontinued and tacrolimus dose was decreased to 2 mg twice a day. One week later, low dose MMF 250 mg twice a day was restarted and target tacrolimus trough level was decreased to 3-5 ng/ml. Renal function was stable during the admission with serum creatinine of 1.8 – 2 mg/dl. She was discharged on hospital day 28 to rehabilitation center with persistent left weakness, dysarthria and cognitive deficits.

Discussion: Cerebral toxoplasmosis has a poor prognosis if not promptly treated, so high index of suspicion and early tissue diagnosis are warranted even in late post-kidney transplantation in order to avoid treatment delays and lessen morbidity and mortality.

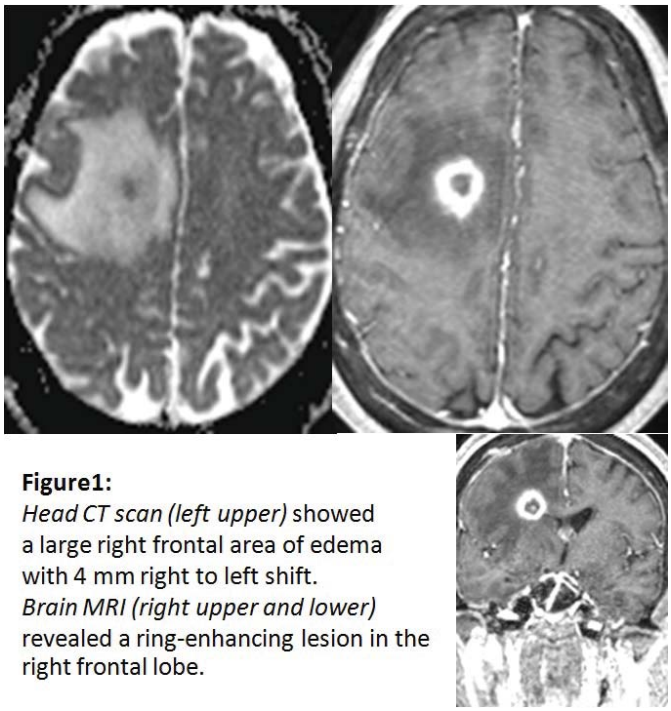


Figure1:
Head CT scan (left upper) showed a large right frontal area of edema with 4 mm right to left shift.
Brain MRI (right upper and lower) revealed a ring-enhancing lesion in the right frontal lobe.

SA-PO740

Foscarnet-Induced Crystal Nephrotoxicity in a Kidney Transplant Recipient Ekamol Tantisattamo,[1] Thomas E. Rogers,[2] Alton Brad Farris,[2] Sundus A. Lodhi.[1] *[1]Renal Div; [2]Dept of Pathology, Emory Univ.*

Introduction: Foscarnet is the treatment of choice for ganciclovir-resistant cytomegalovirus (CMV) infection. Renal injury from crystalline nephropathy is a serious side effect and can challenge the treatment of CMV disease.

Case Description: A 38-year-old CMV seronegative man with ESRD underwent kidney transplantation with a CMV seropositive deceased donor. He was on valganciclovir 450 mg daily for CMV prophylaxis. Six weeks post transplant, he developed primary CMV infection with serum PCR of 4800 IU/mL. Maintenance immunosuppression was decreased, and valganciclovir was increased to 900 mg bid with subsequent reduction in CMV PCR to 580 IU/mL. Three months post transplant he developed abdominal pain, diarrhea, and CMV PCR elevation to 199,000 IU/mL. Genetic mutation testing of UL97 confirmed ganciclovir-resistant CMV. Foscarnet and normal saline were initiated. CMV PCR decreased to low positive within 1 month. However, serum creatinine (SCr) rose to 2.35 mg/dL from the baseline of 1.1 mg/dL. A kidney allograft biopsy was performed and revealed unstained polarizable crystalline material in the tubules and in the hila of involved glomeruli (Figure1). IV fluid was increased and foscarnet was discontinued. Five months later, SCr improved to 1.5 mg/dL with low positive CMV PCR. Serum CMV PCR initially

rebounded to a peak of 50,000 IU/mL, but he did not have evidence of organ involvement. The viremia was managed with a combination of maribavir and CMV IVIG, ultimately leading to a negative serum CMV PCR.

Discussion: As a successful treatment for ganciclovir-resistant CMV, foscarnet is a highly nephrotoxic medication. Here we present a rarely reported case of crystalline nephropathy in the renal allograft. Medication discontinuation and hydration are the mainstays of treatment. Subsequent treatment of CMV disease requires judicious use of medications.

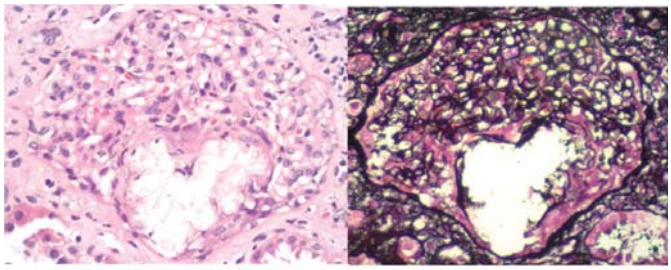


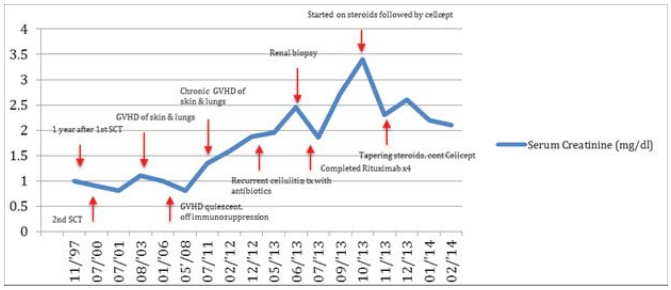
Figure1: A glomerulus has a prominent collection of crystalline material at the hilum (hematoxylin and eosin, left, and Jones silver stain, right, both 400x original magnification).

SA-PO741

Thrombotic Microangiopathy of the Kidney in a Hematopetic Stem Cell Transplant Recipient Successfully Treated with Cellcept: A Presumed Graft versus Host Disease of the Kidney Naveed S. Anwar,[1] Maen Abdelrahim,[2] Ala Abudayyeh.[2] *[1]Nephrology, The Univ of Texas Medical School at Houston, Houston, TX; [2]Nephrology, The Univ of Texas MD Anderson Cancer Center, Houston, TX.*

Introduction: The causes of AKI in HSCT could be due to prerenal, nephrotoxins, engrafment syndrome, veno-occlusive disease of the liver, thrombotic microangiopathy(TMA),and calcineurin inhibitors. A rare late cause of renal dysfunction in allogenic SCT patients is GVHD of the kidney.We will be presenting TMA of the kidney as the first reported case of probable GVHD of the kidney successfully treated with cellcept.

Case Description: A 37-year-old male with a history of chronic myelogenous leukemia underwent allogeneic SCT fourteen years ago. Conditioning regimen included cyclophosphamide and total-body radiation.Post-transplant course was complicated by GVHD of the skin, liver and lungs. He has been off immunsosuppression for over 5 years and his creatinine started to rise from 1.0mg/dl to 1.96mg/dl with proteinuria of 5gm. He underwent a renal biopsy that revealed thrombotic microangiopathy. Initially he was treated with rituximab with a partial response. He later relapsed with creatinine peaked at 3.7mg/dl and proteinuria of 8gm.The patient was initiated on steroids and Cellcept with dramatic improvement in his serum creatinine at 2.0mg/dl and proteinuria at 3gm for the last eight months



Discussion: Recently, nephrotic syndrome occurring in allogeneic SCT patients has been considered to be a possible presentation of chronic GVHD of the kidney. The pathogenesis is yet to be defined, but it has been shown that 52% of patients with HCT-associated membranous nephropathy had active GVHD at the time of diagnosis.This is the first case to report TMA as a presentation of possible kidney GVHD post stem cell transplant successfully treated with cellcept.

SA-PO742

Acute Pyelonephritis of Transplant Kidney with False Positive C4d Staining Mimicking Acute Humoral Rejection Shehpar Khan,[1] Mumnoon Haider,[1] Bruce A. Jones,[2] Jerry Yee,[1] K.K. Venkat.[1] *[1]Div of Nephrology, Henry Ford Hospital; [2]Dept of Pathology, Henry Ford Hospital, Detroit, MI.*

Introduction: Circumferential diffuse (in more than 50% of peritubular capillaries [PTC]) C4d staining is a key diagnostic marker for both Acute Humoral Rejection (A-HR) and Chronic Humoral Rejection (C-HR) of renal allografts. Complement C4 activation in HR generates C4 breakdown products of which C4d has the longest half-life and strongest tissue binding affinity, making it a good marker for C4 activation. We report the occurrence of false positive C4d staining in a patient with allograft pyelonephritis with AKI.

Case Description: A 40 y.o. woman with ADPKD and stage-5 CKD underwent preemptive living unrelated donor (friend) renal transplant (bazliximab induction, tacrolimus/mycophenolate/prednisone maintenance). Nadir serum creatinine (SCr) posttransplant was ~ 2.0 mg/dL. One month after completing six months of trimethoprim-sulfa prophylaxis, fever, oliguria and AKI (SCr 8.43 mg /dL) developed. Urinalysis showed pyuria, hematuria, and positive nitrite test. Allograft biopsy revealed interstitial and tubular wall infiltration ("tubulitis") by PMNs and lymphocytes with many tubular intraluminal PMNs. The C4d immunoperoxidase stain was diffusely positive (90-95% of PTC) with atypical granular PTC wall staining and frequent intraluminal staining. Treatment for A-HR was being considered but positive urine and blood cultures for *Klebsiella pneumoniae* were reported soon after the biopsy. Donor-specific anti-HLA antibody (DSA) was negative. SCr returned to baseline within 1 week with intravenous antibiotic therapy alone and remained between 1.8 and 2.1 mg/dL over the subsequent 7 months. Repeat biopsy 1 month after AKI resolved was negative for C4d.

Discussion: Recovery from severe AKI without added (or change in maintenance) immunosuppression and the negative DSA test confirm that our patient did not have A-HR. Thus, allograft pyelonephritis may cause false and strongly positive C4d staining in PTC, suggestive of A-HR and may result in unnecessary aggressive immunosuppressive therapy. Atypical, granular and intraluminal C4d-PTC staining, in the setting of acute pyelonephritis, should be interpreted with caution.

SA-PO743

Severe Acute Cellular (ACR) and Humoral Rejection (AHR) After Renal Transplant: A Case Report Snigdha Reddy, Jerry Yee, Anita K. Patel. *Div of Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Introduction: Human leukocyte antigen (HLA) donor-specific antibodies (DSA) have been widely associated with poor graft survival. The role of non-HLA antibodies with antigenic targets such as various minor HLA antigens, vascular receptors, adhesion molecules, and intermediate filaments is not clearly understood. We report a case of severe acute cellular and endothelial precursor cell (EPC) antibody-mediated rejection.

Case Description: A 49 year-old immigrant male with end-stage renal disease of unknown etiology with a history of a failed living-related renal transplant underwent a second deceased-donor kidney transplant from a 58 year-old male with a serum creatinine (SCr) of 1.1 mg/dL and a Kidney Donor Profile Index (KDPI) of 70%. The patient received thymoglobulin (TMG) for induction, but developed delayed graft function. The immediate postoperative course was complicated by anuria and revision of the renal artery anastomosis. Allograft biopsy revealed focal infarction. Postoperatively, the urinalysis showed numerous dysmorphic RBCs, cellular casts and urine protein-to-creatinine ratio of 3 g/g. DSA were negative. An intraoperative biopsy on day 11, done during surgery for small bowel obstruction, revealed acute T-cell mediated rejection, Banff 2A, glomerulitis, peritubular capillaritis and endothelialitis. C4d staining was absent. Electron microscopy demonstrated podocyte injury with effacement and endothelial cell detachment from the glomerular basement membrane. In the absence of HLA DSA, the histocompatibility lab flow crossmatch was positive for EPC IgM and IgG antibodies. The patient was treated for severe ACR and AHR with TMG, steroids, and plasma exchanges with intravenous immunoglobulin (IVIG) per institution protocol. His urine output improved and hemodialysis was discontinued as kidney function improved, with a nadir SCr of 1.65 mg/dL.

Discussion: Post-transplant rejection may be mediated by simultaneous ACR and AHR. Investigation for non-HLA antibodies should be carried out when HLA DSA are absent. A better understanding of effector mechanisms between HLA and non HLA antibodies is required to improve targeted therapy.

SA-PO744

Primary Graft Dysfunction Secondary to Early Cell Mediated Rejection after Zero Mismatch Kidney Transplantation Nissreen S. Elfadawy,[1] Gabriela Soledad Diaz,[2] Andres G. Chiesa-Vottero,[1] Emilio D. Poggio,[1] Richard A. Fatica,[1] Saul Nurko,[1] Brian R. Stephany,[1] Stuart M. Flechner,[1] Medhat Askar.[1] *[1]The Kidney Transplant Program, Cleveland Clinic, Cleveland, OH; [2]Unidad de Inmunología e Histocompatibilidad, Hospital Durand, Buenos Aires, Argentina.*

Introduction: We report on a primary graft dysfunction and early acute cell mediated graft rejection (ACR) in a highly sensitized recipient after receiving a zero mismatch (0MM) kidney transplantation.

Case Description: The recipient is a 40-year-old male, with end stage renal disease secondary to IgA nephropathy with previous 2 failed transplants. He subsequently presented with high level of HLA allsonsitization including multiple HLA-DP antibodies (cPRA=100%). The third allograft was a deceased donor (DD) 0MM graft for all HLA including DP (he was allele level mismatch at DQB1) with negative DSA and flow cytometry crossmatch. Donor was a 27-year-old; the implant biopsy showed no sclerosis and normal architecture of the allograft. Despite initially diuresing graft immediately after revascularization alongside induction with IL2-RB and maintenance immunosuppression with Tac/MMF/pred, the patient remained anuric, and he required dialysis on postoperative day 1 (POD1) due to hyperkalemia. Graft biopsy was done on POD9 which showed ACR BANFF grade 2A. The peritubular capillaries stained negative for C4d, and DSA were negative. Combination therapy consisting of antithymoglobulin (ATG), plasmapheresis and intravenous immunoglobulin (IVIG) failed to improve the graft function. Graft biopsy was repeated on POD50 and showed chronic active T-call mediated rejection. Another cycle of ATG was given. By 3.5 months post-transplant, he had no clinical signs of graft recovery, and remained dialysis dependent.

Discussion: This case is the first to report primary graft dysfunction and evidence of cell mediated rejection in a high sensitized patient after 0MM kidney transplantation. Our findings lend support to the notion that highly sensitized kidney transplant candidates are

at increased risk of cell mediated rejection and graft loss even in the context of 0MM DD kidney transplantation, and in the settings of prospective negative DSA and flow crossmatch.

SA-PO745

High-Dose Chemotherapy-Induced Severe Thrombotic Microangiopathy (TMA) in an Autologous Stem Cell Transplant (SCT) Recipient for Post-Renal Transplant B Cell Lymphoma:Critiical Therapeutic Role of Plasma Exchanges (PE) and Eculizumab Despite No Tumor Recurrence or Mutations in the Complement Pathway Proteins (CPP) Usman Z. Bhutta,[1] Kai Lau.[1,2] *[1]Nephrology, Univ of Oklahoma, OKC, OK; [2]Medicine, VAMC, OKC, OK.*

Introduction: Differential diagnoses of TMA in renal transplants include calcineurin inhibitor toxicity, radiation, rejection, cancers, sepsis, thrombotic thrombocytopenia purpura, hemolytic uremic syndrome (HUS) and the increasingly recognized mutations in CPP, producing atypical (a) HUS. Recently we cared for a 36-year-old man with post-transplant B cell lymphoma for which he underwent resection, followed by rituximab, high-dose chemotherapy with cytoxan, adriamycin, vincristine and autologous SCT. Despite complete lymphoma remission and full SCT engraftment, a few mon later, he showed persistent and worsening thrombocytopenia, hemolytic anemia and acute allograft failure.

Case Description: History, clinical course and lab findings were reviewed to gain insights in potential pathogenesis and management.

Serum (S) creatinine (cre) rose relentlessly from 1.4 to 2.8 mg%. Renal biopsy showed severe TMA and no antibody-mediated rejection. Donor-specific HLA antibodies were negative. Despite steroid pulses and omitting tacrolimus, S cre steadily rose to peak at 7.5. Platetets (Plt) fell from 156 to 46 k/mm3; Hgb from 11 to 5.6 g%. LDH rose from 287 to 870 U/L. ADAMTS 13 activity was normal. Genetic testing and studies of CCP showed no defects. He received plasma exchanges (7 courses each ~6 sessions in 5 mon) reducing mean LDH by 207 U/L and raising haptoglobin by 26 mg%. Though C3 [71 (nl >85 mg%)] and C4 [8 (nl >13 mg%] were mildly low, his rapidly deterioration forced us to try eculizumab, monoclonal antibodies versus C5. After 11 doses in 5 mon and with extra rituximab, his plt rose to 200 K/mm3, Hgb stabilized at 8.9 g% without transfusion, LDH <500, S cre ~ 5 mg%. He was asymptomatic and able to work full time.

Discussion: By exclusion, we attribute his TMA to high-dose chemotherapy though deemed very rare in autologous (versus allogenic) SCT without prior radiation. Previous reports suggest poor prognosis but our patient illustrates possible sustained remission over time by combining PE and eculizumab.

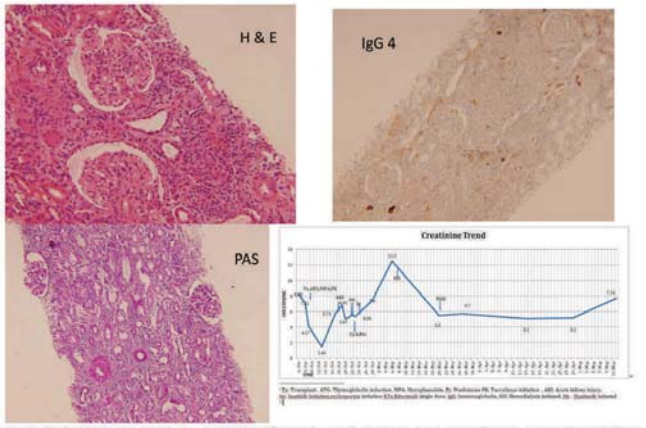
Funding: NIDDK Support, Veterans Affairs Support, Private Foundation Support

SA-PO746

Novel Use of Masitinib for Recurrent Familial Interstitial Nephritis Post Renal Transplantation Siddiq Anwar, Ravkiran Kaur Khurana, Helen Liapis, Timothy A. Horwedel, Daniel C. Brennan. *Washington Univ School of Medicine.*

Introduction: Recurrence of familial interstitial nephritis(FIN) post kidney transplantation (KT) is previously reported. We report a fulminant course of acute interstitial nephritis(AIN) post KT.

Case Description: 32 yr. white man with FIN underwent his third KT; 0-A,2-B, 1-DR Mismatch deceased donor(DD)KT with thymoglobulin induction, prednisone taper, myfortic 360mgBID, Prograf - aim 5-7ng/L plus Valgancylcovir, Bactrim and fluconazole prophylaxis. Creatinine (SC) was 1.4mg/dl at discharge.Represented week later with SC of 5.2mg/dL needing dialysis (HD). Biopsy showed severe AIN with negative C4d. Bactrim, aspirin +omeprazole were stopped and high dose prednisone commenced + Rituximab given in view of 1 donor specific antibody (B7:MFI 12, 817). We commenced him on Imatinib(Tyrosine kinase inhibitor-TKI). As his insurance would not pay, Masitinib 300mg Qd was commenced 4 weeks after his initial presentation after obtaining on compassionate grounds. As he remained on HD after 5 weeks MB was discontinued



Sample contained about 17 glomeruli, of which none are globally sclerosed and are unremarkable. The tubules show extensive tubular injury. There is mild tubulitis. There is diffuse severe acute interstitial nephritis with eosinophils and focal vague granulomas. There is mild interstitial fibrosis and tubular atrophy. The arteries and arterioles are moderately thickened. Arteriolar hyalinosis is not seen. At least two medium to large sized arteries are present and show no endothelialitis. Peritubular capillaritis is not present. PAS x2 shows minimal tubulitis with the majority of inflammation in the interstitum. The trichrome stain highlights no interstitial fibrosis. The findings show mild tubulitis. IgG4 staining was performed and was negative

His 1st KT from his mother was lost due chronic rejection (CAMR) after 15 yrs and lost his 2nd KT (DD) in 6 months due to AIN. Sister's KT from her eldest brother was lost due to CAMR after 15yrs and lost her 2nd KT(DD) early due to AIN. Brother received KT from his father which has lasted > 15 yrs.

Discussion: We believe recurrence of FIN post KT is autoimmune process that occurs in DDKT due to introduction of foreign antigens however living related KT provides relative protection as the tubular epitope was similar. Recurrence has been reported within weeks to years post KT. TKI have immunomodulating and antifibrotic properties. In this case though ineffective in reversing his inflammatory process it was well tolerated with no untoward events. Further control studies are needed to define its use in kidney diseases. Genetic workup is underway in this family cohort.

SA-PO747

Wunderlich Syndrome in a Transplant Recipient Venkatesh Kumar Ariyamuthu,[1] Tanya Tocharoen Tang,[1] Muhammad Omer,[1] Daniela P. Ladner,[2] Mohammed Javeed Ansari.[1] *[1]Nephrology, Feinberg School of Medicine, Northwestern Univ, Chicago, IL; [2]Surgery, Feinberg School of Medicine, Northwestern Univ, Chicago, IL.*

Introduction: Spontaneous rupture of renal cell cancer (RCC) is rare yet well-recognized entity. Wunderlich syndrome is spontaneous non-traumatic renal hemorrhage and we report one such case in a renal transplant recipient caused by Papillary RCC (PRCC).

Case Description: 54 year old African American male who had been on hemodialysis for 6 years for End Stage Renal Disease secondary to hypertension underwent a deceased donor kidney transplant in 2006 which failed and received another kidney transplant in 2011. He presented with sudden onset right flank and right lower quadrant abdominal pain. Computed tomography of abdomen was initially reported as consistent with a large right retroperitoneal hemorrhage later revised as an intra-renal hematoma. The patient underwent transcatheter embolization of the right native kidney and subsequently underwent exploratory laparotomy with right native nephrectomy due to high clinical suspicion of underlying RCC in light of the dialysis vintage. This suspicion was further heightened by the fact that the patient's hemoglobin was 15 g/dl despite CKD stage 4, which hinted towards paraneoplastic erythrocytosis from RCC. Pathological examination revealed Fuhrman's Grade 1 PRCC, type 1. A CT of chest and PET scan were negative. Immunosuppression was lowered given the malignancy.

Discussion: Wunderlich syndrome is commonly caused by tumors, vasculitis and cysts. Patients may present with the classic Lenk triad, including acute flank or abdominal pain, palpable flank mass, and hypovolemia. CT is 100% sensitive for detection of retroperitoneal hemorrhage and better than ultrasound for identification of an underlying renal mass, however CT can miss underlying tumors because of smaller size of mass, presence of surrounding hematoma and acquired cysts. Incidence of RCC is increased in both ESRD patients and transplant recipients. Erythrocytosis is a presenting feature of RCC. Currently there are no guidelines for screening for RCC in ESRD patients. Our case reiterates the significance of routine screening for RCC in renal transplant recipients.

SA-PO748

Plasmapheresis as Bridging Therapy for Primary Non-Function following Liver Transplantation Anja H. Bienholz,[1] Bartosz Tyczynski,[1] Ali Canbay,[2] Andreas Kribben,[1] Andreas Paul,[3] Fuat H. Saner.[3] *[1]Dept of Nephrology, Univ Hospital Essen, Univ Duisburg-Essen, Essen, Germany; [2]Dept of Gastroenterology and Hepatology, Univ Hospital Essen, Univ Duisburg-Essen, Essen, Germany; [3]Dept of General, Visceral and Transplant Surgery, Univ Hospital Essen, Univ Duisburg-Essen, Essen, Germany.*

Introduction: Primary non-function (PNF) following liver transplantation (LTX) is a rare, but severe complication of unknown cause. The only life-saving therapy consists of a second LTX within a narrow time frame. To bridge the time to organ allocation and to ensure a clinical condition which seems to make a successful LTX feasible is a special difficulty.

Case Description: We report on two 41 and 56 years old patients (pre-operative MELD-Score 11 and 16) with PNF following LTX were plasmapheresis ensured retransplantation.

Initial LTXs were without complications and free of blood product transfusions. Despite ultrasound doppler proofed regular perfusion, both patients developed PNF immediately following surgery with transaminase elevation GOT/GPT >10,000/>7,500 U/l, severe coagulopathy and lactate levels >17 mmol/l. Hemodynamic instability required vasopressors in cumulative concentrations of >5 resp. >1.7 µg/kg/min. Both patients developed anuric acute kidney failure requiring renal replacement therapy. Patients were listed "high urgency" for retransplantation. Lacking further therapeutical options patients received two resp. one plasmapheresis with replacement of plasma volume by 3.4-4.0 l fresh frozen plasma during organ allocation. Besides an adequate treatment of coagulopathy, transaminases were reduced by >50% and lactate levels to <3 mmol/l. Already during treatment demand for vasopressors declined to concentrations of <1,5 und <0,4 µg/kg/min. Plasmapheresis stabilized the clinical conditions of the patients and allowed a second, successful LTX. Histological work-up of both cases indicated massive necrosis (>80%) of the first grafts.

Discussion: Plasmapheresis as bridging therapy for PNF following LTX seems to be an option especially for hemodynamic stabilization during organ allocation for renewed LTX. The underlying mechanisms going beyond plasma infusion are unknown by today.

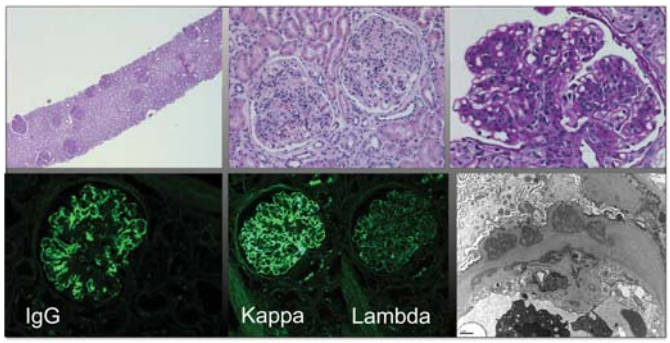
Funding: Private Foundation Support

SA-PO749

Proliferative Glomerulonephritis with Monoclonal IgG in Renal Allografts Laith Farah Al-Rabadi, Sandeep Ghai, Joel M. Henderson, Jean M. Francis. *Nephrology, Boston Univ Medical Center.*

Introduction: Various glomerular diseases are associated with the deposition of monoclonal IgG. The differential diagnosis of monoclonal IgG glomerular deposition include type 1 cryoglobulinemia, light and heavy chain deposition disease, and immunotactoid glomerulonephritis. Proliferative GN with monoclonal IgG deposits (PGNMID) is a newly described entity that does not conform to any of those subtypes. It represents a form of proliferative GN with electron-dense deposits localized to the glomeruli and the finding of monoclonal IgG deposits on immunofluorescence. Recurrent and de novo PGNMID in the renal allograft have been described in few case series with total of nine cases reported. Herein, we present the clinical-pathologic features of three cases of recurrent PGNMID in the renal allograft showing the variable course of this disease.

Case Description: The 3 patients did not have any bone marrow involvement and there was no evidence of monoclonal protein in the urine and serum immunofixation studies making disease activity monitoring difficult. Case 1 was treated only conservatively with ACE inhibitors but eventually developed worsening renal function. Patient opted to resume chronic dialysis with no further therapy. Case 2 was complicated with the loss of the graft within the first year after transplant, and case 3 (Biopsy below) is actively being treated with bortezomib in the setting of worsening graft function and nephrotic range proteinuria.



Discussion: PGNMID is becoming a more recognized entity. Evaluating those patients for transplant eligibility has not been previously discussed and may be challenging in the absence of distinct data of remission given that most patients have normal bone marrow biopsy and no monoclonal band on serum and urine testing. Data is scarce in terms of follow up and treatment. Some immunosuppressive medications in the new era of transplant may be promising.

SA-PO750

Secondary Oxalosis: An Under Diagnosed Cause of Delayed Graft Function After Kidney Transplantation Varsha Pathak, Iris J. Lee, Avrum Gillespie, Serban Constantinescu, Mythili Ghanta. *Temple Univ School of Medicine.*

Introduction: Secondary oxalosis is associated with malabsorptive states, ethylene glycol poisoning, and excessive vitamin C ingestion. It can be clinically silent in end stage renal disease patients on dialysis. If left untreated after kidney transplantation, secondary oxalosis can lead to oxalate deposition and allograft loss. Primary renal allograft non-function has also been reported as a consequence of secondary oxalosis. We report a case of secondary oxalosis that contributed to prolonged delayed graft function (DGF).

Case Description: 55-year-old male with ESRD on hemodialysis received a deceased donor kidney from a 50-year-old male with hypertension and stroke. Donation was after cardiac death with terminal Cr of 1.2 mg/dl. Post transplant, the recipient required dialysis. Allograft biopsy ten days after transplant showed minimal tubular injury and no rejection. Second biopsy three weeks after transplant due to ongoing dialysis dependency showed extensive deposition of oxalate crystals in the tubules. Review of recipient history suggested enteric hyperoxalosis secondary to chronic pancreatitis and malabsorption. Therapy with oral calcium carbonate was initiated, along with a strict low oxalate diet and adherence to pancreatic enzyme replacement therapy. Renal allograft function recovered four weeks after transplant. 24 hr urine studies showed hyperoxaluria (50ng/dl) and also hypocitraturia, hypomagnesuria. Repeat biopsy eight weeks post transplant showed decreasing burden of oxalate in biopsy sample. Allograft function stabilized with Cr at 2.4 mg/dl at 6 months.

Discussion: Enteric oxalate nephropathy may lead to allograft dysfunction and should be considered in the differential for DGF in kidney recipients with history of chronic malabsorptive states. This case emphasizes the need to identify potential causes of secondary hyperoxaluria at the time of initial transplant evaluation, which can lead to prevention or early diagnosis post transplantation.

SA-PO751

Successful Transplantation of Deceased Donor Kidneys From an Anuric Dialysis Dependent Donor with Acute Kidney Injury Varsha Pathak, Akshta Pai, Mythili Ghanta, Serban Constantinescu, Iris J. Lee. *Temple Univ School of Medicine.*

Introduction: Kidneys from donors with acute kidney injury (AKI) are often under utilized for transplantation. Despite studies showing comparable outcomes (3-5 year follow-up) to a standard criteria donor kidney, donor AKI remains the second leading cause of organ discard after harvest. Donor features in AKI that lead to center refusal include: 1. Dialysis dependency, 2. Oliguria or anuria, 3. Patients with disseminated intravascular coagulopathy (DIC) and 4. Terminal serum creatinine (Cr) >2.

Case Description: We report two successful kidney transplants from a donor with severe anuric AKI, dialysis dependency and DIC at the time of organ recovery. The donor was a 17 year old white male, with KDPI of 21%, with an admission Cr of 1mg/dl. Procurement biopsies showed moderate to severe acute tubular necrosis with no evidence of interstitial fibrosis or glomerulosclerosis. The organs were perfused after recovery with acceptable pump numbers. Recipient 1 had delayed graft function (DGF) requiring dialysis for 17 days, but achieved stable allograft function with a Cr of 1.4mg/dl at three months. Recipient no 2 had DGF requiring dialysis for 30 days, with recovery of the Cr to 1.5mg/dl at three months.

Discussion: With a growing wait list, increased acceptance of carefully selected kidneys from donors with AKI is needed. Elevated terminal creatinine and dialysis dependency are strong risk factors for kidney discard, however further studies are needed to optimally identify AKI donor features that are associated with safe and favorable outcomes.

SA-PO752

Minimal Change Disease Superimposed on Chronic Calcineurin Inhibitor Nephrotoxicity in a Stem-Cell Transplant Recipient with Chronic Graft versus Host Disease Egbert C. Liquete,[1] Josephus Sanjorjo,[1] Jerry Yee,[1] Nalini Janakiraman,[2] K.K. Venkat.[1] *[1]Nephrology and Hypetension, Henry Ford Hospital, Detroit, MI; [2]Hematology/Oncology, Henry Ford Hospital, Detroit, MI.*

Introduction: Late renal complications in Stem-Cell Transplant Recipient (SCTR) include CKD from Chronic Calcineurin Inhibitor Nephrotoxicity (CNIT) or CNI-related thrombotic microangiopathy and Graft versus Host Disease (GVHD)-related membranous nephropathy or Minimal Change Disease (MCD).

Case Description: We report the rare, simultaneous occurrence of CKD from CNIT and AKI on a background of GVHD-related MCD. A 40 year-old man, an allogenic SCTR for thalassemia intermedia, developed cutaneous Graft versus Host Disease 1 month after SCT. He was treated with tacrolimus, sirolimus and prednisone and developed CKD (serum creatinine [SCr] 1.5 mg/dL) within 1.5 years after treatment. The SCr increased to 2.04 mg/dL 3 years post- transplant; tacrolimus and sirolimus were discontinued, and prednisone was maintained at 15 mg daily. Within 8 weeks, the patient gained 30 lb and developed anasarca, and the SCr increased to 3.28 mg/dL, with a 24-h urine protein of 30 g and serum albumin of 1.2 g/dL. A kidney biopsy revealed nodular afferent arteriolar hyalinosis, 32% glomerular obsolescence, tubular atrophy, interstitial fibrosis, diffuse podocyte fusion, thickened basement membranes, and no glomerular electron dense deposits. Anasarca resolved, SCr declined to 1.52 mg/dL and the urine protein-to-creatinine ratio was 1.5 g/g, following rituximab administration (375mg/m^2/wk× 4) and an increase in prednisone dosage to 30 mg daily.

Discussion: The severe, abrupt appearance of nephrotic range proteinuria and salutary response to rituximab and prednisone supports a diagnosis of MCD. Its rapid development after tacrolimus and sirolimus discontinuation implies that MCD was a manifestation of GVHD, and superimposed on CNIT-induced CKD. This report demonstrates that MCD may be superimposed on CNIT and establishes the efficacy of rituximab treatment in GVHD-related MCD.

SA-PO753

Rapid Recurrence of C3 Glomerulonephritis after Transplantation in Setting of Delayed Graft Function Neetika Garg,[1] Eliyahu V. Khankin,[1] Anne Nicholson-Weller,[2] Christin Rogers,[3] Isaac Ely Stillman,[4] Martha Pavlakis.[1] *[1]Nephrology, Beth Israel Deaconess Medical Center (BIDMC); [2]Immunology, BIDMC; [3]Pharmacy, BIDMC; [4]Pathology, BIDMC.*

Introduction: C3 glomerulonephritis (C3GN) is a recently described entity resulting from dysregulation of the alternate complement pathway (ACP). Recurrence rate is high (up to 67%), typically occurring months to years after transplantation, and frequently leading to graft loss. Here, we describe a patient with C3GN whose disease recurred in the allograft soon after transplantation, in setting of delayed graft function (DGF).

Case Description: 51 year old Caucasian male on hemodialysis (HD) for 4 years underwent deceased donor renal transplantation. Native renal biopsy was consistent with C3GN. Serum was negative for C3 nephritic factor, anti factor H autoantibody, hepatitis C and monoclonal gammopathy. Genetic evaluation revealed mutations in one allele each of CFH and CFI, encoding regulatory complement factors H and I respectively. Immunosuppression regimen consisted of antithymocyte globulin (6 mg/kg), steroids (withdrawal over 6 days), mycophenolate (2 grams/day) and tacrolimus (target trough 10 – 12 ng/mL). Due to acute kidney injury in donor and prolonged cold ischemia time, DGF was not unexpected. However, despite improving urine output over the next two weeks, he remained HD dependent for solute clearance. He had low C3 level (54 mg/dL), normal C4 level and heavy proteinuria (urine protein to creatinine ratio (PCR) 8 to 15). Allograft biopsy on POD 14 was consistent with acute tubular necrosis and recurrent

C3GN. Following booster vaccinations and antibiotic prophylaxis for various encapsulated organisms, treatment with eculizumab was initiated. After his first two doses, he has been able to come off HD with current creatinine of 2.7 mg/dL and urine PCR down to 0.1.

Discussion: To our knowledge, this is the first report of C3GN recurrence soon after transplantation, in a patient with known complement gene mutations. Ischemic reperfusion injury related complement activation contributing to DGF may have played a role. Terminal complement blockade with eculizumab, when used early, may be effective in limiting renal injury from both these processes.

SA-PO754

Renal Recovery of Pediatric Liver Transplant Patients after Prolonged Renal Replacement Therapy Adnan Safdar,[1] Karen Eldin,[2] Ryan Himes,[3] Ayse Akcan Arikan,[1,4] Moreshwar Desai,[4] Michael C. Braun,[1] Poyyapakkam Srivaths.[1] *[1]Renal Section, Texas Children's Hospital; [2]Dept of Pathology, Texas Children's Hospital; [3]Gastroentrology and Liver, Texas Children's Hospital; [4]Critical Care Medicine, Texas Children's Hospital, Houston, TX.*

Introduction: The RIFLE classification for acute kidney injury (AKI) defines end stage renal disease (ESRD) as the persistence of renal failure for greater than 3 months. In pediatrics the impact of prolonged AKI on renal recovery has not been described in detail. We report renal recovery in a case series of pediatric liver transplant recipients (OLT) with AKI who received a renal replacement therapy (RRT) for more than four weeks.

Case Description: This is a retrospective chart review of all patients less than 21 years of age who received OTL at Texas Children's Hospital and developed AKI requiring RRT for more than 30 days, between June 2013 to June 2014.

Five patients were identified during this period. The etiology of their AKI was multifactorial including hypotension, sepsis, capillary leak, hepato-renal syndrome, and multiple nephrotoxic medications. Despite aggressive medical management, they developed significant fluid overload (>15%) with oliguria/anuria requiring RRT. All five were initially treated with continuous venovenus hemodiafiltration (CVVHDF) and later transitioned to intermittent hemodialysis. Renal biopsy in the two patients with AKI for more than 8 weeks was consistent with ATN. All five patients were eventually discharged home without chronic dialysis.

Case	Age (M)	Creatinine on Admission (mg/dL)	Days on RRT	Creatinine on Discharge (mg/dL)	Creatinine at Last Followup (mg/dL)	eGFR (Modified Swartz) ml/min/1.73 m^2
1	9	0.21	31	0.46	0.29	108
2	13	0.20	34	0.29	0.22	142
3	9	0.20	62	0.12	0.12	261
4	4	0.13	81	0.46	0.34	83
5	7	0.13	157	0.99	0.51	55

Discussion: In this case series, all five patients with after OLT who developed loss and/or ESRD per RIFLE, and required RRT for more than four weeks had significant recovery of renal function; none were dialysis dependent. Our data prompts reconsideration of the RIFLE ESRD classification, since recovery was observed even after three months of dialysis.

SA-PO755

Treatment of Recurrent *Clostridium difficile* Infection with Fecal Microbiota Transplantation in a Renal Transplant Recipient Vishwas Raghunath, Michael G. Suranyi, Angela Makris. *Nephrology, Liverpool Hospital, Liverpool, Sydney, New South Wales, Australia.*

Introduction: Clostridium difficile associated diarrhoea can be devastating in solid organ transplant recipients with a strong tendency for recurrence. The novel use of a known therapy - fecal microbiota transplantation has shown promise in recurring and refractory cases, with minimal complications in this susceptible population, as we illustrate in this case of a renal transplant recipient.

Case Description: We report the case of a 62yr deceased donor renal transplant recipient on standard immunosupression, who had multiple hospital admissions either as a result of, or complicated by Clostridium difficult associated diarrhoea. She was treated with multiple courses of specific antibiotics (vancomicin, metronoidazole and rifaximin) but proved to be refractory to medical therapy. She had a total of 20 hospital admissions across the health district in the period from October 2011 to February 2014, resulting in a total of 397 days spent in hospital. She underwent a fecal microbiota transplant, which resulted in resolution of diarrhea, improvement in well being and has kept her out of hospital.

Discussion: Clostridium difficile is more prevalent in immunocompromised patients, resulting in significant patient morbidity and strain of the health care resources. This novel therapy has the potential to decrease hospitalization rates and length of stay in future especially with early application. To date there are only very few reported cases of the use of this therapy in solid organ transplant patients.

SA-PO756

Case of Acquired Pure Red Cell Aplasia with Synthetic Erythropoietin That Resolved after Renal Transplantation Vishwas Raghunath, Michael G. Suranyi, Angela Makris. *Nephrology, Liverpool Hospital, Liverpool, Sydney, New South Wales, Australia.*

Introduction: Pure red cell aplasia is a rare hematological disorder characterized by severe anemia and a striking absence of erythroid precursors in the bone marrow. In the renal population an important cause is synthetic erythropoietin, causing progressive anemia despite escalating doses of erythropoetin.

Case Description: We report a case of darbepoetin induced pure red cell aplasia in a 52-year male with chronic kidney disease presumed secondary to recurrent renal calculi on maintenance dialysis. He developed transfusion dependent anemia while on synthetic erythropoietin therapy. The diagnosis was confirmed with a bone marrow aspirate, which showed absent erythroblasts. He was found to have antibodies to the generic erythropoetin epitope and darbepoetin epitope. The initial anti-erythropoetin alfa antibody level was 25.40 mcg/mL and anti-darbepoetin antibody level was 24.40 mcg/mL (reference positive of >0.25 mcg/mL). He was treated with a series of immunosuppressive medications including prednisolone, cyclophosphamide, intravenous immunoglobulin, mycophenolate and rituximab over a period of 16 months, without success. He required 2-3 units of packed cells per week to maintain his hemoglobin, resulting in iron overload and attempts at intraperitoneal and oral chelation. He underwent a deceased-donor kidney transplantation following which his hemoglobin stabilized. The antibody levels were reduced at 2 weeks and undetectable by 4 weeks post transplantation. He has remained transfusion-free post transplant other than 2 units of packed cells required for severe haemorrhagic adenoviral cystitis at 12 weeks. His current immunosuppressive regimen includes prednisolone, tacrolimus and mycohenolate. He has developed post transplant diabetes but maintains a stable graft function with a creatinine of 103 umol/L.

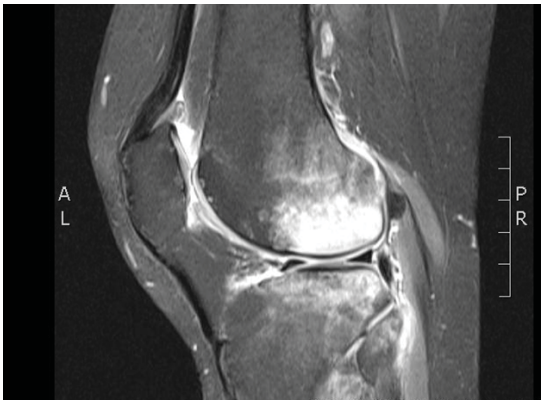
Discussion: The efficacy of immunosuppressive regimen for acquired pure red cell aplasia in end stage kidney disease is suboptimal .We recommend earlier renal transplant to minimize complications of multiple blood transfusions and serial immunosuppression.

SA-PO757

Calcineurin Inhibitor-Induced Pain Syndrome Post-Kidney Transplantation Mohammad Sharif, Thanh Hoang. *Loma Linda Univ Medical Center.*

Introduction: Musculoskeletal pain is a common problem after kidney transplant. It is most often a manifestation of steroid therapy, renal osteodystrophy, hypophosphatemia, gout or avascular necrosis. Here, we present a case of a young female who presented with disabling bone pain post kidney transplant.

Case Description: A 30 year old female with past medical history of hypertension and end stage renal disease secondary to presumed hypertensive nephrosclerosis had a deceased donor kidney transplant (DDKT). She was started and maintained on Tacrolimus, Mycophenolate Mofetil and Prednisone. Ten months post-transplant, she presented with bilateral knee pain. The pain was mainly when she stood up and interfered with her activity. Patient denied any trauma or muscle pain. The pain worsened and involved other joints. Subsequently she became wheelchair bound. Extensive work up was done including: knee x-ray, joint aspiration, serum electrolytes – including phosphate, calcium and magnesium, parathyroid hormone and rheumatological work up. All were negative so knee magnetic resonance imaging was done



It showed extensive bone marrow edema involving distal femur, proximal tibia, and fibula. Therefore, diagnosis of Calcineurin inhibitor-induced pain syndrome (CIPS) was concluded. Tacrolimus was gradually weaned off. Her joint pain slowly improved over time. Interestingly, her grandfather had CIPS when he was taking a Calcineurin inhibitor (CNI) after transplant. This might indicate underlying genetic or familial predisposition to this disease.

Discussion: CIPS is thought to be secondary to hypervascularization, hyperperfusion and hypermetabolism. It is a diagnosis of exclusion. It requires prompt attention from physicians because it is a rare but disabling condition. Management includes reduction or replacement of CNI therapy and administration of calcium-channel blockers to help ameliorate symptoms and improve quality of life.

SA-PO758

Non-HIV Collapsing Glomerulopathy in Living Kidney Transplants: A Case Series Etti Deborah Zeldis,[1] Girish N. Nadkarni,[1] Rebecca L. Kent,[1] Fadi Salem,[1] Ioannis Konstantinidis,[1] Achint Patel,[2] Madhav C. Menon,[1] Michael J. Ross.[1] *[1]Icahn School of Medicine at Mount Sinai, New York, NY; [2]School of Public Health at Mount Sinai.*

Introduction: Collapsing glomerulopathy (CG) is increasingly recognized in non-HIV patients. There have been case-series of CG in allografts in predominantly deceased donor kidney transplants (DDKT). We describe a case series of 8 patients developing CG post living kidney transplant (LKT).

Case Description: We reviewed non-HIV CG cases after LKT from 2005-2013 and abstracted demographic, clinical, laboratory, follow-up and outcome data.We had complete data on 8 patients. The age range was 25-65 years with 5/8 (62.5%) males. Recipient race was African American (AA) (3/8), Caucasian (2/8), Hispanic/Other (3/8), and donor race was AA (2/8), Caucasian (2/8) and Hispanic/Other (4/8). One recipient had a history of FSGS prior to transplant. Four of eight patients were on tacrolimus, and 4/8 were on cyclosporine. Duration from LKT to biopsy varied from 9 months to 15 years (mean 6.25 years). At time of biopsy, the mean urine protein/creatinine ratio was 5.4± 4.6 gms and mean creatinine was 2.3+1 mg/dL. Pathology revealed a mean of 12.6± 5.1 glomeruli per biopsy with 18±11.6% of glomeruli demonstrating collapsing features. One patient underwent plasmapheresis, and one was treated with high dose steroids; both patients progressed to ESRD. Graft failure [defined as End stage renal disease(ESRD)] occurred in 6/8 patients after a mean of 14±(22.3) months post biopsy; 5/6 patients progressed to ESRD within 12 months of diagnosis. Overall graft survival of those who progressed to ESRD was 6.6(+/2.9) years.

Discussion: We describe one of the largest case series of non-HIV CG in LKT. The prognosis remains poor with most patients progressing rapidly to graft loss. Interestingly, the two patients with CG who did not experience graft loss were Caucasian with Caucasian donors. Larger series including multi-institutional registries are needed to elucidate its natural history, identify risk factors for progression, and to determine strategies to improve clinical outcomes.

SA-PO759

Unique Case of Extremely High Tacrolimus Level Nivin Haroon, Atul Singh, Zeenat Yousuf Bhat. *Nephrology, Wayne State, Detroit, MI.*

Introduction: Tacrolimus (Fk) is an effective immunosuppressive agent derived from the fungus Streptomyces tsukubaensisde.Despite being a very potent immunosuppressant, its use is complicated by narrow therapeutic range, significant individual variation in pharmacokinetics and complex and significant drug-drug interactions.We report a case of very high Fk level (>120ng/ml) in a patient who was on antiretroviral medication and Fk.

Case Description: The patient is a 40 year old who underwent a deceased donor renal transplant in 2009.His immunosuppressant regimen includes Fk 0.2mg once a week, Cellcept 500mg twice daily and prednisone 5mg daily.His significant past medical history include human immunodeficiency (HIV) infection diagnosed in 1993 and on combination antiretroviral therapy (cARV) since diagnosis. His regimen included ritonavir 100 mg daily atazanavir 300 mg daily and lamivudine-zidovudine 150 mg in morning and 300 mg in evening.Patient was admitted with significant complaints of head ache,malaise and diarrhea of 3 day duration. His clinical exam was unremarkable including detailed neurologic exam, He did not have any tremors.Lab work showed acute kidney injury with a peak creatinine (Cr) of 2.5 mg/dL from a baseline Cr around 1.4 mg/dL.The FK level was reported as more than 120 mg/dL.Repeat on subsequent days were also reported as more than 120 mg/dl persistently.Despite these patient only complaints was that of his headache. His FK toxicity was attributed to a combination of compounding error from pharmacy and the significant drug interaction with cARV medications keeping the levels higher for over a week.He was treated by withholding FK and cARV medications for that one week duration.His renal function and symptoms of headache improved paralleling the FK levels.

Discussion: To our knowledge there are only a handful of literature on Fk toxicity and treatment of overdose.The highlight of this case report is an extremely high Fk level with negligible clinical outcomes and complete renal recovery despite the postulates of severe central nervous system adverse effects.We also would like to draw attention to the severe drug interaction and the conservative approach to manage such high FK levels.

SA-PO760

Ureteral Herniation into the Inguinal Canal Causing Transplant Obstructive Uropathy Chelsea Estrada, Deepika Eve Slawek, Doris Chan, Frank Darras, Patrick Gerard Lynch, Nand K. Wadhwa. *Nephrology and Transplantation, Stony Brook Medicine, Stony Brook, Ny.*

Introduction: Obstructive uropathy is a reversible cause of allograft dysfunction. Etiology ranges from ureteral clot, ureterolithiasis, ureteral stricture, hematoma or lymphocele. We report a rare case of an inguinal herniation of the transplant ureter leading to acute kidney injury from urinary obstruction.

Case Description: A 44 year old man with ESRD due to HUS received his first kidney transplant in 1976, which failed secondary to chronic allograft nephropathy in 1991. He remained on hemodialysis until 1998 when he received his second deceased donor transplant. He had one episode of acute rejection treated with methylprednisolone in 1999. He was managed by an outside institution and reported a baseline serum creatinine of 2.0 mg/dL which gradually increased to 3.1 mg/dL over the past one year. He recently presented for a routine follow-up visit and he had no complaints. Physical examination revealed BP of 130/84 mmHg, HR 70 beats a minute and temperature of 36.5C. Chest examination was normal. Abdomen was obese, and the left lower quadrant allograft was non-tender. He had no leg edema. Immunosupression included tacrolimus 1 mg twice a day, azathioprine 50 mg daily and prednisone 5 mg daily. His BUN was 70 mg/dL, serum creatinine 3.4 mg/dL, serum potassium of 5.3 mg/dL, WBC 8.9k/mcL, Hgb 13.3g/dL, and platelets 123 k/mcL. Urinalysis showed no protein, blood or cells. Serum tacrolimus level was 4.8 ng/mL. Serum BK virus PCR was negative. A CT scan of the abdomen and pelvis was done due to rising creatinine and revealed hydroureteronephrosis due to herniation of the transplant ureter into the left inguinal canal. He underwent left inguinal hernia repair, ureter repair and re-implantation with stent placement. At outpatient follow-up 1 week later his serum creatinine improved to 1.8 mg/dL.

Discussion: Transplant ureteral herniation into the inguinal canal leading to hydroureteronephrosis and acute kidney injury is rare. Our patient underwent emergent surgical correction with an improvement in his allograft function. Early recognition and prompt intervention of this entity is needed.

SA-PO761

Successful Eculizumab Treatment of Recurrent Atypical Hemolytic Uremic Syndrome after Kidney Transplantation Katherine Garlo,[1] Douglas M. Dressel,[2] John P. Vella.[1] *[1]Div of Nephrology and Transplantation, Maine Medical Center, Tufts Univ School of Medicine, Portland, ME; [2]Dept of Pathology, Maine Medical Center, Tufts Univ School of Medicine, Portland, ME.*

Introduction: Atypical hemolytic uremic syndrome (aHUS) is a rare disorder of the alternative pathway of the complement system. Fifty percent of aHUS cases are linked to genetic mutations, 5% to autoimmune disease, and the remainder are incompletely understood.

Case Description: The patient first presented to an outside hospital at age 28 years with aHUS that developed after a first delivery. In spite of treatment with plasma exchange, she required two years of hemodialysis before receiving a living donor kidney transplant. Two years later, she presented to our institution. She was pregnant at 26 weeks gestation with hypertension and proteinuria. After premature delivery, she developed hemolytic anemia, thrombocytopenia, and oliguric kidney injury requiring dialysis.

Lab Test	Patient's Result (Normal range)
Hb	6.5 (11.8-15.8) g/dL
MCV	96 (80-100) fL
Cr	7.1 (0.50 - 1.30) mg/dL
PLTs	89 (140-440) Thou/uL
AST	35 (0-37) U/L
ALT	19 (0-40) U/L
ALK PHOS	70 (39-117) U/L
LDH	975 (94-250) U/L
Hapto	< 20 (25-200) mg/dL
Blood Smear	2+ Schistocytes (none expected)
ADAMTS13	75% (50-160%)

There was no response to plasma exchange, but Eculizumab led to successful resolution of hemolysis and AKI. Three months after therapy was stopped she relapsed and again needed dialysis. At that time, an allograft biopsy revealed thrombotic microangiopathy. Eculizumab was resumed without plasma exchange leading to resolution of aHUS and acute kidney injury. Her baby survived. She remains on maintenance Eculizumab therapy with a Cr of 1.5mg/dl.

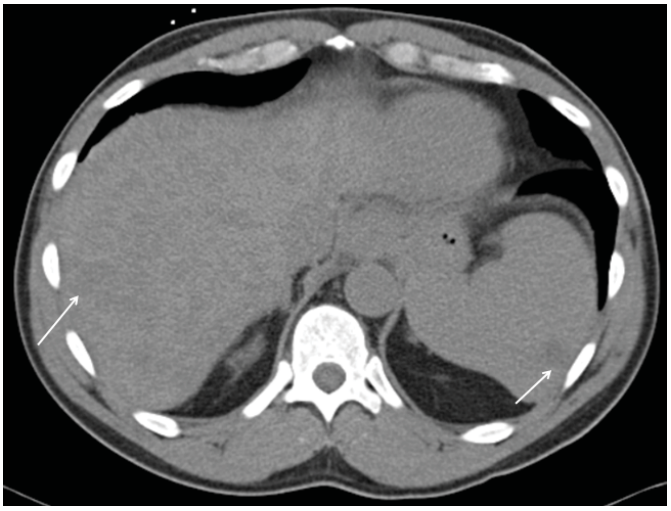
Discussion: Postpartum aHUS is rare but severe disorder with an incidence of 1:25,000 gestations. It usually presents within 48 hours of delivery and is associated with pre-eclampsia, prematurity, and mortality. Many maternal survivors develop ESRD and recurrence after transplantation. Conventional management is often ineffective. Eculizumab is a monoclonal antibody to C5 and suppresses complement amplification. It was FDA approved for treatment of aHUS in 2012.

SA-PO762

Post-Transplant Lymphoproliferative Disorder Presenting as Fever of Unknown Origin Anyeri K. Peguero,[1] Hani Wadei.[2] *[1]Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL; [2]Transplant Nephrology, Mayo Clinic, Jacksonville, FL.*

Introduction: Post-transplant lymphoproliferative disorder (PTLD) is a rare but serious post-transplant complication and can have various presentations. We hereby present a case of PTLD presenting with fever of unknown origin (FUO) in a kidney transplant recipient.

Case Description: 58-year-old African American man received a deceased donor renal transplant with induction immunosuppression consisting of anti-thymocyte globulin (ATG) followed by prednisone, mycophenolate mofetil (MMF) and tacrolimus. At the time of transplant, both donor and recipient tested positive for previous EBV exposure. His post-transplant course was complicated with recurrent rejections requiring parenteral steroids and later ATG. 9 months post-transplant he presented with 2 weeks of pleuritic chest pain, productive cough, fevers, and generalized malaise. He had been treated with azithromycin and later levofloxacin for presumed pneumonia in another institution without remission of his symptoms. Further evaluation showed both blood and urine cultures were negative, chest x-ray failed to show consolidation but he did have transaminitis and detectable plasma EBV PCR. Abdominal CT showed a mottled liver (Figuere 1 left arrow) and a new splenic hypodense lesion (Figure 1 right arrow) but no lymphadenopathy.



Liver biopsy was significant for diffuse large B cell lymphoma which stained positive for CD20 and EBV. MMF and tacrolimus were discontinued and after consultation with oncology; rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP) were started. Two weeks later his symptoms resolved and fever disappeared.

Discussion: PTLD can present as fever in up to 50% of cases. This case highlights the importance of considering PTLD in kidney transplant recipients presenting with FUO.

SA-PO763

Regression of Immunoglobulin (IG) Deposits in Renal Amyloidosis after Successful Autologous Stem Cell Transplant (ASCT) Sami Safadi,[1] Ahmed Saad,[1] Nelson Leung,[1] Paul J. Kurtin,[2] Samih H. Nasr.[2] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Anatomic Pathology, Mayo Clinic, Rochester, MN.*

Introduction: Clinical remission of IG related amyloidosis is achieved with chemotherapy and ASCT. However, the effect on the amyloid kidney mass is unclear. Previous reports showed persistence of deposits in light-chain amyloidosis(AL). In contrast, we present a case of heavy-light-chain amyloidosis(AHL) in which monoclonal IG deposits regressed after successful treatment.

Case Description: A 64 y/o male presented with AHL involving kidney and heart. He had monoclonal IgG-lambda on serum and urine immunofixation. His bone marrow(BM) biopsy showed 10% lambda-restricted plasma cells. His kidney biopsy showed extensive Congo-red positive glomerular amyloid deposits on light microscopy(LM) that were composed of randomly-oriented fibrils on electron microscopy(EM). The amyloid deposits stained for IgG and lambda, but was negative for kappa, IgA and IgM. Laser microdissection/ tandem mass spectrometry(LMD/MS) showed abundant spectra for IgG, lambda, SAP, ApoE. He underwent ASCT. His early course was complicated by ATN requiring transient dialysis. 4 years later, he developed renal failure, and worsened proteinuria. He was still in hematologic remission with no identifiable monoclonal protein in the serum or urine and a negative BM biopsy. A repeat biopsy was done. LM again showed typical Congo red positive glomerular amyloid deposits with fibrillary substructure on EM. One glomerulus had a sclerotic lesion. Surprisingly, the amyloid deposits did not stain for IgG, kappa or lambda on IF. LMD/MS detected SAP and ApoE but not IG heavy or light chains.

Discussion: AHL is an uncommon form of amyloidosis. It is more common in older patients, has less cardiac involvement, better patient, and renal outcomes. In this case, we hypothesize that the patient's worsening renal function is due to secondary FSGS from previous injury. The amyloid deposits on the second biopsy did not stain for IgG, kappa or lambda on IF, which suggests that the amyloid deposits are old. In conclusion, monoclonal IG deposits can regress in amyloidosis after treatment whereas the other structural proteins (SAP and ApoE) do not.

SA-PO764

Recurrent Immunotactoid Glomerulopathy in a Transplanted Kidney Koyal Jain,[1] Akanksha Gupta,[2] Kawan A. Swain,[1] Patrick H. Nachman,[1] Karin A. True.[1] *[1]Nephrology, Univ of North Carolina Kidney Center, Chapel Hill, NC; [2]Nephropathology, Univ of North Carolina, Chapel Hill, NC.*

Introduction: Immunotactoid glomerulopathy (ITG) is found in only 0.06% of native kidney biopsies. It is characterized by Congo-red-negative glomerular deposits of microtubules staining for IgG and complement. It has been commonly associated with lymphoproliferative disorders and cryoglobulinemia. We present a patient with early recurrence of ITG within 9 months after kidney transplantation.

Case Description: Our patient is a 71 year old man with a history of end-stage kidney disease due to ITG 5 years prior to kidney transplantation. Work-up for malignancy at the time of first diagnosis was negative. A kidney biopsy for BK viremia 9 months after transplantation revealed scant polyclonal ITG deposits (Urine Protein/Cr 0.05) along glomerular basement membranes. 3 months later, the patient developed proteinuria (Urine Protein/Cr 5.5). Repeat biopsy demonstrated overt MPGN (membrano-proliferative glomerulonephritis) pattern with immunotactoid deposits. Laboratory data revealed creatinine 1.8 mg/dL (baseline 1.2), low C3 and C4, and rheumatoid factor 119.7 IU/mL. HIV, Hepatitis B/C, ANA, RPR, ANCA, echocardiogram and PET scan were negative. A monoclonal IgG Lambda was detected on serum and urine electrophoresis. Bone marrow biopsy showed monoclonal fixation consistent with monoclonal gammopathy, leading to a diagnosis of "monoclonal gammopathy of renal significance" (MGRS). Treatment for MGRS is being initiated with Cyclophosphamide, Bortezomib and Dexamethasone.

Discussion: ITG has been reported to recur after transplantation in 47-64% of patients. Patients have been treated with a combination of Cyclophosphamide, Rituximab and steroids, with varied outcomes. This case illustrates the clinical emergence of monoclonal gammopathy 6 years after the initial diagnosis of ITG. Clinical vigilance and repeat work up for underlying lymphoproliferative or infectious disorders is therefore warranted.

SA-PO765

An Unusual Case of Renal Failure After Liver Transplantation Amanda K. Hall,[1] Jo Abraham,[1] Monica Patricia Revelo Penafiel,[2] Kalani L. Raphael.[1] *[1]Nephrology and Hypertension, Univ of Utah, Salt Lake, UT; [2]Anatomic Pathology, Univ of Utah, Salt Lake, UT.*

Introduction: A 61 year old woman who received a liver transplant in 2011 for end stage liver disease due to hepatitis C virus(HCV) and hepatocellular carcinoma was evaluated for worsening renal function and nephrotic proteinuria.

Case Description: After transplantation, HCV recurred but was successfully treated with negative viremia. One month prior to nephrology consultation, she had left eye pain, blindness, and serum creatinine of 2.8mg/dL(baseline 1.7mg/dL). She was diagnosed with optic perineuritis and began oral prednisone 60mg daily. Other medications included cyclosporine, lisinopril and furosemide. Urine microscopy showed dysmorphic hematuria. Workup revealed ANCA(1:40) with atypical p-ANCA staining, 5.4 g/g proteinuria, negative cryoglobulins, normal C3 and C4, and negative HCV RNA by PCR. There were 13 glomeruli on renal biopsy;>50% showed membranoproliferative features, 5 cellular crescents, 2 had focal fibrinoid necrosis, and 2 with segmental sclerosis. Immunofluorescence showed granular mesangial and capillary wall staining with C3, IgG, IgA and IgM, and kappa and lambda light chains.

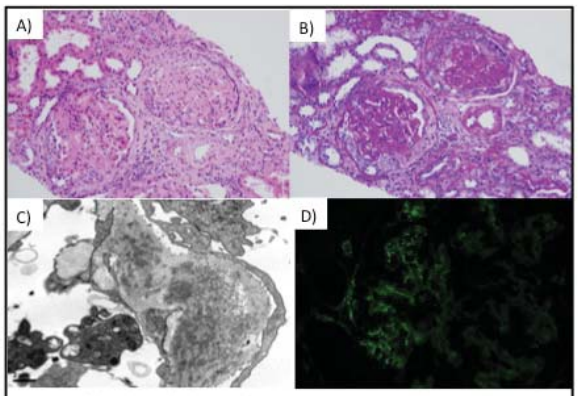


Figure 1: A) The glomeruli show moderate increase in mesangial matrix. The capillary loops appear thickened. Endocapillary proliferation is seen segmentally. B) Segmental cellular crescents. C) The glomerular basement membranes appear thickened with intramembranous electron dense deposits with randomly oriented fibrils measuring 16 to 23 nm in diameter. D) 3+ granular mesangial and capillary wall staining with C3.

Electron microscopy(EM) revealed randomly arranged 16-23nm fibrils. Congo red stain was negative. The diagnosis of crescentic fibrillary glomerulonephritis was made and oral cyclophosphamide was added. Renal function continued to deteriorate and hemodialysis began for refractory volume overload.

Discussion: Fibrillary glomerulonephritis is a rare cause of kidney disease. Pathognomonic findings include randomly arranged fibrils of 12-22nm in size on EM, which are Congo-red negative. It is commonly idiopathic or secondary to malignancy, autoimmune diseases, HIV, or HCV. Like most with fibrillary glomerulonephritis, this patient presented with renal insufficiency that did not respond well to immunosuppression.

SA-PO766

A Rare Case of Tuberculosis in Bilateral Native Polycystic Kidneys following Renal Transplantation Dhaval Sureja, Farhan Zahid, Ahmed Abdulrahman, Dong Heun Lee, Adrianne M. Ortega, Suganthi Soundararajan, Gregory Malat, PharmD, Karthik M. Ranganna, Gary S. Xiao, Alden Michael Doyle. *Nephrology, Drexel Univ, Philadelphia, PA.*

Introduction: The risk of tuberculosis (TB) in dialysis and renal transplant recipients is higher than general population. Clinical presentation of TB in dialysis patients can be atypical and reliance on positive purified protein derivative (PPD) test and chest x-ray (CXR) screening may miss significant extra pulmonary disease as it may require invasive procedure for diagnosis. We report a rare case of extra pulmonary tuberculosis in native bilateral polycystic kidneys in a renal transplant recipient.

Case Description: A 60 year old Cambodian female with history of polycystic kidney disease on peritoneal dialysis, successfully received deceased donor kidney transplant. Six months after transplant, she presented with fever, flank pain and dysuria, and was treated

for E. Coli pyelonephritis. Despite appropriate antibiotics, her fever and back pain recurred. CT scan of abdomen was unremarkable except for stable polycystic kidneys. Patient underwent bilateral native nephrectomy to manage pain and probable recurrent polycystic kidney infections. Intraoperatively, multiple nodules in the peritoneum were discovered. Biopsy revealed caseating granulomas with rare acid fast bacilli which was confirmed as Mycobacterium Tuberculosis by culture. Native kidneys showed tuberculous granulomatous interstitial nephritis. Allograft function was stable. Post operatively, patient was started on anti-tuberculous therapy and remained afebrile. This case presented as disseminated TB likely due to reactivation in the background of BCG vaccination in Cambodia, positive PPD and a negative CXR.

Discussion: Diagnosis in our case was challenging and it underscores the limitations of standard dialysis and pre-transplant testing for TB. It requires low threshold of suspicion with proper screening tests for early diagnosis. Our program has adopted widespread use of direct testing for TB antigens using T-spot and QuantiFERON-TB gold tests. We suggest that the nephrology and transplant communities adopt this improved testing methodology, especially in patients at increased risk of TB.

SA-PO767

Management Issues following Successful Kidney Transplantation for a Patient with Alström Syndrome Dhaval Sureja, Gregory Malat, PharmD, Karthik M. Ranganna, Stephen Guy, Alden Michael Doyle. *Nephrology, Drexel Univ, Philadelphia, PA.*

Introduction: Alström Syndrome is a rare, autosomal recessive disorder affecting less than 1:5,000,000 live births caused by mutation in ALMS1 gene. Alstroöm syndrome is associated with childhood obesity, early onset type II diabetes, retinal degeneration, sensory-neural hearing loss, hypertriglyceridemia, liver dysfunction, dilated cardiomyopathy, and progressive nephropathy with glomerulosclerosis. Although it often results in end-stage kidney disease, there are only few reports of affected patients undergoing successful kidney transplantation.

Case Description: Our patient is a 20 year old male found to have Alstroöm syndrome after being diagnosed with early childhood hypertriglyceridemia and hyperinsulinism. At the age of 14, he was found to have elevated serum creatinine with significant proteinuria. He also has a history of retinal degeneration with photophobia and vision loss, sensory neural hearing loss, hypertension, anemia, and hyperparathyroidism. He received a living related kidney transplant from his mother and is maintained on tacrolimus, mycophenolate mofetil, and low dose prednisone. His post-transplant course has been complicated by severe hypertriglyceridemia, with triglyceride levels of 3000's despite high dose statins, fibrates, and omega-3 fatty acids. He has had persistent liver dysfunction, with moderate transaminitis and elevated alkaline phosphatase levels. He has persistently low platelet counts (80,000). Although his cardiac function has remained stable, it requires regular monitoring as affected patients can develop symptoms at any time during the disease course. He is legally blind and attends a university that caters to the visually impaired. He requires specific arrangements at school including timer system for meds and special attention to his fitness.

Discussion: Alstroöm syndrome is a rare genetic disorder associated with progressive kidney disease that can be successfully treated with kidney transplantation as in our case; however providers who care for these patients should be aware of multiple manifestations of Alstroöm syndrome as they are likely to complicate post-transplant management.

SA-PO768

Use of ACTH Gel (Acthar) as Rescue Therapy for Transplant Glomeruloapthy (TG) in Two Kidney Transplant Recipients: Short and Long Term Follow-Up Maybel M. Tan, Laurel W. Yap, Mariana S. Markell. *Renal Diseases, SUNY Downstate Medical Center, Brooklyn, NY.*

Introduction: Chronic allograft nephropathy (CAN) is a leading cause of allograft failure. TG is a particularly virulent form of CAN. At the present time there is no good treatment once it has been established. Our Center has been using ACTHAR as rescue therapy since 2010. We present long-term follow up of the first patient treated for TG and the clinical course of a second case.

Case Description: Case 1: A 36 year old woman with history of systemic lupus erythematosis, underwent Deceased Donor Renal Transplant in 2013 with baseline creatinine of 1.1, maintained on tacrolimus and prednisone. 10 months post-transplant she developed severe proteinuria despite telmisartan. Kidney biopsy suggested early TG. At initiation of 80mcg twice weekly Acthar, her urine protein was 9.8 gm/l and decreased to 2.8 g/L and 1.4g/l respectively after 2 and 4 months of therapy. Her current creatinine is 1.29 mg/dl. Case 2: A 42 year old woman with a history of medullary cystic disease received a second living related kidney transplant in 2004. In 2010 she developed rapid progression of proteinuria to a peak of 12 gm with albumin 2.9 and cholesterol 350. Transplant biopsy revealed TG. Creatinine was 1.9. Acthar was initiated at 80mcg twice weekly in June 2011 and proteinuria fell to 8 gm by Dec, 5.3 gm by April 2012, and 2.4 gm with normalization of albumin and cholesterol by June 2012. She was tapered to 80mcg once weekly and has refused to stop the medication. Repeat biopsy in 2013 showed no interval change from her previous biopsy. It was negative for C4d staining. She is now 4 years post initiation of treatment with a recent creatinine of 2.8 and proteinuria of 3.7 gm. Her cholesterol is 220mg on Ezetimibe and her albumin is normal.

Discussion: TG carries an ominous prognosis for kidney allograft survival, and is complicated by features of severe nephrotic syndrome. Acthar has been used to treat nephrotic syndrome in native kidneys. We suggest that long term use of Acthar should be considered in patients with transplant glomerulopathy for whom no other therapy is available, based on the efficacy and long term safety of the patients reported here.

SA-PO769

Acute Myeloid Leukemia in a Renal Transplant Patient Deepti S. Moon, Lisa K. Prince. *Nephrology, Walter Reed National Military Medical Center, Bethesda, MD.*

Introduction: Acute myeloid leukemia is rare post transplant but associated with poor survival. This case highlights importance of high degree of suspicion and early referral for evaluation of leukopenia in renal transplant patient.

Case Description: 58 year old Caucasian male with history of Crohn's disease, autoimmune hepatitis, primary sclerosing cholangitis and is status post liver/renal transplant in October 2012 presents with new onset leukopenia. Patient denied any complaints and ROS was negative. Physical exam was unremarkable. Laboratory tests revealed white blood cell count of 2.3, patient had normal count 4 months before. Patient's mycophenolate mofetil was decreased to 250mg twice a day but no improvement. Patient was evaluated by hematology/oncology and noted on peripheral smear to have blasts. Bone marrow biopsy revealed findings consistent with acute myeloid leukemia. Patient's immunosuppressant regimen was modified to tacrolimus with trough goal 2-3, and prednisone 10mg. He completed induction chemotherapy with idarubicin and cytarabine and is doing well with renal and liver function at baseline.

Discussion: Organ transplant recipients are at 2-fold overall increased risk for cancer with Non Hodgkin's lymphoma, skin cancers, and cancers of the lung, liver, and kidney most common. But leukemia accounted for 2.7% of non-cutaneous tumors in one study, but AML made up 43%. Another study found recipients have standardized incidence ratio of 2.7 for AML. Screening for breast, colon, skin, and cervical cancers is routine part in transplant patient's care, providers need to have high degree of suspicion for myeloid malignancies in appropriate circumstances. Especially with new onset leukopenia when timely referral to hematology/oncology is needed if patient's leukopenia fails to improve. Another contributing factor to development of myeloid disorders is choice of immunosuppressant. Correlation between use of azathioprine has been noted to increase frequency of AML and a study found 60% of kidney transplant patients with AML had received azathioprine as immunosuppressant. Interestingly this patient was placed on MMF and tacrolimus post-transplant, he had been on azathioprine for his Crohn's for 2 years prior to transplantation.

SA-PO770

Thermal Ablation as an Alternative to Traditional Surgical Resection for Renal Cell Carcinoma in a Transplanted Renal Allograft Rajat Lamba, Nandita Singh, Maureen E. Brogan, Anita Kaul. *Nephrology, Westchester Medical Center, Valhalla, NY.*

Introduction: Renal cell carcinoma in renal transplant recipients is usually in the native kidneys, particularly in patients who have been on dialysis for many years. Renal tumors are rare in the transplanted kidney but when they do occur the treatment options include allograft nephrectomy, partial nephrectomy or more recently reported option of thermal ablation. We are reporting a case of papillary cell carcinoma in the renal allograft that we treated with thermal ablation.

Case Description: A 61 year old female with history of hypertension, right native nephrectomy for renal cell carcinoma 5 years prior, ESRD status post dual pediatric en bloc kidney transplant in the right lower quadrant 18 years ago, with baseline creatinine of 0.6, on tacrolimus and Post Transplant Lymphoproliferative Disorder treated with Rituximab, was found to have a 3.1cm mass in the lateral allograft on an MRI. A biopsy showed fuhrman grade 2 of 4 papillary renal cell carcinoma. Lateral transplant nephrectomy was considered high risk due to the complicated anatomy. Considering the low tumor grade, a CT guided microwave ablation at 140 W for 10 minutes of the lateral transplanted renal mass was performed via per cutaneous route. Post operatively, the patient had severe pain at the ablation site and was noted to have right lower extremity weakness from femoral nerve injury requiring physical therapy. Her creatinine acutely increased post procedure and stabilized at a new baseline of 1.4. She had to be rehospitalized within a week of her discharge for a urine leak from a nephrocutaneous fistula and a urinary tract infection which was treated with intravenous antibiotics and a Foley catheter placement.

Discussion: Thermal ablation has been reported in small case series as a safe and effective nephron sparing treatment in the renal allograft for tumors less than 4cm in diameter, specifically in patients at high surgical risk. While making treatment decision, it should be kept in mind that although transplant nephrectomy is associated with surgical risks, thermal ablation can also lead to complications like femoral nerve damage, acute kidney injury and nephrocutaneous fistula.

SA-PO771

Leukemoid Reaction in Acute Antibody-Mediated Rejection with Thrombotic Microangiopathy Vesh Srivatana,[1] Thangamani Muthukumar,[1] Surya V. Seshan,[2] Choli Hartono.[1] *[1]Dept of Nephrology and Hypertension, Weill Cornell Medical College, New York, NY; [2]Dept of Pathology, Weill Cornell Medical College, New York, NY.*

Introduction: Fever and leukocytosis are common characteristics of systemic infection. However, a predominantly neutrophilic leukemoid reaction occurring in a transplant patient on immunosuppression is rare and may have diagnostic, therapeutic, and prognostic implications.

Case Description: We report a patient with end stage renal disease of unknown etiology who received a one haplotype-matched live-donor kidney transplant. Despite low levels of donor-specific antibodies (DSA), the pre-transplant crossmatch was negative. She received induction therapy with rabbit-antithymocyte globulin. Corticosteroids were rapidly tapered to a daily dose of 20mg of prednisone at 5 days after transplant with maintenance tacrolimus

and mycophenolic acid. The patient's serum creatinine was 1.34mg/dl at discharge. She presented 8 days later with fever, creatinine of 2.13 mg/dl, and white blood cell count (WBC) of 44.4x10(3)/ul with 83% neutrophils and 13% bands. Blood and urine cultures obtained prior to empiric antibiotic coverage were negative. Biopsy of the kidney allograft showed intense peritubular capillary C4d staining with thrombotic microangiopathy (TMA) consistent with acute antibody-mediated rejection (AMR) in the presence of a rising DSA titer. The patient was treated with high-dose corticosteroids, plasmapheresis, and bortezomib which promptly reversed the leukemoid reaction to WBC of 9.3x10(3)/ul. The renal function returned to baseline within 2 weeks and remained stable at one year follow-up.

Discussion: Review of the literature showed that leukocytosis is uncommon during acute rejection. We propose that complement activation on the graft endothelium produced tissue inflammation and ischemic injury. In response to local hypoxia, damaged-associated molecular pattern molecules (DAMPs) are recognized by toll-like receptors (TLR). Both complements and TLRs are capable of recruiting neutrophils, possibly causing the leukemoid reaction in our patient. To our knowledge, this is the first reported case of a leukemoid reaction elicited by AMR and TMA in the modern era of immunosuppression.

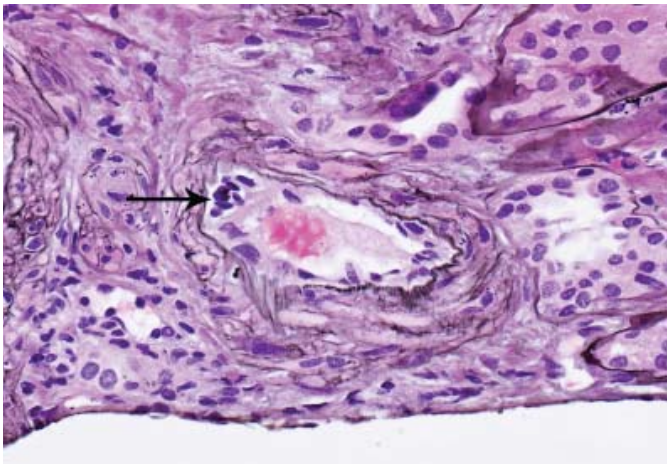
SA-PO772

Isolated V Lesions: Uncertain Prognostic Significance Irfan K. Moinuddin,[1] Machaiah M. Madhrira,[1] Amy Nicole Sussman,[2] Erika R. Bracamonte.[3] *[1]Div of Nephrology, Univ of Arizona Medical Center, Tucson, AZ; [2]Dept of Nephrology, Univ of Arizona at Tucson, Tucson, AZ; [3]Dept of Pathology, Univ of Arizona at Tucson, Tucson, AZ.*

Introduction: Isolated V lesions, in renal transplant patients, are characterized by endothelialitis with minimal tubulitis and interstitial inflammation. The significance of isolated v lesions remains unclear with opinions ranging from no prognostic significance to severe vascular rejection requiring accelerated immunosuppression.

Case Description: We report a case study of isolated v lesion in a 70 year old patient with ESRD due to HTN who received a deceased donor kidney transplant. Postop, patient developed atrial fibrillation and hypotension requiring transient pressors; patient also had to be taken to the OR for repair of a bowel leak. Patient had delayed graft function and oliguria. Patient had abdominal fluid with Cr 18.8 and was taken to OR for repair of urinary leak and biopsy of the transplant kidney.

On kidney biopsy, there is no significant glomerulitis or capillaritis. No segmental sclerosis. There is a mild chronic interstitial inflammatory infiltrate. Rare tubules contain 1-2 lymphocytes per cross section. Two muscular arteries show endotheliitis characterized by small clusters of mononuclear cells beneath the endothelium. No fibrinoid necrosis or transmural inflammation is seen. SV40 stain is negative. Stain for C4d is negative. Banff scoring: i1, t1, g0, v1, ci1, ct1, cg0, cv0, mm1, ah0,c4d0. Patient was not thought to be a candidate for accelerated immunosuppression. Persistent urinary leak lead to placement of nephrostomy tubes with subsequent downtrending of creatinine and good graft function on a regimen of MMF 500mg bid, prednisone 5mg qd, and program 2mg qam and 1.5mg qpm.



Discussion: Isolated V lesion, in this case, did not require accelerated immunosuppression and may not be of prognostic significance. This case points out that other etiologies of DGF such as urinary leak should be sought out and treated before subjecting the patient to the risk of increased immunosuppression.

SA-PO773

Effects of Serum Creatinine Calibration on Estimated Glomerular Filtration Rate and Chronic Kidney Disease Determination in African Americans: The Jackson Heart Study Wei Wang,[1] Michael Griswold,[1] Adolfo Correa,[1] Bessie A. Young,[2] L. Ebony Boulware,[3] Tibor Fulop,[1] Ian H. de Boer,[2] Ronit Katz.[2] *[1]Univ of Mississippi Medical Center, Jackson, MS; [2]Univ of Washington, Sealttle, WA; [3]Duke Univ, Durham, NC.*

Background: The calibration of serum creatinine values to Isotope Dilution Mass Spectroscopy (IDMS) traceable creatinine is essential for valid use of the new Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to estimate the glomerular filtration rate (GFR).

Methods: For 5,210 participants in the Jackson Heart Study, serum creatinine concentration was measured with a multipoint enzymatic spectrophotometric assay at the baseline study visit (2000-2004). Serum creatinine was re-measured using the Roche enzymatic method, traceable to IDMS in a subset of 206 subjects. The 206 samples were divided into three disjoint sets - training, validation, and test - to select an appropriate calibration model, estimate true errors, and assess performance of the final calibration equation. The selected calibration equation was applied to serum creatinine measurements of all 5,210 subjects to estimate GFR and the prevalence of CKD.

Results: According to the true error estimate among four potential calibration models and justifiable assumption made, the final selected Deming regression model showed a slope of 0.968 (95% CI:0.904, 1.053; $P < 0.001$) and intercept of -0.0248 (95% CI: -0.0862, 0.0366; $P = 0.430$) with $R^2 = 0.9527$. Applying the calibration equation to the unused test set (50 samples), calibrated serum creatinine concentration showed high agreement with actual measurements (concordance correlation coefficient 0.934, 95% CI: 0.894, 0.960). The baseline prevalence of CKD in the Jackson Heart Study (2000-2004) defined by eGFR less than 60 ml/min/1.73 m^2 was 6.30% using calibrated serum creatinine concentrations, compared with 8.29% using non-calibrated serum creatinine values with CKD-EPI equation ($p < 0.001$).

Conclusions: A Deming regression model was generated and validated to calibrate baseline serum creatinine concentrations in the Jackson Heart Study to values traceable to IDMS and accurately estimate the prevalence of CKD.

Funding: Other NIH Support - HLBI/NIMHD HHSN268201300046C1 Correa (PI)

SA-PO774

Inter-laboratory Variability of Serum Creatinine Assays Despite Assay Calibration Standardization Elizabeth S. Lee, Christine A. White. *Medicine, Queen's Univ, Kingston, ON, Canada.*

Background: Inter-laboratory variation in analyte measurement exist due to differences in assay methods, manufacturers, heterogeneous reference materials and other inherent measurement errors. The aim of this study was to examine the extent of inter-laboratory variability in serum creatinine (sCr) and its impact on GFR estimation in the post-IDMS standardization era.

Methods: Serum samples from 53 ICU patients at Kingston General Hospital, ON, Canada were obtained. Samples were split and an aliquot from each patient was sent to 12 participating laboratories (7 using enzymatic and 5 using Jaffe methods) for sCr measurement. Glucose, total bilirubin and protein were also measured. For each patient, we calculated all labs mean sCr and the mean sCr for both Jaffe and Enzymatic labs. We also determined the all labs mean CKD-EPI eGFR (SD) assuming 65 year old non-African American woman. For each laboratory and patient, we determined the absolute percentage (| (%) difference | from the mean for sCr and eGFR. The overall mean (| (%) difference | and coefficient of variation (COV) were then calculated. This analysis was repeated after stratifying by the median study sCr of 121.4 umol/L.

Results: On average, Jaffe results were 5.8±7.0 umol/L higher than enzymatic results resulting in 2.3±4.7 mL/min/1.73m^2 lower mean eGFR results. Table 1 shows the inter-laboratory variability results. Greater variability is seen at lower sCrs and in the presence of high levels of bilirubin and glucose as evidenced by greater % difference from the mean and higher COV.

	Mean l% difference in sCrl (SD)	Mean % COV	Mean l% difference in eGFRl (SD)	Mean % COV
All (n=53)	4.7±4.3	6.2	4.3±2.8	5.5
sCr (n=27) <121.4umol/L	6.4±5.4	8.5	4.9±3.6	6.4
sCr (n=26) >121.4umol/L	2.9±1.2	3.8	3.8±1.3	5.7

Conclusions: Significant inter-laboratory variability of serum Cr measurement persists despite IDMS-standardization and this results in variability in estimated GFR. Inter-laboratory differences are due in part to the presence of interfering substances. Manufacturers need to improve assay specificity in order to reduce measurement error and variability between labs.

Funding: Private Foundation Support

SA-PO775

Does the BIS2 Equation Best Predict Mortality in the Elderly?
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Background: Recently a new serum cystatin C and SCr-based eGFR equation, the Berlin Initiative Study-2(BIS2), was developed for the community-dwelling elderly. However, it is unclear whether BIS2 improves prediction of mortality in the elderly when compared with existing equations.

Methods: We conducted a prospective study of 2994 community-dwelling elderly men in the MrOS Sleep Study who had serum cystatin-C and SCr measured at baseline(2003-2005). We used Cox regression and net reclassification improvement(NRI) to compare the ability of BIS2, CKD-EPI$_{cysc,cr}$, CKD-EPI$_{cysc}$ and MDRD in 4 eGFR categories (≥90, 75-89, 60-74, <60) to predict all-cause mortality. For NRI analyses, CKD-EPI$_{cysc,cr}$ was the reference.

Results: Mean age was 76±6y; 91% were white. Mean BMI was 27±4 kg/m^2. Mean follow-up was 7±2y. Mean eGFR(ml/min/1.73m^2) was: CKD-EPI$_{cycscr}$ 70±17, BIS2 62±14, MDRD 73±17, CKD-EPI$_{cysc}$ 68±20. Only 2% had eGFR≥90 by BIS2 eGFR versus MDRD(14%), CKD-EPI$_{cysc}$(15%), CKD-EPI$_{cysc,cr}$(12%). However, 42% had eGFR<60 by BIS2 versus MDRD(22%), CKD-EPI$_{cysc}$(36%), CKD-EPI$_{cysc,cr}$(28%). BIS2 eGFR<60 was associated with a trend for higher mortality versus BIS2 eGFR≥90, after adjustment for age, race, BMI, HTN, DM(HR 1.2, 95%CI[0.6,2.3], p trend 0.007). Results were stronger for CKD-EPI$_{cysc,cr}$<60(HR 1.7, 95%CI[1.2,2.4], p trend<0.001) and CKD-EPI$_{cycs}$<60(HR 1.9, 95%CI[1.4,2.6], p trend<0.001);there was no association for MDRD<60(HR 1.1, 95%CI[0.8,1.3], p trend 0.17). In NRI analyses, both BIS2 and MDRD misclassified more subjects with respect to mortality risk compared to CKD-EPI$_{cysc,cr}$(BIS2 NRI -0.13, 95%CI[-0.17, -0.09], p<0.001; MDRD NRI -0.22, 95%CI[-0.27, -0.17], p<0.001). CKD-EPI$_{cysc}$ correctly classified subjects more often than CKD-EPI$_{cysc,cr}$(NRI 0.05, 95%CI[0.01, 0.08], p=0.02).

Conclusions: In a cohort of elderly community-dwelling men, BIS2 identified a higher proportion as having CKD. However, BIS2 performed somewhat worse with respect to mortality risk prediction than current equations used for clinical and research purposes.

Funding: Other NIH Support - NHLBI R01 HL070837, Veterans Affairs Support

SA-PO776

Comparison of the Japanese, CKD-EPI, and MDRD Study Equations for GFR Estimation in a Multicenter Korean Population
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Background: A new glomerular filtration rate estimation equation for the Japanese population was proposed using measured inulin clearance. To expand its applicability to other Asian populations, we performed a comparative study of the Korean population.

Methods: Inulin clearance was measured in 166 patients who were selected from seven participating medical centers in Korea. Patient sera and urine were collected, and baseline clinical characteristics were measured to provide an estimated glomerular filtration rate by the Japanese GFR equation using inulin clearance (Japanese 2009 equation), the Modification of Diet in Renal Disease (MDRD) study 2006 equation, and the Chronic Kidney Disease - Epidemiology Collaboration (CKD-EPI) 2009 equation. We compared the equation results to determine which equation best estimated the measured GFR (mGFR).

Results: Accuracy (95% CI) within 30% of mGFR by the Japanese 2009 equation, the CKD-EPI 2009 equation and the MDRD study 2006 equation were 66 (58-72), 51(43-58), and 55 (47-62)%, respectively. Bias (mGFR minus eGFR) were 3.4 22.4, -12.0 22.1, and -9.7 23.8 ml/min/1.73m^2, respectively. The accuracy of the Japanese 2009 equation was significantly better than MDRD study 2006 equation in subjects with mGFR <60 ml/min/1.73m^2 and in total subjects. The bias of the Japanese 2009 equation was significantly smaller compared with other two equations in total subjects. The coefficient (95% CI) for the Japanese 2009 equation, the CKD-EPI 2009 equation, and the MDRD study 2006 equation in Korean subjects were 1.067 (1.014-1.118), 0.847 (0.808-0.886), and 0.858 (0.816-0.901), respectively, indicating significant overestimation of GFR by MDRD study 2006 equation and CKD-EPI 2009 equation. There were no adverse events associated with inulin administration during the study.

Conclusions: The Japanese 2009 equation shows a higher accuracy in estimating GFR in Korean populations.

SA-PO777

Comparison of Estimated Glomerular Filtration Rate By the Chronic Kidney Disease Epidemiology Collaboration Equations with and without Cystatin C for Predicting Clinical Outcomes in Elderly Women
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Background: Reduced estimated glomerular filtration rate (eGFR) using the cystatin-C derived equations might be a better predictor of cardiovascular disease (CVD) mortality compared with the creatinine-derived equations, but this association remains unclear in elderly individuals. The aims of this study were to compare the predictive values of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)-creatinine, CKD-EPI-cystatin C and CKD-EPI-creatinine-cystatin C eGFR equations for all-cause mortality and CVD events (hospitalizations±mortality).

Methods: Prospective cohort study of 1165 elderly women aged >70 years. Associations between eGFR and outcomes were examined using Cox regression analysis. Test accuracy of eGFR equations for predicting outcomes was examined using Receiver Operating Characteristic (ROC) analysis and net reclassification improvement (NRI).

Results: Risk of all-cause mortality for every incremental reduction in eGFR determined using CKD-EPI-creatinine, CKD-EPI-cystatin C and the CKD-EPI-creatinine-cystatic C equations was similar. Areas under the ROC curves of CKD-EPI-creatinine, CKD-EPI-cystatin C and CKD-EPI-creatinine-cystatin C equations for all-cause mortality were 0.604 (95%CI 0.561-0.647), 0.606 (95%CI 0.563-0.649; p=0.963) and 0.606 (95%CI 0.563-0.649; p=0.894) respectively. For all-cause mortality, there was no improvement in the reclassification of eGFR categories using the CKD-EPI-cystatin C (NRI -4.1%; p=0.401) and CKD-EPI-creatinine-cystatin C (NRI -1.2%; p=0.748) compared with CKD-EPI-creatinine equation. Similar findings were observed for CVD events.

Conclusions: eGFR derived from CKD-EPI cystatin C and CKD-EPI creatinine-cystatin C equations did not improve the accuracy or predictive ability for clinical events compared to CKD-EPI-creatinine equation in this cohort of elderly women.

Funding: Government Support - Non-U.S.

SA-PO778

Limitations of Creatinine- and Cystatin C-Based GFR Estimation in Assessing CKD Risk in Obese Persons: NHANES 1999-2004
Stephen P. Yang, Deep Sharma, Matthew K. Abramowitz. *Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.*

Background: Higher BMI has been associated with increasing risk of CKD using GFR estimated by creatinine (eGFR$_{Cr}$) or cystatin C (eGFR$_{CysC}$). However, obesity can affect the accuracy of eGFR$_{Cr}$ and eGFR$_{CysC}$. We investigated the role of body composition in assessing CKD risk in obese persons.

Methods: We identified subjects from the National Health and Nutrition Examination Survey 1999-2004. Lean body mass (LBM) and percent total body fat (%TBF) were measured by dual-energy X-ray absorptiometry. Moderate and severe obesity were defined as 35>BMI≥30 and BMI ≥35, respectively. We defined CKD as eGFR <60 ml/min/1.73m^2 and excluded participants with eGFR <15 ml/min/1.73m^2. Logistic regression models were created to examine the association of obesity with CKD after adjustment for age, sex, and race/ethnicity.

Results: Greater obesity was associated with higher LBM, %TBF, and prevalence of CKD. Moderate and severe obesity were associated with CKD defined by eGFR$_{Cr}$, with odds ratios (OR) of 1.66 (1.17 – 2.34) and 2.05 (1.21 – 3.49), respectively. This association was not seen after further adjusting for LBM (OR 1.24 (0.81 – 1.92) and 1.21 (0.65 – 2.25) in moderate and severe obesity, respectively), but was unchanged after adjustment for %TBF. There was a non-significant trend towards an association of higher %TBF with CKD defined by eGFR$_{Cr}$ (OR per 10% TBF 1.25 (0.98 – 1.61)), which was substantially attenuated after adjusting for LBM (OR 1.03 (0.81 – 1.32)). Moderate and severe obesity were also associated with CKD defined by eGFR$_{CysC}$ (OR 2.28 (1.56 – 3.33) and 4.28 (2.49 – 7.36), respectively). This association was independent of LBM, but was attenuated by adjustment for %TBF (OR 1.66 (1.11 – 2.50) and 2.70 (1.17 – 6.22), respectively).

Conclusions: Obesity is associated with high levels of muscle mass, which can confound the diagnosis of CKD in obese patients. Although eGFR$_{CysC}$ is independent of muscle mass, higher %TBF may result in higher serum cystatin C levels and also confound eGFR$_{CysC}$. Whether this results in overdiagnosis of CKD deserves further study.

Funding: Private Foundation Support

SA-PO779

Slope Estimates of Glomerular Filtration Rate in a UK Population with Diabetes Mellitus: Assessing Progression of Chronic Kidney Disease in Relation to Modeling for Cardiovascular Outcomes
Claudia S. Cabrera,[1] Alison Lee,[2] Marita Olsson,[1,3] Sergio Eslava.[4] *[1]AstraZeneca R&D, Mölndal, Sweden; [2]AstraZeneca R&D, Macclesfield, United Kingdom; [3]Dept of Mathematical Sciences, Chalmers Univ of Technology, Gothenburg, Sweden; [4]AstraZeneca R&D, Gaithersburg.*

Background: The interaction between type II diabetes mellitus (T2DM) and chronic kidney disease (CKD) on cardiovascular disease (CVD) is not well understood, in part due to a lack of consensus regarding the optimal measure for nephropathy progression in observational studies. We describe CKD progression in a representative UK population.

Methods: Within the Clinical Practice Research Datalink (CPRD), a CKD cohort of adults (≥18 yrs) was selected among prevalent T2DM subjects (Jan 1 1995–Dec 31 2013). CKD was defined by the Chronic Kidney Disease Epi Equation (CKD-EPI) and READ codes. Due to the long and heterogeneous follow-up, computing a single eGFR slope per patient appeared to be imprecise. Instead, multiple slopes were calculated over varied time periods per patient: one slope over the full follow-up period "no-window model", slope for 3 yr windows with an 18 month overlap "3-yr model", and slope for 1 yr windows with a 6 month overlap "1-yr model".

Results: Among 56,671 adults with ≥4 eGFR measures and follow-up ≥1 yr (mean age 69 yrs, 52% women), 22% had >10 yrs prevalent diabetes at Index Date. The follow-up period ranged between 1 and 19.5 yrs [mean (SD), 4.9 (1.8) yrs]. Heart failure was the most prominent CVD outcome. The % per slope type was: no window 5.02% (n=56,671); 3-yr 5.13% (n=51,972); and 1-yr 6.36% (n=32,688). The rate (per 1000 person-yrs) of incident CVD in general was: heart failure 11.5 (95% CI 11.08–11.93); ischemic stroke 2.06 (1.89–2.24); and myocardial infarction 3.41 (3.18–3.65).

Conclusions: In this large CKD population, the 1-yr window model appeared to capture a greater proportion of CVD outcomes versus other windows, indicating that progression of CKD may be better assessed using a 1 yr time window to estimate the impact of renal function in subjects with extensive follow-up time. One single slope or a 3 yr slope may only dilute the possible association between eGFR and CVD outcomes.

Funding: Pharmaceutical Company Support - AstraZeneca

SA-PO780

SOMAscan™–Based Discovery of More Precise Measures of Early Decline in GFR

Anders G. Christensson,[1] Steve Williams,[2] Laila Bruun,[1] Robert E. Mehler,[2] Britta Singer,[2] Barry H. Smith,[3] Daniel Levine,[3] Thomas Parker,[3] Robert Kirk Delisle.[2] *[1]Dept of Nephrology, Skåne Univ Hospital, Malmö, Sweden; [2]SomaLogic Inc, Boulder, CO; [3]The Rogosin Inst, New York, NY.*

Background: Glomerular filtration rate (GFR) is defined as the volume of fluid filtered by the kidney per unit time and, as an index of total functioning renal mass, is the best measure of renal function. Clearance by the kidney of certain exogenous substances from the blood allows measurement of GFR but is not suitable for routine clinical practice. Endogenous clearance markers creatinine and cystatin C are attractive because they offer simpler means of estimating GFR. However, these estimates are relatively imprecise, especially over the clinically relevant range of 60-100 ml/min/1.73m^2. Measuring both markers does not resolve this limitation. The result is that early significant loss of functioning renal mass may go unrecognized, and the opportunity for timely, focused intervention is lost.

Methods: Using SOMAscan™, a high-throughput, multiplexed proteomic assay that simultaneously measures >1100 proteins from small volumes of biological samples, we assayed 183plasma samples from male subjects with GFR > 60 ml/min/1.73m^2 as measured by iohexol clearance. The samples were collected at Skåne University Hospital, Malmö, Sweden. Models were developed to assess the ability of protein levels to predict GFR within the range of 60-120 ml/min/1.73m^2.

Results: The performance of these models were compared to those of creatinine, of cystatin C, and of both, and found to better predict measured GFR above 60 ml/min/1.73m^2 (predictive correlation of 0.70 compared to 0.55). In each model cystatin C was a strong contributor, but 7 other proteins in various combinations with cystatin C enhanced each model's performance. Four of those proteins were found to be increased as GFR went down, and 3 were decreased.

Conclusions: We conclude that it will be possible to develop a clinically useful blood test that measures GFR above 60 ml/min/1.73 m^2, thus enabling detection and intervention in cases of early, clinically relevant renal function loss.

Funding: Government Support - Non-U.S.

SA-PO781

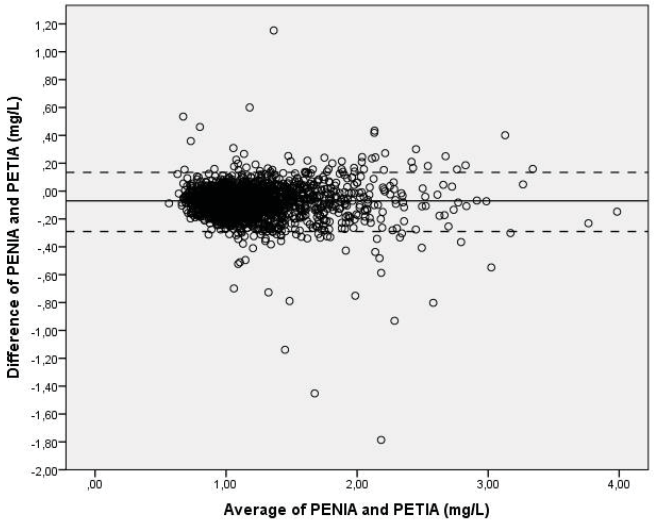
Systematic Analysis of Two Cystatin C Assays (PENIA and PETIA) Using Samples of 2057 Older Adults from the Berlin Initiative Study (BIS)

Natalie Ebert,[1] Pierre Delanaye,[2] Peter Martus,[3] Olga Jakob,[1] Jan Bartel,[4] Mirjam Schuchardt,[1] Etienne Cavalier,[2] Elke Schaeffner.[1] *[1]Charité, Berlin; [2]Univ, Liège; [3]Univ, Tübingen; [4]Limbach Lab, Heidelberg.*

Background: The role of cystatin C (cysC) as renal biomarker has gained importance. At present the two most commonly used methods to quantify cysC are nephelometry (PENIA) and turbidimetry (PETIA). If and how much cysC results may differ depending on the analytical method has been a longstanding debate.

Methods: We performed both, PENIA and PETIA cysC analyses in 2057 baseline serum samples from participants aged ≥70 of the Berlin Initiative Study. PENIA was performed using a BN™II System nephelometer and N Latex assay (Siemens Health Care Diagnostics). PETIA was performed with a Roche/Hitachi Cobas S system and Tina-quant assay. Modified Bland-Altman analysis (median, empirical 2.5% and 97.5% percentiles) was used for method comparison and Deming regression in order to account for random measurement errors.

Results: Modified Bland Altman analysis of both cysC assays in 2057 samples revealed a median difference between PENIA and PETIA of -0.0740 mg/L (95% CI: -0.079 to -0.070) and a standard deviation of 0,1269 mg/L. Non parametric limits of agreement were: -0.2890 to 0.1370mg/L (fig.1).



21 extreme outliers were excluded and Deming regression with inclusion of coefficient of variation (PENIA: 4.6%, PETIA: 5.57%) was performed revealing the following regression equation: PETIA = 0.0925 + 0.9879* PENIA. Details of the regression equation can be found in Table 1:

Parameter	Coefficient	Std. Error	95% Conf. Interval
Intercept	0.09246	0.009017	0.07478 to 0.1101
Slope	0.9879	0.008389	0.9714 to 1.0043

Conclusions: We demonstrated a good agreement between PENIA (Siemens) and PETIA (Roche) cysC assays in a cohort of elderly individuals. However a systematic difference of -0.0740 mg/L between both assays was observed.

Funding: Private Foundation Support

SA-PO782

Impact of Two Cystatin C Assays (PENIA and PETIA) on Estimating GFR in a Population-Based Cohort of Older Adults, the Berlin Initiative Study (BIS)

Natalie Ebert,[1] Peter Martus,[2] Olga Jakob,[1] Jan Bartel,[3] Mirjam Schuchardt,[1] Elke Schaeffner.[1] *[1]Charité, Berlin; [2]Univ, Tübingen; [3]Limbach Lab, Heidelberg.*

Background: Cystatin C (cysC) as a renal biomarker is gaining importance and a variety of cysC-based GFR estimating equations have been developed recently. However, differences in cysC assays may have a strong impact on accuracy and precision of cysC-based GFR estimates.

Methods: We performed nephelometric (PENIA) and turbidimetric (PETIA) cysC analysis in 2057 baseline serum samples from participants aged ≥70 of the Berlin Initiative Study (BIS). PENIA was performed with the Siemens BN™ II System and PETIA with the Roche/Hitachi Cobas S System. CysC-GFR was estimated with BIS3 and CKD-EPIcys equations. Linear regression with PENIA as goldstandard was performed to correct PETIA values (PETIA corrected).

Results: Mean GFR values for BIS3 with PENIA were significantly higher ($p<0.001$, t-test) than with PETIA (60 versus 56 ml/min/1.73m^2), the same held true for CKD-EPI (64 for PENIA versus 59 ml/min/1.73m^2 for PETIA, respectively). Linear regression with PENIA values as goldstandard revealed the regression equation: PENIA=0.002+0.933 x PETIA. This regression equation was obtained by ordinary least squares and is different to the one with Deming regression for analysis of agreement. Median bias, Interquartile range (IQR), P_{30} and P_{15} values for BIS3 and CKD-EPIcys equations with PETIA as well as the corrected PETIA values and PENIA as goldstandard are shown in Table 1:

Name of Equation	Median Bias	IQR	P_{30} (%)	P_{15} (%)
BIS3 (PETIA)	-3.57	6.28	98.6	86.7
BIS3 (PETIA corrected)	-0.12	5.88	98.6	93.4
CKD-Epi CysC (PETIA)	-4.93	8.90	97.6	73.4
CKD-Epi CysC (PETIA corrected)	-0.15	8.26	97.8	83.6

Conclusions: GFR estimates with BIS3 and CKD-EPIcys equations vary significantly depending on the cysC assay (PENIA or PETIA). This difference of up to 4 ml/min/1.73m^2 may have a limited clinical relevance only. By correcting PETIA values for systematic assay bias, the precision and accuracy of GFR estimates of both equations with PENIA as goldstandard improved considerably. In conclusion the present study shows that both cysC assays can be used in BIS and CKD-EPI equations almost interchangeably.

Funding: Private Foundation Support

SA-PO783

Determination of Fluid Status in the General Population Using Bioimpedance Techniques Fansan Zhu, Samer R. Abbas, Peter Kotanko, Nathan W. Levin. *Renal Research Inst.*

Background: Normal fluid status (NFS) can be estimated by bioimpedance hydration markers (HM) in the general population. A population average HM could be used but it is likely that the HM measure is influenced by age and comorbidities. This study evaluates the effect of age and systolic blood pressure (SBP) on HM.

Methods: A general population (n=213, males 106) was studied. Whole body and calf bioimpedance spectroscopy measurements were made with subjects in the supine position (Hydra 4200 device). Body weight, height and SBP were measured as well as calf normalized resistivity (CNR), extracellular (ECV) and intracellular (ICV) volumes. ECV/total body water (TBW) were calculated. Subjects were stratified by age; G1: 18 to 35; G2: 36 to 60, G3: 61 to 80 years. One-way ANOVA was used for group comparisons.

Results: Body mass index (BMI), CNR, ICV and ECV/TBW differed significantly between age groups and genders (Table 1). Decrease in CNR (Fig.1a) and increased SBP (Fig.1b) were associated with older age. CNR and SBP were negatively correlated with all subjects (R^2=0.12, p<0.0001; Fig.1 c). CNR in G1 was at the same levels as in 36% of subjects in G2 and 12.5% of subjects in G3, respectively. Of note, in those G2 and G3 subjects with CNR comparable to G1 subjects, SBP was lower than in the remainders of the respective age groups.

Conclusions: Our study in subjects from the general population shows that CNR declines and ECV/TBW increases with age. Although CNR in about one third of the subjects older than 35 years was in same level as in G1, CNR in most subjects (>35 years) was lower which was associated with higher SBP because of fluid overload, indicating that CNR reflects the relationship between volume expansion and blood pressure regardless of age. Identifying hypertensive patients with volume expansion may inform decision making with respect to drug therapy, such as the preferential use of diuretics.

Table 1

	G1: 18 to 35		G2: 35 to 60		G3: 60 to 80	
	Male n=37	Female n=34	Male n=49	Female n=51	Male n=20	Female n=22
Age, (year)	29.1±4.2	28.6±4	46.6±6.6	47.2±6.6	65.4±7.8	66.7±7.8
BMI (kg/m^2)	25.6±5.5	23.0±3.5	27.1±4.3	26.3±4.5	28.1±4.1*	26.3±5.8**
SBP (mmHg)	119.3±12§	110.5±11	123.1±17	119.7±19	130.2±19	122.9±24*
CNR (10^{-2}Ωm^3/kg)	20.3±2	21.4±2.9	17.4±2.6	18.1±3.1	14.4±2.3**	17.4±2.9**
ECV (L)	18.1±2.3	13.3±1.7	18.4±2.6	14.6±2.1	18.5±1.9	13.8±2.2*
ICV (L)	28.3±5.5	19.2±4.4	26.8±5.3	20.3±4.7	25.0±3.1*	17.6±2.3*
ECV/TBW	0.39±0.04	0.41±0.04	0.41±0.03	0.42±0.05	0.43±0.02**	0.44±0.02*

*or ** indicate significant difference (p<0.05 or p<0.01) between groups by One-way ANOVA analysis
§ indicates significant difference between G1 and G3.

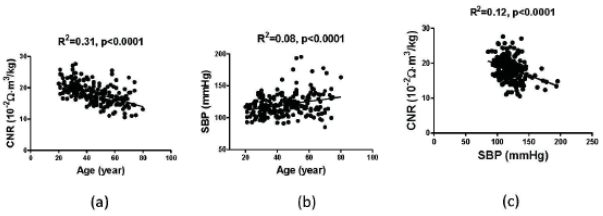


Fig.1

SA-PO784

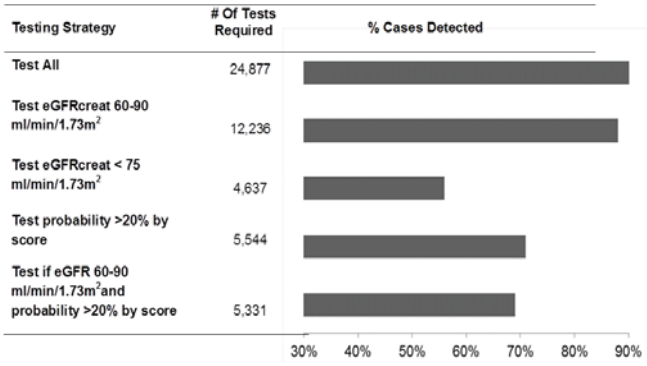
A Risk Score to Guide Cystatin C Testing to Detect Occult Chronic Kidney Disease Carmen A. Peralta,[1] Paul Muntner,[2] Rebecca Scherzer,[1] Suzanne E. Judd,[2] Michael Shlipak.[1] *[1]School of Medicine, Univ of California San Francisco, San Francisco, CA; [2]Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Persons with occult chronic kidney disease (CKD), defined as eGFR< 60 ml/min/1.73m^2 by serum cystatin C but not creatinine, are at high risk for complications. Tools are needed to guide cystatin C testing among persons without CKD by creatinine.

Methods: We developed and validated a risk score to estimate an individual's probability of having occult CKD among 24,877 Black and White adults with eGFRcreat >60 ml/min/1.73m^2 in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. Occult CKD was defined as eGFRcys < 60 ml/min/1.73m^2 by the 2012 CKD-Epi equation. We used multivariable logistic regression to model the probability of occult CKD, including variables readily available to clinicians. Performance characteristics were assessed using calibration and discrimination measures. The score was externally validated in the Third National Health and Nutrition Examination Survey III (NHANES).

Results: Mean age was 64 years, 41% were Black, and 13.5% had occult CKD by cystatin C. The final risk function included age, eGFRcreat, race, diabetes, hypertension, cardiovascular disease, smoking, and BMI. The risk function had excellent calibration and discrimination (c-statistic 0.87 in REGARDS, and 0.84 in NHANES). Using the risk score, 71% of occult CKD cases could be detected by measuring cystatin C in 22% of participants. For comparison, using eGFRcreat cutpoints alone, 49% of participants would need to be tested to detect a similar proportion of cases.

Figure 1. Yield of Testing Strategies for Occult CKD based on eGFRcreat or risk score



Conclusions: A risk score using characteristics readily accessible in clinical practice can identify the majority of persons with occult CKD. The cost-effectiveness of this approach to guide cystatin C testing requires further research.

Funding: NIDDK Support, Private Foundation Support

SA-PO785

Liver Stiffness Is Independently Associated with Estimating GFR Using Serum Cystatin C in Chronic Kidney Disease Patients with Chronic Liver Disease Hoon Young Choi, Tae Hoon Kim, Ah Ran Choi, Miok Cho, Sung Chang Bae, Sung-Kyu Ha, Hyeong Cheon Park. *Dept of Internal Medicine, Gangnam Severance Hospital, Yonsei Univ School of Medicine, Seoul, Korea.*

Background: Liver disease, especially nonalcoholic fatty liver has been reported to associate with an increased chronic kidney disease (CKD) risk. The prognosis and management of chronic liver disease (CLD) depends strongly on the degree of liver fibrosis. Recently, FibroScan (Echosens, Paris, France) has been accepted as a highly reproducible technique that measures liver stiffness in CLD. We carried out a retrospective study to investigate the association between liver stiffness and kidney function represented asestimating GFR using serum cystatin C (cystatin C-eGFR) in CKD with CLD patients.

Methods: Estimated glomerular filtration rate was calculated using cystatin C level (Cystatin C-GFR) [77.239 X (1.0675 X cystatin C - 0.1)$^{-1.2623}$]. Liver stiffness measurement (LSM) using FibroScan® was performed by a well-trained physician.

Results: A total of 512 CKD with CLD patients were enrolled. Average level of LSM and cystatin C-eGFR were 13.1 ± 14.8 kPa and 96.8 ± 47.1 ml/min. Age adjusted partial correlation analysis showed that LSM (r=-0.294, p=0.000), uric acid level (r=-0.216 p=0.001), alkaline phosphatase level (r=-0.229, p=0.000), and CRP level (r=-0.282, p=0.012) significantly correlated with cystatin C-eGFR negatively. WBC count (r=0.394, p=0.000) and hemoglobin level (r=0.375, p=0.000) showed the significant positive correlation. Serum albumin, AST and ALT level, Hepatitis B or C positive status did not show the significant correlation with cystatin C-eGFR. On multiple regression analysis, LSM was only the independent risk factor affecting cystatin C-eGFR.

Conclusions: Our results suggest that the increase LSM is the potential risk factor for kidney dysfunction in CKD with CLD patients.

SA-PO786

Impact of Cystatin c on Metformin Use among Adult Veterans with Diabetes Delphine S. Tuot, Rebecca Scherzer, Luciana Mendiola, Michael Shlipak. *Univ of California, San Francisco.*

Background: Current recommendations for metformin use are dependent on eGFR category: eGFR >45 ml/min/1.73m^2 – "first-line agent"; eGFR 30-44 – "use with caution"; eGFR<30 – "do not use". Nationally, metformin is underused among persons with eGFR 30-60. Misclassification of metformin eligibility by creatinine-based MDRD GFR estimates (eGFRcr) may contribute to underuse. We investigated the impact of cystatin c estimates of GFR (eGFRcys) on metformin eligibility.

Methods: Metformin use was assessed in a randomly selected cohort of 550 adult Veterans with diabetes in San Francisco. eGFR categories were defined as <30, 30-44, 45-60, and >60. We defined discrepancy between eGFRcys and eGFRcr as cases where the eGFRcys category was more severe than the eGFRcr category with an absolute difference >5 ml/min/1.73m^2. Multivariable relative risk regression was used to model predictors of metformin use and of discrepancy between eGFRcys and eGFRcr.

Results: Cohort subjects were 95% male, diverse (45% White, 22% Black, 11% Asian, 22% unknown) and had a median age of 68 years. Metformin use differed by eGFR: 8% in eGFRcr <45, 45% in eGFRcr 45-60, and 63% in eGFRcr >60. Compared to eGFRcr >60, likelihood of metformin use was much lower for persons with eGFRcr 45-59 (aRR=0.77, 0.59-0.99) and eGFRcr 30-44 (aRR=0.18, 0.08-0.41). eGFRcys reclassified 36% of persons, including 20% for whom metformin eligibility changed.

eGFRcys	eGFRcr <30 "do not use" (n=31)	eGFRcr 30-45 "caution" (n=58)	eGFRcr 45-60 "first line" (n=93)	eGFRcr >60 "first line" (n=368)
<30 "do not use"	**27 (90%)**	23 (40%)	8 (9%)	1 (1%)
30-45 "caution"	3 (10%)	**31 (53%)**	38 (41%)	32 (9%)
45-60 "first line"	0	4 (7%)	**35 (38%)**	76 (21%)
>60 "first line"	0	0	12 (13%)	**259 (70%)**

Factors associated with eGFRcys reclassification to a worse eGFR category were older age (aRR=1.38 per decade, 1.44-1.82) and albuminuria > 30mg/g (aRR=1.40, 1.10-1.77).

Conclusions: Metformin use is low among Veterans with CKD. eGFRcys may serve as a confirmatory estimate of kidney function to allow safe use of metformin among patients with CKD, particularly among older individuals and those with albuminuria.

Funding: NIDDK Support

SA-PO787

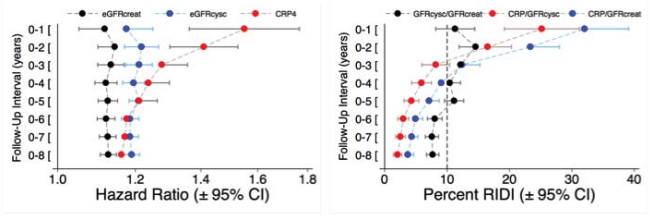
Adding C-Reactive Protein Improves the Risk Prediction for All-Cause Mortality with Estimating Equations for Glomerular Filtration Rate

David G. Warnock,[1] Nancy Jenny,[2] William M. McClellan,[3] Richard J. Glassock.[4]
[1]UAB, Birmingham, AL; [2]Univ of Vermont, Burlington, VT; [3]Emory Univ, Atlanta, CA; [4]UCLA, Los Angeles, CA.

Background: Cystatin-C based estimated GFR (eGFR) outperform creatinine-based eGFR for assessing all-cause mortality (Shlipak et al 2013), but not complication of chronic kidney disease ((Rule et al 2013). C-reactive protein (CRP) is associated with early deaths (Jenny et al 2007). We assessed the the mortality risk among participants in the REGARDS (Reason for Geographic and Racial Differences in Stroke) study when CRP quartiles were added to multivariable regression models based on eGFRcysc or eGFRcreat.

Methods: Regression models with 25,734 REGARDS participants; there were 3,020 deaths over 8 years. The performance of CRP quartiles (CRP4) was assessed with relative integrated discrimination improvement (rIDI; Pencina et al 2011). Covariates included demographics, cardiovascular risk factors and urine albumin to creatinine ratio (ACR).

Results: Average age was 65 (SD 9.4) years; 46% males, 40% Black; 22% had previous cardiovascular events; 21% type 2 diabetes; 14% current smokers; average systolic blood pressure was 127 (SD 17); total cholesterol, 192 (SD 40); body mass index, 29.3 (SD 6.1); and eGFRcreat, 85 ml/min/1.73 m^2 (SD 20). CKD with eGFRcreat less than 60 was present in 10.7%, and 14.6% had ACR ≥30 mg/g.



Hazard ratios (HR) for eGFRcreat and eGFRcysd were constant over 8 years follow up; while HR for CRP4 showed marked attenuation after 3 years. Addition of CRP4 improved performance for eGFRcysc and eGFRcreat models (RIDI significantly ≥10%) and was significantly better than the performance of the eGFRcysc model compared to the eGFRcreat model for the first 2 years of follow up.

Conclusions: Adding CRP quartiles significantly improves risk prediction for all-cause mortality when added to regression models based on eGFRcreat or eGFRcysc.

Funding: Other NIH Support - NINDS, Pharmaceutical Company Support - Amgen

SA-PO788

Correlation between Renal Apparent Diffusion Coefficients and Glomerular Filtration Rate in Chronic Kidney Disease

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Background: Studies have shown diffusion weighted imaging (DWI) can sensitively detect the diffuse movement of the water molecules, and we can learn the internal structure and composition of the diseased tissue by measuring the apparent diffusion coefficient (ADC) values. The objectives of the present study were to explore the correlation between renal apparent diffusion coefficient (ADC) values and glomerular filtration rates (GFR) in patients with chronic kidney disease (CKD).

Methods: 64 CKD patients (128 kidneys) were included as CKD group in our study, and 18 healthy adults (36 kidneys) were enrolled as the control group. The cortical and medullary ADC values were tested by diffusion weighted imaging (DWI). According to the single kidney glomerular filtration rate (SKGFR) calculated with 99Tcm-DTPA scintigraphy. The 128 kidneys of CKD group were divided into 2 groups: normal renal function (48 kidneys) and renal impairment (80 kidneys). The correlation of the cortical and medullary ADC values and SKGFR were analyzed.

Results: The cortical ADC values were significantly higher than the medullary ADC values in the control group and the CKD group (P<0.05). The cortical and medullary ADC values of normal renal group and renal impairment group were all significantly lower than the control group (P<0.05), ADC values of the renal impairment group were lower than those of normal renal function group (P<0.05). Pearson correlation analysis showed that the cortical and medullary ADC values were positively correlated with SKGFR (r=0.716(cortical), P<0.01, r=0.813(medullar), P<0.01).

Conclusions: DWI is a new noninvasive technique. Our study shows ADC values tested by DWI can reflect GFR, and it can be used as a marker for early detection of renal disfunction in CKD patients.

SA-PO789

New Index Adjusted U-Pro$_{1.0}$ Is Useful to Estimate Urinary Protein by Eliminating the Effect of Protein Intake

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Background: Urinary protein excretion (U-Pro) is a major target for the treatment of Chronic Kidney Disease (CKD). However, U-Pro is remarkably affected by the daily changes of protein intake (PI). Estimation of U-Pro with elimination of the effect of PI would be useful to evaluate the renoprotective effect of medication such as RAS blockers. For this purpose, we propose the new index, adjusted proteinuria$_{1.0}$ (adjusted U-Pro$_{1.0}$), which estimates urinary protein excretion in an assumption with protein intake of 1.0g/kg BW/day.

Methods: We collected the chemistry data of blood and 24hr urine sample from 151 patients with CKD (total points = 3882). Daily PI was calculated by the Maroni, Steinman, and Mitch method as follows. PI (g/day) = [Urine volume (UV;ml) x Urinary urea nitrogen (UUN;mg/dl)÷10^5 + Body Weight (BW;kg) x 0.031] x 6.25. Urinary urea nitrogen (mg/dl) adjusted by a protein intake of 1.0g/kg BW/day (UUN$_{1.0}$), was calculated by substitution of PI to BW in this formula (since PI=BW at protein intake of 1.0g/kg BW/day) and adjusted U-Pro$_{1.0}$ was estimated.

Results: The relation between UUN and UUN$_{1.0}$ is shown in Figure1. The adjusted U-Pro$_{1.0}$ was defined by following formula. Adjusted U-Pro$_{1.0}$ (g/gCr) = measured U-Pro (g/gCr) x UUN$_{1.0}$ / measured UUN. U-Pro$_{1.0}$ was larger than measured U-Pro (Figure2), which indicate that patients with CKD were under proper restriction of protein intake (average: 0.883±0.004 g/kg BW).

Conclusions: U-Pro is useful to examine the effects of low protein diet while adjusted U-Pro$_{1.0}$ is useful to examine the effect of RAS blockers on urinary protein excretion.

Figure1

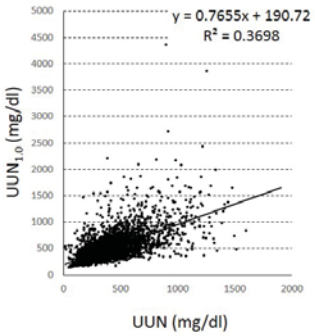
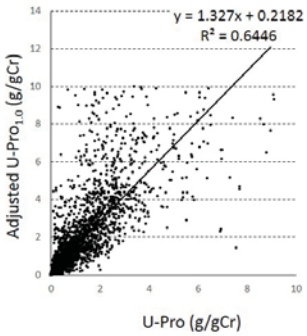


Figure2



SA-PO790

Evolution of Renal Function in Anorexia Nervosa

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Background: Anorexia nervosa (AN) is a frequent eating disorder that may be associated with chronic kidney disease (CKD) as described by the term "hypokalemic nephropathy", an ill-defined entity. The aim of this study was to clarify the evolution of glomerular filtration rate (GFR) in these patients and its determinants.

Methods: In a retrospective study, we analyzed the electrolyte disturbances and the evolution of GFR assessed on 104 patients in hospital who have AN by using the Cockcroft-Gault formula (CG). The CKD was defined as two GFR < 60ml/min for at least 3 months without subsequent return to normal value. We used the Chi2, Fisher, Student, Welch-Satterthwaite and Pearson tests.

Results: The cohort included 98 women and 6 men with a mean age 27 ± 11 years, and a BMI of 14.6 ± 3.04 kg/m2. Initial data were obtained from patient serums: K = 3.84 ± 0.72 mmol / L, Cl = 99.96 ± 8.16 mmol / L, HCO3 = 28.4 ± 6.07 mmol / L and creatinine = 73.22 ± 33 83 µmol / L. For 55 patients, we had a follow-up of serum creatinine over a period of 20.28 ± 10.2 months. 18/55 patients (32.7%) were diagnosed in CKD. They did not differ from other patients for the K + (3.75 ± 1.27 versus 3.94 ± 0.49 mmol / L; p = 0.73) and HCO3 - (32 ± 11.3 versus 28 ± 3.2 mmol / L; p = 0.15) initials. However, the initials serum chloride and BMI were significantly lower, respectively 93 ± 13.6 versus 101 ± 5.6 mmol / L (p = 0.007) and 12.69 ± 2.36 versus 15.36 ± 3.16 kg/m2 (p = 0.01).

Conclusions: The assessment of renal function in these patients with reduced muscle mass is difficult, but Delanaye et al [1] showed that the CG overestimates measured GFR but is the most reliable, compared with the others equations based on creatinine or cystatin C. Approximately one third of patients were diagnosed in CKD, and those are the ones in which AN was the most severe. The hypochloremia probably reflects the complexes electrolyte

disturbances of these patients. Our results strongly suggest that a regular follow-up of renal function should be recommended in AN. [1] Delanaye P et al, Clin Nephrol, 2009.

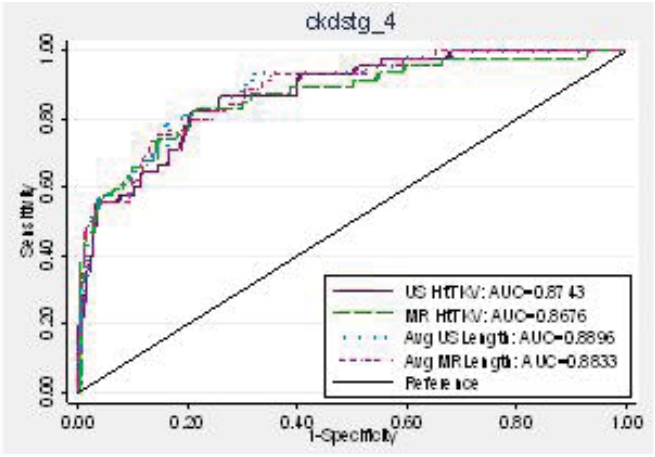
SA-PO791

Ultrasound (US) and Magnetic Resonance (MR) Renal Imaging Predict Future Chronic Kidney Disease in Autosomal Dominant Polycystic Kidney Disease (ADPKD) Arlene B. Chapman,[1] Harpreet Singh Bhutani,[1] Frederic F. Rahbari-Oskoui,[1] Vicente E. Torres,[2] Alan S.L. Yu,[3] Kyongtae Ty Bae,[4] Peter C. Harris,[2] Michael F. Flessner,[5] William M. Bennett,[6] Jared J. Grantham,[3] Patrice Gibbs,[4] Doug Landsittel.[4] *[1]Emory; [2]Mayo; [3]KUMC; [4]UPitt; [5]NIDDK; [6]Legacy.*

Background: ADPKD is characterized by increasing cyst burden and height corrected total kidney volume (htTKV) decades prior to loss of kidney function. We determined if kidney length (KL) by US or MR could predict future CKD stages 3,4 and 5 as well as htTKV.

Methods: The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease is a prospective, observational, longitudinal, multicenter study of 241 ADPKD adults with preserved kidney function. Demographic, physical, lab and imaging data were collected at baseline and follow up visits. CKD stage was determined using iothalamate clearance. Subjects were followed for up to 12 years. ROC curves were developed for each CKD Stage for baseline htTKV and KL from US and MR. A logistic regression model was used adjusting for age and serum creatinine. Optimal correct classification and the cut point associated with these were determined.

Results: As of June 2014, 31, 47, 70 and 99 participants reached CKD Stage 5,4, 3b and a respectively. ROC analyses for each CKD stage demonstrated similar AUC. As a single example, Figure 1 demonstrates ROCAUC for CKD stage 4. Table 1 shows the cut points associated with optimal combined sensitivity and specificity for each imaging method for CKD Stage 4.



Modality MR or US	Sensitivity (%)	Specificity (%)	Correct classification (%)	Cut point ml/m or cm
MR htTKV (ml/m)	73	73	73	650
US htTKV (ml/m)	75	74	74	750
MR Kidney Length (cm)	73	75	74	17
US Kidney Length (cm)	76	78	78	18

Conclusions: Both KL and htTKV strongly predict future CKD stages 3,4 and 5 in ADPKD, over a decade prior to their occurrence. US and MR performed equally well in predicting all CKD stages.

Funding: NIDDK Support

SA-PO792

Urinary mRNA for Vimentin Could Serve as a Potential Novel Biomarker of Renal Fibrosis Yuhan Cao, Linli Lv, Lihong Ding, Bi-Cheng Liu. *Inst of Nephrology, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Renal fibrosis is a common histologic outcome of primary glomerular diseases (PGD) progression. However, it is still a challenging issue to find a sensible parameter which could predict or reflect the development of renal fibrosis. Here, we demonstrated that detection of urinary mRNA for vimentin (a major cytoskeletal component of mesenchymal cells which play a critical role in fibrosis generation) might serve as a novel biomarker for evaluation of renal fibrosis in PGD.

Methods: In screening set, we collected urine samplesfrom 26biopsy-proven PGD patients and 8 healthy controls. Urinary mRNA array of 86 genes were quantified and analyzed the correlation with clinical parameters and fibrosis grades. The potential biomarkers were confirmed in validation set with 32 glomerular disease patients and 8 controls.

Results: In screening study, urinary mRNA profiles showed a total of 12 mRNAs were differentially expressed between PGD patients and controls(P<0.05), among which vimentin mRNA was significantly increased by 6.63 fold in patients group. Spearman's rank correlation showed that mRNA levels of vimentin associated with serum creatinine and eGFR (r_s=0.623, P<0.001; r_s=-0.618, P<0.001, respectively). Additionally, vimentin mRNA correlated with glomerulosclerosis (r_s=0.418, P=0.033), severity of tubulointerstitial fibrosis (TIF) (r_s=0.533, P=0.006) and could discriminate PGN from controls with AUC of 0.938 (P<0.001). Moreover, vimentin could distinguish moderate–severe fibrosis from none–mild with AUC of 0.830 (P=0.006). Higher expression of vimentin in fibrotic kidney disease was confirmed by immunohistochemical test. In validation phase, urinary vimentin mRNA also performed well in discriminating PGD from controls(AUC=0.953, P<0.001) and could predict degree of TIF with AUC of 0.864(P=0.001)which confirmed the dignostic potential of vimentin mRNA inscreening study.

Conclusions: In both screening and validation study, urinary vimentin mRNA showed great potential as biomarker of PGD for monitoring both renal function and fibrosis.

Funding: Government Support - Non-U.S.

SA-PO793

Trends in the Prevalence of Decreased GFR in an Adult Korean Population: Comparison of the CKD Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) Study Equations Jung-Woo Noh, Ajin Cho, Youngki Lee, Ja-Ryong Koo, Eun Jung Kim, Jang Won Seo. *Internal Medicine, Hallym Kidney Research Inst, Hallym Univ College of Medicine, Seoul.*

Background: The estimated glomerular filtration rate (eGFR) calculated using the Modification of Diet in Renal Disease (MDRD) study equation is a representative index for the prediction of the glomerular filtration rate (GFR). Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was developed, and the $eGFR_{CKD-EPI}$ was more accurately accurate and predictive, particularly for individuals with normal or mildly impaired kidney function. In this study, we evaluated the difference in the prevalence of decreased GFR determined using the two equations in the Korean general population.

Methods: Data from the Korea National Health and Nutrition Examination Survey (KNHANES) I (1998), II (2001), III (2005), IV (2007-09) and V (2010-12) were analyzed. A total of 55066 participants aged 20 or older with serum creatinine data were included. Decreased GFR was defined as estimated GFR < 60 mL/min/1.73m^2.

Results: The mean age in KNHANES I-V was 45±16, 45±15, 42±19, 49±16 and 51±16 years, respectively. The mean estimated GFR in KNHANES I-V was 87±15, 79±13, 81±19, 90±19 and 93±18 ml/min/m^2 using MDRD study equation and 91±16, 83±16, 85±19, 91±18 and 94±17 ml/min/m^2 using CKD-EPI equation, respectively. The prevalence of reduced GFR in KNHANES I-V was 2.2%, 7%, 6.8%, 3.8% and 2.8% using MDRD study equation and 2.5%, 6.8%, 6.8%, 4.3%, 3.3% using CKD-EPI equation, respectively. After adjusted by age, sex, BMI, diabetes and hypertension, prevalence ratio of reduced GFR in the later survey versus earliest survey (KNHANES I) were 4.0 (95% CI, 3.3-4.9), 4.0 (95% CI, 3.3-4.8), 1.1 (95% CI, 0.9-1.3) and 0.7 (95% CI, 0.6-0.9) using MDRD study equation and 3.5 (95% CI, 2.9-4.2), 3.7 (95% CI, 3.0-4.4). 1.0 (95% CI, 0.8-1.2), 0.7 (95% CI, 0.6-0.8) using CKD-EPI equation, respectively.

Conclusions: The mean $eGFR_{CKD-EPI}$ was higher than the mean $eGFR_{MDRD}$ in KNHANES I-V. The prevalence of reduced $eGFR_{CKD-EPI}$ in an adult Korean population has decreased since 2001 confirming the decrease observed in the prevalence of reduced $eGFR_{MDRD}$.

SA-PO794

The Applicability of Different Equations for Estimating Glomerular Filter Rate (eGFR) in Chinese Elderly Patients Wei Liu, Wenke Hao, Wenna He, Feng Yu, Wenxue Hu, Yanhua Wu, Yuanjuan Qian, Xiaowu Fang, Wei Shi. *Div of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: The objective of this study was to evaluate the applicability of different equations compare with standard GFR (sGFR) measured by ^{99m}Tc-DTPA clearance using a dual plasma sampling method in elderly Chinese patients.

Methods: A total of 297 patients (205 men and 92 women) were enrolled, which aged 75.1±6.7 (range, 65-91) years. GFR estimated using the Cockcroft-Gault (formula 1), MDRD (formula 2) and CKD-EPI equations based on SCr (formula 3), Cys C (formula 4) and combining SCr with Cys (formula5). Bias was assessed as the median of the differences between sGFR and eGFR, and precision was assessed as the interquartile range (IQR) for the differences. Accuracy was assessed as the percentage of estimates within 30% of sGFR (P30). The consistency was tested by correlation analysis and Bland-Altman analysis.

Results: The averages of sGFR and eGFRs were 32.57±19.82, 32.36±22.63(formula 1), 36.89±28.01(formula 2), 36.59±27.36(formula 3), 31.76±23.06(formula 4) and 33.27±24.60(formula 5)ml/min/1.73m^2, respectively(P=0.904, 0.031, 0.041, 0.646 and 0.705 versus sGFR). The median differences were successively 0.31(formula 3), -0.84(formula 5), 0.95(formula 2), -1.54(formula 4) and -1.69(formula 1) ml/min/1.73m^2. The IQRs were 11.76(formula 5), 11.85(formula 4), 11.96(formula 1), 17.55(formula 3) and 18.42(formula 2)ml/min/1.73m^2, respectively. P30 for all equations were 60.9%(formula 1), 59.6%(formula 4), 57.6%(formula 5), 49.2%(formula 2) and 47.5%(formula 3), respectively. Significant correlation was found between sGFR and eGFRs calculated from formula 5(r=0.860), formula 2 (r=0.860), formula 3 (r=0.856), formula 1 (r=0.837) and formula 4 (r=0.806) (all P=0.000). Bland-Altman analysis also showed the mean differences of different equations were -0.21(formula 1), 0.7(formula 5), -0.8(formula 4), 4.0(formula 3) and 4.3(formula 2) ml/min/1.73m^2, respectively.

Conclusions: The CKD-EPIequation based on SCr and Cys had less bias, higher accuracy and precision in Chinese elderly patients. It could be preferentially applied to assess GFR in Chinese elderly patients.

Funding: Government Support - Non-U.S.

SA-PO795

System Dynamic Modeling for ESRD Fiscal-Impact and Outcomes Projections

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Background: Current end-stage renal disease (ESRD) projections utilize Markov models but do not readily allow for simulating scenarios of policy change. System dynamic modeling (SDM) can quickly simulate such scenarios and are well suited for complex systems but have not yet been applied to ESRD.

Methods: We developed a causal diagram from critical care pathways in the ESRD continuum, including dialysis treated ESRD, transplantation and conservative care. We employed SDM using AnyLogic software (Version 7.0.3) and data from USRDS, specifically the prevalence rates from 2010 and averaged incidence, mortality and other flow rates from 2007-2011. We used age and primary diagnosis specific mortality rates to create 35 specific cohorts. We tested the ESRD SDM by running historical scenarios using data from 2000-2010 and 2005-2010 for 2010 projections. To forward simulate likely resource utilization, outcomes and cost, the ESRD SDM was calibrated against the output of the USRDS prediction for year 2020.

Results: To test the robustness of the model we ran three extreme scenarios: 200% increase in transplant rate, 30% increase in conservative care for patients 75 and older, and a 6-fold increase in peritoneal dialysis use (30% of dialysis patients). Projected results for 2020 from SDM and USRDS (2009 ADR) were similar: incidence rate was 136,623 versus 142,858; prevalence rate 838,906 versus 774,386; mortality rate 108,906 versus 118,617. Each of the scenarios ran successfully in the model and yielded expected results, e.g. a doubling of the transplant rate appropriately increased prevalence of ESRD and lowered the annual mortality rate to 92,965.

Conclusions: ESRD SDM is a viable approach to predicting and illustrating growth of the ESRD population in real time. ESRD SDM may be useful for modeling the impact of fiscal and policy changes to identify areas, ex ante, that could be made more efficient over a specified time horizon.

Funding: Clinical Revenue Support

SA-PO796

Risk Factors of Renal Functional Decline in Patients Receiving Antiviral Agents for Chronic Hepatitis B

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Background: Renal functional decline frequently seen in patients receiving antiviral agents for chronic hepatitis B may limit aggressive treatment using antiviral agents and exert adverse effects on overall prognosis. In this study, we analyzed the risk factors of renal functional decline in chronic hepatitis B patients receiving antiviral agents.

Methods: Between January 1, 2008 and December 31, 2013, medical records of chronic hepatitis B patients treated with oral antiviral agents in Samsung Medical Center were retrospectively analyzed. End points were end-stage renal disease requiring renal replacement therapy, liver transplantation, death, and switching of antiviral agents.

Results: A total of 4178 patients (M:F=2892:1286) were recruited and the mean duration of follow-up period was 26.9 months. Antiviral agents included lamivudine (17.0%), entecavir (70.4%), tenofovir (4.0%), clevudine (4.3%), telbivudine (0.6%), or adefovir (3.7%). Estimated glomerular filtration rate (eGFR) of 706 (16.9%) patients decreased by more than 25% from baseline eGFR during total follow-up period. Age, hypertension, diabetes, history of liver or kidney transplantation, and concomitant treatment of diuretics showed increased hazard ratio (HR). Both entecavir and clevudine groups compared to the lamivudine group showed decreased HR. A total of 341 (17.4%) patients among 1959 patients, whose kidney function were measured at 1 year after starting antiviral agents, showed decreased eGFR by more than 5mL/min/1.73m^2 and was defined as rapid decline group. Baseline serum levels of albumin, uric acid, and cholesterol were lower in the rapid decline group. Age, diabetes, liver or kidney transplantation, and underlying chronic kidney disease (CKD) were identified as risk factors for rapid decline group.

Conclusions: Age, hypertension, diabetes, liver or kidney transplantation, concomitant treatment of diuretics, and underlying CKD were identified as risk factors for renal functional decline in chronic hepatitis B patients receiving antiviral agents.

SA-PO797

Progression of Diabetic Nephropathy and Tubulointerstitial Nephritis: Role of Asymmetric Dimethylarginine and Oxidative Stress

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Background: Etiology of chronic kidney disease (CKD) is an important factor of progression. Asymmetric dimethylarginine (ADMA) and oxidative stress are also connected with progression of CKD. The aim of our study was to compare changes of ADMA and oxidative stress in patients with diabetic nephropathy (DN) and tubulointerstitial nephritis (TIN).

Methods: In a one-year follow-up (examinations at baseline, month 6 and 12), we measured plasma ADMA, advanced oxidation protein products (AOPP) and advanced glycation end-products (AGE) in 72 patients with CKD stages 3-5 (30 pts with DN, 42 pts with TIN). Glomerular filtration rate (eGFR) was estimated using CKD-EPI 2012 cystatin C formula.

Results: We observed a more rapid decrease of eGFR in DN than in TIN. Levels of AGE rose significantly in both groups, while AOPP rose in DN only. We did not find differences in ADMA values between the groups. Results are summarized in the table.

	baseline	month 6	month 12	*P* (DN *vs.* TIN)
eGFR DN (mL/min)	19 (16-28)	18 (14-25)	15 (12-24)	0.0004
eGFR TIN (mL/min)	22 (18-33)	24 (17-33)	21 (16-23)	
ADMA DN (μmol/L)	0.90 (0.85-0.99)	0.84 (0.73-0.93)[a]	0.87 (0.70-1.05)	0.9
ADMA TIN (μmol/L)	0.89 (0.81-0.98)	0.82 (0.74-0.91)[a]	0.96 (0.850-1.06)[b]	
AGE DN (FU/g of protein)	1.7 (1.3-1.8)	1.9(1.5-2.3)[a]	1.9 (1.6-2.3)[c]	0.7
AGE TIN (FU/g of protein)	1.6 (1.4-2.0)	1.8 (1.6-2.1)[a]	1.8 (1.6-2.1)[c]	
AOPP DN (μmol/L)	147 (136-172)	137 (130-155)	161 (142-190)[b,c]	0.01
AOPP TIN (μmol/L)	143 (126-177)	137 (117-155)	130 (116-174)	

Data are presented as median (interquartile range); Mann-Whitney and Friedman tests; significance ($p<0.05$): [a]=month 6 versus baseline, [b]=month 12 versus 6, [c]=month 12 versus baseline.

Conclusions: Diabetic nephropathy was associated with more rapid decline of eGFR and higher levels of AOPP than TIN. Differences in the rate of progression of CKD between DN and TIN are not caused by ADMA. Increase of AGE in both groups and AOPP in DN group only can be interpreted as general presence of oxidative stress in CKD that is more pronounced in DN patients and can contribute to more rapid eGFR decline.

Funding: Government Support - Non-U.S.

SA-PO798

CKD Prevalence and Use of Formulas in Oldest Old Individuals

Antonello Pani,[1] Doloretta Piras,[1] Marco Masala,[4] Alice Atzeni,[1] Silvana Urru,[4] Alessandro Delitala,[2] David Schlessinger,[3] Francesco Cucca.[2] *[1]Ospedale Brotzu, Cagliari, Italy; [2]CNR, Cagliari, Italy; [3]NIA- NIH, Baltimore; [4]CRS4, Pula, Italy.*

Background: Equations to estimate GFR have not been well validated in the elderly, and may lead to misclassification of subjects with chronic kidney disease (CKD). We examined the prevalence of CKD and the degree of agreement between different eGFR equations in a cohort of nonagenarians.

Methods: All of 250 individuals living in 13 villages in the central Sardinia and aged ≥ 90 years were invited to participate in this study. About 89% accepted to join; serum creatinine (sCr) and cystatin C (Cys) were measured in 185 volunteers, and Pr/Cr ratio in 151. eGFR was estimated by CKD-EPI sCr, CKD-EPI Cys, CKD-EPI sCrCys, Berlin Initiative Study Equation (BIS) 1 (sCr based), and BIS2 (sCr and Cys based). CKD prevalence was assessed according to the KDIGO classification. Agreement of the estimating equations in identifying people with different eGFR categories (> 90; 60-90; 45-60; 30-45; 15-30; < 15 ml/min) was evaluated using the k statistic. CKD-EPI sCrCys was used as reference equation.

Results: Median age was 91.9 (interquartile range: 90.7- 94.1 ys). CKD prevalence was similar when evaluated by the different CKD-EPI formulas, but higher if calculated by the BIS equations. However, when GFR was estimated on single individuals, the eGFR category varied depending on the equation used: the agreement of CKD-EPI sCrCys was fair with BIS1 (K 0.37, 95% CI 0.28-0.46; total eGFR categories matches 53.6%); moderate with CKD-EPI Cr (K 0.54, 95% CI 0.45-0.64; total matches 68.9%) and with BIS2 (K 0.60, 95% CI 0.51-0.69; total matches 70.8%); and more substantial with CKD-EPI cys (k 0.67, 95% CI 0.58-0.76; total matches 76.2%).

Conclusions: CKD prevalence according to KDIGO classification was very high in individuals aged ≥ 90 years, as generally expected from the physiologic decline of renal function. It suggests care in identifying very old people as having CKD, especially because the equations used for estimating GFR have a significant impact on CKD classification in the elderly. Further studies using a gold standard GFR measurement are required to establish which equation fits the oldest old best.

Funding: Other NIH Support - National Institute on Aging (NIA)

SA-PO799

Public Health Impact of Chronic Kidney Disease Risk Stratification Estimates Annette Von Thun,[1] Maura A. Watson.[2] *[1]Navy Medical Personnel Development Command, United States Navy, Bethesda, MD; [2]Nephrology Service, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Chronic kidney disease (CKD) patients have increased mortality, morbidity, and healthcare utilization. Kidney Disease: Improving Global Outcomes (KDIGO) guidelines advocate a 2-dimensional matrix and the CKD Epidemiology Collaboration (CKD-EPI) equation versus Modification of Diet in Renal Disease (MDRD) for risk stratifiaction.

Methods: Community based, cross-sectional survey (National Health and Nutrition Examination Survey (NHANES), 2001-2010) of CKD prevalence, population differences and healthcare utilization using the KDIGO matrix, CKD-EPI and MDRD equations. Subjects: non-pregnant adults >20 years, not on dialysis and completed NHANES. Glomerular filtration rate and albuminuria estimated from blood and urine samples. Demographics, vitals, and comorbidities obtained by standardized survey. CKD stage and projected care utilization were calculated per KDIGO guidelines. Prevalence estimates were extrapolated to the 2000 U.S. census population to evaluate overall disease burden and U.S healthcare system impact.

Results: CKD prevalence was 12.5% (CKD-EPI) versus 15.5% (MDRD). Of those diagnosed with CKD by both equations, 12% had a more severe CKD stage by MDRD. MDRD equation use with the KDIGO matrix led to 5.6 million over-diagnoses, 730,000 excess referrals, and 8 million excess healthcare visits annually, extrapolated to the U.S. population. This cohort was more likely to be female, older, obese, and have CKD risk factors. By multivariate analysis, the over-diagnosed group was more likely to have hypercholesterolemia (15%), hypertension (20%) and cardiovascular disease (38%) after adjusting for age, sex and albuminuria.

Conclusions: Estimation of CKD by CKD-EPI identified 37% fewer subjects at risk versus MDRD. Appropriate diagnosis of CKD can impact healthcare availability and utilization. Subjects identified with CKD by the MDRD equation were associated with residual cardiovascular risk, warranting further investigation. The views expressed do not reflect the official policy of the Department of Defense or U.S. Government.

SA-PO800

Prevalence of Chronic Kidney Disease in the Primary Care Hypertensive Patients in Minhang District, Shanghai Yong Gu,[1,3] Xianwu Ye,[1] Yanping Zhao,[2] Jianying Niu.[1] *[1]Div of Nephrology, The Fifth People's Hospital of Shanghai, Shanghai, China; [2]Minhang Center for Disease Control and Prevention, Shanghai, China; [3]Div of Nephrology, Huashan Hospital, Shanghai, China.*

Background: Chronic kidney disease (CKD) is associated with adverse renal and cardiovascular outcomes. Its early identification greatly depends on the awareness of general practitioners (GPs). So far, no study has evaluated CKD prevalence among hypertensive patients in primary care in China.

Methods: Based on the electronic health record system in Minhang District of Shanghai (total population 2.5million), 111,067 hypertensive patients (47,090 men; age 22-103 years) with essential medical information including demographic data, BP values, serum creatinine, dipstick urinalysis/albumin-creatinine ratio (ACR) and the use of antihypertensive medications between 1 Oct.2012 and 31 Sep. 2013 were included in our study. eGFR was calculated by using the simplified MDRD formula, and CKD was defined as either an eGFR<60 mL/min/ 1.73 m^2 or proteinuria, which was defined as either ACR≥30 mg/g or dipstick urinalysis quantified urine protein≥1+.

Results: The prevalence of CKD was 32.43%, but the proportion of patients with a kidney disease diagnosed by GPs was only 1.5%. 87.65% of the CKD patients achieved BP<140/90 mmHg, while those who achieved BP<130/80 mmHg were only 21.47%. Multivariate logistic regression analysis showed the risk factors associated with CKD were diabetes mellitus (OR 1.90, 95%CI:1.85-1.95), female (OR 1.62, 95%CI:1.57-1.67), hypercholesterolemia (OR 1.62, 95%CI:1.57-1.67), hypertriglyceridemia (OR 1.21,95%CI:1.18-1.24), cardiovascular disease(1.14, 95%CI:1.10-1.89), obesity (OR 1.15, 95%CI: 1.10-1.20), smoking (OR 1.11, 95% CI:1.17-1.16) and older age (OR 1.04, 95% CI:1.03-1.04 per year), while BP<130/80 mmHg (OR 0.95, 95%CI:0.92-0.98) was a protective factor for CKD.

Conclusions: Our results suggest that hypertensive patients are frequently affected by CKD, and most of them failed to achieve strict BP control goal. However, GPs' awareness of CKD is still too low. It should be a high priority to prevent and control hypertension in reducing the burden of CKD in China.

SA-PO801

Incidence of Chronic Kidney Disease in the Irish Health System and Population: Findings from a National Surveillance Programme Austin G. Stack,[1,2] Hoang Thanh Nguyen,[2] Liam F. Casserly,[1,2] Cornelius John Cronin,[1,2] Howard Johnson,[3] Ailish Hannigan,[2] Rajiv Saran,[4] Gemma M. Browne,[5] John P. Ferguson.[2] *[1]Nephrology, Univ Hospital Limerick, Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland; [3]Health Intelligence, Health Services Executive, Ireland; [4]Kidney Epidemiology and Cost Centre, Univ of Michigan; [5]Dept of Epidemiology, Univ College Cork, Ireland.*

Background: Improved detection and surveillance of chronic kidney disease (CKD) in the population may lead to improved disease management programmes and better clinical outcomes. We determined the incidence of new CKD and period trends in the Irish population.

Methods: We analysed data from a provincial health system between 2005 and 2011 (n=174,786). Estimated glomerular filtration rates (eGFR) were determined from the CKD-EPI equation from standardised serum creatinine values, excluding episodes of acute kidney injury (KDIGO definition). Incident CKD (ml/min/1.73m^2) was defined as new CKD cases per 100 persons at risk with median eGFR < 60 in the current year and > 60 or missing in previous year. Population at risk was defined from national census data. Incident CKD in the health system was defined as the number of adult patients with eGFR < 60 in current year and eGFR > 60 in previous year, per 100 patients at risk. Poisson regression modelled population incidence while logistic regression modelled hospital incidence.

Results: Incidence rates per 100 persons-at-risk ranged from 0.03 for subjects aged 18-40 years to 29.3 for subjects > 80 years in the population. Rates were significantly higher in women (1.78, 95% CI 1.70-1.90) than men (1.35, 95% CI1.30-1.40), varied by county and were relatively constant over time. In the health system, incidence rates per 100 patients-at-risk were 4.5 (95% CI 4.3-4.7) and multivariate analysis identified increasing age, county of residence, women, and acute kidney injury (Odds Ratio=2.70, 95% CI 2.47-2.95) as significant determinants.

Conclusions: Passive surveillance systems are effective in measuring the growth and trajectories of CKD in the Irish health system. These data are essential in planning preventive strategies and in determining future resource allocation.

Funding: Government Support - Non-U.S.

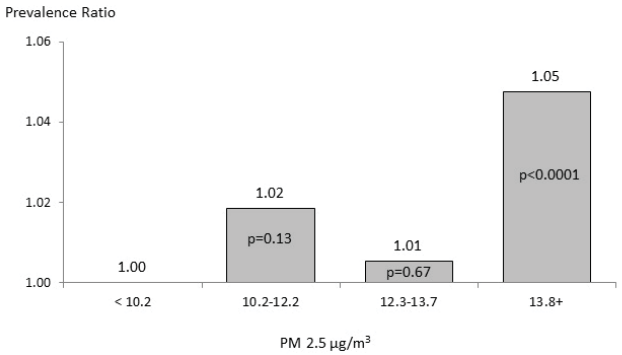
SA-PO802

County-Level Air Quality and the Prevalence of Diagnosed Chronic Kidney Disease in the U.S. Medicare Population Jennifer L. Bragg-Gresham,[1] Hal Morgenstern,[1] William M. McClellan,[2] Sharon Saydah,[3] Desmond Williams,[3] Neil R. Powe,[4] Delphine S. Tuot,[4] Yi Li,[1] Rajiv Saran.[1] *[1]Univ of Michigan, Ann Arbor; [2]Emory, Atlanta; [3]Centers for Disease Control, Atlanta; [4]Univ of California, San Francisco.*

Background: Considerable geographic variation exists in the prevalence of CKD across the U.S. Although part of this variation is explained by differences in individual-level risk factors, additional variability may be explained by environmental factors such as air quality.

Methods: Using data on 1.1 million persons from the 2010 5% Medicare sample and EPA air-quality data, we examined the association between a county-level measure of air pollution, particulate matter ≤2.5 μm (PM2.5) and the prevalence of diagnosed CKD, excluding individuals with ESRD, based on claims, for all U.S. counties. Modified Poisson regression was used to estimate associations (prevalence ratios [PR]) between county PM2.5 and individual CKD prevalence, adjusting for potential individual-level confounders: age, sex, race, hypertension, and diabetes.

Results: The estimated prevalence of diagnosed CKD ranged from 0% to 60% among counties (median = 16%). Treating PM2.5 level a continuous variable, the adjusted PR for an increase of 4 μg/m^3 was 1.03 (95% CI = 1.02, 1.05; p < 0.001). The results when categorizing PM2.5 by quartiles suggest a possible detrimental threshold effect at air quality index levels well below those typically considered unhealthy for sensitive groups, eg. the elderly (~40 μg/m^3).



Conclusions: We found that poorer air quality was associated with higher prevalence of CKD. However, the cross-sectional design and lack of individual exposure data precludes causal inference. Future investigations should include lab-based diagnosis of CKD, longitudinal observation, measures of multiple air pollutants and individual exposure, as well as more extensive control of confounding.

Funding: Other U.S. Government Support

SA-PO803

Association of Body Composition and Estimated GFR in Elderly Males and Females Hrefna Gudmundsdottir,[1,2,3] Olafur S. Indridason,[1] Margret B. Andresdottir,[1] Runolfur Palsson,[1,3] Tamara Harris,[4] Vilmundur Gudnason,[3,5] Andrew S. Levey,[6] Thor Aspelund.[3,5] *[1]Landspitali - The National Univ Hospital of Iceland; [2]Icelandic Medicines Agency; [3]Univ of Iceland; [4]National Inst on Ageing; [5]Icelandic Heart Association; [6]Tufts Medical Center.*

Background: Changes in body composition (BC) and kidney function are commonly seen with aging, but limited data is published on their association in the elderly. The goal was to determine if there was an association between BC, strength and estimated GFR (eGFR) in elderly males (M) and females (F).

Methods: The data were obtained from the population-based AGES I - Reykjavik Study. A total of 940 (421 M, 519 F) of the 5764 participants had complete data for eGFR using the creatinine - cystatin C-based CKD-EPI equation. Creatinine and cystatin C assays were traceable to standardized reference materials. Body mass index (BMI) and abdominal circumference (AC) were measured using standardized protocols, fat% by bio-electrical impedance and leg muscle strength (LMS) by using a dynamometer chair. Descriptive statistics and linear regression adjusted for age, hypertension (HTN), diabetes (DM) and smoking were performed.

Results: All participants were white and 55% were F. The mean (SD) age was 76 (4) years, eGFR 74 (17) ml/min/1.73 m^2, 81% had HTN, 10% DM and 13% smoked. Mean LMS and BC measures and their change associated with 10 ml/min/1.73m^2 lower eGFR for M and F are shown in table. A significant interaction with sex was found for the association of eGFR with BMI, fat% and AC.

	n	Mean (SD)	M	F	Beta M *vs.* F
			Beta ± SE	Beta ± SE	
LMS (Newton)	889	327 (112)	-3.85 ± 2.98	-3.86 ± 2.08	
BMI (kg/m^2)	939	27 (4)	-0.16 ± 0.11	0.51 ± 0.12◊	◊◊
Fat (%)	587	29 (8)	-0.60 ± 0.21**	0.58 ± 0.15◊	◊◊
AC (cm)	939	100 (11)	-0.13 ± 0.29	1.41 ± 0.33*	◊

*p<0.01, **p<0.005, ◊p<0.0005, ◊◊p<0.0001

Conclusions: Measures of BC are associated with eGFR in older adults but the direction of this relationship may be different for men and women. These findings may reflect confounding by non-GFR determinants of serum creatinine and cystatin C and need to be confirmed using measured GFR.

Funding: Other NIH Support - NIA, Government Support - Non-U.S.

SA-PO804

Association of HCV with CKD: A Retrospective Study Ajay Singh Rathore,[1] Linda D. Green,[1] Xiaoxiao Lu.[2] *[1]Internal Medicine, Prince George's Hospital Center, Cheverly, MD; [2]MPH, Univ of Maryland, Greenbelt, MD.*

Background: Hepatitis C (HCV) is being identified in more patients and its impact on kidney function is being clarified. Previous studies have reached different conclusions about the risk of CKD in the presence of HCV. We sought to determine whether HCV was an independent risk factor for CKD in a largely African American(AA) population with other traditional risk factors.

Methods: A retrospective chart review study using EMR (Athena) in the Glenridge Cliinic reviewed records from Jan 2004 till Jan 2014. Patients with HIV were excluded. 106 HCV positive patients and 131 HCV negative patients were identified. CKD stage 3-5 was defined by the MDRD calculation or National Kidney foundation's KDOQI defination. Demographics included age, gender and race. Clinical factors included diabetes mellitus, hypertension, hyperlipidemia, anemia, smoking, alcohol use, cardiac diseases and illicit drug use.

Results: Mean age of study group was 57.4 ± 6.5 years. 78.1% were AA. Prevalence of CKD stage 3-5 in HCV infected and HCV negative patients was 17 (16.0%) and 8 (6.3%) respectively with p< 0.0158. Diabetes and smoking were less prevalent in HCV-infected cases compared with controls, 39 (29.8%) versus 35 (33.0%) and 37 (34.9%) versus 64 (48.9%) respectively. This reinforces the importance of HCV or other unknown factors on CKD. Univariate analysis showed that HCV infection had an OR, 2.87: 95% CI, 1.18-6.94. Multivariate logistic regression analysis showed that HCV had an OR, 5.04; 95% CI (1.46-17.44) and supports our hypothesis that HCV is an independent risk factor for CKD stage 3-5. Limitations include small sample size and predominantly AA population.

Conclusions: Our study results provide evidence that HCV infection is correlated with increased CKD prevalence and also potentially with the severity of CKD stage 3-5, beyond the well-known independent CKD risk factors. These findings strengthen the need for the prevention and treatment of HCV infection in patients with or without CKD.

SA-PO805

Prevalence and Complications of Chronic Kidney Disease in a Representative Elderly Population in Iceland Aghogho A. Okparavero,[1] Meredith C. Foster,[1] Hocine Tighiouart,[1] Vilmundur Gudnason,[2,3] Olafur S. Indridason,[4] Hrefna Gudmundsdottir,[3,4] Gudny Eiriksdottir,[2] Lesley Inker,[1] Andrew S. Levey.[1] *[1]Tufts Medical Center; [2]Icelandic Heart Association; [3]Univ of Iceland; [4]Landspitali Univ Hospital.*

Background: CKD is common in the elderly, but data are limited on the distribution of GFR and albuminuria and the prevalence of related complications.

Methods: We performed a cross-sectional study in 3173 adults (42% male, mean age 80 years) from the Age, Gene/Environment Susceptibility Reykjavik study. We evaluated the distribution of eGFR (CKD-EPI 2012 creatinine-cystatin C equation) and albumin-creatinine ratio (ACR from a spot urine sample), prevalence of CKD (eGFR<60mL/min/1.73m^2 or ACR>30 mg/g) and complications [anemia (hemoglobin g/dl<12 in women, <13.5 in men) and hypoalbuminemia (<3.5g/dL) in the overall cohort, and acidosis (bicarbonate<21mmol/L), increased anion gap (≥12mmol/L), hyperphosphatemia (≥4.5mmol/L), and hyperparathyroidism (intact PTH>70pg/mL) in a subcohort (n=773)] by CKD status.

Results: eGFR and ACR varied widely (**Table**), mean (SD) eGFR was 64 (18) mL/min/1.73m^2 and median (IQR) ACR was 8 (5-17) mg/g. The prevalence [95%CI] of eGFR<60, ACR>30 and CKD was 40% [38-41], 14% [12-15], and 45% [43-47], respectively.The prevalence of complications was higher among those with versus without CKD: anemia (26 versus 14%), hypoalbuminemia (19 versus 13%), acidosis (5 versus 1%), increased anion gap (36 versus 24%), hyperparathyroidism (38 versus 15%) (p≤0.001 for all), except hyperphosphatemia (1 versus 1%).

Table: Prevalence N (%) of eGFR and Albuminuria Categories. Shaded boxes represent CKD.

eGFR Categories (mL/min/1.73m^2)	Albuminuria Categories (mg/g)			Total
	< 10	10 - 30	> 30	
≥ 90	118 (3.7)	59 (1.9)	14 (0.4)	191 (6.0)
75 - 89	461 (14.5)	181 (5.7)	48 (1.5)	690 (21.7)
60 - 74	649 (20.5)	271 (8.5)	115 (3.6)	1035 (32.6)
45 - 59	456 (14.4)	218 (6.9)	117 (3.7)	791 (24.9)
30 - 44	161 (5.1)	98 (3.1)	86 (2.7)	345 (10.9)
< 30	38 (1.2)	32 (1.0)	51 (1.6)	121 (3.8)
Total	1883 (59.3)	859 (27.1)	431 (13.6)	3173 (100)

Conclusions: There is a high burden of CKD among community dwelling elderly Icelandic adults. The wide range of eGFR and ACR suggests heterogeneity in processes leading to CKD.

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SA-PO806

Optimal Screening Interval of Renal Function for CKD in Healthy People Keita Hirano,[1,2,3] Goki Eriguchi,[3] Osamu Takahashi,[2] Yasuhiro Komatsu,[3] Motoko Yanagita.[1] *[1]Dept of Nephrology, Kyoto Univ Graduate School of Medicine; [2]Center for Clinical Epidemiology, St.Luke's Life Science Inst; [3]Dept of Nephrology, St. Luke's International Hospital.*

Background: It is recommended to calculate eGFR from sCr once or twice a year in healthy people by KDIGO guideline 2012. However, there is little evidence to show the optimal intervals for the analysis of sCr as an indicator of the function of kidney for people with normal renal function. we aim to evaluate optimal screening interval of renal function for adequately early detection of CKD.

Methods: We planned a retrospective cohort study involving patients with normal renal function (eGFR≥60ml/min/1.73m^2) who had a health check up at the first time at St Luke's International Hospital from 2005 to 2007 and followed up to 9 years. In this study, we classified them into three groups by renal function; high risk (60≤eGFR<75), intermediate risk (75≤eGFR<90), and low risk (90≤eGFR). As univariate analysis, we analyzed the relationship of developing CKD to age, gender, proteinuria, and medical history such as diabetes and proteinuria. Optimal screening interval of renal function is defined as the estimated time until developing CKD for 5% of the patients. Applying accelerated failure time model, we analyzed the transitions to CKD and estimated the optimal intervals in each renal function group.

Results: 44,911 patients were involved and 53% of them were male. The mean age was 45.6 ± 11.8, serum Cr 0.72 ± 0.15 mg/dl, and eGFR 84.1 ± 14.4 ml/min/1.73m^2. The patients with urine protein-positive and diabetes were 323(0.7%) and 1,149(2.6%), respectively. The estimated optimal screening intervals of the renal functions for high, intermediate, and low risk groups were 0.6, 4.0, and 18.4 years, respectively. In addition, the estimated intervals were 0.4, 2.6, and 12.1 years for the patients with proteinuria and 0.4, 2.4, and 11.0 years for the patients with diabetes.

Conclusions: This study shows that approximately 18 years follow up time was needed in low risk group of renal function. People with diabetes or proteinuria need shorter intervals than that without these diseases. In the future, Cost-effectiveness analysis remains to be studied.

Funding: Private Foundation Support

SA-PO807

Associated of Traditional Cardiovascular Disease Risk Factors with Kidney Damage Indicators in 33308 Adults Receiving Physical Examination Hao Zhang, Wei Li, Guo Xu, Jing Huang, Juan Mao, Ke Zhang. *Dept of Nephrology, The Third Xiangya Hospital of Central South Univ, Changsha, Hunan, China.*

Background: The traditional cardiovascular disease(CVD) risk factors (age, BMI, smoking, hypertension, diabetes, hyperlipidemia)was closely connected to the kidney damage indicators (proteinuria, albuminuria and eGFR), However, no survery has been done incorporating both kidney damage indicators and traditional cardiovascular disease risk factors among the healthy physical examination population in China.

Methods: We did a cross-sectional survey of 33308 Chinese adults who received healthy physical examination in the health management center of central south university third xiangya hospital from June 2013 to February 2014. Exclude hospitalized people within one year. Factors associated with the indicators of kidney damage analysed by multiple stepwise regression analysis.

Results: 33308 people were invited to participate, male-female ratio is almost 1.5:1, the average age was 44.7±14.5 years, about 89.8% for college grads, the prevalence of smoking was 30.1%, the detection of obesity,hypertension,diabetes mellitus,hyperlipidemia and hyperuricemia were 11.0%, 19.9%, 6.2%, 25.7% and 10.7%, respectively. Specific demographic characteristics had been found in the healthy physical examination population compared to the survery wich was published in the Lancet in 2012.The detection of proteinuria, albuminuria,eGFR less than 60 mL/min per 1·73 m², hematuria and abnormal renal ultrasound were 2.5%, 18.5%, 1.1%, 3.7% and 0.8%, respectively. Multiple factors Logstic stepwise regression analysis showed that: Diabetes, male, hypertension, smoking, hyperuricemia, high TG, age, BMI were independent risk factors for the proteinuria; Diabetes, hypertension, male, BMI, high TG, smoking are independent risk factors for the development of microalbuminuria; Hyperuricemia, age, low HDL - C, high LDL - C, hypertension, diabetes were independent risk factor for decline in eGFR.

Conclusions: Specific demographic characteristics had been found in the healthy physical examination population. Attention should be paid to people with traditional cardiovascular disease(CVD) risk factors in healthy physical examination.

SA-PO808

Renal Function in the Cardio Renal Syndrome: Dramatically Overestimated Sandrine Lemoine,[1,2,3] Florence Sens,[1] Fitsum Guebre-Egziabher,[1,2] Laurence Dubourg,[3] Aoumeur Hadj-Aissa,[3] Laurent Juillard.[1,2] *[1]Nephrology Dept, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France; [2]INSERM Carmen 1060, Univ Lyon 1, Univ de Lyon, France; [3]Renal Function Dept, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France.*

Background: Most of patients with a cardio-renal syndrome 2 (CRS2) eligible for ultrafiltration present with a chronic kidney disease (CKD) not requiring dialysis initially. The muscle mass loss could alter the estimation of renal function thus delaying the ignition of dialysis. The aim of this study is to test if MDRD, CKDEPI and Donatio formulas may estimate correctly renal function in CRS2 patients compared to a reference method.

Methods: Renal function was measured prospectively by inulin clearance (mGFR) during one year in 31 patients with CRS2. We measured the mean bias (eGFR-mGFR) and the accuracy 30%. The lean body mass was estimated simultaneously by impedancemetry. We tested a new formula developed by Donatio : dGFR = [lean mass (kg) x 2.554/Pcr (mg/dL) – 0.776] x 1.73/body surface area in women; [Lean mass(kg) x 2.700/Pcr (mg/dL) – 2.920] x 1.73/body surface area in men.

Results: mGFR was 23 ± 14 mL/min/1.73 m² eGFR was à 39 ± 17 mL/min/1.73 m² with MDRD and 39 ± 18 with CKD-EPI. The biases were 15 ± 16 and 15 ± 17 mL/min/1.73 m² for MDRD and CKD-EPI, respectively. Accuracy 30 was low, 33 and 40 % respectively. The lean body mass was low, estimated at 19 kg (27% of total body weight). The Donadio formula estimated the renal function more accurately: dGFR 27 ± 7 mL/min/1.73 m², with a bias of -3.9 mL/min/1.73 m².

Conclusions: Patients with CRS2 have a worse renal function than predicted by the MDRD and CKD-EPI formulas. This overestimation is explained probably by the diminished lean body mass due to the cardiac failure. If a reference method is not accessible, the Donatio formula should be used preferentially in patients with CRS2 for an optimal orientation towards ultrafiltration, dialysis or heart-kidney transplantation.

Funding: Private Foundation Support

SA-PO809

Cystatin C Based GFR Equation Does Not Outperform Creatinine Base Formulas in Obese CKD Patients Sandrine Lemoine,[1,2] Marine Panaye,[1] Caroline Pelletier,[1] Laurent Juillard,[1] Laurence Dubourg,[2] Aoumeur Hadj-Aissa,[2] Fitsum Guebre-Egziabher.[1] *[1]Nephrology Dept, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France; [2]Renal Function Study Dept, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France.*

Background: Recent findings suggest that cystatin C is independently associated with obesity, body mass index and waist circumference. Hence, cystatin C based equation could overestimate the chronic kidney disease (CKD) prevalence for overweight and obese patients. The objective of this study was to assess the relation of cystatin C and body composition and evaluate the accuracy of cystatin C based equation for glomerular filtration rate (GFR) estimation.

Methods: This prospective study included 140 patients with BMI > 25 kg/m² in Lyon for measurement of gold standard clearance by inulin or iohexol (mGFR). Patients were separated in two groups, a group with a GFR < 60 mL/min/1.73m² and a group with GFR ≥ 60 mL/min/1.73m². All patients underwent bioelectrical impedancemetry for body composition analysis and cystatin C measurement. GFR was also estimated by equations derived from creatinine ($eGFR_{CKD-EPI}$), cystatin ($eGFR_{cyst}$) or both ($eGFR_{cyst-creat}$). Mean Bias (mGFR – eGFR) and accuracy 30% were calculated for the three equations.

Results: The mean age was 57 ± 15, mean BMI was 32 ± 4 (65% of BMI > 30) and mGFR was 62±2 mL/min. Cystatin c was positively correlated with fat mass (r=0.35, p=0.04) in the GFR ≥ 60 mL/min/1.73m² group, while no relation was found in the group with GFR < 60 mL/min/1.73m². In the group with GFR < 60 mL/min/1.73m², mGFR was 40.6 ± 1.5, $eGFR_{CKD-EPI}$ 44.8 ± 1.9, $eGFR_{cyst}$ 43.7 ± 2.6 and $eGFR_{cyst-creat}$ 43.7 ± 2.1 mL/min/1.73m². We found no difference between biaises of $eGFR_{CKD-EPI,}$ $eGFR_{cyst}$ and $eGFR_{cyst-creat}$ (-2.1. ± 4 versus -6 ±1, p=0.05 and -2.5 ± 0.7 mL/min/1.73m², p=0.1 respectively); The accuracy 30% was 87% versus 75% and 90 % for $eGFR_{CKDEPI}$, $eGFR_{cyst}$ and $eGFR_{cyst-creat}$ respectively.

Conclusions: Body fat is only a determinant of cystatin C in obese and overweight CKD patients with a GFR > 60 mL/min/1.73m², but not in patient with GFR < 60 mL/min/1.73m². $eGFR_{cyst}$ and $eGFR_{cyst-creat}$ could be used as an accurate estimation of GFR, but do not outperformed $eGFR_{CKDEPI}$.

SA-PO810

Prediction of GFR Trajectories in Autosomal Dominant Polycystic Kidney Disease Patients with Normal Renal Function Michal Mrug,[1] Sylvie Mrug,[1] Doug Landsittel,[2] Vicente E. Torres,[3] Kyongtae Ty Bae,[2] Peter C. Harris,[3] Lisa M. Guay-Woodford,[4] Michael F. Flessner,[5] William M. Bennett,[6] Alan S.L. Yu,[7] Jared J. Grantham,[7] Arlene B. Chapman.[8] *[1]U AL Birmingham; [2]U Pittsburgh; [3]Mayo Clinic; [4]Children's Nat Med Ctr; [5]NIH; [6]Legacy Good Samaritan Med Ctr; [7]U Kansas; [8]Emory U.*

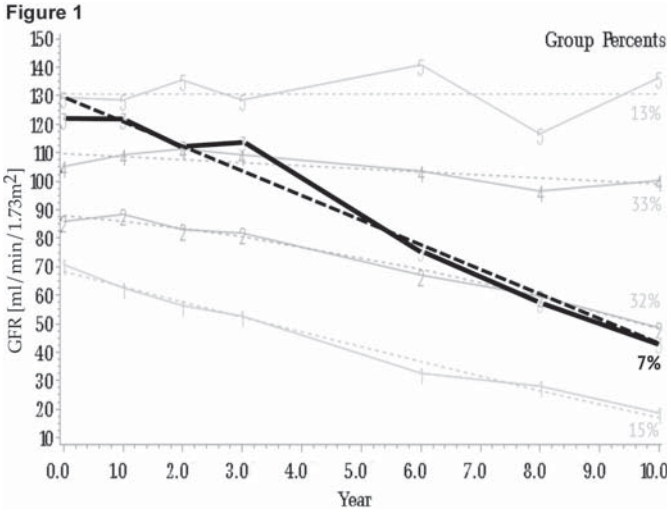
Background: There is limited information on predictors of distinct renal function trajectories in patients with ADPKD.

Methods: We developed group-based models of renal function trajectories using data collected by the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP), a prospective, observational, longitudinal, multicenter study of 241 ADPKD adults with preserved renal function.

Results: The best fitting iothalamate clearance (GFR) trajectory model included five trajectory groups (1 quadratic, 3 linear and 1 intercept only). Three of these groups involved different levels of initial GFR (70, 85 and 106 mL/min/1.73m²) followed by gradual GFR decline (by 53, 36 and 6 mL/min/1.73m²). One group had high initial GFR (131) and no GFR loss over the next decade. Another group with comparably high initial GFR (122) had highest GFR decline (by 78 mL/min/1.73m²) over the next decade (Figure 1, bold line). Logistic regression analyses compared the rapidly declining group with the top two groups that had similar initial GFR but preserved GFR over time. Five baseline variables were identified as predictors of membership in the rapidly declining GFR group (age, gender, cyst number, height adjusted total kidney volume and systolic blood pressure). ROC analyses demonstrated an area under the curve (AUC) of 0.81 for a multivariable model with these predictors.

Conclusions: Group modeling of GFR trajectories has identified a subset of CRISP participants with rapidly declining GFR and led to development of predictive models to identify the high-risk ADPKD patients.

Figure 1



Funding: NIDDK Support

SA-PO811

Prevalence and Baseline Characteristics of Pulmonary Hypertension in Different CKD Stages Zhilian Li, Xinling Liang, Yuanhan Chen, Shuangxin Liu, Zhiming Ye, Ruizhao Li, Lixia Xu, Wenjian Wang, Wei Shi. *Dept of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China.*

Background: Pulmonary hypertension (PH) was recently recognized as a common complication of end-stage renal disease (ESRD) that causes an increased risk of mortality. Epidemiological data for this disorder in earlier stages of chronic kidney disease (CKD) and its association with cardiovascular (CV) morbidity are scarce.

Methods: We retrospectively analyzed 2,351 Chinese CKD patients with complete clinical records and echocardiography data between Jan 2008 and May 2012. The patients were divided into the following 6 groups: CKD Stages 1-4; Stage 5 for those not on or initiated on hemodialysis for <3 months; and Stage 5D for the patients undergoing hemodialysis for ≥3 months. The prevalence of PH and CV morbidity was investigated, and their association was evaluated with a logistic regression model.

Results: PH was detected in 426 patients (18.1%). Mild, moderate and severe PH was diagnosed in 12.1%, 4.9% and 1.1% of the patients, respectively. Severe PH was detected in CKD Stages 5 and 5D. CV morbidity was found in 645 patients (27.4%). Compared with the non-PH group, the PH group had a higher risk for cardiac disease but not for cerebrovascular disease risk. PH severity was associated with cardiac morbidity risk [odds ratio (95% CI) for mild PH: 1.79 (1.30-2.47); moderate PH: 2.75 (1.73-4.37); severe PH: 3.90 (1.46-10.42)].

Conclusions: Our study showed the epidemiology profile of PH across the spectrum of CKD. Mild-to-moderate PH occurs with more frequency in advanced CKD, and severe PH is scarce in non-ESRD CKD. PH might be a risk factor for cardiac disease but not for cerebrovascular disease in CKD patients. PH severity affects cardiac outcomes. Evidence from prospective studies addressing PH in this population is needed to predict cardiac events.

Funding: Government Support - Non-U.S.

SA-PO812

Risk Factors for Postoperative Renal Impairment after Adrenalectomy in Patients with Primary Aldosteronism

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Background: Primary aldosteronism (PA) may induce significant renal structural damage. However, renal functional impairment can be masked in patients with PA before surgical treatment because of glomerular hyperfiltration and aldosterone escape. In this study, renal functional changes after unilateral adrenalectomy in patients with PA and the risk factors of postoperative renal impairment were analyzed.

Methods: A total of 558 patients who received unilateral adrenalectomy between January 2002 and June 2013 were included: 422 patients with pheochromocytoma, Cushing's disease, or adrenal cysts (control group) and 136 patients with PA (PA group). Baseline characteristics and postoperative changes in estimated glomerular filtration rate (eGFR) were analyzed.

Results: There was no difference in preoperative eGFR between groups, but patients with PA were more likely to have hypertension and hypokalemia. Patients in the PA group showed a significant decrease in eGFR at 3 days, 2 weeks and 6 months after surgery compared to the control group. In the PA group, 60 patients (44.1%) showed postoperative eGFR decline by more than 25% of baseline. Multivariate regression analysis identified longstanding hypertension [$p<0.001$, $\beta=0.037$], lower BMI [$p=0.011$, $\beta=-0.036$] and higher levels of uric acid [$p=0.046$, $\beta=0.054$] as risk factors for significant renal functional impairment. Among the 89 patients with preoperative eGFR > 60mL/min/1.73m^2, 22 patients (24.7%) developed chronic kidney disease (eGFR < 60mL/min/1.73m^2) postoperatively. Lower preoperative eGFR [$p<0.001$, $\beta=-0.110$], longstanding hypertension [$p=0.034$, $\beta=0.182$], and higher levels of cholesterol [$p=0.036$, $\beta=0.019$] were associated with the development of chronic kidney disease.

Conclusions: Our study suggests that primary aldosteronism patients with risk factors are at increased risk of renal functional impairment after surgical treatment.

SA-PO813

Geographic Difference in the Prevalence of Proteinuria and Albuminuria in Japan: Okinawa versus Ibaraki

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Background: The prevalence of chronic kidney diseases (CKD) which is defined by reduced estimated glomerular filtration rate in Okinawa prefecture (South in Japan) is significantly different from that in Ibaraki prefecture (East in Japan). However, Reasons for the difference in CKD prevalence in Japan remain speculative and regional differences in prevalence of proteinuria and albuminuria, that are risk of progressive CKD, is poorly evaluated.

Methods: We examined the prevalence of proteinuria and albuminuria among community-based screened populations from 40 to 80 years old in Okinawa (n=11,882) and in Ibaraki (n=2,596). Prevalence of proteinuria or microalbuminuria was defined as (+) or more, or more than 30 mg/g cre, respectively. The statistical analyses were performed by categorization with age by 10 years old.

Results: The participants in Okinawa exhibited higher body mass index, higher serum creatinine value than those in Ibaraki. Prevalence of proteinuria in subjects with 40-49 years old is significantly higher in Okinawa (2.43%) than in Ibaraki (0.49%). Interestingly, higher prevalence of that with 70-80 years old in Ibaraki (5.26%) was observed than in Okinawa (2.27%). Otherwise, prevalence of albuminuria in Okinawa (3.55% in age of 40-49 and 8.75% in age of 70-80) is lower than Ibaraki (8.96% in age of 40-49 and 30.86% in age of 70-80), regardless of their age.

Prevalence (%)	Proteinuria		Albuminuria	
Age category	Okinawa	Ibaraki	Okinawa	Ibaraki
40-49	2.43	0.49	3.55	8.96
50-59	2.49	2.26	5.12	16.52
60-69	2.81	3.68	6.62	22.99
70-80	2.27	5.26	8.75	30.86

Conclusions: The regional difference in prevalence of proteinuria and albuminuria may underlie the variation in CKD prevalence observed in Japan.

SA-PO814

Urinary Uromodulin as a Marker of Renal Mass and Function: Data From a Swiss Population-Based Study

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Background: Uromodulin (Tamm-Horsfall protein) is a protein exclusively produced by tubular cells lining the thick ascending limb of the loop of Henle, suggesting that it could be a marker of renal mass and function. We therefore analyzed the association of renal function markers, renal length and volume with urinary uromodulin.

Methods: In a multicentre Swiss population-based cohort including randomly selected adults (SKIPOGH), urinary uromodulin excretion rate (UER) was measured in a 24h urinary collection and renal ultrasound performed. Uromodulin was measured using a validated ELISA assay. Glomerular filtration rate was estimated with the CKD-EPI formula (eGFR) and with urinary 24h creatinine clearance (uGFR).Subjects with renal cysts were excluded from the analysis.

Results: We included 817 participants from SKIPOGH (53% women), with a mean age (±SD) of 45±7y, eGFR 98±17 ml/min/1.73m^2, renal length 11.0±0.8 cm, volume 137±34ml and UER of 43.9±22 mg/24h. The linear regression analysis adjusted for centre, age, gender, height and body weight, showed positive associations between square-root transformed UER and the dependent variables kidney length, kidney volume, eGFR, uGFR and urinary osmolality (all: $p<0.01$).

Conclusions: The positive associations between urinary uromodulin excretion, eGFR, uGFR, urinary osmolality, and ultrasound-assessed renal length and renal volume all suggest that urinary uromodulin excretion can be considered as a marker of renal function and tubular mass.

Funding: Government Support - Non-U.S.

SA-PO815

Chronic Kidney Disease with Chronic Obstructive Pulmonary Disease: Does Decline in Forced Expiratory Volume Correlate with Loss of Glomerular Filtration Rate?

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Background: Chronic obstructive pulmonary disease (COPD) is associated with increased risk of both acute and chronic renal failure. Renal-endocrine mechanisms, tissue hypoxia and vascular rigidity all have a role in the pathophysiology. Chronic kidney disease (CKD) is under-recognized in this population and microalbuminura is a risk factor for overall mortality. We aim to determine if there is a relationship between the severity of airflow obstruction (forced expiratory volume - FEV1) and decline in glomerular filtration rate (GFR).

Methods: We conducted a retrospective review of oxygen-dependent COPD patients currently attending the respiratory service in a tertiary referral centre in southern Ireland. Patient records and clinical laboratory data were interrogated. For each oxygen-dependent COPD patient, we documented serum creatinine, estimated GFR (eGFR), spirometry, medications, Charlson comorbidity index, heart function and annual exacerbation frequency. eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula.

Results: 38 patients in receipt of long-term oxygen therapy (LTOT) for a mean duration of 2.3 years (SD 1.9 years) were identified. The mean age of the group was 72.2 years (SD 7.5 years) and mean FEV1 was 49.7% predicted (SD 21.3%). 13.2% were diabetic and 21% had known ischaemic heart disease. 34.3% had frequent (3 or more) yearly exacerbations. The mean current eGFR was 80.1ml/min/1.73m2 (SD 26.3ml/min/1.73m2). 8 patients (21.1%) had stage 3 CKD (defined as eGFR<60ml/min/1.73m2) and no patient had stage 4 or 5 disease. There was a positive correlation between annual change in eGFR and FEV1 ($r = 0.384$, $r^2=0.148$, $n=28$, $p=0.043$).

Conclusions: Our study indicates that preserving lung function in COPD may benefit long-term renal survival. GFR has not previously been shown to correlate with the severity of airflow obstruction by spirometry. The relatively low level of advanced chronic kidney disease in this cohort may reflect the positive impact of LTOT on renovascular resistance and filtration fraction.

SA-PO816

Medication Prescribing Patterns among Pre-Dialysis Malaysian Patients Muhammad Salman,[1,2] Amer Hayat Khan,[1,2] Syed Azhar Sulaieman,[2] Azreen Syazril Adnan,[1] Fauziah Jummaat,[3] Nurul Jannah Ambak,[1] Tauqeer Hussain Mallhi. *[1]Chronic Kidney Disease Resource Center, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia; [2]Dept of Clinical Pharmacy, Univ Sains Malaysia, Penang, Malaysia; [3]Dept of Obstetrics and Gynecology, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.*

Background: Chronic kidney disease (CKD) is associated with numerous co-morbidities and complications which necessitate its management by complex therapeutic regimens. Therefore, it is imperative to evaluate medication prescribing patterns for effective treatment of CKD.

Methods: A retrospective, cross-sectional study was conducted on 612 pre-dialysis adult patients at Hospital Universiti Sains Malaysia from 2010-2013. Medical records were reviewed and data were extracted. Individual drugs were classified based on Anatomical Therapeutic Chemical (ATC) classification. Comparison was made between age groups (≤ 50 or > 50 years), gender, diabetic status (diabetes mellitus (DM) versus non-DM) and stages of CKD.

Results: There were 6960 prescriptions to 612 patients. Patients were 63.81 ± 12.04 years old, and prescribed 11.37 ± 4.39 (range 2-25) drugs. The top five prescribed medication groups were lipid modifying agents 78.6%, calcium channel blockers 63.9% and anti-thrombotic agents 62.7%, anti-diabetic drugs 56.7% and diuretics 54.9%. Underutilization of certain medications (erythropoietin, non-calcium/aluminum based phosphate binders, etc.) was apparent. Patient age and gender did not influence the number of medication used (p=0.523 and p=0.06, respectively). Diabetic patients were prescribed more drugs than non-diabetics (11.90 ± 4.36 versus 10.26 ± 4.26; p < 0.001). Furthermore, the number of prescribed drugs substantially increased with the declining GFR with the hierarchy (stage 2and 3a < stage 3b < stage 4 < stage 5).

Conclusions: Multiple medication use was observed in pre-dialysis patients. This audit also identified appropriate and questionable utilization of medication that indicated possible areas for improvement in prescription practices.

SA-PO817

Association of Dietary Sodium and Potassium Intake with Prevalence of Chronic Kidney Disease in Korea and U.S. NHANES Sang-Woong Han,[1] Jennifer L. Bragg-Gresham,[2] Hal Morgenstern,[2] Joo-Hark Yi,[1] Hyaejin Yun,[1] Meda E. Pavkov,[3] Desmond Williams,[3] Tanushree Banerjee,[4] Neil R. Powe,[4] Rajiv Saran.[2] *[1]Hanyang Univ, Seoul, Korea; [2]Univ of Michigan, Ann Arbor; [3]Centers for Disease Control, Atlanta; [4]Univ of California, San Francisco.*

Background: Evidence suggests that dietary intake of macrominerals is associated with CKD. We estimate and compare these associations in large representative population samples from the U.S. and Korea.

Methods: Study included 5,350 participants from U.S. (2009-2010) and 4,759 from Korea (2011), ≥20 years old. CKD was defined as: estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m²; elevated urinary albumin to creatinine ratio (ACR ≥30 mg/g); or either CKD indicator. Dietary intake was estimated by 24-hour dietary recall. Associations between Na and K and CKD were computed as prevalence ratios (PR) per 1000 mg/day intake, using modified Poisson regression models. Interaction terms between macromineral and country were included to test for country differences in these associations.

Results: CKD prevalence was higher in the U.S. than Korea (low eGFR: 5.4% versus 3.0%; high ACR: 7.9% versus 6.3%; combined indicators: 11.6% versus 8.5%). Na and K intake was higher in Korea versus U.S. (Na: 5,232 versus 3,605 mg/day; K: 3,144 versus 2,796 mg/day). In the U.S. only, low eGFR associated with high Na intake (PR=1.13) and low K intake (PR=0.81). High ACR and combined CKD indicators were inversely associated with K in both countries and unassociated with Na.

Outcome measure (per1,000 mg/day)	Korea		US		P for macro-mineral* country interaction
	PR	95% CI	PR	95% CI	
Low eGFR					
Na	0.96	0.91-1.02	1.13	1.04-1.22	0.001
K	1.01	0.90-1.13	0.81	0.72-0.92	0.006
High ACR					
Na	0.99	0.95-1.03	1.00	0.93-1.08	0.76
K	0.86	0.79-0.95	0.88	0.80-0.97	0.81
Combined					
Na	0.98	0.95-1.02	1.03	0.98-1.01	0.11
K	0.92	0.86-0.99	0.87	0.81-0.94	0.32

Adjusted for age, sex, race, BMI, DM, hypertension, total calorie intake.

Conclusions: In the U.S., low eGFR was associated with high Na and low K intake; in both countries, low K intake was associated with high ACR and with CKD defined by both ACR and eGFR. Further research may explain these associations in the context of dietary patterns in the two countries.

Funding: Other U.S. Government Support

SA-PO818

Febuxostat Reduce Renal Function Progression in Taiwanese Patients with Stage 3 to Stage 5 CKD Ming-Ju Wu, Kuo-Hsiung Shu. *Div of Nephrology, Dept of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan.*

Background: Hyperuricemia is very common in advanced chronic kidney disease (CKD). However, the treatment of hyperuricemia is often inadequate because of frequent allopurinol related side effects. Febuxostat is a new, non-purine based-xanthine oxidase inhibitor with much less adverse reaction.

Methods: We conducted a 3 months open label prospective study to evaluate the efficacy of serum urate lowering and the impact of eGFR change with fixed dose 40mg/day febuxostat in patients with history of gout and stages 3 to 5 CKD.

Results: Totally, 461 patients complete the study. The distribution of the stage of CKD 3a, 3b, 4 and 5 were 16.6%, 21.1%, 31.6%, 30.8%, respectively. All patients aged 20 to 93 years old, with mean age of 63.6±13.3% and 50.8% aged over 65 years old. Seventy percent of patients were male. Before the use of febuxostat, 34.2% and 35.7% patients received treatment of allopurinol and benzbromazone. Three months febuxostat significantly decreased the serum uric acid from 9.3±2.3 mg/dL to 6.8±2.6 mg/dL, P < 0.0001. Febuxostat lead to serum uric acid less than 6.0 mg/dL in 44.4% patients. Besides, febuxostat improved the trend of eGFR decrease, in 3 months period before the treatment, from -2.4 to +0.8 ml/min/1.73m² (P=0.007).

Table 1. Febuxostat treatment improved the change of estimated GFR in patients with stage 3~5 chronic kidney disease

CKD Stage	Total	3A	3B	4	5
-3 Months	28.4±12.9	54.1±11.3	39.9±8.2	24.9±8.7	11.8±5.1
O Month	26.0±11.3	50.6±8.8	37.2±4.4	22.0±4.4	9.2±3.4
Change from -3 to 0 month	-2.4	-3.5	-2.7	-2.9	-2.7
+3 Months	26.8±10.9	52.4±9.1	39.0±7.0	22.1±6.5	9.4±3.8
Change from 0 to 3 month	+0.8	+1.8	+1.8	+0.1	+0.2
P value (+3~0 vs 0~+3)	0.007	0.026	0.002	0.005	<0.0001

(ml/min/1.73 m²)

Only 2.8% patients experienced recurrent episode of gout. The efficacy is more significantly in patients with stage 3 CKD. The most common side effects of febuxostat were elevated liver function and skin itching.

Conclusions: In summary, our results support aggressive treatment of hyperuricemia in patients with advanced CKD.

Funding: Private Foundation Support

SA-PO819

Incidence and Clinicopathological Characteristics of Nephrotic Syndrome following Allogeneic Hematopoietic Stem Cell Transplantation: 27-Year Experience in a Single Cancer Center Takeshi Tokoroyama,[1,2] Minoru Ando,[1,2] Ken Tsuchiya,[2] Kosaku Nitta.[2] *[1]Dept of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; [2]Dept of Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan; [3]Dept of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan.*

Background: Nephrotic syndrome is a manifestation of graft-versus-host disease (GVHD) and is usually seen in the context of several organ manifestations of GVHD following hematopoietic stem cell transplantation (HCT). However, This type of renal involvement remains an ill-defined clinical entity.

Methods: Clinicopathological features of patients who manifested nephrotic syndrome after allogeneic HCT were retrospectively studied, using the data documented in electronic medical charts.

Results: The incidence of nephrotic syndrome was 0.068% (8 out of 1,175 allogeneic HCT) in the period of 27 years, from 1986 to 2013. Six patients were men, and mean age was 44.9 ± 15.1 years. Renal biopsy proved that 7 had membranous nephropathies (MNs) (87.5%) and 1 minimal change disease (12.5%). IgG_1 and IgG_4 were the predominant IgG subclasses in the glomerular deposits of MN. In addition, the glomerular deposition of C_3 was observed in 4 MN cases (50%), and that of both C_4 and C_{1q} only in 1 MN case. Seven (87.5%) were positive for anti-nuclear antibody in serum. Administration of prednisolone and/or cyclosporine reduced proteinuria and contributed to the complete or almost complete remission of the insult. No patients developed end-stage renal disease.The nephrotic syndrome occurred at the median time of 26 months [range, 14 to 40 months] following HCT and accompanied the mild relapse of chronic GVHD (cGVHD) including skin eruption, possibly due to the early cessation or decrease of immunosuppressant medications. This may indicate that the spectrum of immunological abnormalities that are associated with cGVHD is involved in the emergence of this renal disease.

Conclusions: Nephritic syndrome is a complication that rarely develops in the late phase following HCT, and the predominant histological change is MN. Nephrotic syndrome may represent a renal manifestation of cGVHD, according to the timing of the onset.

SA-PO820

Pregnancy Outcomes in Patients with IgA Nephropathy Xiaole Su, Jicheng Lv, Youxia Liu, Sufang Shi, Lijun Liu, Hong Zhang. *Renal Div, Peking Univ First Hospital, Beijing, China.*

Background: The outcomes of pregnancy in patients with IgA nephropathy(IgAN) remain much uncertain. Our prior study has suggest pregnancy in IgAN didn't increase the risk of kidney progression. In this extended study we aim pregnancy on the kidney function and also to identify risk factors for adverse pregnancy outcomes.

Methods: Female patients who had been with at least one pregnancy and with follow-up time more than 1-year from one large prospective IgAN database in North China were enrolled in this study. Pregnancies prior to the onset of kidney disease were excluded. We assessed the slope of estimated GFR decline, kidney diease progression and adverse pregnancy outcomes during the follow-up. Kidney disease progression was defined as eGFR halving or developing end-stage kidney disease. Adverse pregnancy outcomes include pre-eclampsia, intrauterine death, embryo damage, fetal malformation, voluntary or spontaneous abortions.

Results: From December 2003 to May 2014, 362 female patients entered the IgAN cohort and among them 79 women had 86 pregnancies. The pregnant patients had a median proteinuria at baseline of 1.04 (range 0.06 to 7.25) g/d and eGFR of 105.5 (range 40.0 to 136.2) ml/min/1.73m^2. During a mean follow-up of 52.2 months, 6/79(8%)patients in the pregnancy and 31/283(11%)in the non-pregnancy group had kidney disease progression events. After adjusted analysis including age, eGFR, MAP, proteinuria in the extended Cox proportion model, pregnancy was not associated with kidney disease progression events (HR=2.0; 95% CI 0.8 to 5.4; *P*=0.15). Similarly a linear regression analysis didn't show there was significant difference of the median rate of eGFR decline in two groups (-2.5 versus -2.9 ml/min/1.73 m^2 per year,95% CI -2.4 to 2.2; *P*=0.9). Adverse pregnancy outcomes were observed in 29patients. Proteinuria at the beginning of pregnancy (OR 2.9; 95% CI 1.3 to 6.6, *P*=0.007) was an independent risk factor of adverse pregnancy outcomes in the multivariate logistic regression model.

Conclusions: Pregnancy in IgAN with preserved or mild impaired kidney function does not seem to accelerate loss of renal function. Proteinuria at the beginning of pregnancy predict a higher rate of adverse pregnancy outcomes.

SA-PO821

The Relationship between Decoy Receptor 2 Expression and Stress Induced Tubular Senescence, Tubular Interstitial Lesion in IgA Nephropathy Bengang Huo, Huanzi Dai, Jurong Yang, Yani He. *Nephrology, Daping Hospital, ChongQing, China.*

Background: SIPS (stress induced premature senescence) plays a very important role in the development of chronic kidney disease. We found that SIPS participated in the progression of the IgA nephropathy. Recently, Decoy receptor 2 (DcR2) expression was found to be increased in senescence premalignant and fibroblast cells, suggesting that it can be involved in SIPS by resistence to apoptosis. This study aim to verify the relathionship between DcR2 expression and SIPS, tubular interstitial lesion in IgA nephropathy.

Methods: 116 primary IgA nephropathy patients aged 18-50 years without treatment and their relevant clinical data were enrolled. Oxford IgA nephropathy scoring system was used for the renal tissue lesion scoring. Renal tissue SA-β-gal expression detection, immunohistochemical detection and immunofluorescence detection were used for for p16 and DcR2.

Results: 1.Cell senescence markers were significant increased in IgA nephropathy patients: DcR2 was only expressed in renal tubular epithelial cells, SA-β-Gal positive cells were mainly tubular epithelial cells, and p16 was expressed in renal tubular epithelial cells, glomerular cells, renal interstitial cells, They all were low expression in normal kidney tissue, and increased with tubular atrophy/interstitial fibrosis score. 2. The correlation between SA-β-Gal,p16, DcR2 and clinical feature, kidney lesion score in IgA nepropathy: the percentage of p16, SA-β-Gal, DcR2 positive RTECs have a positive correlation with and blood pressure, urine NAG, uric acid, urea nitrogen, serum creatinine, urine protein to creatinine ratio, but a negative correlation with eGFR in IgA nephropathy patients. The percentage of p16, SA-β-Gal, DcR2 positive RTECs were positively correlated with segmental sclerosis/adhesion, interstitial fibrosis, arcuate arteriosclerosis/interlobular arteriosclerosis, arteriolar hyalinosis.

Conclusions: Our study confirms renal tubular epithelial cell senescence may lead the progression of IgA nephropathy. DcR2 as a marker of renal tubular epithelial cell senescence is initially proved, the expression levels of DcR2 is an important indicator for judging IgA nephropathy progression.

SA-PO822

Mitochondrial Nephropathy in Adults: Genetic, Clinical and Pathologic Features Zhihong Liu, Shuzhen Wen, Huimei Chen, Shutian Xu, Honglang Xie, Shaoshan Liang, Caihong Zeng. *National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, Jiangsu, China.*

Background: Mitochondrial nephropathy is a rare form of kidney diseases and caused by the defects in mitochondrial DNA (mtDNA). Currently its prevalence and genetic, clinical and pathologic features have not been well studied.

Methods: Mitochondrial nephropathy was diagnosed based on the presence of multisystemic involvements, ultrastructural renal lesions, and mtDNA defects. Its frequency and clinical and pathologic features were assessed. Literature review was performed for a comparison between adults and children.

Results: Review of 5,181 adults with biopsy-proven renal damage identified 21 (0.41%) patients as mitochondrial nephropathy candidates. Complete mtDNA sequence analysis identified 10 known pathogenic mtDNA mutations with 1/10 being a deletion and 3/10 3243 A>G mutation. Their heteroplasmy in kidney was over 50% and higher than that in blood. Eventually, mitochondrial nephropathy was diagnosed in 8 patients, resulting in an estimated frequency of 1.54 in 1,000 adult kidney disease patients, and prevalence of ~ 0.17 in 1,000 Chinese people. There were no sex and age preference in the distribution of the disease. The most common feature in these patients was glomerular proteinuria which correlated with renal dysfunction in half of the cases. Histological studies in kidney revealed focal segmental glomerulosclerosis (FSGS), abnormal mitochondrial morphology, and reduced activity of cytochrome c oxidase and succinate dehydrogenase in tubuli. The multisystemic involvements varied and lacked distinctive manifestations. Based on published studies, the percentage of patients with glomerular proteinuria, FSGS lesions or mtDNA mutations is higher in adult than children. Other clinical manifestations in adults included diabetes and ear/eye lesions, but were nonspecific.

Conclusions: mtDNA mutations often cause mitochondrial nephropathy in adults characterized with glomerular defects and mitochondrial abnormity. The genetic and clinical features of the disease are diverse, and the awareness and correct diagnosis of this disease entity are needed for effective treatment.

Funding: Government Support - Non-U.S.

SA-PO823

Food Access and Kidney Disease in the U.S. Jonathan J. Suarez,[1] Tamara Isakova,[2] Cheryl A. Anderson,[4] L. Ebony Boulware,[3] Myles S. Wolf,[2] Julia J. Scialla.[1] *[1]Univ of Miami; [2]Northwestern Univ; [3]Duke Univ; [4]UC San Diego.*

Background: Diet is a risk factor for hypertension and CKD. CKD prevalence demonstrates geographic clustering and socioeconomic disparities. Geographic areas with limited access to grocery stores (i.e. food deserts) may promote unhealthy diets and contribute to CKD disparities.

Methods: We linked data from adults in the 2003–2010 National Health and Nutrition Examination Survey (n=21,281), a survey of the non-institutionalized U.S. population to food desert data (www.ers.usda.gov) by census tracts. Food deserts are defined by the USDA as low-income tracts in which ≥500 people or 33% of the population lives >1 mile (urban) or >10 miles (rural) from a supermarket or large grocery store. We summarized dietary intake (24h recall) and behaviors as weighted means and frequencies. We used weighted linear and logistic regression to assess the association of food desert residence and income (i.e. ratio of family income to the poverty line) with mean systolic blood pressure (SBP) and odds of CKD.

Results: Participants living in food deserts consumed less protein (79.4 versus 83.3 g; p=0.03) and potassium (2544 versus 2728 mg; p=0.002), with higher net endogenous acid production (60.7 versus 58.4 mEq; p=0.03), among other differences. Participants with lower income displayed similar intake patterns. Availability of fruit at home was lower in food deserts; availability of fruits and vegetables was lower with low income (each p<0.001 by χ^2). Living in a food desert was associated with higher SBP, whereas low income was associated with higher SBP and greater odds of CKD.

	Odds Ratio of CKD (95% CI)‡	Difference in SBP (95% CI)§
Residence* †		
Non-Food Desert	Ref	Ref
Food Desert	1.20 (0.96, 1.49)	**1.53 (0.41, 2.66)**
Ratio of Income to Poverty Line*		
≤1	**1.79 (1.50, 2.13)**	**2.12 (1.25, 3.00)**
1–1.5	**1.75 (1.48, 2.07)**	**2.67 (1.84, 3.51)**
1.5–2	**1.49 (1.22, 1.81)**	**2.07 (1.06,3.09)**
2–3	**1.27 (1.11, 1.45)**	**1.00 (0.07, 1.94)**
>3	Ref	Ref

*Adjusted for age, sex, race/ethnicity
†Additionally adjusted for income
‡CKD defined as eGFR<60 mL/min/1.73m^2 or urine albumin to creatinine ratio >30 mg/g
§Difference in SBP represents the absolute difference in mean SBP in each category compared to the reference category.
Boldface indicates p<0.05

Conclusions: SBP is higher in food deserts independent of demographics and income, but poverty may be more strongly associated with higher SBP and prevalence of CKD. Food access and poverty may each represent targets for CKD prevention.

Funding: NIDDK Support

SA-PO824

Electronic Health Record Patient Portal Use: Correlates and Outcomes Manisha Jhamb,[1] Mark L. Unruh,[2] Khaled Abdel-Kader.[3] *[1]Renal Div, Univ of Pittsburgh; [2]Div of Nephrology, Univ of New Mexico; [3]Div of Nephrology, Vanderbilt Univ.*

Background: Electronic health record (EHR) portals allow patients to access health information and communicate with their care team. While portals support patients with chronic diseases, inequities in internet access (ie, a digital divide) may reinforce care disparities. We are not aware of publications on nephrology portal use.

Methods: In 2009, an outpatient nephrology clinic implemented an EHR portal allowing patients to perform 'active' (eg, communicate with providers) and 'passive' tasks

(eg, review labs). We describe portal use in non-dialysis outpatients seen >=2 times from 1/1/2010-12/31/2012. We also examine sociodemographic correlates of portal use and associated process of care outcomes using multivariable logistic regression.

Results: Of 2,640 patients, 1,020 (39%) used the portal and 316 (31% of users) communicated with the renal team. 'Active' users most commonly requested medical advice (70%) and medication refills (35%). Correlates of portal use are shown.

Variable	Adjusted OR (95% CI)
Age (per 10 years)	0.81 (0.75 - 0.86)
Race	
Black vs. white	0.42 (0.33 – 0.54)
Other vs. white	0.82 (0.46 – 1.45)
Sex (Female vs. Male)	1.16 (0.97 - 1.38)
Marital Status (Married vs other)	1.75 (1.46 – 2.11)
Insurance	
Medicaid vs. Private	0.58 (0.40 – 0.85)
Medicare vs. Private	0.73 (0.60 – 0.90)
Self-pay/uninsured	0.67 (0.47 – 0.97)
HTN	1.38 (1.02 – 1.87)
Hyperlipidemia	1.47 (1.21 – 1.79)
Kidney transplant	1.65 (1.29 – 2.11)
Tobacco (active vs. other)	0.60 (0.47 – 0.77)
eGFR (per 10ml/min/1.73m^2)	1.04 (1.01 – 1.08)
Baseline proteinuria (>1+ on dipstick)	0.71 (0.59 – 0.86)

Portal use was not associated with ACEi/ARB use in proteinuric patients or with optimal BP control in adjusted analyses.

Conclusions: Many (39%) nephrology outpatients used an EHR portal and 31% of users communicated with the renal care team. Portal use associated with younger age, white race, and private insurance, but not with processes of care. As health systems enhance online communication tools, they should devote attention to access for vulnerable populations and ensure that portals reduce care disparities.

Funding: NIDDK Support, Private Foundation Support

SA-PO825

Rates of Cardiovascular Events and Death in African-Americans with CKD: The Jackson Heart Study

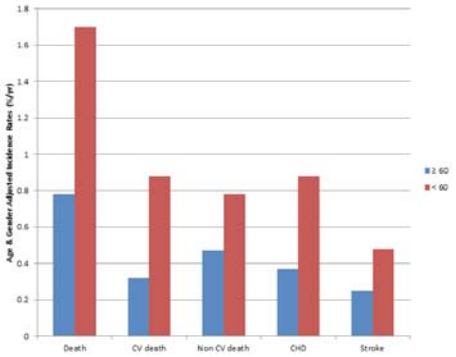
Nisha Bansal, Ronit Katz, Bessie A. Young, Jonathan Himmelfarb, Bryan R. Kestenbaum, Ian H. de Boer. *UW.*

Background: Chronic kidney disease (CKD) and cardiovascular disease (CVD) are particularly common among African-Americans. The extent to which CKD contributes to specific CVD risk is not well defined in this population.

Methods: We studied 5,187 participants in the Jackson Heart Study, a community-based cohort of African-Americans. CKD was defined at baseline as eGFR <60 mL/min/1.73m2 (CKD-EPI equation). Subsequent stroke, coronary heart disease (CHD) and death were adjudicated. Poisson regression was used to calculate adjusted incidence rates (IR) and incidence rate ratios (IRR) of each outcome in participants with and without CKD.

Results: At baseline, the mean age was 55 (13) years, 37% of participants were men, 22% had diabetes, 61% had hypertension and mean eGFR was 95 (22) ml/min/1.73 m2. Participants with CKD (N=307) had a mean eGFR of 46 (12) ml/min/1.73 m2. With adjustment for age and gender, those with CKD had higher rates of death, incident CHD and stroke compared to those without CKD (Figure). Adjusting for age, gender, family history of CVD, prevalent CVD, hypertension, diabetes, BMI, tobacco use, plasma lipid concentrations, and pertinent medications, IRR comparing participants with CKD to those without CKD were 1.83(1.02, 3.27) for stroke, 1.45(0.84, 2.49) for CHD, 1.96 (1.30, 2.95) for non-CVD death, 1.76(1.15,2.68) for CVD death and 1.88(1.41, 2.51) for all-cause death.

Conclusions: Among a community-based cohort of African-Americans, rates of CVD events and death were significantly greater among participants who had CKD. Targeted CVD risk reduction may improve outcomes among African-Americans with CKD.



Funding: NIDDK Support

SA-PO826

Effects of Race and Sex on Measured GFR in the Elderly: Multi-Ethnic Study of Atherosclerosis

Lesley Inker, Tariq Shafi, Aghogho A. Okparavero, Hocine Tighiouart, John H. Eckfeldt, Ronit Katz, Craig Johnson, Zarqa Tariq, Imene Benayache, Ann Talbot Martz, Wendy S. Post, Josef Coresh, Andrew S. Levey, Michael Shlipak. *MESA-Kidney.*

Background: Blacks and men have higher incidence of end stage renal disease (ESRD). No prior studies have measured GFR in a community-based cohort of older adults with adequate representation of Blacks and Whites. We conducted MESA Kidney, an ancillary study within MESA, to compare measured GFR in a sample of older Black and White men and women from a representative cohort.

Methods: MESA Kidney participants were recruited from the Johns Hopkins University MESA site after the 5th examination cycle. 670 participants were invited, 294 completed the protocol and 292 have been analyzed to date. GFR was measured using plasma clearance of iohexol computed from 5-6 blood samples and a two compartment model. Using linear regression, we compared GFR, indexed to body surface area (BSA), in Blacks versus Whites and men versus women; models were unadjusted and adjusted for age, diabetes, hypertension and sex/race.

Results: Mean (SD) GFR was 73 (19) ml/min/1.73 m^2, age was 71 (9) years, 47% were Black, 47% were women, 25% were diabetic, and 64% were hypertensive. Mean GFR was slightly, but not significantly, higher in Blacks compared with Whites. Men had higher GFR than women, even after adjustment (Table). Differences were greater in magnitude when GFR was not indexed for BSA; after adjustment, Blacks had a 4.3 ml/min (-0.7, 9.4; p=0.09) and men a 23.2 ml/min (18.3, 28.1; p<0.001) higher GFR.

	Mean difference (95% CI) in mGFR (ml/min/1.73 m^2)	
	Blacks vs. White	Men vs. Women
Unadjusted	3.0 (-1.3, 7.3)	**9.2 (5.0, 13.4)**
Adjusted for age, sex or race	1.2 (-2.5, 4.9)	**10.4 (6.7, 14.1)**
Adjusted for age, sex or race, diabetes status and hypertension status	2.3 (-1.6, 6.2)	**11.0 (7.2, 14.8)**
Bold indicates p <0.05. P-values >0.4 for sex/race interactions.		

Conclusions: Mean GFR was similar in Blacks compared to Whites and higher in men compared to women in community-based older adults. Mean GFR was not lower in the demographic groups with the highest risk of ESRD. The higher incidence of ESRD in Blacks and men cannot be explained by levels of average GFR in the population.

Funding: NIDDK Support, Other NIH Support - National Center for Advancing Translational Sciences (NCATS) UL1 RR 025005

SA-PO827

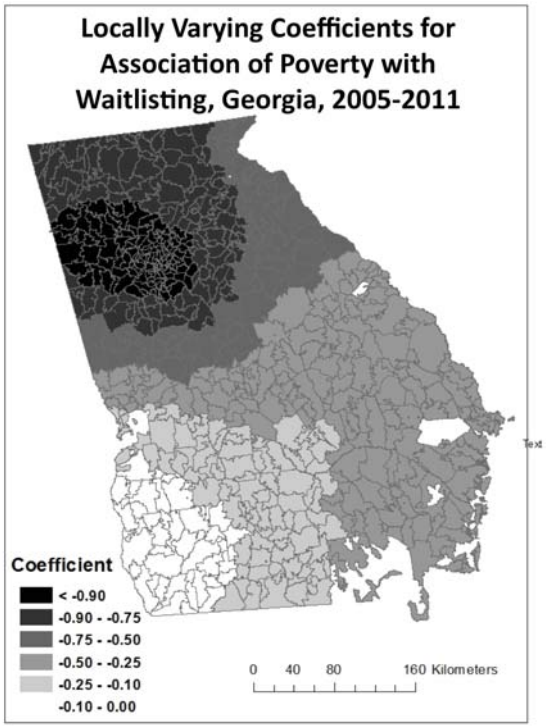
Area Poverty and Access to Kidney Transplantation in Georgia: Multilevel versus Spatial Approach

Laura Plantinga, Curtis Travers, Juan L. Rodriguez, Rachel E. Patzer. *Emory Univ, Atlanta, GA.*

Background: Georgia has the lowest kidney transplantation rates in the nation. While area poverty is a known barrier to transplant access in the Southeast, the contribution of spatial patterns of poverty to this association remains relatively unexplored.

Methods: Using linked United States Renal Data System (2005-2011) and American Community Survey (2007-2011) data, we examined the association of zip code tabulation area (ZCTA)-level poverty (% living below federal poverty threshold) with waitlisting (within 1 year of ESRD start), among U.S. incident black and white dialysis patients aged 18-69 (*n*=12,130) in multilevel mixed-effects models. Geographically weighted regression (GWR) of the association of poverty and waitlisting (both ZCTA-level) was then used to assess potential effect modification by spatial patterns of poverty.

Results: With adjustment and allowing for random effects, patients living in ZCTAs with greater poverty (mean, 20.2%) were ~30% less likely to be waitlisted for each increase in ZCTA poverty of 10% (OR=0.73, 95% CI, 0.67-0.79). Modest spatial autocorrelation of ZCTA poverty (Moran's I = -0.10, P<0.001) was eliminated with GWR using 30-km bandwidth kernel density estimation, and the median change in % ZCTA-level waitlisting per 1% increase in ZCTA-level poverty was -0.60 (IQR =-1.19,-0.22). Compared to ordinary least squares regression with no spatial component, no substantial improvement in model fit was seen with GWR.



Conclusions: In Georgia, greater area poverty is associated with significantly decreased likelihood of waitlisting. While coefficients indicated the greatest effect of poverty in northwest Georgia (including the Atlanta area) and least effect in rural southeast Georgia, accounting for spatial patterning did not contribute significantly to the association between area poverty and waitlisting in Georgia.

Funding: Other NIH Support - NIMHD, Private Foundation Support

SA-PO828

CKD Risk Factor Control in the Urban Healthcare Safety Net

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Background: Socially disadvantaged patients with CKD are often invisible to much of the U.S. healthcare system unless and until they reach ESRD. We sought to examine CKD risk factor management and determine the degree to which it influences disease progression and mortality in an urban safety net healthcare setting.

Methods: We conducted a cohort study of 12,123 patients with moderate-to-advanced CKD who received ambulatory care during 2000-2009 within a regional safety net health system and who were followed for death and ESRD through 2011. We examined the associations of CKD risk factor control and rates of death and progression to ESRD using proportional hazards regression.

Results: Overall, hypertension was the most common risk factor (47%) for CKD progression followed by diabetes (23%). Patients at highest risk (fifth quintile, n=2,425) for progressing to ESRD were significantly younger and had a higher prevalence of hypertension (70%), diabetes (41%), substance abuse (49%) and chronic viral disease (29%) than lower risk counterparts. Only 27% and 45% of high-risk patients achieved BP (<140/90 mmHg) and glycemic (A1c<8%) control, respectively, compared with 39% and 56% of the larger CKD cohort. Proportionately fewer high-risk patients received RAAS inhibitors and nearly half of high-risk patients were subsequently lost to follow-up. High-risk patients who did not achieve BP control and who were lost to follow-up experienced significantly higher rates of death and ESRD than counterparts who achieved BP control or remained in care.

Conclusions: In this public health setting, we observed missed opportunities to mitigate risks of death and progression of CKD. Intervention approaches aimed at identifying and retaining high-risk patients in care may facilitate care delivery and reduce CKD morbidity and mortality among vulnerable populations.

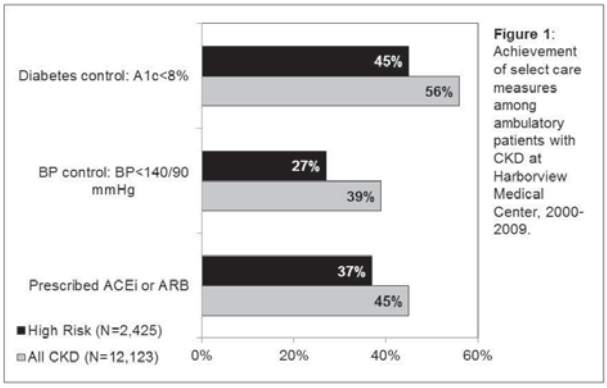

Figure 1: Achievement of select care measures among ambulatory patients with CKD at Harborview Medical Center, 2000-2009.

Funding: NIDDK Support

SA-PO829

KDIGO Screening and Treatment among CKD Stage G3A Patients with Uncontrolled Hypertension

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Background: For patients with eGFR 45-59 ml/min/1.73 m^2 (CKD Stage G3a), KDIGO guidelines recommend yearly albuminuria screening, one-time dyslipidemia screening, statin treatment for patients ≥50 years, and cystatin C testing for patients with <30 mg albumin/g creatinine. Uptake of these guidelines is unknown. High-risk patients in particular may benefit from evidence-based screening and care.

Methods: We retrospectively analyzed adherence to KDIGO guidelines in the adult primary care population within the Geisinger Health System (a rural, integrated health care system in Central PA) for the period January 1, 2013 through December 31, 2013. We limited the analysis to "high-risk" patients, defined as those with CKD Stage G3a and an average outpatient systolic or diastolic blood pressure reading ≥150 or 85 mm Hg, respectively. We excluded patients with <2 blood pressure readings.

Geisinger high-risk cohort characteristics (N=341)	
Age (years), median (IQR)	69.6 (24.0, 72.0)
Male sex, %	40.8
Smoking status (current), %	15.2
Diabetes, %	17.3
History of congestive heart failure, %	7.0
History of myocardial infarction, %	4.4
History of stroke or TIA, %	10.9
Hospitalized between 1/1/13-12/31/13, %	32.3
PCP visits between 1/1/13-12/31/13, mean (SD)	2.5 (1.7)
Systolic blood pressure (mm Hg), mean (SD)	151 (21.9)
Diastolic blood pressure, mm Hg, mean (SD)	81 (12.8)
Last reported eGFR (ml/min/1.73m^2), mean (SD)	53.0 (3.9)
Last reported UACR, mg/g, mean (SD)	113 (56)
Last reported LDL (mg/dL), mean (SD)	107 (39)

Results: Of 70,847 active primary care patients, 341 patients met the definition of high risk. Albuminuria and dyslipidemia screening were performed in 25.2% and 48.7% in the prior 12 months, respectively. Among the 3 patients with < 30 mg albumin/g creatinine, none had cystatin C testing. Of the 309 patients over 50, 49.8% were treated with a statin.

Conclusions: KDIGO guideline-based screening and treatment of high risk stage G3a CKD patients is suboptimal in this integrated health care system. Novel strategies to improve guideline-based care are warranted.

SA-PO830

Added Value of Screening for Chronic Kidney Disease Among Elderly or Persons with Low Socioeconomic Status

Priya Vart,[1] Sijmen A. Reijneveld,[1] Ute Bültmann,[1] Ron T. Gansevoort.[2] *[1]Health Sciences; [2]Nephrology, UMC Groningen, Netherlands.*

Background: For chronic kidney disease (CKD) screening, it is not known whether adding elderly or persons with low socioeconomic status (SES) to the traditional high risk groups is effective. Therefore, we aimed to compare three screening approaches (i.e. Approach 1, traditional, screening only persons with known diabetes mellitus, hypertension or cardiovascular (CV) disease history; Approach 2, as approach 1 + elderly; and Approach 3, as approach 1 + persons with low SES) on their ability to detect CKD cases and, to identify those CKD cases that have a higher rate of incident CV events and renal function decline.

Methods: A sample of 3,421 participants representative for the general population in the Netherlands was examined. Persons with age >60 years were classified as elderly. SES was assessed from educational level and classified as low, medium and high. The presence of CKD was determined during examination at an outpatient clinic. Participants were followed for 9.4 ± 2.6 years during 5 screening rounds.

Results: At baseline, the percentage of the general population to be screened was 16%, 29% and 25% in Approaches 1, 2 and 3, respectively. From a total of 325 CKD cases at baseline, 38% were identified by the traditional screening approach. Adding elderly to the traditional CKD screening groups detected the largest number of CKD cases (65%),

whereas adding the low SES group detected more than half of the CKD cases (53%). Rate of incident CV events, when compared to participants without CKD, was significantly higher in detected and undetected CKD cases in Approach 2 while the rate was only significantly higher in detected CKD cases in Approach 3. Rate of renal function decline, when compared to participants without CKD, was significantly higher in detected and undetected CKD cases in Approach 2 and Approach 3, and was lower in undetected CKD cases in screening Approach 3 compared to undetected CKD cases in Approach 2.

Conclusions: Adding persons with low SES, rather than adding elderly, to the traditional high risk groups may be of help for detecting CKD cases that have a higher risk for future CV events and renal function decline.

SA-PO831

Socioeconomic Disparities in Chronic Kidney Disease: A Systematic Review and Meta-Analysis Priya Vart,[1] Ron T. Gansevoort,[2] Michel M. Joosten,[2] Ute Bültmann,[1] Sijmen A. Reijneveld.[1] *[1]Health Sciences; [2]Nephrology, UMC Groningen, Netherlands.*

Background: Evidence on the strength of the association between low socioeconomic status (SES) and chronic kidney disease (CKD) (defined as low eGFR, high albuminuria, low eGFR/high albuminuria or end-stage renal disease) is sparse and sometimes conflicting. Therefore, we aimed to summarize the strength of the associations between SES and CKD in a systematic review and meta-analysis, and to identify study level characteristics related to the strength of the SES-CKD association.

Methods: We conductedasystematic review and meta-analysis of observational studies published in Medline and EMBASE until January 2013. Association measures from individual studies were pooled using random-effects models. Meta-regression analysis was used to identify study level characteristics related to the strength of the association between SES and CKD, with SES defined using individual/household/family income, educational attainment, occupational class/status, composite or area based SES measures.

Results: Out of 2472 citations, 35 studies were found to be relevant (with 3,632,531 individuals including 832,948 CKD cases).Compared to high SES, low SES was associated with low eGFR (OR=1.27, 95% CI: 1.10 – 1.43), high albuminuria (OR=1.52, 1.22 – 1.82), low eGFR/high albuminuria (OR=1.38, 1.03 – 1.74) and renal failure (OR=1.55, 1.40 – 1.71). Different definitions of SES were not related to the strength of association between low SES and any of the CKD measures (for low GFR: p=0.63, high albuminuria: p=0.29, for low eGFR/high albuminuria: p=0.54 and renal failure: p=0.31). Regarding study level characteristics, variations in the strength of the association were only related to the level of covariate adjustment for low eGFR (p<0.001) and high albuminuria (p<0.001). Study design, geographic setting, baseline risk of study population and number of SES categories had no impact.

Conclusions: Socioeconomic disparities in CKD were fairly strong, irrespective of how SES was measured. Variations in the strength of the associations were only related to the level of covariate adjustment in case of low eGFR and high albuminuria.

SA-PO832

Mediators of the Association between Low Socioeconomic Status and Chronic Kidney Disease in the United States Priya Vart,[1] Ron T. Gansevoort,[2] Deidra C. Crews,[3] Sijmen A. Reijneveld,[1] Ute Bültmann.[1] *[1]Health Sciences; [2]Nephrology, UMC Groningen, Netherlands; [3]Johns Hopkins Univ School of Medicine.*

Background: The factors linking low socioeconomic status (SES) and Chronic Kidney Disease (CKD) are poorly understood. We, therefore, investigated potentially modifiable factors linking SES to CKD for their magnitude of mediation.

Methods: A samlple of 9,823 participants in the 2007-2008 and 2009-2010 National Health and Nutritional Examination Surveys (NHANES) was examined. SES was defined using tertiles of the poverty income ratio (PIR). High, middle and low PIR tertiles were denoted as high, middle and low SES, respectively. Main outcome was CKD, defined as eGFR <60 mL/min/1.73m2 (CKD-EPI) and/or urinary albumin-creatinine ratio (ACR) ≥30mg/g. Mediation analyses tested the health-related behaviors (smoking, alcohol intake, diet, physical activity and sedentary time), comorbid conditions (diabetes, hypertension, obesity, abdominal obesity and hypercholesterolemia) and factors related to health care access (health insurance and routine visits for health care)for their contribution to the SES-CKD association. Mediation analysis was performed using counterfactual approach.

Results: Compared to high SES, low SES was associated with an increased odds of CKD (OR=1.92, 95% CI: 1.64, 2.26). Except sedentary time and diet, all examined health-related behaviors, comorbid conditions and factors related to health care access mediated the SES-CKD association, and contributed 20%, 32% and 11%, respectively, to this association. In ethnicity specific analyses, the identified mediators tended to explain a greater proportion of the SES-CKD association in non-Hispanic blacks than in other ethnic groups.

Conclusions: Potentially modifiable factors like health-related behaviors, comorbid conditions and health care access contribute substantially to the association between low SES and CKD in the U.S., especially among non-Hispanic blacks.

SA-PO833

Prescribing Metformin According to eGFR Rather Than Serum Creatinine Can Enhance Its Use, Particularly among Younger Non-Hispanic Black Men Delphine S. Tuot,[1] Feng Lin,[1] Michael Shlipak,[1] Jerry Yee,[2] Rajiv Saran,[3] Sharon Saydah,[4] Desmond Williams,[4] Neil R. Powe.[1] *[1]Univ of California, San Francisco; [2]Henry Ford Hospital; [3]Univ of Michigan; [4]Centers for Disease Control and Prevention.*

Background: Recent guidelines recommend using eGFR rather than serum creatinine (sCr) to determine metformin eligibility among adults with diabetes mellitus (DM). Compared to sulfonylureas, metformin is associated with less cardiovascular risk, CKD progression and death. We examined the potential impact of these guidelines on metformin eligibility among U.S. adults.

Methods: Metformin eligibility was assessed among 3902 adults with DM who participated in the 1999-2010 National Health and Nutrition Examination Surveys and reported routine access to health care. We compared eligibility using conventional sCr cutoffs (eligible if < 1.4mg/dL for women; < 1.5mg/dL for men) and eGFR cutoffs (eligible if >45ml/min/1.73m^2; contraindicated if <30ml/min/1.7m^2), using different estimating equations: 4-variable MDRD, CKD-EPIcr, CKD-EPIcys and CKD-EPIcr-cys and Cockcroft-Gault to estimate creatinine clearance (CrCl). DM was defined by self-report or glycosylated hemoglobin ≥6.5%. We used logistic regression to estimate odds of newly eligible populations adjusted for age, race/ethnicity and sex. Results were weighted to the U.S. adult population.

Results: Among adults with sCr above conventional cutoffs, those newly eligible for metformin by MDRD eGFR were predominantly male (aOR=33.3, 95%CI 7.4-151.5), <60 years old (aOR=66.3, 1.26-31.7) and non-Hispanic black (aOR versus whites=14.8, 4.27-51.7). No individuals with sCr below prior cutoffs had an MDRD eGFR <30ml/min/1.73m^2. The population eligible for metformin expands when using any of the equations estimating GFR or CrCl: +86,883 (CKD-EPIcr); +104,248 (MDRD); +113,943 (CKD-EPIcys); +141,472 (CKD-EPIcr-cys); +834,740 (Cockcroft-Gault).

Conclusions: Using estimated GFR or CrCl to determine metformin eligibility rather than sCr expands the adult population with DM for whom metformin is considered safe to use, particularly among younger non-Hispanic black men. Enhancing metformin use in these populations may help mitigate disparities in diabetes and CKD outcomes.

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

SA-PO834

Directly Measured Kidney Function Is Linked to Frailty and Frailty Predicts Mortality Independent of GFR Cynthia Delgado,[1,2] Barbara A. Grimes,[4] David V. Glidden,[4] Michael Shlipak,[5] Mark J. Sarnak,[3] Kirsten L. Johansen.[1,2] *[1]Nephrology Section, San Francisco VA Medical Center, CA; [2]Div of Nephrology, Univ of California, San Francisco, CA; [3]Div of Nephrology, Tufts Medical Center, Boston, MA; [4]Dept of Epidemiology and Biostatistics, Univ of California, San Francisco, CA; [5]Medicine, Univ of California, San Francisco, CA.*

Background: Frailty is prevalent in patients with CKD, including non-elderly patients. Most studies of frailty and CKD have estimated GFR using creatinine in cohorts not enriched for CKD. We evaluated the association of measured GFR with frailty among patients with known CKD and determined whether frailty was associated with death after including mGFR.

Methods: We ascertained death in those enrolled in the randomized Modification of Diet in Renal Disease (MDRD) study (1989-1993) (baseline GFR 33 ±12 ml/min/1.73m2) through 2007 with NDI and USRDS linkage. GFR was measured using iothalamate (mGFR), and we estimated GFR using the CKD-EPI (eGFR) equation. We defined frailty using questionnaire data on exhaustion, physical function and activity, and weight loss. Frailty was defined by a score of ≥3 points. We used multivariable logistic regression models to evaluate the association of GFR with frailty and Cox models to assess the association of frailty with death after adjusting for mGFR or eGFR and other covariates.

Results: 812 MDRD participants (96%) had complete data on frailty and GFR. 16% were frail. Median follow-up time was 17 (IQR 11-18) years with 371 deaths. Higher mGFR was associated with lower odds of frailty (OR 0.76, 95% CI 0.64-0.90 per 10ml/min/1.73m2). Similarly, eGFR was inversely linked to frailty (OR 0.80, 95% CI 0.69-0.94 per 10ml/min/1.73m2). Frailty was associated with a higher risk of death (HR 1.67, 95% CI 1.24-2.20), which did not differ when mGFR (HR 1.46, 95% CI 1.10-1.97) or eGFR (HR 1.50, 95% CI 1.10-2.00) was included as an indicator of kidney function (covariate).

Conclusions: We found an inverse association between kidney function and frailty that was similar for mGFR and eGFR. Frailty was associated with higher risk of death after adjustment for multiple covariates including GFR.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO835

Lower Dialysis Facility Staff to Patient Ratio Associated with Kidney Transplant Referral in Georgia Rachel E. Patzer,[1,6] Laura Plantinga,[6] M. Ahinee Amamoo,[2] Eric M. Gibney,[4] Stephen O. Pastan.[5] *[1]Surgery, Div of Transplantation, Emory Univ School of Medicine, Atlanta, GA; [2]Southeastern Kidney Council of ESRD Network 6, Raleigh, NC; [3]Piedmont Hospital, Atlanta, GA; [4]Medicine, Renal Div, Emory Univ School of Medicine, Atlanta, GA; [5]Epidemiology, Emory Univ, Atlanta, GA.*

Background: Georgia (GA) has the lowest kidney transplant rates in the U.S. and worst racial disparities in these rates. Prior studies have shown that fewer dialysis facility staff may be associated with reduced transplant access. We examined whether facility patient-to-staff ratio was associated with kidney transplant referral among GA dialysis patients.

Methods: We collected patient-level transplant referral data on all kidney transplant referrals to each transplant center in GA from 2008 to 2011 as part of our RaDIANT (Reducing Disparities in Access to kidNey Transplantation) Community study. We linked these data to 2008-2011 Centers for Medicare and Medicaid Services (CMS) Dialysis Facility Report (DFR) data to determine number of staff and calculated 4-year annual kidney transplant referral (% of facility patients aged <70 referred in calendar year) for GA dialysis facilities (n=273). We examined whether the patient-to-staff ratio was associated with kidney transplant referral using linear regression models.

Results: A total of 273 dialysis facilities treating >12,000 patients, who were 56% African American and had a mean age of 60.6 years, were examined. The mean number of full-time staff was 11.5 (SD: 5.8; range: 0-43) and mean patient-to-facility staff ratio was 4.5 (SD: 1.5). Median 5-year kidney transplant referral was 14.8% (IQR: 9.2-21.3%). Each unit increase in patient-to-staff ratio was associated with ~1.5% lower kidney transplant referral within GA dialysis facilities (β = -0.01471, SE: 0.00372, p<0.0001).

Conclusions: Identifying characteristics of facilities associated with low transplant referral rates could help improve access to kidney transplantation. For example, targeting interventions among dialysis facilities with high patient-to-staff ratios could help improve referral for kidney transplantation.

Funding: Other NIH Support - National Institute of Minority Health and Health Disparities

SA-PO836

Abstract Withdrawn

SA-PO837

Access to the Preemptive Registration on the Waiting List for Cadaveric Renal Transplantation: A Hierarchical Modeling Approach Natacha Riffaut,[1] Thierry Lobbedez,[1] Marc Hazzan,[2] Dominique Bertrand,[3] Gabriel Choukroun,[4] Bruno Hurault de Ligny.[1] *[1]CHU Clémenceau, Caen, France; [2]CHRU Lille, France; [3]CHU Bois Guillaume, Rouen, France; [4]CHU Amiens, Saloul, France.*

Background: It has been shown that preemptive kidney transplantation (KT) is associated with both higher patients and graft survival compared with transplantation performed after dialysis. Therefore, we believe, like others, that patients should be registered on the waiting list before dialysis onset when feasible. This study was carried out to estimate the association between the renal units and the preemptive registration on the waiting list for first cadaveric KT in a French network of care.

Methods: From 2008 to 2012, 1529 adult-patients followed in 48 centers of the French North-West network, and registered on the waiting list for a first cadaveric KT, were included. We used a mixed logistic regression with renal unit as random-effects term for statistical analysis.

Results: Of the 1529 patients included in the study, 407 were placed on the waiting list preemptively. The non-adjusted mixed model showed a significant variability across renal units (variance 0.452; SD 0.672). In multivariate analysis, factors independently associated with preemptive registration were cardiovascular disease (OR 0.47, 95% CI 0.41 to 0.78), social deprivation (OR 0.75, 95% CI 0.58 to 0.98), and characteristics of the units (Ownership of the facility: academic hospital, reference – community hospital, OR 0.43, 95% CI 0.22 to 0.80 – private hospital, OR 0.34, 95% IC 0.16 to 0.69; and Transplant center; p<0.05). Variability between renal units was reduced after taking into account for their characteristics (proportional change in variance, 45%) but was not influenced by patients characteristics.

Conclusions: Preemptive registration is associated with renal units, transplant centers, social deprivation, and can be partly explained by disparities in practices. In view of these findings, public health actions should be implemented to facilitate the preemptive access to the waiting list, especially among the most disadvantaged populations.

SA-PO838

Using Hypothetical Patient Scenarios to Assess Nephrologists' Perceptions Regarding Choice of Treatment for End Stage Renal Disease Ankita Tandon,[1] Ming Wang,[2] Kevin C. Roe,[1] Surju Patel,[1] Nasrollah Ghahramani.[1] *[1]Dept of Medicine, Penn State Milton S. Hershey Medical Center, Hershey, PA; [2]Dept of Public Health Sciences, Penn State College of Medicine, Hershey, PA.*

Background: Kidney transplantation (KT) is the most effective treatment for end stage renal disease (ESRD). Factors related to nephrologists and their perceptions of suitable patient characteristics are likely contributors to patients referred for KT.

Methods: Using an online survey, 250 nephrologists were asked whether they would recommend dialysis or kidney transplant (KT) for hypothetical patients with ESRD. The 4 scenarios varied in race, living situation (alone or with spouse), and rural location. 215 nephrologists provided complete responses.

Results: In multivariate analysis, rural patients (OR:0.71, p= 0.005) were less likely to be recommended for KT. Nephrologist factors associated with lower likelihood of recommending KT included lack of academic affiliation (p=0.01), and longer time since completing fellowship (p<0.01). Adjusted for rural location, attending 4 or more national nephrology meetings (p=0.01) and longer time practicing in the current location (p<0.01) were associated with significantly higher likelihood of recommending KT; practice as a non-transplant nephrologist (p<0.01) was associated with lower likelihood of recommending transplant (p<0.05).

Conclusions: Rural patients are less likely to be referred for KT. Lack of academic affiliation, length of time since fellowship, frequency of attendance to national meetings and whether the nephrologist is a transplant nephrologist significantly impacts the decision regarding KT referral.

Funding: Other NIH Support - K23DK084300

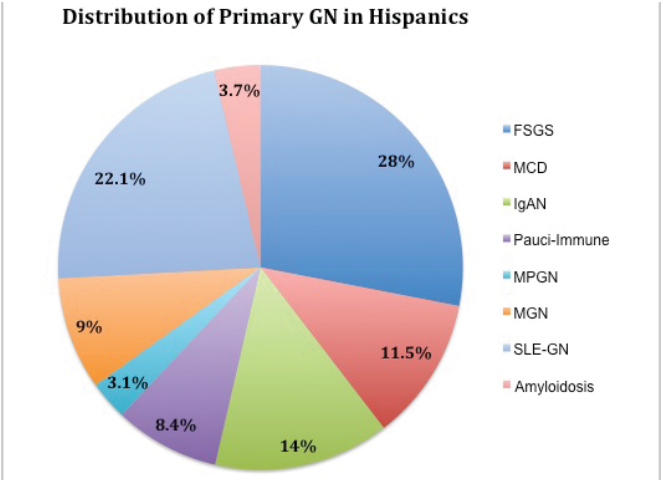
SA-PO839

Distribution of Primary Glomerulonephropathies among Hispanics Taurino N. Avelar,[1] Aviv Hever,[1] Michael Batech,[1] Teresa N. Harrison,[1] Sejal Vora,[2] Leslie Vollenweider,[2] Anna Pavlova-Wolf,[2] John J. Sim.[1] *[1]Internal Medicine, KP LAMC, Los Angeles, CA; [2]Questcor Pharmaceuticals, Inc.*

Background: Few U.S.-based studies have described primary glomerular diseases in the Hispanic population. Small observations suggest Hispanics are more likely to be affected by focal segmental glomerulosclerosis (FSGS) and IgA nephropathy/ Henoch-Schönlein Purpura nephritis (IgAN). We sought to describe the distribution of primary glomerulonephropathies (GNs) amongst Hispanics in Southern California.

Methods: Retrospective chart review study of all renal biopsy reports within Kaiser Permanente Southern California from 1/1/2006 - 12/31/2009. Results for Hispanics were categorized based on the primary diagnoses. Primary GN's [FSGS, minimal change disease (MCD), IgAN, pauci-immune crescentic glomerulonephritis/vasculitis (pauci-immune), membranoproliferative glomerulonephritis (MPGN), membranous glomerulonephritis (MGN), lupus glomerulonephritis (SLE-GN), and amyloidosis] were identified and patients were characterized within those GN's.

Results: A total of 321 Hispanics had a primary GN diagnosis. The mean age was 38 years, 51.7% were female, and 49.2% had a median household income $50,000 - $99,999. The primary GN distribution was: FSGS n=90 (28%), SLE-GN 71 (22.1%), IgAN 45 (14%), MCD 37 (11.5%), MGN 29 (9%), pauci-immune 27 (8.4%), amyloidosis 12 (3.7%) and MPGN 10 (3.1%).



FSGS was most common among males and SLE-GN among females. Hypertension was most commonly associated with MPGN and least with IgAN. Diabetes was most commonly associated with pauci-immune and least with IgAN. Systemic lupus erythematous was also commonly associated with FSGS.

Conclusions: In a large Hispanic population in Southern California, FSGS, SLE-GN and IgAN were the most common GN's. This cohort may provide future insights on comparative outcomes in CKD and among different races/ethnicities.

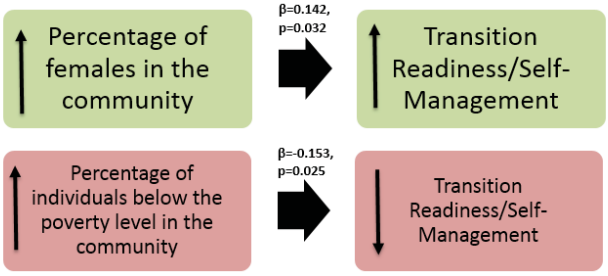
SA-PO840

Ecological Disparities as Predictors of Transition Readiness/ Self-Management Among Adolescents/Emerging Adults Seen in the Adult and Pediatric Nephrology Clinics at the UNC Kidney Center
Karina Javalkar, Meredith Johnson, A. V. Kshirsagar, Karin A. True, Randal K. Detwiler, Sofia Ocegueda, Maria E. Ferris. *UNC Kidney Center, Univ of North Carolina, Chapel Hill, NC.*

Background: The influence of ecological factors on the readiness to transition from pediatric to adult care and/or disease self-management skills among adolescents/emerging adults (youth) with chronic conditions is yet to be characterized.

Methods: IRB-approved consents/assents were signed by youth ages 12 to 29 in both the adult and pediatric nephrology clinics at the UNC Kidney Center. Transition readiness/self-management was measured by the UNC TRxANSITION™ scale, a provider-administered 33-item scale. Age, gender, race, and insurance type were used as control variables. Ecological factors (e.g. percent individuals below poverty level in the community, percent females in the community) were identified for each participant's zip code using the published U.S. Census data. We conducted linear regression analyses in SPSS.

Results: We enrolled 169 participants with the following characteristics:59% females; 37% White; 46% African American; 33% private insurance; 57% public insurance and 10% self-pay; mean age 21 years (±5). Their diagnoses included:CKD (39%), Transplant (28%), lupus nephritis (21%) and other diagnosis (12%). Participants whose communities had a greater proportion of females had greater transition readiness/self-management of their disease (β=0.142, p=0.032). However, in communities with a greater percentage of individuals below the poverty level, participants had lower transition readiness/self-management of their disease (β= -0.153, p=0.025).



Conclusions: Ecological factors and community characteristics appear to be important factors in the transition from pediatric to adult health care and/or in disease self-management. Our findings highlight disparities that need further exploration.

Funding: Private Foundation Support

SA-PO841

Apolipoprotein L1 (*APOL1*) Renal Risk Alleles and Glomerular Causes of Chronic Kidney Disease (CKD) in Children
Robert Woroniecki,[1] Derek Ng,[2,4] Sophie Limou,[3] Cheryl Ann Winkler,[3] Craig S. Wong,[4] Mark Mitsnefes,[4] Susan L. Furth,[4] Bradley A. Warady,[4] Jeffrey B. Kopp,[5] Frederick J. Kaskel.[4,6] *[1]SUNY at Stony Brook, NY; [2]Johns Hopkins Univ, Baltimore, MD; [3]NIH/NCI, Frederick, MD; [4]CKiD Study; [5]NIH/NIDDK, Bethesda, MD; [6]Children's Hospital at Montefiore, Bronx, NY.*

Background: While studies demonstrated strong recessive association of *APOL1* G1 and G2 genetic variants with glomerular and vascular disease progression in African-American (AA) adults, there is limited information on *APOL1* role in children with CKD.

Methods: We investigated children with CKD in the Chronic Kidney Disease in Children (CKiD) cohort. *APOL1* G1 (rs73885319, S342G) and G2 (rs71785313, NY388-389 deletion) renal risk variants were genotyped in AA patients. Linear mixed effects models with random intercepts and slopes were used to describe changes in renal function. The main effects of AA with low risk (LR) *APOL1* (0 or 1 risk allele, n=68) and AA high risk (HR) *APOL1* (2 risk alleles, n=21), and their interactions with time were included, with non-AA (no *APOL1* risk allele, n=675) as the reference group.

Results: Glomerular disease (GD) was a cause of CKD in 26% (175 out of 675) non-AA children, 19% (13 out of 68) LR, and 81% (17 out of 21) HR *APOL1* AA subjects (p<0.001). GD subjects data:

	non-AA (n=175)	LR (n=13)	HR (n=17)	P-value
Age at entry	14 [10,15]	13 [10, 15]	14 [13, 15]	0.33
Female	46% (81)	46% (6)	53% (9)	0.91
Abnormal birth history	23% (38)	40% (4)	41% (7)	0.14

HR were more likely to have a smaller proportion of life with CKD, despite being about the same age: 34% non-AA, 83% LR, 25% HR (p= 0.06). While not significant, HR tend to have a lower GFR at entry than LR and non-AA (54, versus 57.3, versus 61 ml/min/1.73m2, p= 0.33) and a faster GFR decline (-11%, versus 8%, versus -8.2%, p=0.50). All groups had similar level of proteinuria at baseline but it increased more in LR than in HR and non-AA (+44%, versus 11.4%, versus 5.5% per year respectively, p<0.001).

Conclusions: HR *APOL1* is strongly associated with an underlying glomerular cause of CKD in children. Further study of larger population of children is required to determine if an accelerated decline in GFR occurs in association with HR, as it does in adults.

Funding: NIDDK Support

SA-PO842

Primary Care Providers Perceptions of Racial/Ethnic and Socioeconomic Disparities in Hypertension Control
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Background: Primary care providers (PCP) play an important role in reducing racial/ethnic and socioeconomic disparities in hypertension control. The purpose of this study was to evaluate the attitudes and perceptions of PCPs regarding racial/ethnic and socioeconomic disparities in hypertension control.

Methods: We conducted an online survey of PCPs at the University of Colorado Hospital and Denver Health Hospital. We included physicians, nurse practitioners and physician assistants. The survey assessed provider recognition and perceived contributors of racial/ethnic and socioeconomic disparities in hypertension control.

Results: 115 providers completed the survey (response rate 64%). Respondents were primarily female (66%), non-Hispanic white (84%) and physicians (80%). Among respondents, 67% and 73% supported the collection of data on the patients' race/ethnicity and socioeconomic status, respectively. Eighty-six percent and 89% agreed that disparities in race/ethnicity and socioeconomic status existed in hypertension care within the U.S. health system. However, only 33% and 44% thought racial/ethnic and socioeconomic disparities existed in the care of their own patients. Providers were more likely to perceive patient factors rather than provider or health system factors as mediators of disparities. However, most supported interventions such as improving provider communication skills (87%) and cultural competency training (89%) to reduce disparities in hypertension control.

Conclusions: Most providers acknowledged that racial/ethnic and socioeconomic disparities in hypertension control exist in the U.S. health system, but only a minority reported disparities in care among patients they personally treat. Increased provider awareness about disparities, provider training and hypertension guidelines at the local level are needed to reduce disparities in hypertension control.

Funding: NIDDK Support

SA-PO843

Late Referral, Physician Practice and Healthcare Seeking in Pre-Dialysis – A Population-Based Study
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Background: Late referral is common in CKD care with poor prognosis and unclear mechanism. We conducted this national survey to clarify the association between late referral with physicians' practice and patients' healthcare seeking.

Methods: All incident log-term dialysis population from 2002 through 2007 at Taiwan is included. The timing, frequency and specialty of medical visits and laboratory tests were evaluated retrospectively every 3 months before dialysis. Missing was defined as no any medical visit (or test) in a 3-month interval. No more than one missing every year and having the rest all done at the same hospital/clinic was defined as regular follow-up. Risk factors were identified for both irregular follow-up of eGFR and medical visit before dialysis.

Results: Total 46,626 patients were included. Near one fourth of the incident patients never had their eGFRs tested until one year before dialysis. In the observation periods of one, two and three years before dialysis, 87, 66, and 50% of patients had regular medical visit, but only 44, 21, and 12% had the regular visit at nephrology. In addition, only 49, 23, and 12% of incident patients had regular eGFR test in the above observation periods. Finally, risk factors of irregular eGFR follow-up included age(95%CI:1.03-1.07), cardiac disorder(1.03-1.24), stroke(1.17-1.44), regular surgery visit(1.07-1.41), orthopedics visit(1.18-1.65), other specialty visit (1.01-1.25) and primary clinic visit(1.22-1.41); while risk factors of irregular follow-up at nephrology were diabetes(1.95-2.20), cardiac disorder(1.47-1.74), stroke(1.70-2.06) and any specialty regular visit(OR range:1.32 to 5.58).

Conclusions: Regular medical visit was common in late CKD patient, but late referral and much less frequent did they receive regular eGFR measurement.

SA-PO844

Risk Factors for Rapid Renal Function Decline and Incident Chronic Kidney Disease (CKD) among African Americans: The Jackson Heart Study (JHS)
Bessie A. Young,[1] Ronit Katz,[1] Bryan R. Kestenbaum,[1] Ian H. de Boer,[1] Wei Wang,[2] Nisha Bansal,[1] Cassianne Robinson-Cohen,[1] Michael Griswold,[2] L. Ebony Boulware,[3] Adolfo Correa.[2] *[1]KRI, UW, Seattle, WA; [2]JHS CC, Univ of Mississippi, Jackson, MI; [3]Medicine, Duke, Durham, NC.*

Background: African Americans (AA) with known CKD are at greater risk for endstage renal disease compared to whites; however, less is known regarding risk factors for rapid renal function (RRF) decline and incident CKD amongst a population-based cohort of AA with preserved kidney function.

Methods: We evaluated RRF decline and incident CKD among a cohort of 2382 AA JHS participants with renal function data from three examinations (2000-2004, 2005-2008, and 2009-2013). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation. RRF decline was defined as an absolute decline of >3ml/min/1.73 m^2

annually. Incident CKD was defined as eGFR decline to <60ml/min/1.73 m^2 and an annual decrease of eGFR >1% over 10 yrs. We quantified the association of baseline risk factors with odds of RRF decline and incident CKD using logistic regression.

Results: At baseline, mean age was 54 (SD=12) years, 37% were male, average body mass index (BMI) was 31.8 (7.0), average systolic blood pressure (SBP) was 125 (17), 18% had diabetes, 59% had hypertension, mean eGFR was 97.0 (20) ml/min/1.73m2, and albumin to creatinine ratio (ACR) was >30mg/g in 10%. RRF decline was found for 350 (14.7%) participants. Factors associated with greater odds of RRF decline were: older age; SBP (OR=1.29, 95% CI 1.12-1.48, per SD); smoking (OR=1.64, 95% CI=1.04-2.59); and doubling of ACR (OR=1.40, 95% CI,=1.23, 1.38. Factors associated with incident CKD were: age (OR=1.86, 95% CI=1.48-2.34, per 10 years); SBP (OR=1.40, 95% CI=1.16-1.69, per SD); and doubling of ACR (1.30, 95% CI=1.17-1.45). BMI was not statistically associated with RRF in the multivariate models (OR=1.14, 95% CI=0.91-1.41) per 7 kg/m2).

Conclusions: Among AA in JHS, age, SBP, and ACR were associated with RRF decline and incident CKD, while the influence of BMI was less supported. Efforts to reduce the burden of traditional risk factors may decrease RRF and the prevalence of kidney disease in African Americans.

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

SA-PO845

Characterizing Barriers to Health Care Among Chinese Immigrant Populations with the Kidney Disease Screening and Awareness Program (KDSAP)

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Background: Culture-specific social and ethnic factors influence access to health care, quality of physician-patient interactions and individual health outcomes among Asian immigrant populations in the U.S. To eliminate immigrant health care disparities, care providers must construct service models that facilitate the dissemination of culturally competent care. The free community health clinic offers a model for gathering demographic, social and health service information that will inform strategies for reducing cultural care barriers.

Methods: A bilingual questionnaire was administered to 82 participants at four KDSAP renal health screenings in Boston's Chinatown from 2011 to 2013. The following topics were addressed: language, education, general health, communication barriers, difficulty of receiving care, insurance, cost of medications, health information sources and usage of herbal/traditional medicines.

Results: Primary languages included Cantonese, Mandarin, Taishanese and Southern Min. 39% of participants attained an education level of grade school or less. 50% rated their health "Fair" or "Poor." One quarter reported experiencing language barriers when communicating with physicians, while 21% experienced "some" to "extreme" difficulty in obtaining care. Primary sources of health information included newspapers (62%), television (30%) and community centers (18%). 29% of participants reported taking herbal/traditional medicines, for which the chief information source was non-health-professional family or friends. 14% denied having insurance coverage while 22% lacked benefits to pay for medications.

Conclusions: Population-specific cultural assessment identified salient characteristics that may inform the design of care tailored to the Chinese immigrant population in Boston by addressing issues of accessibility, linguistic diversity, dissemination of health information and the physician-patient communication gap. Health clinics serving ethnic groups nationwide must prioritize the collection of relevant social and cultural information to enhance local health services design and effect policy changes toward eliminating disparities in immigrant health care.

Funding: Private Foundation Support

SA-PO846

Socio-Economic Status Influences Survival in CKD

Beng Hock So,[1,3] Shona Methven,[4] Jamie P. Traynor,[2] Mario D. Hair,[1] Alan G. Jardine,[3] Mark Steven Macgregor.[1] *[1]Univ Hospital Crosshouse; [2]Monklands Hospital; [3]Inst of Cardiovascular Medicine and Sciences, Univ of Glasgow, United Kingdom; [4]Univ of Bristol.*

Background: Socio-economic status(SES) is associated with poor outcomes and has had limited attention in chronic kidney disease(CKD). We hypothesised that lower SES is associated with poorer outcomes (mortality, initiation of RRT).

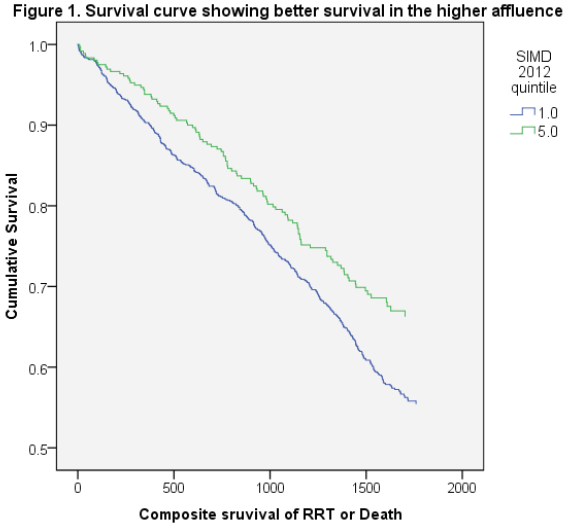
Methods: We identified all CKD patients from our centralised laboratory database between 2009-2012(n=21,035) with an algorithm to confirm chronicity. Data were linked to regional Scottish Index of Multiple Deprivation quintiles(SIMD: 1=most, 5=least deprived), hospitalisations, and date of death for the following 5 years; obtained from Information Services Division Scotland. To avoid lead-time bias, patients were 'enrolled' when eGFR fell to less than 45mL/min/1.73m^2(n=3,122). Multivariate Cox hazard models were generated.

Results: There were 1,238 deaths and 95 who started RRT. Demographics and comorbidity for SIMD quintile 1 and 5 are shown in table 1.

	SIMD 1	SIMD 5	p-value(t-test, X^2)	Exp(B)	95% CI
Age(mean±SD)	75±11	76±12	0.28	1.53	1.39, 1.69
eGFR(mean±SD)	34±9	33±9	0.15	0.96	0.95, 0.97
Female(%)	65	61	0.17	1.47	1.24, 1.75
Statin use(%)	52	41	<0.05	0.68	0.57, 0.82
HTN(%)	49	38	<0.05	0.97	0.81, 1.17
CHD(%)	30	22	<0.05	1.51	1.25, 1.83
DM(%)	22	15	<0.05	1.17	0.94, 1.45
CVA(%)	10	9	0.53	1.25	0.96, 1.63
PVD(%)	6	4	0.14	1.06	0.75, 1.52

Survival analysis was carried out after adjustment for the factors listed. SIMD 5 versus SIMD 1 was associated with a hazard ratio of 0.71 (95% CI 0.57,0.89).

Figure 1. Survival curve showing better survival in the higher affluence quintile



Conclusions: We demonstrated an association between SES and survival in a community-based prevalent cohort with CKD. These associations might be explained by prevalence of other comorbidities (eg smoking, alcohol), or other unknown effects of deprivation. Further work will be required to differentiate between these possibilities.

Funding: Private Foundation Support

SA-PO847

Transition Readiness Outcomes following Establishment of Formal Transition Nephrology Program

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Background: Outcomes in adolescents and young adults (AYA) with chronic kidney disease (CKD) following transfer to adult practitioners remains poorly defined.

Methods: We evaluate the effect of a formal Nephrology Transition program on outcomes in AYA with pediatric-onset CKD. Patients between ages 12 and 22 years were identified and referred for enrollment into formal transition. STARx survey tools: Physician Survey, Transition Readiness Survey and Scoring, Realm Literacy tool, Parent survey of Transition Burden and Quality of Life (QOL) tools were used to evaluate patients during transition process.

Results: Forty-seven patients from a single academic pediatric nephrology practice were identified as eligible, and 22 (46.8%) were referred for Transition. Patients were average on average 18 ± 1.67 years old, 64 % female, 73% Black, 18% White, 9% Hispanic. Half of referred patients were evaluated in Nephrology Transition clinic between April 2013 and January of 2014. Four patients refused transition because of resistance to provider transfer and increased travel burden. The remaining 7 patients had not yet engaged in Transition. The average patient transition readiness score was 7.17 out of 10, with parent score of 8.66. Patients averaged 4-6th grade reading skills and parents averaged 7th-8th grade reading skills. Patient and parental QOL scores averaged 75, 71, 65, 29 and 87.9, 68.7, 65.3, 37.3 out of 100 for physical, emotional, social and school arenas. Use of assistive tools for medication management improved with ongoing transition.

Conclusions: Social factors including low literacy, travel constraints and resistance to leaving the pediatric setting remain major barriers to successful management and transition of AYA with CKD. CKD in AYA also significantly affects school performance. On-line self-assessment tools and patient portals can promote self-efficacy and facilitate distance transition in this age group. Improved engagement of pediatric and adult practitioners remains key to a successful transition program.

SA-PO848

Access to Pre-ESRD Nephrology Care in Rural Areas: A Nationwide Perspective Brendan P. Lovasik,[1] Hua Hao,[2] Stephen O. Pastan,[1] Howard Chang,[2] Rachel E. Patzer.[1,2] *[1]Emory Univ School of Medicine, Atlanta, GA; [2]Emory Univ Rollins School of Public Health, Atlanta, GA.*

Background: Rural areas are disproportionally affected by the nationwide physician shortage. ESRD patients residing in rural areas have geographic and economic barriers that limit access to specialized nephrology care; regional studies have shown lower rates of pre-ESRD nephrology care in rural areas.

Methods: 3,819 dialysis facilities across the U.S. were geocoded into rural and non-rural categories according to the U.S. Office of Management and Budget Metropolitan Statistical Area and National Center for Health Statistics. A marginal mixed generalized estimating equation model was used to estimate the association of facility-level characteristics and the proportion of patients within a facility who received pre-ESRD nephrology care.

Results: Rural dialysis facilities show a similar proportion of patients receiving any pre-ESRD nephrology care as non-rural facilities (70.11% versus 68.16%, OR=1.007, P=0.085); this was consistent for care greater than 6 months (46.26% versus 47.59%, OR=0.998, P=0.472) and care greater than 12 months (24.27% versus 22.33% OR=1.007, P=0.098). Rural facilities show higher rates of erythropoiesis-stimulating agent use (29.11% versus 22.39%, OR=1.017, P<0.001), but no difference in arteriovenous fistula use (P=0.442). There was no difference in rural versus non-rural dialysis facility Standardized Mortality Ratio (P=0.549). Medicare coverage was higher in rural facilities (21.54% versus 19.32%, OR=1.009, P=0.048), while Medicaid coverage (P=0.178) was similar between rural and non-rural facilities.

Conclusions: Dialysis facilities in rural areas show similar rates of pre-ESRD nephrology care as non-rural dialysis facilities on the national level. Rural facilities have high rates of outcome-improving measures, including erythropoiesis-stimulating agent and arteriovenous fistula use, that show strong primary medical management of ESRD. However, access to nephrology care in this patient population should be carefully monitored as the rural physician shortage intensifies.

Funding: Other NIH Support - National Institute on Minority Health and Health Disparities (R. Patzer)

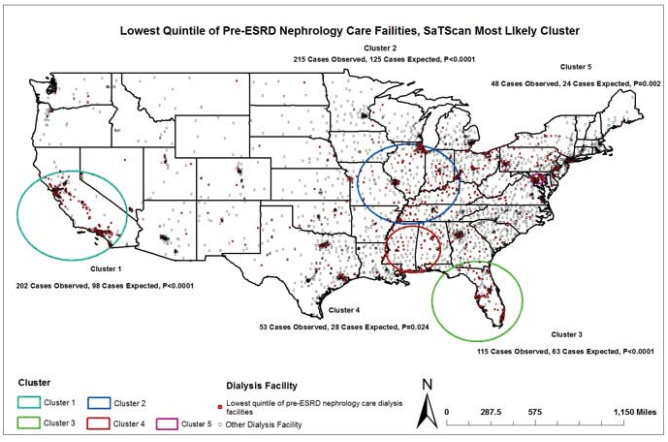
SA-PO849

Geographic Determinants of Low Pre-ESRD Nephrology Care in the United States Brendan P. Lovasik,[1] Hua Hao,[2] Stephen O. Pastan,[1] Howard Chang,[2] Rachel E. Patzer.[1,2] *[1]Emory Univ School of Medicine, Atlanta, GA; [2]Emory Univ Rollins School of Public Health, Atlanta, GA.*

Background: Pre-end stage renal disease (ESRD) nephrology care is crucial for optimizing clinical outcomes for patients with ESRD. Geographic variation of pre-ESRD nephrology care coverage has not been studied nationally.

Methods: A marginal mixed generalized estimating equation model was used to estimate the association of the proportion of patients within a facility who received pre-ESRD nephrology care and facility-level neighborhood characteristics among 5,387 dialysis facilities across the United States. SaTScan testing was utilized to detect geographic clusters of dialysis facilities with low pre-ESRD nephrology care coverage.

Results: Dialysis facilities in the lowest quintile of pre-ESRD nephrology care were geographically clustered in 5 distinct areas (P<0.05), including San Francisco, Los Angeles, Chicago, Miami, and Baltimore and along the corridors of Mississippi and Ohio River.



Facilities in the lowest quintile of pre-ESRD nephrology care were more likely to be located in inner cities compared to those in the highest quintile (45.8% versus 21.8%, OR=1.88, P=0.014). Lowest quintile facilities were significantly more likely to be in high-poverty neighborhoods (24.2% versus 16.6%, OR=1.96, P=0.030). The proportion of racial minorities within a neighborhood was not associated with pre-ESRD nephrology care rates (P=0.929).

Conclusions: The proportion of patients receiving access to pre-ESRD nephrology care within a facility varies by geographic region. Policy makers and ESRD Networks should target these low-pre-ESRD facilities and regions to improve access to nephrologist care with interventions and specific pilot programs aimed at improving patient outcomes. Further examination of what factors make geographic regions better or worse at providing pre-ESRD nephrology care is warranted.

Funding: Other NIH Support - National Institute on Minority Health and Health Disparities (R. Patzer)

SA-PO850

Incidence of Diabetes-Related End-Stage Renal Disease Declined but Racial/Ethnic Disparities Remained Nilka Rios Burrows, Israel Hora, Desmond Williams, Linda S. Geiss. *Div of Diabetes Translation, Centers for Disease Control and Prevention, Atlanta, GA.*

Background: In the United States, diabetes is the leading cause of end-stage renal disease (ESRD), a condition associated with premature death that disproportionately affects racial/ethnic minorities. To assess progress in closing the disparity gap, we examined racial/ethnic-specific trends in incidence of diabetes-related ESRD in the United States.

Methods: From the U.S. Renal Data System, we obtained the number of non-Hispanic white (White), non-Hispanic blacks (Black), and Hispanic adults aged ≥18 years who began therapy for ESRD with diabetes listed as primary cause (ESRD-DM) between 1997 and 2011. Incidence was calculated using racial/ethnic-specific estimates of the adult population with self-reported diabetes from the National Health Interview Survey, and age-adjusted to the 2000 U.S. standard population. Joinpoint regression was used to analyze trends.

Results: From 1997 to 2011, the total number of White, Black, and Hispanic adults who began ESRD-DM therapy increased from 33,286 to 46,090, but the overall age-adjusted ESRD-DM incidence rate decreased by -3.7% per year (276.2 to 197.7 per 100,000 diabetic population) (p<0.001). Throughout the period, age-adjusted rates declined by -4.2% per year among Whites (243.9 to 148.4 per 100,000 diabetic population), and by -2.3% per year among Blacks (449.1 to 352.8 per 100,000 diabetic population) (both p<0.001). Among Hispanics, after an initial period of no significant change, age-adjusted rates declined by -3.9% per year from 2000 to 2011 (385.4 to 247.2 per 100,000 diabetic population) (p<0.001). In 2011, compared with Whites, the age-adjusted ESRD-DM incidence rate was 2.4 times higher among Blacks and 1.7 times higher among Hispanics.

Conclusions: The declining ESRD-DM incidence in the population with diabetes is likely due in part to a reduction in prevalence of ESRD risk factors, improved treatment and care, and other factors. However, compared with Whites, ESRD-DM continues to disproportionately affect Blacks and Hispanics with diabetes. Continued efforts might be considered to sustain and improve these encouraging trends, particularly among U.S. minority populations.

SA-PO851

The Differential Effect of Socio-Economic Status on Body Mass Index among Aboriginal Children and Adolescents Siah Kim,[1,2] Petra Macaskill,[2] Louise Alison Baur,[2,3] Elisabeth M. Hodson,[1,2] Jennifer Daylight,[1] Rita Angeline Williams,[1] Rachael Kearns,[1] Nicola Vukasin,[1] David M. Lyle,[4] Jonathan C. Craig.[1,2] *[1]Centre for Kidney Research, The Children's Hospital at Westmead, Westmead, NSW, Australia; [2]School of Public Health, Univ of Sydney, Camperdown, NSW, Australia; [3]Discipline of Paediatrics and Child Health, Univ of Sydney, Camperdown, NSW, Australia; [4]Dept of Rural Health, Univ of Sydney, Camperdown, NSW, Australia.*

Background: Obesity is a known risk factor for chronic kidney disease (CKD), and earlier onset of obesity associated with increased risk of CKD in adult life. Aboriginal children have a higher prevalence of overweight and obesity with the contribution of socioeconomic status (SES) unclear. We aimed to determine changes in BMI between Aboriginal and non-Aboriginal children as they move through adolescence into young adulthood.

Methods: A prospective cohort study of Aboriginal and non-Aboriginal school children commenced in 2002 across 15 different screening centers involving 213 high schools across urban, regional and remote NSW. SES was recorded at study enrollment and participants BMI were measured every 2 years. We fitted a series of mixed linear regression models adjusting for age, sex, Aboriginality and socioeconomic status.

Results: 3418 (1949 Aboriginal) participants were screened over a total of 11, 387 participant years follow up. The prevalence of obesity was 14.2% (mean age 11 years) at baseline, and increased to 17.2% at eight years follow up (mean age 16 years). The mean BMI SDS increased with age and we found that SES had a differential effect on mean BMI for Aboriginal children compared to non-Aboriginal children (P for interaction = 0.01). Aboriginal children of highest SES status had a BMI SDS 0.16 higher than non-Aboriginal children, whilst Aboriginal children of lowest SES had mean BMI SDS 0.17 lower than non-Aboriginal children.

Conclusions: Socio-economic status has a differential effect on adiposity for Aboriginal children, similar to that seen in low middle income countries. Interventions to reduce the prevalence of obesity are needed, and within the Australian Aboriginal community they need to also focus on those who are of socioeconomic advantage to maximise their impact.

Funding: Government Support - Non-U.S.

SA-PO852

Prevalence of Chronic Kidney Disease in a Brazilian Former Slave Population: Preliminary Findings from the Prevrenal Study

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Background: In Brazil, ethnic minority populations, such as indigenous and former slave communities, still live in isolated regions, which results in lack of awareness of chronic kidney disease in these groups. The PREVRENAL study was established to investigate the prevalence of CKD in a Brazilian former slave population.

Methods: The PREVRENAL was designed as a cohort study and has been conducted by a multidisciplinary staff from the Federal University of Maranhão and in technical cooperation with the Federal University of São Paulo Brazil. PREVRENAL studied afro-descendant patients with restricted access to public health system. This population was recruited from 32 remaining quilombos communities of the municipality of Alcântara, state of Maranhão, northeast Brazil. Firstly, all patients underwent extensive clinical and laboratory evaluation, including GFR estimation using the CKD-EPI formula. The diagnosis of systemic hypertension (SH) was based on arterial blood pressure ≥ 140/90 mmHg or documented antihypertensive drug therapy. We considered as reduced the eGFR lower than 60ml/min/1,73m² and as albuminuria the spot urine creatinine/albumin ratio >30 mg/g.

Results: A total of 1.539 participants were enrolled with mean age ± SD of 44.3 ±17.6 yr; 51.4% were women, and 21.3% had an age ≥ 60 yr. Approximately 30% of the patients were identified as hypertensive and 4.5% had diabetes. Reduced eGFR and albuminuria were found in 6.4% and 7.3% of the patients, respectively.

Conclusions: Although, roughly one-third of the participants are hypertensive, the prevalence of CKD was lower than those described in other population studies. Particularities related to the lifestyle of the current population may explain this finding.

Funding: Government Support - Non-U.S.

SA-PO853

Prevalence of Chronic Kidney Disease among Persons with Serious Mental Illness

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Background: The prevalence of CKD in persons with serious mental illness (SMI) is uncertain, but likely under-recognized in spite of their increased risk of cardiovascular disease, use of nephrotoxic medications (e.g., lithium), and premature mortality.

Methods: The baseline prevalence of CKD among 288 overweight/obese adult ambulatory participants with SMI in a multi-center behavioral weight loss intervention study was compared with age, sex, race and overweight/obesity matched participants of the 2009-2010 National Health and Nutrition Examination Survey. CKD was defined as eGFR of 15-59 mL/min/1.73 m². SMI was defined by a diagnosable (DSM III-R) mental, behavioral, or emotional disorder that resulted in functional impairment or limited major life activities.

Results: Persons with SMI had a mean age of 45 years. 50% were male and 38% were non-Hispanic black. 58% of the participants had schizophrenia or a schizoaffective disorder, 22% had bipolar disorder, and 12% had major depression. The prevalence of CKD among persons with SMI was 9.5% (95% CI 3.7-10.5) compared with 3.2% (CI 2.2-4.3) in NHANES. Participant characteristics by CKD status in the SMI cohort and NHANES indicate that CKD in SMI occurred at earlier age and in persons without diabetes (see Table).

Participant Characteristics by CKD Status						
	SMI Cohort			NHANES 2009-2010		
	Non-CKD *n=259*	CKD *n=29*	p value	Non-CKD *n=3516*	CKD *n=236*	p value
Mean age (years)	44.5	53.5	<0.0001	45.2	63.1	<0.001
Male (%)	50	55	0.58	51	41	0.197
Non-Hispanic Black (%)	39	28	0.245	37	47	0.003
Mean BMI (kg/m²)	36	36	0.85	32	35	0.18
Diabetes (%)	29	21	0.35	12	43	0.042
HTN (%)	57	79	0.02	41	83	<0.001
HIV (%)	0	4	0.002	0.5	9	0.35
Hepatitis B or C (%)	5	10	0.21	3	5	0.72
Lithium (%)	12	10	0.96			

Conclusions: The prevalence of CKD among persons with serious mental illness may be more than 3 times that of the general population, and is present at a younger age. Studies examining causal/temporal relationships between SMI and CKD are warranted to guide risk modification in this high risk population.

Funding: Other NIH Support - National Institute of Mental Health

SA-PO854

Racial Variation in Renal Conditions after Living Kidney Donation

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Background: Recent studies have raised concerns for increased risks of renal failure among African American (AA) and biologically related living kidney donors (LKD).

Methods: We examined a novel database that links national U.S. transplant identifiers for LKD (1987-2007) to billing claims from a private health insurer (2000-2007 claims) to identify post-donation categories of non-infectious renal diagnoses as reported by ICD-9 coding. Cox regression with left and right-censoring was used to estimate the cumulative incidence of diagnoses over time after donation, and associations (adjusted hazards ratios, aHR) of donor traits with study outcomes.

Results: Among 4650 sampled LKD, 13.1% were AA and 76.3% were white race; 81% were biologically related to their recipient (including 88% of AA, and 79% of white LKD). Median time from donation to end of insurance data was 7.7 yrs. Diagnosis of any renal condition was reported in 14.9% of AA versus 9% of white LKD by 7 yrs post-donation. Compared with white LKD, AA LKD experienced trends towards increased likelihoods of all the renal diagnoses after donation; statistical significance (P<0.05) was reached for nephrotic syndrome (aHR 15.7), chronic kidney disease (aHR 2.32), proteinuria (aHR 2.27) and the composite of any renal diagnosis (aHR 1.71) (**Table**). Associations persisted, and also included coded renal failure (aHR 2.28), after adjustment for biological relationship to recipient. AA race was also associated with increased renal risk among samples limited to related LKD.

RACIAL VARIATION IN RENAL CONDITION DIAGNOSES AFTER LIVING KIDNEY DONATION

	Age & Sex-Adjusted 7 yr Incidence		Adjusted Impact of AA vs White Race
	White	African American	
	(%)	(%)	aHR (95% CI)
Nephrotic syndrome	0.1	1.3	15.7 (2.97-83.0)†
Nephritis & nephropathy	0.9	1.7	1.99 (0.73-5.44)
Acute kidney failure	1.0	1.7	1.69 (0.57-5.66)
Chronic kidney disease	5.6	12.6	2.32 (1.48-3.62)†
Renal failure, unspecified	1.6	2.6	2.23 (1.00-4.98)*
Disorders from impaired renal function	0.9	1.8	2.14 (0.77-5.94)
Proteinuria	3.6	5.7	2.27 (1.32-3.89)†
Any renal diagnosis	9.0	14.9	1.72 (1.23-2.41)†

Incidence adjusted to average sample age & sex distribution at donation
aHR, adjusted hazards ratio; * P=0.05; † P <0.05

Conclusions: AA race is associated with increased likelihood of renal diagnoses after living kidney donation, independent of biological relationship to the recipient. Future research to identify prognostic markers for renal outcomes among higher risk LKD is warranted.

Funding: NIDDK Support

SA-PO855

Onset of *APOL1* Kidney Disease Manifests as Incident Albuminuria and Declining Glomerular Filtration Rate but Does Not Fully Explain Race Differences

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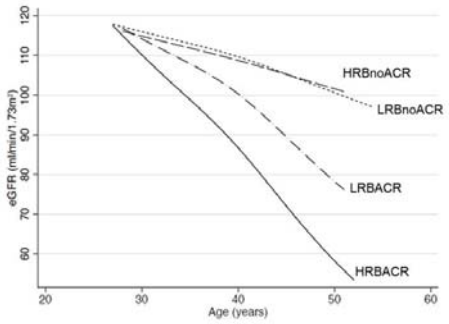
Background: The role of *APOL1*, socioeconomic position (SEP) and traditional risk factors in explaining race differences in albuminuria and kidney function decline in a community-based sample of young healthy adults is unknown.

Methods: We included 3,031 Blacks and Whites with repeated measures of cystatinC (eGFRcys) and ACR in CARDIA (Years 10, 15, 20). We categorized blacks as high risk (HRB) if they had 2 *APOL1* risk alleles and low risk (LRB) if 0/1 alleles. We examined differences in incident albuminuria (ACR ≥ 30 mg/g) and annualized rates of eGFRcys decline, by generalized models adjusting for age, gender, income, education, caretaker education, smoking, BMI, systolic blood pressure, and diabetes.

Results: At baseline, mean age was 35±3.6 and eGFRcys 111 ± 12 ml/min/1.73m². 13.2% of Blacks had high risk *APOL1*. Risk of incident albuminuria was increased in both HRB (HR4.83, 2.80-8.35) and LRB (HR2.06, 1.45-2.93), compared with whites (age and sex adjusted). Associations were moderately attenuated after full adjustment in HRB (HR3.60, 2.02-6.41), and lost significance in LRB (HR 1.41, 0.95 to 2.10). *APOL1* status was associated with eGFRcys decline only among blacks with albuminuria during follow up (p-interaction *APOL1**ACR 0.005).



Age	High risk with albuminuria vs. low risk no albuminuria	High risk no albuminuria vs. low risk no albuminuria	Low risk with albuminuria vs. low risk no albuminuria
30	0.001	0.96	0.02
35	0.04	0.98	0.01
40	<0.001	0.59	<0.001
45	0.02	0.54	<0.001
50	<0.001	0.55	<0.001

eGFRcys decline was fastest in HRB with albuminuria: (3.4% ml/min/1.73m^2 (2.9%-3.8%) per year), followed by LRB with albuminuria, 2.1% (1.9%-2.4%), compared with 0.8% (0.7-0.9%) in whites.

Conclusions: Onset and progression of *APOL1* kidney disease is characterized by albuminuria and reduced eGFR. However, reduction of race disparities in CKD requires strategies for detection and management among Blacks with and without *APOL1*.

Funding: NIDDK Support, Private Foundation Support

SA-PO856

Association of Perceived Racial Discrimination and Kidney Function Decline among African Americans and Whites Angedith Poggi-Burke,[1] May A. Beydoun,[1] Alan B. Zonderman,[1] Michele Kim Evans,[1] Deidra C. Crews.[2] *[1]NIA, NIH; [2]Johns Hopkins U.*

Background: Psychosocial factors such as perceived racial discrimination (PRD) have been associated with chronic diseases including hypertension, however, little is known about the relationship of PRD with kidney function decline (KFD).

Methods: We examined whether PRD was associated with KFD over 5 years of follow-up in the Healthy Aging in Neighborhoods of Diversity across the Life Span study (Baltimore, MD). A total of 1,574 participants (whites=630; African Americans (AAs)=944) aged 30-64 years at baseline were included. PRD was defined using an adaptation of the Experience of Racial Discrimination questionnaire. Mixed-effects regression models with random intercepts and slopes were used to compare linear trends in mean estimated GFR (eGFR), with adjustment for age, sex, race, education, poverty status, tobacco use, body mass index, hypertension and diabetes status.

Results: A total of 319 (20%) participants reported 'a lot' of PRD, and these persons were more likely to be AA, male, more educated, but were less likely to have diabetes mellitus than those reporting none or 'some' PRD. Overall, PRD was significantly associated with KFD. Sex and race stratified models revealed PRD was associated with decline in eGFR only among AA women.

Change in eGFR over 5 Years of Follow-Up (95% Confidence Interval) by Baseline Perceived Racial Discrimination

RD	N	Overall (N=1574)	Whites (N=630)	AA (N=944)	AA Men (N=382)	AA Women (N=562)
Not at all	459	Ref	Ref	Ref	Ref	Ref
A little/ Some	796	-0.5 (-2.4, 1.5)	-0.1 (-2.3, 2.0)	-.7 (-3.8, 2.5)	0.6 (-5.3, 6.4)	-0.9 (-4.7, 2.9)
A lot	319	-2.5 (-5.0, -0.1)*	0.8 (-3.5, 5.1)	-3.0 (-6.5, 0.5)	0.1 (-6.0, 6.2)	-4.9 (-9.4, -0.5)*

* p-value < 0.05

Note: Sex stratified results provided only for AAs, as association NS among whites.
Models were adjusted by sociodemographic, lifestyle and health-related factors.
Baseline systolic blood pressure mediated 14.9% (95% CI 11.4, 19.4%) of this association.

Conclusions: Perceived racial discrimination, a psychosocial stressor, may impact risk of KFD, particularly among AA women. Stressors such as PRD, are worthy of further study regarding their potential contributions to disparities in kidney disease.

Funding: Other NIH Support - National Institute on Aging Intramural Research Program

SA-PO857

Racial and Ethnic Differences in Pre-ESRD Care in U.S. Counties Guofen Yan,[1] Keith C. Norris,[2] Alfred K. Cheung,[3] Wei Yu,[1] Jennie Z. Ma,[1] Tom Greene.[3] *[1]Univ of Virginia; [2]UCLA; [3]Univ of Utah.*

Background: While prior studies document that racial and ethnic minorities are less likely to receive specialist care before ESRD, our understanding of the geographic contribution to this disparity is limited. We therefore examined whether this national difference exists within small geographic units, such as individual counties (i.e., county-level disparity).

Methods: We identified incident patients in the USRDS aged ≥18 years who initiated first dialysis between 2005 and 2010. The analysis for black-white comparison included 1270 counties that had ≥5 patients of each race, resulting in 346,368 patients (39.3% non-Hispanic blacks and 60.7% non-Hispanic whites). The Hispanic-white analysis included 613 counties with ≥5 patients of each race, resulting in 224,286 patients (25.7% Hispanics and 74.3% non-Hispanic whites). We examined two pre-ESRD care indicators (yes/no): nephrologist care at least 12 months before ESRD and arterio-venous fistula (AVF) at first outpatient dialysis. Using two-stage logistic regression, we obtained the disparity estimate for each county, expressed as county-specific odds ratio (CSOR) of black (or Hispanic) versus white in receiving such care.

Results: Overall, the percentage of patients who received pre-ESRD nephrologist care was lowest in Hispanics (20.0%), intermediate in blacks (23.8%), and highest in whites (30.0%). There was a similar disparity for AVF with 11.6%, 12.1%, and 14.7%, respectively. After covariate adjustment, the significant disparity for blacks versus whites in receiving nephrologist care was evident in 66.9% (849/1270) of counties (CSOR ranging from 0.46 to 0.9). The significant disparity for Hispanics versus whites in nephrologist care was evident in 96.7% (593/613) of counties (CSOR from 0.52 to 0.9). Counties with larger disparity level (CSOR<0.8) tended to be of lower socio-economic status and healthcare resources, and were more likely to be located in the South and large metropolitan areas.

Conclusions: Although disparities in pre-ESRD care were more likely in certain geographic areas, they existed in diverse locations and in most U.S. counties. Therefore, efforts to improve pre-ESRD care should be implemented nationally rather than regionally.

Funding: NIDDK Support

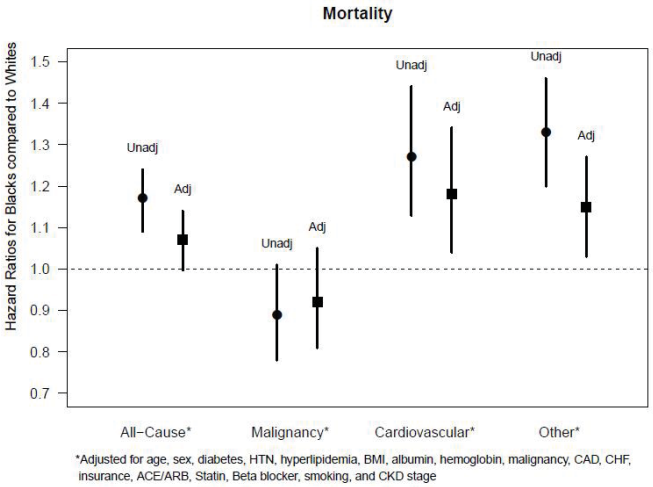
SA-PO858

Causes of Death Are Different between Whites and Blacks with Non-Dialysis Dependent Chronic Kidney Disease Sankar D. Navaneethan,[1] Jesse D. Schold,[2] Susana Arrigain,[2] Victoria Konig,[2] Stacey Jolly,[3] Joseph V. Nally.[1] *[1]Nephrology, Cleveland Clinic; [2]Quantitative Health Sciences, Cleveland Clinic; [3]Medicine, Cleveland Clinic.*

Background: Chronic kidney disease (CKD) is associated with a higher risk of death overall, but cause specific death is less known. Using a large CKD registry and state death data, we examined the leading causes of death and assessed if racial differences existed among a non-dialysis CKD population.

Methods: We obtained death and cause-specific mortality details from the Ohio state death index for 46,785 white and black patients who had CKD (two estimated GFR <60 ml/min/1.73 m^2 90 days apart or had an ICD-9 code for various kidney disease) and resided in the state of Ohio between January 2005 and September 2009. We classified the causes of death as reported by the Center for Disease Control and racial differences were explored using Cox Proportional hazards model.

Results: During the study period, 7358/46,836 CKD patients died during a median follow up of 2.3 years. Overall, malignant neoplasms (31.9%) and heart disease (28.1%) were the leading causes of death. Among whites, malignant neoplasm (33.1%) is the leading cause of death while among blacks the leading cause of death is heart disease (30.3%). In the adjusted models, blacks had similar overall-mortality hazard ratios compared to Whites. However, blacks had higher hazard ratios for cardiovascular death and deaths due to other causes (non-cardiovascular and non-malignant) compared to whites in both unadjusted and adjusted models.



Conclusions: Malignancy and cardiovascular diseases were the leading causes of death among those with CKD. Blacks were more likely to die of cardiovascular disease and due to non-malignant, non-cardiovascular reasons compared to whites. Future studies are needed to understand the reasons for these differences so that interventions can be designed to improve outcomes.

Funding: Pharmaceutical Company Support - Development of CCF CKD registry was supported by an unrestricted educational grant to the Department of Nephrology and Hypertension from Amgen

SA-PO859

Assessment of Racial Disparity in Mortality Among Patients with End Stage Renal Disease due to Systemic Lupus Erythematosus
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Background: A recent study showed an increased risk for mortality in African American (AA) patients with end-stage renal disease (ESRD) secondary to lupus nephritis (LN). Our study aim is to assess the impact of socioeconomic factors and kidney transplantation on the racial disparity in survival of this patient population.

Methods: Using the United States Renal Data System database, we identified 12,350 patients with LN as the cause of ESRD in a retrospective cohort of 1,125,365 patients, initiated on chronic dialysis between January 1, 1995 to December 31, 2006, followed until December 31, 2010. We merged data on median household income from the United States Census based on the ZIP code.

Results: In multivariate Cox regression analyses, AA patients with LN (versus non-AA) had an increased risk of death (adjusted hazard ratio [AHR], 1.18; 95% confidence interval [CI], 1.11-1.25). Adjusting for income and insurance status, the AHR was 1.09 (95% CI 1.02-1.15). Further adjustment for kidney transplantation as a time-dependent covariate resulted in an AHR 0.99 (95% CI 0.93-1.05). AA patients were significantly less likely to receive a kidney transplant than non-AA with LN (29.5% versus 41.8%, p<0.001).

Conclusions: Contrary to the lower mortality rate among AA patients in the general ESRD population, AA with LN were at increased risk of death which was attenuated by income and kidney transplantation. The apparent higher risk of death among AA with ESRD due to LN appears mediated substantially by lower rates of kidney transplantation. *The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy of the Department of the Army, the Department of the Navy, the Department of Defense, or the U.S. government.*

SA-PO860

Profound Impact of Community Risk Factors on Time to Death and Dialysis Initiation among Patients with Chronic Kidney Disease
Jesse D. Schold, Sankar D. Navaneethan, Stacey Jolly, Susana Arrigain, Victoria Konig, Joseph V. Nally. *Cleveland Clinic, Cleveland, OH.*

Background: Numerous factors explain outcomes in the general population beyond individual demographic characteristics and clinical diagnoses. We hypothesized that community risk factors were independently associated with outcomes of Chronic Kidney Disease(CKD) patients.

Methods: The study population derived from a single center registry of CKD patients within our state identified by laboratory measurements and diagnosis between 2005-09(n=46,735), follow up to 2011. We merged data with a national registry(CountyHealthRankings) which compiles risks for >25 medical and social characteristics for each U.S. county derived from the CDC, U.S. Census, Dartmouth Health Atlas(among others) based on patients' primary zip code. We generated a community risk score and evaluated the independent association with time to death and dialysis(validated by USRDS) using multivariable Cox models.

Results: Patients' community risk score ranged from 5(0=lowest risk) to 40(highest risk); mean=25,std=6. Patients in high risk communities(>=30) were more likely young, African American, male, lower eGFR at CKD diagnosis and Medicaid primary insurance. Following adjustment for baseline eGFR, numerous comorbid conditions, demographics and insurance status, patients from highest risk communities had independent significant risks for death(Adjusted Hazard Ratio[AHR]=1.26,95% C.I. 1.15-1.37), dialysis initiation(AHR=1.71,95% C.I. 1.32-2.21) and composite endpoint of death or dialysis(AHR=1.34,95% C.I. 1.21-1.48). Risks were slightly attenuated but remained highly significant with adjustment for zipcode level household income.

Conclusions: CKD patients from high risk communities have significantly higher rates of death and dialysis initiation. Risk factors in patients communities are likely a proxy for multiple factors including underlying comorbid conditions, socioeconomic status, access to and quality of healthcare and environmental and behavioral risks. Tailored treatment for CKD patients from high risk communities is an important aspect of patient-centric care and further understanding of mechanisms for these associations are needed to identify effective interventions.

Funding: Clinical Revenue Support

SA-PO861

Association of Age and African American Race with Incidence of CKD
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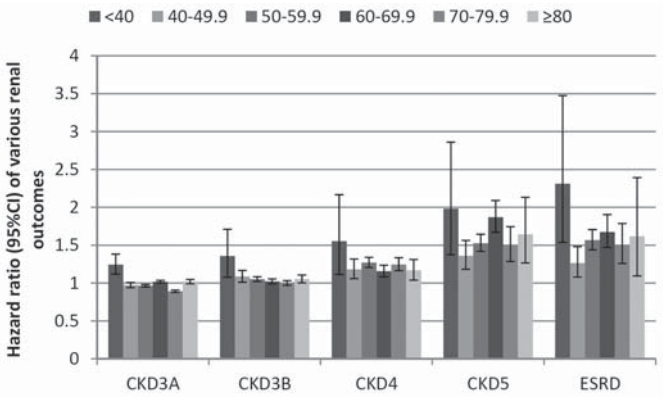
Background: African Americans (AA) are disproportionately affected by CKD. It is unclear if the risk of CKD associated with AA race is different in younger versus older individuals.

Methods: We examined the incidence of CKD stages 3a, 3b, 4, 5 and ESRD in a nationally representative cohort of over 2.8 million U.S. veterans (n=517,980 AA and n=2,355,829 white) with baseline eGFR >=60 ml/min/1.73m^2. Associations were examined in Cox models, adjusted for age, gender, baseline eGFR, comorbidities, BP, BMI, markers of socioeconomic status, adherence with medical interventions, and medication use. The effect of age on the association of AA race with CKD incidence was examined by categorizing patients into 6 age groups (<40 (N=226,522); 40-49.9 (N=369,269); 50-59.9 (N=900,811); 60-69.9 (N=651,711); 70-79.9 (N=521,530) and >=80 years (N=202,083)).

Results: Patients' mean (SD) age was 60.0 (13.6) years, 94% were male, and the mean (SD) baseline eGFR was 84 (16) ml/min/1.73m^2. Events occurred in 586,611 (CKD3a); 136,210 (CKD3b); 27,560 (CKD4); 8,705 (CKD5), and 6,756 (ESRD) patients. AA race was associated with overall adjusted HRs (95%CI) of 0.96 (0.95-0.97), 1.04 (1.02-1.06), 1.25 (1.21-1.29), 1.61 (1.54-1.70) and 1.58 (1.49-1.68) for CKD stages 3a through ESRD, respectively. The HR (95%CI) of various CKD stages associated with AA race in the different age categories was highest in patients <40 years old and tapered with advancing age (Figure).



Conclusions: AA race is associated with increasing risk for more advanced stages of CKD. At all stages (3a-ESRD) the risk of CKD associated with AA race is highest in younger individuals. Screening and preventive efforts aimed at lowering CKD incidence in AA individuals should focus on the youngest age groups.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO862

Non-Pharmacological Interventions for Improving Sleep Quality in CKD Patients: Systematic Review and Meta-Analysis
Bo Yang, Zhiguo Mao. *Dept of Nephrology, Changzheng Hospital, Second Military Medical Univ, Shanghai, China.*

Background: Growing evidence indicates favourable effects and less adverse events of non-pharmacological interventions (NPIs) on primary sleep disorders. But the results of clinical research in CKD population were conflicting. We conducted the meta-analysis to quantify the effects of NPIs for improving sleep quality in CKD patients.

Methods: We searched PubMed, EMBASE, WOS, Cochrane Library and clinicaltrials.gov (up to Dec. 2013) for RCTs and prospective cohort studies comparing effects and safety of NPIs versus standard control in CKD patients. Meta-analyses were used to compute changes in mean values for PSQI score (including its constituent) and relative risks for binary outcomes.

Results: We identified 12 RCTs including 709 patients and one prospective cohort study involving 14 patients. There was a significant global PSQI score reduction in the NPI groups over the control (SMD 1.50, 95% CI 0.91 to 2.09). All three types of NPIs could result in a greater PSQI score reduction: 1) cognitive-behavioural therapy (CBT) (SMD 0.85, 95% CI 0.37, 1.34); 2) physical training (PE) (SMD 3.36, 95% CI 2.16, 4.57) and 3) acupuncture and its variants (ACU) (SMD 1.77, 95% CI 0.80, 2.73). CBT may shorten sleep latency, alleviate sleep disturbance and reduce the use of sleep medications. ACU may ameliorate subjective sleep quality, shorten sleep latency, elongate sleep duration, increase habitual sleep efficiency and improve daytime function of patients. Intervention of CBT and ACU can significantly alleviate fatigue, but PE could not alleviate subjective feeling of fatigue. The finding of the cohort study suggested sleep quality improvement for intradialytic aerobic exercise training on HD patients with restless leg syndrome.

Conclusions: NPIs can improve the general sleep quality in CKD patients. However, the evidence is confined to the ESRD patients on dialysis. CBT, furthermore, could shorten sleep latency, alleviate sleep disturbance and reduce the use of sleep medications. ACU and PE are promising interventions but the results in these subgroups are needed to be interpreted cautiously due to the concern of methodological quality and potential confounding factors.

Funding: Government Support - Non-U.S.

SA-PO863

The Impact of Socioeconomic Status as an Unmeasured Confounder for Claims Based, Comparative Analysis of Dialysis Provider Type and Its Effect on Dialysis Unit Economics
Fadi Almachraki,[1] Michael Tuffli,[1] Paul Lee,[1] Mark Desmarais,[2] Huai-Che Shih,[2] Allen R. Nissenson,[1] Mahesh Krishnan.[1] *[1]DaVita HealthCare Partners, Denver, CO; [2]The Moran Company, Arlington, VA.*

Background: Socioeconomic (SES) factors demonstrably affect health care outcomes (worse outcomes are associated with poverty and rural/urban areas). For dialysis patients, outcomes have been shown to vary by SES. Yet, previous research comparing provider types does not adjust for potential SES confounding. We used geomapping to demonstrate the distribution of SES factors by dialysis provider types.

Methods: Using the Dialysis Facility Compare files (Centers for Medicare and Medicaid Services) and business rules from the Dialysis Outcomes and Practice Patterns Study, dialysis providers were categorized as large (LDO), medium (MDO), small (SDO), hospital/university/government (HUG), for-profit (FP), and non-profit (NP). Poverty by zip code was obtained from 2012 census data. Geography definitions were obtained from the Office of Management and Budget. The Local Indicator of Spatial Association was calculated for poverty in each county. By county, the index of spatial autocorrelation was calculated based on bordering counties.

Results: Variability existed between dialysis provider types and SES factors. A majority of clinics were owned by LDOs (69%; MDO 12%; SDO 10%, HUG 10%) and FP providers (82%; NP 15%; unknown 3%). While providers were concentrated in urban areas, LDOs disproportionately (65%) served rural areas as did FP providers (78%). In high poverty areas, LDOs owned a majority of clinics (metropolitan, 69%; micropolitan, 75%; rural, 75%) and 66% of clinics in extremely poor areas. Similarly, FP providers owned a majority of clinics in poor areas (metropolitan 84%; micropolitan 85%; rural 90%) and 87% of clinics in extremely poor areas.

Conclusions: Due to negative impact of low SES on dialysis-related outcomes plus the over representation of FP LDOs in areas of low SES, the issue of unmeasured confounding emerges. Results suggest that dialysis provider types should be compared using adjustments for SES confounders. Furthermore, in urban, rural, and poverty areas, FP LDOs operate a disproportionate number of clinics.

Funding: Pharmaceutical Company Support - DaVita HealthCare Partners, Inc.

SA-PO864

Associations of Obstructive Sleep Apnea and Cardiovascular Outcomes in Patients with End Stage Renal Disease: A Systematic Review and Meta-Analysis Manoj Das, Shashikumar Yellappa, Qasim Malik, Michael Saleeb, Louis Romel Crevecoeur, Anita A. Kumar, Ghanshyam Palamaner Subash Shantha. *Internal Medicine, The Wright Center For Graduate Medical Education, Scranton, PA.*

Background: Obstructive sleep apnea (OSA) increases risk for cardiovascular outcomes in the general population. Small observational studies that assessed these associations in patients with end stage renal disease (ESRD) lacked precision. Hence, this systematic review and meta-analysis is an effort to pool all available evidence to identify the associations of OSA and cardiovascular outcomes in patients with ESRD.

Methods: Medline, Embase, Cochrane central library, and electronic databases were searched for relevant studies in all languages and without time restriction. Studies were included if: 1) they studied patients with OSA and ESRD, and 2) reported cardiovascular outcomes; stroke, coronary artery disease (CAD) and cardiovascular mortality. Two investigators independently reviewed retrieved citations and assessed eligibility. Discrepancies were resolved by consensus. Data were pooled using a random-effects model.

Results: From a total of 3977 retrieved citations, 5 observational studies were included, representing 1850 patients with ESRD (37% with OSA and 67% without OSA). All 5 were sleep clinic based studies, 4 used apnea-hypopnea index (AHI) > 5 events/hour to define OSA, while 1 defined OSA if AHI was > 15 events/hour. In the pooled analysis, ESRD patients with OSA were at a higher odds for stroke (OR: 1.97, 95% CI: 1.58 – 2.85, P = 0.03, I^2: 48%, 5 included studies), but not CAD (OR: 0.91, 95% CI: 0.57 – 1.51, P = 0.56, I^2: 74%, 4 included studies) or cardiovascular mortality (OR: 1.11, 95% CI: 0.71 – 1.48, P = 0.31, I^2: 63%, 4 included studies) when compared to ESRD patients without OSA. Factors responsible for high heterogeneity between studies could not be assessed due to lack of relevant data.

Conclusions: ESRD patients with OSA are at higher odds for developing stroke. Possible determinants in this association; atrial fibrillation and obesity, need assessment in future studies. Since, all were sleep clinic based studies, population based studies are needed to eliminate selection bias in this association.

SA-PO865

The SNORE Study: Baseline Data from a Longitudinal Study of Sleep Apnea and CKD Progression Nicole Kay,[1] Areef Ishani,[2] I. David Weiner,[1,3,4] Richard Berry,[1,4] Rebecca Beyth,[1,4] Muna T. Canales.[1,3,4] *[1]Malcolm-Randall VAMC; [2]VAMC, Minneapolis, MN; [3]Div of Nephrology; [4]Dept of Medicine, Gainesville, FL.*

Background: Identification of new modifiable risk factors to slow progression of CKD could substantially improve clinic outcomes. We hypothesize that sleep apnea(SA), through recurrent episodes of hypoxia, hypercapnia and arousal during sleep, may be a novel risk factor for CKD progression and poor quality of life(QOL).

Methods: The SNORE Study is an ongoing prospective study of 250 veterans with eGFR of 15-44 ml/min/1.73m^2. At baseline, veterans undergo sleep study, renal function measures, KDQOL-SF and Epworth Sleepiness Scale (ESS). Renal function and QOL are re-assessed annually. SA was defined by the apnea-hypopnea index (AHI, at cut-points of 5, 15, 30 events/h for mild, moderate, severe). Outcomes of the study are time to doubling of SCr or ESRD, and change in KDQOL-SF scores. Enrollment will occur over 2y with 3y follow-up for each veteran.

Results: Enrollment began 3/18/14; as of 5/1/14, 134 subjects have been enrolled. Mean age was 75±9y; 98% were male; 82% were Caucasian; Mean BMI was 30±5 kg/m^2. 95% had HTN, 46% had DM; mean eGFR was 34±9 ml/min/1.73m^2, median [IQR] ACR, 43[12-210]. By KDQOL-SF, 25% reported excellent or very good health. Physical and Mental Health Component Summary Scores were 38.9±10 and 51.3±10, respectively. Burden of kidney disease and sleep (pre-specified domains) were 81±22 and 62±19, respectively. 44% had excessive daytime sleepiness(ESS>10). From the 73 scored studies to date, median[IQR] AHI was 11[4-22]; with no, mild, moderate, severe SA in 30%, 29%, 25%, 16%, respectively. Baseline eGFR did not correlate with severity of SA(p trend 0.67). An ACR≥30 was present in 41% without SA versus 65% with any SA(p=0.06).

Conclusions: The SNORE Study is on track to complete enrollment as planned. The prevalence of SA and daytime sleepiness is high among veterans with CKD. Veterans with SA are more likely to have albuminuria than those without SA. QOL is comparable to other studies of non-veteran populations with CKD. Once complete, the SNORE Study will provide much needed longitudinal data regarding the impact of SA on CKD progression and QOL in veterans with CKD.

Funding: Veterans Affairs Support

SA-PO866

The Factors Associated with Arrhythmia During Dialysis Course in Maintained Hemodialysis Patients Lixia Xu, Liyi Mo, Lu Cai, Wei Dong, Yiming Tao, Ruizhao Li, Jianxun Wu, Chenggen Xiao, Xinling Liang, Wei Shi. *Hemodialysis Center, Guangdong General Hospital, Guangdong Academy of Medical Science, Guangzhou, Guangdong, China.*

Background: Cardiovascular mortality and morbidity are high in maintained hemodialysis patients. Arrhythmia is known to predispose to sudden cardiac death. This study was conducted to assess the occurrence of arrhythmia during dialysis course and analyze the factors of arrhythmia in these patients.

Methods: Two hundred and forty-eight maintained hemodialysis patients were assessed by standard examination including heart rate, 15-lead electrocardiography and laboratory tests like electrolytes (Na (+), K (+), Ca (++), Mg(++) phosphate), urea, and creatinine, uric acid, blood glucose at beginning, 2h, ending of HD. The PR intervals, QRS duration and QT intervals for each lead were measured manually by one observer using calipers. Patients with arrhythmia at beginning of dialysis were excluded.

Results: Arrhythmia is found in 128 patients during dialysis course. The age, baseline heart rate and heart rate variability during dialysis course were comparable between the two groups. The mean PR intervals, QRS duration of pre, 2h and post dialysis increased significantly in arrhythmia group (pre 178.35±37.71ms versus 169.95±30.35ms and 103.01±61.88ms versus 87.68±9.45ms, 2h 171.79±31.20ms versus 164.31±22.39ms and 98.52±19.08ms versus 88.98±9.62ms, post 174.64±36.84ms versus 162.91±24.27ms and 102.42±34.44ms versus 90.33±9.31ms respectively $p<0.05$). The mean of QT dispersions and the corrected QT interval dispersions changed were similar in two groups. The baseline urea, electrolytes, uric acid were comparable between two groups, only the creatinine level of arrhythmia group was lower than the other one (956.06±287.31mmol/L versus 1033.01±317.91mmol/L $p<0.05$). The rate of serum phosphate descent at 2h of dialysis course was lower in arrhythmia group (45.29±11.88% versus 48.85±8.50%).

Conclusions: PR interval and QRS duration prolongation were correlated with attack of arrhythmia in maintained hemodialysis patients. Serum phosphate clearance increase maybe depresses the arrhythmia.

Funding: Government Support - Non-U.S.

SA-PO867

Impact of Ventricular Arrhythmia in Chronic Kidney Disease Patients after 24 Months of Follow-Up Fabiana Oliveira Bastos Bonato,[1] Marcelo Lemos,[1] Jose Luiz Cassiolato,[2] Renato Watanabe,[1] Maria Eugenia F. Canziani.[1] *[1]Nephrology, Federal Univ of Sao Paulo, Sao Paulo, SP, Brazil; [2]Cardiology, Cardios, Sao Paulo, SP, Brazil.*

Background: In general population, ventricular extrasystoles are associated with increase in the risk of death. Although arrhythmia is a common finding in chronic kidney disease (CKD) patients, the role of ventricular extrasystoles in this population remains unknown. In this study, we aimed at evaluating the impact of ventricular arrhythmias (VA) on cardiovascular events, hospitalization and mortality in a nondialyzed CKD population followed for 24 months.

Methods: This prospective study evaluated 109 CKD patients (eGFR 34.8±16.1 mL/min/1.73m², 57±11.4 years, 61% male, 24% diabetics). VA was assessed by 24-hr electrocardiogram. Echocardiogram, multi-slice computed tomography, 24-hr ambulatory blood pressure monitoring and laboratory parameters were performed. The occurrence of cardiovascular events, hospitalization, and death was recorded over 24 months.

Results: At baseline, VA was found in 34% and complex VA in 14% of the patients. During the follow-up, the occurrence of 15 cardiovascular events, 15 hospitalizations, and 4 deaths was registered. The presence of VA was associated with a greater number of deaths ($p = 0.012$) and these patients tended to have more cardiovascular events ($p = 0.088$). The group with complex VA had a superior mortality rate ($p = 0.008$) and a higher frequency of cardiovascular events ($p = 0.006$) and hospitalization ($p = 0.033$). During the 2-yr follow-up, a lower survival was observed in patients with VA ($p = 0.005$) and complex VA ($p < 0.001$). Patients with complex VA had also a shorter cardiovascular ($p = 0.001$) and hospitalization ($p = 0.018$) event-free times. Adjusting for several confounders, complex VA remained as an independent risk factor for cardiovascular events (HR = 16.66, 95%CI = 3.16 - 87.93, $p = 0.001$) and hospitalization (HR = 5.44, 95%CI = 1.26 - 23.52, $p = 0.023$).

Conclusions: The presence of VA in nondialyzed CKD patients should be considered as an adjuvant factor to the risk stratification in this population.

SA-PO868

Comparative Effectiveness of Coronary Revascularization Procedures for Multivessel Coronary Artery Disease in Patients with Chronic Kidney Disease John K. Roberts,[1] Sunil V. Rao,[1] Linda K. Shaw,[1] Dianne Gallup,[1] Oscar C. Marroquin,[2] Uptal D. Patel.[1] *[1]Duke Clinical Research Inst, Duke Univ Medical Center, Durham, NC; [2]Dept of Medicine, Univ of Pittsburgh Medical Center, Pittsburgh, PA.*

Background: Patients with CKD are at increased risk for cardiovascular disease and death. Although some studies have compared revascularization strategies in patients with CKD, few account for anatomic differences. We sought to evaluate whether reductions in mortality differed by coronary revascularization strategies across patients with different severity of CKD and multivessel coronary artery disease.

Methods: We created a cohort of 4,687 adults who underwent cardiac catheterization and were found to have multivessel coronary artery disease (CAD; ≥50% stenosis). We used Cox-proportional hazard regression weighted by the inverse probability of treatment to examine the association between four revascularization strategies (medical management, PCI-BMS, PCI-DES, and CABG) and mortality across all strata of eGFR (CKD-EPI) at a median follow up of 5.1 years.

Results: CABG was associated with a reduced risk of death compared with medical management for patients across all levels of eGFR except those on dialysis. Although trends were similar, there were no significant differences in mortality between PCI and CABG except for a decreased risk of death in patients with severe CKD who received CABG relative to bare metal stents. (see table 1)

eGFR (N)	CABG vs Med	CABG vs BMS	CABG vs DES
≥ 60 (3,144)	**0.47 (0.37, 0.6)**	0.78 (0.59, 1.04)	0.99 (0.77, 1.26)
45-59 (760)	**0.59 (0.40, 0.86)**	0.67 (0.43, 1.02)	0.79 (0.54, 1.14)
30-44 (408)	**0.43 (0.29, 0.63)**	0.67 (0.42, 1.06)	0.65 (0.42, 1.0)
< 30 (240)	**0.44 (0.28, 0.68)**	**0.42 (0.24, 0.75)**	0.65 (0.41, 1.02)
Dialysis (135)	0.81 (0.43, 1.53)	0.94 (0.37, 2.38)	0.85 (0.45, 1.64

Conclusions: Compared with medical management, surgical revascularization for multivessel CAD was associated with reduced mortality in patients with CKD who were not on dialysis. Similar, but non-significant trends toward lower mortality were also observed with CABG when compared with PCI. A prospective, randomized trial in patients with CKD is warranted to confirm these findings.

Funding: NIDDK Support

SA-PO869

Geriatric Nutritional Risk Index Predicts Cardiovascular Events in Patients with Pre-Dialysis Chronic Kidney Disease Midori Hasegawa, Hiroshi Takahashi, Kazuo Takahashi, Hiroki Hayashi, Shigehisa Koide, Yukio Yuzawa. *Nephrology, Fujita Health Univ School of Medicine, Toyoake, Aichi, Japan.*

Background: We investigated whether geriatric nutritional risk index (GNRI), developing as a simplified marker of the protein-energy wasting (PEW), predicts adverse cardiovascular disease (CVD) events in pre-dialysis chronic kidney disease (CKD) patients.

Methods: A total of 406 patients with CKD (stage 3; 141, stage 4; 159 and stage 5; 106) were enrolled. The GNRI was calculated using formula as follows; GNRI = (14.89 × serum albumin) + [41.7 × (body weight / body weight at BMI of 22)]. The patients were divided into tertiles according to GNRI levels; tertile 1 (T1): < 95.5, T2: 95.5 – 99.8 and T3: > 99.8, and were followed up for 4 years. CVD events were defined as hospitalization or death due to acute coronary syndrome, worsen heart failure or stroke. To assess additive incremental value of GNRI into an established risk model for predicting of CVD events, C-index, net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were also evaluated.

Results: During follow-up period (median: 31months), 79 CVD events occurred and 32 patients died. Four-year event-free survival rates were 56.9%, 65.0% and 87.9% for CVD events (p<0.0001), and were 79.7%, 90.9% and 95.1% for mortality (p=0.0006) in T1, T2 and T3, respectively. After adjustment for significant risk factors such as age, diabetes, history of CVD, cystatin C, UACR, hemoglobin and hs-CRP on univariate analysis, the GNRI [hazard ratio (HR) 2.84, 95% confidence interval (CI) 1.31-6.13, p=0.0079 for T1 versus T3], history of CVD (HR 3.42, 95%CI 2.09-5.61, p<0.0001) and cystatin C (HR 1.34, 95%CI 1.01-1.78, p=0.042) were identified as independent predictors for CVD events. The addition of the GNRI to a prediction model based on significant risk factors for CVD events had effect on model discrimination as measured by the C-index (0.728 to 0.779, p=0.045) and improved the NRI (0.619, p<0.0001) and the IDI (0.074, p<0.0001). Similar results were also obtained for all-cause mortality.

Conclusions: Declined GNRI levels could strongly predict CVD events and mortality in pre-dialysis CKD patients.

SA-PO870

Annual Decline in Estimated Glomerular Filtration Rate Is a Risk of Cardiovascular Events Independent of Proteinuria Kei Nagai,[1] Kunihiro Yamagata,[1] Chie Saito,[1] Koichi Asahi,[2] Kunitoshi Iseki,[2] Kenjiro Kimura,[2] Toshiki Moriyama,[2] Ichiei Narita,[2] Shouichi Fujimoto,[2] Kazuhiko Tsuruya,[2] Tsuneo Konta,[2] Masahide Kondo,[2] Tsuyoshi Watanabe.[2] *[1]Nephrology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Steering Committee for "Design of the Comprehensive Health Care System for Chronic Kidney Disease (CKD) Based on the Individual Risk Assessment by Specific Health Checkups", Japan.*

Background: Chronic kidney disease is a risk factor of the development of cardiovascular disease (CVD). However, it is not clear whether decline of glomerular filtration rate (GFR), not reduced GFR, is a risk factor for the occurrence of CVD independent of proteinuria.

Methods: By using a population-based 521,123 person-years longitudinal cohort receiving the Japanese national health program from 2008 to 2011, we examined whether the annual decline of estimated GFR is a risk factor for CVD development independent of proteinuria by Cox-hazard model.

Results: During the follow-up period, there were 4,426 stroke events and/or 8,298 cardiac events. The median annual changes in eGFR from eGFR at the baseline year were -0.45 (10, 25, 75, or 90 percentile; +8.65, +1.34, -5.33, or -12.43, respectively) % per year among males and -0.44 (10, 25, 75, or 90 percentile; +10.34, +0.21, -4.60, or -13.85, respectively) % per year among females. Rapidly decreasing over 10% per year in estimated GFR was a significant and independent risk factor for the incidence of stroke and cardiac events with covariant adjustment for proteinuria and reduced estimated GFR.

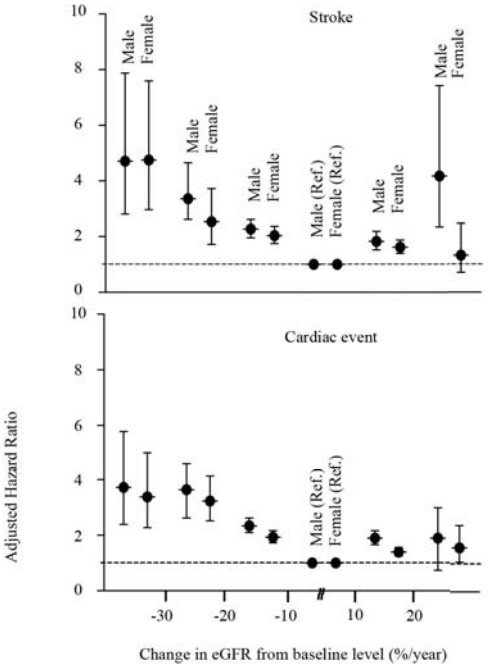


Figure. Multivariable adjusted hazard ratio for the incidence of CVD in subpopulation based on annual change of eGFR.

Adjusted for age, BMI, hypertension category, dyslipidemia, taking anti-dyslipidemia drugs, hyperglycemia, taking hypoglycemic drugs, body mass index, reduced eGFR at baseline, proteinuria and smoking. Bars indicates 95% C.I.

Conclusions: Annual decline of GFR is an independent risk factor for CVD.

SA-PO871

CKD Measures and Future Risk of Peripheral Artery Disease Hospitalizations: The Atherosclerosis Risk in Communities (ARIC) Study Kunihiro Matsushita, Yingying Sang, Shoshana Ballew, M. Grams, Elizabeth Selvin, Bernard G. Jaar, Josef Coresh. *Johns Hopkins Univ, Baltimore, MD.*

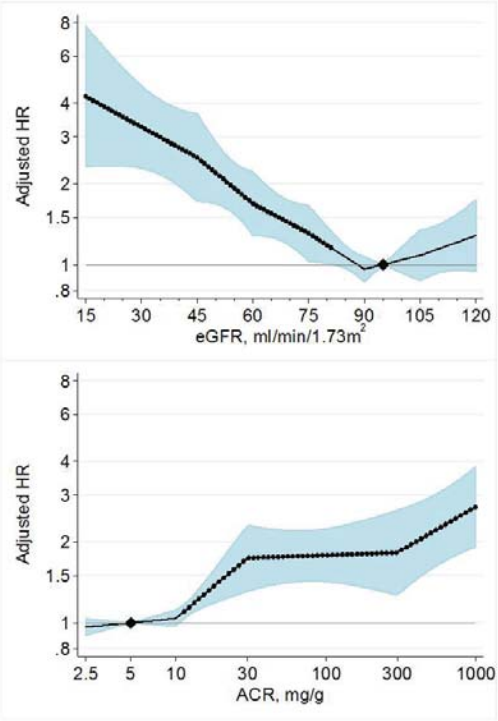
Background: Several studies have reported an association of CKD with peripheral artery disease (PAD). However, most of them were cross-sectional and/or investigated either eGFR or albuminuria, but not both, leaving uncertainty in their respective prospective contributions to PAD risk.

Methods: We studied 9,936 ARIC participants without a history of PAD at baseline (1996-98). Cox models were used to quantify the associations of creatinine-based eGFR and urine albumin-to-creatinine ratio (ACR) with clinically significant PAD-related hospitalizations with adjustment for each other and traditional risk factors.

Hospitalizations were considered "PAD-related" when there was a discharge diagnosis of leg revascularization procedure, ischemic ulcer, gangrene, or amputation. We also evaluated whether eGFR and/or ACR improved PAD risk prediction.

Results: There were 728 PAD hospitalizations during a median follow-up of 14 years (5.8 per 1,000 person-years). Both low eGFR and high ACR were significantly associated with PAD hospitalization risk (Figure). Of note, both eGFR and ACR significantly improved PAD risk discrimination beyond the model with traditional risk factors alone (c-statistic difference: 0.011 (95% CI: 0.004-0.018) with the addition of eGFR; 0.009 (0.001-0.016) with ACR; and 0.016 [0.007-0.025] with both). Results were largely consistent when restricted to the most severe PAD outcomes (ischemic ulcer, gangrene, or amputation).

Conclusions: Both low eGFR and high ACR were independently associated with future PAD hospitalization risk and significantly improved its prediction, suggesting the usefulness of CKD measures to identify high risk patients who may benefit from more intensive PAD risk reduction interventions.



Adjusted for age, sex, race, smoking, diabetes, systolic blood pressure, antihypertensive drugs, total and high-density lipoprotein cholesterols, history of coronary disease, stroke, and heart failure, and each CKD measure.

Funding: Other NIH Support - The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), Private Foundation Support

SA-PO872

eGFR, Dipstick Proteinuria, and Cause-Specific Mortality in a Large Population-Based Cohort from Korea Yejin Mok,[1,2] Kunihiro Matsushita,[1] Yingying Sang,[1] Shoshana Ballew,[1] M. Grams,[1] Josef Coresh,[1] Sun Ha Jee.[2] *[1]Johns Hopkins Univ, Baltimore, MD; [2]Yonsei Univ, Seoul, Korea.*

Background: The link of chronic kidney disease (CKD) to cardiovascular disease (CVD) mortality is well known. However, its link to mortality due to other causes like cancer is less clear.

Methods: We studied 367,932 adults (20-93 years) in the Korean Heart Study (health check-up database from 14 centers in Korea with baseline between 1994-2004 and follow-up until 2011) and assessed the associations of creatinine-based eGFR and dipstick proteinuria with mortality due to CVD (1,315 cases), cancer (4,035 cases), and other causes (3,445 cases) after adjusting for potential confounders (demographic, lifestyle, and clinical risk factors including history of cancer and CVD).

Results: Low eGFR was significantly associated with CVD, other-cause, and all-cause mortality (Table). Cancer mortality risk demonstrated a J-shaped association with the lowest risk at GFR 45-59 and higher risk at eGFR <45 (reached significance with eGFR 45-59 as a reference [HR 1.64, 95% CI 1.12-2.42]). High proteinuria was consistently associated with mortality due to CVD, cancer and other causes. Examining finer causes of death, low eGFR (<60 versus ≥60) was significantly associated with deaths due to coronary heart disease, oropharyngeal cancer, and diabetes, whereas proteinuria (≥trace versus negative) was related to mortality from coronary disease, stroke, stomach cancer, liver cancer, lung cancer, diabetes, infectious disease, liver disease and chronic obstructive pulmonary disease.

Mortality	eGFR			
	≥60	45-59	30-44	<30
	N=364,290	N=3,136	N=338	N=168
CVD	Reference	1.35 (1.08-1.67)	1.73 (1.07-2.79)	1.81 (0.79-4.14)
Cancer	Reference	0.80 (0.67-0.96)	1.33 (0.90-1.97)	1.29 (0.61-2.74)
Other causes	Reference	1.45 (1.24-1.71)	2.68 (1.98-3.64)	6.19 (4.52-8.48)
All-cause	Reference	1.15 (1.03-1.27)	1.94 (1.56-2.41)	3.62 (2.76-4.73)
	Dipstick			
	None	Trace	+	≥++
	N=323,827	N=12,894	N=25,942	N=5,269
CVD	Reference	1.42 (1.13-1.77)	1.58 (1.35-1.85)	2.37 (1.87-3.01)
Cancer	Reference	1.21 (1.05-1.38)	1.20 (1.09-1.32)	1.54 (1.29-1.84)
Other causes	Reference	1.46 (1.27-1.68)	1.32 (1.19-1.46)	2.61 (2.26-3.01)
All-cause	Reference	1.33 (1.22-1.46)	1.29 (1.21-1.37)	2.10 (1.90-2.32)

Conclusions: Both low eGFR and high proteinuria were independently associated with mortality due to CVD, cancer, and other causes. These findings suggest the need for multidisciplinary prevention and management strategiesin individuals with CKD.

SA-PO873

A Low Baseline Glomerular Filtration Rate Predicts Poor Clinical Outcome 3 Months After Acute Ischemic Stroke Jwa-Kyung Kim,[1] Myung Jin Choi,[2] Jung-Woo Noh,[3] Jiwon Ryu,[2] Ji Suk Han.[1] *[1]Internal Medicine, Hallym Univ Sacred Heart Hospital, Seoul, Republic of Korea; [2]Internal Medicine, Hallym Univ Chuncheon Sacred Heart Hospital, Seoul, Republic of Korea; [3]Internal Medicine, Hallym Univ Gangnam Sacred Heart Hospital, Seoul, Republic of Korea.*

Background: Chronic kidney disease (CKD) is an established risk factor for cardiovascular disease including stroke. However, the effect of decreased estimated glomerular filtration rate (eGFR) on stroke outcome remains controversial. In this study, we evaluated the association between eGFR and 3-month stroke outcomes and assessed whether CKD and its severity could affect clinical outcomes or not.

Methods: In this prospective cohort study with a hospital-based stroke registry, 1,373 patients with acute ischemic stroke were enrolled. Patients were divided into 4 groups (eGFR ≥60, 45–59, 30–44, and <30 mL/min/1.73m^2) according to the CKD Epidemiology Collaboration equations. Primary endpoint was 3-month death or dependency as poor functional outcome [mRS (modified Rankin Scale) score ≥3], and secondary endpoints were neurological deterioration (increased NIHSS ≥4 at discharge compared to baseline NIHSS) or mortality during hospitalization.

Results: Mean eGFR was 84.5 ± 20.8 mL/min/1.73m^2. The distribution of baseline renal impairment was as follows: 1,218 patients with eGFR ≥60 mL/min/1.73m^2, 82 with eGFR 45 - 59 mL/min/1.73m^2, 40 with eGFR 30 - 44 mL/min/1.73m^2 and 33 with eGFR <30 mL/min/1.73m^2. At 3 month after the stroke, 476 (34.7%) patients showed poor functional outcome, and that was more frequently occurred at more advanced stages of CKD (31.9%, 53.7%, 55.0% and 63.6% in CKD stage 1-2, 3a, 3b and 4-5, respectively, $p < 0.001$). Multivariate analysis showed that a baseline eGFR <30 mL/min/1.73m^2 increased the risk of poor functional outcome by 2.37-fold ($p = 0.047$). In addition, baseline renal dysfunction was also closely associated with neurological deterioration during hospitalization and in-hospital mortality, too.

Conclusions: A low baseline eGFR was highly predictive of both poor functional outcome 3 months after ischemic stroke and neurological deterioration/mortality during hospitalization.

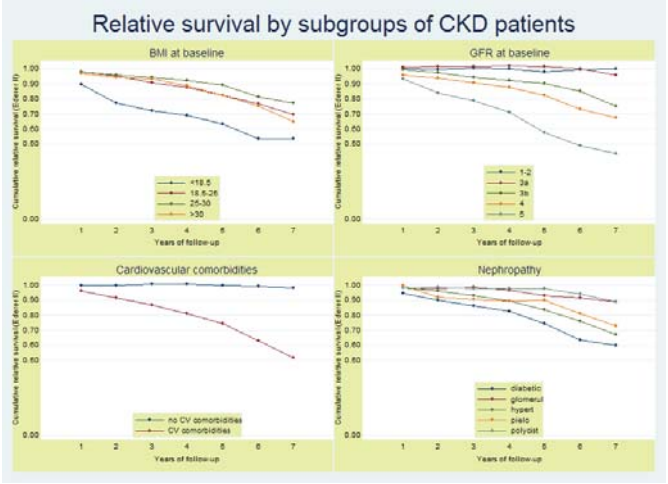
SA-PO874

An Estimation of CKD Cause-Specific Mortality Using Relative Survival Dino Gibertoni,[1] Stefano Torroni,[1] Marcora Mandreoli,[2] Antonio Santoro.[3] *[1]Dept of Biomedical and Neuromotor Sciences, Alma Mater Studiorum - Univ of Bologna, Bologna, Italy; [2]Nephrology and Dialysis Unit, Ospedale S.Maria della Scaletta, Imola, Italy; [3]Nephrology, Dialysis and Hypertension Unit, Policlinico S.Orsola-Malpighi, Bologna, Italy.*

Background: The estimation of cause-specific mortality is usually based on cause of death recordings, that may be incomplete or inaccurate. Relative survival (RS) estimates the attributable mortality as a ratio between the observed survival and the expected survival in the general population obtained by matching mortality tables.

Methods: We applied RS to a cohort of 2179 patients who entered the PIRP registry between 2006 and 2011 in Emilia-Romagna, a North-Eastern Italian region. The PIRP registry collects clinical data on CKD patients who receive pharmacological and dietary treatment aimed to reduce CKD progression. Matching with general population survival by calendar year, gender and age was made using regional ISTAT mortality tables. Patients were followed up for at least one year until 2012 or death. Excess mortality rate ratios (EMRR) among risk groups were found using a multiple flexible parametric survival analysis.

Results: Overall 7-years RS estimate was 0.712. In the multivariate analysis, factors significantly associated with excess mortality attributable to CKD were: previous CV events (EMRR=4.3), CKD stage (EMRR=2.1 for stage 5 versus stage 3b), underweight (EMRR=3.5 versus normal BMI), proteinuria (EMRR=2.3), diabetes (EMRR=1.74), higher phosphate (EMRR=1.46).



Conclusions: Our findings further underline that CKD patients are a particularly frail population with a 28.8% excess mortality over the general population. Because the majority of patients are in CKD stages 3 and 4, our results provide additional useful information for primary care physicians and nephrologists about factors to be monitored to decrease the risk of death from the onset of the disease onwards.

SA-PO875

Interactions of Frailty and CKD with Mortality Xiaorui Chen,[2] G. Wei,[2] E. Constantz,[2] R. Boucher,[2] Tom Greene,[2] Srini Beddhu.[1,2] *[1]VAMC SLC; [2]Univ of Utah.*

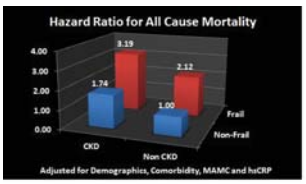
Background: Frailty is common in CKD population. It is unclear whether 1. the combination of frailty and CKD is additive or multiplicative for mortality risk and 2. the associations of frailty with mortality are accounted for by muscle mass and inflammation.

Methods: 2136 participants in the 1999-2002 National Health And Nutrition Examination Survey aged 50 yrs or older were included. 5 frailty criteria were: Unintentional weight loss (>5 lbs) in the past year, exhaustion (difficulty walking from one room to another room in the same level), physical activity (↓ compared to last yr), slowness (time to complete 20 ft walk), and ↓ muscle strength (assessed by dynamometer). Mortality data was through 12/31/2006. As even the presence of 1 or more conditions was associated with mortality (table), non-frail was defined as the presence of 0 conditions. Mid-arm muscle circumference (MAMC) was used as an indicator of muscle mass. HsCRP was used as inflammatory marker. Cox regression models were used to relate CKD and frailty with all-cause mortality.

Results: Mean age was 67.9, 45.8% were male, 6.9% were black, 18.8% had CKD. Prevalence of the number of frailty conditions and the unadjusted mortality rate in non-CKD and CKD are summarized in the table.

# Frailty conditions	Prevalence (%)		Morality rate (deaths/ 100 pt years)	
	Non-CKD	CKD	Non-CKD	CKD
0	56.2	46.4	1.13	2.92
1	31.6	34.2	2.22	7.26
2	9.4	11.4	4.28	5.54
≥3	2.8	8.0	4.52	13.84

Adjusted for demographics and comorbid conditions, compared to non-frail, mortality risk for frail was higher in non-CKD and CKD which persisted after adjustment for MAMC and CRP (figure).



A multiplicative interaction term for CKD and frailty was non-significant (p=0.47).

Conclusions: Frailty and CKD are ~ additive but not synergistic mortality risk factors. These associations are independent of muscle mass and inflammation.

Funding: NIDDK Support

SA-PO876

Chronic Kidney Disease Outcomes in Patients Risk Stratified Using an End Stage Renal Disease Risk Calculator Candace D. Grant,[1] Shayan Shirazian,[1] Navdeep Tangri,[2] Joseph Mattana.[1] *[1]Medicine, Winthrop-Univ Hospital, Mineola, NY; [2]Medicine, Univ of Manitoba, Winnipeg, MB, Canada.*

Background: Accurate determination of the risk of development of end stage renal disease (ESRD) can be helpful in preparing patients with chronic kidney disease (CKD) for renal replacement therapy (RRT) as well as avoiding such preparation in those at low risk for this event. ESRD risk calculators have been developed which provide risk estimations based on age and other variables but have not seen widespread application to date. In this study we risk stratified a cohort of patients with stage 4 CKD using a validated risk assessment tool and carried out an observational study to assess outcomes.

Methods: 189 patients with stage 4 CKD had their ESRD risk determined using the risk calculator developed by Tangri et al. (JAMA 2007;305:1553-9) which incorporates age, gender, eGFR, urine albumin to creatinine ratio, calcium, phosphorus, albumin, and bicarbonate. A 2-year risk of progression to ESRD of <10% was considered low risk (LR) and ≥ 10% was considered high risk (HR). Over the following year development of ESRD, access creation and death were recorded.

Results: The average age of the patients was 71 years, 50% were men, 78% were white, mean eGFR was 23 ml/min/1.73m^2 and 43% were determined to be LR. For the group as a whole, vascular access was placed in 13%, 12% reached ESRD, and 13% died during the one year time period. When the LR and HR groups were compared, we found that 1% of the LR group reached ESRD versus 21% of the HR group, vascular access was placed in 6% of the LR group versus 19% of the HR group and there was no difference in mortality rate (13% each).

Conclusions: These preliminary findings suggest that using this validated risk calculator to assign patients to LR and HR groups appears to help predict clinical outcomes, with a very low incidence of ESRD in LR patients despite being stage 4 CKD. The use of ESRD risk calculation might be a useful tool in individualizing risk of ESRD and helping guide proper selection of patients for preparation for RRT.

SA-PO877

Abrupt Decline in eGFR prior to Initiating Hemodialysis (HD) Predicts Mortality after ESRD: Results from the CRIC Study Raymond K. Hsu,[1] Jason Roy,[2] Boyang Chai,[2] Amanda Hyre Anderson,[2] Nisha Bansal,[3] Harold I. Feldman,[2] Alan S. Go,[4] Jiang He,[5] Edward J. Horwitz,[6] John W. Kusek,[7] James P. Lash,[8] Akinlolu O. Ojo,[9] James H. Sondheimer,[10] Raymond R. Townsend,[2] Min Zhan,[11] Chi-Yuan Hsu.[1] *[1]UCSF; [2]UPenn; [3]UWash; [4]KPNC; [5]Tulane; [6]Case Western Reserve; [7]NIH; [8]UIC; [9]UMich; [10]Wayne State; [11]UMaryland.*

Background: Sudden transition to ESRD may partly explain why some patients initiate HD with catheters, receive little or no nephrology care prior to ESRD, or experience adverse outcomes. Thus the rate of loss of kidney function during the transition from CKD to ESRD may be an important risk factor for outcomes after HD initiation.

Methods: Among the 3939 participants in the Chronic Renal Insufficiency Cohort (CRIC) Study, we studied 740 who initiated maintenance HD during follow-up. We incorporated annual eGFR's into mixed effect models to estimate patient-specific GFR's at 3 months prior to start of HD. Abrupt decline was defined as having an extrapolated GFR of >30 ml/min/1.73m^2 at that time point. Proportional hazards models were used to assess the association between abrupt decline and all-cause mortality after HD initiation.

Results: Among the 740 incident cases of HD, 72 (9.8%) experienced abrupt decline in eGFR, and 192 died after initiating HD. Abrupt decline in eGFR was associated with a higher rate of all-cause mortality (unadjusted hazard ratio [HR] 1.85, 95% CI 1.25-2.75, p<0.05). After adjustment for age at ESRD, sex, race-ethnicity, diabetes and cardiovascular disease, abrupt decline remained independently associated with death (HR 1.87, 95% CI 1.25-2.80, p<0.05). 75 (39%) of 192 deaths occurred within one year of HD start. There was an even stronger association between abrupt decline in eGFR and one-year mortality: unadjusted HR 3.34 (95% CI 1.99-5.91, p<0.001) and adjusted HR 3.62 (95% CI 2.09-6.29, p<0.001).

Conclusions: Abrupt decline in eGFR prior to ESRD occurs in a significant minority of patients and is an independent risk factor for death—especially early death—after onset of ESRD. Factors responsible for this rapid decline need to be identified.

Funding: NIDDK Support

SA-PO878

Comorbidity and Survival in CKD Stage 3 Simon D.S. Fraser,[1] Natasha J. Mcintyre,[2] Richard J. Fluck,[2] Chris W. McIntyre,[3] Paul J. Roderick,[1] Maarten W. Taal.[3] *[1]Academic Unit of Primary Care and Population Science, Southampton Univ, Southampton, Hampshire, United Kingdom; [2]Dept of Renal Medicine, Derby Hospital, Derby, Derbyshire, United Kingdom; [3]Div of Medical Sciences and Graduate-Entry Medicine, Nottingham Univ, Derby, Derbyshire, United Kingdom.*

Background: In ageing populations, increasing multimorbidity prevalence has implications for patients, clinicians and policy makers. CKD is often considered in isolation but commonly occurs with other chronic conditions. Little information exists on the prevalence and prognostic implication of comorbidity combinations in moderate CKD. Aim: to investigate comorbidity prevalence and survival in a cohort of people with CKD stage 3.

Methods: People with eGFR 59-30ml/min/1.73m^2 on 2 occasions prior to inclusion were recruited from Primary Care. Medical history was obtained and participants underwent clinical assessment, urine and serum biochemistry tests. Comorbidities included

hypertension (HT), diabetes, ischaemic heart disease, heart failure, peripheral vascular disease, and cerebrovascular disease. Cox proportional hazards models were used to compare survival in those with CKD+/-1 comorbid condition with those with CKD+2 or more conditions (CKD+2).

Results: 1741 people with CKD were recruited.Mean age was 73±9 years and baseline eGFR 52±10ml/min/1.73m^2. Prevalence of isolated CKD, CKD+1 and CKD+2 were 9%, 50% and 41% respectively. The comorbid condition was HT in 95% of those with CKD+1. After a median 3.6 years 63 (6%) of those with CKD+/-1 had died compared to 112 (16%) of those with CKD+2 (p<0.001).Cox regression identified CKD+2 or more comorbidities as an independent predictor of all-cause mortality

	HR (95% CI)	p
CKD+2 (vs CKD+/-1)	1.84 (1.34-2.51)	<0.001
Age (years)	1.06 (1.04-1.09)	<0.001
Sex (M vs. F)	1.36 (1.00-1.84)	0.05
eGFR (ml/min/1.73m^2)	0.97 (0.95-0.98)	<0.001
Log urine ACR (mg/mmol)	1.16 (1.04-1.30)	0.01

Conclusions: Isolated CKD was rare even in this cohort recruited from primary care. Having more comorbidities was independently associated with mortality. CKD should therefore not be managed in isolation. Further research should focus on methods to reduce risk across multiple comorbidities in people with CKD while considering treatment burden.

Funding: Pharmaceutical Company Support - Roche Products plc.

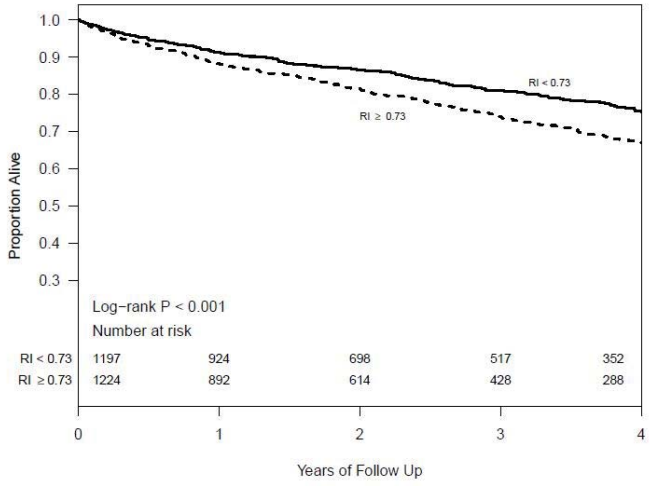
SA-PO879

Renal Resistive Index Is Associated with Increased Mortality in Chronic Kidney Disease Ma Clarisse M. Toledo, George Thomas, Jesse D. Schold, Susana Arrigain, James F. Simon, Joseph V. Nally, Sankar D. Navaneethan. *Cleveland Clinic Foundation, OH.*

Background: Renal resistive index (RRI) measured by Doppler ultrasonography associates with worse outcomes in hypertensive, diabetic and elderly patients. However, its role in predicting mortality in CKD is unknown. We studied the factors associated with high RRI and whether high RRI is associated with increased mortality in CKD patients without renal artery stenosis.

Methods: We included 2,421 patients with eGFR 15-59.9 ml/min/1.73 m^2 and RRI measured using renal Doppler ultrasonography (performed in the same department/ institution) between January 2005 to October 2011 from an existing CKD registry. Participants with renal artery stenosis >60% were excluded. High RRI was defined as RRI >0.73 (median value of the study population). Multivariable logistic regression model was used to study factors associated with high RRI and its associations with mortality was studied using Kaplan-Meier plots and Cox Proportional Hazards model.

Results: Patients with and without higher RRI differed on various factors. In the multivariable logistic regression, older age, female gender, diabetes mellitus, and use of ACEI/ARB (p<0.05) were associated with higher odds of having a high RRI. During a median follow-up of 2.3 years, 570 patients died. After adjusting for age, gender, race, hypertension, diabetes, smoking, BMI, use of ACEI/ARB and statins, RRI >0.73 was associated with increased mortality (adjusted HR 1.30, 95% CI, 1.09-1.54). Results were similar when we compared RRI >0.70 to those with RRI <0.70.



Conclusions: Higher RRI is associated with increased mortality in CKD patients without renal artery stenosis after accounting for other significant risk factors. Its evaluation may allow early identification of those who are at risk thereby potentially preventing or delaying adverse outcomes.

Funding: Pharmaceutical Company Support - Development of CCF CKD registry was supported by an unrestricted support from Amgen to the Department of Nephrology and Hypertension

SA-PO880

Kidney Function and Mortality Risk in Community Dwelling Individuals: Hierarchical Importance of Threshold Values Donal J. Sexton, Scott Reule, Robert N. Foley. *Div of Renal Diseases and Hypertension, Dept of Medicine, Univ of Minnesota, Minneapolis, MN.*

Background: We attempted to identify threshold values of measures of kidney function and other classic risk factors to maximally discriminate all-cause mortality in community dwelling individuals.

Methods: We retrospectively identified eGFR (CKD-EPI equation), and urinary albumin-creatinine ratio (ACR) thresholds to maximize sensitivity and specificity (Max Sn/Sp) for death in NHANES III participants from 1988 to 1994 with mortality follow up through 2006. Classification tree methodology was used to rank eGFR and ACR thresholds in addition to; age, sex, race, smoking status, blood pressure (BP), serum LDL and HDL cholesterol, body mass index, waist-hip ratio (WHR), high sensitivity C-reactive protein, hypertension, diabetes, and cardiovascular disease.

Results: N = 6285 subjects were identified for inclusion, with mean (SD) age 47.4 (19) yrs., eGFR 99.9 (24) ml/min/1.73m^2 and median (25-75th centile) ACR 6.1 (3.6-11.9) mg/g. 10.5% were African American, 4% were Hispanic, and 53.1% were female. Mean follow up was 13.4 years. The Max Sn/Sp of eGFR (0.71/0.70 for 93 ml/min/1.73m^2) and ACR (0.62/0.65 for 7 mg/L) thresholds exceeded those of many classic factors. Sn/Sp were (0.01/0.99) for eGFR ≤ 30, (0.2/0.99) for ≤ 60 ml/min/1.73m^2 and (0.2/0.95) for ACR ≥ 30 mg/g. In a classification tree with exclusion of variables used at parent nodes in subsequent nodes; age 54 yrs. was initially selected, WHR and systolic BP appeared in the second round and systolic BP and ACR in the third.

Conclusions: Urinary ACR and eGFR may be at least as useful for mortality risk triage as most other classic risk factors in community dwelling adults.

SA-PO881

The Crude Annual Mortality Rate of Patients with End Stage Renal Disease Slightly Decreased and the Crude Incidence Rate of End Stage Renal Disease Continued to Increase in the Disaster Area in the following Year of the Great East Japan Earthquake 2011 Masaki Ohsawa. *Hygiene and Preventive Medicine, Iwate Medical Univ, Iwate Prefecture, Japan.*

Background: The Great East Japan Earthquake and Tsunami (March 11th 2011) caused significant damage to people in Iwate Prefecture (North-east area in Japan). Patients with end-stage renal disease (ESRD) are thought to be vulnerable for disasters and prolonged sedentary lifestyle during evacuation may contribute to worsening diabetic and hypertensive status of the refugees. We reported a preliminary study using dataset of 2010 and 2011 from people living in northern part of Iwate Prefecture last year. This year we will report results using data from all people in Iwate Prefecture from 2010 to 2012.

Methods: The Iwate ESRD registry program based on inventory survey was initiated in 2010 and this program has been continued to the present. We obtained data from the database of Iwate ESRD registry program from Iwate Medical Association. We counted total annual number of deaths in ESRD patients and total annual number of incident ESRD in Iwate Prefecture in 2010, 2011 (disaster year) and 2012.

Results: The results are shown in the table.

Date	2009/12/31	2010/1/1 ~2010/12/31	2010/12/31	2011/1/1 ~2011/12/31	2011/12/31	2012/1/1 ~/12/31	2012/12/31
total population of adults	1,094,712		1,091,233		1,077,559		1,077,437
No. of ESRD	2,399		2,645		2,852		3,010
point prevalence of ESRD (/million)	2,191		2,424		2,647		2,794
total number of deaths		316		444		411	
annual crude mortality rate (/1000 patient-years)		131.7		167.9		155.3	
total number of incident ESRD		337		386		418	
annual incidence rate of ESRD(/1000 person-years)		0.308		0.354		0.388	

Conclusions: The crude annual mortality rates in patients with ESRD increased in 2011 (disaster year) and somewhat decreased in 2012. The crude annual incidence rate of ESRD continued to increase in the following year of the disaster.

Funding: Government Support - Non-U.S.

SA-PO882

Middle-Aged Patients with Pediatric End-Stage Renal Disease Are Extremely at Risk for Squamous Cell Carcinoma

Sophie Ploos van Amstel,[1] Judith Leonoor Vogelzang,[1] Markus Starink,[3] Kitty J. Jager,[2] Jaap Willem Groothoff.[1] *[1]Pediatric Nephrology, Academic Medical Center, Amsterdam, Netherlands; [2]Dept of Medical informatics, Academic Medical Center, Amsterdam, Netherlands; [3]Dept of Dermatology, Academic Medical Center, Amsterdam, Netherlands.*

Background: End-stage renal disease (ESRD) is associated with an increased risk for malignancies, but little is known if this effect is enhanced if ESRD exists since childhood. We analysed the prevalence of cancer in patients with paediatric onset of ESRD after median 25 years of follow-up.

Methods: All Dutch patients, born <1979 who started Renal Replacement Therapy in 1972-1992 were followed up until 2010. Data on occurrence of malignancies were retrieved form 37 hospitals in 2000 and 2010 and compared with the national registry for malignancies. Incidence rate and incidence rate ratios were calculated.

Results: A total of 5709 patient years were recorded. No patient was los to follow up until 2000; thereafter 2 patients migrated. The median follow-up was 25.33 years per patient. For 72 patients (29%) follow up was more than 30 years. Median age at transplantation was 12.67 years. Time on transplant was more than three times as long as time on dialysis with a maximum of 39.30 years. Of all patients, 95 died at median age of 22.8 years of whom 20.5% from malignancies. We found 105 primary malignancies in 54 out of all 249 patients after a mean RRT time of 22.9 years (0.25 – 39.90). Patients with cancer developed on average 2.7 tumours, leading to a total of 300, including metastases. The cumulative incidence of having cancer in patients alive after 30 years was 42%. Squamous Cell carcinoma was by far most prevalent. PTLD was the second most common malignancy. The mean age of developing a malignancy was 36.89 (range 11.06 – 50.38, median 37.63) years. For all tumours, non-melanoma skin cancer (NMSC) and non cutaneous malignancies, IRRs were 16.5, 437.6 and 9.8, respectively, for patients aged 25-30 years, and, respectively 81.5, 2610 and 4.1 for patients aged 45-50 years.

Conclusions: Squamous cell carcinoma is extremely prevalent among patients with pediatric ESRD after 23 years of follow-up with a high rate of recurrence.

Funding: Private Foundation Support

SA-PO883

Aortic Stiffness and Kidney Disease in an Elderly Population

Katherine H. Michener,[1] Gary F. Mitchell,[2] Farzad Noubary,[1] Margret B. Andresdottir,[3] Runolfur Palsson,[3,4] Vilmundur Gudnason,[4,5] Andrew S. Levey.[1] *[1]Tufts Medical Center, Boston, MA; [2]Cardiovascular Engineering Inc., Norwood, MA; [3]Landspitali- The National Univ Hospital of Iceland, Reykjavik; [4]Univ of Iceland, Reykjavik; [5]Icelandic Heart Association, Kopavogur.*

Background: The causes of chronic kidney disease in older people are not well understood. Aortic stiffness increases with age and results in transmission of increased pulsatility into the kidney microvasculature, potentially causing damage and contributing to the pathophysiology of kidney disease in older populations.

Methods: We utilized data from the Age, Gene/Environment, Susceptibility-Reykjavik Study, a community-based prospective cohort study of cardiovascular disease (CVD) in Iceland. Associations of carotid pulse pressure (CPP) and carotid femoral pulse wave velocity (CFPWV) with estimated glomerular filtration rate based on creatinine and cystatin C (eGFR) and urine albumin-creatinine ratio (ACR) were assessed using linear regression, adjusting for demographics and CVD risk factors.

Results: We studied 940 participants (mean age 76 years, mean eGFR 68 ml/min/1.73m^2, median ACR 3 mg/g). In participants with CPP greater than 85 mmHg (N=197), higher CPP was associated with lower eGFR in unadjusted analyses but not after adjustment. CPP was significantly associated with higher ACR in fully adjusted models. Higher CFPWV was associated with lower eGFR and higher ACR in unadjusted analyses but not after adjustment.

Relationship of aortic stiffness and kidney disease measures

	CPP (SD)		CFPWV* (SD)	
	eGFR (ml/min/1.73 m^2)	ACR* (mg/g)	eGFR (ml/min/1.73 m^2)	ACR* (mg/g)
Unadjusted	**-4.54 (-8.30, -0.78)**	**0.16 (0.08, 0.25)**	**-1.87 (-2.89, -0.85)**	**0.21 (0.12, 0.29)**
Adjusted	-2.72 (-6.23, 0.79)	**0.14 (0.03, 0.24)**	-0.29 (-1.46, 0.87)	0.01 (-0.09, 0.11)

Results are β (95% CI).
CPP and eGFR used a 2-slope model due to nonlinearity. Results shown for CPP > 85 mm Hg.
Adjusted models include sex, age, MAP, HDL, HbA1c, CRP, height and heart rate.
*CFPWV modeled as the negative inverse, ACR modeled as the natural log.
Bold results are statistically significant.

Conclusions: Greater aortic stiffness may be associated with higher levels of albuminuria in the elderly. The association with lower eGFR may be confounded by age and CVD risk factors.

Funding: NIDDK Support, Other NIH Support - National Institutes of Health (R01 DK082447), contract from the National Institute on Aging(N01-AG-1-2100), Government Support - Non-U.S.

SA-PO884

No Association between Central Parameters of Arterial Stiffness and Early Chronic Kidney Disease in the CARTaGENE Cohort

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Background: Advanced CKD, through various potential mechanisms, has been linked to vascular stiffness. However, recent findings in patients with early CKD have cast doubt over the association between CKD and vascular stiffness. We examined the association between parameters of vascular stiffness and early CKD.

Methods: We used data from the CARTaGENE survey, a randomly selected cohort from the general population, aged 40 to 69 years. Pulse wave analysis was performed by tonometry on radial artery and central pulse wave profile was obtained by generalized transfer function(Sphymocor SCOR-Px) on 17 464 subjects. Central pulse pressure (cPP), PP amplification (PPamp), augmentation index (Aix), augmented pressure (AP), timing of reflected wave (Tr) and forward pressure (Pf) were assessed. CKD was defined by an eGFR <60ml/min/1.73m^2(CKD-EPI equation).

Results: After application of stringent criteria to insure the validity of measurements, 6741 subjects were included for analysis. Mean age was 55±8 years, 44% of participants were women, 14% active smokers, 24% were treated for hypertension, 10% had diabetes, and 6% had known cardiovascular disease. CKD stage 3 to 5 was present in 275 patients(4%). **Table 1** shows the association between parameters of arterial stiffness and CKD.

	Univariable		Multivariable*	
Parameter	*ß coefficients*	*P*	*ß coefficients*	*P*
cPP(mmHg)	0.060	<0.001	0.004	0.646
PPamp	-0.017	0.154	0.006	0.434
Aix(%)	0.018	0.130	-0.004	0.665
AP(mmHg)	0.034	0.006	-0.008	0.332
Pf(mmHg)	0.067	<0.001	0.014	0.171
Tr(ms)	-0.048	<0.001	-0.014	0.185

*variables included: age, gender, BMI, smoking status, diabetes, total cholesterol, statin treatment, anti-hypertensive medication, cardiovascular disease, heart rate, and central mean pressure.

Conclusions: In this large cohort from the general population, prevalent early CKD was not independently associated with arterial stiffness. The impact of kidney function on arterial stiffness may only be manifest at advanced stages of kidney disease.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO885

Compliance Index, a Marker of Peripheral Arterial Stiffness, Predicts Decline in Renal Function in Patients with Chronic Kidney Disease

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Background: Compliance index derived from digital volume pulse (CI-DVP), measuring the relationship between volume and pressure changes in fingertip, is a surrogate marker of peripheral arterial stiffness. The aim of this study was to investigate if CI-DVP can predict the risk of renal function progression, cardiovascular events and mortality in patients with chronic kidney disease (CKD).

Methods: In this prospective observational study, 149 CKD patients were included for final analysis. CI-DVP and brachial-ankle pulse wave velocity (baPWV) were measured using photoplethysmography and ankle brachial index-form device, respectively. The eGFR was determined according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations. Decline in renal function was assessed by the estimated glomerular filtration rate (eGFR) slope.

Results: The baseline serum creatinine and eGFR were 1.5 ± 1.1 mg/dL and 60 ± 28 mL/min/1.73m^2, respectively. The mean decline rate of eGFR and duration of follow up were 2.63 ± 2.50 mL/min/1.73m^2/year and 51 ± 12 months, respectively. Patients in CKD stage 3b to 5 had a higher baPWV and a lower CI-DVP value than those in patients with CKD stage 1 to 3a. The decline rate of eGFR was higher in the advanced CKD group than that in the early CKD group (3.24 ± 2.16 versus 2.28 ± 2.64 mL/min/1.73m^2/year, P =0.0275). Multivariate linear regression analysis showed that lower CI-DVP (P =0.0033) and greater proteinuria (P =0.0006) were independent determinants of higher eGFR decline rate. If CI-DVP was replaced by baPWV in multivariate regression analysis, higher baPWV was also a significantly independent determinant of higher eGFR decline rate (P =0.0112).

Conclusions: The present study demonstrated that compliance index (CI-DVP), a marker of peripheral arterial stiffness, is significantly associated with renal function decline in patients with CKD. A higher CI-DVP predicts a slower eGFR decline.

SA-PO886

Apparent Treatment-Resistant Hypertension Related to Chronic Kidney Disease in the Elderly Jean Kabore,[1,2] Marie Metzger,[1,2] Catherine Helmer,[3] Ziad Massy,[4,5] Benedicte Stengel.[1,2] *[1]Inserm U1018, CESP, Villejuif, France; [2]Univ Paris Sud 11, Paris, France; [3]Inserm U897, ISPED, Bordeaux, France; [4]Ambroise Paré Univ Hospital, Boulogne-Billancourt, France; [5]Paris-Ouest Univ-UVSQ, Paris, France.*

Background: The impact of chronic kidney disease in the occurence of resistant hypertension in the ederly has been poorly investigated. We assessed the prevalence of apparent treatment-resistant hypertension (aTRH) defined as BP ≥ 140/90 mmHg despite use of 3 or more antihypertensive drug classes or use of 4 or more regardless of BP level, and its relation with kidney function level, kidney function decline over time, and albuminuria.

Methods: This study includes 4265 participants over 65 years treated for hypertension (HT) from a population-based cohort. At baseline, adjusted odds ratios (OR) for aTRH and for uncontrolled non-TRH (ucnTRH) associated with estimated glomerular filtration rate (eGFR) level were estimated by multinomial logistic regression using controlled HT (cHT) as reference. ORs associated with eGFR decline and albuminuria (ACR ≥3 mg/mmol or PCR ≥30 mg/mmol) were studied in a subsample with 4-year follow-up.

Results: Baseline mean age was 74 ± 5 years, 40% of the participants were men, 10% had diabetes, and 13% were obese. Mean eGFR was 76 ± 16 ml/min/1.73m². Prevalences of aTRH, ucnrHT and cHT were 6.4%, 62.3 % and 31.2 %, respectively. Each 5 mL/min/1.73 m² decrease in baseline eGFR was associated with a significant increase in the OR of aTRH: 2.04 [1.51 to 2.75] independent of age, gender, obesity and diabetes, but not for ucnTRH. The mean decline in eGFR was -1.5 ± 2.9 ml/min/1.73m²/year. In the subsample, each 2 ml/min/1.73m² per year decrease in eGFR was associated with a significant increase in the adjusted OR for aTRH (1.23[1.08 to 1.41]), but not for ucnTRH. In addition, aTRH was associated with albuminuria (2.58[1.23 to 5.38]) independent of eGFR decline, diabetes, age, gender, mean eGFR and BMI.

Conclusions: This study shows that aTRH affects one elderly out of 15 treated for hypertension. Low eGFR level, rapid eGFR decline and albuminuria are strongly related to aTRH in this population, regardless of other known risk factors for resistant HT.

Funding: Pharmaceutical Company Support - Sanofi-Aventis, Government Support - Non-U.S.

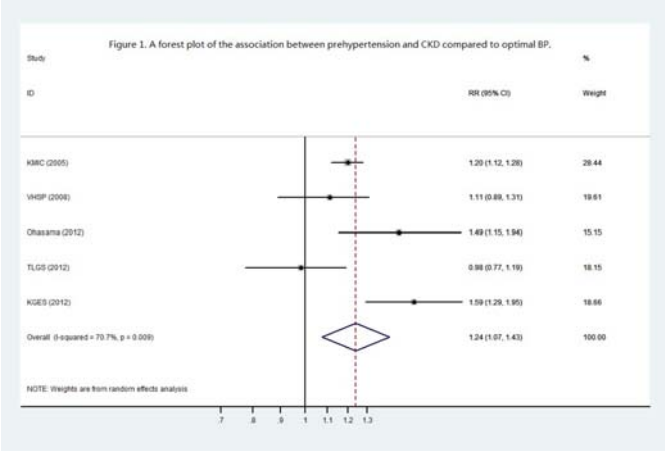
SA-PO887

Prehypertension Predicts Chronic Kidney Disease: A Meta-Analysis Yang Li, Jing Wang, Limeng Chen. *Dept of Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: Recent studies have shown the association of prehypertension with increased risk of end stage renal disease, however there are conflicting evidences of the relationship between prehypertension and chronic kidney disease(CKD). This meta-analysis aims to find the association between prehypertension and the incidence of CKD, and whether gender or ethnical differences impact the connection.

Methods: MEDLINE, EMBASE, Cochrane Library (through May 2014) and article reference lists were searched for relevant studies about blood pressure and CKD. Prehypertension was defined with BPs ranging from 120-139/80-89mmHg. CKD was defined by eGFR of lower than 60 ml/min/1.73m² or proteinuria. Two reviewers extracted the data independently, and assessed the quality of studies included in meta-analysis using the Newcastle-Ottawa Scale (NOS). We performed the meta-analysis using Stata/SE 12.0(StataCorp LP). Random-effect models were chosen for heterogeneous analyses.

Results: Retrieving from 3,216 potentially relevant articles, we identified 5 cohort studies for 189,724 subjects by predefined selection criteria. Three were from Asia, and the other two were from Europe and Iranian, with the average age from 42.2 to 52.1 years, and the proportion of female from 46.4% to 52.0%. The median follow-up period was 2 to 10 years. Compared to optimal BP, prehypertension showed an increasing risk of CKD (pooled RR 1.24, 95% CI 1.07,1.43; P=0.003; I^2=70.7%). In subgroup analysis, we found the same trend only in men (pooled RR 1.17, 95% CI 1.08,1.28; P<0.01; I^2=0.0%), but not in women. And the effect was only seen in Asia (pooled RR 1.39, 95% CI 1.13,1.70; P=0.002; I^2=76.3%) rather than non-Asia people.



Conclusions: Prehypertension can be considered as one of the potential causes of CKD. There are gender and ethnical differences in this association.

Funding: Government Support - Non-U.S.

SA-PO888

Elevated Systolic Blood Pressure Is Associated with Increased Incidence of Chronic Kidney Disease but Not Mortality James W. Lohr,[1,2] Mojgan Golzy,[3] Randy L. Carter,[3] Pradeep Arora.[1,2] *[1]Nephrology, Buffalo VA, Buffalo, NY; [2]Medicine, SUNY at Buffalo, Buffalo, NY; [3]Biostatistics, SUNY at Buffalo, Buffalo, NY.*

Background: The optimal blood pressure to prevent development of chronic kidney disease (CKD)and mortality in the elderly is unclear. the objective of this study was to determine the effect of differing levels of blood pressure on incidence of CKD and mortality in elderly veterans.

Methods: This is a retrospective cohort study of 16,221 individuals > 70 years of age without CKD (eGFR> 60 ml/min) seen in primary care clinic in VISN 2 between 2001 and 2008. Cox proportional hazard model with time dependent covariates was used to examine the association of explanatory variables on hazard ratios for each outcome of interest: incident CKD and death. Time independent variables included observations of sex, race, age, BMI, and occurrence of COPD, cancer, diabetes, and vascular disease. Time dependent covariates included HDL, LDL, TRIG, HGB, SBP,and DBP. The PHREG procedure in SAS with counting process style of input, was used for the analysis of each model.

Results: Overall, 16.0% of individuals with baseline eGFR > 60 ml/min/1.73m2 developed CKD during follow-up. Compared to reference of systolic BP of 130-140 systolic, there was an increased hazard of development of CKD with systolic blood pressure of 140-150 or higher. The following factors were associated with an increased hazard of developing CKD: higher serum triglycerides, age, female gender, use of antihypertensive medications, cancer, COPD, vascular disease, and presence of diabetes. Higher Hb was associated with a decreased hazard of incident CKD. Overall, a total of 1133 (7%) patients died during the mean follow up time of 1185 days This produced a mortality rate of 57 per 1000 patients per year. As compared to a reference range of 130-140 mmHg systolic blood pressure, the relative risk of mortality was higher in the range of 120-130 mmHg systolic BP or less.

Conclusions: The optimal systolic blood pressure in elderly patients to prevent the development of CKD is < 140 mmHg. However, lowering the systolic blood pressure below 130 mmHg is associated with increased mortality.

SA-PO889

Stature Is Related to Renal Impairment and Higher Pulse Pressure Only in Women Ivana Vukovic-Lela,[1] Vanja Ivkovic,[1] Zivka Dika,[1] Sandra Karanovic,[1] Joelle L. Nortier,[2] Jelena Kos,[1] Mario Laganovic,[1] Tomislav Teskera,[3] Ana Vrdoljak,[1] Bojan Jelakovic.[1] *[1]Dept of Nephrology, Hypertension, Dialysis and Transplantation, UHC Zagreb, Croatia; [2]Dept of Nephrology, Erasme Hospital, Belgium; [3]Dept of Nephrology, General Hospital Slavonski Brod, Croatia.*

Background: It was reported that short adult stature might be a risk factor for premature cardiovascular diseses.However,data on association of height and kidney function are sparse. Our aim was to analyze this association in general population with normal kidney function.

Methods: In this cross-sectional survey conducted in general population from continental area out of original cohort of 2412 subjects, 644 subjects (324 W;320 M) (age 40, 35-43) with eGFR MDRD>60ml/min/1.73m2 and ACR<30mg/g were enrolled.Blood pressure (BP) was measured according to the ESH/ESC guidelines.Men and women were separately divided into height quartiles (Q1 the lowest and Q4 the highest height).

Results: There were no differences in age and BMI between height Qs in both gender. Women in the tallest height Qs were more obese (p<0.001),had lower eGFR(79±10 versus 85±12, p=0.005) and lower pulse pressure (PP) (42±12 versus 48±13,p=0.032) compared to those in shortest height Q.There were no differences in BP,ACR and alpha1CR between height Qs.On univariate regression height was associated with eGFR (B=-0.34, SE=0.10;p=0.001) and ACR (B=0.13,SE=0.05;p=0.008).The association remained significant after adjustment for age,PP,BMI, ACR and alpha1CR for eGFR (B=-0.61,SE=0.13;p<0.001) and ACR (B=0.17,SE=0.06;p=0.008).There were no differences in eGFR,ACR and BP values between height Qs in men.On univariate regression we failed to find association of height with eGFR and ACR in men.

Conclusions: In the group of subjects without CKD,short stature is related to lower eGFR,higher ACR and higher pulse pressure only in women.Additionally,PP adjusted adult stature was related to renal impairment only in women.Further investigations are needed to confirm our findings and analyze whether higher PP could be related to the early renal impairment in subjects with taller stature.

Funding: Government Support - Non-U.S.

SA-PO890

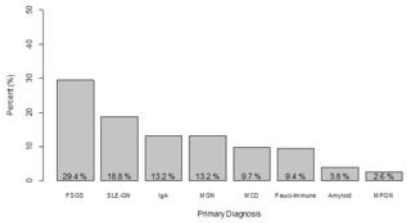
Distribution of Glomerulonephritis among an Ethnically Diverse United States Population John J. Sim,[1] Michael Batech,[1] Aviv Hever,[1] Teresa N. Harrison,[1] Sejal Vora,[2] Leslie Vollenweider,[2] Anna Pavlova-Wolf,[2] Kamyar Kalantar-Zadeh.[3] *[1]Nephrology and HTN, Kaiser Permanente LAMC, Los Angeles, CA; [2]Questcor Pharm; [3]UCI Med Ctr.*

Background: The incidence and prevalence of glomerulonephritis (GN) has varied across race/ethnicities and countries worldwide. This underscores the role of both innate biology and environment to occurrences of GN and its different types. We sought to describe the distribution of the most common non-diabetic GN's identified from a large ethnically diverse contemporary population.

Methods: This was a cross-sectional study conducted within Kaiser Permanente Southern California, an integrated health delivery system with 3.7 million members across 14 medical centers, in the period 1/1/2006-12/31/2009 using native renal biopsies results. Chart reviews of renal biopsy pathology reports were performed and the GNs were categorized based on the primary diagnosis on the renal pathology report. Data on age, sex, race/ethnicity, socioeconomics, comorbidities, and laboratories were extracted from administrative databases.

Results: In total, 879 cases of the most common GN's were identified. The mean age was 46 years and nearly half (47%) were females. The most common GNs were FSGS (29.4%), SLE (18.8%), IgAN (13.2%), membranous (13.2%) minimal change disease (9.7%), pauci-immune (9.4%), amyloid (3.8%), and membranoproliferative (2.6%). Among primary etiologies, IgAN was the second most common primary GN in Hispanics and Asians, while membranous GN was the second most common primary GN in whites and blacks.

	White	Black	Hispanic	Asian	Missing/unknown	Total
FSGS	58	70	90	37	31	258
SLE	25	40	71	25	34	165
IgAN	30	0	45	35	20	116
Membranous	41	30	29	13	10	116
Minimal Change	28	14	37	4	6	85
Pauci-immune	34	16	27	5	6	83
Amyloidosis	14	2	12	4	1	33
Membranoproliferative	9	3	10	0	5	23
Total	239	175	321	123	113	879



Conclusions: Among a diverse population, we observed FSGS as the most common primary GN across all races/ethnicities and ages. The distribution of GNs demonstrated that Hispanics and Asians had similar patterns of GN while whites and blacks were similar. Our findings suggest that GN's may occur differently within U.S. race groups compared to similar races in other countries.

Funding: Pharmaceutical Company Support - Questcor Pharmaceuticals

SA-PO891

Effect of Statins on Survival in Patients with Vascular Access for End Stage Renal Disease Paola De Rango, Basso Parente, Beatrice Fiorucci, Luca Farchioni, Enrico Cieri. *Vascular and Endovascular Surgery Unit, Hospital S.M. Misericordia; Univ of Perugia, Perugia, Italy.*

Background: The benefit of statins therapy in patients with advanced chronic kidney disease remains unsettled. Many patients with end stage renal disease (ESRD) are not under statins. This study aimed to investigate the impact of statins use on survival of patients with vascular access performed at a single vascular surgery center.

Methods: Data from consecutive patients with ESRD admitted for vascular access surgery from 2006 to 2013 were reviewed. Data were collected prospectively. Information on therapy was retrieved and patients under statins therapy were compared to those without. Primary end-point was 5-year survival. Cox regression analysis with backward stepwise selection of covariates (age, gender, hypertension, cardiac disease, chronic obstructive pulmonary disease, obesity, diabetes,statins) was used to analyze independent predictors of mortality.

Results: Three hundred fifty-nine patients (230 males;mean age 69.2y) receiving 565 vascular accesses during the study period with available information on statins use were analyzed. One hundred twenty seven patients (35.4%) were on statins. Use of statins was more frequent in patients with hypertension (P=.034), hyperlipidemia (P<.0001), coronary disease (P=.43) diabetes (P=.001) and obesity (P<.001). Kaplan Meier survival rates at 3 and 5 years were 77.0% and 65.1% for patients without statins, and 84.4% and 75.9% for those under statins (P=.17). Cox regression analysis selected age as independent positive predictor (Odds Ratio,OR,1.04;95% Confidence Interval, CI, 1.02-1.05;P<.0001) and statins therapy as independent negative predictor (OR 0.57; 95%CI 0.33-0.98;P=.043) of mortality. There was a trend for higher primary patency rate at 3 years of the vascular access procedures performed in patients under statins but without statistical relevance (patency: 36% in patients without statins and 50% for those under statins:P=.45).

Conclusions: Statins therapy may reduce the risk of mortality in patients with ESRD including those receiving dialysis. Use of statins may be implemented in patients with vascular access.

SA-PO892

BK Virus as a Significant Predictor of Chronic Kidney Disease and Overall Survival in Hematopoietic Stem Cell Recipients Ala Abudayyeh,[1] Amir Hamdi,[1] Maen Abdelrahim,[1] Aimaz Afrough,[2] Yan Heather Lin,[1] Jeffrey J. Tarrand,[2] Elizabeth J. Shpall,[2] Katy Rezvani.[2] *[1]Nephrology, The Univ of Texas MD Anderson Cancer Center, Houston; [2]Stem cell Transplantion, The Univ of Texas MD Anderson Cancer Center.*

Background: BK virus nephropathy is an evolving challenge in hematopoietic stem cell transplantation (HSCT) recipients. In contrast to kidney transplantation, where there are screening protocols for early detection and prevention of BK virus, there is no such guideline in HSCT. The aim of this study was to evaluate the role of BKV in renal function and survival of HSCT survivor population.

Methods: We analyzed all patients undergoing first allogeneic HSCT at MD Anderson Cancer Center between January 2004 and December 2012. We evaluated the renal outcome and survival of these patients as well as factors that contribute to them. BKV positivity was defined as BKV detection in urine by PCR testing. CKD was defined by constant decrease of 25% or more in GFR compared to the baseline at the time of transplant.

Results: We identified a total of 2477 patients with BK viruria in 25% (n=629). The median time from transplantation to BK viruria development was 42 days. Conditioning regimen consisted of a myeloablative regimen 19%,reduce intensity regimen 67%, and non-myeloablative regimen 14%. The median follow-up time for CKD was 690 days after transplant. A total of 1086 patients developed CKD (43.8%). In multivariate analysis, with the adjustment of age, gender, acute GVHD, chronic GVHD, preparative conditioning regimen, graft source, tacrolimus serum level, BKV showed significant association with CKD (HR)= 1.599 (1.386, 1.845), *P*-Value <0 .0001, and a significant association with overall survival (HR= 1.293 (1.134, 1.475), *P*-Value <0 .0001.

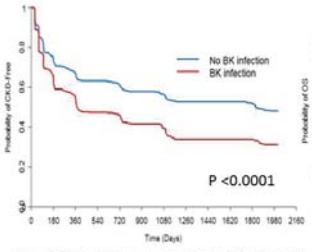


Figure 1a: Kaplan-Meier curve progression to CKD related to BK infection

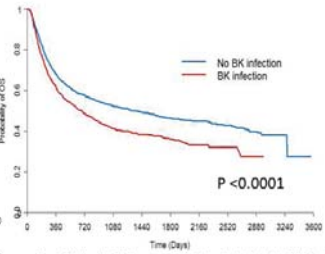


Figure1b: Kaplan-Meier curve overall survival related to BK infection

Conclusions: To our best of knowledge, this is the first study to identify BKV as a strong independent predictor of CKD and survival after HSCT.

Funding: Private Foundation Support

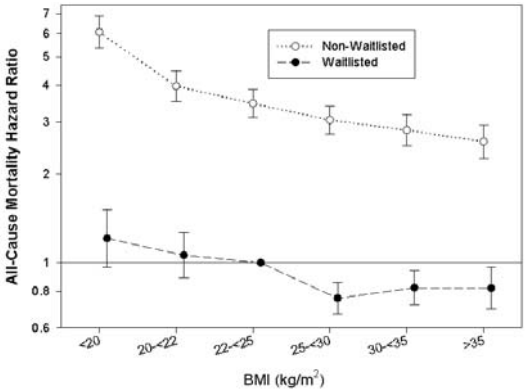
SA-PO893

Comparing the Associations of Body Mass Index with Survival in Kidney Transplant Waitlisted and Non-Waitlisted Hemodialysis Patients Rochelle Rogers,[1] Elani Streja,[1] Connie Rhee,[1] Csaba P. Kovesdy,[2] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Memphis VA MC, Memphis, TN.*

Background: Maintenance hemodialysis (MHD) patients who are waitlisted for kidney transplantation are often asked to lose weight if their body mass index (BMI) is in the obesity range despite the well-known obesity paradox in these patients. Few studies have compared the BMI-death association in non-waitlisted versus waitlisted MHD patients.

Methods: Using a large national dialysis database, we created a 1:2 propensity-matched cohort of 8,474 waitlisted and 11,989 non-waitlisted MHD patients who underwent treatment from 2001-2009. Associations between all-cause mortality and 6 groups of BMI were analyzed using Cox models in the total cohort and separately in non-waitlisted and waitlisted patients.

Results: Patients were 54±15 yrs old, 39% female, 53% diabetic, and 29% African American. In case-mix adjusted models, compared to the reference group (BMI of 22-25kg/m^2 waitlisted patients), both waitlisted and non-waitlisted patients showed improved survival with BMI>25kg/m^2. In each BMI increment, waitlisted patients had 4-6 times lower risk of death than non-waitlisted matched counterparts.



Conclusions: Both waitlisted and non-waitlisted HD patients exhibit evidence of an obesity paradox. Non-waitlisted patients showed an incrementally lower death risk with higher BMI, but had a 4 to 6 times higher death risk compared to waitlisted matched patients in the same BMI category. Studies are warranted to examine the need to change the current BMI recommendations for waitlisted patients.

Funding: NIDDK Support

SA-PO894

Outcomes of Prepared versus Non-Prepared of Dialysis in Chronic Kidney Disease Patients Yi-Chih Lin, Wen-Chih Chiang, Chih-Kang Chiang. *Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan.*

Background: The creation of fistulas or grafts before starting dialysis is recommended, but whether it reduces survival and clinical outcomes still unknown. In clinical practice, there are may variations to decide when to start dialysis in stage 5 chronic kidney disease. Early preparation and then early dialysis were favored in the world without definite evidence, especially in Asia. In this study, we observed whether prepared dialysis has better survival rate and clinical outcoms among 440 patients with advanced chronic kidney disease in one medical centers in Taiwan.

Methods: We observed retrospectively 440 patients 18 years of age or order with advanced chronic kidney disease and divided these patients in two groups, prepared dialysis access and non-prepared before chronic dialysis starts.

A non-prepared patient occurred if the patient had at least one outpatient consultation with nephrology prior to dialysis start, and if the patient initiated dialysis using a double lumen catheter. The primary outcome was all cause mortality.

Results: We observed retrospectively a total of 440 adults and excluded 60 patients due to unknown preparation of dialyis and loss follow up(mean age 60.6 years old, 209 men and 172 women, 164 diabetes) between April 2007 and March 2011 via electronic medical records in National Taiwan University Hospital (NTUH). Almost randomized characteristics in these two group except eGFR while dialysis, DM patient number, HbA1c and LDL levels. Up to Dec 2013, 33 of 202 patients in prepared group and 24 of 178 patients in non-prepared group died. There was no significant difference between the groups in survival rate.

Conclusions: In this study, patients in advanced CKD stage with prepared dialysis access were not associated better survival or clinical outcomes.

Funding: Other NIH Support - National Taiwan University hospital

SA-PO895

Effects of Educational Intervention on Renal Outcome of Early- to Moderate-Stage Chronic Kidney Disease: A Cluster-Randomized Trial Kunihiro Yamagata,[1,2] Hirofumi Makino,[2] Kunitoshi Iseki,[2] Sadayoshi Ito,[2] Kenjiro Kimura,[2] Eiji Kusano,[2] Kimio Tomita,[2] Ichiei Narita,[2] Tomoya Nishino,[2] Yoshihide Fujigaki,[2] Tsuyoshi Watanabe,[2] Takashi Wada,[2] Seiichi Matsuo.[2] *[1]Dept of Nephrology, Univ of Tsukuba, Tsukuba, Ibaraki, Japan; [2]On Behalf of Study Group for Frontier of Renal Outcome Modifications in Japan, FROM-J, Japan.*

Background: This study was designed to prove the effect of multidisciplinary treatment on the outcome of early- to moderate-stage CKD.

Methods: In this stratified open cluster-randomized study, we recruited 49 local medical associations (clusters); 26 clusters were randomly assigned to intervention group A (standard intervention) and 23 clusters were assigned to intervention group B (advanced intervention). The patients in groups A and B consisted of 1195 and 1184 patients. All patients were managed in accordance with the current CKD guidelines; in addition, group B patients received educational intervention every 3 months. The primary outcome measures were 1) the rate of continued consultation, 2) the collaboration rate between general practitioners and nephrologists, and 3) the progression of CKD.

Results: The rate of continued consultation was significantly higher in group B (16.2% of group A and 11.5% of group B ceased regular visits for 6 months, p=0.0121). Significantly higher referral and co-treatment rates were observed in group B (p<0.0001). Average speed of eGFR deterioration tended to be lower in group B (group A: 2.6+5.8 ml/min/1.73 m^2, group B: 2.4+5.1 ml/min/1.73 m^2, p=0.068). A significant difference in eGFR deterioration speed was observed in subjects with CKD stage 3 (group A: 2.4+5.9 ml/min/1.73 m^2, group B: 1.9+4.4 ml/min/1.73 m^2, p=0.028), while eGFR deterioration speeds were identical in other CKD stage subjects.

Conclusions: The educational intervention could achieve significantly higher rates of continued consultation referral and co-treatment. The progression of CKD stages was retarded by this multidisciplinary treatment in CKD stage 3 patients.

Funding: Government Support - Non-U.S.

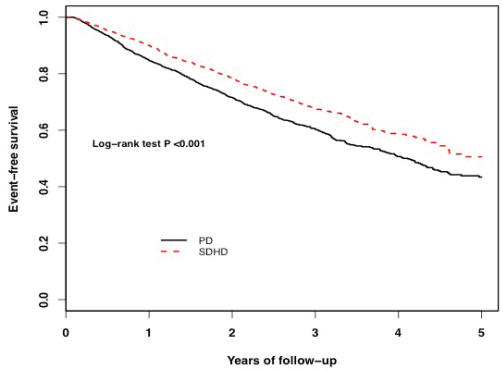
SA-PO896

Comparative Effectiveness of Home Dialysis Therapies Gihad E. Nesrallah,[1,2] Lihua Li,[2] Rita Suri.[2,3] *[1]Nephrology Program, Humber River Hospital, Toronto, ON, Canada; [2]Dept of Medicine, Western Univ, London, ON, Canada; [3]Dept of Nephrology, Centre de Recherche, Centre Hospitalier de l'Univ de Montréal, Montreal, QC, Canada.*

Background: Increasingly, patients must choose between daily home hemodialysis (DHD) and peritoneal dialysis (PD), yet the comparative effectiveness of these therapies is unknown.

Methods: We linked a large U.S. dialysis organization (LDO) database to the USRDS for the years 2004 through 2011. We identified 3359 consecutive adult patients initiating DHD (≥5 days/week for ≥3 hours/day) through the LDO's home dialysis programs and matched them by propensity score to contemporaneous USRDS patients receiving home PD, yielding 2668 matched pairs. Comorbidities, demographics, and outcomes for both exposure groups were ascertained in the USRDS. We used Cox regression including the robust covariance estimator to compare all-cause mortality between groups. All patients were followed to October 31, 2012 or death; transplants were censored.

Results: After matching, between-group standardized differences for all baseline variables were <10%. Mean age was 51 years, 66% were male, 72% were white, and 29% had diabetes. During 10221 patient-years, 1493/5336 patients died. Compared to PD, DHD was associated with a significantly lower risk of death (PD versus DHD: 16.7 versus 12.7 deaths per 100 patient-years; HR 0.75 (95% CI 0.68– 0.82); p<0.001). During follow-up, 20% of DHD and 59% of PD patients switched to another therapy. Censoring modality switches at 90 days yielded HR 0.83 (95% CI 0.74–0.95; p=0.005) favoring DHD. Similar results were noted in multiple subgroups and with the use of several different analytic methods.



Conclusions: In this observational study, we found that among adult patients with end-stage renal disease, there was a significant long-term survival advantage among patients who underwent DHD as compared with patients who received PD.

SA-PO897

Lower Risk of Hospitalization in Daily Home Hemodialysis versus Peritoneal Dialysis Patients Eric D. Weinhandl,[1] Kimberly M. Nieman,[1] Allan J. Collins.[1,2] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]School of Medicine, Univ of Minnesota, Minneapolis, MN.*

Background: Frequent hemodialysis and peritoneal dialysis (PD) each offer unique benefits and risks pertaining to cardiovascular morbidity and infection. We aimed to compare the risk of hospitalization in U.S. patients initiating daily home hemodialysis (DHHD) or PD.

Methods: We identified new DHHD patients, Jan 1, 2007-Jun 30, 2010, from a registry of NxStage System One users and linked them to United States Renal Data System (USRDS) records; we identified new PD patients from USRDS records. We retained the subset of these patients with Medicare coverage. For each DHHD patient, we selected 1 matched PD patient according to the date of home dialysis initiation and a 33-factor propensity score of DHHD initiation. We followed patients from home dialysis initiation to the earlier of death or Dec 31, 2010. Admissions were ascertained from Medicare claims and causes of admission from principal diagnoses.

Results: We identified 3560 DHHD and 3560 matched PD patients. All-cause hospitalization rates per patient-year for DHHD versus PD were 1.71 versus 1.96 admissions and 10.2 versus 12.2 days. From Prentice-Williams-Peterson regression, the all-cause admission hazard ratio (HR) for DHHD versus PD was 0.92 (95% CI, 0.89-095); the HR was 0.89 (0.85-0.94) for first admission and 0.93 (0.89-0.96) for all subsequent readmissions (after first discharge). For admissions related to cardiovascular disease and to infection, HRs were 0.84 (0.79-0.89) and 0.89 (0.85-0.94), respectively. For admissions due to heart failure and to hypertensive disease, HRs were 0.80 (0.71-0.91) and 0.77 (0.69-0.87),

respectively. For admissions due to sepsis and to access infection (i.e., vascular access infection or peritonitis), HRs were 1.25 (1.11-1.41) and 0.88 (0.79-0.98), respectively.

Conclusions: DHHD was associated with lower risk of hospitalization than PD in multiple dimensions, including first admission; subsequent readmissions; admissions due to cardiovascular disease, including heart failure; admissions due to infection, including access infection but excluding sepsis; and cumulative days.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

SA-PO898

Differences in Medication Use among Daily Home Hemodialysis, Peritoneal Dialysis, and In-Center Hemodialysis Patients

Eric D. Weinhandl,[1] David T. Gilbertson,[1] Allan J. Collins.[1,2] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]School of Medicine, Univ of Minnesota, Minneapolis, MN.*

Background: Anemia, hyperphosphatemia, and hypertension are common complications of end-stage renal disease. We aimed to compare use of medications for these indications in U.S. patients undergoing daily home hemodialysis (DHHD), peritoneal dialysis (PD), or in-center hemodialysis (IHD).

Methods: We identified new DHHD patients, Jan 1, 2007-Jun 30, 2010, from a registry of NxStage System One users and linked them to United States Renal Data System (USRDS) records; we identified new PD and prevalent IHD patients from USRDS records. We retained the subset of these patients with Medicare coverage. For each DHHD patient, we selected 1 matched PD and 5 matched IHD patients according to propensity scores of DHHD initiation. We followed patients to the earliest of dialytic modality change, kidney transplant, death, or Dec 31, 2010, and ascertained medication use from Medicare claims.

Results: We identified 3560 DHHD, 3560 matched PD, and 17,800 matched IHD patients. In DHHD, mean ESA dose increased, the percentage of patients using phosphate binders was stable, and the percentage of patients using antihypertensive agents decreased. Compared with DHHD, mean ESA dose was significantly lower in PD and IHD by month 12 of follow-up, and the percentage of patients using antihypertensive agents was significantly higher by month 1.

	DHHD	PD	IHD
ESA dose (IU per month)			
Month 1	48,500	39,900*	61,800*
Month 6	57,900	35,600*	57,800
Month 12	61,700	34,700*	57,600*
Month 24	63,200	40,700*	57,600*
Phosphate binder use (%)			
Month 1	51.9	49.0	50.0
Month 6	48.3	46.4	50.7
Month 12	47.7	42.4*	51.1*
Month 24	47.7	49.9	50.3
Antihypertensive agent use (%)			
Month 1	62.1	68.6*	66.0*
Month 6	53.4	63.5*	65.6*
Month 12	49.1	61.8*	65.3*
Month 24	48.5	63.7*	63.9*
**P < 0.05, compared with DHHD*			

Conclusions: DHHD initiation was followed by changes in ESA dose and antihypertensive agent use, but no change in phosphate binder use. Reasons for increased ESA use in DHHD are unclear; this merits further study. Lower antihypertensive agent use in DHHD likely reflects improved fluid control.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

SA-PO899

Lower Risk of Technique Failure in Daily Home Hemodialysis versus Peritoneal Dialysis Patients

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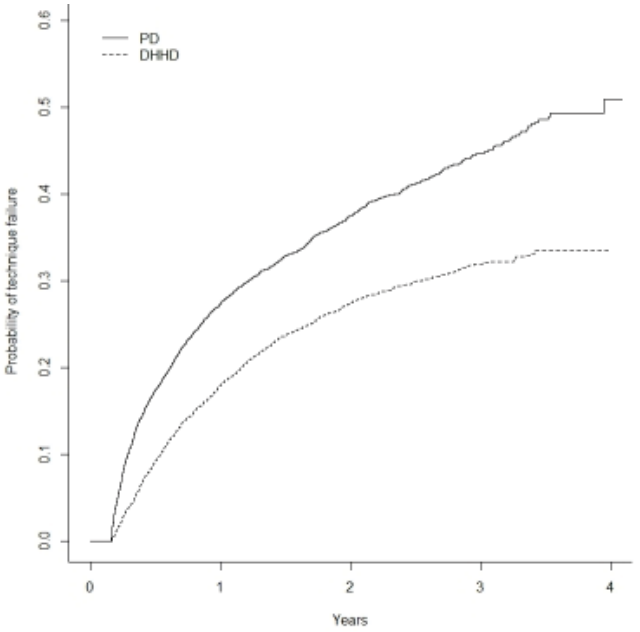
Background: Patient losses due to technique failure are an important impediment to the growth of home dialysis programs. We aimed to compare the risk of technique failure in U.S. patients initiating daily home hemodialysis (DHHD) or peritoneal dialysis (PD).

Methods: We identified new DHHD patients, Jan 1, 2007-Jun 30, 2010, from a registry of NxStage System One users and linked them to United States Renal Data System (USRDS) records. We identified new PD patients from USRDS records. For each DHHD patient, we selected 1 matched PD patient according to the date of home dialysis initiation and a 33-factor propensity score of DHHD initiation. We followed patients from home dialysis initiation to the earliest of technique failure, kidney transplant, death, or Dec 31, 2010; technique failure was declared at 2 months after the last home dialysis session.

Results: We identified 4460 DHHD and 4460 matched PD patients. Cumulative incidence of technique failure for DHHD versus PD was 9.1% versus 17.3% at 6 mo, 17.9% versus 27.3% at 1 yr, 27.3% versus 37.5% at 2 yr, and 31.9% versus 44.7% at 3 yr (figure). From Fine-Gray regression of cumulative incidence, the technique failure hazard ratio (HR) for DHHD versus PD was 0.62 (95% CI, 0.58-0.67). In patients (n = 1368 per group) initiating home dialysis within 6 mo following ESRD onset, the technique failure HR was 0.70 (0.60-0.82).



Conclusions: DHHD was associated with lower risk of technique failure than PD; this association was evident even in patients who initiated home dialysis within 6 mo following ESRD onset. Further study is needed to identify key differences in the distribution of causes of technique failure in DHHD and PD patients and whether relative hazards of technique failure vary by dialysis provider.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

SA-PO900

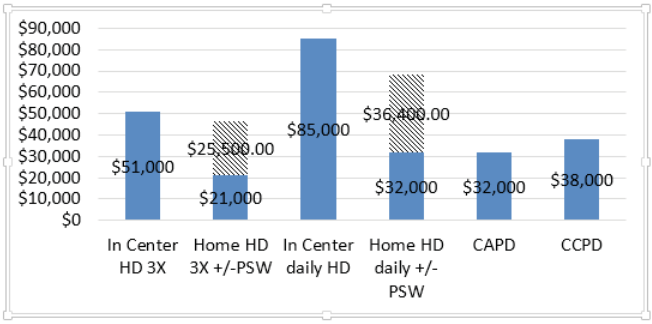
Use of Personal Support Workers for Home Hemodialysis – A Major Paradigm Shift

Andreas Pierratos,[1,2,3] Gokulan Kandasamy,[3] Graham Woodward.[3] *[1]Nephrology, Humber River Hospital, Toronto, ON, Canada; [2]Medicine, Univ of Toronto, Toronto, ON, Canada; [3]Ontario Renal Network, Toronto, ON, Canada.*

Background: Despite good clinical outcomes and low cost, home hemodialysis (HHD) is underutilized, mostly due to the lack of home patient support. Patient-based bundle funding in Ontario is provided by the Ontario Renal Network (ORN) supporting frequent dialysis both in-center and at home.

Methods: A pilot study examined the feasibility, cost and outcomes from the use of personal support workers (PSWs) to assist with HHD. Funding was secured initially by industry and then by the ORN. To allow future wider adoption and economies of scale, a non-hospital based industry partner assumed the human resource organizational role and risk. Three employed PSWs were trained by Humber River Hospital to support 3 patients. The project was approved by the hospital risk management and legal teams and insurance provider.

Results: Three patients have been dialyzed at home for 18 months by 3 PSWs. Three more patients received respite care when the PSWs were available. No clinical problems were encountered. There was a high level of satisfaction among patients and nurses. The hospital team provided back-up support as needed. The projected yearly cost, from payor perspective, assuming economies of scale of 30 patients is provided below (PSW cost in hatched columns).



Conclusions: Based on this pilot, use of PSWs to provide HHD is feasible, and based on ORN funding rates, is projected to be **less expensive than in-center HD for similar dialysis frequency**. This is being expanded to include enough patients to confirm the cost projections, when economies of scale are achieved and help understand patient eligibility and outcomes. It is predicted that significantly more patients can be dialyzed at home with the aid of PSWs, with significant implications in the provision of dialysis.

Funding: Pharmaceutical Company Support - Janssen, Government Support - Non-U.S.

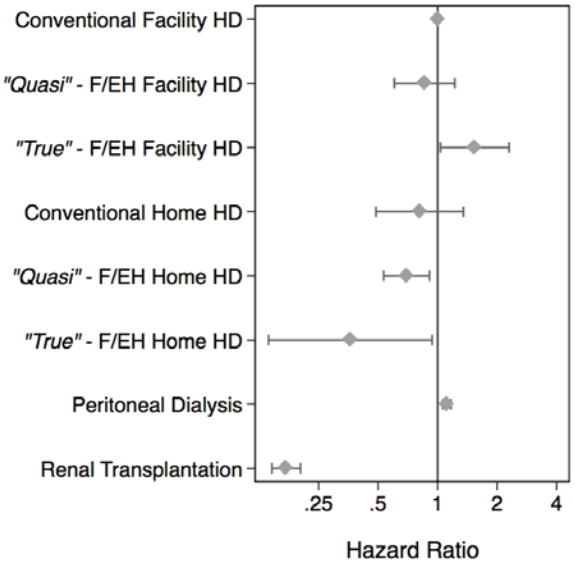
SA-PO901

Frequent/Extended Hour (F/EH) Hemodialysis (HD) and Mortality Risk in the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA) Mark R. Marshall,[1] Carmel M. Hawley,[3] Peter G. Kerr,[4] Kevan Polkinghorne,[4] John W. MacD. Agar,[5] Stephen P. McDonald.[2] *[1]Faculty of Medicine and Health Sciences, Univ of Auckland, Auckland, AKL, New Zealand; [2]Australia and New Zealand Dialysis and Transplant Registry, The Royal Adelaide Hospital, Adelaide, SA, Australia; [3]Dept of Nephrology, Princess Alexandra Hospital, Brisbane, QLD, Australia; [4]Dept of Nephrology, Monash Medical Centre, Clayton, VIC, Australia; [5]Renal Unit, Geelong Hospital, Geelong, VIC, Australia.*

Background: A previous study using ANZDATA showed that Home HD was associated with lower mortality risk than Facility HD and PD, although F/EH regimens per se were not associated with change in mortality risk. That analysis was limited by the absence of time varying-co-morbidity for some patients (only available in ANZDATA from 31 Mar 2001), informative censoring for renal transplantation (RT), and categorization of alternate-day HD with more frequent HD.

Methods: We repeated analyses in an updated, larger inception cohort from 31 Mar 1996 to 31 Dec 2011 (n=38773, 2054990 pt-mths, 18130 deaths), with time-varying comorbidity available for the vast majority, modelling RT as a competing risk, and categorizing HD regimens as - Conventional (3/week, ≤6 hrs/Rx); "Quasi" F/EH (>conventional, but <"True"); and "True" F/EH (≥5 x a week, any hrs/Rx). Marginal structural modelling was implemented with time-varying modality and co-morbidity via multinomial logit, adjusting for age, gender, race, eGFR at dialysis inception, late referral, DM, primary renal dis, coronary artery dis, peripheral vascular dis, cerebrovascular dis, lung dis, smoking.

Results: Results are below



Conclusions: RT is associated with the lowest overall mortality risk, "True" and "Quasi" F/EH Home HD with the next lowest, and "True" F/EH Facility HD the highest (possibly due to residual selection bias). Conventional Home and Facility HD are associated with similar mortality risk.

Funding: Government Support - Non-U.S.

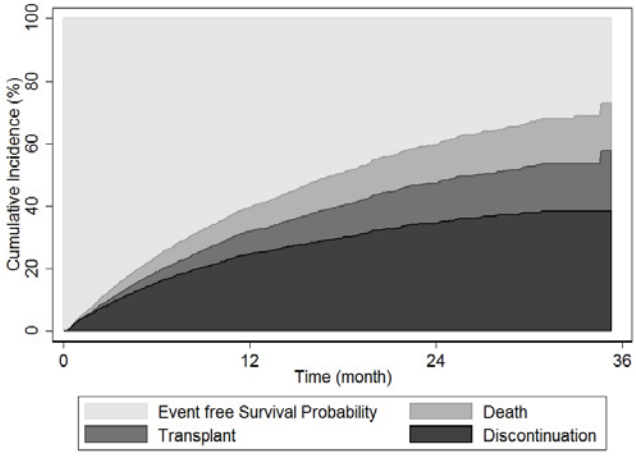
SA-PO902

Factors Associated with Discontinuation of Home Hemodialysis Rebecca Kurnik Seshasai, Michael Chaknos, Nandita Mitra, Jiaqi Li, Christopher Wirtalla, Dan Negoianu, Joel D. Glickman, Laura M. Dember. *Univ of Pennsylvania, Philadelphia, PA.*

Background: Home hemodialysis (HHD) is associated with improved clinical and quality of life outcomes compared with in-center hemodialysis but remains an underused modality in the U.S. Discontinuation from HHD is an important contributor to the low utilization of this modality. The rate, timing and contributing factors to HHD discontinuation are largely unknown.

Methods: Using DaVita clinical data, we identified a nationally representative cohort of patients who initiated HHD from 2007 - 2009 (n=2925). This cohort was linked with 1) USRDS data to obtain demographic and treatment history information, 2) 2000 U.S. Census data to estimate income, and 3) Rural Urban Commuting Area (RUCA) data to characterize the rural/urban nature of residence. Dialysis discontinuation was defined as ≥60 days with no HHD treatments; death and transplant were treated as competing risks. Competing risks regression was used to identify socio-demographic and clinical variables associated with HHD discontinuation.

Results: Mean age was 52.1±14.2 yr; 70.1% were white and 65.9% were male. Median duration of HHD was 253 days. 1-yr discontinuation and 1-yr mortality were 24.6% and 7.6%, respectively. Diabetes and smoking/alcohol/drug use were associated with increased risk of HHD discontinuation [HR (95% CI) 1.33 (1.07-1.66) and 1.4 (1.07-1.83), respectively]. Listing for kidney transplant and rural residence (RUCA ≥ 7) were associated with decreased risk of HHD discontinuation [HR (95% CI) 0.73 (0.61-0.87) and 0.76 (0.59-1.0), respectively].



Conclusions: Patients with diabetes, substance use, non-listing for transplant and urban residence are at greater risk for HHD discontinuation. Incorporating patient factors into HHD modality selection and targeting high-risk patients for increased support from clinical teams are potential strategies for reducing HHD discontinuation.

Funding: NIDDK Support

SA-PO903

Time to Recovery from Haemodialysis – Does Dialysis Location Matter? Anuradha Jayanti, The Basic-Hhd, Sandip Mitra. *Dept of Nephrology, Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: Patient recovery time from the effects of hemodialysis (HD) has been proposed as an indicator of the impact of HD on quality of life (QoL), in recent studies. Studies engaging HD patients, receiving institutional care and those self-caring, are limited by small patient numbers. We examined associations between patient-reported recovery time and patient characteristics in home and hospital HD patients.

Methods: In a cross-sectional study design, demographic and clinical data were obtained from a multicentre study in the UK (BASIC-HHD study). Hospital HD (N) = 213; Self-care facility-based patients (7)/Home HD (93) = 100. Patients responded to the question, 'Typically, how long does it take you to recover from a hemodialysis session'? Recovery time has been treated as a continuous variable in this analysis.

Results: Mean time to recovery from HD amongst hospital and home HD patients is significantly different ($p<0.01$). On controlling the cohort to which the patients belonged, in the univariate analysis, variables that were associated with lesser recovery time include male gender, no heart failure, higher albumin, higher systolic blood pressure and Beck depression score <15. Lower recovery time (RT) was associated with higher physical component score on SF-36, a quality-of-life questionnaire. Greater than 3 dialysis sessions/week, dialysis vintage, comorbidity index, diabetes, ethnicity and BMI were not found to be a significantly associated with a lower RT trend in this study. In the multivariate regression analysis, being a home haemodialysis recipient reduced the time to recovery by 103 minutes, having heart failure increases recovery time by 139 minutes, and each unit increase in systolic blood pressure (SBP) reduces recovery time by about 3 minutes-the range of SBP recorded (69 to 219mmHg).

Conclusions: Home as the destination for hemodialysis impacts RT positively. Self-care HD independent of dialysis prescription variables influences perceived quality of life.

SA-PO904

Evaluation of Non-Linear Heart Rate Variability (HRV) Parameters and Their Associations to Cardiac Function, Fluid Accumulation and Physical Performance in the Frequent Hemodialysis Network (FHN) Daily Trial Jochen G. Raimann,[1] Manuela Ferrario,[2] Brett Larive,[3] Andreas Pierratos,[4] Stephan Thijssen,[1] Sanjay Rajagopalan,[5] Tom Greene,[6] Gerald J. Beck,[3] Christopher T. Chan,[4] Peter Kotanko,[1] The FHN Trial Group.[7] *[1]Renal Research Inst; [2]Politecnico di Milano; [3]Cleveland Clinic; [4]Univ Health Network Toronto; [5]Univ of Maryland Medical Center; [6]Univ of Utah; [7]NIDDK, NIH.*

Background: An association between HRV and cardiovascular outcomes is known. More frequent hemodialysis (HD) decreases left ventricular (LV) mass, extracellular volume (ECV) and HRV, and also improves physical functioning and health (PF, PH; FHN Trial Group). We analyzed non-linear HRV indices and their correlates at baseline in the FHN Daily Trial.

Methods: Spectral Power Slope (SPS), Detrended Fluctuation Analysis (DFA), Multiscale Entropy Slope (MSE), Multiscale Sample Entropy (MSE SampEn), Multiscale Approximate Entropy (MSE ApEn), ApEn and SampEn were computed from 24-hours Holter recordings prior to randomization. Correlations between HRV parameters and left

ventricular ejection fraction (LVEF), left ventricular end-diastolic volume (LVEDV), ECV/total body water (TBW), PH and PF (both assessed with SF-36) were tested in diabetic and non-diabetic subjects (D, ND) using linear regression.

Results: We obtained the following measurements in 210 subjects [49.8±13.5 years, 62% males, 42% D, 56.7±11.2 % LVEF]: ECV/TBW 0.52±0.07, DFA 1.07±0.07, SPS 1.32±0.22, MSE SampEn 0.01±0.06, MSE ApEn 0.01±0.07, ApEn 0.91±0.37, and SampEn 0.64±0.34. In ND, MSE SampEn and MSE ApEn correlated positively with LVEF, PF and PH, and inversely with LVEDV and ECV/TBW (correlation coefficient r ranging from 0.20 to 0.32; P<0.05). SPS correlated positively with ECV/TBW (r=0.27). In ND subjects, irregularity measures (MSE ApEn, MSE SampEn) correlated positively with LVEDV (r=0.19 and 0.20). Ds showed an inverse correlation between MSE SampEn and ECV/TBW.

Conclusions: This to date largest assessment of nonlinear HRV parameters in HD patients indicated associations between cardiac function, fluid status, physical condition and HRV indices in HD patients. HRV in D was less affected by differences in clinical parameters.

Funding: NIDDK Support

SA-PO905

Efficacy and Safety of Tinzaparin Anticoagulation of the Extracorporeal Circuit with a Single Bolus Administration in Nocturnal Home Hemodialysis

Robert Zoël Bell, Vincent Pichette, Jean-Philippe Lafrance, Martine Leblanc, Georges Ouellet, Linda Nolin, Sarah Bezzaoucha, Annie-Claire Nadeau-Fredette, Joannie Lefebvre, Caroline Lamarche, Michel Vallee. *Nephrology, Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.*

Background: Tinzaparin (T) has shown practical benefits over unfractionated heparin for extracorporeal circuit anticoagulation during in-center hemodialysis treatments. However,efficacy and safety of anticoagulation with T has not been substantiated in patients with extended dialysis sessions, as in nocturnal home hemodialysis (NHD). The aim of the study was to evaluate the efficacy and safety of the use of T as a single bolus injection into the arterial line of the extracorporeal circuit of patients requiring 8 hours of anticoagulation in NHD.

Methods: 23 hemodialysis patients (NHD group) admitted to our NHD program between 2009 and 2014 had their first 8-hour in-center dialysis session receiving twice the T dose that gave adequate anticoagulation for their usual 4-hour dialysis treatment. Safety and dose/response were assessed by measuring anti-Xa levels at time 0 and at 15,30,60,120,240,360 and 480 minutes after administration of the T bolus. Anticoagulation efficacy was evaluated visually by assessing clot formation in both the dialyzer and the venous bubble trap through a simple scoring system. 7 chronic hemodialysis patients (HD group) paired for weight, age and sex receiving 4 hours of dialysis also had serial anti-Xa measurements at time 0 and at 15,30,60,120 and 240 minutes after administration of the T bolus.

Results: In terms of efficacy, the appearance of the dialyser and the bubble trap for the 8-hour dialysis sessions were similar to those of the 4-hour dialysis sessions. In terms of safety, there was no minor nor major bleeding events during the 8-hour dialysis sessions. The mean dose of T for 8 hours of hemodialysis was 111+/- 20 anti-Xa units/kg before and 113+/-15 anti-Xa units/kg after dose adjustments for patients with stage 3 coagulation.

Conclusions: Our study is the first to demonstrate that a single weight-based bolus injection of tinzaparin into the arterial line of the extracorporeal circuit in nocturnal home hemodialysis is effective and safe.

Funding: Private Foundation Support

SA-PO906

Economic Evaluation of High Dose Hemodialysis versus Conventional in-Center Hemodialysis in the Netherlands

Raymund Zinck,[1] Frank Xiaoqing Liu,[2] Cees Adriaansen,[1] Anna Trisia Beby.[1] *[1]Baxter, Utrecht, Netherlands; [2]Baxter, Deerfield.*

Background: Evidence shows that clinical and humanistic outcomes of hemodialysis (HD) improve by increasing the dose from conventional in-center HD (three times a week). The best results are seen when there are no consecutive days without dialysis and the weekly Kt/V is at least 3.0 (high dose HD). This analysis explores the cost-effectiveness of high dose HD versus conventional in-center HD in the Netherlands.

Methods: A Markov model was developed from payer perspective to compare high dose HD performed in-center and at home over a 10-year period to conventional in-center HD. Inputs included: epidemiology (incidence, prevalence, transplant rates), costs (dialysis, complications, transport, monitoring, medications), complications (all-cause hospitalizations), utilities (EQ-5D) and survival. Data from Dutch registries (such as Renine) or the ERA-EDTA registry, official tariffs and medical literature were used to populate the model. One-way and probabilistic sensitivity analyses were performed to test the robustness of the conclusions.

Results: In the Netherlands, the willingness-to-pay lies between €20,000 and €80,000 per QALY depending on the underlying disease. Using the latest available tariffs, doing high dose in-center shows an incremental cost-effectiveness ratio (ICER) of €76,142 per QALY over 10 years. In contrast, high dose HD at home costs €42,930 less while producing 0.669 more QALYs than conventional in-center HD over 10 years.

Conclusions: This preliminary analysis shows that high dose HD when performed in-center is cost-effective in the Netherlands, almost reaching the limit of willingness-to-pay. However, when performed at home high dose HD provides better outcome with less costs. This indicates that there is room to increase the current weekly tariff for high dose HD at home in order to compensate for its additional production costs without compromising the cost-effectiveness of this regimen.

Funding: Pharmaceutical Company Support - Baxter

SA-PO907

Impediments to Adaptation of Home Hemodialysis (HHD)

Mary Gellens,[1] Catherine Firanek,[1] Lucy Barker Todd,[1] Ann Mancini,[1] Sara L. Garza,[2] Karen Lattrel,[3] Ann Robar,[4] James A. Sloand.[1] *[1]Baxter Healthcare Corporation, Deerfield, IL; [2]US Renal Care, Plano, TX; [3]Greenfield Health Systems, Detroit, MI; [4]Satellite Healthcare, San Jose, CA.*

Background: According to the United States Renal Data Systems (USRDS), as of 2011 92% of prevalent dialysis patients in the United States were on in-center hemodialysis (ICHD) while only 1.3% were on HHD. Many ICHD patients would benefit from HHD and Medicare "Conditions for Coverage" mandates that at least annually all patients be educated on the HHD option. Knowledge and perceptions of HHD by ICHD staff may impact patient use of HHD.

Methods: A validated, on line survey was developed to assess knowledge of evidence based clinical outcomes and perceptions about home dialysis. Nursing directors of 4 dialysis chains sent the survey to 580 dialysis health care professionals (DHCP). The survey took 10 minutes to complete and was conducted from 2/24/14 through 4/18/14.

Results: 274 individuals completed the survey (47% response rate). Thirty two percent of the respondents were ICHD nurses and technicians, 31% were peritoneal dialysis (PD) nurses, 24% were administrators/managers and 13% other dialysis staff. On average, respondents thought that 17% of US dialysis patients used HHD. When asked what dialysis modality they would choose for themselves, 56% chose PD and only 34% chose HHD. The majority of respondents felt that HHD was superior to ICHD in the following clinical categories: blood pressure control, survival, hospitalization rate, phosphate control, left ventricular mass, dialysis recovery time and cognitive function. They felt that the number one and two primary obstacles to patients starting HHD were fear of performing dialysis on their own and lack of patient education respectively. The majority felt that other obstacles to HHD included unwillingness to leave the ICHD space, lack of motivation and concern with the home set up.

Conclusions: ICHD staff has a good understanding and positive perception of HHD. They do not feel that clinical outcomes are an impediment to HHD but patient knowledge and patient perception of the therapy are. To increase use of HHD robust patient support programs and education are necessary.

Funding: Pharmaceutical Company Support - Baxter Healthcare

SA-PO908

Self-Reported Experiences with Short Daily Hemodialysis: Interim Results from the FREEDOM Study

Fredric O. Finkelstein,[1] Hocine Tighiouart,[2] Diane Wuerth,[3] Susan Finkelstein,[1] Bertrand L. Jaber.[4] *[1]Yale Univ School of Medicine, New Haven, CT; [2]Inst of Clinical Research and Health Policy Studies, Tufts Medical Center, Boston, MA; [3]Yale New Haven Hospital, New Haven, CT; [4]Tufts Univ School of Medicine, St. Elizabeth's Medical Center, Boston, MA.*

Background: Standardized surveys have been used extensively to examine the self-reported benefits of more frequent dialysis. However, the use of open-ended questions has received little attention. The FREEDOM study is a prospective cohort study investigating the clinical benefits of at-home short daily hemodialysis.

Methods: At month-4 (M4) and month-12 (M12), study participants were asked the following open-ended question: "What is the one thing that has changed the most for you since starting daily dialysis?" Two dialysis social workers classified the responses into 5 positive and 5 negative categories of self-reported experiences.

Results: Of the 499 enrolled patients, 289 completed the question at M4 and 199 at M12. At M4 and M12, 78% and 76% of responses reflected positive experiences with daily hemodialysis, respectively, and 22% and 24% of responses reflected negative experiences. The categories of positive and negative experiences are shown in the table:

	M4 (n=362)	M12 (n=235)
Positive		
1. Improved physical function	20%	19%
2. Feeling better	18%	17%
3. Flexibility of therapy	18%	19%
4. Improved overall quality of life	12%	17%
5. Physiological improvements	13%	13%
Negative		
1. Burden of treatment	4%	3%
2. Burden of treatment time	5%	6%
3. Disrupted daily routine	2%	1%
4. Decreased physical function	1%	2%
5. Physiological declines	0.3%	2%
Unclassifiable	4%	8%
No response	20%	15%

Results presented in rows are not mutually exclusive.

Conclusions: In summary, these data provide confirmation of the positive impact of at-home daily hemodialysis on quality of life, using individual patient responses as opposed to standardized questionnaires. Negative responses were cited by only 22 to 24% of patients, the most important involving burden of treatment and time. These findings should be incorporated into patient education programs, and clinicians should address the burden of therapy for patients receiving home dialysis.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

SA-PO909

On-Site, Short Daily Hemodialysis Is an Effective Renal Replacement Therapy at Skilled Nursing Facilities Marvin V. Sinsakul. *Circle Medical Management, Chicago, IL.*

Background: As the number of patients with ESRD admitted to skilled nursing facilities (SNF) continues to grow, there remains a need to provide dialysis for patients while at these facilities. We present our early experience with using short daily hemodialysis prescribed 5 days weekly on-site at skilled nursing facilities using the NxStage System One Home HD device.

Methods: Data of monthly QAPI reports from six SNF with on-site, SDHD programs was aggregated and reviewed.

Results: From January 2014 through April 2014, 2187 treatments were provided for a total of 41 patients. The average number of patients per facility was 7 (range 3-11) with a mean of 53.08±3.73 treatments per patient. The average length of stay was 10.4 weeks. The percentage of patients completing the prescribed number treatments during the course of their stay was 97%, with a mean occurrence of 13.17 missed treatments and 1.83 shortened treatments per facility. Evaluation of clinical data revealed that 81% percent of patients achieved the targeted kt/V of 2.0, while only 2% of patients had a hemoglobin of <9.0gm/dl. Sixty-one percent of patients had TSATs between 20-40% and 47% of patients had a ferritin of >1000ng/mL. Regarding MBD parameters, 45% of patients had iPTH <300, 69% had a serum calcium between 8.4-10.9mg/dL and 70% had a serum phosphorus between 3-6mg/dL. Only 32% of patients in the program had a serum albumin of >3.5gm/dL. The average distribution of patients with AVF, AVG and catheters were 34%, 26% and 27%, respectively.

Conclusions: We conclude that providing short daily hemodialysis (5 days weekly) is an effective modality for ESRD patients admitted to SNFs. The use of a home HD device alleviated the need to install a water treatment system at these facilities. The patients ultimately completed a high percentage of prescribed treatments without the disruption leaving the facility for off-site dialysis. Based on the relatively low serum albumin and high ferritin of these patients, this sub-acute, post-hospitalization population remains more ill than the ambulatory, in-center HD population. The relatively short stay of these patients may make goals that require titration of the dialysis prescription or medication more difficult to achieve.

SA-PO910

No Improvement in Arterial Stiffness or Pro-Inflammatory Cytokines with Quotidian Haemodialysis in End-Stage Renal Disease Patients Kenneth Yong,[1,2] Gursharan K. Dogra,[1] Neil Boudville,[1,2] Wai Hon Lim.[1] *[1]Dept of Renal Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia; [2]School of Medicine and Pharmacology, Univ of Western Australia, Perth, Western Australia, Australia.*

Background: Pro-inflammatory cytokines such as interleukin(IL)-12 and IL-18 are implicated in the pathogenesis of cardiovascular disease (CVD) in end-stage renal disease (ESRD) patients. Increased serum IL-18 levels are independently associated with vascular stiffness and CVD mortality in ESRD patients. Quotidian HD improves parameters such as left ventricular hypertrophy, which may be related in part to enhanced clearance of pro-inflammatory mediators and improved arterial stiffness.

Methods: To assess the effects of quotidian haemodialysis (HD) upon clearance of pro-atherogenic cytokines and arterial stiffness. **Methods:** We conducted a longitudinal study of 14 stable conventional HD patients commencing quotidian home haemodialysis (HHD) between 2012 and 2013. We measured arterial stiffness [augmentation index (AIx), pulse wave velocity (PWV), pulse pressure (PP)] inflammatory markers (hs-CRP, IL-12, IL-18) and bioimpedance analysis (ECW:ICW ratio) prior to commencement of quotidian HD and between 3-6 months following commencement of quotidian HD.

Results: The mean±SD age was (51.9±9.2) years and 79% were males. The median (IQR) duration of conventional HD was 11(1.5-34.5) months. After commencement of HHD, the median hours of dialysis hours per week increased from 12.5(12-13) to 16(16-17). HHD did not significantly reduce inflammatory mediators such as hs-CRP (5.3±1.4 versus 41.±1.4g/L), IL-12 [240(181-349) versus 222(156-392)pg/mL], IL-18 [379(328-573) versus 336(306-435)pg/mL] or arterial stiffness such as PP (66±6.1 versus 61±5.4mmHg), PWV (7.6±1.1 versus 7.8±1.1m/s) and AIx (20±3.5 versus 18±4.2%) or ECW:ICW ratio (0.89±0.04 versus 0.80±0.08) (all p>0.05).

Conclusions: Changing to quotidian HD did not improve arterial stiffness or inflammatory markers in this small cohort of stable patients on conventional HD. Extension of this study to a larger cohort and longer follow-up period is warranted to determine if quotidian HD provides improved CVD outcomes.

Funding: Government Support - Non-U.S.

SA-PO911

Is Progression of Arteriosclerosis in ESRD Patients Inhibited By Nocturnal Hemodialysis or Renal Transplantation? One Year Results From the NOCTX Study Franka E. Van Reekum,[1] Marianne C. Verhaar,[1] Petrus F. Vos,[2] Akin Ozyilmaz,[3] Brigit C. Van Jaarsveld.[4] *[1]Nephrology, Univ Medical Center Utrecht, Utrecht, Netherlands; [2]Dianet Dialysis Center, Utrecht, Netherlands; [3]Dialysis Center Groningen, Groningen, Netherlands; [4]VU Medical Center, Amsterdam, Netherlands.*

Background: Coronary artery calcification (CAC) is associated with increased cardiovascular events and mortality. The objective of this prospective cohort study is to determine the influence of dialysis modality and renal transplantation on CAC progression in ESRD patients.

Methods: The NOCTX study is scheduled to measure CAC scores (CACS), expressed as the standardized Agatston score, at baseline and after 1, 2, and 3 yr with multislice CT of the heart in 4 different groups: pts treated with conventional hemodialysis (HD 3-4x/week, 3-4 hr), peritoneal dialysis (PD), incident nocturnal HD (5-7x/week, 6-8 hr) and kidney transplant after dialysis. Inclusion aim will be a total of 160 pts. We analyzed available one-year results, to study CACS progression in the different patient groups.

Results: At present, 112 pts are included in the study by 7 centers. So far, 73 patients underwent multislice CT at baseline and after 1 yr of treatment. In these pts, overall mean (SD) and median CAC score increased from 649 (1397) and 86 (IQR 0-642) at baseline to 812 (2126) and 103 (IQR 1-830) at 1 yr. Of the 23 pts with no calcifications at baseline, 78% remained without calcification. Of the 49 pts with baseline CACS > 10, 35% had an increase in CACS of >15%: 50% in the HD group, 33% in the PD group, 27% in the nocturnal HD group and 29% in the transplant group (NS). We performed a transformation of the CACS to ln(CACS+1) to enable more detailed comparison between the groups. As expected, with the present number of pts no significant difference in progression of CACS between the 4 groups could be found.

Conclusions: In this preliminary analysis of NOCTX data, most pts with CACS=0 at baseline remained without calcification, and of pts with calcification 35% progressed >15% in CACS. With the present number of inclusions, no difference in progression of CACS could be observed between pts treated with HD, PD, nocturnal HD or transplantation after 1 yr of follow-up.

Funding: Pharmaceutical Company Support - Amgen, Fresenius, Baxter, Shire, Roche, Private Foundation Support

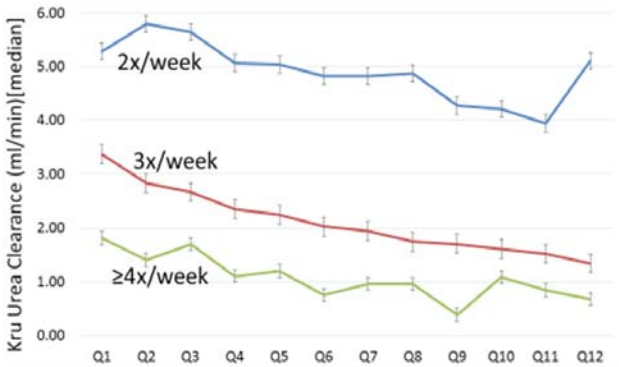
SA-PO912

Comparing Residual Kidney Function Pattern among Patients with Twice-Weekly, Thrice-Weekly, or More Frequent Hemodialysis Treatments Kamyar Kalantar-Zadeh,[1] Elani Streja,[1] Vanessa A. Ravel,[1] Connie Rhee,[1] Csaba P. Kovesdy,[4] Steven M. Brunelli,[3] Rajnish Mehrotra.[2] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Harborview MC, Univ of Washington, Seattle, WA; [3]DaVita Clinical Research, Minneapolis, MN; [4]Memphis VA MC, Memphis, TN.*

Background: ignificant differences in residual kidney function (RKF) may exist among different hemodialysis (HD) frequency regimens, but the RKF pattern across contemporary HD practices is less well-known. We hypothesized that RKF is higher in HD patients (pts) with less frequent HD.

Methods: Among all pts who started in-center HD during 2007-2011 and were treated for at least 60 days in a dialysis chain, each in-center HD pt was assigned one of 3 HD frequencies (2x/wk, 3x/wk, ≥4x/wk) for each 91-day (quarter) period from the date of first HD up to 3 yrs (12 quarters).

Results: We identified 1173 2x/wk, 125,751 3x/wk, and 126 ≥4x/wk HD pts who were 69.6±13.7, 62.1±15.2 and 55.0±18.5 yrs old and included 49%, 43% and 49% women, 13%, 31% and 21% Blacks, and 54%, 57% and 59% diabetic, respectively. In 673, 42,251, and 53 of these pts, respectively, the RKF was documented by Kru and was 6.0±3.7, 4.2±3.6 and 2.4±2.2 ml/min, respectively, in the first quarter. These differences in Kru maintained over a 12 qtr observation period, in that 2x/wk HD pts continued to exhibit significantly higher Kru than 3x/wk HD patient in each of the 12 qtrs, (p<0.001 for all differences in all qtrs) whereas ≥4x/wk HD pts had lower Kru than 3x/wk HD pts. Figure shows median trends:



	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12
2x/wk	673	454	557	398	418	307	295	245	180	155	127	102
3x/wk	42251	17124	18898	12913	12099	9159	8284	6553	5618	4479	3605	2918
≥4x/wk	53	47	63	49	58	48	53	39	42	40	29	17

Conclusions: Selection of HD frequency for an in-center HD pt by nephrologists in the U.S.A. is associated with consistent differences in Kru, in that 2x/wk HD pts exhibit the highest Kru both at the start of HD and up to 3 yrs thereafter, while ≥4x/wk HD pts have consistently the lowest Kru. Whether HD frequency affects preservation or loss of RKF cannot be answered by observational studies and requires RCT.

Funding: NIDDK Support

SA-PO913

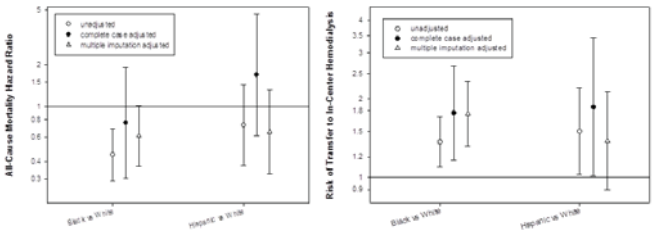
Association of Race with Risk of Mortality and Transfer to In-Center Hemodialysis in Home Hemodialysis Patients

Melissa Soohoo,[1] Vanessa A. Ravel,[1] Elani Streja,[1] Sooraj Kuttykrishnan,[2] Miklos Zsolt Molnar,[1] Kamyar Kalantar-Zadeh,[1] Rajnish Mehrotra.[2] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Univ of Washington, Seattle, WA.*

Background: Previous studies have reported that Blacks and Hispanics undergoing hemodialysis (HD) have a lower risk for death than whites. Home hemodialysis (HHD) is a convenient treatment modality for chronic kidney disease and increasingly being used. However, there are no data on racial differences in survival and time on therapy for patients undergoing HHD.

Methods: We examined the probability of treatment with HHD among Blacks, Hispanics, and Whites from among 162,671 patients who received dialysis in a large dialysis organization in calendar years 2007 through 2011 and the risk for all-cause mortality and rate of transfer to in-center HD for a cohort of 2,523 HHD patients from a large dialysis organization (553 Blacks, 144 Hispanics, and 1,826 Whites).

Results: Blacks and Hispanics were less likely to use HHD (Blacks, OR: 0.50; Hispanics, OR: 0.27) in comparison to Whites. Among Blacks, Hispanics, and Whites treated with HHD, the mean+SD age was 47+13, 48+15, and 55+14 years and included 33%, 34% and 45% women; and 60%, 67% and 60% diabetics, respectively. Compared to Whites, there was no significant difference in risk for death for Blacks or Hispanics, even after adjusting for case mix and laboratory parameters. However, there was a significantly higher risk for Blacks treated with HHD to transfer to in-center hemodialysis (HR: 1.8, 95% CI: 1.31-2.34) compared to Whites; there was no significant difference in the risk for Hispanics.



Conclusions: Racial-ethnic minorities are less likely to use HHD for treatment. There are no racial differences in survival of patients treated with HHD. However, Blacks have a significantly higher risk for transfer to in-center hemodialysis. Additional studies are needed to examine these racial-ethnic differences.

Funding: NIDDK Support

SA-PO914

Technique Survival and Remoteness in Home Haemodialysis

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Background: Home haemodialysis (HHD) is associated with significantly improved quality of life, survival and fewer hospitalisations compared with in-centre HD. However, little is known about how long patients remain on HHD, and whether this is associated with remoteness.

Methods: Patients who commenced chronic renal replacement therapy between 2000 – 2012 were classified by postcode as either major city or other, using the Australian Bureau of Statistics' Remoteness Area Classification. Uptake of HHD was analysed using Cox models, censored for death, transplantation or PD. Technique failure (TF, dialysis modality change lasting more than 30 days) similarly censored at death or transplantation. Results were presented both as univariate and after adjustment for age, race, gender, BMI and primary kidney disease.

Results: Uptake of HHD was not associated with remoteness in adjusted or unadjusted analyses (P≥0.6). Of 8064 non-indigenous patients who commenced HHD, 5481 (67.9%) lived in major cities. Patients outside major cities were generally older, had higher BMI and more comorbidities. Five years after commencing HHD, 28.2% of major city patients had TF compared with 32.5% of other patients. Associations between remoteness and TF varied with time on HHD. There was no difference in TF in unadjusted analyses within the first 3 years (hazard ratio [HR] versus major city 0.90, 95%CI 0.72-1.12, P=0.35), but patients outside major cities were more likely to suffer subsequent TF (HR 1.72 95%CI 1.16-2.54, P = 0.007). Adjustment made little difference (HR during first 3 years 0.82 95%CI 0.65-1.03 P = 0.095; HR after 3 years 1.69, 95%CI 1.11-2.55, P = 0.012).

Conclusions: Contrary to the belief that HHD is more prevalent in remote areas due to lesser availability of satellite HD advantage, our data suggests otherwise. Patients outside major cities were also more likely to cease HHD beyond 3 years. Understanding the reasons for this may facilitate strategies to improve technique survival in remote patients.

SA-PO915

Errors in Self Cannulation and Access Manipulation Identified by Vascular Access Audit Is Associated with Infections in Home Hemodialysis

Mathieu Rousseau-Gagnon, Karlien Francois, Christopher T. Chan. *Nephrology, Univ Health Network, Toronto, ON, Canada.*

Background: Vascular access-related infection is an important adverse event in home hemodialysis (HHD). We hypothesize that errors in self-cannulation or manipulation of dialysis access is associated with increased incidence of access-related infection.

Methods: We conducted a retrospective cohort study of all prevalent HHD patients at the University Health Network. All vascular access-related infections were recorded from 2006 to 2013.

Results: Selected patient characteristics are presented in Table. Median HHD vintage differed between patients with and without appropriate vascular access technique. The overall rate of infection between patients with and without appropriate vascular access technique was similar. Among patients who were identified with errors in dialysis access manipulation, patients with≥ 5 errors were associated with higher rate of access related infection (0.65 versus 0.24 infection/patient-year, p=0.017).

Variable	Error (n=49)	No error (n=43)	p value
Age at vascular access audit	46.4 ± 11.7	46.2 ± 12.8	0.938
Sex			0.486
Male	33 (67%)	25 (58%)	
Female	16 (33%)	18 (42%)	
Home Hemodialysis Vintage	5.8 (1.5-9.4)	2.3 (0.9-5.0)	0.005*
Dialysis session length	8 (7-8)	8 (7-8)	0.785
Weekly number of dialysis sessions	5 (4-5)	5(5-5)	0.479
Vascular access at vascular access audit			0.105
AV fistula using rope ladder cannulation	6 (12%)	11 (25%)	
AV fistula using buttonhole cannulation	25 (51%)	12 (28%)	
AV graft	3 (6%)	2 (5%)	
Tunneled catheter	15 (31%)	18 (42%)	
Charlson Comorbidity Index	3 (2-4)	3 (2-4)	0.828
Use of immunosuppressive drugs			0.374
Yes	14 (29%)	17 (40%)	
No	35 (71%)	26 (60%)	
Requirement of caregiver to dialyze			0.544
Yes	9 (18%)	5 (12%)	
No	40 (82%)	38 (88%)	

*: Statistically significant.

Conclusions: Patients with a longer median HHD vintage are at higher risk of inappropriate vascular access technique. Patients with multiple erroneous steps in vascular access technique are at high risk of dialysis access related-infection. Prospective evaluation of the impact of vascular access audit on recurrent infection is warranted.

SA-PO916

Protein-Bound Uremic Toxins, Carbonyl Stress, and Advanced Glycation End-Products in Conventional and Extended Hemodialysis and Hemodiafiltration

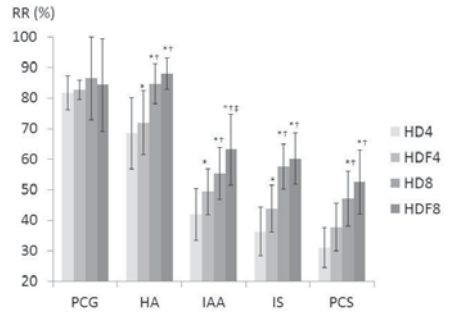
Tom Cornelis,[1] Sunny Eloot,[2] Griet Lrl Glorieux,[2] Karel M. Leunissen,[1] Raymond C. Vanholder,[2] Frank van der Sande,[1] Casper Schalkwijk,[1] Jeroen Kooman.[1] *[1]Nephrology, Maastricht Univ Medical Centre, Maastricht, Netherlands; [2]Nephrology, Ghent Univ Hospital, Ghent, Belgium.*

Background: Protein-bound uremic toxins (PBUT), carbonyl stress and advanced glycation end-products (AGEs) are associated with cardiovascular disease in dialysis. We aimed to study reduction ratio (RR) and total solute removal (TSR) of these toxins in extended hemodialysis (HD) and hemodiafiltration (HDF).

Methods: Thirteen conventional HD patients randomly completed 4h HD (HD4), 4h HDF (HDF4), 8h HD (HD8) and 8h HDF (HDF8). RR and TSR of PBUT [indoxyl sulphate (IS), p-cresylsulphate (PCS), p-cresylglucuronide (PCG), 3-carboxyl-4-methyl-5-propyl-2-furanpropionic acid (CMPF), indole-3-acetic acid (IAA), hippuric acid (HA)], AGEs [N^{ε}-(Carboxymethyl)lysine (CML), N^{ε}-(Carboxyethyl)lysine (CEL), N_{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1), pentosidine], as well as dicarbonyl compounds [glyoxal (GO), methylglyoxal (MG), 3-deoxyglucosone (3-DG)] were measured.

Results: Extended HD and HDF increased TSR (mg) and RR of total (Figure 1) and/or free PBUT (except CMPF), as well as carbonyl stress and free AGEs.

	PCG	***HA***	***IAA***	***IS***
TSR_{HD4}	108.0±139.0	622.7±411.4	14.6±10.9	148.2±80.3
TSR_{HDF4}	133.5±183.5	657.7±450.3	16.5±10.0	176.5±91.2
TSR_{HD8}	169.0±182.5*	965.0±802.2*†	22.4±13.9*	239.5±128.4*
TSR_{HDF8}	180.7±235.1*	945.5±615.7*†	19.6±11.9*	249.1±130.0*
* P <0.05 versus HD4; † P <0.05 versus HDF4				



* P<0.05 versus HD4; † P<0.05 versus HDF4; ‡ P<0.05 versus HD8

Conclusions: Increased dialysis time had a major effect on RR and/or TSR of most PBUT, as well as on RR and TSR of carbonyl stress and free AGEs. Convection had an additive effect on the RR of selected PBUT, free CML and CEL, as well as on TSR of 3-DG (in HDF8).

SA-PO917

The Wnt Signaling Pathway Is Activated During Peritoneal Fibrosis

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Background: Peritoneal fibrosis is a complex process with multiple pathways involved. The wnt signaling pathway is involved in cellular transition and has been associated with fibrosis in other organ systems. We investigated the regulation of the wnt signaling pathway in an animal model of peritoneal fibrosis.

Methods: C57BL/6 mice were treated with an adenovirus expressing active transforming growth factor beta (AdTGFB) or control adenovirus (AdDL). We evaluated histology, downstream proteins of the wnt pathway by western blot, and gene expression by Nanostring 4 and 10 days after infection.

Results: There were no histologic changes to the peritoneal membrane after exposure to control adenovirus. After exposure to AdTGFB, we observed cellular proliferation, angiogenesis, submesothelial thickening and fibrosis. Gene expression of Wnts 2 and 4 were significantly upregulated by TGFB, as was the receptor FZD1 and the co-receptor ROR2. Other co-receptors LRP5 and 6 were unchanged by TGFB. Wnt 7b and 8a were both significantly downregulated in mice treated with AdTGFB. By western blot, we found that b-catenin was significantly upregulated and GSK3B phosphorylation was increased by TGFB. The common post-receptor signaling molecule DVL was also increased after exposure to AdTGFB.

Conclusions: There is evidence that wnt signaling pathway is active in the setting of experimental peritoneal fibrosis. Elements of both canonical and non-canonical wnt signaling appear to be upregulated by TGFB.

Funding: Private Foundation Support

SA-PO918

Twist Accelerates Peritoneal Membrane Fibrosis by Regulating YB-1

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Background: Both E-box–binding transcription factor Twist and YB-1 controls gene expression through both transcriptional and translational mechanisms. We have previously shown that Twist is overexpressed in high glucose induced human peritoneal mesothelial cells (HPMCs) and is involved in peritoneal membrane (PM) fibrosis. Structural analysis of the YB-1 promoter reveals that several E-boxes, as Twist may participate in the regulation of YB-1 expression.

Methods: To investigate Twist regulate YB-1 impacts on growth of HPMCs and EMT-derived fibrosis in PM, we isolated HPMCs from the effluents of patients with end-stage renal disease (ESRD) to observe the response of PM to Twist and YB-1.

Results: Here, our results demonstrated the overexpression and activation of Twist and YB-1 of HPMCs under extensive periods of PM fibrosis ex vivo. In immortal HPMCs and in HG-induced PD animal model, high glucose (HG, 60 mmol/L) also stimulated Twist and YB-1 overexpression and a transformed fibroblastic phenotype of HPMCs. Evidence from chromatin immunoprecipitation and reporter assays further supported that Twist transcriptionally regulated YB-1 by directly binding to its promoter. Collectively, these data suggest that YB-1 is a major downstream target of Twist. Re-expression of Twist led to decrease HPMCs growth, induced cell cycle arrest and increase PM fibrosis. Silencing of Twist could also down-regulate YB-1 expression, promote cell cycle progress of HG-induced HPMCs growth and also inhibited PM fibrosis.

Conclusions: Our data suggested that activation of Twist/YB-1 pathway might contribute to the growth retardation of HPMCs and the progressive PM fibrosis during PD.

Funding: Government Support - Non-U.S.

SA-PO919

Primary Cultured Peritoneal Pericyteand Tests of Its Function

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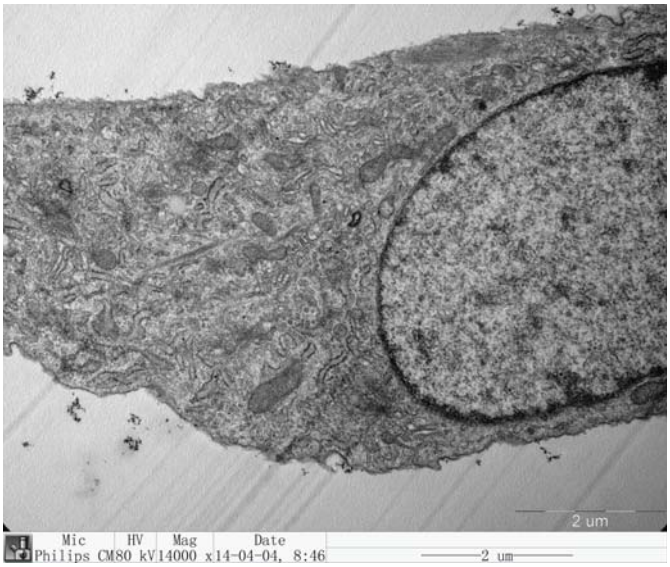
Background: This research is to establish a method of primary cultured peritoneal pericytes, providing a new strategy for the study of angiogenesis in UF.

Methods: Using the traditional enzymatic digestion, we successfully cultured and purified primary peritoneal pericytes from visceral peritoneum, and light microscopy, immunofluorescence, electron microscopy, flow cytometry, western blotting were used to identify and detect its function.

Results: Using electron microscopy, the primary cells presents pericyte's characteristics,while cell length reaches 50-70um, collagen does not exist between the cells.



Immunofluorescence was used to detect the surface biomarkers expression of primary cells repeatedly: pericyte in normal SD rats, surface biomarkers are PDGFR-ß (+), integrinα3 (+), α-SMA (+),CD248(+),Desmin(-/+),NG_2(+/-),while in uremic rats are PDGFR-ß(-/+), integrinα3(-/+),α-SMA(+),CD248(+), Desmin(+~++), NG2(+).The flow cytometry confirmed uremic state can affect the relationship between pericytes and basement/ endothelial, the presence of glucose enhanced this phenomenon, also confirmed by western blotting.

Conclusions: Using the traditional enzymatic digestion, we successfully cultured, purified primary peritoneal pericytes in visceral peritoneum. State of uremia can change the surface biomarkers of pericytes. Uremic state affect therelationship between pericytes and basement /endothelial cells, The existence of glucose enhanced this phenomenon.

SA-PO920

Histological Study and Analysis of Vascular Calcifications in Peritoneal Biopsies: Adipocytes as New Players in Encapsulating Peritoneal Sclerosis?

Joelle L. Nortier,[2] Tooulou Monika,[1] Pieter Demetter,[2] Anwar A. Hamade,[2] Agnieszka Anna Pozdzik.[1] *[1]Nephrology, Erasme Hospital, ULB, Brussels, Belgium; [2]Pathology, Erasme Hospital, ULB, Brussels, Belgium.*

Background: Histological study and analysis of vascular calcifications in peritoneal biopsies: Adipocytes as new players in encapsulating peritoneal sclerosis?

Methods: We approached both hypotheses by studying tissue expression of AE1/AE3 (mesothelial cells), calretinin (adipocyte marker), α-smooth muscle actin [α-SMA] (mesenchymal cells) in peritoneal tissue samples from 3 patients suffering from EPS. We also assessed interstitial inflammation and neoangiogenesis (CD3, CD4, CD8, CD20, CD68 and CD31 immunostainings, respectively) and analyzed the mineral composition of vascular calcifications (infrared microspectroscopy).

Results: Three patients (1M/2F; age 17, 64 and 39 years, respectively) developed EPS after 21, 90 and 164 months of PD therapy.Mesothelial cells expressed AE1/AE3 and calretinin in controls but this expression disappeared and there was no migration of mesothelial cell into the interstitium in all EPS patients. In comparison with normal tissue biopsy, the adipose tissue with calretinine+ adipocytes was replaced by severe sub-mesothelial fibrosis. In these areas, besides αSMA+ cells (as expected), we found a huge number of calretinin+ fusiform interstitial cells. We observed a neoangiogenesis in all EPS patients but vascular calcifications were detected in only 1 patient (8 PD-related bacterial peritonitis), which contained mainly carbapatite.

Conclusions: Despite the small number of this series, our results suggest that: (1) the involvement of MMT in peritoneal fibrosis remains unproven, (2) resident adipocytes

may represent underestimated sources of peritoneal fibroblasts and (3) the infrared microspectroscopy of tissue calcifications could be helpful in studying the link between peritoneal infections and vasculopathy in EPS.

SA-PO921

Contemporaneous Utilization of Biocompatible and Standard Peritoneal Dialysis Fluids Is Associated with a Similar Risk of Peritonitis M. Auxiliadora Bajo, David Menendez, Gloria Del Peso, Rafael Selgas. *Nephrology, Hospital U. La Paz. IdiPAZ. REDinREN.IRSIN, Madrid, Spain.*

Background: Peritoneal dialysis (PD)-related peritonitis is an important cause of morbidity, mortality and technique failure in PD patients. The risk of peritonitis depending on the peritoneal solution type is controversial, with data suggestive of a higher risk for those patients using more-biocompatible solutions. The peritoneal membrane protection associated with these solutions might be challenged. The aim of this study was to compare peritonitis risk in patients contemporaneously using more biocompatible (BC) solutions and standard solutions (SS) at one experienced PD center.

Methods: This retrospective cohort study included incident patients on PD at one center from January, 1999 to December, 2013. All patients used the same peritoneal dialysis solution during their whole follow-up. Multicompartimental solutions were defined as BC. 324 patients with a mean follow-up of 24.7±18 months were included. 151 patients (47%) used BC solutions and 173 patients (53%) SS. In the BC group, there was a higher prevalence of diabetes (26 versus 15%, p=0.015), but no differences in age, gender and time on PD were observed between groups.

Results: 131 patients had 229 peritonitis episodes. Patients with peritonitis were older and showed longer PD treatment, more comorbidity and were more frequently on CAPD. No difference in the incidence of peritonitis was observed with both fluids (44% in BC and 37% in SS, NS). A non-significant higher catheter loss was observed in the SS group (25% versus 12%, p=0.053), with no differences in hospitalization or peritonitis-associated death. The time to the first peritonitis was shorter in BC group, but with no statistical significance (HR 1.35 (95% CI 0.96-1.91), p=0.088). In multivariate analysis, only age, time on PD and CAPD use (versus APD) were independently associated with peritonitis, but not the type of solution used.

Conclusions: In this experience,the contemporaneous utilization of biocompatible and standard peritoneal dialysis fluids was associated with a similar risk of peritonitis. The use of biocompatible solutions cannot be questioned by this reason.

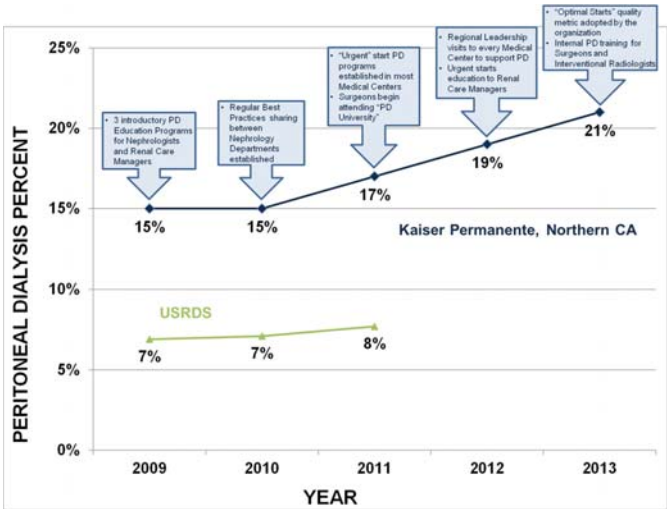
SA-PO922

Multidisciplinary Approach to Improve PD Prevalence in an Integrated Health Care Delivery System Leonid Pravoverov, Sijie Zheng. *Nephrology, The Permanente Medical Group, Oakland, CA.*

Background: The Permanente Medical Group (TPMG) recognized that Peritoneal Dialysis (PD) is an underutilized method of Renal Replacement Therapy (RRT). Through multiple organizational changes, TPMG has greatly increased PD prevalence and incidence.

Methods: Since 2009, the Kaiser Permanente Northern California (KPNC) region has developed a multidisciplinary approach to PD promotion in, including : **Patient / Family Education**. **Provider and Organizational Education**. **Operational Improvements Monitoring and continuous improvement**.

Results: Since 2009, KP Northern California saw a sustained growth in incident (31% of PD starts in 2013) and prevalent PD patients from 15% in 2009 to 22% by the end of 2013. This corresponds to growth from 630 to 975 prevalent PD patients in KP Northern California.



Conclusions: As an integrated health care system, Kaiser Permanent Northern California, has achieved higher prevalence and incidence of PD in our ESRD populations compare with the rest of the nation.

SA-PO923

Percutaneous Endoscopic Gastrostomy Feeding in Children on Peritoneal Dialysis: Experiences and Outcome Rainer Büscher, Anja K. Büscher, Julia Mohr, Peter F. Hoyer. *Pediatric Nephrology, Univ Children's Hospital, Essen, Germany.*

Background: Percutaneous endoscopic gastrostomy (PEG) feeding is common in children receiving chronic peritoneal dialysis (PD) but rarely used in adult PD patients. Only few studies on its effectiveness are available. The aim of our retrospective analysis was to obtain data on complications and outcome of PD children with PEG in a large pediatric dialysis center.

Methods: In this retrospective single center study we report our experience of PEG feeding in 24 children (14 males) on PD during an 8-year study period. All PEGs were placed under general anaesthesia and prophylactic antibiotics with or without antifungals were given at induction. Double-cuff straight Tenckhoff catheters were placed as a separate procedure and patients were on continuous cycling PD (n=11) or continuous ambulatory PD (n=13). Complications such as spontaneous peritonitis, number of Tenckhoff catheters or indications for change or removal of PEG/button gastrostomy were recorded.

Results: Eighteen out of twenty-four children, median age 2.5 (0.5–12.1) years had a PEG before starting PD and six children, age 0.6 (0.5–5.5) years, had a PEG after PD catheter insertion/start of PD. Most patients were malnourished, with standard deviation score (SDS) for body weight between –3.6 and –0.5 (median –2.4). In 18 patients (75%, 14 patients with PEG insertion before start of PD) no problems were encountered within the first year of observation. In the remaining 6 patients major complications were early bacterial peritonitis in 4 (17%) patients and 2 cessations of PD and temporary change to hemodialysis. Early peritonitis could effectively be treated with intraperitoneal antibiotics. In 6/24 patients, PD was successfully reinitiated shortly after PEG insertion.

Conclusions: Complication rates were lower in our patients than previously described. PEG insertion following PD initiation is possible but carries a risk for peritonitis and PD failure. Antibiotic and antifungal prophylaxis and a temporary stop of PD for 2 – 3 days are highly recommended. However, if possible, PEG insertion should be performed prior to the time of initiating PD.

Funding: Clinical Revenue Support

SA-PO924

Below 0.8g/kg/Day of Protein Intake Restriction Succeeds Favorable Control of Serum Phosphate Levels without Induction of Malnutrition in Patients Treated with Peritoneal Dialysis Hironori Tayama, Tomoaki Miyazaki, Yoshikuni Nagayama, Eri Kawashima, Kiyoko Inui, Yoshihiko Inoue, Ashio Yoshimura. *Div of Nephrology, Dept of Medicine, Showa Univ Fujigaoka Hospital, Yokohama, Japan.*

Background: 0.9-1.2 g/kg/day of daily protein intake is recommenced to patients treated with peritoneal dialysis (PD) according to PD guidelines, because of the supplement for protein loss to PD water fluid. We studied the efficacy of further restriction of protein for clinical state of PD patients, because we have already reported that protein restriction in pre-dialysis has induced favorable peritoneal function at the start of PD treatment.

Methods: Two groups of PD patients, such as 0.9-1.2 g/kg/day of protein intake (HPD, n=12) and 0.5-0.8g/kg/day (LPD, n=14), were studied. All patients of LPD has been continuing protein intake restriction from several years before PD induction. Two groups were compared in residual renal function, urine volum, PD ultrafiltration, index for nutrition (total protein, albumin, Hb), serum phospahe, serum calcium, blood pressure, and amount of protein in PD water after treatment.

Results: Serum phosphate level was siginificantly low in LPD compared to HPD (4.8±1.0 mg/dl versus 5.8±0.9, p<0.03, mean±SD). LPD also induced lower Kt/V (1.52±0.24) than HPD (2.1±0.46, p<0.03). There was no significant difference in amount of protein in PD water after treatment between two groups. There was no siginificant difference in index for nutritional state and other indices studied between two groups.

Conclusions: Below 0.8g/kg/day of protein intake restriction succeeded in better control of serum phosphate levels without induction of malnutrition in patients treated with PD. There was no significant increase in protein loss to PD fluid. Control of serum phosphate levels is critically important for dialysis patients, therefore protein restriction (0.5-0.8g/kg/day) should be recommended to PD patients.

SA-PO925

A Retrospective Observational Study of Peritoneal Dialysis Associated Peritonitis and Encapsulating Peritoneal Sclerosis During Therapy with a Twin-Bag System and Biocompatible Peritoneal Dialysis Solutions Masatsugu Nakao, Izumi Yamamoto, Nanae Matsuo, Yukio Maruyama, Yudo Tanno, Ichiro Ohkido, Masato Ikeda, Keitaro Yokoyama, Takashi Yokoo. *Internal Medicine, The jikei Univ School of Medicine, Tokyo, Japan.*

Background: The clinical relevance of single-bag versus twin-bag systems and conventional versus biocompatible peritoneal dialysis (PD) solutions are unclear. This study investigated PD-associated peritonitis prevalence, its causative microorganisms and encapsulating peritoneal sclerosis (EPS).

Methods: The study included 527 patients who received PD between January 1980 and December 2012 at a single center.We divided patients undergoing PD into three groups according to the type of PD system used, namely single-bag and conventional PD solutions (n = 145), twin-bag and conventional PD solutions (n = 171) and twin-bag and

biocompatible PD solutions (n = 211), and analysed PD-associated peritonitis and EPS incidences. The independent risks of PD-associated peritonitis and EPS were determined by univariate and multivariate logistic models.

Results: PD-associated peritonitis and EPS incidences (times per patient-months) were 1/54.1, 1/102.2 and 1/106.8, respectively, and 13.1%, 12.3% and 0%, respectively. Only the twin-bag system and PD duration were associated with PD-associated peritonitis. Further, peritonitis episode numbers and PD durationas well as β2 microglobulin (β2MG) level and dialysate-to-plasma creatinine ratio were risk factors for EPS. The duration of peritonitis, rather than PD duration, was independently associated with EPS risk in patients with PD-associated peritonitis.

Conclusions: Use of the twin bag system and PD duration, rather than the use of biocompatible PD solutions, decreased PD-associated peritonitis risk. The duration of peritonitis was a risk factor for EPS in addition to β2MG level and D/P Cr.

SA-PO926

Serum Asymmetric-Dimethylarginine, Apelin and N-Terminal Prohormone of Brain Natriuretic Peptide Levels in Dialysis Patients Hulya Taskapan, Recep Bentli. *Nephrology, Inonu Univ, Malatya, Turkey.*

Background: Asymmetric-dimethylarginine (ADMA) is an L-arginine analogue that inhibits endothelial nitric oxide synthase. The physiological effects of apelin are opposite to the actions of angiotensin-II. It may prevent excessive fluid retention by its actions on central vasopressin release. The relationships among serum apelin, Asymmetric-dimethylarginine (ADMA), N-terminal probrain natriuretic peptide (NT-proBNP) levels, and blood pressures in dialysis patients are not well known.

Methods: In age and sex matched 30 hemodialysis (HD), 30 peritoneal dialysis (PD) patients and 20 healthy controls the relationships among serum apelin-36, ADMA, NT-proBNP levels, and blood pressures were evaluated.

Results: Serum ADMA levels in HD patients were significantly higher than in PD patients and controls (0.57±0.17, 0.38±0.067, 0.40±0.06 μmol/L respectively; p: 0.000), ADMA levels in PD patients was not different from in the controls. HD patients had significantly higher serum apelin levels than those of PD patients (0.48±0.53 versus 0.23±0.47pg/mL). There was no difference between controls (0.28±0.34 pg/mL) and PD patients according to serum apelin levels (p>0.05). NT-proBNP levels were higher in both HD and PD patients (5949.66±7617.72; 11357.46±12321.52 pg/mLrespectively) compared to in controls (45.39±43.48 pg/mL) (p:0.000). In multiple regression analyses the predictors of serum apelin levels were BMI (β:0.281,p:0.021), ADMA (β:0.253,p:0.038) and systolic blood pressure (β:-0.243,p:0.046). The predictors of serum ADMA levels were being on HD (β:-0.579,p:0.000). The predictors of serum NT-proBNP levels were serum albumin (β:-0.416,p:0.000)and higher systolic blood pressure (β:0.414,p:0.000).

Conclusions: Being on HD is predictor of high ADMA levels. It may possible that ADMA removal rate by HD is lower. It seems that there is a relationship between serum apelin levels and blood pressure in dialysis patients. Further studies with larger populations are needed in order to clarify the relationship of serum apelin and blood pressure in dialysis patients.

SA-PO927

No Increase in Small-Solute Transport in Peritoneal Dialysis Patients Treated without Hypertonic Glucose for Fifty-Four Months Dominique C. Pagniez,[1] Celia Lessore,[1] Jean-Baptiste Beuscart.[2] *[1]Nephrology, Centre Hospitalier Univ, Lille, France; [2]Geriatrics, Centre Hospitalier Univ, Lille, France.*

Background: Glucose is widely used as an osmotic agent in peritoneal dialysis (PD) patients, but exerts untoward effects on the peritoneum, starting with an increased small-solute transport. The potential protective effect of a reduced exposure to hypertonic (3.86%) glucose solutions has never been investigated.

Methods: The cohort of PD patients attending our center, which tackled the challenge of a restricted use of hypertonic glucose solutions, has been prospectively followed since 1992. Small-solute transport was assessed using an equivalent of the glucose peritoneal equilibration test after 6 months, and then every year. Study was stopped on July 1st, 2008, before the use of biocompatible solutions became common. A linear regression of the D4/D0 ratios with a variance analysis using a marginal mixed model was applied in patients treated with PD for 54 months.

Results: In the study period, 44 such patients were treated for a total of 2376 months, 2058 without hypertonic glucose. There was one episode of peritoneal infection every 18 patient-months. The mean of slopes of the regression lines for D4/D0 ratios was found to be significantly positive (Student's test, p<.001). This result reflected a significant decrease of small-solute transport.

Conclusions: In this large series of long-term PD patients, avoiding hypertonic glucose solutions was associated with an overall decrease of small-solute transport within 54 months, despite a high rate of peritoneal infections.

SA-PO928

The Role of Telephone Follow-Up in the Management of Anemia in Patients with Peritoneal Dialysis Yan-Ru Chen, Hui Peng, Xun Liu, Hui-Qun Li, Wenbo Zhao, Tan-Qi Lou. *Nephrology Div, Dept of Medicine, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Regularly checking hemoglobin of dialysis patients and adjusting EPO dose in time are very important to keep hemoglobin in the target range (110~120g/L). However, since peritoneal dialysis (PD) is mostly performed at home,it is difficult to monitor hemoglobin regularly and timely adjust the EPO dose. The aim of the study is to find whether telephone follow-up could improve the adherence of EPO administration and anemia in peritoneal dialysis (PD) patient.

Methods: based on the previous data of 95 PD patients in our center, we found that patients with longer follow-up interval had lower level of hemoglobin. In order to improve anemia in these patients, we developed telephone follow-up intervention project: telephone follow-up was arranged every month when hemoglobin≥ 110 g/L; in those patients who was the first time using EPO or need to adjust EPO dose, telephone follow-up was arranged at the first 2 weeks, and then every month. And the nurse would urge patients to check routine blood and adjust the dose of EPO according to the results.

Results: In the study there were 37 patients adhering to the PD on–site clinic monthly while 58 with follow-up interval of 2 ~ 6 months. The average hemoglobin is 105.87 ±16.84 g/L before the intervention with target rate at 39%. The dosage of EPO is 103.5 ±39.6 IU/Kg per week. After six months of telephone follow-up intervention, hemoglobin reached 108.78 ±14.01g/L with the target rate at 56%, and dose of EPO was 105.5 + 105.5 IU/Kg per week. There was no difference in the level of hemoglobin or dose of EPO between before and after intervention. However, the patients had a significantly increased target rate after intervention(P = 0.020).

Conclusions: patients on PD in urban of China are difficult to take regularly clinic on-site follow-up. Our results show telephone follow-up would help nurses and doctors in PD center monitor the hemoglobin situation of PD patients and timely adjust their dosage of EPO. It could help to improve anemia of PD patients in China.

SA-PO929

Temporal Changes in Depression and Quality of Life in Incident Peritoneal Dialysis Patients Hye Eun Yoon, Eun Nim Kim, Bum Soon Choi, Yong-Soo Kim. *Internal Medicine, The Catholic Univ of Korea, College of Medicine, Seoul, Republic of Korea.*

Background: Temporal changes in depression and health-related quality of life (HRQOL) over time are not well described in peritoneal dialysis (PD) patients.

Methods: A multicenter prospective observational study was conducted in 343 incident PD patients. Beck's Depression Inventory (BDI), medical outcomes study short form 36-item quality of life instrument, and kidney disease quality of life short form were used to evaluate depression and HRQOL, respectively, at baseline and after 12 months. Bioimpedance analysis was also performed.

Results: The BDI score significantly increased after 12 months (P = 0.039). Scores of physical role significantly increased, but scores of kidney disease-related symptom/problem list, sleep and social support significantly decreased after 12 months. Increase in serum albumin significantly predicted decrease in BDI score (β = -3.7, P = 0.011). Charlson comorbidity index (β = -3.51, P = 0.028) and increase in plasma hemoglobin (β = 5.01, P = 0.001) and serum albumin (β = 11.29, P = 0.048) were independently associated with increase in physical QOL scores. Decrease in adipose tissue index (β = -0.83, P = 0.006) and increase in serum albumin (β = 6.88, P = 0.001) significantly predicted increase in mental QOL scores. Charlson comorbidity index (β = -1.35, P = 0.035) and decrease in peritoneal KT/Vurea (β = -2.7, P = 0.007) significantly predicted increase in kidney-disease related QOL scores.

Conclusions: In conclusion, depressive symptom is aggravated and HRQOL is impaired during 12months in incident PD patients. Changes in nutrition, anemia, adipose tissue and dialysis adequacy are associated with changes in depressive symptoms and HRQOL.

SA-PO930

The Association of Overhydration Status with Depression and Health-Related Quality of Life in Peritoneal Dialysis Patients Hye Eun Yoon, Eun Nim Kim, Bum Soon Choi, Yong-Soo Kim. *Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea.*

Background: This study was to determine the association of overhydration (OH) status with depression and health-related quality of life (HRQOL) in peritoneal dialysis (PD) patients.

Methods: A multicenter study was conducted in 628 PD patients. Body composition monitor, Beck's Depression Inventory (BDI), medical outcomes study short form 36-item quality of life instrument, and kidney disease quality of life short form were used to evaluate OH status, depression and HRQOL, respectively. Patients were divided into two groups according to the OH status; -2L≤OH≤2L group and OH>2L group.

Results: The BDI score did not differ between the two groups. However, the OH>2L group showed significantly lower physical, mental, and kidney-disease related quality of life (QOL) scores compared with the -2L≤OH≤2L group. The OH>2L group showed significantly lower scores in all of the physical QOL subcategories (physical functioning, role-physical, bodily pain, and general health) than the -2L≤OH≤2L group. Among the mental QOL subcategory, scores of social function were significantly lower in the OH>2L group than the -2L≤OH≤2L group. Among the kidney disease-related QOL subcategory, scores of effect of kidney disease, burden of kidney disease, and cognitive function were

significantly lower in the OH>2L group than the -2L≤OH≤2L group. In a multivariate analysis, OH status was not an independent predictor for BDI score and physical and mental QOL scores. However, OH status independently predicted kidney disease-related QOL score (β = -2.354, *P* = 0.012).

Conclusions: In conclusion, OH negatively affects HRQOL in PD patients. Intensive efforts to control fluid overload may improve HRQOL of PD patients.

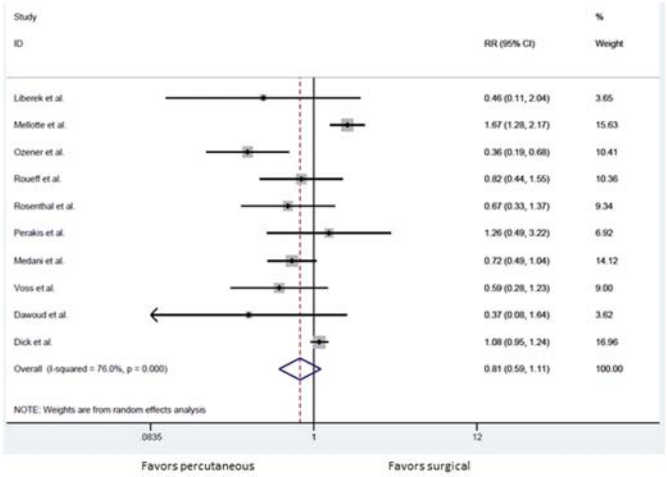
SA-PO931

Percutaneous versus Surgical Insertion of PD Catheters in Dialysis Patients: A Meta-Analysis Kevin Chapla,[1] Ning Fu,[1] Lamya Boujelbane,[1] Alexander S. Yevzlin,[1] Jung-Im Shin,[1,2] Brad C. Astor,[1,2] Micah R. Chan.[1] *[1]Nephrology, Univ of Wisconsin, Madison, WI; [2]Population Health Sciences, Univ of Wisconsin, Madison, WI.*

Background: Several small studies have suggested that the percutaneous method of PD catheter insertion is effective and has a lower complication rate than surgical techniques, though no randomized, controlled study has compared these methods. Our objective was to compare percutaneous PD catheter insertion versus surgical placement in terms of 1-year catheter survival, catheter dysfunction, fluid leak and incidence of peritonitis.

Methods: We searched Medline for English-language literature from 1966 through June 2014, along with national conference proceedings and reference lists of all included publications to identify relevant studies. Random effects models were used to derive the pooled risk ratios, differences in patency and their variations.

Results: Thirteen studies with a total of 2749 subjects met the inclusion criteria. There was no significant difference in 1-year catheter survival in percutaneous versus surgical PD catheter placement (relative risk [RR]=0.81; 95% confidence interval [CI]: 0.59-1.11, p=0.19). Catheter dysfunction also did not differ significantly between the groups (pooled odds ratio [OR]=0.86; 95% CI: 0.57-1.29, p=0.46) respectively. The prevalence of peritoneal fluid leak also was similar for percutaneous and surgical groups (OR=1.10; 95% CI: 0.58-2.09, p=0.77). However, there was a significant lower incidence of peritonitis among those with percutaneous placement (incidence rate ratio [IRR]=0.77; 95% CI: 0.62-0.96, p=0.02).



Conclusions: Our results suggest that there is no significant difference in catheter survival between percutaneous versus surgical placement of PD catheters. Whether there are significant benefits from percutaneous placement in terms of peritonitis rates requires further robust studies.

Funding: Clinical Revenue Support

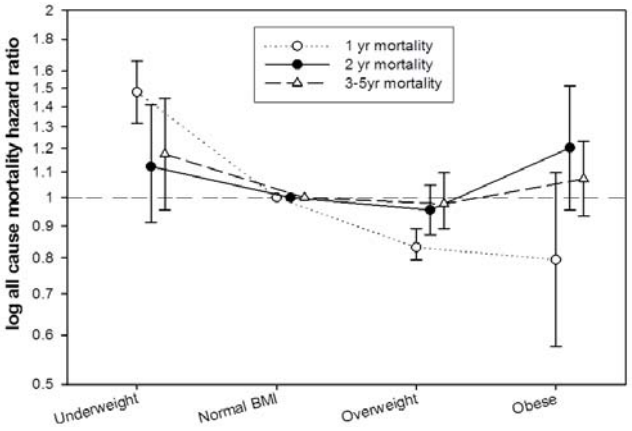
SA-PO932

Association of Body Mass Index with Mortality in Peritoneal Dialysis Patients: A Systematic Review and Meta-Analysis Seyed-Foad Ahmadi,[1] Golara Zahmatkesh,[1] Elani Streja,[1] Rajnish Mehrotra,[2] Connie Rhee,[1] Csaba P. Kovesdy,[3] Daniel L. Gillen,[1] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Univ of Washington, Seattle, WA; [3]Memphis VA MC, Memphis, TN.*

Background: We aimed to synthesize the results from all large and robust studies that have evaluated the association of body mass index (BMI) with mortality in patients receiving peritoneal dialysis (PD).

Methods: We searched MEDLINE, EMBASE, Web of Science, CINAHL, and Cochrane CENTRAL, and screened 7,123 retrieved studies for inclusion. Two investigators independently selected the studies using predefined criteria and assessed each study's quality using the Newcastle-Ottawa Quality Assessment Scale. We meta-analyzed the results of the largest studies with no overlap in their data sources.

Results: We included eight studies (total "n": 90,181) in the systematic review and four studies in the meta-analyses. The largest included study revealed that BMI might be differentially associated with one-, two-, and three-year mortalities. Our similar meta-analyses showed that being underweight was associated with trends towards higher one-, two-, and three-to-five year mortalities, while obesity was associated with trends towards a lower one-year mortality and higher two- and three-to-five-year mortalities.



Conclusions: Lower BMIs are associated with higher mortality over both shorter and longer duration while higher BMIs are associated with a lower mortality over shorter duration but a higher mortality over longer duration in PD patients. A plausible mechanism is that obesity favorably impacts short-term outcomes by attenuating malnutrition, while adversely impacting long-term mortality via cardiovascular sequelae.

Funding: NIDDK Support

SA-PO933

Visit-to-Visit Variabilitiy of Systolic Blood Pressure and Outcomes of Peritoneal Dialysis Patients Hyung Ah Jo,[1] Jung Nam An,[1,2] Jung Pyo Lee,[1,2] Chun Soo Lim,[1,2] Kook-Hwan Oh,[1] Yun Kyu Oh.[1,2] *[1]Dept of Internal Medicine, Seoul National Univ Hospital, Seoul, Korea; [2]Dept of Internal Medicine, Seoul National Univ Boramae Medical Center, Seoul, Korea.*

Background: Hypertension is common among patients with end stage renal disease and often remains poorly controlled in peritoneal dialysis (PD) patients. Many PD patients suffer of cardiovascular complications. Recent studies have suggested that a high visit-to-visit systolic blood pressure (SBP) variability is associated with an increase in subsequent cardiovascular events and complications. However, there are few studies about visit-to-visit SBP variability and outcomes in PD patients.

Methods: We reviewed 249 PD patients in Seoul National University Hospital from May 2005 to March 2011. Inclusion criteria were patients receiving PD at least 15 months. Subjects were followed up for an average of 42 months, during which time cardiovascular and mortality events were recorded. Among them, 39 patients were excluded due to short PD duration time and 2 patients had no SBP data. We retrospectively reviewed office SBP data up to two years later from PD start date. The subjects were grouped as quartile based on their office based SBP calculated using standard deviation. Primary outcomes were composite events of cardiovascular events and all cause mortality. Secondary outcome was residual renal function defined as anuria event.

Results: High SBP variability patients had significantly higher proportion of smoking history and C reactive protein and lower albumin and had more anti-hypertensive medications. During the follow-up, 15 patients had primary outcome. Although not significantly due to small event size, high visit-to-visit SBP variability patients had more cardiovascular events and all cause mortality. High SBP variability patients had significantly more experienced anuria events (Q1:5, Q2:8, Q3:13, Q4:17, p=0.010). Cox proportional hazard model revealed that fourth quartile of SBP variability patients had 5.223 hazard ratio than one quartile group.

Conclusions: Larger variability of visit to visit SBP in PD patients had more cardiovascular risk factors and correlated with renal function decline.

SA-PO934

Uric Acid and Mortality in Peritoneal Dialysis Patients Eunjin Bae, Hyunjeong Cho, Sunhwa Lee, Ji In Park, Nara Shin, Dong Ki Kim, Kwon Wook Joo, Yon Su Kim, Hajeong Lee. *Internal Medicine, Seoul National Univ Hospital, Seoul, Korea.*

Background: Although hyperuricemia as well-known risk factor for hypertension, coronary heart disease and chronic kidney disease, however, a role of uric acid (UA) in chronic dialysis patients is still scarce. We explored the association between UA level and mortality according to dialysis mode.

Methods: Among 1,682 prevalent dialysis patient enrolled in Clinical Research Center for End Stage Renal Disease (CRC-ESRD) in Korea, we included patients with available UA level at the time of enrollment. UA levels were categorized as follows: <5.5, 5.5–6.4, 6.5–7.4, 7.5–8.4 (reference group), and ≥8.5 mg/dL. Cox regression analysis was used to calculate the hazard ratio (HR) of all-cause mortality according to UA group.

Results: A total of 908 hemodialysis (HD) and 637 peritoneal dialysis (PD) patients were included in final analysis. UA level was significantly higher in HD (median [IQR], 7.2 [6.1–8.3]) than in PD (6.7 [5.8–7.6]) patients (p<0.001). Interestingly, UA level showed different relation with mortality according to dialysis mode. In PD patients, UA was negatively associated with mortality rate (p<0.001), however, it could not showed significant association in HD patients (p=0.739). In the Kaplan-Meire curve, we proved the lowest UA group (UA< 5.5 mg/dL) showed highest mortality rate in PD patients during median 18.7

months of follow up (Log-rank P<0.001). Compared with UA 7.5–8.4 group, UA <5.5 mg/dL predicted all-cause mortality even after adjusted by age, sex, body mass index, dialysis vintage, systolic blood pressure, diabetes, hemoglobin, hs-CRP, albumin level (HR; 4.408, 95% confidence interval; 1.490–13.042, p=0.007) in PD patients.

Conclusions: In this study, we elucidated that UA level of chronic dialysis patients had a different influence on mortality according to dialysis mode. Interestingly, we found paradoxical associations between UA level and mortality in PD patients, not in HD patients. Further prospective studies are warranted to validate these findings.

SA-PO935

Diastolic Dysfunction Is Related to Thyroid Hormone in Incident Patients on Dialysis Marcela Avila,[1] Virginia Sanchez,[2] Oscar Orihuela,[3] Carmen María del Prado,[1] Francisco Martinez-Baca,[4] Carmen Josefina Mora,[1] Teresa Renata Romero,[1] Ramon Paniagua-Sierra.[1] *[1]Unidad de Investigacion Medica en Enfermedades Nefrologicas, Hospital de Especialidades, CMN S XXI IMSS, Mexico, D.F., Mexico; [2]Medicina Interna, Hospital General de Zona 1 Venados, IMSS, Mexico, D.F., Mexico; [3]Servicio de Cardiologia, Hospital de Especialidades, CMN SXXI IMSS, Mexico, D.F., Mexico; [4]Hospital de Cardiología, CMN SXXI IMSS, Mexico, D.F., Mexico.*

Background: Diastolic Dysfunction (DD) is a frequent findingin patients on dialysis. Thyroid hormones (TH) are related to anatomical cause of DD; however, information about the relationship with DDis scarce. Our aim was to know if this association holds true in incident patients on dialysis.

Methods: A cross-sectional study design with 197 incident patients on dialysis programs was performed. Clinical and demographic data were registered. Diastolic dysfunction was evaluated using early diastolic mitral and annulus inflow velocities (cm/s); e´, E/e´, E/A ratios, with pulsed-wave Doppler echocardiography.Plasma determination from TSH, total and free T3 (t/f), T4 (t/f) were measured by RIA Diagnostic Kits.

Results: Mean age was 47.1 years, 64% men, BMI 25.3 Kg/m^2, SBP and DBP; 135.2 and of 82.6 mm Hg respectively; 42% were diabetic (DM) and 58% were on CAPD. Plasma tT3 <0.6 ng/mL in 30.2% of dialysis patients,TSH ≥ 4.5 µIU/mL in 28.1%, TSH (4.5-10µIU/mL+ fT4 (7-18pg/mL) in 32%.DD was presented in 62%;grade I 18%; grade II 8% and grade III 36%. The patients with DD were older and more had DM (all p<0.001) than not. On multiple regression analysis, (t/f) T3was inversely associated with and E/é (r=-0.20, p<0.05 and r=-0.24 p<0.01). tT4 with E/é (r=-0.24, p<0.001) and with E/A (r=-0.22 p<0.01) fT4 with ejection fraction (EF), (r= -0.157, p<0.03). On multiple logistic regression analysis; OR (95%CI,); tT3; 8.72 (1.2-63,p<0.03) tT4; 0.96(0.94-0.98,p<0.002), tT4; 1.26 (1.06-1.49,p<0.007) and age; 0.89(0.85-0.93,p<0.001), were independently risk factor for DD, gender 0.57(0.22-1.41,p<0.22) and DM 0.66(0.29-1.48p<0.31) were not.

Conclusions: Our study showed high prevalence of diastolic dysfunction in incident patients on dialysis; the independent risk factors for DD were Total T3, Total and free T4, and age.

Funding: Government Support - Non-U.S.

SA-PO936

Stability of Intraperitoneal Antibiotics in Extraneal 7.5% Icodextrin Peritoneal Dialysis Bags Dwarakanathan Ranganathan,[1] Saiyuri Naicker,[2] Steven C. Wallis,[2] Jeffrey Lipman,[1,2] Sharad K. Ratanjee,[1] Jason A. Roberts.[1,2] *[1]Renal Medicine/Critical Care Medicine, Royal Brisbane and Women's Hospital, Herston, Brisbane, Queensland, Australia; [2]Burns, Trauma and Critical Care Reserch Unit, Univ of Queensland, Herston, Brisbane, Queensland, Australia.*

Background: The standard treatment of peritoneal dialysis (PD)-associated peritonitis is intraperitoneal administration of antibiotics into the PD solution. Effective empiric antibiotics include vancomycin (VCM) in combination with gentamicin (GTM). Patients with PD-associated peritonitis may be advised to store PD bags with pre-mixed antibiotics at home. The purpose of this study was to assess the stability of pre-mixed antibiotics in PD bags when stored at different temperatures over a 14 day period.

Methods: 27 Baxter 7.5% Icodextrin (Extraneal) PD bags were dosed with GTM alone (n=9), VCM alone (n=9) and GTM and VCM in combination (n=9). The concentration of GTM was intended to be 20 mg/L and VCM 1000 mg/L in all experiments. Bags were stored in triplicate at 37°C, room temperature (25°C) and refrigeration (4°C). Samples from the bags were taken at 0, 6, 24, 48, 96, 168 and 336 hours. Antibiotic concentrations were quantified using a validated chromatographic method for each antibiotic. The initial concentration of the 0 hour sample was considered as 100%. A storage interval was considered unstable if the concentration of the antibiotic dropped <90% of the initial value.

Results: GTM alone was found to be stable for 14 days at all temperatures. VCM alone was stable for 4 days at 37°C and for 14 days at both 25°C and 4°C. The combination of GTM and VCM was stable for 4 days at 37°C and for 14 days at 25°C and 4°C.

Conclusions: Icodextrin PD bags pre-mixed with GTM and VCM alone and in combination with each other can remain stable when stored at room temperature and refrigeration for up to 14 days. At an elevated temperature, 37°C, whilst GTM remained stable for 14 days, VCM was only stable for 4 days, as was the combination of the two. To our knowledge, the stability of VCM in combination with GTM in Icodextrin solution for 14 days has not been previously assessed.

Funding: Pharmaceutical Company Support - Baxter Healthcare Pty. Ltd,Australia(Provided Icodextrin bags)

SA-PO937

Stability of Cephalosporin Antibiotics in Extraneal 7.5% Icodextrin Peritoneal Dialysis Bags Dwarakanathan Ranganathan,[1] Saiyuri Naicker,[2] Steven C. Wallis,[2] Jeffrey Lipman,[1,2] Sharad K. Ratanjee,[1] Jason A. Roberts.[1,2] *[1]Renal Medicine/Critical Care Medicine, Royal Brisbane Hospital, Brisbane, Queensland, Australia; [2]Burns ,Trauma and Critical Care Reserch Centre, Univ of Queensland, Brisbane, Queensland, Australia.*

Background: The standard treatment of peritoneal dialysis (PD)-associated peritonitis is intraperitoneal administration of antibiotics into the PD solution. Effective empiric antibiotics include cefazolin (CZL) in combination with gentamicin (GM) or ceftazidime (CTD). Patients with PD- associated peritonitis may be advised to store PD bags with pre-mixed antibiotics at home. The purpose of this study was to assess the stability of pre-mixed cephalosporin antibiotics in PD bags when stored at different temperatures over a 14 day period.

Methods: 36 Baxter 7.5% Icodextrin (Extraneal) PD bags were dosed with CZL alone (n=9), CTD alone (n=9), CZL and CTD (n=9), and CZL and GM in combination (n=9). The concentrations of CZN and CTD were intended to be 500mg/l and GM at 20mg/l. Bags were stored in triplicate at 37°C, room temperature (25°C) and refrigeration (4°C). Samples were taken at 0, 6, 24, 48, 96, 168 and 336 hours. Antibiotic concentrations were quantified using validated chromatographic method for each antibiotic. The initial concentration of the 0 hour sample was considered at 100%. A storage interval was considered unstable if the concentration of the antibiotic dropped ≤90% of the initial value.

Results: CZL alone was stable for 24 hours at 37°C, 7 days at 25°C and 14 days at 4°C. CTD alone was found to be stable for only 6 hours at 37°C, 2 days at 25°C and 14 days at 4°C. The combination of CZL and CTD was stable for 24 hours at 37°C, for 2 days at 25°C and for 14 days at 4°C. The combination of CZL and GM was stable for 1 day at 37°C, for 4 days at 25°C and for 14 days at 4°C.

Conclusions: Icodextrin PD bags pre-mixed with CZL alone, CTD alone, combination of CZL and CTD, and combination of CZL and GM can remain stable if refrigerated for up to 14 days. At room temperature, CZL stability approaches 7 days whilst CTD stability is only 2 days. To our knowledge, the stability of CZL in combination with CZL and GM in icodextrin solution for **14 days** has not been previously assessed.

Funding: Pharmaceutical Company Support - Baxter Healthcare Pty. Ltd, Australia (Provided Icodextrin bags)

SA-PO938

Inflammatory Biomarker Pairs as Outcome Measures in Peritoneal Dialysis Tiane Dai, Ying Wang, Sharon G. Adler. *Nephrology, Harbor-UCLA Medical Center, Torrance, CA.*

Background: We showed JAK/STAT pathway activation in the peritoneal membrane of rats receiving peritoneal dialysis (PD) with 4.25% Dianeal; concomitant PD with a JAK1/2 inhibitor attenuated inflammation, fibrosis, and hypervascularity. We also showed that Long-term (LT) PD patients had higher mean levels of the inflammatory and fibrotic STAT-regulated biomarkers MCP-1 and periostin than New PD patients (KI, in press). High MCP-1 and periostin levels in LT versus New patients were observed in a subset, but not all LT patients.

Methods: We measured inflammation/injury biomarkers in PD effluent and performed an analysis of pairs of baseline PD effluent inflammatory biomarkers in LT (n=8) and New (n=8) PD patients to identify pairs that separated LT patients with higher levels of inflammation/injury from New and LT patients with lower values. We then determined whether these pairs predicted a composite outcome of death and/or PD membrane failure (defined by the subsequent need for icodextrin or transfer to hemodialysis due to membrane failure) after a follow-up period of 29 +2months.

Results: Of 83 analytes tested, 28 could be reliably measured by electrochemiluminescence (Meso Scale Discovery, Rockville, MD). In this small sample, some analyte pairs separated LT from New PD patients. The best biomarker pairs included secreted protein acidic and rich in cysteine (SPARC, aka osteonectin) and were SPARC/Cadherin-3; SPARC/ICAM1; SPARC/TNFα; SPARC/CA125; and SPARC/MCP-1. Graphic representation of these relationships show a clustering of a subset of four LT patients whose PD effluent values distinguished them from other LT and New patients. These LT PD patients had a statistically significantly higher risk for the composite outcome than comparator patients (Chi square, p=0.02). The pairs associated with risk better than SPARC alone.

Conclusions: These data suggest that pairs of PD effluent inflammatory biomarkers may reflect evolving inflammation and fibrosis in PD patients. The concentration of SPARC in PD effluent, particularly in combination with MCP-1, may be useful in predicting membrane failure and/or death in LT PD patients.

SA-PO939

Rate of Decline of Residual Kidney Function before and after the Start of Peritoneal Dialysis Lian He,[1,2] Zita C. Abreu,[2] Tushar Malavade,[2] Charmaine E. Lok,[2] Joanne M. Bargman.[2] *[1]Peking Univ 3rd Hospital, Beijing, China; [2]Univ of Toronto, Toronto General Hospital, Toronto, ON, Canada.*

Background: There is a paucity of information on whether peritoneal dialysis (PD) slows the decline of residual kidney function (RKF)compared to the natural slope of RKF decline prior to dialysis start. Confounding factors may contribute to the change in RKF in patients who receive PD. Our aim was to analyze the course of RKF decline before and after initiating PD, and to determine the main factors affecting RKF decline during the PD period.

Methods: In this retrospective study, we determined individual glomerular filtration rates (GFR) (ml/min/ 1.73m^2 per month) for the 12 months before and 12 months after initiating PD in 77 new PD patients in a large academic medical center (2008-2012). The GFR was estimated by the MDRD equation in the predialysis period and by averaging 24-hour urine creatinine and urea clearances in the PD period. The rate of RKF decline was calculated using unadjusted linear regression analysis. Wilcoxon signed rank test was used to compare RKF decline before and after peritoneal dialysis initiation. Multivariate linear regression was used to identify independent risk factors for RKF decline.

Results: A slower mean rate of RKF decline was observed in the PD period compared with the predialysis period (–0.21±0.30 versus –0.59 ± 0.55 ml/min/1.73 m^2/month, P< 0.01). Higher baseline RKF, higher serum phosphate and older age were independently associated with faster decline of RKF (all P<0.01). There were no other clinical associations with the rate of RKF decline (e.g. gender, comorbidities, use of ACEI/ARB or use of diuretics during the 1styear of PD).

Conclusions: In patients with advanced chronic kidney disease, initiating PD was associated with a slower mean rate of RKF decline compared to the rate in the predialysis period. Higher RKF, higher serum phosphate and older age at PD initiation are independent factors for a faster decline of RKF in PD phase.

SA-PO940

Carotid Artery Changes in Uremic Patients Undergoing Peritoneal Dialysis Treatment Damir Rebic, Senija Rasic. *Clinic for Nephrology, University Clinical Center of Sarajevo, Sarajevo, Bosnia and Herzegowina.*

Background: We compared the structural and hemodynamic changes in the common carotid arteries (CCA) between end-stage renal disease (ESRD) patients before turning on the renal replacement therapy and after 18 months of peritoneal dialysis (PD) treatment with the objective to identify predicting risk factors for atherosclerosis and the impact of PD treatment on vascular remodeling.

Methods: This longitudinal study included 50 patients before start and 18 months after PD treatment. It was used B-mode ultrasonography to study changes CCA, were divided into groups of subjects according Mannheim consensus for carotid ultrasonography (groups AS0, AS1, AS2, AS3). A simplified peritoneal equilibration test was performed after 6 months of PD treatment, and at the end of the observation period. A multiple regression analysis was applied to examine the relationship between CCA ultrasound parameters (intima—media thickness (IMT), plaque score, CCA diameter, peak systolic velocity (PSV) and end-diastolic velocity (EDV)), parameters of PD and a set of clinical and laboratory parameters.

Results: After 18 months of PD treatment IMT, plaque score, PSV and EDV was significantly lower than patients in ESRD. The adequacy of dialysis, compared to other observed group was better in AS0 group of patients at the beginning, but at the end of the study. The value of endothelin-1 (ET-1) at baseline were higher in comparison with value after 18 months PD (6.32 versus 4.0 pg/mL), in contrast to nitric oxide (NO), which showed an inverse relationship (40.72 versus 48.0 µmol/L). At the beginning of the study 44% of patients had severe atherosclerosis (AS3) until after 18 months of PD treatment 26% of patients had AS3 (p <0.016).

Conclusions: Homocysteine, ET-1, low-density lipoprotein, high-density lipoprotein, lipoprotein(a), C-reactive protein and product CaxP are an independent risk factor for atherosclerosis and was significantly correlated with IMT CCA. The noninvasive carotid ultrasound can be used to monitor atherosclerosis in PD patients. The treatment with PD, with the regulation of these vasoactive molecules and other vascular risk factors, importantly impedes vascular remodeling.

SA-PO941

Prognostic Value of Predialysis Indices for Early Technique Failure in Peritoneal Dialysis Patients Masaru Matsui, Yasuhiro Akai, Katsuhiko Morimoto, Ken-Ichi Samejima, Yoshihiko Saito. *First Dept of Internal Medicine, Nara Medical Univ, Kashihara, Japan.*

Background: Peritoneal dialysis (PD) offers several advantages in preserving residual renal function and improving survival compared hemodialysis (HD). Early PD technique failure is defined as the switch from PD to HD in relatively early phase after instituting PD. This failure is often due to poor volume control and antibiotics resistant peritonitis. A number of predictors of early PD technique failure were reported but these factors were mostly obtained after starting PD, while predialysis predictors for early technique failure have remained unclear. We conducted this study to investigate the prognostic value of predialysis indices for early technique failure in PD patients.

Methods: We recruited consecutive 183 PD patients who were treated at Nara Medical University Hospital between April 1, 1997 and December 31, 2012. Forty-two patients were excluded because of transition from HD and withdrawal from PD within three months, leaving 141 patients for analysis. Clinical characteristics and laboratory data within three months BEFORE PD initiation were analyzed. The primary outcome was the composite of time to combined use of HD and transition to HD within two years after PD institution.

Results: Overall, mean age of the patients was 61±14 years and 89 patients (63%) were male. During study period, primary outcome was observed in 38 patients. Although diabetes, blood pressure and hemoglobin levels were similar between participants with and without outcome events, obesity and serum levels of albumin and corrected calcium were significantly different. Multivariate followed by univariate Cox proportional hazard regression analysis revealed that older age [hazard ratio (HR) 2.83(1.42-5.92)], obesity [HR 3.01(1.53-5.79)], low serum levels of albumin [HR 2.78 (1.40-5.55)] and corrected calcium [HR 2.78 (1.37-5.69)] were independent predictors of early PD technique failure.

Conclusions: Pre-dialysis older age, obesity and low serum albumin and calcium could be associated with early technique failure in PD patients.

SA-PO942

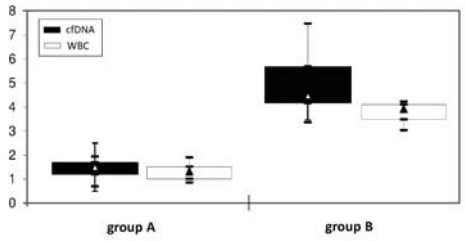
Cell-Free DNA: A New Method to Determine Acute Cell Damage in Peritoneal Membrane Grazia Maria Virzì, Sabrina Milan Manani, Alessandra Brocca, Massimo de Cal, Roberto Zambon, Chiara Pasqualin, Ilaria Tantillo, Carlo Crepaldi, Claudio Ronco. *Nephrology-IRRIV, St. Bortolo Hosp.*

Background: Peritonitis remains a frequent complication of peritoneal dialysis (PD). Cell-free DNA (cfDNA) is a circulating extracellular DNA fragment and originates from necrotic and apoptotic cells. cfDNA is present in the peritoneal effluent of PD patietns (pts). The objective of this study was to compare cfDNA levels in the peritoneal effluent in PD pts with and without peritonitis.

Methods: We enrolled 53 PD pts: 30 pts without history of systemic inflammmation and peritonitis in the last 3 months (group A, 17 male, 66±14 years) and 23 pts with acute peritonitis (group B, 14 male, mean age 68 ±16 years). CfDNA was extracted from peritoneal effluent and was quantified by Real time PCR in Genome Equivalent (GE)/ml for β-globin gene.

Results: The median concentration of cfDNA in the peritoneal effluent in group A was 31 GE/ml (16-51), as previosly reported. The concentration in group B was 25523 GE/ml (IQR 18831-459541). Quantitative analysis of cfDNA showed significantly higher levels in pts with peritonitis compared with pts without (Figure 1) (p<.01), similarly as WBC increasing during acute peritonitis. A significant positive correlation was observed between cfDNA and WBC values (rho=.885, p<.01). In group B, there is no difference in cfDNA levels between Gram+/- peritonitis, pts with single episode and relapsing peritonitis, but there is a significantly difference in cfDNA concentration between PD pts with positive and negative outcomes (62137, IQR 12850-119541 versus 122793, IQR 20159-607353; p<.01).

Conclusions: Our data demonstrated that cfDNA is increased in peritoneal effluent of PD pts with acute peritonitis. We speculate that cfDNA quantification in peritoneal effluent could be an innovative method to determine acute cell damage *in vivo* origined by apoptosis and necrosis in peritoneal cells.



Funding: Private Foundation Support

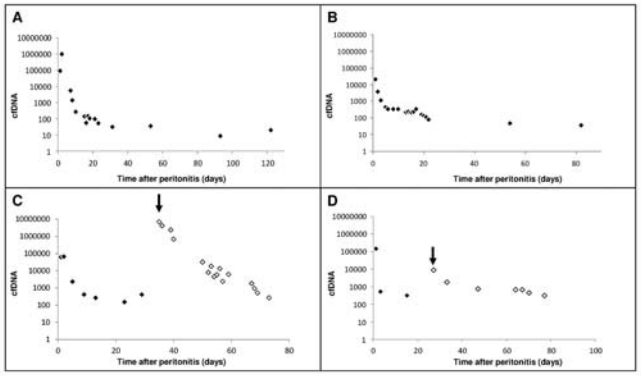
SA-PO943

Cell-free DNA Trend in 23 PD Patients with Peritonitis Sabrina Milan Manani, Grazia Maria Virzì, Alessandra Brocca, Silvia Pastori, Chiara Pasqualin, Massimo de Cal, Ilaria Tantillo, Carlo Crepaldi, Claudio Ronco. *Nephrology-IRRIV, St. Bortolo Hosp.*

Background: Peritonitis is still the major complication associated with peritoneal dialysis (PD). Perionitis treatment should aim for rapid resolution of inflammation. Cell free DNA (cfDNA) is present in the peritoneal effluent of PD patients (pts). There is no data on cfDNA in PD pts with peritonitis. We present a case series of 23 PD pts with peritonitis.

Methods: 23 PD pts (14 M; mean age 63.1±16.3) were enrolled. We collected peritoneal effluents from the first day of peritonitis until the 120th day. WBC counts were routinely measured. CfDNA was extracted from peritoneal effluents and quantified by Real time PCR for the β-globin gene (genome equivalents (GE)/ml).

Results: 17 pts were treated with CAPD and 6 with APD. The average length of PD treatment was 21 months (minimum: 3.6–maximum: 132.9). All pts were treated and clinically recovered from peritonitis after 13.5 days±5.4. 18 pts had a first episode of peritonitis and responded to first-line antibiotics. 5 had a relapsing episode of peritonitis; subsequently, responded to another course of intra-peritoneal antibiotics. We observed a similar cfDNA trend in all patients. The cfDNA concentration in the peritoneal effluent at the first day of peritonitis was very high, concordaly with WBC. Then, cfDNA level tended to progressively decrease during follow up for each patient. CfDNA level diminished slowerly than WBC count. In 5 relapsing peritonitis, at the first day, we observed a new rapid increased of cfDNA level (consistent with WBC). We reported 4 pts'trends.



Conclusions: We hypothesized that cfDNA in peritoneal effluent could be a specific indicator of peritonitis. However, the exact characteristics of cfDNA kinetics remained uncertain. Further studies are warranted to clarify the precise mechanism and clinical significance of elevated cfDNA in this population.

Funding: Private Foundation Support

SA-PO944

Peritoneal Dialysis Effluent and Serum Angiopoietin-Like Protein 2 Levels in Peritoneal Dialysis Patients Shinichi Abe,[1] Yoko Obata,[1] Tomoya Nishino,[1] Kumiko Muta,[1] Takehiko Koji,[2] Shigeru Kohno.[1] *[1]Second Dept of Internal Medicine, Nagasaki Univ School of Medicine, Nagasaki City, Japan; [2]Dept of Histology and Cell Biology, Nagasaki Univ Graduate School of Biomedical Science, Nagasaki City, Japan.*

Background: Angiopoietin-like protein 2 (Angptl2) is a protein which is structurally similar to angiopoietin, and is primarily secreted by adipose tissue. Recent reports suggest that Angptl2 induces chronic inflammation and angiogenesis via promoting the production of inflammatory cytokines such as IL-6. Meanwhile, chronic inflammation concerns the increase of peritoneal permeability in peritoneal dialysis (PD) patients, and there have been no studies which assess PD effluent or serum Angptl2 levels in PD patients. We measured Angptl2 levels in the PD effluent (P-Angptl2) and serum (S-Angptl2), and investigated the relationship between Angptl2 and peritoneal function.

Methods: Subjects were 33 PD patients in our hospital. PD effluent and serum samples of each patient were collected at the time of peritoneal equilibration test, which was performed at 12 and 24 months after the start of PD. Angptl2 and IL-6 levels were measured by ELISA, and retrospectively analyzed the relationship with D/P creatinine or other laboratory data.

Results: A positive correlation was observed between P-Angptl2 level and D/P creatinine, both at 12 and 24 months (12 months later: r=0.58, p=0.04, 24 months later: r=0.86, p<0.01). Meanwhile, S-Angptl2 level had no correlation with P-Angptl2 level or D/P creatinine. P-Angptl2 and PD effluent IL-6 levels showed positive correlation only at 24 months (12 months later: r=0.45, p=0.12, 24 months later: r=0.84, p=0.02). The comparison between P-Angptl2 and S-Angptl2 levels indicated that Angptl2 is locally secreted in peritoneal cavity.

Conclusions: Our results indicate that P-Angptl2 level may become an indicator of peritoneal function in PD patients. In addition, it is suggested that Angptl2 is locally secreted in peritoneal cavity and involved in the increased peritoneal permeability through IL-6 induction in PD patients.

SA-PO945

Incidence of Encapsulating Peritoneal Sclerosis: A Single-Center Experience with Long-Term Peritoneal Dialysis in Saudi Arabia Naveed Aslam, Seddeg Younis, Dildar Ahmed Laghari, Ebadur Rahman. *Nephrology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.*

Background: Encapsulating peritoneal sclerosis (EPS) is a serious complication of long-term peritoneal dialysis (PD). The reported incidence varies between 0.5% and 4.4% and increases with length of time on PD. According to our knowledge there is no data available on Epidemiology of EPS in Saudi Arabia. The aim of the present study was to assess the incidence of EPS and outcome in a tertiary care hospital in Riyadh.

Methods: In all the patients started on Peritoneal Dialysis from Jan 2000 to Dec 2013 in Prince Sultan Military Medical City and maintained on PD for 5 or more years, clinical symptoms were documented and abdominal computed tomography (CT) findings were reviewed. Patients were tracked whether they remained on PD, transferred to hemodialysis (HD), underwent transplantation or died.

Results: Among the total of 350 patients retrieved from data base, only 12 patients met the inclusion criteria (dialysis duration 5 or more years). 2 of them were transplanted, 2 were shifted on haemodialysis and 8 patients are still on PD.

Patient Age(Years)/Sex	Duration on PD (Years)	CT Score	Albumin (g/l) (38-51)	ESR (mm/hr) (nr 0-15)	CRP (mg/l) (nr 0-6)	CrCl	Kt/V
40/F	14	8	37	10	2	65	2.1
59/M	11	6	35	8	4	62	2.3
24/M	9	1	45	15	6	58	2.4
57/M	9	1	38	12	2	59	2.3
78/M	7	2	33	11	8	48	1.9
78/F	6	1	39	20	1	55	2.1
43/M	6	1	41	13	3	66	2.2
39/M	5	1	42	17	7	64	2.4

Abdominal CT imaging was done in all patients; only 2 patients had radiological features consistent with EPS, with a radiological score of 8 and 6; others had localized peritoneal calcification. None of the patients met the 2000 criteria of International Society of Peritoneal Dialysis for a diagnosis of EPS; all having good clearance and normal nutritional and inflammatory markers.

Conclusions: The present study suggests that, in patients maintained on PD for 5 or more years at our center, the incidence of EPS is nill. Localized Peritoneal calcification could be part of atherosclerotic changes. These findings need to be confirmed in other centers in Saudi Arabia.

Funding: Government Support - Non-U.S.

SA-PO946

Clinical Characteristics and Outcome of Elderly Patients on Peritoneal Dialysis: A Retrospective Single Center Experience Naveed Aslam, Dildar Ahmed Laghari, Seddeg Younis, Ebadur Rahman. *Nephrology, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.*

Background: The number of elderly ESRD patients is on rise in Saudi Arabia. A few studies specifically investigating elderly patients on PD (peritoneal dialysis) show great uncertainty on the factors involved in the vital prognosis; however, PD has recently become clinically acceptable as an appropriate therapy for these patients. The objective of this study is to describe outcome, in elderly patients (age > 65 years), in terms of technique and patient survival and possible correlation with peritoneal transport status (PTS), anthropometric data, biochemical data and dialysis adequacy (Kt/V).

Methods: In a retrospective, single-center study, we evaluated a total of 96 patients, aged 65 years and above, newly started on PD during January 2006 to December 2013. Serum albumin, hemoglobin, b2-microglobulin, CRP, triglyceride, creatinine, calcium, phosphate, PTH were assessed. Withdrawal from PD, peritonitis and death were recorded.

Results: The average age of patients starting PD was 78.3 ± 7.2 and the percentage of diabetes was 35%. The 2 and 5-year rates of patients survival were 86% and 63% respectively. Technique survival rates were 64% and 26% respectively. Only 10% of the patients could perform their PD therapy without the assistance of any family member or nurses. 78% of the patients showed high peritoneal transport property (High: 28%, High-Average: 50%, Low-Average: 20%, Low: 2%). Only 50% of the patients were on CAPD. Albumin level and CRP were negatively associated with patient survival.

Conclusions: Overall elderly patient survival was not different compared to younger patients. The high transport property is a contributory factor in low serum albumin levels. We consider that PD in elderly patients is safe with good efficacy and quality of life. There are no medical concerns to introducing PD therapy in elderly ESRD patients.

Funding: Government Support - Non-U.S.

SA-PO947

Hair Zinc Concentration Correlates with Cardiac Contractility in Peritoneal Dialysis Patients Hideki Yamahara,[1] Takanobu Imada,[1] Yoshiki Okuno,[1] Maiko Seo,[1] Tatsuyori Morita,[1] Keiko Kono,[1] Hiroya Masaki,[2] Mitsushige Nishikawa,[1] Ichiro Shiojima.[1] *[1]Dept of Medicine II, Kansai Medical Univ, Hirakata, Osaka, Japan; [2]Dept of Laboratory Medicine and Clinical Sciences, Kansai Medical Univ, Hirakata, Osaka, Japan.*

Background: Renal failure patients often have trace elements deficiency due to dietary restriction. Zinc is an essential micronutrient, and its deficiency is associated with many diseases including cardiovascular disease. Because serum zinc concentration shows a diurnal variation, we selected the zinc concentration in the hair as a long-term measure of body zinc content. Peritoneal dialysis (PD) is an important renal replacement therapy for end stage renal disease patients, since it improves their quality of life and reduces dietary restriction. Nevertheless, the association between various diseases and trace elements kinetics in PD patients are not well elucidated.

Methods: Hair zinc concentration (HZn) was measured in 16 PD patients using inductively coupled plasma mass spectorometry (La Belle Vie Inc. Japan). We also measured eGFR, serum zinc concentration (SZn), and body fluid volume markers (hANP, BNP, and NT-proBNP), and performed transthoracic echocardiography. Because fluctuation range is large in hair zinc concentration, common logarithm was used for statistical analysis.

Results: HZn in PD patients showed a distribution similar to normal control group (Average 165,948 ppb, -1SD 123,310ppb, +1SD 223,357ppb). There was no significant correlation between HZn and SZn (r=-0.149, p=0.589). HZn negatively correlated with NT-proBNP (r=-0.592, p=0.0183), but not with hANP or BNP. HZn positively correlated with ejection fraction (r=0.535, p=0.0385), and negatively correlated with left ventricular dimensions in end-diastole (r=-0.518, p=0.047) and end-systole (r=-0.552, p=0.0315). SZn did not correlate with any of these factors.

Conclusions: In PD patients, hair zinc concentration correlates with NT-proBNP and cardiac contractility. NT-proBNP was previously reported to be a predictor of cardiovascular

disease in renal failure patients. Whether body zinc content can be a predictor of cardiovascular disease in patients with end stage renal disease awaits further investigation.

SA-PO948

Epidemiological Characteristics and Modality Impact on Long-Term Survival in a Cohort of Elderly Incident PD Patients
Natalia Maria da Silva Fernandes,[1,2,3] Marcia R.G. Franco,[1,2,3] Abdul Rashid Tony Qureshi,[4,5] Jose C. Divino-Filho.[4] *[1]Dept of Internal Medicine, School of Medicine, Federal Univ of Juiz de Fora, Juiz de Fora, Brazil; [2]NIEPEN (Núcleo Interdisciplinar de Ensino e Pesquisa em Nefrologia), Federal Univ of Juiz de Fora, Juiz de Fora, Brazil; [3]IMEPEN Foundation, Juiz de Fora, Brazil; [4]Div of Renal Medicine, CLINTEC, Karolinska Instt, Stockholm, Sweden; [5]Baxter Novum Div of Renal Medicine, CLINTEC, Karolinska Instt, Stockholm, Sweden.*

Background: As the number of elderly patients starting renal replacement therapy (RRT) increases, it is important to understand their clinical characteristics as well as the most appropriate RRT for this group of patients**.** To describe a cohort of elderly incident PD patients and to evaluate the modality impact on the long-term survival.

Methods: Multicenter prospective cohort (Dez/2004-Oct/2007) with 2144 elegible patients; 762 ≥ 65 years old. Patients were followed up until transfer to HD, recovery of renal function, renal transplantation, death or loss to follow-up. Demographic and clinical data were evaluated at study enrollment and described as mean±SD, median or percentage. As RR is not proportional along the time, a time-dependent Cox analysis was performed, with PD modality (APD versus CAPD) as dependent variable.

Results: Mean age 74.6 ±6.7 yrs, 53% women, 69% Caucasians. Pre-dialysis nephrological care was given to 56% of the patients, 54 % received dialytic modalities information and only 23% chose PD as an option. In relation to family income, 31% earned up to 2 minimum wage per month, 58.1% had basic education and the most frequent co-morbidity were hypertension (78%) and diabetes (50%) with 47% presenting Davies score >=2. Death occured in 31% of the patients.

Follow-up time (Reference APD vs CAPD)	HR (CI)
<18months	1.11(0.45-1.46)
>18months	0.25(0.73-0.86)

Conclusions: Death risk changes along time in elderly incident PD patients, according to the modality. Up to 18 months there was no difference, but beyond this point in time APD is a protective factor.

SA-PO949

The Impact of Body Mass Index (BMI) and Longitudinal Body Weight (BW) Changes on Survival of Elderly Incident PD Patients: A Cohort Analysis
Natalia Maria da Silva Fernandes,[1,2] Marcia R.G. Franco,[1,2] Abdul Rashid Tony Qureshi,[3,4] Jose C. Divino-Filho.[3] *[1]Dept of Internal Medicine, School of Medicine, NIEPEN-Federal Univ, Juiz de Fora, Brazil; [2]Fundacao IMEPEN, Juiz de Fora, Brazil; [3]Dept Renal Medicine CLINTEC, Karolinska Instt, Sweden; [4]Baxter Novum, Karolinska Instt, Sweden.*

Background: The impact of BMI and BW changes on survival of elderly patients on PD is controversial. **GOAL**: To evaluate the impact of BMI and BW changes on survival of elderly patients on PD.

Methods: Multicenter prospective cohort (Dec/2004-Oct/2007) with 2144 elegible patients; 762 ≥ 65 years old and 733 with at least two BMI measurements. Patients were followed up until transfer to HD, recovery of renal function, renal transplantation, death or loss to follow-up. They were divided in two groups: PD as first therapy (PD-first: 333) and those transferred from HD (HD-first: 400). Comparison in between groups (PD and HD first) and, sociodemographic and clinical data compared among the patients classified according to BMI using ANOVA, Kruskal Wallis or Chi-square. Survival analysed with Kaplan Meier curve and Cox regression adjusted for confounding variables, besides longitudinal changes in BW during follow-up.

Results: Patients with higher BMI were older, had more comorbidities, higher levels of blood pressure and glycemia. Table 1- Analysis of the impact of BMI on survival (Cox regression adjusted and non-adjusted)

Non Adjusted	Malnourished	1.74(0.99-3.07)	1.88(1.00-3.55)
	Overweight	0.64(0.42-0.98)	0.89(0.55-1.43)
	Obese	0.67(0.37-1.19)	0.72(0.34-1.53)
Adjusted	Malnoursihed	1.24(0.66-2.33)	2.09(1.06-4.1)
	Overnight	0.70(0.45-1.10	1.02(0.60-1.72)
	Obese	0.61(0.33-1.14)	0.76(0.35-1.68)

Conclusions: Malnourished PD-first patients presented higher mortality. Those patients who gained BW during the first year in both groups had lower mortality.

SA-PO950

Serum Gamma-Glutamyltransferase Levels Predict Mortality in Peritoneal Dialysis Patients
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Background: Increased gamma-glutamyltransferase (GGT) is associated with oxidative stress and all-cause mortality in the general population. However, its role in peritoneal dialysis (PD) patients is unclear. The aim of this study is to determine the association between serum GGT levels and mortality in PD patients.

Methods: PD patients were selected from Clinical Research Center registry for End Stage Renal Disease cohort in Korea. Patients were categorized into three groups by tertiles of serum GGT levels as follows: Tertile 1, GGT < 17 U/L ; Tertile 2, 17 ≤ GGT ≤ 27 U/L; Tertile 3, GGT > 27 U/L. Cox regression analysis was used to calculate the adjusted hazard ratio (HR) of mortality with a serum GGT levels of tertile 1 as the reference.

Results: A total of 757 PD patients were included. The median follow-up period was 28 months.The multivariate Cox proportional hazard model showed that the higher tertiles of serum GGT levels were associated with highermortality (Tertile 2: HR 2.10, 95% confidence interval (CI), 1.13-3.88, p=0.018, Tertile 3: HR 1.99, 95% CI, 1.10-3.61, p=0.023) after adjustment for clinical variables.

Conclusions: Our data showed that higher serum GGT levels were associated with increased mortality in PD patients. These findings suggest that serum GGT levels are useful predictor for mortality in PD patients.

SA-PO951

Peritoneal Dialysis in Patients with Refractory Congestive Heart Failure
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Background: Refractory congestive heart failure (RCHF) is a major reason of mortality and morbidity. Leading cause of hospitalization even in patients receiving treatment. Diuretics as firstline therapy for RCHF are linked with worsening kidney function, and progression of heart failure. Peritoneal dialysis (PD) can be a good option. Our aim is to describe relative risk benefit ratio based on data reported for use of PD in RCHF.

Methods: Electronic database MEDLINE and relevant articles are included in the database from 1951 to February 2014 to identify studies on PD in the RCHF. References were screened for relevant articles. Eligibility criteria:(1)Prospective or retrospective(2) Age≥18years(3)RCHF(4)At least 10 PD treatments.

Results: Within 864 citations, 21 studies (n=673 patients) were identified including 14 prospective studies and 7 retrospective studies. The mean age is 66.9 years; the mean percentage of males is 71.5% (28.5% females). After PD, hospitalization days significantly declined (6.22 *versus* 1.19, p=0.0008), heart function improved significantly (LVEF:34.38 *versus* 39.07, p=0.0027; NYHA score: 3.55 *versus* 2.17, p=0.0000). No change in renal function at 1% significance level, but at 5% (p=0.0322). Body weight decreased (73.28 *versus* 69.54, p=0.0024) and diuretic use hold (229.20 *versus* 248.64p=0.7890). Yearly average peritonitis was13.9%, average mortality was 21.5%.

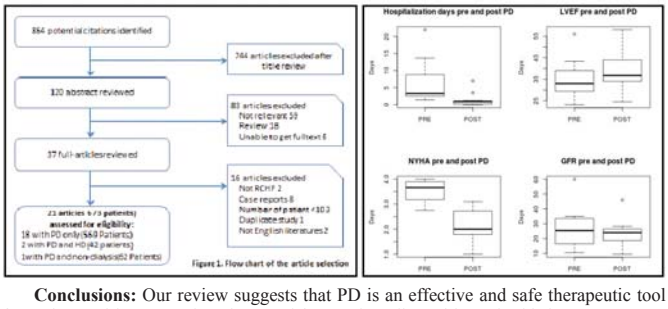

Figure 1. Flow chart of the article selection

Conclusions: Our review suggests that PD is an effective and safe therapeutic tool for patients with RCHF.There is a need for good-quality evidence in this important area.

SA-PO952

Report of Sudden Decrease in Rates of Culture Negative Peritonitis with Adoption of a New Policy: Single Center Experience in an Urban Inner City Peritoneal Dialysis Program
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Background: High rates of culture negative (CN) peritonitis are reflective of inadvertent administration of antibiotics and erroneous methods of collection of peritoneal fluid. Tracking of peritonitis rates with a focus on CN peritonitis rates was performed at our

center. Our focus was to curb the rising rates of CN peritonitis. Below we report the CN rates at our program and suggest a possible method to decrease these by strict adherence to a policy of collecting peritoneal fluid culture.

Methods: Comparison of peritonitis rates before and after the adoption of policy for collection of peritoneal fluid, mentioned below: 1) 50ml of effluent from an overnight dwell was placed in a sterile container, sent to the laboratory in 6 hours without refrigeration. 2) 5 to 10ml was also collected in anaerobic and aerobic blood culture bottles 3) For patients that were dry for the day, 1 liter of 1.5% dextrose was allowed to dwell for 2 hours before sample collection as described.

Results: 1) Peritonitis rates prior to the policy from January 2013 to August 2013: total peritonitis rates 8 episodes in 151 patient months 2) 62.% of them were culture positive, 37.5 culture negative 3) The most common organism was Enterococcus fecalis (60%) 3) Rates of culture negative peritonitis after instituting this policy in August 2013 until May 2014: 0 episodes.

Conclusions: Root-cause analysis for the explanations of our incidence of CN peritonitis was done. We noticed a dramatic decrease in the number of CN peritonitis once the home program adhered to the policy instituted. Since implementation we have had only two episodes of peritonitis both that were culture positive and no episodes of CN peritonitis thus far. In conclusion,the method of collection of peritoneal fluid appears crucial in reducing the rates of CN peritonitis and obtaining maximum yield of cultures from peritoneal fluid. Adoption of this policy with emphasis to its adherence resulted in a significant decrease in the episodes of CN peritonitis thus far at our urban inner city peritoneal dialysis center.

SA-PO953

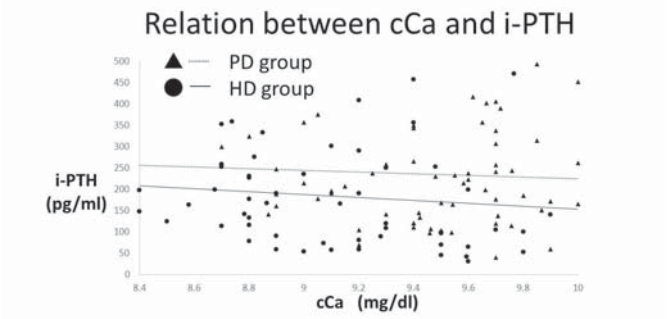
The Acid-Base Differences Affect the Calcium-PTH Axis of Dialysis Patients: Comparison Between Peritoneal Dialysis and Hemodialysis

Masamitsu Morishita, Masatsugu Nakao, Nanae Matsuo, Izumi Yamamoto, Yukio Maruyama, Yudo Tanno, Ichiro Ohkido, Masato Ikeda, Keitaro Yokoyama, Takashi Yokoo. *Div of Nephrology and Hypertension, Dept of Internal Medicine, Jikei Univ School of Medicine, Tokyo, Japan.*

Background: Serum ionized calcium (iCa) is the biologically active component of Ca. The ratio of serum iCa in corrected total Ca (cCa) has been already identified to be changed according to the serum acid-base status. Because peritoneal dialysis (PD) patients tend to be under metabolic alkalosis status due to the characteristics of PD dialysate, iCa levels of PD patients are expected to be lower than those of hemodialysis (HD) patients. We hypothesized that PD patients may present lower iCa levels than HD patients, and that lower iCa levels may affect their parathyloid hormone (PTH) levels.

Methods: We recruited 209 patients (101 on PD, 108 on HD) in multicenter. Their acid-base status, iCa, albumin, and intact-PTH (i-PTH) were measured, and cCa was calculated by Payne compensation formula recommended in Japanese guideline. The factors associated with Ca-PTH axis were assessed statistically.

Results: HCO_3 levels of PD group were significantly higher than those of HD group (26.8±2.4 versus 22.5±2.2mmHg), and HCO_3 levels and iCa/cCa ratio had inverse correration between them. The serum iCa levels and iCa/cCa ratio of the PD group were significantly lower than those of the HD group (iCa; 1.07±0.10 versus 1.12±0.09mmol/l, P<0.05: iCa/cCa; 44.6±3.1 versus 49.6±3.2%, P<0.05). After the adjustment of the dialysis vintage and the prescription of cinacalcet, i-PTH levels of the PD group were significantly higher than those of HD group (235±129 versus 184±129 pg/ml, P<0.05).



Conclusions: Because PD patients often present with metabolic alkalosis, they tend to have lower iCa levels, and in turn have higher i-PTH levels despite their cCa levels being within the recommended range.

SA-PO954

Retrospective Review on the Outcome of PD Associated Peritonitis Caused by Acinetobacter in a Single Center

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Background: PD associated peritonitis caused by Acinetobacter has low incidence rate of 2-5% but notorious in treatment difficulty, poor response and high morbidity. We perform this review to look into the magnitude of the problem and the predictors of poor outcome.

Methods: Single centre record from 1 Jan 2003 to 31 May 2014 were analyzed. Logistic regression of the predictors was performed for outcome prediction. Non-responders include relapse, failed treatment with PD catheter removal and death due to peritonitis.

Results: There are 55282.5 patient months during the review period, with 1207 episodes of peritonitis, rendering a rate of 1 in 45.8 months. Distribution of organisms were: 555(46%) gram positive organisms, 527(43.7%) gram negative organisms, 46 fungus, 27 episodes mycobacterium and 52 culture negative. 29 episodes were caused by Acinetobacter with 20 confirmed Acinetobacter baumanii and 9 episodes were due to Acinetobacter species but no multi-resistant strain was found. The characteristics of the patients were summarized in Table 1.

	Mean/Frequency (%), n = 29
Age	64.6
Male	17 (58.6%)
Presentation before 2008	22 (75.9%)
Polymicrobial peritonitis	10 (34.5%)
Antibiotics sensitivity, cefoperazon + sulbactam	29 (100%)
Sensitivity, ciprofloxacin	25 (86.2%)
Sensitivity, cotrimoxazole	26 (89.7%)
Sensitivity, gentamicin	26 (89.7%)
Initial antibiotics, cefazolin	24 (82.8%)
Initial antibiotics, gentamicin	24 (82.8%)
DM	16 (55.2%)
IHD	5 (17.2%)
Prior peritonitis within 30 days	9 (31%)
Day 1 WCC	1954/ml
Day 3 WCC	956/ml
Day 3 WCC after appropriate antibiotics	208/ml

Non-responders accounted for 34.5% of the episodes. Logistic regression found that older age, day 3 cell count after appropriate antibiotics (cut off 500) were independent predictors of treatment response (p-value=0.012 and 0.042 respectively).

Conclusions: Acinectobacter peritonitis has a very high rate of poor outcome while older age and day 3 cell count after appropriate antibiotics were independent predictors of poor outcome. Although the sample size of this study is small, the number of cases is actually among the largest from the case series reported so far. Prompt action to escalate treatment in high risk group could possibly improve outcome.

SA-PO955

A Comparison of Two Methods on Replacing Peritoneal Dialysis Transfer Set

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Background: Peritoneal dialysis (PD) transfer set is one key part for the patients on peritoneal dialysis, and it should to be replaced at intervals not longer than 6 months or more frequently as specified by a physician. In China, however, there was no details about this operation in the Peritoneal Dialysis Standard Operating Procedure (SOP).The objective of this study was to evaluate the effect of two methods on replacingPD transfer set.

Methods: Two types of operation procedure on replace PD transfer set were showed and 80 patients (45 male, 35 female) were involved in this study. The mean age was 43 (from 18 to 75). All the patients were randomly divided into two groups. The traditional method was used in the control group (n=40) and the modified method was used in the observational group (n=40). The traditional method included the procedure of soaking the set twice in the iodine disinfectants, and the modified method included soaking only once.

Results: It took less operating time (p=0.005) in the observational group (24.8±1.6 minutes) to replace PD transfer set than in the control group (38.1±2.8 minutes). The cost of the materials in the observational group was obviously reduced (p=0.005).Results also showed that the shorter operation process made the patient more satisfied (p=0.001) and the occurrence of peritonitis in the two groups was 0.

Conclusions: This study shows that the modified method on replacing PD transfer set is more economical, more efficient, and the important thing is that it is safe for patients and easy to be accepted.

SA-PO956

Increased Aortic Stiffness Evaluated by MRI-Based Pulse Wave Velocity in Patients with Peritoneal Dialysis: A Cross-Sectional and Longitudinal Study

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Background: Pulse wave velocity (PWV) is a biomarker directly related to arterial stiffness that has the potential to provide information on early atherosclerotic disease. MRI has shown good agreement with PWV determined with invasive intra-aortic pressure measurements (Grotenhuis et al. J Magn Reson Imaging, 2009). To date, very few studies have reported on MRI-based PWV in patients with chronic kidney disease (CKD), especially peritoneal dialysis (PD) patients. In the present study, we measured MRI-based PWV in patients with moderate to severe CKD including PD patients and examined what factors impact on higher PWV including PD versus non-dialysis dependent CKD (NDD-CKD) in those patients.

Methods: In a cross-sectional study, 181 CKD patients (NDD-CKD, n=106; PD, n=75) were recruited and underwent cardiovascular MRI. Among them, 107 patients (NDD-CKD, n=70; PD, n=37), who underwent a second cardiovascular MRI after 2 years, were recruited in a longitudinal study. Using cine and phase contrast sequences, the cross-sectional area for distensibility and average blood flow were measured between the ascending and the

proximal descending aorta and between the proximal descending aorta and the abdominal aorta just proximal to the iliac bifurcation. We constructedlinear mixed-effects models for the association of various independent factors with PWV.

Results: In the cross-sectional study, increasing age, male gender, diabetic, higher systolic blood pressure, higher logarithmic brain natriuretic peptide and PD patients were significantly associated with higher PWV. The association of increasing age, higher systolic blood pressure, and PD patients with higher PWV were also significant even after multivariable confounding factors. In the longitudinal study, these associations remained unchanged in the linear mixed-effects model.

Conclusions: Aortic stiffness is significantly increased in PD patients compared with NDD-CKD independent of age and blood pressure.

SA-PO957

Survival Differences Between Home Dialysis Therapies and In-Center Haemodialysis: A National Cohort Study Austin G. Stack,[1,2] Waleed Mohammed,[1,2] Mohamed Elsayed,[1,2] Cornelius John Cronin,[1,2] Liam F. Casserly,[1,2] John P. Ferguson.[2] *[1]Nephrology, Univ Hospital Limerick, Limerick, Ireland; [2]Graduate Entry Medical School, Univ of Limerick, Ireland.*

Background: Increasing use of home dialysis therapies may result in better survival for all patients who need dialysis treatment. It is unclear, however, whether home therapies offer superior survival to standard in-center haemodialysis for all new patients. We compared mortality between home therapies and in-center dialysis in a national cohort.

Methods: National data were available on 585, 911 patients from the U.S. Renal Data System recruited between 1/1/2005 to 31/12/2010 and followed until until death, transplantation, lost to follow-up, or 31/12/2010. Patients were classified as receiving continuous ambulatory peritoneal dialysis (CAPD) (n= 23, 166), continuous cycling peritoneal dialysis (CCPD) (n = 15,679), In-center HD (n = 543,851) or home HD (n = 3, 215). In-center HD was limited to 3 days per week, while Home HD was categorised as 3, 4, 5, or 6 times per week. Multivariable Cox regression evaluated the relative hazard of death (HR) by dialysis modality using intent to treat analysis adjusting for demographic characteristics, clinical conditions, lifestyle and functional factors, insurance status, erythropoietin use pre-dialysis, and laboratory factors (albumin, haemoglobin and eGFR at dialysis initiation using the CKD EPI equation).

Results: Compared to In-center HD, the adjusted HR over all years were significantly lower for patients treated with CCPD or CAPD and for patients treated with Home HD x 6 times per week

Dialysis Modality Type	Hazards Ratios and 95% confidence Intervals
In-center HD (referent)	1.00
CAPD	0.91 (0.88-0.93)
CCPD	0.88 (0.86-0.91)
Home HD (x3/week)	1.47 (1.40-1.55)
Home HD (x4/week)	1.37 (0.98-1.92)
Home HD (x5/week)	0.98 (0.72-1.34)
Home HD (x6/week)	0.74 (0.55-0.99)

Conclusions: Peritoneal dialysis and frequent Home HD (x 6 per week) conferred the greatest patient survival compared to In-center HD while Home HD (x 3 per week) conferred the poorest survival. Peritoneal dialysis and frequent Home HD should be considered as first-line dialysis therapies for all suitable dialysis patients who approach end stage kidney disease.

SA-PO958

Changes in pH during Hemodialysis Determine Indoxyl Sulfate Binding and Limit Its Removal Jonathan Chun, Sumit Kumar, Prabhjot Singh, Sachin Jhawar, Jerome Lowenstein. *Medicine, NYU Langone Medical Center, New York, NY.*

Background: There is increasing evidence that indoxyl sulfate(IS), as a uremic toxin, may be implicated in accelerated arteriosclerosis in ESRD. Protein binding limits the removal of IS by hemodialysis. In an earlier study (JASN 24:FRPO191) we observed that the protein-bound fraction of IS averaged 73% in pre-dialysis plasma and > 95% at termination. In this study we have examined the hypothesis that the pH increase during dialysis results in more avid binding of IS to protein (albumin) thereby further limiting the dialyzer's removal of IS.

Methods: Blood samples from the afferent dialysis line were drawn at the onset, midpoint, and end of a standard 3-3.5 hour hemodialysis session in 10 patients with stable chronic kidney disease. Blood pH was measured with a Siemens ABL90 blood gas analyzer. IS in plasma and ultrafiltrate (Amicon) was measured by HPLC.

Results: This study showed that pH increased from 7.39 to 7.52 in the first half of dialysis, then remained essentially unchanged for the remainder of dialysis. The IS binding increased from 80% to 94% by the midpoint and to 97% by the end of dialysis. Concomittantly, the amount of IS removed in the first half of dialysis as a fraction of all IS removed throughout dialysis was greater than in the second half ($p<0.025$).

	pH	HCO_3^- meq/l	IS_{total} µgm/ml	*$IS_{bound/total}$	Δ IS_{total} %
pre-dialysis	7.39	24.18	47.98	80.69	---
mid-point	7.52	31.06	37.42	93.94	62.63
post dialysis	7.55	33.00	29.86	97.14	37.36

*some values of free IS were below the limits for detection

Δ IS_{total} % represents the fraction of IS removed in the first half of dialysis vs the second half of dialysis

Conclusions: The data lead us to conclude that the dialyzer clearance of indoxyl sulfate, a major uremic solute, is limited by the pH increase during standard dialysis. The data suggest that the efficency of hemodialysis in removing indoxyl sulfate might be significantly enhanced by modifying the pattern of bicarbonate replacement during dialysis.

Funding: Private Foundation Support

SA-PO959

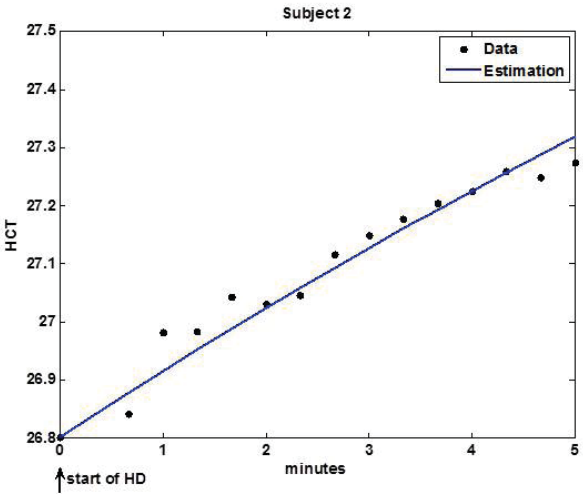
Estimating Intravascular and Interstitial Volume at the Start of Hemodialysis Session Cheng-Hung Chen,[1] Joseph Horowitz,[1] Christopher V. Hollot,[1] Rajiv P. Shrestha,[3] Michael J. Germain,[2] Yossi Chait.[1] *[1]Univ of Massachusetts; [2]Western New England Renal and Transplant Associates, PC; [3]Octet Research Inc.*

Background: Intradialytic hypotension is the most common hemodialysis (HD) complication and a significant cause of morbidity. Recent models of absolute blood volume in HD patients are based on bioimpedance, tracer dilution, relative blood volume measurements or combination thereof. The goal of this study was to investigate the feasibility of estimating pre-HD intravascular and interstitial volumes using Crit-Line data during routine HD conditions.

Methods: We describe fluid volume dynamics during HD using a two-compartment model comprising intravascular and interstitial volumes. We used retrospective hematocrit (HCT) data (Crit-Line) collected every 20 seconds from 24 HD patients. Nonlinear regression was used to estimate model parameters over the initial HD period.

Results: Pre-HD volumes were successfully estimated for all 24 data sets.

Pre-Hd	Mean	Median	SD	Min	Max
HCT (%)	28.71	28.57	2.88	22.60	33.77
Plasma (L)	4.03	4.05	0.39	3.02	4.64
Intravascular (L)	5.65	5.67	0.45	4.52	6.43
Red Blood Cells (L)	1.62	1.66	0.18	1.21	1.86
Interstitial (L)	11.92	10.99	2.22	10.00	16.13



The mean (SD) time period required for successful estimation was 6.3 (3.4) min. The mean (SD) root mean square estimation error of HCT was 0.13 (0.16).

Conclusions: Crit-Line HCT data can be used to estimate pre-HD intravascular and interstitial volumes in HD patients. The minimal length of data required for estimation depends on the initial refill profile driven by unknown osmotic and hydrostatic pressures. These results should be tested in a study with a larger number of patients, and validated against gold standards of absolute blood volume measurements. These results may be used to better estimate dry weight for dialysis dose prescription and red cell mass in anemia management.

Funding: NIDDK Support

SA-PO960

Dialysate Sodium: Do We Get What We Order? Sriram Narsipur,[1,4] L. A. Arbeit,[2,4] Philip Zager,[3,4] S. Paine.[4] *[1]SUNY, Syracuse, NY; [2]SUNY, Stony Brook, NY; [3]UNM, Albuquerque, NM; [4]DCI, Nashville, TN.*

Background: There is considerable controversy surrounding the optimal dialysate sodium (DNa) concentration. Recently, leaders of several dialysis providers have urged their medical directors to reduce DNa concentrations to the range of 134 to 138 mEq/L in an effort to reduce interdialytic weight gain (IDWG), blood pressure, hospitalization and mortality. However, in the effort to accomplish this, there has been little attention directed to comparing ordered versus measured DNa concentrations.

Methods: We compared ordered DNa to DNa concentration measured at the start of dialysis. The study was conducted in 340 patients across five facilities operated by Dialysis Clinic Inc., (DCI) and located in Albuquerque, Stony Brook and Syracuse, utilizing Fresenius, Cobe, and Gambro dialysate delivery machines respectively. The inline clearance feature was turned off during the study. All DNa values shown are expressed as mEq/L.

Results: The percentiles of ordered DNa, measured DNa and the absolute differences are shown in the table. The absolute difference was ≥ 4 and 6 in 25% and 10% of patients, respectively. Ordered DNa was ≤ 135 in 33.8%, 136 to 139 in 17.4% and ≥ 140 in 48.8% of patients. Among the 115 patients who had ordered DNa ≤ 135, 64 (55.7%) had a measured DNa ≥ 136. Among the 59 patients who had ordered DNa 136-139, 40 (67.8%) had a measured DNa ≥ 140. Overall among the 174 patients with an ordered DNa < 140, 46 (26.4%) had measured DNa levels ≥ 140. It was only among the patients with an ordered DNa ≥ 140 that the majority (95.2%) fell within the prescribed range. Results were similar across clinics.

Distribution of Dialysate Sodium: Ordered, Measured and Difference (mEq/L)			
DNa (percentile)	**Ordered**	**Measured**	**Difference**
1st	130	132	0
5th	134	134	0
10th	134	134	0
25th	134	137	1
50th	138	141	2
75th	140	143	4
90th	140	145	6
95th	140	146	7
99th	140	151	11

Conclusions: The majority of patients who had dialysis prescriptions for a DNa <140 appear to have been dialyzed with a higher DNa concentration. The effort to reduce DNa should include measurement of DNa and additional attention to preparing dialysate and machine calibration.

Funding: Clinical Revenue Support

SA-PO961

Barriers to Dietary Sodium Restriction among Patients on Hemodialysis

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Background: The low sodium (LS) diet is a key intervention for blood pressure (BP) and volume management in hemodialysis (HD). However, adherence to LS diet is difficult. We sought to assess potential barriers to following LS diet in HD.

Methods: As part of an ongoing randomized clinical trial of LS diet in HD, stable adults receiving outpatient HD treatment, were assessed for salt taste recognition using taste strips impregnated with 0-1.6% sodium chloride; mean sodium (Na) intake was estimated from 3-day food diary. A dietary sodium survey (DSS) was administered to assess Perceived Behavior Control (PBC) measured on a scale 0-(Not a barrier) to 4-(Extreme barrier), and Perceived Dietary Impact (PDI) on a scale 1-(Definitely True) to 5-(Definitely False). Depression symptom (DS) score was measured on a scale 0-(Not at all) to 3-(Nearly every day).

Results: 19 patients (11 male, 9 black, 7 white, mean age 57 ±14) are currently enrolled. Mean monthly pre-HD systolic BP was 151 ± 15 mmHg. Mean Na intake was 2.5 g/day (range: 1.3-5.2). A higher salt taste recognition threshold was associated with higher Na intake (r=0.37, p=0.14), particularly in patients who did not recognize salt taste at 1.6% compared to those who recognized salt taste (3.2 versus 2.2g/day; p=0.07). Of the 15 patients who completed the DSS, 13 reported following LS diet for a mean of 6.6yrs without much difficulty. Over half (8) reported ≥5 PBC barriers and 6 reported ≥6; the majority (9) expressed lack of knowledge. A higher DS score was significantly associated with a higher PDI score (i.e., less knowledge).

Conclusions: PDI, PBC, taste impairment and mood serve as potential barriers to following a LS diet. While all patients indicated the importance of following a LS diet, many were not aware of its relation to fluid overload and cardiovascular problems. Lack of availability of LS foods, food preference, taste and resolve were among the top barriers. The inability to recognize the taste of salt and DS also factor into patient ability to adhere to LS diet. Further study of these factors is warranted.

Funding: Private Foundation Support

SA-PO962

Association of Bioimpedance Spectroscopy-Based Volume Estimation with Postdialysis Hypotension in Hemodialysis Patients

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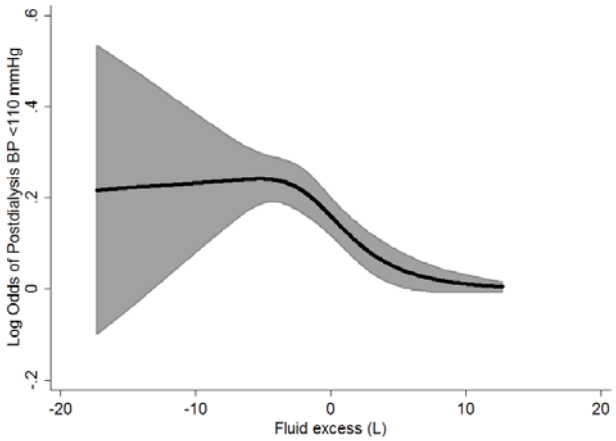
Background: Clinical examination to determine the dry weight of patients on dialysis has been problematic, with studies showing discordance between physician assessment and objective measures of volume status.

Methods: We studied the association between predialysis bioimpedance spectroscopy (BIS)-based estimates of fluid excess and postdialysis hypotension in 635 patients in the USRDS ACTIVE/ADIPOSE study receiving hemodialysis in the San Francisco and Atlanta areas recruited from July 2009 to August 2011. We recorded pre- and postdialysis weight and blood pressures over three consecutive dialysis sessions, and performed BIS before a single session. Using a previously reported method of estimating dry weight in hemodialysis patients from BIS data, we estimated fluid excess in liters in our population. We used multivariable logistic regression with extracellular water/total body water (ECW/TBW) or BIS-based fluid excess as the primary predictor and one or more postdialysis systolic blood pressures less than 110 mmHg as the primary outcome. Multivariable models were adjusted for ultrafiltration rate, body weight, congestive heart failure, sex, ESRD vintage, diabetes, age, African-American race, and albumin.

Results: Higher ECW/TBW was associated with significantly lower odds of postdialysis hypotension (OR 0.34, 95% CI 0.14-0.85 per 0.1 higher, p=0.02). Every liter of BIS-based fluid excess was associated with a lower adjusted odds of postdialysis hypotension (OR 0.88, 95% CI 0.82-0.95, p=0.001).



Conclusions: Prospective studies are needed to determine whether this application of BIS could improve current clinical efforts to minimize episodes of postdialysis hypotension without leading to volume overload.

Funding: NIDDK Support, Other NIH Support - K24DK085153

SA-PO963

Reducing the Sodium Concentration in Dialysate Has a Favourable Effect on Interdialytic Weight Gain without Major Adverse Events

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Background: In chronic hemodialysis patients, high interdialytic weight gain and uncontrolled hypertension are still a management conundrum. Low dialysate sodium baths have been associated with hypotension and hyponatremia. We report the efficacy and safety results of reducing the dialysate sodium concentration in 24 patients at a dialysis clinic.

Methods: This was a quality improvement project in a single dialysis facility with 24 patients undergoing thrice-weekly conventional hemodialysis. Data was collected pre and post-intervention. The intervention was reduction in the sodium bath to 138, 136 or 134 meq/l from the standard of 140meq/l. The lower sodium bath of 134 or 136meq/l was chosen for patients with IDWG% of more than 3%. The measured primary outcomes were IDWG, IDWG % (indexed to dry weight), pre-dialysis and post-dialysis SBP, DBP and MAP; and lowest intradialytic SBP, DBP and MAP. The secondary outcomes were serum sodium concentration and the number of antihypertensive medications. These measurements were taken as averages in 6 dialysis treatments preceding the intervention and 6 dialysis treatments after 12 weeks of the intervention. Paired t-tests were used in IBM SPSS software.

Results: A statistically significant reduction in the post-intervention IDWG%, in comparison to the pre-intervention IDWG% was found with a P value of 0.033 and standard deviation of 1.21. The reduction in IDWG also reached near significance (P 0.063, SD 0.8049). We found no statistically significant differences between the pre and post-intervention blood pressures, measured as pre and post dialysis and lowest intradialytic SBP, DBP and MAP respectively. However, there was a significant reduction in the number of antihypertensive medications post intervention (P 0.001, SD 0.6). No significant hyponatremia occurred post- intervention.

Conclusions: Modest lowering of the dialysate sodium bath is a safe and effective tool in the management of high interdialytic weight gain.

SA-PO964

Comparison of the Impact of High-Flux Dialysis on Mortality in Hemodialysis Patients with and without Residual Renal Function

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Background: The effect of flux membranes on mortality in hemodialysis (HD) patients is controversial. Residual renal function (RRF) has shown to not only be as a predictor of mortality but also a contributor to β2-microglobulin clearancein HD patients. Our study aimed to determine the interaction of residual renal function with dialyzer membrane flux on mortality in HD patients.

Methods: HD Patients were included from the Clinical Research Center registry for End Stage Renal Disease, a prospective observational cohort study in Korea. Cox proportional hazards regression models were used to study the association between use of high-flux dialysis membranes and all-cause mortality with RRF and without RRF. The primary outcome was all-cause mortality.

Results: This study included 893 patients with 24h-residual urine volume ≥ 100 ml (569 and 324 dialyzed using low-flux and high-flux dialysis membranes, respectively) and 913 patients with 24h-residual urine volume < 100 ml (570 and 343 dialyzed using low-

flux and high-flux dialysis membranes, respectively). After a median follow-up period of 31 months, mortality was not significantly different between the high and low-flux groups in patients with 24h-residual urine volume ≥ 100 ml (HR 0.86, 95% CI, 0.38-1.95, P = 0.723). In patients with 24h-residual urine volume < 100 ml, HD using high-flux dialysis membrane was associated with decreased mortality compared to HD using low-flux dialysis membrane in multivariate analysis (HR 0.40, 95% CI, 0.21-0.78, P = 0.007).

Conclusions: Our data showed that HD using high-flux dialysis membranes had a survival benefit in patients with 24h-residual urine volume < 100 ml, but not in patients with 24h-residual urine volume ≥ 100 ml. These findings suggest that high-flux dialysis rather than low-flux dialysis might be considered in HD patients without RRF.

SA-PO965

Relationship between Subjective and Objective Cognition Deficits in Patients Receiving Haemodialysis Anuradha Jayanti,[1] Alison J. Wearden,[2] The Basic-Hhd,[1] Sandip Mitra.[1] *[1]Dept of Nephrology, Manchester Royal Infirmary, Manchester, United Kingdom; [2]Dept of Psychology, Univ of Manchester, Manchester, United Kingdom.*

Background: Cognitive impairment is common in haemodialysis (HD) patients. It is associated with increased morbidity and mortality. Assessment of subjective cognition may provide an easier way of implementing cognitive testing in routine clinical practice. Accordingly, we assessed beliefs about one's cognition using the metacognition questionnaire. It has two components- metamemory (5Q) and metaconcentration (4Q). The questions have no specific age focus and are applicable to the HD population.

Methods: In a cross-sectional, multi-centre study (BASIC-HHD) cohort (n=313), patients receiving HD were administered neuropsychometric tests and the metacognition questionnaire (99% return). Demographic and clinical variables were ascertained from records. Patients completed the Beck Depression Inventory and two objective cognition tests, Modified Mini Mental State (3MS-global cognition) and TMT B (executive function).

Results: Both metamemory and metaconcentration scales have significant relationships with the cognitive impairment outcome variable (3MS≤80 or 3MS>80) at the 5% significance level (N=244, with exclusion of missing data). In a linear regression model that adjusts for age, gender, education, BDI score, stroke and number of weekly dialysis sessions, the p-value is **0.002** (metamemory scale) and **0.04** (metaconcentration scale). Adjusted OR (95% CI) is 0.80 (CI 0.70, 0.92) for metamemory and 0.82 (CI 0.68, 0.99) for metaconcentration suggesting that, an increase in metamemory score or the metaconcentration score by one, reduces the likelihood of being cognitively impaired by 20% and 18% respectively, adjusting for other variables. Both scales did not have a significant relationship with the TMT B outcome variable.

Conclusions: Subjective cognition assessment of HD patients periodically, may provide an indication of the change in global cognitive status of HD patients and is relatively easily employed. The twin components of the metacognition questionnaire should be validated as a determinant of neurocognitive performance in patients with chronic kidney disease across all stages.

Funding: Pharmaceutical Company Support - Baxter Clinical Evidence Council has supported this investigator initiated and investigator led study

SA-PO966

The Effect of the Interdialytic Interval on Cognitive Functioning in Hemodialysis Patients Shayna L. Henry,[2] Larry D. Jamner,[2] Sarah E. Choi,[3] Madeleine V. Pahl.[1] *[1]Nephrology, Univ of California, Irvine, CA; [2]Psychology and Social Behavior, Univ of California, Irvine, CA; [3]Nursing, Univ of California, Irvine, CA.*

Background: Cognitive problems are common among hemodialysis (HD) patients and may be associated with a variety of factors including fluid and electrolyte abnormalities, effect of uremic toxins, and the HD procedure. The long interdialytic interval (IDI) may be particularly challenging for patients as compared to short IDIs. The aim of the present study was to examine the relationship between cognitive dysfunction, social support and IDI length.

Methods: Stable patients maintained on HD for > 90 days were administered a neuropsychological battery of tests to address treatment adherence, affect, personality traits, health locus of control, social support and cognitive function before the short and long IDI. Cognitive function measures included the Mini-Mental Status Exam, Digit Span, California Verbal Learning, Benton Visual Retention and Trail-Making Tests. Patients were engaged in one week of smartphone-based monitoring in which 5 times a day they were prompted to report on their activities, social context, diet, cognitive function, and perceived support.

Results: 22 HD patients (mean age 39.5 yrs, 13 women) completed the study. CD was minimal, and diary reports of perceived support did not appear to differ significantly between HD days and between long and short IDIs. During the long IDI, patients reported CD in 12% of smartphone entries, compared to 26% during the short IDIs, and CD was significantly lower on long IDI than short IDIs for all diary measures of CD, including reaction time (M_{Long}=.11, *SD*=.36; M_{Short}=.38, *SD*=.88; *t*=-3.16, *p*=.002), trouble thinking (M_{Long}=.02, *SD*=.13; M_{Short}=.26, *SD*=.59; *t*=-4.66, *p*<.0001), and confusion (M_{Long}=.04, *SD*=.19; M_{Short}=.14, *SD*=.42; *t*=-2.52, *p*=.013).

Conclusions: We conclude that CD is mild in stable, young ESRD patients. CD is significantly worse after the HD treatment during the short IDI as measured by self-reported entries, reaction times, trouble thinking and confusion. These abnormalities improve during the long IDI. These findings suggest the HD procedure may exacerbate CD in ESRD patients.

SA-PO967

Cognitive Depression and Functional Impairment Status Are Associated Factors of Malnutrition in Elderly Patients in Maintenance Hemodialysis Nasser Abdel Polanco Flores, Erika Lopez, Belen Meltiz, Manolo Ramos Gordillo, Jose C. Pena. *CEDIASA, México, D.F., Mexico.*

Background: The relationship between cognitive depression, functional status and malnutrition in an elderly population in maintenance hemodialysis is very complex and not yet fully understood. Currently, there are no studies to establish this association. We evaluated cognitive depression and quality of life (QOL), with malnutrition in elderly chronic hemodialysis patients.

Methods: We evaluated cognitive depression, anxiety and depression and quality of life (QOL), with malnutrition in elderly chronic hemodialysis patients. A cross-sectional study was conducted in 107 patients, 55 men (51.4%) and 52 women (48.6%), mean age 70.05±4.6 for men and 71.26±5.7 for women (p=0.234). They received 3 to 4 hours high flux hemodialysis 3 times per week. Cognitive impairment was classified as, severe, moderate and mild with the mini-mental state examination score (MMSE); for emotional state was used the Hospital Anxiety and Depression scale (HADS) and for QOL the short term-12 (SF-12) Karnofsky index (KI). Nutritional status was classified also as severe, moderate and mild with, Subjective Global Assesment (SGA), body mass index (BMI) and biochemical parameters that included (sodium, potassium, cholesterol, triglycerides, albumin, uric acid, intact parathyroid hormone (iPTH) and KT/V.

Results: A severe cognitive impairment was found in 49.7% patients, moderate in 39.8%; mild in 6.9% and 3.4% showed no cognitive impairment. Severe malnutrition was present in 4.7 %, moderate in 65.4% and mild in 29.9%. The statistical analysis displayed a significant correlation between; nutrition status and MMSE r= 0.428 (p<0.001), and between QOL and nutrition r=0.361 (p<.001).

Conclusions: In our population elderly patients in maintenance hemodialysis showed a significant correlation between cognitive impairment, QOL and malnutrition. However, this association is very complex and more studies are needed to support this contention. We also were unable to demonstrate if cognitive impairment is the cause of malnutrition or viceversa.

SA-PO968

Comparison of Common Screening Tools for Depression in Haemodialysis Patients Ayman Guirguis,[1,2] Karin Friedli,[2] Clara Day,[3] Naomi Fineberg,[2,4] Michael K. Almond,[5] Andrew Davenport,[6] Maria Da Silva-Gane,[1] David Wellsted,[2] Joseph Chilcot,[7] Ken Farrington.[1,2] *[1]East and North Hertfordshire NHS Trust, United Kingdom; [2]Univ of Hertfordshire, United Kingdom; [3]Univ Hospitals Birmingham NHS Foundation Trust, United Kingdom; [4]Hertfordshire Partnership Univ NHS Foundation Trust, United Kingdom; [5]Southend Univ Hospital NHS Foundation Trust, United Kingdom; [6]Royal Free London NHS Foundation Trust, United Kingdom; [7]King's College London, United Kingdom.*

Background: Depression is common in haemodialysis (HD), its diagnosis is complicated by overlapping symptoms.The Beck Depression Inventory-II (BDI-II) is a useful screening tool with good psychometric properties. Cut-off score ≥ 16 indicates probable depression in this setting,with few data on other commonly used tools.We determined cut-off values for the Patient Health Questionnaire 9 (PHQ-9) and Patient Health Questionnaire 2 (PHQ-2), corresponding to BDI-II ≥ 16 in this setting.

Methods: HD patients at 3 UK renal centres. Inclusion criteria:dialysis vintage >3 months, aged >18 years and English proficiency.Patients screened for depression with the BDI-II and PHQ-9. We used ROC analysis to determine the cut-off points for the PHQ-9 and PHQ-2 corresponding to a BDI-II cut-off ≥ 16. We also determined levels of agreement.

Results: 494 patients (61% males) median interquartile range for the BDI-II, PHQ-9 and PHQ-2 were 10(5-19),5(2-11) and 1(0-2) respectively. Using the BDI-II cut-off, area under the curve in the ROC analysis for the PHQ-9 and PHQ-2 were 0.94 (CI 0.92-0.96) and 0.89 (CI 0.86-0.93) respectively optimal cut-off points of ≥ 8 for PHQ-9 and ≥2 for PHQ2 (sensitivity 87% and 84% and specificity 88% and 86% respectively).Proportion of patients with BDI ≥16 was 34%, with PHQ-9 ≥8 was 38% and PHQ-2 ≥ 2 was 38%. Levels of agreement of PHQ-9 ≥ 8 and PHQ-2 ≥2 with BDI-II ≥ 16 were substantial (κ = 0.724 and 0.678 respectively).

Conclusions: The PHQ-9 and PHQ-2 are acceptable screening tools compared to the BDI-II in dialysis patients. Cut-off scores for PHQ-9 ≥ 8 and PHQ-2 ≥2 compare well to a BDI-II cut-off ≥ 16. Use of simple screening tools may help to detect depression and improves clinical outcomes in HD patients.

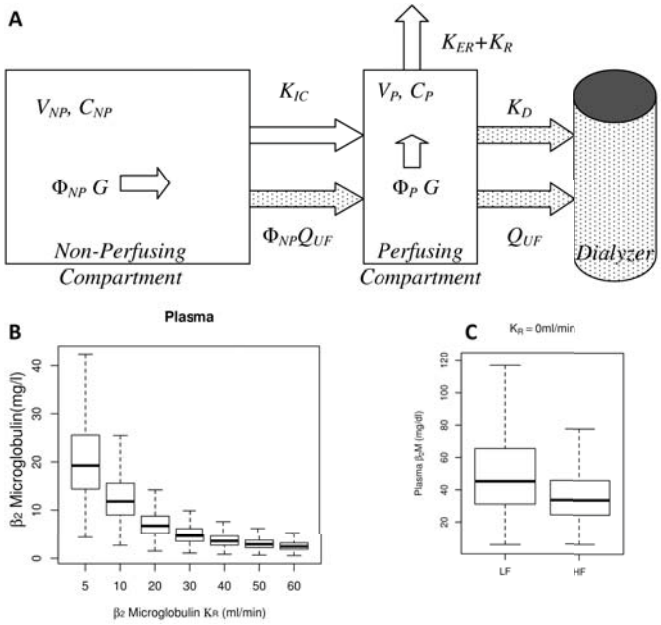
Funding: Clinical Revenue Support, Government Support - Non-U.S.

SA-PO969

Systematic Review of Beta 2 Microglobulin Population Kinetics Christos Argyropoulos,[1] Maria-Eleni Roumelioti,[1] Thomas D. Nolin,[2] Mark L. Unruh.[1] *[1]UNM, Albuquerque, NM; [2]Univ of Pittsburgh, Pittsburgh, PA.*

Background: Beta 2 Microglobulin (β_2M) is a middle molecule associated with a higher risk of death in hemodialysis (HD) and chronic kidney disease (CKD) patients. We lack a quantitative understanding of β_2M at the population level.

Methods: We did a meta-analysis of studies with patient level data about β_2M generation, distribution and elimination and we developed a population kinetic bicompartmental model (PM) for β_2M in CKD and HD



[A].

Results: Ten papers reporting 9 separate studies fulfilled the criteria for inclusion. Most of the patients (74/106) were on HD with minimal residual renal function (RRF). Renal function is the major determinant of total body β_2M clearance and the estimated median value was 90.4 versus 2.9 ml/min for HD patients. In the table we summarize estimates for the population distribution values (median, upper, Q95 and lower, Q025, 2.5% tail). In simulations from the PM, β_2M increased as renal function declined [B]. In anuric HD pts, β_2M was higher in those on low flux dialysis, but there was substantial overlap [C].

			Population Distribution Values		
Kinetic Parameter	**Number (studies)**	**Number measurements/ patients**	**Median**	**Q025**	**Q975**
Generation Rate (mg/kgr/day)	8	146/96	3	1.6	5.8
Intracompartmental Rate Transfer (ml/min)	6	73/63	68.5	39.4	119.3
Extrarenal Clearance, Cl (ml/min)	6	59/56	2.9	1.4	6.25
Total Body Clearance in controls (ml/min)	3	19/19	90.4	62.1	131.7
Total Volume Distribution, TVD (L)	6	79/69	11.1	6.6	18.9
TVD (BW%)	5	69/59	17.7	11.1	28.2
Perfusing Compartment Volume, PCV (BW%)	5	69/59	4.7	3.5	6.3
Ratio of Non-perfusing to PCV	5	69/59	2.7	1.8	4.15

Conclusions: A PM β_2M based on estimable parameters of generation, distribution and clearance, highlights the importance of RRF and may allow personalization of HD prescription.

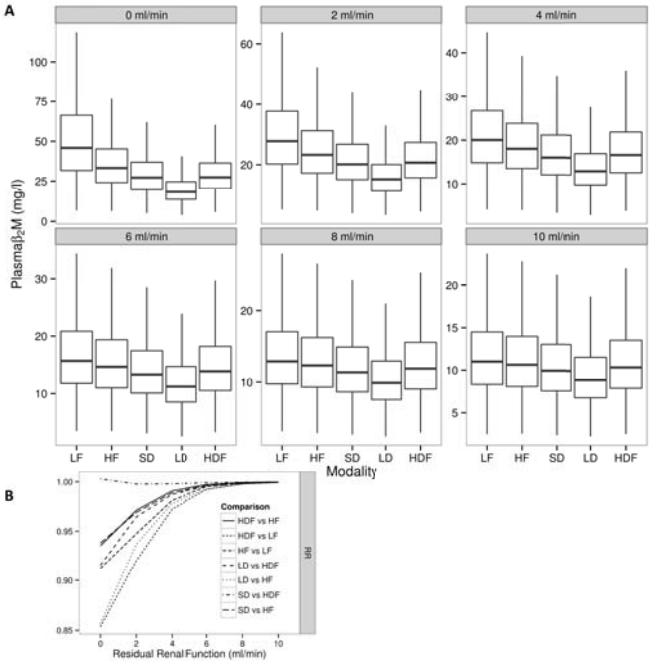
SA-PO970

Large Scale Modeling of Beta 2 Microglobulin Population Kinetics Biomarker for In-Silico Hemodialysis Trials Christos Argyropoulos,[1] Maria-Eleni Roumelioti,[1] Kamyar Kalantar-Zadeh,[3] Thomas D. Nolin,[2] Mark L. Unruh.[1] *[1]Renal Div, Univ of New Mexico, Albuquerque, NM; [2]Pharmacy and Therapeutics, Univ of Pittsburgh, Pittsburgh, PA; [3]Univ of California Irvine, School of Medicine, Orange, CA.*

Background: Beta 2 Microglobulin (β2M) is a middle molecule uremic toxin associated with a higher risk of death in hemodialysis (HD) patients. Nevertheless, a quantitative understanding of the relative role of residual renal function (RRF) and dialytic clearance on β2M levels is lacking.

Methods: We adopted a population kinetic model (PKM) for the generation, distribution, extrarenal, renal and dialytic removal of β2M. Predialysis concentration was related to survival using the observed relation in the HEMO trial. Subsequently we used the PKM model to simulate β2M concentrations and relative survival in a population of ESRD patients with different levels of RRF. In these simulations we evaluated the intervention protocols utilized in randomized controlled trials (RCTs) of HD patients: low flux (LF) and high flux (HF) membranes in conventional thrice weekly HD, and HFHD in short (SD) and long daily (LD) sessions and on-line hemodiafiltration (HDF).

Results: Only when RRF was <2 ml/min, higher dialytic removal materially affected β_2M exposures



[A]. In patients initiating conventional HFHD total loss of RRF was associated with a relative risk (RR) of >20%. HDF and daily HD may decrease this RR by 10%[B]. Only frequent long sessions consistently reduced mortality risk by 15% in anuric pts[B].

Conclusions: These assessments highlight the potential importance of individualizing treatment regimes based on RRF in this era of cost-utility consciousness. Preservation of RRF may be an important treatment goal when managing HD patients, since RRF is a very important determinant of β2M concentration in HD patients. RCTs of interventions to preserve RRF are needed.

SA-PO971

Accuracy of Point of Care Glucometer in Stable Hemodialysis Patients Using Different Blood Samples Eng Kuang Lim,[1] Lee Ying Yeoh,[2] Yan Zhang,[3] Fei Fu,[4] Si Chanjuan.[5] *[1]Renal Medicine, Khoo Teck Puat Hospital, Singapore; [2]Renal Medicine, Khoo Teck Puat Hospital, Singapore; [3]Renal Center, Khoo Teck Puat Hospital, Singapore; [4]Renal Center, Khoo Teck Puat Hospital, Singapore; [5]Renal Center, Khoo Teck Puat Hospital, Singapore.*

Background: Samples for glucose monitoring in hemodialysis(HD) population is often obtained interchangeably from HD bloodline (BL) and capillary prick(CP). However, use of BL sample in Point Of Care Glucometer (POCG) has not been validated. ISO 15197 guideline requires 95% of POCG measurements to be within ±15 mg/dl margins of reference results at glucose concentrations < 75 mg/dl and within ±20% if glucose concentration ≥ 75 mg/dl. The purpose of this study was to determine the reliability of CP and BL glucose measurement using POCG compared to standard laboratory measurement.

Methods: This was a prospective observational study of consecutive inpatient HD sessions for stable diabetic ESRD patient. Patients dialysed using temporary dialysis catheter, or unable to provide consent are excluded. Haematocrit (HCT), Mean Arterial Pressure (MAP), Ejection fraction (EF), Serum Albumin and finger oedema were recorded. Paired Sample of blood obtained from CP and BL will be tested on POCG concurrently. Concurrent bloodline sample is sent to laboratory as control.

Results: A total of 53 patients with 149 dialysis sessions were included in the study. Mean laboratory plasma glucose, POCG CP, and POCG BL were 132.6±34.8mg/dl, 134.2±35.1mg/dl and 133.5±33.2mg/dl respectively. 19.5% of patients were septic, 4 % have Congestive Cardiac Failure but none has finger oedema or on vasopressor. Mean haematocrit was 30.4%. Bland Altman plot showed that POCG CP and POCG BL have good agreement with laboratory glucose although both have imprecision of 6.8% respectively. POCG BL will give 93% of result within the ISO 15197 margin whereas POCG CP, 86%. Regression analyses showed that CP glucose was not influence by HCT, MAP, EF, or Serum Albumin.

Conclusions: POCG glucose measurement using hemodialysis bloodline sample gave better precision and less bias compare to capillary prick sample in stable hemodialysis patients. We recommend that POCG measurement to be carried out using hemodialysis bloodline sample.

Funding: Private Foundation Support

SA-PO972

Dose-Dependent Glycemic Control with Teneligliptin, a Novel DPP-4 Inhibitor, in Diabetic Hemodialysis (HD) Patients Assessed by Continuous Glucose Monitoring (CGM)

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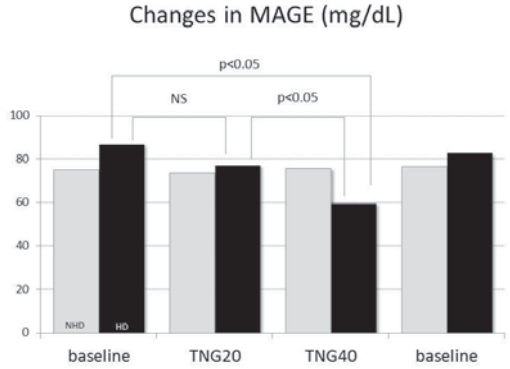
[1]Nagasaki Kidney Center, Nagasaki, Japan; [2]Nagasaki Univ, Nagasaki, Japan.

Background: Teneligliptin (TNG) is a novel DPP-4 inhibitor that does not require dose adjustment for HD patients. We conducted a prospective study to assess the efficacy of TNG in controlling glycemic variability, and the dose-dependency of this agent on DM patients on HD.

Methods: Ten relatively well-controlled (GA<25) DM patients on HD were enrolled in this study. All the subjects were already treated with various types of DPP-4 inhibitors not including TNG (alogliptin, vildagliptin or linagliptin) monotherapy as the baseline treatment. Subjects were switched to 20mg/day of TNG for 2 weeks, 40mg/day of TNG for 2 weeks, then baseline treatment one by one. Plasma glucose, GA and the mean amplitude of glycemic excursions (MAGE) were measured at the end time point of each arm including baseline-treatment on both HD-days and HD-free days.

Results: As shown in Figure 1, the average MAGE on HD days in 40mg TNG treatment group was significantly reduced compared with the baseline treatment (86.4±26.3 mg/dL versus 59.5±20.0 mg/dL).



Notably, significant decrease in average MAGE on HD days in 40mg of TNG compared to 20mg of TNG from 77.5±16.7 mg/dL to 59.5±20.0 mg/dL. It is of importance that MAGE was the same after switching back to baseline therapy, indicating these changes in MAGE were not due to physical or environmental factors, but the pharmacological effect of each agent. No symptomatic hypoglycemia was observed.

Conclusions: In our study, monotherapy of TNG at 40 mg/day as was superior to 20 mg/day in controlling glucose variation on HD-days in the treatment for diabetic HD patients. This is the first report to demonstrate clinical dose-dependency of DPP-4 inhibitor.

Funding: Private Foundation Support

SA-PO973

Continuous Reduction of Protein-Bound Uremic Toxins with Improved Oxidative Stress by Oral Charcoal Adsorbent in Maintenance Hemodialysis Patients; an Additional Blood Purification Therapy

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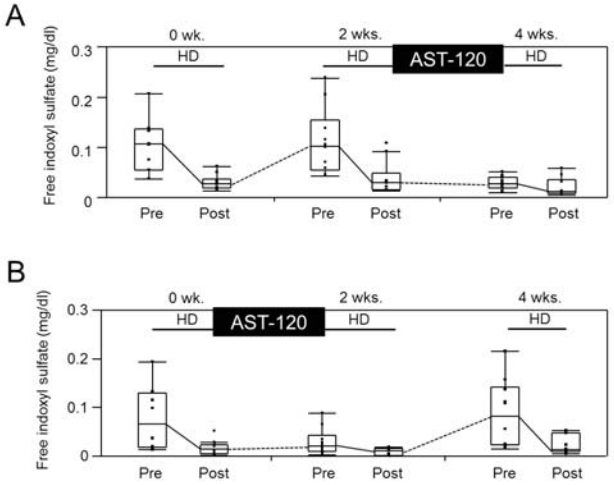
[1]Niigata Univ Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Omori Clinic, Niigata, Japan; [3]Kumamoto Univ Graduate School of Pharmaceutical Sciences, Kumamoto, Japan.

Background: An accumulation of protein-bound uremic toxins is one of the causes for the development of uremia-related complications. We examined whether oral charcoal adsorbent, AST-120, shows an additional effect on removal of protein-bound uremic toxins in patients undergoing maintenance hemodialysis treatment.

Methods: A crossover trial was conducted with AST-120, 6 g/day, for 2 weeks and non-treatment for 2 weeks in maintenance hemodialysis patients (n=20). Serum level of total and protein unbound, free, indoxyl sulfate at pre/post dialysis session before and after the AST-120 treatment were measured. Oxidative stress markers, such as oxidized albumin and 8-isoprostane, were also measured. Data were presented as medians (interquartile range), and Wilcoxon signed- rank test was used for the statistical analysis.

Results: Use of AST-120 6 g/day for two weeks induced dramatic reduction both of total and free indoxyl sulfate [total 45.7 (33.2-50.5)%, p<0.001, and free 70.4 (44.8-79.8)%, p<0.001.



In addition, use of AST-120 induced significant reduction of oxidized albumin [63.4 (60.3-69.0)% versus 68.5 (63.0-74.3)% in before AST-120 treatment, p=0.041] as well as 8-isoprostane [458.1 (380.7-615.6) versus 642.4 (450.7-880.6) pg/ml in before AST-120 treatment, p=0.035].

Conclusions: Oral charcoal adsorbent induced a continuous removal of protein-bound uremic toxins with improvement of oxidative stress in patients undergoing maintenance hemodialysis treatment. This adsorption will be an additional blood purification of regular dialysis treatment.

SA-PO974

Increasing the Dialytic Clearance of the Protein-Bound Solute P-Cresol Sulfate Does Not Achieve a Proportional Reduction in Plasma P-Cresol Sulfate Levels

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[1]Stanford and VA, Palo Alto, CA; [2]Case Western, Cleveland, OH.

Background: The dialytic clearance of protein-bound solutes can be increased out of proportion to that of urea by increasing the dialysate flow (Q_d) and the dialyzer mass transfer area coefficient (K_oA). This study tested whether increasing the clearance of the bound solute p-cresol sulfate (PCS) by these means would reduce its plasma levels.

Methods: Studies were performed in patients on nocturnal hemodialysis so that Q_d and K_oA could be manipulated over a wide range while maintaining adequate Kt/Vurea. Six patients were studied during two periods of two weeks duration. The prescriptions for these periods were designed to provide similar urea clearances but widely different bound solute clearances. The *Low* K_oA-Q_d period used a small dialyzer, Q_d of 300 ml/min, and blood flow of 350 ml/min. The *High* K_oA-Q_d period used a larger dialyzer, Q_d of 800 ml/min, and blood flow of 270 ml/min. At the end of each period PCS and ureaN were measured in plasma and spent dialysate. PCS clearances were calculated in terms of the free unbound PCS concentration.

Results: Results (mean±sd; [a] p<0.05)

		Low K_oA-Q_d	*High* K_oA-Q_d
UreaN	Clearance (ml/min)	200 ± 20	219 ± 18 [a]
	plasma predialysis (mg/dl)	43 ± 11	47 ± 12
	Removed in dialysate (g)	18 ± 3	20 ± 3
PCS	Clearance (ml/min)	234 ± 32	535 ± 76 [a]
	plasma predialysis (mg/dl)	0.19 ± 0.12	0.18 ± 0.09
	Removed in dialysate (mg)	142 ± 84	236 ± 135 [a]

The *High* K_oA-Q_d prescription increased ureaN clearance by an average of only 10 percent so that plasma ureaN levels as well as the amounts of ureaN removed in the dialysate at the end of the periods remained similar. In contrast, the *High* K_oA-Q_d prescription more than doubled the clearance of free PCS. This large increase in clearance was accompanied by a marked increase in the amount of PCS removed in the dialysate but the plasma PCS level was not significantly reduced.

Conclusions: An increase in PCS production may prevent plasma PCS levels from falling in proportion to increases in PCS clearance achieved by changing the dialysis prescription.

Funding: Pharmaceutical Company Support - Baxter CEC

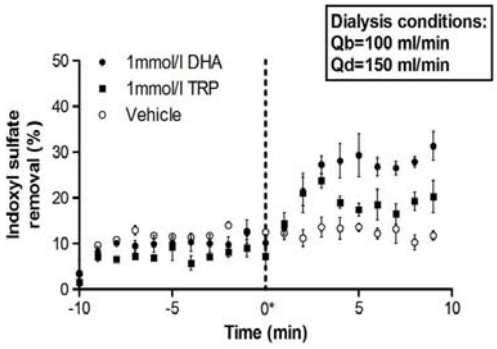
SA-PO975

Enhanced Indoxyl Sulfate Dialyzer Clearance with Use of Binding Competitors: An *In Vitro* Proof-Of-Concept Study Xia Tao,[1] Stephan Thijssen,[2] Nathan W. Levin,[2] Peter Kotanko,[2] Garry J. Handelman.[1] *[1]Clinical Laboratory and Nutritional Sciences, Univ of Massachusetts Lowell, Lowell, MA; [2]Renal Research Inst, NY, NY.*

Background: Indoxyl sulfate (IS) is a uremic toxin that accumulates in chronic kidney disease patients and is associated with multiple-organ toxicity. Because of its high-affinity binding to human serum albumin (HSA), the dialytic clearance of IS is comparatively low. Here we propose a method for increasing the dialytic clearance of IS by using competitors of its binding to albumin.

Methods: Potential displacers of IS on HSA were tested in rapid equilibrium dialysis (RED) cartridges. The ability of 1mM tryptophan (TRP) and docosahexaenoic acid (DHA) to increase IS removal was evaluated with an *in vitro* dialysis model. HSA preloaded with 100uM IS, was circulated on the blood side (100 ml/min) of a F40S dialyzer with single-pass counter-current dialysate flow (150 ml/min). After a 10-min baseline test of clearance, 1mM TRP or DHA were infused into the blood-side circuit upstream of the dialyzer for 10 min, and the effects on IS removal were determined by HPLC.

Results: The free fraction of IS increased 1.4 fold with addition of TRP and 3 fold with DHA in RED device, while no significant change was seen with addition of antipyrine. Baseline IS removal in the in vitro dialysis model was 10.0±0.3 % ml/min (mean ± SEM), which increased to 19.5 ± 0.8 % with infusion of TRP and 27.3±1.0% ml/min with infusion of DHA.



* Start of displacer infusion, Mea ± SEM, N=3

Conclusions: This study shows that TRP and DHA effectively displace IS from albumin and increase its removal when evaluated in an *in vitro* dialysis model. The results suggest that the concept of using binding competitors to enhance the dialytic clearance of protein-bound uremic toxins is a potential therapeutic approach that can be applied to current hemodialysis technology.

Funding: Pharmaceutical Company Support - Renal research institute

SA-PO976

Oxidative Stress in Maintenance Hemodialysis Patients Siren Sezer,[1] Mehtap Erkmen Uyar,[1] Ayse Zeynep Bal,[1] Nilüfer Bayraktar,[2] Bahar Gurlekdemirci,[1] Burak Sayin.[1] *[1]Dept of Nephrology, Baskent Univ Faculty of Medicine, Ankara, Turkey; [2]Dept of Biochemistry, Baskent Univ Faculty of Medicine, Ankara, Turkey.*

Background: Advanced glycation end products (AGEs) are a group of highly oxidant compounds with pathogenic significance in diabetes and in several other chronic diseases. Various antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase. The aim of this study is to evaluate the association between the levels of the components of the oxidative stress process and clinical and biochemical parameters including pulse wave velocity in maintenance hemodialysis (MHD) patients.

Methods: One hundred thirty six maintenance hemodialysis patients (MHD) (59 female, 51.8±14.3 years) were enrolled into the study. Monthly assessed biochemical parameters were recorded.Office systolic and diastolic blood pressure levels and dry weights were also recorded. Pulse wave velocity (PWv) was determined from pressure tracing over carotid and femoral arteries using the SphygmoCor system and change in PWv levels through 1 year was evaluated with ΔPWV. Serum AGE, malondialdehyde and SOD levels were determined by ELISA method.

Results: Patients were divided into two groups from the median value of serum AGE; group 1, n:68, mean AGE 2.76 u/mL and group 2, n:68, mean AGE 6.21 u/mL. Groups were similar in means of demographic characteristics but group 2 patients had longer dialysis duration (p:.0026).Patients with higher serum AGE levels had significantly higher systolic (p:.046) and diastolic (p:.041) BP than group 1 patients. Group 2 patients had also lower dry weights (p:.013) and serum bicarbonate levels (p:.005) and; higher comorbidity scores (p:.038) and serum CRP levels (p:.003). Both PWv levels and ΔPWV were significantly higher in group 2 patients than group 1 patients (p:.001 and 0.001 respectively). Serum AGE levels were positively correlated with serum CRP (r:.164, p:.046) and negatively correlated with serum SOD (p:.008, r:-.212) levels.

Conclusions: We suggest that increased AGE levels appear to be associated with arterial stiffness and hypertension; and could be a potential biomarker that can be assessed as a cardiovascular risk in MHD patients.

SA-PO977

Dietary Fiber Intake: Its Relation with Glycation End Products and Arterial Stiffness Siren Sezer,[1] Bahar Gurlekdemirci,[1] Burak Sayin,[1] Irem Olcay Eminsoy,[2] Emre Tutal,[1] Eyup Kulah.[3] *[1]Dept of Nephrology, Baskent Univ Faculty of Medicine, Ankara, Turkey; [2]Dept of Nutrition, Baskent Univ Faculty of Medicine, Ankara, Turkey; [3]Dept of Nephrology, Baskent Univ Faculty of Medicine, Istanbul, Turkey.*

Background: Dietary fiber intake is associated with protection against cardiovascular diseases as well as anti-inflammatory and metabolic effects that were pointed out in recent studies. Advanced glycation end products (AGEs) are a group of highly oxidant compounds. The pathologic effects of AGEs are related to oxidative stress and inflammation. The aim of this study is to evaluate the association between the dietary fiber intake and the components of oxidative stress and inflammation as CRP, superoxide dismutase (SOD) and AGEs accompanied by pulse wave analysis.

Methods: One hundred thirty maintenance hemodialysis patients (73 male, 51.9±14.3 years with 9.1±5.4 years dialysis duration) were enrolled into the study. Daily dietary fiber intake was assessed using a validated food frequency questionnaire for 6 consequence days. Monthly assessed biochemical parameters were recorded. Serum AGEs and SOD levels were determined by ELISA method. Pulse wave velocity (PWv) was determined from carotid and femoral arteries using the SphygmoCor system. Patients were divided into 2 groups according to mean value of dietary fiber intake.

Results: Mean dietary fiber intake were 16.5±3.0 g/day in patients with group 1 (n: 64) and 8.8 ± 2.1 g/day in group 2 (n: 66). However, mean values of dietary water, carbohydrates, lipids and protein deals were similar in both groups. Patients in group 1 had significantly lower CRP (p: 0.002) and AGE (p: 0.001) and higher SOD (p: 0.04) levels when compared with group 2. PWv levels were higher in patients with group 2 ($p<0.05$). In regression analysis dietary fiber intake and SOD levels were the detected as the predictors of AGE.

Conclusions: We conclude thatdietary fiber intake is independently correlated with CRP and AGEs, in addition decreased fiber intake is resulted in improved arterial stiffness. Thus, adequate fiber intake could prevent cardiovascular events and inflammatory processes in patients ongoing hemodialysis.

SA-PO978

Expanding the List of Uremic Solutes Using an Established Metabolomics Platform Hisae Tanaka,[1] Tammy L. Sirich,[1] Daniel S. Weaver,[2] Timothy W. Meyer.[1] *[1]Dept of Medicine, Stanford and VA, Palo Alto, CA; [2]SRI International, Menlo Park, CA.*

Background: Metabolomic studies using mass spectrometry have increased the number of solutes known to accumulate when the kidneys fail. The current study was designed to identify additional uremic solutes using an established metabolomics platform.

Methods: Samples of plasma and plasma ultrafiltrate from 6 maintenance hemodialysis patients (HD) and 6 normal subjects (NL) were analyzed on a platform capable of detecting more than 3000 known compounds by the combination of liquid and gas chromatography with mass spectrometry. Solutes were classified as uremic based on the finding of significant differences between their concentration in HD and NL with a false discovery rate <0.05 and an HD/NL concentration ratio greater than 2.5 or detection of the solute in at least 5 of 6 HD patients but no more than 1 normal subject. Solutes were classified as novel uremic solutes when reports of their accumulation in humans with CKD or ESRD could not be found in PubMed or the EUTox database. Potential microbial contributions to uremic solute production were identified by searching the BioCyc collection of databases for microbial metabolic pathways capable of producing each solute.

Results:

459	solutes detected in the plasma and/or plasma ultrafiltrate of HD patients
126	of these solutes classified as uremic
60	of the uremic solutes classified as novel based on the lack of prior reports in PubMed or EUTox
66	of the uremic solutes potentially produced by microbes, including 54 by known colon microbes

Solutes which had not been previously identified as uremic included 14 amino acids which had undergone N-acetylation or other chemical modification and 6 phenyl compounds with had undergone sulfate conjugation.

Conclusions: These results add significantly to the number of known uremic solutes. A significant portion of uremic solutes may be produced by microbes. Increased knowledge of uremic solutes and their sources should help improve treatment for renal failure patients.

Funding: NIDDK Support, Veterans Affairs Support

SA-PO979

Steady State Kinetic Analysis of Low Molecular Weight Protein Removal Using Only Intradialytic Concentration Measurements Baris U. Agar,[1] Richard A. Ward,[2] Werner Beck,[3] Markus Storr,[3] J. Ken Leypoldt.[1] *[1]Baxter Healthcare Corporation, Deerfield, IL; [2]Univ of Louisville, Louisville, KY; [3]Gambro (Subsidiary of Baxter International, Inc), Hechingen, Germany.*

Background: Evaluation of low molecular weight protein kinetics during extracorporeal treatments is optimal when both intradialytic and postdialytic changes in protein concentrations and the protein concentration before the next treatment are

determined. Such study designs are often impractical due to excessive time commitments by patients and clinical staff. We evaluated the effect of more limited sampling schedules on the accuracy of kinetic parameters for protein removal.

Methods: Kinetic parameters for beta-2-microglobulin (b2m) were determined using 3 different methods: 1) an optimal method using concentrations measured over a complete intra- and interdialytic cycle (Optimal Model); 2) a steady state method not requiring a concentration before the next treatment (SS Model); and, 3) a steady state method using only intradialytic concentrations (ID Model). Kinetic data from 10 patients treated for 240 min by thrice weekly hemodiafiltration were analyzed using a two-compartment kinetic model containing 4 unknown parameters: generation rate (G); total distribution volume (V_T); central distribution volume (V_C); and intercompartmental clearance (K_{12}).

Results:

Model	G (mg/min)	V_T (L)	V_C (L)	K_{12} (mL/min)
Optimal	0.129 ± 0.026	18.7 ± 4.3	5.7 ± 1.0	73.5 ± 18.9
SS	0.129 ± 0.033	19.0 ± 3.8	5.8 ± 0.9	72.0 ± 19.3
ID	0.131 ± 0.035	20.6 ± 5.3	5.3 ± 1.9	72.2 ± 40.3

There is little loss of accuracy when using the SS model (median absolute differences between the Optimal and SS models were 0.022 mg/min for G, 1.0 L for V_T, 0.3 L for V_C and 4.0 mL/min for K_{12}). Similar overall accuracy is obtained using the ID Model except for higher variability in V_T and K_{12} (median absolute differences between Optimal and ID models were 0.024 mg/min for G, 1.8 L for V_T, 0.4 L for V_C and 13.8 mL/min for K_{12}).

Conclusions: Steady state kinetic parameters for low molecular weight protein removal during extracorporeal treatments can be obtained using only intradialytic concentration measurements.

SA-PO980

The Role of Uremic Toxin Accumulation in Adipose Tissues in Lipodystrophic Phenotype in Mild Chronic Kidney Disease

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Background: Lipodystrophic phenotype has been described in early chronic kidney disease (CKD) that includes adipose tissue atrophy, systemic insulin resistance (IR), dyslipidemia and ectopic lipid accumulation. To elucidate its pathogenesis, we investigated the role of an endogenous nitric oxide synthase inhibitor, asymmetric dimethyarginine (ADMA) and indoxyl sulfate (IS) that are among uremic toxins and affect insulin sensitivity.

Methods: We rendered six-week old SD rats CKD by 5/6th nephrectomy (Nx) and compared various phenotypes between Nx and sham-operated rats. Cultured 3T3L1-fibroblasts were differentiated into mature adipocytes with or without ADMA. Transgenic (TG) mice overexpressing each isoform of ADMA degrading enzyme, dimethylarginine dimethylaminohydrolase (DDAH)-1 and DDAH-2 were subject to Nx and their phenotypes were investigated. By using our cohort with non-diabetic G1-G3a CKD patients, associations between eGFR and various clinical parameters were examined.

Results: OGTT and ITT revealed that IR was evident in Nx. Insulin stimulation failed to activate downstream molecules of insulin receptor, PDK and Akt in adipose tissues in Nx. As a consequence of IR, adipose tissue weight, adipocyte size and adipocyte differentiation marker expressions decreased in Nx. Tissue lipid content in the liver and muscle increased in Nx. Tissue levels of ADMA, IS and oxidative stress increased in the adipose tissue of Nx. Both DDAH1 and DDAH2 expressions decreased and a putative IS receptor, arylhydrocarbon receptor (AhR) expression increased in the adipose tissue of Nx. ADMA inhibited adipocyte differentiation, triglyceride accumulation and insulin signaling, which were reversed by pretreatment with cGMP. Both in DDAH1 and DDAH2 TG mice with Nx, all the lipodystrophic phenotypes were reversed. In our CKD cohort, eGFR levels were significantly associated both with serum ALT and triglyceride levels.

Conclusions: In mild CKD, uremic toxin accumulations in the adipose tissue trigger lipodystrophic changes including ectopic fat depositions, which might explain the recently-recognized clinical association between CKD and fatty liver.

Funding: Government Support - Non-U.S.

SA-PO981

Chronic Kidney Disease (CKD) Disruption of Macrophage Lipid and Inflammatory Handling and Effects of Liver X Receptor (LXR) Agonism

Ryohei Kaseda, T. Alp Ikizler, Sergio Fazio, Valentina Kon. *Vanderbilt.*

Background: Macrophage foam cell formation, a key feature of atherosclerotic cardiovascular disease (CVD), reflects an imbalance between uptake and efflux of cellular cholesterol. Advanced CKD requiring dialysis disrupts efflux, but little is known about foam cell formation in moderate CKD which has increased CVD risk. We evaluated cellular lipid handling as well as inflammatory response to lipoproteins from CKD patients and assessed potential benefits of activating cellular cholesterol transporters by LXR agonism.

Methods: LDL and HDL were isolated by sequential density ultracentrifugation of blood specimens from CKD patients (stage 3-4, N=50) and normal controls (N=20). Human THP-1 cells were used to measure uptake of LDL and efflux to HDL with gas chromatography which determined cellular lipid content. HDL effects on cytokines (MCP-1, *TNF-α, IL-1b)*, Toll like receptor-2 (TLR-2), and cholesterol transporters (ABCA1) were assessed by RT-PCR and western blot.

Results: There was no difference in macrophage uptake of $LDL^{Control}$ versus LDL^{CKD}. By contrast, compared to $HDL^{Control}$, cholesterol efflux to HDL^{CKD} was reduced (24.6±1.7 versus 19.8±0.8, p=0.008) and macrophage inflammatory cytokine response was enhanced (IL-1ß 1.09±0.06 versus 1.60±0.09 p<0.001, TNF-α 1.11±0.10 versus 1.55±0.09 p=0.001, MCP-1 0.98±0.05 versus 1.18±0.06 p=0.021). Cellular exposure to LXR agonist (T0901317) significantly increased macrophage ABCA1 gene and protein expression and improved efflux not only to $HDL^{Control}$ but also to HDL^{CKD} (24.6±1.7 versus 28.7±1.3, 19.8±0.8 versus 22.8±0.8, both p<0.001). However, LXR agonist further amplified the cytokine response and increasedHDL^{CKD} effect on TLR-2 (1.07±0.06 versus 1.28±0.13 p=0.008).

Conclusions: CKD-induced potentiation in macrophage foam cell formation does not reflect increased LDL uptake, and instead denotes reduced efflux due to impairment in cholesterol acceptor function of HDL^{CKD}. LXR activation in cellular cholesterol transporters improves efflux to HDL^{CKD}. Thus, even in the face of dysfunctional HDL, activation of cellular transporters can abrogate pro-atherogenic lipid handling, a benefit offset by heightened pro-inflammatory effect of HDL^{CKD}.

Funding: NIDDK Support, Pharmaceutical Company Support - Merck Pharmaceuticals

SA-PO982

Evaluating Observed versus Expected Mortality Rates in U.S. Dialysis Patients Amid Secular Declines

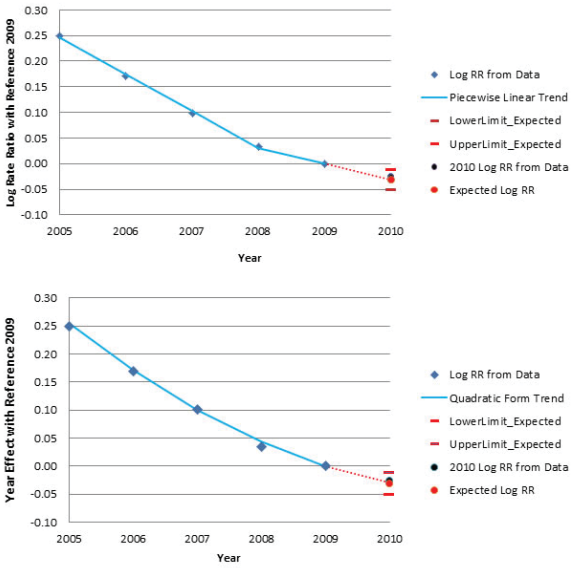
Jiannong Liu,[1] Keri Monda,[2] Charles A. Herzog,[1] M. Alan Brookhart,[3] Til Stürmer,[3] Brian D. Bradbury,[2] Kenneth J. Rothman,[4] Glenn M. Chertow.[5] *[1]Chronic Disease Research Group, Mpls, MN; [2]Amgen, Inc., Thousand Oaks, CA; [3]UNC-CH, Chapel Hill, NC; [4]Research Triangle Inst, Raleigh, NC; [5]Stanford Univ, Palo Alto, CA.*

Background: Effects on mortality and CV events in dialysis patients (pts) of the 2011 ESA label and reimbursement changes have not been carefully examined. We modelled recent mortality trends in U.S. dialysis pts, as a first step in a before/after comparison.

Methods: Using the CMS ESRD database, we included all adult (≥18) pts on dialysis for ≥9 mos, 2005-2010, with Medicare as primary payer (MPP) for ≥6 mos. Pts were followed from Jan 1 or day 1 of the first calendar mo after criteria were met, each yr, to the earliest of death, loss of MPP, modality change, transplant, or yr end. Time trends were modeled using Poisson regression with log link for 2005-2009, adjusted for pt characteristics. We used a piecewise linear function of yr with 2008 as the node, and a quadratic polynomial function for the time trend. The linear trend from 2008-2009 and the quadratic function were extrapolated to 2010; we used bootstrapping to estimate the standard error and 95% CI. The expected 2010 mortality rate was calculated using the extrapolation and 2010 cohort characteristics, and compared with the observed rate.

Results: Both the piecewise linear and quadratic functions fit the 2005-2009 trend fairly well and predicted the 2010 death rate accurately. The expected 2010 death rate was 20.0 per 100 pt-yrs from both functions; the observed rate was 19.9.

Figure. Observed, Fitted, and Expected Log Rate Ratios



Conclusions: Accurately predicting temporal mortality trends in dialysis pts is possible. This sets the groundwork for analyses examining deviance of observed 2011/2012 mortality rates from rates expected based on complete 2005-2010 data in a context of secular decline.

Funding: Pharmaceutical Company Support - Amgen, Inc.

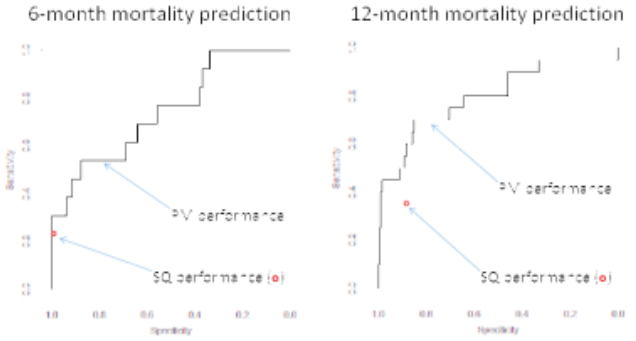
SA-PO983

Performance of Surprise Question Compared to Prediction Models in Hemodialysis Patients Stephan Thijssen,[1] Yuedong Wang,[2] Rebecca H. Apruzzese,[1] Paul Balter,[3] Qingqing Xiao,[1] Len A. Usvyat,[3] Peter Kotanko.[1] *[1]Renal Research Inst, New York, NY; [2]Statistics and Applied Probability, Univ of California, Santa Barbara, CA; [3]Fresenius Medical Care North America, Waltham, MA.*

Background: The surprise question (SQ) ("Would you be surprised if this patient were still alive in X months?") is a significant predictor of patient survival on hemodialysis (HD). We compared the SQ with novel statistical prediction models (PM) for mortality prediction.

Methods: We developed a set of 2 statistical models (with different sets of predictor variables to cover the majority of patients) for 6- and 12-month mortality prediction in a large cohort of U.S. chronic HD patients (1/2 for training, 1/4 for parameter selection, 1/4 for validation). 6- and 12-mo SQ survey responses were obtained from attending nephrologists for 215 prevalent HD patients from 5 U.S. clinics. Patients were prospectively followed for 12 mo and their survival compared to model predictions using ROC analysis.

Results: The SQ's sensitivity and specificity were 0.2308 and 0.9897 @ 6 mo and 0.35 and 0.8817 @ 12 mo. The PM's area under the ROC curve was 0.75 and 0.8 for 6- and 12-mo prediction, resp. The PM outperformed the SQ for both 6- and 12-mo mortality prediction (greater sensitivity @ the same specificity, and vice versa; see figure: SQ well below PM's ROC curve).



Conclusions: The SQ has a low sensitivity for mortality prediction in HD patients and is not well suited for identifying patients at risk. Statistical PM can deliver superior sensitivity and specificity compared to the SQ. Further, they provide a death probability and, thus, more detailed information than the binary SQ. With adequately chosen cutoffs for PM, the tradeoff between sensitivity and specificity can be optimized to suit the clinical need in the context of HD patient care.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

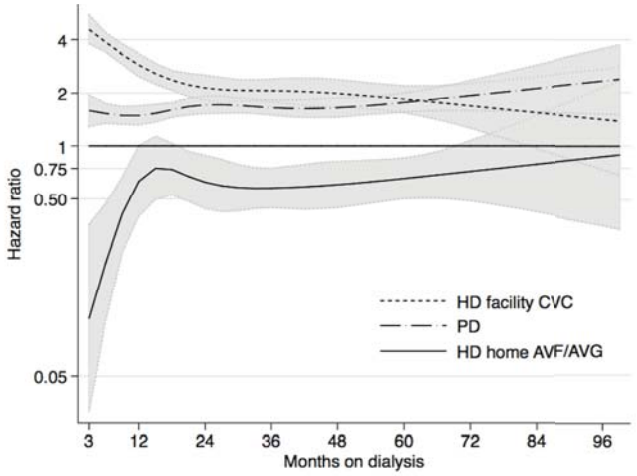
SA-PO984

Dialysis Modality and Mortality: Applying Marginal Structural Models to Data from Australian and New Zealand Dialysis and Transplant (ANZDATA) Registry Kevan Polkinghorne,[1,3] Jessica Kasza,[1] Rory Wolfe,[1] Mark R. Marshall,[2,3] Stephen P. McDonald.[3] *[1]Epidemiology and Preventative Medicine, Monash Univ, Melbourne, Victoria, Australia; [2]Nephrology, Middlemore Hospital, Auckland, New Zealand; [3]ANZDATA Registry, Royal Adelaide Hospital, Adelaide, South Australia, Australia.*

Background: The optimal dialysis modality for patients with end stage kidney disease remains unclear. Without adequate randomized studies, sophisticated analyses of observational data must inform decision-making. Previous analyses comparing dialysis modalities have failed to account for vascular access (VA) type in the hemodialysis patients.

Methods: All incident adult patients who commenced dialysis between October 1, 2003 and December 31, 2011 were assessed. The exposure of interest was time-varying dialysis modality (PD, facility HD, or home HD), with facility and home HD sub-classified by VA type (CVC, or AVF/AVG). Discrete-time Cox proportional hazards marginal structural models for death were fit. Each patient's observation at each period was weighted using stabilized inverse probability of treatment and censoring weights, estimated using propensity score models, which account for exposure and censoring history.

Results: 20,191 patients with mean 2.5 years follow-up were included. 30% of patients had at least one change in dialysis modality. Relative to facility HD AVF/AVG (HR=1, figure) the risk of death for patients treated with home HD with AVF/AVG was much lower early, waning over the first year and stabilizing around 0.75 from 24 months. HD CVC and (to a lesser extent) PD were associated with an ongoing higher risk of death.



Conclusions: Relative to facility HD with permanent VA, home HD has improved survival while facility CVC has the highest risk. PD has a lower risk of death compared to facility CVC but was consistently higher than facility HD.

SA-PO985

Vascular Access and Survival in a Large Catheter-Dominant Dialysis Program Damien Ashby, Neill D. Duncan, Albert J. Power, Sophie Kathirgamanathan. *Renal and Transplant, Imperial College London, London, United Kingdom.*

Background: Compared to other forms of access, arteriovenous fistulae are associated with improved survival in haemodialysis patients, and are widely believed to be superior, but the evidence base is entirely observational, and therefore subject to bias. Centre level data avoids some of this bias, so the outcome in centres with unusual practice patterns can be informative.

Methods: The largest UK dialysis centre(currently 1390 haemodialysis patients) is also an extreme outlier in terms of access practice with 80% of prevalent patients dialysing on tunneled catheters. This retrospective study examined survival after 90 days, in a cohort of patients who started dialysis during a 3 year period, with respect to vascular access.

Results: Between 01.12.2005 and 01.12.2008, 746 incident haemodialysis patients (aged 18–91, 60.1% male, 47.2% caucasian) were included. The dominant form of access was a pair of single-lumen tunneled catheters, used in 85.4% of patients at 90 days. Over a mean observation period of 1178 days 34.7% of patients died, with fistula access associated with longer survival (median 2721 versus 2298 days, p=0.019). In a Cox regression model, the independent predictors of survival were age, ethnicity, comorbidity and renal diagnosis, with fistula access only weakly associated with outcome (HR 0.717, p=0.116). In the catheter group, one year(after 90 day) survival was 91.5%, after adjustment to age 60.This compares favourably with the UK age adjusted average of 89.0% for the whole 2008 incident cohort (all access types).

Conclusions: The advantage of fistula access is diminished in this catheter-dominant program, where outcome in catheter patients is at least equivalent to the national average. This could be because catheter outcomes are improved incentres where they are more frequently used, or because a substantial fraction of the fistula "advantage" is actually a result of selection bias.

SA-PO986

Association between Catheter Anatomy and Fatal Cardiac Arrest in Hemodialysis Kevin Chan, Ann Mooney, Len A. Usvyat, Franklin W. Maddux. *Fresenius Medical Care - NA, Waltham, MA.*

Background: Mortality risk doubles for ESRD patients who dialyze through a catheter vascular access when compared to patients who dialyze through an arteriovenous fistula(Chan et al., 2011). The pathophysiological mechanism for this catheter-related excess mortality remains unclear. As the tip of the catheter sits in the right atrium where the outflow of blood is deposited, we hypothesized the tip's proximal location is pro-arrhythmogenic because it directly exposes the heart to electrolyte shifts.

Methods: Case-control study of all hemodialysis patients with a catheter vascular access discharged from the Fresenius Medical Care North America (FMCNA) Dialysis Network from January 1997 to April 2014. The patient outcome was determined by the reason for the patient's discharge: death versus other (transfer of care, recovery of renal function, or transplantation). The exposure variable was the anatomical location of the catheter: upper body (internal-jugular or subclavian) or femoral. Logistic regression was used to determine the association of catheter location with cardiac mortality.

Results: We identified 310,801 hemodialysis patients with a catheter vascular access who were discharged from the FMCNA Dialysis Network. 3.5% of these patients were discharged with a femoral vascular access. Using logistic regression, we found patients with a femoral catheter associated with a significant 18% increased risk of fatal cardiac arrest when compared to patients with an internal-jugular or subclavian vascular access (Table 1). Femoral catheter patients were at even greater risk of dying from sepsis.

	Events (%)		OR (95% CI)
	Femoral	Upper Body	
Cardiac Arrest	19.8%	17.3%	1.18 (1.13-1.23)
Intra-dialytic cardiac arrest	1.36%	1.40%	0.97 (0.85-1.19)
Sepsis	7.7%	4.8%	1.67 (1.57-1.78)

Conclusions: Given significantly greater risk of cardiac arrests and sepsis episodes, femoral catheters should be the vascular access of last resort.

SA-PO987

High-Flux versus Low-Flux Hemodialysis in Patients with Higher Dialysis Dose: Insights From the MPO Trial Martin Wagner,[1,2] Franziska Koenigsbauer,[1,3] Navdeep Tangri,[4] Jan Beyersmann,[3] David M. Kent,[5] Peter U. Heuschmann,[2] Christoph Wanner,[1] Francesco Locatelli.[6] *[1]Div. of Nephrology, Univ Hospital Wuerzburg, Germany; [2]Inst of Clinical Epidemiology and Biometry, Comprehensive Heart Failure Center, Univ of Wuerzburg, Germany; [3]Inst of Statistics, Univ of Ulm, Germany; [4]Div. of Nephrology, Univ of Manitoba, Winnipeg, Canada; [5]Center for Predictive Medicine Research, Tufts Medical Center, Boston, MA; [6]Dept of Nephrology, Allessandro Manzoni Hospital, Lecco, Italy.*

Background: Mortality risk varies among hemodialysis (HD) patients. High-flux membranes were thought to improve survival, but evidence remains unclear. We investigated the effect of high-flux versus low-flux HD on mortality in patients of the MPO trial with higher versus lower dialysis dose, considering individual's baseline mortality risk.

Methods: Incident HD patients of 9 European countries were randomized to high-flux versus low-flux and followed for up to 7.5 yrs if they achieved a single-pool Kt/V >1.2 after a 4-12 wks run-in phase. Primary endpoint was death of all causes. Baseline mortality risk was determined by a novel clinical score that we developed in the UK Renal Registry and validated in DOPPS. Dialysis dose was classified as Kt/V≥1.4 or <1.4.

Results: As determined by the score, patient characteristics (n=637) varied across risk quartiles (age, BMI, residual renal function, vascular access, diabetes, CVD, hematocrit, albumin, creatinine, all p<0.05). In patients with Kt/V≥1.4 (n=221), high-flux was associated with a beneficial effect on mortality (HR 0.59, p=0.058) independent from baseline risk (HR 1.07, p<0.01). We detected evidence suggestive for variation of treatment effect by high-flux according to baseline risk, but the interaction of risk*flux was not statistically significant (p=0.1). No effect was found for high flux (HR 1.05, p=0.8) in the Kt/V<1.4 group (n=416) while baseline risk was associated with increased risk of mortality (HR 1.09, p<0.01).

Conclusions: Our clinical risk score accurately stratified incident HD patients according to mortality risk. The results suggest that high-flux HD may prolong survival in patients treated with higher dialysis doses, independent from baseline mortality risk.

SA-PO988

Different Impact of Hemodialysis Vintage on Cause-Specific Mortality in Long-Term Hemodialysis Patients in Japan Keiichi Sumida,[1] Kunihiro Yamagata,[2] Kunitoshi Iseki,[2] Yoshiharu Tsubakihara.[2] *[1]Nephrology Center, Toranomon Hospital Kajigaya, Kawasaki, Kanagawa, Japan; [2]Renal Data Registry Committee, Japanese Society for Dilysis Therapy, Tokyo, Japan.*

Background: Along with the improvement of hemodialysis care, the proportion of patients with a longer hemodialysis (HD) vintage has been increasing. Several studies have shown that the HD vintage is associated with increased mortality risk in patients receiving HD. However, no significant effect of HD vintage on cause-specific mortality in HD patients has been shown, especially among long-term HD survivors.

Methods: To elucidate the effect of HD vintage on cause-specific mortality, we used the standard analysis file (JRDR-12002) and conducted a nationwide registry-based retrospective cohort study of 192,760 maintenance HD patients who had been on hemodialysis more than one year and who could be followed for a year between 2008 and 2009. The HD vintage was divided into eight subgroups: 1–<2, 2–<5, 5–<10, 10–<15, 15–<20, 20–<25, 25–<30, and ≥30 years.The study outcomes were 1-year all-cause and cause-specific mortality.

Results: The mean age and hemodialysis vintage of study participants were 66.2 ± 12.4 and 8.3 ± 6.9 years. During the study period, 15,998 deaths occurred from all causes, which included 6302, 3019, and 1459 deaths, respectively, from CVD, infection-related, and cancer causes. In multivariate analysis (vintage 1–<2 years as referent), there was a significant association between the HD vintage and the odds ratio (OR) of all-cause mortality with an increasing trend of OR in each vintage (vintage ≥30, OR = 1.84, 95% CI 1.50–2.27). A similar association was observed between the HD vintage and infection-related mortality (vintage ≥30, OR = 3.11, 95% CI 2.10–4.61). The OR of CVD mortality remained significant and fairly constant up to 20–<25 years of category (OR = 1.14 to 1.37) followed by reductions in OR to 1.28 (95% CI 0.94–1.75) and 1.13 (95% CI 0.78–1.65) for HD vintages of 25–<30 and ≥30 years, respectively. No significant association was found between the HD vintage and cancer mortality.

Conclusions: The HD vintage has a different impact on cause-specific mortality in HD patients. This impact was more pronounced among long-term HD patients.

SA-PO989

Inflammation and Oxidative Stress in Hemodialysis Patients with Sleep Apnea Syndrome Olga Nikitidou,[1] Vassilios Liakopoulos,[1] Euphemia Daskalopoulou,[2] Nicholas V. Dombros,[1] Aikaterini A. Papagianni.[3] *[1]Nephrology and Hypertension, 1st Dept of Internal Medicine, AHEPA Univ Hospital, Medical School, Aristotle Univ of Thessaloniki, Thessaloniki, Greece; [2]Sleep Laboratory, Dept of Internal Medicine, St. Paul General Hospital, Thessaloniki, Greece; [3]Nephrology Dept, Hippokration General Hospital, Medical School, Aristotle Univ of Thessaloniki, Thessaloniki, Greece.*

Background: Sleep apnea syndrome (SAS) is an established cardiovascular risk factor in the general population related to inflammation and oxidative stress and is very common among hemodialysis patients. The aim of the present study was to investigate the role of SAS in the promotion of inflammation and oxidative stress in hemodialysis patients.

Methods: 37 hemodialysis patients (23 males) participated in the study. The night between two consecutive midweek hemodialysis sessions, they underwent an overnight polysomnography study with a "SOMNOscreen™" device. The following morning blood samples were obtained for TNF-α, IL-6, MPO and oxLDL.

Results: We investigated the correlation of patients' markers of inflammation and oxidative stress with their characteristics (age, BMI, duration of hemodialysis) and their sleep parameters (total sleep time, AHI–Apnea-Hypopnea Index, RDI-Respiratory Disturbance Index, DI-Desaturation Index, mean and minimum SpO_2 and percentage of sleep time with SpO_2<90%). TNF-αcorrelated positively with BMI (r=0.510, p<0.0001) and total sleep time(r=0.370, p=0.027). IL-6 correlated positively with age (r=0.363, p= 0.027), AHI (r=0.385, p=0.018), DI (r=0.336, p=0.042) and percentage of sleep time with SpO_2<90% (r=0.415, p=0.012) and negatively with mean SpO_2 (r=-0.364, p=0.027). MPO correlated positively with AHI (r=0.385, p=0.018), DI (r=0.380, p=0.02) and percentage of sleep time with SpO_2<90%(r=0.388, p=0.019). Finally, oxLDL correlated positively with BMI (r=0.443, p=0.007), AHI (r=0.395, p=0.015), RDI (r=0.328, p=0.048) and total sleep time with SpO_2<90%(r=0.389, p=0.019).

Conclusions: These results indicate that, in hemodialysis patients, the severity of SAS and nocturnal hypoxia correlate with markers of inflammation and oxidative stress and may contribute to their augmented cardiovascular risk.

Funding: Government Support - Non-U.S.

SA-PO990

Dental Health and Mortality in People with End-Stage Kidney Disease Treated with Hemodialysis: A Multinational Cohort Study Giovanni F.M. Strippoli.[1,2,3,4] *[1]On Behalf of the ORAL-D Study Investigators; [2]Diaverum; [3]Univ of Sydney; [4]Amedeo Avogadro Univ of Eastern Piedmont.*

Background: Clinical outcomes for adults treated with hemodialysis are poor and have not substantially improved, with typical mortality rates of 15% per annum. Interventions evaluated to date have largely been ineffective so novel, testable determinants of health outcomes for dialysis patients are needed.

Methods: We did a prospective multinational cohort study (the ORAL-D study) conducted from 2010 to 2012 **t**o investigate whether dental disease and health practices are associated with early mortality in adults with end-stage kidney disease on hemodialysis. We included 4205 patients in Europe and Argentina. We assessed edentulousness (complete tooth loss), dental disease (number of decayed, filled and missing teeth), frequency of teeth brushing and changing a toothbrush, use of dental floss and mouthwash, time spent on oral hygiene, age at first dental visit and time elapsed since last dental visit. The outcomes were all-cause and cardiovascular mortality.

Results: Over an average of 22.1 months (7737 person-years) follow-up, 942 deaths occurred including 477 from cardiovascular causes. Edentulousness (adjusted hazard ratio [HR] 1.28, 95% CI 1.11–1.48) and decayed, missing, or filled teeth score ≥ 14 (1.67, 1.33–2.10) were associated with early all-cause mortality whilst dental flossing (0.52, 0.33–0.82), brushing teeth daily (0.77, 0.60–0.97), spending at least two minutes oral hygiene daily (0.82, 0.70–0.96), changing a toothbrush at least every three months (0.79, 0.67–0.95), and visiting a dentist within the past six months (0.79, 0.65–0.95) were all associated with better survival. Results for cardiovascular mortality were similar, although associations with brushing teeth and time elapsed since a recent dental visit were not significant. These associations were independent of socioeconomic and clinical prognostic factors.

Conclusions: In adults with end-stage kidney disease treated with long-term hemodialysis, poorer dental health was associated with higher risk of death, including cardiovascular mortality, whereas preventive dental health practices were associated with better survival.

Funding: Pharmaceutical Company Support - Diaverum

SA-PO991

Periodontitis and Early Mortality in Adults with End-Stage Kidney Disease treated with Hemodialysis Giovanni F.M. Strippoli.[1,2,3,4] *[1]On Behalf of the Oral Diseases in Dialysis (ORAL-D) Study Investigators; [2]Diaverum; [3]Univ of Sydney; [4]Amedeo Avogadro Univ of Eastern Piedmont.*

Background: Periodontitis is associated with cardiovascular events in the general population and represents a testable health determinant of dialysis patient outcomes. We aimed to evaluate whether periodontitis is associated with early mortality and cardiovascular death in adults treated with hemodialysis.

Methods: The ORAL-D study is a prospective multinational cohort study of oral health in adult hemodialysis patients in Europe and Argentina treated between 2010-2012.

We included 4205 patients who underwent a standardized oral examination at baseline. We assessed for periodontitis according to World Health Organization and Centers for Disease Control definitions. We calculated the hazards of all-cause and cardiovascular mortality using a random-effects Cox proportional hazards analysis fitted using a shared frailty model to account for clustering within countries and adjusted for sociodemographic and clinical variables.

Results: During 6150 person-years of follow-up, 650 deaths occurred of which 325 were cardiovascular. Periodontitis was evaluable in 3338 dentate participants of which 1355 (40.6%) had moderate to severe periodontitis. In crude analyses, moderate to severe periodontitis was associated with lower risks of all-cause (HR 0.78, 95% confidence interval 0.67 to 0.92) and cardiovascular mortality (0.70, 0.56 to 0.89). There was evidence of decreasing mortality risk with more severe periodontal disease (P=0.02 for trend). However, when analyses were clustered by country and adjusted for other prognostic clinical and socio-demographic factors, periodontitis was not associated with all-cause (0.86, 0.71 to 1.05) or cardiovascular mortality (0.77, 0.59 to 1.02). Similar results were observed when the Centers for Disease Control (CDC) definition for periodontitis was used.

Conclusions: Unlike in other settings, periodontitis is not an independent risk factor for early mortality or cardiovascular death for adults treated with hemodialysis.

Funding: Pharmaceutical Company Support - Diaverum

SA-PO992

Geriatric Nutritional Risk Index Predicts Quality of Life and Clinical Outcome in Hemodialysis Patients No Better Than Its Individual Components Ilia Beberashvili,[1] Ada Azar,[2] Inna Sinuani,[3] Gregory Shapiro,[1] Leonid Feldman,[1] Zhan Averbukh.[1] *[1]Nephrology, Assaf Harofeh Medical Center, Zerifin, Israel; [2]Nutrition, Assaf Harofeh Medical Center, Zerifin, Israel; [3]Pathology, Assaf Harofeh Medical Center, Zerifin, Israel.*

Background: Geriatric Nutritional Risk Index (GNRI) has been reported as a predictor of clinical outcomes in maintenance hemodialysis (MHD) patients. The aim of our study was to test whether GNRI provides an improved specificity for adverse prognosis in this population than its individual components.

Methods: Atwo-year prospective observational study performed on 261 MHD outpatients (38.7% women) with a mean age of 68.6±13.6 years. Prospective all-cause and cardiovascular (CV) hospitalization and mortality, geriatric nutritional risk index (GNRI) and short form 36 (SF-36) quality-of-life (QoL) scores were measured.

Results: For each one unit increase in baseline GNRI levels, the first hospitalization hazard ratio (HR), after adjustments for confounders, was 0.98 (95% confidence interval (CI), 0.97 to 0.99) and first CV event HR was 0.96 (95% CI, 0.93 to 0.99); all-cause death HR was 0.96 (95% CI, 0.93 to 0.98) and CV death HR was 0.95 (95% CI, 0.92-0.99). Adjusted HRs for each 0.1 g/dl increase in baseline albumin levels were 0.94 (95% CI, 0.91 to 0.98), 0.89 (95% CI, 0.82 to 0.97), 0.86 (95% CI, 0.80 to 0.91) and 0.87 (95% CI, 0.79 to 0.95), respectively. The accuracy of albumin was also stronger than GNRI in predicting of above clinical outcomes according to the receiver operating characteristic (ROC) curve analysis. Albumin was related to self-reported QoL with higher strength and magnitude than GNRI. This difference was prominent in total score (r=0.24, P<0.001 versus r=0.15, P=0.01), mental health (r=0.23, P<0.003 versus r=0.15, P=0.02) and physical health (r=0.23, P<0.001 versus r=0.16, P=0.02) dimensions, and in most scales of the SF-36. Body weight/ ideal body weight, another component of GNRI, performed worse than GNRI as a predictor of clinical outcomes and QoL.

Conclusions: Despite the strong relationship with clinical outcomes and QOL, GNRI doesn't improve or even worsens performance of albumin. This questions the clinical value of GNRI as a prognostic tool in MHD population.

SA-PO993

A Pilot Evaluation of ZS-9 as a Potential Emergency Treatment for Hyperkalemia William Peacock,[1] Zubaid Rafique,[1] Alex Yang,[2] Philip T. Lavin,[3] Henrik S. Rasmussen,[4] Phillip D. Levy.[5] *[1]Baylor College of Medicine, Houston, TX; [2]Xelay Acumen, Inc., Belmont, CA; [3]Boston Biostatistics Research Foundation, Framingham, MA; [4]ZS Pharma, Inc., Coppell, TX; [5]Wayne State Univ, Detroit, MI.*

Background: Hyperkalemia is a common problem, with higher levels requiring emergent intervention. Non-invasive treatments are limited by a slower rate of potassium (K+) reduction, with more rapid therapy (i.e., renal replacement therapy [RRT]) being both invasive and expensive, with estimated costs of up to $140,000 per episode. ZS-9, an orally dosed zirconium-based potassium (K^+) trapping agent, may lower serum K^+ at a sufficient rate to avoid the need for emergent RRT in selected pts. Our purpose was to evaluate the rate of reduction and dose relationships of ZS-9 as a potential therapy for pts with baseline K^+ >6.0 mEq/L.

Methods: Data from a large prospective phase 3 study, designed to evaluate different doses of ZS-9 for acute and chronic treatment of moderate hyperkalemia, are abstracted. Pts were included if they had a baseline K^+ >6.0 and repeated K^+ measurements.

Results: Thirteen pts met entry criteria, of whom 54% were male, 31% black and 69% white. Mean age (SD) was 69.7 (10.7) yrs in the 5g group and 67.0 (5.2) yrs in the 10g group. Comorbidities included 46% chronic kidney disease, 46% congestive heart failure, and 69% diabetes mellitus. 9 pts (69.9%) had a K^+<6.0 by 4 hrs (Table) with an effect on K^+ that was dependent upon ZS-9 dosing.

Potassium, mEq/L	ZS-9 dosage	
	5g (n=7)	10g (n=6)
K^+ at baseline, mean (SD)	6.1 (0.07)	6.2 (0.2)
K^+ at 4 hrs post-treatment, mean (SD)	5.6 (0.5)	5.3 (0.9)
K^+ change over 4 hrs, mean (SD)	-0.5 (0.5)	-0.8 (0.8)
No. (%) pts with K^+ <6.0 mEq/L by 4 hrs	5 (71.4%)	4 (66.7%)

Conclusions: ZS-9 may be an effective emergency treatment for hyperkalemia and can potentially be used to avoid acute RRT, translating to substantial cost savings. This should be further evaluated in a randomized prospective trial.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

SA-PO994

Insulin Resistance Is Associated with New-Onset Cardiovascular Events in Non-Diabetic Peritoneal Dialysis Patients Chang-Yun Yoon, Young Su Joo, Eunyoung Lee, Jung Tak Park, Seung Hyeok Han, Tae-Hyun Yoo. *Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea.*

Background: Chronic exposure to high glucose-containing peritoneal dialysis (PD) solution and consequent abdominal obesity are potential sources of insulin resistance (IR) in prevalent PD patients. The aim of this study was to elucidate the prognostic values of IR on new-onset cardiovascular (CV) events in non-diabetic prevalent PD patients.

Methods: A total of 201 non-diabetic prevalent PD patients were recruited. IR was assessed by homeostatic model assessment of IR (HOMA-IR) using fasting plasma insulin and glucose levels. The primary outcome was new-onset CV events during the follow-up period. Cox proportional hazard analysis was performed to ascertain the independent prognostic value of HOMA-IR for the primary outcome.

Results: The mean age was 53.1 years and male was 49.3% (n=99). The mean HOMA-IR was 2.6±2.1. HOMA-IR was positively associated with body mass index (BMI) and serum concentrations of triglyceride (TG) and calcium, whereas negatively associated with high-density lipoprotein cholesterol. Moreover, previous CV diseases were significantly more prevalent in the high HOMA-IR group. In multivariate linear regression, BMI (β=0.222, P=0.002), TG (β=0.421, P<0.001), and previous CV diseases (β=0.151, P=0.032) were still significantly associated with HOMA-IR. During a mean follow-up duration of 40.2 months, the primary outcome was observed in 36 patients (17.9%). When patients were divided into tertiles according to HOMA-IR, the highest tertile group showed significantly higher incidence rates of new-onset CV events compared to the lower two-third group (P=0.029). Furthermore, multivariate Cox analysis revealed that HOMA-IR was a significant independent predictor of the primary outcome (hazard ratio=1.18, 95% confidence interval=1.03-1.35, P=0.014).

Conclusions: IR measured by HOMA-IR was an independent risk factor for new-onset CV events in non-diabetic prevalent PD patients.

SA-PO995

Glycated Albumin Has a Stronger Association Than Hemoglobin A1C with Mortality in ESRD Patients in the 4D Trial Christina Chen,[1] Christiane Drechsler,[2] Christoph Wanner,[2] S. Ananth Karumanchi,[1] Anders H. Berg.[1] *[1]Beth Israel Deaconess Medical Center; [2]Univ Hospital Würzburg.*

Background: Hemoglobin $A1_c$ (Hb $A1_c$) may not be an accurate index of glycemic control in patients with renal insufficiency due to factors such as uremia and epogen that affect the lifespan of red cells, and these effects may confound the relationship between Hb $A1_c$ and prediction of outcomes. This study compares the risk of death associated with glycated albumin (GA) versus Hb $A1_c$ in ESRD patients.

Methods: The Deutsche Diabetes und Dialyse Studie (4D study) was a prospective, randomized control trial (RCT) involving 178 dialysis centers in Germany that investigated the cardiovascular benefit of atorvastatin use in 1255 patients with ESRD with median time of follow up of 4 years. Hb $A1_c$ and GA were measured in samples obtained at month 6. Cox regressionanalysis was used to determine the hazard ratio (HR) for all-cause mortality over 42 months for GA as well as Hb $A1_c$.

Results: GA was studied as a continuous variable using univariate Cox proportional HR and found to be significant (HR 1.274; 95% CI 1.009-1.610, P-value 0.042). It remained significant after using multivariate analysis to adjust for age, sex, atorvastatin treatment, systolic relative risk, body mass index, coronary artery disease, heart failure, peripheral artery disease, duration of diabetes, hemoglobin, phosphate, and c-reactive protein (HR 1.291; 95% CI 1.004-1.659, P-value 0.046). In contrast, univariate analysis of Hb $A1_c$ values found no significant risk(HR 1.007; CI 0.937-1.082, P-value 0.954) and was also not significant when using multivariate analysis of the above variables (HR 1.029; 95% CI 0.951-1.114, P-value 0.474).

Conclusions: This is the largest study to date comparing the prediction of risk by GA and Hb $A1_c$ tests for glycemic control in patients on hemodialysis.These results suggest that GA is more closely associated to mortality than Hb $A1_c$, in agreement with previous smaller studies. Additional studies are necessary to confirm that targeting lower GA levels improves survival.

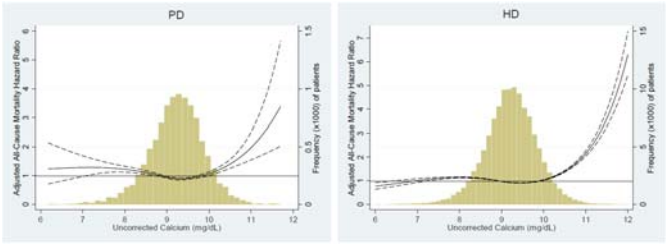
SA-PO996

Time-Averaged Uncorrected Serum Calcium: Relationship with Dialysis Modality and Cause-Specific Mortality Matthew B. Rivara,[1] Vanessa A. Ravel,[2] Kamyar Kalantar-Zadeh,[2,3] Elani Streja,[2] Wei Ling Lau,[3] Allen R. Nissenson,[4,5] Bryan R. Kestenbaum,[1] Ian H. de Boer,[1] Jonathan Himmelfarb,[1] Rajnish Mehrotra.[1] *[1]Kidney Research Inst, Seattle, WA; [2]Harold Simmons Ctr for Chronic Dis Research and Epi, UC Irvine Med Ctr, Irvine, CA; [3]Nephrology, UC Irvine Med Ctr, Orange, CA; [4]DaVita, Inc., El Segundo, CA; [5]David Geffen Sch of Med at UCLA, Los Angeles, CA.*

Background: Uncorrected serum calcium concentration is the first mineral metabolism metric planned for use as a quality measure in the U.S. dialysis population. Few studies in patients undergoing peritoneal dialysis (PD) or hemodialysis (HD) have assessed the association of uncorrected serum calcium with outcomes.

Methods: This observational cohort study uses data from 129,076 dialysis patients (PD 10,066; HD 119,010) treated in a large dialysis organization from 2001-2006. We performed Cox proportional hazards survival analyses to determine the relationship of time-averaged uncorrected serum calcium with all-cause, cardiovascular, and infection-related mortality.

Results: Following adjustment for potential confounders, uncorrected calcium <8.5 and ≥10.2 mg/dl was associated with excess all-cause mortality in both PD and HD patients (comparison group: calcium 9.0 to <9.5 mg/dl). Further adjustment for serum albumin attenuated the all-cause mortality hazard ratios (HRs) associated with calcium <8.5 mg/dl (HR 1.29: 95% CI 1.16, 1.44 for PD; HR 1.17: 95% CI 1.13, 1.20 for HD), and amplified the HRs associated with calcium ≥10.2 mg/dl (HR 1.65: 95% CI 1.42, 1.91 for PD; HR 1.59: 95% CI 1.53, 1.65 for HD). Calcium ≥10.2 mg/dl was also associated with increased risk for cardiovascular mortality (HR 1.45: 95% CI 1.15, 1.84 for PD; HR 1.64: 95% CI 1.55, 1.73 for HD).



Conclusions: In a large nationally representative cohort of dialysis patients, high concentrations of uncorrected serum calcium were associated with increased mortality risk, irrespective of dialysis modality.

Funding: NIDDK Support

SA-PO997

High Dialysate Calcium Concentration Promotes Low Parathyroid Hormone Status, which Is an Independent Risk Factor for Short-Term Cardiovascular Mortality in Hemodialysis Eric Daugas,[1,2] Emilie Merle,[1,2] Hubert Roth,[2] Gerard M. London,[2] Guillaume Jean,[2] Thierry P. Hannedouche,[2] Jean-Louis Bouchet,[2] Tilman B. Drueke,[2] Denis Fouque.[2,3] *[1]Nephrology, APHP, Bichat Hospital, Paris, France; [2]French Calcium and Phosphate Observatory, France; [3]Nephrology, Hospices Civils de Lyon, France.*

Background: To detail low parathyroid hormone (PTH) status and mortality association in incident patients on hemodialysis.

Methods: Multicenter prospective cohort study including 2164 incident hemodialysis patients at M0 in October 2010, prospectively followed until M24. Patients were classified according to their K/DIGO PTH status at M0 or M12 or both. M12 to M24 all-cause mortality, cardiovascular (CV) mortality and non-CV mortality were evaluated for each PTH status using adjusted Cox analysis. Factors of M0 to M12 PTH variability were analyzed by multivariate logistic regression.

Results: A decrease of PTH to a low status between M0 and M12 was independently and strongly associated with M12-M24 CV mortality (HR 2.01; 95%CI: 1.2-3.37; p =0.008; respectively). Among patients with a high or normal PTH status at M0, M6 independent predictive factors for a decreased PTH to a low status at M12 were hypoalbuminemia (OR: 0.96; 95%CI: 0.93 -0.99; p=0.01), a high dialysate calcium concentration use (1.75 mmol/L) (OR:2.02; 95%CI :1.37 to 2.99; p<0.0001) and to a lesser extent the use of native vitamin D (OR:1.02, 1.01-1.03, p = 0.014), whereas non calcium-based phosphate binders were protective (OR: 0.92, 95%CI: 0;86 to 0.99; p=0.031 for sevelamer and OR: 0.97, 95%CI: 0.95 to 0.99; p=0.04 for lanthanum). In the high CV mortality risk subgroup with an acquired low PTH status at M12, M12 independent factors associated with M12-M24 cardiovascular mortality were age, a C-reactive protein higher than 10mg/L and again a high dialysate calcium concentration (OR:5,43; 95%CI: 2.17-13.62; p<0.0001).

Conclusions: Patients with decreased PTH levels to a low status one year after hemodialysis initiation are at high risk of short-term CV mortality. High dialysate calcium concentration use is an important contributor of PTH decrease and its continuation is a major predictor of CV mortality the year after. Therefore, use of high dialysate calcium concentration should be restricted in hemodialysis patients.

Funding: Pharmaceutical Company Support - SANOFI

SA-PO998

Non-Oxidized PTH (n-oxPTH) Is Associated with Cardiovascular Events and All-Cause Mortality in Patients with Secondary Hyperparathyroidism Undergoing Hemodialysis Who Participated in the EVOLVE Trial Berthold Hocher,[1] Michael Godes,[1] Christoph Reichetzeder,[1] Oleg Tsuprykov,[1] Glenn M. Chertow,[2] Patrick S. Parfrey,[3] Jürgen Floege,[4] Yumi Kubo,[5] Bastian Dehmel,[5] Tilman B. Drueke.[6] *[1]Univ of Potsdam; [2]Stanford Univ School of Medicine; [3]Memorial Univ; [4]RWTH Univ of Aachen; [5]Amgen Inc.; [6]Inserm Unité 1088.*

Background: Oxidized PTH (oxPTH) has no PTH/PTHrp receptor (PTH1R)-stimulating properties, whereas non-oxidized PTH (n-oxPTH) is an agonist at PTH1R. However, PTH oxidation has been ignored in the development of PTH assays: the intact PTH (iPTH) assays currently used, recognize both n-oxPTH and oxPTH. We recently developed an assay that separately measures oxPTH and n-oxPTH.

Methods: To test the hypothesis that n-oxPTH, but not oxPTH or iPTH, has predictive value for cardiovascular events and all-cause mortality, we analyzed baseline plasma samples from 2867 participants of the EVOLVE trial (ClinicalTrials.gov: NCT00345839). The patients were followed for up to 64 months. The primary composite end point (PCEP) was the time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event.

Results: Median (10^{th} and 90^{th} percentiles) baseline iPTH was 497 (247, 1246) pg/mL, oxPTH was 438 (215, 1111) pg/mL and n-oxPTH was 58 (27, 151) pg/mL. Pearson's correlation analyses showed a strong relationship between iPTH and oxPTH (r=0.996; p<0.001) and a weaker relationship between iPTH and n-oxPTH (r=0.82; p<0.001). A multivariate Cox regression model adjusted for patient characteristics, cardiovascular comorbidities and baseline labs on PCEP revealed that n-oxPTH, but not oxPTH or iPTH, was associated with the PCEP (hazard ratio: 1.078; 95%CI: 1.012, 1.148; p=0.020), cardiovascular mortality (hazard ratio: 1.111; 95%CI: 1.014, 1.218; p=0.024), and all-cause mortality (hazard ratio: 1.113; 95%CI: 1.038, 1.193; p=0.003).

Conclusions: Only n-oxPTH, but not iPTH or oxPTH, was associated with cardiovascular events and mortality as well as all-cause mortality in the baseline samples of the EVOLVE trial.

Funding: Pharmaceutical Company Support - Amgen

SA-PO999

The Effect of Cinacalcet on Calciphylaxis Events in Haemodialysis Patients in the EVOLVE Clinical Trial Jürgen Floege,[1] Yumi Kubo,[2] Glenn M. Chertow,[3] Patrick S. Parfrey.[4] *[1]RWTH Univ of Aachen, Germany; [2]Amgen Inc., Thousand Oaks, CA; [3]Stanford Univ School of Medicine, Palo Alto, CA; [4]Memorial Univ, St. John's, NF, Canada.*

Background: Uncontrolled secondary hyperparathyroidism (sHPT) in hemodialysis (HD) patients is a well recognized risk factor for calciphylaxis (calcific uremic arteriolopathy). The effect of calcimimetics on the incidence of calciphylaxis is unknown. Non-adjudicated, adverse events collected during the Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial were used to define the frequency of calciphylaxis events in HD-patients with sHPT receiving cinacalcet or placebo.

Methods: A multicenter, global, randomized, double-blind, placebo-controlled clinical trial assessing mortality and non-fatal cardiovascular events in 3883 HD patients with secondary hyperparathyroidism (baseline median parathyroid hormone (iPTH) concentration 693 pg/mL (10%, 90% range 363 - 1694 pg/mL). Calciphylaxis adverse events were collected while patients were receiving study drug.

Results: Within the study safety analysis set (n= 3861) 24 patients developed calciphylaxis, 18 in the placebo group and 6 in the cinacalcet group (hazard ratio 0.30, 95% confidence interval [CI] 0.12-0.76; log-rank, p=0.007). In a multivariate model, predictors associated with an increased risk of calciphylaxis included treatment with placebo, female sex, higher body mass index, higher diastolic blood pressure, history of peripheral vascular disease, history of parathyroidectomy, as well as current and former tobacco use at baseline. The cumulative event rates (95% CI) at year 4 were 1.5% (0.9%, 2.6%) in placebo patients and 0.7% (0.3%, 1.5%) in cinacalcet patients. Median (10%, 90% range) plasma iPTH at the time of calciphylaxis was 796 (225, 2093) pg/mL in placebo patients and 410 (71, 4957) in cinacalcet patients. Calcium was within the KDIGO target range in most patients. Immediately prior to calciphylaxis, vitamin K antagonists were used by 9/18 placebo patients and 2/6 cinacalcet patients.

Conclusions: In the EVOLVE trial, treatment of sHPT with cinacalcet led to a decreased risk of calciphylaxis. ClinicalTrials.gov number: NCT00345839.

Funding: Pharmaceutical Company Support - Amgen

SA-PO1000

Effect of Paricalcitol and Atorvastatin Combined Treatment on Inflammatory and Oxidative Stress Markers in Chronic Hemodialysis (hD) Patients with Permanent Subcutaneous Catheter (psc). NCT1820767

Ricardo Mouzo,[1] Valter Ruggero Maria Lombardi,[2] Jose Carlos Diez Baylon,[3] Herless Rodrigo Avellaneda Campos,[1] Jose Paniagua De la Riva,[1] Carmen Perez Nieto,[1] Fernando Simal,[1] Benjamin De Leon Gomez,[4] Mario Prieto Velasco1.[4] *[1]Nephrology, Hospital El Bierzo, Ponferrada, Spain; [2]Biotechnology, EBIOTEC, La Coruna, Spain; [3]Hemodialysis, PONFEDIAL, Ponferrada, Spain; [4]Nephrology, Hospital Leon, Leon, Spain.*

Background: Cardiovascular disease remains the most common cause of morbidity and mortality in CKD patients related to oxidative stress, inflammation and endothelial dysfunction. In addition, PSC is an inflammatory stimulus in these patients. The aim of the current study was to evaluate the effect of different oral treatments, paricalcitol (P), paricalcitol plus atorvastatin (P+A), and atorvastatin (A) alone on the release of pro-inflammatory cytokines and oxidative stress.

Methods: 30 patients age 71.21 ±16.88 in HD treatment 3 times per week for 33.93 months ± 35, were randomized into a 12 weeks period study. Group 1 (n=10) was treated with P; Group 2 (n=11) was treated with P+A; Group 3 (n=9) received A alone. Blood samples were collected two weeks before treatment (T-2), at baseline (T0), 6 weeks (T6) and 12 weeks after treatment (T12) and 2 weeks post treatment (T14). CD3, CD4, CD8, CD19, CD25, CD56, CD69 and CD95 lymphocytes blood markers and serum levels of IL-2. IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-1β, TNF-β, TNF-α, and IFN-γ were analyzed by flow cytometry. Serum levels of PEG-2, COX-2, iNOS and FGF-23 were analyzed by ELISA.

Results: Treatment with P+A significantly reduced the expression of CD25, CD56, CD25/CD56 double positive, and CD69 compared with the treatment with P and A alone. A reduction in the release of IFNγ, IL-1β, IL-2 and IL-5 was also observed, mainly in Gr. 1 and 2; and a reduction in COX-2 p<0.012 in T0 versus T12.Moreover treatment with P+A significantly reduced the plasma level of FGF23 (T0 versus T12: p=0.044).

Conclusions: The combined treatment with P+A elicits an early and significant decrease of inflammation and oxidative stress in HD patients. These effects could have a considerable impact in reducing the risk of progressive atherosclerotic cardiovascular disease in patients using psc.

SA-PO1001

Beneficial Effects of Parathyroidectomy on Proximal Aortic Stiffness in Hemodialysis Patients with Secondary Hyperparathyroidism: A Pilot Study

Haiming Li, Shensen Li, Chuanming Hao, Yong Gu, Jing Chen. *Nephrology, Huashan Hospital Fudan Univ, Shanghai, China.*

Background: Aortic pulse wave velocity is a strong predictor of all--cause and cardiovascular mortality in chronic dialysis patients. Increased PWV is associated with both traditional and nontraditional factors in dialysis patients. The effects of parathyroidectomy (PTX) on aortic stiffness remains unclear.Our aim was to evaluate the specific changes of proximal aortic stiffness after successful PTX.

Methods: Forty five patients undergoing PTX were studied at baseline and during a follow-up period of 12 months regarding changes of proximal aortic stiffness using echocardiographic methods(PTX group). We evaluated the pulse wave velocity (PWV) in the region of aortic arch. The clinical data was also recorded at baseline and one year's follow up. 45 age, sex and dialysis vintage matched hemodialysis patients were enrolled as control group. Both the PWV and the clinical data were also recorded in control group. ΔPWV is calculated by PWV(one year after PTX) minus PWV at baseline.

Results: Thirty nine patients in PTX group were analyzed while six patients were excluded (5 due to poor image of echocardiography, 1 due to relapse of hyperparathyroidism within one year). 37 patients were finally analyzed in control group. The pre-dialysis blood pressure in PTX group was dramatically decreased from 158±16 mmHg to 124±13 mmHg in systolic BP and from 92±11 mmHg to 71±12 mmHg in diastolic BP with reduced numbers of anti- hypertension drugs at one year's follow up. The pre-dialysis BP remained similar in the control group after one year's follow up. None of the patients was prescribed Vit D in the PTX group while 31 patients were taking vit D in control group. ΔPWV is significantly lower in the PTX group (0.2±0.03 m/s versus 0.89 ± 0.16 m/s, p<0.001) than in the control group. No difference was observed in both groups when considering the ejection fraction and LVMI.

Conclusions: Parathyroidectomy might slow the progress of aortic stiffness in hemodialysis patients with hyperparathyroidism. The benefits of PTX may be due to decreased serum calcium, iPTH level,blood pressure and prescription of active vit D.

Funding: Government Support - Non-U.S.

SA-PO1002

Sevelemar Decreases Serum Uric Acid Levels and Improves Arterial Stiffness in Hemodialysis Patients

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Background: Sevelamer has an unique effect as a resine that can act as a prebiothic. Avoiding calcium intake, there might be a differential influence of sevelamer in terms of cardiovascular risk profile. The aim of this study was to evaluate the effects of phosphorus binders on serum uric acid, HbA1c and lipid profile levels and the progression of arterial stiffness.

Methods: A total of 151 patients (mean age: 53.0 ± 14.4 years; mean duration of dialysis: 9.5 ± 1.2 years) undergoing maintenance hemodialysis and using the same phosphorus binders under three years follow-up were enrolled into the study. Patients were divided into two groups according to usage of phosphate binders (PB) as sevelamer based PB (group 1; n: 99) and calcium based PB (group 2; n.52). Biochemical parameters were assessed from monthly clinical visits in three year follow up period. Arterial stiffness determined at the initial and at the end of the 3rd year by aortic pulse wave velocity (PWV) (Sphygmo-Cor).

Results: : Serum calcium, phosphorus, parathyroid hormone, CRP and URR levels were similar in two groups. Serum LDL-C levels and PWv values were significantly lower in group 1 (p<0.005). Mean üric acid levels had significantly decreased in group 1 where remained stable in group 2 in three years follow up period. 22.4% of patients in group 1 and 3.8% of patients in group 2 showed a reduction more than 2 mg/dl in mean üric acid levels. Fasting plasma glucose (p:0.001) and HbA1c (p<0.005) levels were significantly lower in diabetic group 1 patients than group 2. PWv values were similar in both groups at the inital of the study. PWv values of group 1 patients were significantly lower than group 2 at the 3rd year of the study (p<0.005).

Conclusions: Despite similar phosphorus levels and dialysis adequency, sevelamer improves the cardiovascular risk by pleiotropic effects through lowing uric acid, LDL-C and HbA1c concentrations Thus, sevelamer improves the cardiovascular risk profile of hemodialysis patients and prevents the progression of arterial stiffness.

SA-PO1003

Changes in Serum Uric Acid Level in Chronic Dialysis Patients prior to Death

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Background: Hemodialysis (HD) patients are subjected to enhanced oxidative damage as a result of reduced anti-oxidant systems and increased pro-oxidant activity. Uric acid has important antioxidant properties.We hypothesized that uric acid levels change prior to death in HD patients.

Methods: In this retrospective cohort study we analyzed a subset of the international MONitoring Dialysis Outcomes (MONDO) initiative (Usvyat, Blood Purification 2013). HD patients from the Fresenius Medical Care (FMC) clinics in Europe who died between 1/1/ 2006 and 12/31/2012 were identified. Only patients with more than one serum uric acid measurement in the 24 months prior to death during the study period were selected. Penalized b-splines were constructed to track the trajectories of uric acid levels in patients before death.

Results: A total of 11,433 incident HD patients (58 % males) were enrolled. Mean serum uric acid was <5.9 mg/dl before death. Males showed higher levels of serum uric acids compared to females. Serum uric acid levels dropped significantly between months 24 and 4 before death, followed by a notable increase in the 3 months prior to death.

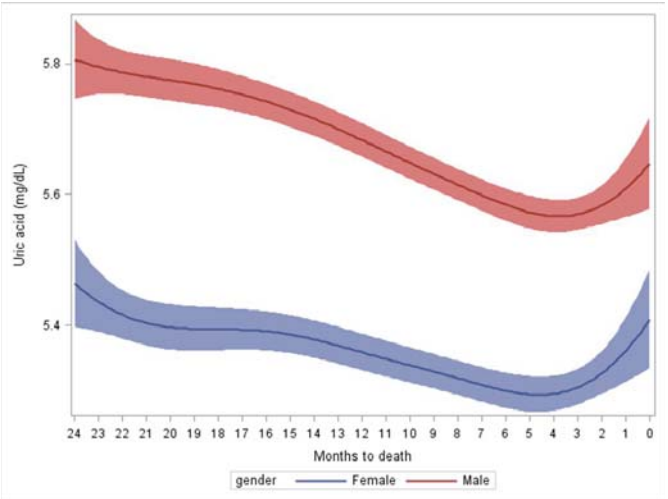

Figure 1: Mean (95% CI) uric acid levels before death.

Conclusions: Our study indicates that uric acid levels present a reverse J-shaped pattern prior to death in incident HD patients, with a significant increase during the last 3 months of life. Additional studies are required to define the biology underlying this dynamic.

SA-PO1004

Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) as an Independent Predictor of Cardiovascular Events in Hemodialysis Patients

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Background: Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which belongs to TNF ligand superfamily, is inversely associated with the severity of atherosclerosis and the prognosis of patients with advanced atherosclerosis. Recent clinical studies have shown that low TRAIL levels were associated with increased mortality in peritoneal and hemodialysis patients. Therefore, we focused on the association between serum TRAIL levels and cardiovascular disease (CVD) events in hemodialysis patients.

Methods: The hemodialysis patients in this study were selected from our prospective observational cohort. Five hundred and sixteen patients were divided into quartile 1(Q1) (lowest) to Q4 (highest) according to their baseline TRAIL level measured by ELISA. The main outcomes were CVD events including ischemic heart disease, stroke, peripheral artery disease, pulmonary edema, and valve disease.

Results: The median (IQR) TRAIL levels were 85.9 (67.7-108.5) pg/mL and did not differ significantly between male and female. During follow-up, 189 CVD events were recorded. A decrease from Q4 to Q1 of TRAIL was associated with a significant increase in CVD events. TRAIL was also associated significantly and inversely with CVD in Cox models adjusted for age and confounding variables including sex, diabetic nephropathy, dialysis vintage, and pre-existing CVD in Q1 versus Q4 of TRAIL (HR 1.79, 95% CI 1.17-2.80, p = 0.007).

Conclusions: Serum TRAIL may be a novel biomarker for predicting CVD events in hemodialysis patients.

SA-PO1005

Association of Oxidized Low-Density Lipoprotein/Low-Density Lipoprotein Cholesterol Ratio, Not Superoxide Dismutase Level, with Cardiovascular Disease and Coronary Artery Calcification in Hemodialysis Patients

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Background: and Aims: Serum malondialdehyde-modified low-density lipoprotein (MDA-LDL)/LDL-cholesterol (LDL-c) ratio, a marker for the degree of LDL oxidation, has been shown to be a risk factor for cardiovascular disease (CVD) and metabolic syndrome in non-hemodialysis (HD) subjects. However, little information is available on the association of this ratio with antioxidant level ([high-density lipoprotein-cholesterol [HDL-c] and superoxide dismutase [SOD]), the presence of CVD, and coronary artery calcification (CAC) in HD patients.

Methods: One hundred and two HD patients and 26 healthy subjects (controls) were enrolled in this cross-sectional study. Serum MDA-LDL/LDL-c ratio and SOD level were examined in all study participants. The CAC scores and their possible associations with clinical/laboratory data, including the presence of CVD, MDA-LDL level, MDA-LDL/LDL-c ratio, HDL-c level, LDL-c level, as well as the mean values of the most recent 3-year levels of phosphate, calcium, and intact parathyroid hormone, were examined in 57 HD patients (CAC group).

Results: Both the all HD patients and CAC group had significantly higher MDA-LDL/LDL-c ratios than the controls. The MDA-LDL/LDL-c ratios were significantly and positively correlated with CAC scores, associated with the frequency of CVD, and significantly and negatively correlated with HDL-c levels, but not with SOD levels in the CAC group. The multivariate logistic regression analysis showed that MDA-LDL/LDL-c ratio (odds ratio [OR], 1.04; 95% confidence interval [CI], 1.01–1.07; P = 0.003), and HD duration (OR, 1.17; 95% CI, 1.04–1.36; P = 0.007) were independently associated with CAC score in CAC group.

Conclusions: The MDA-LDL/LDL-c ratio rather than the SOD level of HD patients could reflect the degree of LDL oxidation, and therefore, could be an important factor for CVD and CAC in HD patients.

SA-PO1006

Hemodialysis and Peritoneal Dialysis Equally Impact Endothelial Function in Patients with End-Stage Renal Disease

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Background: Cardiovascular disease (CVD) is the major cause of disability and death in dialysis patients with end-stage renal disease (ESRD) while endothelial dysfunction is a major pathogenesis of CVD. It has been shown that hemodialysis improves endothelial function by removing dialysable endothelial toxins but not peritoneal dialysis. Here we investigated the factors affecting the ET-1/NO system in ESRD patients receiving dialysis to compare the effects of hemodialysis (HD) and peritoneal dialysis (PD) on the endothelial dysfunction.

Methods: Forty HD and 80 PD patients without diabetes and 12 normal healthy adults were enrolled. Radioimmunoassay was adopted to assay the concentration of ET-1. Colorimetric nitrate reductase assay was conducted to measure the activities of endothelial nitric oxide synthase (eNOS). Clinical characteristics of patients were recorded following standard procedure.

Results: Higher systolic blood pressure (SBP), Uric Acid (UA) and incidence of CVD while lower cholesterol (TC) and LDL were found in HD group (P<0.05). However, more patients with residual renal function (RRF) ≥1ml/min were found in PD than in HD group (50% versus 20%, P<0.05). Circulating ET-1 levels increased significantly in both HD and PD patients compared to that in controls (138.92±31.65pg/ml, 140.89 ±29.5pg/ml and 16.11±1.24pg/ml respectively, P<0.05) but no difference was found between HD and PD groups (P>0.05). eNOS activities, decreased dramatically in both dialysis groups (12.65±2.36U/ml in HD,12.34±1.60U/ml in PD and 14.74±1.60U/ml in control respectively, P<0.05) but without difference between HD and PD patients. Multiple linear regression analysis showed that SBP, UA and LDL were the independent risk factors for decreased eNOS activities while SBP and TG for increased ET-1 levels.

Conclusions: Our results suggest both hemodialysis and peritoneal dialysis have equal effects regarding improving endothelial function given that both groups had same levels of eNOS activities and ET-1, which is probably due to the facts that the HD patients had higher SBP and UA while peritoneal patients had better RRF.

Funding: Government Support - Non-U.S.

SA-PO1007

Association between Inflammatory Markers, Left Ventricular Systo – Diastolic Dysfunction and Right Heart Involvement in CKD Patients

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Background: Chronic kidney disease (CKD) is characterized by an increased mortality and morbidity due to cardiovascular involvement. Both left ventricular systolic and diastolic function are affected since CKD early stages. Pathophysiology of heart failure in CKD patients involves left ventricular hyperthophy, dilated cardiomiopathy, arrythmias and cardiac fibrosis together with widespread inflammatory status accountable for early atheroembolic disease.

Methods: We have enrolled 146 patients (96 males and 50 females aged 68 ± 9 years with mean dialytic age of 18 ± 0.4 months) on hemodialysis treatment and 120 patients (72 males and 48 females aged 57 ± 8 years) on stage III – V CKD. They underwent trans – thoracic ecocardiography and screened for inflammatory markers (CRP, IL-6, TNF-α).

Results: Hemodialysis patients showed significant correlations between IL – 6, CRP and TNF-α blood levels and systo - diastolic dysfunction parameters such as E/E' ratio. Therefore, they also showed strong and significant correlation between IL-6, CRP levels and right ventricular dysfunction indexes, such as TAPSE (tricuspid annulus plane systolic excursion) and systolic pulmonary artery pressure (PAPs). On the other hand, CKD patients showed no correlations between inflammatory asset and right heart dysfunction.

Conclusions: Our data confirm close correlation between systolic dysfunction and inflammatory markers in CKD and hemodialysis patients. Therefore, our findings underline close relationships between inflammatory markers and right heart dysfunction parameters in hemodialysis patients such as TAPSE and PAPs reflecting right heart involvement in the development of cardio – renal syndrome.

SA-PO1008

Factors Associated with Intradialytic Hypotension in HD Patients with Pre-Dialysis BP >140/90

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Background: Intradialytic hypotension(IDH) can precipitate ischemic events, cardiac stunning and death. Patients with a low BP at the start of dialysis are prone to IDH. We examine factors associated with IDH in patients with normal-to-elevated BP at dialysis start (SBP >140 mm Hg).

Methods: The study is based on HD patients enrolled in baseline of the Blood Pressure in Dialysis Study, a RCT assessing 2 levels of BP control. During weekly study visits, treatment sheets were reviewed and patients were questioned about symptoms during dialysis. IDH was defined as symptoms (e.g. dizziness) or a drop in systolic BP to <90 mm Hg requiring intervention. Cramps were excluded as they may be due to different factors than reduced effective circulating volume (ECFV). Cardiac MRI was performed at end of baseline.

Results: There were 170 episodes of IDH (excluding cramps) requiring intervention over 8 weeks in 95 patients. Selected factors across groups by IDH frequency are shown in the table.

% or Mean	No IDH or cramps only (n=50)	1-3 IDH Episodes (n=28)	4+ IDH Episodes (n=19)
Interdialytic Weight Gain (% EDW)	4.3	4.1	4.5
Ultrafiltration Rate (UFR) mls/kg/h	9.3	10.1	9.4
Delivered Time (h)	3.8	3.6	3.6
Dialysate-Serum Na Gradient	2.0	2.8	1.8
Pre-dialysis SBP (mm Hg)	148	153	151
Pre-dialysis DBP (mm Hg)	80	77	73 *
Pulse Pressure (mm Hg)	68	76	79 *
Hx Coronary Artery Disease (CAD)	4%	4%	27%
LV Mass Index (g/m2)	36	33	32
Ejection Fraction	54	58	57
IVC diameter (mm)	23	19	24

*p ≤ 0.05

Conclusions: Among patients with normal to elevated pre-dialysis BP and preserved EFs, we find that that lower diastolic BP relates to IDH. This association may reflect cardiac ischemia, with DBP dropping below a level at which there is adequate coronary perfusion. In keeping with this, we find a higher prevalence of CAD in patients most prone to IDH. Alternatively, a lower diastolic pressure, which equates to a higher pulse pressure, may reflect arterial non-compliance and inability to autoregulate with declining ECFV. More attention should be paid to diastolic BP when considering BP goals in HD patients, especially those with CAD.

Funding: NIDDK Support

SA-PO1009

Skin Autofluorescence (SAF) and Tissue–Advanced Glycation End Product Concentration (AGES) in Hemodialysis Patients. A New Tool to Detect Cardiovascular Risk (CVR) Secundino Cigarran,[1] Walter Luis López Alarcón,[1] Juan Latorre,[1] Montserrat Pousa,[1] Anna Minasyan,[3] Jesus Calvino,[2] Lourdes Gonzalez-Tabares,[2] Miguel A. Garcia-Gonzalez.[3] *[1]Nephrology, Eoxi Lugo-Cervo- Monforte, Burela, Lugo, Spain; [2]Nepphrology, Eoxi Lugo-Cervo- Monforte, Lugo, Spain; [3]Nephrology, Health Research Inst of Santiago de Compostela (IDIS), Santiago de compostela, La Coruña, Spain; [4]Nephrology, Health Research Inst of Santiago de Compostela (IDIS), Santiago de Compostela, La Coruña, Spain.*

Background: The prevalence of cardiovascular disease in patients on HD remains high,because combinations of classical and non classical risk factors. AGEs assessed by SAF as measure of cumulative metabolic stress correlates with outcomes in HD pts.Our aim is to assess and compare tissue AGEs levels with nutritional,inflammation and CVR markers in HD.

Methods: 40 caucasian, stable, HD pts were included cross transversal study (mean age 72 yo),67% men,40% diabetes status.At midd week day session blood biomarkers were drown.Anthropometric measures were obtained.Inflammation (Epo resistance index (ERI) C Reactive protein),nutritional (nPNA,Prealbumin,Albumin) and CV R markers (Fibrinogen,Troponin us) and adequacy parameters were measured. AGEs were evaluated with SAF (AGE Reader, DiagnOptic s, Groningen, The Neetherlands) by 3 consecutive measurements at slightly different skin sites on the same forearm. Arms with graft or vascular access were avoided. SAF is a validated cutaneous device using ultraviolet source at sepcific range of wavelengths (350-420 nm).

Results: 85% pts showed at high CVR by SAF. 77% had previous CV events (stroke, AMI). SAF levels had a significant positive correlation with: Charlson comorbidity index (r: 0.440, p<0.005),troponin us (r: 0.368, p=0.023), CRP (r 0.338, p= 0.033), ERI (r=0.463, p=0.03, Fibrinogen (r 0.380; p=0.038) and eKT/V (r: 0.383; p= 0.015).A significant negative correlation with:Vit D levels (r=-0.315;p=0.025) and triglycerides (r= -0.322; p= 0.042).No other significance was met.

Conclusions: AGEs´skin accumulation are directly associated with, comorbidity, inflammation, cardiovascular and coronary artery disease risk biomarkers in caucasian pts on HD. SAF becomes a non invasive novel tool to assess CVR.

Funding: Other NIH Support - Galician Health Service

SA-PO1010

Skin Autofluorescence (SAF) to Assess Advanced Glycation End Products (AGEs) and the Association with Renal and Cardiovascular Risk Factors (CVR) in Chronic Kidney Disease (CKD) Stage 1-5ND Secundino Cigarran,[1] Juan Latorre,[1] Ana Isabel Fernandez-Alonso,[1] Jesus Calvino,[2] Montserrat Pousa,[1] Emilio E. Gonzalez-Parra,[3] Saray Lopez-Prieto,[1] Lourdes Gonzalez-Tabares,[2] Miguel A. Garcia-Gonzalez.[4] *[1]Nephrology, Eoxi Lugo-Cervo-Burela, Burela, Lugo, Spain; [2]Nephrology, Eoxi Lugo- Cervo- Burela, Lugo, Spain; [3]Nephrology, Fundacion Jimenez Diaz, Madrid, Spain; [4]Nephrology, Health Research Inst of Santiago de Compostela (IDIS), Santiago de Compostela, La Coruña, Spain.*

Background: AGEs accumulation is a novel CVR and is a measure of cumulative metabolic stress. Assessment by SAF may be a useful marker of CKD progression.Aim is to compare tissue AGEs levels with nutritional,inflammation and CVR markers in CKD stage 1-5ND pts.

Methods: 259 caucasian pts were included (mean age 72 yo),61.4% men,37.1% diabetes status. Comorbidity by Charlson Index, CKD risk formula at 5 yr, CKD-EPI formula, urine and biochemistry tests, fluid overload(FO) by spectroscopic bioimpedance (BCM,Fresenius Bad Homburg, GER),Arm/branch test and carotid atheromatosis were assessed.AGEs were evaluated with SAF (arbitray units) (AGE Reader,DiagnOptics, Groningen, The Neetherlands) by mean of three readings. Data were analyzed with univariate Spearman´s rank correlation as appropiate. "p" value <0.05 was considered significant.

Results: 50.3 % showed moderate-severe CVR.Univariate analysis revealed significant correlations between SAF levels and some potential CVR and CKD progression. SAF were high in males (3.15± 0.8 versus 2.71± 0.6), PAD (3.34±0.8 versus 2.84± 0.7),Carotid atheromatosis (3.17±0.8 versus 2.5 ±0.6) SAF had a significant positive correlation with: Charlson comorbidity index (r: .337, p<0.001),CKD risk formula (r: .312, p<0.001), FO (r=.332, p<0.001),P excretion index (r=.326, p <0.001),iPTH(r= .284, p <0.001),ACR (r= .184, p=0.004).A negative significant correlation with Hb (r:- .321, p<0.001),GFR (r=- .448, p<0.001),nPNA (r=-.156, p<0.001),Albumin (r= -.249, p<0.001),Cholesterol (r= -.235, p<0.001).No significance with DM status.

Conclusions: SAF is associated with nutritional, CVR,carotid atheromatosis,peripheral artery disease,comorbidity and risk to initiate renal replacement therapy in CKD pts.SAF may be used as outcome after measures taken.

Funding: Other NIH Support - Galician Health Service

SA-PO1011

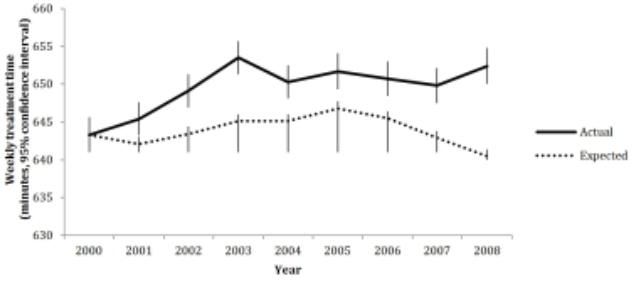
Trends in Treatment Time for In-Center Hemodialysis Susan P.Y. Wong,[1] Ann M. O'Hare.[2] *[1]Univ of Washington; [2]Univ of Washington.*

Background: The majority of patients treated for end-stage renal disease receive in-center hemodialysis, which demands substantial time commitment.

Methods: Using data from the Clinical Performance Measures Project--a Centers for Medicare and Medicaid Services quality improvement initiative conducted in Medicare-certified dialysis facilities across the U.S.--we performed an observational study to describe temporal trends and determinants of prescribed dialysis treatment time among a national sample of prevalent adults receiving chronic hemodialysis from 2000-2008. We evaluated temporal trends in actual versus expected treatment times based on 2000 practices.

Results: From 2000-2008, mean weekly treatment time increased from 643±98 to 652±110 minutes (trend, p<0.001). After adjusting for changes in patient characteristics over time, patients surveyed in 2008 received an additional 12 minutes (95% confidence interval [CI] 9, 14) of dialysis every week as compared with similar patients surveyed in 2000.

Figure 1. Actual versus expected time spent on dialysis each week based on 2000 practices



Weekly treatment times increased by more than 20 minutes for black patients, those residing in the South, those with a serum hemoglobin level <10g/dL and those prescribed ultrafiltration volumes ≥3L per treatment. There was heterogeneity in the magnitude of discrepancy between actual and expected weekly treatment times, especially across groups defined by race (black versus other racial minority groups; 24 additional versus 1 additional minutes), age (45-64 years versus≥80 years; 19 additional versus 1 fewer minutes), cause of ESRD (diabetes mellitus versus other causes; 19 additional versus 1 fewer minutes), facility ownership (for-profit versus non-profit; 3 fewer versus 15 additional minutes), census region (3 fewer in the Northeast versus 24 additional minutes in the South), dialysis sp*Kt*/*V* dose (<1.2 versus≥1.2; 15 fewer versus 13 additional minutes) and ultrafiltration volume per treatment (<3L versus≥3L; 4 fewer versus 23 additional minutes).

Conclusions: From 2000-2008, there was an increase in treatment time for patients receiving in-center hemodialysis and growing heterogeneity in treatment time across patient subgroups. In 2008, patients spent an average of 10 additional hours on dialysis as compared with similar patients in 2000.

Funding: NIDDK Support

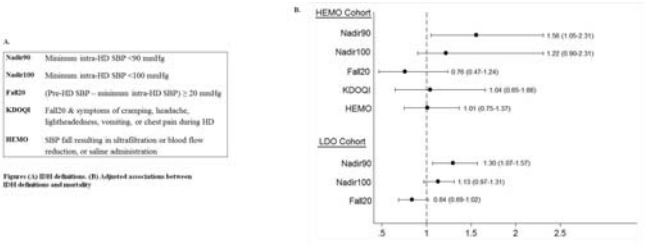
SA-PO1012

Empirically Defining the Associations of Mortality and Various Definitions of Intradialytic Hypotension Jennifer E. Flythe,[1,2] Gary C. Curhan,[2] Steven M. Brunelli.[2,3] *[1]Univ of North Carolina Kidney Center; [2]Brigham and Women's Hospital; [3]DaVita Clinical Research.*

Background: Intradialytic hypotension (IDH) is a serious and frequent complication of hemodialysis (HD); however, there is no consensus evidenced-based definition of IDH. As a result, coherent evaluation of the effects of IDH is difficult. We examined the associations of commonly employed IDH definitions and mortality in two cohorts.

Methods: We analyzed data from 1,409 HEMO study patients and 10,392 patients from a single large dialysis organization (LDO). IDH definitions (Figure A) were selected *a priori*. For each definition, patients were characterized as having IDH if they met the corresponding definition in at least 30% of baseline exposure period treatments and were characterized as control otherwise. Odds ratios (OR) for 2-year mortality (HEMO cohort) and 1-year mortality (LDO cohort) across binary IDH definitions were estimated by fitting logistic regression models. Analyses stratified by pre-HD systolic blood pressure (SBP) were performed in the LDO cohort.

Results: Overall, Nadir90-defined IDH was most potently associated with mortality (Figure B). Within the subgroup of patients with pre-HD SBP ≥160 mmHg, Nadir100 was most potently associated with mortality: adjusted OR (95% CI) 1.29 (1.07-1.56). IDH definitions that considered intra-HD SBP fall, symptoms, and interventions were not associated with outcome (Figure B) and, when added to nadir SBP, such criteria did not accentuate mortality associations (not shown).



Secondary analyses indicated a dose-response trend in the association of Nadir90-defined IDH (<5%, 6-29%, 30-49%, and ≥50% sessions with IDH) with mortality (not shown).

Conclusions: Our results suggest that nadir-based definitions best capture the association between IDH and mortality.

Funding: Other NIH Support - National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award

SA-PO1013

Interdialytic Weight Gain Has Declined over Last Decade Internationally

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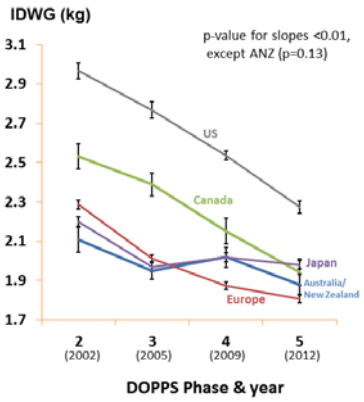
Background: High interdialytic weight gain (IDWG), high ultrafiltration rate, and short treatment time are associated with adverse outcomes in hemodialysis (HD) patients. Based on preliminary indication of a declining trend over time in IDWG, we examined this trend and evaluated facility practices that could explain it.

Methods: Data were from 25,601 patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) phase 2-5 (2002-2012), with HD vintage ≥1 year. The mean of the absolute IDWG between the 1st and 2nd session and the 2nd and 3rd session of the week was compared across U.S., Canada, Europe, Japan and Australia/New Zealand (ANZ). Linear regression was used to assess the association between IDWG and patient-level and facility-level factors.

Results: IDWG declined across regions, ranging from -0.2kg (ANZ and Japan) to -0.7kg (U.S.) (figure). The % of patients with high IDWG (>5.7% of post-HD weight) also declined. Younger age, male sex, lack of residual renal function, higher nPCR, and lower pre-HD serum sodium were significantly associated with higher IDWG. Higher facility mean dialysate sodium (DNa) was associated with higher IDWG; other facility practices (table) were not significant. DNa declined over the same time; U.S. had the largest decline (-1.9 mEq/L).

Mean IDWG, by DOPPS Phase and Region



Facility practices associated with IDWG*

	Adjusted linear association with IDWG (kg), (95% confidence interval)
Avg. dialysate sodium (per mEq/L)	0.09 (0.08, 0.11)
Avg. treatment time (per 30 min)	0.02 (-0.02, 0.06)
Target pre-HD SBP (per 5 mmHg)	0.01 (0, 0.03)
Target pre-HD DBP (per 5 mmHg)	-0.02 (-0.05, 0.01)
Dietitian consult for high IDWG (+ likely)	0.01 (-0.01, 0.03)
Diuretic use (per 20%)	-0.01 (-0.04, 0.01)

*adjusted for patient characteristics

Conclusions: The steady decline in IDWG is a provocative finding that may affect clinical outcomes. Of the facility factors analyzed, DNa had the strongest association with IDWG, accounting for ~0.1kg per 1mEq/L DNa. In the U.S., DNa explains ~0.2kg of the decline in IDWG, but future investigation needs to consider other factors such as dietary sodium restriction and achieving measured "dry weight" post-HD.

Funding: Pharmaceutical Company Support - The DOPPS program is supported by grants to Arbor Research from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, Ltd. Additional support for specific projects is provided in Canada by Amgen, BHC Medical, Janssen, Takeda, Kidney Foundation of Canada (for logistics support); in Germany by Hexal, DGfN, Shire, WiNe Institute; for PDOPPS in Japan by the Japanese Society for Peritoneal Dialysis; for PDOPPS by Fresenius Medical Care

SA-PO1014

Do We Need to Reduce Dialysate Sodium Now?

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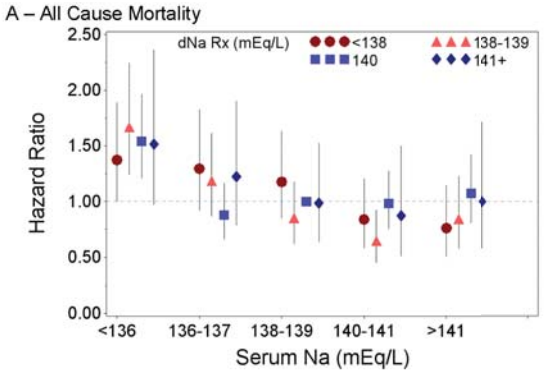
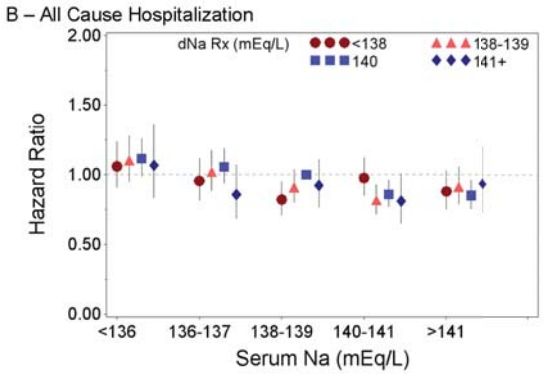
Background: There is controversy surrounding the optimal dialysate sodium (DNa). Several dialysis providers have urged their medical directors to reduce dNa to 134 to 138 mEq/L to reduce interdialytic weight gain (IDWG; [% EDW]) and BP. However, recent DOPPS data suggests that the impact of DNa on IDWG and BP is modest and that calls for reductions in DNa may be premature. Therefore, we assessed the relationships of DNa to predialysis serum sodium (SNa), systolic BP (SBP), ΔSBP during dialysis, IDWG, hospitalization and mortality in DCI.

Methods: We studied HD patients who dialyzed in 2013 with a stable DNa for ≥ 90 days (n=9350). We computed least square means for SNa, IDWG, SBP and ΔSBP. In a second analysis, we and constructed Cox models to assess the relationships of SNa, DNa and DNa-SNa (NaG) to mortality and hospitalization in prevalent patients in 2009-2012 (n = 12,728). We adjusted for age, sex and race in both analyses.

Results: SNa were similar at DNa ranging from ≤ 136 mEq/L (137.4 mEq/L) to ≥141 mEq/L (138.6 mEq/L). IDWG was 2.8%, 3.4%, 3.0% and 2.9% at DNa ≤ 136, 139, 140, and ≥141 mEq/L, respectively. Predialysis SBP did not differ by DNa. The ΔSBP (mm Hg) was similar at DNa concentrations of ≤ 136 (10), 137 (9.5), 138 (10.5), 139 (9.6), 140 (8.3) and ≥141 mEq/L (9.3). Hyponatremia and a NaG >6 mEq/L were associated with increased mortality. However, DNa, when stratified by SNa, was not associated with changes in mortality or hospitalization.





Conclusions: The apparent impact of DNa on IDWG, SBP, and ΔSBP was modest. Differences in DNa were not associated with differences in mortality or hospitalization. Until we have trial data it may be premature to further reduce DNa.

SA-PO1015

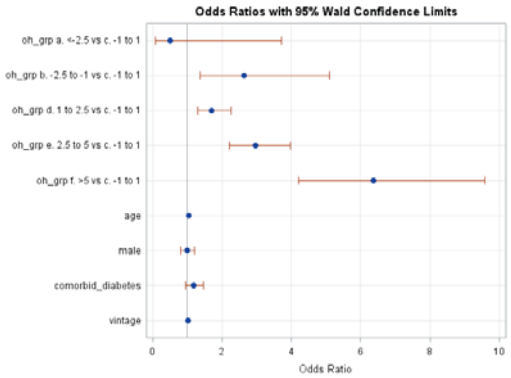
Hydration Status and Mortality: Results From a Large International Study

Marijke J.E. Dekker,[1,2] Len A. Usvyat,[3,6] Peter Kotanko,[3] Frank van der Sande,[1] Constantijn Konings,[2] Daniele Marcelli,[4] Yuedong Wang,[5] Nathan W. Levin,[3] Bernard J. Canaud,[4] Karel M. Leunissen,[1] Jeroen Kooman.[1] *[1]Dept of Internal Medicine, Div of Nephrology, Univ Hospital, Maastricht, Netherlands; [2]Dept of Internal Medicine, Catharina Hospital, Eindhoven, Netherlands; [3]Renal Research Inst, New York; [4]Fresenius Medical Care, Bad Homburg, Germany; [5]Univ of California, Santa Barbara; [6]Fresenius Medical Care, Waltham.*

Background: In hemodialysis (HD) patients fluid overload (FO) is a strong predictor for all-cause mortality. Fluid depletion (FD) can lead to intradialytic hypotension, associated with increased mortality. The relation of different levels of FO and chronic FD on outcome has not yet been studied. In this study we evaluated the effect of both FO and FD on patients outcome.

Methods: We studied all prevalent HD patients from the European Fresenius network (17 countries). Hydration status was assessed by Body Composition Meter (BCM) measurements. We included patients with ≥ 1 BCM measurement between 1/2011 and12/2011 and recorded their survival between 1/2012 and 6/2012 (follow up). Logistic regression was constructed to evaluate the association between FO/FD and survival.

Results: We included 9,463 patients (mean age 63 years, 57.4% male, mean dialysis vintage 5.1 months). During follow up, 476 patients (5.03%) died. FO was associated with an incrementally elevated risk of mortality, OR 1.71 (95% CI 1.29-2.26) with 1.0-2.5L FO and OR 2.96 (95% CI 2.20-3.98) with 2.5L-5.0L FO. Also FD (pre-dialytic -2.5 to -1.0L) was associated with mortality (OR 2.64 (95% CI 1.36-5.11)).



Conclusions: Our data indicate that FO and FD are associated with an increased mortality risk in a dose-dependent fashion. This study showed for the first time that FD is associated with an increased risk of death. Achieving euvolemia may improve outcomes in chronic HD patients.

SA-PO1016

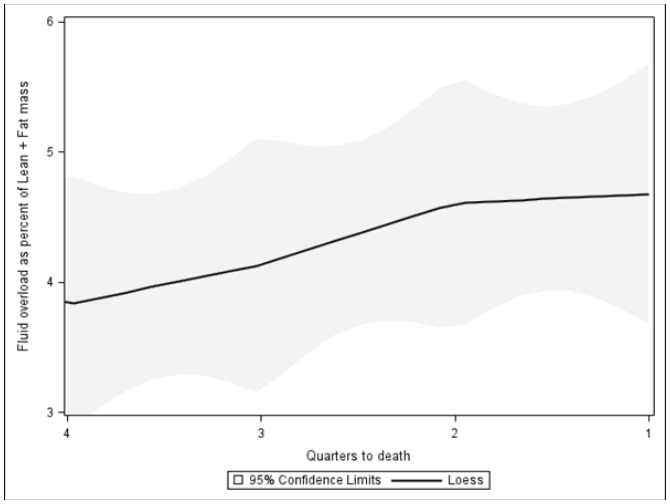
Body Composition in Hemodialysis Patients in the Final Year before Death: Results from the International MONDO Initiative Adrian M. Guinsburg,[1] Marcelo D. Ferder,[1] Aileen Grassmann,[2] Xiaoqi Xu,[3] Len A. Usvyat,[4,5,6] Jeroen Kooman,[5] Frank van der Sande,[5] Peter Kotanko,[6] Franklin W. Maddux,[4] Michael Etter,[3] Daniele Marcelli,[2] Cristina Marelli,[1] Bernard Canaud.[2] *[1]Fresenius Medical Care, Buenos Aires, Argentina; [2]Fresenius Medical Care, Bad Homburg, Germany; [3]Fresenius Medical Care, Hong Kong, Hong Kong; [4]Fresenius Medical Care, Waltham, MA; [5]Maastricht Univ Medical Centre, Maastricht, Netherlands; [6]Renal Research Inst, New York, NY.*

Background: Lean tissue mass (LTM), fat mass (FM), and fluid overload (FO) have been linked to survival in hemodialysis (HD) patients. Here we describe changes in body composition (BC) in the final year of life in a large HD cohort.

Methods: This analysis was conducted in a subset of MONitoring Dialysis Outcomes (MONDO) database [Usvyat, Blood Purif 2013]. Patients treated in Europe(E), Asia(A) and South America(SA) who died between Jan06-Dec12 with at least one BC measurement each in the 1-3 months and 9-12 months before death were enrolled. BC was assessed by bioimpedance spectroscopy (BCM, Fresenius Medical Care).

Results: Data from 1,141 patients were available (E 885,SA 195,A 61). Age 70.1±13, male 59%, diabetic 35%. Post-dialysis weight (W), LTM and FM declined significantly in the final year of life, while FO increased.

	9-12 months before death (MBD)	1-3 MBD	Diff (mean/95CI)
W(kg)	68.2 (16.0)	66.0 (16.1)	-2.2 (-2.5/-2.0)
LTM(kg)	30.8 (8.8)	30.0 (8.7)	-0.8 (-1.1/-0.4)
FM(kg)	26.6 (11.4)	25.2 (11.5)	-1.4 (-1.7/-1.0)
FO(kg)	2.1 (1.9)	2.5 (2.1)	+0.3 (0.2/0.4)
FO% of LTM+FM	4.0 (3.5)	4.8 (3.8)	+0.8 (0.6/1.0)



Conclusions: This is the first international research to assess BC in final year before death. Data indicate a clinically relevant decrease in LTM and FM, and a concurrent increase in FO. These findings might aid in earlier identification and design of interventional strategies for patients at high risk for adverse outcome.

SA-PO1017

Urea Kinetic Parameters Confounded by Volume Overload in HD Patients Richard Amerling,[1] Len A. Usvyat,[3] Qingqing Xiao,[2] James F. Winchester,[1] Peter Kotanko,[2] Nikolas B. Harbord.[1] *[1]Nephrology and Hypertension, Mount Sinai Beth Israel, New York, NY; [2]Renal Research Inst, New York, NY; [3]Fresenius Medical Care, Waltham, MA.*

Background: Low KT/V urea and urea reduction ratio (URR) have been linked to poor outcomes in HD patients in observational trials. Both parameters are affected by the patients' volume status. We conducted a retrospective data analysis to explore this relationship.

Methods: We analyzed data from 5,162 Renal Research Institute in-center HD patients who had at least one in-center treatment in August 2010 (study month). Treatment parameters: post-dialysis weight (PW) was computed as average for the study month. URR and kt/V, were obtained from monthly urea kinetic modeling. Target weight (TW) is weight prescribed by the patients' physicians. As surrogate for pre-dialysis volume status, we used the difference between the achieved post-dialysis weight and the estimated target weight (PW-TW).

Results: Patients with KT/V <1.2 (Fig. 1), and URR <65% had significantly greater values of PW-TW, suggesting they were volume overloaded.

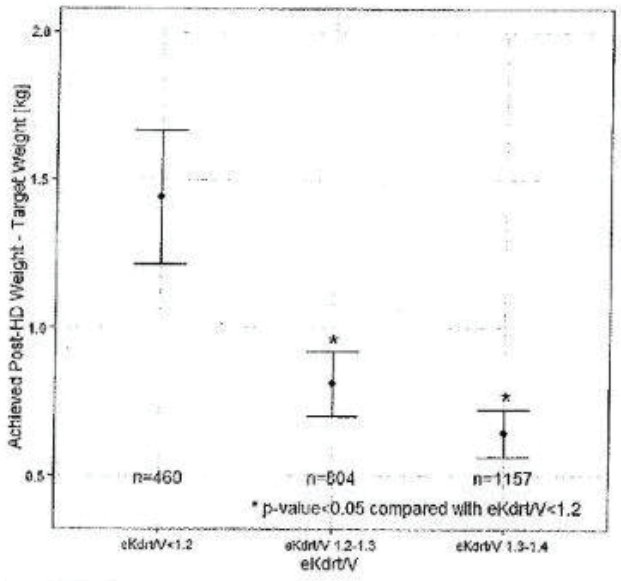


Figure 1. Relationship between Urea Kinetics and PW-TW

Conclusions: Low KT/V and URR could reflect volume overload, as opposed to decreased small solute clearance. Since volume overload is a known cause of morbidity and mortality in HD patients, this observation calls into question the use of small solute kinetics to guide dialysis prescriptions. Greater attention to volume removal and control, via longer and/or more frequent treatments, might be preferred.

SA-PO1018

Missing Hemodialysis (HD) Treatments: International Variation and Associations with Predictors and Outcomes in the DOPPS
Issa A.L. Salmi,[1] Maria Larkina,[2] Lalita Subramanian,[2] Hal Morgenstern,[3] Stefan H. Jacobson,[4] Raymond M. Hakim,[5] Francesca Tentori,[2] Rajiv Saran,[3] Takashi Akiba,[6] Natalia A. Tomilina,[7] Friedrich K. Port,[2] Bruce M. Robinson,[2] Ronald L. Pisoni.[2] *[1]Royal Hosp, Oman; [2]Arbor Research; [3]Univ MI; [4]Danderyd Hosp, Sweden; [5]Vanderbuilt Univ; [6]Tokyo Women's Med. Univ, Japan; [7]Moscow St. Univ, Russian Federation.*

Background: Missed HD treatments (MT) are known to be associated with mortality and hospitalization. Here we report new DOPPS results on frequency of MT in 20 countries and associations with patient (pt) predictors and outcomes.

Methods: Data were from 8,667 DOPPS 5(2012-14) pts on dialysis >120 days and prescribed 3X/week HD. Logistic and linear regression were used to estimate associations (adjusted odds ratios [OR] or differences [Δ], with p<0.05 shown) for pts with ≥1 MT versus no MT in 1st 4 study months (excluding hospitalizations), adjusting for case mix and country.

Results: MT prevalence varied >10 fold across countries (Table). MT was more likely in younger pts(OR=1.19 per 10 yrs younger), had a depression symptom score (CESD)>10 (OR=1.48), or travel >1 hour to HD unit (OR=2.35). MT was not strongly linked with CV-related comorbidities. MT pts had poorer control of serum phosphorus (OR=1.27 for P>5.5mg/dl), PTH (OR=1.26 for PTH> 300 pg/mL), and hemoglobin (OR=1.52 for Hb<10g/dL). With the KDQOL, MT pts reported higher perceived burden of kidney disease (Δ=4.35), worse general health (Δ=5.73), and worse physical component score (Δ=1.25, p=0.06).

Conclusions: Our findings support prior studies, highlighting that longer travel times, higher perceived disease burden and lower self-reported physical wellness relate to HD adherence. While MT is most common in the U.S., the tremendous variation across DOPPS countries implies that MT may be modifiable. Focus on strategies to lessen MT in the U.S. and elsewhere is needed. Improvement in MT could be one metric to assess impact of efforts, such as renal ACOs, to coordinate care in the U.S.

Country	N Patients[A]	Percent of HD Patients Missing ≥1 HD treatments		
		During any of the 4 months[A]	During 1 month[B]	During each of 4 months[A]
United States[1]	1172	23.0	11.5	2.3
GCC[2]	587	22.0	10.5	4.0
Russia	348	14.0	6.7	1.9
Australia/New Zealand	354	10.0	4.5	1.4
Canada	333	9.6	4.6	1.4
United Kingdom	295	8.8	2.9	0.0
Sweden	285	6.7	3.1	1.3
Turkey	288	6.6	2.3	0.7
China	779	2.8	1.0	0.0
Germany	495	2.8	0.9	0.0
Belgium	365	2.5	0.7	0.0
Spain	455	2.4	0.7	0.0
Italy	363	0.8	0.3	0.0
Japan	1422	0.4	0.3	0.2

[1] Data not available from one large dialysis organization in US
[2] Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, United Arab Emirates
[A] Among patients for whom complete information about missing HD treatments was reported for each of the 4 study months
[B] Averaged over 4 month period

Funding: Pharmaceutical Company Support - The DOPPS program is supported by grants to Arbor Research from Amgen, Kyowa Hakko Kirin, AbbVie, Sanofi Renal, Baxter Healthcare, and Vifor Fresenius Medical Care Renal Pharma, Ltd. Additional support for specific projects is provided in Canada by Amgen, BHC Medical, Janssen, Takeda, Kidney Foundation of Canada (for logistics support); in Germany by Hexal, DGfN, Shire, WiNe Institute; for PDOPPS in Japan by the Japanese Society for Peritoneal Dialysis; for PDOPPS by Fresenius Medical Care, Government Support - Non-U.S.

SA-PO1019

What Treatment Benefits Are Important to Patients with ESRD on Hemodialysis?
Mona L. Martin,[1] Matthew J. Wolfe,[1] Anna Ryden.[2] *[1]Health Research Associates Inc., Seattle, WA; [2]AstraZeneca R&D, Mölndal, Sweden.*

Background: There are no published data on patient perceptions of condition-specific treatment success in individuals with end-stage renal disease on hemodialysis (ESRD-HD). Results from qualitative interviews helped to identify concepts important to this patient group in defining treatment success.

Methods: Semi-structured interviews were conducted by telephone as part of a phase 2 clinical trial of tenapanor (AZD1722; a minimally absorbed inhibitor of the Na^+/H^+ exchanger NHE3) for patients with ESRD-HD and chronic fluid overload (NCT01764854). Interviews were conducted prior to treatment initiation and at the end of treatment, in order to document patients' experiences and capture their thoughts on what constitutes a successful treatment. Patients responded to open-ended questions such as "Can you describe your current thoughts about what makes a treatment successful?", followed by questions probing into the changes in symptoms and impacts on daily life that they would like to experience as a result of treatment. The data reported below are from patient responses to the above questions during pre-treatment interviews.

Results: Twenty-one patients from eight sites across the U.S.A. completed the pre- and post-treatment interviews and are included in this analysis. The main emergent themes for treatment success arising from the initial open-ended questions were: reduction in the amount of fluid removed during dialysis and less fluid retention between dialysis sessions, fewer side effects following dialysis, and feeling better after dialysis. When asked which changes in symptoms would define treatment success, fluid retention was the most frequently discussed theme. Other themes included relief from constipation and thirst, and increased energy. When asked about impact on daily life, the main emergent theme was regarding activity levels (e.g. the ability to travel and perform everyday tasks).

Conclusions: Patients with ESRD-HD considered that a key element of a successful treatment should be reducing the amount of fluid removed during dialysis. Therapies able to reduce chronic fluid overload may have a role to play achieving this outcome.

Funding: Pharmaceutical Company Support - AstraZeneca

SA-PO1020

Weight Gain after Starting Peritoneal Dialysis: Prevalence, Possible Causes and Prognostic Significance
Shin Man Choy, Cheuk-Chun Szeto. *Div of Nephrology, Prince of Wales Hospital, Hong Kong, Hong Kong.*

Background: Observational studies suggested that high body mass index is associated with improved survival in dialysis population. Weight gain is common amongst patients newly put on peritoneal dialysis (PD). However, the prevalence, risk factors and long term implications of body weight gain in patients newly started on PD have not been explored.

Methods: We studied 444 consecutive patients with end stage renal disease newly started on PD therapy. Body weight, measured when the patient was clinically euvolemic, at the time of initiation of PD and 1 year later were reviewed. Clinical factors affecting weight changes were explored.

Results: Patients were followed up for 60.9 ± 32.8 months. The mean weight change after one year of PD was 1.34± 3.27 kg. Patients without any peritonitis episodes during the first year of PD had significantly more weight gain than those who had peritonitis (1.58 ± 3.17 versus 0.16 ± 3.56 kg, P = 0.001). The number of peritonitis episodes during the first year of PD had inverse correlation with weight gain during this period (r = -0.174, P = 0.0002). Moreover, weight change had a modest but statistically significant correlation with the concomitant change in residual renal function (r = 0.137, P = 0.004). However, there were no significant relations between body weight change and glucose load, peritoneal transport characteristics, fasting plasma glucose, HbA1c, dialysis adequacy, or baseline residual renal function. Patients with weight gain >3.0 kg had similar overall survival, technique survival and peritonitis-free survival as compared to those with stable body weight. On the other hand, patients with weight loss >0.5 kg had worse technique survival (P = 0.03) and peritonitis-free survival (P = 0.005) than the others.

Conclusions: Weight gain is common among Chinese patients during the first year of PD. Weight change is related to peritonitis and decline of residual renal function during the same period. Weight gain within the first year of PD is not associated with any adverse clinical outcomes, while weight loss more than 0.5 kg is associated with worse technique survival and peritonitis-free survival subsequently.

SA-PO1021

Variation in Focus of Treatment Outcome Preferences According to Renal Replacement Therapy Modality
Zoe C.L. Pittman,[1] Chris W. McIntyre.[1,2] *[1]Royal Derby Hospital; [2]Univ of Nottingham.*

Background: Individual patient preferences for treatment outcomes vary between symptom control and survival, but the relationship with treatment modality in renal replacement therapy (RRT) has not been previously investigated. We systematically explored RRT patients' outcome preferences, utilising established methodology to compare and quantify the subconscious trade off in decision making.

Methods: Prevalent haemodialysis (HD), peritoneal dialysis (PD) and transplant (Tx) patients completed an outcome preference set comprising 25 possible treatment outcome profiles each scored for overall acceptability. All profiles contained the same 6 attributes (pain, tiredness, breathing, depression, survival and treatment burden (TB)), spread across 4 severity levels. Standard full profile conjoint analysis was used to derive importance weights for each attribute, allowing their relative contribution to be determined for every individual. Patient demographics and treatment history were collected.

Results: 44 patients were studied (HD 24 PD 19 Tx 11), mean age HD 63.3±14.6, PD 60.1±9.9, Tx 61.6±9.1yrs (p=0.800), % male HD 62.5, PD 66.7, Tx 54.5 (p=0.855). Overall importance weights (%) were highest for survival (24.1) and lowest for TB (11.2). The balance varied with modality; HD patients ascribed highest weights to pain (19.9) and breathing (19.6), PD rated survival (21.9) and breathing (19.6) and Tx survival (36.1) and depression (16.9). TB rated least for both HD and PD patients. There was a significant difference between all groups for pain (p=0.006), and between Tx and HD for survival (p=0.049). 7 patients completed 1 year follow up with no significant change in preference distribution. At patient level there was a clear separation in preference patterns; either predominant preference for survival, or aversion to symptoms and indifference to survival ± indifference to increased TB.

Conclusions: We have demonstrated that the balance of outcome preferences change by modality irrespective of age or gender suggesting that the modality itself may influence the focus. Understanding these preferences and how they change with time and modality may lead to additional targets for improving the quality of life of our patients.

SA-PO1022

Patient Characteristics and Outcomes by Glomerulonephritis (GN) Subtype in End-Stage Renal Disease (ESRD) Michelle M. O'Shaughnessy, Maria E. Montez-Rath, Richard A. Lafayette, Wolfgang C. Winkelmayer. *Stanford Univ School of Medicine, Palo Alto, CA.*

Background: Patients with ESRD due to GN have rarely been the focus of outcomes based research. We examined patient characteristics by GN subtype in a national ESRD cohort and identified independent associations between GN subtype and post-ESRD mortality.

Methods: Data on patients with ESRD attributed to 6 major GN subtypes [focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), IgA nephropathy (IgAN), lupus nephritis (LN), membranoproliferative GN (MPGN), vasculitis] who initiated ESRD therapy from 1996 to 2011 were extracted from the U.S. Renal Database System. Demographic variables were tabulated. Mortality hazard ratios were compared using Cox regression.

Results: In this national ESRD cohort (n=83,740), demographic variables differed considerably among GN subtypes.

	FSGS, n=34,071	LN, n=16,385	IgAN, n=12,912	MN, n=7,121	MPGN, n=5,148	Vasculitis, n=8,103
Age, mean	51.4	40.7	45.9	58.0	52.6	63.1
Male sex, %	61.7	18.3	67.6	66.0	60.2	52.4
Race, %						
- White	59.0	43.5	75.1	69.5	74.6	88.8
- Black	36.1	48.6	6.6	26.0	17.9	7.5
- Other	4.9	7.9	18.3	4.5	7.4	3.7
Hispanic, %	8.7	16.3	12.5	10.1	10.7	9.0
Medicaid eligible, %	18.5	32.0	13.5	16.6	20.4	12.0
Geographic region, %						
- Northeast	19.6	15.8	19.3	20.1	20.6	19.2
- Mid-west	23.4	18.5	23.1	23.6	24.8	27.2
- South	39.7	44.2	30.4	38.1	33.0	33.2
- West	16.7	20.4	26.7	17.0	20.7	20.0
Incident ESRD therapy, %						
- Hemodialysis	79.0	85.9	70.6	82.6	81.6	91.8
- PD	14.6	10.8	17.0	12.9	11.7	6.5
- Transplant	6.0	3.1	11.8	4.2	6.3	1.5
Mortality HR (95% CI)						
- Unadjusted	2.0 (1.9-2.1)	2.1 (2.0-2.2)	1.0 (referent)	2.5 (2.4-2.7)	2.3 (2.2-2.5)	4.0 (3.8-4.2)
- Demographics-adjusted	1.4 (1.3-1.5)	2.3 (2.2-2.5)	1.0 (referent)	1.3 (1.3-1.4)	1.6 (1.5-1.7)	1.7 (1.6-1.8)

Mean age ranged from 40.7 (LN) to 63.1 (vasculitis), % male from 18.3 (LN) to 67.6 (IgAN), % black from 6.6 (IgAN) to 48.6 (LN), % peritoneal dialysis (PD) as initial modality from 6.5 (vasculitis) to 17.0 (IgAN). Adjusted mortality referent to IgAN was highest for LN (HR 2.3, 95% CI 2.2-2.5).

Conclusions: These preliminary data demonstrate substantial heterogeneity among GN subtypes even after ESRD development, both in patient characteristics at presentation to ESRD therapy and in subsequent patient survival.

Funding: Private Foundation Support

SA-PO1023

Glycemic Markers and 1-Year Hemodialysis Outcomes in Non-Diabetic Patients from the GIDE Study Neal Mittman,[1] Lin Ma,[2] Brinda Desiraju,[1] Mark E. Williams,[3] Julia I. Brennan,[4] Curtis D. Johnson,[4] Franklin W. Maddux,[2] Eduardo K. Lacson.[2] *[1]Kidney Care of Brooklyn and Queens, Brooklyn, NY; [2]Fresenius Medical Care, North America, Waltham, MA; [3]Joslin Diabetes Center, Boston, MA; [4]Spectra Laboratories, Rockleigh, NJ.*

Background: We previously reported elevated glycemic indices, fructosamine (F) and glycated albumin (GA), in non-diabetic (NDM) ESRD patients, as confirmed by hemoglobin A1c (HgbA1c) <6.5%. From the multi-year prospective observational Glycemic Indices in Dialysis Evaluation (GIDE) Study, we report 1st year outcomes in NDM HD patients exceeding hyperglycemic thresholds for these alternative glycemic markers per the literature and/or manufacturer recommendation.

Methods: As of April 1, 2013, 946 active NDM-HD patients from 26 FMCNA facilities with glycemic markers from Jan-Mar 2013 were followed until Mar 31, 2014. Baseline albumin-adjusted and unadjusted fructosamine (AlbF; F) and glycated albumin (GA) or percent GA (%GA) divided patients into high/low glycemia by thresholds of: AlbF≥974 µmol/g, F>285 µmol/L, %GA>15.7%, and GA>300 µmol/L. Cox models with and without adjustment for age, sex, race, ethnicity, vintage (log), BMI, HD catheter, and baseline comorbidity were utilized to determine associations between each dichotomized glycemic index to death and to hospitalization.

Results: Elevated glycemia was indicated in 4% (AlbF), 60% (F), 29% (%GA) and 16% (GA) of NDM cohort patients. Only high AlbF was significantly associated with both 1-year death risk (HR=2.3, p=0.04; adjusted HR=2.6, p=0.04) and hospitalization risk (HR=1.5, p<0.05; adjusted HR=1.6, p=0.03). A paradoxical association with lower death risk was noted with elevated F (HR=0.5, p=0.005) and GA (HR=0.4, p=0.04).

Conclusions: Preliminary findings in HgbA1c-confirmed NDM HD patients indicated an association between poor glycemic control determined from AlbF with 1-year survival and hospitalization risk. Elevated F and GA were both associated with better survival. To our knowledge, this is the first prospective indication that NDM glycemic status may have prognostic implications in HD patients. The GIDE study is ongoing; more detailed evaluation and longer term follow-up is planned.

SA-PO1024

Glycemic Control and Mortality in Diabetic Patients on Dialysis: Effect of Age and Dialysis Type Ji In Park,[1,2] Kyung Don Yoo,[1,2] Seung Seok Han,[1,2] Jung Pyo Lee,[1,2] Dong Ki Kim,[1,2] Kwon Wook Joo,[1,2] Yon Su Kim,[1,2] Yong-Lim Kim,[2] Shin-Wook Kang,[2] Chul Woo Yang,[2] Hajeong Lee.[1,2] *[1]Seoul National Univ Hospital; [2]Clinical Research Center of End Stage Renal Disease in Korea.*

Background: Though the improved glycemic control prevents diabetic nephropathy progression and lowers mortality in the chronic kidney disease, it remains unclear whether glycemic control is beneficial in the ESRD. In this study, we evaluate the effect of HbA1c on mortality in the diabetic patients on dialysis according to the age and dialysis types.

Methods: Among 3,302 patients with ESRD enrolled in Clinical Research Center for End Stage Renal Disease in Korea between 2008 and 2013, we included patients with underlying diabetes mellitus or initial HbA1c>6.5%. Age was categorized as <55(young), 55-64(middle) and ≥65(old) years old. We adjusted in proportional hazards models for age, sex, MCCI, hemoglobin, primary renal disease, BMI, and dialysis duration.

Results: Among a total of 1,239 patients, 873 patients received HD, and 366 did PD. During the mean follow-up of 19.1 months, 141 patients (11.4%) died. HbA1c≥8% group showed worse survival than <8 % groups (HR 2.2, 95% CI 1.48-3.29, P<0.001). In the subgroup analysis, same results were shown in HD (HR 1.9, 95% CI 1.08-3.21, P=0.025) and PD group (HR 2.7, 95% CI 1.38-5.20, P=0.004). However, in the age groups, HbA1c≥8% was a significant predictor in the young (HR 4.3, 95% CI 1.78-10.41, P=0.001) and middle group (HR 3.3, 95% CI 0.81-2.69, P=0.002), but not in the old group. Further analysis was carried out on age subgroups of each dialysis modalities. It revealed that among HD patients, HbA1c≥8% was a significant factor only in the young group, while among PD, it was in the young and middle groups. Mortality rates due to infection were higher in PD group than in HD (32.2, 20.1/1000-patient-year, respectively) and among PD patients, deaths from infection were significantly higher in HbA1c≥8% group than in <8% group (P=0.024).

Conclusions: In this study, we elucidated that target of glucose control should be individualized according to age and dialysis type in diabetic patients on dialysis. Improving glycemic control is beneficial only in young dialysis patients and middle aged PD patients.

Funding: Government Support - Non-U.S.

SA-PO1025

Use of Insulin Pump in Patients with End Stage Renal Disease on Dialysis Hossein Ghofrani, Elaine Kaptein. *Nephrology, LAC/USC Medical Center, Los Angeles, CA.*

Background: Among patients with end stage renal disease (ESRD) on hemodialysis (HD) or peritoneal Dialysis (PD), about 5-10% have diabetes mellitus type 1. Prolonged insulin half-life as well as insulin resistance alters insulin dosing in diabetics with ESRD. Although use of insulin pumps in type 1 diabetics leads to more favorable metabolic control and less hypoglycemic events, there are no data or guidelines on insulin dosing in dialysis patients.

Methods: We retrospectively report diabetes control, insulin dosing and dose adjustments in a series of 5 ESRD patients (4 on HD or 1 on PD) who are currently using insulin pump, compared to the same patients prior to pump use. We recorded and compared periodic HgA1C and incidence of hypoglycemia episodes before and after pump use, the initial basal insulin dose estimated from standard formulas to estimates for our ESRD patients to avoid hypoglycemia and hyperglycemia, and actual total daily dose (TDD) of insulin to achieve acceptable glycemic control with TDD calculated with the formula used for general diabetic population (based on pre-pump insulin amount).

Results: The initial basal dose of insulin required to start the pump was only 10-15% of the recommended dose for general insulin pump users. Average TDD of insulin was lower while using the pump compared to pre-pump dosing (32.9±6.5 units versus 38.3 ± 4.7 units; not statistically significant). After dose adjustment, the actual TDD to achieve an acceptable glycemic control was on average 16% less than calculated TDD. HgA1C level did not show significant change before and after pump use. Average monthly number of recorded hypoglycemia episodes decreased significantly (3.1±0.9 on pump versus 5.8±1.6 pre-pump, p=0.04).

Conclusions: We have successfully used insulin pumps in five dialysis-dependent patients, and found that the initial basal dose was about 10% of recommended, although total daily dose was 90% of expected.Experience in our ESRD patients may provide some guidance for calculating initial, maintenance, basal and bolus dosing of insulin while using an insulin pump in ESRD patients.

SA-PO1026

Monthly Variability in Serum Bicarbonate in Hemodialysis Patients

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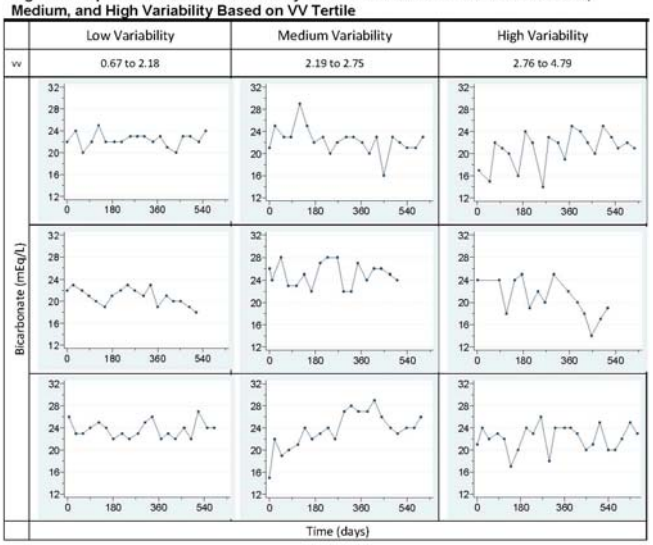
Background: The optimal management of acid-base status in hemodialysis (HD) patients remains unclear. Previous studies have associated both low and high serum bicarbonate with adverse outcomes. However, a standardized approach to individualizing therapy does not exist. An important yet overlooked factor is the month-to-month variability in serum bicarbonate.

Methods: We retrospectively studied consecutive patients initiating HD from 1/2008–6/2012 at a single dialysis center with a uniform dialysate bicarbonate concentration. Clinical cutpoints were used to classify each serum bicarbonate as low (<22), normal (22-26), or high (>26 mEq/L). Each monthly observation *i* was assigned a Variability Value (VV): $VV = \sqrt{(([HCO_3]_i - [HCO_3]_{i-1})^2 + ([HCO_3]_i - [HCO_3]_{i+1})^2)/2)}$. VV tertiles were used to define low, medium, and high variability. Factors associated with VV over time were examined using linear mixed models.

Results: 181 patients were included, with mean serum bicarbonate 22.6±3.0 mEq/L and mean VV 2.50±1.60. VV distinguished low versus high month-to-month variability.

Figure 1. Representative Plots of Monthly Serum Bicarbonate in Patients with Low, Medium, and High Variability Based on VV Tertile



Among low bicarbonate values, 41% returned to normal the subsequent month. Among high values, 60% returned to normal. Compared with normal bicarbonate levels, both low and high serum bicarbonate were associated with higher VV (0.50 (0.39-0.61) and 0.92 (0.74-1.09), respectively) after multivariable adjustment including medication use, protein intake, dialysis adequacy, and interdialytic weight gain.

Conclusions: Substantial month-to-month bicarbonate variability exists in a subset of HD patients. Even if clinicians take no action, a bicarbonate value outside the normal range has approximately a 50% chance of returning to normal the following month. There is little utility to basing treatment decisions on a single monthly bicarbonate level.

Funding: Private Foundation Support

SA-PO1027

Serum Potassium and All-Cause Mortality in Dialysis Patients: A Nationwide Prospective Observational Cohort Study in Korea

Sunhwa Lee,[1] Hajeong Lee,[1] Dong Ki Kim,[1] Kwon Wook Joo,[1] Yong-Lim Kim,[2] Shin-Wook Kang,[3] Chul Woo Yang,[4] Yon Su Kim.[1] *[1]Internal Medicine, Seoul National Univ Hospital; [2]Kyungpook National Univ Hospital; [3]Yonsei Univ College of Medicine; [4]Seoul St. Mary's Hospital.*

Background: Abnormalities of serum potassium concentration has been suggested to be a risk factor of mortality in chronic kidney disease or dialysis patients. We investigated the impact of serum potassium level on survival according to dialysis modalities.

Methods: A nationwide prospective observational cohort study for end stage renal disease patients has been ongoing since August 2008. Among the participants, patients had been selected who checked serum potassium level at least twice between 3 and 18 months. Time-averaged potassium level was calculated by an arithmetic mean, and its relationship with mortality was analyzed using Cox proportional hazard model.

Results: A total of 1,686 patients (hemodialysis (HD) 62.3%, peritoneal dialysis (PD) 37.7%) were included in the final analysis. Serum potassium levels were significantly lower in PD than in HD patients (4.31±1.06 versus 4.79±1.05 mg/dL, $P<0.001$). During the mean 2.2-year of follow up, 142 patients died of mainly cardiac arrest (n=39) and infection (n=46). In survival analysis, hypokalemia was a significant predictor of mortality. Moreover, hypokalemic PD patients showed higher mortality even after adjustment of age, history of congestive heart failure, diabetes, hypoalbuminemia, and hypouricemia (Hazard ratio 2.98, 95% confidence interval 1.68–5.28, $P<0.001$). However, serum potassium did not show any association with mortality in HD patients. Hypokalemic PD patients died more due to cardiovascular events than normo~hyperkalemic PD patients ($P<0.001$). Subgroup analysis showed that hypokalemic PD patients had still higher mortality rate than others regardless of sex or presence of diabetes.

Conclusions: Hypokalemic PD patients have higher mortality risk. Cardiovascular death is the major cause of mortality in PD patients with hypokalemia. However in HD patients, serum potassium level was not associated with mortality.

SA-PO1028

Continuous Glucose Monitoring System in Glucose Profile Assessment and Insulin Pumps in Diabetic Nephropathy Patients with Hemodialysis

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Background: Good glycemic control is often difficult to maintain in diabetic patients treated with hemodialysis. However, recent studies have suggested that diabetic patients who use insulin pump has been shown to reduce glycated hemoglobin levels without an increased risk of hypoglycemia, as compared with a regimen of multiple daily insulin injections, but results in diabetic nephropathy patients with hemodialysis have not been reported. The continuous blood glucose monitor (CGMS) has recently offered an opportunity to monitor blood glucose at 5-minute intervals for 72 continuous hours in diabetic patients.

Methods: There were 14 diabetic patients on hemodialysis using free glucose dialysate in the study. All participants underwent 72-hour CGMS and HbA1c evaluation. Continuous subcutaneous insulin pump insulin intensive therapy group for the ultra-short effect of human insulin analogues - insulin lispro, conventional subcutaneous insulin Humalog before meals program for the Addition 21:00 Novolin N treatment. There was the whole area under the curve (AUC) of each 24-hour glucose profile. Glucose area above high limit is 10mmol/L, and glucose area below low limit is 3.9mmol/L.

Results: Diabetic nephropathy patients with hemodialysis 12 cases with first use of continuous subcutaneous insulin pump therapy to strengthen, then the routine use of subcutaneous insulin program. Duration above high glucose limits are 34% ± 12%, duration within limits are 65 ± 23%, and duration below limit are 1% ± 1% in continuous subcutaneous insulin pump therapy group, while duration above high glucose limits are 65% ± 32%, duration within limits are 35% ± 12% in the conventional subcutaneous insulin program, $p< 0.05$.

Conclusions: In diabetic nephropathy with hemodialysis in insulin pump therapy compared with conventional subcutaneous insulin therapy, blood glucose is more stable standard. CGMS technology can effectively evaluate the patient's blood glucose with hemodialysis.

Funding: Government Support - Non-U.S.

SA-PO1029

Higher Alkaline Phosphatase Level as an Independent Risk Factor for All Clinical Fractures in Hemodialysis Patients: A Prospective Cohort Study

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Background: Risk factors of bone fracture in hemodialysis (HD) patients have not been fully clarified. Recently, higher alkaline phosphatase (ALP) level was reported as a predictor of hip fractures in a Japanese cohort study (Maruyama et al. Nephrol Dial Transplant 2014); however, its follow-up period was short (one year) and incidence was examined retrospectively. Using prospective cohort data, we investigated the relationship between ALP level and all clinical fractures in HD patients.

Methods: We examined data of 1,551 HD patients (mean age 67±13 years, males 57.8%, diabetes 24.0%, and median HD vintage 74 months) in the Miyazaki Dialysis Cohort Study (MID study), and evaluated ALP level and the incidence of all clinical fractures during the 48-month follow-up using multivariate Cox regression analysis.

Results: During the follow-up, 157 (10.1%) patients were newly diagnosed with clinical fractures (hip fracture 27.4%, vertebral fracture 19.7%, and other fractures 52.9%). The higher ALP tertile group was older, more likely female, had a longer HD vintage, and had more past hip fractures. Patients in the higher ALP tertile were more likely to have lower levels of albumin, calcium, and phosphate, and a higher level of intact parathyroid hormone (iPTH). Multivariate Cox regression analysis showed that being in the highest ALP tertile (269 to 1180 IU/L) was a significant risk factor for all clinical fractures, even when the middle tertile (196 to 268 IU/L) was set as a reference [hazard ratio (HR) 2.02, 95% confidence interval (CI) 1.06 to 3.85] after adjustment for age, sex, primary disease, calcium carbonate, oral vitamin D, corticosteroid, statin, past hip fracture, hemoglobin, albumin, calcium, phosphate, and iPTH.

Conclusions: In this cohort study, higher ALP level was an independent risk factor for all clinical fractures in HD patients.

SA-PO1030

The Relationship between the Spine Deformity Index Biochemical Parameters of Bone Metabolism and Vascular Calcifications: Results from the Epidemiological Vertebral Fractures Italian Study in Dialysis Patients (EVERFRACT Study) Maria Fusaro,[1] Marianna Noale,[1] Giovanni Tripepi,[2] Claudia Torino,[2] Marianna Alessi,[3] Luciana Bonfante,[3] Antonio Piccoli,[3] Rosalba Cristofaro,[3] Maurizio Gallieni.[4] *[1]CNR, Padua, Italy; [2]CNR IFC, Reggio Calabria, Italy; [3]Nephrology Unit, Padua, Italy; [4]Nephrology Unit, Milan, Italy.*

Background: The Spine Deformity Index (SDI) is a summary measure of the VFs. The aim of this study was to evaluate the relationships SDI, laboratory parameters (EVERFRACT study).

Methods: 387 hemodialysis patients, aged 64.2 ± 14.1 years, we determined: 25(OH) vitamin D, total Bone Gla Protein (BGP), undercarboxylated BGP (ucBGP) and total Matrix Gla Protein (MGP). We performed L-L x-Rays of the spine (T5 to L4) to evaluate VFs and aortic (AoVC) and iliac (IaVC). We divided the SDI score by the number of fractures, to obtain a more precise index of fracture severity (corrected-SDI: c-SDI).

Results: 55.3% VFs. SDI was 1.4 ± 1.74 while c-SDI was 0.74 ± 0.75. VFs had a grade of severity higher through T11-L3. The severity of VFs was highlighted only by c-SDI (see Table).80.6% AAoC and 55.6% IAC. SDI was significantly associated with AAoC (OR=1.15, p=0.023). A SDI >1 was significantly associated with: sex (male OR 1.86, p=0.007), age (OR 1.03, p=0.0003) and albumin ≥ 3.5 g/dL (OR 0.54, p=0.026). c-SDI score was significantly associated with AAoC (OR=1.48, p=0.0009) and with IAC (OR=1.54, p=0.025). c-SDI >1 was significantly associated with: age (OR 1.05, p<0.0001), LDL Cholesterol ≥ 90 mg/dL (OR 1.74, p=0.0354) and ucBGP ≥ 17.2 mcg/L (OR 0.35, p=0.0025).

Vertebra	Mild VF	Moderate VF	Severe VF	SDI	c-SDI
T5	24 (6.0%)	7 (1.7%)	0 (0.0%)	38	1.23
T6	33 (8.3%)	14 (3.5%)	1 (0.2%)	64	1.33
T7	38 (9.6%)	12 (3.0%)	0 (0.0%)	62	1.24
T8	35 (8.8%)	10 (2.5%)	0 (0.0%)	55	1.22
T9	26 (6.5%)	8 (2.0%)	1 (0.2%)	45	1.29
T10	19 (4.7%)	8 (2.0%)	0 (0.0%)	35	1.30
T11	30 (7.5%)	14 (3.5%)	3 (0.7%)	67	1.43
T12	35 (8.8%)	17 (4.2%)	2 (0.5%)	75	1.39
L1	19 (4.7%)	12 (3.0%)	2 (0.5%)	49	1.48
L2	4 (1.0%)	3 (0.7%)	2 (0.5%)	16	1.78
L3	2 (0.5%)	3 (0.7%)	0 (0.0%)	8	1.60
L4	13 (3.2%)	4 (1.0%)	0 (0.0%)	21	1.24
Tot	278	112	11	Sum 535	Average 1.38

Conclusions: Only c-SDI score performed the grade of VFs severity and it showed a stronger association with vascular markers. This is the first time that the association of SDI with biochemical parameters and VC.

SA-PO1031

Epidemiology of Hip Fracture in ESRD Dialysis Patients: Taiwan National Cohort Study Chih-Chiang Chien. *Dept of Nephrology, Chi-Mei Medical Center, Tainan City, Taiwan.*

Background: Chronic kidney disease increases the risk for hip fractures. Hip fractures are associated with increased mortality, decreased quality of life, and higher economic burden. To determine whether dialysis modality is associated with a higher incidence of hip fractures in end-stage renal disease (ESRD) dialysis patients.

Methods: In the Taiwan National Health Insurance Research Database, we examined records of ESRD patients who initiated dialysis between 1999 and 2005. Patients were followed until death, transplant, dialysis withdrawal or 31 December 2008. The cumulative incidence rate of hip fracture was calculated using Kaplan-Meier methods. Predictors of hip fracture was calculated using Cox models.

Results: A total 51,473 dialysis patients were examined in this study. During the study period, 1903 patients had hip fracture. The overall incidence rate of hip fracture was 89.21 per 10,000 patient-years. Patients' age was 67.91±10.45 and 59.27±14.08, and they included 60.6% and 51.8% women in patients with and without hip fracture, respectively. Female gender, old age, receiving hemodialysis, a prior history of hip fracture and having baseline comorbidities were risk factors for hip fracture in the dialysis patients. Patients on hemodialysis had a 31% higher incidence of hip fracture than those on peritoneal dialysis (HR 1.31, 95% CI: 1.01-1.70). Patients ≥ 65 years had a more than 13-fold increased risk of hip fracture compared to those aged 18-44 years old (HR 13.65, 95% CI: 10.12-18.40). The overall in-hospital mortality rate was 3.2%. The cumulative survival rates after a hip fracture were 74.6% at one year and only 29.6 % at seven years.

Conclusions: Our findings supported the notion that being on HD is a risk for hip fracture. Additionally, age was also a significant risk for a hip fracture in patients with ESRD and undergoing dialysis.

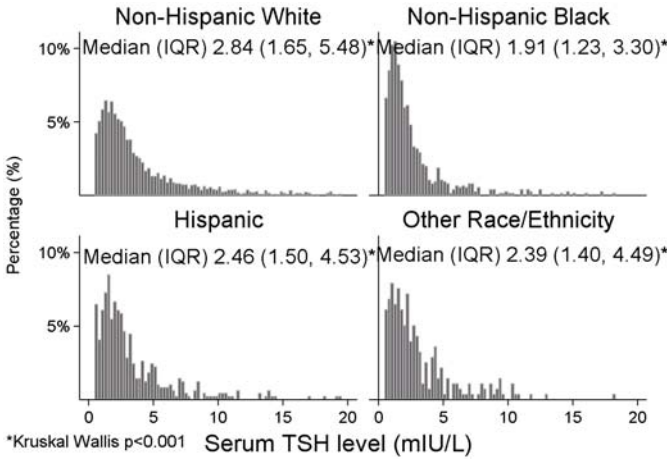
SA-PO1032

Thyroid Function Patterns across Race/Ethnicity in a National Incident Hemodialysis Cohort Connie Rhee,[1] Jiaxi Wang,[1] Bahattin T. Oztan,[1] Steven B. Kim,[1] Daniel L. Gillen,[1] Rajnish Mehrotra,[2] Elani Streja,[1] Steven M. Brunelli,[3] Csaba P. Kovesdy,[4] Gregory Brent,[5] Kamyar Kalantar-Zadeh.[1] *[1]UCI, Irvine, CA; [2]Univ of Washington, Seattle, WA; [3]DaVita Clinical Research, Minneapolis, MN; [4]Memphis VAMC, Memphis, TN; [5]UCLA, Los Angeles, CA.*

Background: Nearly one-quarter of hemodialysis (HD) patients have hypothyroidism (HT), defined by an elevated serum thyrotropin (TSH). Given racial/ethnic differences in TSH distribution in the general population, we compared the baseline distribution and longitudinal trajectory of TSH levels across race/ethnicity in HD patients from a large national dialysis organization.

Methods: Among a 5-year cohort (1/2007-12/2011) of adult incident HD patients with ≥3 TSH levels, we examined the TSH distribution in non-Hispanic whites, non-Hispanic blacks, Hispanics, versus other race/ethnicities (former 2 groups referred to as whites and blacks). We then examined the longitudinal trajectory of TSH across racial/ethnic subgroups using unadjusted and multivariable linear mixed models adjusted for age, sex, and diabetes.

Results: Among 3946 patients, the baseline median (IQR) of serum TSH was 2.54 (1.49, 4.80) mIU/L. Compared to whites, blacks and Hispanics had lower median (IQR) baseline TSH. In unadjusted linear mixed models, blacks and Hispanics had significantly lower baseline serum TSH versus whites (Δ=-0.90 and -1.15mIU/L), which were attenuated in adjusted models. In unadjusted and adjusted linear mixed models, TSH decreased over time in whites (-0.38mIU/L per year in both models), with similar rates of decline observed in blacks, Hispanics, and other race/ethnicities.



Conclusions: Black and Hispanic HD patients have lower TSH distributions versus whites, but similar rates of TSH decline over time. Further studies are needed to determine the genetic, autoimmune (i.e. thyroid autoantibodies), and environmental factors for differential TSH distributions across race/ethnicity.

Funding: NIDDK Support

SA-PO1033

Sex Steroid Levels in Chronic Kidney Disease, Dialysis and Kidney Transplant Recipients: Associations with Disease Severity and Prediction of Mortality Matthew Allan Roberts,[1] Francesco L. Ierino,[2] Rudolf Hoermann,[3] Mark Ng Tang Fui.[3] *[1]Eastern Health Clinical School, Monash Univ, Australia; [2]Nephrology, Austin Health, Australia; [3]Medicine, Univ of Melbourne, Australia.*

Background: Levels of circulating sex steroids change with stages of chronic kidney disease (CKD) and may be associated with clinical outcome.

Methods: We prospectively recruited patients with (a) CKD III-IV, (b) undergoing chronic dialysis and (c) kidney transplant recipients (KTR) from a single centre in 2003 and 2004. Two stored samples taken 3 months apart were analyzed for serum testosterone and other sex hormones using liquid chromatography/tandem mass spectrometry and the mean of the two was used for analysis. We also measured novel cardiac biomarkers troponin T and NT-BNP. Patients were followed until death, transplant or June 30, 2013, and survival analysis performed.

Results: Sex hormones were measured in 49 patients with CKD III-IV, 102 patients receiving dialysis and 70 KTR. In males, median (IQR) testosterone levels were lowest in dialysis patients and highest in KTR, whereas in females, testosterone was lowest in KTR. Over a median follow up of 8.5 years 52 men (36%) died.

Testosterone ng/dL	CKD III-IV	Dialysis	KTR	P
Male	309 (242-372); n=38	277 (193-398); n=61	372 (274-467); n=44	0.005
Female	7.5 (6.3-21.1); n=11	7.5 (4.9-12.1); n=41	4.9 (1.2-10.1); n=26	0.04

In Cox proportional hazard regression models, testosterone predicted mortality independent of baseline age, body mass index, stage of renal disease, and circulating levels of brain

natriuretic peptides or cardiac troponin T. An increase in testosterone by 29ng/dL (1 nmol/L) was associated with a 9.8% (95% confidence interval 3.1-16.3%) decrease in mortality. By contrast, sex steroid levels were not associated with mortality in females.

Conclusions: Levels of testosterone vary by CKD stage and in males, predict mortality independent of established and novel predictors.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

SA-PO1034

The Soluble Urokinase Receptor (suPAR) Predicts Mortality in End-Stage Renal Disease Bjorn Meijers,[1] Ruben Poesen,[1] Markus Storr,[2] Kathleen Claes,[1] Pieter Evenepoel,[1] Bert Bammens,[1] Dirk R. Kuypers.[1] *[1]Nephrology, Univ Hospitals Leuven, Leuven, Belgium; [2]Gambro Dialysatoren GmbH, Hechingen, Germany.*

Background: The soluble urokinase receptor (suPAR) is a candidate biomarker for focal segmental glomerulosclerosis (FSGS). The clinical usefulness however is questioned as suPAR accumulates parallel to loss of kidney function. In non-FSGS CKD patients suPAR is an independent predictor of cardiovascular disease. Whether suPAR is associated with outcome in dialysis patients has not been studied to date.

Methods: We measured suPAR concentrations in patients with end-stage renal disease using the human uPAR enzyme-linked immune sorbent assay (R&D systems™). Associations with overall mortality were explored using Kaplan-Meier estimates and multivariate Cox proportional hazards analyses.

Results: We determined suPAR concentrations in 186 prevalent patients with end-stage renal disease (hemodialysis patients (HD) n=125; peritoneal dialysis patients (PD) n=61). suPAR concentrations in HD and PD were similar (median 5918 versus 6372 ng/mL, Wilcoxon P NS). suPAR concentrations were associated with overall mortality (P 0.001). In multivariate analysis, after correction for age, sex, albumin, c-reactive protein, haemoglobin and PTH, suPAR remained independently associated with mortality (P 0.01).

Conclusions: suPAR is directly and gradually associated with overall mortality in patients with end-stage renal disease. This extends observations in patients with mild-to-moderate CKD. These observations question whether suPAR is a selective biomarker for FSGS and suggest a role for the urokinase receptor signalling pathway in CKD.

Funding: Pharmaceutical Company Support - Gambro Dialysatoren GmbH, Hechingen, Germany

SA-PO1035

Major Bleeding in Incident Hemodialysis Patients Suzanne H. Forbes, Neil Ashman. *Dept of Nephrology and Transplantation, Royal London Hospital, London, United Kingdom.*

Background: End-stage renal disease (ESRD) is associated with increased risk of bleeding related to uremia, platelet dysfunction, hypergastrinemia and dialysis anticoagulation. In prevalent hemodialysis (HD) patients in our centre the rate of major bleeding is 3.3 per 100 patient years (comparable to North American data at 2.5). All published analyses looking at bleeding risk in HD, however, exclude incident (<90day) patients. We hypothesized the risk of major bleeding at this time of maximal biochemical disturbance would be elevated.

Methods: We performed an observational retrospective study of all HD new starters of any cause over a 74 month period ending March 2013. Those dialysing for <1week, for delayed graft function, or transfers already established on HD were excluded. Bleeding was defined as 'major' using International Society on Thrombosis and Haemostasis criteria, or as any bleed requiring admission. Minor bleeds were also captured.

Results: 1540 patients were analysed. The majority dialysed for a minimum 90 days, equalling 364 patient-at-risk years. All patients were anticoagulated on HD with tinzaparin. The median age was 57 with a pre-HD eGFR of 6.5ml/min/1.73m^2. 973 were male and 517 diabetic. Starting access was a catheter in 1256 patients and 730 were on at least one antiplatelet agent. Within the first 90 days of HD there were 188 bleeding events across 177 patients; 95 were major, 3 fatal. The overall rate for a major bleed was 26.1 per 100 patient years. Contrary to published prevalent data, gastrointestinal bleeds constituted just 60% of the major bleeds, with intracranial, intraocular and retroperitoneal bleeds also significant. Analysis of time from initiation of HD to major bleeding event showed the risk to be maximal in the first 2 weeks, falling quickly thereafter to that seen in the prevalent population.

Conclusions: In comparison with prevalent patients, we demonstrate that incident HD patients have an 8-fold increase in major bleeding events, exaggerated particularly in the first 2 weeks. This data should prompt increased caution with antiplatelets and dialysis anticoagulation at this time. Furthermore this data forms a proposal to examine registry-wide incident bleeding across the UK.

SA-PO1036

Outcomes of Warfarin Use in Haemodialysis Suzanne H. Forbes, Emma O'lone, Neil Ashman. *Nephrology and Transplantation, Royal London Hospital, United Kingdom.*

Background: Haemodialysis (HD) patients have increased bleeding risk. There is debate about warfarin use in these patients. A 4-fold increased risk of major bleeding in prevalent HD patients taking warfarin is published. Non-renal patients taking warfarin are closely monitored in anticoagulation clinics whereas HD patients engaging with renal services thrice weekly are more likely to have their warfarin dosed there. In a population with elevated bleeding risk, tight INR control is key. We examined whether this was achieved.

Methods: We examined all patients known to our services taking warfarin for any reason whilst on HD and calculated combined time on HD and warfarin, examining bleeding outcomes, death and calciphylaxis. For patients with >3 INR results available we used the Rosendaal linear interpolation formula to calculate time in the therapeutic range (TTR, based on target INR). We also expanded this to a wider therapeutic window (INR 1.5-4), outwith which a thrombotic or bleeding event is known to be more likely, and noted peak INR results.

Results: We identified 214 HD patients taking warfarin, totalling 510 patient years. Reasons for warfarin are shown.

Indication	n (%)
AF	61 (29)
DVT	55 (26)
Valve	42 (19)
PE	26 (12)
Thrombus	10 (5)
Access	8 (4)
Other	12 (5)

There were 81 bleeding events, overall bleed rate of 15.9 per 100 patient years (versus published 3.57 in non-HD patients). 6 patients developed calciphylaxis (4 fatal). TTR was 36.5% days (37.9% tests) in range for target INR. Expanding the range to 1.5–4 gave 68.7% days (70% tests) in range. 40% of patients had at least one INR>5, and 10%>10. Of those with a bleed, median peak INR was 7.4 versus 5 in those without and TTR correlated with outcome.

Conclusions: We show significant bleeding in HD patients taking warfarin. We also show INR control in these patients is suboptimal compared with standards expected in haematology clinics, with significant time spent outwith a safe INR range. There are few indications for warfarin in HD that have a solid evidence base. This data reinforces that HD patients should avoid warfarin. When it is absolutely indicated, INR control should be rigorously monitored and there may be a role for a joint anticoagulation service for these patients.

SA-PO1037

Clinical Characteristics Associated with High Post-Dialysis Hemoglobin in Chronic Hemodialysis Patients Linda H. Ficociello,[1] Paul Balter,[1,2] Dong (Winnie) Hua,[1] Claudy Mullon,[1] Paul M. Zabetakis,[2] Jose A. Diaz-Buxo.[1] *[1]Fresenius Medical Care North America, Waltham, MA; [2]Renal Research Inst, NYC, NY.*

Background: Hematocrit-derived hemoglobin (hb) was captured during a quality improvement project on fluid management across multiple dialysis (DL) units, including beginning (pre-DL) and end (post-dL) of DL. Post-DL hb is not routinely measured, so its clinical utility has not been fully explored.

Methods: Hb was captured via Crit-Line Monitors (CLM) in over 41,000 treatments from 1099 patients (pts) during 2 years. Pre-DL hb is measured after saline prime, post-DL hb before rinse-back, and measurements include multiple observations/pt.

Results: 66% of treatments (n=27,285) had post-DL hb<12, 29% (n=11,834) had a post-DL hb between (b/t) 12-13.9, and 5% (n=2,131) had post-DL hb ≥14g/dL [2.9% b/t 14-14.9, 1.4% b/t 15-15.9, 0.7% b/t 16-16.9, and 0.2% 17+g/dL]. Table categorizes pts based on highest post-DL hb. On average, pts with post-DL hb ≥14g/dL had higher pre-DL hb, steeper % change in blood volume, lower pre and post-DL SBP, greater pre and post-DL weight, and lower epo doses.

	Post-DL hb<12	Post-DL hb 12-13.9	Post-DL hb≥14	p-value
Number of pts (n,%)	253 (23%)	578 (53%)	268 (24%)	
Cause of ESRD (%): Diabetes Hypertension Polycystic Kidney %Other	36 35 2 27	41 32 3 24	34 34 2 30	0.24
Monthly/Bi-weekly Pre-DL hb (g/dL)	10.3	11.5	12.5	<0.0001*
CLM Pre-DL hb (g/dL)	10.0	11.7	13.0	<0.0001*
CLM Post-DL hb (g/dL)	10.7	13.0	15.0	by design
%Change Blood Volume	-6.3	-10.0	-13.5	<0.0001*
Pre-DL SBP (mmHg)	145.7	145.8	139.0	0.003**
Post-DL SBP (mmHg)	138.7	134.8	125.5	<0.0001*
Pre-DL Wt (kg)	78.2	80.7	86.6	<0.0001**
Post-DL Wt (kg)	76.0	78.1	83.3	0.003**
% administered Epo	77.5	62.5	31.7	<0.0001
Epo per Epo treatment (units)	6604	4231	4541	<0.0001*
Epo per treatment (units)	5108	2523	1343	<0.0001*

* All group means differ
** ≥14 group means differ from other groups

Conclusions: Clinical characteristics of pts with post-DL hb≥14 differ from pts with lower post-DL hb. Clinical relevance of high ending hb should be further explored, such as cardio and cerebrovascular hospitalizations. Future projects may consider inter-dialytic hb changes in addition to intra-dialytic changes.

Funding: Pharmaceutical Company Support - Fresenius Medical Care NA

SA-PO1038

Cancer Incidence Among U.S. Medicare End-Stage Renal Disease Patients on Hemodialysis, 1996-2009 Anne Mobley Butler, A. V. Kshirsagar, M. Alan Brookhart. *Univ of North Carolina at Chapel Hill, Chapel Hill, NC.*

Background: Patients with end-stage renal disease (ESRD) receiving dialysis have increased risk of cancer. Yet, contemporary cancer burden estimates in this population are sparse, and estimates that account for the high competing risk of death in this population are non-existent.The objective of this study is to quantify the burden of incident cancer among U.S. ESRD patients, overall and among subgroups.

Methods: Using data from the United States Renal Data System (USRDS), a national registry of patients in the Medicare ESRD program, we identified patients ≥ 18 years receiving dialysis between April 1995 and December 2010 with Medicare as primary payer and parts A and D coverage. Site-specific cancers required ≥ 2 ICD-9-CM diagnosis codes within 6 months. For overall and site-specific cancers, we calculated 5-year cumulative incidence since dialysis initiation using competing risk methods and standardized mortality ratio (SMR)-weighted annual incidence rates.

Results: Between 1996-2009, we observed constant rates of incident cancers for all sites combined, from 3923 to 3860 cases per 100,000 person years [annual percentage change, 0.1; 95% confidence interval (CI), -0.4, 0.6], but identified increasing and decreasing rates for some common site-specific cancers. Of 482,510 patients in the cumulative incidence analysis, 37,128 patients were diagnosed with cancer within 5 years after dialysis initiation. The 5-year cumulative incidence of any cancer was substantially lower in the analysis that did not censor deaths (9.48%; 95% CI, 9.39% to 9.57%) compared to the analysis that censored deaths (13.86%; 95% CI, 13.71% to 14.01%). Accounting for case-mix characteristics and the competing risk of death, the 5-year standardized cumulative incidence of any cancer was higher among the following subgroups: older age; males; non-whites; non-Hispanics; primary ESRD cause other than diabetes; recent dialysis initiation; and history of kidney transplant evaluation.

Conclusions: These results suggest a high burden of cancer in the dialysis population, with varying patterns of cancer incidence in subgroups.

Funding: NIDDK Support

SA-PO1039

Physican Bias in Initiating Chronic Hemodialysis in Major Psychiatric Illness Roshni Upputalla, Andi Qipo, Siddhartha Yedla, Vivek Reddy, Njideka Oragunye, Kisra Anis. *Nephrology, Jacobi Medical Center, Bronx, NY.*

Background: Patients with major psychiatric illnesses are a challenging population in the initiation and maintenance of chronic hemodialysis (HD). These patients require frequent care and need more allocation of resources due to their underlying psychiatric diagnosis. There is a lack of literature addressing the many issues related to initiation of chronic HD treatment in this group.

Methods: We retrospectively looked at 503 patients, who started dialysis at Jacobi Medical Center from 1998 to 2012. There were 41 patients with diagnosis of major depression, bipolar disorder and schizophrenia (study group), and 462 patients with no mental illness (control group).The prevalence of patients with mental illnesses in our group (8.8%) reflected the prevalence of the same diagnosis in general population (9-10%). Data included age, gender, smoking, alcohol, drug history, eGFR (MDRD), BUN, creatinine, albumin, and type of dialysis access used at first HD session.

Results: Control group had a higher percentage of males (53%) compared to study group (28%).The study group started on HD at a higher level of GFR of 9.4 versus 7 (p=0.012), lower BUN 65 versus 92 mg/dL (p<0.0001) and creatinine 5.3 versus 7.3 mg/dL (p<0.0001), and higher albumin 3.6 versus 3.4 g/dL (p=0.05). At initiation of HD the study group had higher percentages of AVF 17% versus 9.5%, AVG 4.8% versus 1.5%,and TDC 60% versus 56% compared to controls but none was statistically significant possibly due to small sample size. There was a statistically significant lower percentage of non-tunneled dialysis catheters in the study group compared to control group 17% versus 32.5% (p=0.041) at HD initiation.

Conclusions: Possible explanations for our findings are that patients with mental illness received medical interventions earlier due to closer follow up. In 20% of cases the decision for HD initiation in the study group was made due to altered mental status, ranging from agitation to lethargy, believed to be due to uremia. Diagnosis of altered mental status due to uremia might represent a challenge in these patients as the underlying mental illness or side effects of medications could affect mental status.

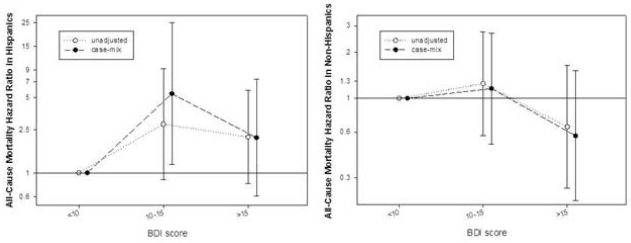
SA-PO1040

Association of Depression and Ethnicity with Survival in Hemodialysis Patients: Findings from the MADRAD Study Melissa Soohoo,[1] Elani Streja,[1] Connie Rhee,[1] Tracy Nakata,[1] Jennie Jing,[1] Csaba P. Kovesdy,[2] Daniel L. Gillen,[1] Kamyar Kalantar-Zadeh.[1] *[1]UCI, Irvine, CA; [2]Memphis VAMC, Memphis, TN.*

Background: Hemodialysis (HD) patients have a disproportionately higher risk of both depression and mortality, but prior studies examining the relationship between depression and mortality have been inconsistent. It remains unknown as to whether there is a differential depression—mortality association among ethnic minority HD patients.

Methods: We conducted a prospective study examining the association between depression and all-cause mortality in 490 HD patients from 13 large dialysis organization centers from the Malnutrition, Diet and Racial Disparities in Chronic Kidney Disease (MADRAD) cohort (entry period starting October 2011 with follow-up through March 2014). Associations between depression, defined by baseline Beck Depression Inventory (BDI) score, and mortality were examined in the overall cohort and within ethnic subgroups (Hispanic versus non-Hispanic) using case-mix-adjusted Cox proportional hazards models.

Results: Among 490 HD patients, the mean age was 54.5±15 years; 44% were Hispanic; and 19% and 29% of patients had mild (BDI 10-15) and moderate-severe depression (BDI>15), respectively. We did not observe a significant association between BDI score and mortality in the overall cohort (ref.: BDI <10): adjusted HRs (aHRs) (95%CI) 1.60 (0.81-3.16) and 1.03 (0.52-2.02) for mild and moderate-severe depression, respectively. However, when stratified by ethnicity, we observed a significant association between mild depression and mortality in Hispanic patients only: aHR (95%CI) 5.45 (1.20-24.8) (Figure).



Conclusions: We observed a 5.5-fold higher risk of death with mild depression among Hispanic HD patients. Further studies are needed to confirm these findings, and to determine if depression may be a potential factor for racial and ethnic survival disparities in HD patients.

Funding: NIDDK Support

SA-PO1041

The Prevalence of Depressive Affect and Associations in Increased Hospitalization Rates in Incident Hemodialysis Patients Kathryn A. McDougall,[1] John W. Larkin,[1] Rebecca L. Wingard,[1] Lin Ma,[1] Mikhail Artemyev,[2] Len A. Usvyat,[1] Eduardo K. Lacson,[1] Franklin W. Maddux.[1] *[1]Fresenius Medical Care North America (FMCNA); [2]Renal Research Inst.*

Background: Depression is common in hemodialysis (HD) patients (Pts), but not well defined in the incident HD (iHD) population. We investigated the prevalence of and associated hospitalization rates for depressive affect (DA) during 1-30 and 121-150 days after initiating HD.

Methods: From Jan-Jul 2013, among 108 randomly selected FMCNA HD clinics, 577 and 543 iHD Pts at 1-30 and 121-150 days after initiating HD were attempted to be contacted by telephone for depression screening using the Patient Health Questionnaire 2 (PHQ2). The PHQ2 has two questions that determine presence of depressed mood and anhedonia in the prior two weeks. PHQ2 scores range from 0-6; a positive DA score is ≥3. Clinical and lab parameters were collected for up to the first 30 and 150 days of HD, respectively. Analyses included Poisson regression and t-test comparisons.

Results: Of 1,120 attempts to perform DA screening, 306 PHQ2 assessments were completed. Responders (30.4%) were: 40.5% females; mean age 64.3±14.8 years; 66.4% diabetic; and 49.0% and 20.0% utilized catheters at the end of 30 and 150 days respectively. PHQ2 indicated DA prevalence at 20.1% and 15.0% for Pts at 1-30 and 121-150 days after starting HD (p=0.24). DA screening was performed at both time points in 35 Pts with 7 and 2 Pts found positive at 1-30 and 121-150 days respectively (p=0.076). Pts with DA had 0.66 more admissions and 4.9 additional hospital days per Pt year when compared to Pts without DA (p<0.001).

Conclusions: The prevalence of DA in iHD Pts is consistently high throughout the incident period, with a trend to be lower after the first 30 days. Increased hospitalization rates were significantly associated with DA in iHD Pts. In a small number of paired Pts screened for DA at both the initiation of and end of the incident period, the occurrence of DA was slightly reduced; while adjustment to HD and Pt selection might have contributed to this finding, early identification of DA and subsequent Pt care interventions could be a factor. Further investigation is needed to evaluate this hypothesis.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

SA-PO1042

Faith, Religion and Mental Health in a Sample of Brazilian Hemodialysis Patients Gildete Barreto Lopes,[1] Gentil Luz Junior,[1] Barbara De Alencar Costa,[1] Jean M. Monteiro,[1] Lucas Resende,[1] Sherman A. James,[2] Antonio Alberto Lopes.[1] *[1]Univ Federal da Bahia, Salvador, Brazil; [2]Duke Univ, Durham, NC.*

Background: Religiosity and faith/spirituality are associated with clinical presentation and outcomes in patients with chronic diseases. We assessed associations between self-declared faith and mental health in a sample of Brazilian patients on maintenance hemodialysis (MHD) from a population of predominantly African descent with a variety of religious orientations.

Methods: We conducted a cross-sectional analysis of data from 784 MHD patients (60.2% males, mean age = 48.9±18.1 yrs; 87% non-White) enrolled in the PROHEMO cohort in Salvador (Bahia), Brazil, during Jan 2010-Jan 2011. Patients were asked to state their religious affiliation (e.g., Catholic, Protestant, other) and how much their faith/spirituality has helped in their adjustment to chronic kidney disease: not helpful; helpful, but not so much; and very helpful. The SF-36 was used to measure mental health on a

scale of 0 (lowest) to 100 (highest). Linear regression was used to assess associations between faith/spirituality and mental health scores, controlling for religious affiliation and several other covariates.

Results: Patients' religious affiliations were: 55.8% Catholic, 33.8% Protestant, 2.0% Spiritism, 0.8% Candomblé, 0.1% Mormon, and 7.4% No Religion. 81.5% indicated that faith was "very helpful" in their adjustment to kidney disease. The "very helpful" response was endorsed by 93.9% of Protestants, 77.8% of Catholics, 69.6% of patients affiliated with other religions, and 56.9% of patients with no religious affiliation. In the statistical models adjusting for religious affiliation, comorbidities, and standard covariates, mental health score was approximately 10 points higher (P<0.001) in patients reporting faith to be "very helpful". In stratified analyses, Catholics endorsing the "very helpful" response scored 12.75 points higher than other Catholics (P<0.001) and Protestants endorsing this response scored 15.48 points higher than other Protestants (P=0.009).

Conclusions: This study provides additional evidence for an important role for spirituality/religious faith in the mental health of MHD patients.

SA-PO1043

Risk Factors Associated with Depressive Affect in Incident Hemodialysis Patients John W. Larkin,[1] Kathryn A. McDougall,[1] Rebecca L. Wingard,[1] Mikhail Artemyev,[2] Lin Ma,[1] Len A. Usvyat,[1] Eduardo K. Lacson,[1] Franklin W. Maddux.[1] *[1]Fresenius Medical Care North America (FMCNA); [2]Renal Research Inst.*

Background: Depression and depressive symptoms in hemodialysis (HD) patients (Pts) are common, yet not well characterized in the incident population. FMCNA is performing initiatives to identify depressive affect (DA) in incident HD (iHD) Pts. This study is an enlarged cohort built upon initial results that investigated the risk factors associated with DA in iHD Pts.

Methods: From Jan-Jul 2013, 577 Pts in their first month of HD at 108 randomly selected clinics were attempted to be contacted for telephonic DA screening using the Patient Health Questionnaire 2 (PHQ2). DA is defined as a PHQ2 score ≥3 out of a range of 0-6. PHQ2 is validated to determine presence of depressed mood and anhedonia during the previous two weeks. Clinical and lab parameters were captured during the 1st month of HD and associations were studied using logistic regression.

Results: In 577 attempts to screen iHD Pts for DA, 214 PHQ2 assessments were performed. Responders were: 39.2% females; mean age 67.2±15.4 years; 65.6% diabetic; and 49.0% utilized dialysis catheters at the end of 30 days. The prevalence of DA was 20.1% in Pts in the first month of HD. Male Pts were observed to have lower risk of DA (OR=0.30, p=0.0089). Higher levels of online dialytic clearance were associated with a tendency for lower risk of DA (OR=0.33, p=0.1172). Although higher log of creatinine (Cr) levels were observed to tend towards greater risk of DA in the original Pt cohort studied; this association was not observed in this analysis with a sufficiently increased sample size. No associations were identified between DA and age, diabetes, access type, ethnicity, race, body mass index, residual renal function, albumin, or interdialytic weight gain.

Conclusions: In this study, DA was observed to have a prevalence of 20.1% in Pts in the first month of dialysis. Males were identified to have a lower risk for DA. Higher Cr levels were not associated with DA, in contrast to previous findings in a smaller cohort. Lower online dialytic clearance may be linked to DA, but further investigation is needed to determine if higher dialytic clearance may ameliorate DA.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

SA-PO1044

Prevalence and Associated Factors of Chronic Itch in Hemodialysis Patients – Results of GEHIS, a Representative Cross Sectional Study in Germany Melanie Weiß,[1] Thomas Mettang,[2] Ulrich Tschulena,[3] Elke Weisshaar.[1] *[1]Dept of Clinical Social Medicine, Univ Hospital Heidelberg, Germany; [2]Clinic for Nephrology, German Clinic for Diagnostics, Germany; [3]Fresenius Medical Care Germany GmbH, Germany.*

Background: Chronic Itch (CI) is an often underestimated symptom in hemodialysis (HD) patients and appears to be a frequently neglected problem. Aiming to investigate the prevalence of CI and associated factors, a representative cross-sectional study was conducted in 25 randomly selected dialysis units in Germany.

Methods: Prevalence of CI was investigated in all participants as well as clinical data, dialysis parameters and the generic health related Quality of Life (HRQOL) (SF12). Characteristics of CI were assessed in HD patients with CI. A visual analogue scale (VAS) was used to assess severity of CI.

Results: 860 patients were included, 42.8% were female. Mean age was 67.2 years (SD: 13.4). Point prevalence of CI was 25.2% with a 95% confidence interval (95%-CI) of 22.4-28.1%. 27.2% (95% CI 24.1-30.3%) experienced CI within the past 12 months, lifetime prevalence was 35.2% (95%-CI 31.9-38.3%). 60.4% of the patients with CI had been suffering from CI up to 10 years, 11.8% since more than 10 years. Patients aged <70 years suffered significantly more frequently of CI than older patients. Though men were more frequently affected by CI (26.2 versus 23.9%), sex was not a significant association. The top 3 localizations were lower limbs (54.6%), back (52.0%) and scalp (43.2%). The shuntarm was affected in 26.6%. Worst severity of CI (VAS) was 6.5, mean severity 4.1. HD patients with CI showed significantly worse values both for the SF-12 physical and mental component summaries. Top 3 dialyzer membranes were polysulfone (59.5%), polyarylethersulfone (24.3%) and polyethersulfon (9.9%). Patients treated with polysulfon membrane showed significantly less CI, whilst polyarylethersulfon was significantly associated with a higher number of affected.

Conclusions: The 1st epidemiological study on the prevalence of CI in HD patients indicates CI to cause a high impairment in HRQOL in affected patients. Though suffering for years and leading to substantial burden, CI still seems to be a medical challenge in HD patients.

Funding: Pharmaceutical Company Support - Fresenius Medical Care Germany GmbH

SA-PO1045

Psychiatric Illness and Mortality in ESRD HD Patients Paul L. Kimmel,[1] Chyng-Wen Fwu,[2] Kevin C. Abbott,[3] Marva M. Moxey-Mims,[1] Paul W. Eggers.[1] *[1]Div of Kidney, Urologic and Hematologic Diseases, NIDDK, NIH, Bethesda, MD; [2]Social and Scientific Systems, Inc., Silver Spring, MD; [3]Walter Reed National Military Medical Center, Bethesda, MD.*

Background: Only limited epidemiologic data exist on psychiatric illness in ESRD patients, and none on pediatric patients.

Methods: We used 1991-2010 incident ESRD patients from USRDS to describe numbers of patients with psychiatric illness having at least 1 hospitalization within a year after starting dialysis. ICD9 codes were identified in Medicare billing data for depression and affective disorders, organic disorders and dementias, drug and alcohol-related disorders, schizophrenia and other psychoses, anxiety, personality and other disorders. We calculated 1-year survival rate from 1st day of hospitalization in adult patients with or without primary psychiatric diagnosis, using Kaplan-Meier analyses.

Results: 1,596 patients with ESRD before age 20 (of 3570, 45%) and 899,869 of 1,448,720 patients (62%) who started ESRD therapy after age 20 were hospitalized within a year after ESRD therapy. 24% of adults and 23% of children had an admission with a secondary psychiatric diagnosis. 2% of adults and 3% of children admitted had a primary psychiatric diagnosis. The most common primary pediatric psychiatric hospitalization was depression/affective disorder. The most common primary adult psychiatric hospitalization was organic disorder/dementia, closely followed by depression/affective disorder. Patients over 65 years were the most likely adults to have a primary psychiatric hospitalization. Although adults with a primary psychiatric hospitalization were younger than patients without (63.0±15.2 versus 67.3±13.4, P<0.001), their 1-year survival rate was worse (56% versus 63%, P<0.001).

Conclusions: Medicare billing data allows identification of comorbid psychiatric illness in ESRD. Primary psychiatric admissions comprise 2-3% of hospitalizations, with dementia and organic disorders in adults and depression and affective disorders in children the most common diagnoses. Greater mortality risk of patients with a psychiatric diagnosis may be related to the disease, poor adherence or concomitant medications.

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

SA-PO1046

HIV Infection in U.S. ESRD Patients Paul L. Kimmel,[1] Chyng-Wen Fwu,[2] Kevin C. Abbott,[3] Paul W. Eggers.[1] *[1]Div of Kidney, Urologic and Hematologic Diseases, NIDDK, NIH, Bethesda, MD; [2]Social and Scientific Systems, Inc., Silver Spring, MD; [3]Walter Reed National Military Medical Center, Bethesda, MD.*

Background: HIV infection has affected primarily African-American men in the ESRD program. We previously showed the incidence of HIV infection in ESRD patients plateaued by 2000, but the prevalence had increased monotonically.

Methods: We used data from 1995-2011 USRDS to describe recent trends of numbers of HIV-infected ESRD patients using two identification methods. Method 1: AIDS nephropathy listed as the primary cause of ESRD in CMS Form 2728. Method 2: Method 1 patients plus patients with at least one hospitalization and/or two outpatient encounters in a one-year observation period with an HIV/AIDS diagnosis (ICD9 codes 042, 043, 044, 07953, and V08) identified from Medicare billing data. Patients ascertained by Method 1 were followed from the date of 1st ESRD service. Patients ascertained by billing data were followed from the date of 1st hospitalization for HIV/AIDS diagnosis or the 2nd HIV/AIDS outpatient encounter after ESRD service, until death or December 31, 2011. Annual mortality rate was calculated for period prevalent HIV/AIDS ESRD patients.

Results: 13,255 and 31,116 patients were identified by Methods 1 and 2, respectively. The number of AIDS nephropathy patients recorded was relatively stable from 810 in 1995 to 813 in 2007, but decreased 34% to 535 in 2011. In contrast, the number of period prevalent HIV/AIDS patients (Method 2) increased linearly almost 3 fold from 3,208 in 1995 to 9,508 in 2011. The mean age of 41.3±10.1 (SD) in 1995 prevalent patients increased to 50.2±11.0 in 2011 prevalent patients. Annual mortality rate in period prevalent HIV/AIDS patients decreased from 547 per 1,000 patient years at risk in 1995 to 146 in 2011. Mortality rates in HIV/AIDS ESRD patients however were higher than general ESRD patients of comparable age.

Conclusions: Medicare billing data enhances identification of HIV infected ESRD patients. The incidence of HIV infection in the ESRD program has decreased, but prevalence has increased as patient mortality has decreased. These findings are likely a result of access to antiretroviral therapy.

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

SA-PO1047

Prospective Memory Is Impaired in Patients with End Stage Renal Disease (ESRD) Daniel J.W. Jones,[1] Laurie T. Butler,[1] John P. Harris,[1] Emma C. Vaux.[2] *[1]School of Psychology and Clinical Language Sciences, Univ of Reading, Reading, United Kingdom; [2]Renal, Royal Berkshire NHS Foundation Trust, Reading, United Kingdom.*

Background: Cognition is known to be impaired in End Stage Renal Disease (ESRD); numerous areas of retrospective memory have been explored in patients with ESRD, suggesting impairments in verbal, visual and episodic memory. Another type of memory,involved with the formation of future intentions and, at present, yet to be explored in ESRD, is that of prospective memory (PM). PM is considered to be essential in the successful completion of day-to-day activities and vital for independent living. It is particularly important for ESRD patients due to the strict diet, medication and treatment programmes thatpatients must follow. It is crucial to determine if patients are cognitively impaired in this area due to the potentially fatal consequences that could arise if strict schedules are not fulfilled.

Methods: In the present study, 18 ESRD patients (Kt/V>1.4) were compared with 18 age and education-matched controls on the Virtual Week task, a measure of PM that emulates typical tasks an individual is likely to carry out on a daily basis (e.g. taking medication). Tasks are irregular (non-repeated), but use prompts to cue an individual as to when to complete a specific task (event-based tasks). The exercise involves patients reading cards aloud whilst making their way around a board with a dice (one loop of a board simulates one day); each day involves remembering to complete a certain number of tasks which must be communicated to the experimenter. Each patient completed the Virtual Week immediately before a haemodialysis treatment session in a quiet room located on the renal ward.

Results: Patients remembered significantly fewer events than the control group, suggesting patients' PM to be impaired in comparison to typical-functioning adults.

Conclusions: This study provides the first piece of evidence to suggest a PM impairment in ESRD, highlighting potential implications that may arise in terms of patients' well-being and quality of life, especially relating to their functional independence whilst away from the dialysis centre. Further work is required to fully understand the extent of this PM impairment.

Funding: Government Support - Non-U.S.

SA-PO1048

Cognitive Decline in Maintenance Hemodialysis Patients David A. Drew, Daniel E. Weiner, Hocine Tighiouart, Urvi Ajay Shah, Amy Kantor, Tammy Scott, Mark J. Sarnak. *Tufts Medical Center, Boston, MA.*

Background: Cognitive impairment is common among dialysis patients, but little information exists on cognitive decline in hemodialysis patients.

Methods: In the Boston Dialysis Study, cognitive function was assessed at baseline in 314 patients using a comprehensive battery of cognitive tests. Using principal component analysis, individual test results were reduced into two domain scores, representing memory and executive function, which have a mean of zero and standard deviation of one. The cognitive battery was re-administered yearly whenever possible from 2005 until 2012. Linear mixed models and joint mixed models (accounting for death, transplant and drop-out) were used to explore the change in cognitive performance over time.

Results: The mean age of study participants at enrollment was 63 years, 53% were men, 22% were African American and 90% had at least a high school education. During median follow up of 1.8 years (25^{th}-75^{th}: 1.1-3.2), 196 had at least one follow up test (median number of tests = 3), 141 deaths occurred, and 40 underwent kidney transplantation. We observed statistically significant declines in multiple cognitive tests (Table). The greatest declines were seen in tests of executive function (-0.09 SD per year [-0.05, -0.12]). Similar results were seen when using joint mixed models accounting for the potential competing risks of death and kidney transplantation.

Table 1. Slope in Tests of Cognitive Function (Change Per Year)*

Test	MMSE	Recall Total	Recognition	Digit Symbol	Trails A (seconds)	Trails B (seconds)	PCA Memory	PCA Executive Function
Linear Mixed Model	**-0.43 (-0.28, -0.58)**	-0.20 (0.10, -0.49)	-0.11 (0.02,-0.24,)	**-0.70 (-0.14, -1.26)**	**6.4 (3.0, 9.7)**	**5.9 (2.5, 9.3)**	0.03 (0.07, -0.01)	**-0.09 (-0.05, -0.12)**
Joint Mixed Model	**-0.46 (-0.30, -0.62)**	-0.22 (0.08, -0.53)	-0.12 (0.01, -0.26)	**-0.73 (-0.14, -1.33)**	**7.6 (4.1, 11.1)**	**6.2 (2.7, 9.8)**	0.03 (0.07, -0.01)	**-0.09 (-0.05, -0.14)**

*Average slope (linear rate of change of the test per year). Linear mixed model includes time, random intercept and slope. For joint model, dropout was accounted for when estimating the slope. A negative coefficient indicates worsening performance for all tests except for Trails A&B, where a positive coefficient indicates worsening performance. Significant results are highlighted in bold.

Conclusions: Hemodialysis patients have significant declines in cognitive performance over time, particularly within tests of executive function. Future studies should evaluate predictors of decline and strategies to maintain cognitive function.

Funding: NIDDK Support, Private Foundation Support

SA-PO1049

Frailty and Cognitive Function in Incident Hemodialysis Patients Mara Mcadams-DeMarco,[1] Jingwen Tan,[1] Megan Salter,[1] Andrew Law,[1] Lucy A. Meoni,[1] Bernard G. Jaar,[1] Wen Hong Linda Kao,[1] Rulan S. Parekh,[2] Dorry L. Segev,[1] Stephen M. Sozio.[1] *[1]Johns Hopkins; [2]Univ of Toronto.*

Background: Cognitive impairment is an important outcome in hemodialysis (HD) patients. While frail older adults without kidney disease are at risk for poor cognitive function, it is unclear whether frail HD initiates are more likely to develop poor cognitive function.

Methods: At cohort entry 323 adult HD patients from PACE were classified into 3 groups (frail, intermediately frail, and nonfrail [Fried *et al*]). Global cognitive function (3MS) and executive function (Trail Making Tests A (TMTA) and B (TMTB)) were assessed at cohort entry and 1-year follow-up. All tests were performed on a non-dialysis day. Associations between frailty and cognitive function were evaluated in adjusted (for sex, age, race, BMI, education, depression and comorbidity at baseline) linear (3MS, TMTA) and tobit (TMTB) regression models.

Results: At baseline, mean age was 55 (SD=13), with 57% male, 73% AA, 22% prior CVD, and 18% prior stroke. Prevalence of frailty was 34.2% and 37.3% intermediate frailty; mean (SD) of 3MS was 89.6 (8.2), of TMTA was 55.6 (30) and of TMTB was 161 (83). Frailty was independently associated with worse cognitive function at baseline and 3MS at 1-year follow-up.

Table: Adjusted Association of Frailty at Hemodialysis Initiation and Cognitive Function

Baseline	Non-Frail	Intermediately Frail	Frail	P for Trend
3MS (n=319)	Ref	-0.97 (-2,89, 0.95)	**-3.01 (-5.00, -1.02)**	**0.003**
Trail Making Test A (n=323)	Ref	5.98 (-1.20, 13.17)	**12.62 (5.09, 20.14)**	**0.001**
Trail Making Test B (n=316)	Ref	19.78 (-2.49, 42.04)	**33.74 (10.34, 57.14)**	**0.005**
1-year follow-up				
3MS (n=155)	Ref	-1.54 (-4.15, 1.08)	**-4.02 (-6.70, -1.33)**	**0.003**
Trail Making Test A (n=174)	Ref	2.74 (-8.44, 13.92)	3.41 (-8.42, 15.23)	0.57
Trail Making Test B (n=170)	Ref	7.15 (-24.16, 38.46)	16.32 (-17.05, 49.68)	0.33

3MS: lower is worse
TMTA and TMTB: higher is worse
Bold is statistically significant (P<0.05)

Conclusions: In adults HD patients, frailty is associated with decrements in cognitive function, particularly global cognitive function. Frail HD patients should be screened for cognitive function and potentially targeted for interventions to improve cognitive function.

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SA-PO1050

Associations of Different Definitions of Frailty with Adverse Outcomes among Prevalent Hemodialysis Patients Kirsten L. Johansen, Lorien S. Dalrymple, Glenn M. Chertow, George A. Kaysen, John Kornak, Barbara A. Grimes, Cynthia Delgado. *USRDS Nutrition Special Studies Center, UCSF, San Francisco, CA.*

Background: Frailty is a state of impaired homeostatic reserve that is associated with vulnerability to adverse health outcomes. Many definitions have been applied among patients with ESRD, and their relative value has not been explored.

Methods: We defined frailty using criteria developed and validated by Fried and colleagues: three or more of weight loss, exhaustion, weakness, slow gait speed, and low physical activity. We compared this "performance-based" definition to one that substituted low self-reported physical function (score <75 on the Physical Function scale of the SF-36) for the performance criteria (weak grip and slow walking) among 709 participants in the USRDS ACTIVE/ADIPOSE cohort study. We used multivariable time-dependent Cox proportional hazards modeling (adjusting for demographics, comorbidity, and type of vascular access) to compare the degree to which each definition served as an independent predictor of mortality. We also evaluated a step-wise approach using both definitions, dividing patients into three groups: not frail, frail by self-report only, and frail by self-report and performance-based definitions.

Results: 33% of patients met the performance-based definition of frailty, and 54% met the self-report-based definition at baseline. Median follow-up was 1.7 (IQR 1.4-2.4) years, and there were 92 deaths. Both definitions of frailty predicted mortality (HR 1.74 [95% CI 1.07 – 2.80] for self-report and HR 2.20 [95% CI 1.42 – 3.40] for performance-based). Considering both definitions together, there was a graded association across the three categories that persisted after multivariable adjustment (HR 1.51 [0.82 – 2.80] for self-report only and 2.40 [1.38 – 4.00] for the group meeting both definitions) compared with the non-frail group.

Conclusions: Our findings suggest that a frailty definition based on self-report measures, most of which are already collected in dialysis facilities, was associated with higher mortality. Testing physical performance among those meeting the self-report-based definition could identify patients at even higher risk.

Funding: NIDDK Support

SA-PO1051

Feasibility of Clinical Studies in Haemodialysis Patients - Influence of Cognition and Frailty Osasuyi A. Iyasere,[1] Graham D. Cole,[2] Jordan Abdi,[2] David Andrew Williams,[2] Megan Griffith,[1] Neill D. Duncan.[1] *[1]Imperial College Renal and Transplant Centre, United Kingdom; [2]Imperial College, London.*

Background: Studies have reported better outcomes in patients who participate in clinical research, but the underlying risk factors are unclear. Cognitive impairment and frailty, common in haemodialysis (HD) patients, often preclude recruitment into clinical studies. The outcomes from such studies may therefore not be generalizable. This observational study evaluated the relationship between the 2 variables, research interest and short term clinical outcomes.

Methods: HD patients were evaluated from a single UK centre. Executive function and frailty were assessed using the CLOX 1 test and the Canadian Study of Health and Aging Frailty scale respectively. An independent assessor evaluated their willingness to take part in research. They were thus classified into three groups- interested in research (IR), not interested (NR) and could not consent due to language barrier or lack of capacity (CC). Hospitalisation and mortality data were collected over 6 months. Regression analysis was used to evaluate the relationship between cognition, frailty and outcomes.

Results: 102 patients (median age - 71 years) were assessed. 57.8%, 31.4% and 10.8% were in the IR, NR and CC groups respectively. There was no significant difference in the prevalence of executive dysfunction (47% IR, 40% NR, 83.3% CC,p=0.16) or frailty (46% IR, 47% NR, 73% CC,p=0.25) between the groups. Multinomial logistic regression analysis showed higher CLOX1 scores where associated with a lower risk of being in the CC group (OR-0.691, p=0.014).Age, sex, comorbidity, dialysis vintage and frailty were not influential. There was no difference in admission rates (p=0.54), length of stay (p=0.92) and time to first admission (p=0.56). There were no deaths in this cohort.

Conclusions: Frailty and executive function did not influence the willingness to participate in research but lower CLOX1 scores were associated with incapacity and language barrier. There were no differences in short term outcomes between those interested in research and those who were not.

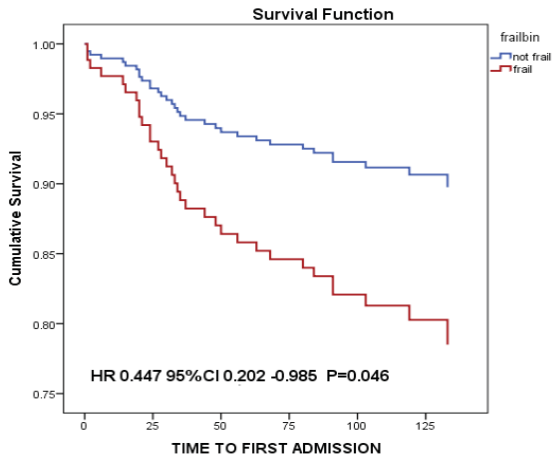
SA-PO1052

Executive Dysfunction and Frailty in a Dialysis Population - Correlates and Outcomes Osasuyi A. Iyasere,[1] Graham D. Cole,[2] Jordan Abdi,[2] David Andrew Williams,[2] Megan Griffith,[1] Neill D. Duncan.[1] *[1]Imperial College Renal and Transplant Centre, London, United Kingdom; [2]Imperial College, London, United Kingdom.*

Background: Cognitive impairment and frailty are common in the haemodialysis (HD) population. They have been linked to mortality and hospitalisation. Executive function is the most affected cognitive domain in HD patients. Little is known about the impact of executive dysfunction on clinical outcomes. We report the results of a 6 month observational study evaluating the risk factors and outcomes associated with executive dysfunction and frailty in HD patients.

Methods: Adult HD patients from a single UK centre, were assessed for executive dysfunction and frailty using the CLOX1 test and Canadian Study for Health and Aging Frailty scale, respectively. Clinical data were collected over 6 months from medical records. Logistic and Cox regression analysis were used to evaluate risk factors and outcomes. Survival and hospitalisation were the clinical outcomes evaluated.

Results: 213 HD patients were evaluated [median age(IQR) 69(22) years, 55% Asian]. 43% had executive dysfunction (CLOX 1 <10) and 53% were frail. Age (OR-1.032,p=0.037) and dialysis vintage (OR- 1.011, p=0.031) were associated with executive dysfunction. Admission rates were higher in the frail group (17%) than in the non frail group (7%, p=0.019). The frail group were less likely to be admission free in a Cox regression model adjusting for age, sex, ethnicity, dialysis vintage and comorbidities.



There was no difference in mortality between the groups but event rates were low (n=3). Executive dysfunction did not affect clinical outcomes.

Conclusions: Age and dialysis vintage were associated with executive dysfunction in HD patients. Frailty was associated with a higher risk of hospitalisation over 6 months, but not executive dysfunction. Event rates were too low to evaluate their influence on mortality.

SA-PO1053

Frail Elderly Patient Outcomes on Dialysis (FEPOD) - A Cross Sectional Comparison of Assisted Peritoneal Dialysis with Haemodialysis Osasuyi A. Iyasere, Lina Johansson, Edwina A. Brown. *Imperial College Renal and Transplant Centre, London, United Kingdom.*

Background: Assisted peritoneal dialysis (aPD) is an emerging alternative to haemodialysis (HD), the prevalent modality for older UK patients. But its use is limited by lack of patient reported outcome (PRO) data. FEPOD part 1, a multicentre cross-sectional study, compared quality of life and physical function on aPD and HD in older patients. It showed that frailty and not dialysis modality, determined outcomes. FEPOD part 2 is an on-going 2 year longitudinal study measuring trajectories of these outcomes .We report the baseline results from the combined group (FEPOD 1 and 2), to evaluate the consistency of our initial findings.

Methods: Patients >60 years old, on dialysis for >3 months were recruited from 23 centres. HD patients (needing hospital transport) were matched to aPD patients (needing assistance to perform PD) by age, sex, diabetes status, dialysis vintage, ethnicity and index of deprivation. Frailty was assessed using the Canadian Study of Health and Aging Frailty scale. PRO assessments included Hospital Anxiety and Depression Scale (HADS), SF-12, Palliative Outcomes Symptom scale (renal), Illness Intrusiveness Rating Scale (IIRS) and Barthel's score.Outcome predictors were determined by regression analysis with p values adjusted for multiplicity.

Results: We recruited 250 patients (129 aPD, median age 75years; 121 HD, median age 76years). Frailty was more common in aPD patients (51.9%) than in HD patients (42.6%). Univariate analysis showed possible depression (HADS >8) was more common in the aPD group (38.8%) compared to HD (23.8%, p=0.051), with no differences in other PROs. Frailty was the predominant predictor of PROs after multivariate analysis using a generalised linear model (SF 12 MCS p=0.0015, p =0.0008 for SF12 PCS, IIRS, Symptom score, HADS, Barthel score respectively). It was also associated with possible depression in logistic regression analysis (p < 0.001).Dialysis mode was not influential.

Conclusions: The results confirm that frailty determines outcomes in older and frail patients, not dialysis modality. Assisted PD should therefore be made more available, allowing patients to choose their optimal dialysis modality.

Funding: Pharmaceutical Company Support - BAXTER Healthcare, Private Foundation Support

SA-PO1054

Frail Elderly Patient Outcomes on Dialysis (FEPOD) Study: A Baseline Analysis of Secondary Outcomes Osasuyi A. Iyasere, Lina Johansson, Edwina A. Brown. *Imperial College Renal and Transplant Centre, London, United Kingdom.*

Background: Assisted peritoneal dialysis (aPD) is an emerging alternative to haemodialysis (HD) for frail older patients in the UK. A lack of outcome data has limited its use. FEPOD study part 1, a multicentre cross-sectional observational study showed a high falls and hospitalisation burden regardless of modality. FEPOD part 2 is the ongoing longitudinal study evaluating the trajectory of these outcomes. We report on the secondary outcomes of falls, hospitalisation and patient satisfaction for the combined cohort (FEPOD 1 and 2) at baseline.

Methods: Patients >60 years old, on dialysis for >3 months were recruited from 23 centres. HD patients (requiring hospital transport) were matched to aPD patients (requiring assistance to perform PD) by age, sex, diabetes status, dialysis vintage, ethnicity and Index of Deprivation. Frailty was assessed using the Canadian Study of Health and Aging Frailty scale. Patients were given a falls questionnaire, the Renal Treatment Satisfaction questionnaire (RTSQ) and a health use proforma to evaluate secondary outcomes.

Results: 250 (129aPD; 121HD) patients were recruited at baseline. 33 % of the entire cohort sustained at least one fall in the preceding 6 months (34% aPD, 32% aPD, p=0.717). 11 patients had subsequent fracture (14% of falls). 46% of all patients were admitted to hospital in the preceding 3 months with 42% being dialysis related admissions. There was no significant difference in admission rates between aPD and HD. The median RTSQ score was higher in the aPD group (55) than in the HD group (51, p= 0.012). Dialysis modality was the only predictor of RTSQ in multivariate analysis (p=0.029).But over 90% of the study group, regardless of modality, would recommend their treatment to others.

Conclusions: These results confirm the high falls, fracture and hospitalisation rates in older patients on dialysis, regardless of modality. The morbidity burden associated with dialysis in this group should be highlighted when discussing renal replacement therapy. Treatment satisfaction was higher in the aPD group, but this did not influence recommendation rates.

Funding: Pharmaceutical Company Support - Baxter Healthcare, Private Foundation Support

SA-PO1055

Frailty and Mortality in Dialysis: Evaluation of a Clinical Frailty Scale Talal A. Alfaadhel, Karthik K. Tennankore. *Dept of Medicine, Div of Nephrology, Dalhousie Univ, Halifax, NS, Canada.*

Background: Frailty is associated with poor outcomes for dialysis patients; however, the severity of frailty is not captured by previous measurement tools. The purpose of this study was to assess if frailty (as defined by a clinical frailty scale) was associated with mortality among incident dialysis patients.

Methods: We conducted a prospective cohort study of incident, chronic dialysis patients between January 2009 and June 2013, (last follow-up December 2013). Based on overall clinical impression, the Canadian Society of Health and Aging Clinical Frailty Scale (CFS) score, was determined for patients at the start of dialysis by their primary nephrologist. The CFS allocates a single point to different states of frailty (1:very fit, 2: well, 3: managing well, 4: vulnerable, 5: mildly frail, 6: moderately frail, 7: severely frail or terminally ill) based on physical, functional and cognitive status. The primary outcome was time to death. Patients were censored at the time of transplantation.

Results: The cohort consisted of 390 patients with completed CFS scores (mean age 63±15 years). The majority were Caucasian (89%) and male (67%) and 30% had end stage renal disease (ESRD) due to diabetic nephropathy. The median Charlson Comorbidity Index (CCI) score was 4 (IQR 3-6) and the median CFS score was 4 (IQR 2-5). There were 96 deaths over 750 patient-years at risk. Using an adjusted multivariable Cox survival analysis

the HR associated with each one-point increase in the CFS was 1.24 (95% confidence interval 1.06-1.46), p=0.009.

Model	Number exposed / Events	HR	95% CI	P
Unadjusted	390 / 96	1.42	1.25-1.61	<0.001
Model 1[a]	390 / 96	1.32	1.15-1.52	<0.001
Model 2[b]	390 / 96	1.30	1.12-1.51	<0.001
Model 3[c]	390 / 96	1.24	1.06-1.46	0.009

a: adjusted for age, race and sex
b: adjusted for variables in a, CCI ≥ 5 and diabetic ESRD
c: adjusted for variables in b, GFR, albumin, BMI and dialysis modality

Conclusions: Frailty as defined by the CFS is associated with mortality in dialysis. The CFS is easy to implement in routine clinical practice and may be a novel prognostic tool for incident dialysis patients.

SA-PO1056

A Comparison of Stroke Incidence between Patients Initiating Hemodialysis and Peritoneal Dialysis in Korea Dong-Ryeol Ryu,[1] Hyunwook Kim,[2] Hee Sung Ko,[1] Shina Lee,[1] Jung-Hwa Ryu,[1] Mina Yu,[1] Seung-Jung Kim,[1] Duk-Hee Kang,[1] Kyu Bok Choi.[1] *[1]Dept of Internal Medicine, School of Medicine, Ewha Womans Univ, Seoul, Korea; [2]Dept of Internal Medicine, Wonkwang Univ College of Medicine Sanbon Hospital, Gunpo, Korea.*

Background: We aimed to compare the stroke incidence between incident hemodialysis (HD) patients and peritoneal dialysis (PD) patients using the Korean Health Insurance Review and Assessment Service database, which enabled us to perform a population-based complete survey.

Methods: We initially identified all of the incident dialysis patients who had started HD or PD and whose age was 18 years or older between January 1, 2005 and December 31, 2008 in Korea. Among them, the patients who were dead or developed any kind of strokes within 90 days from the date of dialysis were excluded; the remaining eligible 30,828 patients were included in the final analyses. Patients who underwent kidney transplantation, who were dead during follow-up period, or who survived until December 31, 2009 were censored.

Results: During the median follow-up period of 24.7 months, incidence rates of total stroke (P=0.0014), hemorrhagic stroke (P=0.0017), and ischemic stroke (P=0.0341) were significant higher in HD patients than those in PD patients by log-rank test. In addition, after adjustments with baseline characteristics in multivariate Cox analysis, hazard ratio of hemorrhagic stroke in HD patients was significantly higher than that in PD patients (HR, 1.217; 95% CI, 1.032-1.434; P=0.0194), while there were no significant differences in hazard ratios of total stroke and ischemic stroke between HD and PD patients.

Conclusions: The risk of hemorrhagic stroke in Korean HD patients was increased compared to PD patients. The possible causes should be evaluated and a countermeasure will be needed.

SA-PO1057

Serum Sodium, Cognition and Mortality in Hemodialysis (HD) Patients Urvi Ajay Shah, Amy Kantor, Hocine Tighiouart, Daniel E. Weiner, Tammy Scott, David A. Drew, Mark J. Sarnak. *Dept of Nephrology, Tufts Medical Center, Boston, MA.*

Background: Cognitive impairment is highly prevalent in HD patients. Hyponatremia has been associated with cognitive impairment in the general population and may be associated with mortality in HD patients.

Methods: The mean of 3 serum sodium (Na) measurements, approximating the timing of cognitive testing, was ascertained in 323 patients. All patients underwent a battery of previously validated cognitive tests. Principal factor analysis (PFA) was used to derive two summary factors representing memory and executive function. Multivariable linear models were used to assess the relation of Na to each PFA score in cross sectional analysis, and multivariable cox models were used to evaluate the association of Na with all-cause mortality.

Results: Mean (SD) age was 62.3 (16.5) years, 46% were women and 22% were blacks. Median (interquartile range) Na was 139.3 (137.3-140.7) mEq/L. Median length of follow up was 2.1 years and there were 154 deaths. There was no significant association between serum Na and cognitive function (p>0.1 for all analyses). Lower Na was however independently associated with higher mortality.

Association of serum sodium with all-cause mortality

	Unadjusted HR (95% CI)	Demographic adjusted* HR (95% CI)	Risk factor adjusted** HR (95% CI)	Adjusted for † HR (95% CI)
Sodium Linear (per 1 SD = 2.6 mEq/L ↑)	0.81 (0.68-0.96)	0.79 (0.67-0.93)	0.77 (0.65-0.92)	0.80 (0.67-0.96)
Quartiles median (p25, p75)				
Q1 136.3 (135.0, 136.7)	1.57 (1.00-2.45)	1.61 (1.03-2.52)	1.65 (1.03-2.64)	1.59 (0.99-2.58)
Q2 138.3 (138.0, 139.3)	0.83 (0.52-1.32)	1.00 (0.63-1.60)	0.83 (0.51-1.36)	0.77 (0.47-1.26)
Q3 140.3 (140.0, 140.7)	1.22 (0.76-1.95)	1.02 (0.63-1.64)	1.04 (0.64-1.68)	1.03 (0.64-1.67)
Q4 142.0 (141.3, 142.7)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)

*Adjusted for age, gender and race
**further adjusted for dialysis vintage, cause of ESRD (diabetes vs rest), dialysis access (fistula vs rest), PVD/CAD/stroke/HF and smoking
†further adjusted for Kt/V, albumin and CRP

Conclusions: Serum Na is not associated with cognitive impairment in HD patients, but lower Na values, even within the normal range, are associated with higher mortality.

Funding: NIDDK Support

SA-PO1058

Rates of Infection in Patients Treated with Dialysis Seth Wright,[1] Mark J. Sarnak,[1] Klemens B. Meyer,[1] David Richard Snydman,[1] Vladimir Ladik,[2] Hocine Tighiouart,[1] Doug Johnson,[2] Daniel E. Weiner.[1] *[1]Tufts Medical Center, Boston; [2]Dialysis Clinic, Inc., Nashville.*

Background: Infections in patients treated with dialysis are associated with substantial morbidity and mortality, but their incidence rates, patterns and potential risk factors are poorly described.

Methods: Infections (defined as a positive blood culture or peritonitis; ≥5 days of in-center antibiotics or an out-of-center oral, IV, or intraperitoneal (IP) antibiotic order; or hospitalization or death with an infection-related code) were evaluated in all patients treated by a large U.S. dialysis provider (DCI) from Oct 2009-Dec 2012. Dialysis modality and vascular access were defined by those in use at the time of an event. Cox regression was used to evaluate risk factors for infection.

Results: A total of 27,394 patients contributed 42,798 pt-years of data. In the study period, 30% of patients died with 13% of the deaths listing infection as a primary or secondary cause. The most common infections causing hospitalization were pneumonia (30%), bacteremia/sepsis (17%), and cellulitis/soft tissue (15%).

Outcome	Catheter	Fistula	Graft	Peritoneal
Antibiotic course	13.07 (12.85-13.28)	5.84 (5.75-6.08)	6.77 (6.61-6.94)	8.49 (7.74-9.28)
Parenteral only	7.92 (7.75-8.09)	2.10 (2.05-2.16)	2.74 (2.64-2.85)	6.23 (6.00-6.46)
Hospitalization-infection	3.44 (3.33-3.55)	1.58 (1.53-1.63)	1.77 (1.69-1.86)	2.38 (2.24-2.53)
Positive blood culture	5.90 (5.75-6.10)	0.66 (0.63-0.69)	0.96 (0.90-1.03)	
Peritonitis				3.23 (3.06-3.39)
Death-infection	0.50 (0.46-0.54)	0.18 (0.17-0.20)	0.25 (0.22-0.28)	0.22 (0.18-0.27)

Rates of infectious outcomes by modality per 100 pt-months (95% CI). For each outcome, rates differed between dialysis modalities (p<0.001). Rates were similar when limited to incident patients only (44%). In multivariable analysis, risk factors for infection (as a composite of the above outcomes; antibiotics limited to IV/IP) included: HD catheter [HR 2.83 (2.73-2.94)] or PD [HR 2.09 (1.98-2.19)] versus fistula; ESRD from diabetes [HR 1.23 (1.19-1.28)] versus hypertension; and white race [HR 1.16 (1.12-1.20)] versus black.

Conclusions: These detailed, modality-specific data provide background information on the rate and type of fatal and non-fatal infections in dialysis patients. Pneumonias and cellulitis made up nearly half of the infectious admissions. Exploring risk factors associated with these specific infections may be of interest.

Funding: NIDDK Support, Pharmaceutical Company Support - Dialysis Clinic, Inc.

SA-PO1059

Efficacy of Interferon Therapy in HCV Positive Patients on Haemodialysis Fizza Kazmi,[1] Hafiz I. Ahmad,[1] Syed Rizwan Bokhari,[1] Arif Asif.[2] *[1]Dept of Nephrology, Allama Iqbal Medical College, Lahore, Pakistan; [2]Div of Nephrology and Hypertension, Albany Medical College, Albany, NY.*

Background: In Pakistan, the prevalence of hepatitis C is very high in dialysis patients than the general population, 38% weighted average (23.7-68%). Pegylated interferon in combination with ribavirin is recommended treatment for most patients with chronic hepatitis C infection without renal dysfunction. Because ribavirin and pegylated interferon are not removed adequately by hemodialysis, only standard non-pegylated interferon alfa (IFNa) is used in ESRD patients. However, limited data are available regarding the efficacy of this approach. We studied the IFNa alone therapy in our chronic hepatitis C patients receiving maintenance hemodialysis.

Methods: All 22 Hepatitis C positive patients undergoing maintenance hemodialysis in our center were included in this study. PCR revealed that 14 patients were positive

for hepatitis C viral RNA. Of these, two were excluded on clinical grounds and 12 were considered suitable for treatment. IFNa in a dose of 3 million i.u. thrice weekly was given to these patients.

Results: Of the 12 patients who received treatment, 2 were dropped out due to treatment side effects mainly pancytopenia and thrombocytopenia, and 3 developed decompensated liver disease during therapy and hence their treatment was stopped. Seven patients completed the treatment. Four patients became PCR negative as an early response and completed 6 months therapy. Three patients received an extended course of IFNa for 8 months and became PCR negative.

Conclusions: Interferon therapy in hepatitis C positive hemodialysis patients is effective. However, the number of patients who are unfit for therapy and the dropout rate due to intercurent illnesses and side effects is quite high.

SA-PO1060

Combination Pegylated Interferon Alpha-2A and Ribavirin in the Treatment of Hepatitis C in End Stage Renal Disease Patients - A Single Centre Experience Sai Krishna Duraisingham,[1] Richard Marley,[2] Raj C. Thuraisingham,[1] Mark Blunden.[1] *[1]Dept of Nephrology, Barts Health NHS Trust, London, United Kingdom; [2]Hepatology, Barts Health NHS Trust, United Kingdom.*

Background: Hepatitis C is a leading cause of liver disease in patients with ESRD with associated morbidity and mortality. Outcomes of renal transplantation in such patients are improved if patients are rendered RNA negative pre-transplant. Historically, treatment with pegylated Interferon (IFN) and Ribavirin (Rib) has been discouraged due to concerns regarding reduced clearance on haemodialysis and the side effect profile. The primary purpose of this study was to assess the success of IFN/Rib in achieving sustained viral remission when treatment was supervised in a joint Renal/Hepatology clinic. Successful renal transplantation was examined as a secondary outcome.

Methods: 39 Patients with HCV were identified from our hospital database. Demographic, dialysis modality, virology, treatment regime, side effect profile and transplantation data were recorded. All patients were followed-up by a hepatitis nurse specialist and supervised by the joint liver-renal clinic. Dose of ribavarin was titrated up to maximal tolerated dose and full blood count was measured weekly. EPO/iron treatment was modified accordingly.

Results: 22 dialysis patients were identified as having been treated, 17 were not treated due to not needing treatment (eg not transplantable or pre-dialysis) or unable to undergo treatment (compliance issues or patient choice). Sustained viral response was achieved in 14 patients which when broken down by genotype were comparable to general population results for interferon and ribavarin regimes. 8 of these patients underwent successful transplantation as did 1 patient who had failed treatment, after a biopsy showed no fibrosis.

Conclusions: With close monitoring ribavirin is safe to use in the ESRD population and allows comparable sustained viral response rates to non-ESRD population. At present newer agents are yet to be fully studied in this group and as yet their promise is mainly in genotype 1 hepatitis C. These agents hold promise of interferon free treatments and will allow post transplant treatment too in a selected sub-group.

SA-PO1061

Positive HBV and HCV Status Exhibit Pronounced Iron Metabolism Disturbances and Higher Phosphate Levels Jolanta Malyszko,[1] Andrzej Milkowski,[2] Teresa Rydzynska.[2] *[1]2 Dept Nephrology, Medical Univ, Bialystok, Poland; [2]Fresenius Medical Care, Poznan, Poland.*

Background: Iron status in patients on dialysis is disturbed. Iron metabolism is regulated by number of factors including inflammation, hepcidin, liver function, diet etc. Viral infection i.e. hepatitis, still present in dialyzed patients may affect liver function. In animal model hepatectomy is related to hypophosphatemia. The aim of the study was to find out whether iron status, phosphate levels and hemoglobin are affected by presence of viral hepatitis in hemodialyzed patients.

Methods: Biochemical parameters and viral status were assessed by standard laboratory methods.

Results: The study was performed on 5058 patients dialyzed in Fresenius Medical Care facilities in Poland. 55 patients were hepatitis B antigen positive, 150 had antibodies against HCV and 6 patients were positive for both viruses. We analysed serum haemoglobin, TSAT, ferritin, albumin, phosphate in prevalent patients. HCV and HBV positive patients were the youngest among studied groups (mean age 42), HBV (mean age 52) and HCV positive patients (mean age 55) were also significantly younger than negative patients (mean age 66 years). HBV and HCV positive patients have significantly higher ferritin (median 883 ng/mL, TSAT (mean 47%), albumin (4.11 g/dL) and phosphate (5.87 mg/dL) relative to negative patients and HBV or HCV positive subjects. HCV positive patients have higher significantly phosphate and haemoglobin levels than negative patients (5.20±1.58 mg/dL versus 4.71±1.56 mg/dL, $p<0.01$ and 11.05±1.45 g/dL versus 10.80±1.91 g/dL, $p<0.05$, respectively), while HBV positive patients had higher albumin than negative patients 4.1±0.5 g/dL versus 3.9±0.4 g/dL, $p<0.05$). TSAT were similar in patients with HBV, HCV and negative for both viruses.

Conclusions: Prevalence of viral hepatitis is very low (below 4%) in total HD population studied, mainly found in younger patients. However, patients with HCV and HBV infection showed a very different pattern of iron metabolism and phosphate levels. Hyperferritinemia in this population may be due to previous blood transfusions and/or enhanced chronic inflammatory process in the liver.

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SA-PO1062

Cystatin C and Risk of Sepsis T. Clark Powell,[4] Orlando M. Gutierrez,[1] Russell Griffin,[2] John P. Donnelly,[4] Monika M. Safford,[3] Henry E. Wang.[4] *[1]Div of Nephrology, Univ of Alabama School of Medicine; [2]Dept of Epidemiology, Univ of Alabama School of Medicine; [3]Div of Preventive Medicine, Univ of Alabama School of Medicine; [4]Dept of Emergency Medicine, Univ of Alabama School of Medicine, Birmingham, AL.*

Background: Chronic kidney disease (CKD) and systemic inflammation are risk factors for sepsis. While often viewed as a marker of chronic kidney disease, Cystatin-C (Cyst-C) may also reflect systemic inflammation. We sought to determine the association between baseline Cyst-C and long-term rates of community-acquired sepsis, and to determine if this relationship is influenced by traditional markers of CKD (eGFR, ACR) and inflammation (hsCRP).

Methods: We used 10 years of data on 30,239 adults ≥45 years old from the REasons for Geographic and Racial Differences in Stroke (REGARDS) cohort, identifying sepsis events through hospital records. Using multivariable Cox regression, we examined the independent association of increased Cyst-C with long-term rates of sepsis, adjusting for sociodemographics, health behaviors, and chronic medical conditions. We also evaluated the influence of eGFR, ACR and hsCRP upon the association between Cyst-C and sepsis rates.

Results: Among 28,095 individuals, mean Cyst-C were: sepsis 1.24±0.72 mg/dL, non-sepsis 1.03±0.45 mg/dL. Over a median follow-up of 6.7 years (IQR 5.2-8.2), 1,405 participants experienced a sepsis hospitalization. Cyst-C≥1.11 mg/dL was independently associated with increased sepsis rates (adjusted HR 1.79; 95% CI: 1.59-2.03). The addition of eGFR<60 mg/min/1.73m^2 (HR 1.53; 95% CI: 1.32-1.77), ACR≥30 mg/dL (1.47; 1.29-1.67), and hsCRP>3.0mg/dL (1.55; 1.38-1.74) only partially influenced the association between Cyst-C≥1.11 mg/dL and sepsis (1.52; 1.32-1.74).

Conclusions: Cyst-C is associated with long-term rates of sepsis, independent of eGFR, ACR or hsCRP. Cyst-C may provide a strategy for long-term sepsis risk prediction and prevention.

SA-PO1063

Senior Patients on Hemodialysis - Are They an Economic Burden? Teresa M. Jeronimo, Anabela M. Guedes, Ana Pocinho Pimentel, André Fragoso, Ana Cabrita, Ana Paula Silva, Viriato J.V. Santos, Idalecio Bernardo, Pedro Neves. *CHA.*

Background: With a longer life expectancy older patients face dialysis. A trend for guarantying only conservative treatment has been advocated, not only due to health care related issues, but also due to financial contingencies. Do these patients portent a higher economic burden? The aim of this study was to evaluate and compare the hemodialysis(HD) related expenditure of a senior population during the first year of treatment.

Methods: This study considered incident HD patients at our Centre, between 2008 and 2011. Serial laboratory data were collected at dialysis initiation and monthly until 12 months of follow-up. Costs were calculated based on the Portuguese capitation system. T-test for variable comparison and Kaplan-Meier for survival were used in the statistical analysis.

Results: A total of 201 patients(77 females,124 males), with mean age of 65.0 years, previous nephrology follow-up time of 32.5 months and one-year survival on HD were included. Group I(n=61), which included patients with 75 years or older, started HD earlier(eGFR 9.7 versus 8.0 mL/min/1.73m2,p=0.003) and presented initial lower PTH levels(525.6 versus 796.1 pg/mL,p=0.011). There were no differences on hemoglobin, phosphorus or albumin levels. This group presented lower global expenditure at 6 and 12 months evaluations(3849.3 versus 4791.6 euros/patient, p=0.045 and 6511.2 versus 8230.0 euros/patient,p=0.031), respectively. These differences result mainly from the mineral metabolism related medication(818.7 versus 1511.4 euros/patient/year,p=0.005), for an equal control of PTH and lower phosphorus levels(3.9 versus 4.1 mg/dL,p=0.007). Anemia related costs are similar and both groups evolved with similar hemoglobin. Lower albumin levels were detected in the group I(3.8 versus 4.1 g/dL,p=0.001). Despite survival being higher in group II(n=124) which included patients younger than 75 years old(log rank=3.9,p=0.049), there were no differences in hospitalization rate or duration.

Conclusions: In conclusion, older patients start dialysis sooner, with lower PTH levels. Through the first year on HD exhibit lower medication cost(mainly due to mineral bone disease related medication) and equal hospital related expenses and morbidity.

SA-PO1064

Diagnostic Utility of Gait Speed for Predicting Hemodialysis Patient Outcomes Nancy G. Kutner,[1,2] Rebecca H. Zhang,[1,3] Yijian Huang.[1,3] *[1]USRDS Rehabilitation/QoL Special Studies Center, Emory Univ, Atlanta, GA; [2]Rehabilitation Medicine, Emory Univ, Atlanta, GA; [3]Biostatistics/Bioinformatics, Emory Univ, Atlanta, GA.*

Background: Impaired mobility has personal and social burdens, and increased mortality risk with gait speed <0.8 meters/second (m/s) has been shown among individuals with chronic kidney disease. In clinical care for older persons, routinely measuring usual walk speed is increasingly advocated, and dismobility defined by gait speed of <0.6 m/s, <0.8 m/s, and <1.0 m/s has been shown to predict a variety of adverse outcomes. Information is needed about the predictive utility of gait speed for dialysis patient outcomes.

Methods: In the USRDS special study ACTIVE-ADIPOSE, we assessed baseline gait speed 2009-2011 in a multi-center cohort of 752 prevalent patients aged 20-92 undergoing maintenance hemodialysis (HD). Baseline gait speed and mortality over a median follow-up of 703 days were investigated in a Cox proportional hazards model, and associations with

hospitalization, need for ADL assistance, and SF-36 Physical Functioning (PF) score at a 12-month follow-up were estimated in multivariable logistic or linear regression models, adjusting for age, sex, and baseline value of the outcome.

Results: Slow gait speed prevalence increases with age and is higher among women, but the difference between HD patients and the general population in prevalence of slow gait speed is much greater at younger ages. Compared with study participants whose walk speed was 0.6 m/s or faster, participants with walk speed <0.6 m/s (n=94) were almost twice as likely to die, and those who were unable to perform the walk test (n=83) were over five times more likely to die. Risks for hospitalization, ADL difficulty, and lower SF-36 PF score after 12 months were greater for those with walk speed 0.6 to <1.0 m/s compared with participants whose walk speed was 1.0 m/s or faster.

Conclusions: Walking places demands on the heart, lungs, circulatory, nervous, and musculoskeletal systems, and gait speed can provide a validated, easily measured and informative marker of dialysis patients' health. The predictive utility of gait speed warrants continued study in this population.

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SA-PO1065

Right TraC Is Associated with Improved Patient Experience of Care during Care Transitions Rebecca L. Wingard,[1] Kathryn A. McDougall,[1] Billie Axley,[1] Andrew D. Howard,[2] Janice B. Sitzlar,[1] Sharon Deluca,[1] John W. Larkin,[1] Len A. Usvyat,[1] Franklin W. Maddux.[1] *[1]Fresenius Medical Care North America (FMCNA), Waltham, MA; [2]Metropolitan Nephrology Associates, Clinton, MD.*

Background: Patient-centered care requires strategies to improve patient (pt) activation. The ongoing Right TraC™ Care Transitions Program includes such strategies aimed at reducing hospital readmissions. This is an interim analysis of Phase I data of the Right TraC Patient Experience Survey.

Methods: As of Apr 2013, 28 HD clinics in the U.S. participate in Right TraC, Phase I. Staff use hospital admit and post-hospitalization checklists, which include enhanced exchange of pt information, rapid anemia management, nutrition supplements, target weight assessment, medication review, pt counseling for attending appointments, "red flag" symptoms of illness and readmission prevention strategies for the same diagnosis. Teach-back technique is used to review why pts were hospitalized and their role after discharge. Clinic secretaries were trained to administer the survey on the third outpatient treatment after discharge. We compared results from Q2 2013 to Q1 2014 using ANOVA.

Results: Pts responded to each question with "yes", "somewhat", or "no." These responses were converted to a scale of 0-100% and summarized by calendar quarter (yes=100%, somewhat=50%, no-0%). Approximately 350 surveys were completed per quarter with an average response rate of 30%.

Question	Q2, 2013	Q3, 2013	Q4, 2013	Q1, 2014	Change from Q2 2013 to Q1 2014	p-value for difference between Q2 2013 and Q1 2014
Can you explain why in hospital?	88.2	95.2	96.3	97.0	9.95%	<.0001
Did nurse teach about new meds in a way you could understand?	81.3	89.0	89.8	91.8	12.90%	<.0001
Know what your meds do for you?	74.3	82.1	83.3	82.4	10.91%	0.005
Can act on Red Flag symptoms?	76.8	84.9	85.6	75.0	-2.29%	NS
Can follow discharge instructions?	85.8	96.4	95.1	95.3	10.98%	<.0001
Can ask questions of healthcare team?	91.6	99.3	98.9	98.9	7.88%	<.0001
Feel well-cared for by staff after hospital stay?	97.5	98.5	98.9	99.2	1.83%	0.056

Conclusions: Ratings of pt experience improved during Right TraC, particularly for medications and discharge instructions. Ratings varied by quarter for Red Flags. Further modification of interventions is needed for continued improvement.

SA-PO1066

Successful Multidisciplinary Quality Initiative to Preserve Arm Veins in CKD/ESRD Populations at a Tertiary Care Center Gauri Bhutani,[1] Mireille El Ters,[4] Sami Safadi,[1] Amy Mahon,[3] Joe L. Klunder,[2] Sandra J. Taler,[1] Amy W. Williams,[1] Andrew Stockland,[2] Marie C. Hogan.[1] *[1]Nephrology, Mayo Clinic; [2]Radiology, Mayo Clinic; [3]Nursing, Mayo Clinic, Rochester, MN; [4]Nephrology, Univ of Kansas, Kansas City, KS.*

Background: Peripherally inserted central venous catheters (PICCs) are associated with higher rates of venous thrombosis and lower rates of functioning AV fistulas (AVFs). Small bore tunnelled central venous catheters (STCCs) may be a safer alternative to help improve rates of successful AVFs.

Methods: Interventions (2011 - 2014): 1. Electronic alert discouraging provider orders for PICC placement if eGFR<30ml/min/1.73m^2 and providing rationale for PICC avoidance 2. Interventional Radiology collaboration to facilitate/ substitute STCCs instead of PICCs. 3. Education of providers (didactics) and CKD patients (onsite patient education, brochures, posters and CKD education classes). We compared number of cases with eGFR<30 who received PICCs before [Jan 2010 (Period 1)], during [Mar - Apr 2013 (Period 2)] and after interventions [Feb-Mar 2014 (Period 3)]. We also evaluated the trends in STCC use in CKD/ESRD. Fisher's exact test was used for statistical analysis.

Results: PICCs placed in ESRD patients declined in Periods (Pds) 2 and 3 compared to Pd 1 ($^{\times,\alpha}p<0.01$). Fewer PICCs were also placed in patients with eGFR<30 (Not ESRD) in Pd 3 ($^{\#}p = 0.015$). This reflects the reduced PICC placement in CKD 4-5 ($^{\#}p <0.01$). We did not detect any change in PICC placement rates in AKI patients.

PICC (by group)	Period 1 (Jan 2010) n = 275	Period 2 (Mar - Apr 2013) n = 580	Period 3 (Feb-Mar 2014) n = 580
ESRD	11 (4%) $^{\alpha,\times}$	**6 (1.03%)** $^{\times}$	**1 (0.01%)** $^{\alpha}$
eGFR <30 ml/min/1.73m^2 (Not ESRD)	35 (12.7%)*	62 (10.7%)	**43 (7.4%)***
-CKD 4 and 5	17 (6.2%)$^{\#}$	36 (6.2%)	**9 (1.5%)**$^{\#}$
-Acute kidney injury (AKI)	18 (6.5%)	26 (4.5%)	34 (5.8%)

STCC use at our center has increased 17 fold (2010-14). Prevalent ESRD cases among all STCCs placed increased from 22.6% in 2010-2012 to 42% by August 2013 (p = 0.04).

Conclusions: A targeted multidisciplinary strategy reduced PICC use in CKD and ESRD patients at our center. STCC use increased, especially in ESRD patients. Future studies will determine if these efforts translate to improved AVF rates.

SA-PO1067

A Multidisciplinary Approach Is Successful in Improving Vascular Access Outcomes in an Underserved Dialysis Population in a Large Indigent-Care Public Hospital Arshad Ali, Ravi R. Rajani, Susan M. Shafii, Vandana Niyyar. *Emory Univ School of Medicine, Atlanta, GA.*

Background: The three basic kinds of vascular access for hemodialysis(HD) are an arteriovenous fistula (AVF), AV graft (AVG),and a central venous catheter (CVC).National Kidney Foundation Kidney Disease Outcomes Quality Initiative recommends AVF and AVG for vascular access and avoidance of CVC if possible.The Fistula First Catheter Last recommends an AVF prevalence rate of 68%. However, 80% of ESRD patients initiate HD with a CVC and 52% dialyze with a CVC at >90 days.In a retrospective review, we studied patient,physician and socio-economic factors as they relate to a specific underserved and uninsured ESRD population at Grady Memorial Hospital–"the undocumented immigrants" who do not have legal access to the U.S. medical system.

Methods: We conducted a retrospective review of the medical records from 11/01/2010 to 04/7/2014 of this patient population. A multipronged approach led by the renal fellows included detailed counseling,emphasizing compliance and involvement of their families. Social workers and interpreters were extensively involved to overcome the socio-economic,cultural and language barriers.There were concerted efforts made for placement of AV access during intermittent admissions for HD.

Results: There were a total of 35 patients in this cohort on 04/07/2014.The average age was 47.8 +/-13.9 years,with a male to female ratio of 1.2:1.They were predominantly Hispanic(74%),African(23%), and Asian(3%).The etiology of the renal failure was HTN(51%), Diabetes(31%),glomerulonephritis(6%),HIVAN(3%) and others(9%).All were initiated on HD with CVC.We found a 77% conversion rate of CVC to AV access,of which 33% were radiocephalic AVF,37% brachiocephalic AVF,26% brachiobasilic AVF and 4% AVG.The average time of conversion from CVC to AV access was 6.3 months +/- 255 days with 48% placed within 90 days of HD initiation. Primary AVF failure rate was 3.7%.

Conclusions: This retrospective review indicates that it is possible to achieve optimal dialysis vascular access in an underserved and uninsured patient population by using a multidisciplinary approach.Renal physicians should take the lead and assume the role of team leaders.

SA-PO1068

Can an Active Intervention Increase Vascular Access Outcome? Marco Oliveira Mendes,[1] David Navarro,[1] Ana Azevedo,[2] Patricia Veigas,[1] Ana Carina Ferreira,[2] Patrícia Matias,[1] Tiago Amaral,[2] Bruno Costa Pinto,[1] Cristina Jorge,[1] Fernanda Gomes,[1] Célia Gil,[1] Manuel A. Ferreira.[1] *[1]Nefrologia, Nephrocare de Vila Franca de Xira, Vila Franca de Xira, Lisboa, Portugal; [2]Nefrologia, Dialverca, Alverca, Lisboa, Portugal.*

Background: Programs of monitoring vascular access (VA), followed by angiographic and/or surgical intervention, have been performed in order to improve VA outcome. Our goal is to assess the impact of an immediate response from a specialized vascular access center (VAC) on the VA of patients (pts) from a large hemodialysis center.

Methods: A retrospective analysis of the VA patency and VA interventions in 2 periods (20 months each), before and after the implementation of an immediate response from VAC, was performed. Both populations were equivalent. 218 pts were included, with mean age ±SD) 67,2±15,4 years, 50% female, 37,2% diabetics, with mean HD time of 61,5±58,4 months. Monthly determinations of VA flow (Qa) using the BTM-Blood Temperature Monitor-Fresenius Medical Care, were performed. Predefined indications for angiography were: A-V fistula (AVF) Qa < 400 ml/min; PTFE graft Qa < 600 ml/min or a Qa drop > 50% in 2 consecutive months; significant high venous pressure (VP), edema of VA limb or thrombosis of the VA.

Results:

	Pre CAV	POS CAV	p value
VA in use			
Central venous catheter (CVC) (%)	28.58	25.7	NS
AVF (%)	42.86	48.2	NS
AVF patency (months)	58,26±59,69	56,57±59,15	NS
PTFE patency (months)	27,50±25,09	34,63±25,56	P <0.01
Kt/v	1.57 (±0.27)	1.94 (±0.35)	P <0.01
Intervention			
Angioplasty without stent (%)	13.47	24.76	NS
Surgical review (%)	18.37	26.98	NS
Outcome			
Failure due to thrombosis (%)	19.42	8.97	NS
Hospitalization related to the VA (%)	11.02	3.17	P <0.01

Conclusions: Introduction of VAC represented a decrease of thrombosis rate (50%), hospitalizations related to the AV (71%), use of CVC and a significant increase in Kt/V. The role of VAC appears to be particularly important in preserving the PTFE patency. In our opinion, these major clinical advantages justify the increased number of VA interventions and the costs associated.

SA-PO1069

Timing of Dialysis Access Creation: A Single Centre Experience Post-Initiating Dialysis Early and Late (IDEAL) Study and following the Creation of a Dedicated Vascular Access Nurse Position Jonathan Eng Ho Ling, Gail Theresa Read, Lisa Shelverton, Elizabeth Nguyen, Richard Yu, Geoffrey S. Kirkland. *Nephrology, Royal Hobart Hospital, Hobart, Tasmania, Australia.*

Background: The Initiating Dialysis Early and Late(IDEAL) study, published in 2010, showed that starting dialysis later in patients with well-controlled chronic kidney disease(CKD) was not associated with a significantly worse mortality rate. In our dialysis service following this publication, we observed an increased time from arteriovenous fistula(AVF) creation to dialysis initiation, decreased primary access failure rates and a lower estimated glomerular filtration rate(eGFR) on starting dialysis.

Methods: A Vascular Access Nurse position was created in 2010 to improve access to surgical AVF creation. Standard practice in our unit is an eGFR of 15 or less is used as a trigger for AVF creation. We analysed data on patients with CKD 5 progressing to dialysis during 2008 to 2013. Data was routinely collected during this period and included dates and eGFR at the time of AVF creation and commencement of dialysis, eGFRs at 1,2 and 5 years before initiation of dialysis, cause of renal disease and co-morbidities. Patients who required emergency initiation of dialysis were excluded from this review.

Results: Results show an increase in average time to dialysis from eGFR of 15 from 5.5 months to 10.8 months and a decrease in the mean eGFR on commencement of dialysis was 9 ml/min to 5 ml/min across the 5 year period. The mean eGFR at AVF creation was 13 in 2008 and 16 in 2013. Despite the wider gap between AVF creation and dialysis commencement post-IDEAL, AVF failure rates decreased from 10 episodes in 2010 to 4 episodes in 2013.

Conclusions: In summary we have shown a significant change in our unit's pattern of access creation and initiation of dialysis resulting in an average patient gaining an extra 5 dialysis-free months with no increased risk of AVF failure.

SA-PO1070

Clinical Utility of Far-Infrared Therapy to Improve Access Blood Flow and Pain Control in Hemodialysis Patients Soo Jeong Choi, Moo Yong Park, Jin Kuk Kim, Seung Duk Hwang. *Internal Medicine, Soonchunhyang Univ Bucheon Hospital, Bucheon, Gyunggi-do, Korea.*

Background: A well-functioning vascular access and minimal needling pain are important goals for achieving adequate dialysis and improving the quality of life for hemodialysis (HD) patients. Far-infrared (FIR) therapy has been used for various medical conditions, including chronic pain control and the treatment of some cardiovascular diseases. Recently, Taiwan researchers reported that FIR therapy improved endothelial function and increased access blood flow and patency in HD patients.The aim of this study is to evaluate the effects of FIR therapy on access blood flow and needling pain in Korean HD patients.

Methods: This prospective clinical trial was conducted at Soonchunhyang University's Bucheon Hospital in Korea between June 2013 and January 2014. A total of 31 HD patients were enrolled in the study. FIR therapy was administered for 40 min during HD three times per week and continued for 3 months. The access blood flow was measured by the ultrasound dilution method, while pain was measured by the numeric rating scale (NRS) at baseline, then once per month.

Results: One patient was transferred to another facility and six patients stopped FIR therapy due to increased body temperature and discomfort, though none of them experienced a burn episode.While hemostasis improved after the first month of FIR therapy, itching sensation of patients did not change. Pain score (4.7 ± 0.5 to 1.5 ± 0.3, $p < 0.001$) and anxiety score (4.4 ± 0.6 to 1.6 ± 0.3, $p < 0.001$) decreased after three months. However, the access blood flow did not increase significantly after a single HD session (878.1 ± 108.1 ml/min to 923.9 ± 102.3 ml/min, $p = 0.317$) nor after 3 months (827.9 ± 139.3 ml/min to 924.2 ± 153.7 ml/min, $p = 0.160$). Patients with an initial access flow under 500mL/min experienced a great increase in flow after FIR therapy than those with an initial flow over 500ml/min (p=0.010, Figure 1).

Conclusions: This study shows that FIR is safe and can improve access flow and needling pain in hemodialysis patients. Future studies with large populations and long-term follow-up are needed to further investigate these findings.

SA-PO1071

Reducing Adverse Effects of Arteriovenous Fistula Cannulation with Doppler Ultrasound Fabiana Dias Carneiro, Ana C.M.S. Ramos, Bruna Gomes Barbeiro, Ewerton Soares Dias, Erika B. Rangel, Maria C.C. Andreoli, Adriano Luiz Ammirati, Nadia K. Guimaraes, Thais Nemoto Matsui, Bento C. Santos. *Centro de Diálise Einstein, Hospital Israelita Albert Einstein, Sao Paulo, Brazil.*

Background: Vascular access function and patency are essential for optimal management of hemodialysis (HD) patients. Failure to detect access dysfunction has consequences on morbidity and mortality. To note, only a small number of studies has been performed prospectively to assess the impact of vascular access surveillance on outcome. Objective: We aimed to report the use of Doppler ultrasound (DU) for guiding arteriovenous fistula (AVF) cannulation in HD patients.

Methods: AVF cannulations were guided by DU and clinical adverse effects related to cannulations were assessed (hematoma and more than two cannulations attempts) from 2004-2014. Surveillance DU was repeated when physical examination detected abnormalities. One-way Anova was used and $P<0.05$ was considered significant.

Results: Demographic data of 157 HD patients comprised 75.2% male patients, with average age of 65,9± 15,6 yo, and 39% diabetic. HD treatment was delivered 3 times per week, average duration of 4h, blood flow of 300 to 350 ml/min, dialysate flow of 500 ml/min, and polysulphone dialyzers were used. Average time of follow-up was 32±28 months (range, 2 to 120 months). Median time from AVF confection to first use was 32 days (range, 20-730 days). DU-guided AVF cannulation reduced the occurrence of AVF hematoma from 2.63 to 1.71 episodes/patient.year (P=0.054) and the requirement for more than two cannulations from 4 to 1.78 episodes/patient.year (P=0.0014).

Conclusions: Doppler ultrasound is a helpful tool in association with physical assessment to minimize adverse effects of AVF cannulation, as well as to detect vascular accesses at risk and to track access complication rates. Furthermore, DU may be implemented to maximize access longevity in HD patients.

SA-PO1072

Real-Time Imaging of Vascular Access to Optimize Cannulation Practice and Education: Role of the Access Procedure Station Rosa M. Marticorena,[1] Latha Kumar,[1] Gurpreet Dhillon,[1] Jo-Ann Tria,[1] Darya Zevart, Sanjeev Sirpal,[1] Ian Smith,[2] Sumeet Suneja,[1] Xi Shan,[1] Sahar Kajbaf,[1] Abdul Aziz Walele,[1] Jasdip S. Sachdeva,[1] Hitesh K. Mehta,[1] Bajinder S. Reen,[1] Sandra M. Donnelly.[1] *[1]Medicine/Nephrology, William Osler Health System, Brampton, ON; [2]Surgery/Vascular Surgery, William Osler Health System, Brampton, ON.*

Background: The Access Procedure Station (APS), a novel healthcare delivery system, was established to optimize the management of vascular access (VA) in CKD patients on hemodialysis. The APS consolidates the management of new accesses, complicated accesses or accesses difficult to cannulate (defined as the use of more than 2 needles for hemodialysis at least once per week), in a controlled environment.

Methods: Eighty-five patients were enrolled in APS; 35 females and 50 males. Patients received a complete evaluation with bedside ultrasound and real-time ultrasound-guided needle insertions by nurses with specialized training in ultrasound use. Admission criteria for APS include, but are not limited to: assessment and cannulation of new and/or complicated accesses, cannulation post procedures, troubleshooting during HD (needle reposition or re-cannulation), assisting with CVC insertions and removals, local thrombolysis and other access procedures.

Results: A total of 516 access procedures were performed in 85 patients admitted in APS over a period of 5 weeks. Of the 85 patients, 25 had a history of multiple needle insertions at least once per week over the 5 weeks prior to admissions. Compared to 125 events of multiple needle insertions reported in the medical chart prior to APS enrollment, during the first 5 weeks of operation of the APS, there were 7 events (*p=0.0001*[/italic][/italic]); 3 cases of a second needle insertion at one of the cannulation sites, 2 episodes of blood extravasation - intramural bleed- during the first needle insertion, and 2 events of flow limiting severe venous spasm after cannulation.

Conclusions: The implementation of the APS has improved access care in our Unit, reduced morbidity, and has streamlined the delivery of cost effective patient care. The APS has also served as an educational opportunity for advanced training in imaging in the specialty of Vascular Access for Nursing Staff.

SA-PO1073

Patency and Complications of Arteriovenous Fistulation in Upper Arm of Hemodialysis Patients with Multiple Comorbidities Xueqin Bian, Hong Ye. *Center for Kidney Disease, Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

Background: In clinical settings, arteriovenous fistula, also called lifeline of dialysis patients, is the preferred access in hemodialysis; however, incidence of fistula dysfunction is extremely high in maintenance hemodialysis patients with multiple comorbidities.

Fistulation in upper arm was recommended in several recent investigations to reduce the occurrence of fistula dysfunction of these patients. Here, the patency of upper arm fistula and complication after fistulation in upper arm of maintenance hemodialysis patients with multiple comorbidities were analysis in this study.

Methods: From January 2009 to December 2012, maintenance hemodialysis patients with multiple comorbidities underwent upper arm fistulation in our hospital were enrolled in this study. Patency of fistula was examined at 6 and 12 months after fistulation by ultrasonography. Complications, including bleeding, infection, steal syndrome, aneurysm, thrombosis and central venous stenosis were evaluated at 12 months after fistulation. Cox proportional hazard models were used for statistic analysis.

Results: A total of 195 times upper arm fistulation were performed in 183 hemodialysis patients with multiple comorbidities. The primary patencies were approximately 90% and 86% at 6 and 12 months, respectively. The cumulative patencies were 95% and 89% at 6 and 12 months, respectively. The incidence of all complications was 13.1% (24 of 183) at 12 month, among which bleeding was 41.6% (10 of 24), infection was 4.1% (1 of 24), steal syndrome was 8.2% (2 of 24), aneurysm was 8.2% (2 of 24), thrombosis was 16.4% (4 of 24), central venous stenosis was 20.8% (5 of 24). In all kinds of comorbidities, diabetes (HR: 2.73; 95% CI: 1.19 to 6.32) and fistulation history (HR: 1.45; 95% CI: 1.06 to 1.97) were responsible for reduction of primary patency.

Conclusions: Arteriovenous fistulation in upper arm of hemodialysis patients with multiple comorbidities had excellent patency and few complications. Diabetes and fistulation history were detrimental factors for patency of upper arm fistula.

SA-PO1074

Pre-Operative Examination for Arteriovenous Access in Hemodialysis: An Insight into Clinical Practice Guidelines Deirdre B. Cassidy,[1] Aurangzaib Khawaja,[2] Javaad Ayub,[3] Nicholas Inston,[2] Eric G. Lancelot,[3] Shona Matthew,[1] John Graeme Houston.[1] *[1]Univ of Dundee, United Kingdom; [2]Queen Elizabeth Hospital, Birmingham, United Kingdom; [3]Guerbet, Paris, France.*

Background: Arteriovenous fistulas (AVF) are the preferred vascular access (VA) for hemodialysis but have a high incidence of primary failure, particularly at the radiocephalic site. Recent studies have shown routine pre-operative vascular ultrasound (US) in addition to clinical assessment improves the AVF patency rates. This study aims to assess the content and consistency of international clinical practice guidelines in terms of pre-operative recommendations.

Methods: Guideline databases and nephrology societies' websites were used to search for VA practice guidelines that included recommendations on pre-operative assessment (up to April 2014). Key guidelines (NDOQI, ERBP, CARI, CNS) were identified and compared using the Appraisal of Guidelines for Research Evaluation (AGREE) II instrument by three observers (DC, AK, JA). All scores were calculated as scaled domain score and between group differences calculated using analysis of variance. Inter rater reliability was assessed by intraclass correlation coefficient (ICC).

Results: Overall, guideline recommendations for pre-operative assessment include physical examination (4/4) and US for planning (3/4). Venography was recommended in suspected cases of central venous stenosis (2/4). The application of magnetic resonance angiography (MRA) and computed tomographic angiography (CTA) have been mentioned in suggestion to care (2/4). Guideline methodology rigour was variable between the guidelines ($p<0.02$). This may be due to different search strategies and different methods of updating guidelines. Agreement was found between observers in overall ranking of the guidelines and inter rater reliability was found to be significant (ICC, $p<0.005$).

Conclusions: Physical examination, US mapping and venography are the main methods available for pre-operative assessment. Further work will include a systematic review of recent preoperative imaging studies with MRA or CTA in order to see if these modalities can offer additional information as compared to the conventional methods recommended in the guidelines.

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SA-PO1075

Time to Get Off the Button-Hole Bandwagon? - A Single Center Experience of Staph Aureus Infection and Vascular Access Type Luxme Nadarajah, Neil Ashman, Ravindra Rajakariar, Mark Blunden. *Renal Unit, Royal London Hospital, London, United Kingdom.*

Background: There has been increasing interest /use in the "button - hole " method of A-V fistula cannulation, with apparent benefits of less pain and bleeding. Concerns have been raised about infectious complications. After a number of serious infections in our institution we reviewed staph Aureus bacteriemia cases in haemodiallysis over a 2 year period and access type, as well as other complications asscociated with access such as bleeding.

Methods: Using our database we analysed all postive blood cultures and bleeding episodes on haemodialysis program over a two year period - accounting for approx 1900 patient years of haemodialysis. All positive cultures for staph Aureus were analysed as to access, complications of the sepsis, inpatient days and co-morbities. Major bleeding was defined as requiring admission and transfusion.

Results: 11 cases of staph aureas sepsis were felt due to staph infection introduced via button hole AVF, 40 cases due to tunnel line, one in a PTFE graft and none due to rope ladder cannulation. 3 button patients had endocarditis and 1 had a mycotic aoritc aneurysm. Mortality was 18%. In a seperate review of bleeding from vascular access over a 8 year period, there were no major bleeding episodes assocaited with button hole, with most bleeding coming from infected PTFE grafts or anuersymal (and often stenotic proximally) native AVFs. Average length of stay was 13.5 days with button hole asscoiated sepsis compared to 14 days for line sepsis.

Conclusions: Button hole cannulation was apparently assoctiated with increased severe infection but not other major complications related to access such as bleeding. Patient selection may have been an issue with infections more likley to happen in diabetics (5 patients) and immuno-compromised patients (2 patients were HIV positive). Button-hole can probably be safely used on a select subgroup but not all patients especially with underlying conditions associated with increased skin infections.

SA-PO1076

Haemodialysis Arteriovenous Autogenous and Prosthetic Angioaccess in 80 Years and Older Patients: Single Centre Study Dinith Prasanna Galabada,[1] Maggi Steele,[1] Mohamed Morsy,[2] Abbas Ghazanfar,[2] Rebecca Suckling,[1] Atul Bagul.[2] *[1]South West Thames Renal Unit, St. Helier Hospital, Carshalton, United Kingdom; [2]Vascular Unit, St. George's Hospital, London, United Kingdom.*

Background: The number of patients over 80 years with end stage kidney disease requiring chronic haemodialysis has been steadily increasing. Autogenous angioaccess is widely considered as the preferred vascular access due to its high patency and low complication rates. Aim of this study was to analyze the primary patency,assisted primary patency and secondary patency rates of autogenous and prosthetic angioaccess in the elderly population over 80 years of age in the South West Thames Renal unit.

Methods: This is a retrosepective observational study, assessing all angioaccess procedures performed between January 2006 and March 2013. Demographic data,Cause of renal failure,Type of angioaccess and Co-morbidities were collected. Primary patency, assisted primary patency and secondary patency rates at 6,12,24 and 36 months were calculated using Kaplan-Meier Survival method.

Results: There were 277 Autogenous (No=263;95%) and prosthetic(brachioaxillary bypass graft) angioaccesses. Among fistulae,50% were radiocephalic,41% brachiocephalics. Mean age at creation of access was 83.7 years (Range 80-95 yrs), 68.8% were males, 55% had a Davies Co-morbidity score of 2 and 75% were de-novo (first angioaccess). More than half of the angioaccess procedures were pre-emptive. primary patency rates were 71.5%,62.5%,46% and 43% at 6,12,24 and 36 month respectively. Assisted primary patency rates were 76%,68.5%,55% and 48.5% at same time points. Secondary patency rates were 78.5%,70%,56.2% and 51% at the respective time points post creation. Functional secondary patency rates were 83.8%,79%,66% and 58% for the same durations. Primary failure of angioaccess rate was 25%,30% died with a functioning access. Thrombosis rate was 0.123 per patient- year. There was no statistical difference between autogenous and prosthetic angioaccess patencies.

Conclusions: Elderly patients aged 80 or over with end stage renal failure should be considered for angioaccess as first line for haemodialysis. Autogenous angioaccess is recommended as a priority in the elderly patients over 80 years.

SA-PO1077

The Economic Burden of Initiating Dialysis with the Wrong Foot – A Matter of Vascular Access Anabela M. Guedes, Teresa M. Jeronimo, Ana Pocinho Pimentel, André Fragoso, Ana Cabrita, Ana Paula Silva, Viriato J.V. Santos, Idalecio Bernardo, Pedro Neves. *Serviço Nefrologia, Hospital de Faro, Portugal.*

Background: Vascular access is primordial in timely preparation for hemodialysis (HD) start for a number of reasons, namely dialysis efficacy, morbidity and mortality. Nonetheless economic reasons should also be ascertained. Is anticipated construction of a definitive blood access cost beneficial? The aim of this study was to compare different initial vascular access related expenditure during the first year on dialysis.

Methods: This study considered incident haemodialysis patients at our Centre, between 2008 and 2011. Serial laboratory data were collected at baseline and monthly until 12 months of follow-up. Costs were calculated based on the Portuguese capitation system. T-test for variable comparison and Kaplan-Meier for survival were used in the statistical analysis.

Results: A total of 176 patients (70 females, 106 males), with mean age of 67.4 years and previous nephrology follow-up time of 43.0 months were included. Group I (n=38 patiens) started HD by catheter and presented higher C reactive protein (54.9 versus 24.5 mg/L, p=0.016), lower albumin (3.5 versus 3.8 g/dL, p= 0.013) and lower eGFR (8.1 versus 9.9 mL/min/1.73m2, p= 0.014) by the time of HD initiation. Both groups had similar demographic and other considered biochemical characteristics. They evolved without significant analytical differences. Group II (n=138), which started HD with a functioning arteriovenous fistula or graft, exhibited lower cumulative cost after 6 months (3920.2 versus 5369.3 euros/patient, p=0.010), mainly due to lower expenditure with anemia related medication (3152.9 versus 4529.1 euros/patient, p=0.007). These disparities fade after 12 months on HD. Higher hospitalization rate and duration was observed in group I (13.9 versus 6.0 days/patient, p=0.002). Survival was higher in group II (log rank = 4.5, p=0.035).

Conclusions: In conclusion, patients presenting with definitive blood access start dialysis sooner, with better albumin levels, probably reflecting the lower grade of inflammation, measured by CRP. These features result in lower medication cost (mainly anemia related medication) and lower hospital related expenses and morbidity.

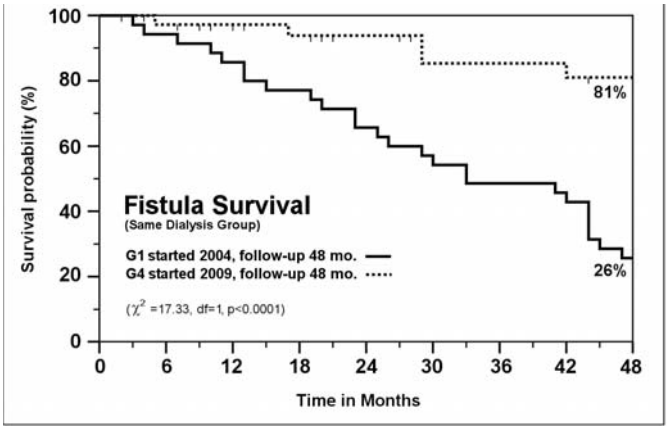
SA-PO1078

A New Method for Vascular Access Life Table Analysis Chaim Charytan,[1] Stephen R. Ash,[2] Anatole Besarab,[3] Farhanah Yousaf,[1] Stanley Frinak,[4] Joan E. Arslanian,[1] Kim Hirschman.[5] *[1]Nephrology, NYH Queens, New York, NY; [2]Nephrology, Indiana U., Lafayette, IN; [3]Nephrology, UCSF, San Francisco, CA; [4]Nephrology, Henry Ford HS, Detroit, MI; [5]QA, Vasc-Alert, Lafayette, IN.*

Background: Dialysis centers are required to have an access surveillance program (ASP), but do ASPs improve outcomes? One way to measure efficacy is to assess the effect of ASPs on access longevity. We describe a new method of survival analyses for AV accesses based on dialysis treatment data (DTD). Rules for extracting life table data from DTD are given. Access survival analysis is then used to assess efficacy of the ASP by comparing the 1st and 5th year of ASP use.

Methods: NY Hospital Queens initiated an ASP in 2003/4 using the Vasc-Alert device to identify patients at risk for access complications. Our method of analysis used first and last dates of access use in DTD to approximate access initial use and loss, confirmed by review of patient records. Fistula (AVF) data in two time periods was analyzed and compared: AVFs first used in the 1st yr of the ASP use, (group G1, 6/1/04 to 5/31/05, N=35), and AVFs first used five yrs later, (group G4, 1/1/09 to 12/31/09, N=42).

Results: Results of Kaplan-Meier analysis of DTD survival data are shown in the figure below. The number of patients in G1 ranged from 35 to 9 at 48 mo, with survival=26%. For G4, patients ranged from 42 to 18 at 48 mo, with survival=81%. There was a significant difference in the survival curves ($p < 0.0001$).



Conclusions: Results show that extracting access survival data from DTD is an excellent approximation of access survival. Kaplan-Meier analysis of DTD can be used as a tool to evaluate the success of an ASP or to compare different ASPs. The data in the figure indicate that an ASP in concert with a vascular access management program can extend the patency of fistulas.

SA-PO1079

Vascular Access and Patient Values: Fistula Refusal May Be Rational and Non-Modifiable Damien Ashby, Azara Janmohamed, Jeremy Crane, Neill D. Duncan, Lina Johansson. *Imperial College Kidney and Transplant Centre, Imperial College, London, United Kingdom.*

Background: Arteriovenous fistulae are associated with improved survival, but some patients refuse to have a fistula. These patients may be better understood by analysis of the decision process, which is influenced by (1) circumstance: factors specific to the decision, including the patient's understanding of it; and (2) disposition: stable patient factors, such as personality or values, which are independent of the decision being made, and largely non-modifiable. The role of values in access preference has not been studied.

Methods: Healthcare values were determined using structured interviews in prevalent haemodialysis patients, with subjects unaware of the vascular access focus of the study. Values were determined by weighted analysis of discrete trade-off decisions relating to a set of non-renal healthcare scenarios, designed to test the following value attributes: (1) survival, (2) pain, (3) appearance, and (4) treatment burden.

Results: Thirty patients were interviewed, with one incomplete, leaving 29 (aged 19–76, 67% male) with completed interviews for analysis. Fourteen patients had a fistula, and 15 were patients with a tunnelled catheter who had refused fistula formation. Compared to the fistula group, patients from the catheter group gave pain a higher priority (mean score 19.1 versus 13.2, p=0.025) and tended to give survival a lower priority (mean score 12.1 versus 16.2, p=0.127). These differences led to altered attribute rankings: pain was the lowest ranked attribute in the fistula group, whereas for the catheter group, survival had the lowest rank. Values were predictive of access grouping: of patients who prioritised pain over survival, 71% were from the catheter group, as opposed to only 33% of those who prioritised survival over pain (p=0.046).

Conclusions: Healthcare values have a significant effect on vascular access decisions, with patients who prioritise pain over survival being more likely to refuse fistula formation. Fistula refusal of this type may be a non-modifiable rational choice, arising from and consistent with a distinct set of healthcare values. The proportion of fistula refusal which is of this type is unknown.

Funding: Clinical Revenue Support

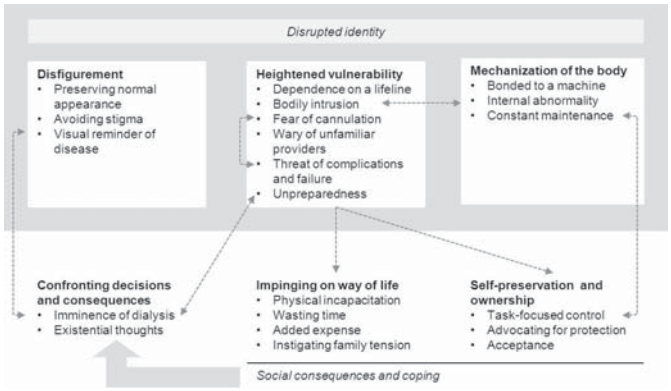
SA-PO1080

Anything but a Simple Surgical Procedure: Patients' Perspectives of Vascular Access for Hemodialysis Jordan R. Casey,[1,2] Camilla Sara Hanson,[1,2] Wolfgang C. Winkelmayer,[3] Jonathan C. Craig,[1,2] Suetonia Palmer,[4] Giovanni F.M. Strippoli,[1] Allison Tong.[1,2] *[1]School of Public Health, Univ of Sydney; [2]Centre for Kidney Research, The Children's Hospital at Westmead; [3]Div of Nephrology, Stanford Univ; [4]Univ of Otago.*

Background: Delayed creation of vascular access may be partly due to patient refusal and is associated with adverse outcomes. Concerns about vascular access are prevailing treatment-related stressors for patients on hemodialysis (HD). We aimed to describe patients' perspectives on vascular access initiation and maintenance.

Methods: In this systematic review, electronic databases were searched to December 2013. Thematic synthesis was used to analyze the findings.

Results: From 46 studies (n=1034 patients) we identified six themes: heightened vulnerability (bodily intrusion, fear of cannulation, threat of complications and failure, unpreparedness, dependence on a lifeline, wary of unfamiliar providers); disfigurement (preserving normal appearance, visual reminder of disease, avoiding stigma); mechanization of the body (bonded to a machine, internal abnormality, constant maintenance); impinging on way of life (physical incapacitation, instigating family tension, wasting time, added expense); self-preservation and ownership (task-focused control, advocating for protection, acceptance); and confronting decisions and consequences (imminence of dialysis, existential thoughts).



Conclusions: Initiation of vascular access signifies kidney failure and imminent dialysis which is emotionally confronting. Patients strive to preserve their vascular access for survival; but describe it as an agonizing reminder of their body's failings and "abnormality" of being attached to a machine disrupting their identity and lifestyle. Timely counseling and building patients' trust in healthcare providers may improve the quality of dialysis and outcomes for patients with CKD requiring HD.

SA-PO1081

Avoiding the Need for Dialysis Lines: Outcomes of Pre-Emptive AVF Formation – A Single Centre Retrospective Analysis Raja Mohammed Kaja Kamal, Nelomi Anandagoda, Ankan Mittal, Maggi Steele, Bhrigu Raj Sood, David Makanjuola. *South West Thames Renal Unit, St. Heliers Univ Hospital NHS Trust, Carshalton, Surrey, United Kingdom.*

Background: The native arteriovenous fistula (AVF) remains the preferred choice, with better outcomes. UK guidelines recommend creation of dialysis access atleast 6 months before the anticipated start of dialysis and that 65% of all incident HD patients should commence dialysis with an AVF.

Methods: This single centre retrospective study analyses outcomes of patient undergoing pre-emptive AVF/graft surgery between Jan 2006 and Oct 2012. Data were collected from our renal and pathology department database. Outcomes were analysed for successful use, need for surgical or radiological intervention prior to use of the AVF/graft and the impact of eGFR at the time of AVF/graft creation.

Results: Number of pre-emptive AVF/graft procedures (N) = 741

Outcome			N (%ge)
AVF successfully used for 1st HD without intervention			**186 (25%)**
AVF successfully used for 1st HD after intervention			**62 (8%)**
AVF became usable after intervention, but after starting dialysis with alternative access			**117 (24%)**
AVF never used (reasons given below)			**310 (42%)**
Excluded due to transfer			**4 (1%)**
Reasons for not using AVF			
AVF/Graft failure			191 (26%)
Patient died before starting dialysis			46 (6%)
Not yet started dialysis			49(6.8%)
Transplanted before starting dialysis			12(1.6%)
Change in modality (Conservative management)			12(1.6%)
eGFR (ml/min) at time of creation of AVF			
	Mean	**Median**	**Range**
AVF used successfully with or without intervention	12.1	12	3-48
AVF never used	14.6	14	4-60

Conclusions: This large single centre study shows that only 33% of AVF/graft were used successfully for first HD, further 24% used, had to start dialysis with a line. 42% were not used and over half of these were due to AVF/graft failure. The eGFR at time of creation of the AVF/graft did not affect outcome.Timely planning is necessary as only 25% of **AVF/graft** were usable at start of dialysis without any intervention, which is comparable to other published literature.The groups who died or switched to conservative management needs to be looked at in greater detail, as it suggests that they may have had an unnecessary procedure performed.

SA-PO1082

Catheter Removal Is Associated with Reduced Blood Stream Infections, All-Cause Hospital Admissions and Myocardial Infarctions

Kathryn Taylor,[3] Jerry W. Jackson,[1] Qingqing Xiao,[2] Julia I. Brennan,[1] John W. Larkin,[1] Len A. Usvyat,[1] Norma J. Ofsthun,[1] Kevin Chan,[1] Jeffrey L. Hymes,[1] Franklin W. Maddux.[1] *[1]Fresenius Medical Care North America, Waltham, MA; [2]Renal Research Inst, New York, NY; [3]Johns Hopkins Medicine Armstrong Inst for Patient Safety and Quality, Baltimore, MD.*

Background: It is known that hemodialysis (HD) dependent end stage renal disease patients with central venous catheter (CVC) vascular accesses are at an increased risk for infection, hospitalization, and death compared to patients with arteriovenous fistulas (AVFs) or arteriovenous grafts (AVGs). Additionally, CVCs have been suggested to contribute to a state of chronic inflammation in HD patients. We investigated the short-term impact on several key outcomes for conversion from a CVC to an AVF/AVG vascular access in HD patients.

Methods: We studied all in-center HD patients that converted from CVCs to AVFs/AVGs between Jan 1, 2013 and Dec 31, 2013. For every patient, we computed rates for all-cause hospital admissions, hospital admissions related to myocardial infarctions, and number of positive blood cultures for 90 days prior to and 90 days after vascular access conversion. Only patients with treatments in the 90 days before and after periods were selected. Comparisons of admission rates and positive blood culture rates between before and after periods were performed using Poisson regression.

Results: We studied 3,543 patients. All-cause hospital admissions decreased by 37%, admissions related to myocardial infarctions decreased by 28% and positive blood culture events decreased by 62% upon transition from CVCs to AVFs/AVGs.

Table 1:	90 days before	90 days after	Percent change	p-value
Number of patients	3543			
All-cause hospital admissions ppy	3.03	1.92	-37%	<0.001
Admissions related to myocardial infarctions	0.012	0.008	-28%	<0.001
Positive blood cultures ppy	0.48	0.18	-62%	<0.001

Conclusions: Among in-center HD patients, transition from a CVC to an AVF/AVG access was associated with significant benefits of reductions in blood stream infection rates, all-cause hospitalization rates and myocardial infarction related hospitalizations. These results emphasize the imperative need for reducing the prevalence of CVCs in this population.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

SA-PO1083

A Retrospective Analysis of Tunnelled Femoral Vein Catheter Outcomes in Haemodialysis Patients

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Background: Permanent vascular access (VA) for haemodialysis can be challenging for some patients and may require the use of tunnelled femoral venous catheters (TFVC), usually as a last resort. Published literature on TFVCs for dialysis is scarce and study populations have been small. Our aim was to examine the clinical outcomes of TFVCs inserted in our unit over a 2 year period.

Methods: In this retrospective case series study we have identified and collected data on all TFVCs inserted between February 2011 and September 2013. Prior to TFVC insertion all patients were referred for consideration of arterio-venous fistula or graft, peritoneal dialysis, and were deemed unsuitable for tunnelled venous catheters in neck veins. Patients without contraindications were started on warfarin anticoagulation therapy at the time of TFVC insertion. Indications, complications and outcomes of TFVCs were obtained from patient records.

Results: 98 TFVCs were inserted in 59 patients during the study period. The median catheter survival was 49 days (range 0 – 771 days). When censored for elective removal and death with catheter in-situ, catheter patency at 2, 6 and 12 months was 70%, 40% and 25% respectively. The indications for catheter removal are shown in table 1. Elective catheter removal after establishing alternative VA was the most common indication for removal (32%). Catheter related blood stream infection contributed to catheter removal in 7% cases. Organisms were predominantly staphylococcus aureus and epidermidis species. One of these patients (who was colonised with a methicillin resistant strain) died as a result of their bacteraemia. 1 patient receiving warfarin therapy died of a spontaneous gastrointestinal bleed. There were no incidences of deep vein thrombosis (DVT) in this study.

Indication for Removal	Number (n)	%	n per 1000 catheter days
No longer required	30	32	3.0
Poor flow	26	27	2.6
Retracted	14	15	1.4
Infection	7	7	0.7
Death with catheter in-situ	6	6	0.6
Bleeding	1	1	0.1

Conclusions: TFVCs can be used as an effective and relatively safe option when there is either no other access options or whilst definitive access is created.

SA-PO1084

Complications and Outcomes of Tunneled Cuffed Catheters in Elderly Hemodialysis Patients

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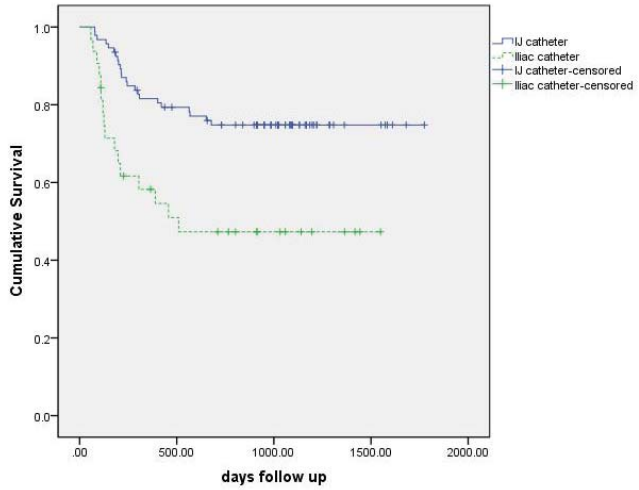
Background: To investigate complications and outcomes of TCCs in elderly patients.

Methods: We reviewed the clinical parameters of 127 patients aged over 65 years with 207 new TCC placements and measured complications and survival of TCCs.

Parameter	IJ TCCs	Iliac TCCs	*P* value
N catheters	155	52	
N pts	94	33	
Age	69±7	70±6	0.527
Sex N (% male)	51(54%)	19(58%)	0.741
Diabetes N (%)	39(41%)	15(45%)	0.157
Hypertension N (%)	23(24%)	7(21%)	0.705
Coronary heart disease N (%)	49(52%)	15(45%)	0.435
Glomerulonephritis N (%)	24(26%)	8(24%)	0.883
Peripheral vascular disease N (%)	34(36%)	11(33%)	0.769
Previous catheter placement	17(18%)	16(48%)	0.001
Complactions			
malnutrition	16(17%)	11(33%)	0.049
tumors	5(5%)	6(18%)	0.024
hypotension	17(18%)	12(36%)	0.031
Kt/V	1.1±0.17	1±0.19	0.006

Results: The average primary catheter patency was shorter in iliac vein TCCs. Patients with iliac vein TCCs underwent frequent exchanges than those with internal jugular vein TCCs. Infection-free survival was similar for both groups, but dysfunction-free survival was significantly poorer in iliac vein TCC. Age, peripheral vascular disease and previous catheter placement were the independent risk factors for TCCs.



Conclusions: There is a high incidence of catheter related infection for elder hemodialysis patients with TCCs. The iliac vein tunneled cuffed catheter is associated with an increased risk of dysfunciton.

Funding: Government Support - Non-U.S.

SA-PO1085

Nosocomial Acute Dialysis Catheter Related Events in Relation to Size and Site: Mayo Clinic ICU Experience in CRRT Patients Pramod Kumar Guru,[1] Abbasali Akhoundi,[2] Kianoush Banaei-Kashani.[3] *[1]Pulmonary and Critical Care, Mayo Clinic, Rochester; [2]Mayo Clinic; [3]Mayo Clinic.*

Background: Optimal size and site of dialysis catheter for proper delivery of CRRT in ICU patients remains a debatable issue. Practices vary according to provider's preferences and patient characteristics. Literature gap is significant to allow appropriate decision making.

Methods: This is a retrospective analysis of adult ICU patients, spanned over 3 year, regarding our CRRT patient outcomes in relation to catheter size and site. We hypothesized that catheter related complications and patient outcomes do not depend on the size or specific site of the catheter in acutely ill ICU patients.

Results: Right internal jugular (IJ) catheter of ≤ 15 cm and Left IJ of ≤ 20 cm are classified as short length; Right IJ ≥ 20 cm and Left IJ ≥ 24 cm as long length group. The baseline characteristics were similar, except higher SOFA score in short length group. The cause of AKI, need for MV, and coagulation parameters didn't differ between the two groups.

Characters	Short length cathters, N= 190, n(%)	Long length catheters, N=137,n(%)	p value
Areterial puncture	1(0.5)	2(1.4)	0.5
Hematoma	2(1)	2(1.4)	1
Minor bleeding	23(12)	29(21)	0.027†
Major bleeding(RBC transfusion)	75(39)	44(32)	0.17
New Anemia	44(23)	54(39)	0.001†
Significant arrhythmia	11(6)	10(7)	0.6
Others	11(6)	10(7)	0.6
No complication	152(80)	95(69)	0.027†
ICU mortality	68(25)	47(34)	0.8
Hospital mortality	89(46)	64(46)	1
Hospital days,median(IQR)	22(11-35)	21.3(11-43.5)	0.63
ICU days,median(IQR)	9 (5-16.6)	10(5-17)	0.8
CRRT time,hours,median(IQR)	103(43-171)	107(43-173)	0.72
Catheter time, hours,median(IQR)	136(63-256)	144(63-254)	0.6

Patients with long catheters had more complications (31%) in comparison to patients with short catheter (20%), p<0.05. None of the other in-hospital patient outcomes were statistically significant. There was no catheter related infection. Analysis for catheters at right IJ, left IJ and femoral site didn't show any difference, except more transfusion requirement on left IJ group.

Conclusions: In this large cohort of ICU patients, our analysis showed that mortality and major complications were unrelated to dialysis catheter size and site.

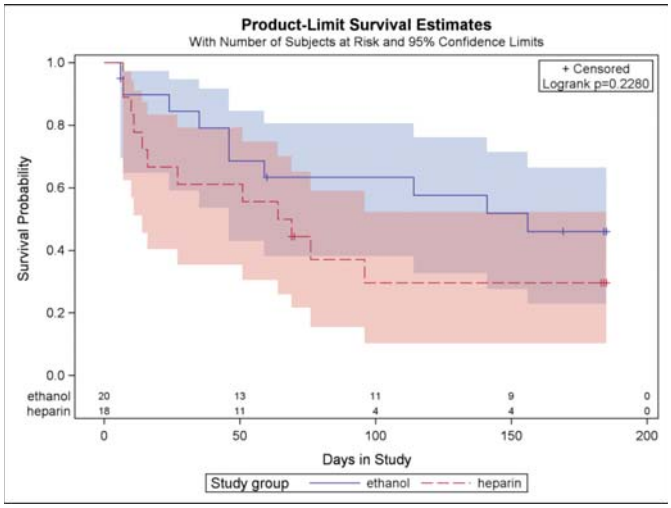
SA-PO1086

Safety and Efficacy of a 30% Ethanol / 4% Sodium Citrate Locking Solution Compared to Heparin to Prevent Hemodialysis Catheter-Related Infections: A Pilot Study Lavern M. Vercaigne,[1,3] Sean Armstrong,[2,3] Don Allan,[2,3] James M. Zacharias,[2,3] Lisa M. Miller.[2,3] *[1]Faculty of Pharmacy, Univ of Manitoba, Winnipeg, MB, Canada; [2]Section of Nephrology, Faculty of Medicine, Univ of Manitoba, Winnipeg, MB, Canada; [3]Manitoba Renal Program, Winnipeg, MB, Canada.*

Background: Catheter-related blood stream infections (CRBSI) are problematic in the hemodialysis population. The reduction of CRBSI and catheter thrombosis can improve patient outcomes. The objective of this pilot study was to collect preliminary data on the safety and efficacy of a 30% ethanol / 4% sodium citrate catheter locking solution versus heparin 1000 units / ml to prevent CRBSI and thrombosis.

Methods: A prospective, randomized, comparative design was used in chronic hemodialysis patients with incident catheters. Forty patients were followed prospectively until they developed a CRBSI, evidence of catheter dysfunction, were censored due to unrelated issues, or reached the full study duration without an endpoint.

Results: There were no serious adverse events directly related to the catheter locking solutions. The rate of CRBSI in the heparin and ethanol / sodium citrate groups were 0.76/1000 and 0/1000 catheter days, respectively. The median time to the first catheter endpoint was 67 days in the heparin group and 156 days in the ethanol / sodium citrate group. Kaplan Meier curves are shown in Figure 1.



The rate of alteplase use was 1.51 / 1000 catheters days in the heparin group and 2.78 / 1000 catheter days in the ethanol / sodium citrate group.

Conclusions: The 30% ethanol / 4% sodium citrate catheter locking solution was safely used in this pilot study. It was effective in eliminating CRBSI and prolonging catheter survival compared to heparin. The pilot study is limited by sample size and a larger appropriately powered RCT is required to confirm these promising results.

Funding: Pharmaceutical Company Support - MedXL Inc.

SA-PO1087

Ethanol Locks for the Prevention of Catheter-Related Bloodstream Infections: A Systematic Review and Meta-Analysis Lisa M. Miller,[1,2] Lavern M. Vercaigne,[3] Sherri Vokey,[1] Rasheda Rabbani,[4] Ahmed Abou-Setta,[4] Ryan Zarychanski.[1,4,5] *[1]Faculty of Medicine, Univ of Manitoba, Winnipeg, MB, Canada; [2]Manitoba Renal Program, Winnipeg, MB, Canada; [3]Faculty of Pharmacy, Univ of Manitoba, Winnipeg, MB, Canada; [4]George and Fay Yee Center for Healthcare Innovation, Univ of Manitoba/WRHA, Winnipeg, MB, Canada; [5]Dept of Haematology and Medical Oncology, Cancer Care Manitoba, Winnipeg, MB, Canada.*

Background: Catheter-related bloodstream infections (CRBSIs) are a serious complication of central venous catheters (CVCs), associated with increased hospitalization and death. The purpose of this systematic review is to examine whether ethanol locking solutions reduce CRBSIs in patients with CVCs.

Methods: Randomized controlled trials (RCTs) from MEDLINE, EMBASE, Cochrane Library, and IPA databases were searched from inception to March 2014. We also searched the World Health Organization's International Clinical Trials Registry Platform, relevant conference proceedings from 2009-2013, and reference lists of relevant articles, and performed Forward searches in Scopus and Web of Science. Two reviewers independently identified RCTs comparing ethanol lock solutions to other locking solutions in the prevention of CRBSIs in patients with a CVC, assessed studies for inclusion/exclusion, and extracted trial level data including population characteristics, interventions, and outcomes. Risk of bias was assessed using the Cochrane Collaboration risk of bias assessment tool.

Results: Three studies with a total of 485 patients, constituting 34 047 catheter days were included. Fixed-effects models were used to estimate pooled rate ratios for outcomes. CRBSIs ranged from 0.28 to 31.2 per 1000 catheter days. The rate ratio comparing ethanol

locks to controls was low in all studies ranging between 0.17 (95% CI 0.02-1.62) and 0.83 (95% CI 0.46-1.49). Ethanol locks did not significantly decrease CRBSI (pooled rate ratio 0.60, 95% CI 0.35-1.00). There was no difference in catheter removal rates between the ethanol and the control groups (pooled rate ratio 0.58, 95% CI 0.26-1.29).

Conclusions: There is insufficient evidence to support the routine use of ethanol locking solutions in the prevention of CRBSIs in patients with central venous catheters.

SA-PO1088

Benefits and Harms of Citrate Locking Solutions for Dialysis Catheters: A Systematic Review and Meta-Analysis Alexa L. Grudzinski,[1] Arnav Agarwal,[2] Gihad E. Nesrallah.[1,3] *[1]Nephrology Program, Humber River Hospital, Toronto, ON, Canada; [2]Dept of Health Sciences, McMaster Univ, Hamilton, ON, Canada; [3]Keenan Research Centre, Li Ka Shing Knowledge Inst, Toronto, ON, Canada.*

Background: Citrate-based locking solutions may be a cost-effective alternative to heparin for hemodialysis catheters. We conducted a systematic review and meta-analysis to compare benefits and harms of these interventions.

Methods: We searched CENTRAL, MEDLINE, EMBASE, and CINAHL. We included randomized, parallel arm clinical trials that enrolled adult patients (>18 years) receiving chronic hemodialysis through central venous catheters using a citrate locking solution. We excluded studies in which citrate was combined with other agents, such as antibiotics. Two reviewers performed data extraction independently and in duplicate with standard data extraction forms. We pooled count data using generic inverse variance with random-effects models, but used fixed-effect models when combining only two studies. Subgroups included low (4% or lower) versus higher (>30%) citrate.

Results: We screened 600 citations. Forty-one proceeded to full-text screen; 5 met inclusion criteria. Studies included between 19 and 291 participants (Median N = 61) followed for a total of 174.6 catheter-years; 2 were multi-centred trials. Three studies assessed all-cause mortality; the pooled relative risk was 0.71 (95% CI=0.42-1.24; p=0.21; I^2=0%). The rate ratio for bacteremic episodes was 0.54 (95% CI=0.23-1.29; p=0.16; I^2=65%) while the rate ratio for bleeding was 0.48 (95% CI=0.3-0.75; p=0.001;I^2=5%), favouring citrate. Pooled rates of catheter exchange/replacement and in-situ thromboslysis were not significantly different between groups. Risk of bias within pooled studies was low; however, imprecision due to small sample sizes and low event rates reduce our overall confidence in the pooled effect estimates.

Conclusions: Compared with heparin, citrate-based locking solutions for dialysis catheters had more favourable effects on rates of bacteremia and bleeding; however, larger trials are needed to confirm these findings. Given its lower unit cost, citrate may represent a dominant treatment strategy over heparin.

SA-PO1089

Outcome of AVF/Graft Formation in Patients Who Started Dialysis with Central Venous Catheter – A Single Centre Retrospective Analysis Nelomi Anandagoda, Raja Mohammed Kaja Kamal, Ankan Mittal, Maggi Steele, Bhrigu Raj Sood, David Makanjuola. *SW Thames Renal Unit, St. Helier Hospital, Carshalton, Surrey, United Kingdom.*

Background: Access related complications cause significant morbidity, therefore, good dialysis access is a key priority. Native arteriovenous fistula(AVF) remains the preferred choice with significantly better outcomes. UK guidelines suggest that 85% of all prevalent patients on haemodialysis should receive dialysis via a functioning AVF and central venous catheters(CVC) should be employed as a method of last resort, to reduce the overall risk of infectious complications.

Methods: This single centre retrospective study analysed outcomes of AVF/grafts created from January 2006 to September 2012 in patients already established on dialysis through CVC. Data was collected from renal and pathology department databases on successful use of AVF/graft and reasons for failure.

Results: Number of access procedures (N)-786;AVF/AVG used for HD–614(78%);AVF/AVG used without need for intervention–487(62%);AVF/AVG used after surgical or radiological intervention-122(16%);AVF/AVG never used–172(22%).

Reason for AVF/Graft never used (172)			
Renal Transplant	0.9%		
Death	3.9%		
Patient Choice	0.3%		
AVF/Graft Failure prior to use (reasons below)	16.7%		
Recovered Function	n=1		
Reason for AVF/Graft Failure (131)			
Bleeding	2.3%		
Thrombosis within AVF/Graft	55.0%		
Failure to mature	13.7%		
Infection	3.8%		
Vascular Compromise	21.4%		
Steal Syndrome	3.8%		
Age(years) at time of creation of AVF/graft			
	Mean	**Median**	**Range**
AVF/graft successfully used for HD	63.1	66	18.9-94.6
AVF/graft not used for HD	63.7	65.7	18.6-95.4

Conclusions: Our data demonstrate that in the majority of patients, the AVF/graft was used successfully but 16% required surgical/radiological intervention to facilitate this. Age at time of creation did not affect success/failure to use the AVF/graft. 16.7% of all AVF/grafts created were never used due to primary failure. In this group and the subgroup of patients who died prior to use of the AVF/graft further analysis is required to see whether there were any factors which would identify them, as they might be a group in whom long-term dialysis through a line would be appropriate.

SA-PO1090

Resistance Index in Ultrasound Vessel Mapping Predicts Successful Maturation of Arteriovenous Fistula without Interventional Assistance Hyeon Seok Hwang,[1] Se Young Kim,[1] Suk Young Kim,[1] Yong-Soo Kim.[2] *[1]Section of Interventional Nephrology, Div of Nephrology, College of Medicine, The Catholic Univ of Korea, Daejeon, Korea; [2]Section of Interventional Nephrology, Div of Nephrology, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

Background: Arteriovenous fistula (AVF) is the most preferred vascular access for hemodialysis and it frequently needs interventional assistance to facilitate maturation. However, there is no reliable indicator for the prediction of unassisted AVF maturation.

Methods: In 175 patients, who had matured AVF with and without interventional assistance, parameters of duplex ultrasonography were analyzed to evaluate whether preoperative mapping predicts unassisted AVF maturation.

Results: Successful AVF maturation without interventional assistance was observed in 142 (81.1%) patients. The area under the receiver-operating characteristic curve used to predict unassisted AVF maturation was 0.620 of resistance index (RI) (P = 0.034) and 0.615 of end diastolic velocity (P = 0.027). The best cutoff value of RI was 0.80 (sensitivity, 0.56; specificity 0.70). However, the peak systolic velocity, arterial and venous diameter did not predict unassisted AVF maturation. The patients with RI ≤0.8 showed faster AVF maturation and received less interventional procedure than those with RI >0.8 (all P < 0.05). In multivariable logistic regression, female gender and history of previous access reduced the odds ratio (OR) for unassisted AVF maturation, but patients with RI ≤0.8 (OR 3.07; P = 0.021) were independently increased the OR of unassisted AVF maturation.

Conclusions: RI value in preoperative ultrasound vessel mapping is an independent predictor for unassisted AVF maturation, and patients with higher RI value should be closely monitored for the necessity of intervention to promote maturation.

PUB001

Role of MRN Complex in DNA Damage of Cisplatin-Induced Acute Renal Failure Youngjung Kim, Tae Won Kim, Sora Park, Hyun Tae Kim, Si Yun Ryu, Ju Young Jung. *Veterinary Medicine and Institute of Veterinary Science, Chungnam National Univ, Daejeon, Korea.*

Background: The cisplatin-induced acute renal failure is a major side effect which limits cisplatin long-term chemotherapy. Mre11, Rad50, and Nbs1 (MRN) complex play critical roles in the pathways involved in the early phase of the cellular response to the DNA double strands breaks (DSBs) and DNA damage repair. The aim of the present study was to explore whether the MRN complex is associated with DNA repair mechanisms in cisplatin-induced ARF.

Methods: The rats were randomly allocated into three groups as follows; control group (sacrificed 10 d after saline intraperitoneal injection), 5D group (sacrificed 5 d after cisplatin injection) and 10D group (sacrificed 10 d after cisplatin injection).

Results: In the 5D group, the level of blood urine nitrogen and creatinine were increased seven times compared to the control group with increased apoptotic cells. In the 10D group, cisplatin-induced histopathological alterations in kidney were ameliorated together with decreased apoptotic cells if compared to that of 5D group. The expression of MRN complex was increased and localized in the nucleus of proximal tubule after cisplatin injection and these increased expressions was reduced in 10D group. As in MRN complex expression, increased DNA repair related signals (ATM, RPA2, H2AX, p53) in 5D group were reduced in 10D group.

Conclusions: Taken together, cisplatin-induced damage might trigger the MRN complex expression which is followed by the DNA repair related proteins expression.

Funding: Government Support - Non-U.S.

PUB002

Direct Effect of LPS on Glomerular Renin Angiotensin System in Extracorporeal Renal Perfusion Luciane Gomes Santana, Waldemar S. Almeida, Nestor Schor. *Medicine, EPM/UNIFESP, São Paulo, SP, Brazil.*

Background: Experimental observations suggest that, at least in the early phases of septic acute kidney injury, changes involving the glomerular hemodynamics results in the loss of glomerular filtration rate (GFR), leading to accented mutate in local renin angiotensin system(RAS). In our laboratory demonstrated the inhibition of enzymatic activity of renin by the direct effect of lipopolysaccharide (LPS) in immortalized human mesangial cell. If reproducible *in vivo*, inhibition of the RAS site by LPS have clinical importance given the role usually played by AngII on glomerular cells and autoregulation of glomerular hemodynamics.

Methods: FITC-LPS were infused into the aorta above the renal arteries of male Wistar rats weighing approximately 250g. 1-2 hours after systemic injection, cortical glomerular cells were isolated through sieving technique. To exclude systemic effects of LPS on the kidneys did the extracorporeal perfusion for 1-2 hours with Krebs Henseleit buffer (KH) and solution KH+LPS of *E. coli* (0.10 mg/ml) and stored at -80°C for determination of AngI/II and renin activity in high performance liquid chromatography (HPLC). Furthermore, fragments of kidney tissue will be fixed and analyzed by immunohistochemistry (IHC) for RAS.

Results: The evaluation of the blades showed absence of blood circulating cells in glomerular homogenate, n=8. The fluorescence in flow cytometry showed a higher mean fluorescence intensity of glomerular cells in the renal cortex from animals treated with FITC-LPS, n=4, compared with animals control groups, n=4. Histological evaluation by light microscopy performed in the control group underwent extracorporeal perfusion showed that tubules and glomeruli had maintained its integrity. Groups exposed to LPS in extracorporeal perfusion for 1h and 2h showed a decrease of 70% (AngII=150ng/mg versus control group 600ng/mg, $P<0.001$, $n=4$).

Conclusions: So far we proved that circulating LPS-FITC is retained in renal glomerular tissue and that LPS isolated on extracorporeal renal perfusion significantly blocked the SRA in glomerular renal tissue. We suggest that reduced levels of AngII may contribute for changes in glomerular microcirculation and GRF decline in severe sepsis.

Funding: Government Support - Non-U.S.

PUB003

A Combination of Delayed Ischemic Preconditioning and Galectin-9 Protects against Renal Ischemic Injury through a Regulatory T Cell-Dependent Mechanism Bingying Zhang, Yi Fang, Hui Zhang, Sheng Wu. *Nephrology, Zhangshan Hospital, Fudan Univ, Shanghai, China.*

Background: To investigates the protective mechanisms of a combination of delayed ischemic preconditioning (IPC) and galectin-9 against kidney injury caused by ischemia/reperfusion (IR).

Methods: The male mice were randomly divided into 4 groups: IR, IPC-IR, IPC-Gal9-IR and sham-sham groups (n=5). IR was introduced by clamping bilateral renal pedicles for 35 min. 18-min ischemia preconditioning was induced 4 days before renal IR, and a daily injection of 5μg galectin-9 or PBS was subcutaneously administered during the 4 days between IPC and IR. The mice were sacrificed after 35min/24h IR. Renal function was assessed based on the serum creatinine (Scr) and renal pathology. The peripheral blood and the spleen were also collected to evaluate the Treg expression by flow cytometric analysis.

Results: A 18-min renal ischemic preconditioning significantly attenuated ischemia-reperfusion injury induced 4 days later (Scr: 47.06±12.06mml/L versus 60.13±4.42mml/L, $P<0.05$; histology score: 2.12±0.13 versus 3.3±0.21, $P<0.05$), while IPC in combination with galectin-9 treatment further improved the renal protection effects(Scr: 47.06±12.06mml/L versus 60.13±4.42mml/L, $P<0.05$; histology score:1.54±0.15 versus 2.12±0.13, $P<0.05$). A reduced infiltration of macrophages and DC in renal tubulointerstitium was also identified in the IPC-Gal9-IR group. Flow cytometric analysis revealed that percent of Treg in peripheral blood, renal tissue and spleen in the IPC-Gal9-IR group increased markedly, compared with the IR or IPC-IR group ($P<0.05$). PC61 treatment, used to decrease Treg cell levels, reduced the renoprotective effects of Galectin-9.

Conclusions: Delayed IPC in combination with galectin-9 administration substantially attenuates IR injury through expansion of Regulatory T cells.

Funding: Government Support - Non-U.S.

PUB004

Differential Susceptibility to Acute Kidney Injury in Heterogenous Stock Rats Praneil D. Mehta,[1] Sarah M. White,[1] Leah C. Solberg Woods,[2] Kevin R. Regner.[1] *[1]Nephrology, Medical College of Wisconsin; [2]Human and Molecular Genetics, Medical College of Wisconsin, Milwaukee, WI.*

Background: Variation in the susceptibility to ischemia-reperfusion injury (IRI) has been demonstrated in several rat strains indicating the presence of genes that promote resistance to acute kidney injury (AKI). Identification of these genes may lead to the discovery of novel biologic pathways that can be targeted therapeutically to treat or prevent AKI in humans. Heterogeneous stock (HS) rats are a novel resource that can be used to study the genetics of renal disease by yielding diverse phenotypes and enabling rapid fine-mapping of quantitative trait loci to small genomic regions. The aim of the present study was to evaluate the range of phenotypes in HS rats following renal IRI.

Methods: Experiments were performed in 8 week old male Sprague-Dawley (SD, n=8) and HS (n=39) rats. Rats underwent 30 min bilateral renal ischemia and 24 hrs reperfusion. Rats were euthanized at 24 hrs and measurement of serum creatinine by LC-MS/MS was performed. Kidneys were collected for histologic analysis and Western blotting. Tubular injury was determined by the proportion of tubular cross sections in the cortex and outer medulla with tubular casts, loss of brush border, flattened epithelium, or sloughing of tubular epithelial cells. Expression of neutrophil gelatinase-associated lipocalin (NGAL), a marker of tubular injury, in kidney lysates was assessed by Western blot.

Results: Survival at 24 hrs after IRI was 100% in both the SD and HS rat groups. Serum creatinine at 24 hrs was 1.89±0.12 mg/dl in HS rats and 3.8±0.38 mg/dl in SD rats ($P<0.0001$). Serum creatinine in 30 of 39 HS rats was below the 95% confidence interval of the mean serum creatinine in SD rats. In HS rats, kidney NGAL expression correlated with serum creatinine ($r=0.7$, $P<0.0001$) and with renal cortex tubular injury scores ($r=0.5$, $P<0.001$).

Conclusions: In summary, these data demonstrate that HS rats exhibit decreased severity of renal dysfunction and renal injury following IRI in comparison to SD rats. These findings indicate that HS rats may harbor resistance alleles for kidney injury and suggest that the HS model may be a useful tool for further genetic studies in AKI.

Funding: NIDDK Support

PUB005

Impact of Comorbidities on Acute Kidney Injury Harlan Sparrow,[1] Carol M. Ashton,[1,2,3] Wadi N. Suki,[1,3,4] David Putney,[1] Nelda P. Wray,[1,2,3] Linda W. Moore,[1,2] A. Osama Gaber.[1,2,3] *[1]Houston Methodist Hospital; [2]Houston Methodist Research Institute; [3]Weil Cornell Medical College; [4]Baylor College of Medicine.*

Background: Acute Kidney Injury (AKI), which occurs in up to 18% of hospitalized patients, doubles the average length of stay (LOS) and results in 12-fold increase in risk of death compared with patients who do not experience AKI. Identification of risk factors that contribute to AKI is important in arriving at strategies to reduce AKI in hospitalized patients. The purpose of this project was to examine the impact of various comorbidities on the rate of AKI.

Methods: The first hospitalization of patients admitted in 2012 to a tertiary academic medical center were included. Patients <18 years, those with preexisting AKI, stage 5 CKD, or dialysis on admission, and those with a maximum serum creatinine (SCr) <=0.4 mg/dL were excluded. Also, patients lacking 2 SCr values within a 72-hour period were excluded. In-hospital AKI was defined as an increase in SCr by >=0.3 mg/dL or >=50% or a decrease in estimated Glomerular Filtration Rate by >=25% over a 72-hour interval. ICD9 codes, either present on admission (POA) or non-POA were used to determine comorbidities.

Results: Demographic and hospital data of 13,951 unique patients were as follows: AKI 18%, 31% had one or more ICU days, 52% were female, and 64% were Caucasian. Patients with AKI were older (66 versus 61 yrs $p<0.001$) and more likely black (22% $p<0.001$). All comorbidities showed a significant increase ($p<0.001$) in the incidence of in-hospital acquired AKI compared to those without the comorbidity except hypertension (NS).

Comorbidity	% Total Population	% AKI with Comorbidity	% AKI without Comorbidity
Chronic Kidney Disease	9	47	16
Congestive Heart Failure	17	37	15
Cardiovascular Disease	18	29	16
Cirrhosis	5	27	18
Diabetes	26	26	16
Hypertension	55	18	19

Conclusions: This retrospective analysis on nearly 14,000 patients shows that five of the six comorbidities have significantly higher in-hospital acquired AKI. Further research is needed to determine if this association is causal or induced by confounders. Regardless, these patients should be targeted for potential intervention to reduce the incidence of AKI.

PUB006

N-Acetylcysteine Ameliorate Heat Stroke Induced Acute Kidney Injury
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Background: The heat-related illness has become more prevalent and contributed to increased morbidity/mortality in the world with global warming. Heat stroke (HS) is the most severe and fetal heat-related illness. However, specific and effective therapies are not yet available to date. ROS plays a central role in HS. We assessed the efficacy of N-acetylcysteine (NAC), a thiol-containing free radical scavenger and antioxidant,therapy for HS induced AKI.

Methods: Adult male Sprague-Dawley rats were used to induce experimental HS. The rats are allocated into four groups. Two experimental HS groups of rats pre-treated with either saline, or NAC and another two as normothermic controls. All physiological and biochemical variables are measured. Disease severity was verified by with serum and urine metabolic profiles and with renal histopathology. The expression of cytokines and oxidative stress markers, cell apoptosis, and the associated mechanisms were also determined.

Results: HS rat treated with NAC displayed a better survival rate and stable hemodynamics. NAC also significantly reduce severity of AKI based on biochemical and histopathlogical evidence. The NGAL-positive cells and TUNEL-positive apoptotic cells in the kidney were significantly reduced in the NAC-treated HS rat. Oxidative stresses markers were significantly reduced in NAC -treated HS rat. Cytokines studies indicated that NAC significantly modulate serum proinflammatory, and anti-inflammatory cytokines. The effect on other organs revealed similar pattern as kidney.

Conclusions: Our studies have demonstrated NAC significantly attenuated organ damage and enhanced survival in HS rats. These protective effects may be associated with its anti-inflammatory capacity and anti-oxidant activity. We suggests that NAC could be potentially therapeutic for HS induced AKI in the future.

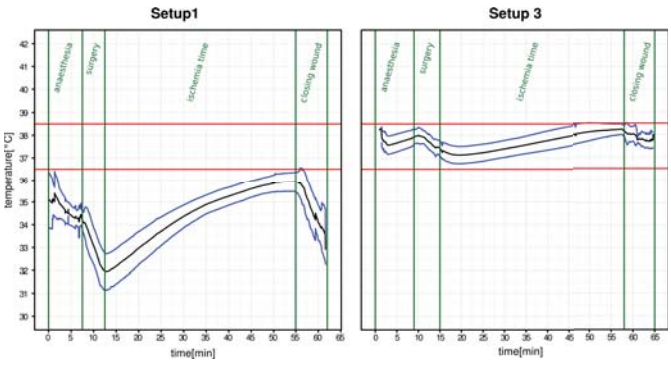
PUB007

Preoperative Heat Supply Improves Temperature Control in Experimental Ischemia Reperfusion Injury
Hannah Schaefer, Julian A. Marschner, Hans J. Anders. *Nephrologisches Zentrum, Klinikum der Univ Muenchen, Munich, Germany.*

Background: Experimental ischemia reperfusion injury (IRI) is widely used to induce AKI in mice. Beyond ischemia time body temperature (BT) is an important determinant of the severity of injury. Online body temperature monitoring (OBTM) in each mouse revealed that in our established surgery setup the BT was not constant within a target range of 36.5-38.5°C. We therefore tested various ways to improve temperature control during IR surgery based on OBTM.

Methods: Our established IRI protocol was changed stepwise. The old setup (1) provided heat supply during ischemia time by a heating pad, while setup 2 extended this also during ischemia time in a heating box. Setup 3 provided also presurgery heat supply in a 2nd heating box. Tubular injury was estimated by PAS-scoring and mRNA expression of tubular injury markers, the immune response was quantified by cytokine expression and neutrophil staining.

Results: Setup 1, 2 and 3 target range temperature readings were 0.69, 34.6 and 99.3%, respectively.



This increased the percentage number of necrotic S3 segment tubules (setup 1: 63.2%, 3: 93.3%) and neutrophil influx (setup 1: 5.7%, 3: 29.2%) as well as KIM-1 and TNFa expression. Testing various ischemia times in setup 3 for the same read-out parameters revealed that the same amount of injury is created with 20 min less ischemia time compared to setup 1. Setup 3 also allowed us to precisely modulate injury with ischemia time length since all parameters displayed significant differences for each time point.

Conclusions: As the animals BT drops quickly upon installing anaesthesia, presurgery heat supply is needed to maintain a target range temperature during IR. Optimized BT control improves the reproducible induction of IRI, as BT has a great impact on tissue injury. Vice versa, reducing BT may be a way to reduced IRI in surgical settings, such as kidney transplantation.

PUB008

Myo-Inositol Oxygenase (MIOX)-Mediated, Gentamicin-Induced Acute Tubular Injury via Oxidative and ER Stress
Ming Zhan, Yashpal S. Kanwar. *Dept of Pathology and Medicine, Northwestern Univ, Chicago, IL.*

Background: Gentamicin-induced acute kidney injury (AKI) has been recognized over decades and the clinical use of this antibiotic is thus limited. Gentamicin nephrotoxicity is associated with increased endoplasmic reticulum (ER) stress and oxidative stress, but the mechanism is still unclear. MIOX, a renal tubular enzyme, modulates tubular cellular redox in diabetes and is also upregulated in the plasma of patients with AKI. Here we investigated the potential role of MIOX in the pathogenesis of gentamicin-induced AKI.

Methods: Adult CD1 mice were injected with gentamicin intraperitoneally for different time periods. The kidney and serum samples were then obtained for the detection of targeted genes and tissue morphology analysis. In HK2 cells treated with various inhibitors, the impact of MIOX on gentamicin-induced stress signaling and mitochondrial injury was evaluated.

Results: CD1 mice with gentamicin injection for 7 days showed elevated levels of serum creatinine and blood urea nitrogen. Compared to the controls, MIOX mRNA and protein expressions were upregulated in the kidneys of gentamicin-treated mice in a dose-dependent manner, which paralleled with the increase of an AKI biomarker Kim-1. These changes were accompanied with elevated expressions of ER stress markers including GRP78, CHOP, p-JNK; as well as oxidative stress indicators Nox4, catalase, and elevated NADPH/$NADP^+$ ratio. In addition, proximal tubules from gentamicin-injected mice exhibited fragmented mitochondrial morphology and altered ER-mitochondrial contacts, along with increased Bax but decreased Bcl-2 levels, mitochondrial cytochrome C release and apoptosis. Following gentamicin treatment in vitro, HK2 cells transfected with MIOX siRNA showed reduced oxidative and ER stress. Importantly, gentamicin-induced mitochondrial dysfunction and apoptosis were partially ameliorated in HK2 cells with MIOX knockdown or cells treated with ROS scavenger or ER stress inhibitor.

Conclusions: Data suggested that ER and oxidative stress triggered by gentamicin in tubular cells is mediated by MIOX, which leads to mitochondrial dysfunction and tubular cell injury and death.

Funding: NIDDK Support

PUB009

Chymase Inhibition Ameliorates Apoptosis and Fibrosis in Hypoxic Injury of Renal Proximal Tubular Epithelial Cells
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Background: Chymase is an alternative pathway for angiotensin converting enzyme (ACE) in Ang II formation, and it also plays an important role in tissue remodeling through the activation of transforming growth factor (TGF)-β1 and endothelin A receptor (ET_AR). However, renoprotective effects of chymase inhibition remain elusive. We investigated the effects of chymase inhibition on the apoptosis and fibrosis induced by hypoxia in human renal proximal tubular epithelial (HK-2) cells.

Methods: HK-2 cells were cultured in hypoxic chamber in the absence or presence of chymostatin, a chymase inhibitor. The protein expressions of chymase, Ang II, Ang II type 1 receptor (AT1R), ACE, ET_AB and ET_BR were determined. We also investigated the effects of chymostatin on the expressions of pro-apoptotic and pro-fibrotic proteins.

Results: In HK-2 cells, hypoxia increased the expressions of chymase, Ang II and AT1R. However, the protein expression of ACE was not changed. The expression of ET_AR was increased while that of ET_BR was decreased. These were counteracted by the pretreatment of chymostatin. In HK-2 cells with hypoxic injury, the protein expression of Bax/Bcl-2, cleaved caspase-3 and TGF-β1 was also increased. In addition, hypoxic treatment resulted in decreased cell viability. These were also ameliorated by the pretreatment of chymostatin.

Conclusions: These findings suggest that chymase inhibition may play a protective role in the hypoxic renal tubular injury through the inhibition of apoptotic and fibrotic pathways.

PUB010

Target Specificity of PTBA Class Inhibitors
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Background: Acute Kidney injury (AKI) is a rapid loss of kidney function that causes irreversible damage and fibrosis. Other than renal replacement there are no post AKI therapies. We identified the histone deacetylase (HDAC) inhibitor phenylthiobutanoic acid (PTBA) from an embryonic renal progenitor cell (RPC) proliferation screen. PTBA Analogues UPHD00025 and UPHD00186 activate proliferation of RPCs and enhance renal function after tubule injury in zebrafish and mouse AKI models. They also reduce post-injury fibrosis in mice. Since therapeutic efficiency is an outcome of target engagement, a correlation to the PTBA cognate target is essential. Using cellular thermal shift assay (CTSA) we propose to identify HDAC targets of PTBA. By pharmacokinetics we also compare PTBA, UPHD00186 and UPHD00263 to describe a mechanism of action.

Methods: Ligand induced thermal stabilization is a measure of target engagement. HEK-293 cell lysates are subjected to thermal denaturation with and without PTBA. After

pelleting the denatured proteins, the supernatants are probed on westerns with specific HDAC antibodies. For DMPK, compounds at 50mg/kg is injected IP into BALB/c mice. Metabolites in blood samples withdrawn are identified by LCMS.

Results: PTBA can stabilize all four class I HDACs from denaturation at 55°C compared to DMSO control. However class II HDACs such as HDAC5 are not stabilized by PTBA. UPHD00025 and UPHD00186 generate PTBA in plasma within 5 minutes or less after IP injections due to hydrolysis. UPHD00263 lacking the amide bond of UPHD00186 does not generate PTBA nor induce RPC proliferation.

Conclusions: Results from CTSA demonstrates that PTBA analogues target the class I HDACs. CTSA will help us to establish therapeutic targets for the PTBA class HDAC inhibitors and define which HDACs are best to target during AKI events. *In vivo* hydrolysis generates PTBA from the analogues to drive activity. Hence UPHD00025 and UPHD00186 functions as reservoirs for extended release of the PTBA. Grant support: DK069403 and DK079307.

Funding: NIDDK Support

PUB011

Role of Tight Junction Proteins in Hydrogen Peroxide-Mediated Regulation of Paracellular Permeability Angelina Voronina, Nikita Shah, Shirin Jaggi, Josephine Axis, Kurt Amsler. *Dept of Biomedical Sciences, NYIT College of Osteopathic Medicine, Old Westbury, NY.*

Background: Oxidative stress increases paracellular permeability of renal epithelia both in vitro and in vivo. Hydrogen peroxide (H_2O_2) treatment of cultured renal epithelial cells is a model for aspects of this pathogenic process. H_2O_2 treatment of MDCK cells produced a concentration-dependent collapse of domes, manifestations of transepithelial salt and water absorption.

Results: Even at concentrations that did not lead to collapse of domes, H_2O_2 produced a concentration-dependent increase in paracellular flux of calcein. In contrast, transepithelial resistance of MDCK cell populations was not affected by treatment with H_2O_2 at any of the tested concentrations. Cell death (necrosis) was observed at higher H_2O_2 concentrations but flux changes could be observed at sublethal H_2O_2 concentrations. The total cellular contents of TJ proteins, occludin, claudin-1, ZO-1 and ZO-2, were unaffected by treatment with either sublethal or lethal concentrations of H_2O_2. There were also no dramatic changes in the contents of these TJ proteins in the Triton X-100-soluble and –insoluble fractions with either sublethal or lethal H_2O_2 treatment. Lethal H_2O_2 concentrations produced altered the localization of occludin, ZO-1 and F-actin. Less dramatic alterations in the localization of ZO-2 and claudin-1 were observed. Despite increasing paracellular permeability, sublethal H_2O_2 concentrations did not markedly alter subcellular localization of any TJ proteins. Knockdown of occludin or ZO-2 increased the sensitivity of MDCK cells to H_2O_2. In contrast, overexpression of occludin or knockdown of ZO-1 diminished the sensitivity of MDCK cells to H_2O_2. Transepithelial resistance was decreased in ZO-2 knockdown cells compared to control MDCK cells but was unaffected by other manipulations.

Conclusions: These results indicate a complex interplay of multiple TJ proteins in the H_2O_2-induced regulation of paracellular permeability.

Funding: NIDDK Support

PUB012

Human Kidney-2 (HK-2) Cells Resist Hypoxic Death Emmett D. Ratigan, Alana Shigeoka, Sashi Kasimsetty, Reza Elahimehr, Dianne B. McKay. *Medicine, Univ of California San Diego, San Diego, CA.*

Background: In vitro hypoxia is an experimental method used to mimic ischemia/reperfusion injury, and is considered a valid model. To better understand the molecular mechanisms of ischemic kidney injury, immortalized renal tubular cell lines have been developed that provide a homogenous cell population allowing for in depth molecular studies of cellular stress. Amongst studies evaluating hypoxic stress, cell death assays have been commonly used as experimental end points. To test the validity of in vitro hypoxia using immortalized cell culture as a model of ischemic renal tubular injury, we subjected the well-characterized HK-2 proximal tubular cell line to severe hypoxic conditions and analyzed their ability to resist cell death.

Methods: HK-2 monolayer cell cultures were grown to 50-70% confluency and exposed to either ambient oxygen (21%) or 1% oxygenation in a Coy® hypoxic chamber for 24, 48, and 72 hours duration. The cells were grown in glucose-free DMEM to allow for aerobic respiration only. After the incubation period, HK-2 cells were harvested and cell death analyzed, using the Cellometer® Annexin V-FITC/PI Apoptosis Assay. The data represent the composite of duplicate experiments with similar results. Chi-square analysis was used with a significance level of 0.05.

Results:

Exposure	Living at 24 hours	Living at 48 hours	Living at 72 hours
Hypoxia	79%	72%	52%
Normoxia	77%	75%	53%
	P<0.01	P<0.01	P=.33

Cultures subjected to hypoxia had less cell death at 24 hours, although at 48 hours the opposite was true. Following 72 hours there was no difference between groups. Overall, the amount of excess cell death in the hypoxic group was either absent or surprisingly low, which suggests caution in interpretation of hypoxic studies using immortalized proximal tubular cell lines.

Conclusions: HK-2 cells were derived through transduction of human proximal tubule cells with HBV 16 E6 and E7 genes, which encode viral proteins acting to degrade p53 and interfere with pRB tumor suppressor activity. We propose that HK-2 cells are refractory to hypoxic cell death due to selective degradation of p53, supporting the potential of apoptotic inhibition for protection from prolonged ischemic renal injury.

Funding: NIDDK Support

PUB013

Stabilization of eEOC Autophagy in Murine Ischemic AKI Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology and Rheumatology, Univ Hospital Göttingen, Germany.*

Background: *Early Endothelial Outgrowth Cells* (eEOCs) protect mice from ischemic AKI. Autophagy, a cellular mechanism mediating endogenous protection can prolong the lifespan of cells. Thus, aim of the study was to analyze consequences of eEOC autophagy stabilization in murine ischemic AKI.

Methods: Male, 8-12 week old C57/Bl6N mice were subjected to unilateral renal ischemia (45 minutes) postuninephrectomy. Animals were systemically injected with either untreated or SAHA (SuberoylAnilide Hydroxamic Acid) or Temsirolimus pretreated syngeneic murine eEOCs at the time of reperfusion. Mice were analyzed 48 hours and 4 weeks later.

Results: Renal ischemia significantly affected renal function. Administration of SAHA pretreated cells elevated intrarenal endothelial levels of Atg8, indicating increased autophagy of the endothelium. Nevertheless, neither the administration of untreated nor SAHA or Temsirolimus pretreated cells protected mice from AKI in the short-term. Renal dysfunction still persisted at 4 weeks and cell administration did not result in any stabilization of renal function at this time.

Conclusions: Although eEOC treatment with autophagy inducers increases endothelial autophagocytic activity in the kidney, mice are nor protected from postischemic acute renal dysfunction in the short- or in the mid-term. Thus, autophagy induction in eEOCs is most likely not an effective strategy to stimulate renoprotective effects of the cells in AKI.

PUB014

Mid-Term Outcome of Murine AKI with versus without Administration of Early Endothelial Outgrowth Cells Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology and Rheumatology, Univ Hospital Göttingen, Germany.*

Background: *Early Endothelial Outgrowth Cells* (eEOCs) protect against murine ischemic AKI in the short-term. These effects can be stimulated by Angiopoietin-2 and Bone Morphogenetic Protein-5 (BMP-5). Aim of the study was to analyze mid-term consequences of eEOC treatment of murine AKI (with versus without Ang-2 / BMP-5 treatment).

Methods: Male, 8-12 weeks old C57/Bl6N mice were subjected to unilateral renal ischemia post-uninephrectomy. Untreated and Ang-2 / BMP-5 pretreated syngeneic murine eEOCs were adminstered at the time of reperfusion. Six weeks later, animals were analyzed for renal function, fibrosis and mesenchymal transdifferentiation of intrarenal endothelial cells (EnMT - Endothelial-to-Mesenchymal Transition).

Results: Renal function of postischemic mice was still significantly affected at six weeks after acute ischemia. Cell administration did not protect mice from renal dysfunction. Ang-2 treated eEOCs tended to reduce interstitial fibrosis and BMP-5 aggravated fibrosis as compared to Ang-2. EnMT remained unaffected by cell therapy.

Conclusions: Early Endothelial Outgrowth cells alone do not protect from postischemic renal damage in the mid-term. Ang-2 and BMP-5 tend to increase renoprotective effects of the cells at 6 weeks. Pharmacological preconditioning of eEOCs can result in aggravation of fibrosis, eEOC priming protocols in AKI must therefore be chosen with caution.

PUB015

Conditioned Medium (CM) from Mesenchymal Stem Cells (MSCs) Protects Human Proximal Tubular Cells (HK2) Lesions by SEPSis Jessica S. Garcia, Marcelo Andery Naves, Fernanda Teixeira Borges, Nestor Schor. *Medicine - Nephrology, UNIFESP - EPM, São Paulo, Brazil.*

Background: Recent studies emphasize the contribution of MSCs in the regeneration related to acute kidney injury (AKI). MSCs mitigate the damage and / or accelerate the repair and participate in the immunomodulation processes, probably by paracrine pathways. This protection involves the CM that contains soluble factors or microvesicles (MVs). We studied the paracrine effect of MSCs in vitro models of AKI induced by LPS in culture.

Methods: HK2 cells were cultured and incubated for 24-96 h with LPS (100mg/ml), with or without CM from MSCs. The CM was also pre-conditioned (PC) by treating MSCs with LPS (100mg/ml, PC_{LPS}) or GENTA (2 mM, PC_{GENTA}) for 48 h before the experiment and CM was also employed in frozen form ($_f$CM). Cell proliferation was evaluated by MTT assay.

Results: Increases in cell proliferation in the CM+LPS group compared with LPS alone (0.12±0.02 versus 0.10±0.02 DO, $p < 0.05$), and the major proliferation happened with the $_f$CM+LPS (0.16±0.04 DO, $p < 0.05$). Evaluating groups with PC-CM, it was observed higher proliferation in PC_{GENTA}-CM+LPS (0.16±0.05 DO, $p < 0.05$) in HK2 than in PC_{LPS}-MC+LPS (0.09±0.02 DO, $p < 0.05$). Comparing only the different CM in HK2 cells in culture, it was measured greater proliferation in PC_{LPS}-MC (0.27±0.07 DO, $p < 0.05$) when compared with CM or $_f$CM (0.21±0.03 and 0.20±0.06 DO, $p < 0.05$, respectively).

Conclusions: These preliminary results suggested that CM from MSCs exerts a protective effect by stimulating cell proliferation during the treatment with LPS. Also, it is also verified potentialization with previous PC, enhancing its effects. It is interesting that a cross-over effect in PC was observed since PC_{GENTA} was more effective than PC_{LPS}

in the isolate cell model of sepsis. Also, surprisingly ₍CM showed higher effect than the fresh form. Thus, results may indicate that it is possible to minimize AKI induced by LPS without cells transplantation but only with their CMs, avoiding potential harmful effects of cell therapy. And finally, it is also possible to stimulate the CM (PC strategy) in order to enhance its effects. Support: CNPq, FAPESP, CAPES, FOR.

Funding: Government Support - Non-U.S.

PUB016

Estrogens Protect against Hemorragic Shock-Induced Acute Kidney Injury in Mice Melodie Cuny,[1] Marie Buleon,[1] Julie Belliere,[1] Ivan A. Tack.[1,2] *[1]I2MC, UMR 1048, INSERM, Univ Toulouse III; [2]CHU Toulouse, France.*

Background: The protective effect of estrogens against chronic glomerular diseases is well admitted but remains unknown during Acute Kidney Injury (AKI). This study aimed to determine the impact of estrogens deprivation, replacement or acute administration during AKI, using a model of hemorrhagic shock in mice.

Methods: Four groups of C57/Bl6 female mice were submitted to a pressure-controlled hemorrhagic shock: control [C]; receiving a single acute intravenous bolus of estradiol (E2) during resuscitation [AE2]; ovariectomized [O]; ovariectomized with chronic E2 replacement [CE2]. Renal impact was studied 1 and 21 days (D1 and D21) following the shock. At D1, renal function, programmed cell death (TUNEL) and endoplasmic reticulum (ER) stress (GRP78 and CHOP expressions) were assessed. Since ER stress can induce pyroptosis, caspases 1 and 11 expressions were studied. Renal immune response was assessed using TLR4 expression and IL1β synthesis. Finally, Glomerular Filtration Rate (GFR) and renal fibrosis (collagen III deposits) were studied at D21.

Results: Mortality was higher in CE2 group. At D1, renal function was similar between groups. However, some mice, from O and CE2 groups only, showed tubular impact (assessed by cystatinuria). In control, hemorrhagic shock induced epithelial cell damages involving ER stress (increase in GRP78 and CHOP) and pyroptotic cell death (increase in caspases 1 and 11) that resulted, at D21, in secondary renal fibrosis without significant decrease in GFR. Estrogen deprivation worsened renal damages but chronic E2 replacement was not beneficial. In AE2 group renal damages were largely prevented by reduction of ER stress, pyroptosis, renal inflammation and fibrosis.

Conclusions: In this model, AKI leads to profibrotic pyropotosis that was worsened by endogenous estrogens deprivation, but not improved by chronic E2 replacement. By contrast, administration of a single bolus of E2 during resuscitation resulted in a striking protective effect, reducing both the severity of cell death related to pyroptosis, renal inflammatory response and secondary fibrosis. This result highlights the importance of studying the " bolus effect " of estradiol in situations of AKI.

Funding: Government Support - Non-U.S.

PUB017

Effects of the Transplantation of Ast-hADSC in Cisplatin-Induced Acute Kidney Injury Nanmei Liu. *Dept of Nephrology, Jimin Hospital of Shanghai, Shanghai, China.*

Background: To observe the effect of the injured renal tissue and contribute to its recovery in mice with transplantation of human adipose-derived mesenchymal stem cells (hADSCs) cultured with Astragaloside IV(Ast) in cisplatin-induces acute kidney injury and explore possible mechanisms.

Methods: Male BALB/C mices were randomly divided into normal group, model group, hADSCs group and Ast-hADSCs group after establishing animal models of cisplatin-induced renal injury. hADSCs and Ast-hADSCs were injected into the transplantation group through caudal vein after 24h of cisplatin injection, and equal physiological saline was also injected into the model group. These mice were sacrificed on the 3d after transplantation. Blood samples were used to test serum creatinine (Scr) and urea nitrogen (BUN). The renal tissues were got, and HE staining was performed to observe the pathological changes, and both VEGF and PCNA staining were also observed by immunhistochemical analysis. The level of TNF-α,IL-6 and RANTES was also detected by enzyme-linked immunosorbentassay (ELISA).

Results: The levels of Scr and BUN of model groups were significantly higher than those in the normal group and intervenient group, comparing with the hADSCs group, the pathological changes in the Ast-hADSCs group was much lower. Furthermore, the Ast-hADSCs group confirmed renal tuble epithelium to be deposited by laser confocal-microscopy, as well as the expression of inflammatory cytokines, such as TNF-α, IL-6 and RANTES and the levels of apoptosis related protein, such as Caspase-3 and Bax were obviously decreased while the expression of VEGF, PCNA and Bcl-2 was increased. Among all those groups, the Ast-hADSCs had a more obviously positive effect on cisplain-induced renal damage.

Conclusions: Transplantation of hADSCs into injured kidney contributes to recovery of cisplatin-induced acute renal injury, and Ast-hADSCs is more effective in repairing acute renal injury.

Funding: Government Support - Non-U.S.

PUB018

Nephrotoxicity of Ifosfamide in Adult Patients Gaël Ensergueix,[1,2] Alexandre Karras,[1] Marie Essig,[2] Eric Thervet. *[1]Nephrology, Dialysis, Hôpital Européen Georges Pompidou, Paris, France; [2]Nephrology, Dialysis, Transplantation, CHU Limoges, France.*

Background: Ifosfamide (IFO) is an alkylating agents used as an antineoplasic drug in several types of solid cancers, especially sarcomas. IFO renal side-effects have moslty been described in pediatric populations. The aim of this study was to describe, in adult patients (pts) the clinical and histological features of the IFO-associated nephrotoxicity.

Methods: We conducted an observational, retrospective study in 4 French nephrology centers. All adult pts admitted for renal failure (eGFR < 60 ml/min/1.73 m2) or tubular dysfunction after IFO therapy were included.

Results: We included 15 pts (7 men and 8 women) with a mean age at diagnosis of 54 (18-75), 93% had received IFO for sarcoma. The mean cumulative dose of IFO was 18.40 g/m². Ten pts received concomitant cisplatin treatment. In 6 pts, the renal presentation was an acute kidney injury (AKI) occurring during the first month following IFO administration, 5 pts had a slowly progressive chronic kidney disease (CKD) detected 6 to 24 months after IFO therapy and 7 pts, a proximal dysfunction (Fanconi syndrome). Three pts had several forms of nephrotoxicity. Renal biopsy was performed in 5 cases, showing marked proximal tubular mitochondrial cytopathy in 4 cases, interstitial inflammation and tubular atrophy/interstitial fibrosis in 2. After a mean follow-up of 16 months, 3 pts reached end-stage renal disease. At the last follow-up, the mean eGFR of the 12 remaining pts was 27 ml/min/1.73m². Among the 4 pts requiring initial hemodialysis only one could stop dialysis. Corticosteroids were used in 2 cases without improvement of renal function.

Conclusions: In adult pts, IFO nephrotoxicity can induce either AKI, progressive CKD diagnosed several months after discontinuation, or proximal tubulopathy. Prospective, large-scale studies are needed to precisely determine the prevalence, the pathophysiological mechanisms and the risk factors associated with this severe side-effect.

PUB019

NGAL and Urine Biochemistry for Early Detection of Acute Kidney Injury Lilian Carmo, Camila Lima, Fernando de Almeida Soares, Vivian Lumi Onusic, Liliany P. Repizo, Emmanuel A. Burdmann, Etienne Macedo. *Nephrology, USP, Sao Paulo, SP, Brazil.*

Background: Early AKI diagnosis with biomarkers of injury is promising advance for improving AKI outcomes. Association of clinical risk factors, urinary indices with these biomarkers could help to improve their performance in the clinical practice. We hypothesized that urinary neutrophil gelatinase-associated lipocalin (NGAL) in association with urinary biochemistry could be a tool to improve early AKI detection in high-risk critical ill patients.

Methods: We performed a prospective study between Jan 2012 and July 2013. We recruited patients admitted in intensive care unit (ICU) with high risk for AKI. Urinary NGAL and urine biochemistry were measured serially every 12 hours during the first two days of ICU stay. Urinary indices including strong ion difference (SIDu), potassium gradient transtubular (TTKG) and fractional excretion of sodium (FENa) were evaluated during this period.

Results: Of the 272 screened patients, 46 met the inclusion criteria. During the observation period, first 7 days of ICU stay, 30(62.5%) patients met KDIGO criteria for AKI. Urinary NGAL was higher at all points in AKI group.

	NGALu basal	NGALu 12h	NGALu 24h	NGALu 36h	NGALu 48h
No AKI	54 (21-148)	38 (22-144)	39 (21-187)	49 (12- 201)	90 (21-222)
AKI	260 (75-2649)	147 (69,7-505)	95 (54-301)	101 (52-510)	123 (57-245)

Urinary NGAL value with best sensibility and specificity to predict the development of AKI was 175 ng/ml (area under ROC curve = 0.738 - IC 95% 0,586-0.890 *p=0.011*). In patients developing AKI by sCr criteria, urinary NGAL would provide an earlier diagnosis in 10 patients (38%). Four (18%) patients with urinary NGAL levels above the cutoff did not developed AKI during the observation period. FENa was <1% in AKI and non-AKI groups. The progression of urinary SID was similar in both groups. In patients that developed AKI TTKG was not statically significantly higher.

Conclusions: Urinary NGAL was an earlier predictor of AKI in high risk patients. The association of urinary biochemistry did not improve the prediction ability of NGAL in this cohort. Further studies incorporating this indices should confirm their role in early diagnosis of AKI.

Funding: Government Support - Non-U.S.

PUB020

The Risk Factors on the Prognosis of Acute Kidney Injury According to Kidney Disease: Improving Global Outcomes Definition and Staging Criteria: A Retrospective, Multicenter Study in Critically Ill Patients Jiaojiao Zhou, Lichuan Yang, Fang Liu, Ping Fu. *Div of Nephrology, Dept of Medicine, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China.*

Background: Despite significant advances in the medical management and therapeutics, acute kidney injury (AKI) is still a familiar and serious complication with enhanced morbidity and mortality in hospitalized patients, especially in intensive care units (ICUs). The primary purpose of this study is to apply the definition proposed by the KDIGO criteria to analyze the morbidity and risk factors on the prognosis of AKI in ICUs.

Methods: In this retrospective study, we collected the data from patients admitted in ICUs of West China Hospital of Sichuan University from July 28, 2009 to April 16, 2010. Logistic regression analysis was performed to investigate the related factors for 28-day mortality of AKI in ICU.

Results: After further exclusion, there were 1036 patients of ICU admissions enrolled in this research. We found AKI occurred in 36.9% and the mortality was 51.3%. The variables that were related to the prognosis of AKI in logistic regression analysis were as follows: sepsis (odds ratio [OR], 21.43), AKI stage 2 (OR, 17.34), severe acute pancreatitis(SAP) (OR, 11.75), AKI stage 3 (OR, 8.41), the timing of AKI in ICU (OR, 4.84), chronic obstructive pulmonary disease(COPD) (OR, 3.46), mechanical ventilation (OR = 2.76).

Conclusions: In these ICU patients, various risk factors including sepsis, SAP, AKI stage 2 and 3, the timing of AKI in ICU, COPD, and mechanical ventilation were considered as independent predictors of 28-day mortality. The KDIGO criterion is a sensitive and scientifically valid definition that provides an early warning signal for renal dysfunction in critically ill patients.

PUB021

AKIN Criteria: A Dynamic Tool Associated with Higher Odds of Hemodialysis Dependence and Death in Patients with Acute Kidney Injury Hugo Pinheiro, Gisele Vajgel Fernandes, Ederson Vidal Moura, Luis H.B.C. Sette, Lucila Maria Valente, Cintia Germana Mergulhão da Costa, Geraldo José de Amorim, André Luiz De Andrade Araújo. *Nefrologia, Univ Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Background: AKIN was planned to standardize the diagnosis and the classification of AKI allowing comparison between clinical studies worldwide and there is a linear correlation between mortality and increasing AKI stage. The aim of this study was to evaluate the association between AKIN classification in the moment of the nephrologist referral and its in-hospital progression to the patient outcomes.

Methods: We conducted a retrospective single center cohort study of in-hospital patients referred to nephrologist consult from Jan 2011 to Dec 2013. AKI was defined and classified according to the AKIN criteria, based on SCr, at the first day of nephrologist consult and consecutively during in-hospital stay. Primary endpoints were mortality and the combined outcome was defined as mortality or dialysis dependence at hospital discharge.

Results: We analysed 778 in-hospital admissions and among them 407 met AKI criteria. The mean age was 54y, 54% were female and patients were classified as follows: AKIN 1 (23.3%), AKIN 2 (25.8%) and AKIN 3 (43.7%) at the moment of nephrology referral. We found that AKIN 1, 2 or 3 needed RRT in 27.3%, 28.5% and 58.4%, respectively (P<0.0005). Worsening AKIN score from 1 or 2 to AKIN 3 was associated with higher odds of mortality and combined outcome of death and permanent need for dialysis when compared to those who maintained AKIN 1 or 2 during follow up, in unadjusted analysis (OR 2.02/CI 1.04-3.94; P= 0.038 and OR 2.22/CI 1.15-4.26; P= 0.017, respectively). Those who were referred to the nephrologist at AKIN 1 or 2 and afterwards changed AKIN to 3 during in-hospital stay had similar rates of mortality and combined outcome as those who maintained AKIN 3 (P= 0.9 and P= 0.57).

Conclusions: Patients whose AKIN rose to 3 during in-hospital stay or were referred to the nephrologist on AKIN 3 had higher rates of death or permanent need for dialysis when compared to those who remained at AKIN 1 or 2. Our results highlight the importance of nephrology referral in earlier stages of AKI.

PUB022

Acute Babesiois Presenting with AKI Ashwin Narasimhan,[1] Nishitha Cherukumalli,[2] Sravan Jasti.[3] *[1]Nephrology, Univ of Pennsylvania Hospitals, Philadelphia, PA; [2]Nephrology, Albert Einstein, Philadephia, PA.*

Background: 60 year male with hx of HepatitisB,recently diagnosed lymes disease presented to ER with sweats,chills,fatigue and fever.

Methods: Patient was diagnosed with lymes disease 1 week prior by primary who noted a target lesion on his right inner thigh and placed on 21 day course of doxycycline.Vitals on admission: BP 136/74,HR-95, RR-18 and T-103.5.Chemistries: Na-141, K-4.7,Cl-113, Co2-24,AST103, ALT78, CPK 43, Cr 2.8 and BUN58 .Hgb was 8.9, Platelets-299000, LDH 528.Micro anaplasma titres and Ehrlichiosis titres were less than assay .Peripheral smear confirmed RBC inclusions consistent with babesia with parasitic load of 6.2%. Renal consulted for AKI.Urinary sediment revealed large number of muddy brown casts and 2-3WBC casts consistent with ATN .Patient was oliguric on admission that improved with hydration. Infectious disease was consulted and given severity of parasitic burden patient underwent RBC exchange transfusion with symptoms of anemia,organ failure and fever.He was started on IV clindamycin and Quinine.His parasitic load came down to <0.01 with overall improvement in symptoms and his discharge creatinine was 1.7 and BUN of 16. He was discharged home on 10 days of atovaquone and azithromycin.

Results: Asymptomatic Babesiois occurs in only 25% of immunocompetent adult hosts. Symptomatic disease may involve multiple organ systems with respiratory distress, acute hepatitis, and AKI causing significant morbidity,including severe dialysis-requiring AKI. Kidney involvement in babesiosis is largely due to ATN from combination of underlying sepsis, hemodynamic instability,and heme pigment induced tubular injury.Rare cases of AIN have been reported.Only a few cases of babesiosis with ATN and AIN have been reported .Interstitial nephritis although not confirmed by biopsy could have existed as sediment contained WBC casts. Prompt identification,aggressive antibiotic therapy, hydration and possible redcell apheresis is treatment .In rare cases with plasma dominant AIN,prednisone may be be required.

Conclusions: Prompt identification of babesiosis with treatment in a timely manner helps avoid serious outcomes like renal failure that is dialysis dependant.

PUB023

Cell Cycle Arrest Biomarkers Accurately Predict Acute Kidney Injury in Diabetics with or without Chronic Kidney Disease Michael Heung,[1] Luis M. Ortega,[2] Lakhmir S. Chawla,[3] John A. Kellum.[4] *[1]U Michigan; [2]Temple U; [3]George Washington U; [4]U Pittsburgh.*

Background: Patients with diabetes mellitus (DM) and chronic kidney disease (CKD) are at high risk for development of acute kidney injury (AKI). However, identification of AKI can be challenging in patients with underlying chronic disease, and biomarkers often perform poorly in this population. We recently reported data from two multi-center studies of 728 (Sapphire) and 408 (Topaz) critically ill patients where a panel of urinary tissue inhibitor of metalloproteinases-2 (TIMP2) and insulin-like growth factor-binding protein 7 (IGFBP7), both markers of cell cycle arrest, were validated for risk stratification for moderate to severe AKI (KDIGO stages 2 and 3). Here we report performance characteristics of this novel biomarker panel ([TIMP2]•[IGFBP7]) in patients with DM with or without CKD.

Methods: We conducted subgroup analyses of patients with DM with and without CKD from the Sapphire and Topaz studies. Subjects were enrolled and samples obtained within 24 hours of ICU admission. We constructed receiver operating characteristic (ROC) curves using urinary [TIMP2]•[IGFBP7] for prediction of moderate to severe AKI within 12 hours. We also examined the relative risk (RR) for AKI using a pre-specified cutoff for [TIMP2]•[IGFBP7] of 0.3.

Results: There were 210 and 116 patients with DM in Sapphire and Topaz, respectively, of which 36 and 14 had CKD. Demographics for the two studies were similar. Overall, 52 patients (16%) developed moderate-severe AKI. For diabetic patients, the area under the ROC curve (AUC) was 0.83 (95%CI 0.77-0.89); the RR for AKI above the 0.3 cutoff was 13 (95%CI 4-40). When analyzed separately, the AUC and RR for diabetic patients without CKD were 0.82 (95%CI 0.75-0.88) and 9 (95%CI 3-30), respectively, and with CKD were 0.89 (95%CI 0.80-0.99) and 22 (95%CI 1-361), respectively. In the absence of AKI, neither DM nor DM with CKD resulted in [TIMP2]•[IGFBP7] distributions above the normal range.

Conclusions: For patients with DM with or without CKD, the urinary [TIMP2]•[IGFBP7] test accurately identifies patients at risk for developing AKI within 12 hours. Interquartile range for normals.

Funding: Pharmaceutical Company Support - Astute Medical

PUB024

ACE - Inhibitors (ACE-I) and Angiotensin Receptor Blockers (ARB) in the Elderly: Not so Safe Andrea Guarnieri, Serena Bainotti, Elisabetta Moggia, Carmela Pino, Paola Inguaggiato, Maria Pia Giorgi di Vistarino, Alfonso Pacitti. *Nephrology and Dialysis Unit, S. Croce - Carle Hospital, Cuneo, Italy.*

Background: ACE-I and ARB are widely prescribed in any age group. The general applicability of the recommendations of the large RAAS blockade trials to especially the older CKD patient population reserves some doubts because several trials were short term studies, often enrolling younger patients with relatively few comorbid conditions and a very strict clinical control. The aim of the study was to evaluate the incidence and course of AKI due, according to clinical data and medical history, to the use of ACE-I or ARB in old patients hospitalized in our renal care unit.

Methods: Retrospective study of all old patients (age > 75 years) admitted from 1/1/2009 to 12/31/2013. 55 patients (18% of total AKI), affected by AKI due to drugs interfering with RAAS, 27 men and 28 women, mean age 82 years, treated with ACE-I (35), ARB (19), ACE-I + ARB (1). Concurrent CV disease 53%, diabete mellitus 29%, pulmonary disease 29%, multiple diseases 33%. AKIN stages: 1 29%, 2 11%, 3 60%. AKI triggered by: enteritic syndrome 49%, use of diuretic with simultaneous reduction of fluid intake 44%, pneumonia 5%, NSAID use 2%.

Results: Average days of hospitalization 8.5 +/- 4.5.

Blood chemistry.

Creatinine IN (mg/dL)	5.2 +/- 3.4
Creatinine OUT (mg/dL)	1.5 +/- 0.6
K IN (mEq/L)	5.3 +/- 1.4
K OUT (mEq/L)	4.3 +/- 0.6

Two patients needed hemodialysis with subsequent functional recovery. Two patients died with AKI without need for dialysis.

Conclusions: Our data underestimate the true incidence of the disease because not infrequently patients with such features are hospitalized in other medical departements. However some considerations are possible: 1. The incidence remained constant over the observed years without seasonal variations. 2. In 44% of cases, only a reduction in fluid intake occured, without any significant intercurrent disease. 3. After all the prognosis was benign, in 96% a functional recovery occurred and the mean length of hospitalization was comparable to the annual average. 4. No absolute controindications to RAAS blockade in old patients, but extreme caution and stop in risk of hypovolemia.

PUB025

Retroperitoneal Fibrosis: A Retrospective Descriptive Study on Clinical Features and Management Ann-Sophie Laroche, Robert Zoël Bell, Caroline Lamarche, Michel Vallee. *Nephrology, Hôpital Maisonneuve-Rosemont, Montréal, QC, Canada.*

Background: Our study was meant to describe the clinical characteristics, diagnostic methods and treatments of patients with retroperitoneal fibrosis (RPF).

Methods: We conducted a retrospective analysis of patients diagnosed with RPF at Maisonneuve-Rosemont Hospital.

Results: We identified 17 patients with RPF between 1998 and 2013. Eight patients were female (47%) and the mean age was 62 years. Eleven cases were idiopathic, four were secondary to a neoplasia, one was thought to be in the setting of an IgG4-related disease and one was caused by radiotherapy. Back pain was the most common symptom. All diagnosis were made based on the finding of a retroperitoneal mass on the computed tomography (CT) imaging. Three patients (17.6%) had histological diagnosis of RPF and seven had unspecific changes on their biopsy. There were no biopsy for 7 patients (41.1%). Ten patients needed double-J stents, 3 had a temporary percutanous nephrostomy and 2 patients had to have a nephrectomy. Only one patient required hemodialysis (5.8%). 10 patients received medical treatment including corticosteroids, tamoxifen, methotrexate and colchicine. Of the 5 patients with complete remission (29.4%), only two received treatment with prednisone, while the other three did not received any medication. There were 7 patients with partial reduction of the inflamed mass and overall improvement with treatment, 3 with prednisone only and 3 with the combination of prednisone and methotrexate. Only one did not receive any treatment. The remaining five patients were non responders – three did not receive treatment, one had one course of prednisone and another was treated with colchicine.

Conclusions: In conclusion, most of our cases of RPF were idiopathic and were diagnosed with CT scans. Most of the treated patients received prednisone and seem to respond, at least partially. There was a lot of heterogeneity in patient management, which makes it difficult to compare treatment effects. There seems to be a better outcome when treatment is adjusted based on acute-phase reactant biomarkers and CT imaging, than only CT imaging. Overall, untreated patients appear to have a worse outcome than treated patients.

Funding: Government Support - Non-U.S.

PUB026

Cisplatin Nephrotoxicity in Children with Solid Tumors Climaco Andres Jimenez Triana,[1] Rodolfo Rivas-Ruiz,[1] Osvaldo D. Castelan_martinez,[1] Bruce Carleton,[2] Mara Medeiros.[1] *[1]Hospital Infantil de Mexico, DF, Mexico; [2]BC Children´s Hospital, Vancouver, Canada.*

Background: Objective: To evaluate the prevalence and severity of cisplatin nephrotoxicity in Mexican children and to determine the impact of nephrotoxicity on height and weight.

Methods: Retrospective study of 54 children treated with cisplatin for solid tumors. From the medical record, the information about height, serum creatinine and electrolytes in each cisplatin cycle and after 12 months of treatment was recorded. Nephrotoxicity was graded as follows, 0: normal renal function, 1: asymptomatic electrolytes disorders in blood work, grade 2: need for electrolyte supplementation and/or increase in serum creatinine 1.5-1.9 times from baseline, grade 3: increase in serum creatinine at 12 months 2-2.9 times from baseline or need for electrolyte supplementation for more than 3 months after treatment completion, grade 4: renal replacement therapy.

Results: 41 patients had nephrotoxicity (NTX) (76%). Hypophosphatemia was found in 35 patients, hypomagnesemia in 22, hypokalemia in 20 patients. 13 patients had no nephrotoxicity, Grade 1 NTX was observed in 18, Grade 2 in 5 patients and Grade 3 in 18 patients. Patients without NTX maintained their z Score for height after 12 months of treatment, whereas those with NTX had worse z Score for height after 12 months of treatment, being more accentuated in those with NTX grade 1 than grades 2 and 3. There was no difference in z Score in weight (basal versus 12 months) in all groups (Figure 1). The cisplatin total dose had a significant negative relationship with magnesium levels at 12 months (Spearman r =-0.527, p=0.0001).

Conclusions: NTX prevalence was 76%. NTX patients were younger and received higher cisplatin doses than those with normal renal function. Patients receiving cisplatin should be monitored during each cycle of treatment for evidence of grade 1-4 nephrotoxicity. Even asymptomatic electrolyte abnormalities can cause clinically significant effects on height. Serum magnesium is likely to fall with increasing cisplatin dose and should be specifically monitored as cumulative cisplatin exposure increases. Hypomagnesemia can cause significant neurological effects.

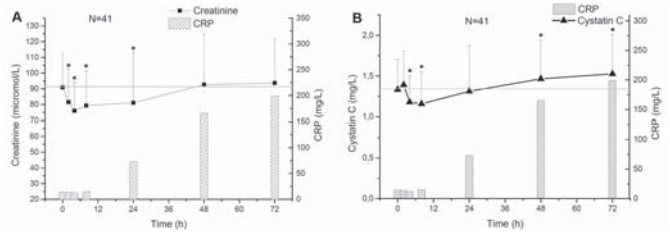
PUB027

Serum Cystatin C as Diagnostic Marker of Acute Kidney Injury after Cardiopulmonary Bypass: A Word of Caution Anders Svensson,[1] Csaba P. Kovesdy,[2,3] John-Peder Escobar Kvitting,[4] Ingemar R. Cederholm,[4] Zoltan Szabo.[4] *[1]Ostergotland Heart Centre Univ Hospital; [2]Memphis VAMC; [3]Univ of Tennessee; [4]Linkoping Univ Hospital.*

Background: Serum cystatin C may be an alternative to serum creatinine to estimate glomerular filtration rate and to diagnose post-operative acute kidney injury (AKI) after cardiac surgery. Serum creatinine and cystatin C may, however, have different postoperative dynamics the reasons for which are unclear.

Methods: Post-operative changes in serum creatinine and cystatin C were followed in 41 patients with normal pre-operative kidney function undergoing cardiac surgery using cardiopulmonary bypass (CPB). Inflammation and AKI were examined by measuring pre- and post-CPB C-reactive protein (CRP) and neutrophil gelatinase associated lipocalin (NGAL), respectively. Intra-individual differences in creatinine and cystatin C were examined using repeated measures ANOVA and Tukey's HSD. The effect of inflammation on intra-individual changes was examined using mixed effects regression.

Results: Both serum creatinine and cystatin C decreased significantly in the first 24 hours post-operatively (p<0.001). Serum creatinine levels returned to pre-operative levels (Figure, Panel A), whereas serum cystatin C continued to rise and were significantly elevated at 48 and 72 hours post-CPB compared to pre-operative levels (Figure, Panel B). CRP levels increased significantly post-CPB and were significantly associated with increases in both serum creatinine and cystatin C in mixed models (p<0.001). AKI occurred in two patients, and their exclusion did not affect the results.



Conclusions: Serum creatinine and cystatin C have different post-CPB dynamics. The reasons for this are unclear, but may include processes unrelated to changes in kidney function.

PUB028

Incidence of Acute Kidney Injury in Critically Ill Patients at an Urban Community Hospital Jiandong Wei, David Kau, Spiro Demetis, Reynaldo G. Tan. *Medicine, Lutheran Medical Center, Brooklyn, NY.*

Background: Acute kidney injury (AKI) is a common condition in critically ill patients and is often associated with poor prognosis. More accurate data about the incidence and early recognition of risk factors for AKI will improve awareness and early prevention of AKI in community-based hospitals.

Methods: Patients who were admitted to the intensive care units (ICUs) at Lutheran Medical Center, an urban community hospital from July 2013 to May 2014 were screened for development of AKI from first ICU day to 7 consecutive days. Patient demographics, co-morbidity and survival status at hospital discharge were compared in patients with or without AKI. Pearson Chi-Square was used for data analysis.

Results: 360 were enrolled in the study. 46 (12.8%) patients developed AKI, 63 (17.5%) did not have AKI, 251 (69.7%) patients did not complete screening due to being transferred out of the ICUs. Patients with age ≥ 75 have higher incidence of AKI than those under age of 75 (18.0% versus 9.0%, p = 0.025). AKI has the highest incidence in medical ICU when compared to surgical ICU and cardiac ICU (16.7%, 7.6% and 11.1% respectively, p = 0.003). There was no difference of AKI incidence in patients among different race, with or without type 2 diabetes mellitus, chronic liver disease or morbid obesity. Hospital mortality was 47.8% in critically ill patients with AKI, 15.9% in those without AKI abd 9.2% in patients who were transferred out of the ICUs in less than 7 days (p < 0.001).

Conclusions: This single-centered prospective observational study shows that AKI is commonly encountered and is associated with high mortality in critically ill patients in an urban community hospital. Patients with advanced age and admitted to the medical ICU have higher risk for developing AKI. Prevention of AKI starts in the community with prompt assessment of those at risk.

PUB029

Discrepancies between Urinary and Plasma NGAL Concentrations in Patients Admitted to the Medical Intensive Care Pierre Delanaye,[1] Bernard Lambermont,[1] Philippe Morimont,[1] Jean-Marie H. Krzesinski,[1] Nicolas Maillard,[2] Guillaume Claisse,[2] Christopher R. Mariat,[2] Etienne Cavalier.[1] *[1]Univ of Liège; [2]Univ Jean Monet, Saint-Etienne.*

Background: Both plasma and urinary NGAL concentrations are presented as useful tests to detect acute kidney injury (AKI) in intensive care units (ICU). However, little is known about the concordance between these two biomarkers at the individual level.

Methods: Urinary and plasma NGAL were measured in ICU patients within the first 24h after admission. The same method was used to quantify NGAL both in urine and plasma (Bioporto, Gentofte, Denmark). For urinary NGAL, both absolute value and ratio to urinary creatinine were considered. Different cut-offs were used for the diagnosis of AKI in the two milieus: 100, 150, 200 or 300 ng/mL. We focused on the cut-off value of 150 ng/mL. Discrepant results were patients with higher values than a defined threshold (for example 150 ng/mL) in the urine but lower in the plasma, or conversely.

Results: We included 98 patients: mean age of 64±15 y, 59% of men. Median [IQR] plasma concentrations was 218 [118-440] ng/mL. Median urinary concentrations (absolute and ratio) were 57 [23-295] ng/mL and 115 [52-479] ng/g. Considering the absolute urinary NGAL concentrations, we observed a concordance in the AKI diagnosis between urinary and plasma results which ranges from 40 to 76%, according to the cut-offs used. The best concordance was observed for a cut-off of 300 ng/mL and 150 ng/mL for the plasma and the urine, respectively. If the cut-off of 150 ng/mL was considered for both milieus, the global concordance was 64%. In the 62 patients with concordant values, NGAL was higher than 150 ng/mL in 53%. In the discordant values, the plasma concentration was higher than 150 ng/mL in 33 patients. Only 2 patients had urinary concentrations higher than 150 ng/mL although plasma concentrations remained lower. Regarding urinary concentration with the ratio, the diagnosis concordance ranged from 45 to 73%.

Conclusions: At best, our study showed that measurement of NGAL in the urine versus plasma was discordant in 25% of ICU patients. In most discrepants, the urinary NGAL was below the defined cut-off whereas the plasma NGAL was above.

PUB030

Incidence and Outcomes of Acute Kidney Injury in Unselected Admissions to a Large Tertiary-Care Mexican Facility Jonathan Chavez, Paulina Albarran, Guillermo Garcia-Garcia. *Nephrology Div, Hospital Civil de Guadalajara, Guadalajara, Jal, Mexico.*

Background: Acute kidney injury (AKI) is a common and serious problem in hospitalized patients. Frequently, AKI is not detected and these cases are commonly not referred to nephrology, resulting in inadequate care. We evaluated the incidence and outcomes of acute kidney injury in unselected admissions, with and without nephrology referral, in a large tertiary-care Mexican facility.

Methods: We reviewed the records of 402 patients admitted to the hospital in a single day. Patients ≤ 15 years old and those admitted to nephrology or obstetrics services were not included. Patients were followed until their demise or discharge. Outcome measures were the presence or absence of AKI using KDIGO criteria and mortality. Student T test and χ^2 were used when appropriate.

Results: 127 (32%) patients were identified with AKI; they were predominantly medical cases (56% versus 33%, p=0.000) male (65% versus 52%, p= 0.01)), older (median age 67 versus 52 y, p=0.05) and diabetic (43% versus 17%, p=0.000) than those without AKI. 47 (37%) cases were KDIGO I, 42 (33%) KDIGO II, and 38 (30%) KDIGO III. Main AKI causes were sepsis (36%), hypovolemic (30%), and nephrotoxicity (18%). Only 14 (11%) cases were referred to nephrology. Results of patients with and without nephrology care are described in table 1.

	Nephrology referral n (%)	No nephrology referral	p
	14 (11)	113 (89)	
Age (y)	69 (46-94)	49 (22-76)	0.02
Diabetes	9 (64)	44 (39)	0.12
Hypertension	8 (57)	29 (25)	0.03
Deaths	6 (43)	19 (17)	0.05
Deaths by KDIGO stage			
KDIGO I	0	0	
KDIGO II	0	10 (9)	
KDIGO III	6 (43)	9 (8)	0.000
SCr double than baseline at discharge	5 (36)	34 (30)	0.92
Outpatient follow up	8 (57)	1 (0.88)	0.05

Conclusions: AKI occurred frequently in hospitalized patients, affecting 32% of unselected admissions to our hospital; the majority of these cases were not referred to nephrology care and AKI resolved spontaneously, but it was associated to an increased risk of death. Also, a large percentage of these patients had a high serum creatinine at discharge and lacked outpatient follow-up. Nephrology referral and intervention may result in better clinical outcomes.

PUB031

Renin Angiotension System Inhibitor in Treatment of Lupus Nephritis with Thrombotic Microangiopathy Chao Li, Hang Li, Yu-Bing Wen, Hai-Yun Wang, Jie Ma, Ying Wang, Bing-Yan Liu, Rui-Tong Gao, Jian-Ling Tao, Yan Qin, Qun-Sheng Yuan, Ying Su, Yang Yu, Ming-Xi Li, Xue-Mei Li, Xuewang Lee. *Nephrology, Peking Union Medical College Hospital, Beijing, China.*

Background: To investigate whether RAS inhibitor can improve the renal function in treatment of lupus nephritis (LN) with thromboticmicroangiopathy (TMA)confirmed by renal pathology.

Methods: 15 LN patients with TMA proven by renal biopsy, from January 2000 to December 2013 in PUMCH, were enrolled. The changes of SCr and BP before and after using RAS inhibitor were analyzed retrospectively.

Results: (1)Male/female ratio is 1:14. All of the patients had renal dysfunction, and median peak value of creatinine was 396μmol/L (160-643μmol/L). 5 cases (33.3%) required acute dialysis during hospitalization. Hypertension occurred in 15 patients, while 6 cases (40.0%) were diagnosed malignant hypertension. (2) Anemia and thrombocytopenia occurred in 15 and 14 cases, respectively. 3 cases (20.0%) and 5 cases (33.3%) were diagnosed MAHA definitely and probably, respectively. (3) Renal biopsy showed class II in 1 case, III in 4, IV-(G) in 2, IV(S) in 5 and IV+V in 3 cases. Active lesions were predominant in glomeruli, as well as renal vasculopathy. (4) All the patients received immunosuppressive therapy, of whom 9 cases were given steroid pulse therapy, 13 case received cyclophosphamide, and the rest 2 cases also receive mycophenloate. Only 5 patients' creatinines (55.6%) decreased after steroid pulse therapy. In 13 patients (86.7%), hypertension ameliorated and SCr decreased within one week after implementing RAS inhibitors, which falled medianly 15.8% and 17.0%, repectively. (5) 11 Patients were followed from 8 to 135 months (median 32 months), although 4 patient were lost to follow up. 5 cases on dialysis during hospitalization didn't require renal replacement therapy, while renal function of other cases improved further.

Conclusions: Patients diagnosing LN with TMA who develop AKI and refractory hypertension should be treated with RAS inhibitors. Improved renal survival and successful discontinuation of dialysis are possible benefits when ACE inhibitors are used to treat LN with TMA.

PUB032

How Important Is GFR in Heart Failure Re-Admission? Mohinder Reddy Vindhyal, Brent A. Duran, Hussam H. Farhoud, Ken James Kallail. *Internal Medicine, KUSM - Wichita, KS.*

Background: Heart Failure is the leading cause of morbidity and mortality, as well as hospitalization rates in the U.S. An impetus has been created to identify improved predictors to prevent hospital readmission. The average life span of patients admitted to the hospital for the heart failure is 5.5 years. The aim of this study was to determine if renal function (GFR) has an association with heart failure readmissions. Even though the DOSE trial validates that the kidney function (GFR and creatinine) will be normal two months after placing them on high dose diuretics in heart failure patients, but doesn't mention about readmissions or the renal function status with in the month. Moreover CKD patients were excluded from the study.

Methods: A retrospective cohort study was performed utilizing data from three community hospitals in the United States. A total of 132 patients with heart failure were evaluated over one year comparing *glomerular filtration rate (GFR)* at admission and discharge and 30-day readmission status.

Results: There is a significant difference by readmission status in the change in GFR from admission to discharge. The GFR of patients readmitted in 30 days had an average decrease in GFR by 2.46 mL/min/1.73 m^2 whereas patients not readmitted in 30 days had an average increase in GFR by 1.92 mL/min/1.73 m^2. In the 28 readmitted patients, 13 (46%) had a decrease in GFR, 6 (21%) had an increase, and 9 had no change (32%). In the 99 patients not readmitted, 33 (33%) had a decrease in GFR, 48 (48%) had an increase, and 18 (18%) had no change.

Conclusions: A decline in renal function over hospitalization in patients with heart failure is associated with an increase in readmission for heart failure. Providers should be cognizant of the need to optimize renal function as well as cardiac function during hospitalization.

PUB033

Efficacy and Morbidity of Urinary Derivation (UD) to Treat Obstructive Uropathy (OU) Related Acute Kidney Injury (AKI) in Cancer Patients Renato Antunes Caires,[1] Veronica T. Costa e Silva,[1] Regis Franca Bezerra,[3] Elerson Costalonga,[1] James Hung,[1] Luis Yu,[1] Emmanuel A. Burdmann,[1] William C. Nahas,[2] Mauricio Dener Cordeiro,[2] Rafael Ferreira Coelho.[2] *[1]Nephrology Div, Sao Paulo State Cancer Institute - Sao Paulo Univ Medical School, Sao Paulo, Brazil; [2]Urology Div, Sao Paulo State Cancer Institute - Sao Paulo Univ Medical School, Sao Paulo, Brazil; [3]Radiology Div, Sao Paulo State Cancer Institute - Sao Paulo Univ Medical School, Sao Paulo, Brazil.*

Background: There is scanty data on efficacy and morbidity of UD to treat OU related AKI in cancer pacients (pcts).

Methods: We prospectively analyzed UD procedures in OU related AKI adult cancer pcts in the Sao Paulo State Cancer Institute, from March 2009 to December 2011. AKI was defined as an increase > 50% in the baseline serum creatinine (SCr). Renal function recovery (RFR) was defined as return to baseline SCr. Radiology tests were assessed by a radiologist with expertise in cancer urinary diseases.

Results: A total of 109 AKI pcts were assessed. Pcts' characteristics were mean age 57.5 +/- 15 y and 56% female. Tumor origins were: gynecologic system 34.8%; genitourinary tract 33%; gastrointestinal tract 24.7%. Metastasis was observed in 49.5% of pcts. Baseline SCr was 1.0 (0.82 – 1.33) mg/dL and six months mortality was 53.2 %. OU was diagnosed by computed tomography in 87.2% of pcts, considered bilateral in 86.2% and graded at level 3 or 4 in 70% of them. Percutaneous nephrostomy (NT) was performed in 81.7% of pcts. At UD, blood tests were: SCr 4.48 (2.91 – 7.13) mg/dL; urea 108 (71 – 150) mg/dL; K 4.5 (5 – 3.9) mEq/L; Hb 8.3 (7.5 – 9.35) g/dL; platelet 326,000 (203 – 403)/mm^3 and INR 1.1 (1.0 – 1.29). Hemodialysis was necessary in 30 (27.7%) pcts. Hematuria requiring blood transfusion was observed in 9.3% pcts in the immediate post UD period. At 30 days, SCr was 1.4 (1.0 – 1.97) mg/dL, RFR was observed in 68% of pcts and only four pcts remained dialysis dependent. During follow-up, NT repositioning was necessary in 12.3% of pcts and pyelonephritis was observed in 23.9% pcts.

Conclusions: UD was an efficient treatment for OU related AKI in cancer pcts, with high rate of RFR and acceptable morbidity.

PUB034

Acute Kidney Injury among Very Elderly Patients Hospitalized in a Single Center: Epidemiology and Outcomes from a Retrospective Cohort Ederson Vidal Moura, Hugo Pinheiro, Cintia Germana Mergulhão da Costa, Gisele Vajgel Fernandes, Luis H.B.C. Sette, Geraldo José de Amorim. *Nefrologia, Hospital das Clínicas - Univ Federal de Pernambuco, Recife, Pernambuco, Brazil.*

Background: Acute Kidney Injury (AKI) is commom condition among in-hospital patients, but only a few studies evaluated the epidemiological profile and outcomes in very elderly patients (VEP).

Methods: We analized medical records of nephrology referrals (NR) from January 2011 to December 2013 in order to identify very elderly patients (older than 75 years) who developed AKI during in-hospital stay. AKI was classified at the first day of NR based on Scr AKIN criteria and CKD was defined based on prior baseline Scr >1.3 mg/dL.

Results: Among 778 medical records analyzed, 80 of them were VEP with AKI and acute on-CKD (A-CKD), with mean age 82y. AKI was found in 55 VEP (68.75%) and

A-CKD was found in 25 patients (31.25%). There was female predominance in AKI group (52.7%) and male predominance in A-CKD group (76%). Ischemic etiology was the main renal injury factor (42% of all cases) and dialysis requirement was higher in AKI group (41.8%) compared with A-CKD group (36%), but without significance (P=0.80). Mean Charlson Commorbitity Index was 6,41 (95%CI: 5.75 - 7.06) in AKI patients and 5,44 (95%CI: 4.52 - 6.36) in A-CKD group.Sepsis was found in 45% of AKI group and 32% of A-CKD patients, and pneumonia was the major cause in 32% and 50% of groups patients, respectively. Overall mortality rate was 41% and unadjusted analysis showed higher mortality in AKI group (36.4%) when compared to A-CKD group (16%), P=0.033. There was no difference in mortality among patients with dialysis requirement compared to non-dialytic patients both in AKI and A-CKD groups: P=0.73 and P=0.53, respectively. At the time of the NR, AKIN 3 was the most common stage of renal injury found in AKI group (30.9%), and AKIN 1 was more prevalent in A-CKD patients (40%) than AKI patients (25.4%), P=0.014.

Conclusions: Very elderly patients have a high incidence of AKI and high mortality rate. When compared to A-CKD patients, the AKI group have more prevalence of sepsis, worse renal dysfunction at time of nephrologist referral and higher mortality rate.

PUB035

Acute Kidney Injury in United Arab Emirates, Incidence, Etiology, and Outcome Bassam O. Bernieh, Yousef Boobes, Mohamed Raafat Al Hakim, Qutaiba Hussain Daoud, Mohamed E.O. Ahmed, Ahmed Ahmed Ahmed, Hanan Eljack, Imran Khan, Ahmed Chaaban, Walaa Dabbas, Osama S.R. Ouda, Amany Hillis. *Nephrology, Tawam Hospital, Al Ain, Abu Dhabi, United Arab Emirates.*

Background: Acute kidney injury (AKI) is a public health burden increasing, morbidity, mortality, Health care costs, and associated with increased risk for the development of CKD and ESRD. The incidence of AKI is not well known in United Arab Emirates (UAE). In the current study, we are reporting the incidence, etiology and the outcome of AKI in our tertiary care institution.

Methods: retrieving from the hospital HIS, all adult patients with the ICD-9 code of AKI, and ARF, for six months period from 01/01/2013 till 30/06/2013. Inclusion criteria: Patients with ICD-9 code of AKI / ARF, and fulfilling the KDIGO definition of AKI. Total number of patients who fulfilled the above criteria was 460. Total number of adult patients admitted in the hospital during the same period was 7352. Number of patients included in the analysis was 388/460 (84%).

Results: total number of patients 388, 221 (57%) were male, mean (SD) age was 63.6 (18) year. 214 (55%) were UAE nationals. Long of stay in the hospital was 14.7 (15.3) days. Incidence of AKI in this cohort was 62.6/ 1000 patient admissions. 21% of the patients were in ICU and 79% in the inpatient wards. Dehydration and sepsis were the etiology of AKI, encountered in 51 and 47% respectively. Hypertension and diabetes were the most common comorbidities, found in 72 and 59% of the patients respectively. Only 53% were referred to nephrology. 73% had KDIGO stage I AKI, 20% stage II, and 7% stage III. 76% had community acquired, and 24% had hospital acquired AKI. 89% were managed conservatively, and 11% required renal replacement therapy. Renal outcome: 45% had full recovery of kidney function, 38% had partial recovery, and 17% did not recover. Patients' outcome: 77% survived, and 23% died. Age> 65, presence of CKD, or malignancy were all significantly associated with bad patients' outcome.

Conclusions: AKI has a high incidence in the UAE community, it carries high morbidity and mortality, affecting mainly old age group. More efforts are required to validate this data at the national level.

PUB036

A Case of Tenofovir Induced Distal RTA Causing AKI Sravan Jasti,[1] Ashwin Narasimhan,[2] Nishitha Cherukumalli.[3] *[1]Nephrology, Albert Einstein, Philadelphia, PA; [2]Medicine, Our Lady of Fatima, Providence, RI; [3]Nephrology, Upenn, Philadelphia, PA.*

Background: 62 year old male with hx of latent syphilis and HIV on Truvada therapy came with generalized weakness. No prior history of autoimmune disease was noted.Admission BP was108/80,Pulse-74, RR-12, T-98 Physical exam showed no crackles, lymphadenopathy, murmurs or organomegaly. Pertinent labs:Hgb-11.7,K-1.6,CO2-13,Cr-4.7,Ca-7.3,Phos-4.1,Alb-1.7,Mg-1.9,CPK-16310,AG-16,Urine Na-98,UrineK-28,UrineCl-124,Urineurea-122,Urine Uric Acid-14,Urine Creatinine-16,Urine Phosphorous<5,UrinaryAG-2.0,UA showed large blood.Baseline creatinine was 0.7 and bicarbonate was 24 prior to HAART therapy.

Methods: With no Glycosuria or elevated fractional excretion of phosphorous and Uric acid,distal RTA was diagnosed.AKI was suspectedfrom underlying Rhabdomyolysis causing ATN .ID was consulted and HAART therapy was discontinued. Pt received 360 meq of KCL on day 1 with a standing order of KCL 40meq every 4 hrs for the next 4 days .Once potassium improved to 3.2 mmol/lit, he was started on Bicitra to correct his acidosis. He was discharged 6 days later with a serum potassium of 3.4 mmol/lit, bicarbonate of 22 mmol/lit. on KCL 40 meq daily with Bicitra 15ml bid. His CPK improved to 10,000 and his creatinine normalized upon discharge.His HAART therapy was put on hold.

Results: Tenofovir disoproxil fumarate is nucleotide reverse transcription inhibitor eliminated via glomerular filteration and active tubular secretion,enters proximal tubule cells via human organic anion transporters 1 and 3 and exits cells via multidrug resistance protein.It causes nephrotoxicitywith increases in creatinine,glycosurea, hypophosphatemia and ATN.Ifosfamide,valproic acid and antiretrovirals such as Tenofovir cause proximal RTA. Distal RTA is generally asymptomatic but may rarely present as hypokalemic paralysis In a study of 271 patients, 10 percent initiated with TDF developed AKI .In a French Study of 222 patients with HIV, 29 had tubulopathy fromTDF.

Conclusions: Although Tenofovir is known to cause proximal RTA this seems to be the first case of Distal RTA .The mechanism of distal RTA is unclear,tenofovir could lead to distal tubular damage with it being a known mitochondrial toxin.

PUB037

Urinary Exosomal miRNA27b in Patients with Acute Decompensated Heart Failure Ping Li,[1] Usman J. Rahmat,[1] Jonathan Street,[2] Ethan Ko,[1] Richard Amdur,[1] Sonya Malekzadeh,[1] Federico E. Mordini,[1] Lakhmir S. Chawla,[1] Peter S.T. Yuen,[2] Carlos E. Palant.[1] *[1]Medical and Research Services, Washington DC VAMC, Washington, D.C.; [2]NIDDK, NIH, Bethesda, MD.*

Background: Previous studies show that development of acute kidney injury (AKI) during inpatient hospitalization for acute decompensated heart failure (ADHF) is associated with increased morbidity/mortality. Urinary biomarkers may become a useful tool to monitor renal function in ADHF patients. Urinary miRNA27b is a marker of AKI in animal models. We hypothesized that increased urinary excretion of miRNA27b might be associated with renal tubular damage in ADHF.

Methods: 21 patients were studied and divided into 3 groups: 8 controls (CON), 5 stable CHF patients, and 9 ADHF hospitalized patients. One urine for each CON and stable CHF patient was analyzed and 3 urine specimens were collected in ADHF patients on consecutive days. Urinary miRNA27b was quantified via multi-step ultracentrifugation and quantitative reverse transcription PCR (Taqman). Urine microRNA27b was normalized with urinary creatinine. Patients with hepatitis B and/or C, HIV infection or urinary infections were excluded. One way ANOVA was used for statistical analysis.

Results: Mean baseline serum creatinines (SC) were: 1.05 ± 0.16, 1.06 ± 0.27 and 1.46 ± 0.52 mg/dL for CON, stable CHF and ADHF patients respectively. Ejection fraction was 24 ± 11% and 22 ± 5.3% for stable CHF and ADHF patients respectively. Urinary miRNA27b were 441 ± 518, 1524 ± 842 zmoles/mg in CON and stable CHF groups respectively. In ADHF patients, microRNA27b levels were 1273 ± 1091, 1352 ± 1471, and 2057 ± 1872 zmoles/mg ($p<0.01$ ADHF day 3 versus CON)and SC levels were 1.46 ± 0.52, 1.53±0.70 and 1.11±0.17 mg/dL on day 1, 2 and 3 following IV diuretics.

Conclusions: miRNA27b is found in the urine of human subjects. In this pilot study, patients with ADHF showed a tendency to excrete higher concentrations of miRNA27b than CON patients. A larger clinical trial seems justified by these preliminary data.

PUB038

Impact of Severe Acute Kidney Injury on Long Term Survival Muhammad Taufiq Dawood, Vivienne Ralph, Christine Sarah Catley, Anthony Chan, Abdelgalil Abdelrahman Ali, Sumith C. Abeygunasekara. *Renal Unit, Mid Essex Hospitals NHS Trust, Chelmsford, Essex, United Kingdom.*

Background: We looked at patient mortality in hospital, at thirty days and at one year after suffering an episode of severe Acute Kidney Injury (AKI), and to determine factors predictive of a poor outcome.

Methods: Data was collected from August to November 2012 of patients who developed AKI stage 3 (RIFLE criteria) during admission to a district general hospital in the UK. The patient demographic data included co-morbidities, drugs and renal function on discharge, as well as the date of death.

Results: A total number of 271 patients with AKI stage 3 (RIFLE criteria) were identified during the 6-month period. There were 48 in hospital deaths. Results showed 95 patients died within thirty days of developing AKI and 144 patients died within one year. Thirty-day all cause mortality was 35%, while twelve month mortality was 53%. The initial analysis of this data has revealed that there is a significant all cause mortality rate associated with these patients who have suffered an AKI.

Conclusions: It is clear from our study that patients who developed severe AKI have very high mortality as inpatients as well as on discharge. Positive predictive factors include advanced age, number of comorbidities and extent of the recovery of renal function prior to discharge. It is becoming increasingly clear that AKI is an important contributor to mortality generally, and that suffering an AKI is a more global process than its name suggests and may reflect a more generalized ischemic state. In the future AKI should not be viewed as simply a reversible process that is forgotten once 'normal' kidney function has returned. Rather it is an indicator of a significant insult to the body, and that it alone may increase mortality.

PUB039

Predictors of Mortality in Patients with Congestive Heart Failure (CHF) Receiving Darbopoeitin (DAR) a Potential Interaction Between DAR and Acute Renal Dysfunction (ARD) Jose I. Iglesias,[1] Savan Ghetiya,[2] Jerrold S. Levine.[3] *[1]Dept Medicine Section of Nephrology, Rowan Univ School of Osteopathic Medicine, Stratford, NJ; [2]Dept of Medicine, Medical Univ of Silesia, Katowice, Poland; [3]Dept of Medicine, Section of Nephrology, Univ of Illinois at Chicago, Chicago, IL.*

Background: Anemia and renal dysfunction commonly coexist in patients with congestive heart failure (CHF) and are known as the cardiorenal anemia syndrome (CRAS). CRAS is associated with adverse clinical outcomes. Although the treatment of CRAS with DAR has been extensively studied, there is a paucity of data regarding the use of DAR in patients admitted with decompensated CHF that is complicated by ARD.

Methods: We retrospectively examined the impact of DAR on 60-day mortality in 465 patients admitted with decompensated CHF of whom 155 developed ARD.

Results: Univariate analysis revealed that DAR use was associated with an increase in mortality (5.5% versus 15%, $p <.0.01$). By forward stepwise regression analysis,

however, DAR was not an independent predictor of mortality. The following variables were independent predictors of mortality: severe ARD (defined as a rise in SCr ≥ 0.5mg/dl)p=0.00001, OR 7.4, 95% 3.8-13 ; lack of renin angiotensin system (RAS) inhibition,p =0.009, OR 0.4, 95% CI 0.2-0.8. and advanced age, p=0.024, OR 1.05, 95% CI 1.007-1.09. When patients were stratified according to DAR use, only severe ARD was associated with mortality in pts receiving DAR. This effect was independent of the effect of anemia. In patients who developed ARD, the following variables were independent predictors of mortality: use of inotropes, p= 0.030, OR 3.49, 95 % CI 1.12-10.82, use of DAR, p= 0.0004, OR 6.29, 95% CI 1.8-21.8 and advanced age, p=0.012, OR 1.08, 95% CI 1.02-1.14.

Conclusions: Our data suggest that although DAR is not independently associated with an increased risk of mortality, the development of ARD in association with DAR usage may confer an increased risk.

Funding: NIDDK Support, Clinical Revenue Support

PUB040

Pharmacogenetics of Cisplatin Acute Kidney Injury (AKI)

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Background: Cisplatin is an inorganic metal-containing chemotherapeutic used to treat solid tumors, with AKI reported in one-third of patients. The current study represents preliminary data from a larger study (n=250) that is testing whether functional single nucleotide polymorphisms (SNPs) in genes relevant to metabolism and transport of cisplatin (*SLC22A2, SLC47A1, ABCC2, GSTA1, GSTP1, GGT1*) and antioxidant response to toxicity (*NFE2L2, KEAP*) associate with differential susceptibility to AKI in cisplatin-treated cancer patients.

Methods: Blood(5mL) was obtained from patients(n=89) who prospectively or retrospectively received cisplatin. Genotyping assessments included candidate SNPs in drug transporter(*SLC22A2, ABCC2, SLC47A1*), metabolism (*GSTA1, GSTP1, GGT1*) and toxicity response (*NFE2L2/KEAP1*) genes. Genotyping was performed using QuantStudio and genotypes were coded [0(wt/wt), 1(wt/var), 2(var/var)]. Glomerular filtration rate(GFR) was calculated from the MDRD equation. Univariate and multivariate analyses were performed using genotyping results and population covariates.

Results: Patient demographics(mean±sd) were age 53±13y, weight 81±21kg, BMI 27±7.4, Caucasian 97%, gender (51%M/49%F), and cisplatin dose (64±23mg/m^2). Univariate assessments demonstrated SNPs in *GSTP1*(rs1695), NFE2L2(rs2706110), and *KEAP1*(rs1048290) predicted GFR with p<0.2. BMI was the only non-genetic univariate covariate that predicted GFR. Two multivariate models predicted GFR(p<0.05). Model 1 included *GSTP1* and *KEAP1*: GFR = 0.0214 + 5.173(*GSTP1*) – 6.397(*KEAP1*); p=0.031. Model 2 included *GSTP1*, *KEAP1*, and *NFE2L2*: GFR = -1.322 + 5.366(*GSTP1*) – 6.638(*KEAP1*) + 4.748(*NFE2L2*); p=0.042. The multivariate models accounted for 12% of the change in GFR demonstrated during treatment with cisplatin.

Conclusions: The current preliminary data demonstrate the role of genetic variants in AKI due to cisplatin treatment. Ongoing studies are further refining the pharmacogenetic analysis and utilizing novel biomarkers as subclinical measures of AKI.

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PUB041

Is "NPO" a Risk Factor for AKI in Hospitalized Patients?

Saisindhu Narala, Linda Awdishu, Dinna Cruz, Yu-Ting Christi Kao, Ravindra L. Mehta. *Univ of California, San Diego, San Diego, CA.*

Background: The American Society of Anesthesiologists suggest that healthy patients should be NPO 6 hours from solids and 2 hours from liquids for certain hospital procedures. However, studies suggest that physicians are still recommending "NPO after midnight." Prolonged fasting regimens lead to increased hospital stay and mortality. We hypothesized that fasting orders would be longer than recommended and would be correlated with an increased incidence of AKI.

Methods: UCSD Medical Center admissions between 1/2011-12/2012 were screened using the EPIC database. Patients <18 years, ICU patients, admission <48 hours, transplant and emergent surgical patients, and patients requiring RRT were excluded. 94 patients with at least one order of NPO during their hospitalization were sampled. Demographics, procedure characteristics, exposures, and outcomes were collected. The specific NPO order restrictions, actual duration, and reasons were retrieved. SPSS 20 was used.

Results: The average ordered NPO duration was 15.0 hours and the actual NPO duration was 21.0 hours. A multivariate logistic regression model with risk factors for AKI revealed the following significant variables: multiple NPO orders during a hospitalization (p = 0.033, OR 3.59 [1.11-11.57]), nephrotoxin exposure (p = 0.017, OR 4.11 [1.29-13.1]), and >25% increase in NPO time above ordered (p = 0.076, OR 2.84 [0.74-9.36]). Multivariate regression for outcomes in the AKI population revealed the following: longer length of stay (p=0.003) and need for RRT (p=0.043).

AKI Stage	**n = 20 (21.3%)**		
Stage 1	n = 17 (85.0%)		
Stage 2	n = 2 (10.0%)		
Stage 3	n = 1 (5.0%)		
Demographic Characteristics	**No AKI (n = 74)**	**AKI (n = 20)**	**p-value**
Age > 60	41 (55.4%)	9 (45.0%)	0.456
Male gender	39 (52.7%)	12 (60.0%)	0.620
BMI > 30 (Morbidly obese)	22 (29.7%)	5 (25%)	0.786
Diabetes	19 (25.7%)	7 (35.0%)	0.411
CHF	8 (10.8%)	5 (25.0%)	0.141
HTN	6 (8.1%)	0 (0%)	0.336
CKD	11 (14.9%)	3 (15.0%)	1.00
Emergency Room Admission	16 (21.6%)	6 (30.0%)	0.552
Multiple NPO Orders	34 (45.9%)	15 (75.0%)	0.025
Contrast Exposure	26 (35.1%)	11 (55.0%)	0.127
Nephrotoxic Medication Exposure	29 (39.2%)	15 (75.0%)	0.006
Surgical Patients	35 (47.3%)	8 (40.0%)	0.620
Total Time NPO (hours)	39.0 (20.8-56.8)	45.0 (28.0-69.8)	0.468
> 25% Increase in NPO Time	38 (51.4%)	15 (75%)	0.076
Outcome Data	**No AKI (n = 74)**	**AKI (n = 20)**	**p-value**
Length of Hospital Stay (days)	5.5 (4.0-10.0)	11.0 (6.3-15.0)	0.003
Need for Renal Replacement Therapy	0 (0%)	2 (10.0%)	0.043
Transfer to Skilled Nursing Facility	11 (14.9%)	3 (15.0%)	0.208
Hospitalization Within 6 Months	33 (44.6%)	3 (15.0%)	0.019

Conclusions: Patients were ordered to be NPO longer than recommended by the guidelines: 15 instead of 6 hours for food. Multiple NPO orders during a hospitalization, increase in the actual duration of NPO over ordered, and nephrotoxin exposure were significantly associated with AKI. AKI was significantly associated with worse outcomes.

Funding: NIDDK Support

PUB042

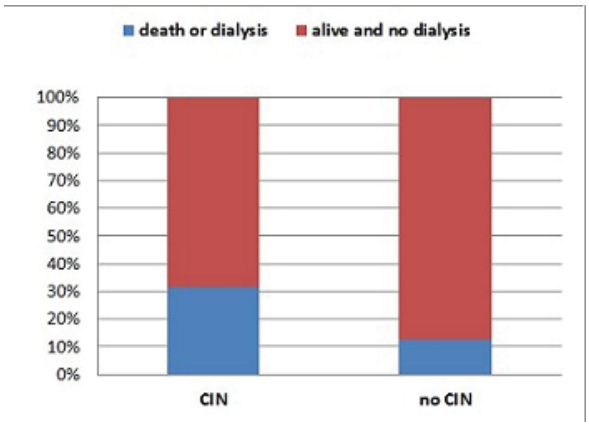
Contrast-Induced Nephropathy – Predictive Biomarkers and Long-Term Effects

Alina Baer,[1] Ralf Schindler,[1] Nadine Koch,[1] Patrick T. Murray,[2] Thomas Otto Joos,[3] Stephen R. Sultana,[4] Natalie M. Otto.[1] *[1]Charite Berlin, Germany; [2]Univ College Dublin, Ireland; [3]Univ of Tuebingen, Germany; [4]Novartis Companion Diagnostics.*

Background: Contrast-induced nephropathy(CIN) is reversible in most cases and rarely leads to dialysis therapy. Most studies concerning CIN prevention concentrate on the acute short-term situation; data on long-term consequences of CIN are scarce.

Methods: We analyzed the long-term effects of CIN in patients with a baseline-eGFR below 60 ml/min who were followed 1 year after contrast exposure. We measured 12 biomarkers after angiography and related them to long-term GFR progression as well as to mortality. 154 patients were followed 1 year after angiography.

Results: Overall, the study comprised 161 patients of which 22 suffered CIN. One year after angiography, mortality could be obtained for 154, the need for dialysis for 130 and renal function could be identified in 40 patients(16 with CIN and 24 non CIN). The mean increase of serum creatinine after one year was 0,77±1,52 mg/dl in the CIN group and 0,02±0,26 mg/dl in the non CIN group(p=0,0047). 3 patients in the CIN(18%) and no patients in the non CIN group developed end stage renal disease. One-year mortality was 18% in the CIN group and 8% in the non CIN group. Figure 1 shows the combined endpoint death or dialysis, being significant different(p=0.034) between CIN and non-CIN patients:



The ROC-Analysis of 12 biomarkers revealed the highest AUC value for combined clinical endpoint death or dialysis for cystatin C(0.85); IL-18(0.77); NGAL(0.77), serum creatinine(0.77) and RBP4(0.75).

Conclusions: Patients with CIN are at higher risk for worsening renal function or mortality 1 year after contrast exposure. Biomarkers measured at the time of CIN have a prognostic value for death or dialysis. Analyzing biomarkers at the time of CIN may prove to be a useful tool for risk stratification.

PUB043

The Incidence, Risk Factors and Clinical Outcomes of Acute Kidney Injury Associated with Scrub Typhus Kyungo Hwang, Eun Ju Lee, Hyun Seop Cho, Hyun-Jung Kim, Se-Ho Chang, Dong Jun Park. *Div of Nephrology, Dept of Internal Medicine, Gyeongsang National Univ Hospital, Jinju, Gyeongsangnam-do, Korea.*

Background: Renal involvement of scrub typhus is clinically various from simple urinary abnormalities to acute kidney injury (AKI) leading to death. Predictors to scrub typhus associated AKI were not well known. This study was done to evaluate incidence, predictors, and prognosis of scrub typhus associated AKI according to RIFLE (risk, injury, failure, loss, end-stage kidney disease) criteria.

Methods: We retrospectively evaluated medical records of patients diagnosed as scrub typhus from January 2001 to November 2013 in Gyeongsang National University Hospital.

Results: Male to female ratio was 48:52 and the mean age was 57.9±18.9 years old. Total 510 patients were diagnosed as scrub typhus during above time and AKI incidence was 35.9% in our study. Patients of risk, injury, and failure were 132 (73.4%), 37 (19.8%), and 14 patients (6.8%), respectively. In comparison with patients in non-AKI group, the patients in AKI group were older (73.9 versus 63.4, p<0.001) and had more co-morbidity such as hypertension, diabetes mellitus (DM), and chronic kidney disease (CKD). AKI frequently occurs in patients with HT taking ARB or ACEi (p=0.002) and diabetes which were higher HbA1c values (p=0.033). Hematuria and proteinuria were more frequent in AKI group. There was no positive relationship between severity of proteinuria and occurrence of AKI. More ICU care and death was present in AKI group. Renal function of most AKI patients recovered without sequelae except for one patient who had underlying DM CKD. Multivariate analysis showed that age, presence of CKD, and serum albumin values were independent risk factors for predicting AKI in patients with scrub typhus (p=0.015, p=0.049, p<0.001).

Conclusions: AKI according to RIFLE criteria in patients with scrub typhus is frequent and may lead to death although AKI in scrub typhus is usually mild and renal recovery occurs in most patients. Physician should suspect scrub typhus and start treatment early if patients with acute febrile disease accompanied with AKI were admitted.

PUB044

Dengue Viral Infection: Culprit of Acute Kidney Injury Yusra Habib Khan,[1] Azmi Sarriff,[1] Amer Hayat Khan,[1,2] Azreen Syazril Adnan,[2] Fauziah Jummaat,[3] Nurul Jannah Ambak,[2] Azhar Amir Hamzah.[2] *[1]Dept of Clinical Pharmacy, Univ Sains Malaysia, Penang, Malaysia; [2]Chronic Kidney Disease Resource Center, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia; [3]Dept of Obstetrics and Gynecology, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.*

Background: Dengue fever is a viral infection that is of potential public health concern. One of the most severe and poorly understood complications of dengue fever is acute kidney injury (AKI).

Methods: We retrospectively reviewed medical records, laboratory findings and comorbidities of dengue infected patients for the last five years i.e. 2008 until 2013 at a tertiary care hospital in Kelantan, Malaysia. Patient records with incomplete demographics and renal function data were excluded.

Results: From January2008 to April 2013, total 93 dengue infected patients (male: 49, female: 44, ratio; 1:1.1) with mean age of 27.37± 14.18 were enrolled in current study. Majority of the enrolled patients had Classical dengue fever while only 1 patient suffered Dengue hemorrhagic fever (DHF). No mortality was observed in our study. AKI was observed in 7 (7.5%) of enrolled patients resulting in elevation of following as compared to those patients without AKI: mean rank of bilirubin (61.41 versus 23.43; p: 0.028). There was no statistically significant difference in platelet count (46.19 versus 63.10; p: 0.194), serum calcium (37.38 versus 52.17; p: 0.170) and serum phosphate (56.38 versus 46.67; p: 0.336) levels between AKI and non-AKI cohort. Furthermore, other laboratory findings revealed proteinuria 3(32%), hematuria 6(65%), hyponatremia 5 (53%) and hypokalemia 5(53%).

Conclusions: Thecurrent findings suggest dengue infection is significantly associated with development of AKI. If untreated it may lead to several life threatening complications. A cautious diagnosis and timely management should be the first and foremost step for management of such patients.

PUB045

Incidence and Characteristics of Acute Kidney Injury in Leptospirosis; a Retrospective 5 Year Study Tauqeer Hussain Mallhi,[1] Amer Hayat Khan,[1,2] Azmi Sarriff,[1] Azreen Syazril Adnan,[2] Fauziah Jummaat,[3] Yusra Habib Khan,[1] Muhammad Salman,[1,2] Nurul Jannah Ambak.[2] *[1]Dept of Clinical Pharmacy, Univ Sains Malaysia, Penang, Malaysia; [2]Chronic Kidney Disease Resource Center, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia; [3]Dept of Obstetrics and Gynecology, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.*

Background: Leptospirosis is most widespread zoonotic disease endemic in warm and humid climate.The incidence of leptospirosis in Malaysia is 13%. Renal involvement is most dreaded complication of leptospirosis and is most common cause of death. Incidence of leptospirosis induced acute kidney injury (AKI) is 40-60% having mortality rate of around 22%.

Methods: A 5 years (2004-2008) retrospective database for patients presenting with leptospirosis was initiated at tertiary care hospital, Kelantan, Malaysia. Patients with concurrent infections, incomplete demographics and renal function tests were excluded from study.

Results: A total of 45 (Male: 40, Female: 5, ratio 8:1) leptospirosis patients with mean age of 38.6 ± 14.31 years were enrolled in this study. According to AKIN classification, overall incidence of AKI was 46.6%. Incidence was higher (61.1 %) in risk groups as compared to non-risk groups (38.9%). AKIN-1 was seen in 6 (16.7%) patients while 7 (19.4%) and 8 (22.2%) patients had AKIN-2 and AKIN-3 respectively. Hypokalemia and hyponatremia was observed in 33.3% and 58.3% of patients. Statistical significant (p<0.001) difference in renal profile and hospital stay was observed in patients with AKI and without AKI at admission e.g. Serum creatinine (Mean rank: 25.17 versus 9.17), blood urea (Mean rank: 25.21 versus 9.10) and hospital stay (Mean rank: 23.52 versus 11.47) respectively. Predictors of AKI in our study were old age and association with risk groups while risk factor of severe form of AKI was age > 40 years. We observed no mortality in our study group.

Conclusions: AKI is common and fatal complication of leptospirosis. Early diagnosis, evaluation of risk factors and prompt treatment can reduce further renal deterioration and hospital stay.

PUB046

Predictors of Acute Kidney Injury in Patients Undergoing Lower Extremity Amputation Nina A. Patel,[1] James Fry,[2] Rajiv K. Dhamija.[1,2] *[1]Nephrology, Ranchos Los Amigos National Rehabilitation Center, Downey, CA; [2]Western Univ of Health Sciences, Pomona, CA.*

Background: Proteinuria is established as an important prognostic factor for the development of acute kidney injury (AKI) in patients undergoing cardiac procedures. In this study, we aim to establish a direct relationship between pre-operative proteinuria and development of AKI postoperatively in patients undergoing lower extremity amputations (LEA).

Methods: We performed a five year single center retrospective analysis from 2009-2013 on all patients undergoing LEA. Data on the patients' demographic characteristics, medical history and laboratory test results were retrieved from medical records. A rise in serum creatinine of 0.3mg/dL or greater, per the Acute Kidney Injury Network (AKIN) guidelines, was used to determine AKI.

Results: A total of 478 LEA surgeries were reviewed out of which 260 were excluded (toe-only amputations, incomplete data, or advanced CKD); 218 surgeries were included. An increased incidence of AKI was found to be associated with moderate preoperative proteinuria using the independent sample T test [p<.009]. Those with CKD III were at an increased risk for AKI [p<.000]. However, when both were correlated using directional measures Somers'd test, those with CKD III and severe proteinuria were at an increased risk for AKI [p<0.029]. The overall incidence rate of AKI was 9.6%.

Conclusions: Individually, both CKD III and moderate proteinuria are independent risk factors for AKI post LEA. When correlated, patients with CKD III and severe proteinuria are at an even higher risk for AKI post LEA in a predominantly Hispanic patient population.This is the first study with a majority Hispanic population that shows proteinuria is a predictor of AKI in patients undergoing LEA. The study population was at high risk for AKI, with 91.7% diabetic, 44.5% with CKD, 69.3% hypertensive, and 77.9% with proteinuria. Existing literature recognizes microalbuminuria, CKD, and post-operative AKI with adverse outcomes in overall morbidity and mortality. To fully understand the extent of risks of AKI development in LEA, a randomized controlled trial needs to be conducted.

PUB047

Are Ace Inhibitors and Angiotensin Receptor Blockers Really Safe in the Elderly? Vivienne Ralph, Muhammad Taufiq Dawood, Christine Sarah Catley, Anthony Chan, Abdelgalil Abdelrahman Ali, Sumith C. Abeygunasekara. *Renal Unit, Broomfield Hospital Mid Essex Hospital Trust, Chelmsford, Essex, United Kingdom.*

Background: AKI accounts for 13-18% of hospital admissions in the UK. A 2009 report published by the National Confidential Enquiry into Patient Outcome and Death highlighted the importance of managing AKI effectively. Despite being diagnosed with AKI, 54% of patients do not have nephrotoxic medications stopped. We identified risk factors for developing Acute Kidney Injury(AKI) in an inpatient cohort, with the objective that awareness of these may prevent AKI.

Methods: Data was retrospectively collected from two cohorts of patients admitted to a District General Hospital in the UK between August and November 2012. The first

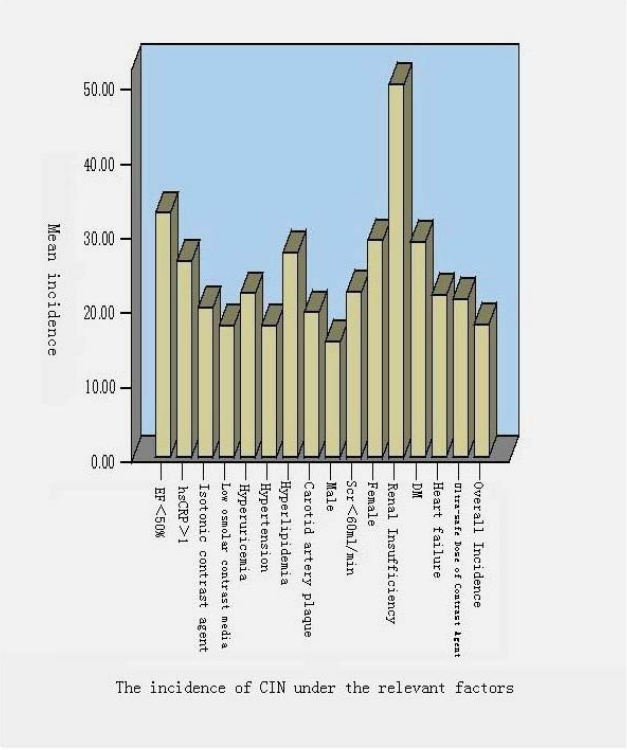
group developed severe AKI (RIFLE criteria stage three) during admission, and the second did not. Potential risk factors compared between these groups included age, creatinine, and medications.

Results: The patients who developed severe AKI were prescribed more nephrotoxic medications than the group that did not develop AKI. The largest difference between the two groups was in the prescription of ACE inhibitors and ARBs, which were almost twice as likely to be prescribed in the AKI group.

Parameter	AKI Patients	Control group
Age	75.85 ±12	67.99 ± 20.7
Serum Creatinine	443.8 ± 240.28	84 ± 29.2
ACEI/ARB risk score	0.44 ± 0.4	0.29 ± 0.46
Diuretics score	0.41 ± 0.33	0.33 ± 0.29
NSAID score	0.04 ± 0.19	0.02 ± 0.15
Metformin	0.11 ± 0.3	0.04 ± 0.2
Medications risk score	1.0 ± 0.9	0.6 ± 0.8
Over all risk score	1.58 ± 1.1	1.08 ± 1.06
Death	0.27 ± 0.44	0.10 ± 0.30

The AKI cohort of patients were found to be of a higher mean age in comparison to the control cohort, and have a higher mortality rate.

Conclusions: Diuretics, ACEI and ARBs were commonly prescribed in the patients who developed AKI. The AKI cohort were of higher mean age compared to the control and which in part may contribute to the increased number of nephrotoxic drugs on admission. The risk factors for developing AKI during hospital admission were identified as age above 65 years and taking nephrotoxic medications. It is imperative that physicians have a higher index of suspicion for AKI in patients who meet these criteria.



The incidence of CIN under the relevant factors

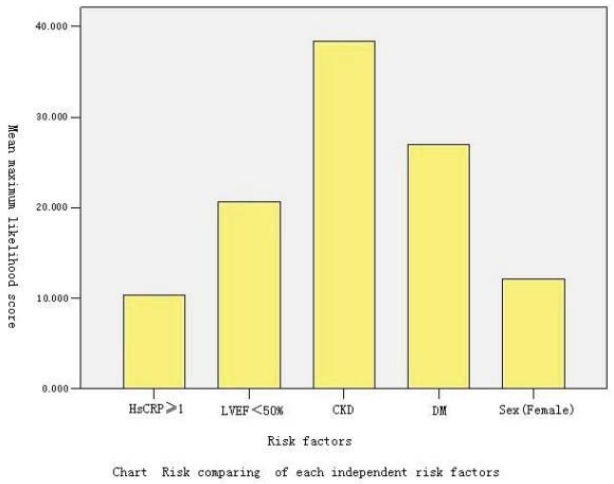


Chart Risk comparing of each independent risk factors

PUB048

The Risk Factors Study of Contrast-Induced Nephropathy in Patients Undergoing Cardiac Surgery of PTCA+PCI

Lin Wei,[1] Wenbo Zhao.[2] *[1]Dept of Neuropathy, Guangdong Province Traditional Chinese Medical Hospital, Guangzhou, Guangdong, China; [2]Dept of Nephrology, the Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: To Confirm the risk factors and estimate the incidence of CIN in patients having PTCA+PCI; To decrease the risks and prevent AKI.

Methods: 60 Patients were enrolled underwent coronary intervention (PTCA+PCI), Serum creatinine Scr was measured at any time a week before the angiography and 48 hours Post Procedure; The definition of CIN is the increase of the serum creatinine of 0.5mg/dl or 25% of the baseline at 48 hours after the use of contrast media. eGFR calculated by CKD-EPI formula. Incidence of CIN undergoing coronary intervention was investigated and multivariate predictors used logistic regression.

Results: 64 cases generated CIN; 6 cases needed to blood purification treatment, and 2 cases to death. Multi-factors logistic regression analysis showed independent risk factors including renal insufficiency, diabetes mellitus LVEF<50%, hsCRP>1, female. The maximum likelihood values were 38.41, 26.95; 20.66, 10.39, 12.11.

Conclusions: The incidence rate of CIN in patients of PTCA+PCI was 17.8%; The Renal dysfunction was the most important risk factors. The patients with renal dysfunction has significantly higher the incidence of CIN; The incidence of CIN in intravenous fluid hydration and non-hydrated patients were no significant difference.

PUB049

A Rare and Emerging Cause of Acute Kidney Injury in a Young Patient

Dilini C. Reyhart,[1] Gordon W. James.[2] *[1]Internal Medicine - Pediatrics Residency, UICOMP, Peoria, IL; [2]Nephrology, Renal Care Associates, Peoria, IL.*

Background: Acute tubular necrosis is the most common cause of intrinsic kidney disease causing acute kidney injury (AKI). It is known to be caused by a multitude of insults with an ischemic event or exposure to nephrotoxic agents being the most common causes. We are reporting a case of AKI secondary to synthetic cannabinoid.

Methods: Our patient was a 20 year old male who presented in Status Epilepticus. He was tachycardia, had dilated pupils and coarse breath sounds at presentation. Neurological exam was limited due to sedation. Patient had 'K2' in his possession. He had no personal or family history of renal disease. Initial labs showed an elevated WBC count of 20,000, Creatinine (Cr) of 1.6, BUN of 10, bicarbonate of 6, anion gap of 42 and lactic acid of 30. UA showed 3+proteinuria, 4+hematuria and 1-5 hyaline casts. Urine protein-Cr ratio was 3.3. CT head was normal. Serum and urine testing for common ingestions were negative. He required propofol drip and versed for seizure control which was weaned off after 24 hours. Workup was done to assess for glomerulonephritis (GN). His C3, C4, ANCA, Anti GBM and ASOT titer were all normal. History revealed no NSAIDS, ACEI, ARB intake to

suggest such medications contributing to AKI. Etiology for AKI was most likely secondary to 'K2' use. AKI was managed with bicarbonate containing fluids. His Cr peaked to 6.7 after which showed improvement and came to baseline by day 20. He never required dialysis.

Conclusions: Synthetic cannabinoids are analogs of naturally occurring chemicals found in marijuana. They are marketed under many different names, including "Spice", "K2", "Spice Gold" etc. Standard rapid urine immunoassays are unable to detect them, thus allowing synthetic cannabinoid users to escape easy detection. In our patient there was no reported history of any other nephrotoxic medication and workup for GN was negative. Prerenal causes were less likely since there was no period of hypotension. Synthetic cannabinoid has the potential to cause AKI, per a limited number of recently reported cases. Therefore it is important to consider synthetic marijuana as a potential cause for AKI in patients with unexplained AKI.

PUB050

Assessment of Kidney Function in Patients Treated with Mild Therapeutic Hypothermia After Cardiac Arrest. A Pilot Study Silvia De Rosa,[1,2] Stefano Marcante,[1] Massimo de Cal,[1] Stefania Aresu,[1] Chiara Pasqualin,[1] Enrico Tonini,[1] Massimo Antonelli,[2] Claudio Ronco.[1] *[1]Nephrology and Dialysis, International Renal Research Institute (IRRIV), San Bortolo Hospital, Vicenza, VI, Italy; [2]Intensive Care and Anaesthesiology, Univ Cattolica del Sacro Cuore, Rome, RM, Italy.*

Background: In animal models and in humans studies, it has been shown that induced hypothermia decreased glomerular filtration rate and renal plasma flow.Currently available trials indicate that therapeutic hypothermia (TH) does not prevent AKI including dialysis requirement but is associated with lower mortality. Unfortunately, the currently trials were not designed to examine the effect of TH on a primary kidney endpoints.The aim of this study was to assess the effect of mild hypothermia on kidney function during cooling and rewarming temperatures.

Methods: We performed a prospective observational study in 19 post-cardiac arrest patients. Inclusion criteria were patients with CA (VF without pulse, asystolia, PEA intra and extra hospital) and Myocardial Infarction. The decision to initiate TH (33–34 °C) was made by the treating physician. Temperature, hemodynamic parameters, serum creatinine, vasoactive medication use, urine output, and intravenous fluid were registered from admission to 24 h after rewarming.

Results: Nineteen patients (84% males; Mean age 66±11) were treated with TH. From admission to 24 h after rewarming, there is no significative difference in serum creatinine and urine output (p<.05;p=NS). Furthermore, there is not a significative modification of hemodynamics parameters, of intravenous fluid requirement (p<.05;p=NS) but also of modified inotrope score and vasoactive inotrope score over 48 hours (p <.05;p=NS).

Conclusions: These findings suggest that renal function was preserved with a normal urine output without a decrease in systolic blood pressure, and a further increase in vasoactive and inotrope medication use in patients treated with mild therapeutic hypothermia. Future studies with bigger study populations will be required to confirm these findings and to further investigate these conclusions.

PUB051

Nephrology Research in a Combat Zone Ian J. Stewart,[1] Jonathan Sosnov,[1] Edward D. Siew,[2] T. Alp Ikizler,[2] Kristen R. Glass,[1] Benjamin D. Morrow,[1] Wayne A. Latack,[3] Kristin K.P. Saenz,[1] Kevin Chung.[4] *[1]San Antonio Military Medical Center; [2]Vanderbilt Univ Medical Center; [3]Kessler Medical Center; [4]U. S. Army Institute of Surgical Research.*

Background: Research is difficult under the best of circumstances. Here we describe the process of translational research in an austere location, the intensive care unit (ICU) at Bagram Airfield (BAF), Afghanistan. Our hypothesis for the study was that urinary biomarkers (NGAL, IL-18, L-FABP, Cystatin C and KIM-1) would predict subsequent acute kidney injury (AKI) in this population.

Methods: We conducted a prospective study examining urinary biomarkers (UB) of acute kidney injury in U.S. service members admitted within 48 hours of traumatic injury, had a Foley catheter placed and required ICU level care. Ten ml of urine at admission and then every morning until evacuation were collected. Samples (10 samples per patient per time point) were stored at BAF in a study dedicated -80ºC freezer until shipped to the U.S. Samples were split into 5 shipments (each with at least one complete set of specimens) with temperature data loggers.

Results: Of 126 patients admitted to the ICU at BAF from Oct 2012 to Dec 2013, 89 were included in the cohort resulting in 166 time points and 1660 samples. Enrollment spanned 3 deployment cycles and involved 6 investigators. Of the 5 shipments, 4 arrived in the U.S. intact. Samples shipped through differing combinations of 7 countries and 3 U.S. states. The one shipment that did not make it intact took 8 days to arrive and the peak temperature was 3.1ºC. The four successful shipments took from 2.5 to 7.6 days to arrive and the peak temperatures were -72.5 to -40ºC.

Conclusions: Nephrology research is feasible in a forward deployed combat environment. Biologic specimens can be kept at low enough temperatures in both the austere environment and in transit back to the U.S. for analysis. We present mitigation strategies for dealing with follow up data across multiple States and suggestions for future research in austere conditions. Furthermore, the association of urinary biomarkers with subsequent AKI will also be reviewed.

Funding: Other U.S. Government Support

PUB052

Successful Treatment of Atipical Hemolytic Uremic Syndrome with Eculizumab: Long Term Remission and Improvement of Renal Function Jorge Alexandre Fares,[1] Carlos Eiji Koga,[1] Andre F.G. Larrubia.[2] *[1]Nephrology, Rede D´Ór - Hospital Sao Luiz Morumbi, Sao Paulo, Brazil; [2]Hematology, Rede D´Ór - Hospital Sao Luiz Morumbi, Sao Paulo, Brazil.*

Background: AAS, 28 yrs-old, female, was admitted to hospital in 02-13-2013, with intense weakness, and muscles pains. Labs at admission showed Coombs negative Hemolytic Anemia, plaquetopenia, ARI and proteinuria. A sample for analysis of ADAMTS13 was drawn, and Plasma Exchange was immediately started.

Methods: ECZ was available for infusion 104 days after diagnosis of aHUS. After the first infusion of ECZ, no PE were required(total:25 sessions).

Results: Labs at admission: Hb:10 g-dl; Plaq: <5.000mm3; Schistocytes + in peripheral blood; LDH:2,700U-l; Indirect Bilirrubin :2,7; D-Dimer : >10,000; C:2.7m-dl; ADAMTS13 (02-13-2013)- Activity Assay >100 %. Daily PE Started on day01. Outcome:kidney function deteriorated and HD sessions were required:BUN:100 mg-dl C:8.0 mg-dl. Discharge on day24 to be submitted to intermitent outpatient PE. Labs at discharge: C:3,5 mg-dl; BUN:59 mg-dl; Plat:113.000 mm3; LDH:789 U-l. Readmission on day38: Hemolytic Anemia relapse: Hb:4.0g-dl; LDH:>2,00U-l; Hapto: undetectable;Worsening of ARI, pt. returned to hemodialysis and PE**.** Discharge on day78 with no hemolysis or plaquetpenia. Labs at discharge: C:2.6 mg-dl. Outpatient PE - twice/week. Last PE session, and first dose ECZ induction on day110. No signs of Hemolysis at the first dose of Eculizumab:Hb:12.4; plat:240.000. D-dimer: 1.200.C:2,2; creatinine clearence 24 h: 39 ml/min. Proteinuria 24 h: 0,9g.Outcome after 4 biweekly doses of Eculizumab : 105 days after the first dose(day215):Hb:12,3; Plat:294.000mm3; Hapto: 140; LDH: 380U-l; Reticulocytes:1,46%, D-dimer:250; Creatinine:1.71mg-dl; BUN:25mg-dl. Creatinine clearence 24h: 49ml-min(10 ml-min improvement) Proteinuria 24h: 0,38g; Hematúria<10.000. Labs in jan-2014. (creatinine;1,7mg-dl); Prot. 24h: <0,300 mg, Hematuria:< 10.000. Current creatinine;1.4 mg-dl; Creatinine clearence 24h: 51 ml-min.

Conclusions: Despite Eculizumab's first infusion was done months after the diagnosis of aHUS, renal function improved, proteinuria became negative, and no relapse occurred.

PUB053

Evaluation of the RIFLE Outcome Criteria Loss and End-Stage in Pediatric Continuous Renal Replacement Therapy Patients Alyssa A. Riley,[1] Mary Neil Watson,[2] Sarah J. Swartz,[1] Poyyapakkam Srivaths,[1] Ayse Akcan Arikan.[1] *[1]Pediatrics, Baylor College of Medicine, Houston, TX; [2]Financial Services, Texas Children's Hospital.*

Background: The original standardized definition of acute kidney injury (AKI) proposed by the Acute Dialysis Quality Initiative, the RIFLE criteria, included two outcome strata: Loss –defined as complete Loss of kidney function for >4 weeks and End-stage kidney disease-defined as complete loss of kidney function >3 months (12 weeks). This definition has never been applied to critically ill pediatric patients with AKI. We aimed to determine short term renal outcomes in pediatric patients who required continuous renal replacement therapy (CRRT) for management of AKI and its sequelae.

Methods: Utilizing our institutional database, we identified 105 patients who received CRRT between 2011-2013. Patients were excluded if they had preexisting chronic kidney disease (n=11), were treated for hyperammonemia (n=8) or ingestion (n=2).

Results: The 84 patient cohort was 46% male with a mean age of 8±7 years and mean weight of 33.4±29.8 kg. Patients were treated with CRRT for a mean duration of 12.6±13.2 days. Additional patient descriptors included hospital length of stay 45±40 days, intensive care unit length of stay 23±20 days; 96% of patients were ventilated with mean length of ventilation 20±16days. Hospital mortality was 63% (n=53). Two patients had recovery of kidney function allowing discontinuation of CRRT, but later died during hospitalization. Of the 31 patients surviving to hospital discharge, 23 patients required less than 28 days total renal replacement therapy (RRT), including CRRT, hemodialysis, and peritoneal dialysis, 5 patients (16%) received 4-12 weeks total RRT (RIFLE-L), 3 patients (9.6%) received more than 12 weeks RRT (RIFLE-E). Two of the RIFLE=E patients had subsequent recovery of kidney function and one patient was declared end-stage renal disease.

Conclusions: Our data suggest that critically ill pediatric patients who meet RIFLE-E criteria for AKI may still demonstrate delayed recovery sufficient to discontinue RRT, thus the RIFLE criteria may warrant redefinition to account for a possible prolonged recovery in pediatric patients.

PUB054

Prevalence and Risk Factors for Aute Diarrhea Positive HUS in Children Judith Sebestyen, Uri S. Alon. *Div of Nephrology, The Children's Mercy Hospital, Kansas City, MO.*

Background: Hemolytic uremic syndrome (HUS) is the most common cause of acute renal failure in childhood. Due to the fact that our hospital is surrounded by rural areas, which it serves as an exclusive referral hospital, we see a higher number of children with diarrhea positive HUS. The primary goals of the study were to identify the demographic characteristics of the patients and to identify risk factors for complicated acute versus favorable course. The secondary aim was to determine the risk factors for development of chronic kidney injury.

Methods: 120 pediatric patients (< 22 years old) who were admitted to our hospital between February 1995 and January 2011 were identified via ICD-9-CM code. Complete information was available in 58 patients who were categorized by age, gender, white blood cell count (WBC), hemoglobin (HGB), thrombocyte number (PLT), antibacterial

treatment prior to admission, urine output (UO) and serum creatinine level as well if the patient required dialysis or not. Follow-up record was reviewed for diagnosis of chronic kidney disease.

Results: Thirty one patients (53.4%) required dialysis. The mean age was 3.76 year (9 month-14 year) with no difference between those needing dialysis or not. Female (57.4%) were more likely to require dialysis ($p = 0.041$), as well who were anuric (UO < 0.5 ml/kg/h) at admission ($p < 0.001$). A trend was noted in those with prior antibiotic therapy ($p = 0.054$). Age of less than 2 years, leukocytosis (WBC> 20,000), low PLT count (<20 000) or extreme anemia on admission (HGB< 6.0) were not risk factors for acute dialysis. The mean serum creatinine at admission was significantly higher among patients who required dialysis (3.074 versus 2.47 95 %CI 2.1-3.4, $p < 0.005$). Risk factors for development of CKD, diagnosed in 5 patients were use of antibiotic therapy ($p < 0.005$) and being anuric at admission ($p < 0.005$).

Conclusions: Being female, anuric and receiving antibiotic therapy prior to development of HUS were risk factors for initiation of acute dialysis, and the latter two factors were associated with development of CKD. Further studies are indicated to learn if patients identified to be at high risk may benefit from closer monitoring and early intervention.

Funding: Clinical Revenue Support

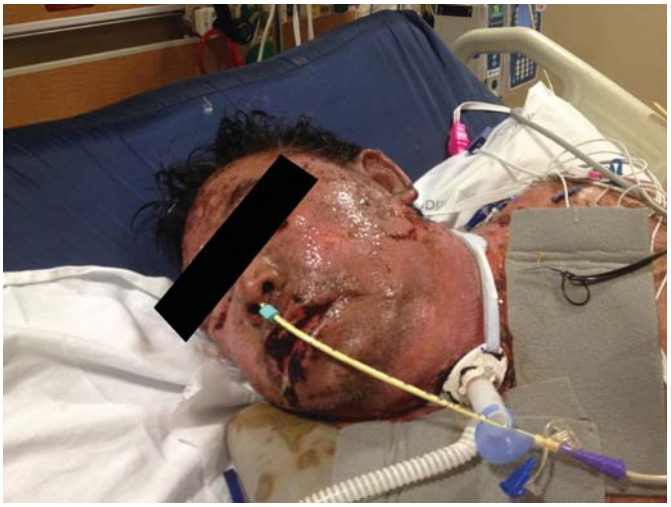
PUB055

Deciding to Get a Flu Shot: Don't Make a Rash Decision

Sunny Mickey Kar,[1] Anuradha Bhavsar,[3] Jafar Mahmood,[2] Pran M. Kar.[2] *[1]Lake Erie College of Osteopathic Medicine; [2]Dr Phillips Hospital; [3]Avalon Univ School of Medicine.*

Background: Rhabdomyolysis can be associated with infections, drugs, hypoxia, and metablic derangements. It can cause acute renal impairment secondary to myoglobinuria. Rhabdomyolysis triggered by an influenza vaccination is rare. Typically, cases of rhabdomyolysis involve multiple risk factors, including the concomitant use of high risk drugs such as statins, fibrates or cyclosporine. A pilot study of 98 patients on statin therapy who received the influenza vaccine did not report any adverse reactions. This may be due to small study size in conjunction with variations in vaccine the formulation. Rhabdomyolysis triggered by an influenza vaccination is an important complication to be aware of, as early recognition can lead to better outcomes in patients.

Methods: A 76 year old male with a history of Chronic Kidney Disease and hyperlipidemia managed on atorvastatin, presented to the emergency room for a new, widely distributed, weeping rash.



He received influenza vaccination three days prior to rash onset. Initial laboratory investigations revealed a creatinine of 4.4 mg/dl, up from his baseline of 2.0 mg/dl. Further, his creatinine kinase levels were found to be elevated at 2610 IU/L. He was admitted to the intensive care unit for renal replacement therapy, and was empirically started Solu-Medrol and diphenhydramine for presumed rhabdomyolysis secondary to Stevens-Johnson Syndrome. The diagnosis was later confirmed by skin biopsy, which showed epidermal sloughing, necrotic keratinocytes and vacuolar interface dermatitis.

PUB056

Rhabdomyolysis Induced Acute Renal Failure Secondary to Severe Hashimoto Hypothyroidism: An Unusual Association

S. Amir Afsharimani. *Medicine, St. Francis Hospital, Evanston, IL.*

Background: Hashimoto's thyroiditis is the most frequent cause of hypothyroidism. The most common neuromuscular manifestations include myalgia, fatigue, proximal muscle weakness and hypoactive tendon reflexes. However, infrequent severe cases of rhabdomyolysis with markedly elevated levels of creatine phosphokinase (CPK) and renal disease have been reported. Association between severe hypothyroidism and acute kidney injury (AKI) is rare.

Methods: We report a case of rhabdomyolysis, acute kidney injury, and myopathy as the only manifestations of severe hypothyroidism secondary to Hashimoto's thyroiditis.

Results: A 32 year old male was admitted to the hospital with bilateral upper and lower extremities progressive pain and swelling. Symptoms started three days prior to admission and became worse the night before admission. Pain was worse with movement. He denied any vigorous activity, trauma, previous medications, and fever. Neurological examination revealed decreased muscle strength symmetrically on lower limb (3 + / 5 +) and upper limbs (4 + / 5 +), and diminished tendon reflexes (1 + / 4 +) symmetrically. Laboratory evaluation revealed the following serum levels: CPK, 2256 IU/L (38-174), creatinine 2.6 mg/dl (0.5-1.20), free thyroxine (FT4) <0.20 ng/dl (0.93-1.70), thyroid stimulating hormone 259.70 uIU/ml (0.400- 5.40), and thyroid peroxidase antibody 452 IU/ml (<35). After ruling out adrenal insufficiency by cosyntropin stimulation test, treatment was started with 125 ug oral levothyroxine. The symptoms gradually improved. The patient was discharged from the hospital five days later, showing only mild muscle pain. CPK levels reduced significantly, and creatinine normalized.

Conclusions: Asymptomatic mild to moderate elevation of creatine kinase frequently develops in hypothyroidism. However,marked creatine kinase elevation with rhabdomyolysis have been reported only in a very small number of cases with undiagnosed hypothyroidism.Although hypothyroidism rarely presents with AKI and rhabdomyolysis, it should be suspected in patients presenting with impaired renal function and high creatine kinase level in the absence of other causes of rhabdomyolysis.

PUB057

Pseudo Renal Injury Associated with Isopropyl Alcohol

Rawan Tayseer Al Odat,[1] Laith Farah Al-Rabadi.[2] *[1]Internal Medicine, Al-Khalidi Hospital, Amman, Jordan; [2]Nephrology, Boston Univ Medical Center, Boston, MA.*

Background: Isopropyl alcohol is a disinfectant and cleaning agent that can potentially suppress CNS when ingested accidentally or in suicide attempts. Isopropyl alcohol (IPA) intoxication is mostly encountered in alcoholics as ethanol substitute. Acute renal injury due to IPA has only been infrequently reported.

Methods: Case report.

Results: Herein, we present a case of 32 year old alcoholic patient who was admitted for alcohol detoxification. He developed doubling of his creatinine level to 1.5 mg/dl in the setting of taking Cal Stat, a rubbing alcohol containing isopropyl alcohol used in medical facilities. Patient was drinking it from containers available on hospital walls to help with his withdrawal symptoms. Osmolar gap was elevated with no evidence of metabolic acidosis. Urine ketone test was positive. Completely normal BUN raised doubts about the validity of the elevation in creatinine. When Creatinine level was obtained from an arterial sample a normal value of 0.7 mg/dl was found. IPA level was elevated. The decrease in osmolar gap and urine ketones level correlate with the decrease in IPA concentration and can serve as surrogate for actual blood levels.

Conclusions: Isopropyl is metabolized through alcohol dehydrogenase to acetone that can't be oxidized to carboxylic acids. Hence, academia does not usually occur and if present, should prompt clinician to look for other coingested substances. Acetone can cause false elevation of serum creatinine by interfering with the colorimetric assay for creatinine. Most analyzers use the Jaffe-alkaline-picrate reaction. Some substances including acetone can react with picric acid and cause spurious elevation in the creatinine level. Measuring creatinine level by enzymatic assay in blood gas analyzer will accurately estimate serum creatinine and avoid the interference of acetone. We emphasize the importance of being aware of the false elevation of creatinine in the setting of IPA intoxication to assist in diagnosis and management and to avoid incorrect diagnosis of acute renal injury.

PUB058

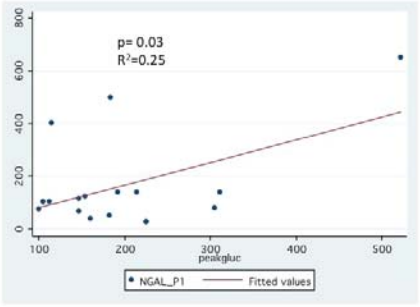
Neutrophil Gelatinase-Associated Lipocalcin (NGAL) and Stress-Induced Hyperglycemia in Critically Ill Children

Roberto Gordillo. *Pediatrics, Univ of Illinois College of Medicine at Peoria, Peoria, IL.*

Background: Hyperglycemia is common in critical ill patients and it has been associated with increased mortality and morbidity in adults and children. However, it is still not clear if stress-induced hyperglycemia correlates with acute kidney injury (AKI). NGAL has been used for early clinical recognition of patients at risk for AKI.

Methods: We conducted a prospective cohort study of 27 critical ill subjects admitted to the pediatric intensive care unit (PICU). No infants and subjects with known kidney disease were included. We determined the intensity of acute kidney injury using the pediatric RIFLE criteria. Two groups were compared, subjects with AKI on admission and subjects without AKI on admission. Urine and serum NGAL were measured on admission.

Results: 27 critically pediatric subjects were included. 8 of those patients had AKI according to the pediatric RIFLE criteria. A positive correlation between peak glycemia during admission and serum NGAL was found, $p = 0.03$. Urine NGAL was not associated with higher glycemia, $p=0.9$. Demographics are shown in table.



	AKI (n=8)	No-AKI (n=19)	P
Age (years)	9.6±7	8.2± 5.5	0.6
Caucasian (%)	50	63	0.6
Length of hospital stay (days)	61.7 ±SEM 41	27.6±SEM 7.5	0.3
Urine NGAL (ng/ml)	358±SEM 324	18.42±SEM 8	0.2

Conclusions: We found a positive correlation between serum NGAL on admission and peak glycemia during PICU admission. Although we found higher levels of NGAL in urine in subjects with AKI, it was not statistically significant. Serum glucose and NGAL are useful tools in treating critically ill pediatric subjects.

PUB059

Modified High-Density Lipoprotein May Contribute to Systemic Inflammation in Patients with Chronic Kidney Disease by Interacting with Polymorphonuclear Leukocytes

Gerald Cohen, Jana Raupachova, Chantal Maureen Kopecky, Marcus Saemann. *Dept of Internal Medicine III, Medical Univ of Vienna, Vienna, Austria.*

Background: Disturbed functions of polymorphonuclear leukocytes (PMNLs), cells of the first-line non-specific immune defense, contribute to cardiovascular disease in patients with chronic kidney disease (CKD). Decreasing renal function is associated with gradual changes in the protein composition of high-density lipoprotein (HDL), such as enrichment of serum amyloid A (SAA), resulting in loss of its anti-inflammatory properties. We tested the effect of HDL from hemodialysis patients (HD-HDL), CKD stage 3 and 4 patients (CKD3- and CKD4-HDL) and healthy subjects (HS-HDL) on different PMNL features.

Methods: HDL was isolated by a one-step density gradient centrifugation. PMNLs were isolated from heparinized blood using discontinuous density gradient centrifugation. Spontaneous apoptosis was assessed by evaluating morphological features of PMNLs with fluorescence microscopy and by measuring their DNA content. The surface expression of CD11b on PMNLs was quantified by flow cytometry.

Results: HD-HDL, CKD3- and CKD4-HDL significantly reduced spontaneous apoptosis of PMNLs from healthy controls, an effect typical for pro-inflammatory mediators. Apoptosis of PMNLs from HD patients was reduced by HD-HDL to the same extent as of PMNLs from HS. SAA alone and HS-HDL spiked with SAA significantly attenuated PMNL apoptosis suggesting that SAA contributes to the observed effect of uremic HDL. Experiments with signal transduction inhibitors suggest that HD-HDL exerts its anti-apoptotic effect by activating pathways involving phospho-inositide 3-kinase and extracellular-signal regulated kinase. The integrin CD11b is involved in the pathogenesis of vascular damage and increased atherosclerotic risk. In contrast to HDL from CKD and HD patients, HS-HDL showed anti-inflammatory properties by significantly reducing expression of CD11b on activated PMNLs.

Conclusions: We conclude that modified HDL may contribute to systemic inflammation in CKD patients by interacting with PMNLs.

Funding: Private Foundation Support

PUB060

Eryptosis and Oxidative Stress Induced By Indoxyl Sulfate and *p*-Cresyl Sulfate Are Attenuated by N-Acetyl Cysteine

Andrea Novais Moreno-Amaral,[1] Natalia Borges Bonan,[1] Gabriela Ferreira Dias,[1] Viktoriya Kuntsevich,[2] Wesley M. Souza,[3] Andréa Marques Stinghen,[4] Lia S. Nakao,[4] Fellype C. Barreto,[1] Peter Kotanko,[5] Roberto Pecoits-Filho.[1] *[1]School of Medicine PPGCS, PUCPR, Brazil; [2]Mount Sinai Beth Israel New York; [3]Dept Clinical Analysis UFPR, Brazil; [4]Basic Pathology Dept UFPR, Brazil; [5]Renal Research Institute New York.*

Background: Uremic toxins play a critical role in chronic kidney disease(CKD)-related anemia inducing eryptosis thorough mechanisms not fully understood. Renal anemia is involved in the pathogenesis of increased oxidative stress in patients, due its association with the overproduction of reactive oxygen species (ROS) and reduction of antioxidant defenses. *N*-acetyl-L-cysteine (NAC) is a cysteine donor with antioxidant properties. The present study explored the potential of indoxyl sulfate (IS) and *p*-cresyl sulfate (*p*CS) in promoting eryptosis and ROS production and the effect of NAC treatment.

Methods: Erythrocytes obtained from healthy donors were incubated for 24h with NAC before 24h incubation with different concentration of IS or *p*CS. Eryptosis was evaluated by positivity of cells expressing phosphatidylserine (PS) by Annexin-V-PE. Intracellular ROS generation was evaluated by DCFH-DA oxidation using flow cytometer.

Results: IS induced eryptosis in a dose-dependent manner, with its highest concentration (236μg/L) resulting in about 35±11% of annexin-V-PE positive erythrocytes. *p*CS (2.6mg/L, higher concentration) induced eryptosis by 25±9.5%. In the presence of NAC, uremic toxins lost their pro-eryptotic activity. We also observed that *p*CS and IS were capable of promoting ROS generation, though in different intensity (20±5.6% at *p*CS 2.6 μg/ml; and 56±19.7% at IS 236mg/L). NAC pre-incubation inhibited the ROS generation by uremic toxins.

Conclusions: Thus, taken together our results suggest that besides the high exposure of PS on erythrocytes surface induced by uremic toxins, these toxins promote increased ROS generation, effects that were attenuated by NAC. These findings indicate the potential role of uremic toxins on eryptosis and parallel an increased ROS generation that may partially explain the systemic vascular effects of anemia in CKD.

PUB061

Overendocytosis of Gold Nanoparticles Increases Autophagy and Apoptosis in Hypoxic Human Renal Proximal Tubular Cells

Fengan Ding, Ping Sheng Chen. *Pathology, School of Medicine, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Gold nanoparticles (GNPs) can potentially be used in biomedical products ranging from therapeutics to diagnostics, and their use will result inincreased human exposure. Many studies have demonstrated that GNPs can be deposited in the kidneys, particularlyin renal tubular epithelial cells. Chronic hypoxia is inevitable in chronic kidney diseases (CKD), and it results in renal tubular epithelial cells that are susceptible to different types of injuries. However, the understanding of the interactions between GNPsand hypoxic renal tubular epithelial cells is still rudimentary.

Methods: Wesynthesized and characterized GNPs by various biophysicalmethods, including transmission electron microscopy (TEM), dynamic light scattering, and UV-vis spectrophotometry. Then we explored the cell viability, autophagy and apoptosis, and reactive oxygen species production both in oxic and hypoxic conditions.

Results: It was found that 5-nm GNPsresulted in cytotoxic effects only in hypoxic human renal proximal tubular cells (HK-2) treated for 24 hwith 5-nm GNPs at a concentration of 50 nMusing theCell Counting Kit-8 (CCK-8) assay. To further understand the cytotoxic mechanism of the smaller GNPs,the cells were exposed to 5-nm GNPs (50 nM) for 24 hunderoxic and hypoxic conditions. The TEM revealed that GNPs were either localized in vesicles or free in the lysosomes in the HK-2 cells, and the cellular uptake of the GNPs was significantly higher under hypoxic than normoxic conditions. In normoxic HK-2 cells, GNP (5 nm) treatment at a concentration of 50 nM could cause autophagy and cell survival; however, in hypoxic conditions, GNP exposure at the same concentration led to the production of reactive oxygen species (ROS), and an increase in apoptosis and autophagic cell death.

Conclusions: Our results provide an important basis for understanding the risks associated with GNP use and provideguidance for the potential GNP-related therapies in CKD patients.

Funding: Government Support - Non-U.S.

PUB062

Docosahexaenoic Acid Attenuates Palmitate-Induced Unfolded Protein Response-Related Changes in Protein Degradation in Muscle Cells

Russ Price,[1,2] Myra Woodworth-Hobbs.[1,3] *[1]Medicine/Nephrology, Emory Univ, Atlanta, GA; [2]Atlanta VAMC, Atlanta, GA; [3]NHS Program, Emory Univ, Atlanta, GA.*

Background: Hyperlipidemia and skeletal muscle atrophy are frequent consequences of chronic kidney disease and diabetes. Accumulation of saturated fatty acids in skeletal muscle causes dysregulation of protein metabolism. In cultured myotubes, the saturated fatty acid palmitate (PA) stimulates protein degradation and decreases cell size, while co-treatment with the omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) prevents the response. PA also induces endoplasmic reticulum (ER) stress and activates the unfolded protein response (UPR) in myotubes, resulting in decreased protein synthesis. ER stress can also activate caspase proteases in some cell types. This study investigated how PA and DHA affect the UPR and components of protein metabolism including caspases and protein synthesis in muscle cells.

Methods: C2C12 myotubes were treated with 500 μM PA and/or 100 μM DHA for 24 h. Proteins and mRNAs associated with ER stress, caspase activation and protein translation were evaluated by immunoblot analysis and qPCR, respectively. Results are were analyzed by ANOVA and post-hoc tests. Results are reported as mean percent of untreated control ± SEM.

Results: PA induced activation of PKR-like endoplasmic reticulum kinase (PERK), as indicated by a 984±321% ($P<0.05$) increase in phospho-PERK:total PERK ratio. PA also increased the phosphorylation (i.e. inactivation) of eukaryotic initiation factor 2α (eIF2α) by 223±52% ($P<0.05$) and the level of Nrf2 protein by 223±52% ($P<0.05$). Co-treatment with DHA attenuated the effects of PA on PERK ratio and restored Nrf2 protein to the control level; DHA did not reduce PA-induced eIF2α phosphorylation. PA also induced CHOP mRNA by 744 ± 45% ($P<0.05$) and the appearance of activated caspase-3 peptide whereas DHA reversed these responses.

Conclusions: DHA attenuates many of the effects of PA on the UPR and caspase 3 but does not appear to counter responses related to global protein synthesis. Changes in CHOP and caspase 3 indicate that DHA restores myotube diameter primarily by inhibiting PA-induced proteolyic responses.

Funding: NIDDK Support, Veterans Administration Support

PUB063

Distinct Cytokine mRNA Expression Pattern in Immunoglobulin G4-Related Kidney Disease Associated with Renal Cell Carcinoma Tetsuhiko Yasuno. *Internal Medicine, Fukuoka Univ, Fukuoka, Japan.*

Background: We treated a 61-years-old man with IgG4-related kidney disease (IgG4-RKD). He had been diagnosed as renal cell carcinoma (RCC) and received left kidney segmental resection at 59 years old. He had allergic diseases and an allergic reaction in past history, and have diagnosed as autoimmune pancreatitis (AIP). According to his clinical course it was clarified that the value of serum amylase and the number of peripheral eosinophils were increased after the development of RCC. We constructed a hypothesis that RCC might induce the development of AIP and IgG4-RKD, and examined extracted RCC tissue. Typical findings of IgG4-RKD attached with RCC were recognized.

Methods: The evaluation of mRNA expression levels of cytokines in the tissues of this case and other ten ordinary RCC cases was performed.

Results: All cases showed sufficient values of IL-10 and great values of TGFβ. Although prominent differences were not observed in Th1, Th17, and Treg cytokines in all cases, concerned with Th2 cytokines only this case showed increased productions of IL-4 and IL-5, and which were not detected in ordinary RCC cases.

Conclusions: Although a mechanism for development of IgG4RKD has not been clarified, Th2 and Treg cells are thought to be predominantly involved in the pathogenesis. It was suggested that immune response against RCC might trigger for the development of IgG4-RKD.

PUB064

Mesangial Tissue Transglutaminase Activity in Patients with IgA Nephropathy: Implications for the Pathogenesis Kazuo Takahashi,[1] Tomohiro Mizuno,[2] Takanori Onouchi,[1] Shin'ichi Akiyama,[4] Stacy D. Hall,[3] Hideki Tatsukawa,[5] Mamoru Kusaka,[1] Yutaka Tsutsumi,[1] Tadashi Nagamatsu,[2] Shoichi Maruyama,[4] Hiroshi Kitamura,[6] Jan Novak,[3] Kiyotaka Hitomi,[5] Yukio Yuzawa.[1] *[1]Fujita Health Univ School of Medicine, Toyoake, Japan; [2]Meijo Univ, Nagoya, Japan; [3]Univ of Alabama at Birmingham, Birmingham, AL; [4]Nagoya Univ Graduate School of Medicine, Nagoya, Japan; [5]Nagoya Univ Graduate School of Pharmaceutical Sciences, Nagoya, Japan; [6]Chiba-East Hospital, Chiba, Japan.*

Background: Tissue transglutaminase (TG2) is essential for mesangial activation by IgA1-containing complexes in a mouse model resembling IgA nephropathy (IgAN). Although TG2 plays an important role in a mouse model of IgAN, its pathological significance for human IgAN is unknown. TG2 is a ubiquitously expressed enzyme and transforms into catalytically active TG2 through extensive conformational changes. Thus, it is crucial to detect catalytically active TG2 to assess the role of TG2 in IgAN.

Methods: We detected TG2 activity and its distribution in renal tissue using FITC-labeled highly reactive substrate peptides and fluorescence microscopy. Renal biopsy specimens were obtained from 50 patients with biopsy-proven IgAN and from 42 disease controls (patients with biopsy-proven kidney diseases without predominant IgA deposition).

Results: TG2 activity was detected in the glomerular vascular pole, but not in the mesangial area in most samples from disease controls. In contrast, 28 of 50 patients with IgAN (56%) exhibited mesangial TG2 activity. Furthermore, IgAN patients with mesangial TG2 activity had higher level of proteinuria (2.3 *versus*. 0.9 g/day, *P*= 0.02) and higher mesangial score (0.54 *versus*. 0.39, *P*= 0.08) defined by Oxford classification than those without TG2 activity in the mesangium.

Conclusions: These results suggest that mesangial TG2 activity may be related to mesangial proliferation in patients with IgAN. Detailed studies of mesangial TG2 expression and activity will provide new information relevant to the pathogenesis of IgAN.

Funding: Private Foundation Support, Government Support - Non-U.S.

PUB065

Henoch-Schönlein Purpura: Assessment of IgG-IgA1 Immune Complexes in Patients with and without Nephritis Ling Yun Lai,[1] Jing Qian,[1] Ping Cheng,[1] Chuanming Hao,[1] Stacy D. Hall,[2] Bruce A. Julian,[2] Jan Novak.[2] *[1]Div of Nephrology, Fudan Univ, Huashan Hospital, Shanghai, China; [2]Dept of Microbiology, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Henoch-Schönlein purpura (HSP) is a vasculitis associated with skin deposits of IgA1-containing immune complexes (IC). Joints and/or gastrointestinal tract may be affected; 40-60% HSP patients develop nephritis (HSPN) 4-6 weeks after onset of purpura. Renal pathology is similar to that of IgA nephropathy, with IgA-containing IC deposits. Circulating IgG-IgA IC likely contribute to the pathogenesis of both diseases.

Methods: Serum was collected from 45 adults (21 females; age range 17-70 yr). 35 patients had HSP and 10 patients had minimal-change disease (MCD). 20 HSP patients with abnormal urinalysis had biopsy-proven HSPN; 15 HSP patients had normal urinalysis for 1 year after onset of HSP. Serum IgA and IgA-IC were purified and IgG and IgA-IgG IC were measured.

Results: All HSP(N) patients had purpura; 53% had arthralgia or abdominal pain. Frequency of joint or gastrointestinal tract involvement did not differ between HSPN patients and HSP patients (p=1.0). Total serum IgA, IgG, or C3 did not differ between HSPN and HSP patients (IgA, 3.33±1.74 *versus* 3.19±0.76 g/L, p=0.84; IgG, 9.72±2.91 *versus* 11.01±2.36 g/L, p=0.29; C3 1.10±0.27 *versus* 1.16±0.22 g/L, p=0.64). For HSPN, HSP, and MCD patients, gender ratio and mean age were similar (p=0.22, p=0.34, respectively); mean serum creatinine was 80.4±37.8, 63.4±14.1, and 74.0±15.8 mmol/L, respectively (p=0.47). Amount of IgG co-purified with IgA in HSPN patients was similar to that for HSP and MCD patients [130 (0, 285) μg/ml, 100 (0, 350) μg/ml, and 105 (0, 293) μg/ml, p=0.906]. Levels of IgG-IgA1 IC were 0.35±0.24, 0.26±0.16, and 0.34±0.19 U/mL in HSPN patients compared to HSP and MCD patients (p=0.43). Moreover, median ratio of IgG complexed with IgA to total serum IgG was 0.0138(0, 0.031) *versus* 0.0076 (0, 0.0195) for HSPN patients *versus* HSP patients (p=0.111).

Conclusions: Patients with HSPN, HSP, and MCD had IgG complexed with IgA1, although HSPN patients tended to have a higher percentage of such IgG. Thus, more specific tests are needed to differentiate HSPN from HSP.

PUB066

Detection of Anti-Phospholipase A2 Receptors Antibodies in the Diagnosis of Primary Membranous Glomerulonephritis Using Immunoassay and Indirect Immunofluorescence A. Bernard Collins, Christie M. Swett, Ivy A. Rosales, Rex Neal Smith, Robert B. Colvin. *Dept of Pathology, Massachusetts General Hospital, Boston, MA.*

Background: The detection of autoantibody against the M-Type phospholipase A2 receptors (PLA2R), a transmembrane glycoprotein expressed on the surface of the glomerular podocyte is diagnostic of primary membranous (MGN). Circulating antibody to the PLA2R receptor is present in most patients with primary MGN but rarely in patients with secondary (MGN) or other glomerular diseases. Quantitation of the level of anti-PLA2R provides important clinical markers for management and treatment.

Methods: This study evaluates the sensitivity and specificity using Immunoassay (ELISA) and indirect immunofluorescence (IIF) compared to the results of WB. Circulating antibody to PLA2R was detected by (IIF) on BIOCHIPs (Euroimmun) on either HEK293 tissue culture cells transfected with a recombinant construct of PLA2R and control transfected cells and by ELISA (Euroimmun) using a purified human recombinant PLA2R receptor from transfected HEK293 cells as a substate. Patients with primary MGN on renal biopsy (n=19) and a peribiopsy serum specimen were tested for anti-PLA2R by WB, ELISA, and IIF. Contols included non-primary MGN immune complex GN; secondary MGN (n=2), immune complex GN (n=5), de novo MGN (n=2), lupus Class 5 (n=1), additional controls included autoimmune sera (n=19); Celiac Disease (n=3), Crest syndrome (n=3), SLE + ANA (n=7), negative ANA (n=3), anti-CCP(n=3), ANCA (n=7), anti-GBM (n=2), and normal blood donors (n=20).

Results:

Diagnosis	Pos WB	Neg WB	WB	Pos ELISA	Neg ELISA	ELISA	Pos IIF	Neg IIF	IIF
MGN (n=19)	14	5	73.6% Sensitivity 100% Specificity	14 (Mean= 588.6 RU/ml) (Range 89->1500 RU/ml)* positive= >14RU/ml	5	76.4% Sensitivity 100% Specificity	10	9	52.6% Sensitivity 100% Specificity
Non Primary MGN Immune Complex	0	10		0	10		0	10	
Control Groups (n=56)	0	48		0	48		0	48	

Conclusions: The detection of anti-PLA2R antibody using ELISA and IIF provides a sensitive and specific tool for detection when compared to WB. The additional advantage of ELISA is quantitation a potential useful marker of treatment response.

Funding: Clinical Revenue Support

PUB067

Oncostatin M Represents a Potent Co-Stimulator of Inflammatory Chemokines in Human Proximal Tubular Cells Rita Sarkozi, Ulrike Corazza, Philipp Osterkamp, Markus Pirklbauer, Gert J. Mayer, Herbert Schramek. *Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical Univ, Innsbruck, Tirol, Austria.*

Background: Increased production of inflammatory chemokines such as MCP-1 and RANTES promotes the accumulation of monocytic cells into injured renal tissue, a common feature observed in diabetic nephropathy. In this context we reported that oncostatin M (OSM) exerts antifibrotic effects in human kidney-2 (HK-2) cells.The present study was performed to investigate OSMs effects on MCP-1 and RANTES mRNA expression in proximal tubular HK-2 cells both in the absence and presence of IL-1β, TNF-α or TGF-β1.

Methods: Cell culture, real-time PCR, RNA silencing.

Results: Administration of 10 ng/ml IL-1β, TNF-α or TGF-β1 led to a rapid, time-dependent induction of MCP-1 mRNA lasting for at least 48 h. OSM (10 ng/ml) stimulated MCP-1 mRNA expression after 30 min and 1 h, which was reduced nearly to basal levels as early as 3 h after incubation. Both, IL-1β and TNF-α led to a slowly building, time-dependent upregulation of RANTES mRNA expression. In contrast, neither TGF-β1 nor OSM significantly affected RANTES mRNA levels for up to 48 h. Treatment of HK-2 cells with IL-1β, TNF-α or TGF-β1 in the presence of OSM for 6 h and 24 h resulted in a

strong additive effect on MCP-1 mRNA expression. Similar additive effects, albeit lower in size, were obtained for RANTES when HK-2 cells were stimulated either with IL-1β or TNF-α in combination with OSM. Furthermore, siRNA-mediated selective silencing of ERK1, ERK2, STAT1 or STAT3 suggests that, in contrast to ERK1/2, both STAT1 and STAT3 are involved in the regulation of basal MCP-1 expression. Most importantly, selective knockdown of these signaling molecules revealed evidence that the additive effect of OSM on IL-1β-induced MCP-1 mRNA expression, at least partially, depends on STAT3 but not on STAT1, ERK1 or ERK2.

Conclusions: OSM enhances the stimulatory effect of IL-1β and TNF-α on mRNA expression of MCP-1 and RANTES in HK-2 cells. OSMs additive effect on IL-1β-induced MCP-1 mRNA expression partially depends on STAT3. The results suggest, that OSM is a co-stimulator of these inflammatory chemokines in human proximal tubular cells.

PUB068

Uric Acid Induces Inflammasomes Formation and Pyroptosis in Human Renal Proximal Tubular Cells Shabirul Haque, Shallu Sharma, Maria Sultana-Syed, Ashwani Malhotra, Pravin C. Singhal. *Medicine, Hofstra North Shore LIJ Medical School, Great Neck, NY.*

Background: The inflammasome is a multiprotein oligomer composed of caspasse-1, PYCARD adm NALPH.The inflammasome promotes the maturation of the inflammatory cytokines such as interleukin (IL)-1ß and IL-18. Uric acid (UA) crystals have been demonstrated to promote gouty arthropathy through inflammasome formation. High serum uric acid has also been considered to promote both acute and chronic tubulointerstitial disease. However, the role of uric acid in the induction of tubular cell inflammasome formation has not been studied. We hypothesized that uric acid enhances tubulointerstitial fibrosis through the induction of tubular cell inflmmasome formation and pyroptosis.

Methods: Human renal proximal tubular cells (HRPTCs) were incubated in media containing either buffer or different concentrations of uric acid for 24 and 48 hours and assayed for pyroptosis through staining with H3342 and propidium iodide. To determine the involved mechanism, HRPTCs treated with UA (100 µg/ml) for 48 h were followed by RNA and protein extraction. cDNA as well as protein blots were probed for NLRP3, IL-1 and caspase-1. To establish causal relationship between caspase-1 activation and pyroptosis, HRPTCs were treated with UA in the presence or absence of caspase-1 inhibitor and then evaluated for occurrence of pyroptosis and inflammasome formation.

Results: Uric acid enhanced tubular cell pyroptosis in a dose and time dependent manner. Uric acid promoted transcription of NLPR3 and pro-caspase-1. Uric acid also enhanced protein expression of IL-1 ß and caspase-1. Since caspase-1 inhibitor not only inhibited tubular IL-1 ß expression but also reduced number of tubular cells displaying pyroptosis, it appears that this effect of uric acid is mediated through caspase-1 activation.

Conclusions: Uric acid enhances tubular cell pyroptosis and inflammasome formation through the activation of caspase-1.

Funding: NIDDK Support

PUB069

Erythropietin Modulates Endothelial Cell From High Glucose Induced Injury Haruka Yasuda,[1] Yasunori Iwata,[2,3] Kengo Furuichi,[3,4] Takashi Wada.[1,3] *[1]Dept of Laboratory Medicine, Kanazawa Univ, Kanazawa, Japan; [2]Div of Infection Control, Kanazawa Univ, Kanazawa, Japan; [3]Div of Nephrology, Kanazawa Univ, Kanazawa, Japan; [4]Div of Blood Purification, Kanazawa Univ, Kanazawa, Japan.*

Background: Diabetic nephropathy (DN) is a major cause of end stage kidney disease and a strong risk factor for cardiovascular diseases. High glucose induces endothelial injury in vasculature, resulting in tissue injury in diabetic condition. Chronic inflammation has been reported to play an important role for the progression of high glucose induced cell injury. Growing data showed that erythropoietin (EPO) protect the tissues from some kind of injury, such as hypoxia and mechanical stress. However, the contribution of EPO to high glucose induced tissue injury remains to be explored. Therefore, we hypothesized that EPO modulates endothelial cells from high glucose (HG) induced injury via the regulation of inflammatory and anti-inflammatory balance.

Methods: To explore this possibility, we performed genome-wide transcriptome profiling in human umbilical vein endothelial cells (HUVEC), which were stimulated by high glucose (HG) with/without EPO treatment and detected the expression of inflammation associated genes.

Results: Hieralchial clustering analysis showed the different pattern of mRNA expression in HG stimulated HUVEC with/without EPO. While inflammatory cytokines/chemokines mRNA expression were increased by the HG stimulation in HUVEC, Th2 related cytokine receptors and intracellular signaling molecules showed the reduced mRNA expression levels. EPO treatment reduced inflammatory cytokines/chemokines mRNA expression and increased Th2 related cytokine mRNA expression levels. Real-time PCR analysis confirmed the increased expression of inflammatory related genes, those were decreased in HG stimulated HUVEC with EPO treatment. Moreover, EPO stimulation increased mRNA expression of EPO receptor and b-common receptor.

Conclusions: Taken together, EPO signaling modulates high glucose induced cell injury by the regulation of immune balance.

PUB070

The Function of Macrophage in Intestinal Tract with Bacterial Translocation in Chronic Renal Failure Model Rats Hongli Jiang,[1] Meng Wang,[2] Hua Liu.[3] *[1]Hemodialysis Center, First Affiliated Hospital of Medical College of Xi'an Jiaotong Univ, Xi'an, Shaanxi, China; [2]Hemodialysis Center, First Affiliated Hospital of Medical College of Xi'an Jiaotong Univ, Xi'an, Shaanxi, China; [3]Hemodialysis Center, First Affiliated Hospital of Medical College of Xi'an Jiaotong Univ, Xi'an, Shaanxi, China.*

Background: Our rencent studies have confirmed that intestinal bacterial translocation is an important reason for micro-inflammatory state exsiting in the patients and rat models with chronic renal failure, which is closely associated with the high mortality rate and a variety of complications. Furthermore, previous studies had shown that the function and status of intestinal macrophages were closely related to the intestinal bacterial teanslocation in many diseases. Therefore, the present study aimed at understanding the function and status of the intestinal macrophage in the rats model with chronic renal failure.

Methods: We used double immunofluorescence staining technique to observe the function and status of intestinal macrophages with specific membrane antigen CD68 and activiated macrophage marker of TLR4, NF-kB and MHC-II between the chronic renal failure group and the sham group.

Results: a. There are more CD68 positive cells in all intestinal segments of the chronic renal failure group than the sham group. b.The fluorescence intensity of TLR4, NF-kB on macrophages of the chronic renal failure group are all stronger than the sham group, especially in ileum. C. Both groups don't express MHC-II on intestinal macrophages.

Conclusions: In all intestinal segments, there are more macrophages infiltrating and activating in the rats model with chronic renal failure, especially in ileum. The activated state of the intestinal macrophages show stronger ability to identify, absorb and engulf pathogens, but no ability to present them. It suggests that the intestinal bacterial translocation appears more in ileum.

Funding: Government Support - Non-U.S.

PUB071

The Expression of Tristetraprolin and Its Relationship with Urinary Protein in Diabetic Nephropathy Patients Zhangsuo Liu,[1] Fengxun Liu,[2] Guo Jia.[3] *[1]the first affiated hospital of zhangzhou Univ; [2]the first affiated hospital of zhangzhou Univ; [3]the first affiated hospital of zhangzhou Univ.*

Background: To investgate the relationship of Tristetraprolin (TTP, a RNA-binding protein) and diabetic nephropathy.

Methods: This study included 128 cases, age (60.7±10.9) years old. A total of 54 cases of patients with diabetic nephropathy, based on the amount of protein divided into microalbuminuria, macroalbuminuria group, choose the same period 33 cases of diabetes, 41 cases of healthy people as the control group. Patient's blood and urine samples was used to analyze the expression of TTP by PCR and ELISA kits.

Results: Urine electrophoresis showed that TTP decreased companied with urinary protein increased in every groups. pairwise comparisons showed proteinuria and microalbuminuria group was significantly lower than that in diabetic group and normal control group ($P < 0.05$), while a large number of proteins no significant difference ($P > 0.05$) between the two groups with urinary microalbuminuria group. Blood samples from the same period of the ELISA assay Tristetraprolin, consistent with the results of the urine specimen. Compared to normal control group (284.77±20.61pg/ml) and simple diabetic patients (196.14±17.11pg/ml),Tristetraprolin in microalbuminuria and proteinuria in patients with a large plasma levels were lower: 140.53 ± 19.22bpg/ml and 81.34 ± 16.51 pg / ml, the difference was statistically significant ($P > 0.05$). Tristetraprolin were negatively correlated with urinary protein excretion, the correlation coefficient was -0.572 ($P < 0.05$) and -0.685 ($P < 0.05$).

Conclusions: 1,TTP involved in the inflammatory response in diabetic nephropathy.

Funding: Government Support - Non-U.S.

PUB072

Xylene Induced Apoptosis and Change of DNA Methylation in HK-2 Cells Wei-Song Qin, Xianghua Cao, Zhao-Hong Chen, Chun-Xia Zheng, Caihong Zeng, Zhihong Liu. *National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, Jiangsu, China.*

Background: Xylene is used as a solvent with wide areas of application. Cortical tubular epithelial cells are a common site of organic solvents induced injury. However, the role and mechanism of xylene induced injuries in proximal tubular cells has not been well documented. We propose to study the role of xylene in inducing the toxicity of HK-2 cells and investigate the change of DNA methylation level of apoptosis associated genes.

Methods: Xylene was dissolved with DMSO and added in the culture medium of HK-2 cells. NGAL, as a marker of renal tubular epithelial cell injury, was detected with ELISA. The apoptosis ratio was analyzed with flowcytometry. Caspase-3 activity was detected using a fluorometric assay kit. Genomic DNA was extracted, bisulfite converted and then hybridized to the HumanMethylation450 Analysis BeadChip. Array methylation results were validated by pyrosequencing.

Results: We found that xylene could induce injury in HK-2 cells in this study. Secretion of NGAL increased while the concentration of xylene and treatment time increased. Xylene could induce the apoptosis of nearly 98% HK-2 cells at the concentration of 2.0mM after 48h. Caspase-3 activity in HK-2 cells was significantly increased after treatment with xylene. Global DNA methylation was analyzed using the Illumina Infinium 450K

microarray platform. Results showed that 243 CpG sites were indicated as differentially methylated, including 109 sites of hypermethylation and 134 sites of hypomethylation. Differential DNA methylation was related to 138 genes involved in cell apoptosis and cell cycle related proteins. Quantitative DNA methylation analysis was performed by pyrosequencing and showed 7% and 4% decrease in methylation of BAX gene and 6.0% decrease in methylation of FAF1 gene.

Conclusions: Xylene could induce the apoptosis in HK-2 cells. The DNA methylation of FAF1 was decreased while the DNA methylation of BAX was increased. The causative effect of these DNA methylation change on the injury of xylene needs to be further explored.

Funding: Government Support - Non-U.S.

PUB073

***Salvia miltiorrhiza* Bunge Protects Renal Function in Partial Ureteral Unilateral Obstruction in Rats** Maria Fatima De Paula Ramos,[1] Josne Carla Paterno,[1] Vera Lucia Rehder,[2] Clara Versolato Razvickas,[1] Nestor Schor,[1] Vicente De Paulo Castro Teixeira.[1] *[1]Medicine / Nephrology Div, Univ Federal de Sao Paulo/EPM, Sao Paulo, Brazil; [2]Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), UNICAMP, Paulinia, Sao Paulo, Brazil.*

Background: Aqueous extracts of *Salvia miltiorrhiza*Bunge root (S. miltiorrhiza) is one of the most commonly used traditional Chinese herbs. The aim of this study was to investigate the effects of aqueous extracts of *Salvia miltiorrhiza* Bungein experimental model of partial ureteral unilateral obstruction in rats.

Methods: Male Wistar rats were submitted to partial ureteral unilateral obstruction and right nephrectomy (PUONx), divided in two groups and studied along eight weeks (n = 5 per group). Control (PUONx): PUONx+ control vehicle (saline); *Salvia miltiorrhiza* (PUONx-S): animals received orally *Salvia miltiorrhiza* aqueous extract (100 mg/kg b.w.) daily by gavage. We measured 24h-proteinuria, microalbuminuria and urine NGAL(neutrophil gelatinase associated lipocalin). The statistical significance of the results was evaluated by ANOVA followed by Tukey test. Values were expressed as mean ± SD.

Results: The orally administration of aqueous extracts of *Salvia miltiorrhiza* ameliorated physiological dysfunctions of 24h urinary protein excretion ($p<0.001$): 12.42 ± 8.3 mg/24h versus 35.34 ± 15.2 mg/24h (PUONx-S versus PUONx) and microalbuminuria ($p<0.007$): 0.55 ± 0.26 mg/24h versus 1.47 ± 0.73 mg/24h (PUONx-S versus PUONx). PUONx-S treated group displayed a significantly ($p<0.02$) reduced loss urinary NGAL than control group (53.72± 40.08 versus 168.20 ± 126.66 pg/mL, respectively).

Conclusions: *Salvia miltiorrhiza*, in turn, had a beneficial effect on early renal function evidenced by normalization of proteinuria and decrease in levels of NGAL in the urine. Our results suggest that *Salvia miltiorrhiza* aqueous extracts could be beneficial in the treatment partial ureteral unilateral obstruction.

PUB074

Propargylglycine Increases Kidney Weight Independently of Effects on Kidney Function Nynke R. Oosterhuis,[1] Anne-Roos S. Frenay,[2] Sebastiaan Wesseling,[1] Marianne C. Verhaar,[1] Harry Van Goor,[2] Jaap A. Joles.[1] *[1]Nephrology and Hypertension, UMC Utrecht, Netherlands; [2]Pathology and Biomedical Biology, UMC Groningen, Netherlands.*

Background: Hydrogen sulfide (H_2S) and nitric oxide (NO) share signaling and vasorelaxant properties and H_2S can increase cell number by effects on proliferation and apoptosis. Blocking NO induces hypertension and proteinuria. However, hypertension and proteinuria induced by blocking production of NO are prevented by concomitant blockade of H_2S production with propargylglycine (PAG) [Wesseling et al. 2014]. We hypothesized that blocking H_2S producing enzymes ameliorates hypertension and proteinuria.

Methods: We studied effects of concomitant PAG in healthy rats and two different models of kidney injury: angiotensin II infusion (AngII) and $^5/_6$ nephrectomy (SNX).

Results: In healthy rats PAG administration did not affect systolic blood pressure (SBP) or proteinuria. PAG reduced SBP and proteinuria in AngII rats compared with vehicle (180 ± 12 versus 211 ± 19 mmHg and 66 ± 35 versus 346 ± 92 mg/24h, $p<0.01$), but not in SNX rats: 159 ± 27 versus 150 ± 27 mmHg, and 141 ± 114 versus 86 ±52 mg/24h. Surprisingly, kidney to body weight ratio was increased in all groups by PAG compared with respective vehicle groups (**Figure 1**). Proximal tubule damage was not affected by PAG in healthy and SNX rats and reduced by PAG in AngII rats ($p<0.05$). PAG did not affect tubular lumen area compared with respective vehicle groups.

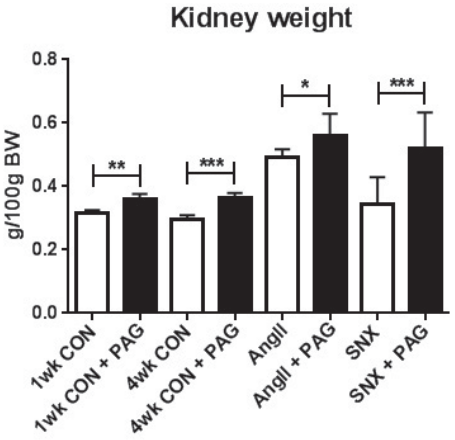


Figure 1. Kidney to body weight ratio was increased in all PAG-treated groups irrespective of effects on blood pressure and proteinuria. *p<0,05 **p<0,01 ***p<0,001.

Conclusions: Blocking H_2S producing enzymes with PAG increased kidney weight independent of effects on SBP and proteinuria. Kidney enlargement was not caused by tubular damage or dilatation, and was possibly due to increased cell number or due to increased intracellular osmoles.

Funding: Government Support - Non-U.S.

PUB075

Dysfunction of the Epithelial Cell Transforming Sequence 2 Gene May Cause Tubulointerstitial Injury Leading to Secondary Focal Segmental Glomerulosclerosis in the Mouse Model of Kidney Injury with Aristolochic Acid Administration Tomoki Miyazawa,[1] Akane Izu,[1] Takuji Enya,[1] Hitomi Nishi,[1] Keisuke Sugimoto,[1] Hidehiko Yanagida,[2] Mitsuru Okada,[1] Tsukasa Takemura.[1] *[1]Pediatrics, Kindai Univ Faculty of Medicine, Osakasayama, Osaka, Japan; [2]Pediatrics, Sakai Hospital Kindai Univ Faculty of Medicine, Sakai, Osaka, Japan.*

Background: Secondary focal segmental glomerulosclerosis (FSGS) follows congenital or acquired tubulointerstitial alterations. Failure of adequate regeneration after tubulointerstitial injury or abnormal tubulogenesis can disturb intrarenal blood circulation, causing excessive glomerular filtration. The product of the epithelial cell transforming sequence 2 gene (*ECT2*) is a transforming protein related to Rho-specific exchange factors and cell cycle regulators. We previously reported two patients with tubulointerstitial disorders, followed by FSGS. These patients carry a nonfunctional genotype of *ECT2*. Alistorochic acid (AA) is known as oncogenic material causing Balkan endemic nephropathy (BEN). One of the locations of DNA injured in BEN, which was located on 3q26.1 to 3q26.3, was consistent with *ECT2* mutation which had been showed in our patients.

Methods: We created the mouse model of kidney injury with AA injection and investigated the relationship between *ECT2* dysfunction and kidney injury. We gave intraperitoneal injection of AA 3 mg/kg to C57BL/6N for 6 weeks once in 3 days from the 6 weeks of their age.

Results: Their histological features of kidney at 20 weeks of their age showed cloudy degeneration, vacuolation of uriniferous tubules, tubular epithelial cell detachment and glomerular swelling. They were identical to finding of *ECT2* mutation. Then, gene expression was screened by the comparative genomic hybridization. Down-regulation of *ECT2* was observed in some mice injected AA. Our results supposed that *ECT2* mutation of our patients congenitally occurred structural and/or functional tubulointerstitial disorder and gradually contributed to the progression of secondary FSGS.

Conclusions: *ECT2* dysfunction could be the primary cause of BEN. Congenital mutation of *ECT2* might be associated with the development of FSGS in some of the patients with FSGS.

PUB076

Plasma Inflammatory Profile in CRS5 Patients: What Is the Cause of Multi-Organ Damage? Alessandra Brocca,[1] Grazia Maria Virzì,[1] Stefano Marcante,[2] Silvia Pastori,[1] Chiara Pasqualin,[1] Massimo de Cal,[1] Claudio Ronco.[1] *[1]Nephrology-IRRIV, S Bortolo Hosp; [2]ICU, Vicenza.*

Background: Cardiorenal Syndrome Type 5 (CRS5) is characterized by the combination of cardiac and renal dysfunction due to systemic disorders. CRS5 mechanism is not well-known, although specific cellular and molecular changes are reported. We examined the inflammatory profile in CRS5 patients (pts) and the *in vitro* response of renal tubular cells (RTCs) treated by CRS5 plasma.

Methods: We enrolled 11 pts with CRS5 (68.4±10.8yrs), and 16 healthy controls (CTR)(52.0±7.7yrs). Plasma from different groups were incubated with RTCs for 24h and, subsequently, cell death was evaluated by Annexin-V/Propidium Iodide assay. Plasma levels of IL6, IL1β, IL8, IL10, IFNγ were measured by ELISA and plasma NGAL (pNGAL) was detected by TRIAGE Alere.

Results: A significantly lower viability, a significantly higher apoptosis and necrosis were observed in RTCs incubated with CRS5 plasma compared to CTR (all p<.05). Plasma pro- and anti-inflammatory cytokines were significantly increased in CRS5 pts compared with CTR (p<.05).

parameters	CRS5	CTRL
IL6 (pg/ml)	64.6 (59.3-83.8)	5.9 (3.5-7.3)
IL10 (pg/ml)	19.6 (8.1-54.6)	3.1 (2.0-3.9)
IL8 (ng/ml)	87.1 (78.0-119.5)	20.3 (12.6-47.6)
IL1β (pg/ml)	80.8 (45.9-273.9)	8.3 (4.3-41.1)
IFNγ (pg/ml)	17.9 (16.9-20.8)	2.0 (1.4-3.2)
pNGAL (ng/ml)	249.0 (134.2-615.7)	60 (60-60)
Viability (%)	82.8 (81.2-85.6)	96.6 (94.8-97.5)
Necrosis (%)	4.2 (2.2-11.5)	1.0 (0.7-1.3)
Apoptosis (%)	15.1 (13.8-17.6)	1.5 (1.2-1.9)

There was a significant positive correlation between RTCs necrosis and IL1β and IL6 levels (rho=.82, rho=.77) and a significant negative correlation between IL6 and cell viability (rho=-.73). An interesting negative correlation between IL1β and cell viability was observed both in CRS5 pts (rho=-.91) and in CTR (rho=-.92).

Conclusions: We observed a strong cytokines dysregulation in CRS5 pts. Furthermore our study demonstrated the induction of cellular death in RTCs incubated with CRS5 plasma. In conclusion, we hypothesize a signals congestion involved in CRS5 pathophysiology: anti-inflammatory cytokines were abundantly released but are insufficient to contain a systemic inflammation that cause multi-organ damage.

Funding: Private Foundation Support

PUB077

Sealing the Slits: A Protective Response of Podocytes against High Shear Stress? Wilhelm Kriz,[1] Kevin V. Lemley.[2] *[1]Medical Faculty Mannheim, Univ of Heidelberg; [2]Dept of Pediatrics, Univ of Southern California.*

Podocytes are lost by detachment from the GBM as viable cells. This is a gradual process, which may begin at a single part of a podocyte and progress to include the entire cell as well as adjacent podocytes. The structural details of this process suggest that shear stress may be a crucial factor. Podocytes are highly susceptible to shear stress (Friedrich et al.: AJP Renal 2006). In vivo, the effect of increased shear stress may directly be seen in podocytes that have come to lie within the urinary orifice before finally undergoing detachment as bottle-shaped cells. The crucial site where podocytes are exposed to shear stress is likely the filtration slits. Without considering the slit diaphragm (SD), the shear stress of filtrate flow acting on foot processes (FPs) has been calculated to be 10-fold higher than that acting on podocyte cell bodies (Endlich and Endlich: Seminars Nephrol 2012). We hypothesize that the SD mitigates shear forces on FPs. Depending on the local situation (comprising FP geometry, slit density, and local filtrate flow rate), the shear forces on FPs may become intolerably high, compromising their attachment to the GBM The transmitted forces likely initiate a process of sealing of the slits. The replacement of SD by occluding junctions has been described in many studies, including several-fold increase of tight junctional proteins in PAN nephrosis (Fukasawa et al.: JASN 2009). This is widely considered as the start of podocyte deterioration called “FP effacement” (FPE) but, in our view, it represents a locally protective response against detachment. This FPE reaction may have three outcomes: (i) reversal, in case the rheological situation improves, (ii) progression to the completed stage of FPE leading to a firm attachment to the GBM and (iii) failure, exposing the FPs to high shear stress, starting the process of detachment. Since at other sites of the same podocyte the lateral connection to adjacent podocytes remains intact, podocytes frequently detach in groups.

Funding: NIDDK Support, Other NIH Support - NIH/ORDR

PUB078

Establishment of a High Throughput Recombinant Protein Synthesis System for Experimental Therapeutics Using Hollow Fiber Kidney and Stable Cell Lines Maria Del Nogal, Hector Donoro Blazquez, Camille E. Mace, Lionel C. Clement, Sumant S. Chugh. *Dept of Medicine Div of Nephrology, Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Cell culture in hollow fiber bioreactors were developed to generate large amounts of recombinant protein. Hollow fiber bioreactors contain artificial porous capillaries with continuously culture medium perfusion that confers a matrix for high density cell culture. Secreted proteins are retained in the extra-capillary space and can be collected for experimental therapeutic use.

Methods: Expression constructs of human Angiopoietin-like 4 (Angplt4) or human Angptl4 mutants were generated in pcDNA3.1V5-His and stable cell lines developed using HEK-293 cells. These stable cell lines were inoculated into the outer dialysate chamber of hollow fiber kidneys housed in 5% CO2 incubators and cell media was recirculated through the hollow fibers using a positive displacement pump. After three weeks of cell growth, fetal bovine serum was removed and substituted with a chemical serum replacement. *N*-acetyl-D-mannosamine was also added to insure adequate sialylation of recombinant protein.

Results: The glucose consumption of the systems was 1 gram per day and 15 ml of extra-capillary space medium containing the recombinant protein were collected every day. Harvested medium were dialyzed overnight in autoclaved PBS and purified over nickel columns. Recombinant protein was quantified using human Angptl4 ELISA, anti V5 ELISA, and Western blot. Each unit produces at least 500 µg of recombinant protein per day. Using a setup of two systems per incubator and 12 functional systems, 6 mg of one or more recombinant protein can be produce per day. Recombinant protein was injected intravenously into animal models to test and confirm in vivo efficacy. In addition, 2D gel electrophoresis and Western blot analysis were conducted to confirm posttranslational changes in the recombinant protein.

Conclusions: Hollow fiber bioreactors are an excellent tool to obtain a large amounts of recombinant protein required to develop and test novel protein based therapeutics for kidney disease.

Funding: NIDDK Support

PUB079

Human Leptospirosis: A Model of Acute Lipotoxicity Caroline Azevedo Martins,[1] Mauro C. Faria,[1] Mauricio Younes-Ibrahim.[1] *[1]Internal Medicine, Univ do Estado do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.*

Background: Leptospirosis is a worldwide acute infectious zoonosis and presenting a peculiar lipid disorder associated to increase of the nonesterified fatty acids. In vitro previous studies showed that both Leptospira’s endotoxin effect on Na/K-ATPase activity and free fatty acid cytotoxicity are aggravated by lower serum albumin levels, supposedly due to albumin adsorptive properties for hydrophobic molecules.

Methods: Prospective observational cohort study was realized in 28 hospitalized patients with Weil’s syndrome. During their hospitalization, the serum concentration of nonesterified fatty acids (NEFA) levels and albumin were performed sequentially. Of these, 20 patients were cured and 8 died. The oleic acid: albumin molar ratio was used as a biomarker of lipotoxicity.

Results: All patients presented at admission significant increase in oleic acid: albumin molar ratio when compared to control. Patients who survived rescued the oleic albumin molar ratio to normal levels, which did not occur in patients who died.

Conclusions: The rescue of oleic acid albumin molar ratio was correlated with good outcome in severe leptospirosis and can be used as a biomarker of lipotoxicity in acute affections.

PUB080

Histological and Clinical Evaluation of Adult Patients with Henoch-Schöenlein Purpura Nephritis from a Single Hospital in China: Review of 114 Cases Ling Yun Lai,[1] Huixian Li,[1] Chuanming Hao,[1] Bruce A. Julian,[2] Jan Novak,[2] Lea Novak.[2] *[1]Div of Nephnology, Fudan Univ, Huashan Hospital, Shanghai, China; [2]Univ of Alabama at Birmingham, Birmingham, AL.*

Background: Henoch-Schönlein purpura nephritis (HSPN) has been less studied in adults than in children. The aim of this study was to evaluate pathological changes and clinical data of adult HSPN patients from a single hospital in China.

Methods: Renal-biopsy slides were collected from 114 adult HSPN patients (63 females; age range 18-78 y) who underwent biopsy from August 2002 to May 2013. Two pathologists graded histological changes. Cellularity of each glomerulus was graded and a mean mesangial score was calculated. Clinical data were collected at time of biopsy. 110 patients with known date of onset of purpura were grouped based on interval from the onset to renal biopsy: Group 1 (G1, <1 mo); Group 2 (G2, 1 – 6 mos), and Group 3 (G3, >6 mos).

Results: All patients had purpura, proteinuria, and microscopic but not macroscopic hematuria. 27% had abdominal pain and 26% had arthralgia. Increased serum IgA level (>3.9 g/L) was detected in 18%. The 3 groups had similar mean 24-h proteinuria, hematuria, mean eGFR, and frequency of ACEI/ARB treatment. The percentages of circulating white blood cells as neutrophils differed (G1 = 71%, G2 = 66%, G3 = 57%, p <0.001). Histology showed mean mesangial score 1.1, presence of segmental sclerosis (18%), global sclerosis (26%), glomerular crescents (56%), glomerular adhesion (26%), tubular atrophy (43%), tubular casts (46%), interstitial fibrosis (39%), and interstitial lymphocytes (51%). Histologic findings of Groups 1-3 did not differ except for median percentage of glomeruli with lymphocytes (G1 = 57%, G2 = 10%, G3 = 21%, p<0.001) and mean percentage of interstitial fibrosis (G1 = 36%, G2 = 31%, G3 = 55%, p = 0.05).

Conclusions: Patients with renal biopsy within 1 mo of onset of purpura more commonly had glomerular lymphocytes. Severity of crescents was not related to the timing of the biopsy. This unique large cohort can serve for comparison with data on adult HSPN patients in other geographic locations.

PUB081

A Recipe for an “All Purpose” Perfusion Fixation of Rodent Kidneys for Morphology and Cytochemistry Sebastian Bachmann, Carsten Dittmayer. *Anatomy, Charité Universitätsmedizin Berlin, Berlin, Germany.*

Background: Current advances in rodent transgenic technology have increased the need for high resolution morphology and histochemistry protocols including suitable fixation recipes. Adequate preservation of the kidney requires particular care owing to the complexity of its parenchyma. Immersion fixation results in poor morphology and inadequate cell biological analysis. Conventional *in vivo* vascular perfusion protocols may be complicated to perform and may require separate approaches for ultrastructural and histochemical investigation, resulting in large animal group sizes and related conflicts with animal welfare regulations. We aimed to establish a simple fixation protocol which permits global structural evaluation at high quality standards within a single experimental animal.

Methods: Retrograde perfusion of the abdominal aorta was done under anesthesia using flushing buffer and aldehyde fixative. Parameters varied were the perfusion pressure, composition of the carrier and fixative solutions, post-fixation protocols and sample processing techniques including cryopreparation. Paraformaldehyde (PFA) fixatives were

compared to standard glutaraldehyde fixation. Variations in carrier osmolalities were adapted to the conditions required for the individual parenchymal zones with particular focus on podocyte preservation. A focus was set on podocyte structural preservation and cytochemical evaluation.

Results: High-end quality was obtained using perfusion at 180 cm H_2O, modified phosphate buffer as carrier solution, 4% PFA as fixative, followed by various post-fixation treatments adapted for cryo- and paraffin sectioning, conventional EM, freeze substitution or Tokuyasu technique, and immuno-EM. State-of-the-art confocal LM and EM techniques including tomography and STEM led to excellent results.

Conclusions: In sum, a universal fixation protocol with appropriate post-fixation treatments of rat and mouse kidney tissues can be easily adapted for global evaluation techniques within a single experimental animal, allowing for high standard glomerular, tubular and interstitial evaluation techniques.

PUB082

Simple Method Ameliorating Phenotypes of Primary Cultured Podocytes

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Background: We have previously established primary culture of rat podocytes which have an arborized morphology without cobblestone-like appearance and retain well podocyte-specific markers including podocalyxin and podocin (Kidney Int 2006, 69:2101). The markers, however, are deeply down-regulated with time during culture. After 8 days of primary culture, nephrin decreases to less than one thousandth of that of isolated glomeruli, and claudin-5 drops sharply to an undetectable level even by PCR. After reviewing many substances, we have found that heparin acts directly on cultured podocytes to improve highly their phenotypes in transcript and protein levels (Nephrology 2014, 19:195). Even so, further improvements are necessary, because expression levels of nephrin, podocalyxin and podocin still remained about 1/60, 1/20 and 1/10 of those of isolated glomeruli, respectively.

Methods: We tried a decrease in the concentration of bovine fetal serum (FBS) and culture on porous membrane, because FBS contains many factors activating podocytes and porous membrane provides the culture condition where media access attachment cells from both their apical and basolateral sides.

Results: Each of the decrease in FBS from 5% to 0.5% and culturing on porous membrane increased nephrin expression significantly. When combined with heparin, 0.5% FBS and culture on porous membrane, cultured podocytes extremely increased expression level of nephrin approximately to 1/3 of that of isolated glomeruli. Those of podocin and podocalyxin expressions reached the same level or more than isolated glomeruli. Especially claudin-5 increased to 1/10 of that of isolated glomeruli. It is in stark contrast to the undetectable level in the original culture condition. Morphologically podocytes extended primary processes which intersected adjacent ones.

Conclusions: These findings suggest that the above simple method takes the intracellular environment of cultured cells closer to that of podocytes *in vivo.*

Funding: Government Support - Non-U.S.

PUB083

Evaluation of Urinary Kidney Injury Biomarkers in the Acute PAN Model: Indication of Both Podocyte and Tubule Injury

Kelly Larson, Lauren Olson, Kathryn Houseman, Deborah Widomski, Bruce Leroy, Andrew J. King, Steve McGaraughty, Zhi Su. *Early Renal Discovery, Abbvie.*

Background: Acute treatment of rats with PAN (puromycin aminonucleoside) is widely used as a model of podocyte injury (acute PAN model). Urinary protein excretion rate (UPER) and electron microscopy (EM) examination of podocytes are major biomarkers/ tools for evaluation of podocyte injury in the acute PAN model. In the current study, we employed a number of novel urinary biomarkers to further evaluate PAN-induced kidney injury in rats.

Methods: The acute PAN model (8-day) was generated by a single subcutaneous injection of PAN (100-150 mg/kg) to male rats (125 -150 grams). In addition to EM, urine sample (24 hrs) was collected at designated time points after PAN injection for analysis of UPER, nephrin, podocalyxin, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), osteopontin (OPN), 8-hydroxy-deoxyguanosine (8-OH dG), and isoprostanes. Serum creatinine (SCr) and urea nitrogen (BUN) levels were also assessed, and a reference compound mizoribine tested.

Results: Foot process effacement, loss of slit diaphragm, large vacuoles, deposition of electron dense granules was observed in podocytes (EM section of kidney cortex) from PAN-injected rats. PAN produced dose- and time-dependent increases in UPER (peaking at day 8-10 post PAN dosing), as well as urinary nephrin and podocalyxin (indicating podocyte injury). Urinary excretion of NGAL, KIM-1, and OPN was also increased in a time-dependent manner (suggesting acute renal tubular injury). However, SCr and BUN were minimally increased in PAN-injected rats. Urinary 8-OH dG and isoprostanes (markers for oxidative stress) were not increased in PAN-injected rats. The increases of urinary biomarkers for podocytes and renal tubules were significantly suppressed by prophylactic treatment with mizoribine (10 mg/kg).

Conclusions: Urinary excretion of both podocyte and renal tubular injury markers is significantly increased in the acute PAN model. These results indicate that in addition to podocyte injury, renal tubules are also subsequently damaged in the acute PAN model.

PUB084

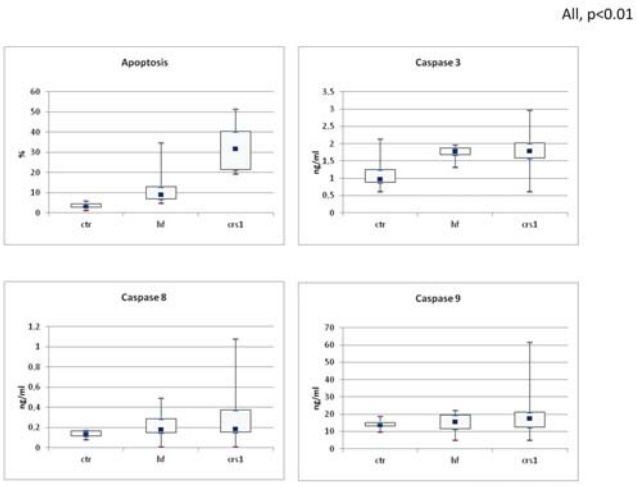
Cardiorenal Syndrome: Activation of Dual Apoptotic Pathways

Grazia Maria Virzì, Alessandra Brocca, Massimo de Cal, Enrico Tonini, Claudio Ronco. *Nephrology-IRRIV, San Bortolo Hospital, Vicenza.*

Background: Cardiorenal syndrome Type 1 (CRS1) is characterized by a rapid worsening of cardiac function leading to acute kidney injury(AKI). CRS1 pathophysiology is complex and an immune-mediated damage, an alterations in the immune response with monocytes apoptosis and cytokine-release have been reported as a potential mechanisms. In this study, we examined the putative role of Renal Tubular Epithelial Cells (RTCs) apoptosis as a pathogenic mechanism in CRS1. In particular, we investigated the Caspase pathways involved in this induced apoptosis.

Methods: We enrolled 29 patients with Heart Failure (HF) (age 73.6±9.5yrs, sCr baseline 0.91mg/dl±.27), 11 patients with CRS1 (74.±13.1yrs, sCr baseline 1.12mg/ dl±.24). Patients who had AKI prior to the episode of HF, or had any other potential causes of AKI, were excluded. Plasma from different groups were incubated with RTCs for 24h. Subsequently, cell apoptosis was evaluated by Flow Cytometer. Renal Tubular cells were assayed for activation of Caspase3, 8 and 9.A *p*-value <.05 was considered statistically significant.

Results: Quantitative analysis of apoptosis by Flow Cytometer showed significantly higher apoptosis rates in CRS1 compare to HF and CTR (p<.01).This increase of apoptosis was confirmed by Caspase3 levels with a strong correlation (rho=.73). Caspase 8 and 9 were significantly higher in CRS1 compare to HF and CTR (p<.01)



Furthermore, Caspase3 levels showed a significantly positive correlation with Caspase8 (rho=.57) and 9(rho=.47).

Conclusions: This study demonstrated the significantly heightened presence of dual apoptotic disequilibrium in CRS1 compared to HF patients and CTR. For the first time we demonstrate evidence for the delineation of 2 distinct apoptotic pathway activations in CRS1. Our findings indicated that apoptosis may have a central role in the mechanism of CRS1 and it could be a potential therapeutic target in this syndrome.

Funding: Private Foundation Support

PUB085

Effects of Fumonisin B1 (FB1) on Cell Cycle in Mouse Kidney and Liver

Sae Jin Lee,[1] Su-Youn Lee,[1] Seikwan Oh,[2] Ki-Hwan Han.[1] *[1]Dept of Anatomy, Ewha Womans Univ, Seoul, Korea; [2]Dept of Molecular Medicine, Ewha Womans Univ, Seoul, Korea.*

Background: Fumonisin B1 (FB1), a mycotoxin produced by Fusarium verticillioides, inhibits the enzyme ceramide synthase. Animal experiments have indicated that FB1 induces both cell death and cell proliferation. The purpose of this study was to examine the expression of cyclin-dependent kinases (CDKs) and CDK inhibitors in FB1-treated kidney and liver.

Methods: C57BL/6 mice were divided into 3 groups and received FB1 (0, 5, and 20mg/kg bw/day, i.p) for 5 days. Kidney and liver tissues were processed for immunohistochemistry and immunoblot analysis.

Results: A 5 mg/kg dose of FB1 did not affect serum AST, BUN, and creatinine levels. However, a 20 mg/kg dose of FB1 significantly increased serum AST and caused extensive liver necrosis. A 20 mg/kg dose resulted in histological changes (e.g. vacuole formation) in the kidney, but BUN and creatinine levels did not rise. Both 5mg/kg and 20mg/kg dose significantly induced proliferation. Hepatic PCNA content was increased 4.69- (5 mg/kg) to 4.86-fold (20 mg/kg) and renal PCNA was increased 15.9- (5 mg/kg) to 16.4-fold (20 mg/kg), respectively. Hepatic and renal expression of CDK2, CDK4, and CDK6 was significantly increased in both 5mg/kg and 20mg/kg dose. Also, expression of CDK associated cyclins (D1 and D3) was increased. Confocal microscopy showed that CDK2 was co-localized with PCNA in the nucleus of many hepatocytes and renal tubular cells in FB1-treated mice. In contrast, hepatic and renal expression of P18Ink4c and P27Kip1 was significantly decreased in FB1-treated mice. Interestingly, localization of

P27Kip1 shifted from the nucleus to the cytoplasm in the liver. In the kidney, the decrease in P27Kip1 expression was more marked in the proximal tubules than the distal tubules.

Conclusions: We conclude that expression and cellular localization of CDKs and CDK inhibitors may play an important role in FB1-induced cell proliferation in the kidney and liver. This work was supported by the National Research Foundation of Korea (NRF-2013R1A1A2058028).

Funding: Government Support - Non-U.S.

PUB086

Comparison of Pharmacological Properties of Antioxidant Drugs, Probucol and Resveratrol, in Adenine-Diet Model Rats of Chronic Kidney Disease (CKD) Yuko Matsushita,[1] Hirofumi Jono,[1,2] Hideyuki Saito.[1,2] *[1]Dept of Clinical Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto Univ, Kumamoto, Japan; [2]Dept of Pharmacy, Kumamoto Univ Hospital, Kumamoto, Japan.*

Background: Oxidative stress is considered to be a key pathogenic event in the kidney of CKD patients. Oxidative stress develops from free radical production evoked by mitochondrial dysfunction and/or accumulation of a uremic toxin indoxyl sulfate (IS). Pharmacotherapies using antioxidants such as alpha-tocopherol and N-acetylcysteine were reported to suppress oxidative stress in CKD patients, but the pharmacological events have been suggested to be diverse.

Methods: In this study, two antioxidants, probucol (lipid-lowering drug) and resveratrol (phytochemical polyphenol with a potent inhibitory effect on hepatic sulfotransferase (SULT)-mediated IS production), were examined for their pharmacological properties in adenine-diet model rats of CKD. Five week-old male rats were given free access adenine-containing diet (0.3% w/w) with or without probucol (1% w/w) or resveratrol (0.05% w/w), and serum creatinine (sCr), BUN, total cholesterol and IS were periodically determined.

Results: sCr and BUN were elevated from 1 week of adenine intake and continued to increase until 8 weeks, and simultaneous intake of probucol had no preventive effect on renal function. Serum cholesterol level increased from 2 weeks of adenine intake, but this increase was significantly suppressed by probucol intake. Probucol failed to reduce the serum cholesterol level when renal dysfunction was aggravated. IS accumulated gradually in serum of CKD rats, but was not attenuated by probucol intake. Simultaneous intake of diet containing resveratrol significantly ameliorated sCr and BUN of CKD rats from 2 weeks of adenine intake. Resveratrol intake caused a significant reduction of serum IS accumulation.

Conclusions: In conclusions, probucol has a cholesterol-lowering effect, but is ineffective in preventing progression of adenine CKD. Resveratrol suppresses serum IS accumulation by inhibiting hepatic SULT activity, thereby reducing IS-inducible oxidative stress in the kidney with nephropreventive action in adenine CKD rats.

Funding: Government Support - Non-U.S.

PUB087

Circulating Mitochondrial DAMPs Are Not Effective Inducers of Proteinuria and Kidney Injury in Rodents Jing He, Hong Xia, Liang Yaojun, Xiao Wang, Chun-Xia Zheng, Zhihong Liu, Shaolin Shi. *National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, China.*

Background: Mitochondria in eukaryotic cells are derived from bacteria in evolution. Like bacteria, mitochondria contain DNA with unmethylated CpG motifs and formyl peptides, both of which have recently been shown to act as damage associated molecular patterns (DAMPs) and induce immune response and cell injury. Based on the facts that the levels of mitochondrial DAMPs (mtDAMPs) are elevated in the circulation of patients of trauma or burn injury who also have proteinuria, that mtDAMPs can activate immune cells which could in turn secrete glomerular permeability factors, that renal intrinsic cells express a variety of DAMP receptors, and that mtDAMPs can increase endothelial cell permeability *in vitro*, we speculate that mtDAMPs may be novel circulating factors capable of inducing proteinuria and kidney injury.

Methods: We tested the hypothesis by directly injecting mtDAMPs samples, i.e., mitochondrial DNA (mtDNA) or mitochondrial debris soluble components (MTD), into rodents via tail vein, and examining urinary protein and kidney histology.

Results: Injection of mtDNA into mice for 20 μg/ml in circulation, which was much higher than clinical range, did not cause any detectable proteinuria or renal lesions, but the injection resulting in 40 μg/ml mtDNA in circulation could cause transient increase of urinary albumin. Injection of MTD to mice for 60 μg/ml MTD protein in circulation (above clinical range) did not result in proteinuria but subtle lung lesions. In rats, the injection resulting in 450 μg/ml MTD protein in circulation, which was much higher than clinical range, caused mild and transient proteinuria, as well as lung lesions that suggested systemic inflammation.

Conclusions: Clinical levels of mtDAMPs in circulation do not cause proteinuria and even the extremely high, hence clinically irrelevant, levels of mtDAMPs could cause only slight and transient increase of urinary protein in rodents, suggesting that circulating mtDAMPs are not effective inducers of proteinuria and kidney injury in patients with trauma, burn injury, or even kidney disease.

Funding: Government Support - Non-U.S.

PUB088

The Expression of Numb in Normal Kidney Tang Li,[1] Jing Nie.[2] *[1]VIP Medical Center, The Third Affiliated Hospital of Sun Yat-Sen Univ, Guangzhou, Guangdong, China; [2]Div of Nephrology, NanFang Hospital, Southern Medical Univ, Guangzhou, Guangdong, China.*

Background: Numb gene is widely expressed in mammalian tissue and organs. Numb not only plays an important role in the development and various diseases of mammalian, but also in the pathogenesis of human carcinoma. We have previously reported that Numb protects renal proximal tubular cells from puromycin aminonucleoside-induced apoptosis through inhibiting Notch signaling pathway. Up to now, there is little report about the function of Numb in renal disease. Therefore, in order to provide the basis for further research, we conduct this study to investigate the expression of Numb in normal kidney.

Methods: Kidneys of normal male Sprague-Dawley rats were collected. Different markers were used to tag the different kinds of intrinsic renal cells, respectively. The expression of Numb protein in normal kidney was detected by double-immunofluorescence and confocal laser scanning microscopy.

Results: Double-immunofluorescence staining showed that Numb labeling was generally exhibited in normal rat kidney. In the glomerulus, the capillary endothelial cell exhibited intense Numb labeling. However, Numb labeling showed no co-localization with markers of the mesangial cell and podocyte. All the segments of renal tubule we detected exhibited Numb labeling, but the distribution and intensity of the labeling were different in various segments. In the epithelium of proximal tubule, intense Numb labeling mainly located in the apical and basolateral membrane. The thin descending limb of Henle exhibited weak Numb labeling. The epithelium of the thick ascending limb of Henle and distal tubule showed dispersive Numb labeling in cytoplasm. The collecting tubule exhibited prominent Numb labeling at the lateral cell-cell contacts.

Conclusions: Numb protein is generally expressed in normal kidney. In the glomerulus, Numb protein is expressed in the capillary endothelial cell. In the renal tubule, the distribution and intensity of Numb protein are different in various segments.

PUB089

Sensing DNA in the Cytoplasm? AIM for the PYHIN Family in Systemic Lupus Erythematosus Allyson Catherine Egan,[1] Michael D. Jones.[2] *[1]Dept of Medicine, Hammersmith Hospital, Imperial College London, United Kingdom; [2]Genomics Laboratory, MRC clinical Sciences Centre, Imperial College London, United Kingdom.*

Background: Aim2 is a novel cytoplasmic DNA sensor, which instigates the caspase pathway and formation of the inflammasome leading to cell death via IL1β and IL18. The protein is a member of the interferon response PYHIN family. Microarray data reported overexpression of family member Ifi202 in lupus prone mouse model BXSB and New Zealand subcongenic B6.Nba2.

Methods: C1 genomic DNA fragments were sequenced by the Wellcome Trust Sanger Institute using Illumina Next Generation Sequencing technology. Fragments were aligned to the mouse reference genome using the Integrated Genome Viewer 2.2.5. Promoter, intronic and exonic sequences were scanned for single nucleotide polymporphisms. Ensemble/ncbi databases, blast searches and clustral sequence comparisons were employed to investigate homology within the region. Real time PCR of Aim2 was compared in autoimmune prone strain BXSB to B10.Yaa control.

Results: This polymorphic region contains multiple SNPs in exonic, intronic and promoter regions in genes Ifi202, Ifi203, Ifi205, Mnda and Aim2. A lysine to glutamine switch occurs in Ifi202, Ifi203 and Ifi205. The start codon in Ifi203 contains a switch from methionine to threonine. Ifi205 has altered charge due to glutamic acid replacement by glycine. In Mnda, glycine is switched to arginine along with a leucine switch to proline, which may cause alteration in protein structure. Exon 1 (E1) appears to be homologous in certain family members. Full length E1 appears in Mnda. Truncated versions occur in Ifi202 and Ifi204. Ifi205 demonstrates 81-83% homology with the other family members. AIM2 E1 shares only 17% homology with Mnda E1. In real-time PCR expression of the autoimmune strains BXSB and subcongenic B10.Yaa.Bxs3 is down regulated in comparison to the control strain B10.Yaa.

Conclusions: Bioinformatic analyses identified homology between the gene family members at E1, with the exception of Aim2. Expression of AIM2 in the lupus prone model BXSB and subcongenic B10.Yaa.Bxs3 in comparison to control B10.Yaa is reduced. Extensive polymorphic changes occurs in the region, which may alter protein structure.

Funding: Pharmaceutical Company Support - Wellcome Trust, United Kingdom

PUB090

Remote Usability Testing of a Mobile Phone-Based Medication Inquiry System (MIS) in Chronic Kidney Disease (CKD) Clarissa Jonas Diamantidis, Marni Zuckerman, Jennifer S. Ginsberg, Lisa Lucas, Jeffrey C. Fink. *Medicine, Univ of Maryland School of Medicine, Baltimore, MD.*

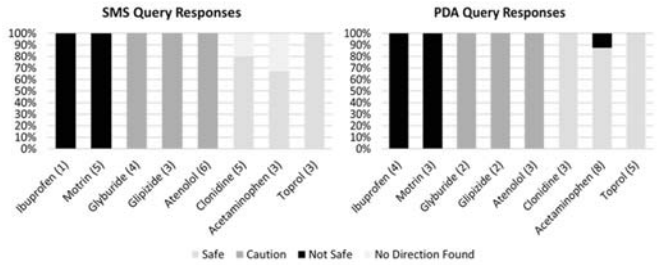
Background: Mobile phones are ubiquitous, and tailored mobile health (mHealth) applications may offer opportunities for innovative safeguards against medication errors observed in CKD. Patients' ability to use such applications requires preliminary study for the tailored designed of effective mHealth applications.

Methods: Participants with CKD enrolled in the Safe Kidney Care (SKC) cohort (ClinicalTrials.gov NCT01407367) were invited to participate in remote usability testing of a mobile phone-based medication inquiry system (MIS) that provides precautionary information on the safety of selected medications in CKD. Participants were randomized to 1 of 2 mobile platforms: 1.) SMS text, or 2.) Personal Digital Assistant (PDA; e.g. iPod

Touch) and were asked to bring the device home for remote testing. Participants were then mailed 3 dummy pill bottles with prescription labels and were asked to input each medication into the MIS and record the responses. Participants completed a user satisfaction survey at study's end.

Results: 20 participants were randomly assigned the SMS (n=10) or PDA (n=10) platform. Participants were predominantly black (80% versus 70%, respectively), with diabetes (60% versus 80%), with > high school diploma (80% versus 60%), and had used the Internet previously (90% versus 60%). 30% of SMS users and 40% of PDA users were > 65 years old. All participants owned a cell phone, but only 30% of SMS users and 10% of PDA users owned a smartphone. Of 60 medication queries inputted into the MIS, there were only 3 reported errors, 2 of which were in the SMS group.

Figure 1. MIS query results by phone platform



Median satisfaction survey score was 7 (strongly agree) for both groups.

Conclusions: Mobile phone search applications have the potential to provide guidance on the proper use of medications and improve patient safety in the CKD population.

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

PUB091

Pore Size Control and Evaluation in Skin Layer of Polysulfone Hollow Fiber for Hemodialysis Changjun Mu, Tiancheng Xu. *Research and Development Dept, WEGO Blood Purification Products Co., Ltd, Weihai, Shandong, China.*

Background: A pore size in skin layer of polysulfone hollow fibers is one of essential factors to dominate functions of dialyzers. By controlling the pore size of polysulfone hollow fibers, dialyzers can be prepared to fit different treatments such as hemodialysis, hemofiltration and hemodiafiltration. Generically, the polysulfone hollow fibers with a homogeneous pore size distribution in the skin layer are preferred to improve the therapeutic performance.

Methods: In this paper, we introduced a series of spinning conditions such like solution composition, coagulation temperature, bore fluid composition, drying temperature and speed of spinning fluid for the pore size control, and as a method to evaluate the pore size in skin layer, a gel permeation chromatography (GPC) was proposed by us. Additionally, as a performance indicator, ultrafiltration coefficients (K_{UF}: mL/hr/mmHg) of the hollow fibers were also derived with a bovine blood at a transmembrane pressure of 50mmHg.

Results: It was found that the pore size in skin layer and the K_{UF} of the hollow fibers increased with a dimethylacetamide concentration in the bore fluid and a narrow pore size distribution could be obtained by changing a speed of spinning fluid.

PUB092

Optimizing Kidney-Targeted Gene Delivery for Polycystic Kidney Disease Sara J. Holditch,[1,2] Vicente E. Torres,[3] Yasuhiro Ikedo.[2] *[1]Mayo Graduate School, Mayo Clinic, Rochester, MN; [2]Molecular Medicine, Mayo Clinic, Rochester, MN; [3]Div of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Background: Polycystic kidney disease (PKD) is an inheritable renal cystic disease, for which there are limited therapeutic options. Adeno-associated virus (AAV)-based vectors are characterized by their efficient transduction of non-dividing cells, proven ability to persist for long term gene expression and low toxicity. Recent successful clinical trials using AAV vectors have paved the way for gene therapy applications for numerous genetic and chronic disorders. However, previous studies have demonstrated variable and suboptimal success in transducing the kidney by AAV vectors.

Methods: Here, we present two studies comparing the transduction efficiency of AAV vectors serotyped 2, 8, and 9, administered at 5.0E+11 gc/kg expressing luciferase. To increase transcriptional kidney-targeted gene delivery, we compared a 2kb- promoter of Acyl-coenzyme A synthetase (ACSM2) against the constitutively expressed viral promoter, CMV.

Results: A) A single injection directly into the renal artery (RAI), delivered 5.0E+11 vector genomes per animal. Upon RAI, we assessed the transduction of AAV2 versus AAV9 vectors through expression of luciferase using the Xenogen non-invasive live imager at 7, 14 and 21 days post injection. We found that administration of an AAV2 vector with a CMV internal promoter efficiently transduced kidney. AAV2-CMV-Luc vector luciferase expression, compared to AAV vectors with ACSM2 promoter or AAV9 capsid, had stronger, and higher renal specificity. B) A single injection directly into the renal pelvis, delivered 5.0E+11 vector genomes per animal. Luciferase expression was monitored as described above. Data is currently being analyzed and will be included in the final presentation.

Conclusions: Although these studies require further optimization, such as a more robust study population, alternate serotypes and routes of administration, and a more thorough interrogation of potential kidney specific promoters, our results demonstrate the unrealized potential for AAV vectors in the treatment of chronic or genetic renal diseases, such as polycystic kidney disease.

Funding: NIDDK Support

PUB093

A Novel Renal Doppler Based Marker to Quantitate Acute Stress Madan Bahadur,[1] Jawahar Jain,[2] Shreyans Gandhi.[3] *[1]Dept of Nephrology, Jaslok Hospital and Research Centre, Mumbai, Maharashtra, India; [2]TTT - Think Tank Team, Samsung Research America, San Jose, CA; [3]Dept of Electrical Engeneering, IIT, Mumbai, Maharashtra, India.*

Background: A medically consequential manifestation of stress is stimulation of renal sympathetic nerve activity. While it has been shown that stress causes an increase in the renal resistive index (RRI), this measure has limited reliability. We present a novel frequency domain method for analyzing renal blood flow (RBF) that appears to have substantially higher signal-to-noise ratio than RRI.

Methods: A pilot study was conducted on 8 healthy volunteers. A baseline renal Doppler was recorded. The participant was then given a analytical test to solve in a limited time. Stress was enhanced by linking it to honorarium award and adding peer pressure. However the questions were so set that it was near impossible to solve them in given time. A renal Doppler was recorded while his unsuccessful performance was being assessed by the examiner. The second part of the stress induction was the anticipation of pin prick however none of the participants were pricked. He was told several times to be ready for the prick generating stress and doppler recorded. A scaled feedback of perceived stress was taken.

Results: The Doppler RBF graphs were digitized and subjected to Fourier transform. This corresponds to the power strength of each signal corresponding to each frequency component.It was seen that the signals corresponding to the baseline and stress phases vary a lot in the low frequency region around 1 Hz. While baseline and stress response could be distinctly identified by their respective RIs, this difference was magnified several-fold in the frequency domain.

Conclusions: RI values do not use the full information available from the doppler waveforms compared to frequency domain analysis. Unlike RI's this method is resilient to noise. Only the low frequencies where maximum change is seen, matters, as a result the high frequency noise does not affect the analysis. This novel analysis of the Doppler RBF spectrum picks up acute stress in a more sensitive and specific manner and may be used as an objective method to document stress.

Funding: Pharmaceutical Company Support - Fujitsu Laboratories of America

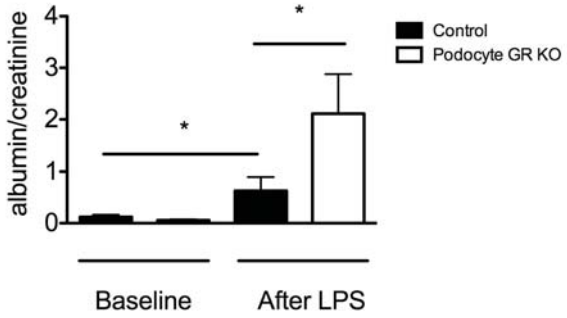
PUB094

Loss of the Podocyte Glucocorticoid Receptor Worsens Proteinuria after Low Dose LPS Julie Goodwin. *Pediatrics, Yale Univ School of Medicine, New Haven, CT.*

Background: Nephrotic syndrome is common in both children and adults and glucocorticoids are a mainstay of treatment in the majority of cases. Despite widespread use of this class of agents the molecular mechanisms by which glucocorticoids induce remission of proteinuria remain unclear. It is known that the glucocorticoid receptor is expressed in podocytes.

Methods: We have created a mouse model with a tissue-specific knockout of the glucocorticoid receptor in podocytes. Proteinuria was induced in control and knockout mice by injection of low-dose LPS. Serum and urine electrolytes, serum creatinine and serum albumin were measured before and twenty four hours after treatment.

Results: At baseline there was no difference in serum creatinine, serum albumin or urine albumin/creatinine ratio between the 2 groups. After LPS treatment control animals showed a modest increase in urine albumin/creatinine ratio (0.12 ± 0.04 before versus 0.63 ± 0.27 after, n=4/group, $p<0.05$). However, podoctye GR KO mice showed a much greater increase in proteinuria following LPS treatment (0.06 ± 0.02 before versus 2.11 ± 0.77 after, n=4/group, $p<0.05$).



Conclusions: These results suggest that:

(1) the podocyte glucocorticoid receptor is important in limiting proteinuria in this model

(2) this receptor may play a role in the development of proteinuria in other states, including nephrotic syndrome.

Funding: NIDDK Support

PUB095

Calcineurin Binding Protein-1 Is Associated with Podocyte Injury Yueqiang Wen,[1] Hui Peng,[2] Wenbo Zhao.[3] *[1]Dept of Internal Medicine, Div of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Dept of Internal Medicine, Div of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [3]Dept of Internal Medicine, Div of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Calcineurin binding protein-1 (cabin1) is a non-competitive inhibitor of calcineurin (CaN), it's N terminus is responsible for interaction with p53, cabin1 renders p53 transcriptionally inactive. Cabin1 plays a critical role in regulating apoptosis in synoviocyte and cancer cell, yet the exact localization and function in kidney is not reported up to now.

Methods: Mouse podocytes were cultivated at 33° to propagate in the presence of 10-20U/ml mouse recombinant γ interferon, then cells were transferred to an incubator at 37° without interferon to allow differentiation for 10-14 days. Differentiation podocyes were divided into three experimental groups: (1) normal control; (2) 10^{-6}M AngiotensionII (AngII); (3) 10^{-6}M AngII plus 10nM tacrolimus. Cells were harvested at 12h, 24h and 48h for protein extraction and immunofluorescence staining(IF).

Results: IF showed well-defined actin stress fibers and strong staining in cytoplasm yet weak staining in nucleus of cabin1 in normal podocytes. AngII induced disruption of F-actin and strong staining in nucleus of cabin1, while tacrolimus stabilized actin filaments and cabin1. Cabin1 protein content significantly increased at 48h which lowered by tacrolimus.

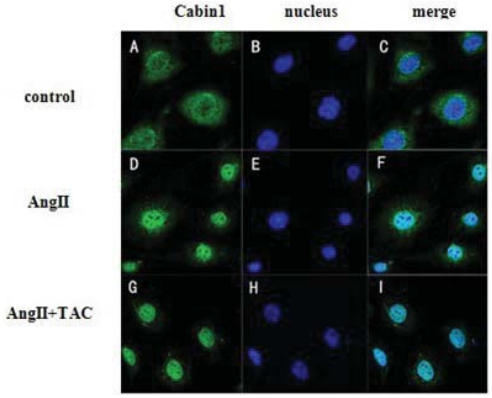


Figure 1: Immunofluorescence staining (fluorescence microscope x 400) showed strong staining in cytoplasm yet weak staining in nucleus of cabin1 in normal podocytes (A and D). AngiotensionII induced strong staining in nucleus of cabin1(D and F), while tacrolimus stabilized cabin1 (G and I).

Conclusions: The expression of cabin1 is increased during podocyte injury, and returned to normal while podocyte recovered. Cabin1 maybe a new target for antiproteinuic drugs.

PUB096

Hypoxia Stimulates the Expression of Tissue Factor and Suppresses Tissue Factor Pathway Inhibitor in Human Podocytes in Culture Ikuyo Narita,[1] Michiko Shimada,[1] Norio Nakamura,[1] Reiichi Murakami,[1] Takeshi Fujita,[1] Moin Saleem,[2] Hideaki Yamabe,[1] Ken Okumura.[1] *[1]Nephrology, Hirosaki Univ, Hirosaki, Japan; [2]Renal Unit, Bristol Univ, Bristol, United Kingdom.*

Background: The role of podocytes in the crescent formation has been suggested. Besides, the coagulation pathways are implicated due to the fibrin deposition. Tissue Factor (TF) is the initiator of extrinsic coagulation pathway and is also related to proliferation and inflammation. We have previously shown the expressions of TF and tissue factor pathway inhibitor (TFPI) in the podocytes, however, their regulations are not well known.

Methods: Conditionally immortalized podocytes were grown at 33°C and then converted to differentiated cells by incubating at 37°C. The differentiated cells were treated in normoxic or hypoxic conditions. The effect of cobalt chloride which mimics hypoxia was also tested for the verification. Total RNA was harvested and mRNA expressions of TF and TFPI were analyzed by quantitative RT-PCR. Additionally, the effect of hypoxia-inducible factor-1α (HIF-1α) small interfering RNA (siRNA) was tested to assess the role of HIF-1α which regulates the numerous changes in hypoxia. The expression of TF was confirmed by immunofluolescent staining, and TFPI in the supernatant was tested by enzyme linked immunosorbent assay (ELISA).

Results: Hypoxia increased the mRNA expression of TF (6h: 2.29 ± 0.05 fold, $p<0.001$, 24h: 5.6 ± 2.4 fold, $p<0.05$) and suppressed TFPI (6h: 0.54 ± 0.04 fold, $p<0.05$, 24h: 0.24 ± 0.06 fold, $p<0.001$) compared with normoxia. Incubation with cobalt chloride 150 μM for 6 - 24h resulted in the similar results. Interestingly, HIF-1α siRNA did not suppress the expression of TF. TF was stained in the cytosol of podocytes and TFPI was detectable in the culture supernatant by ELISA, although the changes by hypoxia were not significant up to 24 h.

Conclusions: Hypoxia increased the expression of TF and reduced TFPI. These changes were both towards pro-thrombotic condition. Increased expression of TF by hypoxia was not suppressed by HIF-1α suggesting other regulating factors in the podocytes. These data suggest that podocytes may contribute to the pro-coagulant changes lead to fibrin deposition.

Funding: Government Support - Non-U.S.

PUB097

Mitochondrial Reactive Oxygen Species Mediated Vancomycin-Induced Apoptosis in LLC-PK1 Cells Takahisa Yano,[1] Yuya Sakamoto,[2] Yuki Hanada,[2] Aki Takeshita,[2] Nobuaki Egashira,[1,2] Satohiro Masuda.[1,2] *[1]Dept of Pharmacy, Kyushu Univ Hospital, Fukuoka, Japan; [2]Clinical Pharmacology and Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: Vancomycin chloride (VCM), a glycopeptide antibiotic, is widely used for the therapy of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, nephrotoxicity is a major concern during VCM therapy. In this study, we investigated the cellular mechanisms underlying VCM-induced renal tubular cell injury using the porcine proximal tubular cell line LLC-PK1 cells.

Methods: LLC-PK1 cells were seeded at density of 1.0×10^4 cells/cm^2 onto plastic plates and used for experiments on the following day, on which they reached 70-80 % confluence. After treatment with VCM (1-5 mM) for 3-24 h, cells were washed with phosphate-buffered saline. Apoptosis was assessed by flow cytometry using the TUNEL staining. Production of intracellular reactive oxygen species (ROS) was measured by using a cell-permeative ROS indicator, carboxy-H_2DCFDA and a specific mitochondrial superoxide indicator, MitoSOX red. Changes in mitochondrial membrane potential were assessed by the JC-1 staining. Mitochondrial cytochrome c was determined by Western blot analysis.

Results: Exposure of LLC-PK1 cells to VCM caused a concentration- and time-dependent increase in the numbers of TUNEL-positive cells, which were reversed by caspase-3-specific inhibitor zDEVD-fmk and a caspase-9 inhibitor zLEHD-fmk. VCM also induced an increase in DCF fluorescent intensity and MitoSOX fluorescent intensity. Vitamin E and mitoTEMPO prevented VCM-induced cell apoptosis and mitochondrial membrane depolarization, while vitamin C or n-acetyl cysteine (NAC) did not show significant protection against VCM-induced cell death.

Conclusions: VCM caused the mitochondrial-mediated ROS generation leading to a concentration- and time-dependent cell apoptosis in LLC-PK1 cells. Moreover, a lipophilic antioxidant vitamin E and a mitochondria-targeted antioxidant mitoTEMPO significantly prevented the VCM-induced mitochondrial dysfunction and apoptosis in LLC-PK1 cells.

PUB098

Hyperglycemia Induces Early Disassembling of Slit-Diaphragm in Podocytes in an Oxidative Stress-Dependent Way Pablo Bautista-Garcia,[1] Brenda Luna,[2] Marisol Martinez,[2] Rafael Rodriguez-Munoz,[1] Maria del Carmen Namorado,[1] Alejandro Perez-Lopez,[1] Jose L. Reyes.[1] *[1]Physiology, Biophysics and Neuroscience, Cinvestav, Mexico City, Mexico; [2]Pharmacology, Cinvestav, Mexico City, Mexico.*

Background: Diabetic nephropathy (DN) is a severe complications of diabetes mellitus (DM). This pathology produces dysfunction of slit-diaphragm leading to the end-stage renal disease. The increase production of advanced glycation end products (AGEs) and the activation of their specific receptors for advanced glycation ends products (RAGEs), stimulate NADPH oxidase, a source of reactive oxygen species (ROS). It has been previously shown that ROS is involved in renal damage during the late phase of diabetes mellitus (DM). Thereby, our aim was to evaluate whether hyperglycemia disassembles the slit-diaphragm proteins during the early phases of DM.

Methods: Diabetes mellitus was induced in rats by a single tail vein injection of streptozotocin (STZ 60 mg/kg Body Weight). Control were injected with equal volume of citrate buffer. Urine was collected for proteinuria determination. Kidney form each group were excised for immunofluorescence to evaluate nephrin and ZO-1 and glomerular isolation from each group were performed to evaluate the expression of RAGEs, nfr-2, NOS-2, nitrotyrosine, nephrin and PCK-β2 by western blot.

Results: By immunofluorescence, we observed that nephrin and ZO-1 are delocalized or absent in glomeruli of diabetic rats. WB demonstrated that initial stages of hyperglycemia increased NOS-2, nitrosylated proteins, PCK-β2 and the activation of transcriptional factors induced by oxidative stress, such as nfr-2. These changes are related to a significant increase of proteinuria at the third week.

Conclusions: Our results suggest that hyperglycemia increase the prooxidant enviroment in glomeruli during the early phases of diabetes. The induction of this deleterious phenomena might provoke delocation of slit-diaphragm proteins through activation of PKC-β2, these changes leading to development of proteinuria an important predictor of evolution of chronic renal disease.

Funding: Government Support - Non-U.S.

PUB099

Bisphenol A Induces Inflammatory Response and Cytokine Release in Renal Mesangial Cells Shing-Hwa Liu,[1] Bo-Lin Chen,[1] Yuan-Siao Chen,[2] Chih-Kang Chiang,[3] Cheng-Tien Wu.[1] *[1]Graduate Institute of Toxicology, National Taiwan Univ, College of Medicine, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan; [3]Dept of Integrated Diagnostics and Therapeutics, National Taiwan Univ Hospital, Taipei, Taiwan.*

Background: Bisphenol A (BPA) is a common ingredient of plastic products, which wildly exists in daily life. In addition to its estrogen-like disrupter effect, reproductive toxicity and renal toxicity have also been implicated. BPA exposure has been demonstrated to be associated with low-grade albuminuria in adults. Here, we investigated the effect and possible mechanism of BPA on glomerular mesangial cells.

Methods: Mouse mesangial cell (MMC) were applied to test the effects of BPA. Cell viability was tested by using MTS assay. PI-Annexin V staining, caspase-3 and PARP cleavages was applied to evaluate the induction of apoptosis. ELISA, Western blotting and kinase assay were applied for evaluating the potential signal pathways.

Results: In this study, MMC was treated with 0~100 μM BPA. The cell phenotyping was not changed during BPA treatment. Cell viability was not affected by 25 and 50 μM BPA after 24 h treatment, but cell viability was decreased to 90% under treatment with 100 μM BPA. BPA 100 μM did not increase the cell apoptosis by PI-Annexin V staining. It also did not change the caspase-3 and PARP cleavages. These data indicated that BPA seems only to decrease the cell growth rate, but does not cause cell death in MMC. After BPA treatment, COX-2 protein expression and PGE2 secretion and nuclear factor κ-B (NFκB)-p65 phosphorylation in MMC were increased in a dose dependent manner. The interleukin-1β levels were also increased in MMC culture medium after BPA treatment. BPA could also activate the AMP-activated protein kinase (AMPK), which is involved in the energy balance and plays an inflammation and oxidative stress regulator, in a dose dependent manner.

Conclusions: Taken together, these results indicate that BPA at non-cytotoxic concentrations is capable of increasing the expression/secretion of COX-2/PGE2 and cytokines in MMC, and imply that BPA may cause inflammatory response in renal glomeruli and influence the renal function.

Funding: Government Support - Non-U.S.

PUB100

Inhibition of Glycogen Synthase Kinase-3β Ameliorates Angsiotensin II-Induced Podocyte Apoptosis through Modulating Inflammation Zhangsuo Liu,[1] Qian Zhang,[2] Guo Jia.[3] *[1]The First Affiliated Hospital of Zhengzhou Univ; [2]The First Affiliated Hospital of Zhengzhou Univ; [3]The First Affiliated Hospital of Zhengzhou Univ.*

Background: Emerging evidence indicates that diabetic nephropathy is an inflammatory disease. Angsiotensin II is a key factor in glomerulosclerosis as well as in podocyte injury. We aimed to investigate whether angsiotensin II(Ang II) induces inflammation in podocytes and whether glycogen synthase kinase-3β is involved.

Methods: Podocytes were exposed to Ang II(10^{-8}mol/L-10^{-5}mol/L) for 48 hours.In another set of experiments,GSK-3β was inhibited by LiCl or scilenced by GSK-3β siRNA before Ang II exposure.Podocyte apoptosis was assessed by flow cytometry.GSK-3β and caspase3 activity was assessed by an activity assay kit. GSK-3β expression was detected by western blot. IL-1β,TNF-α in the supermatant was assessed by ELISA.

Results: Podocyte apoptosis,IL-1β,TNF-α secretion and GSK-3β activity was increased after Ang II stimulation in a dose-dependent manner($P<0.05$).GSK-3β inhibition by LiCl or siRNA suppressed IL-1β,TNF-α secretion,caspase3 activation as well as podocyte apoptosis caused by Ang II($P<0.05$).

Conclusions: Our experiments reveal that GSK-3β is involved in AngII-induced podocyte apoptosis through modulating inflammation and AngII-induced effects could be alleviated by GSK-3β inhibition.

Funding: Government Support - Non-U.S.

PUB101

The Effects of Endothelial Microparticles on the Neointimal Hyperplasia Formation and Vascular Smooth Muscle Cells Proliferation Jung-Hwa Ryu, Shina Lee, Hee Sung Ko, Dong-Ryeol Ryu, Duk-Hee Kang, Kyu Bok Choi, Seung-Jung Kim. *Dept of Nephrology, Ewha Womans Univ, School of Medicine, Seoul, Republic of Korea.*

Background: Vascular access stenosis occurs frequently and predominantly as a result of neointimal hyperplasia (NH) formation due to migration and proliferation of VSMCs at the venous anastomosis site. However, effective preventions or treatments for NH formation in this setting are lacking. Increased endothelial microparticles (EMPs) in uremic condition are closely associated with vascular dysfunction and atherosclerosis. The aim of this study is to investigate the effects of EMPs on proliferation of VSMCs and NH formation.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured and stimulated by indoxyl sulfate (IS; 250μg/mL). The pellet obtained via ultracentrifugation of the culture media at 100,000 Xg was resuspended and analyzed by flow cytometry. The absolute count of EMPs was defined by Trucount tube. Human aortic smooth muscle cells (VSMCs) were treated by collected EMPs. Western blot analysis for TGF-β sub-signaling; Akt, ERK, p38, and Smad3 and BrdU cell proliferation assay were performed. Ex vivo experiments were also performed on porcine internal jugular veins (n=5) with vehicles, EMPs (2.0×10^6), and TGF-β (5ng/mL) for 12 days, respectively. The tissues were stained by H&E, Masson's trichrome and immunostained by α-smooth muscle actin (α-SMA).

Results: The EMPs were well produced by IS and the quantity of them was similar to the amount generated by positive control, TNF-α (10ng/mL). IS-induced EMPs stimulated the TGF-β sub-signalings and the proliferation of cultured VSMCs in a dose-dependent manner. In *ex vivo* culture, NH was significantly developed in EMPs-treated veins compared to controls, which was similar with the patterns developed by stimulation of TGF-β (5ng/mL). Luminal narrowing was noted in EMP and TGF-β treatment group and the intima/media ratio was significantly increase in EMPs-treated vessel.

Conclusions: IS-induced EMPs directly stimulate the proliferation of VSMCs and significant NH formation in porcine vein. Further investigation is needed to demonstrate the fine mechanism and active role of EMPs on vascular access stenosis.

PUB102

Association of Intracellular Localization of Slit–Diaphragm Proteins with Size Selectivity in Idiopathic Nephrotic Syndrome Kiyonobu Ishizuka,[1] Yutaka Harita,[2] Haruko Tsurumi,[2] Noriko Sugawara,[1] Hiroko Chikamoto,[1] Yuko Akioka,[1] Motoshi Hattori.[1] *[1]Dept of Pediatric Nephrology, Tokyo Women's Medical Univ, Tokyo, Japan; [2]Dept of Pediatrics, Graduate School of Medicine, Univ of Tokyo, Tokyo, Japan.*

Background: While slit diaphragm (SD) proteins are critical components of glomerular filtration, detailed expression and localization of SD proteins in idiopathic nephrotic syndrome (INS), and its association with glomerular size selectivity, remains unclear.

Methods: Using a confocal microscopy, we analyzed intracellular localization of Nephrin, Podocin, Neph1 and ZO–1 in podocytes in renal specimens from patients with INS (MCNS: 9, FSGS: 12). Five samples from posttransplant recurrence of FSGS were also analyzed. All the patients had proteinuria at renal biopsy.

Results: Expression levels of Nephrin, Podocin, Neph1, and ZO–1 in podocytes of INS patients did not alter compared to those of controls. 15 samples (71.4%) showed alteration of intracellular localization of either Nephrin (4 samples), Podocin (4 samples) or both (7 samples). In these specimens, Nephrin or Podocin altered their localization from slit–diaphragm to cytoplasm. In all the samples from posttransplant recurrent FSGS, both Nephrin and Podocin were translocated to cytoplasm in as early as 1 hour after transplantation and part of them was localized to Golgi apparatus. Alteration of cytoplasmic localization of Neph1 was not observed. Neither the change of localization of Nephrin nor that of Podocin could distinguish histopathological diagnosis. However, there was significant difference in selectivity index (the clearance ratio of lgG/ transferrin) between cases with Nephrin alteration and ones without ($p=0.024$), and in the amount of urinary protein between cases with Podocin alteration and ones without ($p=0.012$).

Conclusions: Changes of intracellular localization, but not of expression level, of SD proteins were observed in INS. In the pathological situations, localization of Nephrin and Podocin were independently regulated, and proper localization of Nephrin at SD, but not of Podocin, provides size selectivity of glomerular filtration.

PUB103

Optimization of Urine Collection for the Isolation of Extracellular Vesicles Harry B. Holthofer,[1] Wenxiao Zeng,[1,2] Luca Musante,[1] Xinyu Liu,[1] Dorota Ewa Tataruch,[1] Giulio Calzaferri.[1] *[1]Centre for BioAnalytical Sciences (CBAS), Dublin City Univ; [2]Blood Purification Center, The Fifth Affiliated Hospital of Sun Yat-sen Univ.*

Background: Urinary extracellular vesicles (UEVs) represent a fascinating new source of biomarkers. However, several shortcomings limit their practical use like protocols for rapid vesicle enrichment or data normalisation. Furthermore, urine collection itself lacks optimized standards. Here we streamline the urine collection protocol and provide new information of the major interfering protein, Tamm-Horsfall Protein (THP).

Methods: Urine was collected from normal subjects using the void 1st and 2nd morning urine in consecutive 50ml tubes. Additionally, the 3rd 4th and 5th urine was collected after intake of a litre of water. All subjects also provided a 24 hour collection. The samples were first centrifuged at 2,000g and UEVs isolated by hydrostatic dialysis (HD). Fractions were analyzed by electrophoresis and Western blots (WB) to detect a specific exosome marker, Tumor Susceptibility Gene 101(TSG101). Images of Coomassie gels and WB were acquired by Odyssey® scanner and relative quantification performed by Odyssey software.

Results: HD proved to be superior to conventional differential centrifugation method without qualitative changes in the resulting UEV protein pattern. A higher amount of TSG101 was recovered in the 1st morning urine with minimal interference of THP. TSG101 decreased progressively in the successive samples with reciprocal THP increase, particularly after overload of water. THP in precipitated form was successfully spun down at 2,000g in the 1st and 2nd and 3rd morning urine samples while no THP was recovered in the same low speed pellet of 4th and 5th collection after hydration. Thus, hydration and prolonged time in bladder appears to trigger THP polymerization.

Conclusions: We showed that the major variability in the UEV and THP recovery depends on time and modality of collection. Caveats with the first morning urine samples have a practical impact on the design of urine collection for discovery research and diagnostics alike, especially if the whole void urine volume is collected so as to avoid the "mid-stream" catch.

Funding: Government Support - Non-U.S.

PUB104

Hormetic Effects of the Nrf2 Inducer, Bardoxolone Methyl (BARD) Analogs, on Chronic Kidney Disease (CKD) Progression
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Background: Oxidative stress and inflammation which play a major role in progression of CKD are, in part, due to impaired activity of Nrf2, the master regulator of endogenous phase 2 detoxifying and antioxidant molecules. Natural Nrf2 activators have been shown to ameliorate kidney disease in experimental animals. However, treatment with potent synthetic Nrf2 inducers, BARD analogs RTA 405 (50 and 100 mg/kg) and dh404 (5-20 mg/kg/day), has been shown to intensify inflammation and CKD progression in diabetic obese Zucker rats (Zoja et al, 2013). Moreover due to adverse outcomes, clinical trial of BARD in patients with diabetic nephropathy was recently halted (De Zeeuw et al, 2013). In contrast treatment with low dose dh404 (2 mg/kg/day) was recently shown to retard CKD progression in 5/6 nephrectomized rats (Aminzadeh et al, 2013). Notably, natural Nrf2 inducers which serve as insecticides to protect the plants against insects are known to have hormetic properties with beneficial effects at nanomolar and toxic effects at higher concentrations. This study was designed to determine if the reported divergent effects of BARD analogs are dose related.

Methods: SD rats were subjected to 5/6 nephrectomy (CKD) or sham operation and randomized to receive either10 or 2mg/kg/day of dh404 or vehicle for 12 weeks.

Results: The vehicle-treated CKD group exhibited glomerulosclerosis, interstitial fibrosis and inflammation, upregulation of NAD(P)H oxidase, activation of NFkB, reduced Nrf2 activity, and down-regulation of catalase, heme oxygenase-1, and glutamate-cysteine ligase. Administration of low dose dh404 restored Nrf2 activity and expression of its target genes, attenuated oxidative stress, inflammation, glomerulosclerosis, and interstitial fibrosis. In contrast at high dosage dh404 caused massive weight loss, intense proteinuria, and exacerbation of kidney lesions and dysfunction.

Conclusions: The data illustrate the dose-dependent dimorphic impact of BARD which is consistent with the known hormetic properties of the natural Nrf2 inducers.

PUB105

Impaired Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Activity Involves in Aristolochic Acid-Induced Renal Tubular Epithelial Cell Injury
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Background: Recently we have demonstrated in vivo that bardoxolone methyl (BARD), the most potent known activator of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, could prevent aristolochic acid (AA)-induced acute kidney injury. In this vitro study, we investigated the role of Nrf2 and related signaling pathway in AAI injury to renal tubular epithelial cells (NRK52E cells).

Methods: NRK-52E cells were incubated with different concentrations of AAI for several time points. The role of Nrf2 was determined in NRK-52E cells through Nrf2 knockdown by its specific siRNA. Cell viability and reactive oxygen species (ROS) were evaluated by MTT and Flow Cytometer assay, respectively. Cell apoptosis was examined by western blotting of cleaved caspase-3 and Flow Cytometer assay. Expression of Nrf2 and its downstream protein hemeoxygenase-1(HO-1), and NAD(P)H quinoneoxidoreductase-1(NQO-1) was analyzed by western blotting and immunofluorescence.

Results: AAI reduced cell viability and increased apoptosis in a concentration- (0, 5, 10, 25, 50 uM) and time- (0, 4, 8, 12, 24 hrs) dependent manner. ROS production was detected as early as 5 min, and early apoptosis as indicated by cleaved caspase-3 reached highest level at 8 hrs after AAI exposure. After AAI (25 uM) treatment for 24 hrs, Nrf2 protein located in cytoplasm of NRK-52E cells was significantly down-regulated and failed to translocate to the nuclear, which indicated impaired Nrf2 activation. This was consistent with our previous finding in vivo. Moreover, knockdown of Nrf2 led to a significant reduction of HO-1 and NQO1 protein expression, and enhanced ROS production and cell apoptosis induced by AAI.

Conclusions: These results indicate that AAI-induced renal tubular epithelial cell injury was associated with impaired Nrf2 activation and expression of its downstream target genes. Activation of Nrf2 signaling pathway may be a potential treatment target for AA nephropathy.

Funding: Government Support - Non-U.S.

PUB106

Screening of Microarray Profile of Kidney Human From Diabetes And/ Or Renal Cell Carcinoma for a New Biomarker
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Background: Recent study from our laboratory showed that patients with diabetes are at a higher risk of developing kidney cancer. In the current study, we have screened the microarray profile of kidney tissue collected from patients with diabetes, RCC or RCC+diabetes for a new biomarker.

Methods: DNA copy number profiling was performed using the Agilent Human CGH microarrays. Microarray data from healthy control (group1), diabetes (group 3), RCC(group 4) and RRC+diabetes (group 2) were further processed using Nexus Copy Number 7.5 (BioDiscovery) with FASST2 segmentation method for copy number analysis. The copy number gain and loss log2 ratio thresholds are 0.4 and -0.5, respectively. The aberrant regions that occurred in at least one sample in each group were selected.

Results: We found that 901 genes gain of copy number and 171 genes loss of copy number in group 2, 669 genes gain of copy number and 307 genes loss of copy number in group 3, 457 gene gain of copy number and 38 loss of copy number in group 4, after removing gain/loss genes obtained from healthy control group (group 1). Cell survival genes including such as AKTs showed gain of copy number in group 2 while AKTs gain in group 3. In cell apoptosis genes including BCL2, PARP and caspases, we found gain of copy number in group 2 while gain in certain PARPs in group 3. In tumor suppressor genes, we found loss of copy number of VHL gene in group 2 and 4. We also found gain in copy number of DNA damage genes including SETD9 and ATR in both group 2 and 3.

Conclusions: These data showed a potential DNA alteration specifically in pathways that important in the early stages of tumor initiation. Our data shed a light on certain candidate target genes that can be beneficial as a biomarker for diagnosis RCC within diabetic patients.

Funding: Veterans Administration Support

PUB107

Intra-Renal Artery Infusion of Fenoldopam Prevents Acute Renal Failure in CKD Patients Post Contrast Media Exposure
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Background: Chronic kidney disease (CKD) patients run a substantial risk for contrast media induced nephrotoxicity with further worsening of their kidney function. To avoid this, intra-renal artery fenoldopam (F) was administered 41 times to 34 distinct patients with peripheral artery disease (PAD) prior to their angiography.

Methods: The patients' estimated glomerular filtration rates (EGFR) were calculated using the Korckoff Gault formula and their creatinine (C) levels were obtained before their angiograms (A) and within 2 days after the A's were performed. Standard interventional methods were used to place a selective infusion system catheter delivering F to both renal arteries catheter and/or placing a Cobra catheter catheter in one renal artery. Low osmolar contrast media was injected to confirm its location in the renal artery and was used for the A's. A F drip was started through the catheter at 0.2mcg/kg/min injected into the renal circulation was continued for 5 minutes and was then increased to 0.4 mcg/kg/min and continued for another 55 minutes. Blood pressure records were closely monitored during the angiogram. Their pre and post A C levels were analyzed using a paired two tailed t-test.

Results: No patient was admitted to a hospital for ARF post A. Their mean EGFR was 42.7 ml/min +/- 12.2 and their mean age was 73.3 years +/- 11.Their C levels are seen in the table below.

Patinet selection	Mean Pre Angiogram Creatinine mg/dl	Mean Post Angiogram Creatinine mg/dl	P value
All F procedures (41)	1.65 +/- .5	1.64 +/- .5	P=.47
Only One Renal Artery with Fenoldopam infusion (34)	1.62+/- .4	1.59 +/-.4	P=.23
Both renal arteries with Fenoldopam infusion (7)	1.84 +/-.9	1.82 +/-.9	P=.04

Conclusions: There were no cases of ARF in our study population nor were there any significant changes in C levels in pre and post A blood in our study patients with CKD who were exposed to contrast media. F infused into the one, or two renal arteries, avoided AFR following contrast media exposure in a elderly population with advanced CKD. This technique allows necessary angiographic studies to proceed in an elderly population of CKD patients with PAD without the fear of developing ARF.

PUB108

Serum Anti-Müllerian Hormone in Young Hemodialysed Women with Chronic Kidney Disease and after Kidney Transplantation
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Background: In women with chronic kidney disease (CKD) infertility is observed. Anti - Müllerian hormone (AMH) is produced by the ovarian follicles and is a paracrine factor inhibiting the excessive recruitment of primordial follicles. Until now serum AMH concentration in women with CKD and after kidney transplantation has not been determined. The aim of the study was to evaluate the serum AMH concentration in young females with CKD treated with hemodialysis (HDP) and in young female patients in early period after a successful kidney transplantation (KTP).

Methods: In 46 female HDP, 14 female KTP and 46 healthy women (HW) aged 18-40 years old serum AMH concentration in HDP and HW was assessed once, and in KTP 4 times: immediately before surgery, in the 14th and 30th day and 6 months after the transplantation. The results are presented as means and 95% CI.

Results: Serum AMH concentration in female HDP (4.16ng/ml; 3.18-5.15ng/ml) and in HW (4.43ng/ml; 3.49 - 5.36ng/ml) did not significantly differ (p = 0.594). Mean AMH serum concentration in female HDP without dysmenorrhea (n=21) was significantly (p=0.018) lower than in HW with regular menstrual cycles (n=40); 2.42ng/ml (1.58-3.26ng/ml) and

4.07ng/ml (3.23-4.90ng/ml),respectively. A significant (p=0.007) decrease of serum AMH concentration from 4.28 ng/ml (2.29-6.26 ng/ml) at baseline to 2.42 ng/ml (1.44 - 3.4 ng/ml) (p=0.048) at 30 days after surgery and to 1.89ng/ml (1.31-2.47ng/ml) (p=0.013) at 6 months after transplantation were found.

Conclusions: 1. The subgroup analysis of regularly menstruating women showed significantly lower AMH serum concentration in HDP than in HW suggesting the reduction of ovarian reserve in the HDP. 2. Successful kidney transplantation leads to further decrease of serum AMH concentration.

PUB109

Evaluation of Endothelium Function, Left Ventricular Structure and Function in Patients with Chronic Kidney Disease Piotr Bartnicki, Beata Franczyk-Skora, Mariusz Kowalczyk, Jacek Rysz. *Dept of Nephrology, Hypertension and Family Medicine, Medical Univ of Lodz, Lodz, Poland.*

Background: CKD is strongly connected with high risk of cardiovascular complications which is appearing already in early CKD stages and is the highest in patients treated with dialyses. The aim of this study was to evaluate endothelium function, left ventricular structure and function in patients with CKD stage IV and V.

Methods: Three study groups included: 30 patients in stage IV, 30 patients in stage V under hemodialysis treatment and 15 patients without CKD - control group. Blood concentrations of ADMA and NOS were assessed with ELISA system. Echocardiographic parameters were evaluated: intraventricular septum systolic diameter (IVSs) and diastolic diameter (IVSd), left ventricular mass (LVM), left ventricular end-systolic diameter (LVESd) and end-diastolic diameter (LVEDd), left atrium diameter (LA), aorta diameter (Ao), ejection fraction (EF), presence of left ventricular hyperthrophy (LVH) and disorders of left ventricular relaxation (LVR).

Results:

CKD	Stage IV (n=30)	Stage V (n=30)	Control group (n=15)
eGFR (ml/min)	22,5 ± 10,7*	10,3 ± 5,9*•	79,6 ± 12,3
ADMA (μmol/l)	0,63 ± 0,38*	0,77 ± 0,43*	0,36 ± 0,12
NOS (pg/ml)	3380 ± 999*	3833 ± 1005*	10000 ± 1950
IVSd (mm)	13 ± 1,3*	15 ± 2,8*•	10 ± 1,6
IVSs (mm)	16 ± 2,3*	17 ± 3,5*•	12 ± 1,4
LVM (g)	287,8 ± 70,1*	358,1 ± 86*	206 ± 42,2
Ao (mm)	32,7 ± 3,7*	31,8 ± 3,6*	26 ± 2,9
LVESd (mm)	41 ± 6,2*	38,9 ± 6,2*	36 ± 5,9
LVEDd (mm)	48,5 ± 6,7*	50,1 ± 5,6*	40 ± 7,4
LA (mm)	41,9 ± 2,7*	42,3 ± 3,2*	31 ± 2,2
EF (%)	50 ± 12*	45 ± 10* •	60 ± 9
LVH (% of evaluated)	100*	100*	20
LVR (% of evaluated)	96,7*	78,6*•	27

* p <0,05 to control group, •p<0,05 to stage IV

Conclusions: Our results confirm vascular endothelium dysfunction and disadvantageous morphological and functional changes of left ventricle in patients with CKD in comparison to control group. Disorders are greater in the stage V but largely without statistical significance compared with the stage IV of CKD.

PUB110

Differences in Nutritional Parameters, Body Composition and Muscle Strength between Diabetics and Non-Diabetic Patients with Advanced Chronic Kidney Disease (CKD) Guillermina Barril, Angel Nogueira Perez, Carmen Sanchez-Gonzalez, Almudena Nuñez Sanchez, Jose-Antonio Sanchez-Tomero. *Nephrology, Hospital U. de la Princesa, Madrid, Spain.*

Background: Assess nutritional parameters, body composition and muscle strength in 253 patients comparing ACKD results in the group of diabetics versus non-diabetics.

Methods: We evaluated 253 patients with ACKD xage±4 y, 60.5 % men They are divided into two groups according to the ethiology: G1-Diabetes Mellitus (DM) 33.7% and G2 other etiologies66.3% Anthropometric data were assessed, body composition by bioimpedance vector (BIVA),biochemical data: albumin, prealbumin, CRP, total lymphocytes, cholesterol and trasferrin.Muscle strength was asses by dynamometry.

Results: The results and differences between G1 and G2 are in the below.

Diabetes	Mean	St Deviation	p
% TSFold Yes No	123,46 115,99	60,35 53,38	0,1
CRP Yes No	0,58 1,12	0,79 3,09	0,05
MDRD Yes No	18,93 20,25	7,28 10,14	0.02
Phase A. Yes No	4,10 4,60	1,17 1,33	0.04
Na/K Yes No	1,53 1,36	0,54 0,51	0,02
BCM% Yes No	38,02 40,87	10,81 10,12	0,05
ECW% Yes No	56,67 53,50	8,96 9,31	0,01
ICW Yes No	43,31 46,19	8,96 9,48	0.03

G1 patients had significantly lower phase angle, but Na / k interchangeable lower, % BCM, increased ECW and lower ICW. Albumin and prealbumin were lower in G1 without statistical significance We found no significant difference between groups in SGA, OGA or MIS. The MDRD was lower in the group G1 versus G2 $p < 0.13$ nevertheless albumin and prealbumin were similar When comparing muscle strength by dynamometry xg1 found that was significantly lower than G2 mainly on the right side in men but not in women. Analyzing the groups according to sex major statistical significances between G1 and G2 shown in men.

Conclusions: 1-The group of patients with Diabetes Mellitus and CKD body composition traits have more ECW than nondiabetic group that is not justified by the reduction in glomerular filtration 2 -Evidence of decreased muscle strength in the diabetic group that correlates with lower BCMI in BIVA.

PUB111

Subendocardial Viability Ratio and Nt-Pro-BNP in Patients with Chronic Kidney Disease Robert Ekart,[1] Benjamin Dvorsak,[2] Masa Knehtl,[2] Sebastjan Bevc,[2] Nina Hojs,[2] Martin Hren,[1] Eva Jakopin,[2] Tina Stropnik Galuf,[1] Radovan Hojs.[2] *[1]Dept of Dialysis, Univ Medical Centre Maribor, Clinic for Internal Medicine, Maribor, Slovenia; [2]Dept of Nephrology, Univ Medical Centre Maribor, Clinic for Internal Medicine, Maribor, Slovenia.*

Background: The subendocardial viability ratio (SEVR) represents a noninvasive measure of myocardial perfusion related to LV function. The aim of our study was to investigate the relationship between SEVR,NT-pro-BNP and other cardiovascular (CV) risk factors in chronic kidney disease (CKD) patients.

Methods: We performed a cross-sectional study in a cohort of 86 nondialysis CKD patients. SEVR was assessed by radial applanation tonometry (SphygmoCor). NT-pro-BNP and CV risk factors were measured. In all subjects 24-h ABPM was performed using a non-invasive ABPM monitor (Schiller). The patients were asymptomatic regarding heart failure and were divided into two groups according to the median value of SEVR:lower SEVR group (SEVR<151%,n=44), higher SEVR group (SEVR>151%,n=42).

Results: Mean age of patients was 60,3±13 years, 65% were men, 44% were smokers, 24% had diabetes. Other descriptive data for all patients are presented in Table 1. Using t test, we found significant difference between both groups in NT-pro-BNP (P<0.05),but not in ABPM and other CV risk factors.

Variable	All patients (n=86)	Lower SEVR group: SEVR<151% (n=44)	Higher SEVR group:SEVR>151% (n=42)	P-value
Age(years)	60.3±13	61.9±13.6	58.7±12.1	0.26
Cystatin C(mg/L)	2.1±0.9	2,3±0.9	2±0.9	0.11
Cholesterol(mmol/L)	5±1.3	4.9±1.3	5.2±1.4	0.29
Triglycerides(mmol/L)	2.4±3.2	2.5±4.3	2.2±1.2	0.71
hs-CRP(mg/L)	6.1±11.5	5.8±9.2	6.3±13.6	0.86
NT-pro-BNP(pmol/L)	121.5±177.2	158.2±200.2	83.1±141.8	**0.048**
BMI(kg/m^2)	28.5±5.3	28.1±5.4	28.9±5.1	0.5
24-h systolic ABPM(mmHg)	135.4±17.6	138.6±17.3	132.1±17.6	0.08
24-h diastolic ABPM(mmHg)	76.1±9.3	74.8±9.3	77.6±9.2	0.17

Conclusions: Results of our study show that higher values of NT-pro-BNP are associated with lower SEVR, and both can be recognised as early markers of LV dysfunction in asymptomatic non-dialysis CKD patients.

PUB112

Clinical Significance of Troponin T in Chronic Kidney Disease Patients Presenting with Acute Coronary Syndrome Nimisha Venugopal,[1] Bhavna Pandya.[2] *[1]Medical School, Univ of Liverpool, Liverpool, Merseyside, United Kingdom; [2]Dept of Nephrology, Univ Hospital of Aintree, Liverpool, Merseyside, United Kingdom.*

Background: Chronic Kidney Disease patients have chronically elevated Troponin T values regardless of a cardiac event. This is a diagnostic difficulty should these patients present with acute chest pain, as clinicians are unsure how to interpret raised Troponin T values. This study aims to find a more appropriate cut-off diagnostic value of Troponin T in CKD patients.

Methods: This retrospective study collected baseline data from 1053 patients for analysis. Troponin T values and corresponding outcomes were collected between January 2011 to December 2012. A cut-off point was devised using a Receiver Operating Characteristic (ROC) curve. A Kaplan-Meier curve was calculated to evaluate survival and outcome predictors were analysed using the Cox Regression analysis.

Results: The ROC curve showed that a cut-off point of 58.5 ng/L had highest sensitivity (79.4%) and specificity (79.7%). Kaplan-Meier survival curves showed that there is a statistically significant decrease in survival in patients with Troponin T values above 41.5ng/L. Cox Regression analysis showed that Troponin T values and a history of ischaemic heart disease were the only outcome predictors that had a significant contribution towards a cardiac event.

Conclusions: This study shows that Troponin T values above 58.5ng/L is a predictor for a cardiac event in moderate- severe CKD patients. Thus, these patients should be monitored carefully and treatment should be started in order to avoid progression of the cardiac event.

PUB113

Relationship between Heart Rate Variability and the Progression of Chronic Kidney Disease Shuhei Watanabe,[1] Keiji Kono,[2] Kentaro Nakai,[2] Shunsuke Goto,[2] Hideki Fujii,[2] Shinichi Nishi.[2] *[1]Div of Nephrology, Akashi Medical Center, Akashi, Japan; [2]Div of Nephrology and Kidney Center, Kobe Univ Graduate School of Medicine, Kobe, Japan.*

Background: Heart rate variability (HRV) is known to be a measure of autonomic tone. Recently, it is reported that low HRV is an independent risk factor for cardiovascular events and mortality in general population. However, the relationship between HRV and the progression of CKD remains unclear. We aimed to examine the association between HRV and clinical characteristics in patients with CKD.

Methods: We included 67 patients with CKD stage 2-5 who admitted to Akashi medical center for CKD educational program from April 2012 to March 2014. All the patients underwent 24 hour ambulatory blood pressure monitoring to evaluate blood pressure and heart rate. For the assessment of HRV, we used heart rate standard deviation (HR-SD) and coefficient of variance (HR-CV), and performed cosinor analysis (HR-midline estimating statistics of rhythm (HR-Mesor) and HR-Amplitude). Laboratory tests were carried out by standardized clinical laboratory methods.

Results: HR-SD and HR-CV were decreased as declining glomerular filtration rate (GFR) and increasing proteinuria. The presence of diabetes mellitus (DM) reduced HR-SD and HR-CV. Furthermore, HR-Mesor had significant positive correlation with proteinuria. HR-Amplitide had significant negative correlation with GFR and proteinuria.

Conclusions: Our results demonstrated that HRV reduced along with the progression of CKD.

PUB114

Skin Advanced Glycation End Products (AGEs) Accumulation and Body Composition by Bioelectrical Impedance (BIA) in Chronic Kidney Disease (CKD) Stage 1-5.Role of Skin Autofluorescence (SAF) Secundino Cigarran,[1] Juan Latorre,[1] Ana Isabel Fernandez-Alonso,[1] Montserrat Pousa,[1] Walter Luis López Alarcón,[1] Guillermina Barril,[3] Jesus Calvino,[2] Saray Lopez-Prieto,[1] Miguel A. Garcia-Gonzalez.[4] *[1]Nephrology, Eoxi Lugo-Cervo-Monforte, Burela, Lugo, Spain; [2]Nephrology, Eoxi Lugo-Cervo-Monforte, Lugo, Spain; [3]Nephrology, Hospital Univ de la Princesa, Madrid, Spain; [4]Nephrology, Health Research Institute of Santiago de Compostela (IDIS)., Santiago de Compostela, La Coruña, Spain.*

Background: AGEs are involved in CKD progression.Major sources of systemic AGES are endogenous, generated in the body, and exogenous AGEs, in foods, particularly proteins of animal origin.Both are eliminated by kidney.In CKD, AGEs are retained and may influence the body composition.Aim is to assess the relationship between AGEs level and body composition by bioimpedance.

Methods: 259 caucasian pts CKD stage 1-5 were included (mean age 72 yo),61.4% men,37.1% diabetes status.Comorbidity (Charlson Index age-adjusted),Body composition assessment was performed by BIA analyzer (EFG. Biomednet. Akern. Fl.Italy) whole,tetrapolar,in supine in non dominant arm and spectroscopy bioimpedance (BCM,FMC,Bad Homburg,GER).AGEs were evaluated with SAF (arbitray units) (AGE Reader,DiagnOptic s,Groningen,The Neetherlands)by mean of three readings. Data were processed with software package SPSS 20.0 (Chicago,ILL,U.S.A.).Spearman´s rank correlation test was used to estimate the relationships between variables.A p value <0.05 was considered significant.

Results: AGEs level has a significant relationship with parameters BIA derived.

VARIABLE	R Spearman	P
AGE	.336	<0.001
GENDER male	-.268	<0.001
COMORBID INDEX (Charlson)	.337	<0.001
Na-K exchange	.334	<0.001
Hydration Index (OH) L	.302	<0.001
TBW (%)	.342	<0.001
ECW(%)	.174	0.008
ICW (%)	-.167	<0.001
Lean Body Mass (%)	.229	<0.001
Fat Mass (%)	-.252	<0.001
Phase Angle (º)	-.172	0.009
GFR-EPI	-.411	<0.001

Conclusions: AGEs levles have relation with body composition. Lifestyle changes (exercise),Diet counselling (avoid salt and additives) and physical activty(increasing lean body mass) may reduce AGEs accumulation,specially in older males .SAF is a useful tool to monitorize outcome. Prospective studies are required.

Funding: Other NIH Support - Galician Health Service

PUB115

Comparison of Subclinical Cardiovascular Damage in Patients with Chronic Kidney Disease Stage G3a and G3b Pawel Strozecki,[1] Agnieszka Pluta,[2] Mariusz Flisinski,[1] Zbigniew Serafin,[3] Jacek Manitius.[1] *[1]Dept of Nephrology, Hypertension and Internal Medicine, Nicolaus Copernicus Univ, Bydgoszcz, Poland; [2]Institute of Public Nursing, Nicolaus Copernicus Univ, Bydgoszcz, Poland; [3]Dept of Radiology and Diagnostic Imaging, Nicolaus Copernicus Univ, Bydgoszcz, Poland.*

Background: KDIGO 2012 Clinical Practice Guideline divided chronic kidney disease stage 3 into G3a and G3b, based on data supporting different outcomes. Left ventricular hypertrophy (LVH), aortic pulse wave velocity (PWV) and carotid intima-media thickness (IMT) are well defined markers of subclinical cardiovascular (CV) damage and predictors of CV events. Left ventricular mass index (LVMI) and PWV increase with progression of CKD. The aim of the study was to compare subclinical CV damage between patients in CKD stage G3a and G3b.

Methods: The study population consisted of 58 CKD patients stage 3, aged 57 ± 12. In all participants echocardiography with LVMI calculation was performed. LVMI ≥ 110 g/m2 in females and ≥ 125 g/m2 in males was considered as LVH. Aortic PWV and common carotid artery IMT were measured; PWV>10 m/s and IMT>0,9 mm were assumed as increased. Systolic (SBP) and diastolic (DBP) blood pressures were measured. Glomerular filtration rate (eGFR) was estimated using CKD-EPI formula.

Results: Mean eGFR in study population was 45±8 ml/min/1,73m2. There were 11 (19%) diabetic patients. LVH, elevated PWV, and elevated IMT was found in: 24(41%), 30(52%), and 10(17%) patients, respectively. Patients with eGFR 45-59 ml/min/1,73m2 and eGFR 30-44 ml/min/1,73m2 were compared. Results are shown in the table below.

	CKD G3a (n=29)	CKD G3b (n=29)	P
Age (years)	57±11	59±12	NS
Male gender (%)	20(69%)	17(59%)	NS
Patients with diabetes	4(14%)	7(24%)	NS
eGFR (ml/min/1,73m^2)	52±4	38±5	<0,001
SBP (mmHg)	136±16	136±26	NS
DBP (mmHg)	81±10	79±10	NS
LVMI (g/m2)	113±26	113±32	NS
PWV (m/s)	10,9±3,1	10,4±2,3	NS
IMT (mm)	0,72±0,17	0,77±0,22	NS

Conclusions: The study did not show differences between G3a and G3b CKD patients in markers of subclinical CV damage. Satisfactory blood pressure control in CKD patients may prevent subclinical CV damage.

PUB116

Non-Transplant Renal Patients on Cyclophosphamide Should Have Cytomegalovirus Surveillance Fenella J. Beynon, Ravindra Rajakariar. *Nephrology, Barts Health NHS Trust, London, United Kingdom.*

Background: Cytomegalovirus (CMV) is a cause of significant morbidity and mortality in the immunocompromised. No guidelines currently exist for CMV surveillance in non-transplant immunosuppressed patients. This service evaluation sought to determine current practice for CMV screening, prophylaxis, and outcomes at a UK tertiary renal unit.

Methods: Electronic records of non-transplant renal patients biopsied from Jan 2010 – Dec 2013 were retrospectively reviewed. Patients were included if they had renal disease (newly diagnosed or relapsed), requiring cyclophosphamide (CYC) treatment. Data was

collected on demographics, diagnoses, other immunosuppression, and CMV DNA testing, treatment and complications. Significant DNAemia was considered to be >3000, based on local guidelines for transplant recipients.

Results: 70 patients were included (40 women, 30 men). Mean age 48 (17-82). Ethnicity: South Asian 31% Afro-Caribbean 27% Caucasian 24% Other 16%. Diagnoses: Lupus nephritis 43% ANCA associated vasculitis 33% Minimal Change 4% Membranous 4% FSGS 4% Other 10%. Of 70 patients started on CYC, 52(74%) were ever tested for CMV DNA. There was variability in timing and frequency of testing: mean no. tests 4 (1-21), mean months to first test 2 (0-11). DNAemia was found in 20, 10 of whom had DNA >3000 (19% of those tested). 4/20 (20%) were only tested once, 8/20 (40%) were positive on their first test. Mean no. months to first DNAemia was 2 (0-6). 7 patients were treated for presumed CMV disease (2 CMV syndrome, 2 colitis, 2 pneumonitis, 1 hepatitis). 6/7 required hospital admission, 1 patient died. 4 were treated for asymptomatic DNAemia. 12 were prescribed valganciclovir prophylaxis on starting CYC, none of whom had significant (>3000) DNAaemia.

Conclusions: The current approach to CMV testing, prophylaxis and treatment is variable. Further prospective research is needed to evaluate the risks and benefits of prophylaxis and treatment of asymptomatic CMV DNAemia. Whilst the true prevalence cannot be determined from this evaluation, a significant number of patients developed CMV DNAemia and disease. We therefore recommend regular surveillance and increased awareness of CMV in non-transplant patients receiving cyclophosphamide.

PUB117

Anemia Management in Chronic Kidney Disease Not on Dialysis (CKD ND). Results from a French Survey M. Touam, F. Chantrel, David Attaf, Jm Hurot, B. Vendrely, A. Testa. *[1]Nephrology, Necker, Paris, France; [2]Nephrology, Centre Hospitalier de Mulhouse, France; [3]Dialysis MA, Fresenius, France; [4]Dialyse, Nephrocare Tassin Charcot, France; [5]Hopital Prive Saint-Martin, Pessac, France; [6]Echo Nantes, Nantes, France.*

Background: Anemia management evolves because of new Erythropoiesis-Stimulating Agents (ESAs) and IV irons, Clinical Trials (TREAT), and guidelines (KDIGO, 2012). Data related to CKD ND are poor. We aim to evaluate medical practices in CKD ND at the time of 2012 KDIGO's guidelines release.

Methods: Survey (PrEPOFer) evaluating medical practices via online questionnaire for nephrologists (NP). It covers **Diagnostic/Treatment/Monitoring** of anemia in CKD ND. Practices are compared with current guidelines.

Results: 428 NP responded to the survey. 35 % delegate management to other physicians or nurses. **Concerning Diagnostic :** 84 and 6 % use Hb+F+CST or HRC/CHr respectively. **Concerning Treatment :** 22, 57 and 37% of patients have an ESA, Iron or ESA+iron, respectively. Biological criteria for ESA/iron initiation : 36, 33 and 25% of NP initiate ESA/iron if Hb <10, <10,5 or <11 g/dl respectively. 74, 12% of NP initiate Iron if CST <20 or < 25% respectively. 57 and 23% of NP start iron if F <100 or < 200 mg/l respectively. 58 and 11% of NP target Hb = [10-12], |9-11] g/dl respectively. 23, 46 or 12% of NP target F < 500, [500-800] or > 800 mg/l respectively. 9, 10 and 61 % of NP target a CST <30, <40 or <50%. 71% of patients have an oral iron as first line. IV iron is given to 12 % of patients, either sucrose (52%), or carboxymaltose (32%) or Dextran (18% of NP). 69, 15 and 7% of NP give an IV iron dose < 300, [300-500] and [500-1000] mg, respectively. IV iron is given at home or in center (data not shown). IV iron is given weekly, monthly, bimonthly or quarterly. If F. is > target most of NP reduce frequency or dose of iron. If Hb > target most of NP reduce frequency or dose of ESA. Definition of ESA resistance is not consensual.

Conclusions: Impact of recent trials and guidelines is significant: lower Hb target, lower frequency or dose of ESA If Hb > target. First line Iron treatment is oral, upper limit of F. not consensual unlike lower limit of F. and CTS. This survey is a representative picture of practices at time of anemia KDIGO 2012's release.

Funding: Pharmaceutical Company Support - FMC VIFOR

PUB118

The Association of Carbamylated Albumin and Mortality in Non-Dialyzed Patients with Chronic Kidney Disease Anders H. Berg,[1] John Danziger,[1] David J. Friedman,[1] Sahir Kalim,[2] Julia Beth Wenger,[2] Ravi I. Thadhani,[2] S. Ananth Karumanchi.[1] *[1]Beth Israel Deaconess Medical Center; [2]Massachusetts General Hospital.*

Background: Carbamylation is a protein modification by urea that is promoted by chronically elevated urea and amino acid deficiencies. Serum carbamylated albumin (C-Alb) levels have been associated with mortality in ESRD patients on hemodialysis. This study tested whether C-Alb levels are associated with mortality in non-dialyzed patients with chronic kidney disease (CKD), and compared the risk of high C-Alb to that of conventional markers of uremia.

Methods: 119 patients with stages 2 – 5 CKD were enrolled. C-Alb was measured in baseline serum samples using LC-MS/MS. Serum was also analyzed for BUN, creatinine, cystatin C, and calculated MDRD, CKD-EPI, and Cystatin C-based estimated glomerular filtration rates. 3.6 years of survival data was collected. The association between mortality and standardized kidney biomarker values was estimated using Cox proportional hazard ratios (HR).

Results: 10 of 97 included subjects died during the study, 22 were lost to follow-up. The median C-Alb value of subjects who died was twice as high as in survivors (7.7 versus 15.5, p<0.001). Univariate Cox proportional hazards analysis demonstrated a significant association between mortality and C-Alb values (HR 3.54, 95%CI 2.19 – 5.73, p<0.001). There were also statistically significant risks associated with serum creatinine, blood urea nitrogen, cystatin C, $eGFR_{MDRD}$, $eGFR_{CKD-EPI}$, and $eGFR_{Cystatin}$. However, when multivariable HR analysis adjusted for age, C-Alb, BUN, cystatin C, eGFR, gender, race, phosphorous, glucose, calcium, and parathyroid hormone levels, C-Alb was the only variable that remained associated with mortality (HR 3.30, 95%CI 1.19 – 9.16, p=0.02).

Conclusions: New uremic biomarkers that are associated with mortality should be tested for their ability to predict which CKD patients will benefit from early initiation of renal replacement therapy. In this study of non-dialysis-dependent CKD patients, we found that elevated C-Alb was strongly associated with risk of future death, and this association appeared more robust compared to other conventional kidney biomarkers.

Funding: NIDDK Support, Pharmaceutical Company Support - Baxter Healthcare, Private Foundation Support

PUB119

Ivabradine Use in CKD and ESRD Patients: A Single Centre Experience Sanjana Gupta,[1] Magdi Yaqoob,[1] Andrew Wragg,[2] Kieran Mccafferty.[1] *[1]Dept of Nephrology, Barts Health NHS Trust, London, United Kingdom; [2]Dept of Cardiology, Barts Health NHS Trust, London, United Kingdom.*

Background: Ivabradine (IVD) is a novel class of drug which specifically inhibits the sinoatrial node through inhibition of the i_f current, leading to a reduction in heart rate and cardiac work. It is indicated for treatment of patients with stable angina or heart failure (New York Heart Association Class II-IV), who are in sinus rhythm, with a heart rate of >75BPM and in whom beta-blockers are contraindicated or not tolerated. Cardiovascular disease is the most common cause of death in patients with advanced CKD/ESRD with up to 1/3 of incident dialysis patients having echocardiographic evidence of heart failure. This is associated with poor long-term outcomes. IVD is safe and effective in patients with mild to moderate CKD however very little data exists on the safety of IVD in severe renal failure, dialysis and transplantation. We sought to examine the prescribing and tolerability of IVD in a tertiary renal unit.

Methods: We performed a retrospective analysis of IVD use in our unit over the last 10 years. We identified patients who had been prescribed IVD and collected their clinical and demographic data.

Results: We identified 56 patients who had received IVD in our unit.

Age	69 (57-76)
Male n (%)	39 (70)
Ethnicity (White/Black/Asian) %	30/7/63
Aetiology of CKD (Diabetes/BP/Ischaemic nephropathy/other) %	45/9/21/25
Modality (CKDI-III/CKDIV-V/HD/PD/Tx)%	29/29/18/13/13
Indication for IVD (ischaemia/heart failure/other) %	61/30/9
Dose of IVD (mg)	10 (5-10)
Duration of IVD Treatment (months)	49 (22-84)
Mean arterial Pressure (pre IVD) mmHg	87 (63-97)
Change in MAP Post IVD mmHg	-3 (-15-10)
Heart rate (on IVD)	74 (65-82)
Side effects (hypotension/dizziness/dyspnoea/headache) %	18/2/2/2

45% of the cohort remain on IVD, of those that are no longer on it the most common reason for this is patient death (44%). Hypotension was seen in 19% of patients, however only 4% had to stop IVD due to this.

Conclusions: IVD appears safe and well tolerated in this cohort, however there is a small risk of hypotension. Future prospective clinical trials in the use of IVD with respect to tolerability and cardiovascular outcomes in ESRD are warranted.

PUB120

Transferrin Saturation Is More Useful Than Ferritin as a Marker for Iron Deficiency in Chronic Kidney Disease Hye Jin Lim,[1] Young-Hwan Hwang,[2] Su-Ah Sung,[2] Hayne C. Park,[3] Kook-Hwan Oh,[3] Dong-Wan Chae,[3] Kyu Hun Choi,[4] Kyu-Beck Lee,[5] Yong-Soo Kim,[6] Soo Wan Kim,[7] Joongyub Lee,[8] Curie Ahn.[3] *[1]Dept of Internal Medicine, Gachon Univ, Gil Hospital, Incheon, Korea; [2]Dept of Internal Medicine, Eulji General Hospital, Seoul, Korea; [3]Dept of Internal Medicine, Seoul National Univ College of Medicine, Seoul, Korea; [4]Dept of Internal Medicine, Yonsei Univ, Severance Hospital, Seoul, Korea; [5]Dept of Internal Medicine, Kangbuk Samsung Medical Center, Seoul, Korea; [6]Dept of Internal Medicine, Catholic Univ, Seoul St. Mary Hospital, Seoul, Korea; [7]Internal Medicine, Chonnam National Univ Medical School, Gwangju, Korea; [8]Medical Research Collaborating Center, Seoul National Univ Hospital, Seoul, Korea.*

Background: Iron deficiency is known to be prevalent in chronic kidney disease (CKD) patients and should be corrected along with the use of erythropoiesis stimulating agents in the management of anemia. Transferrin saturation (TSAT) and ferritin are widely used as markers for iron deficiency, however, ferritin may reflect inflammatory state at the same time.

Methods: We examined the prevalence and risk factors of anemia in CKD stage 1-5 (non-dialysis) patients, using the baseline data obtained from a prospective cohort study (KNOW-CKD). Anemia was defined as hemoglobin (Hb) <13 in male and <12 g/dL in female. Iron deficiency was defined when transferrin saturation (TSAT) was <20% or ferritin level < 100 ng/ml.

Results: Anemia was identified in 45% of 1,416 subjects at baseline. Iron deficiency by current criteria was found in 30-50% of male and 50-70% in female CKD patients. Hb

level decreased significantly once TSAT lowered to < 20%. In contrast, high ferritin level (>500 ng/ml) instead of low ferritin (<100 ng/ml), was associated with low Hb. TSAT was independently associated with anemia (OR 3.9 with TSAT 10-19% compared to 30-50%; OR 3.0 with TSAT < 10%, both P<0.01), when adjusted for gender, age, diabetes, serum albumin and logCRP. There were no significant correlation between serum ferritin and C-reactive protein (CRP) or serum albumin.

Conclusions: Low TSAT (<20%) was associated with anemia of CKD, whereas low serum ferritin level was not correlated to Hb level.

Funding: Government Support - Non-U.S.

PUB121

The Relation between Pulmonary Hypertension, Peripheral Vascular Calcification, and Major Cardiovascular Events in End Stage Renal Disease Patients Undergoing Dialysis

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Background: Pulmonary hypertension (PHT) is a recently recognized complication of chronic kidney disease and end-stage renal disease (ESRD). In this study we investigated the association between pulmonary hypertension, peripheral vascular calcifications (VCs), and major cardiovascular events.

Methods: In this retrospective study, we included 172 ESRD patients undergoing dialysis (HD=84, PD=88) who had enrolled previous cross-sectional study in March 2009. PHT was defined as an estimated pulmonary artery systolic pressure of more than 35mmHg using echocardiography. Simple vascular calcification score (SVCS) was measured using plain radiographic films of both hand and the pelvis.

Results: The prevalence of PHT was significantly higher in HD patients (57.1% versus 25.0%, p<0.001). ESRD patients with PHT had significantly higher prevalence of severe vascular calcifications (SVCS≥3), and mitral valve disease. They were older, and had higher left ventricular (LV) mass index. In multivariate analysis, the presence of severe VCs (OR, 2.42, p=0.02) and mitral valve disease (OR, 7.11, p<0.001), undergoing hemodialysis (OR, 3.72, p<0.001), and higher LV mass index (OR, 1.01, p=0.04) were independent risk factors for PHT. The patients with PHT had a significantly shorter event-free survival of major cardiovascular events (Log Rank test, p<0.01). In a multivariable Cox regression model, high parathyroid hormone group (HR 4.73, p=0.046), lower hemoglobin levels (HR 0.131, p=0.021), and the presence of severe VCs (HR 4.45, p=0.007) and PHT (HR 3.80, p=0.020) were significant predictors for major cardiovascular events.

Conclusions: The prevalence of PHT was higher in HD patients and is associated with higher major cardiovascular events. The presence of severe vascular calcifications is thought to be independent risk factor for predicting PHT in ESRD patients. Therefore, in ESRD patients with PHT, careful attention should be paid to the presence of vascular calcifications and the occurrence of major cardiovascular events.

PUB122

Risk Factors for Discontinuation of Cinacalcet

Diane Reams,[1] Paul Dluzniewski,[2] Thy P. Do,[2] Brian D. Bradbury,[2] A. V. Kshirsagar,[1] M. Alan Brookhart.[1] *[1]UNC, Chapel Hill, NC; [2]Amgen Inc., Thousand Oaks, CA.*

Background: Predictors of cinacalcet discontinuation and levels of parathyroid hormone (PTH) and calcium (Ca) prior to discontinuation have not been previously reported. We examined predictors of cinacalcet discontinuation and biochemical levels prior to discontinuation in a large population of patients receiving chronic hemodialysis (HD).

Methods: Using data from the United States Renal Data System merged with clinical data from a large dialysis provider, we identified new users of cinacalcet from 2007 through 2010 using Part D prescription claims. Patients received in-center HD and were 18 years or older with continuous Medicare coverage during the study period. New users were identified as patients with at least one 30-day cinacalcet prescription fill and no cinacalcet use 6 months prior to the initial prescription. Covariates, including cardiovascular and renal risk factors, laboratory values, and medications, were assessed in 30-day intervals following cinacalcet initiation. Movement between quintiles of laboratory distributions was examined to determine changes in biochemical levels prior to discontinuation. We calculated hazard ratios (HR) and 95% confidence intervals (CI) for the risk of cinacalcet discontinuation.

Results: We identified 17,791 eligible cinacalcet initiators who contributed 101,147 30-day follow-up intervals. Over half of all patients discontinued cinacalcet by month 4. Proximal PTH levels <150pg/mL were associated with discontinuation: HR = 1.23 (95% CI: 1.11-1.36), whereas low Ca (<7.5 mg/dL) was only weakly associated, HR = 1.10 (95% CI 0.92-1.32). Entering the Medicare Part D gap period increased discontinuation risk: HR = 1.19 (95% CI: 1.00-1.42), and low-income subsidy status decreased the risk of discontinuation: HR = 0.77 (95% CI 0.69-0.86). Increasing PTH and Ca levels: HR = 1.15 (95% CI: 1.08-1.23) and HR = 1.23 (95% CI: 1.15, 1.31), respectively, may be early markers of discontinuation.

Conclusions: Early discontinuation following cinacalcet initiation is common, and occurs frequently for clinical or economic reasons. It also appears that an unanticipated increase in biochemical levels may be an early marker of patient discontinuation.

Funding: Pharmaceutical Company Support - Amgen, Inc. Thousand Oaks, CA

PUB123

Low Plasma Level of Cathelicidin Is Associated with Decreased eGFR

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Background: Infectious complications are a frequent cause of hospitalization in patients with chronic kidney disease, and the second leading cause of mortality after cardiovascular disease. Infectious mortality rates increased with lower kidney function. This study assessed plasma cathelicidin, 25-OH vitamin D, and natural killer (NK) cell which play an important role in innate immunity.

Methods: The study cohort included175 patients who have a variety of estimated glomerular filtration rate (eGFR) at the Chonnam National University Hospital. Plasma catehlicidin levelwas measured by ELISA and absolute count of NK cell was determined by flow cytometry. NK cell was identified phenotypically as CD3-CD56+.

Results: The study population was divided into 3 groups according to the eGFR:Group I, eGFR ≥ 60 mL/min/1.73 m^2, n=33 (18.9%); group II, 15 ≤ eGFR < 60 mL/min/1.73 m^2, n=47 (26.8%); group III, eGFR < 15 mL/min/1.73 m^2, n=95 (54.3%), respectively. Plasma cathelicidin level was decreased with lower eGFR (189.3 ± 18.21 ng/ml in group I, 182.6 ± 17.69 ng/ml in group II, and 179.7 ± 17.76 ng/ml in group III, P=0.032, respectively). On the other hand, percentages and absolute numbers of NK cells were significantly higher in the peripheral blood of patients wtih lower eGFR(1897.7 ± 1584.05 in group I, 2110.8 ± 2168.37 in group II, and 2833.8 ± 2149.65 in group III, P=0.034, respectively). A 25-OH Vitamin D was not distinguish in these groups, but after an exclusion of pateints with hemodialysis, 25-OH vitamin D level showed decreasing tendency with lower eGFR (16.4 ± 6.22 ng/ml in group I, 12.4 ± 4.74 ng/ml in group II, and 11.2 ± 4.96 ng/ml in group III, P=0.166, respectively).

Conclusions: The plasma cathelicidin level was significantly decreased with lower eGFR while an absolute count of NK cell was increased with lower eGFR, which might be associated with dysfunction of NK cell. After an exclusion of patients with hemodialysis, 25-OH vitamin D concentiration showed decreasing tendency with lower eGFR. Further research is needed to determined NK cytotoxicity and understand the mechanisms of decreased innate immunity affecting infectious mortality in chronic kidney disease.

Funding: Clinical Revenue Support

PUB124

Predicting Coronary Artery Disease in Chronic Kidney Disease Patients Referred for Kidney Transplant Using a Novel Risk Score

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Background: Evaluation for CAD is an important aspect of the pre-operative assessment of transplant candidates. Currently, there is no well-established risk calculator to assess the likelihood of CAD in CKD patients. We examined traditional and non-traditional risk factors in CKD patients referred to the University of North Carolina Transplant Clinic to create a simple risk score to predict CAD in kidney transplant candidates.

Methods: The risk score was derived from a cohort of CKD patients referred for cardiac evaluation prior to possible kidney transplant. The average age of our cohort (n = 1028) was 56 ± 11 years; 56% were male and 60% were African-American. 779 (76%) patients had Stage-5D CKD and 205 (20%) had CAD.

Results: In a multivariate logistic regression analysis, the following variables were significant (p<0.05) independent predictors of CAD: smoking history [OR 2.0 (CI 1.5-2.8)], presence of peripheral arterial disease (PAD) or cerebrovascular disease (CVD) [OR 1.8 (CI 1.3-2.6)]; white race [OR 2.2 (CI 1.6-3.0)], diabetes [OR 2.0 (CI 1.5-2.9)], and end-stage-renal-disease (ESRD) status [OR 1.9 (CI 1.3-2.8)]. Variables that were not significant independent predictors of CAD included: gender, BMI, duration of diabetes, systolic and diastolic blood pressure, lipid levels, C-reactive protein, and homocysteine. Since the independent contributions of smoking history, PAD or CVD, white race, diabetes, and ESRD to the risk of CAD were roughly equivalent, we assigned one point to each of these risk factors and defined the risk score (ranging from 0 to 5) as the sum. In our derivation cohort, the prevalence of CAD was 0% when the risk score was 0; 9% with a score of 1; 17% with a score of 2; 25% with a score of 3; 43% with a score of 4; and 69% with a score of 5.

Conclusions: Using our cohort of patients, we derived a simple risk score for CAD prediction in CKD patients. If validated in other populations, this score may prove useful in guiding the evaluation of patients referred for kidney transplantation.

PUB125

N-Terminal Pro-B-Type Natriuretic Peptide and Proadrenomedullin Reflect Volume Status in Hemodialysis and Peritoneal Dialysis Patients

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Background: Although control of normal hydration state is a key parameter for cardiovascular mortality in dialysis patients, the question for biomarkers of volume excess continues. Body composition monitor (BCM; Fresenius Medical Care, Bad Homburg, Germany) has been proven as a non-invasive, quantitative method for measuring body fluid status. In addition, N-terminal pro-B-type natriuretic peptide (NT-proBNP), myeloperoxidase (MPO), copeptin and proadrenomedullin are associated with cardiac dysfunction and systemic blood volume. Present study investigated the relationship between body fluid status and volume markers in dialysis patients.

Methods: Cohorts of pre-dialysis (pre-D), hemodialysis (HD) and peritoneal dialysis (PD) patients and age-and gender-matched healthy individuals were recruited in the study (N=80). In all patients BCM and echocardiography were performed. HD patients were measured at the midweek session before dialysis and PD patients were measured with a full abdomen. Also NT-proBNP, MPO, cepeptin and proadrenomedullin were measured. Clinical overhydration was defined as an overhydration-to-exracellular water ratio of > 15%.

Results: No difference was found the groups in terms of total body water, extracellular water and intracellular water. Clinical overhydration was more prevalent in HD and PD patients compared to control and pre-D patients significantly. This was associated with significantly ($p<0.001$) higher NT-proBNP and proadrenomedullin levels in HD and PD patients than in the control and pre-D groups. However, no significant difference was found in levels of MPO and copeptin in the study groups. In multivariate models, clinical overhydration was directly related to NT-proBNP and proadrenomedullin concentrations in the study population ($r=0.454$ [$p<0.001$] and $r=0.505$ [$p<0.001$]).

Conclusions: NT-proBNP and proadrenomedullin levels increase in association with systemic blood volume in HD and PD patients with cardiovascular disease, however, MPO and copeptin are not volume markers.

PUB126

Renal Insufficiency Evolution in Patients with Relapsed Multiple Myeloma: Preliminary Results of a Large Observational Study Enrique Morales,[1] Antoni García,[2] Fernando Escalante,[3] Soledad Duran,[4] Mercedes Gironella,[5] Tomas J. Gonzalez,[6] Manuel Perez,[7] Ignacio Espanol,[8] Elena Cabezudo,[9] Javier De la Rubia.[10] *[1]H 12 Octubre; [2]H U Arnau de Vilanova; [3]H Leon; [4]H U Ciudad de Jaen; [5]H Vall d´Hebron; [6]H U de Burgos; [7]C H U de Santiago; [8]H Santa Lucía; [9]H Althaia; [10]H la Fe.*

Background: Patients(pts) with RRMM have commonly(20-40%) impaired renal(IR) function, associated with a shorter survival. In RRMMpts, renal response to anti-myeloma drugs according to MDRD, CKD-EPIformulas has not been described prospectively. Aims: Describe renal response in RRMM pts with IR function(CrCl<50ml/min), myeloma response rate(IMWG-criteria), overall survival, safety, and health resource use. We show a descriptive analysis of the first 100pts.

Methods: Spanish observational, prospective, multicenter ongoing study:300RRMM pts with moderate(50-30ml/min)(n=225)/severe (<30ml/min)(n=75)IR function. Planned follow up: 36 months after end of therapy.

Results: 50 out of the first 100pts presented moderate IR function and 50 severe. At study entry 50%, 34% and 16% of pts were in 1st, 2nd and ≥3rdrelapse, respectively. At relapse, 64%pts had anemia, 37%bone lesions, 19%hypercalcemia, and 11%extramedullary plasmacytomas. Anti myeloma therapies were based on lenalidomide, 37%; or bortezomib 39%.

N=100		
Age, mean SD		73.6(8.5)
Male:Female		55:45
Myeloma type,%		
IgG		57
IgA		20
Bence-Jones		49
non-secretor		3
Other		2
Not available		1
Comorbidities,%		
Hypertension		47
Diabetes mellitus		23
Neurophaty		21
Concomitant neoplasms		17
Heart failure		10
Renal Funcition, mean SD		
CrCl (Cockroft-Gault) mL/min		31.4(11.9)
eGFR	MDRD-4mL/min	32.7(15.1)
	CKD-EpimL/min	32.6(15.3)
Renal insufficiency, n(%)		
Moderate(30-50ml/min/1.73m^2)		45(51.7)
Severe(<30ml/min/1.73m^2)		42(49.4)
Stage ISS, %		
3		47
2		27
1		8
NA		18

Conclusions: In a high percentage, RRMMpts with IR are elderly with concomitant diseases(IR-associated) that affect diagnosis and further evolution. It is essential to apply glomerular filtration formulas to properly assess renal function in these pts. As in non-IR pts, most frequent anti MM therapies are based on lenalidomide or bortezomib. Further analysis will be available when more pts were included.

PUB127

Simple Renal Cysts Were Associated with Renal Dysfunction and Hypertension Jian Hui Yang, Qiang He, Yiwen Li. *Nephrology, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China.*

Background: The relationship between simple renal cysts are not well understood. Present study was to investigate the impact of simple renal cysts on renal function and blood pressure.

Methods: 99Tcm-DTPA renal dynamic imaging were performed in sixty-eight patients with spiral computed tomography proven single simple renal cysts. Forty healthy people were used as normal control. Their single renal glomerular filtration rate, fractional blood flow, time to peak secretion, time to half excretion, location of cysts, diameter of cysts were recorded. All the clinical features,especially blood pressure and serum creatinine, were monitored before and after ultrasound-guided ethanol sclerotherapy.

Results: Sixty-five patients' renal cysts located inside kidney area. Their diameters of cysts ranged from 4.6cm to 16cm. Their fractional blood flow (FRB) and single glomerular filtration rate (sGFR) in the intact kidney of patients were not significantly different from those in normal controls(47±9 versus 45±8ml/min, 55±6 versus 53±5%), but significantly higher than those of affected kidney(38±8ml/min, 35±7%). The time to peak secretion and time to half excretion were obviously pre-longed in affected kidney compared with intact kidneys(3.4±0.7 versus 4.6±0.6min; 10.1±7.1 versus 15.3±8.2min). FRB and sGFR were negatively relative to the diameter of renal cysts. After ethanol sclerotherapy, their serum creatinine level and diastolic blood pressure decreased obviously(from 95±11 to 88±10 μmol, from 84±12 to 80±11 mmHg). However, there were three patients whose simple renal cysts located outside the kidney areas. Their FRB and sGFR did not differ between affected and intact kidneys.

Conclusions: Simple renal cysts might affect renal function and blood pressure, they should be treated in time.

PUB128

Sleep Disorder and mRNA Expression Profile of Sleep Related Gene on Peripheral Blood Cells in CKD Patients Shinji Kitajima, Yasuyuki Shinozaki, Yasunori Iwata, Norihiko Sakai, Miho Shimizu, Kengo Furuichi, Takashi Wada. *Div of Nephrology, Kanazawa Univ Hospital, Kanazawa, Ishikawa, Japan.*

Background: Although uremic substances would be candidates involved in sleep disorder, detailed mechanisms remain unclear so far. In this study, we evaluated polysomnography analysis and mRNA expression profile on peripheral blood cells (PBC) in CKD/HD patients.

Methods: Polysomnographic analysis was performed in 9 patients with CKD and 6 healthy subjects. Sleep and awakening related genes were evaluated by RNA microarray in 19 patients, including CKD/HD patients and control subjects.

Results: Polysomnographic analysis revealed that the magnitude of REM/non-REM phase in total sleep time was different between CKD patients and healthy control. In mRNA microarray evaluation, hierarchial clustering analysis showed the different pattern of sleep related gene expression in the patients with HD. The mRNA expression levels of GABA receptor, noradrenaline receptor, dopamine receptor and histamine receptor showed an inverse correlation with renal function. Moreover, orexin and its receptor mRNA expression also showed an inverse correlation with renal function.

Conclusions: These data raise the possibility that sleep related gene expression on PBC in CKD/HD patients may be associated with sleep disorder.

PUB129

Concomitant Intravenous Iron Use Drives Change in Iron Indices Across Geographical Regions in a Sucroferric Oxyhydroxide Study Stuart M. Sprague,[1] Adrian Covic,[2] Jürgen Floege,[3] Markus Ketteler,[4] Bruce S. Spinowitz,[5] Jaco Botha,[6] Anjay Rastogi.[7] *[1]NorthShore Univ Health System; [2]'Gr. T. Popa' Univ of Medicine and Pharmacy, Romania; [3]RWTH Univ Hospital Aachen, Germany; [4]Coburg Clinic and KfH-Dialysis Center, Germany; [5]New York Hospital Queens; [6]Vifor Pharma, Switzerland; [7]Univ of California.*

Background: A randomized Phase 3 study assessed sucroferric oxyhydroxide (SFO; VELPHORO®/PA21), an iron-based phosphate binder, versus sevelamer carbonate (SEV) in dialysis patients. This *post hoc* analysis compared iron indices in patients with or without concomitant intravenous (i.v.) iron across regions: EU, U.S.A. and other countries.

Methods: 1,059 patients were randomized to SFO (1.0–3.0 g/day) or SEV (2.4–14.4 g/day) for 12 weeks' dose titration plus 12 weeks' maintenance. Eligible patients enrolled in a 28-week extension study.

Results: 549 patients completed the extension study. A higher proportion of U.S.A. patients received i.v. iron: SFO (93.2%) and SEV (92.5%) versus EU (69.4%; 77.8%) and other countries (37.8%; 45.1%). Changes in mean iron indices by region are shown in the Table.

Table: Iron indices by i.v. iron use.

	SFO n=322		SEV n=227	
	i.v. iron n=237	No i.v. iron n=85	i.v. iron n=183	No i.v. iron n=44
Δ Ferritin, ng/mL				
EU	139.7* ± 322.6	35.2 ± 331.9	175.7 ± 464.1	−72.1 ± 118.3
USA	240.5*# ± 362.7	−52.9 ± 450.5	81.7 ± 455.3	−166.0 ± 477.6
Other countries	158.2* ± 326.2	40.0 ± 362.1	73.8 ± 268.0	31.4 ± 160.9
Δ TSAT, %				
EU	3.7 ± 18.7	7.9 ± 17.0	2.4 ± 15.8	0.7 ± 6.8
USA	5.3* ± 21.5	−6.6 ± 15.7	1.4 ± 17.0	−4.9 ± 8.0
Other countries	6.0*# ± 14.4	3.3 ± 13.6	−0.4 ± 11.6	3.3 ± 13.1
Δ Hb, g/L				
EU	3.3*# ± 10.6	−1.4 ± 12.4	−2.1 ± 11.2	−6.1 ± 6.9
USA	−3.3* ± 12.6	−3.9 ± 17.3	−3.0* ± 10.9	0.6 ± 10.2
Other countries	4.8* ± 18.4	7.7* ± 19.2	1.2 ± 13.6	6.0 ± 17.4

Data are mean changes (baseline to endpoint [last post-baseline non-missing value]) ± standard deviation.
*P<0.05 baseline to endpoint change; #P<0.05 SFO vs SEV in i.v. iron groups.
TSAT, transferrin saturation; Hb, hemoglobin.

Conclusions: Changes in iron indices across regions in both study arms were mainly attributable to concomitant i.v. iron use. Differences between treatment groups may be due to minimal iron uptake from SFO, although no signs of iron accumulation or overload were observed.

Funding: Pharmaceutical Company Support - Vifor Pharma

PUB130

Active Involvement of Erythrocyte Asymmetric Dimethylarginine in Renal Anemia Miyuki Yokoro,[1] Seiji Ueda,[1] Daisuke Saigusa,[2] Nana Obara,[1] Yosuke Nakayama,[1] Ryotaro Ando,[1] Takaaki Abe,[3] Seiya Okuda.[1] *[1]Div of Nephrology, Dept of Medicine, Kurume Univ School of Medicine, Kurume, Kurume City, Fukuoka, Japan; [2]Dept of Integrative Genomics, Tohoku Medical Megabank Organization, Tohoku Univ, Sendai City, Miyagi, Japan; [3]Dept of Clinical Biology and Hormonal Regulation, Tohoku Univ, Sendai City, Miyagi, Japan.*

Background: Asymmetric dimethylarginine (ADMA) have been reported to be accumulated and play important role in vascular injury in chronic kidney disease (CKD) patients. Recently, we have reported that rat erythrocyte could be one of modulator of plasma ADMA levels. In addition, L-arginine was reported to be an essential factor for erythrocyte maturation. However, erythrocyte ADMA metabolic system and its involvements in renal anemia in CKD patients are still unknown.

Methods: We collected blood samples of 16 healthy subjects and 25 non-dialysis CKD patients. The expression of DDAH, ADMA-degrading enzyme, and PRMT, ADMA-producing enzyme, were determined by western blotting. ADMA concentrations were measured by LC-MS/MS.

Results: We found that DDAH and PRMT were expressed in human erythrocytes. Compared to healthy control, DDAH expressions in erythrocytes were significantly decreased and ADMA levels were markedly higher in CKD patients. Ex vivo analysis revealed that cultured erythrocyte can immediately take in ADMA, which was completely abolished by L-lysine, a competitive inhibitor of cationic amino acid transporter, suggesting that erythrocyte may regulate circulating ADMA through cationic amino acid transporter. In addition, erythrocyte ADMA levels were found to be negatively correlated to RBC count ($r = -0.467$, $p < 0.05$), and tended to be positively correlated to serum ferritin level ($r = 0.392$, $p = 0.087$) and plasma hepcidin level ($r = 0.463$, $p < 0.05$).

Conclusions: Although further evidence is needed, the present study suggests that erythrocyte could be a modulator of ADMA in CKD patients and erythrocyte ADMA accumulation may have a role in the development of renal anemia.

PUB131

Anti-Müllerian Hormone Levels and Sperm Quality Are Decreased in Men with Terminal Renal Failure Anders G. Christensson, Laila Bruun, Dag Eckersten. *Dept of Nephrology, Clinical Sciences, Malmö, Sweden.*

Background: Male reproductive function is impaired in end-stage renal disease (ESRD). Disturbances in the hypothalamic-pituitary-gonadal axis is one of the major causes. Earlier studies have shown elevated levels of prolactin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH).It has also been demonstrated decreased levels of testosterone. Anti-müllerian hormone (AMH), produced by the Sertoli cells in the testes inhibits the production of androgens, and is a marker for Sertoli cells and testicular function. AMH in healthy men is correlated with non-obstructive azoospermia. It has not been studied in men with ESRD. This study is aimed at studying AMH and inhibin B in men with ESRD.

Methods: Twenty male patients on hemodialysis, median age 40 (26-48) years, were analysed for follicle-stimulating hormone, luteinizing hormone, prolactin, sex hormone-binding globulin, testosterone, estradiol, anti-müllerian hormone and inhibin B levels. Furthermore, semen quality parameters were analyzed in 7 of these patients. We used 144 proven fertile men, median age 32 (19-44) years, as a control group to find differences by using multiple linear regression.

Results: Males with end-stage renal disease demonstrated higher mean values for prolactin, 742 versus normals 210 mIE/L (95%CI 60.3, 729), luteinizing hormone, 8.87 versus normals 4.5 IE/L (95%CI 2.75, 6.14), and estradiol 89.7 versus normals 79.0 pmol/L (95%CI -1.31, -0.15). Mean value for anti-müllerian hormone was lower, 19.5 versus normals 47.3 pmol/L (95%CI -37.6, -11.6). There were no differences regarding follicle-stimulating hormone, sex hormone-binding globulin, inhibin B and testosterone. The seminal analyses revealed low sperm count and decreased motility.

Conclusions: We conclude that the most important difference was found for anti-müllerian hormone, a marker of Sertoli cell function in the testes, that was decreased by close to 60% compared to controls. Combined with an increase in luteinizing hormone, these findings may indicate a dysfunction of Sertoli cells and an effect on Leydig cells contributing to a potential mechanism of reproductive dysfunction in men with end-stage renal disease.

Funding: Government Support - Non-U.S.

PUB132

Low Activities of Daily Living Related to Be Unstable on Hemodynamicsduring Dialysis Therapy Atsushi Ueda,[1] Aki Hirayama,[2] Kei Nagai,[3] Chie Saito,[3] Kunihiro Yamagata.[3] *[1]Nephrology, Hitachi General Hospital, Hitachi, Japan; [2]Integrated Medicine, Tsukuba Univ of Technology, Tsukuba, Japan; [3]Nephrology, Univ of Tsukuba, Tsukuba, Japan.*

Background: In hemodialysis (HD) patients, hemodynamics is unstable during dialysis therapy. Improvements of activities of daily living (ADL) may affect beneficial effects on hemodynamics during the HD therapy. We investigated the change of blood pressure during HD session and laboratory data divided into ADL levels.

Methods: We employed retrospectively 47 HD patients treated regularly. They were divided into three groups. 1)Wheelchair group (n=9); in need ofawheelchairwhen moving, 2) DM group; diabetes mellitus and a free standing ADL (n=16), 3) NDM; non-diabetes and a free standing ADL (n=22).a) Systolic blood pressure at the start of dialysis, b) lowest systolic blood pressure during dialysis, c) systolic blood pressure difference between a) -b) were measured on recent dialysis session. Daily urinary volume and laboratory data were measured between at the time of initial dialysis therapy and after one year respectively.

Results: There were significant differences in NDM group (22.5±14.6 mmHg) and the wheelchair group (69.6±35.3) on the systolic blood pressure difference c). However, there were no significant differences on ultrafiltration volume. The urine volume in the wheelchair group was dramatically decreased one year after the initiation of dialysis. Serum albumin in the wheelchair group was significantly decreased at initial dialysis period, however the difference had disappeared after one year. As for serum creatinine, there was no difference among the three groups in the introduction of dialysis therapy, however that in the wheelchair group was significantly decreased (8.2 ± 2.4 mg/dL) compared to that of the NDM groups (11.5 ± 2.9) one year after the initiation.

Conclusions: The wheelchair group with reduced ADL decreased markedly in systolic blood pressure during the dialysis session. Despite of this group showed an improvement of serum albumin level after one year of dialysis, serum creatinine was reduced. This suggests that a decrease in motor function leads to a decrease in muscle mass, which leads to blood pressure regulation declined during dialysis.

PUB133

Iron Status and Inflammation in Early Stages of Chronic Kidney Disease Jolanta Malyszko, Ewelina Lukaszyk. *2nd Dept Nephrology, Medical Univ, Bialystok, Poland.*

Background: One of the potential mechanisms of anaemia in chronic kidney disease is functional iron deficiency, where iron availability for erythropoiesis is reduced despite normal iron resources in the body. Iron status is regulated by number of factors including inflammation, hepcidin production, dietary intake etc. Due to subclinical inflammation accompanying chronic kidney disease there is a need to look for pathomechanisms responsible for developing this kind of anaemia, which may contribute to improve the effectiveness and rationalization of aneamia treatment in this population. The aim of the study was to assess iron status in patients in early stages of chronic kidney disease, prevalence of anaemia in this population, iron correlation with inflammation parameters and others potential iron concentration regulators.

Methods: The study group consisted of 80 patients with the diagnosis of early stage chronic kidney disease in 46% of cases. We assessed plasma and serum levels of following parameters: creatinine, urea, haemoglobin, iron concentration, transferrin saturation (TSAT), ferritin, high sensitive C-reactive protein (CRP), fibrinogen, hepcidin, hemojuvelin, GDF-15, interleukin-6, sTfR and performed statistical analysis.

Results: In over 42% of cases in patients in early stages of chronic renal disease iron concentration was below normal values. In the half of this cases iron deficiency was classified as functional. Iron deficiency (absolute and functional) was more often associated with increased fibrinogen concentration (p=0,02) and CRP concentration (p=0,001). We also revealed that concentration of GDF-15 (p=0,029) and sTfR (p=0,001) is higher in patients with functional iron deficiency. However, there was no statistically significant difference in the level of hepcidin, hemojuvelin and interleukin-6 level in groups with and without iron deficiency.

Conclusions: Iron deficiency may parallel deterioration in kidney function. Moreover, functional iron deficiency is associated with inflammation, which is reflected by elevated CRP and fibrinogen hence it requires the evaluation for inflammation before treatment.

Funding: Government Support - Non-U.S.

PUB134

Stenosis Diameter Predicts Outcome after Intervention for Renal Artery Stenosis Neeraj Kohli, Wladyslaw M. Gedroyc, Neill D. Duncan, Damien Ashby. *West London Renal and Transplant Centre, Hammersmith Hospital, London, United Kingdom.*

Background: Renovascular disease is increasingly recognised as a cause of renal impairment but the role of endovascular intervention in this setting is unclear. Recent multi-centre trials have established that revascularisation does not improve renal prognosis in the average patient, but many clinicians believe that there is a subgroup of patients who benefit.

Methods: In this retrospective single-centre cohort study we included all patients who had renal angioplasty during a 2 year period for whom 12 month follow-up data were available. Patient outcomes were defined according to change in creatinine in the 3 years post intervention, with ordinal outcome scores assigned: 1= improved by 20umol/l; 2= no change; 3= declined by 20-100umol/l; 4= declined by more than 100umol/l, died or started dialysis during follow-up.

Results: Sixty-two patients (aged 51-85, 74% male) underwent angioplasty which was bilateral or to a single functioning kidney in 33% of cases. Outcome scores 1 to 4 were seen in 12.9, 43.5, 24.2 and 19.4% of patients. Absolute stenosis diameter increased across outcome group (mean 1.2, 2.2, 2.4 and 2.4mm) with stenosis diameter significantly correlated with outcome group (R=0.324, p=0.010). Percentage stenosis diameter was not related to outcome. Outcome score 1 was seen in 24% of patients with diameter less than 2mm, and only 5% of patients with diameter over 2mm (p=0.067).

Conclusions: Heterogeneity of outcome can be expected after intervention for renal artery stenosis. Patients benefitting from the procedure have tighter absolute stenosis diameter.

PUB135

Appetite, Body Mass Index and Energy Intake in Predialysis Patients Anita Saxena,[1] Amit Gupta,[1] Anil Bhalla,[2] Georgi Abraham.[3] *[1]Nephrology, SGPGIMS, Lucknow, Uttar Pradesh, India; [2]Nephrology, Sir Ganga Ram Hospital, New Delhi, UT, India; [3]Nephrology, Madras Medical Mission, Chenai, Tamil Nadu, India.*

Background: Dietary intake and nutritional status of CKD patients on first visit to a nephrology center was assessed to evaluate when malnutrition sets in.

Methods: Three days dietary recall of 141 patients in CKD stage 3 and 4 (GFR <60ml/min1.73 m^2) was taken.

Results: Serum albumin was 2.87± 2.10 and 3.56±0.58; total serum protein and proteinuria was 2+ in males and females respectively. Out of 140 patients, 74 (52.8%) had normal BMI, 21 (15%) were underweight, 8 (5.71%) were severely underweight and 38 (27.1%) overweight. Weight (p 0.000), appetite (0.004), dietary intake, fluid intake (0.001), energy (0.004) carbohydrate (0.003), potassium (0.019), phosphorus (0.036) and iron (0.011) between four BMI groups was significantly different. Energy intake based on BMI was 19.4 ±7.92/18.6±13.5 in normals, in underweight 14.5±11.0 and 12.46±10.6, in severely underweight 16.2±14.9 and 18.4±6.5 and in overweight 19.4±8.8 and 18.9±6.0 kcal/kg/d in males andfemales. In normals protein intake was 0.73 ±0.31 and 0.65±0.35; in underweight 0.55±0.63 and 0.42.±0.56 and, in severely underweight 0.57±.53 and 0.87±.31, overweight 0.62±.21 and 0.67±.18. Appetite significantly correlated (p 0.000) with weight, BMI, dietary energy protein, fat, serum albumin and creatinine. BMI was significantly correlated (0.000) with dietary energy, protein and carbohydrate, creatinine and serum total protein and proteinuria. Based on appetite energy and protein intake in male patients with normal appetite was 29.9±4.6 and 1.0±.38, with average 20.7±3,9 and 0.79±.2; poor 18.8±6.4 and 0.69±0.24 and anorexic in 0.39±0.36 and 9.9±6.1 respectively. In females energy and protein intake in patients with normal appetite was 31.3 ±8.2 and .97±.16, in average 26.3 ±8.6 and .94±.49, in poor 17.6±4.9 and 0.69 ±0.17 and in anorexic12.2±5 and 0.64±0.45.

Conclusions: Inadequate dietary intake, body mass index (BMI) and low serum albumin and protein are markers of malnutrition in predialysis population. Appetite, BMI and albuminuria are associated with low energy intake and have synergistic effect on low serum and protein levels in CKD stage 3 and 4 patients.

PUB136

HA Clinical Study of the Efficacy of YiShenXieZhuo Decoction in Patients with Chronic Kidney Disease and Hyperuricemia Lin Wang,[1] Pan Gan,[2] Shenfu You,[1] Xianwen Zhang,[1] Yueyi Deng,[1] Niansong Wang,[3] Min Yin,[4] Lanping Du,[1] Yiping Chen.[1] *[1]Longhua Hospital, Shanghai, China; [2]Shanghai Univ of TCM, China; [3]Sixth People's Hospital, Shanghai Jiao Tong Univ, China; [4]Xuhui District Central Hospital, China.*

Background: Hyperuricemia (HUA) is associated with the development and progression of chronic kidney disease (CKD). YiShenXieZhuo Decoction (YS) is a Chinese herbal medicine prescription used to treat chronic kidney failure accompanied by HUA in Long Hua Hospital for decades. To evaluate the efficacy of YS, we conducted retrospective cohort and prospective cohort studies.

Methods: In the retrospective study, 66 patients were treated with YS only and 49 patients were treated with YS plus allopurinol. Serum creatinine (SCr), estimated glomerular filtration rate (eGFR), uric acid (UA), and blood urea nitrogen (BUN) levels were measured and compared in these two groups 1-year. 2-years and 3-years after treatment. The decrease in eGFR-slope was subjected to hazard function analysis. In the prospective cohort study, fifty patients were treated with YS (treatment group, N = 27) or allopurinol (control group, N = 25) for 12 weeks. The SCr, eGFR, UA and BUN of the two groups were measured and compared.

Results: In the retrospective cohort study, both treatment groups showed significantly decreased SCr levels and increased eGFR levels at all points evaluated, compared to baseline. There was no difference in the UA levels of the two groups at one year. eGFR-slope of both groups increased significantly after 1 year of treatment and slightly more in the following 2 years. However, the hazard ratios of eGFR-slope decrease of the two groups were not statistically different. In the prospective cohort study, patients in the treatment group had a significantly lower SCr and UA levels and improved eGFR, compared to the control group. The UA levels of the control group decreased significantly, but the SCr and eGFR levels did not improve.

Conclusions: YS significantly improved the renal function of CKD patients with HUA. The effect of YS was not mediated by its UA lowering properties. Allopurinol lowered serum UA levels but did not improve renal function.

PUB137

The Need for a Dedicated Nephro-Oncological Evaluation of Nephrectomized Cancer Patients Receiving or Not Active Oncological Treatment Laura Cosmai,[1] Camillo Porta,[2] Marina Foramitti,[1] Wanda Liguigli,[3] Fabio Malberti.[1] *[1]Nephrology, Istituti Ospitalieri, Cremona, Italy; [2]Medical Oncology, IRCC San Matteo Hospital Foundation, Pavia, Italy; [3]Medical Oncology, Istituti Ospitalieri, Cremona, Italy.*

Background: nephrectomized cancer pts have an increased risk of developing chronic kidney disease (CKD); such a risk is higher in those under active Oncological treatment (aTx) due to its possible renal toxicity. Our limited knowledge of these complications and the data suggesting that the survival of kidney donors is greater than that of non-donors, has led us to follow, in a dedicated outpatient Onco-Nephrology Ambulatory, nephrectomized cancer pts with normal kidney function but with concomitant risk factors and nephrectomized cancer pts with CKD; furthermore, pts were either under aTx or just followed-up.

Methods: To date, 127 pts have been referred to us; 51 had stage I-III CKD, 32 stage IV CKD (17 being under aTx) and 23 stage V CKD (13 being on aTx). Pts were evaluated every 3 months for 1 year after nephrectomy if renal function was normal or in case of stage I-III CKD, and then every 6 months (for both followed-up and treated pts); pts with stage IV and V CKD were seen every 30-60 days.

Results: In stage I-III CKD pts no cases of CKD progression were recorded, but 1 case of reversible AKI was observed, together with 2 cases of proteinuria from aTx (which was stopped). Of stage IV CKD pts, 1 developed acute kidney injury (AKI) after heart failure induced by cancer therapy (which was stopped), while 2 others had a worsening of their CKD with the need for aTx dose adjustment. Finally, among stage IV CKD pts, 2 started dialysis treatment, but continued aTx, and 2 more had a reversible episode of AKI due to dehydration.

Conclusions: The role of the Nephrologist in the management of nephrectomized cancer pts, either under aTx or not, is key, potentially leading to relevant benefits: reduced progression of pre-existing CKD, reduced risk of developing de novo CKD, continuation of life-prolonging aTx even in pts with severe CKD or receiving dialysis. Further development of Onco-Nephrology (including dedicated ambulatories) is warranted.

PUB138

Allopurinol Dosing in Stage 4-5 Chronic Kidney Disease Betzaida Rodriguez,[1] Lindsey Norris,[1] Bela Alex Kosztaczky,[2] Zsolt Lengvarszky,[3] Mohit Agarwal,[1] Tibor Fulop.[1] *[1]Dept of Medicine, Univ of Mississippi, Jackson, MS; [2]Dept of Medicine, Danville VAMC, Danville, IL; [3]Dept of Mathematics, Louisiana State Univ, Shreveport, LA.*

Background: Current dosing guidelines recommend major dose reduction of allopurinol in advanced (stage 4-5) Chronic Kidney Disease (CKD). To achieve target uric acid levels, clinician may have to use larger than customary doses in the context of race, obesity and certain dietary habits of the current era.

Methods: We retrospectively reviewed our CKD clinic patients over the last 3 years, where information on both uric acid level and allopurinol dosing were simultaneously available. We collected data on multiple clinical and laboratory parameters for 83 consecutive CKD clinic patients with stage 4-5 CKD (race-adjusted MDRD estimated Glomerular Filtration Rate (eGFR) < 30 mL/min/1.73m^2; 22.9% receiving renal replacement therapy), representing a local urban cohort from the Southeast U.S. Data were analyzed with SPSS v19 and expressed with means (SD) and percent (%).

Results: Mean age of the cohort was 60.6 (12.1) years, weight 93.1 (22) kg with and BMI 33.7 (8.6) kg/m2; 57.8% was female, 77.1% African-American, 49.4% had diabetes and 53% had the diagnosis of gout. Aspirin was used in 51.8% of participants. Mean race-adjusted MDRD eGFR was 21.7 (6.5) mL/min/1.73m^2 [date purged for dialysis patients]. Overall metabolic control was acceptable: potassium 4.2 (0.5) mEq/L; HCO3 24.2 (3.6) mEq/L, phosphorus 4.3 (1.4) mg/dL, hemoglobin 10.8 (1.8) g/dL, albumin of 4.1 (1.2) g/dL; white blood cell count 7.3 (2.3) x1000/mm^3. Mean allopurinol dose was 164 (83) mg/day [median: 100; 25-75%: 100-200 mg/day]; serum uric acid 7.6 (2.5) mg/dL in the entire cohort. Uric acid did not correlate with allopurinol dose, weight, BMI, race or MDRD eGFR in this cohort of patients.

Conclusions: Conventional dosing recommendations for allopurinol dosing in advanced CKD are unlikely to suffice to reach target serum uric acid goals. In our cohort, larger-than-usual allopurinol doses were well tolerated without hematologic side effects.

PUB139

Association of PreS1-Ag, HBeAg, HBV-DNA in the Serum of Patients with Hepatitis B and Chronic Kidney Disease Chenggang Shi, Xun Liu, Cailian Cheng, Qiong-Li Yin, Mei Li, Weizhao Mo. *Dept of Nephrology, the Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: To investigate the relationship between the HBV antigens and hepatitis B and chronic kidney disease (CKD).

Methods: Baseline data, biochemical data and serum virological indicators were analyzed retrospectively between hepatitis B with CKD group and without chronic kidney disease group.

Results: 178 patients were enrolled as the CKD group, and 156 patients as the control group (without CKD). Age, liver functional indicators, renal functional indicators, incidence of liver cirrhosis and serum albumin had significant differences. The percentage of high HBV-DNA copy (>1×10^{5}IU/ml)(P=0.001), the positive rates of HBeAg(P<0.001) had significant differences between the two groups. The positive rates of PreS1 were high both two group(69.1% in experimental group, 65.4% in control group, P=0.54), but not significant differences between the two groups.

Conclusions: The percentage of high HBV-DNA copy (>1×10^{5}IU/ml)and the positive rates of HBeAg is higher of the patients with hepatitis B and kidney injury, and high positive rates of PreS1 of patients with hepatic lesion or kidney injury. The percentage of high HBV-DNA copy(>1×10^{5}IU/ml)and the positive rates of HBeAg tend to the occurrence of chronic kidney disease.

Funding: Government Support - Non-U.S.

PUB140

Predialysis Patients Intramuscular Hepatitis B Vaccination Response Andre F. Charest, Nimesh Patel, Bharat Nathoo. *Medicine/Div Nephrology, Mackenzie Health Hospital, Richmond Hill, ON, Canada.*

Background: Seroconversion rate to Hepatitis B virus (HBV) vaccination remains low in dialysis patients.It is unclear if HBV vaccination in earlier stages of chronic kidney disease would affect response rate. We conducted a retrospective study to determine which clinical factors predict seroconversion, and effects of booster dose on antibody (Ab) titres.

Methods: Patients were screened for previous HBV vaccination and infection. Patients (n = 153) received a 4-dose Intramuscular (IM) vaccination at 0, 1, 2, and 6 months with 40 mcg recombinant Engerix-B IM and a subset received a single IM booster when Ab levels were <100 IU/L. Ab titres were measured a month after and *responders* were grouped—**low** (10-99 IU/L), **good** (100-999 IU/L), **excellent** (>1000 IU/L).

Results: Clinical factors and eGFR values were compared between *responders* (>10 IU/L) and *nonresponders* (<10 IU/L). Seroconversion occurred in 75%. *Nonresponders* were significantly older (76.8±8.8 versus 68.2 ± 12.8 y;p<0.001) and had greater eGFR (19.7 ±5.6 versus 17.3±5.5 ml/min/1.73m^2;p=0.03) at vaccination. After controlling for age (OR: 0.92 (95CI: 0.88-0.97)) in a multivariate logistical regression, only eGFR at vaccination significantly contributed to seroconversion (OR: 0.92 (95CI: 0.86-0.99)). A subset(n=37) had repeat Ab titres with over 60% of patients still immune after 60 months. Baseline factors did not significantly predict loss of immunity. Ninety percent of patients(**n=19**) receiving a booster dose achieved greater peak Ab titres. Of those with initial **low** titres, 62.5% (5/8) improved to **good** response levels, while 63.6% (7/11) with good initial titres improved to excellent response levels and 36.4% (4/11) remaining at good titres.

Conclusions: We found that older age decreases seroconversion rate.However, higher eGFR did not associate with better outcomes when adjusting for age. All but two patients that received a booster dose achieved greater Ab titres. As higher antibody titres correlate with longer duration of immunity, it is expected that patients with excellent response levels to a booster dose would likely be immune at dialysis initiation. More studies are required with larger cohort.

Funding: Clinical Revenue Support

PUB141

Effect of Tolvaptan on Patients with Chronic Kidney Disease due to Diabetic Nephropathy with Heart Failure Eiichi Sato,[1] Tsukasa Nakamura,[2] Mayuko Amaha,[2] Yoshihiko Ueda.[1] *[1]Dept of Pathology, Dokkyo Medical Univ, Koshigaya Hospital, Koshigaya, Saitama Prefecture, Japan; [2]Div of Nephrology, Dept of Internal Medicine, Shinmatsudo Central General Hospital, Matsudo, Chiba Prefecture, Japan.*

Background: The efficacy of tolvaptan for treating heart failure has already been shown. Adequate data relating to the effect of tolvaptan on the correlation of water balance in renal disease are not available. A retrospective study was conducted on the efficacy and adverse reactions of tolvaptan for treating nephrotic syndrome.

Methods: The subjects of this study were 26 patients with chronic kidney failure of diabetic nephropathy with heart failure who were administered tolvaptan and seen between December 2011 and October 2013. The endpoints were urinary outputs, physical findings, and blood analyses. The expression of aquaporin-2 in the collecting duct which is related to the action of tolvaptan was investigated by immunohistochemistry using the kidney tissues obtained for the diagnosis.

Results: Responders were seen in 19 of the patients. In the histopathological investigation there was severe glomerulosclerosis in patients with diabetic nephropathy, but the responders were noticeable in showing mild tubulointerstitial damage. Non-responders exhibited profound tubulointerstitial damage. The expression of aquaporin-2 was conducted in 8 patients, of which 7 were responders and tested positive for aquaporin-2. The remaining one case was a non-responder and showed noexpression of aquaporin-2.

Conclusions: Tolvaptan is considered effective for some cases of nephrotic syndrome. There are no clear parameters for predicting an effect, but the present study showed that aquaporin-2 was expressed in the epithelial cells of collecting duct of tolvaptan responders.

PUB142

N-Terminal Pro-Brain Natriuretic Peptide and Troponin T Are Linked to Cardiac Dysfunction in Pre-Dialytic and Dialytic Stages of Chronic Kidney Disease Belda Dursun,[1] Hilmi Dogu,[1] Dogu Kilic,[2] Halil Tanriverdi,[2] Rota Simin.[3] *[1]Nephrology, Pamukkale Univ Medical School, Denizli, Turkey; [2]Cardiology, Pamukkale Univ Medical School, Denizli, Turkey; [3]Biochemistry, Pamukkale Univ Medical School, Denizli, Turkey.*

Background: Left ventricular systolic dysfunction, left ventricular hypertrophy and left atrial/ventricular dilatation are determinants of cardiovascular events. Aim to assess the relationship between echocardiographically determined cardiac dysfunction and N-terminal pro-brain natriuretic peptide (NT-proBNP) as well as cardiac troponin T as cardiac-derived biomarkers in pre-dialytic and dialytic chronic kidney disease (CKD) patients.

Methods: 50 nondiabetic patients stage 3-5 CKD, 49 hemodialysis patients and 50 controls were enrolled. Serum NT-pro BNP was measured by ELISA assay. Cardiac troponin T was measured by immunoassay. Left ventricular ejection fraction, left ventricular mass index (LVMI) and volume diameters, left atrial (LA) diameter were echocardiographically measured.

Results: NT-proBNP (pg/ml) was higher in dialysis (1069±763, p=0.000) and predialysis (632±748) patients than controls (379±722, p=0,000). Troponin T (ng/ml) was higher in dialysis (0.024±0.045, p=0.000) and predialysis patients (0.005±0.019, p=0.002) than controls (0). LA volume index (ml/m^2) was higher in dialysis (23.7±7.9, p=0.000) and predialysis patients (21±8.3, p=0,016) than controls (17.4±5.5). LV volume index w(ml/m^2) was higher in dialysis (72.3±16) and predialysis patients (67±13.5; p=0.000) than controls (60.5±14.4, p=0.028). LVMI (g/m^2) was higher in dialysis (132.4±43, p=0.000) and predialysis patients (111.9±42.3, p= 0.003) than controls (87.5±33.4). In predialysis patients, NT-proBNP levels showed positive correlations with LV volume index, while Troponin T levels showed positive correlations with LVMI, LV volume index. In dialysis patients, NT-proBNP levels showed negative correlations with LA volume index, whereas Troponin T levels showed positive correlations with LA diameter, LVMI.

Conclusions: Increased levels of NT-proBNP, and Troponin T levels in both predialytic and dialytic stages of CKD are related to cardiac dysfunction, and these biomarkers may have prognostic value in these patients.

PUB143

Relationship Between Levels of Urinary Full-Length Megalin and Histological Findings in Patients with IgA Nephropathy Takuto Seki,[1,2] Katsuhiko Asanuma,[2,1] Rin Asao,[1] Kanae Nonaka,[1,2] Fumiko Kodama,[1] Yu Sasaki,[1] Miyuki Takagi,[1] Hiroyuki Kurosawa,[3] Yoshiaki Hirayama,[3] Satoshi Horikoshi,[1] Akihiko Saito,[4] Yasuhiko Tomino.[1] *[1]Div of Nephrology, Juntendo Univ Faculty of Medicine, Japan; [2]TMK Project, Medical Innovation Center, Kyoto Univ Graduates School of Medicine, Japan; [3]Reagents Development Dept, Denka Seiken Co. Ltd, Japan; [4]Dept of Applied Molecular Medicine, Niigata Univ Graduate School of Medicine and Dental Sciences, Japan.*

Background: Megalin is highly expressed at the apical membranes of proximal tubular cells. Urinary full-length megalin (C-megalin) assay is linked to the severity of type2 diabetic nephropathy. It is still unknown whether urinary C-megalin is associated with histological findings in IgA nephropathy (IgAN) patients. In this study, we examined the relationship between urinary levels of C-megalin and histological findings in IgAN.

Methods: Urine samples voided in the morning on the day of renal biopsy were obtained from 73 adult patients with IgAN (29 men and 44 women). All renal biopsy specimens were analyzed histologically. Pathologic variables of IgAN were analyzed by the Oxford classification of IgAN and Shigematsu classification. Levels of urinary C-megalin were measured by sandwich ELISA.

Results: Histological analysis based on the Oxford classification revealed that the levels of urinary C-megalin were correlated with tubular atrophy and interstitial fibrosis in IgAN patients without reduced eGFR, but not in all patients. There was a significant correlation between levels of urinary C-megalin and severity of chronic extracapillary abnormalities according to Shigematsu in all patients group and patients without reduced eGFR group.

Conclusions: It appears that the levels of urinary C-megalin are associated with histological abnormalities in adults IgAN patients.

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PUB144

Audit of Influenza and Pneumococcal Vaccination in Haemodialysis Patients Qurat Tak, Ajay Prabhakar Dhaygude. *Nephrology, Royal Preston Hospital, Preston, United Kingdom.*

Background: Haemodialysis (HD) is associated with significantly increased mortality, cardio-vascular aetiology being the leading cause. According to U.S. and UK data infection is the second leading cause of death in the dialysis population[1,2]. Respiratory infections including pneumonia is the commonest source and is potentially preventable with vaccinations. The National Health Service offers free Influenza and Pneumococcal Vaccination to patients with chronic kidney disease and patients are actively encouraged and primary care physicians are educated about this. Influenza vaccine is offered every winter

and the Pneumococcal Vaccine should be administered once every five years. In this study we assessed the compliance of our HD patients with the Influenza and Pneumococcal Vaccine.

Methods: We contacted primary care physicians of all HD patients to ascertain vaccination uptake of all prevalent HD patients.

Results: In total there were 115 HD patients. Information was available for 95 patients. Results are presented in Table. Pneumococcal vaccine in last 5 years Pneumococcal vaccine >5 years ago Pneumococcal vaccine declined by pts Influenza vaccine given this season Influenza vaccine given>12 months ago Vaccine declined 15/95 (16%) 15/95 (16%) 6/95 (6.0%) 55/95 (58%) 5/95 (5%) 6/95 (6.0%).

Conclusions: These results suggests

· Pneumococcal vaccine uptake remains poor in HD patients

· Influenza vaccine uptake is better but there is room for further improvement.

Discussion: Improving patients and primary care physicians education is crucial to reduce morbidity and mortality in HD patients of these preventable diseases.

References:

(1) United States renal Data System www.usrds.org

(2) Renal Registry Report 2012 www.renalreg.com/Reports/2012.

PUB145

Serum sFas Levels Identify Chronic Kidney Disease Patients at High Risk for Red Blood Cell Transfusion

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Background: Soluble Fas (sFas) levels are increased and associated with anemia in chronic kidney disease (CKD) patients. **Objective:** To evaluate the prognostic value of serum sFas levels for red blood cells (RBC) transfusion in CKD patients.

Methods: A prospective observational cohort study was conducted over 10-year period. A total of 52 CKD patients (32 male) were followed up until year 10 or death. Hemoglobin concentration (Hb), iron status, glomerular filtration rate (GFR), serum levels of sFas and IL-6 were performed at baseline. We observed the necessity of RBC transfusion from baseline. We perform comparisons between the RBC transfusion (group1) and non-RBC transfusion. Binary logistic regression and ROC curve in the group 1 to evaluate the prognostic value.

Results: Seven patients (3F:4M; 52 ±12 years) required RBC transfusion and 45 patients non-RBC transfusion (57±12 years; p=0,34). At baseline we observed that group1 had lower Hb concentration at baseline (11.1±1.5, 12.8±2.2 g/dl; p=0.04) and GFR (25±10, 34±10 ml/min; p=0.04). We observed higher sFas levels (4885±1261, 2847±938;p<0.001) and transferrin saturation(28±11, 22±4%; p=0.02) in group1. We did not observe any difference in serum ferritin and IL-6 levels between the two groups. Serum sFas was an independent predictor of the need for RBC transfusion in binary logistic regression (95%CI=1.003, 1.000-1.005; p=0.03). ROC curve analysis ascertained that the threshold value of sFas with the highest sensibilty/specificity was 3888, with 85.7% and 89%, respectively (AUC 0.937; p<0.001).

Conclusions: These results suggest that initial serum sFas levels could be helpful in identifying CKD patients at high risk of RBC transfusion.

PUB146

Clinical Outcomes of IgA Nephropathy Patients with Different Proportions Crescents

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Background: Several clinical characteristics and pathologic parameters have been established to be predictors of renal progression in patients with IgA nephropathy (IgAN), but the prognostic significance of extracapillary proliferation in IgAN is still controversial. We aimed to investigate the effect of different proportions of crescents on the outcome of IgAN patients.

Methods: A retrospective cohort enrolled 565 primary IgAN patients presenting crescents on renal biopsy, and registered in IgAN database of The First Affiliated Hospital of Sun Yet-sen University (http://igan.medidata.cn) from January, 2000 to December, 2011. The endpoint was defined as a composite of (1) doubling of baseline serum creatinine, (2) ESRD (maintenance hemodialysis/ maintenance peritoneal dialysis/renal transplantation), or (3) death.

Results: After a median follow-up time of 44 months (range 12 to 154 months), 84 patients (14.9%) developed the adverse outcomes. The 5-year, 10-year survival rates of IgAN patients with crescents was 83.2%, 53.3% respectively. Crescent proportion (increase by 5%: HR =1.12, 95%CI 1.00-1.24, P=0.048), impaired renal function at baseline (eGFR<60 ml/min per 1.73 m^2: HR=5.27, 95%CI 2.22-12.53, P<0.001), hypertension (HR=2.15, 95%CI 1.13-4.09, P=0.019), proteinuria (HR=1.46, 95%CI 1.18-1.81, P= 0.001), endocapillary hypercellularity (HR=2.22, 95%CI 1.09-4.51, P=0.028), and segmental sclerosis (increase by every 5%: HR =1.25, 95%CI 1.11-1.40, P<0.001) were revealed to be independent predictors of adverse outcomes in multivariate Cox proportional hazard model.

Conclusions: Every 5% increment in crescent proportion carried additional 12% risk of progression in IgAN, which confirmed the unfavorable predictive value of crescent for IgAN patients.

Funding: Government Support - Non-U.S.

PUB147

Mortality and Causes of Death in Patients with the Syndrome of Rapid Onset End Stage Renal Disease versus "Classic" ESRD in a Mayo Clinic Northwestern Wisconsin Hemodialysis Unit

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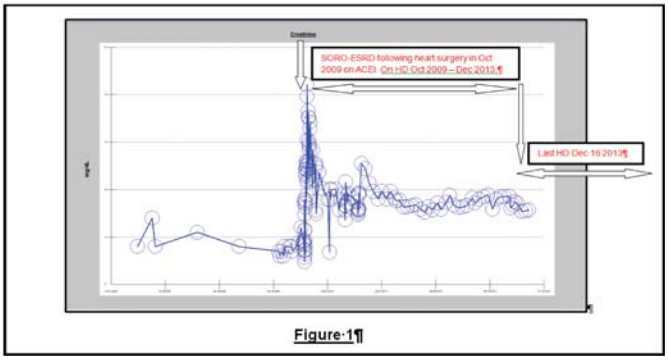
Background: We first described the syndrome of rapid onset end stage renal disease (SORO-ESRD) in 2010. This is acute precipitate yet irreversible ESRD after AKI versus "classic" ESRD where CKD-ESRD progression was progressive and predictable. SORO-ESRD accounts for ~30% of incident U.S. adult ESRD but its impact on ESRD mortality is unknown.

Methods: A retrospective three-year interval analysis of mortality in patients with SORO-ESRD versus "classic" ESRD.

Results: Twenty-two of 31 (71%) SORO-ESRD patients versus 42 of 60 (70%) "classic" ESRD had died. Other findings are shown in table including common causes of death.

Characteristic Examined	SORO-ESRD	"Classic" ESRD
Total (n)	31	60
Number dead (%)	22 (71%)	42 (70%)
Number alive (%)	9 (29%0	18 (30%)
Number remaining on RRT (%)	5 (16%)	12 (20%)
Number that came off RRT (%)	3 (10%)	2 (3%)
Number with new kidney transplant (%)	1 (3%)	4 (7%)
Death from failure to thrive (%)	3 (10%)	7 (12%)
Death from sudden death/cardiac arrythmia (%)	3 (10%)	6 (10%)
Death from septic shock (%)	3 (10%)	5 (8%)
Death from pneumonia (%)	1 (3%)	3 (5%)
Death from respiratory failure (%)	-	3 (5%)
Death from COPD (%)	1 (3%)	2 (3%)



Figure·1¶

Conclusions: We demonstrated no mortality difference between SORO-ESRD and "classic" ESRD after another three years follow up. More patients came off HD in SORO-ESRD versus "classic" ESRD, p=0.16, NS. More "classic" ESRD patients were transplanted, p=0.44, NS. The causes of death were similar with sudden cardiac death due to arrhythmias and septic shock being the leading causes. Larger studies are warranted.

PUB148

Serum Phosphorous and Anemia in Patients with Stage 4-5 CKD

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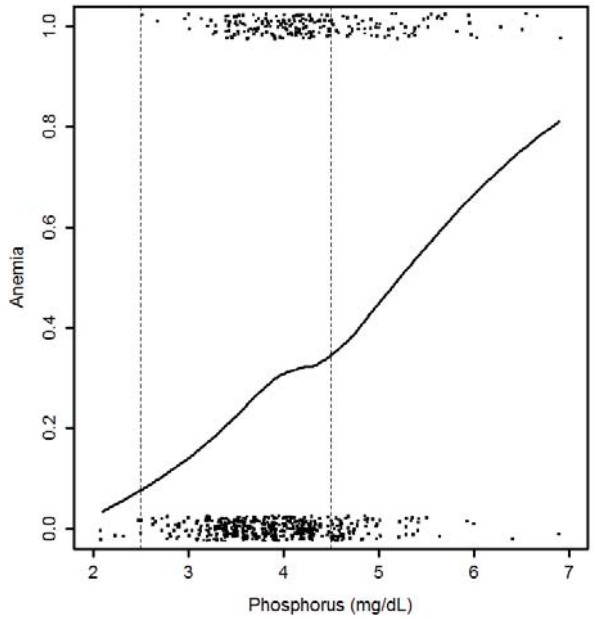
Background: Hyperphosphatemia and anemia are common and associated with adverse outcomes in maintenance dialysis patients. Among patients with CKD not on dialysis, erythropoiesis diminishes as CKD stage increases, and serum phosphorus typically increases. We aimed to examine hemoglobin and serum phosphorus levels in later-stage CKD patients aged 20-64, and to examine serum ferritin levels in a subset of these patients.

Methods: We used MarketScan data to identify patients with CKD stages 4-5, Oct 2009-Sep 2010, using ICD-9 codes and calculating eGFR for those with lab data available. A further subset had both phosphorus and hemoglobin measurements available. Anemia was defined in these patients as hemoglobin < 11 g/dL, and the phosphorus normal range as 2.5-4.5 mg/dL. A final subset included patients whose serum ferritin values were also available.

Results: We identified 661 CKD stage 4-5 patients with phosphorus and hemoglobin levels available for analysis, and 246 with serum ferritin values also available. Mean age was 55 years; 20% of patients had phosphorus levels above the upper limit of normal (4.5 mg/dL), and 30% had hemoglobin levels < 11 g/dL. The figure, displaying phosphorus levels (x-axis) and the probability of anemia (y-axis), shows a monotonically increasing

relationship between phosphorus and the likelihood of anemia (c-statistic = 0.65); 45% of patients with hyperphosphatemia were anemic. For patients with available ferritin levels, average values for those below and above the phosphorus upper limit of normal were 247 and 196 ng/mL, respectively.



Conclusions: In patients with later stage CKD the linear relationship between serum phosphorus and the likelihood of anemia is apparent across the range of phosphorus levels, even within the normal range. Serum ferritin is inversely associated with serum phosphorus.

Funding: Pharmaceutical Company Support - Keryx Biopharmaceuticals, Inc.

PUB149

Prevalence of Hypertension, Proteinuria, and Nocturia in the Rural Middle Belt of Ghana Jerica Johnson,[1] Easmon Otupiri,[2] Adjase Emmanuel Teye,[3] Andrea M. Nelson,[1] Martin C. Gregory.[1] *[1]Internal Medicine, Univ of Utah, Salt Lake City, UT; [2]KNUST, Kumasi, Ghana; [3]College of Health and Well-Being, Kintampo, Ghana.*

Background: There are few studies on the prevalence of chronic kidney disease (CKD) in rural Ghana even though this is an increasingly recognized health problem in sub-Saharan Africa. However, there is limited access to laboratories where serum creatinine can be measured to make a definitive diagnosis of CKD. This favors the use of less expensive screening measures, such as evaluation of hypertension (HTN), proteinuria, and nocturia. The primary aim of this cross-sectional study was to determine the prevalence of HTN, proteinuria, and nocturia in rural Ashanti and Brong-Ahafo areas in Ghana.

Methods: Community health nurses administered surveys to collect demographic information and relevant health information in 695 participants. Blood pressure measurements and dipstick urinalyses were collected for all participants. Outcome measures were defined by: HTN (SBP≥140 or DBP≥90 for ages 18 years and older, ≥95th percentile by age and gender for ages 17 and younger); proteinuria (≥2+); and nocturia (≥2 awakenings per night).

Results: Overall prevalence of HTN was 25%. Prevalence of HTN was 9.2% in ages 7-18, 12.4% in ages 19-39, and 40% in ages 40-60. The overall prevalence of proteinuria was 6.8%. Prevalence of proteinuria was 8.7% in ages 7-18, 6.4% in ages 19-39, and 6.2% in ages 40-60. However, there was no difference in age between those with and those without proteinuria (p=0.38). Systolic blood pressure was significantly higher in those with proteinuria (p=0.013). The overall prevalence of nocturia was 63%. Prevalence of nocturia was 46% in ages 7-18, 66% in ages 19-39, and 76% in ages 40-60. There was no association between proteinuria and nocturia (p=0.65).

Conclusions: Our study showed higher than anticipated rates of HTN, proteinuria, and nocturia. The high prevalence of these outcome measures as well as the youth of those affected justifies further study of renal disease in rural Ghana. Screening for HTN, proteinuria, and nocturia may be used in the community for early detection of renal disease.

PUB150

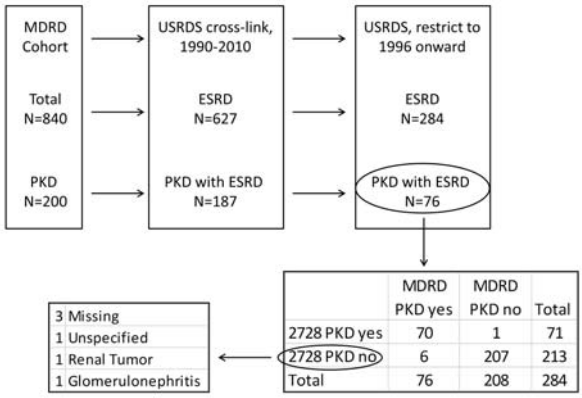
Does the USRDS National Registry Accurately Reflect the Burden of PKD as a Cause of ESRD? Meyeon Park,[1] Barbara A. Grimes,[1] Elaine Ku,[1] Kirsten L. Johansen,[1] Mark J. Sarnak,[2] Chi-Yuan Hsu.[1] *[1]UCSF; [2]Tufts.*

Background: Polycystic kidney disease (PKD) is an important cause of ESRD. Whether or not the U.S. Renal Data System (USRDS) national registry accurately captures the burden of PKD as a cause of ESRD, based on Medical Evidence Report (2728 form), is unknown. Prior studies have shown that the 2728 form may be inaccurate for diagnosing glomerulonephritis and insensitive for capturing comorbid conditions.

Methods: The Modification of Diet in Renal Disease (MDRD) study is an NIH-sponsored clinical trial that enrolled ~25% of participants with PKD as the underlying cause of CKD. We cross-linked the MDRD dataset with USRDS (data complete through December 2010). We analyzed data of patients who developed ESRD from January 1, 1996 onward, as the current 2728 form was implemented in 1995. We assessed the test characteristics of a diagnosis of "Polycystic kidneys, adult type (dominant)" on the 2728 form relative to the gold-standard diagnosis of PKD from MDRD. We used "glomerulonephritis" (GN) as a comparison group.

Results: Out of 840 individuals in MDRD, 627 developed ESRD by the end of 2010 (Fig). Of the 76 with PKD and ESRD, 70 had "Polycystic kidneys, adult type (dominant)" listed as etiology of ESRD on the 2728 form (sensitivity 92.1%, PPV 98.6%). The 6 patients who had PKD based on MDRD that was not captured by 2728 form had primarily missing diagnoses (Fig). Compared to a diagnosis of GN, sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, and kappa were all higher for PKD (Table).

Figure. Derivation of study population.



2728 Form Diagnosis	Sensitivity	Specificity	PPV	NPV	Kappa
"Polycystic kidneys, adult type (dominant)"	92.1%	99.5%	98.6%	97.2%	0.94 (0.89-0.98)
"Glomerulonephritis"	31.4%	91.4%	61.4%	75.4%	0.27 (0.15-0.38)

Conclusions: Although there may be an under-estimation of about 5-10%, the USRDS national registry captures the burden of ESRD due to PKD relatively well.

Funding: NIDDK Support

PUB151

Bioelectrical Impedance as Non Invasive Screening Tool for Preventing Radionuclear Contrast Nephropathy in Chronic Kidney Disease Anita Saxena,[1] Amit Gupta,[1] Georgi Abraham,[2] Anil Bhalla.[3] *[1]Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India; [2]Nephrology, Madras Medical Mission, Chenai, Uttar Pradesh, Iraq; [3]Nephrology, Sir Ganga Ram Hospital, New Delhi, Union Territory, India.*

Background: Radio contrast nephropathy (RCN) is 5-10% in diabetic kidney disease compared to 3% in chronic kidney disease. To evaluate use of bioelectrical impedance (BIA) as a screening tool for kidney disease in general population using creatinine clearance (Crcl) and glomerular filtration rate (GFR).

Methods: Cross-sectional screening study conducted on 52 subjects from general population for presence of CKD. Maltron BIOSCAN derived GFR was caliberated with ^{99m}Tc-DTPA derived GFR (95.0ml/min p 0.001%), a comparative study done on voluntary kidney donors. There was no significant difference between GFR derived from two methods. Hume *et al 's* equations were used to check BIA accuracy in estimating total body water (TBW). There was no significant difference between two methods (33.7 ± 6.6 L and 34.8 ±6.18 L 0.189, p 0.001%).

Results: Mean serum creatinine for males was 0.94 ± 0.14 and for females was 0.91± 0.84 mg% and BIA derived Crcl was 97.3 ± 28.98 and 107.6 ± 34.0 ml/min. Based on BIA derived GFR (male 74.1±25.9 female 65.17±21.14 ml/min), subjects were classified into CKD stages. 15.5% were in CKD stage 1, 44.2% in stage 2, 34.6% in stage 3, 1.9% in stage 4 and 1.9% in stage 5. Microalbuminuria was present in 7.0 %. 36% had normal blood pressure, 15.5% were in prehypertension stage, 30.7% were in stage 1 hypertension, 11.5% were in stage 2 hypertension and 3.8% were in crisis hypertension.

Conclusions: All subjects screened had CKD but they were not symptomatic. CKD has a latent period during which the disease is present but is asymptomatic. Screening for CKD identifies people with renal injury so that early intervention can prevent morbidity and delay progression to end stage renal disease. GFR is index of kidney function. BIA can estimate GFR without use of radionuclear agents. If BIA instrument is validated against radionuclear method, it is a handy and inexpensive tool for measuring GFR and screening CKD in general population and preventing RCN.

PUB152

Comorbidity and Outcomes in Chronic Kidney Disease Simon D.S. Fraser,[1] Maarten W. Taal,[2] Paul J. Roderick.[1] *[1]Academic Unit of Primary Care and Population Sciences, Southampton Univ, United Kingdom; [2]Div of Medical Sciences and Graduate Entry Medicine, Nottingham Univ, United Kingdom.*

Background: CKD is often considered in isolation but commonly occurs in conjunction with other chronic conditions. Little information exists on the prognostic implication of multiple comorbidities in CKD. We aimed to investigate the association of degree of comorbidity with several key outcomes in a large cohort of people with CKD.

Methods: A retrospective cohort of people with eGFR <60ml/min/1.73m^2 on 2 occasions was identified from a routine data repository. Comorbidities included hypertension, diabetes, ischemic heart disease, heart failure, peripheral vascular disease, cerebrovascular disease, hypothyroidism, liver disease, and chronic anti-inflammatory use (reflecting musculoskeletal conditions).Descriptive statistics, Poisson and Cox regression models were used to compare those with CKD+2 or more comorbidities with those with CKD only or CKD+1 and their emergency room (ER) attendance, emergency hospital admission, new renal replacement therapy (RRT), acute kidney injury (AKI) and mortality.

Results: 24,021 people with CKD were identified. Mean follow up 5.3 years. Mean age was 77±11 years and baseline eGFR 47±12ml/min/1.73m^2. Prevalence of isolated CKD,CKD+1,CKD+2 and CKD+3 comorbidities were 13%, 36%, 30% and 21% respectively. During follow up 37% of those with CKD+3 died compared to 27% of those with CKD+2 and 22% of those with CKD only or CKD+1 (p<0.001).CKD+2 or more comorbidities independently predicted ER attendance, hospital admission, new RRT and mortality

Outcome	Univariate		Multivariable*	
	RR	p	RR	p
Attend ER	1.20 (1.16-1.24)	<0.001	1.13 (1.09-1.17)	<0.001
Emergency admission	1.37 (1.24-1.52)	<0.001	1.27 (1.15-1.41)	<0.001
RRT	1.69 (1.33-2.15)	<0.001	2.06 (1.60-2.67)	<0.001
AKI	1.44 (1.11-1.88)	0.01	1.29 (0.99-1.70)	0.06
Mortality	1.53 (1.45-1.60)	<0.001	1.27 (1.20-1.33)	<0.001

*adjusting for age, sex, deprivation index, baseline eGFR

Conclusions: Isolated CKD was rare in this elderly population. Greater comorbidity was associated with several adverse outcomes. Further research should focus on managing disease burden and reducing risk across multiple comorbidities in people with CKD.

PUB153

Inflammatory Marker MRP8/14 Is Correlated with Body Mass Index and Modulates Disease Progression in Japanese Patients with CKD Tatsuki Matsumoto,[1] Yoshinori Taniguchi,[1] Kazu Hamada,[1] Yoshiko Shimamura,[1] Kosuke Inoue,[1] Koji Ogata,[1] Taro Horino,[1] Kenji Yuasa,[2] Shinpei Fujimoto,[1] Yoshio Terada.[1] *[1]Kochi Univ, Nankoku, Japan; [2]Kochi-Takasu Hospital, Kochi, Japan.*

Background: Inflammatory marker, Myeloid-Related Protein 8/14 complex (MRP8/14), is an endogenic ligand of toll-like receptor (TLR)-4. Although it has been reported that MRP8/14 related to arteriosclerosis and coronary lesion in type 2 diabetes, there are few reports about the relationship between MRP8/14 and chronic kidney disease (CKD). We studied the association between MRP8/14 levels and several parameters in CKD.

Methods: A total of 432 patients (mean age 60±17) with CKD were enrolledand a longitudinal follow-up study for 12 months was performed. Serum samples were collected, and MRP8/14 levels were measured by using ELISA kit. Serum creatinine (Cr), urine protein/Cr ratio, and the other parameters were also measured. This study was approved by Kochi Medical School review board. All patients provided written informed consent.

Results: MRP8/14 levels were positively associated with serum Cr (p=0.007, r=0.135), BUN (p<0.001, r=0.175), UA (p=0.011, r=0.127) levels, urinary protein/Cr ratio (p<0.001, r=0.212) and especially Body Mass Index (BMI) (p<0.001, r=0.189), and were inversely associated with eGFR p=0.006, r=-0.137). Moreover, MRP8/14 levels in CKD patients with diabetes and hypertension were significantly increased (p<0.05), compared to patients without diabetes and hypertension. Stepwise multiple regression analysis showed that MRP8/14 levels correlated well with BMI. During the study period of 12 months observation, MRP8/14 levels showed significantly inverse correlation to the decline rate of eGFR for 12 months in sub-group with BMI >25% (p<0.05), although not in all patients.

Conclusions: Serum MRP8/14 positively correlated with BMI in Japanese patients with CKD, and especially in BMI >25% group, is critical for disease progression in CKD.

PUB154

IgA Nephropathy with Nephrotic Syndrome in Children Yuko Shima,[1] Koichi Nakanishi,[1] Taketsugu Hama,[1] Masashi Sato,[1] Hironobu Mukaiyama,[1] Hiroko Togawa,[1] Hiroshi Kaito,[2] Kandai Nozu,[2] Ryojiro Tanaka,[3] Kazumoto Iijima,[2] Norishige Yoshikawa.[1] *[1]Pediatrics, Wakayana Medical Univ., Wakayama, Japan; [2]Pediatrics, Kobe Univ. Graduate School of Medicine, Kobe, Hyogo, Japan; [3]Pediatric Nephrology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Hyogo, Japan.*

Background: Nephrotic syndrome (NS) is rare in IgA Nephropathy (IgAN) in children. Clinical characteristics and long term outcomes of IgAN with NS have not been fully clarified yet.

Methods: We analyzed retrospectively consecutive 427 children. There were 31 IgAN children (7.3%) with NS (NS-IgAN) at onset. We compared clinical and pathological findings between 31 patients with NS-IgAN and the remained 396 patients to clarify the characteristics of NS-IgAN in children.

Results: Onset modes of 31 children with NS-IgAN were annual school screening (9), gross hematuria (5), acute nephritic syndrome (AN, 1), NS alone (10), and AN and NS (6). Logistic analyses showed that male (OR: 3.1, P=.006), gross hematuria (OR: 3.2. P=.004), duration from onset to renal biopsy (1.5 versus 8.4M, P=.001), mesangial hypercellularity score (1.3 versus 0.6, P<.0001), ratios of endocapillary hypercellularity (27.3 versus 3.9%, P<.0001), tubular atrophy/interstitial fibrosis (5.2 versus 3.1, P=.01) nd crescents (12.7 versus 4.3%, P=.0001) were significantly related to NS. Twenty-three children of NS-IgAN (74%) were treated with PSL or combination therapy including PSL and immunosuppressant. Mean observation period was 6.2±3.2 yrs. Complete remission (CR) occurred in 22 (71%) patients. All patients with CR maintained remission during the observation period. Three cases (9.7%) reached chronic renal failure (eGFR<60) at the latest observation. All 3 cases were treated with PSL or combination therapy. They demonstrated heavy proteinuria after their 2-year initial treatments. The Kaplan-Meier analysis showed that the cases with NS-IgAN demonstrated significantly lower renal survival curve than the rest (P=.04). Cox analysis showed that NS was a significant factor for renal failure with adjusting by PD (HR: 5.6 [95%CI 1.2-18.9], P=.03).

Conclusions: Patients with NS-IgAN who were refractory to treatments showed poor renal survival. On the other hand, once they achieved CR, they had favorable outcome.

PUB155

The Incidental Relationship of Microalbuminuria with the Degree of NAFLD in Non-Diabetic Korean Men Dong-Young Lee,[1] Beom Kim,[1] Kyoung Hyoub Moon,[1] Sung Keun Park,[2,3] Sangyoon Lee.[4] *[1]Internal Medicine, Veterans Health Service Medical Center, Seoul, Korea; [2]Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan Univ, Seoul, Korea; [3]Depts of Preventive Medicine, School of Medicine, Kyung Hee Univ, Seoul, Korea; [4]Dept of Occupational and Environmental Medicine, Haeundae Paik Hospital, Inje Univ, Busan, Korea.*

Background: Epidemiological researches have demonstrated that risk of microalbuminuria was significantly associated with nonalcoholic fatty liver disease (NAFLD) in diabetic population. However, there is only limited information about this association in non-diabetic population. Thus, this study was conducted to investigate the incidental relationship of microalbuminuria with the degree of NAFLD in non-diabetic Korean men.

Methods: A prospective cohort study was conducted on 2,855 non-diabetic Korean men without microalbuminuria in 2005. They were categorized into 3 groups by the degree of NAFLD (normal, mild, moderate-to-severe), and followed-up from 2005 to 2010. The incidences of microalbuminuria were evaluated, and Cox proportional hazard model was used to measure the hazard ratios (HRs) for the development of microalbuminuria according to the degree of NAFLD.

Results: Total 123 cases of microalbuminuria (4.3%) developed during 5 year follow-up. Participants with microalbuminuria were older with higher BMIs, BP measurements, lipid profiles, and insulin resistance than those without microalbuminuria. In the unadjusted model, HR for microalbuminuria was 1.69 in mild NAFLD and 4.98 in moderate to severe NAFLD group (P<0.001). These associations were attenuated but remained statistically significant after adjusting for epidemiologic and chemistry characteristics (mild NAFLD: 1.03, moderate to severe NAFLD 2.61, P= 0.002).

. Hazard ratios for microalbuminuria in each NAFLD category

	HR (95% Confidence Interval)	
	Unadjusted	Model 1
NAFLD		
Normal	1.00 (reference)	
Mild	1.69 (1.15 - 2.47)	1.03 (0.66 - 1.61)
Moderate to severe	4.98 (2.57 - 9.68)	2.61 (1.20 - 5.71)
Age		1.03 (1.01 -1.05)
BMI		1.03 (0.96 - 1.12)
HOMA-IR		1.56 (1.33 -1.82)

Conclusions: The degree of NAFLD is independently associated with the development of microalbuminuria in healthy Korean men.

PUB156

Incidence of Biopsy-Defined Glomerulonephritis in Northern Iraq
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Background: The incidence of specific types of glomerular diseases can be analyzed by renal biopsies obtained from a population of known size and age distribution. Ratios relative to membranous glomerulonephritis (MGN) have been used to evaluate whether IgA nephropathy (IGAN), focal segmental glomerulosclerosis (FSGS), and lupus nephritis (LN) vary among different ethnic and racial groups or change over time. In the Middle-East, there is little data on the frequency of specific types of renal disease except their relative proportions in biopsy series. This study estimates the incidence of subtypes of glomerulonephritis (GN) in a Middle-East region with active nephrology services and a well characterized population.

Methods: In 2012-2013, all renal biopsies performed in the Kurdish region of Northern Iraq were studied by light and immunofluorescence microscopy. Of 744 native renal biopsies, 561 were satisfactory for a specific diagnosis. The 2011 update of the World Health Organization (WHO) 2002 regional census was used to determine the biopsy rate and WHO world standardized age and gender adjusted annual incidence rates per 100,000 population for MN, FSGS, IGAN, LN, and all GN (without minimal change disease).

Results: Biopsies of native kidneys were performed on 369 females and 370 males. The annual biopsy rate was 7.59/100,000. For specific diagnoses, the number of cases (2 years), the male to female ratio, and the age and gender adjusted annual incidence rates were as follows: MGN (n=69, 1:1.2, 1.01), FSGS (n=121, 1.2:1, 1.49), IGAN (n=26, 1.9:1, 0.31), LN (n=51, 1:5.3, 0.50) and all GN (n=349, 1:1, 4.47).

Conclusions: With biopsy rates that are somewhat, but not exceedingly low, compared to the U.S., the incidence of MN and LN is similar to that seen in European Americans (EA) but without the marked male predominance for MN. FSGS well exceeds any other type of glomerulonephritis at an incidence and proportion of biopsies seen in EA populations after the 1990s. The rate of IGAN is very low, findings previously reported in nearby Arab countries, but distinctly different from most populations of European ancestry. This may reflect regional biopsy practices or a low epidemiologic risk for IGAN.

PUB157

Visceral Adiposity Index, Hypertriglyceridemic Waist Phenotype, and Risk of Chronic Kidney Disease
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Background: To explore the relationships between visceral adiposity index(VAI), hypertriglyceridemic waist phenotype (HW phenotype) and chronic kidney disease(CKD).

Methods: A cross-sectional study was conducted in Zhuhai city from June to October 2012. Participants aged 18 years and older (n=2142) were recruited. The relationship between VAI, HW phenotype and CKD were determined. Logistic regression was used to evaluate the associations between VAI, HW phenotype and CKD.

Results: After adjustment for age, VAI was significantly associated with CKD (OR 2.16, 95% CI 1.23 to 3.74, P=0.006, comparing the highest to the lowest quartile) in female subgroup. But this association was not seen in male(OR1.72, 95% CI 0.95 to 3.13, P=0.08). Further adjusted for potential confounders, the association was still significant in female subgroup(OR 1.91, 95% CI 1.06 to 3.41, P=0.03, comparing the highest to the lowest quartile). The association was abolished when adding diabetes and hypertension to the model(OR 1.68, 95% CI 0.91 to 3.10, P=0.096). The age-adjusted OR (95% CI, P) of CKD associated with HW phenotype was 2.21 (1.29 to 3.76, 0.004) and 2.54 (1.53 to 4.22, <0.001) for men and women, respectively. Further adjusted for potential confounders, the associations were still significant in female subgroup and male subgroup. The OR were 2.24 (95% CI 1.28 to 3.92, P =0.005) and 2.30 (95% CI 1.28 to 4.12, P =0.005), respectively. When further adjusted for diabetes and hypertension, the association of HW phenotype and CKD was significant (OR 1.91, 95% CI 1.05 to 3.59, P=0.035) in female subgroup. But the association between HW phenotype and CKD was abolished when diabetes and hypertension were added to the model in male subgroup(OR 1.53, 95% CI 0.82 to 2.84, P=0.66).

Conclusions: Our results suggested that both VAI and the HW phenotype were significantly associated with CKD in female. HW phenotype was significantly associated with CKD in male. But VAI was not associated with CKD in male. Compared with VAI, the HW phenotype might be a better predictor of CKD in Chinese men and women.

PUB158

In a Cohort of Older Patients with Confusion, Admission Renal Function Predicts Poor Discharge Outcomes
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Background: Delirium is often misdiagnosed as an acute confusional state in hospitalized patients. Delirium affects up to 10-20% of all hospitalized adults and is associated with adverse outcomes including decreased functional status, fall risk, prolonged hospital stay, and increased use of physical and chemical restraints. Chronic kidney disease has been suspected, but not yet linked, to risk of confusion. The purpose of this study was to explore the relationship between estimated glomerular filtration rates (eGFR) among older patients with confusion, to two outcomes: 1) length of stay (LOS) and 2) discharge destination.

Methods: We examined all clinical records of inpatients at a large tertiary teaching hospital in the southwestern U.S., who had any diagnosis reflecting confusion during calendar year 2011. Demographic information, serum creatinine, LOS, and discharge destination were collected. Data were analyzed using ANOVA to explore the relationship of eGFR and LOS and Spearman's rank correlation coefficient to describe the relationship between eGFR and discharge destination.

Results: We identified 1292 cases out of 37,850 admissions; 670 in patients age 65 or older. Mean age was 79, +8.9, range 65-110 years. Mean creatinine was 1.63 (+ 1.65) and ranged from 0.4-20 mg/dL. Mean eGFR was 52 (+ 24.5) ml/min/1.73 m2 and ranged from 2-112. Mean LOS was 10.6 days, +8.9, range 1-76 days. The sample was 71% Caucasian, 22% black, and 4% Hispanic. LOS was significantly related to eGFR (F=1.52, df=47, p<.017). Lower eGFR was associated with worse discharge destination (extended care or death) (r=.119, p<.002).

Conclusions: Older hospitalized patients with any diagnosis of confusion who had lower eGFR were more likely to have longer LOS, not be discharged home, or die during hospitalization.

PUB159

Effect of Serum Phosphorus Level on Progression of Kidney Disease: A Systematic Review and Meta-Analysis
Xinfang Xie,[1] Jing Jing Da,[1,2] Myles S. Wolf,[3] Sinee Disthabanchong,[4] Jinwei Wang,[1] Jicheng Lv,[1] Luxia Zhang,[1] Hai Yan Wang.[1] *[1]Renal Div, Dept of Medicine, Peking Univ First Hospital, Beijing, China; [2]Renal Div, Dept of Medicine, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China; [3]Div of Nephrology and Hypertension, Dept of Medicine and Div of Biostatistics, Dept of Epidemiology and Public Health, Univ of Miami Miller School of Medicine, Miami, FL; [4]Div of Nephrology, Dept of Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand.*

Background: Recent studies indicated that phosphorus might have an independent pathogenic role in the progression of chronic kidney disease (CKD), but some of those studies were underpowered and yielded inconsistent results.

Methods: Studies assessing the risk ratio of serum phosphorus on kidney failure and mortality for patients with non-dialysis dependent chronic kidney disease published between January 1950 and March 2013 were included following systematic searching of the MEDLINE, EMBASE databases and the Cochrane Library.

Results: Twelve cohorts with 25,546 patients, 1,442 (8.8%) of patients developed kidney failure and 3,089 (13.6%) patients died. Overall, every 1 mg/dl increase of serum phosphorus was independently associated with an increased risk of kidney failure (relative risk, 1.36 [95% confidence interval [CI], 1.22-1.51]) and mortality (relative risk, 1.20 [95% CI, 1.08-1.33]). The risk of kidney failure increased gradually with blood level of phosphorus, starting from 3.87 mg/dl.

Conclusions: This is a first meta-analysis reveals an independent association between serum levels of phosphorus and kidney failure and mortality among non-dialysis-dependent CKD patients, and suggests that large-scale, randomized controlled trials should target disordered phosphorus homeostasis in CKD.

Funding: Government Support - Non-U.S.

PUB160

Post-Operative Complications for Patients Treated by Hemodialysis Undergoing Orthopedic Procedures
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Background: The in-hospital course for patients with chronic kidney disease (CKD) treated by hemodialysis (HD) undergoing orthopedic procedures documents morbidity up to 100% and mortality up to 28.5%; our patients experience superior outcomes.

Methods: We identified 60 patients between 2004-2008 and reviewed electronic records for age, sex, HD vintage, cause of CKD, comorbidities, and complications. Comparisons were performed using Chi-square test or t-test.

Results: Patients undergoing hip (39) or knee (21) surgery experienced 15 post-operative complications; the most common were tachycardia (6.7%) and altered mental status (6.7%). HD vintage averaged 5.9 +/- 8.5 years. Causes of CKD included hypertension (23.3%), collagen vascular disease (18.3%), and diabetes mellitus (15.0%). Common comorbidities were hypertension (75.0%), anemia (31.7%), valvular disease (20.0%), depression (11.7%), CHF (10.0%), and pulmonary circulation disorders (10.0%). Many had multiple comorbidities (23.3% had Charlson comorbidity index of 2, 36.7% 3, 16.7% 4+). Complication rates did not vary with Charlson comorbidity index. Patients experiencing complications had lower pre-operative hemoglobin (mean 11.1 g/dL versus 12.5 g/dL, p=0.013); patients requiring blood transfusions were more likely to suffer complications (80.0% versus 37.8%, p=0.007). Patients with complications had longer hospital courses (mean 12.1 days versus 7.8 days, p=0.044). There were no in-hospital deaths.

Conclusions: Our results do not corroborate the high morbidity and mortality rates reported for similar populations even among patients with the highest comorbidity scores. We found statistically significant relationships between lower pre-operative hemoglobin values, need for blood transfusion, and complication rates. More study is warranted to guide the management of peri-operative anemia to improve outcomes.

PUB161

Chronic Hemodialysis in Far Rockaway, New York Heino R. Anto, Yan Dorneich, Volodymyr Chornyy, Tatyana Tolchinsky. *Dept of Medicine, Saint John's Episcopal Hospital, New York, NY.*

Background: The U.S. Census Bureau reports that Far Rockaway has a population of about 60,000 people, of which 1/3 are below poverty level. In addition, approximately 25 % of the adult population, mainly 45-64 years old, are said to be without health insurance.We examined how these factors impacted on the trends of new chronic hemodialysis patients, generated through the only hospital in Far Rockaway,St. John's Episcopal Hospital (SJEH), from 2011-2013. We also examined the average lenght of stay (ALOS) between insured and non-insured patients.

Methods: 122 hospital admissions from 2011-2013 which coded for acute or chronic renal failure were reviewed.

Results: From 2011 thru 2013, fifty-eight patients started maintenance hemodialysis with a mean age of 63.4 years. Approximately 9 % had no health care coverage, while 91 % had medicaid, medicare, both medicare and medicaid, or " other " health insurance.The ALOS for non-insured was 27 days, and 18.8 days for insured patients.

Conclusions: Since most of the non-insured in the Far Rockaway area are said to be in the 45-64 years age group, we were surprised that 91 % new hemodialysis patients generated at SJEH, with a mean age of 63.4 years, had health care coverage.In addition, since 25 % of the Far Rockaway adult population is reported to be non-insured, we expected to find more non-insured in our study. This low figure for non-insured could stem from patient fear of having to pay for their own medical expenses, thereby avoiding medical attention. Furthermore, some non-insured may have left the Far Rockaway peninsula and sought medical care elsewhere. ALOS was not affected by severity of illness; since severity was similar in both insured and non-insured cases. The longer ALOS for the non-insured was likely impacted by an inability to place such patients into a for profit dialysis center because of facility reimbursement issues.

PUB162

Tenofovir Exposure Is Associated with Biomarkers of Proximal Tubular Injury in HIV-Infected Men Vasantha Jotwani,[1] Rebecca Scherzer,[1] Michelle M. Estrella,[2] Lisa P. Jacobson,[2] Michael Shlipak.[1] *[1]UCSF; [2]Johns Hopkins.*

Background: Tenofovir disoproxil fumarate (TDF) is widely prescribed for treatment of HIV infection, but has been associated with proximal tubular damage. The proximal tubular injury markers, urine interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1), may detect tubular damage among HIV-infected individuals exposed to TDF who have normal kidney function.

Methods: This cross-sectional study of 884 HIV-infected men from the Multicenter AIDS Cohort Study evaluated associations of cumulative TDF exposure with urine IL-18, KIM-1, and albumin-creatinine ratio (ACR). We used the LASSO procedure to determine which of multiple antiretroviral (ARV) agents were associated with each biomarker. Multivariable robust regression analysis was then used to model associations of the selected ARVs while controlling for traditional and HIV-related factors.

Results: Duration of TDF use was independently associated with 3-4% higher levels of IL-18 and KIM-1 per year of exposure in adjusted analyses (**Figure 1**). No other individual ARV was associated with higher levels of both IL-18 and KIM-1. TDF exposure was associated with 3.8% higher ACR per year of exposure, but the association was attenuated and no longer statistically significant after adjustment for LASSO-selected ARVs.

Figure 1: Association of cumulative duration of TDF use with biomarker levels

Model	IL-18 % Estimate[1] (95%CI)	KIM-1 % Estimate[1] (95%CI)	ACR % Estimate[1] (95%CI)
Multivariable-adjusted[2]	3.9 (1.4, 6.4)	3.0 (0.66, 5.4)	3.8 (0.56, 7.2)
Multivariable-adjusted +other ARVs[3]	3.3 (0.75, 5.8)	3.4 (1.1, 5.7)	1.4 (-2.0, 5.0)

[1]Outcomes were log-transformed for analyses and back-transformed to produce estimated % differences in biomarker levels attributable to each year of TDF exposure
[2]Adjusted for demographics, traditional kidney risk factors, and HIV-related risk factors
[3]Other LASSO-selected ARVs include zidovudine, efavirenz, lopinavir, nevirapine, and ritonavir

Conclusions: Cumulative TDF exposure is associated with proximal tubular injury markers with weaker effects on albuminuria. Specific urine markers may be required for early detection of TDF-associated nephrotoxicity.

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PUB163

Retinopathy Is a Better Predictor for Renal Outcomes Than Echocardiographic Parameters in Patients with Chronic Kidney Disease Hyeon Seok Hwang,[1] Se Young Kim,[1] Yoon-Kyung Chang,[1] Chul Woo Yang,[2] Suk Young Kim,[1] Hye Eun Yoon.[2] *[1]Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Daejeon, Korea; [2]Div of Nephrology, Dept of Internal Medicine, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

Background: Retinopathy and cardiac abnormalities are prevalent in chronic kidney disease (CKD) patients. Echocardiographic parameters are known to be associated with progression of CKD. However, the clinical impact of retinopathy on renal outcomes is less clear in CKD patients.

Methods: Four hundred and two patients with nondialysis-dependent CKD stage 3-5 were included, who were evaluated with presence of retinopathy and echocardiography. We investigated whether retinopathy was predictive for CKD progression and whether retinopathy is more predictive for renal outcome than echocardiographic parameters.

Results: Retinopathy was observed in 181 CKD patients (45.0%). The rate of estimated glomerular filtration rate (eGFR) decline was significantly faster in patients with retinopathy compared with those without retinopathy (-6.6 ± 10.0 ml/min/1.73m^2/year versus -3.5 ± 9.6 ml/min/1.73m^2/year; P = 0.001). Patients with retinopathy showed a lower dialysis-free survival rate than those without retinopathy (P = 0.02). The echocardiographic parameters about systolic and diastolic dysfunction did not differ between patients with and without retinopathy. The presence of retinopathy, lower ejection fraction and higher left ventricular mass index was associated with rate of eGFR decline. In a multivariate analysis, retinopathy was an independent predictor for renal function decline (β = -2.23, P = 0.047), but ejection fraction and left ventricular mass index did not show significant associations with rate of eGFR decline.

Conclusions: Retinopathy is independently associated with renal outcomes in patients with CKD, and the examination of retinopathy is more useful than echocardiopraphic parameters to identify high-risk patients for CKD progression.

PUB164

Clinical Features and Renal Outcomes of Elderly Chronic Kidney Disease Patients – A Cohort Study From a Single Chinese Center Yu Wang, Hui Zhao, Luxia Zhang, Fang Wang, Xiaomei Li, Liqiang Meng. *Nephrology, Renal Div, Dept of Med., Peking Univ First Hospital, Beijing, China.*

Background: Despite the high prevalence of chronic kidney disease (CKD) among the elderly, few studies have described clinical features and renal progression of elderly CKD patients under routine follow-up. In this study, the data from CKD clinic of Peking University 1st Hospital was analyzed to determine the clinical features and renal outcomes of the CKD in the elderly.

Methods: Patients aged 65 or older with baseline CKD stage G1-G4 transferred to our CKD clinic from June 2006 to February 2014 and followed up for more than 3 months were retrospectively studied. Estimated glomerular filtration rate (eGFR) was measured by MDRD formula modified for Chinese. Progression of renal disease was defined as the decline in eGFR categories accompanied by a 25% or greater drop in eGFR from baseline, and/or a doubling of serum creatinine and/or initiation of renal replacement therapy.

Results: A total of 172 patients (85 men, mean age 73.8 years) were enrolled. Compared to those young- and middle-aged CKD patients recruited in the CKD clinic, the elderly patients were more likely to have early presence of anemia and hypophosphatemia. Forty-nine elderly patients (28.4%) had progression of their renal function during an average study period (22.5 months). Compared with the non-progressive patients, those progressed had a lower baseline eGFR (30.1 versus 42.0ml/min/1.73m^2, p<0.001), and a higher percentage of A3 category of proteinuria (63.3% *versus* 35.8%, p=0.004). A higher systolic blood pressure (142.3±11.8 *versus* 135.1±12.6mmHg, p=0.001) and phosphate level (4.06±0.65 *versus* 3.50±0.44mg/dl, p<0.001) on treatment were also observed. Multivariate Cox regression showed that baseline eGFR (HR 0.962, 95% CI: 0.930~0.995), proteinuria (HR 1.630, 95% CI: 1.041~2.552) and systolic blood pressure on treatment (HR 1.437, 95% CI: 1.076~1.919) were independent risk factors for progression of CKD, while using RASI was a protective factor (HR 0.424, 95% CI: 0.198~0.905).

Conclusions: Blood pressure control, especially systolic blood pressure and RASI usage are important to prevent renal progression among elderly CKD patients.

Funding: Government Support - Non-U.S.

PUB165

The Association between Blood Pressure and Change of Renal Function in a Community-Based Population: A Longitudinal Survey of a Nationwide Cohort in Japan Atsushi Hirayama,[1] Hiroko Sato,[1] Keita Kamei,[1] Kazunobu Ichikawa,[1] Tsuneo Konta,[1] Shouichi Fujimoto,[2] Toshiki Moriyama,[2] Kunitoshi Iseki,[2] Kunihiro Yamagata,[2] Kazuhiko Tsuruya,[2] Kenjiro Kimura,[2] Ichiei Narita,[2] Masahide Kondo,[2] Koichi Asahi,[2] Tsuyoshi Watanabe.[2] *[1]Dept of Cardiology, Pulmonology, and Nephrology, Yamagata Univ School of Medicine, Yamagata, Japan; [2]Steering Committee of Research on Design of the Comprehensive Health Care System for Chronic Kidney Disease (CKD) Based on the Individual Risk Assessment by Specific Health Checkup, Fukushima, Japan.*

Background: High blood pressure is a risk for adverse renal outcomes in chronic kidney disease. This study investigated the effect of blood pressure on renal function in a community-based population.

Methods: We used a nationwide database of 141,514 subjects (aged 29-74, male 39%), participated in an annual health check, "The Specific Health Check and Guidance in Japan" between 2008-2010, and examined the relationship between blood pressure level at baseline and 2-year change of estimated GFR obtained by the Japanese equation.

Results: The analysis of variance (ANOVA) showed the change of eGFR was inversely correlated with systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure levels (P <0.001). However, in multiple linear regression analysis with the adjustment for possible confounders, the eGFR change was inversely correlated with SBP only, not DBP and pulse pressure, and the regression coefficient of per 10 mmHg increase in SBP was –0.17 (95% confidence interval [-0.13, -0.22], P <0.001). There was a significant interaction between SBP and several parameters including age, gender, alcohol consumption and the presence of diabetes and proteinuria. In subgroups analyses the decline of eGFR (regression coefficient of per 10 mmHg increase in SBP) was especially faster in the subjects with proteinuria (-0.59), diabetes (-0.41), history of heart disease (-0.38), and elderly (-0.25).

Conclusions: This study showed that an increase in SBP is independently associated with faster eGFR decline in the Japanese community-based population and the effect of SBP on renal function might be different depending on the characters of subjects.

Funding: Government Support - Non-U.S.

PUB166

The Different Kinetics of Dickkopf-1 and Sclerostin in Japanese Patients with Chronic Kidney Disease Kazu Hamada, Yoshinori Taniguchi, Yoshiko Shimamura, Tatsuki Matsumoto, Koji Ogata, Kosuke Inoue, Taro Horino, Yoshio Terada. *Endocrinology, Metabolism and Nephrology, Kochi Univ, Nankoku, Japan.*

Background: The serum proteins Dickkopf-1 (Dkk-1) and Sclerostin are soluble inhibitors of canonical Wnt signaling. The Wnt signaling pathway may play a pivotal role in bone and vascular disturbances seen in chronic kidney disease (CKD). However, the clinical significance of Dkk-1 and Sclerostin in CKD is unclear. We studied the association between DKK-1 and Sclerostin levels and several parameters in CKD.

Methods: A total of 404 patients with CKD were enrolledand serum samples were collected, and Dkk-1 and Sclerostin levels were measured by using ELISA kit. Serum creatinine (Cr), urine protein/Cr ratio, and the other parameters including soluble prorenin receptor (s(P)RR) were also measured. This study was approved by Kochi Medical School review board. All patients provided written informed consent.

Results: Dkk-1 levels were inversely associated with serum Cr (p<0.05), BUN (p<0.05), UA (p<0.05), PTH (p<0.05) levels, and were positively associated with eGFR (p<0.05). Notably, Dkk-1 levels were significantly decreased in CKD stage 3-5 compared with stage 1-2 (p<0.05). In contrast, Sclerostin levels were positively associated with serum Cr (p<0.05), BUN (p<0.05), UA (p<0.05), PTH (p<0.05) levels, and were inversely associated with eGFR (p<0.05). Sclerostin levels were positively associated with stage of CKD (p<0.05). Sclerostin levels were positively associated with s(P)RR levels (p<0.05), known as an adaptor between Wnt receptors and the vacuolar H+-adenosine triphosphatase complex, but Dkk-1 not associated. Stepwise multiple regression analysis showed that Sclerostin levels correlated well with eGFR, but Dkk-1 not.

Conclusions: Same two soluble inhibitors of canonical Wnt signaling, Dkk-1 and sclerostin, show different kinetics in the stage of Japanese patients with CKD.

PUB167

The Utility of Diagnostic Testing Employed in the Initial Evaluation of Chronic Kidney Disease Mallika L. Mendu, Sushrut S. Waikar. *Div of Renal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.*

Background: A range of laboratory and imaging tests are available to nephrologists evaluating patients with chronic kidney disease (CKD), but their diagnostic yield is unknown. We sought to identify the most commonly ordered tests for CKD and assess their utility in a retrospective cohort study of 1019 patients.

Methods: We conducted a detailed chart review of patients referred for initial evaluation of CKD to renal clinics affiliated with two academic medical centers. Patients were excluded if they were referred for a second opinion, had an existing diagnosed etiology of CKD, or were referred for another kidney related condition.

Results: Renal ultrasound was the most commonly ordered test (70.5%) other than routine chemistries. 24% of renal ultrasounds showed abnormal findings (most commonly cortical thinning and echogenic kidneys), and 6.4% led to a specific diagnosis (most often due to renal artery stenosis or newly diagnosed atrophic kidney). Serum protein electrophoresis (SPEP) was obtained in 69.1% and led to a diagnosis of paraprotein-related kidney disease in 1.9%. ANA was obtained in 29.5% of patients, abnormal (> 1:40 titer) in 62%, but led to a new diagnosis of lupus nephritis in only 1.3%. ANCA and Anti-GBM were ordered in 12.6% and 3.8%, respectively; no patients were diagnosed with ANCA vasculitis or anti-GBM nephritis.

Conclusions: The diagnostic yield of many commonly ordered tests in the evaluation of CKD is low, and further study is needed to develop an evidence-based approach to diagnostic testing.

PUB168

Outcomes and Perspectives on the Opt-Out Recruitment Strategy in the SNORE Study Muna T. Canales,[1,2,3] Nicole Kay,[1] Areef Ishani,[4] I. David Weiner,[1,2,3] Richard Berry,[1,3] Rebecca Beyth.[1,3] *[1]Malcom-Randall VAMC; [2]Div of Nephrology; [3]Dept of Medicine, Gainesville, FL; [4]VAMC, Minneapolis, MN.*

Background: The opt-out recruitment strategy involves contact of patients by letter with follow-up phone call for non-response. This strategy is highly effective and ideal for scientifically sound and generalizable studies but has been criticized as cold-calling. To date, participant and potential participant views on this method of contact are unknown.

Methods: With IRB approval, we identified veterans age 18-89 in the NF/SGVHS (2/12/12-present) with eGFR 15-44 for enrollment in the SNORE Study, a prospective study of sleep apnea and kidney disease. Target enrollment=250 over 2y. After contacting every PCP in the NF/SGVHS for permission to contact their patients, we invited eligible veterans to participate via letter with follow-up call 3-5 days later if the patient had not responded. Letters referenced their PCP and provided a 1-800 number and email for ease of communication. Beginning 7/22/13, we asked participants and potential recruits their views of this method of by asking Q1) "How comfortable were you with our method of reaching you?" Participants were also asked Q2) "If we called you first, were you comfortable with us calling you?" Comfort was rated on a Likert scale with 10=most and 0=least comfortable.

Results: Of 161 PCPs, 143 gave approval, 2 refused, 12 wanted to be contacted for each patient, 4 did not respond. As of 5/20/14, 1,676 letters have been mailed; of these, 419 veterans called in; we contacted the remaining 1257. Of the 1676, 193 scheduled study visits, 306 were interested but not now, 326 could not be reached by phone, 851 were not interested. Of those who scheduled a study visit, 71% completed enrollment, 2% are pending study visit, 7% will reschedule, 20% cancelled. 148 potential recruits were asked Q1;146 responded with mean 9.1±2.0. 84 participants were asked and responded to Q1, mean 9.8±0.8. For Q2, 80/84 participants responded and rated their comfort with our calling them 9.6±1.4.

Conclusions: The opt-out recruitment strategy met IRB standards and has provided adequate enrollment for our study.Participants and potential recruits appear to be comfortable with this method of contact.

Funding: Veterans Administration Support

PUB169

CKD Education Classes Do Not Have an Impact on Outcomes of Dialysis Modality, Transplant or No Treatment Vikash Kumar Sinha,[2] Lumi Stutz,[1] Ying Zhou,[1] George C. Kim.[1] *[1]Div of Nephrology and Hypertension, Northshore Univ Healthsystems, Evanston, Illinois; [2]Div of Nephrology and Hypertension, Univ of Chicago, Chicago, IL.*

Background: At our institution, nurse practitioners (NP) administer chronic kidney disease (CKD) clinic that provides ESA treatment, hypertension management, bone mineral disorder management and addresses all aspects of CKD education and treatment. Medicare provides for education in Stage 4 CKD for a variety of reasons. To meet this need, we follow topic oriented lessons provided by the National Kidney Foundation by the NP's which includes education on peritoneal dialysis (PD), hemodialysis (HD), transplant and no treatment option. We attempted to determine if these classes versus standard physician and NP care have any impact on these choices.

Methods: We examined the outcomes of patients who were provided services in our chronic kidney clinics run by NPs (Clinic group) versus a Class group who took educational classes in 2012 and 2013. We examined demographics, clinical characteristics and outcomes after a minimum of 6 months follow-up. In the Class group, 47 of 78 pts reached an outcome and in the Clinic group, 54 of 170 pts reached an outcome. Outcomes were HD, PD, transplant and death without dialysis or transplant (DDT). We conducted a chi-square and t-test analysis on demographic and clinical variables. Multinomial logistic regression was used to compare among outcomes between Class and Clinic groups with adjustment of patient age.

Results: There was a difference in age between Class versus Clinic groups. There was no difference in choice of dialysis modality. When including age in the model, there was a higher likelihood that patients in the Clinic group would die versus go onto PD or transplant versus those patients who were in the Class group.

Group	OR	P value
HD vs PD	1.029 (0.995-1.063)	0.091
DDT vs PD	1.135 (1.053-1.221)	<0.001
DDT vs HD	1.013 (0.988-1.038)	0.318
DDT vs Transplant	1.206 (1.094-1.329)	<0.001

Conclusions: Dedicated education does not appear to have any impact on dialysis modality. Despite additional education on a no treatment option in the Class group, there was no increased incidence.

PUB170

Chronic Kidney Disease, Inflammation, and Risk of Mortality in Rheumatoid Arthritis Masako Kochi,[1] Kentaro Kohagura,[1] Kunitoshi Iseki,[2] Yusuke Ohya.[1] *[1]Cardiovascular Medicine, Nephrology and Neurology, Univ of the Ryukyus School of Medicine, Japan; [2]Dialysis Unit, Univ Hospital of the Ryukyus, Japan.*

Background: Rheumatoid arthritis (RA) increases the risk of mortality. The effects of chronic kidney disease (CKD) and inflammation on mortality are unknown. Thus, the aim of this study was to investigate the impact of CKD and C-reactive protein (CRP) levels on mortality in RA.

Methods: We included in our analyses 502 RA outpatients in this study. Of these patients, we selected 412 patients who could be followed more than 6 months and whose clinical data was available. We retrospectively investigated whether the presence of lower eGFR (estimated glomerular filtration rate of < 45 mL/min/1.73 m^2) and elevated CRP levels were independently associated with increased risk of mortality, and we also examined the interaction between lower eGFR and CRP for this risk.

Results: Mean age was 61 years, and 20 patients had lower eGFR. Over a median follow-up of 8 years, 40% (8/20) of those with lower eGFR and 6% (23/392) of those without lower eGFR died (p<0.0001). Either lower eGFR or CRP ≥ 0.5mg/dl were significantly associated with increased risk of mortality. Moreover, lower eGFR was further associated with increased risk of mortality when it was accompanied with elevated CRP ≥ 0.5mg/dl (adjusted hazard ratio, 15.6; 95% confidence interval, 4.4–59.1; p < 0.0001).

Conclusions: CKD had an inflammation-augmented association with increased mortality risk in RA. The impact of therapy for CKD and inflammation on mortality outcomes warrants further investigation.

PUB171

Prevalence and Misdiagnosis Analysis of Hospitalized Patients with Renal Failure Chen Yu, Wu Yitai. *Dept of Nephrology, Tongji Hospital, Tongji Univ, Shanghai, China.*

Background: To realize the prevalence of renal failure in our hospitalized patients; analysis of their morbidity and etiology constitutes; diagnosis and misdiagnosis distribution in each department in order to improve the doctor's knowledge level of renal failure in related department.

Methods: We collected the blood biochemistry results of all hospitalized patients in one year period, screened the clinical data of the patients whose serum creatinine exceeded the upper limit, and performed a restropective analysis.

Results: A total of 1402 patients had renal failure, which accounted for 3.4% of all hospitalized patients at the same term. 63.4% of them were male (n=889), 36.6% of them were female (n=513). The department distribution of renal failure patients was analyzed; 42.2% in nephrology department (n=592), 25.7% in other medicine department (n=360), 32.1% in surgery department (n=450). 26.8% of patients had acute renal failure (n=376) and 3.2% had chronic renal failure (n=1026). Misdiagnosis rates were 36.7% in medicine system and 71.2% in surgery system. The nephrology department consultation rates were 33.4% in medicine system and 20.8% in surgery system.

Conclusions: Renal failure was distributed universally in non-nephrology medical departments. The misdiagnosis rate of renal failure was very high in non-nephrology department, especially in surgery department.

PUB172

Association of Serum Adiponectin, and Leptin Levels with Chronic Kidney Disease in Asian Adults Charumathi Sabanayagam,[1,2] Boon Wee Teo,[3] Qi Chun Toh,[3] E. Shyong Tai,[3] Sunil Sethi,[4] Tien Yin Wong.[1,2] *[1]Singapore Eye Research Institute, Singapore; [2]Office of Clinical Sciences, Duke-NUS Graduate Medical School, Singapore; [3]Medicine, National Univ of Singapore, Singapore; [4]Pathology, National Univ of Singapore, Singapore.*

Background: Adiponectin and leptin, two of the key cytokines secreted by adipocytes have been shown to be associated with cardiovascular disease. However, the association of these adipocytokines with chronic kidney disease (CKD) is not clear. We examined the association of serum adiponectin and leptin levels with CKD in a population-based sample of Asian adults.

Methods: We conducted a case-control study (463 CKD cases and 936 matched controls) involving Chinese and Indian adults aged 40-80 years who participated in the Singapore Epidemiology of Eye Diseases Study (2007-2011). Controls were matched to cases on age, sex and ethnicity. CKD was defined as an estimated glomerular filtration rate <60 mL/min/1.73m^2 from serum creatinine. Serum adiponectin and leptin levels were measured using commercially available ELISA. Odds ratio of CKD associated with elevated adiponectin and leptin levels were estimated using unconditional logistic regression models adjusted for education, smoking, body mass index (BMI), diabetes, blood pressure, total and HDL cholesterol.

Results: Compared to controls, CKD cases had higher levels of adiponectin (mean [SD] = 9.2 [4.2] versus 10.4 [7.4], p=0.001) and leptin (9.6 [11.5] versus16.9 [20.1] ng/mL; p<0.0001). In multivariable models, compared to those in the lowest quartile, the OR (95% confidence interval) of CKD among those in the highest quartile were: 1.94 (1.32-2.85) for adiponectin and 6.31 (3.75-10.60) for leptin. Similar positive association was also observed when adiponectin and leptin were analyzed as continuous variables. This positive association between serum adiponectin, leptin and CKD was consistently present in subgroups of gender, ethnicity, diabetes, hypertension and overweight status (all P-interaction >0.1).

Conclusions: Higher levels of serum adiponectin and leptin were positively associated with CKD independent of traditional risk factors in this Asian population.

Funding: Government Support - Non-U.S.

PUB173

Drug Prescription in CKD Patients of Moderate Severity: Results from the German Chronic Kidney Disease (GCKD) Study Silvia Huebner,[1] Stephanie Titze,[1] Matthias Schmid,[4] Thomas Dienemann,[1,3] Anna Kottgen,[2] Kai-Uwe Eckardt.[1] *[1]Dept of Nephrology and Hypertension, Univ Erlangen-Nuernberg, Germany; [2]Div of Nephrology, Univ Medical Center Freiburg, Germany; [3]Biostatistics and Epidemiology, Perelman School of Medicine, Pennsylvania; [4]Medical Biometry, Informatics and Epidemiology, Univ of Bonn, Germany.*

Background: CKD Patients suffer from a high burden of co-morbidities, including hypertension, bone and mineral disease and diabetes, many of which are amenable to drug therapy. Polypharmacotherapy can result in a cumbersome daily pill intake, hamper compliance, and result in complex pharmacological interactions with adverse side effects. However, data on daily drug burden in pre-ESRD patients are scarce. We examined medications and prescription patterns in the GCKD Study.

Methods: At the time of enrollment into the GCKD Study patients had to be 18 to 74 years and have an eGFR of 30-60 ml/min x 1,73m^2 or an eGFR ≥ 60 in the presence of overt proteinuria. Thorough phenotyping was performed according to standardized protocols. Medication intake was assessed by patient-reporting during structured interviews and validated with medical records. Active substances were classified using ATC codes.

Results: The baseline examination was completed by 5217 patients (mean age 60 years, 40% female, 35% with diabetes, 20% with CVD history). Less than 1% of patients did not take any medication at all and the maximum number of medications was 30. Median daily intake amounted to 7.6 substances. The most commonly administered drug classes at baseline were beta blockers (55%), ACE inhibitors (52%), HMG CoA reductase inhibitors (48%) and loop diuretics (39%). The prevalence of hypertension was 95%. Despite almost ubiquitious treatment only 48% met blood pressure targets of 140/90 mmHg.

Conclusions: Patients with moderately severe stages of CKD have a high burden of daily medication raising questions about drug adherence, side effects and interactions. Despite a large number of prescriptions, treatment targets are not consistently met. Management strategies in CKD need to take into account medication burden and treatment priorities.

Funding: Government Support - Non-U.S.

PUB174

Simultaneous Evaluation of Serum Albumin and Body Mass Index Predicts Chronic Kidney Disease Progression: A Prospective Cohort Study Hiroaki Kikuchi,[1] Eiichiro Kanda,[2] Soichiro Iimori,[1] Teiichi Tamura,[3] Sei Sasaki,[1] Eisei Sohara,[1] Tomokazu Okado,[1] Tatemitsu Rai,[1] Shinichi Uchida.[1] *[1]Tokyo Medical and Dental Univ, Tokyo, Japan; [2]Tokyo Kyosai Hospital, Tokyo, Japan; [3]Yokosuka Kyosai Hospital, Kanagawa, Japan.*

Background: The relationship between nutritional status and renal function has been shown in several studies. Although serum albumin is a widely used biomarker of nutritional status, its validity to evaluate nutritional status is controversial especially in chronic kidney disease (CKD) patients with body fluid imbalance and proteinuria. We evaluated a hypothesis that low albumin level and low body mass index (BMI) were associated with CKD progression in this prospective cohort study.

Methods: 710 patients (CKD stage 2-5) were included in this study, who newly visited our facilities. They were categorized into 4 groups according to their albumin levels and BMI: group 1, low albumin level (<4 g/dL) and low BMI (<23.4); group 2, high albumin level (≥4 g/dL) and low BMI; group 3, low albumin level and high BMI (≥23.4); group4, high albumin level and high BMI. The primary outcome was 15% decline in estimated glomerular filtration rate (eGFR) within 1 year. The secondary outcome was the annual GFR decline, ΔeGFR (mL/min/1.73m^2/year).

Results: The average age and eGFR (SD) were 67.3 (13.4) years and 34.3 (17.4) ml/min/1.73 m^2, respectively. Logistic regression analysis adjusted for baseline characteristics (reference, group 4) showed that group 1 was associated with a high risk of CKD progression; adjusted odds ratio (aOR) 2.055 [95% confidence interval (CI) (1.119, 3.776)]; group2, aOR 1.64 (95% CI 0.907, 2.944); group3, aOR 1.58 (95% CI 0.853, 2.920). A multivariable regression analysis adjusted for baseline characteristics showed that significant difference in ΔeGFR was observed between group 1 and group 4 (β=-2.76, p=0.003).

Conclusions: This study suggested that CKD patients with low albumin level and BMI have a high risk of CKD progression, and that simultaneous evaluation of albumin level and BMI may be helpful for the assessment of nutritional status and prediction of CKD progression.

PUB175

Association of Metabolic Syndrome with Chronic Kidney Disease According to Menopausal Status in a Chinese Population Yongqiang Li, Hequn Zou. *Dept of Nephronology, Institute of Nephronology and Urology, The Third Affiliated Hospital of Southern Medical Univ, GuangZhou, China.*

Background: To explore the relationship between metabolic syndrome (MS) and chronic kidney disease (CKD) according to menopausal status.

Methods: A cross-sectional study was conducted in 1346 community-based women participants from June to October 2012 in Zhuhai city. The prevalence of MS (as defined by the International Diabetes Federation) and CKD (defined as an estimated glomerular filtration rate of <60 mL/min per 1.73 m^2 and/or albuminuria) was determined in premenopausal women and postmenopausal women, respectively. The association between MS and CKD was analyzed using SPSS software(version 19.0).

Results: MS was significantly associated with CKD in the unadjusted analyses as well as after adjustment for potential confounders in premenopausal women. The unadjusted odd ratio(OR) and adjusted OR for MS were 3.10 (95% CI 1.32 to 7.28, P=0.009) and 4.09 (95% CI 1.63 to 10.32, P P=0.003). When adjusted for diabetes and hypertension, there was no association between MS and CKD in premenopausal women (OR 3.25, 95% CI 0.96 to 11.02, P=0.058). MS was associated with CKD (P<0.001) in the unadjusted analyses in postmenopausal women. Further adjustment for potential confounders, MS was still significantly associated with CKD. The OR for MS was 2.60 (95% CI 1.69 to 3.99, P<0.001). When adjusted for diabetes and hypertension, the association of MS and CKD was still significant (OR 1.80, 95% CI 1.09 to 2.98, P=0.022). After adjusting for potential confounders, higher blood pressure, higher serum triglyceride level and higher fast glucose were signicently associated with CKD in postmenopausal women. The OR for elevated blood pressure, elevated serum triglyceride levels and elevated fasting glucose were 2.28 (95% CI 1.22 to 4.26, P=0.01), 1.71 (95% CI 1.03 to 2.86, P=0.039) and 2.25 (95% CI 1.36 to 3.73, P=0.002), respectively.

Conclusions: These findings suggest that MS is associated with CKD in premenopausal women and the association was dependent of diabetes or hypertension. This study also suggest that MS is associated with CKD in postmenopausal women and this association was independent of diabetes or hypertension.

PUB176

Severity of Anemia as a Predictor of Hospital Length of Stay and Readmission in Patients with Chronic Kidney Disease

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Background: Anemia is highly prevalent in patients with CKD. Descriptive studies suggest that anemia is an independent risk factor for increased hospital length of stay and readmission rate. We report the prevalence of severe anemia in hospitalized patients with CKD stage 3-5 and examine its relationship to hospital length of stay and readmission rate.

Methods: This was a retrospective cohort study of patients admitted to Maine Medical Center over 13 months with eGFR < 60mL/min/1.73 m^2 and not on dialysis. Patients with kidney transplant, acute kidney injury, GI bleeding, cancer, pregnancy, and surgery were excluded. The cohort was split into severe (hemoglobin </= 9 g/dl) versus non-severe anemia. The groups were compared for differences in continuous variables using ttests and categorical variables using chisquare tests or Fisher's exact tests. A p value of 0.05 was significant and two tailed tests were used.

Results: A total of 1,141 patients were included. Of these, 156 (13.7%) had severe anemia (mean 8.1 gm/dl, SD 0.8). Severe anemia was associated with increased hospital length of stay as compared to control; mean 6.4 (SD 6.0) days versus mean 4.5 (SD 4.0) days (P < 0.001) and not associated with increased readmission rate; mean 11.5% versus 10.2% (P = 0.7).

Characteristic	Hb< /= 9.0 gm/dl (n = 156)	Hb > 9.0 gm/dl (n = 985)	P Value
Hemoglobin (gm/dl) mean (SD)	8.1 (0.8)	11.8 (1.6)	<0.001
Age, years (SD)	72.9 (14.3)	76.7 (13.2)	0.001
Female, n (%)	91 (58.3)	566 (57.5)	0.91
Non-Caucasian, n (%)	8 (5.1)	25 (2.6)	0.12
Diabetes, n (%)	82 (52.6)	636 (64.6)	0.005
Heart Failure, n (%)	59 (37.8)	376 (38.2)	1.0
eGFR, mean (SD)	32.6 (16.7)	43.9 (13.3)	<0.001
30 day readmission rate, n (%)	18 (11.5)	100 (10.2)	0.70
Hospital length of stay in days, mean (SD)	6.4 (6.0)	4.5 (4.0)	<0.001

Conclusions: Severely anemic patients with CKD are at risk for increased hospital length of stay. Improved transfusion strategies, iron replacement, and erythropoieitin stimulating agent use, has the potential to improve hospital outcomes.

PUB177

Impact of Type of Referral and Dialysis Start on Clinical Outcomes and Final Renal Replacement Therapy in a Multicenter Integrated Care Setting

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Background: Early referral, choice of RRT modality and planned start may increase patient survival, however international reports show disparities between desirable patterns and practice. **Objectives:** To analyze the effects of Integrated Care and education on dialysis start (planned versus non-planned) and RRT modality choice.

Methods: Retrospective analysis of 547 incident patients starting dialysis in 23 HD/PD Diaverum clinics in Poland, Hungary and Romania during 2012. Scheduled initiation of dialysis with a permanent vascular or peritoneal access was considered as planned start.

Results: Population: 30% DM, mean age 64 y., 84% with previous medical care of renal disease, 49 % late referral, 58 % unplanned start, 92% on HD as modality. Half of those with unplanned start had previous Nephrology follow-up. Patients (n=332) with GFR <30 ml/min were followed up mainly by "general nephrologists" (68%) and 29% in structured predialysis units Modality information (80% of all patients) and general renal education (87%) were more frequent (p<0.001) in planned start. Half of patients were involved in therapy choice whereas informed and dialysis start consents were signed by 57% and 77%. The median time from information to dialysis start was 2 months. Unplanned start (p≤0.05) correlated with nephropathy of uncertain origin, worse clinical status, shorter time from information to RRT start and less PD use. Patient non-compliance (36%) and unexpected GFR loss (19%) contributed to unplanned start. "Optimal care" defined as combination of Nephrology follow-up (> 3 months), modality information and planned start occurred in 22% of the patients.

Conclusions: Despite the high rate of late referral, information and education were widely provided. Unplanned start was frequent and may underlie the low frequency of PD choice. Measures such as implementation of structured predialysis units may facilitate better and timely referral and improve well-being and planning of RRT start as well as increased PD use.

PUB178

Evaluation of Type of Dialysis Access for Incident Patients in HD/PD Integrated Care Clinics in Poland, Romania and Hungary

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Background: HD catheters are associated with higher morbi-mortality and cost. **Objectives:** To analyze the dialysis access at initiation of dialysis and its relationship with the predialysis referral and care.

Methods: Retrospective analysis of 547 patients starting dialysis in 23 HD/PD clinics in 2012. Early referral patients were known for ≥ 3 months in Nephrology prior to dialysis initiation. Scheduled initiation of dialysis with a permanent access was considered planned.

Results: Population: 30% diabetes, mean age 64 years, 84% with previous medical care of renal disease, 49% late referral, 80% received information about dialysis modalities, 58% unplanned start, HD: 92% as first dialysis and 89% as first chronic dialysis. No permanent access at dialysis start (p≤0.05) correlated with shorter time from information to dialysis start, higher sCr and lower at request of dialysis access, lower number of pre-dialysis visits, worse clinical status, 49% permanent HD catheter use (p<0.001) and as first chronic HD access (34%), and less PD. PD was used mainly for early referred and planned start patients.

1st Initial Dialysis Accesses Subgroups n (% vs.row)	Patients (n=547)	Permanent HD catheter (n=267)	Temporal HD catheter (n=47)	A-V Fistula (n=188)	Peritoneal catheter (n=45)	p
Early referral (ER) + Planned (P)	168 (30)	3 (2)	0 (0)	130 (78)	35 (21)	<0,001
ER + Unplanned (NP)	113 (20)	99 (87)	10 (9)	1 (1)	3 (3)	
Late referral (LR) + P	63 (12)	2 (3)	0 (0)	56 (89)	5 (8)	
LR + NP	203 (38)	163 (80)	37 (18)	1 (0,4)	2 (1)	

Conclusions: Regardless type of referral, A-V fistula was largely use for HD planned patients. By contrast, a large proportion of unplanned patients persisted with permanent HD catheters as first chronic vascular access. Adequate planning may decrease HD catheters prevalence whilst PD could be used as a therapy for urgent start patients.

PUB179

The Clinical Examination of Prognostic Factor in Nephrosclerosis

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Background: In recent years, nephrosclerosis is an important chronic kidney disease (CKD), as arteriosclerosis and population aging increase. Although hypertension is major prognosis factor, various factors such as obesity, metabolic syndrome, diabetes mellitus, and hyper uricemia can be also implicated to CKD progress. We examined prognostic factors in biopsy-proven nephrosclerosis.

Methods: We retrospectively examined 53 patients who were diagnosed with nephrosclerosis by kidney biopsy in Tokyo Woman's Medical University Hospital since May to June 2013. The clinical parameters assessed at the time of the renal biopsy were age, gender, mean arterial blood pressure (MAP), body mass index (BMI), proteinuria (grams per day), eGFR uric acid, triglyceride and total cholesterol. Subjects with the duration of follow-up less than 1 year were excluded. Prognostic variables for renal survival were examined by using the simple and multivariate Cox proportional hazards method. The renal endpoint of the present study was defined as a 50% eGFR decline or end stage kidney disease (ESKD). We also investigated the correlations between uric acid and baseline clinical data using regression analysis.

Results: In simple Cox analysis, proteinuria (Hazard ratio=2.61, p=0.0002), uric acid (Hazard ratio=1.65, p=0.007) and BMI (Hazard ratio=1.15, p=0.04) were significantly related to e-GFR decline or ESKD. A multiple regression analysis by the Cox method showed that uric acid was significantly associated with e-GFR decline or ESKD (Hazard ratio=2.72, p=0.04). Uric acid was positively correlated with proteinuria (R=0.37, p=0.007) and male (R=0.36, p=0.009) and negatively correlated with e-GFR (R=-0.28, p=0.04) in a simple regression analysis.

Conclusions: The results of this study suggest that uric acid can be clinical prognostic predictor, which has correlations with proteinuria, male and e-GFR, in patients with nephrosclerosis.

PUB180

HDL Quality and CKD Progression in Patients with Heart Failure with Preserved Ejection Fraction (HFPEF) Chantal Maureen Kopecky,[1] Marlies Antlanger,[1] Marcus Saemann,[1] Diana Bonderman.[2] *[1]Dept of Internal Medicine III, Clinical Div of Nephrology and Dialysis, Medical Univ of Vienna, Vienna, Austria; [2]Dept of Internal Medicine II, Clinical Div of Cardiology, Medical Univ of Vienna, Vienna, Austria.*

Background: Heart failure with preserved ejection fraction (HFPEF) is a complex disease with several contributing factors including systemic inflammation. Advanced disease stages are characterized by post-capillary pulmonary hypertension and chronic kidney disease (CKD), which in turn may propagate disease progression. CKD and HFPEF may share common pathophysiological pathways both indicative and causally involved in these diseases. The protein composition of high-density lipoprotein (HDL) has been demonstrated to be severely altered in high cardiovascular risk diseases including CKD. Therefore, we assessed HDL quality by analysis of two critical HDL proteins, serum amyloid A (SAA) associated with systemic inflammation and surfactant protein B (SP-B) involved in pulmonary congestion, to represent indicative clinical and diagnostic HFPEF features.

Methods: We developed a simple, laboratory assay to measure HDL-bound SAA and SP-B levels directly from serum. HFPEF patient samples were subjected to this assay at the time of clinical diagnosis. SAA and SP-B levels were correlated with functional and clinical parameters in the cohort, grouped by occurrence of cardiac events during the follow-up period of up to 3 years.

Results: HFPEF patients had profoundly increased levels of HDL-bound SAA and SP-B compared to controls. SAA(HDL) was found to correlate with kidney function and inflammation., whereas SP-B(HDL) was inversely associated with distinct pulmonary functions. Importantly, these correlations were independent of HDL-cholesterol levels and only found in patients who experienced cardiac events after inclusion.

Conclusions: Substantial enrichment of SAA and SP-B on HDL reflect distinct pathophysiological changes in HFPEF, providing potential novel biomarkers of diagnostic value. Moreover, SAA(HDL) emerges as predictor of CKD progression in this patient group, advancing our understanding of potential causal processes in HFPEF.

PUB181

Lessons Learned from Pyridorin Pilot Study to Design the Pivotal Clinical Trial Jamie P. Dwyer,[1] Kausik Umanath,[2] Bob Peterson,[3] J. Wesley Fox,[3] Julia Lewis,[1] Mohammed Sika.[1] *[1]Nephrology, Vanderbilt, Nashville, TN; [2]Nephrology, Henry Ford Hospital, Detroit, MI; [3]NephroGenex, Inc., Durham, NC.*

Background: Pyridorin™ blocks pathways in the progression of diabetic nephropathy. The Pyridorin pilot was designed to test entry criteria and outcomes. Subjects had SCr 1.3 to 3.5 mg/dL, ≥1200 PCR with a surrogate outcome of 52 wk ΔSCr. Subjects had to be on a max tolerated dose of ACE/ARB for 3 mos; stable other antihypertensives for 2 mos; stable diuretic dose for 2 wks, and a BP ≤160/90 mmHg; or enter a Pharmaco-Stabilization Phase (PSP). This pilot failed to detect an effect on ΔSCr in intent-to-treat analysis.

Methods: We queried the locked trial database.

Results: The pre-specified subgroup analyses revealed that subjects not requiring PSP and those with entry SCr <2.0mg/dL had a treatment effect associated with Pyridorin. Subjects who entered the PSP required more changes in antihypertensive medications after randomization and experienced a larger ΔSCr over 52 wks. Initiation/increase in ACE/ARB can have acute effects on GFR. Time required for these to stabilize is unknown. Our data would suggest that it is longer than 3 mos. Subjects in our pivotal trial must be on ACE/ARB for 6 mos. PSP subjects entering the pilot with BP>140/90 mmHg had no treatment effect, whereas those entering with BP ≤140/90 did. Subjects requiring frequent adjustments of antihypertensives, including diuretics, could experience changes in their SCr not related to the progression of CKD. In our pivotal trial, subjects must have a BP≤150/90 and on stable doses of antihypertensives for 26 weeks, or ≤140/90 and on stable antihypertensives for 13 wks. ΔSCr over 52-wks averages the creatinine of all subjects, many of whom have small increases or decreases of SCr related to acute events other than the progression of CKD. In our pivotal trial, our primary outcome is a time to event analysis of baseline SCr to at least a 50% increase in SCr or ESRD. This substantial change in SCr is protected from noise, is clinically relevant, and has been endorsed recently by the FDA-NKF Endpoints Conference.

Conclusions: The Pyridorin pilot provided critical information to guide the design of the pivotal trial.

Funding: Pharmaceutical Company Support - NephroGenex, Inc

PUB182

Epidemiology of Chronic Kidney Disease in an Adult Malaysian Population Muhammad Salman,[1,2] Amer Hayat Khan,[1,2] Syed Azhar Sulaieman,[2] Azreen Syazril Adnan,[1] Fauziah Jummaat,[3] Nurul Jannah Ambak,[1] Tauqeer Hussain Mallhi.[2] *[1]Chronic Kidney Disease Resource Center, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia; [2]Dept of Clinical Pharmacy, Univ Sains Malaysia, Penang, Malaysia; [3]Dept of Obstetrics and Gynecology, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.*

Background: Information concerning etiologies of chronic kidney disease (CKD) - an escalating public health problem worldwide- is scarce in Malaysia, which is prerequisite to reduce the disease burden.

Methods: A retrospective analysis of medical record data of adults (≥18 years old), hospitalized due to CKD at Hospital Universiti Sains Malaysia (HUSM), Kelantan, Malaysia between January 2009 and December 2013 was made. Data regarding socio-demographics, etiology, and stage of CKD were recorded using a standard data collection tool.

Results: A total of 851 eligible patients were recruited with male to female ratio being 1.75: 1 (541:310) and mean age of 61.18 ± 13.37 years. CKD stage 5 was accounted in 39.1% of the cases whereas stage 4, 3b, 3a, 2 and 1 cases were28.2%, 21.9%, 8.7%, 1.8% and 0.4%, respectively. The leading etiologies of CKD identified in our study population were diabetic nephropathy (DN) (44.9%), hypertension (HPT) (24.2%), obstructive uropathy (OBS) (9.2%) and glomerulonephritis (GN) (6.7%). Unknown etiology constituted 9.5% of our patients. The difference in the incidence of CKD due to DN, HPT and GN between patients ≤ 50 years old and > 50 years old was statistically significant ($P = 0.008$, $P < 0.001$ and $P < 0.001$, respectively).

Conclusions: Diabetic nephropathy is the leading cause of CKD –accounting for approximately 50% of CKD- followed by hypertension in Northeast Peninsular Malaysia.

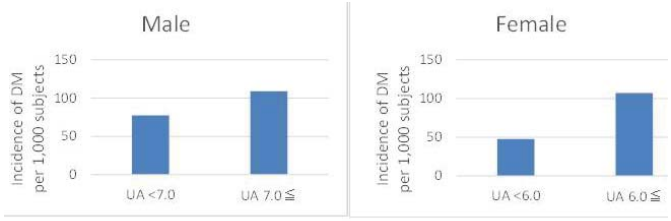
PUB183

Uric Acid as a Risk Factor of Developing Diabetes Mellitus in a Large Community Based Screening Participant Shoji Tsuneyoshi,[1] Kunitoshi Iseki.[2] *[1]Total Renal Care And Team Medicine, Univ Hospital of the Ryukyus, Nishihara, Okinawa, Japan; [2]Dialysis Unit, Univ Hospital of the Ryukyus, Nishihara, Okinawa, Japan.*

Background: Hyperuricemia is common among hypertension and diabetes mellitus (DM). However, the epidemiological studies on the effect of serum uric acid on the incidence of DM are still controversial.

Methods: We examined the subjects who participated twice at 1993 and 2003 screening by the Okinawa General Health Maintenance Association (currently Okinawa Health Promotion Foundation) in Okinawa, Japan. All of them have data both serum uric acid (UA) and fasting blood sugar (FBS) (n=12,458). Hyperuricemia wan defined by UA≥7.0mg/dL in male, and ≥6.0md/dL in female. DM was diagnosed as FBS≥126mg/dL. Adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated using age, sex, serum creatinine, obesity, hypertension, and hypertriglyceridemia, by logistic analysis.

Results: Among the subjects in 1993 survey, 453 (3.6%) was diagnosed as DM, and 12,005 (96.4%) was non DM subjects. DM developed 560 (6.0%) subjects in non hyperuricemic (n=9,329), and 290 (10.8%) subjects in hyperuricemic (n=2,676) (OR 1.26, 95% CI 1.07-1.48). In male, DM developed 298 (7.8%) subjects in non hyperuricemic (n=3,844), and 199 (10.9%) subjects in hyperuricemic (n=1,828) (OR 1.18, 95% CI 0.96-1.44). In female, DM developed 262 (4.8%) subjects in non hyperuricemic (n=5,485), and 91 (10.7%) subjects in hyperuricemic (n=848) (OR 1.44, 95% CI 1.09-1.88).



UA (mg/dL)	<7.0	7.0≦	UA (mg/dL)	<6.0	6.0≦
number	3,844	1828	number	5485	848
new DM	298	199	new DM	262	91

Conclusions: Result supports the notion of the role of hyperuricemia on the development of DM. DM is a leading cause of end-stage renal disease (ESRD). Therefore, early detection of hyperuricemia would be a useful strategy on the prevention of ESRD. Further studies are needed to investigate the effect of treatment of hyperuricemia.

PUB184

The Canadian Assessing Inflammatory Markers to Predict Events in Nephrology Study: The CAN-AIM Study Design, Rationale, and Baseline Dataset Description Tabo G. Sikaneta,[1,4] Fernando Camacho,[2] Anita Kit Seung Ng,[1] Ishwarlal Jialal,[3] George G. Wu,[1] Bharat Nathoo,[1] Paul Y. Tam.[1,4] *[1]Institute of Kidney Lifescience Technologies, Scarborough, ON, Canada; [2]Damos Incorporated, Toronto, ON, Canada; [3]Dept of Pathology and Laboratory Medicine, UC Davis Medical Center, Sacramento, CA; [4]Internal Medicine, The Scarborough Hospital, Scarborough, ON, Canada.*

Background: The increased risks of end stage kidney disease (ESRD), cardiovascular (CV) morbidity, and death in patients with chronic kidney disease (CKD) can only partly be predicted. We plan to assess the temporal relationship between serum FGF-23, high-sensitivity C-reactive protein, and these outcomes.

Methods: Planned prospective observational cohort of 2500 patients followed in three CKD clinics in Ontario for 36 months or until ESRD or death ensues. Recorded data include 6-monthly clinic evaluations with estimates of proteinuria and glomerular filtration (eGFR) rates, and medication and co-morbidity updates, yearly electrocardiograph tracings, and trans-thoracic echocardiograms at study entry and completion. Blood samples are being stored for additional inflammatory markers and for genetic analysis of undetermined

and potentially confounding genetic variables. The primary outcome is need for renal replacement therapy, with secondary outcomes being a composite of CV events and CV mortality, and all-cause mortality.

Results: Of 2530 screened and consenting patients, 2256 completed visit one, with 2164 having provided consent for DNA testing. Mean age was 69.2 (+/-12.3) years, with 66% being male, 49% Asian, 41% White, and 8% Black. A history of diabetes mellitus was documented in 48%, hypertension in 93%, and ischemic heart disease in 24%. Mean enrollment C-reactive protein was 3.58 (+/-6.87) mg/L, FGF-23 170.5 (+/-324) RU/mL, urine albumin:creatinine 50 (+/-102.9) mg/mmol, and eGFR 41.4 (+/-13.3) ml/min/1.73m^2. Enrolment blood pressures were 132.7(+/-18.9)/73.7(+/-10.4) mmHg, and body mass index 28.1 (+/-5.6).

Conclusions: The study is expected to close on December 31st 2016.

Funding: Pharmaceutical Company Support - Janssen

PUB185

The Prognostic Impact of Renin-Angiotensin Aldosterone System Blockades on Survival in Cancer Patients with Chronic Kidney Disease

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Background: Angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) are commonly prescribed to the huge number of chronic kidney disease (CKD) patients. However, recent report about the harmful effect of ACEi/ARB had raised concerns about their safety in cancer patients. Therefore, we determined the association between the antihypertensive medications and mortality in cancer patients and compared the effect on patients with and without CKD.

Methods: We retrospectively reviewed the cases of 28,047 cancer patients with one or more serum creatinine measurements from January 1, 2000 to December 31, 2013. Cox proportional-hazards regression were used to compare the overall survival of the ACEi, ARB, calcium channel blockers (CCB), beta blockers (BB), or diuretics users and non-users in patients with and without CKD.

Results: In all cancer patients, ACEi shows beneficial effect on overall survival (HR 0.82, 95% CI: 0.71-0.95, p= 0.007). While CCB and diuretics are associated with an increased mortality, ARB and BB have no significant effect on survival. In the subgroup analysis, ACEi still shows a benefit on overall survival (HR 0.77, 95% CI: 0.62-0.97, p= 0.022) only in the CKD patients. While diuretics are associated with significant risk for mortality in both CKD (HR 1.62, 95% CI: 1.40-1.87, p< 0.001) and non-CKD (HR 1.82, 95% CI: 1.62-2.05, p< 0.001) patients, ARB, CCB and BB have no significant effect on survival in both CKD and non-CKD patients.

Conclusions: The use of ACEi may be associated with improved survival in cancer patients, especially with CKD. Our results provide some reassurance that there is no need to hesitate to prescribe these medications in cancer patients diagnosed with CKD.

PUB186

Management and Prognosis of Polycystic Kidney Disease: Results of the Italian ALaMMU Study

Marco Galliani. *On Behalf of ALaMMU Working Group, UOC Nephrology, Pertini Hospital, Rome, Italy.*

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease and relentlessly progress to end stage renal failure. A specific treatment is not yet available. Moreover, it is a disease with protean clinical features, especially in the predialytic stage, and little is known about the management still highly different among the nephrologists.

Methods: The aim of the present survey is to describe the different approaches and study its clinical course, also in relation to the genetic variants, in order to identify the factors that contribute to its progression. We studied 437 patients from 28 nephrologic centers of ALaMMU Working Group of central Italy, with a median age of 35aa (F/M = 57.6%/42.4%).

Results: The heritability was 34% paternal and 40% maternal, while it was unknown in the remaining 26%. The mode of presentation was familiar in 54% of cases, occasional in 28% and 18% linked to the onset of symptoms. The main clinical symptoms were represented by hypertension in 60% of cases, urinary tract infection in 28%, nephrolithiasis in 22% and microscopic hematuria in 16%. Renal function at onset is described in the table.

Presentation modality	%	GFRml/min	GFR<60ml/min(%)
Familial	54	98	30
Occasional	28	65	49
Symptoms	18	90	21

The distribution of ACE inhibitors and/or ARBs in hypertension therapy was: ACE:50%, ARB:33%, ACE+ARB:17%. The evaluation of renal size was performed with MRI in 10% of cases and by CT in 2%.

Conclusions: This study will give a contribution to the knowledge of the ADPKD phenotypic aspects, about the clinical course and will throw light on the approach at the disease management. The next phase of the study, beyond the increase in the number of cases, will also include the genetic analysis of patients, and will allow to make genotype/phenotype correlations that will be the foundation for future therapeutic approaches.

PUB187

Cardiovascular Risk Factors in the German Chronic Kidney Disease (GCKD) Study

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Background: CVD is the leading cause of mortality in patients with diabetes mellitus (DM) and chronic kidney disease (CKD). It is not clear whether DM just amplifies the risk or rather modifies the characteristics of CKD patients leading to differences in risk.

Methods: 5217 patients aged 18 to 74 years having an eGFR between 30-60 (mL/min/1.73 m^2) or overt proteinuria were enrolled across 169 renal outpatient centres in Germany.

Results: Baseline data of patients with and without DM are shown.

	Patients with DM n=1842 (35%)	**Patients without DM** n=3375 (65%)	***P-Value***
Age (years)	65 ± 8	58 ± 13	<0.001
Male gender	1228 (67)	1904 (56)	<0.001
Systolic/ Diastolic blood pressure	142 ± 22/ 76 ± 12	138 ± 20/ 81 ± 12	<0.001
BMI (kg/m^2)	32 ± 6	28 ± 5	<0.001
eGFR (mL/min/1.73 m^2)	45 ± 16	48 ± 17	0.07
CRP (mg/L)	3.0 (1.4-6.4)	2.0 (0.9-4.4)	<0.001
Hemoglobin (g/dL)	13.5 (12.3-14.5)	13.7 (12.6-14.7)	<0.001
HbA1c (%)	7.0 (6.8-7.9)	5.8 (5.6-6.0)	<0.001
UACR (mg/g)	47 (9-371)	54 (9-397)	0.45
Current smokers	269 (15)	559 (17)	0.006
LDL-C (mg/dL)	101 (78-129)	121 (96-149)	<0.001
HDL-C (mg/dL)	44 (36-55)	51 (41-64)	<0.001
Triglycerides (mg/dL)	191 (131-268)	159 (112-224)	<0.001
Use of statins (%)	1217 (66)	1444 (43)	<0.001
History of CVD	866 (47)	814 (24)	<0.001

Conclusions: The treatment quality of DM was satisfactory under nephrological care. The higher prevalence of CVD in patients with DM is likely to be related – in addition to DM – to an increased impact of established CVD risk factors, but excluding hypercholesterinemia and albuminuria.

PUB188

Identifying Opportunities to Increase Angiotensin Inhibitor Use in Chronic Kidney Disease Shanza Mujeeb, Sairah Sharif, Candace D. Grant, Pooja Kumari, Milind K. Bhagat, Shayan Shirazian, Joseph Mattana. *Medicine, Winthrop Univ Hospital, Mineola, NY.*

Background: Angiotensin inhibitors (AIs) have a substantial impact on slowing and preventing progression of chronic kidney disease (CKD) to end stage renal disease (ESRD) yet they are often underutilized. It is plausible that in many instances the reasons for nonuse may not necessary preclude using an AI. The objective of this study was to determine reasons for nonuse of AIs in CKD patients in whom it is indicated and to identify potential opportunities to maximize their use.

Methods: The electronic health records of 862 consecutive adult patients with CKD stage 3 and 4 seen in the nephrology faculty practice at our institution were reviewed. Estimated GFR (eGFR) and urine albumin creatinine ratio (ACR) were recorded and CKD stage determined. We considered an indication for an AI to be present for patients with diabetes and ACR >30 mcg/mg and for patients without diabetes and with ACR >300mcg/mg. Medication lists were reviewed for AI use. For patients not on an AI, an exhaustive review of the record was carried out to ascertain the reason.

Results: Of 557 patients with stage 3 and 4 CKD who met the study criteria, 28% (n=155) were found not to be on an AI. The category with the most patients (n=79, 51%) was the "No Reason" category, meaning that there was no explanation provided in the medical record. The next three categories were history of AKI (n=21, 13%), hyperkalemia (n=21, 13%) and hypotension (n=11, 7%). Other reasons noted included allergy, advanced CKD, renal artery stenosis and other. The category ranking was consistent for patients in each stage of CKD.

Conclusions: This study suggests that a large percentage of patients with CKD and an indication for AI therapy may not be not receiving such treatment. However, our findings also suggest that there may be a substantial opportunity to increase use of AIs. An increased understanding of the reasons for nonuse of AIs in patients where it is indicated may be helpful in designing interventions to optimize their use and delay or prevent the development of ESRD.

PUB189

Statins Role in Kidney Disease André Fragoso, Ana Paula Silva, Pedro Neves. *Nephrology Dept, Hospital de Faro, Faro, Portugal.*

Background: Patients with chronic kidney disease (CKD) have a high risk of cardiovascular disease (CVD). The effects of statins therapy in patients with CKD remain uncertain. The purpose of this study was to evaluate the role of statins in CKD progression and CVD.

Methods: In this observational study we included 349 CKD patients (f=191, m=158) with a mean age of 74.7 years and a mean eGFR (MDRD) of 19.9 ±9,9 ml/min followed in our outpatient predialysis clinic during 124 weeks. At baseline, the patients underwent a complete clinical history and physical examination and several laboratory parameters were analyzed. Our population was divided in two groups: G-I (n= 192) without statins therapy and G-II (n=157) with statins.

Results: We found that patients under statins therapy (G-II) showed higher levels of eGFR (MDRD 21.58 versus 18.53; p=0.004), calcium (9.35 versus 9.11; p=0.004), albumin (4.04 versus 3.91; p=0.018) and lower levels of phosphorus (4.01 versus 4.18; p=0.044), iPTH (206.08 versus 280.68; p=0.0001) and pulse pressure (59.64 versus 64.13; p=0.012) at baseline. In a multivariate cox proportional hazard stepwise model to identify independent risk factors of renal survival male gender (HR=2.41, 95% CI, 1.39 to 4.17; p=0.002), Hg (HR=0.623, 95% CI, 0.50 to 0.78; p=0.0001), eGFR (HR=0.891, 95% CI, 0.81 to 0.98; p=0.015), albumin (HR=0.663, 95% CI, 0.46 to 0.96; p=0.028) and statins (HR=0.607, 95% CI, 0.544 to 0.91; p=0.007) were found to predict renal survival. We also found that CVD (HR 4.08, 95% CI, 2.06 to 8.09; p=0.0001), Hg (HR 0.65, 95% CI, 0.48 to 0.87; p=0.004), iPTH (HR 1.171, 95% CI, 1.00 to 1.40; p=0.044), albumin (HR 0.482, 95% CI, 0.31 to 0.75; p=0.001) and statins (HR 0.615, 95% CI, 0.30 to 0.76; p=0.032) predict overall survival. Using a Kaplan Meier analysis we found that the overall survival of the G-I and G-II at 124 weeks was respectively 78,6% and 91,7% (Log-rank= 5.968; p=0.015) and that renal survival of G-I and G-II was respectively 60,4% and 84,1% (Log-rank= 6.639; p=0.01).

Conclusions: Our results suggest that statins therapy reduce overall mortality and extend renal survival in CKD patients. Further studies are needed to validate the clinical role of statins therapy.

PUB190

Evaluation of Anemia Management in Nephrology Fellow Chronic Kidney Disease Clinic Vikas K. Kalra, Yuvraj Sharma, Charuhas V. Thakar. *Nephrology, Univ of Cincinnati, Cincinnati, OH.*

Background: When defined as hemoglobin (Hb) < 13g/dL in males and < 12g/dL in females, the prevalence of anemia is >60 % in patients with Chronic Kidney Disease (CKD) stage IV or V, according to Kidney Early Evaluation Program (2012) data. Untreated iron deficiency is an important cause of hypo-responsiveness to erythropoiesis stimulating agent (ESA) treatment. Supplementation of iron, when indicated, may potentially decrease the need for ESA, which is important given the potential safety concerns associated with ESA use. As a part of clinical quality improvement project, we assessed Fellows' CKD clinics, within two different healthcare systems, for parameters of anemia management in patients with CKD stage IV or V.

Methods: We retrospective selected a cohort of first 120 patients (60 patients per location) with CKD stage IV or V, who were seen by Nephrology Fellows from March 2014 to May 2014. Patients on dialysis were excluded. Data was reviewed to answer two questions: 1.) Was Hb measured within a period 3 months prior or 1 week after the Renal Clinic visit; 2.) In patients with anemia, were iron stores evaluated within a period 6 months prior or 1 week after the clinic visit? Physicians conformity to these questions across clinic sites was assessed by Fisher's exact tests.

Results: The cohort was 73 % men, mean age (+/- standard deviation) was 65.2 (+/- 13.4) years, mean estimated glomerular filtration rate was 19.7 mL/min and 5 patients were on ESA. Hb was checked in 96 out of 120 patients (80%) of which 85 patients (88.5 %) had anemia. Within the target period, iron stores were not evaluated in 36 out of 85 (42.4 %) patients with anemia. Average Hb for patients whose iron stores were evaluated versus not evaluated was 9.8g/dL versus 11.3g/dL. There was statistically insignificant difference among the two clinics in the proportion of patients with Hb checked (p= 0.25), and iron panel checked when anemic (63.2% versus 50 %, p= 0.27).

Conclusions: There is room for improvement in physician's conformity to anemia management guidelines regardles of healthcare systems. Peer review of performance during fellowship, and provider education may be the key in achieving desired quality improvement.

PUB191

Proton Pump Inhibitors Are Associated with Increased Odds of Development of Chronic Kidney Disease and Death Pradeep Arora,[1,2] Nilang G. Patel,[3] Mojgan Golzy,[4] Rajiv Ranjan,[1,2] Randy L. Carter,[4] James W. Lohr.[1,2] *[1]Medicine, VAMC, Buffalo, NY; [2]Medicine, SUNY at Buffalo, Buffalo, NY; [3]Medicine, VAMC, Richmond, VA; [4]Biostatistics, SUNY at Buffalo, Buffalo, NY.*

Background: Proton pump inhibitors (PPI) are associated with increased risk of AKI due to acute interstitial nephritis (AIN). As clinical manifestations of PPI induced AIN may be subtle, many patients who develop AKI with PPI are missed. These patients may present later with chronic kidney disease (CKD). However, data is lacking on the association of PPI use and CKD.

Methods: This retrospective study was conducted using 2001-2008 data extracted from the VA Health Care Upstate New York (VISN 2). Out of 99,381 individuals with at least one observed outpatient eGFR, we excluded patients with eGFR<60 at entry (CKD patients). To estimate the effect of PPI on outcome (development of CKD), we used case-control logistic regression modeling with PPI covariate, controlling for age, sex, race, time history, and comorbidities(HTN, DM, Vascular, Cancer and COPD) and all two way significant interactions of comorbidities. We also controlled for time history which is the history time prior to developing CKD. Similarly we performed multivariate case-control logistic regression modeling for death as an outcome.

Results: The sample size for analysis of CKD outcome is 71,438 individuals. There are 24,149 individuals in case group (developed CKD), and 47289 in control group (with no CKD). Total of 20,657 were on PPI (6,214 in case group and 14,443 in control group). PPI use was associated with increased odds of CKD and death.

Variable	Odd ratios for CKD with 95% CI	Odd ratios for Death with 95% CI
Age (1Year Increase)	1.05 (1.04-1.50)	1.06 (1.059-1.064)
Female vs Male	1.25 (1.15-1.37)	0.63 (0.54-0.73)
Black (W)	0.88 (0.82-0.93)	1.35 (1.24-1.48)
PPI	1.16 (1.11-1.21)	1.58 (1.50-1.66)
Vascular Disease	1.43 (1.22-1.65)	1.52 (1.44-1.60)
DM	7.52 (6.6-8.43)	1.50 (1.40-1.57)
HTN	4.01 (3.80-4.30)	1.60 (1.48-1.71)
COPD	1.41 (1.22-1.60)	2.26 (2.14-2.39)
Cancer	1.46 (1.27-1.64)	2.34 (2.25-2.49)

Conclusions: Use of PPI is associated with increased odds of CKD and death. Judicious use of PPI may decrease incidence of CKD.

PUB192

Serum Albumin, but Not Glycated Albumin Was a Potent Factor Affecting the Performance of GFR Equation Based on Serum Creatinine Masaru Horio,[1] Enyu Imai,[2] Yoshinari Yasuda,[3] Tsuyoshi Watanabe,[4] Hitoshi Yokoyama,[5] Hirofumi Makino,[6] Seiichi Matsuo.[3] *[1]Osaka Univ Graduate School of Medicine; [2]Nakayamadera Imai Clinic; [3]Nagoya Univ Graduate School of Medicine; [4]Fukushima Medical Univ; [5]Kanazawa Medical Univ School of Medicine; [6]Okayama Univ Graduate School of Medicine.*

Background: In CKD patients with diabetes, hgh glycated albumin (GA) was assumed to a major factor in overestimation of GFR by serum creatinine (Cr) (Diabetes Care. 2014 37:596). Effect of GA levels on the estimated GFR (eGFR) by Japanese GFR equation (Eq-cr) was evaluated in 715 Japanese subjects.

Methods: GFR was measured by inulin clearance (Cin). The subjects were divided into two groups by upper limit of the GA reference range (GA-1: GA <16.3% and GA-2: GA>16.4%). Factors affecting the ratio of eGFR to Cin (eGFR/Cin) was evaluated using multivariate analysis. New equations based on creatinine and albumin (Eq-cr-alb) and based on creatinine, albumin and GA (Eq-cr-alb-ga) were developed from 382 subjects and validated in 333 subjects.

Results: Correlation coefficients between eGFR and Cin were 0.839 and 0.914 in GA-1 and GA-2, respectively. Slopes (95 % CI) of the regression lines with zero intercepts

were 1.013 (0.991 to 1.036) and 0.997 (0.951 to 1.043), respectively. Both slopes were not significantly different from 1.0. Biases (eGFR minus Cin) were -2.3±19.0 and 0.2±11.7 ml/min/1.73m^2, respectively. There was no significant difference in biases between the two groups, indicating a reasonable accuracy of Eq-cr in GA-1 and GA-2. Multiple regression analysis showed that lower serum albumin and higher GA were associated with higher eGFR/Cin. Albumin was a more potent factor affecting eGFR/Cin than GA. Precisions of Eq-cr-alb were significantly better compared with Eq-cr.There was no significant diference of precision between Eq-cr-alb and Eq-cr-alb-GA.

Conclusions: Eq-cr has a reasonable accuracy in GA-1 and GA-2. Lower serum albumin and higher GA were significantly associated with higher eGFR/Cin. The former was a more potent factor affecting eGFR/Cin. Eq-cr-alb showed better performance compared with Eq-cr, suggesting Eq-cr-alb should be used in patients with low serum albumin.

Funding: Government Support - Non-U.S.

PUB193

Beliefs and Experiences of Pregnancy in Women with Chronic Kidney Disease: Systematic Review of Qualitative Studies Allison Tong,[1,2] Shilpa Jesudason,[3,4] Jonathan C. Craig,[1,2] Wolfgang C. Winkelmayer.[5] *[1]School of Public Health, The Univ of Sydney, Australia; [2]Centre for Kidney Research, The Children's Hospital at Westmead, Australia; [3]Central and Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Australia; [4]Dept of Medicine, Univ of Adelaide, Australia; [5]Div of Nephrology, Stanford Univ.*

Background: Achieving parenthood in women receiving renal replacement therapy is challenging due to reduced fertility and the substantially higher risk of adverse outcomes. We aimed to describe the perspectives and experiences of pregnancy in women across all CKD stages.

Methods: Electronic databases were searched to April 2014. Studies were imported into HyperRESEARCH and synthesized thematically.

Results: From 11 studies (n=250 women with CKD), we identified five themes. *Failure and blame* described the guilt of being unable to conceive and fulfil a social role which diminished their sense of self-worth. *Fear of birth defects* was mostly attributed to the potential side effects of immunosuppression in kidney transplantation. *Insecurity in decision making* encompassed the uncertainties of prioritizing pregnancy as this meant sacrifices had to be made in their family life and work to minimize their risk of pregnancy complications. Kidney transplant recipients were concerned about the increased risk of graft loss. For patients with autosomal dominant polycystic kidney disease, the chance of genetic transmission influenced decisions regarding childbearing. *Withholding emotional investment* was protecting against the disappointment of inability to conceive, miscarriage or stillbirth. *Control and autonomy* reflected their capacity to choose to accept the risks of pregnancy, while some felt traumatized when advised against pregnancy.

Conclusions: Decisions about pregnancy can be emotionally complicated by the threat to their own health, burden on their family, and the perceived risk of delivering a malformed baby. Multidisciplinary care involving nephrologists, reproductive and obstetrics specialists, and access to psychological support, are suggested to help patients resolve decisional conflict and improve their confidence in managing pregnancy issues in CKD.

PUB194

Chronic Kidney Disease in Familial Dysautonomia: Role of Nocturnal Hypotension and Proximal Tubule Injury Howard Trachtman,[1] Suzanne M. Vento,[1] Horacio Kaufmann,[2] Lucy J. Norcliffe-Kaufmann,[2] Cristina Fuente Mora,[2] Debra J. Morrison,[1] Rachel Brody.[3] *[1]Pediatrics, NYU Langone Medical Center, New York, NY; [2]Neurology, Dysautonomia Center, NYU Langone Medical Center, New York, NY; [3]Pathology, NYU Langone Medical Center.*

Background: With improved survival of patients with familial dysautonomia (FD), new complications have emerged. Approximately 40% of young adults with FD develop chronic kidney disease (CKD). This study was designed to identify prognostic biomarkers of early renal injury and to distinguish between glomerular and tubular damage.

Methods: Eligibility criteria included: (1) confirmation of a genetic mutation in IKAP or ELP-1 associated with FD; (2) Age ≥2 yr; (3) eGFR≥60 mL/min/1.73 m^2. Follow-up included a baseline assessment and visits every 6 months. Clinical assessment included height, weight, BP, 24-hr ambulatory blood pressure monitoring (ABPM). Laboratory testing included serum creatinine and cystatin C levels, eGFR, urine total protein, albumin, and creatinine concentration. Urinary KIM-1, NGAL, and TGF-β excretion (pg/mL) were determined by ELISA.

Results: 33 patients (18 M: 15 F), age 24±11 yr, were included in this analysis. The serum creatinine, cystatin C, and eGFR were 0.94±0.42 mg/dL, 1.07±0.34 mg/L, and 82±34 mL/min/1.73 m^2, respectively. The degree of elevation in urinary KIM-1 excretion was correlated with the magnitude of the nocturnal drop in diastolic BP (P=0.024) and with diastolic blood pressure at night (P=0.040) in the ABPM testing. NGAL, and TGF-β urinary levels did not correlate with diastolic blood pressure at night or with diastolic BP dipping. There was minimal evidence of glomerular barrier dysfunction because only 2 patients had microalbuminuria.

Conclusions: Our findings indicate that patients with FD have evidence of proximal tubular injury based on elevated urinary KIM-1 excretion prior to the development of stage 3 CKD. The primary locus of injury appears to be the tubulointerstitium and the damage may be mediated by excessive decline in nocturnal BP. This 24-hour BP pattern, which is unique to FD compared to other causes of autonomic dysfunction, may represent a potentially remediable target for therapeutic intervention, to prevent CKD.

Funding: NIDDK Support, Private Foundation Support

PUB195

Research Priority Setting in Kidney Disease: A Systematic Review Allison Tong,[1] Shingisai Alice Chando,[1] Sally Crowe,[2] Braden J. Manns,[3] Wolfgang C. Winkelmayer,[4] Brenda Hemmelgarn,[3] Jonathan C. Craig.[1] *[1]School of Public Health, The Univ of Sydney; [2]Crowe Associates Ltd; [3]Dept of Medicine and Community Health, Univ of Calgary; [4]Div of Nephrology, Stanford Univ.*

Background: Research priority setting typically lacks transparency and patient engagement. This has raised concerns that scarce resources may not be efficiently allocated and important areas of research most relevant to patients are potentially neglected. We aimed to describe research priorities of patients with kidney disease, their caregivers and healthcare providers, and policy makers involved in their care.

Methods: Medline, Embase, PsycINFO, and CINAHL were searched to May 2014. Studies that assessed patient, healthcare provider and policy maker priorities for research in kidney disease were included.

Results: We included 16 studies (n=1923 participants) conducted in the United States (8 studies), internationally (5 studies), Netherlands (1 study), Australia (1 study), and Canada (1 study). Three (19%) studies involved patients with kidney disease and/or caregivers. Priority setting methods included the Delphi technique, expert panels, consensus conference, ranking or voting surveys, focus groups and interviews; of which the process was described in detail by 11 (69%) studies. The priority areas for research most frequently identified across studies were prevention of acute kidney injury, prevention of CKD progression, fluid and diet restrictions, improving vascular access, graft survival, access to transplantation, patient education, and psychosocial impact of CKD.

Conclusions: Research priority setting has covered a broad range of topics pertinent to kidney disease but patient involvement in research priority setting is rare and the prioritization processes are varied and sometimes opaque. Establishing research priorities using a transparent process that engages patients, caregivers, healthcare providers are needed so that resources are invested into areas that address the shared priorities and needs in kidney disease.

PUB196

The Survey on General Well-Being of Recipients after Spousal Donor Kidney Transplantations: A Case Control Study Ma Dengyan, Ping Fu. *Nephrology Dept, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China.*

Background: To investigate the general well-being of recipients after spousal donor kidney transplantations (SDKT) and to discuss influence factors might involved in.

Methods: From January 2006 to May 2012, 100 recipients who received kidney transplantations (KT) were assessed utilizing the General Well-being Schedule (GWBS). All recipients were divided into 2 groups according to the source of graft: Group 1, the SDKT recipients (n=41); Group 2, the living related donor kidney transplantation (LRDKT) recipients (n=59). A complete demographic profile of the samples was taken.

Results: The score of GWBS has a significant difference between two groups: recipients in group 1(SDKT) has a higher mean score than group 2. Of the six factors in GWBS, the third factor (the energy level) has a significant difference between the two groups. There were no significant differences between two groups with other 5 factors.

Conclusions: SDKT recipients had a higher score of GWB, which might be associated with the stable relationship and the common family and healthy goal.

PUB197

Serum Beta Trace Protein Before and After Traumatic Amputation in Male Soldiers: A Case-Control Study Dustin J. Little,[1] Sonia Q. Doi,[2] Alison L. Pruziner,[3] Verena Gounden,[4] Brett J. Theeler,[5] Stephen W. Olson.[1] *[1]Nephrology, Walter Reed National Military Medical Center, Bethesda, MD; [2]Nephrology, Uniformed Services Univ, Bethesda, MD; [3]DoD-VA Extremity Trauma Center of Excellence, Walter Reed National Military Medical Center, Bethesda, MD; [4]Clinical Center, National Institutes of Health, Bethesda, MD; [5]Neurology, Walter Reed National Military Medical Center, Bethesda, MD.*

Background: In a previous study, we determined serum creatinine (SCr) levels to be significantly decreased following traumatic amputation in otherwise healthy male subjects; suggesting that SCr-based equations may overestimate glomerular filtration rate (GFR) in this population. We used specimens from our original analysis to compare pre and post amputation serum beta trace protein (βTP) levels, since βTP is produced predominantly in the central nervous system and traumatic amputation would thus not be expected to result in a loss of βTP producing cells.

Methods: We used the Department of Defense Serum Repository to compare pre and post traumatic amputation serum βTP levels in 33 male soldiers. Results were analyzed for small (3-5.9% estimated body weight loss), medium (6-13.5%), and large (>13.5%) amputation subgroups; and for a control group matched 1:1 to the 12 large amputation subjects on the basis of age, sex, race, and timing of serum collection. Serum βTP was measured using the N Latex βTP assay (Siemens Diagnostics). Paired Student's t-test was used for comparisons. Osterkamp estimation using DEXA scan measurements was used to establish %ESBL.

Results: Mean serum βTP (mg/L) levels were unchanged in controls; all amputees (0.57±0.18 versus 0.55±0.16; p=0.48); and the small (0.51±0.19 versus 0.49±0.20; p=0.63), medium (0.55±0.14 versus 0.60±0.14; p=0.38), and large (0.65±0.18 versus 0.57±0.15; p=0.06) amputation subgroups. Mean SCr (mg/dL) was significantly lower post

amputation overall (1.04±0.18 versus 0.84±0.14; p<0.001) and in the medium (1.09±0.17 versus 0.84±0.12; p<0.001) and large (0.97±0.17 versus 0.76±0.11; p<0.001) amputation subgroups; and unchanged in controls and following small %ESBL amputation.

Conclusions: Serum βTP may be superior to SCr as a filtration marker for the estimation of GFR in adult male amputees without comorbidities.

Funding: Other U.S. Government Support

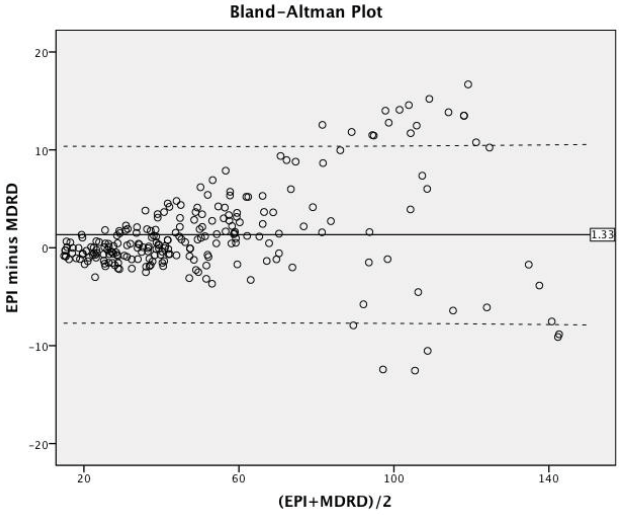
PUB198

Comparison of Creatinine-Based eGFR Equations as Predictors of Renal Events in a Portuguese Cohort of CKD Patients Andreia Campos, Jorge Malheiro, Sofia Santos, Josefina S. Lascasas, A. Castro, António Manuel Nunes Cabrita. *Nephrology Dept, CHP, Porto, Portugal.*

Background: Glomerular filtration rate (GFR) correct assessment is crucial for therapy guidance and decision-making in patients with chronic kidney disease (CKD). We compare the ability of creatinine-based MDRD and CKD-EPI equations as predictors of renal events in a cohort of patients with CKD 1-4.

Methods: A cohort of 234 patients followed in our Nephrology ambulatory unit was selected and eGFR was calculated using MDRD and EPI equation at baseline. Patients were followed until last consultation appointment with a serum creatinine evaluation. Renal event(E) was defined by an increase of 30%in creatinine serum. Category-free net reclassification index(cfNRI) was calculated for EPI in comparison with MDRD.

Results: During the follow up period (median 5 years), 65E(28%)were recorded. The mean baseline eGFR according to MDRD and EPI was respectively 42 versus 44. EPI reclassified 9% to less advanced GFR categories and 2% to more advanced ones.



Event cfNRI was +20%(95%CI 11-29) and non-event(NE) cfNRI was +24%(95%CI 18-31), when EPI equation was compared with MDRD. Mean annual event-rate(AER) was 6,4% patient/year, being greater in patients with diabetes(8%), hypertension(7%) and proteinuria(10%). Considering MDRD equation, there was no significant difference between the AER in stage 2 and 3a (3.7%/4.3% p>0.05). According to EPI, the AER was greater in stage 3a than stage 2 (6,3%/2,2%, p0.04). Considering patients classified as stage 3a by MDRD, those reclassified by EPI to stage 2 had an AER reduction from 5.2 to 0.35 patient/year(p0.004), in comparison with those not reclassified.

Conclusions: Reclassification by EPI, particularly in stage 2-3a, was clinically meaningful. Use of EPI alternatively to MDRD in mild-to-moderate CKD may allow a more adequate referral to a nephrology evaluation, with improvement in means allocation.

PUB199

Normative Surveillance and Treatment Patterns in Real World Chronic Kidney Disease Practice Scott Sibbel, Carey Colson, Mahesh Krishnan, Steven M. Brunelli. *DaVita Clinical Research, Minneapolis, MN.*

Background: There is a paucity of normative data regarding frequency of follow up, surveillance laboratory testing, and drug treatment for chronic kidney disease (CKD) patients in real-practice settings. We sought to clarify the frequency of such data from nephrology practices using the Falcon CKD electronic health record (EHR) system.

Methods: Data were obtained from 4,291 patients treated at 44 qualifying practices who had at least 2 visits after an eGFR<60 ml/min/1.73m^2, excluding those with acute kidney injury and end-stage renal disease. Patients were followed until the last nephrology visit occurring before 01Mar2012-30Jun2013. Patient time and events were ascribed to CKD stage based on the most antecedent eGFR and expressed as rate per 90 days.

Results: Higher CKD stage was associated with older age, male sex, black race, and lower BMI. Nephrologist visit rates (per 90 days) were between 1.06 and 1.25 for stages 1-3B, 1.48 for stage 4, and 3.31 for stage 5 CKD. Rates of eGFR measurement ranged between 0.53 to 0.60 for stages 1-3B, 0.75 for stage 4, and 0.80 for stage 5; potassium measurement frequency was similar to eGFR except for stage 5 where it was higher (1.77 per 90 days). Hemoglobin, phosphate, and PTH measurement were less frequent than eGFR; all were more common in stage 5 versus other stages. Treatment with phosphate binders and erythropoiesis-stimulating agent was rare except in stage 5 (14.9% and 4.2% of patients treated, respectively).

Conclusions: Frequency of testing and medication use are consistent with national CKD guidelines (Kidney Disease Quality Index, 2012 update, Kidney Disease: Improving Global Outcomes 2012 Clinical practice guideline). However, because adoption of the Falcon EHR is not universal, analyses should be updated when additional data become available. We acknowledge the contributions of the Physician Research Consortium.

Table. Patient Characteristics, Surveillance, and Treatment Frequency Among Patients With CKD Stages 1-5

	Stage 1 eGFR ≥ 90 n = 56 89.1 pt-q	Stage 2 eGFR 60-89 n = 253 435.2 pt-q	Stage 3A eGFR 45-59 n = 10,366 1678.6 pt-q	Stage 3B eGFR 30-44 n = 1,516 2511.5 pt-q	Stage 4 eGFR 15-29 n = 1,169 1913.6 pt-q	Stage 5 eGFR < 15 n = 261 447.8 pt-q
Mean age, y	51.4	62.9	67.5	72.5	71.6	65.8
Mean BMI, kg/m^2	32.6	31.1	31.4	31	30.6	30.4
Female , %	60.70%	50.80%	44.90%	54.50%	57.10%	55.60%
Diabetes, %	32.10%	41.90%	34.10%	40.50%	40.30%	36.80%
Hypertension, %	64.30%	87.00%	88.00%	91.40%	90.30%	89.30%
Black, %	35.70%	31.20%	19.90%	15.40%	18.10%	28.00%
Nephrologist Visits, Rate	1.25	1.22	1.07	1.36	1.48	3.31
eGFR Measurements, Rate[a]	0.57	0.6	0.53	0.55	0.75	0.8
Hb Measurements, Rate[a]	0.28	0.34	0.28	0.34	0.58	1.99
PTH Measurements, Rate[a]	0.08	0.06	0.13	0.2	0.36	1.08
Phos Measurements, Rate[a]	0.19	0.23	0.25	0.29	0.48	1.56
K Measurements, Rate[a]	0.57	0.61	0.56	0.57	0.84	1.77
Phos binder use, %[b]	0	0	0	0.26%	1.28%	14.90%
ESA use, %[b]	0	0	0.39%	0.92%	2.14%	4.21%

a. Per 90 days.

b. Point in time cross-sectional prevalence.

Abbreviations: BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; PTH, parathyroid hormone; Phos, Phosphosphate; K, potassium; ESA, erythropoiesis-stimulating agent

Funding: Pharmaceutical Company Support - DaVita Clinical Research

PUB200

Novel Prediction Model of Postoperative Renal Function From Automated Renal Volumetry with Preoperative Multi Detector Computed Tomography Shuji Isotani,[1] Hirofumi Shimoyama,[2] Masaki Kimura,[1] Hisamitsu Ide,[1] Satoru Muto,[1] Shigeo Horie.[2] *[1]Urology, Teikyo Univ, Tokyo, Japan; [2]Urology, Jyuntendo Univ, Tokyo, Japan.*

Background: The predictive model of postoperative renal function may impact on planning nephrectomy. To develop the novel predictive model using combination of clinical indices with computer-volumetry to measure the preserved renal cortex volume (RCV) using multi-detector computed tomography (MDCT), and to prospectively validate performance of the model.

Methods: Total 60 patients undergoing radical nephrectomy from 2011 to 2013 participated, including a development cohort of 39 patients and an external validation cohort of 21 patients. RCV was calculated by voxel count using software (Vincent, Fujifilm). Renal function before and after radical nephrectomy was assessed via the estimated glomerular filtration rate (eGFR).

Results: The postoperative eGFR value was associated with age, preoperative eGFR, preserved renal parenchymal volume (RPV), preserved RCV, % of RPV alteration, and % of RCV alteration (p<0.01). The significant correlated variables for %eGFR alteration were %RCV preservation (r=0.58, p<0.01) and %RPV preservation (r=0.54, p<0.01). We developed our regression model as follows: postoperative eGFR = 57.87 – 0.55(Age) – 15.01(BSA) + 0.30(preoperative eGFR) + 52.92(%RCV preservation). Strong correlation was seen between postoperative eGFR and the calculated estimation model (r=0.83; p<0.001). The external validation cohort (n=21) showed our model outperformed previously reported models. Main limitations were retrospective small cohort, and requirement of semi-automated segmentation in 13% cases.

Conclusions: Combining CT renal volumetry and clinical indices might yield an important tool for predicting postoperative renal function.

PUB201

Neurogenic Lower Urinary Tract Dysfunction and Chronic Kidney Disease in Spinal Cord Injury Patients Hyunjin Na, Hye Min Choi, Dong-Jin Oh. *Dept of Internal Medicine, Myongji Hospital, Goyang-si, Korea.*

Background: It is believed that patients with neurogenic lower urinary tract dysfunction (NLUTD) have a significantly higher risk of developing chronic kidney disease (CKD) than the general population. However, data are limited except a few studies that examined the incidence of renal failure in spina bifida or myelomeningocele in pediatric patients. In addition, serum creatinine is not a reliable marker for renal function in NLUTD patients because they present muscle wasting due to disuse or denervation. We examined the prevalence of CKD in NLUTD patients from spinal cord injury using cystatin-C, and the risk factors for progression to CKD.

Methods: This was a cross sectional study in Korea workers' compensation and welfare Hospital, which is a specialized center for patients from industrial accident. Patients with NLUTD were under regular examination including regular laboratory test and urologic study such as urodynamic study, Sonography and voiding cystourethrography. Patients who visited urology department for routine check-up underwent additional measurement of serum cystatin-C for 3 mo.

Results: Serum cystatin-C was checked in 314 patients (mean age 58.1±8.8 yr, mean time period after injury 18.9±9.3yr). Detrusor hyperreflexia and areflexia accounted for 66.0% and 22.2%, respectively. The overall prevalence of CKD, defined as estimated

glomerular filtration rate (eGFR) < 60ml/min/1.73m^2 was 22.4% and 8.0% by cystatin-C and creatinine-based CKD-EPI equation, respectively, and was greater than age-matched general population (Korean National Health and Nutritional Examination Surveys). Initial eGFR, co-morbid diabetes, proteinuria, bladder volume and the presence of recurrent urinary tract infection (UTI) were the independent risk factors for CKD development in the multivariable analysis.

Conclusions: The prevalence of CKD is higher in NLUTD patients than in the general population, and cystatin-C was more sensitive than creatinine for detecting CKD in NLUTD patients. Co-morbid diabetes, proteinuria, bladder volume and the presence of recurrent UTI seem to be the important risk factors for CKD development in NLUTD patients.

PUB202

High-Density Cholesterol Subclass Distribution in Patients with Chronic Kidney Disease Eliza Samouilidou, Chrisoula Pipili, Kiriaki Vasiliou, Manolis Papamanolis, Eirini Boudou, Eirini Grapsa. *Nephrology, Aretaieion Univ Hospital, Athens, Greece.*

Background: Dyslipidemia and alteration in serum lipoprotein subclass distributions contribute considerably to atherosclerosis in patients with chronic kidney disease (CKD). The shift in the size of HDL-C from larger and more atheroprotective HDL2-C to small dense HDL3-C subclass, predicts the development of oxidative stress in CKD. The aim of this study was to compare HDL-C subclasses in non-dialyzed (stages 2-5) and hemodialysis (HD) patients.

Methods: : Twenty-seven patients with CKD (51±17 years, mean ±SD, 14 males, 13 females), thirty- four patients on HD for 73±16 months (58±15 years, mean ±SD, 19 males, 15 females) and 21 controls (NC) (49±10 years, mean ±SD, 11 males, 10 females) were studied. HDL2 and HDL3-C subclasses were isolated from serum according to a single-step precipitation method following by homogenous HDL-C assay.

Results: Total cholesterol and LDL-C was increased only in non-dialyzed patients compared to NC ($p<0.01$), whereas triglycerides were high both in non-dialyzed (122±62 mg/dl) and HD (138±52 versus 82±32 in NC). HDL-C was decreased in patients compared to NC but this decrease was more profound in HD patients (40±12 md/dl) compared to non-dialyzed patients (53±16, $p<0.01$). Regarding HDL-C subclasses, HDL3-C was dramatically lower in HD patients (11±3 mg/dl) compared to non-dialyzed patients (25±3) and NC (23±4, $p<0.001$). HDL2-C did not differ between patient groups, but was marginally lower in non-dialyzed patients (24±13) compared to NC (33±9, $p<0.05$).

Conclusions: In non-dialyzed CKD patients, a progressive increase of total cholesterol and LDL-C together with a decrease of HDL-C values are observed compared to patients on HD. Hemodialysis does not seem to affect HDL2-C subclass levels, however the significant decrease in HDL3-C may be relevant of a persistent loss of antioxidant capacity of HDL-C in this modality.

PUB203

Standardization Program in Chaco, Argentina: Difficulties in Random Error Correction Maria Eugenia V. Bianchi,[1] Nicolás Ernesto Meier,[1] Mayra Florencia Valdez,[1] Ana M. Cusumano,[3] Gustavo A. Velasco.[2] *[1]Physiology, School of Medicine. Northeast National Univ, Corrientes, Corrientes, Argentina; [2]Biochemical Clinic Studies, Ministry of Health . Chaco Province., Resistencia, Chaco, Argentina; [3]Univ Institute, CEMIC, CABA, Buenos Aires, Argentina.*

Background: Early diagnosis of chronic kidney disease (CKD) is based on the estimation of glomerular filtration rate (eGFR) by MDRD formula, but it is recommended that the determination of creatinine in clinical laboratories must be standardized previously, so that the variations inherent to the laboratory do not affect the result of eGFR and patient classification. The aim of this paper is to describe the creatinine standardization program in Chaco (Argentina) which began in 2010.

Methods: 39 laboratories have participated (public and private), serum panels with certified concentrations of creatinine between 0.65 and 2.28 mg / dl were used. In the first stage, in 2011,the Random Error (RE), Systematic Error (SE) and Total Error (TE) were determined according to EP-10Clinical and Laboratory Standards Institute (NCCLS).

Results: An average of 15.2CV %was obtained, it was concluded then that the most important problem of the program was the RE. In the second stage, during 2012, 37 laboratories participated in the evaluation of the total imprecision of the determination, according to the NCCLS protocol EP -5A, as well as in the reduction of it. In 2013 the laboratories that participated in both stages were categorized: Group I, 19 (48.7%) laboratories that met the desired objectives for EA and Group II, 15 (38.5 %) who failed to reduce imprecision maintaining RE values ≥4.0 %. The mean ± SD values of RE in Group I was 3.99 ± 0.92 in Group II and 11.9 ± 6.31 ($p=0.0001$). Analyzed the strengths,we found that Internal and External Control tools were used in 12 (63.2 %) laboratories in the GI and 4 (36.4 %) in the GII ($p = 0.018$); as well as 10 (27 %) GI laboratories had Homogeneous analytical systems.

Conclusions: After three years of the program only 48.7% of the laboratories can move towards standardization itself, however, the results allow us to state that the lack of control tools and analytical homogeneous systems are the main difficulties.

Funding: Private Foundation Support

PUB204

Primary and Recurrent Focal Segmental Glomerulosclerosis Closely Link to Serum Soluble Urokinase Receptor Levels Juan Jin,[1] Yiwen Li,[1] Qiang He.[1] *[1]Nephrology, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China; [2]The Kidney Disease Center, The 1st Affilliated Hospital, Medical College, Zhejiang Univ, Hangzhou, Zhejiang, China.*

Background: Serum soluble urokinase-type plasminogen activator receptor (suPAR) is implicated in the pathogenesis of native and recurrent FSGS. It is elevated in two-thirds of subjects with primary FSGS, but not in people with other glomerular diseases which can differentiate FSGS from other glomerular diseases.

Methods: We measured soluble urokinase receptor levels in the resum of patients with primary focal segmental glomerulosclerosis (FSGS) and determined their association with clinical and pathological data in 86 patients with primary FSGS, 5 repeat renal biopsy FSGS and 6 recurrent FSGS posttranplantation, healthy controls and patients with minimal change disease, andmembranous nephropathy were used as controls. The resum-soluble urokinase receptor levels, measured by commercial ELISA kits.

Results: Of patients with primary FSGS (median:4232, interquartile range 1299-9714 pg/ml) were significantly higher than those of patients with minimal change disease (median 2784pg/ml), membranous nephropathy (median 3478 pg/ml), andhealthy individuals (median 1994 pg/ml). There was no significant difference in suPAR levels between the 65 patients with minimal change disease and 85 patients with membranous nephropathy. The soluble urokinase receptor levels increased in the 5 repeated renal biopsy FSGS and 6 recurrent FSGS posttranplantation. The suPAR levels were significantly but positively correlated with FSGS, not only primary FSGS but also recurrent FSGS posttransplantation, but negatively correlated with other flomerular diseases.

Conclusions: Thus, suPAR levels can differentiate primary FSGS from other glomerular diseases.

PUB205

Markers of CKD in HIV+ Patients Pedro Pereira Campos,[1] Clara Dias,[3] Fernando G. Pereira,[1] Alberto Ortiz,[2] Ana Luisa Papoila,[3] Karina Soto.[1,3] *[1]H. Fernando Fonseca, Portugal; [2]Fundacion Jimenez Diaz, Madrid; [3]Univ Nova Lisboa.*

Background: Traditional markers of kidney function are not accurate for early diagnosis of CKD in HIV patients. Cystatin C (SCysC) was claimed as a good GFR marker. Fractional excretion of phosphate (FePi) and uric acid (FeUrAc) were proposed as tubular markers for early dysfunction together with the urinary albumin/creatinine ratio (UACR). Aim: Determine the usefulness of these associated markers on CKD diagnosis.

Methods: Cross-sectional analysis in 946 consecutive HIV+ outpatients. Demographic, clinical and laboratorial data were recorded. Primary endpoint: CKD (GFR<60ml/min/1.73m2 as assessed by CKD-EPI based on serum creatinine (SCr) or SCysC or by MDRD. McNemar, Chi-squared test and a logistic regression model were used**.** Significance was set at $\alpha=0.05$.

Results: Among 946 patients, mean age 46.1±11.5; 54.4% male; 45.9% Africans; 92.5% HIV1; 23.3% Hypertensive; 11.1% HCV+ and 7.4% Diabetic. Median CD4 count=478 cells/μL and viral load=309 cop/mL. Median eGFR=103 (84-118)mL/min/1.73m^2. CKD distribution was 72.3; 23.8; 3.4; and 0.5% for I; II; III; and IV+V stages respectively. CKD rates were 3.8, 3.9 and 7.5% with MDRD, CKD-EPI-SCr and CKD-EPI-CysC, respectively. Univariate logistic regression showed that FePi [OR 1.1 (CI 1.0-1.1) p= 0.002]; UACR [OR 7.6 (1.1-46.7) p=0.040]**;** HCV+ [OR 3.6 (1.3-9.9) p=0.013] and age [OR 1.1 (1.0-1.1) $p<0.001$] were associated with CKD. For each 1.0 of FePi increase, the odds of CKD increase by 8.6%. There was an inverse correlation between FePi and eGFR based on SCysC. A trend was observed for Atazanavir [OR 2.5 (1.0-6.4) p=0.054]. No association was found with other antiretroviral drugs. In multivariable regression analysis excluding UACR only FePi remained a significant determinant for CKD (p=0.002). Tenofovir was not associated with variations on eGFR, nor FePi, FeUrAc. Although UACR was not included in multivariable analysis, there is a significant difference between CKD and no-CKD patients, $p<0.001$.

Conclusions: Prevalence of CKD in HIV patients is higher than in the general population. Atazanavir, a drug associated with crystaluria, had the closest association with CKD. Of tubular markers, FePi, but not FeUrAc, was associated with CKD.

Funding: Government Support - Non-U.S.

PUB206

Classification Tree Models for Predicting the Risk of the Progression of Diabetic Nephropathy Wenbo Zhao,[1] Lin Wei,[2] Ming Li,[1] Jun Zhang,[1] Hui Peng.[1] *[1]Dept of Nephrology, The Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China; [2]Dept of Neuropathy, Guangdong Province Traditional Chinese Medical Hospital, Guangzhou, Guangdong, China.*

Background: To establish models for predicting the risk of progression of diabetic nephropathy, and to explore the related factors for type 2 diabetes patients with early renal damage using the classification tree model.

Methods: The 256 cases of the hospitalized patients with type 2 diabetes are enrolled. According to GFR installments and urine albumin quantitative,the patients were divided into proteinuria group (73 cases) and early diabetic kidney damage group (183 cases). The clinical data of the patients were recorded to analyze the main factors for the progression of diabetic nephropathy using the Exhaustive CHAID classification tree algorithm.

Results: Classification Tree model has screened out four important explanatory variables from the 34 candidate variables, including course of hypertension, CysC levels, WHR(Waist/Hip Ratio) and ALB(serum albumin). CysC levels and WHR (Waist/Hip

Ratio) were the main factors. The patients with microalbuminuria as CysC>1.53 mg/L and WHR>0.99 with high risk, occurred macroalbuminuria; The risk of disease progression was also significantly increased in CysC>1.53 mg/L and ALB<39.5 g/L group. CysC was 1.13-1.53 mg/L and duration of hypertension 0-3 years group significantly increasing; The model predicted progression of diabetic nephropathy patients from microalbuminuria to macroalbuminuria; The correct rate is 85.9%. The probability of misclassification(Risk values) was 0.207.

Figure 1 Classification Tree Model

(Microalbuminuria, mAlb; Albuminuria, Alb; WHR, Waist/Hip Ratio)

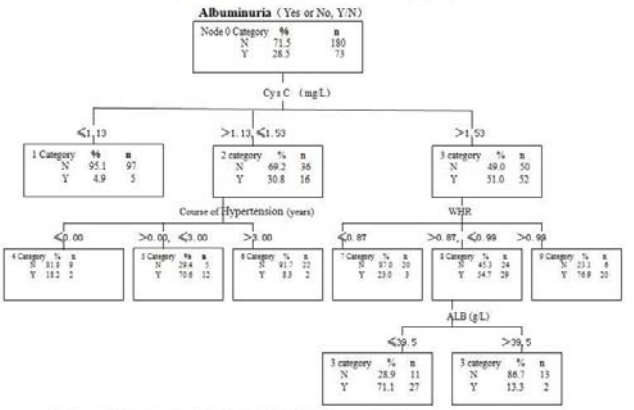


Table 1 Misclassification Matrix and Classified Forecast Table

Classification

Observed	predicted		
	mAlb	Alb	Percent Correct
mAlb	161	22	88.0%
Alb	14	59	80.8%
Overall Percentage	68.4%	31.6%	85.9%

Growing Method: EXHAUSTIVE CHAID
Dependent Variable: Albuminuria

Conclusions: The classification tree model could screen out the major effecting factors of the progression of diabetic nephropathy effectively, and it could help to develop the prevention and treatment methods. Classification Tree Models can be for predicting the risk of the progression of diabetic nephropathy.

PUB207

Multiple Myeloma with Amyloidosis Aggravates Patients' Survival in Plasma Cell Disorder Seon Deok Hwang, Ji Hyun Yu, Wooyeong Park, Byung Ha Chung, Bum Soon Choi, Chul Woo Yang, Yong-Soo Kim, Cheol Whee Park. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, Seoul, Korea.*

Background: Renal failure is a common complication of multiple myeloma and other plasma cell dyscrasias. The presence of coexistent kidney disease is associated with increased mortality. The aim of this study is to investigate the survival effect of multiple myeloma with amyloidosis, renal failure in plasma cell disorder patients.

Methods: Between Dec. 2001 and Feb. 2013, 285 plasma cell disorder Diagnosed patients were included. The median age was 57.32 ± 9.563 years. Male was 126 patients (52.9%). The median GFR 69.26±33.72. 47 patients were excluded because missing data. 10 patients was Amyloid light chain (AL) amyloidosis, 11 patients was multiple myeloma (MM) with amyloidosis, 212 patients MM without amyloidosis and 5 patients others. Kaplan Meier survival curve was used to compare mortality between different groups.

Results: Between AL amyloidosis and MM with amyloidosis, no difference was found in baseline characteristics such as age, sex, transplantation, Hb, MDRD-GFR. The Kaplan Meier showed that AL amyloidosis group were associated with higher survival as compare to MM with amyloidosis group(P-value: 0.013). MM with Amyloidosis patients groups was lower survival in multiple myeloma patients (MM with amyloidosis mortality; 7/11(63.6%) versus MM without amyloidosis; 50/212(23.6%), P<0.001). And also 75 renal failure patients were lower survival than 137 non-renal failure patients in MM without amyloidosis patients (Non renal failure mortality 25/137(18.2%) versus Renal failure 25/75(33.3%), P=0.002).

Conclusions: In plasma cell disorder patients, the prognosis of MM patients with amyloidosis was worse than those of AL amyloidosis patients and MM patients without amyloidosis. MM patients without amyloidosis and AL amyloidosis patients were not associated with the worsening of kidney function. But, MM patients with amyloidosis were associated with the worsening of kidney function and also Renal failure (GFR<60ml/min) is important factor of mortality in MM patients without amyloidosis.

PUB208

Application of Iohexol Plasma Clearance in Chinese Chronic Kidney Disease Children Baobao Wang, Yuan Wu, Mengchun Gong, Xuemei Li, Yan Qin. *Dept of Nephrology, Peking Union Medical College Hospital, Chinese Academic Medical Science and PekingUnion Medical College, Beijing, China.*

Background: Iohexol Plasma Clearance has been validated as a satisfactory reference method for GFR measurement in adult and child. However it has not been applied in China.We therefore propose to develop the method for the quantification of iohexol in serum by high performance liquid chromatography. And then to evaluate the accuracy and practicability for iohexol plasma clearance from the serum and dried bood spots in chronic kidney disease (CKD) children.

Methods: After obtaining informed consent, forty-five CKD children were included and examined by ^{99m}Tc-DTPA plasma clearance and iohexol plasma clearance simultaneously. Blood samples were obtained at 2,4 and 5hours after injection respectively. In the meantime, we also evaluated the efficacy of single blood sample method and dried blood spots method in iohexol plasma clearance. The study was approved by the ethics committee of the Peking Union Medical College Hospital.

Results: Our study established and optimized the measurement of the serum iohexol concentration by HPLC. The standard curve showed a good linearity (the correlation coefficient r>0.999). The RSD% of intra-assay and inter-assay were lower than 5%, the recovery rate was more than 96%. The correlation coefficient between ^{99m}Tc-DTPA plasma clearance and iohexol plasma clearance was 0.94. The correlation between iohexol single sample plasma clearance and double samples was also strong (r=0.96). The iohexol clearance by dried blood spots showed a good correlation with the serum iohexol clearance (r=0.96). Only one 14-year-old boy showed slight scattered red rash in 12 hours after iohexol injection, no other adverse reaction was observed.

Conclusions: The method based on HPLC to measure the serum iohexol concentration is accurate and stable. Iohexol plasma clearance showed a good agreement with ^{99m}Tc-DTPA plasma clearance, which will be an ideal method to measure GFR in CKD children. The single sample method and dried blood spots method make iohexol plasma clearance more convenient and practical.

PUB209

Multiple Myeloma with Amyloidosis Aggravates Patients' Survival in Plasma Cell Disorder Seon Deok Hwang, Ji Hyun Yu, Wooyeong Park, Byung Ha Chung, Bum Soon Choi, Chul Woo Yang, Yong-Soo Kim, Cheol Whee Park. *Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, Seoul, Korea.*

Background: Renal failure is a common complication of multiple myeloma and other plasma cell dyscrasias. The presence of coexistent kidney disease is associated with increased mortality. The aim of this study is to investigate the survival effect of multiple myeloma with amyloidosis, renal failure in plasma cell disorder patients.

Methods: Between Dec. 2001 and Feb. 2013, 285 plasma cell disorder Diagnosed patients were included. The median age was 57.32 ± 9.563 years. Male was 126 patients (52.9%). The median GFR 69.26±33.72. 47 patients were excluded because missing data. 10 patients was Amyloid light chain (AL) amyloidosis, 11 patients was multiple myeloma (MM) with amyloidosis, 212 patients MM without amyloidosis and 5 patients others. Kaplan Meier survival curve was used to compare mortality between different groups.

Results: Between AL amyloidosis and MM with amyloidosis, no difference was found in baseline characteristics such as age, sex, transplantation, Hb, MDRD-GFR. The Kaplan Meier showed that AL amyloidosis group were associated with higher survival as compare to MM with amyloidosis group(P-value: 0.013). MM with Amyloidosis patients groups was lower survival in multiple myeloma patients (MM with amyloidosis mortality; 7/11(63.6%) versus MM without amyloidosis; 50/212(23.6%), P<0.001). And also 75 renal failure patients were lower survival than 137 non-renal failure patients in MM without amyloidosis patients (Non renal failure mortality 25/137(18.2%) versus Renal failure 25/75(33.3%), P=0.002).

Conclusions: In plasma cell disorder patients, the prognosis of MM patients with amyloidosis was worse than those of AL amyloidosis patients and MM patients without amyloidosis. MM patients without amyloidosis and AL amyloidosis patients were not associated with the worsening of kidney function. But, MM patients with amyloidosis were associated with the worsening of kidney function and also Renal failure (GFR<60ml/min) is important factor of mortality in MM patients without amyloidosis.

PUB210

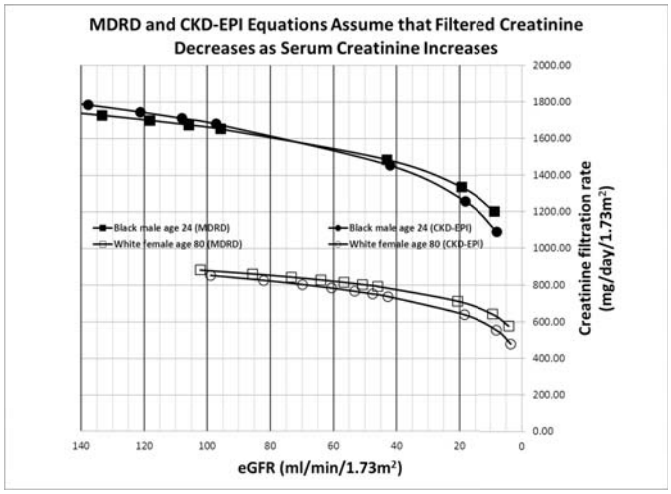
Modeling of Creatinine Kinetics by MDRD and CKD-EPI Equations Includes Non-Renal Creatinine Disposition Karo Torosian,[2] Robert W. Steiner.[1] *[1]Medicine, Univ of California, San Diego, School of Medicine, San Diego, CA; [2]Medicine, Scripps Mercy Hosptial, San Diego, CA.*

Background: The MDRD and CKD-EPI equations are computer-derived, best-fit expressions that predict eGFR from serum creatinine (Cr) and race, gender, and age. We studied how these equations model Cr physiology.

Methods: We compared MDRD and CKD-EPI eGFRs when both were calculated using the same Cr, age, race, and gender, as each of these variables was uniquely varied. We then calculated Cr filtration rates (CrFR, mg/day/1.73m2) as the product of the calculated eGFRs and the serum Cr values used to determine those eGFRs.

Results: At age 24, CKD-EPI estimated up to a 10% higher eGFR than did MDRD, particularly in nonblacks. At age 80, MDRD eGFRs were up to 10% higher, particularly in blacks. Calculated CrFRs varied from 800 to 1700 mg/1.73m2/day at normal eGFRs. At

low eGFRS it was 85-90% of total urine Cr excretion as derived from CKD populations (Ix, CJASN,2011) that we size-normalized for comparison. Both equations predicted an accelerated decrease in CrFR at an eGFR of about 30 ml/min/1.73m2. This curve was closely approximated by subtracting a load-dependent extrarenal Cr clearance of 3L/day,perhaps by bowel flora (Mitch, Nephron,1978), from a constant rate of Cr production.



Conclusions: MDRD and CKD-EPI equations use age and race somewhat differently when estimating eGFR. Both assume an accelerated decrease in CrFR as serum Cr rises. The decrease in CrFR parallels similar decreases in total urinary Cr that are observed CKD populations. Extrarenal Cr disposition at low eGFRs may explain the nonlinearity in eGFR equations, which are challenged to reflect changing Cr kinetics as serum Cr increases. As results were size-normalized and unaffected by age, gender, or race, a major influence of muscle mass is unlikely. The Cr physiology that is implicit in these expressions deserves further study.

PUB211

Is the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation Useful in Chinese Diabetic Patients? Jin-Xia Chen,[1,4] Yanni Wang,[1] Xun Liu,[1,2] Chenggang Shi,[1] Zhenda Zheng,[3] Tan-Qi Lou.[1] *[1]Div of Nephrology, Dept of Internal Medicine, the Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [2]Div of Nephrology, Tufts Medical Center, Boston, MA; [3]Dept of Cardiology, the Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [4]Institute of Nephrology, Affiliated Hospital of Guangdong Medical College, Zhanjiang, China.*

Background: The prevalence of diabetic kidney disease(DKD) is escalating and it has become the main cause of end stage renal diseases in western countries. Thus it is essential to present an accurate and convenient glomerular filtration rate estimation for diabetes. Many studies indicated that the Cockcroft-Gault equation and Modification of Diet in Renal Disease equation showed great bias in diabetes. Yet whether the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation demonstrates less accuracy in diabetes is still controversial. In this study We aim to compare performance of the CKD-EPI equation between diabetic and non-diabetic population.

Methods: 1189 subjects were enrolled, and were divided into diabetic group (n=548) and non-diabetic group (n=641). 99mTc-DTPA-GFR revised by dual sample method was referred as the gold standard. Multivariate logistic regression was used to analyze the factors affecting the 30% accuracy of the CKD-EPI equation. The predictive performance of the equation was assessed by bias, precision and 30% accuracy.

Results: Renal dynamic imaging-GFR was revised as the following equation: Dual plasma sampling 99mTc-DTPA-GFR (ml/min/1.73 m^2)= 3.706+1.039×99mTc-DTPA renal dynamic imaging-GFR (ml/min/1.73m^2) (R^2=0.879, P<0.001). Multivariate logistic regression indicated that diabetic status affected the 30% accuracy of the CKD-EPI equation. The diabetic and non-diabetic group were matching to sex, age, body mass index (BMI) and chronic kidney disease (CKD) categories. After matching, the performances of the CKD-EPI equation in the diabetic group were worse in both precision (inter-quartile range of median difference, 28.4 versus 19.9 ml/min/1.73m^2, P<0.001) and accuracy (30% accuracy, 58.3 versus 67.1%, P=0.02) comparing with the non-diabetic group.

Conclusions: The CKD-EPI equation is less accurate in Chinese diabetic patients comparing with the non-diabetes.

Funding: Government Support - Non-U.S.

PUB212

Developing New Glomerular Filtration Rate Estimating Models for Chinese Diabetic Patients Jin-Xia Chen,[1,4] Xun Liu,[1,2] Linsheng Lv,[3] Chenggang Shi,[1] Yanni Wang,[1] Tan-Qi Lou.[1] *[1]Div of Nephrology, Dept of Internal Medicine, the Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [2]Div of Nephrology, Tufts Medical Center, Boston, MA; [3]Operating Room, the Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [4]Institute of Nephrology, Affiliated Hospital of Guangdong Medical College, Zhanjiang, China.*

Background: The estimation of GFR in diabetes is of great significant. However, reports have identified the current equations all showed less accurate in the diabetes.

Methods: 519 diabetic patients were enrolled. The development dataset was based on patients enrolled from January 2005 through December 2012. And data were randomly divided into the training data set (276) and testing set (138). Data obtained from January 2013 to June 2013 were used as the external validation dataset (n=105). 99mTc-DTPA-GFR revised by dual sample method acted as the gold standard. Multiple regression analysis was used to establish new regression equations and MATLAB 2011A software was adopted to establish the artificial neural network (ANN) models.

Results: Subjects who undergone 99mTc-DTPA renal dynamic imaging and dual plasma sampling ^{99m}Tc-DTPA in the same day were enrolled. 99mTc-DTPA-GFR was revised by the following equation in diabetic patients: Dual plasma sampling ^{99m}Tc-DTPA-GFR (ml/min/1.73m^2) =6.442+1.071×^{99m}Tc-DTPA renal dynamic imaging-GFR (ml/min/1.73 m^2) (R^2=0.703, P<0.001). 8 new regression models and 8 new artificial neural network models were established on the base of sex, age, serum creatinine and by adding BMI, HbA1C, and ACR separately or in combination. Performance of all new models in diabetes were compared with the CKD-EPI equation in the external validation group. Bias between new predicting models and the CKD-EPI equation were not significant. All models had higher precision (P<0.001) than the CKD-EPI equation despite ANN6 (P<0.001). Only ANN3 had higher accuracy than CKD-EPI equation (30% accuracy, 88.6 versus 80.6%, P=0.02). In each original external validation data set, 30% accuracy of the ANN3 had improvements of 4.5% versus the CKD-EPI equation and 8.0% versus the MDRD equation.

Conclusions: ANN3 based on sex, age, serum creatinine and BMI seemed to offer a more accurate and convenience GFR estimation in diabetic patients.

Funding: Government Support - Non-U.S.

PUB213

Burden of Chronic Kidney Disease (CKD) in Peru Elizabeth Francis,[1,2] Chin-Chi Kuo,[2] Antonio Bernabé Ortiz,[3] Lisa C. Nessel,[4] William Checkley,[2] Bob Gilman,[2] Harold I. Feldman,[4,5] Jaime Miranda.[2,5] *[1]Weill Cornell Medical College; [2]Johns Hopkins School of Public Health; [3]La Univ Peruana Cayetano Heredia; [4]Univ of Pennsylvania School of Medicine; [5]Co-Senior Authors.*

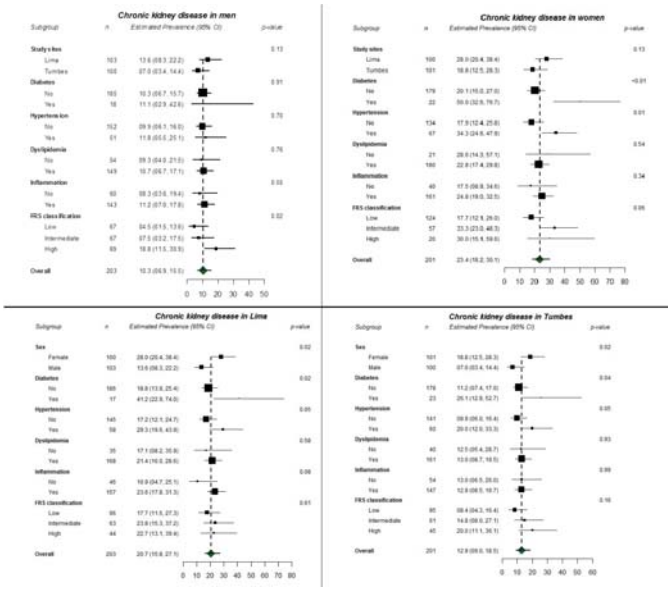
Background: CKD's silent progression, association with chronic disease, and high treatment costs make it a global public health concern.

Methods: The Peruvian CKD burden has yet to be fully described. To provide insight, we completed a cross sectional study of CKD prevalence among 404 participants in Lima and Tumbes between 2/11-6/11 who were enrolled in the Peruvian CRONICAS Cohort Study. Factors potentially associated with CKD were explored with Poisson regression.

Results: In the total study population, the diabetes and hypertension prevalence was 9.9%(95% CI 7.4-13.3%) and 29.2%(95% CI 25.1-34.0%), respectively, median age was 55.34yrs. 68(16.8%, 95% CI 13.5-20.9%) met CKD criteria; proteinuria 60(15%), eGFR <60 ml/min/1.73m^2 4(1%), or both 4(1%). A greater CKD prevalence was noted in Lima, females, and older age median 59.6 yrs, IQR 50.5-69.2). Among participants with CKD, the prevalence of diabetes and hypertension was 19.1% and 42.7%, respectively. After multivariable adjustment, CKD remained associated with older age, female sex, greater wealth, living in Lima, diabetes and hypertension.

Conclusions: The high prevalence of CKD identified in Lima and Tumbes is similar to that in the U.S. These findings highlight the need to identify occult CKD and implement strategies to prevent disease progression and secondary morbidity.

Age (years)	Male (case/n)	Estimated prevalence (95% CI)	Female (case/n)	Estimated prevalence (95% CI)
35-44	3/51	5.8 (1.9-17.8)	5/51	9.8 (4.2-22.7)
45-54	5/54	9.2 (4.0-21.5)	12/51	23.5 (14.3-38.8)
55-64	6/51	11.8 (5.5-25.1)	9/48	18.8 (10.3-34.0)
≥ 65	7/47	14.9 (7.4-29.7)	21/51	41.2 (29.6-57.3)
Overall	21/203	10.3 (6.9-15.5)	47/201	23.4 (18.2-30.1)
p-value for trend	0.13		0.001	



Funding: Other NIH Support - The CKD study was funded by University of Pennsylvania. The CRONICAS Cohort Study was supported by the National Heart, Lung, and Blood Institute Global Health Initiative under the contract Global Health Activities in Developing Countries to Combat Non-Communicable Chronic Diseases (project number 268200900033C-1-0-1)

PUB214

Is Obstructive Sleep Apnoea a Risk Factor for Chronic Kidney Disease?

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Background: Obstructive sleep apnoea (OSA) is associated with intermittent hypoxaemia which leads to activation of a number of pathways including oxidative stress, sympathetic nervous system, endothelial dysfunction and inflammation which in turn lead to hypertension and atherosclerosis. There is some evidence that the same process may predispose to renal dysfunction. In this study, we have compared a group people with hypertension and OSA on continuous positive airways pressure therapy (CPAP) with matched control group not known to have OSA.

Methods: Patients with known OSA and the age, sex and BMI matched control groups were selected retrospectively from a hypertension clinic database. The 2 cohorts were compared in terms of mean 24 hour blood pressure (BP, mmHg), mean serum creatinine (micromol/l) and mean urine albumin creatinine ratio (ACR. mg/mmol).

Results: Forty eight patients were identified with confirmed OSA on CPAP; 6 were excluded due to insufficient data. Of the 42 remaining patients, 34 were male. The mean age was 53.5, the mean BMI was 34.6 kg/m^2. The mean 24 hour systolic BP was 143 (±25.6) in patients with OSA and 135 (±16.7) in the controls (p=0.097). The mean serum Creatinine was 94.0, median 95.0 (range 110) in patients with OSA and mean 93.4, median 93.0 (range 99) in the control (p=0.981). The mean ACR was 2.50, median 0.0 (range 33) in patients with OSA and mean 0.63, median 0.00 (range 7.6) in the control group (p=0.577).

Conclusions: This case control study shows that there is no significant difference in blood pressure, Creatinine or ACR in people with treated OSA and people without although there were trends towards higher BP and ACR in the OSA group. This may be due to CPAP treatment reducing the oxidative stress caused by hypoxaemia. A larger prospective study looking at parameters before and after treatment is needed to confirm this hypothesis.

PUB215

Racial Disparities in End-of-Life (EOL) Preferences in Chronic Kidney Disease (CKD) Patients due to Low Health Literacy

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Background: Few patients with CKD document what their preferences are for EOL care and minority and low-income populations are disproportionately impacted when accessing effective supportive care. Additionally low health literacy, which contributes to numerous disparities throughout healthcare, has been shown to affect up to 32% of patients with CKD. We investigated whether health literacy is a mediator of racial disparities in EOL preferences and knowledge.

Methods: Cross-sectional study of CKD patients in nephrology outpatient clinics affiliated with two large academic centers in Boston, MA. Inclusion criteria include: i) Age ≥ 50 years, ii) English-speaking during clinical encounters, iii) self-identification with Black or White race, and iv) Stage 4 or 5 CKD (defined by eGFR ≤ 30 ml/min/1.73m^2). Patients listed for kidney transplantation or with history of dementia are not eligible. Confidential structured interviews are performed with all participants. Health literacy is assessed via the validated Rapid Estimate of Adult Literacy in Medicine. Variables of interest include: 1) health literacy (predictor), 2) preference for care at the EOL, and 3) advance care planning (ACP) preferences. Information regarding spiritual/cultural influences on EOL preferences, hospice knowledge and physician distrust are also collected.

Results: 44 participants have completed the study. Black and White patients differ on health literacy and many aspects of EOL care preference.

	Black (N=12)	White (N=32)
Low Health Literacy	75%	13%
Mean age (years)	70 (+/- 12)	72 (+/- 8)
Male	58%	66%
CPR - Prefer to be revived	92%	88%
Critically ill - Prefer to stay in hospital	34%	25%
Critically ill - Prefer comfort care	50%	69%
Completed living will/Do-not-resuscitate form	17%	53%
Completed health care proxy form	67%	69%

Note - Frequencies listed as percentages. Means listed as (+/- SD).

Conclusions: Black and White patients prefer different care at the EOL with less Black patients having completed ACP. Future analysis will determine whether health literacy contributes to disparities and potentially sets the premise for interventional studies aimed at improving EOL care for all CKD patients.

Funding: Private Foundation Support

PUB216

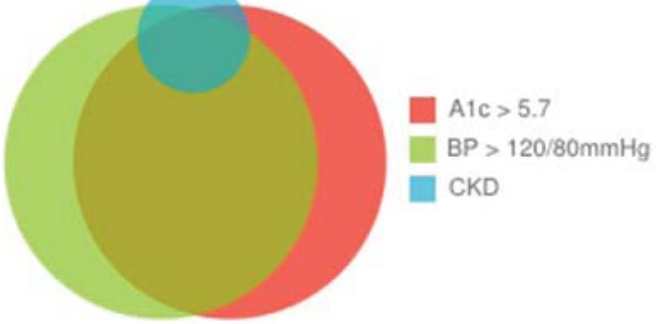
Prevalence of Chronic Kidney Disease in India and Implications for Cardiovascular Disease: The Cardiometabolic Risk Reduction in South Asia Surveillance Study

Shuchi Anand,[1,2] Roopa Shivashankar,[2] Mohammed K. Ali,[3] Dimple Kondal,[2] B. Binukumar,[2] Maria E. Montez-Rath,[1] V.s. Ajay,[1] R. Pradeepa,[4] M. Deepa,[4] Ruby Gupta,[2] V. Mohan,[4] K.M. Venkat Narayan,[3] Nikhil Tandon,[5] Glenn M. Chertow,[1] Dorairaj Prabhakaran.[2] *[1]Medicine/Nephrology, Stanford Univ School of Medicine, Palo Alto, CA; [2]Center of Excellence-CARRS, Centre for Chronic Disease Control, New Delhi, India; [3]Rollins School of Public Health, Emory Univ, Atlanta, GA; [4]Madras Diabetes Research Foundation, Chennai, India; [5]Endocrinology, All India Institute of Medical Sciences, New Delhi, India.*

Background: Data on the prevalence of chronic kidney disease (CKD) in India are sparse.

Methods: Using data from the Center for Cardiometabolic Risk Reduction in South Asia surveillance study—a population-based survey of Delhi and Chennai (n=12,271)—we estimate the overall, and the age-, sex-, city-, and diabetes-specific prevalence of CKD. We present the distribution of the population per the KDIGO CKD classification. We compare ten-year likelihood of a cardiovascular event in participants with and without CKD using the Framingham Risk Score.

Results: 9797 participants (80%) had complete data on serum creatinine and albuminuria. Age-standardized prevalence of CKD and albuminuria were 8·8% (95% CI: 8·1-9·6%) and 7·1 (95% CI 6.5-7·7%). Eighty percent of patients with CKD had HbA1c in the diabetes or pre-diabetes range.



Using KDIGO criteria, 7%, 1%, and 0·6% of study participants are at moderate, high, or very high risk for experiencing CKD-associated adverse outcomes. Ten-year probability of cardiovascular events was high for 43% (95% CI 38-48%) of participants with CKD, compared with 15% (95% CI 13-17%) of participants without CKD.

Conclusions: One in twelve people living in two of India's major metropolitan cities have evidence of CKD, with features that put them at high risk for CKD progression and cardiovascular events.

Funding: NIDDK Support, Other NIH Support - Fogarty and NHLBI

PUB217

Management Differences of Acute Coronary Syndrome among Patients with Chronic Kidney Disease, End Stage Renal Disease, and Normal Kidney Function Geovani Faddoul, Ninad D. Parekh, Marc M. Saad, Youssef El Douaihy, Iskandar Barakat, Suzanne E. El Sayegh. *Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Background: Chronic kidney disease (CKD) and acute coronary syndrome share common pathophysiology, intersecting management and use of medication like angiotensin converting enzyme inhibitor. This study looks at the management of CKD and end stage renal disease (ESRD) patients in the setting of myocardial infarction (ST and non-ST elevation types: STEMI and NSTEMI) with regards to percutaneous coronary intervention (PCI), coronary artery bypass (CABG) and medical therapy.

Methods: Retrospective cohort analysis of all acute coronary syndrome charts between January 2005 and December 2012 of patients aged more than 18. Exclusion criteria were non cardiac chest pain, AKI at time of admission (history of CKD included). The primary outcome was interventions. The interventions were PCI, CABG and change in medication regimen. The secondary outcomes were length of stay, mortality and left ventricular function ejection fraction percentage (LVEF).

Results: We analyzed 334 patients with STEMI (50.3%) and NSTEMI (49.7%) and compared subgroups of normal glomerular filtration rate (GFR), CKD (III to V) and ESRD. The patients with STEMI had an left ventricle ejection fraction of 37%±10 ($p<0.05$). The length of stay was not significantly different between the different subgroups. CKD patients had less PCI in a STEMI setting and ESRD patients had less PCI overall ($p<0.05$). ESRD patients also had less CABG interventions than normal GFR group ($p<0.05$). The analysis did not however show any difference in medical therapy (aspirin, beta-blocker, ACE inhibitor or angiotensin receptor blocker, clopidogrel or equivalent, statins). Results about mortality were controversial since they showed worse mortality for CKD compared to normal GFR in STEMI and compared to ESRD in NSTEMI.

Conclusions: The CKD and ESRD subgroups benefit less of PCI and CABG than normal GFR patients but have the same medical therapy.

PUB218

Chiral Amino Acid Metabolomics for Novel Biomarker Screening in CKD Yoshitaka Isaka,[1] Tomonori Kimura,[1] Ryohei Yamamoto,[1] Hiromi Rakugi,[1] Masashi Mita,[2] Kenji Hamase.[3] *[1]Dept of Geriatric Medicine and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Shiseido Co., Ltd, Tokyo, Japan; [3]Graduate School of Pharmaceutical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: D-Amino acids are the enantiomers of L-amino acids, and increasingly recognized as novel physiologically active molecules and biomarkers. Their distribution and regulation are totally different from those of L-forms, however, the amounts of D-amino acids are usually small in humans, and the determination is frequently interfered by various intrinsic substances. Therefore, chiral amino acid profile remains unknown in CKD patients.

Methods: More than 100 patients recruited from a single center comprised of chronic kidney diseases stage 3-5. Serum D-amino acids were analyzed using a two-dimensional HPLC system with fluorescence detectors and an ESI-tandem mass spectrometer. For the improvement of detection sensitivity and also of chromatographic retention, amino acids were pre-column derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). The NBD-amino acids were separated by areversed phase column (capillary monolithic ODS, 0.53 mm i.d. x 1000 mm) in the first dimension, and the D- and L forms of target amino acids were separated by enantioselective columns (1.5 mm i.d. x 250 mm) in the second dimension.

Results: The 2D-HPLC-FL/MS/MS system could successfully determine all proteinogenic amino acid enantiomers without interference from intrinsic substances in human serum. We found small amounts of D-Ala, D-Pro and D-Ser in the sera of healthy volunteers. On the contrary, CKD patients exhibited significantly increased amounts of serum D-amino acids, and these values were correlated with their creatinine and estimated glomerular filtration rate.

Conclusions: D-Amino acids estimated using 2D-HPLC-FL/MS/MS system may be novel biomarkers in CKD patients.

PUB219

Hemodialysis Staff and Caregivers Burnout a Single Center Experience Konstantina Trigka,[1] Periklis Dousdampanis.[1] *[1]Hemodialysis Unit, Kyanous Stayros Patron, Patras, Achaia, Greece; [2]Univ New Mexico.*

Background: The number of dialysis patient's increases and the trend is towards extended hours of HD sessions, the psychological strain by personnel and caregivers is important. We investigated the impact of job psychological stress of HD personnel by obtaining measurements of burnout, examined the burden of family members who provide care for HD patients and correlations with patient's co-morbidities and depression.

Methods: We administered ProQOL5 questionnaire to HD staff, American Medical Association's Caregiver Health Self-assessment Questionnaire to family members and applied the BDI questionnaire to the aforementioned patients and measured their co-morbidities utilizing the Charlson Co-morbidity Index.

Results: The majority did not experience burnout (mean score 23.2±4.9), without statistical difference between physicians and nurses. 2 stressors categories emerged: duration of working practice and age of HD providers. Staff with higher qualifications, working full-time were more likely to be dissatisfied. Staff's sex, marital status and number of children were insignificantly related. 48.45% caregivers responded -mean age: 59±1). Found no significant difference between caregiver's age and stress degree. 70.2 % of caregivers - mean age 58±13, experienced moderate stress. The remaining 29.8 %-mean age 64±12, experienced higher degree stress. Statistical analysis did not indicate correlation of caregivers' and staff"s burnout levels with patients' co-morbidities- CCI or patients' depression- BDI score. There was no correlation regarding inter-dialysis weight gain, dialysis modality, number of daily administered-or psychiatric drugs, dialysis shift, cerebrovascular or cardiac diseases, causes of CKD, anemia, renal osteodystrophy, albumin, cholesterol, arterial hypertension or diabetes mellitus.

Conclusions: HD workers are not exposed to above-average levels of stress. Older personnel with greater length of service and HD staff with occupational seniority have higher burnout levels. Older caregivers tend to report greater degrees of stress, whereas no relationship was revealed between caregiver's stress degree and patient's depression level or co-morbidities.

PUB220

Associations of Depression and Pain with Quality of Life and Satisfaction in Chronic Hemodialysis Patients Linda Yick Belayev,[1] Maria K. Mor,[3,4] Paul M. Palevsky,[1,2] Robert M. Arnold,[5,6] Steven D. Weisbord.[1,2,3] *[1]Renal-Electrolyte Div, Univ of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Renal Section, Medicine Service Line, VA Pittsburgh Healthcare System, Pittsburgh, PA; [3]Center for Health Equity Research and Promotion, VA PIttsburgh Healthcare System, Pittsburgh, PA; [4]Dept of Biostatistics, Univ of Pittsburgh, Pittsburgh, PA; [5]Div of General Internal Medicine, Univ of Pittsburgh School of Medicine, Pittsburgh, PA; [6]Div of Palliative Care, Univ of Pittsburgh School of Medicine, Pittsburgh, PA.*

Background: The impact of depression and pain on health related quality of life (HRQOL) and satisfaction with care is not fully understood in hemodialysis (HD) patients. We sought to characterize the associations of these symptoms with HRQOL and satisfaction with care.

Methods: We prospectively assessed depression using the Patient Health Questionnaire-9 and pain using the Short-Form McGill Pain Questionnaire on a monthly basis in patients on chronic HD. We assessed HRQOL and satisfaction with care quarterly using the Short Form 12 and Patient Satisfaction Questionnaire, respectively. Random effects linear regression was used to analyze associations.

Results: Among 286 patients, depression was reported on 18% and pain on 79% of monthly assessments. Depression was independently associated with decreased SF-12 physical health scores (B= -0.31; 95% CI: -0.43, -0.20); decreased SF-12 mental health scores (B= -0.91; 95% CI: -1.02, -0.79); and decreased satisfaction with care (B = -0.14; 95% CI: -0.17, -0.12). Pain was independently associated with decreased SF-12 physical health scores (B= -0.23; 95% CI: -0.29, -0.17); decreased SF-12 mental health scores (B= -0.16; 95% CI: -0.23, -0.09); but not with decreased satisfaction with care (B = -0.03; 95% CI: -0.01, 0.04).

Conclusions: Depression and pain are associated with reduced physical and mental HRQOL. Depression is also associated with decreased satisfaction of care. Symptom alleviating interventions may improve these patient-centered outcomes.

Funding: NIDDK Support

PUB221

Cardiovascular and Renal Risk Factors in Wichi Aborigines from Chaco, Argentina Maria Eugenia V. Bianchi,[1] Germán Darío López,[1] Lucas Leonardo Suarez,[1] Katya Carolina Polischuk,[1] Ana M. Cusumano,[2] Gustavo A. Velasco.[3] *[1]School of Medicine., National Northeast Univ, Corrientes, Corrientes, Argentina; [2]Univ Institute, CEMIC, CABA, Buenos Aires, Argentina; [3]Biochemistry, Hospital Julio C Perrando, Resistencia, Chaco, Argentina.*

Background: The Province of Chaco, Argentina, was inhabited since ancient times with gathers and hunters ethnic groups. Wichí aborigines (ab) live near Bolivia, far from urban settlements. The contact with white population and salt began in late 19th century. There are no official records about anthropometric characteristics, blood pressure and social economic state of this ab. National Northeast University designed a program to educate about cardiovascular and renal risk factors (CVRRF). The wichi ab in community meetings were encouraged to create neologisms for diabetes mellitus, hypertension (HT) and Chronic Kidney Disease and trained ab to detect CVRRF. The aim of this work is to show the results obtained by wichi ab health workers in detection of CVRRF.

Methods: This is a descriptive, observational, cross-sectional study. Data collection was conducted using a semistructured survey translated to their own language in presence of medical doctors. VII JC and WHO classifications were adopted for HT and Central Obesity.

Results: A total of 156 ab were surveyed 91(58,3%) men and 65(41,7%) women, mean age of 34,26±11,8 years (range 18-76). The mean (sd) weight was 72,79±14,6 and mean height of 1,63±0,1. Nutritional status showed: overweight in 26,92%(CI95% 21,1-34,6), obesity in 39,1%(CI95% 31,4-47,2), central obesity, 40,15%(CI95% 31,7-49), sedentarism 83,33 %(CI85% 78,2-93,8). HT, 10,9%(CI95% 6,5-16,9), Glycemia > 200mg/dl in only one ab and Proteinuria in 14,6%(CI95% 8,21-23,3). The socioeconomic status was mainly marginal 61,73%(CI95% 50,2-72,3). There were differences by sex in central obesity: 50,9% versus 32,5% (p=0,033) and height 1,56±0,14m versus 1,67±0,06m (p=0,00) for women and men respectively.

Conclusions: These are the first data describing CVRRF in wichi ab from Chaco Argentina. They showed a high rate of obesity with low rates of HT. The cohort will be followed to observe the evolution of CVRRF.

Funding: Private Foundation Support

PUB222

Absence of a Strong Correlation Between Obesity and Systolic Hypertension in Korean and Chinese Populations of Greater Boston Michael Ho-Young Ahn,[1] Andrew J. Pan,[1] Ryan Lindeborg,[1] Laura C. Polding,[1] Enchi K. Chang,[1] Anita Xu,[1] Rayhnuma Ahmed,[1] Supisara Tintara,[1] Tzongshi Lu,[2] Jennie Kuo,[2] Li-Li Hsiao.[2] *[1]Harvard College; [2]Renal Div, Brigham and Women's Hospital.*

Background: Obesity is an established risk factor for developing hypertension. Yet very few studies have examined the relationship between obesity and hypertension in Asian populations in North America. This observational study investigates the relationship between obesity (as quantified by BMI) and systolic hypertension in Korean and Chinese participants of free kidney health screenings hosted by the Kidney Disease Screening and Awareness Program (KDSAP).

Methods: In Korean and Chinese populations in Greater Boston, KDSAP held kidney health screenings that included body mass index (BMI) and blood pressure (BP) measurements. Participants were asked to report their ethnicity. Subsequent data analysis accounted for different BMI standards for Asian populations, with a BMI of 24 or above classified as "overweight" and a BMI of 27 or above as "obese". A systolic BP of 140 or above was considered to be hypertensive.

Results: Among 138 Korean participants, 32 had systolic hypertension, of which 8 were overweight and 8 obese. Among 137 Chinese participants, 55 had systolic hypertension, of which 19 were overweight and 15 obese. BMI and BP were weakly correlated in both Asian populations (r = 0.215 for Koreans; r = 0.254 for Chinese). Differences between mean hypertensive and non-hypertensive BMIs were insignificant in Koreans (p = 0.13) and significant in Chinese (p = 0.015).

Conclusions: Our findings contradict those found for BMI and BP in non-Asians by demonstrating a weak correlation. Further studies are warranted to assess the influence of other factors such as diet, smoking, and socioeconomic status on hypertension in Asians. KDSAP fills a unique niche in collecting data from these populations as an organization dedicated to reducing health disparities in medically underserved communities. Identifying the key social determinants and risk factors for chronic kidney disease and elucidating their relationships will be crucial to improve preventive screenings.

Funding: Private Foundation Support

PUB223

Patients with Chronic Kidney Disease and Their Intention to Use Electronic Personal Health Records Tyrone Harrison,[1] James Wick,[1] Min Jun,[1] Braden J. Manns,[1] Sofia B. Ahmed,[1] Robert R. Quinn,[1] Marcello Tonelli,[2] Brenda Hemmelgarn.[1] *[1]Dept of Medicine, Univ of Calgary, Calgary, AB, Canada; [2]Dept of Medicine, Univ of Alberta, Edmonton, AB, Canada.*

Background: Strategies to enable patient self-management have been advocated in a movement towards patient-centered care. Electronic personal health records (ePHRs) give patients access to their health information, and may facilitate their self-management. The purpose of our study was to describe the perceptions of patients with chronic kidney disease (CKD) about ePHRs, and characteristics associated with their intent to use ePHRs.

Methods: We surveyed consecutive outpatients with non-dialysis CKD (eGFR <30 mL/min/1.73m^2) in Calgary, Canada over a three-month period. Patients were invited to complete a paper-based survey at their routine nephrology clinic visit. Data obtained included patient demographics, intent to use an ePHR, and perceived benefits and concerns about ePHR use. Multivariable logistic regression was used to determine characteristics associated with intent to use an ePHR.

Results: 67 patients with CKD completed the survey. Most patients were over 65 years old (55%), male (74%), and had access to the internet (72%). The majority of respondents (82%) agreed that patients should have access to their personal health information and 70% indicated they would use an ePHR if available. Perceived benefits of ePHR access included greater involvement in personal health care (50%), and better access to lab results (72%), though 40% reported concerns about privacy of their health information. In a multivariable analysis, only "interest in regular monitoring of their health" was associated with an increased likelihood of intent to use ePHR (OR=11.7, 95%CI: [1.2-110], p=0.031).

Conclusions: Patients with CKD are interested in accessing their personal health information and intend to use ePHRs if offered. Factors such as security, anxiety about results, and lack of internet access did not affect their intent to use ePHRs. Our results are limited by the small sample size and single centre nature of the study. Further research is needed to determine whether use of ePHRs improves patient outcomes.

Funding: Government Support - Non-U.S.

PUB224

Comparison of Quality-of-Care Indicators in Patients with End-Stage Renal Disease due to Lupus Nephritis versus Other Causes Laura Plantinga, Rachel E. Patzer, Cristina Drenkard, Stephen O. Pastan, Jason Cobb, William M. McClellan. *Emory Univ, Atlanta, GA.*

Background: Patients with end-stage renal disease (ESRD) due to lupus nephritis (LN) may be treated by multiple specialty providers and, thus, have greater opportunities for high-quality pre-ESRD care than patients with other causes of ESRD.

Methods: In U.S. patients initiating treatment for ESRD (7/05-9/11), we examined whether quality-of-care indicators (receipt of pre-ESRD nephrology care, access to transplant, and permanent access placement for dialysis) were better among those with ESRD due to LN (*n*=6,594) versus other causes (*n*=669,295) using national surveillance data (United States Renal Data System). Logistic and Cox proportional hazards models were used to estimate odds ratios (ORs) and hazard ratios (HRs) of each quality-of-care indicator, with adjustment for age, sex, and race.

Results: Among those with ESRD due to LN versus other causes, 71% versus 66% received pre-ESRD nephrology care; 85% versus 79% were informed of transplant options; and rates of waitlisting were 206 versus 96 per 1000 patient-years. With adjustment, those with LN were more likely to receive pre-ESRD care (OR=1.52, 95% CI 1.49-1.56), be informed of transplant options (OR=1.12, 95% CI 1.04-1.21), and be waitlisted (HR=1.46, 95% CI 1.40-1.53) than those with ESRD due to other causes. However, only 24% versus 36% patients with ESRD due to LN versus other causes had a permanent vascular access (fistula or graft) used or in place at the start of dialysis; with adjustment, those with ESRD due to LN were 40% less likely to have a permanent access (OR=0.62, 95% CI 0.58-0.66).

Conclusions: Patients with ESRD secondary to LN are more likely to receive pre-ESRD care and have better access to transplant—but are less likely to have a permanent vascular access for dialysis—than patients ESRD due to other causes. While there is room for improvement in all quality-of-care indicators among LN patients approaching ESRD, further studies are warranted to specifically examine barriers to permanent vascular access placement and morbidity and mortality associated with temporary access in this population.

Funding: Other NIH Support - NIMHD, Private Foundation Support

PUB225

Ace Inhibitors Slow Down Renal Damage Progression Even in Chronic Kidney Disease Stage 5 Pietro Claudio Dattolo, Elena Romoli, Fiammetta Ravaglia, Elisa Ferrari, Marco Allinovi, Giuseppe Ferro, Marco Amidone, Francesco Pizzarelli. *Nephrology, Internal Medicine, S.M.Annunziata Hospital, Firenze, Italy.*

Background: It is a time-honoured knowledge that ACEi slow the progression of kidney damage; if this effect persists in patients with CKD as advanced as stage 5 is less well known. To verify this, we analyzed clinical and laboratory data of patients referred to our outpatient CKD stage 5 clinics from 2001 to 2010.

Methods: We analyzed data of 313 patients (M 60 %, age 72 ± 14 years), 65% with cardiovascular disease, 35% with diabetes and 50% with other comorbidities (cancer, chronic diseases). At first visit the average eGFR (MDRD formula) was 9.5 ml/min/1,73m2 (IQR: 7.9-11.4), and proteinuria was 1.5 g/day (IQR: 0,6-3). During a median 2 year follow-up, 62 patients died and 198 started dialysis. Data are expressed as mean ± standard deviation, median and interquartile range; primary end point was dialysis start. We used ANOVA, t-test and Cox regression for statistical analysis.

Results: The 195 patients treated with ACEi showed a flat eGFR slope significantly lower than 118 patients not treated with ACEi (0,48 ± 1,08 ml/min/1,73 m2/year versus 3,12 ± 3,00 ml/min/1,73 m2/year, p=0.04). Cox regression analysis identified the use of ACEi and age as the only independent factors of protection from renal injury, while proteinuria and phosphorus were independent factors of progression.

	WALD	p	Hazard	95% CI
ACEi (yes)	6,7	0,013	0,69	0,50-0,92
AGE (years)	15,6	0,000	0,98	0,97-0,99
PHOSPHATE (mg/dl)	9,1	0,002	1,20	1,10-1,30
U-PROT (g/die)	35,0	0,000	1,21	1,15-1,30

Conclusions: Our study shows that treatment with ACEi slows down the renal damage progression in patients with advanced CKD stage 5, allowing us to safely delay the start of dialysis treatment.

PUB226

Catheters May Be Associated with Modestly Increased Risk of Poor Outcomes in Patients Transplanted Early Laura Plantinga, Rachel E. Patzer, Stephen O. Pastan. *Emory Univ, Atlanta, GA.*

Background: Clinical guidelines recommend placement of a permanent vascular access (fistula or graft) in all incident dialysis patients, but providers may rely on temporary catheters when patients are expected to receive a transplant imminently. Whether this practice is associated with increased risk of poor outcomes among these patients, as in the overall ESRD population, is unknown.

Methods: Using United States Renal Data System data, we examined mortality (all-cause, infectious), hospitalizations, and transplant failure in 8,617 U.S. incident dialysis patients (7/05-5/11) aged 18-69 who received a transplant within 1 year of their dialysis start date. Follow-up was defined from ESRD start (mortality, hospitalizations; median, 3.5 years) or transplant (transplant failure; median, 2.7 years) to death or end of follow-up (9/11). Catheter use was defined as not having a fistula or graft in place at start of dialysis. We used Cox proportional hazards and Poisson models with adjustment for age, sex, and race to estimate hazard ratios (HRs) and incidence rate ratios (IRRs) for catheter use versus permanent vascular access used/in place.

Results: Overall, 4,861 (56.4%) started dialysis with a catheter. After adjustment, catheter use was associated with 16% and 53% increased all-cause and infectious mortality risk among these patients, respectively, although the associations were not statistically significant (HR=1.16, 95% CI, 0.97-1.39; and HR=1.53, 95% CI, 0.86-2.71). Only 4 (0.7%) of 609 total deaths (5.4% of 74 infectious deaths) were attributed to vascular access. The association between catheter use and hospitalizations was modest, at 5% increased hospitalization rate for patients with catheters versus permanent access (IRR=1.05, 95% CI, 1.01-1.08). Finally, transplant failure was 16% more likely among those recipients starting with a catheter (HR=1.16, 95% CI, 1.01-1.32).

Conclusions: More than half of patients who receive a transplant within 1 year of starting dialysis do not have permanent vascular access in place. Providers should weigh potential modestly increased risks of mortality and morbidity in shared decision making regarding vascular access for dialysis patients who are likely to receive a transplant early.

Funding: Other NIH Support - NIMHD, Private Foundation Support

PUB227

Timely Data Reporting Is Associated with Improved Blood Pressure Control and Reductions in Interdialytic Weight Gain in Dialysis Patients

Patricia McCarley, Karen G. Butler, Alex J. Rosenblum, Portia R. Scott, Terry Ketchersid, John W. Larkin, Len A. Usvyat, Norma J. Ofsthun, Jeffrey L. Hymes, Evan Norfolk, Franklin W. Maddux. *Fresenius Medical Care North America, Waltham, MA.*

Background: Uncontrolled blood pressure and fluid overload are challenging to clinically manage in the renal dialysis patient population. In December 2013, Fresenius Medical Care North America (FMCNA) initiated a Weekly Snapshot Report pilot program designed to identify uncontrolled systolic hypertension and fluid overload in dialysis patients. This analysis investigated whether the provision of weekly data for elevated systolic blood pressure (SBP) and fluid overload is associated with improvements in clinical management.

Methods: This study analyzed data from two clinics enrolled in the Weekly Snapshot Report pilot program since December 30, 2013. Clinics participating in the program had weekly identification of patients with high SBP (>160 mmHg pre- and post-dialysis) and fluid gains (i.e. interdialytic weight gain (IDWG) > ultrafiltration volume (UFV)) during the preceding week. Patient clinical data was captured for 30 days before and after enrollment. Comparisons were performed using paired t-test analysis.

Results: Overall, 119 patients were enrolled into the Weekly Snapshot Report program. This investigation found the potential impacts of the program to be reductions in the percent of treatments with post-dialysis SBP >160 mmHg and IDWG > UFV. However, no comparisons were identified to be statistically significant.

Figure 1:	30 days before	30 days after	Percent change
IDWG (kg)	3.33 (SD±1.48)	3.26 (SD±1.23)	-2.2%
Pre-dialysis SBP (mmHg)	150.51 (SD±24.82)	149.59 (SD±23.69)	-0.6%
Treatment time (mins)	207.69 (SD±37.86)	213.9 (SD±50.06)	3.0%
Post weight (kg)	85.04 (SD±23.08)	84.93 (SD±23.28)	-0.1%
% with pre-dialysis SBP>160	37.3%	36.0%	-3.3%
% with post-dialysis SBP>160	17.6%	15.5%	-12.0%
% of tx with IDWG>UFV	48.8%	45.4%	-6.9%

Conclusions: Timely data reporting through the Weekly Snapshot Report pilot program had trending associations with improvements in uncontrolled systolic hypertension and fluid overload. Further studies are needed with larger patient numbers to better identify the potential impacts of timely data reporting.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

PUB228

Bridging the Gap between Face-to-Face and Telephonic Case-Management: Use of Telehealth Visits

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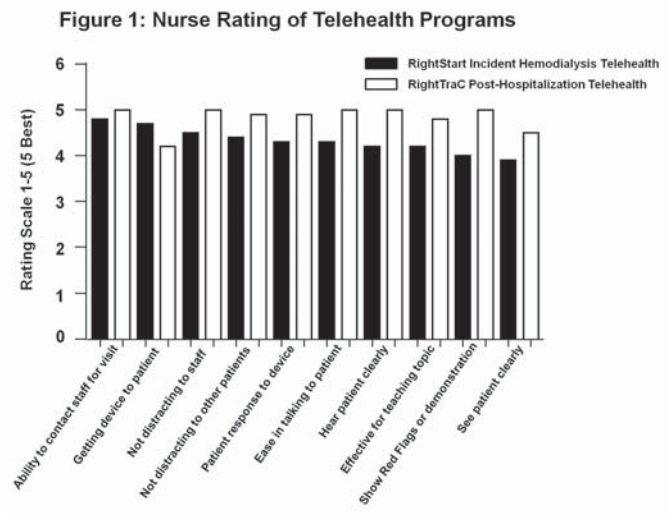
Background: One in three dialysis patients are readmitted within 30 days of discharge from the hospital according to USRDS data. Since March 2014, this challenge is being addressed via the Right TraC™ (RT) care transitions program, which is a population based case-management approach for patients post-hospitalization. The aims of this pilot study were to understand if telehealth is effective for engaging patient management.

Methods: The RT telehealth visit initiative is based upon framework from the Right Start (RS) program, whereby case managers provide specialized management and education to 1-120 day incident dialysis patients. In the RT program, case managers telephonically deliver targeted interventions designed to promote 0-30 day post-hospitalization patients' self-management skills and interdisciplinary healthcare. This process utilizes a video- and tele-communication device configured to allow for use of a secured Skype application. Comparisons have been performed between the objective ratings in key elements of the RS and RT telehealth programs in 48 and 11 patients respectively.

Results: Results from the RS and RT televisit nurse rating of key elements to assist in its evaluation can be seen in Figure 1. Performance ratings in both programs were similar with ranges from 3.9-5.0, with 5.0 as the best possible score. The RT telehealth visits were found to have excellent ratings for key areas of performance.



Conclusions: Based upon the RT case managers' evaluations, use of telehealth technology can successfully enhance the ability of the case manager to engage their patients through virtual evaluation and integrated self-management tools. Use of telehealth visits may be the bridge in the gap between face-to-face encounters and telephonic case management.

Funding: Pharmaceutical Company Support - Fresenius Medical Care North America

PUB229

Direct Healthcare Costs Related to End Stage Renal Disease in Patients New to Haemodialysis: The FARO2 Study

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Background: Objective of this analysis was to estimate direct healthcare cost of end stage renal disease (ESRD) in naïve haemodialysis (HD) patients enrolled in the FARO2 study.

Methods: The FARO2 retrospective observational study conducted in Italy aimed to evaluate patterns of treatment of SHPT and healthcare costs in a subgroup of patients who started HD during the FARO study. Observational period: 2006-2008. Costs were measured in 2008 Euros; no discount rate was applied. Resource consumption evaluated for the first two semesters of HD were: reimbursed SHPT drugs, phosphate binders and erythropoietin stimulating agents (ESA), HD sessions and ESRD-related hospitalizations. The analysis was performed from the Italian National Health Service (INHS) perspective.

Results: 567 patients had at least two semesters of observation (average age: 65.5 years). For the first year after HD entrance average direct healthcare costs accounted for 31,677€/patient.

	Semester 1 (€)	Semester 2 (€)
Vitamin D+cinacalcet	324	381
Phosphate Binders	283	364
ESA	3,005	2,941
HD	11,276	11,695
Hospitalizations	749	659
Total	15,637	16,040

These costs were estimated considering: average weekly frequency of HD, for drugs the percentage of patients treated and the average daily dosages and for inpatients the percentage and type of hospitalizations.

Conclusions: Patients naïve to HD have a significant impact on INHS expenditure even if, such in this study, only costs related to treatment of ESRD and its complications were considered. The major cost drivers were HD and ESA (91% of total costs) while SHPT drugs and phosphate binders accounted for only 4% of direct healthcare expenditure.

Funding: Pharmaceutical Company Support - AbbVie

PUB230

An Updated Assessment of CKD Management in the Primary Care Setting David M. Dewolfe,[1,2] George P. Bayliss.[1,2] *[1]Medicine, Rhode Island Hospital, Providence, RI; [2]Alpert Medical School, Brown Univ., Providence, RI.*

Background: We sought to determine factors associated with nephrologist referral and whether nephrology co-management of CKD patients had a positive effect on adherence to KDOQI guidelines for CKD management and short-term clinical outcomes.

Methods: A retrospective cohort study of 147 patients receiving continued care at the Rhode Island Hospital Resident Medical Clinic between Feb 1 2012 and Feb 1 2014 with an eGFR <60 ml/min/1.73m² on two lab draws at least 3 months apart. Variables include age, gender, CKD severity, presence of CKD ICD-9 code, nephrology referral, consultant correspondence; KDOQI guideline annual assessments include serum Cr, albuminuria, nutrition, bone health, electrolytes, ACE/ARB use and anemia; Clinical outcomes include average blood pressure and rate of decline of GFR.

Results: Of the patients analyzed, 90% had stage III and 10% had stage IV CKD; only 26% were co-managed by a nephrologist. Variables associated with referral to a nephrologist were higher serum Cr, lower eGFR, higher CKD stage and presence of a CKD ICD-9 code in the patient's problem list. Patients co-managed by a nephrologist were more likely to be screened annually for albuminuria (P< 0.0102), electrolyte/bone health (Ca P= 0.0012; Phos P= 0.0012; Vit D P= 0.003; PTH P< 0.001) and anemia (P = 0.031). From Feb 2012 to Feb 2014, median decline in eGFR was 4% for all patients. For those co-managed by a nephrologist the median decline in eGFR was 11.5% versus 2.1% for those not managed by a nephrologist (P= 0.0328), and this difference remained on multivariate analysis. 63% of patients co-managed by a nephrologist met the KDOQI blood pressure goal of <130/80 while only 50% of patients not co-managed did, though this difference did not reach statistical significance (P= 0.15).

Conclusions: Nephrology co-management was associated with greater short-term decline in eGFR despite increased compliance with KDOQI guidelines, in part explained by more advanced CKD at baseline in referred patients, but also perhaps indicating that patients are being referred too late in the clinical course of kidney disease to alter outcomes. Earlier referral may be an area for improvement.

Funding: Clinical Revenue Support

PUB231

Association Between Oral Hygiene Behavior and Hypertension Prevalence, Awareness, Treatment and Urine Sodium Excretion Jun-Beom Park,[1] Hye Min Choi.[2] *[1]Dept of Periodontics, Seoul St Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Myongji Hospital, Goyang, Gyeonggi-do, Republic of Korea.*

Background: A positive association has been reported between hypertension, kidney disease and periodontal disease. Oral health promotion activities appeared to produce improvements in periodontal health, and may result in changes to systemic markers of inflammation and endothelial function. Therefore, this study was performed to assess the association of oral health behaviors, hypertension and sodium intakes in a large probability sample of the Korean population using the data from Korea National Health and Nutrition Examination Survey (KNHANES).

Methods: The data from KNHANES conducted between 2008 and 2010 were used. The tooth brushing frequency and the use of secondary oral products were evaluated. Multivariate logistic regression analyses were used to assess the associations of oral hygiene behaviorwith the hypertension prevalence, awareness, treatment and sodium intakes.

Results: An association between hypertension and oral hygiene behavior could be seen after adjustment for variables. Adjusted odds ratios An association between hypertension and oral hygiene behavior could be seen after adjustment for variables. Adjusted odds ratios and their 95% confidence intervals of hypertension prevalence were 1.231 [1.040-1.457] for individual who brushed none for controlling for age and sex, smoking, drinking, exercise, education, income, energy intake, fat intake and periodontitis. An association between urine sodium excretion and oral health behaviors could be seen in female. Adjusted odds ratios and their 95% confidence intervals of tooth brushing ≥ 3 per day in the highest quartile for urine sodium excretion group were 0.783 [0.608-1.010] after controlling for variables.

Conclusions: Individuals with poor oral hygiene habit were more likely to have higher hypertension prevalence. This study also showed the association between urine sodium excretion and oral health behaviors in female. Within the limits of this study, oral hygiene habit may be considered a potential risk indicator for hypertension.

PUB232

Impact on Estimated Glomerular Filtration Rate (eGFR) in Treatment of Multidrug-Resistant Tuberculosis (MDR-TB) Gustavo Alejandro Casas-Aparicio,[1] Isabel Maria Leon-Rodriguez,[1] Gustavo Reyes-Teran.[1] *[1]CIENI, Instituto Nacional de Enfermedades Respiratorias, México D.F., Ciudad de Mexico, Mexico; [2]CIENI, Instituto Nacional de Enfermedades Respiratorias, México, D.F., Ciudad de México, Mexico; [3]CIENI, Instituto Nacional de Enfermedades Respiratorias, México, D.F., Ciudad de México, Mexico.*

Background: MDR-TB treatment is nephrotoxic, due to use of injectable aminoglycosides(aIY).

Methods: A retrospective study of MDR-TB patients treated with aIY agent at National Health Respiratory Institute in Mexico City between 2009-2013 was performed. Demographics, serum chemistry, hematological testing were recorded at baseline(BL) and End of treatment. Nephrotoxicity was defined as increase in the Creatinine(Cr) level >0.3 mg/dL compared with BL. CKD-EPI was used to calculate the eGFR, KDOQI was used to stage CKD. Results are expressed by means (min, max), Fisher´s exact test and U Mann-Whitney were performed to compare categorical and continues variables respectively. Analysis were obtained using SPSS v15.0, p <0.05 were considered significant.

Results: We evaluated 41 cases with diagnosis MDR-TB confirmed. Age 42 years (19,70). Male 26(62%), Diabetes Mellitus 20(48%), Hypertension 5(12%), HIV 1(2.4%), Kidney Transplant 1(2.4%), Smoke 13(31%). BMI 24.5(13. 34), Antihypertensives 6(14%), AINES 1(2.4%). Anti-TB aIY drugs used were Amikacina 21(51.2%), Kanamicina 2(4.9%), Capreomicina 17(41.5%). Months with aIY drugs 6.29(5, 8), Glucose 146(70, 525), Albumin 3.53 (2.06-4.56), Hemoglobine 13.3(6.9, 18.5). Cr 0.75(0.32, 1.70), eGFR CKD-EPI 108 ml/min/1.73m2 SC(40.5, 150.8). K-DOQI at BL were: Stage 1: 31 (75.6%), 2: 8 (19.5%), 3: 2 (4.9%). Decrease renal function was observed at the end of treatment: CKD-EPI 108.18 (100.51-115.84) versus 91.62 (83.09-100.17) ml/min/1.73 m2 SC p=<0.001. Cr 0.75(0.61-0.78) versus 0.94(0.81-1.06) mg/dL p=0.01. At the end of treatment K-DOQI stages were: Stage 1: 22(55%), 2: 12(30%), 3: 6(15%). Nephrotoxicity was present in 23(56%) of cases, 7 (30.4%) recovered BL renal function. Factors associated with toxicity or loss of eTFG were not found. At analysis all cultures were negative with treatment.

Conclusions: MDR-TB treatment is effective but highly nephrotoxic. Strategies to prevent loss of eTFG are necessary.

PUB233

Improving Quality of Life in Elderly Hemodialysis Patients: A Single Center Prospective Study Sangeetha Murugapandian,[1] Omid Bakhtar,[1] Natasha Sharda,[1] Bijin Thajudeen,[1] Amy Nicole Sussman.[1] *[1]Nephrology, Univ of Arizona Medical Center, Tucson, AZ; [2]Nephrology, Southern Arizona VA Health Care System, Tucson, AZ.*

Background: Quality of life (QOL) is an important factor determining morbidity and mortality in Elderly Hemodialysis patients. Identification of factors like functional decline, frailty, depression, delirium, cognitive decline and malnutrition early in the course of end-stage renal disease (ESRD) helps in improving QOL.

Methods: We did a prospective interventional study in patients on hemodialysis above the age of 65 at SAVAHCS, Tucson, AZ. The following information was obtained at baseline and 6 months.

Baseline	6 months
Physical Exam (PE), Frailty Phenotype (FP)	PE, FP
Surveys:Qualiy of life (QOL), Activities of daily living (ADL),Nutritional Status (NS)	QOL,ADL,NS
Mini-mental status Examination (MMSE) confusion asessment method (CAM), Geriatrics Depression scale(GDS)	MMSE,CAM,GDS

Depending on initial assessment patients were referred to physical rehab, psychology, psychiatry,neurology or dietician as needed.

Results: A total of 14 patients were enrolled in the study with mean age 75.23±8.89 years. We used CAM, ADL, MMSE, GDS, nutrition and Handy Helper score at baseline and 6 months. Initial scores were 3.42±1.02, 0, 80.10±4.44, 27.35±2.82, 6.64±1.40, 22.57±4.67,45.71±7.07 respectively. At six months the corresponding scores were 3.14±1.19, 0, 86.92±3.38, 26.35±3.53, 4.50±1.41, 23.42±3.53 and 48.92±7.25 respectively. GDS was the only variable which showed a statistically significant difference (p value =0.044). QOL assessment at 6 months showed improvement in all variables physical functioning, physical role, emotional role, energy, general health and emotional well-being) except social functioning.

Conclusions: A comprehensive evaluation of all factors which can affect the QOL in ESRD patients should be carried out early and at regular intervals with timely interventions. Depression is a major under diagnosed problem in patients and early recognition and treatment of depression could improve QOL especially in elderly patients.

PUB234

Prevention of Iodinated Contrast Media Nephrotoxicity in Patients with Renal Insufficiency in an Outpatient Basis Maite Rivera, Nuria Rodriguez Mendiola, Victor Burguera, Rodrigo Hernandez Loyola, Viviana Raoch Michaels, Fernando Liano, Carlos Quereda. *Nephrology, Hospital Ramón y Cajal. UAH Univ. IRyCis, Madrid, Spain.*

Background: Prophylaxis of contrast media nephrotoxicity consists of intravenous hydration before and after the radiological procedure, which carries the need of hospitalization. Our aim was to analyze the efficiency of a preventive protocol consisting of a unique dose of intravenous saline hydration and intravenous acetylcysteine before the administration of the iodinated contrast on an out-patient basis.

Methods: Between January and December 2013, 28 patients with chronic renal insufficiency (GFR<60 ml/min/1,73 m2) were treated with this preventive protocol. The radiological exploration in all of them was a computed tomography (CT). Contrast dose:100 ml of Iohexol (concentration of 350 mg/ml). All patients had a stable renal function. Serum creatinine (SCr) before and in the following ten days was recorded. The explorations with iodinated contrast were programmed in the afternoon. During the previous morning, the patients received between 500 and 1000 ml of 0.9 percent saline (according to their hydration grade and / or cardiologycal situation) plus 1 gram of acetylcysteine. Hydration after the iodinated contrast was not prescribed. The treatment was administered in our Nephrology Day hospital, therefore patients were not hospitalized.

Results: Mean age: 62 years. 71% male. 82% hypertense, 39% diabetic and 25 % had vascular peripheral disease. The average Charlson index:6.1 ± 2.5 (2-11). 74% had treatment with ACE i orARB II. 9 patients (32%) had renal chronic disease stage 3; 7 patients (25%) stage 4; and 12 patients (43 %) stage 5. Mean basal SCr was 3.9 ± 2.7 mg/dl and 4.2 ± 2.9 mg/dl after the CT (p=0.09). According contrast nephrotoxicity criterium by KDIGO (relative increase of at least 25 % on the basal level of creatinine) only one patient worsened renal function, that recovery 60 days later.

Conclusions: The administration one dose of 0.9 percent saline with acetylcysteine in an outpatient basis before iodinate contrast in patients with renal insufficiency and risk factors, without posterior hydration, is sure and cheaper since it avoids hospitalization.

Funding: Government Support - Non-U.S.

PUB235

Infections in the Chronic Kidney Disease Population Sameena Z. Iqbal,[1] Jiajia Liu,[2] Paul E. Barre,[1] Murray L. Vasilevsky.[1] *[1]Medicine, McGill Univ Health Centre, Montreal, QC, Canada; [2]Medicine, McGill Univ, Montreal, QC, Canada.*

Background: Chronic Kidney Disease (CKD) is associated with an increased risk of infections. Infections are one of the leading cause of death in end stage kidney disease. The prevalence of infections in Stage 3-5 CKD and the effect of infections on the rate of GFR decline was assessed.

Methods: In a retrospective cohort study of a university center, we randomly identified 202 patients followed in the CKD clinics between 2006 and 2012. Eleven subjects were excluded because of acute kidney injury. Study population demographic data, date of diagnosis of CKD, cause of renal disease, mean change in eGFR using CKD epi formula, any infection requiring antibiotics, genitourinary infections and Charlson comorbidity score (CCS) obtained from hospital charts. All subjects were divided into two groups, those who had at least one infection and those who had no infections during the study period. Generalized estimating equations with poisson distribution were applied to assess the association of GU infections and rate of decline in renal function.

Results: One hundred and ninety one subjects were followed for 2.8 (0.5-11.1) years. The median age was 73 (19-107), 59.7% were male and the median CCS was 4 (1-12). The overall rates of all infections treated with antibiotics and GU infections were 30.7 (26.3-35.1 and 22.8 (19-26.5) per 100 patient years, respectively. The median rate of eGFR decline was 3.9 ml/min/1.73m^2/year (-25-37). The occurrence of at least one or more GU infection was associated with an increased rate of eGFR decline (RR1.5: 95%CI 1.3-1.8) unadjusted and (RR 1.4: 95%CI 1.2-1.6) adjusted for CCS, age, baseline eGFR, renal disease and gender.

Variable	RR (95% CI)
Baseline eGFR per 1 ml/min/1.73m^2	1.016 (1.010-1.022)
Age	0.985 (0.981-0.989)
Gender	0.88 (0.77-1.00)
CCS	1.10 (1.07-1.13)
Ttubulointerstitial disease (ref Diabetes and hypertension)	0.49 (0.38-0.64)
at least one GU infection	1.4 (1.2-1.6)

Conclusions: Based on these results a significant association of GU infections and the rate of decline in eGFR is noted. Future studies should assess the role of the prompt treatment of such infections and the potential contribution to renal protection.

PUB236

Analysis of Clinical Features in Relapsing MPO-ANCA-Positive Eosinophilic Granulomatosis with Polyangiitis Soko Kawashima, Shinya Kaname, Kazuhito Fukuoka, Yoshinori Komagata, Yoshihiro Arimura. *First Dept of Internal Medicine, Kyorin Univ School of Medicine, Mitaka, Tokyo, Japan.*

Background: Eosinophilic granulomatosis with polyangiitis (EGPA) is characterized by precedent bronchial asthma, eosinophilia and vasculitis, with about half of the cases being MPO-ANCA positive. However, clinical features of relapsed cases with EGPA are not well investigated.

Methods: Fifteen patients with MPO-ANCA -positive EGPA (8 male and 7 female) who were admitted to our hospital after 1998 were followed up. At the onset, all patients fulfilled the Chapel Hill Consensus Conference (CHCC) classification criteria for EGPA. The relapse was defined as the occurrence of >1 BVAS items caused by active vasculitis after having achieved remission. We analyzed the clinical features including renal and neurological involvement at the time of relapse with respect to the presence or the absence of MPO-ANCA and eosinophilia.

Results: During the study period, 15 relapses were observed in 8 of the 15 patients investigated, with an average time interval from the onset for 47.8 months. At the time of relapses, serum MPO-ANCA was positive in 13/15 (87%), associated with (7/13; 54%) or without (6/13; 46%) eosinophilia. There were two relapses with negative MPO-ANCA titers (2/15 relapses), one of which associated with eosinophilia. Bronchial asthma was seen in 6/15 episodes, and peripheral neuropathy in 8/15 episodes. In 5 patients who initially complicated crescentic glomerulonephritis, one patient led to end-stage renal failure and 4/5 patients achieved renal remission, only one of which (1/4) showed 2 episodes of renal relapses with MPO-ANCA positive and negative, respectively.

Conclusions: The patients with MPO-ANCA-positive EGPA may see relapses within several years by variable clinical findings and even in the absence of an increase in MPO-ANCA titers and eosinophilia.

PUB237

Anti-Phospholipase A2 Receptor Antibody Is a Useful Biomarker for Screening of Hidden Idiopathic Membranous Nephropathy behind Diabetes with Nephrotic Syndrome in Japanese Patients Reiko Muto, Shin'ichi Akiyama, Shoichi Maruyama, Seiichi Matsuo. *Nephrology, Nagoya Univ Graduate School of Medicine, Nagoya, Japan.*

Background: We have shown that the anti-phospholipase A2 receptor antibody (aPLA2R) was also powerful differential biomarker of idiopathic membranous nephropathy (iMN) in Japanese patients. On the other hand, in the usual case of Japan, a patient with diabetes mellitus and nephrotic syndrome (DM-NS) is diagnosed with diabetic nephropathy solely based on clinical features such as a presence of retinopathy or long duration of diabetes without renal biopsy. Therefore, we infrequently overlooked the hidden iMN in DM-NS. Here, we tried to screen the iMN behind DM-NS by the aPLA2R test.

Methods: This study recruited three patients with DM-NS (case 1-3). Case 1 was a-48-year-old female under medication with DPP-4 inhibitor who had 5-year-diabetic history and no retinopathy. Her HbA1c and urinary protein were 6.5% and 5.26g/gCr, respectively. Case 2 was a-49-year-old male. His diabetes history was unknown, but HbA1c was 15.4% at the initial visit. He had proliferative retinopathy and 14.7g/gCr of urinary protein. Case 3 was a 54-year-old male with 9-year-diabetic history and simple retinopathy. His HbA1c and urinary protein were 7.2% and 6.75g/gCr, respectively. An aPLA2R in plasma samples from these patients were tested by Western blot analysis with recombinant PLA2R protein under non-reducing condition.

Results: We specifically identified aPLA2R in plasma from case1. Then, we performed a renal biopsy. Light microscopy revealed capillary wall thickening and spikes. Immuno fluorescence (IF) showed a fine granular and peripheral capillary loop staining for IgG and C3. Electron microscopy showed sub-epithelial immunedeposits. Additionally IF showed IgG4-dominant deposition in the glomerular basement membrane. Gastrofiberscopy and thoraco-abdominal computed tomography showed no abnormalities. On basis of these results, she was diagnosed with iMN.

Conclusions: This study demonstrated that the aPLA2R test enabled us to discover the hidden iMN behind DM-NS in Japanese patients and suggested that the aPLA2R test was noninvasive diagnostic methodology for iMN which was complicated with DM-NS.

PUB238

The Level of Urinary α1 Microglubulin Excretion Is a Useful Marker to Indicate Existence of Peritubular Capillaritis in Antineutrophil Cytoplasmic Antibody Associated Vasculitis Naro Ohashi,[1] Tomoyuki Fujikura,[1] Takayuki Tsuji,[1] Yukitoshi Sakao,[2] Akihiko Kato,[2] Hideo Yasuda.[1] *[1]Internal Medicine 1, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan; [2]Blood Purification Unit, Hamamatsu Univ School of Medicine, Hamamatsu, Shizuoka, Japan.*

Background: Antineutrophil cytoplasmic antibody (ANCA) associated vasculitis causes a vasculitis of small vessels in the kidney (i.e. arterioles, glomerular or peritubular capillaries, or venules). Although crescentic glomerulonephritis is a common histological finding, the incidence of peritubular capillaritis (PTC) or arteriolitis has not been fully known. Moreover, the laboratory data that reflects the degree of renal histological damage and that distinguishes PTC or arteriolitis has not been clarified.

Methods: We investigated the laboratory data and histological findings in 11 patients (2 men and 9 women, mean age: 70.3±3.3 years) whose renal biopsies were performed from 2009 to 2014 and whose disease was diagnosed as ANCA associated vasculitis. PTC was defined by the histological findings that infiltrated cells were accumulated in the peritubular capillary associated with the disruption of the capillary wall that was stained by anti-CD34 antibody.

Results: Myeloperoxidase (MPO)-ANCA was positive in all patients. PTC and arteriolitis were detectedin 6 patients (54.5%), respectively. There was only significant positive relationship between the levels of urinary α1 microglobulin (u-α1MG) excretion and the percentage of tubular atrophy and interstitial fibrosis (r=0.67, p=0.035) between the laboratory data and the histological findings. No significant differences were found among the laboratory data with or without arteriolitis. However, the levels of u-α1MG excretion with PTC were significantly higher than those without PTC (75.2±19.5 mg/dl versus 15.0±3.6 mg/dl, p=0.035).

Conclusions: PTC or arteriolitis is caused at a high rate independently of crescentic glomerulonephritis in ANCA associated vasculitis patients. The levels of u-α1MG excretion reflect the degrees of tubular atrophy and interstitial fibrosis. Moreover, high levels of u-α1MG excretion suggest that it is possible that PTC not arteriolitisexists in ANCA associated vasculitis patients.

Funding: Government Support - Non-U.S.

PUB239

Can Rhabodomyolysis Be the First Manifestation of HIV? Rhea Bhargava,[1] Abdelrahman Abdallah Abohashem Aly,[1] Christine Corbett,[2] Jim I. Mertz,[2] Reem Mustafa.[2] *[1]Internal Medicine, Univ of Missouri, Kansas City, MO; [2]Nephrology, Univ of Missouri, Kansas City, MO.*

Background: Renal complications in patients infected with HIV are usually secondary to the virus infectivity or toxicity from the HIV treatment. Renal tubular acidosis (RTA) is a known complication of some of the antiretroviral medications. The presence of RTA in HIV patients without treatment is rare with very few cases reported.

Methods: A 49-year-old Caucasian woman with no significant past medical history except admitting to abusing intravenous drugs in the past. She presented to the emergency room with a 3 week history of muscle cramps, weakness and dark urine along with nausea, vomiting and decreased urine output in the last 3 days. She was not taking any medications at home. Physical examination was unremarkable, except for diminished reflexes in the lower extremities. Initial Laboratory data revealed extremely low K of 1.3, Na of 124, Cl of 83, HCO3 of 13, Cr of 6.7, BUN of 65 and CK of 54,275. ABG showed pH 7.38, PaCO2 27, PaO2 64.8. Urine pH was 6.5 and urine anion gap was positive. The patient's rapid HIV testing was positive and UDS was positive for amphetamine. Patient was admitted to the ICU and treatment was started with aggressive hydration, IV bicarbonate and potassium replacement along with temporary hemodialysis with high K bath. Even after resolution of her rhabdomyolysis, her electrolyte levels never fully corrected and she was discharged on bicarbonate and potassium supplements. Unfortunately, the patient was readmitted for severe hypokalemia 3 months later but without rhabdomyolysis or renal failure and was discharged with a higher dose K replacement. Diagnosis of acquired distal RTA was made in the follow up visits due to the persistent hypokalemia, normal anion gap metabolic acidosis, alkaline urine, with the absence of features suggestive of chronic RTA.

Conclusions: To the best of our knowledge, based on literature review, this is the first case of HIV diagnosed with an initial presentation of rhabdomyolysis. We believe that acute renal failure was due to hypokalemia precipitating rhabdomyolysis caused by HIV-induced dRTA which was further exacerbated by amphetamines.

PUB240

Eculizumab in Pregnancy-Associated Atypical Hemolytic Uremic Syndrome

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Background: Atypical hemolytic uremic syndrome (aHUS) is a rare disease resulting from dysregulation of the alternative complement pathway and is characterized by microangiopathcic hemolytic anemia, thrombocytopenia and renal failure. Pregnancy-associated aHUS (P-aHUS) is rare, occurring in about 4% of cases, with outcome being poor for both mother and fetus. The monoclonal antibody Eculizumab (ECU) has induced durable response in treatment of nonpregnant patients with aHUS. We report a case of P-aHUS that benefited from ECU.

Methods: 30-year-old 22 weeks pregnant woman with past medical history of hypertension and systemic lupus erythematosus presented with 1 week history of abdominal pain and leg swelling. She denied any recent diarrhea. On exam, patient had 3+ pitting edema of her legs. Laboratory findings were notable for Hemoglobin 4 and Platelets of 54,000. Reticulocyte count was 4.8%, INR 1.0, PTT 29. LDH was 4664 with Creatinine 9.5, K+ 5.6 and GFR 6. Patient's kidney function was normal 4 months before. Liver enzymes, C3 and C4 levels were normal. ADAMTS13 activity was normal at 67%. ANA titer was 1:80 and DNA antibody was negative. Due to the concern of aHUS in setting of volume overload, anuria and a history of SLE, plasma exchange (PEX) and hemodialysis (HD) were started along with red cells transfusion and IV steroids. Following 2 weeks of treatment, patient remained anuric, had elevated LDH and thrombocytopenia. ECU therapy was started with rapid recovery of platelet count, LDH and Hemoglobin. Patient delivered a healthy 25 week baby by C-section and was discharged on ECU and prednisone taper.

Conclusions: Thrombotic microangiopathies during pregnancy are rare and life-threatening with poor response to PEX. In our case, treatment with ECU was well tolerated by both mother and fetus and resulted in cessation of the disease process, supporting the use of ECU as a therapeutic option in P-aHUS. Role of timing of ECU therapy in preventing need for HD in P-aHUS remains to be elucidated.

PUB241

Surimposed Paraneoplastic Minimal Change Disease with Nephrotic Syndrome in an Elderly Woman on the Course of a Sarcoma : A Rare Association

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Background: Minimal Change Disease (MCD) accounts for 20% of all cases of idiopathic nephrotic syndrome (NS) in elderly patients (pts) .And about 25% of pts have an underlying malignancy, must often lymphomas.We report the case of a NS with MCD associated with a sarcoma in an elderly woman.

Methods: An 72- year old white female with a history of hypertension and thyroid insufficiency developped a rhabdomyosarcoma in June 2012 on her inner face of the left thigh and was treated by curative surgical removal and radiotherapy.Two pulmonary nodules were present with a deep venous thrombosis of the inferior vena cava treated .In October 2013 she exhibits NS with heavy proteinuria (10 gr/day), microscopic hematuria, severe hypoalbuminemia (15g/L) and an increased GFR (hyperfiltration).The Bence-Jones proteinuria was négative.Possible causes of primary or secondary proteinuria were ruled out.A kidney biopsy was performed and showed minimal glomerular changes (MCD). Methylprednisolone (0.5 mg/kg/day) was initiated (30 mg/day) with low molecular-weight heparin and diuretics.

Results: Sustained remission was induced and the pt was clear of symptoms except persistant microscopic hematuria and hyperfitration syndrome with actually 10 mg /day of prednisone with a serum Albumin of 40 g/L and proteinuria of 0.15g /day.

Conclusions: Few cases of Paraneoplastic MCD have been reported in pts with solid tumors (1).The pathogenic linkage between the two diseases is a possible tumor-induced elaboration of glomerular damaging factors.On the other hand, MCD is frequent and accounts for 20 % of idiopathic NS in Caucasian adults .The overall prevalence of malignancy in pts with NS is 22 % .The association of Hodgkin's disease with MCD is well documented .Sarcomas rarely,if ever,are a cause of MCD(2) and makes this case unsual. (1)Meyrier A and al : Minimal change NS revealing solid tumors,Nephron.1992,61 (2):220-3 (2)RichardJ.Glassock :Secondary MCD, Nephrol Dial Transplant(2003) 18 [Suppl 6]:vi52-vi58.

PUB242

Lupus Nephritis and Autoantibody Characteristics of a Single Center Cohort of Male Pediatric SLE Patients

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Background: Males represent 4–22% of all SLE patients, but it is unclear if gender should influence management. Studies of mostly adult patients suggest genetic, hormonal and environmental aspects of gender differences contribute to differences in presentation, clinical course and outcome, including a positive association between male gender and nephritis and a negative association with disease activity and outcome. While differences may exist, these findings are supported by limited evidence, particularly for children and adolescents with SLE.

Methods: We retrospectively reviewed records of the nearly 297 pediatric SLE patients followed in the TCH rheumatology and renal clinics between 1990 and 2014 to identify males with pSLE. Autoantibody features and renal involvement were collected.

Results: We identified 39 males (44% Hispanic, 21% Black, 15% Asian). The mean age of onset of disease was 11.9 ± 3.3 yrs old. The cumulative incidence of positive autoantibodies included 51% anti-dsDNA, 70% anti-phospholipid, 44% wanti-RNP, 33% anti-Ro (SS-A), 16% anti-Smith, 10% anti-La (SS-B), and 5% rheumatoid factor. Nearly half (n=20, 56%) developed lupus nephritis (LN), the majority of whom had class V lesions (70%). Class IV lesions were seen in 9 patients, including 4 with mixed histopathology. LN was a presenting symptom in all patients. Of these, 30% had acute kidney injury and 2 required hemodialysis. The proportion of boys with LN and anti-dsDNA antibodies was not significantly different from boys without LN (74% versus 50%, $p = 0.17$).

Conclusions: Although SLE predominately affects females, males with SLE represent an important subset of patients about which little is known. In our male cohort, rates of kidney disease and autoantibody positivity were similar to those published for females. However, autoantibody profiling may not be as useful in predicting kidney involvement in boys. Additional research is of upmost importance to improve quality of care and outcomes of male SLE patients.

Funding: NIDDK Support

PUB243

Un Unusual Presentation of Gaucher Disease with Renal Envolvement

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Background: Gaucher Disease(GD) is an autosomal recessive disorder due to β-glucocerebrosidase deficiency wich results in lysosomal accumulation of glucosylceramide,leading to hepatosplenomegaly,blood cytopenia,and bone,pulmonary and neurological manifestations.Renal involvement reports are limited.We present a GD case with proteinuria as first manifestation of illness.

Results: 45-year-old man with proteinuria detected in World Kidney Day renal disease screening,GD and endogamy family history. He complained of left hip pain and spontaneous bruising in the past. Unremarkable physical examination. Laboratory:platelet count 95.000/mm3, normal renal function, uric acid(Ua)5.1 mg%,calcium(Ca) and phosphate(P)9.1 and 2.1 mg% respectively,IgG 1973 mg/dL,IgA 362 mg/dL,IgM 302 mg/dL, β_2 microglobulin1.6 µg/mL, Chitotriosidase 627.3nmol/l/h. 24-hour urine(U) collection: 1.31 g of proteinuria, P 1.69 g,Ca 220 mg,Ua 700 mg, P tubular reabsortion 70.2, ratio UCa/creatinine 0.18. Diagnosis of incomplete Fanconi Syndrome was done. S protein electrophoresis:elevated monoclonal γ protein and elevated β_1 globulin. Enzymatic diagnosis by dried blood spot test:βglucosidasa 1.5 nmol/l/h. Leukocytes-βglucosidasa activity:3.5nmol/mg/h. Genotyping: Patogenic variants in exon 9, p.N409S, heterozygous. S immunofixation electrophoresis: Monoclonal immunoglobulins IgG and IgA kappa (λ) light chains. U Immunofixation: No abnormal chains. Bone marrow biopsy:Gaucher Cells infiltration and myelofibrosis. Treatment with enzyme replacement therapy (Imiglucerase,60 U/Kg dosis) was initiated and 6 months after,renal biopsy disclosed no abnormalities (optical,immufluorescence and electronic microscopy).

Conclusions: We report an unusual GD presentation,with proteinuria and renal tubular functional alterations.The absence of tubular changes in renal biopsy could show that manifestations of tubular alteration were not due to disproteinemia but GD and the Imiglucerase therapy could improve potential accumulation of glucocerebroside in the lysosomes of tubular renal cells.

PUB244

Clinical Efficacy of Glucocorticoids and Immunosuppressive Treatment for Patients with Lupus Podocytopathy Zhihong Liu, Weixin Hu, Ying-Hua Chen, Hao Chen, Lihua Zhang, Zheng-Zhao Liu. *National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing Univ School of Medicine, Nanjing, Jiangsu, China.*

Background: Lupus podocytopathy is a newly recognized subtype of lupus nephritis, buttheclinical efficacy of glucocorticoids (GC) and immunosuppressant treatment for the patients have not been well assessed.

Methods: Thirty-seven SLE patients (34 females, 3 males, mean age 31.1 ± 11.6 years) with nephrotic syndrome and biopsy-provenpodocytopathy were recruited. The patients were given prednisone alone (GC group) or GC plus immunosuppressant (Combination group) during induction or maintenance treatment. The remission and relapse rates were retrospectively analysed.

Results: Initial treatment led to remission in 97.4% of thepatients. The complete remission (CR, defined by normallevels of urine protein, serum albumin and SCr) rates were similar in the two groups (81.5% versus 81.8%), while the median treatment duration to achieve CR was much shorter in GC group (4 versus 12 wk in combination group, p<0.05). In the first maintenance phase, the relapse rate was much higher in GC group (81.8% versus 38.5% in combination group, p<0.05). All relapsed patients achieved remission after thesecond GC or combination therapy, and the CR rate was 83.3%. The patients were followed up for 6 to 125 months (median 62), during which 20 patients (52.6%) experienced one or more relapses. In the totalof68 maintenance treatments (16 in GC group, 52 in combination group) during the follow-up, relapse rate was much higher in GC group (75.0% versus 40.4% in combination group, p<0.05). Allfour patients with GC+MMF maintenance treatmentdid not relapseduringthe24-46mfollow-up,incontrastwiththe relapse ratesinthe patientswith GC plus FK506(58.3%), AZA(50%), Tripterygium wilfordii(42.9%) or Leflunomide(33.3%). Re-biopsy in 9 patients after relapse showed histologic changes in 2 patients and notin the other 7.

Conclusions: GC may be the first-line therapy for remissioninduction in thepatients, while GCcombined with immunosuppressant may be used in the maintenance for a lowerrelapse rate. Whether GC+MMF is an optimal maintenance regimen needs prospective studies.

Funding: Government Support - Non-U.S.

PUB245

Anti-C5 Monoclonal Antibody - Eculizumab: Use in the Treatment of Life Threatening ANCA Associated Vasculitis with Pulmonary Hemorrhage and Profound Anemia John Niles, Karen A. Laliberte, Andrew P. Murphy, Katherine M. Cosgrove, Robert Makar. *Nephrology /Medicine, Massachusetts General Hospital, Boston, MA.*

Background: We report the first case of active vasculitis treated with anti-C5 monoclonal antibody (anti C5). Animal models show the importance of C5 in the development of ANCA injury. We describe the results using anti-C5 to treat a patient with immediate life threatening ANCA vasculitis. Due to religious beliefs, she refused blood products and severe anemia prevented the use of cyclophosphamide and plasma exchange.

Methods: Case description: 61 yo woman with new ANCA vasculitis (MPO), diffuse alveolar hemorrhage, glomerulonephritis and life threatening anemia. Her creat was 0.95, hct 20.2, UA with RBCs and protienuria. She required high flow O2 to maintain an O2 sat > 90%. Treatment: In place of cyclophosphamide and plasma exchange, she was given methylpredisolone 250mg IV Q6 hours x 9.5 days, Rituximab 1000mg IV on days 0 and 49, followed 8 hours later with the 1st dose of Eculizumab 900mg IV. She received a total of 3 doses of Eculizumab: #2 on day 7 and #3 on day 14. Blood draws were limited. Aminocaproic acid 1000mg IV /hour was given days 1-5. EPO 40K units SC x4 doses, and Venofer 1000mg IV were given to stimulate erythropoiesis.

Results: Clinical course: Her pulmonary function remained precarious, but did not progress. Her hematocrit held at 17.4%. On day 5 her oxygenation improved. Her anemia improved to 23.8% at discharge. Her last dose of anti-C5 was day 14. Daily cyclophosphamide 100 mg was added on day 18. She was discharged on day 19. Between day 18 and day 49 her creat rose from 1.04 to 3.03. ANCA level remained high. Her B cells remained zero.

Conclusions: The patient was treated for 3 weeks with Rituximab, Eculizumab and steroids, during which time her lungs improved, her Cr remained stable and anemia started to improve, but her ANCA remained high. After fininshing Eculizumab, her renal function deteriorated despite cyclophosphamide, continued B cell depletion and continued high dose steroids. The findings suggest that Eculizumab was highly effective in blocking active vasculitis, but that active vasculitis returned rapidly when Eculizumab was discontinued.

PUB246

Lupus Vasculitis with Clinical Features of HUS/TTP Akinari Sekine, Yoshifumi Ubara, Junichi Hoshino, Masahiro Kawada, Koki Mise, Kenmei Takaichi. *Nephrology, Toranomon Hospital, Minato-ku, Tokyo, Japan.*

Background: D'Agati classified the vascular lesions of lupus nephritis into 5 categories in Heptinstall's pathology of the kidney. Noninflammatory necrotizing vasculopathy (called lupus vasculopathy) and thrombotic microangiopathy were distinguished from each other as the vascular lesions of lupus nephritis. In both types, there is severe narrowing or occlusion of preglomerular arterioles. The former type shows positive staining for immunoglobulin G (IgG) and/or complement components (C3 or C1q), and clinically progresses to severe renal insufficiency. The latter type is negative for IgG or complement, and presents with distinct clinical syndromes such as HUS/TTP.

Methods: We evaluated 4 patients with histological lupus nephritis (Class III in 1, Class IV in 2, and Class V in 1) and clinical features of TTP/HUS such as acute renal failure, microangiopathic hemolytic anemia, thrombocytopenia, fever, and neurologic symptoms.

Results: Immunosuppressive therapy with plasmapheresis was performed for 4 patients. Two patients progressed to end-stage renal failure requiring dialysis and one of them died, while the disease subsided in two patients. The two refractory patients showed positive staining for IgG and C3 along the walls of small arteries, while intraluminal material was negative for IgG and C3 (although it showed positive staining for PTAH indicating fibrin deposition). The two patients who responded to therapy showed positive staining of intraluminal material for IgG, C1q, and C3, as well as staining along small arterial walls, while intraluminal material was negative for PTAH.

Conclusions: These findings indicate that immunosuppressive therapy can control this disease when intravascular thrombosis is closely related to immune complexes associated with activation of the early complement components C1q and C4. However, when intravascular thrombosis is not closely related with these factors and involves fibrin deposition, immunosuppressive therapy will not be effective. Lupus vasculitis with clinical features of HUS/TTP syndrome should be kept in mind.

PUB247

Usefulness of Birmingham Vasculitis Activity Score after Initial Treatment as a Predictor for Renal Prognosis in ANCA-Associated Vasculitis Ryusuke Yotsueda,[1] Akihiro Tsuchimoto,[1] Shunsuke Yamada,[1] Kosuke Masutani,[1] Kazuhiko Tsuruya,[1,2] Takanari Kitazono.[1] *[1]Depts of Medicine and Clinical Science, Kyushu Univ, Fukuoka, Japan; [2]Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu Univ, Fukuoka, Japan.*

Background: Birmingham vasculitis activity score (BVAS) is used to evaluate activities of ANCA-associated vasculitis (AAV). However, it is not clear whether BVAS can be a useful predictor of renal prognosis in AAV patients.

Methods: In this retrospective study, we reviewed 70 AAV patients with 32 males and 38 females, who were treated in our hospital from 1989 through 2013. We identified BVAS ver. 3 at admission and at one month after diagnosis, and evaluated the association between BVAS at each points and renal prognosis.

Results: At diagnosis, 68 patients showed renal impairment and 35 showed pulmonary complications. The median value (interquartile range) of BVAS at admission was 16 (13-20). Fifty-nine and 11 patients were treated with steroid pulse therapy followed by oral corticosteroid and oral corticosteroid only, respectively, while 45 patients were treated with additional immunosuppressants. After the initial treatment, BVAS at one month decreased to 5 (5-5). During a median (interquartile range) follow-up of 731 (58-2,324) days, 17 patients reached end-stage renal disease (ESRD). The likelihood of reaching ESRD within one year was not associated with BVAS at diagnosis, but significantly associated with BVAS at one month (area under the curve 0.78, sensitivity 56%, specificity 91%, cut off 6). In the Cox's hazard models, the group with BVAS at one month ≥6 showed a significantly higher risk of ESRD, independent of renal function at diagnosis; hazard ratio (95% confidence interval), 4.0 (1.4-11.3) (p = 0.010).

Conclusions: BVAS at one month was a useful predictor for renal prognosis in AAV patients. This finding suggests that evaluation of disease activity using BVAS after initial treatment might be important to determine the maintenance treatment for AAV.

PUB248

Percutaneous Kidney Biopsy Performed by Nephrology Fellows in Academic Center: High Efficacy and Low Complication Rates Alcino Pires Gama, Elerson Costalonga, Lilian Carmo, Renato Antunes Caires, Janaina De Almeida Mota Ramalho, Vivian Lumi Onusic, Cristiane B. Dias, Luis Yu, Lectícia Jorge, Viktoria Woronik. *Nephrology, Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Previous studies showed that complications occur in about 15% of percutaneous kidney biopsies. In University of Sao Paulo, fellows perform all kidney biopsies under supervision. The aim of this study is to evaluate incidence of biopsy-related complications performed by non-experienced professionals in an academic center.

Methods: Kidney biopsies between 2009 and 2013 were retrospectively analyzed. All procedures were performed on an inpatient basis, ultrasound guided and subjects fulfilled these criteria: BP<140/90mmHg, Hb>10g/dl, platelet>100k, urea<140mg/dl and INR>1.2. After the procedure, the patients were observed for 24h.

Results: 668 procedures were performed and 94% of the samples were satisfactory. The main clinical indication was nephrotic sd.(38%), followed by nephritic sd.(19%), and lupus nephritis(12%). A post biopsy Hb decline≥1.5g/dL was observed in 69 subjects(10%). This group had a higher serum creatinine levels(sCr) and an increased risk of major complications(hematuria, symptomatic hematoma or blood transfusion). Three subjects(0.45%) underwent arteriography and there was no death related to the procedure.

	ΔHb≥1.5 (n=69)	ΔHb<1.5(n=599)	p
Age/y	41.8±19	40.5±16	ns
%Male	35%	41.2%	ns
Hbpre(g/dl)	11.9±1.7	11.8±1.7	ns
sCr(mg/dl)	2.57±1.7	2.07±1.6	0.015
%Major complication*	26.2%	6.3%	<0.001

Table 1. Clinical features on subgroups.
* symptomatic hematoma, macroscopic hematuria or blood transfusion.

Based on the ROC curve (AUC 0.81, CI 0.78-0.84), the best SCr cutoff value to identify patients with increased bleeding risk was 1.5 mg/dl.

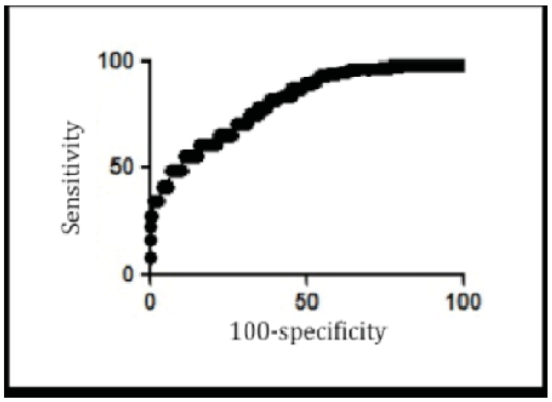


Figure 1: Serum creatinine vs ΔHb≥1.5 g/dl. When sCr≥1.5mg/dl S:87% e E:55%. AUC 0.81.

Conclusions: Renal fellows can safely perform percutaneous kidney biopsy with low rates of complications.

Funding: Government Support - Non-U.S.

PUB249

Tonsillectomy and IgA Nephropathy: Insights Into the Modifications of Innate Immunity and Regulatory T Cells Induced By Tonsillectomy in Children with ENT Indications to Tonsil Ablation Luca Vergano, Elisa Loiacono, Licia Peruzzi, Alessandro Amore, Massimiliano Bergallo, Rosanna Coppo, Vincenzo Landolfo, Paolo Tavormina, Roberto Albera. *Citta della Salute, Turin, Italy.*

Background: The role of tonsillectomy as immune suppressive tool in IgA nephropathy is debated. Comparison of results between Asian and European cohorts is biased since tonsillectomy is performed because of the diagnosis of IgAN in Asia, while in Europe it strictly follows Otolaryngology (ENT) indications.

Methods: To address the effect of tonsillectomy "per se" in subjects with ENT indication to tonsillectomy we investigated mucosal immunity activation, including Toll-like receptors (TLRs 2,3,4,9), which trigger T cell response via proteasome (PS) to immunoproteasome (iPS) switch and T regulatory cells (FoxP3/Th17) in mononuclear cells from 25 children before (T0) and 15 days (T1) after tonsillectomy and 30 healthy controls (HC).

Results:

mRNA	HC	T0	T2	Kruskall-Wallis test p
TLR2	0.98 (0.59-1.68)	1.86 (1.19-2.85)	1.30 (1.06-2.72)	P 0.0061
TLR3	0.86 (0.52-1.43)	1.96 (1.31-2.99)	1.45 (1.07-2.66)	P 0.0004
TLR4	1.03 (0.65-1.64)	4.07 (2.43-6.38)	3.47 (1.36-9.10)	p< 0.0001
LMP2/ β1	0.78 (0.60-0.97)	3.00 (1.17-3.67)	1.96 (1.08-4.07)	p< 0.0001
LMP7/ β5	1.03 (0.83-1.29)	2.18 (1.54-2.38)	2.01 (1.45-2.83)	p< 0.0001
MECL1/β2	0.96 (0.75-1.16)	1.31 (0.83-1.61)	0.99 (0.87-1.41)	P 0.047
IL17	0.85 (0.24-1.56)	3.08 (0.62-4.53)	2.15 (0.85-6,65)	P 0.003

A strong activation of innate immunity with increased expression of TLR2, TLR3, TLR4, TH17 and switch Ps-iPS was observed at T0, with significant improvement at T1.

Conclusions: Tonsillectomy can improve over a short time the hyperactive mucosal immune system which is detected in patients with ENT indication to tonsillectomy. Whether this benefits will last over years during the follow-up of IgA nephropathy and contribute to final clinical outcome remains a matter to be elucidated.

Funding: Private Foundation Support

PUB250

The Long-Term Prognosis of Idiopathic Membranous Nephropathy Patients Treated By New Algorithm Including Ponticelli Protocol Daigo Nakazawa, Saori Nishio, Junya Yamamoto, Sekiya Shibazaki. *Dept of Medicine II, Hokkaido Univ School of Medicine, Sapporo, Hokkaido, Japan.*

Background: Idiopathic membranous nephropathy (IMN) patients are treated by the combination therapy with steroid and immunosuppressive agents based on the prognosis in the world. Contrastively, most IMN patients in Asia including Japan reveal good prognosis and steroid alone therapy is empirically used. However, the treatment is not effective for some patients, and the immunosuppressive therapy should be used restrictively. We decided a new restrictive algorithm including modified Ponticelli protocol (PCP) in Japanese patients with IMN.

Methods: We have identified all 35 patients with biopsy-proven idiopathic membranous nephropathy in 3 Hokkaido university affiliated hospitals from 2004 to 2013. We established therapeutic algorithm that IMN patients predicted poor prognosis are treated with PCP (methylprednisolone 0.5 g intravenously for 3 days followed by oral prednisolone; 0.5 mg/kg for 27 days alternated every other month either with cyclophosphamide; 1.2 mg/kg for 30 days. The whole treatment lasted 6 months). Poor prognosis patients defined as patients who had renal insufficiency or intolerable edema. In addition, we referred to poor prognosis factors (male, older age, hypertension, urinary IgG,β_2MG, FSGS and tubulointerstitial fibrosis). While, good prognosis patients are treated with supportive therapy. We retrospectively evaluated the renal survival rate and remission rate.

Results: 19 IMN patients who were diagnosed as poor prognosis treated with PCP and the complete remission (CR), partial remission (PR), and stable disease (SD) rate at the last visit was 42.1%, 52.6%, 5.3%, respectively. 16 IMN patients who were diagnosed as good prognosis treated with supportive care and the CR, PR, and SD rate at the last visit was 37.5%, 37.5%, 33.3%, respectively. In both groups, 10-year survival rate was 100%.

Conclusions: This is the first report that modified Ponticelli protocol could improve the long-term prognosis for Japanese patients with severe IMN and this algorithm could lead to the avoidance of unnecessary immunosuppressive agents for good prognosis IMN patients.

PUB251

Clinical Significance of Glomerular IgG Deposits in IgA Nephropathy Anthony Alvarado,[1] Sergey V. Brodsky,[2] Tibor Nadasdy,[2] Alice Hinton,[3] Brad H. Rovin.[1] *[1]Faculty of Medicine, Nephrology Dept, The Ohio State Univ, Columbus, OH; [2]Pathology Dept, The Ohio State Univ, Columbus, OH; [3]Div of Biostatistics, College of Public Health, The Ohio State Univ, Columbus, OH.*

Background: It has been suggested that the prognosis of IgA nephropathy (IgAN) is adversely affected if there is significant co-deposition of IgG in the glomeruli. We conducted this study to test whether Ig G deposition may help guide therapy by indicating poor clinical outcomes.

Methods: IgAN biopsies and their associated clinical data found in our biopsy archives between 2004 and 2013 were retrospectively reviewed. Patients were divided into groups showing only IgA deposition in their glomeruli and patients who had IgA+IgG deposition. Change in serum creatinine (SCr) over time, need for renal replacement therapy, and death were assessed in these groups. Covariates used were: proteinuria at biopsy, length of follow-up, treatment, degree of interstitial fibrosis, sex, age at biopsy and race. Univariate differences were evaluated with T-tests or univariate logistic regression. Multivariate linear regression was used to model changes in SCr over time.

Results: This cohort had 29 patients with IgA deposits and 18 patients with IgA+IgG depositis. The patients were mostly male (66%) and Caucasian (79%). Only 13% were Asian. On univariate analysis there were no differences in SCr, initiation of dialysis, kidney transplant or death between the groups. The change in SCr over time was not different between the groups using multivariate modeling (P=0.35), however proteinuria (P<0.001), length of follow-up (P<0.001) and treatment with corticosteroids and/or a cytotoxic agent (P=0.011) were all significant covariates for change in SCr.

Conclusions: Co-deposition of IgG with IgA in the glomeruli of IgAN patients did not affect the prognosis or outcome of the disease. A potential explanation of the difference between this study and other studies that did find an association of IgG deposits with worse outcomes may be that our cohort was predominantly Caucasian and others included 50 to 100% Asian population.

PUB252

Diffuse Extracapillary Crescentic Glomerulonephritis: Analysis of 106 Cases. A Single Center Experience Federico Varela,[1] Gustavo Cristian Greloni,[1] Diego Serra,[1] Griselda Bratti,[1] German Barrera Hugalde,[1] Silvia Beatriz Christiansen,[2] Guillermo Javier Rosa Diez.[1] *[1]Nephrology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; [2]Pathology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.*

Background: Rapidly progressive glomerulonephritis (RPGN) is defined by increase of creatinine twice from baseline in a no longer period than three months. Diffuse extracapillary crescentic glomerulonephritis (DECG) that cause RPGN has crescent formation in more than 50% of glomeruli. Objetive: Analyze prevalence, clinical findings, treatment and outcome of DECG in our rneal biopsies data.

Methods: Data was obteined from medical record during the period 1/1/1990 to 31/12/2011. We used a minimun count of 5 glomeruli for DECG diagnosis. They were classified into 3 types described by Glassock. In each group we analyzed, prevalence, degree of proteinuria and serum creatinine at renal biopsy. Variables were considered in 3 age groups: GI (< 40 years), GII (40-65 years) and GIII (> 65 years).

Results: We performed 2098 renal biopsies in this period. Prevalence of DECG was 5.2 %. Mean age was 53 +/- 21 years and 65% were female. DECG type I represent 5.6 % (6 cases), type II 50% (53 cases) and type III 44% (47 cases). Higher levels of creatinine were observed in DECG type I (mean 10 mg/dl) (p 0.0334) as well as alveolar hemorrhage and a greater number of affected glomeruli ($p < 0.05$). Value of proteinuria in nephrotic levels was observed in DECG type II (mean 4.4 gr/day) (p 0.0069). We found an increased frequency of diagnosis DECG type III (57,04 %) in elderly people and DECG type II in person under 40 years (45.2%) ($p>0.001$) We can´t found any variable statistics with anti-nuclear antibodies, rheumatoid factor or cryoglobulins. Treatment performed consisted steroids (92% IV), cyclophosphamide in 80% of patients and plasmapheresis in 8 %. The year of biopsy 45.8 % of patients were out of dialysis and 34.8 % remained on dialysis. Mortality was 17 % and there was no difference between the mortality by type.

Conclusions: Prevalence DECG in our renal biopsies data was similar that reported in the literature. Although clinical findings are similar to others authors, DECG type II was the most frequently found.

PUB253

Learning Points From 2 Cases of Eculizumab Responsive, Pediatric C3Nef Positive C3 Glomerulopathy Basema I. Dibas,[1] Hsiao Ling Lai,[2] Deborah E. Westbrook,[3] Leonard Curtis Hymes,[1] Benjamin L. Laskin,[4] Guillermo Hidalgo.[1] *[1]Pediatrics, ECU, Greenville, NC; [2]Internal Medicine and Pediatrics, ECU, Greenville, NC; [3]Pediatric Nephrology Section, VMC, Greenville, NC; [4]Pediatrics, Children's Hospital of Philadelephia, Narberth, PA.*

Background: We describe treatment success of 2 children with C3 glomerulopathy with eculizumab.

Methods: Case 1. 12 yrs old AAF who presented with stage 2 hypertension, anasarca and secondary enuresis. A renal biopsy showed exclusively C3 GN. She received methylprednisolone followed by prednisone.(HD) was initiated (s.cr 2.24 mg/dL, BUN 127mg/dL).

Labs	Admission labs (normal range)	Labs (in the interim) Day #	Current labs
BUN	29 mg/dL	↑ 127mg/dL (D #20)	19 mg/dL
s.Creatinine	1.58 mg/dL	↑ 2.45 mg/dL (D #20)	1.03 mg/dL
S. Albumin	1.9 gm/dL	Fluctuates	2.5 gm/dL
Urine protein/creatinine	4.6 mg/mg (<0.2)	↑17 mg/mg (mean ~6mg/mg)	2.3 mg/mg
C3	12 (80-150)	persistently low	30
C5	3.8 (6-20)	Normal then high	46.5
CH50	<10 (31-60)		<10
C_3Nephritic factor	0.69 (0.0-0.30)	Normal	0.28

She received 3 PE sessions. She had no improvement,D#29 she received 900 mg Eculizumab q wk for 3 doses and dose increased to 1200 mg.After 2 doses of eculizumab, she did not need HD or PE.

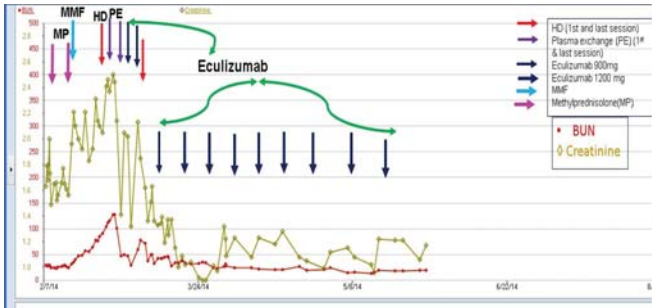


Figure 1. Serum Creatinine, BUN, Hemodialysis, Plasma exchange, Mycophenolate Mofetil, Methylprednisolone and Eculizumab treatment .

Results: Case2. 10 yrs old WF who presented at 6 yrs of age with acute post Strept. GN. Kidney biopsy showed dense deposit disease. First Lisinopril maintained normal u. P/C and renal function for 2.5 yrs. However, she developed new onset severe nephrotic syndrome and her kidney function worsened. She received MP, CRRT, PE and MMF. She received eculizumab,her kidney function recovered.

Conclusions: Eculizumab can be used in treatment of C3 glomerulopathy in pediatric patients and help recovery of renal function. Eculizumab dose, frequency and eculizumab trough level still need to be determined.

PUB254

A Pooled Analysis of Early Use of Adrenocorticotropic Hormone in Nephrotic Syndrome Kenneth V. Lieberman,[1] Anna Pavlova-Wolf.[2] *[1]Hackensack Univ Medical Center, Hackensack, NJ; [2]Questcor Pharmaceuticals, Inc., Hayward, CA.*

Background: The emergence of adrenocorticotropic hormone (ACTH) as a treatment for nephrotic syndrome (NS) in the late 1940s dramatically altered NS management. The current analysis synthesizes the efficacy and safety of ACTH in NS presented in this early work.

Methods: MEDLINE was searched using the MeSH terms 'adrenocorticotropic hormone' and 'nephrotic syndrome,' with the limits of 1945 (the era of ACTH introduction) to 1965 (ACTH was largely replaced by synthetic oral steroids by this time) and English. Relevant studies also were identified from the reference lists of reviewed papers. From 61 papers found, 14 studies met the inclusion criteria of >5 patients treated, defined remission criteria, and report of safety outcomes. The early clinical experience with short-term ACTH treatment is reported in 9 studies, and 5 studies depict longer-term maintenance treatment. The primary short-term outcome of attaining remission assessed diuresis. The primary long-term aim of sustained remission examined proteinuria.

Results: The patients were predominantly pediatric (88%; 349/395 with ages given) across the 14 studies. The 9 studies examining short-term ACTH treatment included 332 patients. ACTH dose ranged from 20 mg/day to 160 mg/day. Mean treatment duration was about 12 days, ranging from 4 to 28 days. Diuresis resulting in loss of edema was reported in 70% (249/356) of patients or ACTH courses. The 5 studies examining long-term ACTH treatment evaluated 96 patients. ACTH maintenance regimen doses typically ranged from 100 mg to 200 mg and included ACTH treatment for 3 successive days repeated weekly. Remission of proteinuria was reported in 66% (63/96) of patients at last follow-up. Complications of treatment included signs and symptoms of hyperadrenocorticism.

Conclusions: There is a re-emergence of interest in ACTH as a second-line therapy for the reduction of proteinuria in the treatment of NS, yet current clinical research is limited. The analysis of the early clinical literature detailing ACTH in the treatment of NS during the 1950s can provide guidance on ACTH efficacy and safety for today's clinicians.

Funding: Pharmaceutical Company Support - Questcor Pharmaceuticals, Inc.

PUB255

Association of PreS1-Ag, HBeAg, HBV-DNA in the Serum of Patients with Hepatitis B Virus-Associated Glomerulonephritis Chenggang Shi, Xun Liu, Cailian Cheng, Qiong-Li Yin, Mei Li, Weizhao Mo. *Dept of Nephrology, the Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: To investigate the relationship between the serum PreS1-Ag, HBeAg, HBV-DNA and Hepatitis B Virus-associated Glomerulonephritis **(**HBV-GN).

Methods: Baseline data, biochemical data and serum virological indicators were analyzed retrospectively among the HBV-GN group, HBV and chronic kidney disease (not HBV-GN) group, chronic kidney disease without HBV group.

Results: 76 patients were enroll as HBV-GN group (experiment group), 85 patients as HBV and chronic kidney disease(control group 1), 70 patients as chronic kidney disease without HBV group(control group 2). Serum albumin and the incidence of ascites had significant differences between the experimental group and control group 1. Gentle, liver functional indicators, serum albumin, the incidence of liver cirrhosis and ascites had significant differences between the experimental group and control group 2; The percentage of high HBV-DNA copy ($>1\times10^5$IU/ml)($P<0.001$), the positive rates of PreS1 ($P=0.005$) and HBeAg($P=0.002$)had significant differences between the experimental group and control group 1. In total, there were 40 cases (52.36%) of membranous nephropathy (MN) in the experimental group, and 20 cases (26.32%) of membranoproliferative glomerulonephritis (MPGN). And in the control group 1, there were 35 cases of IgA nephrosis(41.18%), in the control group 2, there were 25 cases of IgA nephrosis(35.71%). Multivariate analysis showed that serum albumin and the positive of PreS1 were the correlation factors for the progress of the disease. The predictive value regression equation: $P = 1/[1+e^{-(0.841+1.309\,X1-0.078\,X2)}]$ The under area of the ROC curve was 0.765. The maximum Value of Youden index was 0.567 and obtained 0.3696454 as the diagnosis point.

Conclusions: The percentage of high HBV-DNA copy ($>1\times10^5$IU/ml)and the positive percentage of HBeAg and PreS1 is higher of patients with HBV-GN. The serum albumin and the positive rates of PreS1-Ag could help diagnose the HBV-GN.

Funding: Government Support - Non-U.S.

PUB256

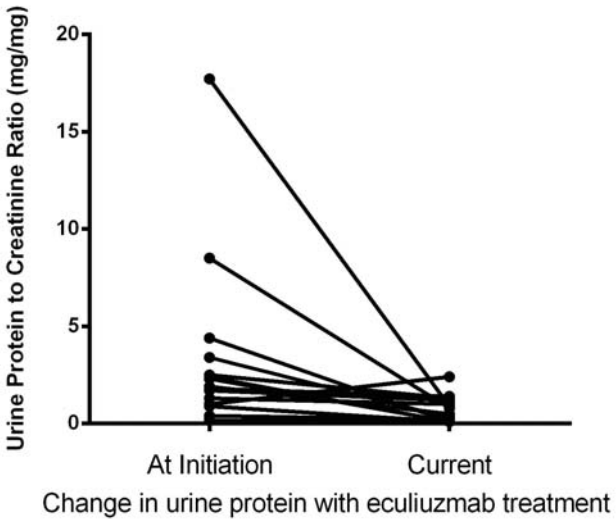
Renal Outcome in Eculizumab-Treated Patients: A Single Center Experience Melissa A. Muff-Luett,[1] Jennifer G. Jetton,[1] Christie P. Thomas,[2] Patrick D. Brophy,[1] Carla M. Nester.[1,2] *[1]Dept of Pediatrics, Univ of Iowa Children's Hospital, Iowa City, IA; [2]Dept of Internal Medicine, Univ of Iowa Hospitals and Clinics, Iowa City, IA.*

Background: Eculizumab ®, a first in class complement blocker, has been shown to be a very effective treatment for atypical hemolytic uremic syndrome (aHUS). Clinicians have begun to use it for other disorders that are believed to involve complement dysregulation.

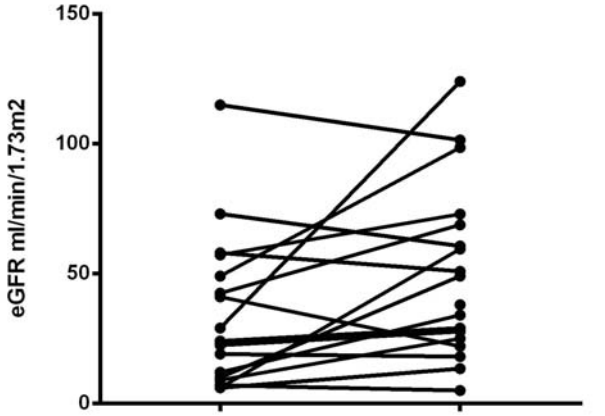
Methods: We performed a retrospective chart review of all patients treated with eculizumab at the University of Iowa. AMR patients were excluded. The major focus was on renal outcomes and patient morbidity. Data collection is ongoing.

Results: The age range of treated patients was 52 days to 64 years. The average length of therapy was 11 months (range 1-47 months) for five diagnoses: 12 with aHUS, 3 with C3G, and 3 with thrombotic microangiopathy from other causes. Nine of the 16 patients

had complement mutations. Two were found to have CFH autoantibodies. The average eGFR at initiation of eculizumab was 43.6ml/min/1.73^2; this improved to 59.1ml/min/1.73^2 at recent evaluation. The urine protein decreased from an average of 3.11mg/mg to 0.71. Anti-hypertensive therapy decreased from an average of 2.7 to 2.1 medications. Reported infections included central line infections, UTIs, parvovirus infection and 1 death following a severe RSV-B infection.



Change in urine protein with eculiuzmab treatment



Change in creatinine with eculiuzmab treatment

Conclusions: Eculizumab appeared to be well tolerated in all age groups. There were no obvious trends in morbidities. Neither the presence nor the type of mutation predicted the renal outcome. aHUS patients demonstrated the most striking improvements. The most significant change for all groups was noted in urine protein reduction.

PUB257

ANCA Vasculitis and Thyroid Disease Treatment in Children: The Chicken, the Eggs or Two Eggs in a Basket: A Case Series of Three Patients Seen at Lebonheur Children's Hospital, TN and West Virginia University Children's Hospital Oulimata K. Grossman, Alimirza Onder. *Pediatric Nephrology, WVUH, Morgantown, WV.*

Background: ANCA vasculitis can be seen during the course of thyroid disease treatment. We report 3 cases in children.

Methods:

	Case 1 –Memphis, TN 2009	Case 2- Morgantown, WV 2012	Case 3- Morgantown, WV 2006
Age-gender-ethnicity	11 y/o F African American	17 y/o F Caucasian	8 y/o F Causasian
Thyroid disease/ Medication	Graves / Methimazole + Propranolol	Hashimoto Thyroiditis/no thyroid medication	Graves / PTU + Propranolol
Presentation	Pericardial effusion	Gross hematuria/ Pulmonary renal syndrome	DVT/ Pulmonary renal syndrome
Anemia requiring transfusion	+	+	+
Weight loss	+	+	+
Fever	-	-	-
Fatigue	+	+	+
Gross hematuria	-	-	+
Serum creatinine (baseline) mg/dl	1.2 (0.86)	1.73 (0.77)	1.6 (na)
Microscopic hematuria	+	+	+
Random protein creatinine ratio	1.5	1.1	2.1
Sedimentation rate mm/h	18	74	86
P-ANCA	High	High	High
c-ANCA	Normal	Normal	Normal
C3, C4	Normal	Normal	Normal
ANA	Positive homgenous	Negative	Positive homogenous speckled
Anti-DS DNA	Negative	Negative	Negative
Lupus anticoagulant	NA	Positive lipoprotein A + Positive dRVVT lasted < 6 weeks	Negative
Kidney pathology: Pauci immune	44 gloms, 65 % crescents	36 gloms, 25% crescent	8 gloms, 50% crescents
Pulse solumedrol with cyclosphosphamide	+	+	+
Induction with monthly cyclosphosphamide	+	+	+
Maintenance	Cellcept	Azathioprine	Cellcept
Thryroid Medication	None	None	None

Results: All 3 patients improved clinically and had a normalization of ANCA levels and serum creatinine as well as thyroid markers. One with Hashimoto thyroiditis and transient lupus anticoagulant never required thyroid medication. None of them had required further thyroid disease treatment after diagnosis and management of vasculitis.

Conclusions: ANCA vasculitis has been well described to be secondary to Methimazole, PTU. The two diseases have coexisted in one patient who was not on thyroid medication and had lupus anticoagulant. Our data is too small however one could start questioning the causality of thyroid medication on concomitant ANCA vasculitis.

PUB258

Anti-Neutrophil Cytoplasmic Antibodi-Associated Vasculitis. Clinical and Survival Study of 84 Patientes from a Single Center Federico Varela,[1] Valeria Scaglioni,[2] Gustavo Cristian Greloni,[1] Griselda Bratti,[1] German Barrera Hugalde,[1] Silvia Beatriz Christiansen,[3] Enrique Roberto Soriano Guppy,[2] Guillermo Javier Rosa Diez.[1] *[1]Nephrology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; [2]Rheumatology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; [3]Pathology, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina.*

Background: Epidemiology of ANCA-associated vasculitis (AAV) is not entirely known. There are few studies published in Latin America. Our objective was to describe the clinical, serological features and outcomes of patients with AAV and renal involvement.

Methods: Electronic medical records from 2000 to 2013 with diagnosis of Wegener's granulomatosis (WG), Churg Strauss (CSS), microscopic polyangiitis (GPA) or renal-limited vasculitis (RLV), were reviewed, and those patients with ANCA-posive.

Results: 84 patients (WG n=34, MPA n = 12, CSS n = 14 and RLV n = 24) were included. Sex, age, progression to renal failure and dialysis, mortality, relapse after induction and remission are summarized.

	WG (n=34)	MPA (n=12)	CSS (n=14)	RLV (n=24)
Women, n (%)	20 (58,8)	10 (83,3)	11 (78,6)	17 (70,8)
Age	58,4	65,7	60,9	66,3
ANCA C +, n (%)	26 (78,8)	1 (8,3)	2 (15,4)	2 (8,3)
ANCA P +, n (%)	3 (9,1)	10 (83,3)	6 (42,3)	21 (87,5)
Involvement, n (%) - Pulmonary - Renal - Pulmonary/Renal	 21 (61,7) 18 (52,9) 3 (8,8)	 4 (33,3) 8 (66,7) 3 (25)	 2 (14,2) 4 (28,6) 0	 0 23 (95,8) 0
ESRD, n (%)	4 (11,8)	0	1 (7,1)	3 (12,5)
Remission after incution, n (%) - No - Partial - Complete Currently treated	 7 (20,6) 10 (29,4) 15 (44,1) 2 (5,9)	 1 (8,3) 6 (50) 4 (33,3) 1 (8,3)	 1 (7,1) 8 (57,1) 4 (28,6) 1 (7,1)	 4 (16,7) 8 (33,3) 11 (45,8) 1 (4,2)
Relapses	13 (39,4)	4 (33,3)	7 (50)	4 (16,6)
Follow up, yeas (DS)	5,5 (6,1)	2,8 (2,3)	4 (2,9)	4,1 (3,8)
Death	3 (8,8)	3 (25)	1 (7,1)	2 (8,3)

Median follow-up was 3.9 years (IQR 1.2 to 7.8). Nine patients died (11%). Overall survival at 10 years was 85% (95% CI 71-93).

Conclusions: The WG and RLV were the most frequent AAV in our cohort. Latter group were at high risk for developing CKD (p = 0.008 versus WG). Acceptable 10-year survival was observed. High percentage of patients showed disease relapse after achieving complete remission. Lower percentage did not achieve remission after the induction.

PUB259

Zoledronic Acid Induced Acute Interstitial Nephritis: Two Cases in One Institute and Literature Review

Titi Chen, Subramanian K. Kumar. *Nephrology, Gosford Hospital, Gosford, NSW, Australia.*

Background: Zoledronic acid is a highly potent intravenous form of bisphosphonate, widely used for treatment of osteoporosis and malignancy associated bone diseases. Its nephrotoxicity, which is dose and infusion rate dependent, has been observed in multiple clinical trials. However, these trials do not usually provide information on renal biopsy findings. A literature search has shown that to date, only eleven patients in four studies had renal biopsies to confirm the etiology of Zoledronic acid induced renal dysfunction. Nine had acute tubular necrosis (ATN), one had focal segmental glomerulosclerosis (FSGS) and one had acute interstitial nephritis (AIN). AIN occurred in a patient with bone metastasis from breast cancer when treated with a high dose of Zoledronic acid (4mg/month).

Methods: We reported two cases of AIN caused by Zoledronic acid, with both patients having normal renal function prior to treatment and both having received the low treatment dose of 4mg/year. Both of these patients developed AIN confirmed by renal biopsy requiring hemodialysis. To our knowledge, these are the first two case reports of biopsy proven AIN requiring dialytic support caused by low dose yearly Zoledronic acid treatment in patients with normal baseline renal function.

Conclusions: Zoledronic acid induced AIN is not uncommon and can occur at a low treatment dose. Renal function should be monitored closely when patients are treated with Zoledronic acid. If acute kidney injury does occur, AIN should be included in the differential diagnosis, in addition to ATN and FSGS as previously reported in the literature. Prompt renal biopsy should be considered to confirm the diagnosis, as the treatment of these conditions are different.

PUB260

Rituximab-Induced Continuous B Cell Depletion for Durable Remission in 32 Consecutive Patients with Idiopathic Membranous Nephropathy

John Niles, Andrew P. Murphy, Katherine M. Cosgrove, Karen A. Laliberte. *Nephrology, Massachusetts General Hospital, Boston, MA.*

Background: Many groups are working on the optimal strategy for induction and maintenance of remission. Immunosuppression may be valuable to preserve renal function and to reduce the exposure to the nephrotic state thus reduce the complications of membranous nephropathy. In the past, induction with cytoxic agents and glucocorticoids were found to be effective; however, as data continues to grow, Rituximab may be even more efficacious in induction and short term maintenance therapy to achieve sustained remission.

Methods: We performed a retrospective analysis of clinical data from patients with biopsy-proven idiopathic membranous treated by our group with continuous B cell depletion.

Results: 32 patients: 16 men and 16 women with a mean age of 56.3 received induction and maintenance therapy for idiopathic membranous nephropathy with Rituximab. All but 1 patient received corticosteroids. 20 patients received cyclophosphamide and rituximab. 2 patients received MMF and rituximab. 12 patients received rituximab without concumitant cyclophosphamide or MMF. Mean duration of therapy was 669 days (22 months). Mean spot urine protein/creatinine ratios went from 11.3 prior to therapy to 1.2 at most recent follow up. One patient in the Rituximab only group died five months into therapy with advanced alzheimer's and one patient in the Rituximab only group developed GCSF-responsive neutropenia.

Conclusions: We have seen an increase in the number of new cases each year. Combined induction therapy with cyclophosphamide, corticosteroids and rituximab followed by 2 years of continuous B cell depletion was effective in achieving and sustaining a complete, long term remission. Safety of Rituximab has been well demonstrated in other patient populations. Proteinuria is reduced, renal function is preserved and complications from membranous nephropathy were absent.

PUB261

Rituximab for Antineutrophil Cytoplasmic Antibody-Associated Vasculitis, Hepatitis C Virus-Associated Cryoglobulinemic Vasculitis, and Membranous Nephropathy: One Center Experience

Elena Zakharova. *Nephrology, City Clinical Hospital n.a. s.P. Botkin, Moscow, Russian Federation.*

Background: Rituximab, which is currently used as a treatment option for several autoimmune diseases, was approved for usage in ANCA-associated vasculitis (AAV) in Russian Federation in March 2013. We use rituximab as a rescue therapy for AAV, HCV-associated cryoglobulinemic vasculitis (CCV), and idiopathic membranous nephropathy (IMN) since 2010, and aimed to evaluate treatment results in our patients.

Methods: Using electronic clinical and pathology database for we searched patients with severe AAV and CCV with biopsy-proven kidney damage, and also IMN, treated with rituximab in our unit during 2010-2013. Treatment regimens were either four weekly infusions (375 mg/m^2), or two 2-weekly infusions (1g per infusion).

Results: Study group included 14 patients, 6 (42.8%) male and 8 (57.2%) female, median age 53.5 [25;79] y.o. Patients presented with rapidly progressive glomerulonephritis (RPGN) or with severe nephrotic syndrome (NS) plus arterial hypertension, those with vasculitis had also multiple life-threatening extra-renal manifestations. Kidney pathology showed crescentic glomerulonephritis (CGN), membranoproliferative glomerulonephritis (MPGN), or membranous nephropathy (MN). Main diagnosis, kidney clinical presentation and pathology, indications for rituximab and short-term outcomes are shown in table.

Main diagnosis	AAV	CCV	IMN	Total
Number of patients	6	4	4	14
RPGN	6	2	0	8
NS	0	2	4	6
CGN	6	0	0	6
MPGN	0	4	0	4
MN	0	0	4	4
Refractory disease	3	0	2	5
Relapse	3	3	2	8
First-line tharapy	0	1	0	1
Complete remission	3	3	0	6
Partial remission	3	0	4	7
No remission	0	0	0	1
Relapses after rituximab	0	1	0	1

Conclusions: Rituximab as a rescue therapy was effective in all patients in our group, treated for refractory or relapsing ANCA-associated vasculitis, HCV-associated cryoglobulinemic vasculitis, and idiopathic membranous nephropathy, and presenting with rapidly progressive glomerulonephritis or severe nephrotic syndrome. First-line therapy used in single case of CCV failed. We intend to use rituximab also for maintenance treatment at least for AAV, and evaluate long-term results.

PUB262

Paraneoplastic Glomerulonephritis with Acute Renal Failure in a Patient with Myeloproliferative Disorder Associated with Eosinophilia

Zeljka Veceric Haler, Spela Borstnar, Andreja Ales Rigler, Nika Kojc, Kovac Damjan, Marko Malovrh, Jelka Lindic. *Nephrology, Univ Clinical Centre Ljubljana, Slovenia.*

Background: We report a rare case of crescentic GN superimposed to membranous nephropathy (MN) in a patient with myeloproliferative disease (MPD) and marked eosinophilia.

Methods: Six years before admission 66-year old woman with nephrotic syndrome (NS) was diagnosed with MN. Secondary causes of MN and systemic disease were excluded, her renal function and blood smear were normal. She achieved spontaneous remission. After 5 years she was diagnosed with myelodisplastic syndrome (MDS). At the time of admission she presented with two months lasting diarrhea, dyspnea, weight loss and extensive sweating, anaemia, trombocytopenia and eosinophilia. She also presented with rapidly progressive GN with concomitant NS and needed hemodialysis. Immunoserologic tests were negative. History of parasitic infection, drug reaction, asthma or allergy was negative. She had acute respiratory failure and her CT revealed dense infiltration of right lower lobe. Bronchoscopy revealed eosinophilic bronchitis, bronchial lavage was sterile. Bone marrow biopsy revealed MDS with blasts in excess. Renal biopsy revealed crescentic GN and mixed cell tubulointerstitial nephritis with acute tubular injury and obturatory erythrocyte casts

superimposed on late stage MN. Immunofluorescent analysis revealed modest IgG and C3 immune complex deposition. Diagnosis of hypereosinophilic syndrome associated with MPD and concomitant paraneoplastic crescentic GN was made. Patient was treated with high dose steroids and intravenous immunoglobulins, diarrhea and respiratory insufficiency disappeared, lung infiltrate partially retracted, but patient still remained dialysis dependent. After azacitidine therapy partial resolution of eosinophilia and complete disappearance of lung infiltrate was achieved and renal function started to improve continually.

Conclusions: In our patient with 6-years long history of MN, paraneoplastic GN with hypereosinophilic syndrome associated with MPD was revealed. Appropriate treatment of the underlying disease resulted in marked improvement of renal function with resolving of NS.

PUB263

Percutaneous Renal Biopsy of Native Kidneys Using Real Time Ultrasound: Adequacy and Complications Rates with 14 versus 16 Gauge Automated Needles Svetha Chunduri, William Luke Whittier, Stephen M. Korbet. *Internal Medicine, Rush Univ Medical Center, Chicago, IL.*

Background: The use of a 16 gauge automated biopsy needle has been recommended when performing percutaneous renal biopsy (PRB) of native kidneys. We compare the adequacy and safety of PRB with a 14-gauge or 16-gauge automated needle.

Methods: PRB of native kidneys was performed in 137 adult patients from 1/2010 to 12/2012 using real-time ultrasound guidance. Clinical, laboratory and outcome data (renal US 1-hr post-biopsy, adequacy and safety) was collected prospectively and compared between biopsies performed randomly with a 14-gauge (82 biopsies) or 16-gauge (55 biopsies) automated needle.

Results: Biopsies done with a 14-gauge versus a 16-gauge needle were compared. There were no differences in age (47±17 versus 48±19 yrs), gender (30% versus 35% male), race (41% versus 49% AA) or blood pressure. The creatinine (2.2±1.8 versus 2.1±1.7 mg/dl), bleeding time (8.0±1.8 versus 7.8±1.8 min), the proportion with a normal bleeding time (83% versus 74%) or PTT (90% versus 82%) were similar at the time of PRB. The pre-PRB hemoglobin (Hgb)(11.3±1.9 versus 11.4±2.0) and post-PRB drop in Hgb (1.0± versus 0.8±0.9) were also similar. There were no differences in the number of passes taken (3.0±0.9 versus 2.7±0.8) or cores obtained (2.3±0.6 versus 2.4±0.6) per biopsy. The number of glomeruli obtained per biopsy (light + fluorescence microscopy) was similar (29±11 versus 31±14) and adequate tissue for diagnosis was obtained in 99% and 100% of biopsies. A hematoma by renal US 1-hr post-PRB was demonstrated more often in biopsies done with the 14-gauge needle (39% versus 22%, P 0.04). However, a hematoma at 1-hr post-PRB had a positive predictive value of only 22% with a negative predictive value of 98% in predicting a post-PRB complication. Thus, the complication (8.5% versus 9.1%), transfusion (7.3% versus 7.2%) and embolization (3.7% versus 1.8%) rates were not significantly different for 14 versus 16-gauge needles.

Conclusions: While a hematoma was observed by renal US 1-hr post-PRB more frequently when using the 14-gauge needle, the overall safety and adequacy of PRB of native kidneys using real-time ultrasound was similar using either a 14 or 16-gauge automated needle.

PUB264

Recurrent Lupus Nephritis in a Kidney Allograft Jianping Lin,[1] Talal A. Khan,[2] Sirma H. Koutzaki,[1] Alden Michael Doyle,[2] Yelena Piazza,[1] Suganthi Soundararajan.[1] *[1]Dept of Pathology and Laboratory Medicine; [2]Dept of Medicine, Div of Nephrology and Hypertension, Drexel Univ College of Medicine, Philadelphia.*

Background: Lupus nephritis (LN) is a common yet serious complication of systemic lupus erythematosus (SLE). It affects 50 percent of SLE patients who may progress to end stage kidney disease (ESKD). Patients who receive renal transplants are thought to be somewhat protected from recurrent lupus nephritis primarily due to the use of immunosuppression. However, the risk of recurrent LN following transplantation is found to be higher in younger African American female patients. Herein we report a case of a biopsy proven recurrent lupus nephritis in a kidney transplant recipient.

Methods: The patient is a 35-year-old Caucasian female with a history of ESKD from lupus nephritis, who received a living unrelated renal transplant in August, 2006. She had induction immunosuppression with basiliximab. She was maintained on standard triple regimen including tacrolimus, mycophenolate and mofetil. The patient was doing well without any complications until August 2013, when she developed kidney allograft dysfunction associated with non-nephrotic range proteinuria. Her urinalysis showed no evidence of RBCs cast, donor specific antibodies were negative, and her complement levels were normal. Her allograft biopsy revealed double contour glomerular basement membrane with electron lucent subepithelial, subendothelial and mesangial deposits. Tubular reticular inclusions were noted. Immunohistochemical stain showed no C4d deposits. All of these observations were consistent with recurrent lupus nephritis against a background of chronic allograft nephropathy. Based on the biopsy findings, patient's mycophenolate dose was increased and high dose prednisone was added. Her proteinuria improved and the kidney function stabilized.

Conclusions: This case illustrates recurrent lupus nephritis in a kidney allograft in a stable kidney transplant recipient who presented with elevated serum creatinine and new proteinuria. Although rare, recurrent LN should be considered in the differential of kidney transplant dysfunction, even in the absence of other clinical evidence of SLE.

PUB265

Morphometry on Baseline Biopsies Predicts Early GFR Change in Nephrotic Syndrome (NEPTUNE) Kevin V. Lemley,[1] S. Bagnasco,[2] Cynthia C. Nast,[3] L. Barisoni,[2] Catherine M. Conway,[4] Stephen M. Hewitt,[5] Peter X.K. Song.[6] *[1]Pediatrics, Univ. of Southern California, Los Angeles, CA; [2]Pathology, Univ. of Miami, Miami, FL; [3]Pathology, Cedars Sinai Med Ctr, Los Angeles, CA; [4]Leica Microsystems, Dublin, Ireland; [5]National Cancer Institute, Bethesda, MD; [6]Biostatistics, Univ. of Michigan, Ann Arbor, MI.*

Background: NEPTUNE is a 21-center consortium undertaking a longitudinal study of nephrotic syndrome due to FSGS, minimal change disease (MCD) and membranous nephropathy (MN) in children and adults.

Methods: From incident diagnostic biopsies, morphometric assessment was done on digital images of 65 cases and correlated with longitudinal change in estimated GFR (eGFR) over 18 months using generalized estimating equations (GEE) method. Three morphometric variables were estimated in the biopsy images on a SlidePath platform (Leica Biosystems): average glomerular tuft cross-sectional area (AG) and cortical glomerular density (NA) were determined by digital planimetry; fractional interstitial area (FIA), a marker of interstitial fibrosis, by point counting. All measurements were done by a single investigator (KVL) who was blind to all other patient characteristics. Eight potential confounding variables (e.g. race, age, sex, baseline level of proteinuria) were included in the GEE analysis.

Results: The subjects studied were 34% African-American, 68% male and 63% adult; disease category was FSGS in 32, MCD in 20 and MN in 11 ('other' in 2). NA and FIA were the strongest predictors of change in eGFR (P=0.003 and P=0.0001), followed by baseline patient age (P=0.019). No other variables had a significant effect in the multivariate longitudinal analysis. AG was significant as a single predictor, but not in the multivariate analysis, possibly due to strong collinearity with NA (r=-0.56, P<0.0001).

Conclusions: All 3 morphometric variables may be considered as markers of glomerulopenia. Thus, their predictive power may reflect the deleterious effects of a congenital low nephron endowment in patients who later develop nephrotic syndrome (low FIA) or reflect preceding glomerular loss, even in these early, diagnostic biopsies.

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PUB266

The NEPTUNE Digital Pathology Protocol Increases Accuracy in Glomerular Number Assessment Avi Z. Rosenberg,[1] M. Palmer,[2] L. Merlino,[3] J. Troost,[4] A. Gasim,[5] S. Bagnasco,[6] C. Avila-Casado,[7] D. B. Johnstone,[8] Jeffrey B. Hodgin,[4] Catherine M. Conway,[9] Jeffrey B. Kopp,[10] Cynthia C. Nast,[11] L. Barisoni,[3] Stephen M. Hewitt.[1] *[1]NCI; [2]U of Pennsylvania; [3]U of Miami; [4]U of Michigan; [5]U of North Carolina; [6]Johns Hopkins Univ; [7]Univ Health Network, Toronto; [8]Temple U; [9]Leica; [10]NIDDK; [11]Cedars Sinai.*

Background: Standardized review of renal biopsies are central to kidney diseases diagnosis/prognosis. Reproducibility is a challenge for basic metrics. The NEPTUNE digital pathology protocol (NDPP) seeks to standardize this by refining scoring reproducibility. Glomerular number (GN) reported by light microscopy (LM) versus enumeration by digital annotation were compared using glomerular annotation of histologic levels on whole slide images (WSI).

Methods: 9,379 glomeruli in 274 cases were annotated at all levels. Total GN/case or average GN/slide, and total number with glomerular sclerosis (GS) were enumerated on WSI; reported GN (total or average) and glomerular number with GS were excerpted from the reports.

Results: The discrepancy between reported total (total and GS) versus annotated glomeruli increased proportionally to the total GN. This difference was disease-dependent for GS glomeruli (IgA NP 5.9; FSGS 3.7; MN 1.6; MCD 0.03 (p<0.01)). There was a mean discrepancy up to 14.6% (<0.0001) in % GS reported versus annotated in 30 - 80% GS bracket.

Summary of variation in glomerular enumeration (#range, mean)

	Reported#	Annotated#	Correlation	Difference#	Significance of difference
Average per slide (n=134)	4 – 125 21.7	1.4 – 135 16.7	0.95	-17 to 15 -1.5	0.002
Total per case (n=140)	2 - 71 18.3	3 – 149 30.8	0.87	-11 to 78 12.4	<0.001
Sclerotic (total, n=140)	0-50 2.7	0-99 4.9	0.89	-6 to 72 2.2	<0.001
% Sclerotic (total, n=140)	0-100 15	1-130 17	0.84	-73 to 93 2.2	0.007

Conclusions: Enumeration of GN on WSI provides an accurate assessment of glomerular lesions. Average glomerular profiles/level are more reproducible, allowing better determination of glomerular injury. Further work into the role of WSI will assess reproducibility/accuracy of involvement by subtler lesions and determine the impact on ennumeration versus estimation has on diagnosis and prognosis.

Funding: NIDDK Support, Other NIH Support - NCI, Private Foundation Support

PUB267

Trefoil Factor 3 Expression in Immunoglobulin A Nephropathy Derya Guler, Izzet Hakki Arikan, Dilek Barutcu Atas, Ebru Asicioglu, Arzu Velioglu, Mehmet Koc, Serhan Tuglular, Cetin Ozener. *Nephrology Div, Marmara Univ Hospital, Istanbul, Turkey.*

Background: Trefoil factor 3 (TFF3) is a small peptide that is secreted from mucous producing cells. It plays an important role in mucosal protection, cell proliferation and migration. Increased plasma or urine TFF3 levels have been reported in patients with acute or chronic kidney disease. However, there is no renal tissue expression in healty kidneys. We aimed to investigate the possible TFF3 expression and its role in pathogenesis of human IgA nephropathy.

Methods: Twenty eight patients with biopsy proven IgA nephropathy (16 women, mean age 37 ± 13.8 years) included to this study. Kidney biopsy materials of these patients were re-evaluated according to the Oxford classification. Patients' demographic, clinical and laboratory data were collected. TNF-α, IL-10, TGF-β, and TFF3 expressions were investigated on renal biopsy specimens. The possible correlations between clinical data and the expressions were assessed by SPSS version 15.0.

Results: The mean follow-up time of the patients was 4.8±2.7 years. None of the patients have reached end-stage renal failure. The uric acid level was higher in patients with messengial hypercellularity (p=0.04). Sitoplasmic TFF3 expression was observed in 19 patients (%67.9). Nuclear TGF-β expression was demonstrated in 20 (71.4%) patients in tubular epithelial cells. IL-10 expression was also observed in majority of the patients (89.3%). There was a positive correlation between TGF-β and TFF3 expression in the tubular epithelial cells (P=0.005). Patients with nuclear TGF-β expression have most likely to show glomerular and tubular IL-10 expression (p=0.015). There were no correlations between clinical or histopathological findings and TFF3 expression.

Conclusions: We have demonstrated TFF3 expression on kidney tissues in patients with IgA nephropathy. The presence of TFF3 expression in tubular epithelial cell but not in glomeruli suggests that TFF3 may contribute in tubular injury rather than spesific pathogenetic role in IgA nephropathy. The correlation between TGF-β and TFF3 expression in tubular epithelial cells may support our hypotesis that TFF could have a role in inflammatory process.

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PUB268

Significance of Intrarenal Arteriolar Lesions in the Patients with IgA Nephropathy with or without Hypertension Tian-Yi Chen, Hong Cheng, Yi-Pu Chen. *Div of Nephrology, Beijing Anzhen Hospital,Capital Medical Univ, Beijing, China.*

Background: To develop quantitative criteria for evaluating intrarenal arteriolar lesions, and to investigate the effect of arteriolar lesions on the patients of IgAN.

Methods: 50 non-IgAN cases with normal small arteries in our hospital were analyzed. patient's arteriolar inside diameters and outside diameters were measured by indirect method. Average value of arteries' inside diameters/outside diameters were calculated and the degrees of severity of arteriolar lesions were evaluated. The criteria for evaluating intrarenal arteriolar lesions were developed, base on the data. According to the criteria, we analyzd 305 cases of IgAN with or without hypertension.

Results: The average ratio of arteriolar inside/outside diameters of all non-IgAN cases was 0.52±0.05. The criteria of arteriolar lesions were defined as follows: >0.48 was normal; 0.45-0.48 was mild; ≤0.45 was severe. Among 305 IgAN, the prevalence of arteriolar lesions was 36.4% and 13.3% of those patients with severe lesions. The cases of intrarenal arteriolar lesions had significantly higher systolic blood pressure (SBP), diastolic blood pressure (DBP), serum creatinine level (SCr), proportion of ischemic glomerulosclerosis (PIG) and lower urine osmotic pressure (UOP) compared with normal cases (P<0.05). The severe lesion group had higher DBP, SCr and PIG, compare with mild lesion group (P<0.05). Through variables selected for the logistic regression model we found that SBP, SCr, UOP and PIG associated with arteriolar lesions independently. Among the 147 IgAN cases with normal blood pressure, 26.5% of them with arteriolar lesions and 7.5% with severe lesions. Compare with normal group, the cases of intrarenal arteriolar lesions had significantly higher SCr and PIG compared with normal cases (P<0.05). The significantly lower UOP only appeared in the cases of severe arteriolar lesions (P<0.05). Among the IgAN cases with normal blood pressure,UOP and PIG associated with arteriolar lesions independently.

Conclusions: Intrarenal arterial lesions appeared in IgAN patients with or without hypertension, and the arteriolar lesions are frequently associated with more severe glomerular and tubular dysfunction.

Funding: Government Support - Non-U.S.

PUB269

RENAL AID, a Newly Established Biorepository Is a Unique Platform for Conducting CKD Research Alan Perlman,[1,2] James M. Chevalier,[1,2] Thomas Parker,[2] Surya V. Seshan,[3] Daniel Levine.[2] *[1]Nephrology, Weil Cornell Medical College, NY, NY; [2]The Rogosin Institute, NY, NY; [3]Pathology, Weil Cornell Medical College, NY, NY.*

Background: RENAL AID (Repository of Novel Analytes Leading to Autoimmune, Inflammatory and Diabetic nephropathies) is a unique dual function data registry and tissue repository designed as a platform to conduct research relating to the correlation between tissue diagnosis, histology patterns and gene expression from renal biopsies relative to subjects clinical characteristics, disease course, serum/urine biomarkers and blood/urine gene expression. The goal of the bio-repository is to collect data from subjects with diabetic, inflammatory and autoimmune renal conditions in order to ascertain factors that predict disease progression, response to therapy and assess for discriminating noninvasive features that predict biopsy findings.

Methods: RENAL AID is able to capture biopsy findings in a retrievable electronic database. Typically, biopsy results are reported in descriptive prose that is useful to the practicing physician but limits the ability to associate biopsy findings with other variables for the purpose of translational research. RENAL AID electronically stores pathology descriptions using a data collection instrument that is objective, quantifiable and retrievable. Pathology details are stored in the same database as all other clinical and laboratory data thereby facilitating straightforward detection of associations between these variables. Follow-up occurs at regular intervals with updated clinical/laboratory data. RENAL AID also serves as a tissue repository for renal biopsy, urine, blood and serum specimens for future comparative analysis between any combination of variables including tissue, blood and urine gene expression, histopathology, pathologic diagnosis, serum/urine biomarkers and clinical variables.

Conclusions: RENAL AID is a unique dual function bio-repository platform designed to study associations between clinical, biochemical, histology and genetic data in subjects with diabetic, inflammatory and autoimmune nephropathies. As data from these sets are stored in a single database, the discovery of novel associations is facilitated.

Funding: Private Foundation Support

PUB270

The Effect of Anemia on Complication Rate after Percutaneous Renal Biopsy William Luke Whittier, Khaleel Sayeed, Stephen M. Korbet. *Nephrology, Rush Univ Medical Center, Chicago, IL.*

Background: A percutaneous renal biopsy (PRB) complication has been defined by bleeding resulting in the need for an intervention, and the degree of anemia pre-PRB has been shown to be a risk factor for a complication. As the most common intervention is a blood transfusion, the purpose of this study was to determine if anemia is truly a risk factor for bleeding, or if anemic patients are simply more likely to receive a transfusion after a PRB.

Methods: PRB of native kidneys was performed in 910 adults using real-time ultrasound and automated 14 gauge biopsy needles from 1/1990 to 4/2014. All patients were prospectively monitored for a bleeding complication in the hospital for 24 hours and evaluated based on the post-PRB drop in hemoglobin concentration (Δ Hgb). A complication was defined by bleeding resulting in an intervention (transfusion, embolization or cystoscopy), re-admission due to post-PRB complication, or death. Patients were divided into 3 groups based on the pre-PRB Hgb (<9, 9-11 and >11 g/dl).

Results: Complications occurred in 71(7.8%) of 910 biopsies, and the majority of these (57/71, 80%) resulted in a transfusion (6.3% overall). Biopsies with complications had a lower pre-PRB Hgb than those without complications (10.3 ± 2.0 g/dl versus 12.0 ± 2.1 g/dl, p < 0.0001) and a greater Δ Hgb (2.1 ± 1.6 versus 1.0 ± 0.8 g/dl, p < 0.0001). Patients with a pre-PRB Hgb < 9 g/dl were significantly more likely to receive a transfusion but had a smaller Δ Hgb and experienced fewer hematomas compared to patients who received a transfusion but started with a higher pre-PRB Hgb.

Pre-PRB Hgb (g/dl)	<9	9-11	>11	P value
N	79	266	565	
Transfusion	19 (24%)	23 (9%)	15 (3%)	< 0.0001
Δ Hgb in Transfusion patients (g/dl)	1.3± 1.0	1.8± 0.8	3.2± 1.6	< 0.0001
Hematomas in transfusion patients	58%	83%	87%	0.04

Conclusions: While severely anemic patients have a higher transfusion rate and thus by definition a higher complication rate, this appears to be due to a lower threshold for transfusing as these patients actually have less clinical evidence of bleeding. Thus, the complication rate may be falsely elevated as an artifact of its definition.

PUB271

Incidental Tubulo-Reticular Inclusions in Allograft Biopsies of Five Kidney Transplant Recipients without Clear Etiology Talal A. Khan,[1] Sirma H. Koutzaki,[2] Alden Michael Doyle,[1] Ying Lu,[2] Suganthi Soundararajan.[2] *[1]Div of Nephrology and Hypertension; [2]Dept of Pathology and Laboratory Medicine, Drexel Univ College of Medicine, Philadelphia.*

Background: Tubulo reticular inclusions (TRIs) are organized subcellular structures within cisternae of endoplasmic reticulum and are considered footprints of interferon activity (IFNα and INFβ). These structures have been described with collagen vascular diseases [SLE(systemic lupus erytheromatosus),systemic sclerosis etc], viral infections (HIV, HBV,HCV) and during systemic treatment with interferons.Incidental findings of TRIs in kidney transplant recipients may suggest underlying pathology. They usually occur in endothelial cells in glomeruli of kidney transplants with underlying SLE or viral infection. However in some cases they occur without any underlying etiology. Herein we report 5 cases of kidney transplant recipients who were found to have incidental TRIs on kidney biopsy specimens examined by electron microscopy.

Methods: We retrospectively reviewed five cases of kidney transplant recipients who had biopsy proven TRIs.Most (4/5) of the patients had biopsies performed to evaluate acute kidney injury, while the other was performed as part of a surveillance. Four were from deceased donors, one was from a living donor. We reviewed these cases for any evidence or history of SLE, HIV, Hepatitis B and C virus infection or BK virus nephropathy.

Results: None of the patients had any evidence or history of SLE, HIV, Hepatitis B and C virus infection or BK virus nephropathy.No patients had been exposed to interferon therapy at any time.Two patients had evidence of chronic rejection, while 1 patient was diagnosed with IgA nephropathy and 1 patient had recurrent FSGS. No patient had evidence of acute rejection.

Conclusions: We report 5 cases of incidental TRIs found in kidney allograft biopsy specimens in patients with no clear reason. We hypothesize that these may be related to the production of cytokines in the recipient's allo-immune response and that these may be of some utility in diagnosis or prognosis. We encourage nephropathologists to be aware of their possible presence and encourage further study into this intriguing histopathological phenomena.

PUB272

Renal Arterial Perfusion Ratio Reflects Cardiac and Renal Functions in Chronic Kidney Disease and Hypertension

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Background: Cardiac hemodynamic function is crucial for maintaining renal perfusion. On the other hand renal tissue alterations corresponding to chronic kidney disease (CKD) ameliorate perfusion of the organ. The aim of the study was to evaluate whether Renal Arterial Perfusion ratio (RAP) could reflect both cardiac and renal functions in patients with stable CKD and hypertension.

Methods: Forty five patients (5 F; 40 M; age 52 ±16) with stable CKD (Cys-CreCKD-EPI 54.4 ±27.8 ml/min/1,73m^2) and hypertension were enrolled in the study. Serum Creatinine (Cre), Cystatin C (Cys), NT-pro brain natriuretic peptide, Troponin I (TnI) were tested. Renal function was estimated according to Cys-Cre based CKD-EPI formula. Echocardiographic examination, ABPM, ultrasound estimation of Intima-Media Thickness (IMT) and Renal Resistive Index (RRI) were collected. RAP was calculated as a ratio of Proximal (PPI) to Distal (DPI) arterial Perfusion Intensity in renal cortex assessed in Color Doppler (PixelFlux, Chameleon Software, Germany).

Results: DPI was lower than PPI (0.077 ±0.072 cm/s versus 0.427 ±0.284 cm/s; p<0.001). Median RAP was 7.00 (range: 0.93 - 12.30). RAP was significantly correlated with mean arterial pressure (MAP)(r=0.33; p=0.031) and left ventricle hemodynamic parameters: stroke volume (LVSV) (r=0.38; p=0.009) and cardiac index (CI) (r=0.37; p=0.014), but not with left ventricular mass index, nor with parameters of renal function. In the model of stepwise multiple regression analysis (Cys, Cys-CreCKD-EPI, TnI, LVSV, IMT, RRI, MAP and PP) LVSV and Cys-CreCKD-EPI as well as RRI and pulse pressure together independently influenced RAP (R^2=0.46; p<0.001).

Conclusions: Renal Arterial Perfusion ratio is significantly influenced by cardiac and renal functions in hypertensive CKD patients. RAP could be considered a new tool in diagnosing cardio-renal interactions.

Funding: Government Support - Non-U.S.

PUB273

Proliferative Glomerulonephritis with Monoclonal IgG Deposit an Atypical Presentation of Acute Post Infectious Glomerulonephritis

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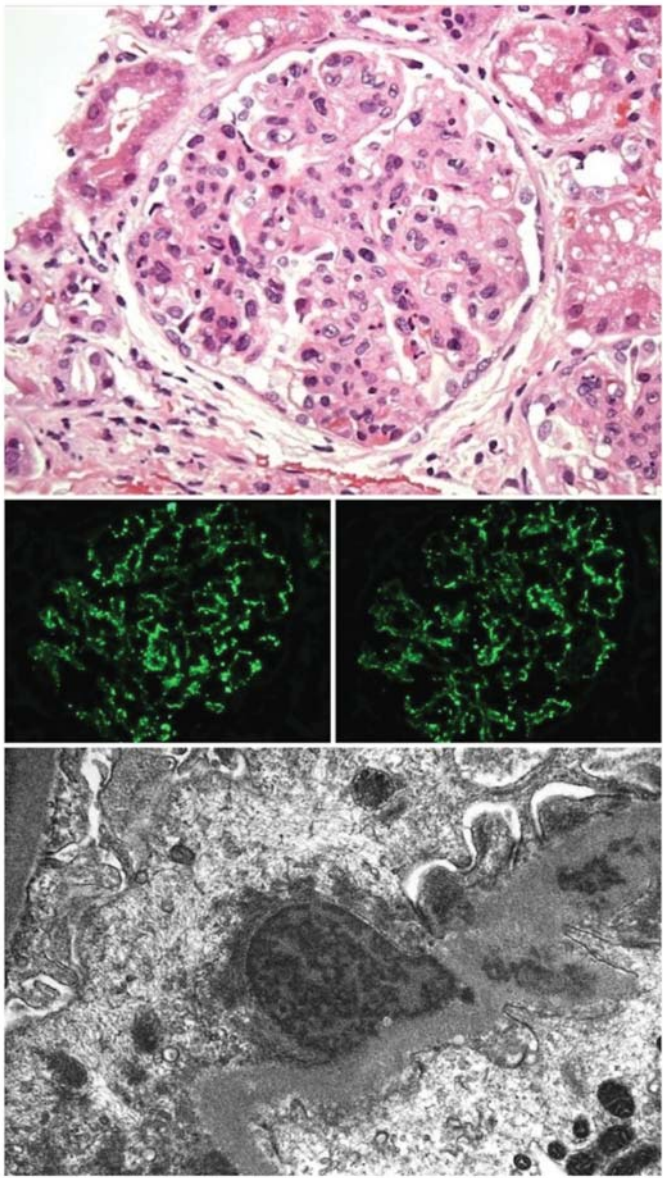
Background: Pathologocal labeling of a glomerulonephritis into a specific entity can be challenging. We present below a rare case.

Methods: This is a case of a 20-yo female with PMH of tonsillectomy presenting for evaluation of proteinuria. She had lethargy, weakness, fever of 102, cough, sputum production 2 weeks prior. She gained 25 lbs,had facial, and lower extremities edema.No hematuria,diarrhea or rash.No OTC medications. No drug abuse. On exam she had +3 edema of lower extremities. The rest was normal. Dipstick showed hematuria with 3+ protein. Her Creatinine was 1.1 mg/dl, CBC was normal .Albumin was 2.2 mg/dl. Urine Pr/Cr was 11 g/g and no RBC casts on urine microscopy, C3, C4 normal, ANA, DsDNA, ANCA, cryoglobulins,RF,viral serologies and cultures were negative.She was started on steroids and a kidney biopsy was done



It showed diffuse proliferative GN with exudative features and IgG3- Kappa deposits. LM shows an acute proliferative and exudative GN and EM shows subepithelial hump-shaped immune type electron dense deposits. These findings are consistent with APIGN. The lack of low C3,C4 argues against that. The IF finding of apparent monoclonal IgG3-Kappa restricted deposits raises the possibility of PGNMID. The patient SPEP, UPEP, serum free light chain were negative.She was started on prednisone at 1mg/kg for a period of 1 month and tapered rapidly over 4 weeks .She is clinically asymptomatic, her last UPr/Cr is 1g/g of creatinine.

Conclusions: Our case is unusual because PGNMID patients tend to be older (80%>40yrs) and the predominance of hump shaped deposits is not characteristic of it. While both APIGN and PGNMID are in the differential, this case appears to be unique and defies definitive classification as a specific entitiy.

PUB274

Renal Involvement as the First Symptom of Multiple Myeloma

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Background: Renal involvement is a common complication of multiple myeloma (MM). However, most studies have focused on renal failure in MM, and little information is available about the other renal manifestations in MM and their association with immunophenotypes.

Methods: We retrospectively analyzed the clinical and laboratory data of 213 MM patients treated in Sichuan Provincial People's Hospital, West China, between January 1990 and May 2012. The patients were divided into a renal involvement group (n = 150) and a non-renal involvement group (n = 63).

Results: In the renal involvement group, 67 (44.7%) patients were diagnosed with MM in the Nephrology Department, and isolated proteinuria, renal failure and nephrotic syndrome were detected in 76 (50.7%), 77 (51.3%) and 44 (29.3%) patients, respectively. 85 patients with renal involvement underwent immunofixation electrophoresis, and IgG,

IgA, IgD, pure light chain and nonsecretory MM were detected in 34 (40%), 25 (29.4%), 1 (1.2%), 22 (25.9%) and 3 (3.5%) patients, respectively. 27 patients without renal involvement also underwent immunofixation electrophoresis, and IgG and IgA MM were found in 15 (55.6%) and 12 (44.4%) patients, respectively. Severe anemia and hypertension and pure light chain disease were more frequent in patients with renal involvement ($P < 0.05$). Serum globulin level was higher in patients without renal involvement ($P < 0.05$).

Conclusions: Renal involvement was common at MM diagnosis and had diverse clinical manifestations. Nephrologists should rule out MM in patients presenting with renal involvement.

PUB275

Identification of Double Negative αβ T Cells in Human Kidney

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Background: We found a large population of the unconventional T cell subset, the TCRαβ+CD4-CD8- double negative (DN) T cell, in mouse kidneys during studies of acute kidney injury (J Leuk Biol 2008). To evaluate the human relevance of the mouse findings, we studied human kidneys removed for renal cell carcinoma (RCC). Furthermore, given the role of immune system in RCC, intra-renal lymphocytes may play an important role in disease pathogenesis. In this study we hypothesized that DN T cells are present in human kidney. We also hypothesized that kidney lymphocyte numbers and function correlate with RCC stage.

Methods: We used a strategy to enrich hematopoietic cells from 6 human kidney modified from our mouse protocol. Briefly, we positively selected pure CD45+ lymphocytes from kidney mononuclear cells. Phenotypic characterization and cytokine analysis of purified CD45+ cells was performed by flow cytometry and data analyzed by Flow Jo software.

Results: We study 6 patient nephrectomy; 4 patients had clear cell, 1 patient had hybrid tumor and 1 patient had papillary RCC. We found DN T cells in all samples (7-55% of T cells). We also analyzed for CD4 (17-49%), CD8 (6-72%) and double positive (DP) CD4+/CD8+ T cells (3-22%). Only one patient had metastasis (patient number 2), who showed the lowest DN T cell infiltration and the highest CD8 T cell infiltration. DN T cells together with CD8 T and DP T cells express high values of IFN-γ (11.5%, 16% and 12% respectively).

Case	Age / Gender	Diagnosis / Tumor Diameter (cm)	% DN	% CD4	% CD8	% DP	Clinical Stage
1	77 M	Clear Cell / 4	**55**	32	6	3	cM0
2	55 M	Clear Cell / 8	**7**	17	72	4	cM1
3	68 M	Clear Cell / 3.5	**13**	49	11	22	cM0
4	63 M	Hybrid (RCC + oncocytoma) / 4	**22**	32	35	4	cM0
5	69 M	Papillary / 3.5	**13**	35	50	6	cM0
6	56 F	Clear Cell / 3.5	**13**	32	33	8	cM0

Conclusions: These data demonstrate that TCRαβ+CD4-CD8- double negative cells are present in human kidneys, validating findings in mouse kidney. Human kidney T cells may participate in regulating RCC.

Funding: NIDDK Support

PUB276

Spectrum of Native and Transplant Histological Features Seen in the Presence of Tubuloreticular Inclusions

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Background: The presence of tubuloreticular inclusions [TRIs] in glomerular endothelial cells is pathognomic of SLE and viral infections, particularly HIV. However, the spectrum of histological findings in patients with TRIs is not well defined. Furthermore, the relevance of TRIs in renal transplant biopsies is not known. The aim of our study is to determine the co-existing histopathological findings in patients with TRIs in native and transplant biopsies.

Methods: We retrospectively analysed all patients who underwent a native or renal transplant biopsy with TRIs on EM examination between 2005 and 2013.

Results: 290 patients had a native renal biopsy with TRIs. 211/290 [72.8%] were females. 83/290 [28.6%] were Caucasoid. The mean age at biopsy was 41.6 ± 15.6 years. The mean follow up was 3.9 ± 2.5 years. Renal survival post biopsy was 91.5%. Lupus was diagnosed on 206/290 [71.0%] biopsies. The remaining diagnoses are shown below:

Non-lupus histological diagnoses			
Immune complex GN	**N=35[12.1%]**	**Non-immune complex GN**	**N=49 [17.9%]**
Membranous	12 [4.1]	TMA	3 [1.0]
IgA	9 [3.1]	DM nephropathy	9 [3.1]
IgM	4 [1.4]	Pauci-immune GN	5 [1.7]
MPGN	5 [1.7]	FSGS	5 [1.7]
C3 glomerulopathy	2 [0.7]	TIN	5 [1.7]
HIVAN/HIVICK	3 [1.0]	TI Scarring	8 [2.8]
		Other	14 [4.8]

40 patients had TRIs on renal transplant biopsy. 16/40 [40.0%] were female. 19/40 [47.5%] were Caucasoid. The mean age at transplant was 52.6 ± 12.9 years. 4/40 [10.0%] had an underlying diagnosis of SLE. The mean follow up post biopsy was 2.3 ± 1.9 years. Allograft survival post biopsy was 50.7%. The distribution of histological diagnosis was as follows: alloimmune injury 17 [42.5%], glomerulonephritis (recurrent or de novo) 11 [27.5%], tubulo-interstitial scarring 6 [15.0%], vascular changes/hyalinosis 4 [10%], pyelonephritis 2 [5%].

Conclusions: This study shows the diversity of native renal pathology which can be seen in patients with TRIs. The lack of 'traditional' associations seen in transplant recipients with TRIs warrants further investigation.

PUB277

Focal Segmental Glomerulosclerosis in Patients Aged 40 Years and Older

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Background: The focal segmental glomerulosclerosis (FSGS) in Brazil corresponds to 29.7% of primary glomerulopathies and 9.7% of biopsies of elderly patients. The aim of this study was to evaluate the clinical, laboratory characteristics and etiologies of FSGS aged 40 years and older.

Methods: This is a retrospective single-center study of FSGS patients diagnosed from 2000 to 2013. There were 257 patients from all ages and 26.8% over 40 years old, while 5.6% were over 60 years old.

Results: Clinical characteristics of FSGS patients over 40 years are in Table 1.

Characteristics	Patients n= 69
Age (years), mean ± SD	52.6 ± 10.8
Serum creatinine (mg/dL), median and interquartile range	1.4 (1.0-4.0)
Proteinuria (g/day), median and interquartile range	4.0 (2.0-7.5)
Serum albumine (g/dL), median and interquartile range	1.4 (1.0-3.1)
Male (%)	63.7
White race (%)	46.3
Nephrotic syndrome (%)	62.3
Hypertension (%)	74
No family history of kidney disease (%)	97
Hematuria (%)	53.6

Eleven patients (16%) showed association with viral infection, 2 patients (2.8%) with long standing hypertension and 1 (1.4 %) with schistossomose. The remaining 55 patients (79.7%) were classified as idiopathic form. Columbia classification was performed in 50 patients, 31 (62%) NOS, 10 (20%) collapsing, 6 (12%) TIP, 2 (4%) perihilar and 1 (2%) celular. Fifteen patients had diagnosis over 60 years and were idiopatic forms.

Conclusions: The patterns of FSGS secondary to metabolic and hypertensive disease were infrequent based on clinical criteria and not indicating kidney biopsy. The idiopathic form occurred in 79.7% of cases and the types of FSGS based on Columbia classification were similar to published data in young patients under 40 years old.

PUB278

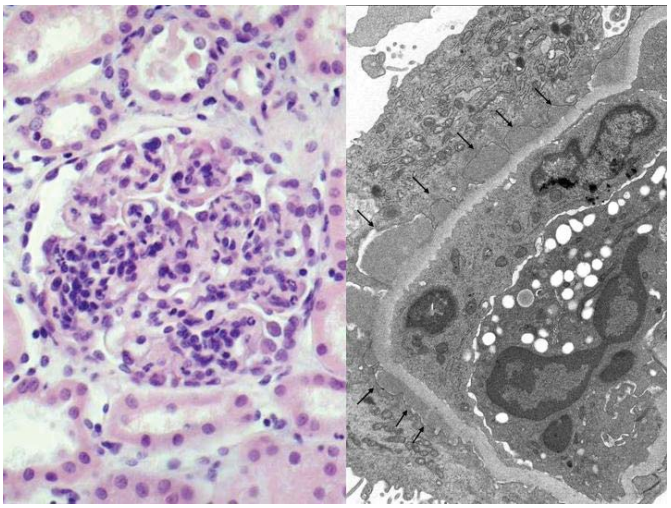
Henoch Schonlein Purpura Renal Biopsy Showing Features of IgA Dominant Post Infectious Glomerulonephritis Requiring Transplantation Followed by an Early IgA Nephropathy in the Renal Allograft

Sami S. Zarouk,[1] Sruthi Jinna,[1] Ping L. Zhang,[2] Michele T. Rooney.[2] *[1]Dept of Nephrology, William Beaumont Hospital, Royal Oak, MI; [2]Dept of Pathology, William Beaumont Hospital, Royal Oak, MI.*

Background: A 38-year-old Caucasian male had lower extremity rash, abdominal pain, and sore throat. Laboratory values revealed acute kidney injury and an elevated anti-streptolysin-O titer, negative anti-nuclear antibody, anti-neutrophil cytoplasmic antibody, and normal complement levels.

Methods: Skin biopsy revealed IgA positive leukocytoclastic vasculitis. Kidney biopsy revealed diffuse "exudative" glomerulonephritis, with endocapillary proliferation

and marginated neutrophils. Immunofluorescence revealed IgA predominance, C3, and light chain with fluorescence for lambda greater than kappa. Electron microscopy showed numerous large epimembranous bell-shaped "hump-like" deposits.



Results: This patient's renal failure progressed in spite of immunosuppressive therapy. Seven months post presentation, he received a preemptive kidney transplant from his wife. He developed hematuria soon after, with findings of IgA nephropathy in his allograft biopsy at one month post transplantation. He is currently 5 years post-transplant with unchanged creatinine at 2.2 mg/dl.

Conclusions: Our case is suspected to be from streptococcus infection as opposed to staphylococcal infection associated IgA nephropathy (APIGN). It highlights the possibility of recurrence of IgA deposit in the renal allograft in a patient with clinical presentation of HSP and renal biopsy compatible with APIGN. The recurrence of IgA deposit in the renal allograft of this patient early on post-transplant is similar to primary cases of IgA nephropathy. Infection has been implicated in the pathogenesis of both of these entities. They may ultimately prove to be ends of a spectrum rather than two distinct entities.

PUB279

Serum LDH Level Is Associated with Decreased eGFR and Proteinuria in Patients with Glomerulonephritis Seong Kwon Ma,[1] Chang Seong Kim,[1] Ha Yeon Kim,[1] Hoon In Choi,[1] Jung Sun Park,[1] Eun Hui Bae,[1] Jongun Lee,[2] Soo Wan Kim.[1] *[1]Dept of Internal Medicine, Chonnam National Univ Medical School, Gwangju, Republic of Korea; [2]Dept of Physiology, Chonnam National Univ Medical School, Gwangju, Republic of Korea.*

Background: Although LDH is expressed in the kidney, the relationship between serum LDH and renal expression of LDH in kidney diseases has not yet been established. We investigated the relationship between serum LDH level and estimated glomerular filtration rate (eGFR) or proteinuria in patients with glomerulonephritis (GN). We also investigated the protein expression of LDH in the kidney of experimental renal ischemia/reperfusion (I/R) injury and puromycin aminonucleoside (PAN)-induced nephrotic syndrome.

Methods: We conducted a retrospective study of patients with GN diagnosed by kidney biopsy (n=104) and control patients (n=28). We analyzed clinical parameters including serum LDH, CKD-EPI eGFR, serum albumin and proteinuria by using one-way ANOVA, post hoc Tukey HSD test, Pearson's correlation coefficient, stepwise multiple linear regression. Renal I/R injury in mice was induced by clamping of both renal pedicles for 25 min. PAN-induced nephrotic syndrome in rats was induced by a single intravenous injection of PAN via the femoral vein.

Results: Serum LDH level is increased in patients with severe proteinuria compared with the control patients (358.4 ± 9.3 versus 515.0 ± 24.0 U/L, $p<0.05$). Serum LDH is also increased in patients with eGFR less than 60 mL/min/1.73 m^2 (358.4 ± 9.3 versus 523.0 ± 26.2 U/L, $p<0.05$). Serum LDH level was correlated with eGFR ($r = -0.433$, $p<0.001$), 24 h urine protein ($r = 0.478$, $p<0.001$), and serum albumin ($r = -0.432$, $p<0.001$). Linear regression analysis showed eGFR and proteinuria were independent predictors of serum LDH level. The protein expression of LDH was increased in the kidney of mice with renal I/R injury and rats with PAN-induced nephrotic syndrome.

Conclusions: In conclusion, serum LDH level is increased in patients with decreased eGFR and severe proteinuria, which may be attributed to increased renal expression of LDH during renal injury.

PUB280

Possible Involvement of von Willebrand Factor in TTP Secondary to SLE Miho Karube, Shinya Kaname, Yoshinori Komagata, Yoshihiro Arimura. *First Dept of Internal Medicine, Kyorin Univ School of Medicine, Tokyo, Japan.*

Background: Although SLE is known to be one of the common causes that may complicate thrombotic microangiopathy (TMA), its pathogenetic mechanism is still unclear.

Methods: We conducted clinical and histological study for two SLE patients with concomitant TMA in the kidney. Immunohistochemical analysis for von Willebrand factor (VWF) was also performed.

Results: Case 1 was a 44-year-old woman with a history of SLE for 12 years with lupus nephritis class V+III. Thrombocytopenia and hemolysis suddenly developed with AKI in the absence of the increased disease activity. ADAMTS13 activity was markedly decreased at 1.6% with an increase in anti-ADAMTS antibody titers, indicating the diagnosis of thrombotic thrombocytopenic purpura (TTP). Renal biopsy revealed class V lupus nephritis with TMA and glomerular endothelial injury. Interestingly, VWF showed strong granular staining along the glomerular capillary loops. After administration of glucocorticoid, TTP findings quickly improved. Case 2 was a 41-year-old woman with a history SLE for ten years. Thrombocytopenia, hemolysis and AKI developed in association with a relapse of SLE, but ADAMTS13 activity was normal. Renal biopsy showed lupus nephritis class IV+V with TMA lesions, but no staining of VWF was observed in contrast to Case 1. The patient was treated with glucocorticoid and plasmapheresis with a good response.

Conclusions: From these results, it is suggested that VWF may be involved in the development of renal TMA in patients with lupus nephritis associated with TTP, but not in those with renal TMA from unknown causes other than TTP.

PUB281

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Conclusions: From these results, it is suggested that VWF may be involved in the development of renal TMA in patients with lupus nephritis associated with TTP, but not in those with renal TMA from unknown causes other than TTP.

PUB282

The Clinicopathologic Study of Renal Diseases in High Altitude Population Zhangxue Hu, Jiachuan Xiong, Zhaoxia He, Ping Fu, Ye Tao. *Dept of Nephrology, West China Hospital, Sichuan Univ, Chengdu, Sichuan, China.*

Background: Little is known about the renal biopsy spectrum of high altitude patients with kidney disease. It is unknown whether high altitude affect the structure of the kidney, too. This study aimed to analyze renal disease spectrum in patients living in high altitude area, and to explore the effect of high altitude on kidney.

Methods: We retrospectively enrolled 111 patients from high altitude area(mainly from Tibet), who underwent renal biopsy in West China Hospital since January,2012 to December, 2012. Thirty MCD patient from normal altitude area were also included. Demographic and clinical information were collected from each patient's clinical record. The glomerular volume and thickness of glomerular basement membrane(GBM) were measured in 41 patients diagnosed minimal chang disease(MCD) and IgA nephropathy(Lee classification 1 grade, M0E0S0T0) from high altitude area and 30 MCD patient from normal altitude area.

Results: In this study, membranous nephropathy (24.3%) was the most common diagnosis in the primary glomerular diseases. Henoch-Schoenlein purpura nephritis (10.8%) accounted the most frequent in the secondary glomerular diseases while lupus nephritis was second cause. High altitude patients have a high prevalence of hyperuricemia and hypertension. Although there was no difference of the glomerular volume between patients with MCD or mild IgAN from high altitude area and those from normal altitude area ($P = 0.27$), GBM was significantly thicker than those from normal altitude area ($P = 0.005$).

Conclusions: There are differences in renal disease spectrum between high altitude population and normal altitude population. Lupus nephritis is not the leading cause in secondary glomerular diaseses. GBM was thicker in high altitude population compared to normal altitude population, which could be the early change caused by high altitude. Further detailed study should be conduct to clarify the mechanism how high altitude circumstances affect renal structure and function.

PUB283

Endothelial Nitric Oxide Synthase Gene Expression Is Associated with Hypertension in Autosomal Dominant Polycystic Kidney Disease

Ismail Kocyigit,[1] Serpil Taheri,[2] Elif Funda Sener,[2] Aydin Unal,[1] Eray Eroglu,[3] Fahir Ozturk,[3] Kezban Korkmaz,[4] Murat H. Sipahioglu,[1] Bulent Tokgoz,[1] Oktay Oymak.[1] *[1]Dept of Nephrology, Erciyes Univ Medical Faculty, Kayseri, Turkey; [2]Dept of Medical Biology, Erciyes Univ Medical Faculty, Kayseri, Turkey; [3]Dept of Internal Medicine, Erciyes Univ Medical Faculty, Kayseri, Turkey; [4]Dept of Medical Genetics, Erciyes Univ Medical Faculty, Kayseri, Turkey.*

Background: Early occurrence of hypertension is the prominent feature of autosomal dominant polycystic kidney disease (ADPKD). The role of angiotensin converting enzyme (*ACE*) gene polymorphism and endothelial nitric oxide synthase (*eNOS*) gene polymorphism on the clinical course ADPKD has not been well understood. Thus, we aimed to investigate both *ACE* and *eNOS* genes polymorphisms and expressions that affect hypertension in ADPKD.

Methods: ACE and eNOS gene polymorphism and their expressions were analyzed in 78 ADPKD patients and 30 controls. The 24-hour blood pressure monitoring was performed for the diagnosis of hypertension in all study participants.

Results: In ADPKD patients, *eNOS* expression and eGFR were found statistically higher in patients without hypertension compared to hypertensive ADPKD patients. Each unit of the increase in *eNOS* expression led to a 0.88fold decrease (95%CI, 0.80-0.96) in multiple logistic regression analysis.

Conclusions: *eNOS* gene expression is independently predicting hypertension in ADPKD population. This study, for the first time, showed a novel link between *eNOS* gene expression and hypertension in ADPKD.

PUB284

Development of ADPKD Cell Lines and CXCL12 Up-Regulation in ADPKD

Sun Ae Han,[1] Joon Young Jang,[1] Ah-Young Kang,[1] Hayne C. Park,[2] Chang-Seok Ki,[3] Young-Hwan Hwang,[4] Curie Ahn.[1,2] *[1]Transplantation Research Institute, Seoul National Univ Medical Research Center, Seoul, Korea; [2]Dept of Internal Medicine, Seoul National Univ Collage of Medicine, Seoul, Korea; [3]Dept Laboratory Medicine and Genetics, Samsung Medical Center, Seoul, Korea; [4]Dept of Internal Medicine, Eulji General Hospital, Seoul, Korea.*

Background: Human primary cells or tissues are also good tools for studying autosomal dominant polycystic kidney disease (ADPKD). Each cyst from one patient is thought to have a different somatic mutation, developing various ADPKD cell lines with a different cyst origin is necessary to understand heterogeneous nature of the disease.

Methods: Primary cells were obtained from several single cysts of 1-3 cm in diameter were transfected with a plasmid pRNS-1 and the SV40 large T antigen by lipofectamine. After establishment of cell lines, expression of SV40 antigen and cytokeratin was identified by immunocytochemistry. cDNA microarray was performed using Agilent SurePrint G3 Human GE 8x60K array and results were analyzed using GeneSpring GX 7.3.1. Candidate genes were validated by using Western blot and immunohistochemistry (IHC).

Results: Three normal cell lines and 6 ADPKD cell lines were established. All of our ADPKD cell lines were found to be LTL-positive and DBA-negative indicating proximal tubule origin. Germline and somatic mutations in PKD1 were identified by direct sequencing of genomic DNA from blood paired with cell lines. In microarray analysis, commonly changed genes in ADPKD cell lines with greater than 2 fold changes were total 842 genes: 319 up-regulated and 523 down-regulated ($p<0.01$). We found top 25 up- and down-regulated genes in ADPKD cell lines compared to nRTEC cell lines. Among them, CXCL12 and CXCR4/7 were examined as a possible candidate pathway. Expression of CXCL12 significantly increased in ADPKD by Western blot and IHC.

Conclusions: All of our PKD cell lines were found to have PKD1 gene defects, and originated from proximal tubules. This study showed up-regulation of CXCL12 in ADPKD, and further study will be required to show its possibility as a therapeutic target for ADPKD.

PUB285

Patient Experience with Pain Related to Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Dorothee Oberdhan,[1] Andrew C. Palsgrove,[2] Jason C. Cole,[2] Jaime Blais,[1] Rebecca Cheng.[2] *[1]Otsuka Pharmaceutical Development and Commercialization, Rockville, MD; [2]Covance Inc., San Diego, CA.*

Background: ADPKD leads to kidney enlargement and worsening kidney function due to cyst development, expansion, infection, and rupture, increasing patients' need for medical care. Patients with ADPKD typically experience pain related to these symptoms (Gabow 1991 and Grantham 1992). The pain experienced by ADPKD patients is complex and multifaceted with acute and chronic episodes which complicates adequate characterization. As a result the ADPKD Pain and Discomfort Scale (ADPKD-PDS) was developed to help assess disease impact and treatment effectiveness as well as inform treatment decisions.

Methods: Focus groups with adult ADPKD subjects (N = 168) and interviews with adolescent ADPKD subjects (N = 22) were performed across Europe, North America, South America, Australia, and Asia. In these interactions, disease symptoms and impacts of ADPKD-related pain on a patient's daily life were discussed.

Results: Adult patients consistently reported three distinct types of ADPKD-related pain: dull pain in the lower back or waist, sharp pain in the kidneys, and fullness or discomfort. They described that pain interfered with routine and leisure activities, relationships, mood, physical activities, and sleep. Adolescents primarily reported the impact of pain and discomfort on their daily activities including physical activities like playing sports, anxiety about their future, and decreased school attendance. Overall pain reported by adolescents was similar in nature to adults but the frequency of their symptoms was more intermittent.

Conclusions: Pain is an important symptom of ADPKD with measurable impacts on the daily lives of adult and adolescent patients. Increasing the understanding of this ADPKD-specific pain and its impact on health related quality of life through the development of tools like patient-reported outcome questionnaires could facilitate the assessment of treatment effectiveness and inform treatment decisions. Such a tool, the ADPKD-pain and discomfort scale, is currently under development.

Funding: Pharmaceutical Company Support - Otsuka Pharmaceutical Development and Commercialization

PUB286

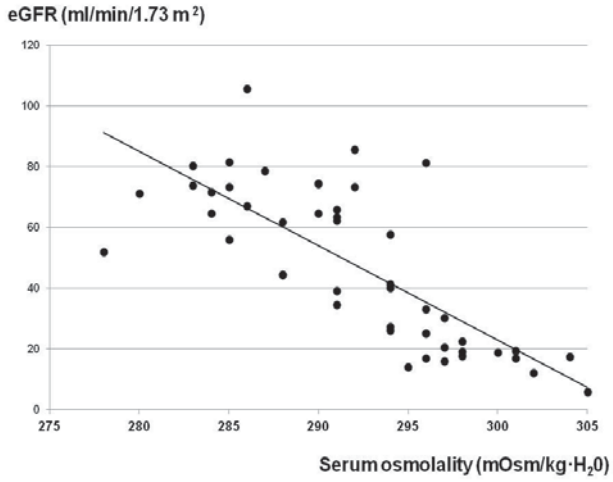
Serum Osmolality Has Much More Effective as Biomarker of Renal Function Than Urine Cyclic AMP or Copeptin in ADPKD Patients

Satoru Muto,[1] Yan Lu,[1] Haruna Kawano,[2] Masaki Kimura,[1] Keisuke Saito,[1] Shuji Isotani,[1] Hisamitsu Ide,[1] Raizo Yamaguchi,[1] Shigeo Horie.[2] *[1]Urology, Teilyo Univ, Tokyo, Japan; [2]Urology, Juntendo Univ, Tokyo.*

Background: In Japan, the novel treatment of V2R antagonist for ADPKD was started at a month ago. In this era, we have a great desire for precise and easy-to-use biomarker of progression of disease associated with arginine vasopressin-cyclic AMP pathway. However, it is unknown whether copeptin concentration is associated with disease severity in patients with ADPKD.

Methods: Urine cyclic AMP and copeptin concentration were measured in 52 ADPKD patients (24 females and 28 males, mean age; 51.8 years) by an immunoassay. Plasma and urinary osmolarity were also measured. To assess disease severity, we compared these markers with BUN, serum Cr, cystatin C, eGFR and total renal volume by magnetic resonance imaging predicted from the conventional ellipsoid method.

Results: The median eGFR and cystatin C were 48.3 ml/min/1.73 m^2 (range; 5.4-105.8) and 1.34 mg/l (range; 0.72-4.81), respectively. The median kidney volumes (TKV) were 1099.8 ml (range; 38.1-16298.2). Although Urine cyclic AMP/urine Cr and copeptin concentration were not significantly correlated with eGFR (cyclic AMP; R^2=0.474, p=0.107, copeptin; R^2=0.427, p=0.218) and TKV (cyclic AMP; R^2=0.132, p=0.273, copeptin; R^2=0.200, p=0.537), serum osmolality was significantly correlated with serum Cr (R^2=0.641, p=0.001), cystatin C (R^2=0.651, p=0.001) and significantly inversely correlated with eGFR (R^2=-0.140, p=0.004).



Conclusions: Although, prognostic and diagnostic properties of copeptin were recently reported in ADPKD patients as a marker of endogenous AVP secretion, serum osmolality may have much more effective as biomarker of renal function than copeptin in ADPKD patients.

PUB287

Clinical Characteristics and Disease Predictors of a Large Chinese Cohort of Patients with Autosomal Dominant Polycystic Kidney Disease

Changlin Mei,[1] Rudolf P. Wuthrich,[2] Shengqiang Yu,[1] Dongping Chen,[1] Yiyi Ma.[1] *[1]Dept of Nephrology, Shanghai Changzheng Hospital, Shanghai, China; [2]Div of Nephrology, Univ Hospital, Zürich, Switzerland.*

Background: The aim of this study was to characterize Chinese patients with autosomal dominant polycystic kidney disease (ADPKD) and to identify the factors which predict the disease progression.

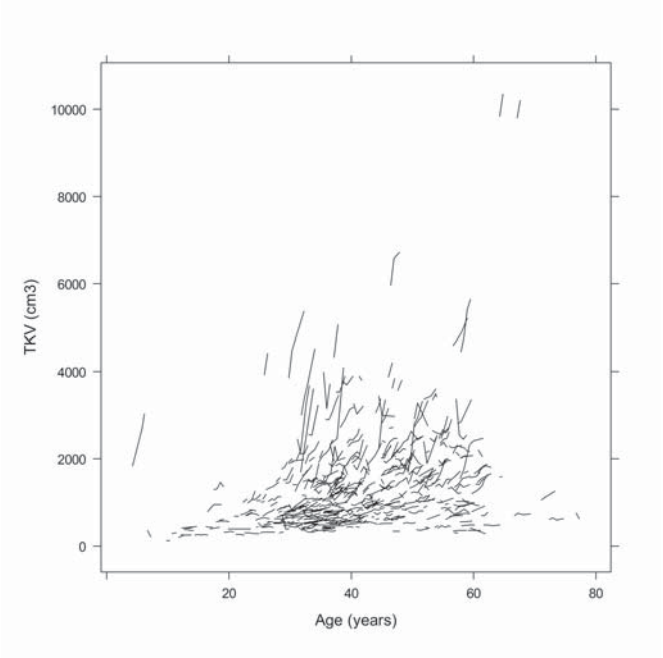
Methods: A prospective longitudinal observational study in a cohort of 541 Chinese patients with ADPKDwas performed. Patients were followed clinically and radiologically with sequential abdominal magnetic resonance imaging (MRI). Clinical characteristics

and laboratory data were related to changes in estimated glomerular filtration rate (eGFR) and total kidney volume (TKV). A linear regression model was developed to analyze the risk factors.

Results: The age range of this unselected cohort ranged from 4 to 77 years. Baseline parameters are listed in table 1.

Characteristics	N	Total
Age (years)	541	39.7±12.1
Height (cm)	539	167.5±9.7
History of hypertension	531	67.2%
Family history for ADPKD	509	75.4
eGFR(ml/min/1.73m)	502	100.4±20.1
Yearly eGFR change(ml/min/1.73m)	416	-0.9±9.6
TKV(cm^3)	532	1265±1002
Yearly TKV growth	421	4.6±10.2%
Protein creatinine ratio	324	0.21±0.32

Median follow-up time was 14.3±10.6 months. Spaghetti plot for TKV over time in individual patients is showed in figure1.



There was a consistent link between eGFR and TKV. Baseline log10-transformed TKV and urinary protein/creatinine ratio were identified as the major predictors for a faster eGFR decline and a higher TKV growth rate. Interestingly, a lower thrombocyte count correlated significantly with lower eGFR (r=0.222) and higher TKV (r=0.134).

Conclusions: This large cohort of Chinese patients with ADPKD provides unique epidemiological data for comparison with other cohorts of different ethnicity.

Funding: Government Support - Non-U.S.

PUB288

Plasma Pentraxin3 and Arterial Stiffness in Autosomal Dominant Polycystic Kidney Disease Abdulmecit Yildiz,[1] Cuma Bülent Gül,[2] Nimet Aktas,[3] Osman Z. Sahin.[4] *[1]Uludag Univ Medical School, Bursa, Turkey; [2]Sevket Yilmaz Research and Training Hospital, Bursa, Uganda; [3]Bilecik State Hospital, Bilecik, Turkey; [4]Recep Tayyip Erdogan Univ Medical School, Rize, Turkey.*

Background: Cardiovascular disease is leading cause of morbidity and mortality in patients with Autosomal dominant polycystic kidney disease (ADPKD), with over 80% of deaths attributable to coronary artery disease. Pentraxin3 (PTX3), a recently discovered inflammatory mediator, is produced abundantly in various cells in atherosclerotic lesions, and therefore, its plasma level could reflect local inflammation at the site of atherosclerotic lesion. The present study evaluated PTX3 levels in in the early stage of ADPKD and sought their association with arterial stiffness.

Methods: 54 ADPKD patients with preserved renal functions and 26 healthy subjects were included. Arterial elasticity was assessed and serum PTX3 level was measured. Comparison of the groups was performed with Student t or Mann Whitney U test and correlation analysis was done with Pearson or Spearman test as needed.

Results: In the ADPKD group, 23 patients were hypertensive (HT). The mean levels of PTX3 were higher in ADPKD patients compared to controls. PTX3 levels were similar in HT-ADPKD and normotensive (NT)-ADPKD patients (n=31). Large and small vessel compliances (C1 and C2, respectively) were lower in the ADPKD patients compared to controls. NT-ADPKD patients had lower C2 and tended to have lower C1 compared to controls. HT-ADPKD patients tended to have lower C1 and C2 compared to controls. HT-ADPKD and NT-ADPKD patients had similar) arterial function values. There was no correlation between arterial elasticity parameters and PTX3 levels.

Conclusions: Vascular inflammation that is evaluated with PTX3 is evident in ADPKD patients with preserved renal function, however vascular inflammation is not associated with arterial stiffness in ADPKD.

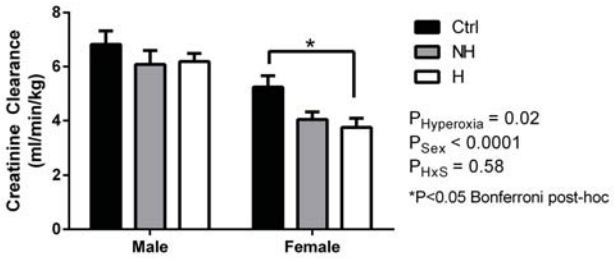
PUB289

The Long-Term Impact of Neonatal Hyperoxia Exposure on Renal Function Megan R. Sutherland,[1,2] Marie-Amélie Lukaszewski,[1,2] Anik Cloutier,[1,2] Mariane Bertagnolli,[1,2] Anne Monique Nuyt.[1,2] *[1]CHU Sainte-Justine Research Center, Montréal, QC, Canada; [2]Univ of Montréal, Montréal, QC, Canada.*

Background: Neonates born preterm are exposed to high oxygen levels relative to the intrauterine environment, when nephrogenesis is ongoing. We have previously shown that hyperoxic gas exposure impairs renal development in neonatal rats and results in a reduced nephron number in adulthood. The aim of this study was to determine the long-term effects of neonatal hyperoxia exposure on renal function.

Methods: Hyperoxia-exposed Sprague-Dawley pups were raised in 80% O_2 from P3 - P10 (H; n=8 litters). To prevent maternal O_2 toxicity, H dams were interchanged every 12 hours with a dam with pups raised in room air (NH; n=8 litters). Control litters were kept in room air (Ctrl; n=8 litters). At 1, 5, and 11 mo (1 male and 1 female /litter/age), animals were placed in metabolic cages with urine collected for 16h overnight; plasma and kidneys were collected the following day.

Results: There was no difference in body or kidney weights between groups. Hyperoxia exposure resulted in a significantly reduced urine output in animals at 5mo of age, but not at 1mo or 11mo. There was no difference in creatinine clearance (Cl_{Cr}) between groups at 1mo and 11mo; at 5mo, however, Cl_{Cr} was significantly reduced following hyperoxia exposure (Figure 1). There was also a trend at 5mo for increased fractional sodium excretion (p=0.09). Urine albumin/Cr levels increased with age, but there was no difference between groups.



Conclusions: In younger and older rats (likely with lower renal functional demand), kidney function was not significantly affected by neonatal hyperoxia exposure. Importantly, however, there was an adverse impact on creatinine clearance at 5 months of age. Overall, these findings suggest that individuals exposed to high oxygen levels during development may have a reduced renal functional capacity in adulthood.

Funding: Government Support - Non-U.S.

PUB290

Amniotic Fluid Stem Cells Transplantation in the Fetal Kidney to Regenerate Nephrons Loss during Nephrogenesis Luc Behr,[1] Sebastien Sammut,[1] Mehrak Hekmati,[1] Agnieszka Anna Ksiazek,[4] Benedikt Weber,[4] Martine D. Lelievre-Pegorier,[2] Kathleen Laborde.[3] *[1]IMMR, Paris, France; [2]INSERM U872, Paris, France; [3]Necker Hospital, Paris-5 Univ, Paris, France; [4]Swiss Center for Regenerative Medicine, Zurich, Switzerland.*

Background: Amniotic fluid stem (AFS) cells are pluripotent and harbour the potential to differenciate upon renal lineages and contribute to the development of primordial kidney structures. The aim of the present study was to investigate AFS cells contribution to the formation of renal tissue when placed in a kidney context of relevance for regenerative medicine, during the window of active nephrogenesis, in a model of subtotal nephrectomy in the fetal sheep.

Methods: Ovine amniotic fluid-derived stem cells (AFSd) were isolated, labelled with GFP and selected using cell surface marker c-kit. When cultured in the absence of LIF, AFSd formed embryonic bodies which cultured in an optimized medium generated renal progenitor population, expressing PAX-2 (AFSr). Subtotal nephrectomy (5/6NX) was performed at 70 days of gestation in 11 fetal lambs among which 5 were treated with intra-renal injection of AFS in the caudal (AFS) and cranial (AFSr) kidney poles. Control fetuses were sham-operated and did not received AFS (SHAM, n=5). At 134 days of gestation, the fetuses were euthanized, the kidneys removed and processed.

Results: Among the fetuses, 3 died before 134 day, one (1/6) in the 5/6NX and 2 (2/5) in the 5/6NX+AFS group. Body weight did not differ significantly between the different groups. Compensatory renal growth was observed in all 5/6NX fetuses, displaying in all cases the striking budding pattern previously described. In spite of AFS injection, catch-up growth was similar in the 5/6NX+AFS and the 5/6NX groups (SHAM: 2.7±0.4; 5/6NX: 4.7±2; 5/6NX+AFS: 5.52±1.44 KW/BW). Renal cranial and caudal budding sizes were similar, and not related to the differenciation status of the AFS injected. GFP cells were present in the kidney parenchyma and appeared integrated in the renal structures.

Conclusions: Amniotic fluid-derived stem cells may represent a potentially source of cells for kidney regeneration but do not seem to restore completely kidney damage, even when injected during nephrogenesis.

Funding: Private Foundation Support

PUB291

Reno Protective Activity of Esculetin in Insulin Resistant and Type 2 Diabetic Rats

Almesh Mallappa Kadakol, Santosh Kumar Goru, Anuradha Pandey, Anil Bhanudas Gaikwad. *Pharmacy, BITS Pilani, Pilani, Rajasthan, India.*

Background: Diabetic nephropathy is the leading cause among the all renal failure in patients. There is a need of better therapeutic agent to prevent initial causes and further worsening of renal failure under type 2 diabetic conditions. Several attempts have been made to improve existing treatment hence, in this study Esculetin which has been recognized as inhibitor of lipoxygenase was used as a therapeutic intervention against type 2 diabetic nephropathy.

Methods: Insulin resistance and Type 2 diabetes was induced by High Fat Diet (HFD) feeding and low dose (35mg/kg, *i.p.*) of Streptozotocin in Wistar rats (140g-150g). In brief, animals were grouped into normal control, high fat diet control, diabetic control and diabetic control treated with Esculetin. The rats were allowed to feed on HFD till end of the study (24 weeks). Rats were treated with Esculetin 50 and 100 mg/kg/day, *per oral* and control with Vehicle (0.5% sodium carboxy methyl cellulose) for 7 days prior to end of the study. Body and organ weights were measured and different biochemical estimations to access insulin resistance, diabetes and diabetic nephropathy were carried out at the end of study.

Results: High fat diet control developed insulin resistance, whereas high fat diet fed animals treated with low dose of STZ exhibited the type 2 diabetes. There was a significant increased in blood urea nitrogen (BUN) level in insulin resistant animals compared to normal control animals. Treatment with Esculetin to diabetic animals was resulted in significant reduction in BUN (18.96±0.37 to 6.52±0.50mmol/l, $p<0.05$) and PCr (11.37±0.05 to 2.42±0.04mg/dl, $p<0.05$), and increase in PA (18.48±1.44 to 37.68g/l, $p<0.05$) levels compared to diabetic control. Further, two folds of the kidney weight and kidney/body weight ratio were also reduce. In addition, the level of MDA (15.73±1.04 to 9.492±0.83, $p<0.001$) was reduced and the level of GSH (1.475±0.21 to 7.420±0.73µg/mg protein, $p<0.001$) level was increased in Esculetin treated HFD+STZ rat kidney compared to HFD+STZ rats.

Conclusions: These observations demonstrated that 100mg/kg dose of Esculetin has better reno protective activity.

Funding: Government Support - Non-U.S.

PUB292

The Effect of Nigella Sativa Extract on Diabetic Nephropathy

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Background: *Nigella sativa* has been studied for its biological impacts and therapeutic potential. We investigated the possible renoprotective effects of this plant seeds on diabetic nephropathy.

Methods: Male Sprague-Dawley rats were randomly divided into:Group1;diabetes (6), group2;diabetes+*N.sativa* (8), group3;only *N.sativa.* (8) and group4;control (8). Diabetes was induced by streptozotocin. 50g *N.sativa* seed was subjected with methanol for 24 hours at 40°C. Methanol was evaporated and extract was obtained with lyophilization at -85°C. Diluted extract was administered 200mg/kg/day i.g for 5 weeks. Serum glucose, urea and creatinine were measured before extract administration and sacrification.Transforming Growth Factor Beta (TGF-β_1)(pg/ml), type IV collagen(ng/ml), superoxide dismutase(ng/ml) and hydroxyproline(ng/L) were measured in the obtained tissue by Elisa.

Results: Glucose and urea levels did not change in all groups throughout the study. Serum creatinine was significantly decreased in groups 3 and 4. Results of tissue parameters and variations between groups are presented in Table1

	[1]TGF-β_1	[2]SOD	[3]Hydroxyprolin	[4]Type 4 collagen	(*p - #p) ,(†p - ‡p), (αp - ∞p)
Group 1	344.5±43.3	2.69±0.78	831.7±41.7	9.74±7.9	[1]0.013-0.001, 0.001-0.000, 0.000-0.105
Group 2	434.4±66.8	2.86±0.12	825.3±36.7	52.32±49.6	[2]0.020-0.001, 0.001-0.038, 0.000-0.000
Group 3	597.8±38.9	3.00±0.42	786.6±19.5	92.25±55.5	[3]0.852-0.029, 0.001-0.015, 0.000-0.000
Group 4	648.1±50.8	3.19±0.06	985.9±76	85.9±73.9	[4]0.059-0.003, 0.001-0.130, 0.234-0.574

TGF-β1Transforming Growth Factor Beta1, SOD superoxide dismutase
*group 1vs group 2, # group 1vs group 3
† group 1vs group 4, ‡group 2vs group 3
α group2vs group 4, ∞ group 3 vs group 4
p less than 0.008 was accepted as significant.

Table1.The results of tissue parameters of all groups and differences according to group analysis.
These parameters were not significantly different between diabetes and diabetes+*N.sativa* group.

Conclusions: Any favorable effect of N. sativa could not be demonstrated on diabetic nepropathy.

PUB293

Effects of SAA and AGE to Activate PKC-β-Mediated Podocyte Inflammation and Apoptosis

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Background: Serum amyloid A (SAA) is increased in the kidneys of humans and mice with diabetic kidney disease (DKD). Advanced glycation end-products (AGE) are metabolic mediators of DKD that produce glomerular damage by podocyte injury and loss. Protein kinase-beta (PKC-β), a central signal for cellular inflammation and death, is activated by ligand engagement with the receptor for AGE (RAGE). The aim of this study was to determine if SAA mediates podocyte inflammatory and apoptotic responses by activation of PKC-β in a manner similar to AGE.

Methods: Mouse podocytes were cultured with human SAA protein or AGE. β-isoform specific (LY-379198) and general (calphostin C) PKC inhibitors were added to media at 100 nM. Inflammatory mediator mRNA expression (SAA3, Cxcl5, Ccl5, Ccl2) was assessed by RT-PCR after 24 hours. Apoptosis was measured by TUNEL assay after 48 hours. PKC-β activation was measured by phosphorylation.

Results: PKC-β inhibition reduced SAA-induced SAA and Cxcl5 mRNA (51±17 %, 68±19 % relative to control; both $p<0.001$, n=8). General, but not PKC-β, inhibition reduced SAA-induced Ccl2 (54±15 % relative to control; $p<0.001$, n=8). However, Ccl5 was not reduced by either inhibitor. PKC-β inhibition reduced AGE-induced mRNA for SAA3, Cxcl5, Ccl2, and Ccl5 (25±15 % relative to control, p=0.019; 75±6 %, $p<0.001$; 27±16 %, p=0.017; 44±20 %, p=0.004, n=6-8). SAA- and AGE-induced podocyte apoptosis were reduced by PKC-β inhibition (93±2 % relative to control, p=0.005; 62±10 %, $p<0.001$, n=6). Phosphorylated-PKC-β was increased by both SAA and AGE.

Conclusions: PKC-β mediated up-regulation of 2 of 4 inflammatory mediators in podocytes exposed to SAA, whereas AGE-induced expression of all 4 mediators was at least partly-dependent on PKC-β. Both SAA and AGE-induced apoptosis were highly-dependent on PKC-β. Effects of SAA and AGE to activate PKC-β-mediated podocyte inflammation and apoptosis are similar but not identical, indicating that SAA acts via pathways that are not entirely comparable to AGE-RAGE interactions.

Funding: Other NIH Support - Providence Sacred Heart Medical Center and Children's Hospital

PUB294

PPARα/γ Agonism Confers Partial Improvement in the BTBRob/ob Mouse Model of Diabetic Nephropathy

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Background: The BTBRob/ob mouse model have been shown to mimic progressive albuminuria and morphologic lesions of advanced human diabetic nephropathy (DN). The enalapril renoprotective effect is limited in the BTBRob/ob mouse whereas leptin replacement was found to reverse damage (1).

Methods: To further investigate renoprotection and improved metabolic control in BTBRob/ob mice the PPARα/γ agonist AZD6610 (10.25mg/kg diet, n=8) were used from 17 to 24 weeks of age and compared with BTBRob/ob without treatment (n=12) or with enalapril treatment (200mg/l water, n=7) from 14 to 24 weeks of age. Lean BTBR mice were used as healthy controls (n=5).

Results: AZD6610 significantly lowered plasma glucose (median 11.3 versus 23.8mM) and triglycerides (median 1.0 versus 2.0mM), and increased liver growth (median 6.5 versus 4.0g) in BTBRob/ob. Urine albumin excretion rate (UAER) at 24 weeks age was increased in BTBRob/ob compared to lean controls (median 1206 versus 39µg/13h). AZD6610 had no effect on UAER whereas enalapril appeared to lower UAER but this did not reach statistical significance. In contrast, morphological semi-quantitative assessment of glomerula demonstrated mesangial matrix expansion in BTBRob/ob that was reduced by AZD6610 treatment but not by enalapril. Immunohistochemical podocyte markers nephrin and WT-1 showed a reduction in BTBRob/ob, but neither enalapril or AZD6610 treatment improved these markers. Furthermore, an expected but slight increase (+10%) in thickness was observed in the glomerular basement membranes in the BTBRob/ob, with no treatment effects.

Conclusions: PPARα/γ agonism improves metabolic control and reduces mesangial matrix expansion, but without positive effects on albuminuria or immunohistochemical podocyte markers. We have reproduced the previously reported effects of enalapril in this mouse model (1) and show that late intervention with PPARα/γ agonism can only partially improve hallmarks of DN.

Reference
1. Pichaiwong, W. et al. (2013) Reversibility of structural and functional damage in a model of advanced diabetic nephropathy. *J Am Soc Nephrol 24 (7):1088-102.*

Funding: Pharmaceutical Company Support - AstraZeneca R&D Mölndal, Sweden

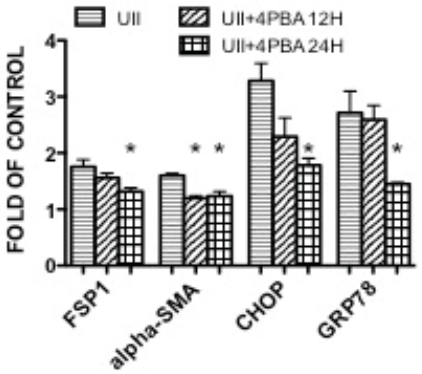
PUB295

Urotensin II Can Induce EMT via Triggering Endoplasmic Reticulum Stress in Diabetic Nephropathy Aihua Zhang, Qiong Bai, Minhua Fan. *Dept of Nephrology, Peking Univ Third Hospital, Beijing, China.*

Background: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease. Urotensin II (UII) and its receptor are highly expressed in the kidney tissue of patients with DN. We presume that UII may cause endoplasmic reticulum (ER) stress. This will in turn induce Epithelial-Mesenchymal Transition (EMT) in renaltubular epithelial cell of DN, which can eventually cause renal tubule injury and interstitial fibrosis.

Methods: Kidney tissues were collected from ten diabeticnephropathy patients and ten healthy control subjects. The expression of UII, UII receptor (GPR14), CHOP, and GRP78 in kidney were detected by immunohistochemistry. HK-2 cells in vitro were cultured and treated with different concentrations of UII, UIIcombined with antagonist of ER stress (4PBA), and PBS solution. Markers of ER stress,CHOP and GRP78, and markers of EMT,α-SMA and FSP1,were then detectedby real-time PCR.

Results: UII,UII receptor (GPR14), GRP78, and CHOP were highly expressed in the kidney tissue of DN than those of normal healthy controls, which were mainly localized in renal tubulointerstitium. Expression of UII was positively correlated with the expression of CHOP and renal fibrosis in DN patients' kidney tissue. In HK-2 cells, the amount of UII present was shown to correspond to the level of induced ER stress and EMT. 4PBA, an antagonist of ER stress, can inhibit the mRNA expression of α-SMA and FSP1 when UII was incubated with HK-2 cells.ID tag.



Conclusions: We first verify the expression of UII and its receptor are positively correlated with ER stress and renal fibrosis in DN, UII can trigger ER stress pathway which will induce EMT in renal tubular epithelial cell in vitro. ER stress pathway of renal epithelial cell may be involved in the UII induced renal fibrosis in DN.

PUB296

CCR2+ Inflammatory Monocytes Contribute to Early Graft Dysfunction of MHC Mismatched Islet Transplants Ke Vin Chow,[1,2,3] Robyn M. Sutherland,[1] Yifan Zhan,[1] Andrew M. Lew.[1] *[1]Immunology, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; [2]Medical Biology, Univ of Melbourne, Parkville, Victoria, Australia; [3]Nephrology, Royal Melbourne Hospital, Parkville, Victoria, Australia.*

Background: Islet transplantation provides a potential cure for patients with type 1 diabetes, and is associated with reduced long-term complications and improved quality of life. Despite this, its use is limited by a lack of supply of donor organs and issues with poor primary engraftment. CCR2+ monocytes are actively involved in the inflammatory response and are capable of differentiating into monocyte derived dendritic cells, which may play a role in islet transplantation.

Methods: To determine the role of CCR2+ monocytes in this setting, we transplanted 400 MHC mismatched islets from BALB/C mice (H-2d) under the kidney capsule of C57BL/6 hosts (H-2b). Host mice had been genetically modified to express the primate diphtheria toxin receptor under the control of the CCR2 promoter (CCR2.DTR) allowing for diphtheria toxin induced conditional depletion of CCR2+ cells. Host mice were rendered diabetic by the administration of streptozotocin prior to transplantation. Nine doses of diphtheria toxin 20 ng/g (DT+) (n=10) or normal saline (control) (n=9) were administered between day -4 and day 15. Graft function was determined by measuring blood glucose (BG) via tail bleed.

Results: DT+ mice showed complete absence of CCR2+ monocytes in peripheral blood with normal populations of CD4+ T cells, CD8+ T cells, CD19+ B cells and Ly6G+ neutrophils. Pre-transplant BGs between DT+ and control mice were equivalent (27.0 ± 1.3 mmol/L versus 29.6 ± 1.1 mmol/L, P = 0.159). DT+ mice achieved higher rates of normoglycemia than control mice at day 1 (11.0 ± 1.8 mmol/L versus 19.1 ± 1.4 mmol/L, P = 0.004) and day 3 (7.1 ± 0.8 mmol/L versus 15.4 ± 2.0 mmol/L, P = 0.003). Despite this, there was no significant difference in long-term graft survival between the two groups.

Conclusions: CCR2+ monocytes appear to be involved in an innate immune response that mediates early graft dysfunction of transplanted islet allografts. Further studies are required to determine the mechanism of this effect and the role of these cells in the adaptive immune response.

Funding: Government Support - Non-U.S.

PUB297

Diabetic Nephropathy Is Attenuated after 8 Weeks of Resistance Exercise Training (RET) Kleiton Augusto Santos Silva,[1] Luciana Jorge,[1] Rafael Luiz,[1] Rodolfo Rosseto Rampaso,[1] Ralmony Santos,[2] Tatiana Sousa Cunha,[2] Nestor Schor.[1] *[1]Medicine, Federal Univ of Sao Paulo, Sao Paulo, SP, Brazil; [2]Science and Technology Institute, Federal Univ of Sao Paulo, Sao Jose dos Campos, SP, Brazil.*

Background: Kidney is impaired in Diabetes mellitus condition. RET normally used against skeletal muscle atrophy has unclear effects on kidney function and thus, we assessed kidney from diabetic rodents under RET.

Methods: Wistar rats were submitted to RET (50 – 80% of maximal load test). After diabetes establishment (Streptozotocin, 50 mg/kg i.v.), animals were divided into four groups. Two control groups (non-exercised and exercised) and two diabetic groups (non-exercised and exercised) in 4 – 6 rats/group. We measured parameters as body weight and proteinuria; we also measured protein level by Western blot and multiplex technology assay.

Results: It was found that RET does not protected skeletal muscle from atrophic process in exercise diabetic group. Extensor digitorium longus weight – EDL were: 0.36±0.20 mg/g versus 0.33±0.53 mg/g ($p>0.05$) in non-exercised and exercised diabetes groups, respectively. Additionally, maximal load test demonstrated no significant different among diabetic groups. Regarding kidney, it was found that KW was preserved in exercised diabetic animals (5.7±2.72 mg/g diabetic versus 4.7±2.01 mg/g exercised diabetic, $p<0.05$); moreover, proteinuria was lower in exercised diabetic group compared to diabetic group ($p<0.05$) and podocin expression was increased in exercised diabetic animals. We assessed the mTOR signaling pathway and found significant decreases in PI3K, p-Akt and p-4EBP1 in exercised diabetic animals ($p<0.05$). Additionally, we found that TGFβ-1 was markedly decreased in exercised diabetic animals (437±13 pg/mL diabetic versus 325.8±21 pg/mL exercised diabetic, $p<0.05$).

Conclusions: Kidney may be functionally protected and mTOR signaling pathway plays important role on renal environment. However, skeletal muscle might have no improvement under this program. Thus, a different effects under RET affect skeletal muscle and kidneys but since a decrease in proteinuria was observed, a potential protection against progression of diabetic nephropathy with RET could be a strategic complementary therapy.

PUB298

Advanced Glycation End Products Impaired Diabetic Muscle Function via AMP-Activated Protein Kinase-Mediated Muscle Atrophy Signaling Pathway Chih-Kang Chiang,[1,2,3] Chen-Yuan Chiu,[1] Yuan-Siao Chen,[2] Shing-Hwa Liu.[1] *[1]Graduate Institute of Toxicology, National Taiwan Univ, College of Medicine, Taipei, Taiwan; [2]Dept of Internal Medicine, National Taiwan Univ Hospital, Taipei, Taiwan; [3]Dept of Integrated Diagnostics and Therapeutics, National Taiwan Univ Hospital, Taipei, Taiwan.*

Background: Advanced glycation end products (AGEs) are the etiology of Diabetes mellitus (DM)-related complications via crosslinking proteins and tissue accumulation. DM has become near a half the primary etiology of incident dialysis. AGEs-induced muscle weakness or wasting has become a critical issue in maintenance dialysis patients. Here, we investigated whether the AGEs accumulation impaired diabetic skeletal muscle function.

Methods: Adult male ICR mice were peritoneally injected with 100 mg/kg streptozotocin to induce type 1 DM. The expression of AGEs in the soleus muscles were determined by immunohistochemistry. Muscle function was examined by the fatigue task using a Rota-Rod, and we also measured the weights of gastrocnemius muscles and soleus muscles in glycerol-induced muscle injury. Moreover, the C2C12 myoblast-differentiated myotubes were used to test the *in vitro* effects of AGEs. The myotubes were determined morphologically by H&E staining. The expressions of proteins (Atrogin-1, MuRF-1, phospho-Akt, phospho-FoxO3a, phospho-AMPK) were determined by Western blotting.

Results: Diabetic mice with/without intra-muscular injection of glycerol had less skeletal muscle mass and lower muscular endurance. To explore the mechanism of AGEs on skeletal muscle atrophy, the myotubes were exposed to AGEs in the differentiation media for 48 h. AGEs significantly and dose-dependently reduced numbers and diameters of C2C12 myotubes at 48 h. As expected, AGEs stimulated AMPK and the muscle atrophy-related protein expressions (Atrogin-1, MuRF-1, phospho-FoxO3a), and inhibited the muscle hypertrophy-related protein expression (Akt phosphorylation).

Conclusions: Modulation of AMPK is linked to AGEs accumulation and AGEs-related impaired diabetic skeletal muscle function. We concluded that AGEs may up-regulate AMPK expression via an atrophy-related signaling pathway, leading to induction of diabetic muscle wasting.

Funding: Government Support - Non-U.S.

PUB299

Atrasentan, a Specific ET-A Blocker, Improves Podocytes Survival and Ameliorates Diabetic Kidney Glomerular Injury in the db/db Mouse

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Background: The endothelin(ET) system is chronically activated in diabetic nephropathy and retinopathy. We have shown that atrasentan administration to db/db mouse ameliorates the retinal vascular pathology while increasing the number of pericytes, a cell with similarities to podocytes. Here we report the effect of atrasentan on glomerular podocyte counts and glomerular injury.

Methods: Diabetic mice, 23 weeks old, were given either atrasentan or vehicle in drinking water for 8 weeks. At the end of the treatment period, kidneys were harvested for PAS staining for morphometric analysis and for WT-1 staining to perform podocyte count. Mesangial matrix expansion, glomerular cellularity and glomerular size were evaluated in PAS stained sections.

Results: In atrasentan-treated db/db mice, glomerular mesangial matrix and glomerular cellularity decreased significantly as compared to vehicle-treated db/db mice. Glomerular size decreased in atrasentan-treated db/db mice (Table). Atrasentan-treated diabetic db/db mice showed significantly higher podocyte counts per glomeruli as compared to vehicle-treated db/db mice ($p < 0.01$).Preliminary work in cultured podocytes suggests that atrasentan reduces glucose-induced apoptosis as a potential mechanism of increased podocyte survival (not shown).

Group	Db/m	Db/db	Db/db, atrasentan
Mesangial matrix expansion	1 ± 0	2.7 ± 0.3*	1.4 ± 0.2†
Glomerular cellularity	1.0 ± 0	2.1 ± 0.1*	1.3 ± 0.2†
Glomerular size	0.0031 ± 0.0001	0.0048 ± 0.0002*	0.0039 ± 0.0006≥†
Podocyte Count (per glomeruli)	13.1± 0.3	10± 0.4*	12.1± 0.4†

Conclusions: Atrasentan ameliorates glomerular diabetic lesions in the db/db mouse and has a protective effect on podocytes, similar to our finding of increased retinal pericyte counts.The dual protective effect of atrasentan for diabetic retinopathy and nephropathy involves enhanced pericyte and podocyte survival.

Funding: Pharmaceutical Company Support - Abbvie

PUB300

Down-Regulation of Transient Receptor Potential (TRP) M6 Channel as a Cause of Hypermagnesiuric Hypomagnesemia in Obese Type-2 Diabetic Rats

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Background: Hypomagnesemia is found in 30-40% of diabetic patients and is associated with the risks of the development of insulin-resistance and the coronary artery disease. The establishment of hypomagnesemia in diabetes is principally caused by the excess excretion of Mg from kidney which is apparent even in the early nephropathy. The purpose of this study is to understand the underlying mechanism causing the increase in excretion of Mg in diabetic nephropathy, and the association with tubulo-interstitial nephropathy (TIN) that is known for the increase in excretion of Mg will be discussed additionally.

Methods: Kidneys were obtained from male OLETF (F) and LETO (L) rats of 16, 24, and 34 weeks-old. Expression profile of Mg transporting molecules and the development of TIN were assessed by immunohistochemistry and RT-PCR.

Results: Renal Mg excretion in F, assessed by UMgV and FEMg, was significantly higher than L in the weeks of 24 and 34. mRNA and protein expression of transient receptor potential (TRP) M6, Mg pathway in DCT, in F was down-regulated in all weeks, which was synchronized with down-regulation of thiazide-sensitive NCC, one of principal regulator of TRPM6 expression. However, expression of claudin-16, the other principal Mg pathway in tight junction of TAL, was not altered in F at all weeks. Development of TIN assessed by the ratio of interstitial area, expression of fibrosis-related molecules was not significant in both F and L at all weeks.

Conclusions: Decreased Mg reabsorption in DCT by the down-regulation of TRPM6 might be a principal cause for hypomagnesemia in diabetes patients being independent from TIN. The down-regulation of NCC might be involved in the altered TRPM6 expression. Assessment of Mg excretion might be useful to evaluate the functional abnormalities of tubules in early diabetes.

PUB301

Role of the Inflammasome and Interleukin-33 in Diabetic Nephropathy Pathogenesis

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Background: Diabetic nephropathy is the leading cause of end-stage renal disease. Therefore, investigations into the mechanisms that underlie intrarenal inflammation may provide new therapeutic targets for anti-inflammatory strategies against diabetic nephropathy. We aimed to explore the roles of NLRP3, the inflammasome and IL-33 in the pathogenesis of diabetic nephropathy in a streptozotocin-induced diabetes mouse model.

Methods: To generate an experimental diabetes model in mice, 50 mg/kg of streptozotocin was administered intraperitoneally. Kidney tissues were obtained via sacrifice in the 1st, 2nd, 3rd, 4th and 6th months. Immunohistochemical staining was performed to visualize IL-33. IL-33, anti-ASC, IL-1β, caspase-1 and NLRP-3 protein analyses were performed in the kidney tissues using the western blot method and ELISA (only for IL-33).

Results: IL-33 expression was typically located in the peritubular and intraglomerular capillaries, according to the results of immunostaining experiments.Consistent with our immunohistochemical data, we found significant 6-fold (diabetic mice 4th month versus control, $p<0.01$) and 5.8-fold (diabetic mice 6th month versus control, $p<0.01$) increases in IL-33 protein expression in western blots of whole kidney tissue and assessment of IL-33 with ELISA also confirmed our results (IL-33 levels 0.409pg/µg diabetic mice 6th month versus 0.164pg/µg control, $p<0.01$). No significant differences in the expression of the anti-ASC, IL-1β, caspase-1 and NLRP-3 proteins were observed between diabetic mice and controls.

Conclusions: These results reveal that IL-33 may participate in the pathogenesis of diabetic nephropathy. The recognition of IL-33 as a significant pathogenic mediator in diabetic nephropathy provides the opportunity to identify new potential therapeutic targets.

PUB302

Role of Innate Immunity Activation in Experimental Diabetic Nephropathy

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Background: Innate immunity activation by hyperglycemia has been pointed out as a potentially important event in the pathogenesis of diabetic nephropathy (DN). We investigated whether innate immunity and inflammasome assembly are activated in streptozotocin (STZ)-induced diabetic rats developing DN, as compared to those failing to develop renal injury.

Methods: Adult male Munich-Wistar rats (N=18) received STZ, 65 mg/kg, and daily insulin injections to keep blood glucose (BG) moderately elevated. Nondiabetic rats (N=5) served as controls (C). At 12 months, urinary albumin/creatinine ratio (U_{alb}/U_{cr}) was determined and kidneys were harvested to determine % glomerulosclerosis (%GS). Diabetic rats with the 4 highest %GS values were considered as DN+, whereas those with the 4 lowest %GS values were considered as DN-, serving as a more specific control. Renal TLR4, Caspase-1 and NLRP3, components of innate immunity, were assessed by qRT-PCR (Tlr4, Casp1, Nlrp3) and Western blotting (TLR4, Caspase-1). In addition, serum MCP-1 was evaluated by ELISA.

Results:

	BG	U_{alb}/U_{cr}	%GS	MCP-1
C	101±2	1.5±0.3	1.5±0.2	1056±60
DN-	430±23[a]	2.7±1.3	1.4±0.2	958±30
DN+	385±32[a]	6.7±4.3	14.6±3.6[ab]	1416±140[ab]

Mean±SE, [a]$p<0.05$ vs. C, [b]$p<0.05$ vs. DN-.

Group DN- showed no significant changes in the expression of innate immunity components compared to C. Despite similar hyperglycemia, glomerular lesions in DN+ were accompanied by a significant ($p<0.05$) increase in Tlr4 (50%), Casp1 (80%) and Nlrp3 (110%) mRNA, compared to C. The renal abundance of TLR4 and Caspase-1 increased numerically and serum MCP-1 was significantly elevated in DN+ ($p<0.05$). Of note, %GS correlated positively with the expression of Tlr4 ($r=0.82$), Casp1 ($r=0.57$), Nlrp3 ($r=0.58$) and serum MCP-1 ($r=0.75$).

Conclusions: Activation of innate immunity and inflammasome assembly may play an important role in the development of DN in STZ diabetes. Such activation may be independent of hyperglycemia. FAPESP/CNPq.

Funding: Government Support - Non-U.S.

PUB303

Immunoglobulin G Fc Receptors with an Immunoreceptor Tyrosine-Based Activation Motif Promote Diabetic Nephropathy in the Early Stage Fei Liu. *Nephrology, West China Hospital of Sichuan Univ, Chengdu, Sichuan, China.*

Background: Diabetic nephropathy(DN) is a low grade inflammatory disease trigged by metabolic disorder. Inflammation has played an important role in the early stage of DN pathogenesis.Inflammatory cytokines, such as C-reactive protein(CRP), have contributed to inflammation through binding immunoglobulin G Fc receptors (FcγRs)in the surface of cell. We previously found that the expression of FcγRs with an immunoreceptor tyrosine-based activation motif(ITAM-FcγRs) increased in the kidney of diabetic CRP-Tg mouse induced by STZ. The present study investigated the potential role of ITAM-FcγRs in the early stage of DN.

Methods: Diabetes was induced by streptozotocin in Sprague Dawley(SD) rats for assessment of kidney injury at 2,4,8weeks by real-time PCR, immunohistochemistry and western blot analysis.

Results: Compared with control, urinary albuminexcretion was significantly increased in diabetic SD rats at 2,4 and 8 weeks after STZ injection. Diabetic rats developed severe renal inflammation with enhanced infiltration of macrophages and T cells, and upregulation of pro-inflammatory cytokines (IL-1β, TNFα). Enhanced renal inflammation in diabetic rats was associated with upregulation of ITAM-FcγRs(CD64, CD16), and over-activation of the nuclear factor κB signalling pathway.

Conclusions: These findings suggested that ITAM-FcγRs(CD64, CD16) may be mediators in the early stage of DN. Enhanced activation of nuclear factor κB signalling pathway may be the mechanism by which ITAM-FcγRs promote renal inflammation under diabetic conditions.

Funding: Government Support - Non-U.S.

PUB304

Atrasentan Ameliorates Proteinuria and Cardiovascular Complications in Diabetic Nephropathy Wai W. Cheung,[1] Shiping Li,[4] Sujana S. Gunta,[1] Nancy Dalton,[2] Katayoon Shayan,[3] Robert H. Mak.[1] *[1]Pediatrics, Rady Children's Hospital, Univ of California, La Jolla, CA; [2]Dept of Medicine, Univ of California, San Diego, La Jolla, CA; [3]Pathology, Rady Children's Hospital, Univ of California, La Jolla, CA; [4]Yangzhou Univ, Yangzhou, Jiangsu, China.*

Background: Diabetic nephropathy (DN) presents with progressive chronic kidney disease (CKD) and cardiovascular complications. We investigate the effects of Atrasentan (AT), an endothelin A receptor antagonist, in mice with DN with CKD.

Methods: DN with CKD was induced by streptozotocin and 5/6 nephrectomy in CD1 male mice. DN/CKD mice were treated with vehicle (V), AT (6 mg/kg/day), enalapril (EN) (10 mg/kg/day) or both AT and EN while sham (S) mice were treated with V. DN/CKD+V mice were fed *ad libitum* while other groups of mice were pair-fed to DN/CKD+V mice for 90 days.

Results: DN/CKD mice had higher BUN and serum creatinine levels than S+V mice ($p<0.001$). Weight gain was reduced in DN/CKD+V mice relative to S+V mice ($p<0.01$) and was normalized in DN/CKD+AT and DN/CKD+AT+EN mice. Urine output was increased in DN/CKD+V mice (6.3±2.1 ml/24hr, $p<0.01$) but was normalized in DN/CKD+AT+EN mice (2.7±0.6 ml/24hr) relative to S+V mice (1.6±0.3 ml/24hr). Urine albumin and urine protein/creatinine ratio were elevated in DN/CKD+V mice relative to S+V mice ($p<0.001$). AT+EN normalized urine albumin concentrations and also reduced urine protein/creatinine ratio in DN/CKD mice. Left ventricular mass/left tibiae length ratio was increased in DN/CKD+V mice (5.1±0.3 mg/mm, $p<0.01$) but was normalized in DN/CKD+AT+EN mice (4.1±0.2 mg/mm) relative to S+V mice (3.7±0.3 mg/mm). Systolic blood pressure was elevated in DN/CKD+V mice (145.4±6.8 mm Hg, $p<0.001$) but was normalized in DN/CKD+AT+EN mice (110.8±4.4 mm Hg) relative to S+V mice (100.2±3.6 mm Hg). TNF-α, IL-6 and IL-1α mRNA were increased in kidney and heart of DN/CKD+V mice than S+V mice ($p<0.001$). AT+EN decreased the expression of these cytokines in DN/CKD mice ($p<0.01$).

Conclusions: Atrasentan alone or in combination with Enalapril ameliorates proteinuria and cardiovascular complications in diabetic nephropathy.

Funding: Pharmaceutical Company Support - Abbott Laboratories/Abbvie

PUB305

mPGES-2 Deletion Attenuates Obesity, Extends Lifespan and Improves Glucose Metabolism in a High-Fat Diet Mouse Model Zhanjun Jia, Ying Sun, Tianxin Yang. *Internal Medicine, Univ of Utah, Salt Lake City, UT.*

Background: By now, there's no report regarding the in vivo phenotypes of PGE2 synthase 2 (mPGES-2). mPGES-2 Arg298his polymorphism is reported to be associated with metabolic syndrome in human subjects. Present study is to elucidate the role of mPGES-2 in obesity.

Methods: mPGES-2 WT (n=39) and KO (n=37) mice were treated with high fat diet (HFD) for 15 months. Then bodyweight, lifespan, glucose metabolism, energy expenditure, and fat tissues were analyzed.

Results: In this mouse strain with a mixed genetic background (C57BL/6X129), 50% mice developed obesity (obesity prone, OP) and 50% remained lean (obesity resistant, OR). The bodyweight (BW) was blocked by more than 60% in KO/OP mice compared to WT/OP group contrasting to the similar BW between KO/OR and WT/OR mice. In line with BW, white fat mass was remarkably reduced in KO/OP contrasting to the increased weight of brown adipose tissue (BAT). In BAT, there is a large amount of lipid accumulation in WT/OP mice but not KO/OP mice. Using metabolic chamber, KO/OP mice showed higher energy expenditure than WT/OP group accompanied with higher UCP1 in BAT and higher UCP3 in skeletal muscle, without affecting food intake and locomotor activity. As control, the OR mice showed no any difference in energy expenditure between genotypes. As expected, the KO/OP mice also displayed improved glucose tolerance test (GTT) and insulin tolerance test (ITT), and such improvement of glucose metabolism was also found in normal diet-treated KO mice in parallel with enhanced plasma insulin level and activated liver insulin signaling. More interestingly, mPGES-2 deficiency led to markedly extended lifespan under long term HFD condition in both OP and OR mice (WT:16 of 39 died; KO: 3 of 37 died) in accordance with markedly up-regulated longevity genes.

Conclusions: mPGES-2 deletion blunted HFD-induced obesity possibly through increasing energy expenditure of BAT, enhanced glucose metabolism possibly via increased plasma insulin and activated liver insulin signaling, and extended lifespan possibly by modulating longevity genes. These findings demonstrated a novel target for the treatment of obesity, type-2 diabetes, and metabolic syndrome.

Funding: NIDDK Support

PUB306

Beta2 Adrenergic Receptor Regulates High Glucose or Diabetes-Induced Pro-Inflammatory Response in Monocytes/Macrophages Hyunjin Noh,[1,2] Mi Ra Yu,[2] Hyun Joo Kim,[2] Jee Wan Wee,[1] Sang Heon Lee,[1] Jin Seok Jeon,[1,2] Dong-Cheol Han.[1,2] *[1]Internal Medicine, Soon Chun Hyang Univ, Seoul, Republic of Korea; [2]Hyonam Kidney Lab, Soon Chun Hyang Univ, Seoul, Republic of Korea.*

Background: Activation of monocytes/macrophages has been shown to be increased in diabetes and correlated with vascular complications. In this study, we investigated whether β2 adrenergic receptor (β2AR) agonists have therapeutic potential targeting monocyte/macrophage activation.

Methods: The effect of β2AR agonists on pro-inflammatory responses was examined using primary rat bone marrow (BM)-derived macrophages (Mph), THP-1 cells, and peripheral blood (PBMC) and BM mononuclear cells (BMMC) from types 1 and 2 diabetic rats.

Results: In Mph and THP-1 cells, high glucose (HG, 30 mM) up-regulated the expression of tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1, interleukin (IL)-1β, and CD36, which were blocked by β2AR agonists, metaproterenol and salbutamol. The activation of nuclear factor κB (NFκB) and phosphorylation of inhibitor of NFκB (IκBα) induced by HG were markedly decreased by both β2AR agonists. The expression of β-arrestin 2 that regulates β2AR signaling and stabilizes cytosolic IκBα and the interaction of β-arrestin 2 and IκBα were significantly decreased by HG. Metaproterenol and salbutamol reversed these changes. Ex vivo treatment of metaproterenol for 16 hours also significantly reduced diabetes-induced TNF-α production in PBMC from streptozotocin-induced diabetic rats. However, metaproterenol failed to reduce TNF-α production when the cells were co-treated with selective β2AR blockade, suggesting that the anti-inflammatory effect of metaproterenol was truly mediated by β2AR. PBMC and BMMC from Zucker diabetic fatty (ZDF) rats showed enhanced expression of CD11b/c and C-C chemokine receptor 2 which was significantly decreased by chronic treatment (12 weeks) with salbutamol. Salbutamol also attenuated monocyte adhesion to endothelium and the expression of CD36 upon lipopolysaccharide stimulation.

Conclusions: These findings suggest that β2AR agonists have novel anti-inflammatory properties and might therefore have protective effects against diabetic vascular complications.

Funding: Government Support - Non-U.S.

PUB307

LncRNAs Expression Profiling in Diabetic Nephropathy Mouse Model Zhigui Li, Zhuo Yang. *College of Medicine, Tianjin Key Laboratory of Animal Models and Degenerative Neurological Diseases, State Key Laboratory of Medicinal Chemical Biology, Tianjin, China.*

Background: Long non-coding RNAs (lncRNAs) are functional RNAs longer than 200 nucleotides in length.LncRNAs play important roles in a variety of biological functions; however, few have been identified that regulate renal function in diabetic nephropathy. In this study, we described lncRNAs profiles in a leptin receptor deficient BKS.Cg-*m*$^{+/+}$ *Lepr*^{db}/J type 2 diabetic nephropathy (T2DN) mouse model by microarray.

Methods: Three Diabetic *db/db* (BKS.Cg-*m*$^{+/+}$ *Lepr*^{db}/J) mice and three age-matched C57BLKS/J mice (10 wk old, male) were included. Body weight, water intake and blood sugar were measured. Expression of lncRNAs and mRNAs in renal cortex were determined using Agilent microarray. 10 aberrantly expressed lncRNAs ($p < 0.05$, FC ≥ 2 and regardless of sample signal value) and 6 aberrantly expressed lncRNAs ($p < 0.05$, FC ≥ 2 and at least one group of sample signal value was greater than 7) were validated by qRT-PCR.

Results: QRT-PCR assay showed that, comparing with the result of lncRNA microarray, only 5 of the 10 lncRNAs ($p < 0.05$, FC ≥ 2 and regardless of sample signal value) were validated to be differentially expressed in *db/db* mice. All of the 6 lncRNAs ($p < 0.05$, FC ≥ 2 and at least one group of sample signal value was greater than 7) were successfully validated. So we defined differentially expressed genes as those having a p value < 0.05, fold change ≥ 2 and at least one group of sample signal value was greater than 7. Comparison between *db/db* and control mice revealed that 951 lncRNAs were differentially expressed. The principal component analysis showed the ability of these lncRNAs to distinguish the two groups. The result of mRNAs microarray revealed that 926 mRNAs were aberrantly expressed in *db/db* mice.

Conclusions: Our data suggested that numerous lncRNAs may function in the development of DN. Identifying potential reasons, especially a potentially interaction with the candidate mRNAs, will require further analyses.

Funding: Government Support - Non-U.S.

PUB308

Roles of CD80, CD86 and CTLA-4 in the Pathogenesis of Mouse Diabetic Nephropathy Sonay Guven Karatas,[1] Hakki Yilmaz,[2] Zehra Firat,[3] Umran Yildirim,[4] Kadir Demircan,[5] Ayse Mukadder Bilgic,[2] Nuket Bavbek,[2] Ali Akcay.[2] *[1]Internal Medicine, Turgut Ozal Univ School of Medicine; [2]Internal Medicine, Section of Nephrology, Turgut Ozal Univ School of Medicine; [3]Biotechnology, Ankara Univ, Institute of Biotechnology; [4]Pathology, Turgut Ozal Univ School of Medicine; [5]Medical Biology, Turgut Ozal Univ School of Medicine.*

Background: Diabetic nephropathy (DN) has become a major health problem in recent years due to the increasing prevalence of diabetes. It is a well known fact that, inflammation plays a key role in the development and progression of DN. Our aim in this study is, to investigate the roles of CD80, CD86 and CTLA-4,which are crucial in T cell-related inflammatory responses, in the pathogenesis of DN.

Methods: To generate an experimental diabetes model, 50 mg/kg of streptozotocin was administered for 5 consecutive days. A total of 36 mice with blood glucose levels >250 mg/dl were included in the study. Kidney tissues were obtained via sacrifice in the 1st, 2nd, 3rd, 4th and 6th months. Diabetic nephropathy was confirmed by examination of histopathology. Immunohistochemical staining was performed to visualize CD80, CD86 and CTLA-4. CD80, CD86 and CTLA-4 protein analysis was performed in the kidney tissues using the western blot method.

Results: CD80, CD86 and CTLA-4 positive immunostaining was observed in the intraglomerular and peritubular capillary areas. Western blot analysis revealed greater CD80 protein levels in the DN group in the 6th month than in the healthy controls (p=0.003). These levels were also greater than those observed in the DN group in the 1st (p=0.006) and 4th months (p=0.009). In addition, CTLA-4 and CD86 protein levels were increased in the 4th and 6th months in the DN group compared to the healthy controls (All p <0.05).

Conclusions: These results suggest that the costimulatory molecules CD80, CD86 and CTLA-4 may be associated with DN pathogenesis and may represent a new treatment approach.

PUB309

Development and Progression of Renal Functional Decline in the ZSF1 Rat Model of Diabetic Nephropathy Kathleen A. Lincoln, Paul Harrison, Damian C. Matera, Hongxing Chen, Hu Sheng Qian, Chung-Wein Lee, Nicholas F. Brown, Susan Goldrick, Steven S. Pullen, Glenn A. Reinhart, Carine Boustany. *Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.*

Background: The ZSF1 obese rat closely approximates the pathophysiological conditions of human diabetic nephropathy (DN). We sought to further characterize disease progression in this model with a particular focus on glomerular filtration rate (GFR) and renal biomarkers.

Methods: Male ZSF1 obese rats and their age-matched lean controls were studied from 11 through 41 weeks of age for the development and progression of DN. Starting from week 25, losartan was administered at 3 doses (3, 10, 30 mg/kg QD) for 16 weeks. Mean arterial pressure (MAP) was assessed from week 25 onwards. Weekly measurements of proteinuria were performed. GFR was measured in the conscious rats utilizing the inulin clearance method. Additionally, histologic quantification of glomerular and interstitial lesions were performed at select ages on a subset of rats (11, 25 and 41 weeks) and specific biomarkers were assessed for mesangial proliferation (PCNA, Ki67), myofibroblast activation (α-SMA), macrophage infiltration (ED-1), podocyte (Glepp-1) and tubular damage (Kim-1) by immunohistochemistry.

Results: Proteinuria developed by 16 weeks of age (Lean: 25 ± 1; Obese: 61 ± 6 mg/day) and continued to increase with age. This coincided with increased incidence of glomerular lesions (Lean: 1 ±0.1; Obese: 20 ±0.6 %) and interstitial lesions (Lean: 0; Obese: 77 ± 3.2 total number foci). Furthermore, there was an increase in ED-1 (Lean: 0.2; Obese: 2 ±0.1 %), KIM-1 (Lean: 0; Obese: 4 ±0.4 %), α-SMA (Lean: 0.4; Obese: 2.9 ± 0.3 %) and a decrease in GLEPP1 (Lean: 37 ± 1.0; Obese: 19 ± 0.7 %) renal expression. Losartan dose-dependently reduced proteinuria and MAP. Importantly, GFR was significantly reduced in obese ZSF1 rats versus lean (Lean: 0.7 ± 0.1; Obese: 0.3 ± 0.1 ml/min/100g). This was partially reversed by losartan.

Conclusions: In summary, the ZSF1 obese rat model displays key hallmarks of early DN and progresses towards overt diabetic nephropathy over the course of 11-41 weeks of age.

PUB310

Urinary Excretion of Neutrophil Gelatinase-Associated Lipocalin (NGAL) in Diabetic Rats Horacio Osorio,[1] Abraham Said Arellano-Buendia,[1] Fernando E. García-Arroyo,[1] Maria L. Loredo,[2] Edilia Tapia,[1] L. Gabriela Sanchez-Lozada.[1] *[1]Nephrology, Instituto Nacional de Cardiología "Ignacio Chavez", Mexico, Distrito Federal, Mexico; [2]Histopathology Laboratory, School of Medicine, Univ Panamericana, Mexico, Distrito Federal, Mexico.*

Background: The excessive production of reactive oxygen species (ROS) has been suggested as a mechanism which leading to diabetic nephropathy. On the other hand recent studies suggest that in the progression to diabetic nephropathy the tubular damage precedes to glomerular damage. Therefore, we evaluated the oxidative stress and urinary excretion of tubular proteins in diabetic rats as markers of tubular dysfunction.

Methods: The groups were control (C), diabetic (D) and diabetic-insulin treated (DI). ROS was assessed by catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and by 3-nitrotyrosine (3-NT), 4-hydroxynonenal (4-HNE) and oxidized protein (OP) levels. The urinary excretion of neutrophil gelatinase-associated lipocalin (NGAL) (uNGAL), osteopontin (uOPN) and N-acetyl-b-D-glucosaminidase (uNAG) were determined.

Results: At 7 day of diabetes, hyperglycemia, glycosuria and uNGAL was elevated, whereas at 30 days of diabetes, hyperglycemia, low body weight, glycosuria, diuresis, creatinine clearance and glomerular filtration rate were significantly increased compared with C. Further at 30 days in D there was an increase in the CAT and SOD activities, in 3-NT levels and 4-HNE and OP levels in cortex and medulla while the GPx activity was low. The NGAL and OPN levels in serum as well as the uNGAL, uOPN and uNAG were increased in D compared with C. All these alterations were associated with tubular damage which was evidenced in samples at 7th days and aggravated to 30th days post diabetes induction.

Conclusions: The results suggest that tubular dysfunction evidenced by increase in urinary excretion of NGAL, precede to the oxidative stress during diabetes.

Funding: Government Support - Non-U.S.

PUB311

High Glucose Increases Formation as well as Pro-Oxidative and Pro-Coagulant Activity of Endothelial Microparticles Dylan Burger, Kevin D. Burns. *Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada.*

Background: Diabetic subjects exhibit elevated plasma levels of endothelial microparticles (eMPs) which predict risk of cardiovascular events. Emerging evidence suggests that eMPs may influence pathophysiological processes in a paracrine fashion. However the impact of high glucose on the formation and biological activity of eMPs is unknown.

Methods: Human dermal microvascular endothelial cells (ECs) were cultured in media containing normal D-glucose (5.6 mM), 15 mM D-glucose or 25 D-mM glucose, with L-glucose as osmotic control. eMP levels were assessed by flow cytometry (Annexin V labeling) and nanoparticle tracking analysis (NTA). eMPs were isolated from the media of ECs subjected to high glucose and effects on endothelial reactive oxygen species (ROS) formation were assessed by dichlorofluorescin fluorescence. Effects of high glucose on eMP pro-coagulant activity were assessed by a procoagulant activity assay.

Results: Exposure of ECs to high D-glucose caused a dose-dependent increase in eMP formation relative to osmotic controls. At 24 hours, there was a ~2.5-fold increase in eMP release associated with 25 mM D-glucose exposure (P<0.01, n=4-6). eMPs formed in 25 mM D-glucose displayed a greater diameter compared with normal D-glucose osmotic controls (25 mM: 260±14 nm versus 5.6 mM: 203±7nm versus P<0.05, n=6). eMPs generated in 25 mM D-glucose were more potent inducers of EC ROS production than eMPs generated in 5.6 mM D-glucose (~6-fold versus ~2.5 fold increase versus control, respectively; P<0.05, n=4). High glucose eMPs caused a ~3-fold increase in pro-coagulant activity compared with an equivalent amount of normal glucose eMPs (P<0.001, n=4).

Conclusions: Our results suggest that elevated glucose is a potent inducer of endothelial MP formation while also increasing the pro-oxidative effects of endothelial MPs. Such effects may contribute to progressive endothelial injury in diabetes.

Funding: Government Support - Non-U.S.

PUB312

The Early Renal Pathologic Changes in Diabetic Rats and the Protective Effects of Traditional Chinese Medicine Qingli Cheng, Yang Liu, Guang Yang, Qinglin Li. *Dept of Geriatric Nephrology, Chinese PLA General Hospital, Beijing, China.*

Background: To explore the early renal pathological changes, the expression of ED-1+ cells and toll-like receptor 4 (TLR4) in the renal tubulointerstitium in diabetic rats and the protective effects of Traditional Chinese Medicine (ShenKang Injection, SK) were investigated in this study.

Methods: Thirty SD rats were divided into three groups: diabetic group (DM), DM+SK group (DM+SK) and normal control group (NC). The type 2 diabetic rat models were induced by fed with high-fat diet for 4 weeks, then intraperitoneal injection with streptozotocin (35mg/kg). The insulin resistance of the rats was evaluated using hyperinsulinemic-euglycemic clamp test. Intervention of SK was given by Intraperitoneal injection for 8 weeks. The body weight(BW), kidney index(KI) were recorded; the level of blood glucose (GLu), serum creatinine (Scr), and the level of microalbuminuria (mALB), urinary NAG were tested. The renal pathological lesions were observed using PAS staining. The renal tubular injury were evaluated with Paller's score. The expression of TLR4 and ED-1+ cells in the renal interstitial were detected using the immunohistochemical method.

Results: Compared with the NC group, the level of glucose infusion rate(GIR) decreased markedly in the DM group. The levels of GLu, mALB, urinary NAG, KI, the glomerular volume and the tubular score in the DM+SK group were significantly lower than those of the DM group respectively (P<0.01). Meanwhile, the expression of TLR4 and ED-1+ cells in the rat renal interstitial of the DM+SK group significantly decreased when compared with the DM group (P<0.01). Insulin resistance was also significantly improved in the DM+SK group when compared with the DM group.

Conclusions: The early renal changes in diabetic rats included glomerular volume increase, tubular vacuolar degeneration, and renal interstitial macrophage infiltration. Traditional Chinese Medicine SK Injection could attenuate diabetic tubulointerstitial damage and delay the progression of diabetic nephropathy, which may be associated with the reduced expression of TLR4 and the inhibition of inflammation-mediated macrophage infiltration.

PUB313

Renal Structure-function Relationship in Aging Obese ZSF1 Rats during Progression of Diabetic Nephropathy Jay Kuo, Suzanne Nodop Mazurek, Chung-Wein Lee, Hu Sheng Qian, Ryan M. Fryer. *Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.*

Background: The obese ZSF1 rat is described as a preclinical model of type 2 diabetic nephropathy (DN) with marked renal dysfunction and structural derangement. However, these hallmarks of disease progression have not been evaluated in the same cohort of animals in an age-dependent manner. This study characterizes temporal changes in renal function (GFR) relative to changes in proteinuria and glomerular and tubulointerstitial structure using morphometric techniques to further understand renal disease progression in this model.

Methods: Male obese ZSF1 rats were aged to 20, 25, 30, 35, or 40 weeks of age (n=5-11/group). At each timepoint, proteinuria (UPCR) and GFR (anesthetized, FITC-inulin, kidney weight (KW) normalized) were measured. The left kidney was processed for histopathological analyses of glomerular lesions (% glomerulosclerosis) and interstitial damage (total foci number, interstitial collagen (SRM), myofibroblast encroachment (a-SMA), tubular injury (KIM-1), and macrophage infiltration (ED-1+)).

Results: GFR remained steady from 20 to 30 weeks of age (avg 0.71±0.06 mL/min/g KW). At 35 and 40 weeks, GFR declined markedly to 0.29±0.11 and 0.23±0.05 mL/min/g KW, respectively. The incidence of glomerulosclerosis increased steadily from 12±1% at 20 weeks to 71±2% by 40 weeks of age as did UPCR, and the number of interstitial lesions, a-SMA, and ED-1. In contrast, interstitial collagen increased modestly from 20 weeks (1.3±0.2%) to 30 weeks (3.7±0.3%). However, at 35 and 40 weeks of age, interstitial collagen markedly increased to 8.3±0.6% and 13.3±0.9%, respectively. KIM-1 increased until 30 weeks (7.5±0.6%) before declining at 40 weeks (6.9±0.7%).

Conclusions: In aging obese ZSF1 rats, increases in UPCR and the incidence of glomerulosclerosis occurred prior to the decline in GFR and further increased as GFR fell. The decreased GFR also corresponded with a marked increase in tubulointerstitial fibrosis (collagen deposition) and injury (declining KIM-1 indicative of excessive tubular damage). These results, consistent with observations in human DN, also support the obese ZSF1 rat as a relevant preclinical model of DN.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim Pharmaceuticals, Inc.

PUB314

Advanced Glycation End Products Induces Myo-Inositol Oxygenase via Activation of PI3K Pathwayin Proximal Tubular Epithelial Cells Rashmi Santosh Tupe, Tatsuya Tominaga, Yashpal S. Kanwar. *Dept of Pathology, Northwestern Univ, Chicago, IL.*

Background: Pathogenesis ofDiabetic Nephropathy is multifactorial, and the advanced glycation end products (AGEs) have been postulated to play a central role in this process. Conceivably, AGEs induce damage to both the glomerular and tubular compartments of the kidney. Myo-inositol Oxygenase (MIOX)is exclusively expressed in the kidney tubules, where it catabolizes myo-inositol and channels it into xylulose-pentose pathway. MIOX up-regulation is associated with an intra-renal myo-inositol deficiency, which is implicated in the development of nephropathy following increased expression of fibronectin (FN). Previously we observed up-regulation of MIOX in proximal tubular epithelial cells (HK-2) via PI3K pathway under high glucose ambience. However, the association of MIOX with AGEs and the receptor of AGEs (RAGE) in cellular signalling is unknown. We investigated the effect of AGEs, derived from albumin, laminin and Collagen IV, on cellular MIOX regulation and examined the underlying mechanisms.

Methods: HK2 cells were subjected to AGEs treatment and processed for MIOX expression and activity, PI3K signalling, and expression of NF-KB, TGF-β and FN, and status of cellular redox. ECM proteins were also glycated and for their effects on the HK-2 cell viability, adhesion and binding to RAGE, were studied. For the latter studies solid phase ELISA assay was used.

Results: In HK2 cells treated with AGEs- albumin (50-400 μg/ml) for 6- 48 h, MIOX expression and activity was increased in concentration and time dependent manner. In addition, increased expression of PI3K signalling pathway molecules (PDK, Phospho-PDK, AKT, Phospho-AKT); NF-KB, TGF-β, FN and cellular oxidative stress was observed. Glycated ECM proteins – laminin and Collagen IV had increased binding to RAGE and cell adhesion, whereas the cell viability was somewhat reduced.

Conclusions: These results suggest the AGEs, derived from glycated albumin and ECM proteins up-regulate cellular MIOX activity via PI3K pathway and triggers downstream events leading to excessive ECM production, and this may be a novel mechanism by which AGEs induce renal damage.

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

PUB315

Mycophenolate Mofetil Attenuates Diabetic Kidney Injury by Modulating Renal Th17 Su-Mi Kim, Jiyun Park, Yang Gyun Kim, Kyung-Hwan Jeong, Sang Ho Lee, Tae Won Lee, Chun-Gyoo Ihm, Ju-Young Moon. *Div of Nephrology, Dept of Internal Medicine, Kyung Hee Univ College of Medicine, Seoul, Korea.*

Background: Recent studies reported that proinflammatory T helper 1 (Th1) and T helper 17 (Th17) cell subsets have been associated with the pathogenesis of metabolic disease. However, the role of Th1 and Th17 cells in the development and progression of diabetic nephroapathy remains unknown. In this study, we investigated the hypothesis that MMF attenuates diabetic kidney injury by modulation of renal T cell proliferation and related cytokine.

Methods: We designed four animal groups as following; 1) C57BL/6 (Control); 2) Control + MMF (30 mg/kg/day); 3) Streptozotocin-induced diabetic mice (STZ); 4) STZ + MMF for 12 weeks. Using immunohistochemistry staining, $CD4^+$, $CD8^+$ T cell infiltration in kidney was measured. Intrarenal $CD4^+$ T cell proliferation was assessed by BrdU stating. IFN-IL-17 producing kidney and spleen mononuclear cell was assessed by flow cytometry.

Results: Urinary albumin excretion, mesangial expansion, and tubulointerstitial fibrosis decreased in STZ + MMF compared to STZ without change of HbA1c level. In immunohistochemistry, increased numbers of kidney interstitial $CD4^+$, $CD8^+$ T cells were identified in diabetic mice. BrdU incorporation was detected by subsequent intracellular staining of single cell suspensions from kidney, and consequently**,** renal $CD4^+$ T cell proliferation was increased in STZ mice and decreased by MMF. In flow cytometry of kidney mononuclear cell, diabetic mice showed an increase of renal Th1 and Th17 cells from 8 weeks after STZ induction. The population of Th1 and Th17 are no difference between control and STZ in spleen. The production of IL-17 was decreased by MMF while no significant difference was observed in IFN- γ production.

Conclusions: Our study results indicated that MMF attenuates diabetic nephoropathy by modulating renal IL-17 production.

PUB316

Vitamin D Receptor Regulates High Glucose Induced Inflammation through Inhibition of NF-κB Pathway Hao Zhang, Jing Huang, Bin Yi, Wei Li. *Dept of Nephrology, The Third Xiangya Hospital, Central South Univ, Changsha, Hunan, China.*

Background: Vitamin D (VD) and its receptor (VDR) have beneficial function on the pathogenesis of DN by anti-inflammation. This study aims to investigate the underlying mechanism for VD and VDR to modulate inflammation caused by high dose of glucose treatment in Human proximal tubules cell line (HK2), focusing on the NF-κB pathway.

Methods: 1. To investigate the effect of vitamin D on the inflammation induced by high glucose in HK2 cells. HK2 cells were divided into 5 groups: control group, high glucose group (HG), high glucose added with VD group (at concentrations of 10-7 M). 2. To investigate the effect of vitamin D receptor on the inflammation induced by high glucose in HK2 cells. HK2 cells were divided into 4 groups: control group, high glucose group (HG), VDR siRNA group(transfected with VDR siRNA) and blank plasmid group (transfected with scrambled siRNA). 3. The mRNA level of VDR, NF-κB was detected by realtime qPCR. The total protein and nuclear protein level of VDR, NF-κB protein were detected by western Blot. The level of MCP-1 and RANTES was detected by realtime qPCR and western Blot. The level of IκBa and IKK was detected by western Blot.

Results: 1. In high glucose group, the mRNA and protein level of MCP-1 and RANTES are increased compared with control group ($p<0.05$). Meanwhile the protein level of IκBa and IKK are also increased ($p<0.01$, compared with control group). Vitamin D can decrease the expression of MCP-1, RANTES, NF-κB, IκBa and IKK ($p<0.01$, compared with high glucose group). 2. Vitamin supplementation significantly ($P<0.05$) decreased the total and nuclear protein abundance of NF-κB/p65, compared those with high glucose group. Vitamin D supplementation significantly promoted the total and nuclear protein abundance of VDR ($P<0.05$). 3. Treatment with VDR siRNA significantly inhibited the expression of VDR from protein and mRNA levels ($p<0.01$, compared with control group), leading the up-regulation of IκBa, IKK and MCP-1 protein ($P<0.05$, compared with scrambled siRNA).

Conclusions: Vitamin D and VDR regulate high glucose induced inflammation by affecting NF-κB pathway.

PUB317

Berberine Ameliorate Epithelial-Messenchymal-Transition (EMT) in Renal Tubular Epithelial Cells Induced by High Glucose plus Advanced Glycation End-Products Nanmei Liu. *Dept of Nephrology, Jimin Hospital of Shanghai, Shanghai, China.*

Background: Increased glucose and advanced glycation end products (AGEs) are proposed to lead to diabetic kidney disease (DKD). The epithelial-messenchymal-transition (EMT) plays an important role in loss of renal function and progressive of DKD. Since berberine, an herbal compound extracted from Rhizoma Coptidis, can lower blood levels of glucose and improve protenuria, we hypothesised that it might attenuate EMT in tubular epithelial cells induced by high concentration of glucose plus AGE.

Methods: Cultured human renal tubular epithelial cell line (HK-2 cells), treated with high concentration of glucose (50mM), AGE(100ng/ml), Berberine (3uM) respectively for 24h, detected the positive deposit of type IV collagen (Col-IV), α-smooth muscle actin (α-SMA) through immunohistochemistry, measured the protein expression of AGE receptor (RAGE), Col-IV,α-SMA and transforming growth factor--β1(TGF-β1) by Western blot.

Results: After incubated with 50mM glucose or AGE, the cells' shape changed from round into fusiform and hypertrophy. The positive optical density (OD) of type IV collagen was increased, with the expression of α-SMA, TGF-β1 and RAGE up-regulated. Berberine significantly decreased the OD value of type IV collagen and inhibited the expression of α-SMA, TGF-β1 and RAGE.

Conclusions: A continuously stimulating with high glucose plus AGE resulted renal tubular epithelial cells hypertrophy and induced extracellular matrix synthesis or execrations, Berberine improved cell hypertrophy and inhibited cell transdifferentiation. The mechanism might be related to down-regulate the expression TGF-β1.

Funding: Government Support - Non-U.S.

PUB318

VDR and NF-κB Can Combine with Nup214 in HK-2 Cells Induced by High Glucose Hao Zhang, Jing Huang, Wei Li, Bin Yi, Wei Zhang. *Dept of Nephrology, The Third Xiangya Hospital, Central South Univ, Changsha, Hunan, China.*

Background: Inflammation plays an important role in the pathegenesis and progression of diabetic nephropathy (DN), recent research has demonstrated that VDR can play anti-inflammatory role by inhibiting the activation of NF-κB: VDR can physically interact with IKKβ, affect IκBα ubiquitination and degradation, form a complex with NF-κB p65 to affect p65 nuclear translocation. These research mostly focus on cytoplasm level, and the condition regarding to cell nucleus level is missing. Our previous clinical research has found significant down-regulation of nuclear VDR expression in type 2 DN patients, which is negatively related with uACR. Nuclear vitamin D receptor(nVDR) protein expressions is negatively associated with NF-κB nuclear protein expression.

Methods: Human proximal tubules cell line(HK2) were divided into 2 groups: control group, high glucose group (HG). Co-immunoprecipitation was used to investigate the correlation between protein and protein.

Results: We found that NF-κB and nVDR can combine with nucleoporin214 (Nup214) in HK-2 cells induced by high glucose, which locates in the nuclear envelope and mediates nucleocytoplasmic transport.

Conclusions: In conclusion, VDR may regulate inflammation by affecting NF-κB pathway during their nucleocytoplasmic transport induced by high glucose. The mechanism about whether there is a competitive inhibition between VDR and NF-κB in the process of nucleocytoplasmic transport remains to be studied further.

PUB319

Activation of RAS in Diabetic Mice as Determined by Increased Urinary Angiotensin II - Two to Tango? Jan A. Wysocki, Minghao Ye, Daniel Batlle. *Div of Nephrology and Hypertension, Northwestern Univ, The Feinberg School of Medicine, Chicago, IL.*

Background: Urinary renin-angiotensin system (RAS) components reflect kidney RAS overactivity and could indicate a risk of developing diabetic kidney disease. Urinary angiotensinogen (AOG) has been found to be increased in urine of diabetic patients with nephropathy. To study RAS activity more directly we measured Ang II, the main active peptide of the RAS, and also urinary renin, another critical component of the RAS cascade.

Methods: We studied urinary angiotensin (Ang) II in conjunction with renin and AOG in the urines from the db/db model of type 2 diabetes at an age when albumin excretion is maximally increased and when glomerular lesions are more established (35-48 weeks of age). Urinary AOG, total renin protein and angiotensin II were measured using ELISA kits. Urinary activity of ACE2 was measured using synthetic fluorogenic substrate.

Results: In db/db mice, there was an almost 10-fold increase in urinary AOG as compared to age-matched db/m controls (165±63 versus 17±5 ng/mg creatinine, $p<0.05$, respectively). Urinary renin in a subset of *db/db* mice of similar age was also significantly increased *as compared to db/m* (1.19±0.74 versus 0.23±0.21 ng/mg creatinine, $p<0.05$, respectively). The increases in urinary AOG and renin levels were paralleled by an increase in angiotensin II in urines from db/db mice as compared to urines from db/m mice (576±230 versus 42±6 pg/mg creatinine, $p<0.05$, respectively). Urinary activity of ACE2, an Ang II-degrading enzyme, was found to be increased in *db/db* mice as well (29±9 versus 6.5±0.8 RFU/ug creat/hr, $p<0.05$, respectively) consistent with our previous findings suggesting a compensatory increase in this enzyme to degrade Ang II more efficiently.

Conclusions: Kidney RAS activation in diabetic mice is best demonstrated from increased levels of urinary Ang II. This results from upstream activation as a result of both increased urinary angiotensinogen and renin.

Funding: NIDDK Support, Private Foundation Support

PUB320

GQ-16, a Partial PPARg Agonist, Decreases Blood Glucose, Visceral Adiposity and Induces the Expression of Beige Fat-Selective Genes in Obese and Diabetic Mice Alexandre Martini, Angélica Amorim Amato, Michela Soares Coelho, Francisco R. Neves, Caroline Lourenço de Lima. *Laboratory of Molecular Pharmacology, Univ of Brasilia, Brasilia, Distrito Federal, Brazil.*

Background: Thiazolidinediones (TZDs) are used for the treatment of type 2 diabetes (T2DM), and their therapeutic actions are mediated via activation of peroxisome proliferator-activated receptor γ (PPARγ). Despite their efficacy, their use is limited by side effects such as weight gain, edema, bone loss, and visceral adiposity. This has prompted the search for novel PPARγ agonists with reduced side effects. We have previously identified a novel PPARγ ligand (GQ-16) with similar anti-diabetic efficacy as rosiglitazone (RSG), yet in the absence of weight gain in obese and insulin-resistant mice. The aim of this study was to evaluate the effects of different doses of GQ-16 on adiposity and expression of PPARγ-dependent genes in mice with obesity and insulin resistance induced by high fat diet (HFD).

Methods: Mice were fed a normal-fat diet (NFD, 10% kcal fat) or HFD (60% kcal fat) since weaning. At the age of 16 wk, they were randomly assigned into six groups and received GQ-16 (5, 10 or 20mgkgd), RSG (4mgkgd) or vehicle by gavage daily for two weeks. Body weight (BW) gain, food and water intake, energy intake, metabolic efficiency, and fasting blood glucose were measured daily or weekly. Two different white adipose depots (subcutaneous and visceral) and interscapular brown adipose tissue (BAT) were harvested for determination of gene expression by quantitative real-time PCR.

Results: BW, BW gain, WAT fat mass content and blood glucose were greater in the HFD group, compared to the NFD group. In HFD-treated mice, weight gain was reduced by all treatments with GQ-16, but increased by. In addition, GQ-16 treatment (20 mg/kg/d) reduced visceral WAT pads, whereas RSG treatment increased them. Brown (UCP1) and Beige (CD40, Tmem26) fat-selective genes were upregulated in WAT in response to GQ-16.

Conclusions: Lower doses of GQ-16 decreased blood glucose and visceral adiposity in mice with obesity and insulin resistance induced by HFD, possibly through the expression of thermogenesis-related genes.

Funding: Government Support - Non-U.S.

PUB321

Post Hoc Analysis of ZEUS and HERAKLES, Two Prospective, Open-Label, Multicenter, Randomized Trials: Onset and Progression of Diabetes in Kidney Allograft Recipients on Standard Cyclosporine or Converted to CNI-Free or CNI-Low Everolimus Regimen Claudia Sommerer,[1,2] Wolfgang Arns,[1,2] Klemens Budde,[1,2] Ingeborg A. Hauser,[1,2] Volker Kliem,[2] Johannes Jacobi,[2] Petra Reinke,[1,2] Barbara M. Suwelack,[1] Anja Susanne Muehlfeld,[1,2] Ute Eisenberger,[1] Rudolf P. Wuthrich,[1] Katharina M. Heller,[1] Hans-Hellmut Neumayer,[1,2] Heiner H. Wolters,[1] Martina Porstner,[3] Daniel Baeumer,[3] Oliver Witzke,[1,2] Martin G. Zeier,[1,2] Frank Lehner.[1,2] *[1]ZEUS Study Group, Germany; [2]HERAKLES Study Group, Germany; [3]Novartis, Germany.*

Background: To compare incidence of NODAT (new-onset diabetes mellitus(DM) after Transplantation(Tx)) and progression of pre-existingDM in de novo kidney(K) allograft recipients after conversion to everolimus(EVR) based regimen and withdrawal or reduction of cyclosporine A(CsA).

Methods: Post hoc analysis on NODAT development or DM progression from ZEUS and HERAKLES, two 12months(Mo), prospective, open-label, multicenter, randomized(rdz) trials. De novo KTx patients (pts) received standard CsA/EC-MPS +steroids since Tx and were rdz to either a) continue CsA regimen or b) convert to EVR/EC-MPS (ZEUS:Mo4.5, HERAKLES:Mo3 post Tx) or c) change to a 3rd arm with low CNI+EVR(HERAKLES).

Results: ZEUS: 8%(25/300) of pts had DM at Tx. To Mo12 NODAT had developed in 8%(22/275) of non-DM pts(EVR14/142, CsA8/133) with 7% before rdz (EVR13/142, CsA7/133); after rzd incidence of NODAT was similar between groups($p=0.97$). Mean blood sugar change from rdz to Mo12 was similar between EVR versus CsA pts, in NODAT and DM subgroups. Mean eGFR (Nankivell[ml/min/1.73m^2]) was similar at rdz and significantly higher at Mo12 for EVR versus CsA pts within all defined subgroups: NODAT: EVR+14.0 versus CsA-9.2; pre-existing DM: EVR+5.5 versus CsA+0.7. HERAKLES results: To Mo12 NODAT occurred in 6.8%(30/438) of all pts (EVR 6.7%[10/149] versus CsA 8.3%[11/133] versus low-CsA 6.3%[9/143]), hence a similar incidence of NODAT in all 3 HERAKLES groups ($p=0.62$).

Conclusions: No difference in NODAT incidence or DM progression after conversion to EVR with CNI elimination or reduction were found until 12Mo post Tx. The benefit on renal function of an early conversion to an EVR-based regimen was seen in the NODAT subpopulation, similar to what was seen in both trials' ITT study results.

Funding: Pharmaceutical Company Support - Novartis Pharma Germany

PUB322

Novel Urinary Biomarkers for Early Detection of Kidney Disease in Type 2 Diabetes: A Pilot Study Veeda O. Landeras,[1,3] Jennifer W. Xu,[1,3] Carla Cavallin,[1,3] Mahboob Rahman,[1,2,3] Donald E. Hricik,[1,3] Michael S. Simonson.[1,3] *[1]Medicine, Div of Nephrolohy and Hypertension, Univ Hospitals Case Medical Center, Cleveland, OH; [2]Medicine, Div of Nephrology and Hypertension, Louis Stokes VA Medical Center, Cleveland, OH; [3]Case Western Reserve Univ School of Medicine, Cleveland, OH.*

Background: Kidney disease in type 2 diabetes is difficult to identify at an early stage when treatment to slow progression of renal insufficiency may be most effective. In a pilot cross-sectional study of patients with type 2 diabetes, we measured candidate urinary biomarkers (endothelin-1, ET-1; interleukin-6, IL-6; and macrophage chemoattractant protein-1, MCP-1) to determine whether they discriminate patients with eGFR < > 90 ml/min/1.73 m^2 more accurately than albumin/creatinine ratio (ACR).

Methods: Spot urine specimens were collected from 56 outpatients with type 2 diabetes, and ET-1, IL-6, MCP-1 and albumin levels were assessed by ELISA and corrected for urinary creatinine. Receiver operating characteristic (ROC) regression (Stata 13, Rocreg) was used to determine area under the ROC curve (AUC) for discrimination of eGFR < > 90ml/min/1.73 m^2 and to adjust for covariates with a non-parametric bootstrap.

Results: Nineteen patients had MDRD eGFR > 90 ml/min/1.73 m^2 (109 + 13, mean + SD) and 37 had eGFR < 90 (63 + 22, $P<0.01$). The AUC for ACR was 0.565 (95% CI, 0.413-0.716). AUCs for ET-1, MCP-1 and Il-6 were 0.752 (0.610-0.895, $P= 0.04$ versus ACR), 0.694 (0.541-0.847, $P=0.02$ *versus*. ACR) and 0.833 (0.715-0.952, $P<0.01$ *versus*. ACR), respectively. AUCs for ET-1, MCP-1 and IL-6 were statistically equivalent (all comparisons P > 0.231). AUCs for ET-1, MCP-1 and IL-6 were not significantly affected by covariate adjustment for age (all $P>0.639$), female sex ($P>0.597$), Black race ($P>0.499$), HbA1c ($P>0.593$) or blood pressure > 140/80 mm Hg ($P>0.415$).

Conclusions: Urine levels of ET-1, MCP-1 and IL-6 discriminate type 2 diabetic patients with eGFR < 90 ml/min/1.73 m2 with higher accuracy than ACR independent of covariates. Further evaluation of these candidate biomarkers for early detection of kidney disease is warranted in cohorts with type 2 diabetes.

Funding: Other NIH Support - NIH RO1 DK096549

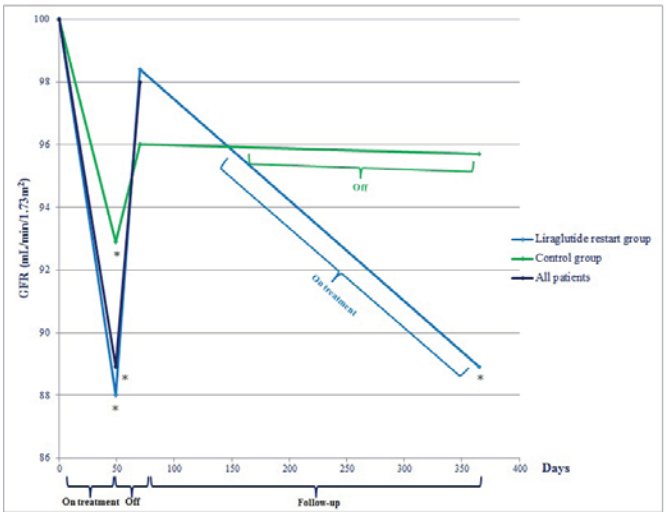
PUB323

Glucagon-Like-Peptide (GLP-1): Long-Term Effect on Kidney Function in Patients with Type 2 Diabetes Bernt Johan Illum von Scholten,[1] Tine Hansen,[1] Frederik I. Persson,[1] Peter Rossing.[1,2,3] *[1]Steno Diabetes Center, Denmark; [2]Aarhus Univ, Denmark; [3]Univ of Copenhagen, Denmark.*

Background: In a short-term study including 31 patients with type 2 diabetes, we observed that glucagon-like-peptide-1 receptor agonist (GLP-1 RA) treatment was associated with a decline in GFR, which was reversed when GLP-1 RA was stopped. The aim of this study was to investigate the effect of long-term treatment on kidney function in patients restarting GLP-1 RA.

Methods: We included 30 patients from our short term study, in an open-label follow-up of one year, 23 restarted with liraglutide and seven control subjects continued without. Primary endpoint was change in GFR (^{51}Cr-EDTA).

Results: Patients were 8 (35%) females, mean (SD) age was 61.5 (10.0) years, HbA_{1c} 60.1 (13.8) mmol/mol and diabetes duration (range) 6 (1-18) years. Baseline GFR was 100.6 (24.9) mL/min/1.73m^2 and treatment was associated with a reduction of 11 (95% CI: 6.6; 15.7) mL/min/1.73m^2 from baseline (p<0.0001), independent of change in 24hour systolic blood pressure (SBP), weight, UAER or HbA_{1c} (p>0.59). Baseline geometric means (IQR) of UAER was 25.5 (9.9-50.9) mg/d and was reduced with 27% (5%-44%, p=0.020) after one year of treatment, independent of change in SBP, weight, GFR or HbA_{1c} (p>0.22). Fractional albumin excretion was reduced by 37% (13%-54%, p=0.007) and 24hour SBP by 8.2 (0.1-16.2) mmHg (p=0.048).



Conclusions: Long-term treatment with GLP-1 RA liraglutide was associated with blood pressure independent changes in measured GFR identical to what was seen during short-term treatment. Moreover, UAER was reduced. Whether this could reflect a renoprotective effect of GLP 1-RA has to be addressed in randomized long-term trials.

PUB324

Relationship Between Urinary N-Acetyl-β-D-Glucosaminidase and 24-Hour Glycemic Profile, or 1,5-Anhydroglucitol in Patients with Type 2 Diabetes Mellitus Motoshi Ouchi,[1] Tatsuya Suzuki,[2] Taro Saigusa,[2] Kenzo Oba,[2,3] Promsuk Jutabha,[1] Naohiko Anzai,[1] Shuichi Tsuruoka.[4] *[1]Pharmacology and Toxicology, Dokkyo Medical Univ School of Medicine, Shimotsuga-gun, Tochigi, Japan; [2]Geriatric Medicine, Nippon Medical School, Bunkyo-ku, Tokyo, Japan; [3]Internal Medicine, Oarai Sea Shore Core Clinic, Higashiibaraki-gun, Ibaraki, Japan; [4]Nephrology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan.*

Background: The serum 1,5-anhydroglucitol (1,5AG) level has been shown to generally reflect postprandial hyperglycemia. Elevated urinary levels of N-acetyl-β-D-glucosaminidase (NAG) indicate proximal tubular damage. The relationship between 1,5AG and urinary NAG levels has been reported by some studies, including our studies, in patients with and without diabetes mellitus (DM). However, no studies have evaluated the relationship between 24-hour glycemic profile and urinary NAG.

Methods: 176 subjects (38-87 years of age) were recruited among inpatients who had received diagnoses of type 2 DM at Nippon Medical School Hospital. Patients who had been brought to our hospital by ambulance were excluded. Other exclusion criteria included severe illness, pregnancy, previous gastrectomy, estimated glomerular filtration rate < 45 (ml/min/1.73 m^2), albuminuria ≥ 300 (mg/g Cr), a history of proteinuria, and kidney disease. The daily profiles of plasma glucose was recorded at 10 time points in the preprandial period (8:00, 12:00, 18:00), and the postprandial period (10:00, 14:00, 20:00), and then at 0:00, 3:00, 6:00, and 8:00 on the next day.

Results: The mean urinary NAG level was 8.24 ± 6.45 U/g Cr. There was weak correlation between urinary NAG and some markers (10:00, 12:00, 18:00 glucose, M-value, mean plasma glucose, area under the curve [AUC] of daily glycemic profile, AUC of postprandial glycemic profile, and 1,5AG). Urinary NAG did not correlate with fasting plasma glucose, HbA1c, and the mean amplitude of glycemic excursions.

Conclusions: This study is the first to show, by means of 24-hour glycemic profile, the relationship between urinary NAG and 1,5AG, or other glycemic markers in patients with type 2 DM. Urinary NAG had stronger associations with 1,5AG, than 24-hour glycemic profile.

PUB325

The Therapeutic Potential of C-Peptide in Kidney Disease: A Systematic Review and Meta-Analysis James A. Shaw, Partha Pradeep Shetty, Kevin D. Burns, Greg A. Knoll. *Div of Nephrology, Dept of Medicine, Kidney Research Centre, Ottawa Hospital Research Institute, Univ of Ottawa, Ottawa, ON, Canada.*

Background: C-peptide, normally released by pancreatic beta-cells along with insulin and in equimolar amounts, has intrinsic biological activity and may be renoprotective. Our major objective was to conduct a systematic review to identify whether C-peptide has an effect on GFR, proteinuria, kidney histology, requirement of renal replacement therapy, and mortality in patients with kidney disease of any etiology.

Methods: MEDLINE, EMBASE, and the Cochrane Central Databases were searched for human and animal studies in which C-peptide was administered and renal endpoints were subsequently measured.

Results: We identified 4 human trials involving a total of 74 patients and 18 studies involving 568 animals, most of which were rat studies. In humans, the renal effects of exogenously delivered C-peptide have only been studied in type I diabetics with either normal renal function, microalbuminuria, or glomerular hyperfiltration, and meta-analysis showed no difference in GFR (mean diff. -1.36 mL/min/1.73 m^2, p=0.72) or renal blood flow (mean diff. 32.53 mL/min/1.73 m^2, p=0.27) in patients receiving C-peptide compared to a control group. One study reported reduced albuminuria in the C-peptide group (p<0.05). Diabetic rodent models revealed C-peptide-induced reduction in GFR (mean diff. -0.62 mL/min, p<0.00001), proteinuria (mean diff. -186.24 mg/day, p=0.05), glomerular volume (p<0.004), and mesangial matrix area (p<0.00001). C-peptide did not affect blood pressure or plasma glucose. Most of the studies were relatively short-term in duration, most falling within a range of 1-2 hours - 4 weeks.

Conclusions: In diabetic animal studies, C-peptide reduces GFR, proteinuria, and mesangial matrix, without affecting blood pressure or plasma glucose. The underlying mechanisms for these effects are unknown. In patients with type I diabetes there is minimal evidence supporting a reduction in albuminuria. Human studies of sufficient sample size and duration are needed to determine if the beneficial effects of C-peptide seen in animal models translate into improved outcomes for patients with chronic kidney disease.

PUB326

Urinary miR-124 Correlated with Albuminuria and Renal Function in Patients with Type 2 Diabetes Mellitus Hui Peng, Meirong Zhong, Yan-Ru Chen, Jun Zhang, Wenbo Zhao, Tan-Qi Lou. *Nephrology Div, Dept of Medicine, the Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: miR-124 may be involved in the pathogenesis of diabetic kidney disease (DKD) by targeting the RhoA/ROCK signal pathway or integrin a3 b1. The study was undertaken to explore the possibility association of urinary miR-124 and renal injury in diabetic mellitus.

Methods: Eighty-three patients diagnosed with type 2 diabetes mellitus were recruited into this cross-sectional study. Urinary miR-124 levels were detected by TaqMan qRT-PCR. Urinary albumin excretion rate, urinary albumin to creatinine ratio and renal functional parameters including the blood urea nitrogen (BUN), serum creatinine, cystatin, β2-microglobulin levels, and estimated glomerular filtration rate (eGFR) were collected as well. They were divided into two groups based urinary albumin to creatinine ratio: patients with albuminuria (n=42) and with normoalbuminuria (n=41).

Results: The miR-124 in urinary supernatant was significantly higher (p = 0.007) in patients with albuminuria than those with normoalbuminuria. However, the expression of miR-124 in urinary sediment was significantly lower (p = 0.021) in patients with albuminuria than others.There was no significant difference in the serum miR-124 level between both groups. The urinary supernatant miR-124 level was significantly correlated with the urinary albumin excretion rate (r = 0.271, p = 0.023), eGFR (r = -0.308, p = 0.005), serum cystatin (r = 0.239, p = 0.042), serum β2-microglobulin (r = 0.307, p = 0.009), BUN (r = 0.239, p = 0.030), and serum creatinine (r = 0.321, p = 0.003).

Conclusions: Urinary supernatant miR-124 is associated with albuminuria and renal function in patients with type 2 diabetes, which may have the potential to serve as noninvasive biomarker for DKD. The implication of miR-124 in the pathogenesis of DKD needs further exploration.

Funding: Government Support - Non-U.S.

PUB327

Glycoalbumin Is an Accurate Indicator for Postprandial Blood Glucose and Fluctuation Evaluated by CGM (Continuous Glucose Monitoring) in Dialysis Patients Kunihiro Ishioka, Hidekazu Moriya, Takayasu Ohtake, Sumi Hidaka, Shuzo Kobayashi. *Nephrology,Immunology, Vascular Medicine, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan.*

Background: Little data is available concerning blood glucose (BG) monitoring in dialysis patients with continuous glucose monitoring (CGM). CGM is ambulatory and plays an important role for indicating every 5 min BG for 48 hrs, which could show blood

glucose fluctuations, postprandial BG, average BG or area under the curve (AUC). Japanese guideline for BG managements in dialysis patients says that glycoalbumin (GA) should be used instead of HbA_1C because of underestimation of BG due to a short survival of RBCs and ESA usage. With CGM, we evaluate what GA indicates, and reveal relationships between GA, HbA_1C and various kinds of blood glucose indicators.

Methods: We performed consecutive 2-days CGM to monitor glucose levels in 16 HD patients (DM 10 pts, non-DM 6 pts), and studied the association of various CGM indicators (average glucose values ; AG, fluctuations of 24h blood glucose ; FL, and area under the curve for glucose level >180mg/dl ; $AUC_{>180}$) with GA, HbA1c,and postprandial BG (pre-dialysis BG), respectively.

Results: The mean HbA1c, and GA of the subjects were 5.4±1.0 %, and 22.0±5.9 %, respectively. GA is significantly associated with 48hrs average blood glucose levels, postprandial BG (pre-dialysis BG), and AUC180, and FL on non-dialysis day ($r=0.65$, $p<0.01$). However, HbA1C is not associated with neither indicator above mentioned. 24h AG and AUC>180 are significantly correlated with postprandial BG and FL significantly is correlated with GA on non-dialysis day in multiple regression analyses ($p<0.03$).

Conclusions: We showed that GA is more accurate and a useful marker than HbA1C in terms of BG management in dialysis patients because GA is well associated with several indicators, all of which include BG excursion from low to high blood levels. Since postprandial or fluctuations of BG is known to play an important role for the development of cardiovascular events, we emphasize that BG should be managed continuously throughout 48 hrs between dialysis sessions using GA.

PUB328

A Multicenter Clinical Trial of Allopurinol to Prevent Early Renal Function Loss (PERL) in Type 1 Diabetes (T1D) David Cherney,[1] Bruce A. Perkins,[1] Michael Mauer,[2] Alessandro Doria.[3] *[1]Univ of Toronto; [2]Univ of Minnesota; [3]Joslin Clinic. On Behalf of the PERL Consortium.*

Background: Despite improvements in glycemic and blood pressure control and renin angiotensin system blockade (RASB), the incidence of diabetic nephropathy (DN) remains high. The Joslin Kidney Study found that that moderately elevated serum uric acid (UA) is associated with a 2-fold increase in GFR loss in T1D, suggesting that UA lowering may prevent or slow GFR loss in T1D.

Methods: To test this hypothesis, we established a consortium of investigators from 8 centers (U.S.A., Canada, Denmark) with expertise in the conduct of DN clinical trials. Recently fully funded by a 5 year grant from the NIDDK and the JDRF, PERL is proceeding with the pivotal 3-year, multicenter, double-blind, placebo-controlled, randomized clinical trial to evaluate the efficacy of allopurinol, compared to placebo, in reducing GFR loss in T1D.

Results: The trial is targeted to T1D patients with high risk features such as elevated albumin excretion rates or GFR decline and serum UA ≥4.5 mg/dl, since these patients may benefit most from UA lowering. Baseline GFR is being measured by the plasma disappearance of iohexol (iGFR) between 45 and 99 ml/min/1.73m2. Patients will receive full dose RASB with blood pressure targeted at <130/80 mmHg. The primary endpoint of the study will be the iGFR (adjusted for the baseline iGFR) at the end of a 2-month allopurinol wash-out period after the 3-year intervention, thereby demonstrating durability of potential benefits. Sample size calculations suggest that 240 subjects in each treatment arm will provide ≥80% power to detect a meaningful reduction in the rate of iGFR decline in the allopurinol versus the placebo group of 1ml/min/year, which would translate to a 7-10 year delay in end stage renal disease (ESRD).

Conclusions: We anticipate that allopurinol may be a valuable adjuvant therapy to prevent or slow GFR loss in T1D patients with established DN. The reduction in morbidity and mortality associated with the prevention of delay of ESRD due to the use of allopurinol would have a major impact on the lives of T1D patients, significantly reducing the human and financial costs associated with this condition.

Funding: NIDDK Support

PUB329

The Clinical and Pathological Analysis and Prognosis of the Renal Disease in Type 2 Diabetes Mellitus Patients Ming Li,[1] Huiqing Chen,[2] Wenbo Zhao,[1] Canming Li,[1] Tan-Qi Lou.[1] *[1]Dept of Nephrology, The Third Affiliated Hospital, SUN Yat-sen Univ, Guangzhou, Guangdong, China; [2]Dept of Pediatric, The First Affiliated Hospital, SUN Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Objective To analyze the pathological and clinical characteristics of renal disease in patients with type2 diabetes mellitus(all receive renal biopsy) and evaluate prognosis.

Methods: The cilnical and pathological data of 42 type2 diabetic patients with abnormal urinalysis or increased serum creatinine in our department were studied, who were followed-up for average 4 years.

Results: Among 42 patients, 27 cases(64.3%)were diagnosed diabetic nephropathy (DN), including 3 cases presenting with diabetic nephropathy complicated with non-diabetic renal disease (NDRD), and 15 cases were diagnosed as NDRD (35.7%). Membranous glomerulonephritis was the most common pathological changes in NDRD(33.3%), followed by minimal-change disease (20%) and IgA nephropathy (13.3%). Difference of diabetic duration, hemoglobin level, whether complicated with diabetic retinopathy (DR) or other DM complications between two groups had statistical significance ($P<0.05$).Logistic regression analysis indicate that DR is the independent risk factor for DN.DR had 66.7% sensitivity and 93.3% specificity for diagnosing DN**.** After 4 years follow-up,totally 10 patients(23.8%) progressed to ESRD,8 in DN group, and 2 in NDRD group.Multiple linear regression model indicated renal interstitial fibrosis was the only independent risk factor for progression to ESRD in two groups**.**

Conclusions: The renal disease occured on the patients with type2 diabets sometimes belongs to NDRD. It is necessary to differentiate the renal pathological changes by history,fundus examination, renal biopsy et al.Compared with NDRD patients, prognosis in DN group is worse,and renal interstitial fibrosis is the only independent risk factor for progression to ESRD.

PUB330

Urine AQP5 Is a Potential Novel Biomarker of Diabetic Nephropathy Wenzheng Zhang,[1] Yiyang Lu,[2] Lihe Chen,[1] Zhou Xiao,[2] Ting Meng,[2] Qiaoling Zhou.[2] *[1]Graduate School of Biological Sciences, Univ of Texas Health Science Center at Houston, Houston, TX; [2]Internal Medicine, Central South Univ, Changsha, Hunan, China.*

Background: Although several urine-based proteins have emerged as potential markers of diabetic nephropathy, none of them have been used clinically. Water channel AQP5 functions in the generation of saliva, tears, and pulmonary secretions, but is barely detectable in normal kidneys. Based on our recent findings, we hypothesize that urine AQP5 is a potential novel biomarker of diabetic nephropathy.

Methods: We performed immnunofluorescence staining of kidney biopsies from 15 patients with minimal change disease as normal controls and 17 patients with diabetic nephropathy. Using an AQP5-specific enzyme-linked immunosorbent assay, we measured serum and urine AQP5 in 84 subjects consisting of normal controls (n=26) and patients with diabetes mellitus (n=25) or diabetic nephropathy (n=33). Correlations between urine AQP5 and clinical parameters were calculated as the Pearson's correlation coefficient, using Microsoft Excel. Analyses of ANOVA with Holm-Sidak test, Receiver Operator Curve (ROC), multiple logistic regression, and Bland-Altman agreements were conducted using SigmaPlot 11 (Systat Software). Differences were considered statistically significant at $p<0.05$.

Results: We found that 1) AQP5 exists inside the lumen of the tubules in patients with diabetic nephropathy, but not in patients with minimal change disease. 2) Urine AQP5/creatinine is significantly higher in patients with diabetic nephropathy than in other two groups, and in diabetic nephropathy stage V than in stage III; 3) correlates with serum creatinine, urine microalbumin, and multiple other known risk factors of the disease; 4) improves the clinical models in distinguishing diabetic nephropathy from normal controls and diabetic mellitus; and 5) has a high stability over prolonged frozen storage.

Conclusions: Our data suggest that urine AQP5/creatinine may possess diagnostic and prognostic values as a biomarker of diabetic nephropathy and offer flexibility for reliable measurement in the clinical setting.

Funding: NIDDK Support

PUB331

Oxalate Nephropathy Causing Irreversible Renal Failure following Roux-En-Y Surgery: Recognizing Patients at Risk Jessica M. Nelson, Jamie Alton Green, Maria C. Bermudez. *Geisinger Medical Center, Danville, PA.*

Methods: We present a case of a 51 year old female with medical history significant for hypertension, type 2 diabetes mellitus (DM), chronic kidney disease (CKD) stage III and morbid obesity (BMI 38) status post bariatric surgery complicated by volume depletion and subsequent acute on chronic kidney injury. During her hospitalization she had persistent decline in her renal function prompting further investigation. The work-up revealed normal- large hyperechogenic kidneys, protein-creatinine ratio of 1 gram per day, bland urinary sediment and negative serologic evaluation. Ultimately, a renal biopsy was performed revealing severe tubulointerstitial inflammation, moderate to severe tubular atrophy and interstitial fibrosis with diffuse tubular deposition of Calcium Oxalate (CaOx) crystals consistent with ON. Urine oxalate levels were not measured given oliguria. Despite institution of a low fat-oxalate diet, calcium supplementation and surgical reversal of her bypass, she rapidly progressed towards dialysis with no evidence of renal recovery.

Conclusions: Oxalate nephropathy (ON) is an unrecognized, devastating phenomenon following Roux-En-Y Gastric Bypass (RYGB). ON is the result of iatrogenic fat malabsorption leading to hyperoxalemia, hyperoxaluria and renal deposition of CaOx crystals. Our case illustrates the associated potential complication of ON with RYGB as previously described in the abandoned jejunoileal bypass. This phenomenon has been observed in a few case series with biopsy-proven oxalate nephropathy reported in the literature. Interestingly, the presence of underlying CKD, DM and volume depletion appear to be common risk factors, all of which were present in this patient. Risk stratification and early recognition are mainstays of improved morbidity from this irreversible condition. Renal biopsy, the goal standard diagnostic tool, should be considered in patients at risk in order to institute early treatment which includes consideration of surgical reversal of the bypass. As recurrence of the disease is anticipated after renal transplantation, objective diagnosis and establishment of preemptive strategies are crucial.

PUB332

Beneficial Effect of Polymyxin-B Direct Hemoperfusion for Septic Shock Patients with Low Central venous Pressure: A Retrospective Cohort Study Hiroyuki Yamada, Tatsuo Tsukamoto, Motoko Yanagita. *Dept of Nephrology, Kyoto Univ Graduate School of Medicine, Kyoto, Japan.*

Background: Although fluid resuscitation is a fundamental therapy for severe sepsis or septic shock, positive fluid balance might be associated with adverse outcomes. The direct hemoperfusion with polymyxin B-immobilized fiber column(PMX-DHP) could improve hemodynamic status of septic shock patients without aggressive fluid resuscitation. In

order to examine the beneficial effect of PMX-DHP in patients with chronic kidney disease or chronic heart failure, whose fluid resuscitation has to be limited, we retrospectively compared the hemodynamic improvement and the mortality of patients treated with PMX-DHP by using central venous pressure (CVP) as an indicator.

Methods: 70 patients received PMX-DHP for septic shock between May 2008 and 2013 April were recruited and divided into low CVP group (n=33, CVP<12mmHg) and high CVP group (n=37, CVP≥12mmHg). PMX-DHP was carried out for at least 120 minutes per session. Primary outcome was the change in mean arterial pressure (MAP) and vasopressor requirement, and secondary outcome was the 28-day mortality.

Results: MAP significantly increased after PMX-DHP (66 to 78 mmHg in low CVP group and 62 to 67mmHg in high CVP group; p<0.01), however, the increased MAP was maintained at 24 hours only in low CVP group (to 76mmHg; p<0.01). Inotropic score significantly decreased at 24 hours in low CVP group (20 to 10; p<0.01), whereas no significant change was observed in high CVP group (25 to 22; p=0.11). Vasopressor dependency index also significantly improved at 24 hours in low CVP group (0.33 to 0.16 $mmHg^{-1}$; p<0.01), but not in high CVP group (0.43 to 0.34 $mmHg^{-1}$; p=0.10). The 28-day mortality rate was higher in high CVP group (57% versus 21%: p<0.01), despite the presence of similar comorbidity diseases between two groups.

Conclusions: Our findings indicate that PMX-DHP could provide beneficial effect on both the hemodynamics and the mortality in patients with low CVP group, whose fluid resuscitation was limited. Further study is required to elucidate the mechanism of action of PMX-DHP on sepsis treatment.

Funding: Private Foundation Support

PUB333

Disparity between Prescribed Dose Based on Effluent Flow Rate and Actual Delivered Dose in Continuous Renal Replacement Therapy Explained

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Background: Continuous renal replacement therapy (CRRT) is a dialytic modality commonly used in patients with acute kidney injury. The recommended minimal CRRT urea clearance rate is 20 ml/kg/hr, and it is critical that the prescribed calculated dose is accurate.

Methods: CRRT urea clearance is calculated as the ratio between the [urea] in the effluent and plasma multiplied by the effluent flow rate (EFR). Due to its low molecular weight, the sieving coefficient of urea is one, i.e. the effluent/plasma (E/P) [urea] ratio is equal to 1. The convective clearance of urea is thus equal to the EFR. Hence, the dose of CRRT has been reported as the EFR, which is equal to the ultrafiltration rate in CVVH and sum of ultrafiltrate and spent dialysate in CVVHD and CVVHDF. However, observational studies have shown that the prescribed dose based on EFR significantly overestimates the measured urea clearance. We hypothesized that this difference is due to the fact that E/P [urea] = 1 holds true only for convective solute transport. In diffusive solute transport, the effluent [urea] will be equal to the equilibrated [urea] at diffusive equilibrium, which will always be less than the plasma [urea]. Thus, the E/P [urea] ratio is < 1 and calculation of urea clearance using the EFR will overestimate the actual urea clearance.

Results: Accounting for the fact that the equilibrated effluent [urea] at diffusive equilibrium is less than the plasma [urea], two new equations are derived to accurately calculate urea clearance: CVVHD urea clearance = $(Q_{BW} \times Q_{effluent})/(Q_{BW} + Q_{DFR})$ and CVVHDF urea clearance = $(Q_{BW} \times Q_{effluent})/(Q_{BW} + Q_{RF} + Q_{DFR})$, where Q_{BW} = blood water flow rate, $Q_{effluent}$ = EFR, Q_{DFR} = dialysate flow rate, and Q_{RF} = predilution replacement fluid rate.

Conclusions: The disparity between prescribed dose based on EFR and actual delivered dose in CRRT can be explained by the lower effluent [urea] at diffusive equilibrium. By accounting for the equilibrated effluent [urea], these new equations will provide clinicians with a more accurate guide for calculating the prescribed CRRT dose.

Funding: Private Foundation Support

PUB334

Implementation of Sustained Low Efficiency Dialysis in a Community Hospital without a Chronic Hemodialysis Program

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Background: Sustained low-efficiency dialysis (SLED) is a hybrid modality for the management of acute kidney injury in the critical care setting which avoids many of the drawbacks of continuous renal replacement therapy. North York General Hospital is a 420-bed community teaching hospital in suburban Toronto with in-patient volumes of 28,000 per year and a 22-bed Critical Care Unit (CrCU). There is no chronic hemodialysis unit on-site.

Methods: We implemented a SLED program in the CrCU in 2009 performed exclusively by CrCU RNs under the supervision of staff nephrologists and intensivists. Training and technical support was provided by Fresenius Medical Care using the 2008K@ home hemodialysis machine. In the last 5 years, a total of 67 patients, average age 68, have undergone 434 SLED sessions in our CrCU.

Results: Overall in-hospital mortality was 48%. The average MODS score on day 1 of SLED was 8.1. 78% of patients were ventilated at dialysis initiation, and 45% were on pressors. 31% of survivors (11 patients) were still dialysis-dependent at the time of CrCU discharge and were transferred to other hospitals for chronic hemodialysis. The average cost per treatment was CAD$250.

Conclusions: Our experience indicates that SLED is a safe, efficacious and cost-effective option for the management of acute kidney injury in a community hospital lacking chronic hemodialysis facilities. To our knowledge, SLED is not widely practiced in such settings. Our approach avoids both the cost of consummables and other disadvantages of CRRT, as well as the added cost of a hemodialysis nurse traditionally employed in the performance of SLED.

PUB335

Clinical Study of CRRT for 56 Cases of Neonate

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Background: Recently we could come to perform CRRT for neonates, because recent CRRT device is accurate. CRRT for neonate does not clarify diagnosis, vascular access and so on.

Methods: From 2002 to 2012, these 56 patients were performed CRRT and neonate in NICU. We confirm diagnosis, vascular access, CRRT device, modality of CRRT, mortality of all and mortality of Sepsis.

Results: There are 56 neonate patients performed CRRT. Vascular access are femoral vein, umbilical vein, internal jugular vein, umbilical artery, radial artery and subclavian vein as frequency series. Technical skills of insertion depend on department of pediatric cardiology, neonatologist, cardiac surgeon, critical care and nephrology. CRRT device were perista pump, ACH-07(QB20ml/hr~) and JUN505(QB1ml/hr~). Concerning with modality of CRRT, there are PD(18 cases), CHF(4 cases), CHD(3 cases), CHDF(35 cases), PMX-DHP(13 cases) and PEX(1 case) were also performed. From 2002 to 2007, there are 18 patients with PD out of 30. From 2007 to 2012, there are 5 patients with PD out of 27. In diagnosis as series of high frequency, there are Sepsis, congenital heart disease, congenital diaphragm hernia, digestive system disease (perforation and so on), congenital metabolic disease, persistent pulmonary hypertension of neonate, meconium aspiration syndrome, transient abnormal myelopoiesis, hyperkalemia, kidney disease and fetal hydrops. Mortality was 59.3% in previous term. In following term, mortality was 55.8%. All of the mortality was 57.4%. Mortality of sepsis was 46.2%.

Conclusions: Team medicine, accuracy of CRRT device and technical skills of blood access insertion make it possible to perform CRRT for neonate.

Funding: NIDDK Support

PUB336

Renal Replacement Therapy in Acute-On Chronic Liver Failure – Outcomes and Predictors of Survival

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Background: Acute-on chronic liver failure (ACLF) is defined as acute deterioration of preexisting chronic liver disease usually related to a precipitating event. Compared to decompensated cirrhotics with refractory hepato-renal syndrome, patients with acute-on chronic liver failure (ACLF) with acute kidney injury (AKI) have a slightly better chance of survival on renal replacement therapy (RRT). But the data on outcomes and predictors of survival in this subset of patients is lacking.

Methods: We retrospectively analyzed the data of adult (> 18 years) ACLF patients with severe AKI requiring RRT, admitted to our institute between May 2009 to September 2013. We excluded following ACLF patients from our study- patients who had pre-existing chronc kidney disease; patients with following co-morbidities - coronary artery disease or cardiomyopathies, significant structural lung disease; malignancies.

Results: About 170 patients of ACLF had severe AKI requiring RRT during the study period. Out of these, only 117 patients qualified for the final analysis. The study population consisted predominantly males. Median age was 46.9 ± 24.2 years.ACLF patients with AKI requiring RRT had severe liver disease (mean MELD 42.3) and high rate of multi-organ dysfunction (Mean SOFA – 12.8). SLEDD was the modality used for RRT. About 53% of the ACLF patients had some form of intra-dialytic complication. The average number of days ACLF patient survived after initiation of RRT was 11.6±2.02 days. On multi-variable stepwise linear regression analysis, the independent predictors of mortality in this subset were – CTP score (Beta- 0.14, standard error – 0.05) and SOFA score (beta – 0.09; standard error – 0.05).

	Beta	Standard error	P value
SOFA score	0.094	0.18	< 0.001
CTP score	0.14	0.05	0.01

Conclusions: ACLF patients with severe AKI requiring RRT have very high mortality. CTP and SOFA score are independent predictors of mortality among these patients. Prospective studies comparing different modalities of RRT in this subset of patients are needed.

PUB337

The Implication of Reticulocyte Hemoglobin Content in Maintenance Hemodialysis Patients

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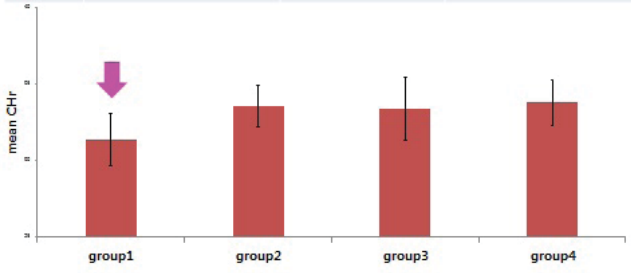
Background: Iron deficiency is one of the most important factors causing erythropoietin hyporesponsivness. According to the K/DOQI guideline, absolute iron deficiency correlates with transferrin saturation(TAST) ≤20% or serum ferritin≤100ng/ml or reticulocyte hemoglobin content (CHr) <29 pg. The aim of this study is to examine the relationship between reticulocyte hemoglobin content and other parameters associated with iron deficiency in maintenance hemodialysis patients.

Methods: Sixty-two patients undergoing hemodialysis were enrolled in this study. Blood samples were obtained prior to the first-of-the-week hemodialysis session. CHr was measured with ADVIA 2120 reticulocyte analysis (SIEMENS, Germany). Correlations among CHr, age, mCHr, Hb, MCV, MCHC, albumin, iron, Kt/V, Hct, ferritin, TIBC and TSAT were analyzed by Pearson correlation coefficient and multivariate linear regression analysis. Patients were divided into 4 groups according to their TSAT and ferritin levels. CHr levels were compared among these 4 groups by ANOVA.

Results: Mean CHr was 33.1±1.9 pg. CHr was related to mCHr($p<0.001$), MCV($p<0.001$), MCHC($p<0.001$), iron($p<0.001$), ferritin($p=0.016$) and TSAT.($p=0.013$) In regression analysis including these variables, MCV($p<0.001$), MCHC($p<0.001$) and iron($p=0.008$) were significantly related to CHr. Mean CHr levels were 31.4 pg(group 1), 33.5 pg(group 2), 33.4 pg(group 3) and 33.8 pg(group 4). The level was significantly lower in group 1. ($p<0.001$).

<Mean CHr of subgrups >

		Mean	Std. Deviation
CHr	Group 1 TSAT<0.2 | ferritin <100	31.4	1.6959
	Group2 TSAT<0.2 | ferritin ≥100	33.5	1.3721
	Group3 TSAT≥0.2 | ferritin< 100	33.4	2.0502
	Group4 TSAT≥0.2 | ferritin ≥100	33.8	1.4642
	Total	33.0	1.9065



Conclusions: Although reticulocyte hemoglobin content is not used in usual clinical practice, it was related to parameters representing iron deficiency in Korean hemodialysis patients. Further research is needed to verify the usefulness of reticulocyte hemoglobin content in predicting iron deficiency in hemodialysis patients.

PUB338

Six Months Intradialytic Aerobic Cycling Program Exerts Beneficial Effects on Haemoglobin Stability in Hemodialysis Patients Treated with ESA

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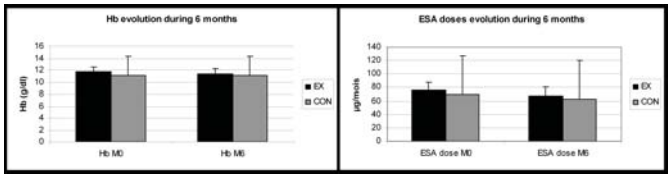
Background: Chronic Kidney Disease (CKD) leads to decrease erythropoietin synthesis. Haemodialysis patients have an unfavourable cardiovascular outcome: the variation of haemoglobin (Hb) is now considered like a cardiovascular risk factor. In clinical practice, the Hb correction with an Erythropoietin Stimulating Agent (ESA) during CKD must be slow and progressive. The morbidity due to quick variations of Hb has been largely studied. This study aimed to determine if physical activity during dialysis (PA) may help to maintain Hb stability in haemodialysis patients treated with Metoxy Polyethylene Glycol Epoetin Beta (MIRCERA*).

Methods: We studied 17 haemodialysis patients treated with MIRCERA in an hemodialysis center. They were voluntarily assigned to either intradialytic exercise training (cycling) group (EX;n=7) or a control group (CON;n=10). Baseline characteristics of the patients:

Groups	%Male	Age	Charlson Index	Time in Dialysis (year)	Diabetes%	Cardiovascular Risk Factors%
EX n=7	71	68 ±10.9	6.4 ± 2.3	4 ± 2.16	57	71
CON n=10	40	67 ± 17.8	5.1 ± 1.66	4.2 ± 2.9	60	70

The Charlson Score is higher in EX: one patient has a score of 11.

Results: Intradialytic training protocol did not modify Hb level, no significant difference between the 2 groups (t=0.21). Hb remains stable in the 2 groups, no supplementary dose during 6 months of follow-up and the doses of ESA diminish in the 2 groups



Conclusions: These results show that: Hb level is stable in the 2 groups, the ESA doses are reduced during the 6 months follow up; no need of supplementary dose. The Hb stability is a protective cardiovascular factor for the haemodialysis patients. It seems that when they practice physical activity it is more stable, so we can encourage patients to do PA. But certainly randomised studies are needed on the subject.

Funding: Pharmaceutical Company Support - ROCHE

PUB339

Anemia Management in the Gulf Cooperation Council (GCC) Countries: International Comparisons from the DOPPS Phase 5 Study (2012-2014)

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Background: Managing anemia and iron stores is an important aspect of care for chronic hemodialysis (HD) patients (pts). Here we provide the first detailed comparison of anemia and iron management (mgmt) in HD pts in 6 GCC countries with that in Europe(EUR), Japan(JPN), and North America (NA).

Methods: Data are from DOPPS 5 (2012-2013) initial cross-section of 742 HD pts on dialysis >90 days at 34 randomly selected facilities in the GCC and compared to 3 other regions: JPN, NA, and EUR. Results are weighted for sampling fraction in each unit.

Results: Mean age of GCC HD pts (53.6 yrs) was 8-11 years younger than in JPN, EUR, or NA, 56% were male, with mean dialysis vintage of 4.2 yrs (versus 4.4, 5.6, 9.3 yrs in NA, EUR, and JPN, respectively). Mean hemoglobin (Hgb) was similar in the GCC, JPN, and NA but varied from 10.3-11.2 g/dL across GCC countries (Table); lower Hgb target varied greatly across GCC facilities. Monthly ESA use was highest in the GCC (89%), ranging from 85-100% across GCC countries; 49%, 48%, and 3% of ESA pts used epoetin, darbepoetin, or Mircera, respectively, but ESA type used varied greatly across GCC countries. Mean TSAT and ferritin in the GCC were similar to that in EUR. Monthly IV iron % use in the GCC was lower than in EUR and NA although mean IV iron doses were the highest among DOPPS countries.

Conclusions: Many aspects of anemia and iron mgmt in the GCC are similar to that in EUR, JPN, and NA. However, considerable differences also are seen across GCC countries. Future studies will examine the relationship of anemia control and iron stores with pt outcomes, and reasons for anemia/iron mgmt practice differences within the GCC.

Measure	EUR	JPN	NA	GCC	Bah	Kuw	Omn	Qat	SA	UAE
N patients [a]	3102	1529	2517	742	26	107	44	53	338	174
Post-dialysis weight, kg	74	56	81	70	73	76	60	71	70	70
Hgb, g/dL	11.4	10.6	10.9	10.8	10.3	11.2	10.7	11.2	10.8	10.8
(std. deviation)	(1.4)	(1.2)	(1.2)	(1.5)	(1.5)	(1.3)	(1.5)	(1.4)	(1.6)	(1.5)
Hgb <10 g/dL, %	13%	27%	18%	25%	35%	15%	32%	19%	24%	28%
ESA prescription, %	85%	88%	83%	89%	100%	86%	94%	97%	85%	91%
EPO dose, u/wk [b]	8730	4032	12501	12789	12286	--	10000	14615	12157	16116
S. ferritin, ng/mL	534	138	726	523	461	N/S	434	564	N/S	608
Ferritin>800 ng/mL, %	18%	3%	39%	17%	15%	N/S	6%	16%	N/S	26%
TSAT, %	27.74	23.18	29.29	28.18	N/S	26.78	N/S	31.61	N/S	N/S
TSAT<20%, %	27%	41%	22%	25%	N/S	24%	N/S	20%	N/S	N/S
IV iron prescription, %	68%	26%	61%	52%	69%	67%	63%	70%	32%	57%
IV iron dose, mg/mo [c]	327	220	315	448	655	347	532	340	603	338
Oral iron prescription,%	1%	3%	6%	22%	20%	9%	5%	0%	46%	13%

Results displayed as mean or %; Countries in GCC are Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, United Arab Emirates; Countries in Europe are Belgium, France, Germany, Italy, Spain, Sweden, UK;
[a] Overall sample N;
[b] Among patients prescribed epoetin;
[c] Among patients prescribed IV iron;
N/S: not shown due to <70% reporting;
ESA and IV iron use was any use during a month;
22 of GCC HD units indicated a Hgb upper target of 12 g/dL for 74% of units, 12.5 or 13 g/dL for 17% of units, and 10.5 or 11 g/dL for 9% of units; Hgb lower target of 10 g/dL for 36% of units, 8.5-9.5 g/dL for 14% of units, 10.5 g/dL for 9%, and 11.0 g/dL for 36% of units.

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PUB340

Non-Anemic Dialysis Patients with No Requirement for Erythropoiesis Stimulating Agents: Who Are They?

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Background: Not only anemia, but also erythropoiesis stimulating agents (ESA) may adversely affect outcome of hemodialysis (HD) patients. Features of ESA-independent non-anemic cases may be useful for defining pathogenesis and related outcomes of anemia in chronic HD patients.

Methods: Data, retrieved from European Clinical Database (EuCliD)-Turkey, on ESA-independent non-anemic (Hb >11.5 g/dl) prevalent HD patients (n:410) were compared with their counterparts, who needed ESA for anemia treatment (n:3891).

Results: Various parameters are provided in table 1.

Table1. Various features of the patients who did, and did not need ESA treatment

Parameter	*No ESA requirement*	*ESA Users*	*P*
Age (yrs.)	54,6±12,6	57,9±14,8	<0,001
Male (%)	78,7	52,8	<0,001
Dialysis vintage (months)	88,8±62,4	61,4±51,7	<0,001
Diabetic (%)	22,7	32,1	<0,001
Hepatitis C (+) (%)	25,1	13,3	<0,001
Dialysis with a-v fistula (%)	86,1±28,4	81,8±32,3	<0,001
URR (%)	73,8±4,7	76,0±5,3	<0,001
Hb (g/dl)	13,2±0,9	11,4±0,9	<0,001
Ferritin (ng/ml)	428,7±469,3	827,0±493,4	<0,001
CRP (mg/dL)	1,3±1,8	1,7±1,9	<0,001
Vit. D usage (%)	66,3	77,2	<0,001
Carnitene usage (%)	49,3	62,9	<0,001

Survival of ESA-independent non-anemic patients was superior as compared to the ESA users.

Conclusions: Favorable effects of several clinical and laboratory parameters as well as elimination of side-effects of ESAs can play a role in better outcome observed in non-ESA requiring patients.

PUB341

Responses to Erythropoietin in Iron Deficient Hospitalized Hemodialysis Patients

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Background: Management strategies to correct anemia in hospitalized hemodialysis (HD) patients include therapy with erythropoietin (EPO). The effectiveness of EPO is known to be limited by iron deficiency. Repeated blood studies and blood loss from various procedures can cause or worsen iron deficiency. There is a paucity of data on iron management in hospitalized ESRD patients.We evaluated the EPO responsiveness in iron deficient patients who did and did not receive iron therapy.

Methods: Hospitalized anemic hemodialysis patients who were treated for more than two weeks over an eleven month period were enrolled. Hemoglobin (Hb) levels, iron status, EPO dosage, use of intravenous iron supplementation and transfusion use were observed.

Results: Fifty-seven patients were enrolled during this period. Anemia was present in 29 patients (50.8%). Of these, 15 patients had transferrin saturation (TSAT) < 23%. Ten (66%) iron deficient patients did not receive intravenous iron because there was concern regarding active infection. Among these 10 patients the average dosage of EPO was 9500 units/HD. In the other 5 patients who were given iron therapy, the average administered EPO dose was 8000 u/HD. The difference was not statistically significant (P=0.35). In patients not given iron initial Hb was 9.0±0.8 gm/dl and discharge Hb was 8.9±0.8gm/dl. Those who did receive iron therapy had initial Hb 8.8±1.7gm/dl and discharge Hb 9.8±1.5gm/dl. The changes between initial and discharge Hb level were not different in the two groups (P=0.30). The discharge Hb levels in both groups were also not statistically different (p=0.15). There was no difference in the incidence of transfusion.

Conclusions: Current practice avoids intravenous iron therapy in the setting of infection. There is insufficient data to assess EPO responsiveness to various levels of iron storage and replacement. In this short term evaluation of iron deficiency anemia in hospitalized ESRD patients we found that Hb levels were not different between patients who received or did not receive iron. Our data suggest that patients are able to maintain some responsiveness to EPO despite iron deficiency.

PUB342

Roxadustat (FG-4592) May Lower Cholesterol Through a Mechanism Related to More Efficient Anemia Correction in Patients with Chronic Kidney Disease

Lynda Szczech, Anatole Besarab, Lona Poole, Brian K. Roberts, Gopal Saha, Kin-Hung Peony Yu, Thomas B. Neff. *FibroGen, San Francisco, CA.*

Background: Iron deficiency is associated with higher lipid levels; with iron repletion, lipid levels fall. Roxadustat, a prolyl hydroxylase inhibitor stabilizing hypoxia inducible factor being developed for the anemia Rx in CKD 3-5, decreases total cholesterol (TC). This analysis explored the potential mechanism for this observation.

Methods: Post-hoc analysis of 2 phase II studies evaluated change in total cholesterol (TC) from baseline across treatment arms stratified by concomitant statin use by Spearman rank correlation.

Results: In ESRD subjects randomized to roxadustat or epoetin alfa, roxadustat reduced TC (assessed as mean change from BL to day 43) irrespective of concomitant statin use (p<0.0001 for both). TC on day 43was significantly lower with roxadustat compared to epoetin alfa (Table 1).

Table 1: TC changes from baseline (mg/dL) by treatment group stratified on use of statins: mean (SD)

	FG-4592	N	epoetin alfa	N	p value
Concomitant statin	-37.10 (45.39)	48	6.52 (29.22)	21	<0.0001
No statin	-36.14 (25.56)	56	0.93 (28.40)	15	<0.0001

Similarly, roxadustat reduced TC from BL to week 9 irrespective of concomitant statin use in CKD (-24.8+30.6, p<0.0001,n=88 and -27.7+28.1, p< 0.0001,n=45 respectively). Among subjects receiving roxadustat and statins, TC inversely correlated with MCV at baseline (slope=-0.24, p=0.03), and a decline in TC was associated with a greater increase in TSAT (slope=0.31, p=0.004). Among subjects receiving roxadustat without statins, greater declines in TC were seen among those with greater TC at baseline (slope=-0.38,p=0.007) and associated with greater increases in MCV from baseline to week 9 (slope=-0.33, p=0.02). Decline in TC was not correlated with either baseline or change in CRP but was associated with increased ferritin (slope=-0.42, p=0.003).

Conclusions: In the two studies analyzed, roxadustat reduced TC in patients with or without concomitant statin therapy. TC reduction was correlated with smaller baseline MCV, a greater rise in MCV during therapy, as well as a rise in TSAT and ferritin suggesting improved availability of endogenous iron as a potential mechanism.

Funding: Pharmaceutical Company Support - FibroGen

PUB343

Glycemic Control and Erythropoietin Resistance in Diabetic Hemodialysis Patients

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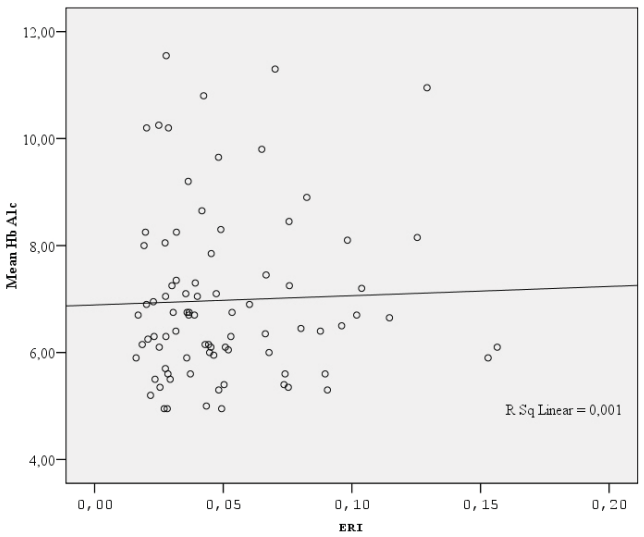
Background: Anemia is very common in patients with chronic kidney disease (CKD) and erythropoiesis stimulating agents (ESA) are often used to treat anemia in these patients. In the course of CKD, diabetic patients tend to develop anemia earlier and to a greater degree than patients without diabetes mellitus. The primary objective of this study was to evaluate the association between glycemic control and ESA dose in diabetic hemodialysis patients.

Methods: This is a cross-sectional study conducted on hemodialysis patients using ESA for anemia. The data between September, 2008 and December, 2008 were investigated retrospectively. Diabetic patients (either type 1 or type 2) receiving darbopoetin alpha and aged ≥18 years were included in the study. To assess ESA responsiveness, an erythropoietin resistance index (ERI) was calculated as weekly ESA dose per kg body weight, divided by the hemoglobin concentration. Erythropoietin resistance was defined as an ERI >0.02 μg/kg/week/g Hb.

Results: A total of 84 diabetic patients of 441 patients on hemodialysis were included in the final analysis. Seventy one patients (84.5 %) had an ERI > 0.02 (patients with ESA resistance). Demographic characteristics were similar in patients with and without ESA resistance. There was a small but significant difference in albumin and Kt/V between the groups (p=0.047, p=0.011, respectively). We found no correlation between ERI and Hb A_{1c} (r= 0.034, p=0.7) (figure1). There was a significant negative correlation between ERI and serum albumin levels (r= -0.273, p=0.012).



Conclusions: We showed that glycemic control may not influence the ESA dose requirement, and there is an association between low serum albumin levels and EPO resistance in diabetic hemodialysis patients.

Funding: Clinical Revenue Support

PUB344

The Sequential Change of Serum Hepcidin-25(HPC) Level and Other Iron Metabolism Markers After Single Intravenous Iron Administration in Hemodialysis (HD) Patients

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Background: HPC is a crucial player of iron metabolism. Iron-overload and inflammation stimulate HPC production, whereas anemia, iron depletion inhibit HPC production. It is not elucidated that the sequential changes of the relation between HPC level and other iron parameters after single intravenous(IV) iron administration in HD patients.

Methods: 23 HD patients were administered ferric oxide (Fe 40mg) intravenously after HD session. We evaluated the following markers before and at 0.5, 1, 2, 4, 6, 20 and 44 hours after iron administration : HPC, transferrin (Tf), 8-oxo-2'-deoxyguanosine (8-OHdG) and standard hematological parameters including high sensitive CRP (hs-CRP). The transferrin saturation (TSAT) value (%) was calculated from the serum iron and Tf values using the formula : serum iron (μmol / L) / Tf (g/L) x 3.8.

Results: 1. TSAT was immediately increase after IV iron administration (before IV was 20(14-27)% and reached the peak after 0.5 hour at 81(65-100)%), and returned to the same level before IV administration at 44 hours. In 6(26%) patients, TSAT had oversaturated. The data are indicated as medians(interquartile range) or numbers(%). 2. HPC level before IV iron administration was 5.1(0.6-20.3) ng/ml, significantly elevated after 4hours at 15.1(0.8-47.3) ng/ml ($p<0.05$), marked the peak after 20 hours at 36.5(5.5-57.6) ng/ml and gradually decreased to 20.7(3.5-41.8) ng/ml after 44 hours. 3. Ferritin level and 8 OHdG level before IV iron administration were 110(49-216) ng/ml and 0.197(0.120-0.268) ng/ml, significantly elevated after 20 hours at 151(83-222) ng/ml ($p<0.01$) and 0.290 (0.196-0.374) ng/ml ($p<0.01$), respectively. 4. Stepwise analysis indicated that HPC level at 4hr, 8OHdG at 0 hr and TSAT at 2 hr were predictors of ferritin level at 20 hrs.

Conclusions: In HD patients, after single IV iron administration, TSAT level was firstly elevated and then HPC level and lastly ferritin level was increased. HPC, 8OHdG and TSAT could be predictor of ferritin level after IV iron administration.

PUB345

Effectiveness of Biosimilar Epoetin Alfa (HX575): Interim Results from the MONITOR-CKD5 Study of Anemia in Hemodialysis

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Background: MONITOR-CKD5 is a European prospective observational multicenter study of practice patterns and outcomes with HX575 in hemodialysis patients.

Methods: Analysis of 2087 CKD5 patients with renal anemia started on HX575 treatment de novo or by conversion in 10 countries. Interim longitudinal results on HX575 dosing and Hb levels in the first 18 months are presented.

Results: Mean age was 64.8±14.9y. Mean time since first dialysis was 3.7±4.7y. Most patients were male (60%) and converted from a prior ESA (82%). Primary CKD5 etiologies were diabetic nephropathy (25%), glomerulonephritis (21%), vascular disease (16%), tubular interstitial nephropathy (11%). Common comorbidities were hypertension (88%), coronary disease (39%), type 2 diabetes (33%). At enrollment median serum ferritin was 559±574 ng/mL; 73% had adequate iron stores, 22% had functional and 5% absolute deficiency. Across visits, iron supplementation was given in 62-67%, and transfusions were given in 2-9%. At enrollment mean Hb was 11.2±1.2 g/dL and mean weekly HX575 dose was 7532±5342 IU/wk. Hb remained stable over 18 months ($p=0.07$) ranging from 11.2±1.2 to 11.3±1.2 g/dL. Changes in HX575 dose ranged from 7385±6769 to 7721±6393 ($p<0.01$), but ESA resistance index (HX575 dose/Hb) remained stable, ranging from 9.3±8.1 to 10.0±9.8 ($p=0.10$).

Conclusions: Interim analysis of this real-world study reveals that biosimilar epoetin alfa (HX575) dosing is effective in maintaining stable longitudinal Hb outcomes, reflecting the same patterns as known with originator epoetin alfa.

Funding: Pharmaceutical Company Support - Sandoz

PUB346

The Cost-Effectiveness of Erythropoietin Stimulating Agents for Treating Anemia in Dialysis Patients: A Systematic Review

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Background: Anemia is common in patients with chronic kidney disease. Erythropoietin stimulating agents (ESAs) are used to treat anemia and reduce the need for blood transfusions. However, recent evidence suggests that ESAs may increase cardiovascular risks and have no significant effects on quality of life. In light of these findings, treatment with ESAs to current hemoglobin targets, may not be cost-effective. Our objective was to evaluate published studies that determine the cost-effectiveness of ESAs based on relevant costs and benefits in adult patients with CKD.

Methods: We conducted a systematic review of studies evaluating the cost-effectiveness of ESAs in patients on dialysis with no restriction on setting. We searched PubMed, EMBASE, and the Cochrane Library ranged from their establishment until June 2013. The primary outcome was the incremental cost-effectiveness ratio (ICER) of treating anemia with ESA as compared to routine blood transfusion, lower Hb target levels, or no ESA treatment.

Results: Seven studies met our inclusion criteria. Four studies compared ESAs and routine blood transfusion as standard of care, two studies compared the cost-effectiveness between different Hb targets, while one studied compared ESA treatment and no ESA treatment. ESAs were found to be less cost-effective than routine blood transfusions (ICERs > $50,000 USD/QALY) in all studies except one. One study found targeting higher hemoglobin levels to be associated with greater clinical benefits at a lower cost and assumed a mortality benefit from increased Hb targets, while a second study found that aiming for higher Hb levels was not cot-effective and assumed an increase in mortality from targeting higher Hb targets.

Conclusions: Our analysis suggests treatment of anemia with ESAs is not cost-effective without assuming large benefits in utility scores or all cause mortality from ESA treatment. Cost-effectiveness models for ESA treatment need to be updated to reflect current clinical evidence on benefits and risks.

PUB347

Improving Erythropoiesis Stimulating Agent Therapy in Haemodialysis Patients. A Clinical Audit of a Novel Bloodline

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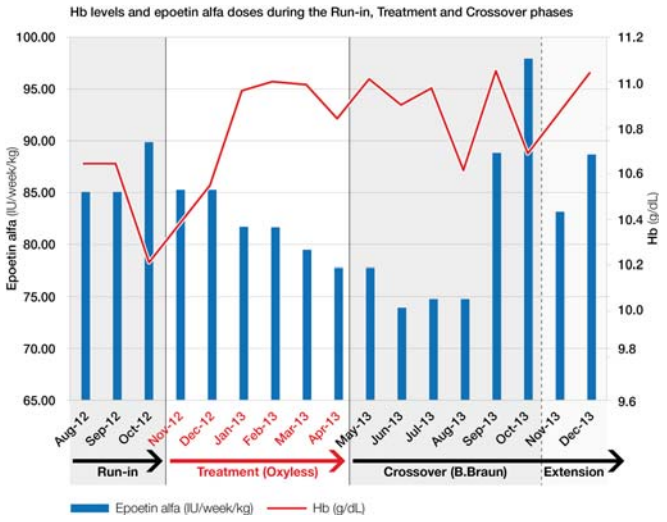
Background: A patented universal bloodline (Oxyless) which greatly reduces the contact between blood and air has been developed for routine haemodialysis (HD). The aim of the investigation was to explore whether the use of the Oxyless bloodline could improve the efficiency of Erythropoiesis Stimulating Agent (ESA) therapy.

Methods: Patients (> 18 years, HD for ≥ 3 months with AV fistulas) were entered into a 12 month open label, single cross-over, cohort prospective pilot audit. Haemoglobin (Hb), epoetin alfa and ESA resistance index (ERI) were reported.

Results: Data from only eight out of fifteen patients were able to be used as a result of drop-outs due to clinical intercurrent events. Haemoglobin increased in the first three months and stabilised during the treatment phase at 11.0 g/dL. Individual patients had Hb increases of more than 1.0 g/dL. Mean peak reduction in epoetin alfa levels were noted in June, ranging from 14% to 67%, representing a total reduction of 12,667 IU/week and a per patient reduction of 2,533 IU/week. In the crossover phase, epoetin alfa levels increased to a mean of 113% of run-in values in response to falling Hb levels (reductions ≤ 1.3 g/dL). These returned to 99%, indicating a rebound effect. Mean Hb returned to 104% of run-in values.



ERI decreased steadily during the treatment phase with a maximum reduction of 16.2%.

Conclusions: There is a small but definite signal that the use of the Oxyless bloodline can improve haemoglobin levels and result in reduced ESA doses. The crossover design allows some confidence in the validity of the data. These results could have potential financial benefits for HD service delivery. A prospective multi-centre European audit (PEMA) is underway to gather more robust clinical and health economic data.

Funding: Pharmaceutical Company Support - Oxyless Ltd

PUB348

Optimizing Anaemia Management and Transfusion Rates in Haemodialysis
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Background: The last decade has seen major changes in anaemia management in end-stage renal disease (ESRD) including different iron regimens, advances in erythropoietin stimulating agents (ESA) and altered haemoglobin (Hb) targets. Despite this, red cell transfusions to maintain target Hb are common in hemodialysis (HD) patients, conferring a burden in cost and nursing time, and risk of sensitization for transplantation. We aimed to quantify transfusion rates in our HD patients at 3 time points reflecting historic changes in our practice, with a cohort of PD patients as control.

Methods: We analysed 4 groups retrospectively. We excluded the first 90 days of HD and patients with sickle cell disease (n=13). For each group we determined the number of units of blood transfused over time on dialysis during the specified year, as well as various haematological parameters and cumulative ESA dose. All ESA doses were subcutaneous until 2012 when policy switched to intravenous.

Results: 1958 patient years were studied. Transfusion rates (units/patient year) between the 4 groups were significantly different (p=0.002) with a clear fall over time to rates seen on PD.

Modality	n (pt yrs)	ESA		Transfusion (u/pt/yr)	TimeAve Results					
		% pts taking Darbepoeitin (cumulative dose mcg/kg/yr)	% pts taking NeoRecormon (cumulative dose mcg/kg/yr)		Hb	Plt	Fer	Tsat	TIBC	CRP
HD 2004	340 (301)	49 (28.4)	51 (4784)	2.42	10.9	233	480	-	-	25
HD 2008	757 (637)	46 (49.4)	38 (8579)	1.66	10.7	230	475	28.4	41.2	23
HD 2012	1071 (917)	89 (22.8)	4 (4488)	1.52	10.7	212	493	27.6	45.5	22
PD 2012	164 (104)	80(14.6)	10 (1156)	1.37	10.9	233	358	28.3	41.6	14

All time-averaged haematological parameters were comparable except significantly lower CRP and ferritin levels in PD. Cumulative ESA dose fell over time, particuarly with iv dosing.

Conclusions: Although occasionally required, blood transfusions in dialysis patients should be avoided, particularly if transplantation is anticipated. We show here that with the use of iv iron (fortnightly to a ferritin target of 500mg/l) and iv darbepoeitin at minimal doses on HD, Hb targets can be met with minimal blood transfusion use, providing safer and more cost-effective care.

PUB349

Sleep-Disordered Breathing Is Associated with Low Serum Klotho in Hemodialysis Patients
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Background: Sleep-disordered breathing (SDB), characterized by nocturnal intermittent hypoxia, is frequently observed in the aged and in patients with end-stage renal disease (ESRD). The anti-aging protein Klotho is mainly expressed in the kidney and released into the blood, but down-regulated in hypoxic conditions or chronic kidney disease. We therefore examined the relationship between SDB and serum Klotho protein level in ESRD.

Methods: Sixty-three maintenance hemodialysis (HD) patients (male: 50.8%, age: 64.2 ± 1.6 years, body mass index [BMI]: 21.3 ± 0.4, duration of HD: 6.7 ± 0.7 years, diabetes: 33.3%) in Koga Red Cross Hospital were subjected to overnight pulse oximetry on a dialysis day. The SDB group was defined as 3% oxygen desaturation index over five events per hour, and the others were designated as the normal group. Serum Klotho levels were measured by a commercially available sandwich ELISA.

Results: Twenty-four patients (38.1%) were classified into the SDB group. Compared to the normal group, the SDB group exhibited lower serum Klotho (SDB 352 ± 23 versus normal 435 ± 34 pg/ml, P=0.040), but higher serum C-reactive protein and higher blood radical formation potential, which denotes oxidative stress. Serum fibroblast growth factor 23, BMI and frequency of diabetes were similar between the groups. After adjusting for age, gender, BMI, duration of HD and diabetes in a logistic regression analysis, SDB was independently associated with low serum Klotho (odds ratio 0.63; 95% confidence interval 0.33–0.99: P = 0.044).

Conclusions: SDB in HD patients was associated with low serum Klotho. This study suggests that nocturnal intermittent hypoxia may contribute to a decrease in renal Klotho expression.

Funding: Private Foundation Support

PUB350

Proton Pump Inhibitors: An Innocent Bystander in Uremic Cardiovascular Disease?
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Background: It has recently been demonstrated that PPIs inhibit dimethylarginine dimethylaminohydrolase, a key enzyme responsible for the metabolism of the eNOS antagonist asymmetrical dimethylarginine (ADMA). There is evidence in animal and ex vivo human models that PPI treatment leads to increased ADMA levels, reduced NO availability and impaired vascular function. ESRD patients have hugely elevated ADMA levels compared to non-uremic patients. High levels of ADMA are associated with cardiovascular death in dialysis cohorts and as such ADMA is considered a 'uremic toxin'. Our hypothesis is that PPI use, through increased ADMA levels may be associated with altered hemodynamic parameters on dialysis.

Methods: We performed a single center observational cross-sectional study, in a teaching hospital HD unit (n=334), collecting demographic and clinical data and stratifying patients according to PPI use.

Results: When compared to patients not on a PPI (n=180), PPI treated patients (n=154) were older (p=0.009), had a lower serum calcium (p=0.02), were more likely to have diabetes (p=0.03) and to receive aspirin (p=0.01), clopidogrel (p=0.004) and β-blockers (p=0.006). There was no difference between the groups in terms history of ischemic heart disease, dialysis vintage, EPO dose and overall antihypertensive use. There was a slight reduction in mean arterial pressure, which approached statistical significance (p=0.06), but no difference in pulse pressure (p=0.32) with PPI treatment.

Conclusions: Our data do not support the hypothesis that PPI use is associated with significant haemodynamic changes in a haemodialysis cohort. Explanations for this result are: at a physiological dose, PPIs may not inhibit ADMA sufficiently to alter blood pressure (a crude surrogate marker of nitric oxide metabolism); other factors (EPO use, hyperglycaemia, oxidative stress) which lead to increased ADMA levels may overwhelm the signal from PPI use, and the colocalization of PPIs and antiplatelet use for heart disease may lead to confounding. Future prospective studies examining PPI use on ADMA levels in patients with CKD may be of great therapeutic potential.

PUB351

Haptoglobin 2-2 Genotype Is Associated with Pulmonary Hypertension in Diabetic Individuals on Hemodialysis
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Background: Haptoglobin (Hp) is an antioxidant protein by virtue of its ability to bind free hemoglobin (Hb) and prevents heme-iron mediated oxidation.The Hp1 protein is superior to the Hp 2 protein in binding to free Hb and neutralizing its oxidative potential. Pulmonary hypertension (PHT) is an elevation of pulmonary arterial pressure, recently been recognized as a complication of chronic kidney disease and Hemodialysis (HD). The higher PHT is an independent predictor of increased morbidity and mortality in HD patients.

Methods: 29 HD patients with DM (mean age, 66.75 ± 8.94 yrs) and 18 HD patients without DM (mean age, 66.58 ± 16.97 yrs)were included. Exclusion Criteria: severe lung disease or known congestive heart failure. Hp typing was performed. Echo measurments of HD patients included left ventricular end-systolic (LVESD), left ventricular end-diastolic diameter (LVEDD) and estimated systolic pulmonary pressure (e-PASP), by Doppler echocardiography.

Results: 1. The prevalence of PHT in Hp 2-2 DM HD patients is much higher (80%) compared with DM HD patients with Hp 2-1 (44%) or Hp1-1 (25%) genotypes. In the non-DM group only 20% of the patients have PHT with no significant differences between the Hp genotypes. **2.** Hp 2-2 DM patients have a significantly higher e-PASP levels (42.17±9.7) compared with Hp 1-1 DM patients (28±7.16) or Hp 2-1 (35.28±5.65) genotypes. In the non-DM patients we found no significant differences in e-PASP values between the different Hp genotypes. **3.** Hp 2-2 DM HD patients had higher e-PASP (42.17±11.69), LVEDD (54.07±6.18) and LVESD (40±3.16) levels compared with non-DM Hp 2-2 HD patients (28.33±3.42, 46.67±6.08 and 33.67±5.05 respectively).

Conclusions: 1. Hp polymorphism affects the prevalence and the severity of PHT specifically in DM HD patients but not in non-DM HD patients. **2**.DM Hp 2-2 HD patients are with the highest risk for CV event. **3.** DM Hp 2-2 HD patients, should be screened by echocardiogram for PHT before entering the HD program.

PUB352

Low Serum Klotho Level Predicts High Mortality in Hemodialysis Patients
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Background: Klotho is a transmembrane protein that is predominantly expressed in the kidney. Its extracellular domain is subjected to ectodomain shedding and released into the blood. Klotho has protective effects against cardiovascular disease (CVD) and death,

but is down-regulated in end-stage renal disease (ESRD). We therefore examined whether low serum Klotho is related to long-term cardiovascular events and mortality in ESRD.

Methods: Sixty three chronic hemodialysis (HD) patients (male 32, age 64.2 ± 1.6 years, BMI 21.3 ± 0.3, follow-up period 60.3 ± 1.6 months) were included in this study. Serum Klotho levels were determined by a commercially available sandwich ELISA. The patients with serum Klotho of less than 350 pg/ml were defined as the low Klotho group, and the others were defined as the high Klotho group. The primary outcome was cardiovascular events and all-cause death. The Kaplan–Meier method and Cox proportional hazard model were used for survival analyses.

Results: Twenty-six patients (41.3%) were classified into the low Klotho group. Age, gender, history of CVD, serum calcium, phosphorus, fibroblast growth factor 23, intact parathyroid hormone, and 1,25-dihydroxy vitamin D3 were similar between the groups. Kaplan–Meier analysis with log-rank test revealed that the low Klotho group had a higher rate of all-cause mortality (P=0.041) and cardiovascular events (P=0.055) than the high Klotho group. In the multivariate Cox regression model including age, gender, serum albumin, and diabetes, low serum Klotho remained an independent predictor for increased mortality (hazard ratio, 3.32; 95% confidence interval, 1.02 to 12.68; P=0.045).

Conclusions: This observational study showed that low serum Klotho is an independent predictor for increased mortality in HD patients. Further studies are required to conclude that low serum Klotho is a cause of poor clinical outcome in HD patients.

Funding: Private Foundation Support

PUB353

Effects of Adherence to Anti-Hypertensive Drugs on All-Cause Mortality and Hospitalizations in a Cohort of Prevalent Hemodialysis Patients

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Background: Poor adherence to anti-hypertensive drugs (AHD) was linked to adverse outcomes in general hypertensive population. However, little is known about effects of adherence to AHD in hemodialysis (HD) patients. We investigated the association between adherence to AHD and all-cause mortality and hospitalizations in a cohort of prevalent HD patients.

Methods: We prospectively studied a cohort of 58 HD patients (mean age 55 years, 96.5% African-Americans, 59.3% males) undergoing HD for at least 12 months under care of a single nephrologist. Adherence to AHD was determined at baseline using analysis of pharmacy dispensation records by calculating proportion of days covered (PDC) in the previous 12 months. Adherence to AHD was considered as good (PDC>80%) and poor (PDC<80%). The association between adherence to AHD and 1-year all-cause mortality and hospitalizations were assessed using univariate analysis and multivariate logistic regression models after adjusting for age, race, gender, and the number of AHD.

Results: At baseline, 10 patients (17.2%) had good adherence to AHD. Among remaining 48 patients, 39.6% had PDC between 40-79% and 60.4% had PDC<40%. During 1-year follow up period, 7 patients (12.1%) died, 41 patients (70.7%) had at least one hospitalization, and 23 patients (39.6%) were hospitalized for cardiovascular causes. In univariate analysis, good adherence to AHD was associated with 89% lower risk of any hospitalizations (OR 0.11, 95% CI 0.03-0.52) and 91% lower risk of all-cause mortality and hospitalization for any cause (OR 0.09, 95% CI 0.02-0.40). In the adjusted analysis, the patients with good adherence to AHD remained at lower risk of any hospitalization (OR 0.16, 95% CI 0.03-0.80) and combined outcome of all-cause mortality and hospitalization for any cause (adjusted OR 0.10, 95% CI 0.02-0.57).

Conclusions: Good adherence to AHD is associated with improved survival and reduced risk of hospitalizations in prevalent HD patients. Therefore, adherence to AHD should be monitored and reinforced in order to improve outcomes in this population.

PUB354

The Correlation of 1,25-Dihydroxyvitamin D with Peripheral Arterial Disease in End Stage Renal Disease Patients on Hemodialysis

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Background: Vitamin D deficiency is recently regarded as an independent risk factor for peripheral arterial disease(PAD) and frequently occurs in end stage renal disease(ESRD) patients on hemodialysis(HD). However, since most studies are conducted on 25(OH) vitamin D (25(OH)D), the effect of 1,25(OH) vitamin D (1,25(OH)2D) on PAD in ESRD patients has not yet been investigated. The aim of this study is to examine the effect of 1,25(OH)2D on the occurrence of PAD in ESRD patients on HD, similarly to other factors related to bone metabolism.

Methods: Preliminary assessment was carried out on adult patients receiving HD. Patients with ankle-brachial index (ABI) less than 0.9 were defined to have PAD. Concentration of 1,25(OH)2D was measured using radioimmunoassay. Many covariates such as sex, age, body mass index(BMI), serum albumin, intact PTH, calcium and phosphate concentration were simultaneously measured.

Results: Serum 1,25(OH)2D concentration was measured for 40 patients out of 44 patients. The mean concentration was 30.9±6.3 pg/mL. ABI of 15 patients was measured, and PAD was confirmed in 20% of patients. Between the patient groups with and without PAD, no difference was found in sex, age (years, 65.0±11.8 versus 69.1±11.2, p=0.57), BMI (kg/m2, 24.8±2.7 versus 24.0±5.5, p= 0.81), serum calcium (mg/dL, 9.1±1.0 versus 8.5±0.5, p=0.19), phosphate (me/dL, 2.8±0.8 versus 4.6±2.3, p=0.29) and intact PTH (pg/mL, 218.6±85.6 versus 199.5±65.4, p=0.68), as well as 1,25(OH)2D (pg/mL, 27.8±7.0 versus 32.3±6.7, p=0.052). An interesting fact is that the female patient group showed direct correlation of serum 1,25(OH)2D concentration with ABI. Such result was statistically significant (p=0.042) after correcting various covariates.

Conclusions: No difference was found in serum 1,25(OH)2D concentration between the patient groups with or without PAD in this study. The female group on HD had a significant correlation of serum 1,25(OH)2D concentration with ABI. Since this study was a preliminary assessment conducted on a small number of participants, future study should be carried out.

PUB355

Walking Speeds for Reduced Cardio-Cerebrovascular Events in Hemodialysis Patients: A Seven-Year Cohort Study

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Background: Deterioration in walking ability characterized by slow walking speed is associated with an increased risk of hospitalization and mortality in hemodialysis (HD) patients. However, few studies have focused on walking speeds associated with reduced clinical events. Here we assessed the benefits of a range of maximum walking speeds (MWS) to reduce cardio-cerebrovascular events in HD patients.

Methods: A total of 188 Japanese outpatients (90 men, 98 women; mean, 65±10 years) undergoing maintenance HD 3 times a week were monitored for 7 years. We measured clinical characteristics (age, sex, body mass index, HD duration, comorbid conditions, serum albumin, and serum C-reactive protein) and MWS at baseline, and followed the patients for clinical events. Patients were divided into quartiles (Q1=lowest, Q4=highest) based on MWS for each sex. Kaplan-Meier analysis and Cox proportional hazards regression were used to assess the contribution of MWS to cardio-cerebrovascular events.

Results: During the follow-up period, cardio-cerebrovascular events occurred in 67 patients. Seven-year cumulative incidence rates were 36%, 32%, 13%, and 9% for Q1 through Q4, respectively, and a significant difference across quartiles of MWS was observed (Log rank, $P<0.001$). While the incidence did not significantly differ between Q1 and Q2 and between Q3 and Q4, Kaplan-Meier curves clearly differed between Q2 and Q3.

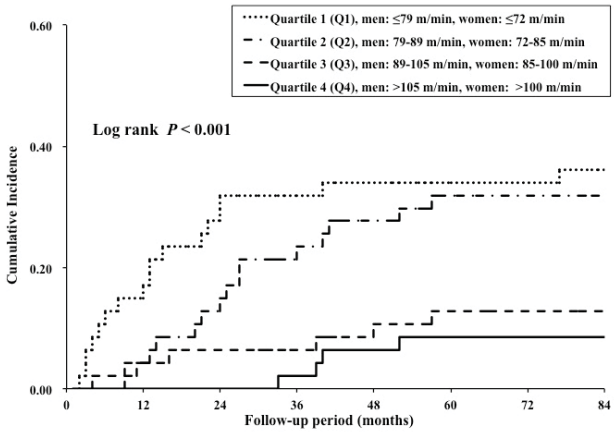

Figure 1. Kaplan-Meier analysis of the number of cardio-cerebrovascular events in 188 hemodialysis patients.

After adjusting for potential confounders, the hazard ratio for events per 10-m/min increases in MWS was 0.71 (95% confidence interval: 0.58-0.86; $P = 0.001$).

Conclusions: Our findings suggest that MWS >89 m/min in men and >85 m/min in women should be maintained in order to reduce subsequent cardio-cerebrovascular events in HD patients.

PUB356

The Effect of Statin for Cardiovascular Prevention in Peritoneal Dialysis Patient

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Background: End stage renal disease is associated with a high risk of cardiovascular event, which is one of the leading causesof death among peritoneal dialysis patients. However, most of the statin treatment randomized studies focus on hemodialysis or chronic kidney disease. The study of statin use for cardiovascular prevention in peritoneal dialysis patients is still sparse and need further investigate. This study aimed to evaluate the efficacy of statin in reducing the subsequent risk of cardiovascular event in a national cohort of Taiwan peritoneal dialysis patients.

Methods: We conducted a nationwide follow-up study, based on the Taiwan National Health Insurance Research Database. We identified incident peritoneal dialysis patients between during 1998 and 2006. Of the peritoneal dialysis patients, 725 received statin with

medication possession ratio (MPR) more than 80% in the first year and 2,114 received statin with MPR less than 80% for hyperlipidemia control. During the 3 years follow up, the primary outcomes of the study was major cardiovascular event, which include hospitalization for acute coronary syndrome and ischemic stroke.

Results: Statin with MPR more than 80% users were not significantly lower hospitalization for major cardiovascular event than statin with MPR less than 80% users (6.34% versus 7.05%), whereas adjusted HR was 0.91 (95% CI: 0.65 to 1.27). The adjusted HRs of hospitalization for acute coronary syndrome and ischemic stroke were 0.9 (95% CI: 0.61 to 1.32) and 0.98 (95% CI: 0.5 to 1.89).

Conclusions: In this retrospective analysis, statin use with MPR more than 80% had no statistically significant effect on the composite end point of hospitalization for acute coronary syndrome and ischemic stroke than statin use with MPR less than 80%.

PUB357

Use of Non-HDL Cholesterol as an Alternative Lipid Panel in Hemodialysis Patients Joannie Lefebvre, Jean-Philippe Lafrance, Vincent Pichette, Martine Leblanc, Robert Zoël Bell, Georges Ouellet, Caroline Lamarche, Anne Devin, Michel Vallee. *Nephrology, Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada.*

Background: Treatment of dyslipidemia is controversial in hemodialysis patient. KDIGO guidelines suggest continuing statin therapy for patients already on statin when starting dialysis. Non-HDL cholesterol (non-HDL C) in contrast to calculated LDL cholesterol (LDL C) can be measure in non-fasting patient but has not been validated in hemodialysis patients. LDL C is obtained by subtracting non-HDL C and triglycerides from total cholesterol and is falsely low in non-fasting patient. Non-HDL C is the difference between total cholesterol and HDL cholesterol. In high risk patients goal of treatment are LDL C ≤ 2.0 mmol/L (70 mg/dl) or non-HDL C ≤ 2.6 mmol/L (100 mg/dl).

Methods: We conducted a cross-sectional study on 360 hemodialysis patients. We analyzed clinical data and measured LDL cholesterol, non-HDL cholesterol and apolipoprotein B (apoB) just before the hemodialysis treatment. The patients dialyzed in the morning (132 patients) were asked to fast for 14 hours prior to blood sampling but this could not be validated. The lipid panel from the patients dialyzed in the afternoon or the evening was obtained without fasting.

Results: Among the 360 hemodialysis patients, 51% were on statin therapy and 45% had coronary heart disease (CHD). Correlation between apoB and non-HDL C was stronger (r=0.939, p<0.0001) than correlation between apoB and non-fasting LDL C(r=0.83, p<0.0001). Forty patients (11 %) had an undetermined or ≤ 2.0 mmol/L non-fasting LDL C combined with a non-HDL C superior to the goal of 2.6 mmol/L. Of these patients, 60% were taking statins and 45 % had CHD.

Conclusions: These results suggest that we may use non-HDL C from the non-fasting lipid profile of hemodialysis patients to adjust lipid-lowering therapy in patients already on statin therapy when starting hemodialysis.

PUB358

Ideal Screening Tools for Coronary Artery Disease in Dialysis Patients: An Angiographic Examination Min-Jee Han, Chae Rim Kim, Do Hyoung Kim, Su Hyun Kim. *Dept of Internal Medicine, Chung-Ang Univ Hospital, Seoul, Republic of Korea.*

Background: Screening for coronary artery disease in dialysis patients is important, because of high cardiovascular mortality in dialysis patients. But there were no defined consensus for screening methods, therefore we studied the clinical effect for coronary angiography (CAG) as a screening tool for coronary artery disease (CAD) in dialysis patients.

Methods: A total of 120 patients who were undergoing dialysis patients (hemodialysis, n= 113, peritoneal dialysis, n= 7) were enrolled in this study. We evaluated the prevalence of coronary artery disease, and diagnostic values of non-invasive test were measured by using coronary angiography as the gold standard, and survival rate of patients.

Results: The patientswere divided into three groups according to ischemic heart disease related symptoms and performance of CAG; Group 1 (symptoms without CAG, n= 58), Group 2 (symptoms with CAG, n=42) and Group 3 (no symptoms with CAG, n=20). Among 62 dialysis patients who underwent CAG (Group 2 and 3), prevalence of CAD was 52(83.9%) and there were no statistically different between two groups. The accuracy of non-invasive diagnostic test was measured by using CAG as the gold standard, regional wall motion abnormality on resting echocardiography showed comparatively good sensitivity (65.4%) and specificity (80%), but its sensitivity was less than clinical symptoms (69.2%). Electrocardiography and chest X-ray showed low sensitivity (40.4%). Specificity of Troponin I (≥ 0.8 ng/mL) showed highest (90%) but low sensitivity (40.4%). Cardiovascular mortality rate was significantly higher in Group 1 than in Group 2 (p=0.049) and Group 3 (p=0.045). Age, performance of CAG, troponin-I, electrocardiography abnormalities, total cholesterol showed independent predictors of cardiovascular mortality. In multivariate Cox proportional hazard analysis, performance of CAG (4.16, 1.44-11.98, p = 0.017) and troponin-I (4.78, 1.55-14.72, p = 0.006) showed significant correlated with cardiovascular deaths.

Conclusions: This report suggests that performance of CAG as a screening test for CAD in dialysis patient may further improve cardiovascular survival rate.

PUB359

Reduced Systolic Longitudinal Strain in Hemodialysis Patients with Preserved Ejection Fraction Is Independent of Symptomatic Coronary Artery Disease Diana Chiu,[1] Nik Abidin,[2] Philip A. Kalra,[1] Darren Green.[1] *[1]Manchester Univ; [2]Salford Royal Hospital, United Kingdom.*

Background: Myocardial strain is a measure of the deformation of tissue from its resting dimensions.Left ventricular (LV) strain abnormalities have been found in hemodialysis(HD) patients with preserved ejection fraction (LVEF),indicating sub-clinical systolic dysfunction.This study compares strain pattern in HD patients with LVEF>50%,with and without symptomatic coronary artery disease (CAD) to establish whether previous findings were as a result of CKD or CAD.

Methods: 2D transthoracic echocardiography was performed in 194 stable HD patients on a non-dialysis day. Strain was assessed by speckle tracking software. The LV was divided into 21 segments and strain determined in each (abnormal strain is ≥-15%). Global longitudinal strain(GLS) was the mean of peak systolic longitudinal strain from the 3 apical views. CAD was defined as previous myocardial infarction,angina or coronary revascularization. Comparison between groups with and without CAD was made with t-test for continuous data and Fisher's exact test for categorical data.

Results: Of the 194 patients,166 had LVEF>50%.Those with CAD were older and more likely to have diabetes.Despite this,there was no difference in LV mass(118 v 113g/m^2,p=0.5),or LVEF(66 v 68%,p=0.2)between groups.Patients with CAD did not have more segments with abnormal strain(12 v 11,p=0.1),nor was there a difference in GLS(-13 v -14%,p=0.1).

Table1.Demographics and results of 2D echocardiography and speckle tracking

	No CAD (n=127)	CAD (n=39)	p
Age(yrs)	59(15)	68(9)	<0.01
Sex(male)	79(62)	29(74)	0.18
Diabetes	39(31)	20(51)	0.02
LVEF(%)	67.8(9.1)	65.7(9.8)	0.24
LVMI(g/m^2)	113.3(35.3)	117.6(32.7)	0.50
GLS(%)	-14.1(3.4)	-13.0(3.8)	0.11
Abnormal segments(n)	11(4)	12(4)	0.10

Data shown as mean (standard deviation,SD)-continuous variables; n(%)-categorical variables
LVMI:left ventricular mass index

Conclusions: Despite preserved LVEF,there were more LV segments showing abnormal longitudinal strain than not.This finding was the same in both groups indicating that symptomatic CAD is not responsible for these abnormalities in HD patients.Rather they are likely to be due to other factors such as uremic cardiomyopathy.

Funding: Other NIH Support - Kidney Research UK charity project grant

PUB360

Time-Dependent Aggravation of Abnormal Serum Sulfatide Levels Associated with Increased Oxidative Stress in Hemodialysis Patients Yuji Kamijo, Makoto Harada, Taro Kanno, Akinori Yamaguchi, Yosuke Yamada, Mai Sugiyama, Koji Hashimoto, Makoto Higuchi. *Dept of Nephrology, Shinshu Univ School of Medicine, Matsumoto, Nagano, Japan.*

Background: Sulfatides are major glycosphingolipids of lipoproteins that are speculated to influence atherosclerosis and blood coagulation. Our previous cross-sectional study of hemodialysis patients showed that serum sulfatide levels decreased markedly with increasing duration of hemodialysis treatment, which may contribute to the development of cardiovascular disease. However, the mechanisms underlying the decrease in serum sulfatide levels in HD patients are currently unknown.

Methods: To confirm the time-dependent abnormality of serum sulfatide levels and to elucidate the underlying mechanisms, we followed 95 hemodialysis patients for 3 years and analyzed correlations between clinical factors and serum sulfatide levels. As previous experimental studies reported an association between abnormal serum sulfatide levels and oxidative stress, we also measured serum levels of an oxidative stress marker, malondialdehyde.

Results: The current study detected a time-dependent decrease in serum sulfatide levels associated with increased malondialdehyde levels, regardless of baseline levels. Multiple linear regression analysis showed a significant correlation only between the time-dependent change in serum sulfatide levels and the time-dependent change in serum malondialdehyde levels.

Conclusions: These findings suggest that abnormal serum sulfatide levels are aggravated in hemodialysis patients in a time-dependent manner, and that oxidative stress is an aggravating factor. This progressively unhealthy situation may result in the development of cardiovascular diseases. As continuation of hemodialysis treatment does not improve abnormal serum sulfatide levels or the increased oxidative stress, development of novel therapeutic strategies may be important.

PUB361

Changes in Cardiac Structure and Function after Treatment with Paricalcitol in Patients on Periodical Haemodialysis. Role of Renin-Angiotensin-Aldosterone System Inhibitors Elvira Bosch,[1] Eduardo Baamonde,[1] Carlos Culebras Caeceres,[3] Fatima Batista,[1] German Perez Suarez,[1] Gloria Anton Perez,[1] Celia Lopez,[2] Mar Lago,[2] Agustin Toledo,[2] Cesar Garcia-Canton.[2] *[1]Centro de Hemodiálisis Avericum - Hospital Univ Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain; [2]Nephrology Service, Hospital Univ Insular de Gran Canaria, Spain; [3]Cardiology Service, Hospital Univ Insular de Gran Canaria, Spain.*

Background: The aim of the study was to analyze changes in cardiac structure and function of patients with secondary hyperparathyroidism at 12 and 24 months after treatment with paricalcitol.

Methods: Observational prospective study with 50 patients on haemodialysis, who started treatment with paricalcitol. Echocardiogram was performed after 12 and 24 months of treatment. Patients with and without treatment with rennin-angiotensin-aldosterone system inhibitors (RAASI) were compared.

Results: After 12 months reductions were observed in the average diameter of the left atrium (LA 42.05 versus 39.9 mm;p=0.029) and the posterior wall of the left ventricle (PW 13.1 versus 12.3 mm; p= 0.048), as well as in the percentage of patients with concentric VH (p=0.009). After 24 months (n:31) reduction in LA diameter (40.04 versus 37.2 mm;p= 0.017), improvement in left ventricular ejection fraction (LVEF 61.7 versus 68.1 %; p= 0.008) and increase in the percentage of patients with normal LV morphology (p=0.041) were observed. **Patients without RAASI treatment:** After 12 months, reductions were observed in DDLV (51.2 versus 47.3 mm;p=0.001), LVM (289.2 versus 243.2 g;p=0.021) and LVMI (148.6 versus 126.3 g/m^2;p=0.022). After 24 month**s** only improvement in LVEF was observed (63 versus 69.2 %;p= 0.043).

Conclusions: Reduction in the LA size and improvement in the LV structure were evidenced after 12 months of treatment with paricalcitol. Such changes were more evident in the group of patients without RAASI treatment, which suggests that the use of RAASI might be masking the cardiovascular effects of paricalcitol. Some of these changes were no longer observed after 24 months of treatment. This finding could be accounted for by the reduced size of the sample.

PUB362

Isolated Diastolic Dysfunction Is an Independent Predictor of Cardiovascular Events in Incident Dialysis Patients Shin-Wook Kang, Chang-Yun Yoon, Young Su Joo, Seung Gyu Han, Seung Hyeok Han, Tae-Hyun Yoo. *Dept of Internal Medicine, College of Medicine Yonsei Univ, Seoul, Korea.*

Background: Diastolic heart failure (HF) is associated with cardiovascular (CV) morbidity and mortality in the general population as well as patients with end-stage renal disease (ESRD). The concurrence of diastolic HF and systolic dysfunction is common among ESRD patients due to recurrent volume excess and CV events. However, it is not clear whether diastolic HF per se is associated with poor CV outcome in this population. This study was aimed to elucidate the impact of isolated diastolic dysfunction with preserved systolic function on CV outcomes in incident dialysis patients.

Methods: A total of 194 incident ESRD patients, who started dialysis between July 2008 and August 2012 and had normal left ventricular (LV) systolic function, were included. The independent prognostic values of echocardiographic parameters for CV events were ascertained by multivariate Cox proportional hazard regression analysis. Kaplan-Meier curves were also constructed, and between-group survival was compared.

Results: During a mean follow-up duration of 27.2 months, CV events occurred in 57 patients (29.4%). Compared to the CV event-free group, LV mass index, the ratio of early mitral flow velocity (E) to early mitral annulus velocity (E') (E/E'), left atrial volume index (LAVI), and right ventricular systolic pressure were significantly higher in patients with CV events. In multivariate Cox proportional hazard analysis, severe diastolic dysfunction (E/E'>15) and severely enlarged LA volume (LAVI>32 mL/m^2) were independent risk factors for CV events [E/E'>15: hazard ratio (HR)=5.40, 95% confidence interval (CI)=2.73–10.70, P<0.001; LAVI>32 mL/m^2: HR=5.56, 95% CI=2.28–13.59, P<0.001]. When patients were divided into four groups according to E/E' and LAVI, Kaplan-Meier analysis revealed that patients with both E/E'>15 and LAVI>32 mL/m^2 had the worst CV outcomes (P<0.001).

Conclusions: Diastolic dysfunction even with preserved LV systolic function was associated with poor CV outcomes in incident dialysis patients.

PUB363

Echocardiographic Parameters as Cardiovascular Mortality Predictors in Chronic Hemodialysis Patients Kosaku Nitta, Ken Tsuchiya. *Dept of Medicine, Kidney Center, Tokyo Women's Medical Univ, Shinjuku-ku, Tokyo, Japan.*

Background: Hemodialysis (HD) patients have high rates of cardiovascular (CV) mortality. Although structural and functional echocardiographic alterations in HD patients have been the subject of several survival analysis studies, the prognostic value of these alterations is not well established. The aim of this study was to determine the prognostic value of echocardiographic parameters in chronic HD patients.

Methods: One hundred eighteen HD patients were clinically evaluated and underwent Doppler echocardiography, being followed for 45.7 ± 13.6 months. The outcome measures were CV mortality. The predictive value of echocardiographic variables was evaluated by Cox regression model and survival curves were constructed using the Kaplan-Meier method and log-rank test to compare them.

Results: CV diseases accounted for 46.4% of all deaths during the follow-up period. We found that the event-free survival rates in one and two years were 96.5% and 83.0%, respectively. Diabetes and E/e' ratio were predictors of CV outcome by multivariate analyses.

Conclusions: Diabetes and diastolic dysfunction are independent predictors of CV mortality in chronic HD patients.

PUB364

Use of Supplementary Oxygen During Haemodialysis Is Not Associated with a Reduction Dialysis Induced Myocardial Stunning Lisa E. Crowley,[1] Helen J. Jefferies,[1] Chris W. McIntyre.[2] *[1]Dept of Renal Medicine, Royal Derby Hospital, Derby, United Kingdom; [2]Faculty of Medicine and Health Sciences, Univ of Nottingham, Nottingham, United Kingdom.*

Background: Myocardial stunning (recurrent segmental ischaemic injury) is a common consequence of haemodialysis (HD) treatment. Arterial hypoxaemia is well described as a consequence of HD. We investigated whether potentially addressing myocardial oxygen supply with supplementary oxygen therapy during HD might reduce the occurrence of HD induced myocardial stunning.

Methods: 9 patients receiving conventional thrice weekly haemodialysis were recruited. All patients dialysed via an arteriovenous fistula. Patients were studied at visit one with pre and peak stress echocardiography. Following this they received 24-28% inspired oxygen via nasal cannulae on 3 consecutive dialysis sessions and then underwent further echocardiographic study (visit 2). Analysis of echo's utilized 2D speckle tracking software for measurement of left ventricular longitudinal strain. A stunned segment was defined as one undergoing a 30% reduction in longitudinal strain.

Results: Median age of subjects was 72.6yrs±9.94 with a median dialysis vintage of 39.8months±24.9. Median ultrafiltration volume at visit 1 was 1.38kg±0.73 and at visit 2 was 1.29±0.83, there were no significant differences in haemodynamic response to HD between the two visits. The mean number of left ventricular segments undergoing a 30% reduction in longitudinal strain was 2.8±1.48 at baseline compared with 3.25±3.45 after 1 week of supplementary oxygen therapy. The mean pre-dialysis global longitudinal strain (GLS/%) was 16.05±6.51 at visit 1 and 16.97±4.67 at visit 2. Post dialysis GLS after visit 2 was 13.56±5.09, lower than that seen after visit 1 (16.7±2.9).

Conclusions: The results of this small study suggest that administration of oxygen during HD is not associated with any improvements in the haemodynamic response to dialysis and does not significantly abrogate myocardial stunning.

PUB365

A Study on Intradialytic Hypertension at Four South African Haemodialysis Units Sajith Sebastian,[1] Christelle Filmalter,[2] Mogamat-Yazied Chothia.[1] *[1]Nephrology, Univ of Stellenbosch, Cape Town, Western Cape, South Africa; [2]Health Science, Central Univ of Technology, Bloemfontein, Free State, South Africa.*

Background: Intradialytic hypertension (IDH) refers to the paradoxical rise in blood pressure (BP) during chronic haemodialysis, and increases morbidity and mortality. The reported prevalence ranges from 5-15% but the prevalence in South Africa is unknown. It has been suggested that IDH may be due to subclinical fluid overload. We set out to determine the prevalence of IDH in our setting and studied its association with potential risk factors.

Methods: A multicentre, cross-sectional study was conducted at four haemodialysis units in the Western Cape, South Africa. Cases of IDH were defined as a rise ≥10 mmHg in systolic BP during dialysis in 4 out of 6 consecutive dialysis sessions. Data were collected on demographics, fluid status using bioimpedance, the haemodialysis procedure and medication.

Results: The prevalence of IDH was 28.4% (n=190). There was a trend toward 'overhydration' in the IDH group as measured by whole body bioimpedance (2.6 L (95% CI 1.7- 3.4) versus 1.8 L (95% CI 1.4-2.1); p=0.06) but no difference in mean ultrafiltration volume (2.4 L versus 2.6 L; p=0.30). Mean age in the two groups was similar (57.1 versus 55.1 years; p=0.42), as was gender (males 53.7% versus 59.5%, p=0.40), time-averaged sodium concentration (138.4 mM versus 138.3 mM; p=0.72), dialysate calcium concentration (1.34 mM versus 1.36 mM; p=0.46), weekly erythropoietin stimulating agent dose (6896 IU versus 6352IU; p=0.38) in the IDH versus control groups respectively. There was a trend to more use of antihypertensive drugs in the IDH group (2.5 drugs (95% CI 2.15-2.87) versus 2.1 (95% CI 1.82-2.3); p=0.05). More participants in the IDH group received calcium channel blocker (54 versus 36, p=0.03). There was no difference in the use of other antihypertensives.

Conclusions: The prevalence of IDH in our treatment centres is much higher than has been previously reported. Subclinical fluid overload may contribute significantly to the development of IDH in our setting. The use of whole body bioimpedence has allowed us to identify patients who may benefit from additional ultrafiltration and potentially treat IDH.

Funding: Pharmaceutical Company Support - Fresenius Medical Care(SA)

PUB366

The Impact of Red Cell Distribution Width on Mortality in Chronic Hemodialyis Patients Myung Jin Choi,[1] Jiwon Ryu,[1] Jwa-Kyung Kim,[1] Youngki Lee,[1] Ja-Ryong Koo,[1] Jung-Woo Noh,[1] Yeo Jin Bang,[2] Miji Jeong.[2] *[1]Dept of Internal Medicine, College of Medicine, Hallym Univ and Hallym Kidney Research Institute, Chuncheon, Republic of Korea; [2]Hemodialysis Center, Chuncheon Sacred Heart Hospital, Chuncheon, Republic of Korea.*

Background: Red cell distribution width (RDW), a measure of the erythrocyte variabiltiy and heterogeneity, is a strong predictor of adverse outcomes in patients with cardiovascular disease. However, no studies have investigated the impact of RDW on cardiovascular complication and mortality in chronic hemodialysis (HD) patients.

Methods: A total of 177 HD patients (age 57.8 ± 12.0 years, male 48.0 %, diabetes 54.8 %, mean dialysis duration 48.4 ± 51.1 months) were enrolled. After baseline evaluation, patients were divided into two groups according to median RDW level. All patients were prospectively monitored for the development of coronary artery disease, cerebrovascular disease and death.

Results: The median RDW was 14.35 % (range 7.86 to 17.30). Patients with higher MPV levels (n=89) had lower levels of anemia, albumin, total cholesterol, triglyceride, LDL cholesterol and HbA1c. During a follow-up period of mean 25 months, 68 composite events (33 deaths, 25 coronary artery disease, 10 cerebral artery disease) occurred. The Kaplan-Meier curve showed significant difference between two groups in the cumulative events of all-cause mortality (11.36 % versus 25.84 %; log-rank test, p=0.006) and cardiovascular mortality (3.41 % versus 14.61 %; log-rank test, p=0.006). In multivariate Cox analysis, RDW was an independent risk factor for all-cause mortality (hazard ratio (HR), 1.499; 95% confidence interval (CI), 1.035 to 2.173; p=0.032) and cardiovascular mortality (HR, 1.857; 95% CI, 1.198 to 2.880; p=0.006). There were no significant differences between the two groups in coronary artery disease and cerebral artery disease.

Conclusions: Higher RDW level was significantly associated with increased all-cause and cardiovascular mortality. RDW may be an independent predictor of death and poor prognosis in chronic hemodialysis patients.

PUB367

Clinical Significance of Progressive Rise in Red Blood Cell Distribution Width in Incident Dialysis Patients Hye Eun Yoon, Bum Soon Choi, Eun Nim Kim, Yong-Soo Kim. *Internal Medicine, The Catholic Univ of Korea, College of Medicine, Seoul, Republic of Korea.*

Background: Red cell distribution width (RDW) represents the variabililty of sizes of circulating erythrocytes. RDW is a robust marker of adverse clinical outcomes in patients with cardiovascular diseases. However the clinical significance of rise in RDW is undetermined in incident dialysis patients.

Methods: Three hundred and thirty seven incident dialysis patients were included. Temporal changes in RDW during 12 months after the start of dialysis were assessed by calculating the coefficient by linear regression. Patients were divided in two groups; RDW-not-increased group included those with negative coefficient values (n = 184), and RDW-increased group included those with positive coefficient values (n = 153). We investigated whether increase in RDW was predictive for cardiovascular events (CVE) and deaths.

Results: The RDW-increased group showed significantly older age and lower total iron binding capacity at baseline compared with the RDW-not-increased group. During a median follow-up for 2.6 years (range, 0.3 – 7.7 years), 66 non-fatal CVE (19.6%) and 82 deaths (24.3%) occurred. The RDW-increased group showed significantly lower event-free survival rate for non-fatal CVE and deaths compared with the RDW-not-increased group (P = 0.009). In multivariate analysis, increase in RDW was an independent predictor for CVE and deaths (hazard ratio, 1.46; *P* = 0.038).

Conclusions: Progressive rise in RDW independently predicted adverse outcomes in incident dialysis patients. Assessing the change in RDW after starting dialysis may be helpful to identify high risk patients for CVE or death.

PUB368

Incident Elderly versus Young: Dialyze in the Same Way? Fatih Kircelli,[1] Ozgur Akin Oto,[2] Murvet Yilmaz,[3] Ercan Ok,[4] Kutay Gunestepe,[1] Cengiz Dogan,[1] Ali Basci,[4] Mehmet S. Sever.[2] *[1]Fresenius Medical Care; [2]Istanbul Univ; [3]Bakirkoy R&E Hospital; [4]Nephrology, Ege Univ.*

Background: The number of incident elderly hemodialysis patients is increasing. Tailor-made prescriptions may be needed in these cases considering varying demographic, clinical and laboratory features.

Methods: Data of 3934 incident patients retrieved from EuCliD-Turkey were used to compare dialytic features of elderly (>75 years) with middle-aged (45-75 years) and young (<45 years) patients.

Results: Incident elderly patients were more diabetic, had lower blood pressure levels, IDWG, processed blood volume, and blood pump rate, but higher Kt/V. All these features were present during the course of 3 years. In the elderly cases, serum creatinine, albumin and phosphorus levels were lower, while hospitalization and mortality rates were higher (3 years mortality: 48 % versus 89%)

Table 1. Demographical, clinical and laboratory data of the incident patients.

Parameters	***>75 years n=613 Elderly***	*<45 years n=592 Young*	P
Age (years)	**80,7±4,6**	34,4±7,3	<0,001
Gender (male,%)	**53,0**	60,3	0,047
Dialysis duration (months)	**0,5±0,8**	0,4±0,8	0,030
Diabetes (%)	**26,6**	12,8	<0,001
AV fistula (%)	**42**	67	<0,001
PBV (liters)	**64±13**	70±11	<0,001
taPBV (ml/min)	**68±12**	75±12	<0,001
BFR (ml/min)	**271±53**	297±43	<0,001
taBFR (ml/min)	**286±49**	311±44	<0,001
KtV	**1,27±0,33**	1,25±0,33	<0,001
taKtV	**1,38±0,30**	1,36±0,27	<0,001
SBP (mmHg)	**126±16**	133±17	<0,001
taSBP (mmHg)	**125±15**	131±17	<0,001
Serum albumin (g/dl)	**3,5±0,5**	3,7±0,5	<0,001
Serum phosphate (mg/dl)	**4,2±1,5**	5,2±1,6	<0,001
Hemoglobin (g/dl)	**10,0±1,4**	9,7±1,7	0,002

ta: time-averaged, PBV: Processed blood volüme, BFR: Effective Blood Flow Rate, SBP: Systolic Blood Pressure. Incident patient is defined as: age>18, on hemodialysis≤3 months

Conclusions: Various clinical and laboratory features differ significantly between elderly and young dialysis patients. Higher mortality and morbidity rates in the elderly can be improved by focusing on correctable factors independent of the age; hence individualized dialysis prescription is strictly needed in these cases.

Funding: Pharmaceutical Company Support - Fresenius Medical Care Turkey

PUB369

Prevalent Elderly versus Young: Treat the Same Way? Fatih Kircelli,[1] Ozgur Akin Oto,[2] Murvet Yilmaz,[3] Ercan Ok,[4] Kutay Gunestepe,[1] Cengiz Dogan,[1] Ali Basci,[4] Mehmet S. Sever.[2] *[1]Fresenius Medical Care; [2]Nephrology, Istanbul Univ; [3]Nephrology, Bakirkoy Research and Education Hospital; [4]Nephrology, Ege Univ.*

Background: The prevalence of elderly population is increasing among chronic HD patients. Tailor-made prescriptions may be needed in these cases considering varying demographic, clinical and laboratory features.

Methods: Data of the 7688 prevalent patients retrieved from EuCliD-Turkey were used to compare dialysis features in the elderly (>75 years) with middle-aged (45-75 years) and young (<45 years) patients.

Results: Elderly patients were more diabetic and had lower blood pressure levels and intradialytic weight gain. Their processed blood volume, blood flow rate and Kt/V was lower over 3 years. Hemoglobin, serum creatinine, albumin and phosphate levels were lower in addition to higher hospitalization and mortality rates (53% versus 94%).

Table. Demographical, clinical and laboratory data of the prevalant patients.

Parameters	***Elderly >75 years n=859***	***Young <45 years n=1594***	P
Age (years)	**80,0±3,9**	34,7±6,7	<0,001
Gender (male,%)	**53,0**	60,5	<0,001
Dialysis duration (months)	**46,8±39,7**	81,3±62,4	<0,001
Diabetes (%)	**25,7**	7,4	<0,001
AV Fistula (%)	**76**	86	<0,001
taPBV (liters)	**74±11**	81±12	<0,001
taBFR (ml/min)	**306±46**	327±47	<0,001
taKt/V	**1,49±0,26**	1,52±0,29	<0,001
taIDWG (%)	**2,6±1,1**	4,2±1,5	<0,001
taPredialysis-SBP (mmHg)	**124±15**	128±19	<0,001
taPredialysis-DBP (mmHg)	**72±8**	77±10	<0,001
taCreatinine (mg/dl)	**6,3±1,6**	9,4±2,1	<0,001
taAlbumin (g/dl)	**3,8±0,3**	4,1±0,3	<0,001
taPhosphate (mg/dl)	**4,2±1,0**	5,3±1,2	<0,001
taHemoglobin (g/dl)	**11,3±1,0**	11,5±1,4	<0,001

PBV: Processed blood volume, BFR: Effective Blood Flow Rate, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, ta: time-averaged. Age, gender, dialysis duration and diabetes are baseline values.
Prevalent patient is defined as: age>18, on hemodialysis>3 months

Conclusions: Prevalent elderly patients differ significantly from the younger population. They have much higher mortality and morbidity rates and should be given special attention. Thus, dialysis prescription has to be individualized for this population.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

PUB370

Relationship Between Thyroid Function and Arterial Stiffness in Hemodialysis Patients Kota Kakeshita, Fumihiro Tomoda, Tsutomu Koike, Hidenori Yamazaki, Hayato Fujioka, Hiroshi Inoue. *Second Dept of Internal Medicine, Univ of Toyama, Toyama, Japan.*

Background: In hemodialysis patients, the decrease in thyroid hormones is frequently observed in spite of normal levels of thyroid stimulating hormone (TSH). This study was designed to explore the association of thyroid dysfunction with vascular structural alterations in hemodialysis patients.

Methods: In 45 hemodialysis patients (67±10 years, male/female= 30/15), cardio-ankle vascular index (CAVI) was automatically measured and calculated by VaSera VS-1000 (Fukuda Denshi, Tokyo, Japan) as an arterial stiffness index. Biochemical parameters and plasma levels of free thyroxine (FT4), free triiodothyronine (FT3) and TSH were also measured.

Results: FT3 and FT4 were lower than the lower limit of normal range (2.3 pg/mL and 0.8 ng/mL in FT3 and FT4, respectively) in 43 patients (96 %) and 7 patients (16 %) patients, respectively. In contrast, TSH was within normal range in all the patients. FT3 and ratio of FT3 to FT4 (i.e., index for the conversion from FT4 to FT3) correlated negatively with CAVI (r= -0.33 and -0.28, respectively; p< 0.05 of each), although FT4 and TSH did not associate with CAVI. Additionally, age, pH and serum b2-microglobulin also correlated with CAVI (r= 0.34, -0.312 and 0.274, respectively; p< 0.05 of each), although blood pressure, pulse rate and parameters for lipid, glucose, calcium, phosphate and parathyroid hormone did not associate with CAVI. Multiple regression analysis revealed that ratio of FT3 to FT4 as well as age and pH were independently associated with CAVI (r^2= 0.561).

Conclusions: In hemodialysis patients, thyroid dysfunction, especially the impairment of conversion from T4 to T3 might contribute to the aggravation of arterial stiffness in addition to aging and acidosis.

PUB371

Comparison of Arterial Stiffness and Aortic Systolic Pressure Measured in Static Conditions by the MOBIL-O-GRAPH and SPHYGMOCOR Devices in Hemodialysis Patients Pantelis Sarafidis,[1] Antonis Karpetas,[2] Panagiotis I. Georgianos,[2] Thanasis Bikos,[2] Pantelis Vakianis,[3] Maria Tersi,[3] Vassilios Liakopoulos,[2] Pantelis Zebekakis,[2] Anastasios Lasaridis,[2] Athanase Protogerou.[4] *[1]Nephrology Dept, Hippokration Hospital, Thessaloniki, Greece; [2]Div of Nephrology, 1st Dept of Medicine, AHEPA Hospital, Thessaloniki, Greece; [3]Therapeutiki Dialysis Unit, Greece; [4]Hypertension Unit and Cardiovascular Research Laboratory, "Laiko" Hospital, Athens, Greece.*

Background: A new oscillometric brachial cuff-based device (Mobil-O-Graph, IEM, Germany) assesses non-invasively aortic systolic blood pressure (aSBP), augmentation index (AIx) and pulse wave velocity (PWV) in ambulatory conditions. The aim of this study was to investigate the agreement of the Mobil-O-Graph with the currently most widely applied tonometric device (Sphygmocor, ArtCor, Australia) in hemodialysis patients.

Methods: In 73 hemodialysis patients, aSBP, heart rate-adjusted AIx (AIx(75)) and PWV were measured with both devices (order: Sphygmocor then Mobil-O-Graph) after 10 min of rest in the supine position. BP for calibration of the Sphygmocor waveform was obtained with a mercury sphygmomanometer.

Results: aSBP and AIx(75) measured with Sphygmocor did not differ from measurements obtained with Mobil-O-Graph (aSBP: 136.3±19.6 versus 133.5±19.3 mmHg, P=0.068; AIx(75): 28.4±9.3 versus 30.0±11.8%, P=0.229, for Sphygmocor versus Mobil-O-Graph). Sphygmocor-derived PWV was higher than PWV measured with Mobil-O-Graph (10.3±3.4 versus 9.5±2.1 m/sec, P<0.01). This marginally insignificant difference for aSBP is explained by the difference in peripheral SBP (146.9±20.4 versus 145.2±19.9 mmHg, P=0.274, for Sphygmocor versus Mobil-O-Graph). All 3 hemodynamic parameters obtained with Sphygmocor showed significant associations with measurements taken with Mobil-O-Graph (r=0.770, P<0.001 for aSBP, r=0.400, P<0.001 for AIx(75) and r=0.739, P<0.001 for PWV). The Bland-Altman Plots for all parameters showed acceptable agreement between the 2 devices.

Conclusions: In hemodialysis patients, aSBP and PWV were slightly underestimated by the Mobil-O-Graph compared to Sphygmocor device. However, acceptable agreement between devices was evident for aSBP, AIx(75) and PWV.

Funding: Private Foundation Support

PUB372

Pulse Wave Velocity in Haemodialysis Patients Is Associated with Survival and Comorbidity Ana Rita Mateus Martins,[1] Maria Gabriela Teixeira,[1] Inês Filipa Moreira,[1] Patricia Quadros Branco,[2] Teresa Adragao,[2] Andre L. Weigert.[1] *[1]Davita Portugal, Davita, Leiria, Portugal; [2]Nephrology Dept, Hospital Santa Cruz, Lisbon, Portugal.*

Background: Chronic kidney disease (CKD) is a risk factor for cardiovascular (CV) mortality. Haemodialysis (HD) patients (pts) have a high risk of death. Arterial stiffness (AS) has been recognized as a strong and independent predictor for CV events in CKD. Our aim was to evaluate risk factors of CV risk in prevalent HD pts.

Methods: Observational prospective study in 149 prevalent portuguese HD pts. We obtained baseline data regarding demographic, blood biochemistry, comorbidities, ankle-brachial index (ABI), pulse wave velocity (PWV), central buckberg sub-endocardial viability ratio (SEVR) and nutritional parameters. PWV and SEVR assessments were performed at baseline, before the second or the third dialysis session of the week.

Results: In our cohort, 93 pts were male, mean age was 67 years, 39% had diabetes (DM), average time on dialysis was 54 months. 9.3% of the patients had previous history of renal transplantation and comorbidity charlson score was on average 4.44±2,3. The follow up period time was about 10 months. During that period, 6 pts died of CV events. Patients with history of renal transplantation had lower pwv (10.79±4.136 VS 15.6±7.138; p=0.001). Multivariate Cox analysis revealed that a higher value of PWV (HR 1.186; IC(95%): 1.03-1.364; p=0.017) and a lower value of albumin (HR 0.22; IC(95%): 0.065-0.752; p=0.016) were significant risk factors for death, in a model adjusted for age and DM. In a linear regression, Charlson comorbidity score was closely related to a higher value of PWV (B=0.768; IC:0.255-1.282; p=0.004). In multivariate analysis (linear regression), there was a significant association between age (B=0.148; IC: 0.065-0.23; p=0.001), DM (B=2.903; IC: 0.559-5.247; p=0.016) and lower albumin (B=1.87; IC: 0.020-3.76; 0=0.05) with PWV, even adjusted for time on dialysis.

Conclusions: Our findings suggested that PWV value was an independent predictor of survival and comorbidity in our cohort of Portuguese HD pts.

PUB373

Measurement of the Sympathetic Nerve Activity Index (LF/HF) in Diabetic and Non-Diabetic Dialysis Patients Fumiko Fukuuchi,[1] Ken Tsuchiya.[2] *[1]Nephrology, Komagome Kyouritsu Clinic, Bunkyou-ku, Tokyo, Japan; [2]Medicine IV, Tokyo Women's Medical Univ, Shinjyuku-ku, Tokyo, Japan.*

Background: Autonomic nervous system dysfunction is very common in dialysis patients. The symptoms of autonomic nervous system dysfunction are remarkably severe especially in dialysis patients with diabetes. Autonomic nervous system dysfunction is concerned with intradialysis hypotension and induced congestive heart failure. The aim of this study is to reveal the differences of sympathetic nerve activation between diabetic groups and non-diabetic groups during hemodialysis.

Methods: Holter electrocardiography power spectrum analysis carried out 24 hour chronometries from the beginning of dialysis. The ratio of low frequency band (LF; sympathetic modulation of heart rate) and high frequency band (HF; vagal modulation of heart rate), LF/HF is considered as the indices of sympathetic activation. We compared the LF/HF of these two groups.

Results: The 24 dialysis patients (eleven diabetics, thirteen non-diabetics) are included in this study. 44 Holter electrocardiography results were analyzed. LF/HF was compared between diabetic hemodialysis and non-diabetic hemodialysis patients from the beginning to 1 hr after hemodialysis treatment. In non-diabetic groups, LF/HF was increased during hemodialysis treatment. In diabetic groups, LF/HF ratio was decreased for 3 hours from the beginning of hemodialysis. Interdialytic weight gain is more in diabetic groups than non-diabetic groups. Although, LF/HF was significantly lower in diabetic groups in 1 hour, 2 hours and 3 hours after starting hemodialysis. Diastolic blood pressure was also significantly lower in diabetic groups in 3 hours and 4 hours after starting hemodialysis. Systolic blood pressure in non-diabetic groups was inversely correlated significantly with LF/HF in 2 hours and 3 hours after starting hemodialysis without intradialytic hypotension.

Conclusions: In diabetic dialysis patients, the sympathetic nerve activity assessed by LF/HF is distinctly disturbed, that is likely to result in intradialytic refractory hypotension.

PUB374

Mortality following Cardiac Valve Replacement in Patients with End Stage Renal Disease Yanilda M. Nunez Germosen, Maria Coco. *Medicine, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.*

Background: Valvular calcifications are common in patients (PTS) with ESRD leading to cardiac surgery. PTs on chronic dialysis who undergo valvular surgery have a high morbidity and mortality. The purpose of this study is to identify risk factors associated with the high mortality in these PTS.

Methods: We conducted an electronic chart review of ESRD PTS who underwent cardiac valve replacement at Montefiore Medical Center between 1999 to 2009, comparing cohorts pre and post 2005, corresponding to the introduction of cinacalcet and lanthanum. We evaluated median survival times post surgery, biochemical parameters of mineral metabolism, demographics, BMI, BSA, Left Ventricular Ejection Fraction, vintage dialysis time, access type, valve used, diabetes, endocarditis. Multivariate models were constructed to determine predictors of survival.

Results: 99 PTS (56M, 43F) age 58.7± 13 yrs had valve replacement. They were on dialysis for 3.1±.89yrs; 41 tunneled catheters, 38 AVF, 13 AVG, 5 peritoneal dialysis, 2 unknown. P was lower post 2005 (4.8±1.5 versus 5.5±2.2, p=.046). CxP was lower post 2005 (42±15 versus 53±25, p=.022). The entire cohort survived a median of 639 days. Median survival pre versus post 2005 was 427 versus 726 days (p=0.016). PTH, Ca, P, Ca X P, access, age and BSA individually impacted survival. Multivariate adjusted models showed that only Ca x P affected survival. When compared pre and post 2005: Ca, P, Ca x P, BSA and endocarditis impacted individually, but in the adjusted model, only P predicted survival pre-2005. Post 2005, PTH, access, type of valve and age were significant in the unadjusted survival model. Post-2005 PTS had been on dialysis for a longer period compared with pre-2005 (3.6 yrs±0.47 versus 2.1±0.6 p=.001).

Conclusions: Mineral metabolism in ESRD has improved since 2005 with new and aggressive treatment targeting P and PTH. PTS who had valvular surgery after 2005 had been on dialysis for a longer period suggesting that better control of mineral metabolism

may have delayed the progression of calcification. In addition, post-operative survival was improved. However, further study is needed to evaluate other factors contributing to the high mortality.

Funding: Clinical Revenue Support

PUB375

Factors Involved in Coronary Artery Calcification in Maintenance Hemodialysis Patients Yoshiko Nishizawa,[1,2] Sonoo Mizuiri,[2] Kyoka Ono,[2] Mariko Asai,[2] Kazuomi Yamashita,[2] Kenichiro Shigemoto,[2] Kohji Usui,[3] Noriaki Yorioka,[4] Yasuhiko Tomino.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Juntendo Univ Faculty of Medicine, Tokyo, Japan; [2]Nephrology, Ichiyokai Harada Hospital, Hiroshima, Japan; [3]Nephrology, Ichiyokai Clinic, Hiroshima, Japan; [4]Nephrology, General Incorporated association Hiroshima Kidney Organization, Hiroshima, Japan.*

Background: Cardiovascular diseases related to coronary artery calcification are common causes of morbidity and mortality in maintenance hemodialysis (MHD) patients. In addition to the well-known traditional risk factors, uremia-specific factors, medications appear to affect coronary artery calcification. The objective of this study was to evaluate coronary artery calcification score (CACS) in MHD patients, and to identify potentially involved factors.

Methods: The study included 207 patients (58 females, 149 males), aged 64±14 years, who were on MHD treatment for 94±88 months (mean±SD). We assessed sex, age, dialysis vintage, presence of diabetes mellitus (DM), smoking history, presence of ≥100 ml urine volume/day, normalized protein catabolic rate (nPCR), geriatric nutritional risk index (GNRI), administration of active vitamin D_3, cinacalcet and phosphate binders, antihypertensive agents, and circulation parameters including creatinine, albumin, corrected calcium and phosphate in sera. CACS was assessed using Agatston Score by multi-detector CT (MDCT).

Results: Coronary artery calcifications were observed in 192 MHD patients (92.8%), and CACS ≥400 Hounsfield units was recognized in 62.8%. In univariate analysis, age, dialysis vintage and presence of DM, serum creatinine, serum albumin and administration of active vitamin D_3 were significant independent factors for CACS ($p<0.05$). In multivariate analysis, CACS showed direct associations with age ($p<0.001$), dialysis vintage ($p<0.001$) and presence of DM ($p<0.005$), and an inverse association only with active vitamin D_3 administration ($p<0.001$).

Conclusions: In conclusion, age, dialysis vintage and presence of DM were risk factors, and the administration of active vitamin D_3 was a modifiable protective factor for coronary artery calcification in MHD patients.

Funding: Private Foundation Support

PUB376

Comparison of Metal versus Tissue Valve Replacements in End-Stage Renal Disease Suzanne H. Forbes, Luxme Nadarajah, Andrea Cove-Smith, Neil Ashman, Mark Blunden. *Nephrology and Transplantation, Royal London Hospital, London, United Kingdom.*

Background: Little evidence guides the choice of valve replacement in end-stage renal disease (ESRD) patients; meta-analyses suggest no survival difference between bio- and mechanical prostheses. The use of metal valves necessitates anticoagulation, usually with warfarin. It is increasingly recognised that haemodialysis (HD) patients have significant increased bleeding risk, and that warfarin should be avoided. We examined outcomes in ESRD patients with both bio- and mechanical valve replacements.

Methods: We retrospectively studied patients known to our services who had a valve replacement, excluding those with insufficient follow up data or without stage 5 CKD. We gathered demographics from the time of replacement and data on anticoagulation. We noted outcomes including major bleeding, endocarditis, tissue valve redo and mortality.

Results: We included 82 patients, equating to 419 patient years. Median age at replacement was 58. 43 had metal valves, 39 tissue. All metal valves were anticoagulated with warfarin. 5 patients with tissue valves also took warfarin. 63% of patients with a metal valve and 59% of tissue valves were on HD or peritoneal dialysis at the time of operation. There were 44 major bleeding events (2 fatal) in 24 patients with metal valves versus 9 in tissue; a bleed rate of 19.6 per 100 patient years versus 4.6 (p=0.004). Rates of endocarditis were higher in metal valves (23% versus 10%,p=0.05). Median time before death was 5.7yrs in metal valves and 3.6 in tissue. 3 patients with metal valves had calciphylaxis (2 fatal). 3 patients with tissue valves required redo operations (1 paediatric valve, 2 due to endocarditis).

Conclusions: The usual indication for metal valves is in younger patients for whom tissue valve lifespan must be considered. Poor 5 year survival in ESRD makes this less relevant. The necessity of future procedures (vascular access, transplantation, biopsy) in a population at increased risk of major bleeding makes anticoagulation hazardous. We demonstrate increased risk of major bleeding, endocarditis and calciphylaxis with metal valves in ESRD with no valve-survival advantage and suggest metal valves be avoided in this population.

PUB377

Correlation of Serum Visfatin Levels with Cardiovascular Disease and Serum Biochemical Parameters in Hemodialysis Patients Nimet Aktas,[1] Abdulmecit Yildiz,[2] Cuma Bülent Gül,[3] Osman Z. Sahin.[4] *[1]Nephrology, Bilecik State Hospital, Bilecik, Turkey; [2]Dept of Nephrology, Uludag Univ Medical School, Bursa, Turkey; [3]Dept of Nephrology, Sevket Yilmaz Research and Training Hospital, Turkey; [4]Dept of Nephrology, Recep Tayyip Erdogan Univ Medical School, Turkey.*

Background: Cardiovascular (CV) diseases constitute a major cause of mortality in chronic kidney disease. We evaluated the role of visfatin; an adipokine, on end-stage renal disease(ESRD), investigated its relation with CV diseases and risk factors.

Methods: There'retwo control groups included participants without diabetes mellitus: healthy control group HC (n=40) and CC (n=28), included patients with normal renal functions, had coronary artery disease. There're two study groups includedhemodialysis patients without diabetes mellitus: HD (n=30), who had no coronary artery disease history and HDC (n=32), who had established coronary artery disease. Biochemical parameters and visfatin of serumwere done in all patients.

Results: The average level of serum visfatin was 26,52 ng/ml (9,10-47,18) in HC group, 33,68 ng/ml (13,25-55,3) in CC group, 35,96 ng/ml (11,62-60,61) in HD group, 35,74 ng/ml (9,21-61,64) in HDC group. Whereas there was statisticaly significant difference between HC and other groups in case of serum visfatin levels ($p<0,005$), no significant difference was found between other groups. In correlation analysis serum visfatin levels were correlated with triglyceride in HC group, with systolic blood pressure, urea, creatinine in CC and with uric acid in HD group. In HDC group no correlation was found with any parameter. Serum visfatin are compared between groups. The level of visfatin were 30,51 ng/ml (9,1-55,3) in control group and 35,745 ng/ml (9,21-61,64) in study group, this's statistically significant ($p =0,014$) (Table). In correlation analysis in control group serum visfatin level correlated with urea, creatinine, uric acid, total cholesterol, triglyseride and parathormone. In study group it was correlated with serum insülin, HOMA-IR, uric acid levels.

Conclusions: Visfatin has effects on CV disease however this effect is less in ESRD.

PUB378

Echocardiographic Abnormalities Associated with Cardiac Death in a Cohort of Dialysis Patients Mauro Castellano, Pablo U. Massari, Javier De Arteaga, Walther Douthat. *Servicio de Nefrología., Hospital Privado Centro Médico, Córdoba, Argentina.*

Background: There is a strong association between end-stage renal disease (ESRD) with cardiovascular (CV) events and death. Echocardiographic studies in these patients have shown a high prevalence of pathological findings.

Methods: We conducted a prospective study to assess CV changes by echocardiography and establish its influence on the survival in a single-center dialysis population. We included 70 patients, age 18 or higher, both incident and prevalent at November 1, 2009.

Results: Mean age was 58.0 ± 14.9 years, male (62.0%). Main causes of ESRD were diabetes and nephroangioesclerosis, 28.2 and 23.5% respectively. 81.7% were on hemodialysis, with an average time from the beginning of therapy of 41.6 months. The follow-up time was 36 months. Just 7.0% of patients had preserved ventricular geometry. 60% of patients with impaired ventricular geometry showed concentric remodeling, 29.8% concentric hypertrophy and 3.5% eccentric hypertrophy. 27.6% showed ventricular systolic dysfunction and 47.6% diastolic dysfunction. During follow-up, 27.5% were transplanted, 34.8% continued on dialysis and 37.7% died. The main cause of death was CV (42.3%). Patients who remained alive had a higher ejection fraction marginally significantly to the deceased ones, especially from CV causes (56.4 ± 9.8 versus 51.4 ± 14.8%, P=0.22). Patients who died from CV causes had a higher proportion of wall motion disorders than the living patients (50.0 versus 16.2%, P=0.10). They also had higher prevalence of mitral and aortic deficiency that living patients (70.0 versus 45.9%, P=0.44 and 30.0 versus 24.3%, p=0.39 respectively).There was statistical differences in the survival curves (Kaplan-Meier analysis) and increased mortality in patients with disorders in ventricular wall motion than those who did not (50.0 versus 81.1%; Logrank p=0.01). In univariate analysis (Cox model) the presence of wall motion disorders represented a 52% increase in the risk of death (p=0.11).

Conclusions: We found a high prevalence of echocardiographic abnormalities, predominating changes in ventricular geometry. The deceased patients showed a tendency to higher wall motion being a factor that influenced survival.

Funding: Clinical Revenue Support

PUB379

Determinants of Metabolic Acidosis in Maintenance Hemodialysis Patients Hironori Nakamura,[1] Anayama Mariko,[1] Yasushi Makino,[1] Makoto Higuchi,[2] Masaki Nagasawa.[1] *[1]Dept of Nephrology, Shinonoi General Hospital, Nagano, Japan; [2]Dept of Internal Medicine, National Hospital Organization, Matsumoto Hospital, Matsumoto, Japan.*

Background: Metabolic acidosis is associated with poor nutritional status and survival in hemodialysis (HD) patients. The K/DOQI clinical practice guidelines recommend that serum bicarbonate level should be maintained at 22 mEq/L as the standard level for HD patients. However, factors involved in metabolic acidosis are not fully clarified.

Methods: The aim of this study was to evaluate the association between predialysis bicarbonate level, clinical parameters, and phosphate binder types. We enrolled 159 consecutive HD patients (at least 1-year follow-up after HD initiation) in this cross-sectional study. Clinical parameters included age, HD vintage, HD time, body weight

(BW) gain, blood pressure, laboratory data, and amount of phosphate binders. Phosphate binders included calcium carbonate, sevelamer hydrochloride, and lanthanum carbonate. Moreover, univariate and multivariate analysis were used. Bicarbonate 30 mEq/L was the dialysate used for all patients.

Results: The mean age was 66.4 years old and HD vintage was 130 months. The mean amount of calcium carbonate was 1.62g, sevelamer hydrochloride was 1.19g, and lanthanum carbonate was 0.41g. Patients who showed a predialysis bicarbonate level of ≤22 mEq/L were 22.3%. Univariate analysis showed that predialysis bicarbonate levels significantly correlated with age (β= 0.249), inversely with HD vintage (β = -0.26), BW gain (β = -0.408), potassium (β = -0.174), phosphate (β = -0.247), intact parathyroid hormone (β = -0.257), nPCR (β = -0.227), calcium carbonate (β = -0.177), sevelamer hydrochloride (β = -0.436), and lanthanum carbonate (β = -0.243). Multivariate analysis showed that HD vintage (β = -0.174), BW gain (β = -0.310), and sevelamer hydrochloride (β = -0.306) were dependent factors for metabolic acidosis. Subgroup analysis without sevelamer hydrochloride treatment indicated that HD vintage (β = -0.261) and BW gain (β = -0.399) were independent factors.

Conclusions: In this study population, HD vintage, BW gain, and sevelamer hydrochloride treatment seemed to be associated with predialysis bicarbonate levels.

PUB380

The Effect of Diet on Deposition of Skin Advanced Glycosylation End Products Assessed by Skin Autofluorescence in Chronic Haemodialysis Patients Arkom Nongnuch,[1,2] Andrew Davenport.[2] *[1]Renal Unit, Faculty of Medicine, Ramathibodi Hospital, Mahidol Univ, Bangkok, Thailand; [2]UCL Centre for Nephrology, UCL Medical School, London, United Kingdom.*

Background: The major cause of death in chronic dialysis population is cardiovascular disease (CVD), owing to traditional and nontraditional risk factors. Amongst the nontraditional risk factors, Advanced Glycosylation End Products (AGEs), a group of heterogeneous compounds derived from the non-enzymatic reaction called Maillard reaction. are renally excreted, thus both serum AGEs and AGEs deposited in tissues are increased in dialysis patients. Previous studies have shown that tissue AGEs had greater association with CVD than serum AGEs. Currently, tissue AGEs deposition can be measured by using skin autofluorescent technique (SAF), reliable and convenient measurement.

Methods: We measure SAF AGEs using DiagnOptics, Groningen, Netherland on non fistular arm in multiethnic chronic haemodialysis population.

Results: The mean age of 332 patients was 65.2 ± 15.1 years, 64% male, 42% diabetes, 66% Caucasian,21% Afro-Caribean, 13% Asian, with a dialysis vintage 38.5 months (1-413), 37% smoker, 8.1% vegetarian, 57% prescribed calcium carbonate, and 15% prescribed lanthanum. In multivariate analysis, woman, Caucasian ethnicity, older age, longer dialysis vintage, diadetes, prescribed calcium carbonate and lanthanum was associated with higher tissue AGEs whereas vegetarian has lower tissue AGEs.

Table 3. Multiple regression analysis of factors associated with SAF of dialysis cohort (R^2 = 0.334, adjusted R^2 = 0.309).

Variable	B (95% CI)	P value
Race C/A/Af	-0.52 (-0.64 to -0.39)	<0.001
Age	0.014 (0.008 to 0.02)	<0.001
Male gender	-0.22 (-0.4 to –0.04)	0.019
Log dialysis vintage	0.30 (0.14 to 0.46)	<0.001
Diabetes	0.23 (0.05 to 0.42)	0.013
Vegetarian	-0.39 (-0.7 to -0.07)	0.019
Calcium carbonate prescription	0.311 (0.12 to 0.5)	0.001
Lanthanum carbonate prescription	0.38 (0.12 to 0.64)	0.005

Conclusions: Caucasoid ethnicity, older age, female gender, longer dialysis vintage, diabetes status, and both prescription of calcium and lanthanum based phosphate binders were associated with increasing skin AGEs deposition. On the other hand, patients eating vegetarian, low AGEs diets had lower tissue AGEs accumulation, and further interventional studies are required to determine whether dietary intervention can modify tissue AGE deposition and CVD risk.

Funding: Private Foundation Support

PUB381

A Decade of Tight Infection Control in Hemodialysis, Did It Make a Difference? Jafar Al-Said, Aimee Pagaduan, Soni Murdeshwar. *Nephrology and Internal Medicine, Bahrain Specialist Hospital.*

Background: Hemodialysis related bacteremia and hemodialysis access infection are the major causes of morbidity and the second cause of mortality in ESRD population. The aim of this study was to determine the outcome, over 10 years, of tight infection control in preventing Hemodialysis related Bacteremia and vascular access infection.

Methods: This study is a retrospective longitudinal analysis for the outcome of the hemodialysis infection protocol srtictly implemented over 120 months from January 2004. All Hemodialysis sessions were included. Patients' demographics and the types of vascular access were collected from the electronic data. The following outcomes variables were calculated: Hemodialysis induced bacteremic infection per 100 patient months, admission rates for Hemodialysis related bacteremia per 1000 patient years, admission for vascular access infection, hemodialysis bacteremic infection per patient, monthly blood infection rates, and infection rate per hemodialysis session.

Results: Total Patients were 138. Total Hemodialysis sessions were 9383. Mean age was 57.6 years (SE 1.3). Male gender were 55%. Types of vascular access were; 56.3% cuffed tunneled catheters, 19.5% AV fistula, 18.2 AV grafts and 5.8% were vascath. Total Hemodialysis related bacteremia occurred in 9 patients. Hemodialysis related blood stream infection per 100 patient month was 0.01. Admission for hemodialysis related bacteremia per 1000 patient year was 0.55. Admission for vascular access infection over 120 months was Zero. Hemodialysis bacteremic infection per patient was 0.06. Hemodialysis bacteremic infection per month was 0.075. Infection rate per HD session was 0.00096.

Conclusions: The tight infection control protocol followed was able to reduce Hemodialysis Bacteremia and eliminate Vascular Access infection. We would like to share our infection control protocol, that we followed for the last decade, to participate in reducing hemodialysis related bacteremia and vascular access infection.

PUB382

Improving Care for Hemodialysis Patients: Rationale and Design of a Patient-Centered Medical Home Model for Patients with End-Stage Renal Disease Anna C. Porter, Michael J. Fischer, Jose A.L. Arruda, Michael L. Berbaum, Sheila R. Castillo, Rani Gallardo, Marian L. Fitzgibbon, James P. Lash, Lisa Sharp, Denise M. Hynes. *Univ of IL at Chicago.*

Background: More than 500,000 patients with end-stage renal disease (ESRD) in the United States depend on chronic hemodialysis (HD) for their renal replacement therapy. Despite receiving this treatment, chronic HD patients incur a disproportionately high burden of morbidity, mortality, resource utilization, and poor quality of life (QOL). Enhanced coordination of care through a patient-centered medical home model has been used to improve outcomes in other complex chronic illnesses, but has not been studied in chronic HD patients.

Methods: The Patient-Centered Medical Home Model for Patients with Kidney Disease (PCMH-KD) will implement a comprehensive multidisciplinary care team within the dialysis unit to improve coordination of primary care for chronic HD patients. The PCMH-KD will expand the existing care team of the dialysis unit (comprised of a nephrologist, dialysis nurse, dialysis technician, social worker, and dietitian) to include a general internist, pharmacist, and health promoter, all of whom will see the patient during dialysis treatments and separately as needed. An approximately 300-patient cohort at the University of Illinois independent freestanding dialysis unit and one freestanding for-profit dialysis unit will be recruited for study participation.

Results: The effect of the PCMH-KD model on the following outcomes will be examined: i)patient- and caregiver- reported outcomes such as QOL and care satisfaction; ii)clinical outcomes such as blood pressure and dietary management; iii)healthcare use such as ER use, hospitalizations; and iv)staff perceptions of care.

Conclusions: Patients with ESRD on HD suffer from a significant burden of hospitalizations, mortality, and poor QOL. Improvement of care coordination with a PCMH-KD provides an opportunity to potentially reduce this burden.

PUB383

Which Reality behind the New Dialyser Fx Cordiax? Matching Study and Comparative Efficacy of Molecules Removal Sebastien Deleuze, Jean Pierre Rivory, Isabelle Selcer, Francois Maurice, Catherine Rouanet. *Nephrology, Nephrocare Castelnau le Lez, Castelnau le Lez, France.*

Background: It seems that the removal of middle molecules solute using the recently developed high flux dialysis membranes in haemodialysis (HD) is close to the results obtained by on line pre (Pre) and post dilution (Post) haemodiafiltration. The subject of this study is to compare the removal of urea, beta 2 microglobulin (β 2 m), and myoglobin (myo) by the new dialyser in these 3 technics.

Methods: We realized an observational study on 200 patients after matching patients by duration of dialysis. Extraction of β 2 m and myo was evaluated by the reduction ratios (RR) using a kinetic model.The dialyser's area was prescribed depending on the body area of the patient (Fx cordiax 800 or 1000 for Post and Pre, Fx cordiax 80,100,120 or Bk for HD).

Results: We matched 35 patients in HD (7 Bk, 28 Fx Cordiax), 35 patients in Post (Fx cordiax 800, 1000), and 35 patients in Pre(Fx cordiax 800, 1000). The mean of volume substitution is 20,09 l in Post, and 45,63 l in Pre. **Comparison HD versus post:** Two groups are similar in duration of dialysis (p=0, 96), blood flow, and volume of treatments. There is no difference between HD and post in predialysis level of β 2 m, myo and Albumin (alb). Post is better than HD group for RR of myo(70, 56% +/- 8, 99 versus 38% +/-9,92; p< 0,0001) and RR of β 2 m (79,8%+/- 8,56 versus 64,3%+/- 14,9; p<0,001), Urea extraction (76,91 % +/- 6,45 versus 73 % +/- 7,26; p= 0,025) and Kt/v e (1,48 +/- 0,32 versus 1,32 +/- 0,3, p=0,021). **Comparison PRE versus POST:** Two groups are similar in duration of treatment (p= 0,51), blood flow, and volume of treatments.There is no difference between Post and Pre in the pre dialysis level of β 2 m, myo, and alb. Post is better than Pre group for RR myo (70, 09% +/- 8, 19 versus 47,55 % +/-11,05; p< 0,0001), RR β 2 m (79,37%+/- 9,67 versus 74,77%+/- 12,01; p<0,039).There is no difference between the 2 groups in urea extraction and Kt/v e.

Conclusions: Our study showed that treatment using Post with FX Cordiax (800 or 1000) remains the best treatment for removing molecules of any size, and in particular for those higher than 17kDa (despite the fact that we used a less than 23 l substitution volume).

PUB384

Validity of Registry Data: Agreement between Comorbidities in ANZDATA Compared to Administrative Data in Australia Sradha S. Kotwal,[1] Angela C. Webster,[2,3] Alan Cass,[1,4] Martin P. Gallagher.[1,5] *[1]The George Institute for Global Health, The Univ of Sydney, Sydney, Australia; [2]Sydney School of Public Health, The Univ of Sydney, Sydney, Australia; [3]Centre for Transplant and Research, Westmead Hospital, Westmead, Australia; [4]Menzies School of Health Research, Charles Darwin Univ, Darwin, Australia; [5]Concord Clinical School, Univ of Sydney, Sydney, Australia.*

Background: Coding quality of administrative data has been considered poor for comorbidity recording. Co-morbidities recorded by ANZDATA are considered reliable, but audit of registries is time consuming. To assess agreement of comorbidity recording between the ANZDATA Registry and the New South Wales (NSW) Admitted Patient Data Collection (APDC).

Methods: ANZDATA identified all incident patients in NSW receiving dialysis or transplant between 01/07/2000 and 31/07/2010. Patients were then linked to APDC (records up to 55 medical conditions impacting upon an admission). We calculated agreement between the two datasets for diabetes, cardiovascular disease (CVD), chronic lung disease (CLD) and peripheral vascular disease (PVD) and agreement beyond chance using the kappa statistic (κ). A kappa of >0.75 indicates excellent agreement, 0.40-0.75 fair to good agreement and <0.40 poor agreement.

Results: ANZDATA identified 11,036 patents, 531 did not match within APDC or had missing data, leaving 10,505 patients with 2,384,218 hospitalisations including 2,282,850 dialysis or daystay admissions. ANZDATA recorded diabetes in 3199 (30.5%) while APDC recorded diabetes in 3086 (29.4%; κ = 0.81) patients. CVD was recorded in 3756 (35.8%) patients in ANZDATA and in 3253 patients in APDC (31.0%; κ=0.46). CLD was recorded in 1564 (14.9%) patients in ANZDATA and in 674 patients in APDC (6.4%; κ=0.28). ANZDATA recorded PVD in 2500 (23.8%) patients while APDC recorded PVD in 548 patients (5.22%; κ=0.09).

Conclusions: Excellent agreement exists between ANZDATA and APDC for the recording of diabetes, good agreement for cardiovascular disease but poor agreement exists for the other co-morbidities tested. These findings have implications for future health care funding models and the ability to use administrative data in clinical risk adjustment tools.

Funding: Private Foundation Support

PUB385

Association between Bacterial Colonization in Tunneled Cuffed Catheter and Residual Renal Function and Mortality in Incident Hemodialysis Patents Jangwon Lee, Sang Heon Song, Ihm Soo Kwak, Soo Bong Lee, Dong Won Lee, Eun Young Seong, Byeong Yun Yang, Il Young Kim. *Dept of Internal Medicine, Pusan National Univ School of Medicine, Busan, Republic of Korea.*

Background: Hemodialysis (HD) patients have compromised immunity that permits bacterial colonization. There are 3 main passages through bacteria can enter the body, that are gut mucosal break, dialysis water, and dialysis access. Influences of the former two on the clinical outcomes of HD patients have long been studied, but there are few reports about the bacterial colonization of tunneled cuffed dual lumen catheter (TCC), mainly used incident HD access.

Methods: Retrospectively we found the clinical data of 155 patients who received TCC removal from Jan. 2005 to Dec. 2012. In Dec. 2013 we collect follow-up data by phone calls which were urine output (UO) at that time and time to anuria or death from HD initiation. Not only the relationship between biochemical, clinical parameters and TCC colonization, but also the survival of kidney and patients according to TCC colonization were assessed.

Results: Of the 155 patients, 33 (21.3%) had bacterial colonization in TCC. Eighty six were followed up by phone calls and 21 of them (24%) were ones in the colonization group(C- group). The rate of decrease in UO was significantly more rapid in the C-group (68.1 ± 75.2 versus 32.9 ± 42.6 mL/month, p=0.012) and the time to anuria was also shorter in the C-group (19.3 ± 18.9 versus 48.6 ± 46.1 months, p=0.011).

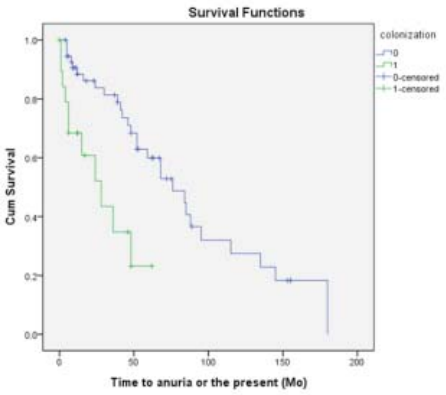


Figure 1. Kaplan-Meier curve showing anuria free survival in incident hemodialysis patients with bacterial colonization of tunneled cuffed dual lumen catheter compared with those without bacterial colonization.

In kidney survival analysis, patients in the C-group had significantly worse estimated anuria-free survival (log rank p=0.001).

Conclusions: Bacterial colonization in TCC was associated with more rapid decrease rate in UO and worse anuria-free survival.

Funding: Private Foundation Support

PUB386

The Establishment of Endtoxin and Bacteria Free Pure Dialysate in South East Asian Developing Countries Tomotaka Naramura,[1,2] Kenichi Kokubo,[1,3] Shunichiro Urabe,[1,3] Moe Kojima,[1,4] Natsumi Abe,[1,5] Emi Kimura,[1,5] Rika Yamanaka,[1,5] Hirokazu Matsubara,[1] Haruki Wakai,[1,5] Sovandy Chan,[6] Sabo Ojano,[6] Fumitaka Nakajima,[1,7] Toru Hyodo.[1] *[1]NGO Ubiquitous Blood Purification International, Yokohama, Japan; [2]Dept of Medical Engineering, Faculty of Health Sciences, Junshin Gakuen Univ, Fukuoka, Japan; [3]School of Allied Sciences, Kitasato Univ, Saamihara, Japan; [4]Clinical Engineering, Tokai Univ Oiso Hospital, Oiso, Japan; [5]Reiseikai Shinagawa Garden Clinic, Tokyo, Japan; [6]Blood Purification Center, Sen Sok International Univ, Phnom Penh, Cambodia; [7]Moriguchi Keijinkai Hospital, Moriguchi, Japan.*

Background: We have reported that dialysis patients and facilities number has been increasing in South East Asian developing countries, but the condition of dialysate was poor [Nephron Extra 2014;4:64-69]. So only low flux dialyzers have been used in dialysis in these countries. In this study, we show the simple method to keep dialysate clean and present that the dialysate purification has been established at 5 dialysis facilities in Myanmar and Cambodia.

Methods: The dialysate survey pertained to the endotoxin(ET) level and bacterial count in tap water, reverse osmosis (RO) system water, dialysate. And we set up ET retentive filters just before dialyzers and continued the dialysate survey for 5 or 6 months in 5 facilities in Myanmar (4) and Cambodia (1).

Results: The ET and bacteria in dialysate were not observed between just after setting up and 5 or 6 months after setting.

			Hosp. A	Hosp. B	Hosp. C	Hosp. D	Hosp. E
At 0 month	Tap Water	ET (EU/mL)	7.09	11.26	18.11	8.94	9.34
		Bacteria (cfu/mL)	48	>300	>300	>300	210
	RO Water	ET (EU/mL)	0.21	0.26	0.71	0.42	0.12
		Bacteria (cfu/mL)	>300	>300	>300	>300	180
Before ETRF setting	Dialysate	ET (EU/mL)	1.40	0.28	0.82	0.37	0.65
		Bacteria (cfu/mL)	>300	>300	>300	>300	>300
Just after ETRF setting	Dialysate	ET (EU/mL)	N.D.	N.D.	N.D.	N.D.	N.D.
		Bacteria (cfu/mL)	N.D.	N.D.	N.D.	N.D.	N.D.
5 or 6 months after ETRF setting	Dialysate	ET (EU/mL)	N.D.	N.D.	N.D.	N.D.	N.D.
		Bacteria (cfu/mL)	N.D.	N.D.	N.D.	N.D.	N.D.

Conclusions: The high-flux dialyzers have been started in Cambodia by this know-how to keep dialysate clean. But more efforts to clean RO water line should be needed.

Funding: Pharmaceutical Company Support - Nipro Co.Ltd., Japan

PUB387

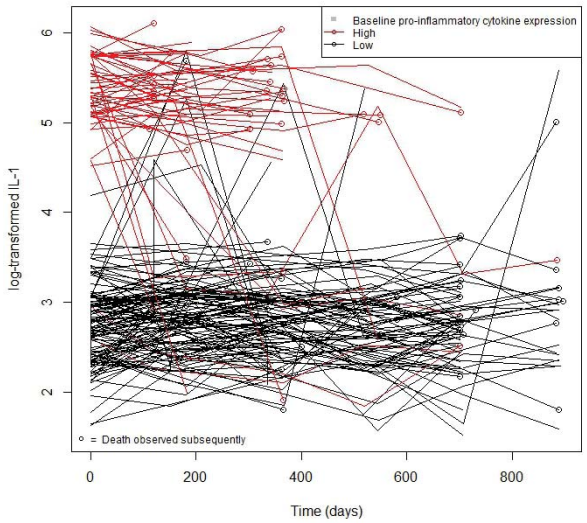
Cytokine Variability in Hemodialysis Patients over Longitudinal Follow-Up

Paul L. Kimmel,[1] Kenneth J. Wilkins,[2] Dawei Xie,[3] J. Richard Landis.[3] *[1]Div of Kidney, Urologic and Hematologic Diseases, NIDDK, NIH; [2]Biostatistics Program, NIDDK, NIH; [3]Dept of Biostatistics and Epidemiology, Univ of Pennsylvania Perelman School of Medicine.*

Background: High baseline levels of circulating pro-inflammatory cytokines have been linked to mortality in several prospective studies of hemodialysis (HD) ESRD patients. Whether cytokine levels in such patients are stable over time is unknown.

Methods: We previously studied interleukin (IL)-1, IL-6 and IL-2 (an anti-inflammatory T-cell mediator) levels in 234 HD patients (75 incident, 159 prevalent), followed for a mean of 3.3 (3.1, 3.3 respectively) years with a mean of 3.9 assessments at 3 centers in Washington, DC. Cytokine levels were skewed, ranging over 6 natural logs. Scatter plots revealed high and low groups for all three cytokines at baseline. This grouping was assessed for each cytokine to have >90% accuracy by established methods (K-means cluster, quadratic discriminant and principal components analyses).

Results: Of the 157 in the low IL-1 group, only 5% exhibited post-baseline values greater than 4 log (54.6 pg/mL). Of the 77 in the high IL-1 group, only 13% similarly exhibited values under 4 log (Figure; no differential misclassification, McNemar's $\chi 2$ p=0.8). A majority's levels remain roughly constant over follow-up, such that 84% of variability (95%CI: 80-87%) is explained by individual mean levels. Similar findings were noted for IL-2 and IL-6.



Conclusions: Cytokine levels exhibit limited variability over time in the majority of HD patients studied. Longitudinal cytokine levels can be characterized as "high" or "low" according to baseline measurements with only modest misclassification rates. Such characterization might be useful in HD patients in designing clinical trials with stratification to enhance event rates. Further study will assess if longitudinal trajectories add prognostic precision.

Funding: NIDDK Support

PUB388

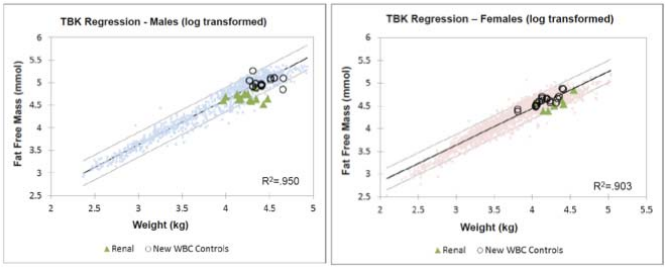
Muscle Wasting in the Emergent Dialysis Population

Biruh Workeneh, Roman Shypailo, William E. Mitch. *Baylor College of Medicine, Houston, TX.*

Background: The care of patients receiving emergent dialysis is a growing public health concern (incident rate estimated to be >6,000/year), and the magnitude of muscle wasting that occurs in this population is not known. The emergent dialysis population is comprised of primarily undocumented individuals who have high rates of employment and productivity, even among ESRD patients, and muscle wasting can affect all aspects of their lives including their socioeconomic status.

Methods: We used a case-control design to determine differences in lean body mass, using total body potassium (K^{40} counter), in emergent dialysis patients and healthy normal subjects. We controlled for 4 variables including age, sex, height and weight and studied 22 subjects matched with controls.

Results: We found that TBK is 18% lower suggesting significant muscle wasting has occurred in the emergent dialysis subjects versus controls (p=.001). Additional analysis found that muscle wasting was more profound among male compared to female subjects (see figure).



Conclusions: We conclude that among other consequences, muscle wasting is a significant concern in the growing emergent dialysis population. This should inform any efforts to rehabilitate these patients and develop uniform policies regarding the management of this vulnerable population.

Funding: Private Foundation Support

PUB389

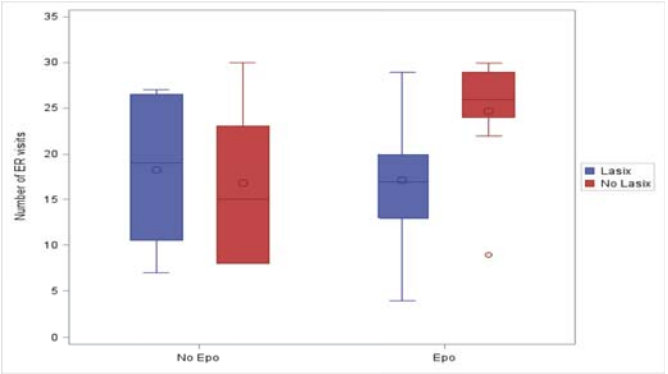
Diuretic Management in Emergent Dialysis Patients

Salman Ahmed, Charles G. Minard, Danielle Guffey, Biruh Workeneh. *Baylor College of Medicine, Houston, TX.*

Background: There are nearly 11 million undocumented immigrants residing in the U.S. and the incidence of ESRD is estimated to be >6,000 /yr. There is no uniform policy regarding the management of these patients, the majority of whom utilize the ER for their dialysis related care. Severe HTN, volume overload and hyperkalemia are commonplace among the emergent dialysis population, and the benefit of diuretic use in this population has never been evaluated.

Methods: We sought to answer whether furosemide use is associated with decreased ER utilization. We reviewed charts of 93 patients at Ben Taub County Hospital in Houston, TX during a 3 month period. We abstracted prescription data, number of ER visits HD sessions and peak potassium values.

Results: The furosemide group had 3.1 (SE=1.8) fewer ER visits compared with the non-furosemide group (p=0.10). We found no significant relationship between furosemide TDD and peak serum K levels. In a multiple regression model, furosemide treated subjects had significantly fewer ER visits after adjusting for EPO and sevelamer use (p=0.007). Among subjects using EPO, patients on furosemide had 7.7 (95% CI: 1.9,13.6) fewer ER visits comparatively (p=0.04) compared with those not on furosemide.



Conclusions: Although our analysis revealed no *independent* association between furosemide dosage and number of ER visits, there was nevertheless a strong trend. We speculate a larger sample size controlling for residual renal function would reveal a significant independent effect of furosemide. The reason for decreased ER utilization among patients on EPO *and* furosemide is unclear, but might be explained by less symptomatology because of EPO. The care of the emergent dialysis population is becoming an increasing public health concern and hospitals systems that cannot offer maintenance dialysis to these patients should explore best practices and consider diuretics as a possible strategy.

Funding: Private Foundation Support

PUB390

Survival on Haemodialysis in Elderly Patients at a Single Center Australian Private Hospital Gavin Lee, Andrew M. Bofinger, Karen Ann Herzig, Rhianna G. Miles. *Nephrology, Greenslopes Private Hospital, Brisbane, Queensland, Australia.*

Background: Increasing numbers of elderly patients with end-stage renal failure are initiated on haemodialysis. This population tends to have more comorbidities and a shorter life expectancy than younger patients on haemodialysis. Our aim was to assess the survival on haemodialysis of patients aged 80 or over at time of dialysis initiation in an Australian private hospital.

Methods: We retrospectively reviewed the records of patients over the age of 80 who were initiated on haemodialysis in a single centre over a 12 year period. Patients were excluded if their duration of haemodialysis was less than 1 month. Data was also collected on cause of renal failure, comorbidities and serum creatinine at time of dialysis initiation.

Results: 82 patients aged 80 or over were commenced on haemodialysis between 1999 and 2010 at Greenslopes Private Hospital in Brisbane, Australia. 52 were aged 80-85 years, 24 were aged 85-90 years and 6 were aged greater than 90 years. The median estimated glomerular filtration rate (eGFR) at commencement of dialysis was 9mL/min/1.73m^2. Median survival on haemodialysis (with 25th and 75th percentiles) was 29.8 (13.0-44.4) months for the whole group, 30.3 (13.9-41.0) months for the 80-85 years group, 26.27 (9.0-43.4) months for the 85-90 years group and 39.28 (19.9-50.5) months for the >90 years group. This is in comparison to the Australian and New Zealand Dialysis and Transplant Registry (ANZDATA) national dialysis survival data of 35.6 (15.6-63.2) months in the 75-85 years age group and 24.4 (8.8-47.6) months in the >85 years age group.

Conclusions: The median survival on dialysis at this centre is comparable to the national data, with particularly extended survival in the patients aged over 90 at time of initiation. Elderly patients can have a reasonable life expectancy on haemodialysis if well selected. Age is not necessarily a contraindication to haemodialysis.

PUB391

Polyoma Viral Replication on Plasma in End - Stage Renal Disease Patients Waiting for Renal Transplantation Mediha Boran,[1] Ertay Boran,[2] Mertay Boran,[3] Hasan Kilic,[4] Ebru Gok Oguz,[5] Derya Yurdakul,[4] Pinar Parlak.[4] *[1]Dept of Nephrology, Hemodialysis and Transplantation, Turkiye Higher Education Hospital, Ankara, Turkey; [2]Dept of Anesthesiology and Reanimation, Duzce Univ School of Medicine, Duzce, Turkey; [3]Dept of Thoracic Surgery, Duzce Univ School of Medicine, Duzce, Turkey; [4]Dept of Microbiology, Turkiye Higher Education Hospital, Ankara, Turkey; [5]Nephrology Dept, Ankara Diskapi Yildirim Beyazit Training and Research Hospital, Ankara, Turkey.*

Background: Progression of BK virus (BKV) infection to BKV-associated nephropathy remains an undesirable because of early graft dysfunction and graft loss in renal transplant recipients. In generally, BKV infection or reactivation has not been searched in pre-transplant hemodialysis (HD) patients.

Methods: In this study, the prevalence of BKV infection on plasma was investigated in 100 HD patients from different hemodialysis units, who were in waiting list as future candidates for renal transplantation and in 25 age- and sex- matched healthy controls. Because these HD patients were without diuresis, only the prevalence of BKV infection on plasma was analysed by quantitative real-time polymerase chain reaction.

Results: A total of 100 HD patients, 41 female (41%) and 59 male (59%), median age 44.7 years (range 23-67), median dialysis age 66.29 months (range 24-132 months) and 25 healthy controls, 10 female(40%) and 15 male (60%), median age 42.84 yrs (range 33-57) were included in the study. BKV replication on plasma of these HD patients and control individuals, was not detected.

Conclusions: We thought that BKV infection in renal transplant recipients maybe due to donor origin and we recommend pre-transplant routine screening for polyoma virus infection in dialysis patients and their living or disease donor at transplantation centres.

PUB392

Influence of Type D Personality on Health Related Quality of Life and Physical Activity in Patients on Dialysis Christopher Susanto,[1] Constantijn Konings.[2] *[1]Dialysis Centre Deurne, Elkerliek Hospital, Helmond, Netherlands; [2]Dialysis Centre Eindhoven, Catharina Hospital, Eindhoven, Netherlands.*

Background: Type D personality, which is defined by both negative affectivity (NA) and social inhibition (SI), has been identified as an independent predictor of health-related quality of life (HRQOL) in patients with cardiovascular disease. An interesting hypothesis is if this relation also exists in patients on dialysis. Besides this, CVD shares several other risk factors with CKD such as lack of physical activity. Aim of this study was to explore the prevalence of type D personality and was to show its influence on HRQOL and physical activity in patients on dialysis.

Methods: 63 patients, who had received maintenance hemodialysis for more than 3 months at outpatient Dialysis Centre Deurne, the Netherlands completed the Dutch version of the DS14 and the kidney disease quality of life instrument (KDQOL-SF™). Physical activity is defined as an accumulation of at least 30 minutes of moderate-intensity physical activity on most—and preferably all—days of the week.

Results: 41 patients were men (65%)and 21 patients were women (35%). The prevalence of type D personality in patients on dialysis was 48% which was indicated by a score≥ 10 on both NA and SI subscales of the DS14. The mean score of the NA and SI scale for all participants with type D personality was 14.7 (SD=3.9) and 14.3 (SD=2.8) respectively. The mean score of NA and SI scale for all participants with non type D personality was 4.7 (SD=5.2) and 9.7 (SD=3.7) respectively. 62% (n=21) of patients with type D personality had lack of physical activity compare with 38% (n=13) of patients with non type D personality (p=0.02). HRQOL in patients with type D personality was lower compared with non type D: 57.5 (SD=11.4) and 65.9 (SD=11.2) respectively (p=0.005).

Conclusions: Type D personality influenced HRQOL and physical activity in patients on dialysis. Future research should address appropriate interventions in order to improve HRQOL and physical activity in patients on dialysis.

PUB393

Emergency Department (ED) and Hospital Admissions in All of New England and New York: A Comparative Study of Fellow (FL) versus Non-Fellow (NF) Managed Hemodialysis Units (HDU) George N. Coritsidis,[1] Marie France R. DeLeon,[1] Jasjit Singh,[1] Aleef M. Rahman,[1] Carol Lyden,[2] Jaya Bhargava,[2] Payam Shakouri.[1] *[1]Nephrology, Icahn School of Medicine at Mount Sinai/EHC, Elmhurst, NY; [2]IPRO.*

Background: Most HDU staff is primarily technicians and nurses supervised by nephrologists. ED visit and hospitalization rates of dialysis patients are important measures of care in end stage renal disease (ESRD) patients. Previously when reviewing New York state data, we found that FL-managed HDU had significantly higher African American patients but without any outcome differences. We were interested to see what impact an academic fellowship program has on outcomes in HDU throughout New England and New York.

Methods: We retrospectively reviewed the de-identified records of all of Networks 1 and 2 (the 7 states) for 2010 regarding demographics, ED visits and hospitalization rates. FL managed HDU were compared to non-fellow managed HDU. Data was corrected for the number of patients per HDU.

Results: There were a total of 46 FL and 342 NF managed HDU. FL-HDU was concentrated in urban settings near their academic medical centers. FL managed HDU had a significantly higher minority (African American and Hispanic) population, but younger with lower diabetes prevalence. History of hypertension, rate of arterio-venous fistula, grafts and catheter were similar in both groups. ED visit/pt, percent admissions from ED and total hospital admissions were similar in both FL and NF managed HDU.

	FL: 7456 Pts	NF: 45335 Pts	P value
Age	59.46	64.11	<0.01
Hispanic %	14.90	12.11	<0.05
African American %	42.29	31.92	<0.01
Minorities %	57.19	44.03	<0.05
DM %	36.10	41.11	<0.05
Catheter > 30 days %	11.66	11.01	ns
ED visits/pt	1.35	1.35	ns
% Hospitalized from ED	43.20	44.10	ns
Total hospitalizations/pt	0.96	0.99	ns

Conclusions: Fellow managed units were comparatively younger with fewer diabetics but a higher minority and urban population. Despite being associated with lower socio-economic status and compliance issues, FL managed HDU had similar outcomes as to hospitalizations and ED visits.

PUB394

Formation of Patient Social Networks in a New Urban Hemodialysis Clinic Avrum Gillespie,[1] Athanasia Polychronopoulou,[2] Sarah Bauerle Bass,[3] Judith R. Greener,[3] Teri Browne,[4] Zoran Obradovic.[2] *[1]Temple Univ School of Medicine; [2]Biomedical Informatics, Temple Univ; [3]Public Health, Temple Univ; [4]Univ of South Carolina College of Social Work.*

Background: Social networks are important in chronic diseases, yet hemodialysis (HD) patient social networks are poorly understood. Building on work that showed the influence of social networks in established clinics, this study characterizes the formation of social networks in a new urban clinic.

Methods: Eighty seven percent of the patients (26/30) admitted to 3 shifts at a new clinic in Philadelphia were enrolled. Exclusion criteria were: in the clinic less than a month and unable to speak English or Spanish at/or above 6th grade level. Patients were surveyed on admission and every 3 months in English (85%) or Spanish (15%). Mean enrollment was 14 months (6-22). Data was analyzed using either an independent t-test or Fischer's exact test.

Results: The mean age was 57.4 (24-80). Patients identified themselves as black (42%), Hispanic (39%), or white (19%). Almost half (47%) had been on HD for less than 6 months. Thirty nine percent of patients identified other patients as part of their social network within 6-9 months of admission. Patients identified the HD staff (58%) and their nephrologist (81%) as part of their social network, usually within a month. Eighty percent of the patients who identified other patients in their network were on the first shift (p=0.04) which had a more consistent seating arrangement (p=0.0001). Spanish speaking patients identified other patients and their doctor but not HD staff as part of their network (p=0.022).

Conclusions: Over a third of HD patients form patient networks within 6 months, most were on the shift with most stable seating. Half of patients identify the staff and 80% their nephrologist in their network usually within 1 month of admission, however, networks continue to form over time. Spanish speaking patients appear not to form networks with staff. Further research is underway to understand the effects of the newly formed hemodialysis patient social networks on adherence outcomes, and transplantation.

Funding: Private Foundation Support

PUB395

Prevalence of Hepatitis C and B in Hemodialysis Patients in United Arab Emirates over Two Decades (One Center Experience) Yousef Boobes, Bassam O. Bernieh, Mohamed Raafat Al Hakim, Ahmed Ahmed Ahmed, Qutaiba Hussain Daoud, Ahmed Chaaban, Hanan Eljack, Mohamed E.O. Ahmed, Imran Khan. *Nephrology/ Medicine, Tawam Hospital, Al Ain, Abu Dhabi, United Arab Emirates.*

Background: Viral hepatitis is a challenging clinical problem in hemodialysis patients of the developing countries in general, and in the Middle East in particular. In order to monitor the size and the trend of this problem, we are reporting the prevalence of hepatitis C and B in our hemodialysis unit for the last 20 years.

Methods: our hemodialysis unit started to function in 1987, with less than ten patients. Since 1993 hepatitis screening is done regularly every three months for all patients. Our laboratory is using the well validated serology kits, and the most updated technics including the PCR. In this study we are reporting the screening results of the years 1993, 2002, and 2014.

Results: in 1993, total number of hemodialysis patients was 25, none of them had HBs Ag (zero prevalence), while ten (40%) patients had anti HCV positive. Action taken was complete isolation of HCV positive patients, in addition to maintaining high adherence to the universal precautions. In 2002, total number of hemodialysis patients was 60, HBs Ag was positive in 1 (1.6%) patient. Eleven patients (18.3%) were anti HCV positive. In March 2014, the total number of hemodialysis patients was 270, six (2.2%) patients had HBs Ag positive, and 26 (9.6%) patients had anti HCV positive. During this 20 years we did not have any single case of seroconversion.

Conclusions: During two decades: 1- the number of hemodialysis patients increased by 11 folds 2-Hepatitis B prevalence remained low and stable all over the time 3- the prevalence of HCV decreased significantly by 4 folds 4- No seroconversion happened during 20 years 5- the exact contribution of isolation policy in addition to the implementation of universal precausions in achieving these excellent results needs further researches.

PUB396

Outcome of ESRD Patients after the Initiation of Emergency Hemodialysis at an Academic Medical Center of a Developing Country Syed Rizwan Bokhari,[1] Hafiz I. Ahmad,[1] Syed A. Khalid,[1] Arif Asif.[2] *[1]Dept of Nephrology, Allama Iqbal Medical College/ Jinnah Hospital, Lahore, Pakistan; [2]Div of Nephrology and Hypertension, Albany Medical College, Albany, NY.*

Background: In developing countries, chronic kidney disease (CKD) patients fail to seek medical attention earlier on and often present to tertiary care hospitals with uremic complications and requiring emergent hemodialysis. Majority of these patients become aware of their CKD during such presentations. The dialysis centers at tertiary care hospitals are overwhelmed with long wait lists. After discharge vast numbers of these patients are unable to receive optimum dialysis treatment and follow up care. We set out to investigate the long-term outcomes of these patients.

Methods: 123 consecutive advanced CKD5 patients admitted for uremic complications over period of 4 months were included in this prospective study. Patients were interviewed according to a standardized questionnaire and were followed via telephone calls monthly for three months, at 6 months and then at 1 year after the initiation of dialysis. Eighteen patients died during the hospital stay due to co-morbid conditions. Thirteen patients at the end of first 3 months and another 8 patients at 6 months were lost to follow up.

Results: 92 patients completed the three-month follow-up, of which 54(58%) were males (mean age of 38±5 years). Forty five (49%) patients were alive at the end of 3 months and only 4 (9%) were getting optimum dialysis. At 6 months of follow-up 22(26%) were alive and at 1 year follow up only 11 (13%) patients were alive. Of the surviving 11 patients, one received renal transplantation, another one initiated CAPD and the remaining 9 patients were maintained on twice-weekly hemodialysis.

Conclusions: Only a small number (<15%) of ESRD patients who initiated hemodialysis on an emergent basis were alive at the end of 1 year and those who had survived were receiving suboptimal treatment. These disturbing outcomes call for a multifaceted comprehensive planning with appropriate funding required to improve the early detection, long-term care and provision of regular and chronic renal replacement therapy.

PUB397

Analysis of Rate, Cause and Cost of Hospitalization of Maintenance Hemodialysis Patients: A Single Center Study Yuan Luo, Hong Ye. *Center for Kidney Disease, Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

Background: Chronic kidney disease (CKD) has become one of leading public health problems nowadays. It was reported recently that the prevalence of CKD is up to 10.8% in China. Most of the CKD patients will inevitably progress to end stage renal disease (ESRD) and need renal replacement therapy, such as hemodialysis. The hospitalization rate of dialysis patients is increasing which will consequently bring heavy economic burden to society. The aim of this study was to analysis the rate, cause and cost of hospitalization of maintenance hemodialysis patients in our dialysis center.

Methods: Patients underwent maintenance hemodialysis in the blood purification center of our hospital were enrolled in this study. The frequency and period of hospitalization, as well as the cause and cost of each hospitalization of these patients from January 2011 to December 2013 were collected and analyzed.

Results: The annual dialysis frequency in our center is more than 900 times. From 2011 to 2013, 252, 285 and 368 patients were enrolled each year. More than 60% were male and most of them were at their age of 50-69 years. Half of these patients had their dialysis ages more than 60 months. Among them, the rates of hospitalization were 464.29, 596.49 and 635.87 per 1000 patients, respectively. Patients who were over eighty-year-old or had dialysis ages of 0-12 months had the highest hospitalization rates. The top three causes of hospitalization were infection, cardio- and cerebro-vascular diseases and vascular access dysfunction. Infection caused 25% of hospitalization, among which 70% were respiratory infection. The hospitalization periods were relevant to the causes. About 35% of the total hospitalization costs were used to treat infectious diseases.

Conclusions: The annual hospitalization rate of maintenance hemodialysis patients in our center has being increased since 2011. Over eighty-year-old and one-year dialysis age patients had higher hospitalization rates. Infection was the most popular cause of hospitalization in our dialysis center, which was also the major cost. This study might help improving clinical care of maintenance hemodialysis patients and reducing medical expenditure.

PUB398

Introspective Examination of Quality of Life in Haemodialysis Patients Daniel J.W. Jones,[1] Laurie T. Butler,[1] John P. Harris,[1] Emma C. Vaux.[2] *[1]School of Psychology and Clinical Language Sciences, Univ of Reading, Reading, United Kingdom; [2]Renal, Royal Berkshire Foundation Trust, Reading, United Kingdom.*

Background: Haemodialysis (HD) is an all-encompassing treatment affecting patients' psychological/emotional well-being, as well as their physical state. This is reflected in the high prevalence of depression amongst patients. Moreover, the rate of depression is found to increase after more than a year living with HD, suggesting that it is more likely to be associated with the treatment process rather than ESRD itself. At present,the literature is limited in its attempt to examine the HD patient experience holistically, from a patient perspective, especially in terms of mental and emotional aspects of living with ESRD and HD.

Methods: We interviewed 20 patients receiving HD three times per week for 3-5 hours per treatment (all had been receiving HD >90days, with Kt/V >1.4). Semi-structured interviews were conducted at the bedside during a patient's HD. A topic guide consisting of 12 open-ended questions was used to direct the discussion: topics included mood, memory and pre/post-dialysis well-being. Elaboration was encouraged to allow patients to express their own personal concerns. Interviews were recorded and transcribed verbatim. Thematic analysis was conducted using ATLAS.ti software.

Results: Four distinct themes emerged from the data, reflecting common stressors associated with HD: fluctuations in mental/physical well-being across the dialysis cycle, restrictions due to treatment schedule, impact of the disease/treatment on relationships and overall emotional reactions to treatment. Findings suggest that identifying and addressing such areas will contribute to better patient welfare and improved quality of life.

Conclusions: This study is the first to provide a detailed understanding of HD patients' personal experiences, which can, in part, help guide treating clinicians and healthcare managers on the negative psychological/physical consequences of HD. The data suggests that a holistic approach is necessary to provide the optimal care for all aspects of patients' lives. Future guidelines need to take this into account to enhance patients' overall quality of life and improve current patient mortality rates.

Funding: Government Support - Non-U.S.

PUB399

Haptoglobin 1-1 Genotype Is Associated with Lower Depression Rate in Hemodialysis Patients Zaher Armaly,[1] Kamal Hassan,[2] Amir Abd Elkadir,[1] Adel Rafik Jabbour,[1] Rawi Ramadan,[3] Bishara Shafik Bisharat.[1] *[1]Nephrology, The Nazareth Hospital-EMMS, Nazareth, Israel; [2]Nephrology, Western Galilee Hospital, Nahariya, Israel; [3]Nephrology, Rambam Health Campus, Haifa, Israel.*

Background: Depression is a common psychological disorder in hemodialysis (HD) patients. HD modality drastically and negatively affects life expectancy and quality of life at both the physical and psychological levels. This is largely attributed to stressors, including medication effects, dietary constraints, fear of death, and dependency upon treatment. Haptoglobin (Hp) genotype (Hp 1-1, 1-2, or 2-2) is associated with risk for cardiovascular

diseases, but its relationship with depression, a growing concern in HD, has not been studied yet. Therefore, the present study examines whether Hp genotype influences the prevalence of depression among the Arab population undergoing HD in Nazareth, Israel.

Methods: The study that included 54 patients in the HD unit with a mean age of 61.9 ± 14.1 years who had undergone HD and 26 relative healthy control subjects with a mean age of 59.3 ± 7.3 years. Beck's Depression Inventory and Hamilton Depression Scale assessments were administered. Blood analysis for Hp genotype was performed. Diagnosis was made using the Diagnostic and Statistical Manual of Mental Disorders scale to correlate depression with Hp genotype.

Results: The prevalence of depression was 35.2% in HD patients. 17% of HD patients were Hp 1-1, 28% Hp 2-1, and 55% Hp 2-2. Hp 1-1 genotype was more prevalent in non-depressed HD patients as compared with depressed HD subjects (23% versus 11%). Similarly, Hp 2-2 genotype was observed in 60.0% of non-depressed HD as compared with 42% in depressed HD patients. In contrast, most of the depressed HD patients were Hp 2-1 carriers as compared with non-depressant HD subjects (47% versus 17%).

Conclusions: Depression is highly prevalent in Arab patients undergoing HD in Nazareth area. Hp Arab patients who carry Hp 2-1 genotype may be at risk to develop depression during HD treatment. In contrast, Hp 1-1 HD patients have a lower prevalence of depression. The mechanisms underlying this phenomenon remain to be explored.

PUB400

Hazard Perception during Driving in Renal Patients Daniel J.W. Jones,[1] Laurie T. Butler,[1] John P. Harris,[1] Emma C. Vaux.[2] *[1]School of Psychology and Clinical Language Sciences, Univ of Reading, Reading, United Kingdom; [2]Renal, Royal Berkshire NHS Foundation Trust, Reading, United Kingdom.*

Background: Lab based studies have found ESRD patients to be impaired in areas of attention, psychomotor speed and executive functioning. However, exactly how such impairments affect patients outside of the lab is still unknown. An area that seems likely to demonstrate the kind of impairments observed in the lab is driving a car; a cognitively demanding task that requires several cognitive processes working in harmony. Areas such as attention, motor control and psychomotor speed are essential for driving, and have all been shown to be impaired independently in the lab. This being said, driving as a whole has not been examined with the same level of scrutiny. Currently, the effects of ESRD on driving ability are simply unknown, and consequently no guidelines are in place.

Methods: We examined one aspect of driving: hazard perception. 24 ESRD patients (Kt/V>1.4) and 24 age, sex and education-matched controls completed the Official UK DSA Hazard Perception task. It involves viewing a series of driving video clips (driver's perspective) on a video monitor. Each clip contains a hazard the participant should respond to within a set response window by clicking the mouse; scores range from five, for responding immediately, to zero, for responding outside of the response window or not at all.

Results: Conventional hazard perception scores did not differ between groups, possibly due to the learning gradient associated with the test. Results analysed in terms of detection rate (hits versus misses) found patients to be significantly worse than controls.

Conclusions: Findings suggest ESRD patients may be processing the hazards in a qualitatively different way to controls and that their hazard perception may be impaired. Due to the complexities of the task we are unable to conclude which cognitive aspects may be contributing to the impairment. Nevertheless, this study does highlight the possibility that patients may be impaired when driving, and are potentially putting themselves, and others, inunnecessary danger. Studies such as this highlight the need for further examination to establish standardised driving guidelines for this population.

Funding: Government Support - Non-U.S.

PUB401

Efficacy of Early Examination of Adequacy of Dialysis Compare with Regular Examination in Korean CAPD Patient Joong Kyung Kim, Jin Ho Lee, Seong Han Yun, Joon Seok Oh, Seong Min Kim, Yong Hun Sin. *Div of Nephrology, Dept of Internal Medicine, BongSeng Hospital, Busan, Korea.*

Background: Examination of adequacy of dialysis(especially, weekly KT/V) is important for initiation and maintaining of continuous ambulatory peritoneal dialysis(CAPD). Generally, because of inaccuracy of the test that was performed immediately after catheter insertion surgery, most of the test was performed 3 months after operation. But, if accuracy of early test was high, it could increase the compliance of patients and reduce costs.

Methods: From June 6, 2007 to October 13, 2013, 100 patients was enrolled in single center of Korea. We retrospectively compared the weekly KT/V at early test and follow up test(mean 16.2days and 516.4days after initiation of CAPD). p value under 0.05 were considered statistically significant.

Results: Female patients were more than male (62 versus 38) and mean age was 53.73 years old. Early test was significantly correlated with regular follow up test in every CAPD patients(2.96 ± 0.95 versus 2.75 ± 0.76, p<0.05). Especially early test and follow up test has statistical significance in DM patients(3.04 ± 0.62 versus 2.59 ± 0.69, p<0.05). But there was no statistical significance in non DM patients(2.89 ± 1.11 versus 2.88 ± 0.8, p = 0.35).

Sex(male/female)	38/62
Age(years)	53.73
Diabetes/non diabetes	36/64
Early weekly KT/V(all patients, 13.8 days after surgery)	2.96 ± 0.95
Follow up KT/V(all patients, 515.9 days after surgery)	2.75 ± 0.76
Early weekly KT/V(DM patients, 19.5 days after surgery)	3.04 ± 0.62
Follow up KT/V(DM patients, 477 days after surgery)	2.59 ± 0.69
Early weekly KT/V(DM patients, 13.8 days after surgery)	2.89 ± 1.11
Follow up KT/V(DM patients, 515.9 days after surgery)	2.88 ± 0.8

Table 1. Characteristics of the Patients(n=100)

Conclusions: Early examination of weekly KT/V in CAPD patients is effective and increase the compliance of patients and reduce costs.

PUB402

Predictors of Life Quality on Dialysis: A Cross-Sectional Study in a Northeastern Brazil Center Douglas Rafanelle Moura de Santana Motta, Kleyton Andrade Bastos. *Medicine, Federal Univ of Sergipe, Aracaju, Sergipe, Brazil.*

Background: Discuss quality of life has gained increasing importance in a scenario of increasing prevalence of patients on dialysis worldwide. The objective of the study is assessing quality of life in dialysis patients, seeking to identify demographic, social, clinical and laboratory predictors.

Methods: This is a cross-sectional study. 356 patients were selected from the largest dialysis center in Aracaju, city located in northeast of Brazil. Socio- demographic and laboratory data were obtained from medical records and supplemented directly with patients at the time of interview. Questionnaires to assess quality of life (KDQOL- SF), depression and anxiety (HADS), sleep disorders (Epworth and Pittsburgh) and sexual dysfunction (FSFI and IIEF) were used.

Results: 273 patients completed the study, 215 on hemodialysis (78.8%) and 58 on peritoneal dyalisis (21.2%). The average age was 50.9 years with a male predominance (61.9 %). Most of the population has low levels of education and economic status. When comparing the 2 main components of KDQOL, we see a worse involvement of the SF -36 (median 61.0) compared to the KDCS (median 69.8). There was no significant difference between dialysis methods, hemodialysis and peritoneal dialysis. In a multivariate multiple regression analysis, we found as independent predictors the presence of anxiety and depression for 2 subscales of KDQOL- SF (KDCS: adjusted R 0.49 $p < 0.001$ and SF-36: adjusted R 0.53 $p < 0.001$). Also in the SF -36, we observed as predictors age and the presence of erectile dysfunction.

Conclusions: No significant difference was found in quality of life between hemodialysis and peritoneal dialysis. The main predictors of life quality were the presence of anxiety disorder and depression. New strategies to approach these two pathologies are necessary.

PUB403

Prevalence of Anti HBc and Occult HBV Infection in Hemodialysis Patients Bassam O. Bernieh,[1] Ahmed Ahmed Ahmed,[1] Waheed Uz Zaman Tariq,[2] Yousef Boobes,[1] Mohamed Raafat Al Hakim,[1] Qutaiba Hussain Daoud.[1] *[1]Nephrology / Medicine, Tawam Hospital in Affiliation with Johns Hopkins Medicine, Al Ain, Abu Dhabi, United Arab Emirates; [2]Laboratory Medicine, Tawam Hospital in Affiliation with Johns Hopkins Medicine, Al Ain, Abu Dhabi, United Arab Emirates.*

Background: The prevalence of HBV infection is low (2.2%) among our hemodialysis (HD) patients. On the other hand we observed out of proportion high prevalence of anti HBc positivity among these patients. This led us to investigate further and look for the presence of occult HBV infection, and to compare the prevalence of anti HBc positive in (HD) with its prevalence in healthy subjects.

Methods: Hepatitis serologyis mandatory for all (HD) patients. In addition to the adherence to the stander precautions measures, we are adopting an isolation policy for both HBV and HCV infected patients. In January 2014, we did HBV DNA PCR for all (HD) patients with HBs Ag negative and anti HBc positive, for possible occult HBV. In addition we did random HBV DNA for other (HD) group. We compared the prevalence of HBs Ag, anti HBc positive, and anti HCV positve in our (HD) patients, with healthy people using the results of blood donors of the month of January 2014.

Results: Total number of (HD) patients screened was 270, total number of control healthy group (blood donors) was 1422 subjects. HBs Ag positive was found in six (2.2%) HD patients, and 3 (0.2%) of the control (p=0.000). 66 (25%) of HD, had HBs Ag negative with anti HBc positive Compared to 35 (2.5%) of the control (P=0.000). HBV DNA detected in one (1.5%) of the (HD) anti HBc positive, compared to zero in the control, and other (HD) groups. Anti HCV positive found in 26 (9.6%) of the (HD), and 2 (0.14%) in the control (p= 0.000). 9 (35%) of the anti HCV positive had anti HBc positive, none of them had HBV DNA detected.

Conclusions: 1- Anti HBc is highly prevalent in our HD patients 2- occult HBV infection detected in one patient with anti HBc positive 3- anti HCV positve is associated

with higher anti HBc positivity 4- the highly significant prevalence of anti HBc in (HD) group compared to healthy group raises the possibility of causal relationship between HBV exposure and CKD which requires further investigations.

PUB404

Multidimensional Frailty in Older Veterans with Advanced Chronic Kidney Disease Katharine L. Cheung,[1] Nicky M. Quinlan,[2] Vj Periyakoil,[2,5] Manjula Kurella Tamura.[3,4] *[1]Nephrology, Univ of Vermont College of Medicine, Burlington, VT; [2]Palliative Care and Hospice Medicine, Stanford Univ School of Medicine, Palo Alto, CA; [3]Nephrology, Stanford Univ School of Medicine, Palo Alto, CA; [4]Geriatric Research and Education Clinical Center, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; [5]General Medicine Disciplines, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA.*

Background: Frailty is common in patients with chronic kidney disease (CKD) and is correlated with poor outcomes. While the concept of frailty is used to describe physical characteristics (weakness, low activity, weight loss, slow walking speed and exhaustion), the emotional, psychosocial and cognitive effects of frailty are unknown.

Methods: Nineteen older adults with CKD with eGFR <20ml/min/1.73m^2, were enrolled between February and June 2013 at a large academic VA Hospital and followed for one year. Patients completed the following tests: SF-36, Mini-Cog, Clock-in-the-box test (CIB), Palliative Grief and Depression Scale (PGDS), Flourishing Scale, Barthel Index for ADL, Lawton IADL scale and Short physical performance battery (SPPB).

Results: Participants were male, age 75.4±6.5 years with low eGFR (12.8±3.1ml/min/1.73m^2) not yet on dialysis, were independent and primarily living at home (16/19). They demonstrated impaired physical functioning (SPPB score 7.8±2.2/12, range 5-12), memory (3 word recall 1.8±1.2, range 0-3) and executive function (CIB score 5.2±2.4/10, range 0-8). The PGDS subscores for depression, restorative oriented grief and loss oriented grief were 1.1 ± 2.4/10, 4±1.1/5, and 1.6±1.8/5, respectively. The flourishing score was 46.1±6.5/56, range 32-56. At one year, eight started dialysis (1 PD, 7 HD), ten were hospitalized, and four died (three withdrew from dialysis and one patient did not start dialysis.)

Conclusions: Older veterans with advanced CKD were found to be frail, had cognitive and functional impairment. Our subjects had intense preparatory grief about their loss of kidney function. More work is needed to better characterize multimorbidity in older adults with CKD as such data may improve informed decision-making and prognostication.

PUB405

Designing a Positive Discussion Environment Where Dialysis Patient and Supporters Support Each Other Atsuhiko Kiyota,[1] Ryoko Hanada.[2] *[1]Dialysis Unit, Kiyota Clinic, Matsubara City, Osaka Pref., Japan; [2]Dept of Psychology, Tokyo Woman's Christian Univ, Suginami-ku, Tokyo, Japan.*

Background: In order to reduce psychosocial burden caused by chronic diseases such as renal failure, we built a program to promote positive discussion that enables supporting of each other in the daily lives by a community consisting of three parties, patients, their family and the medical staff (physician, nurse etc.) serving them. We have assigned a "psychological counselor" as the key person that ties the three parties in an organic way in our research.

Methods: Objects are 11 cases. We have decided to develop a method where the psychological counselor listens to the patient's discussion during a dialysis treatment and analyzes the problematic patterns and avoid them to have more positive discussions.

Results: Following are the problematic patterns that were identified. 1. Organic symptoms and negative "feelings" (such as not wanting to undergo dialysis) were mixed, making it difficult for the staff to understand their complaints and to address them. 2. When the patient expresses his/her "feeling", they were unable to verbalize them well making the staff to misunderstand them as monster patient. By having the psychological counselor explaining that such problematic patterns exist, the community consisting of patient-family-medical staff was able to promote positive discussion among each other and as the result, the psychosocial burden caused by the disease was reduced.

Conclusions: Through this research, we tried to understand the patients' "feelings" in an empathic way with the support of the psychological counselor. With this, the patients felt that their existence was respected and as the result, they would re-identified "their uniqueness" enabling the positive and good communication environment among patient-family-medical staff.

PUB406

Renal Ultrasound and Other Procedural Experience of U.S. Adult Nephrology Fellows Mala Sachdeva, Daniel W. Ross, Hitesh H. Shah. *Medicine, Div of Nephrology; North Shore-LIJ Health System, Great Neck, NY.*

Background: Procedures are a key component to nephrology fellowship training. Current renal ultrasound (R-US) and other procedural experience of U.S. adult nephrology fellows is not known. In addition, it is uncertain if nephrology fellows undergo formal training in R-US during fellowship. To gain a greater insight into nephrology fellows' experience regarding R-US and other nephrology procedures, we conducted this study.

Methods: An anonymous online survey was electronically mailed to U.S. adult nephrology fellows through nephrology training program directors and coordinators in May 2014.

Results: So far, 125 nephrology fellows have responded to the survey (14% response rate). Half of the respondents were first-year fellows. 41% were graduating fellows. 34% of the respondents were U.S. medical graduates. 6% of the respondents had formal R-US training during residency. 83% of the respondent's fellowship program did not offer formal clinical training in R-US during fellowship. Only 34% had formal didactic R-US experience during fellowship. 64% of the respondents described their ability to identify normal anatomy on R-US as either fair or poor. Over three-fourths (78%) of the respondents described their ability to identify pathological findings on R-US as either poor or fair. Majority (96%) of the respondents felt that fellowship programs should offer a formal training in R-US. Nearly one-fifth plan to undergo R-US training outside their fellowship programs. None except one respondent had placed peritoneal dialysis catheters during fellowship training. While 28% of the respondents had placed <5 temporary femoral hemodialysis (HD) catheters, another 30% had placed none. In addition, 30% fellows had not placed a temporary internal jugular HD catheter. R-US guided native or transplant kidney biopsies were more commonly performed than either CT guided or blind technique kidney biopsies.

Conclusions: Most fellowship programs did not offer formal clinical training in R-US, although fellows believe this should be an important part of their curriculum. Measures to enhance R-US and other procedural experience of nephrology fellows should be considered by the training community.

PUB407

Development of a Telenephrology Chronic Kidney Disease Education Class Lucile Parker Gregg,[2] Raimund H. Pichler,[1,2] Bessie A. Young.[1,2] *[1]Medicine/Div of Nephrology, Veterans Affairs Puget Sound Health Care System, Seattle, WA; [2]Medicine, Univ of Washington, Seattle, WA.*

Background: CKD education has been shown to improve outcomes for patients approaching the need for renal replacement thereapy (RRT). Barriers to education of rural patients include lack of awareness of kidney disease and lack of nephrologist in rural areas prior to the need for RRT. In response to that need, we developed a telemedicine CKD education class for Veterans in the rural Pacific Northwest. In order to determine barriers to participating, we developed a patient survey that will be used in both pre and post assessment of patients asked to participate in the class.

Methods: As part of a quality improvement project, we developed a survey to determine barriers to participation in a telenephrology CKD education class. CKD classes were designed to inform patients about dialysis modalities and transplant options prior to the need for dialysis. A literature search was performed to determine barriers to CKD education and summarized. Questions were developed and refined based on the literature and reviewed by the authors for content and completeness.

Results: A one page questionnaire was developed. Needs assessment questions included: How many miles do you live from the VA; Do you have access to a computer; Do you use MyHealtheVe (secure messaging)t if necessary; What are your barriers to participation in a class:, would you be willing to attend class telecast from a regional VA center; Would you be willing to attended class by telecast from your personal computer at home. Surveys will be piloted with patients who participate both on-site and distance CKD education classes. Patients will be queried as to the ease of participation and surveys will be sent using either mail or Veterans Affairs secure email, MyHealtheVet.

Conclusions: Telenephrology is potentially a novel way to educate rural Veterans about CKD and RRT. A pilot study will be conducted to determine feasibility of either at home or off site education via telemedicine modality. Results will be used to further refine the CKD-distance education class, which could be a model for long-distance CKD education across the country.

Funding: Veterans Administration Support

PUB408

Extracellular Matrix Regulates Polycystin Localization and Function in *Caenorhabditis elegans* Deanna M. De Vore, Maureen M. Barr. *Cell and Developmental Biology, Rutgers Univ, Piscataway, NJ.*

Background: PKD2 encodes a transient receptor potential polycystin (TRPP) channel receptor protein found in primary cilia of mammalian cells and sensory cilia of *C. elegans* neurons. In humans, PKD2 mutations result in Autosomal Dominant Polycystic Kidney Disease (ADPKD). Given the ancient and evolutionarily conserved role for polycystin-2 in cilia, we are using *C. elegans* as a model to identify new genes required for ciliary receptor localization. We recently found that extracellular matrix components are important for *C. elegans* PKD2 localization and function. ECM formation, secretion, and integrity have been shown to be a primary factor in ADPKD (Mangos 2010). Transmembrane and ECM proteins such as cadherins and galectins act in cilium retraction and elongation also play a role in cell-to-cell junction signaling via the ECM which is important since ADPKD is a ciliopathy (Seeger 2012, Rondanino 2011). *mec-1*, *mec-5*, and *mec-9* encode ECM components that play a role in mechanosensation and degenerin/epithelial sodium channel (DEG/ENaCs) localization (Du 1996, Emtage 2004). mec-1 and mec-9 contain multiple EGF and Kunitz domains; mec-5 is a collagen (Du1996, Emtage 2004). I hypothesize that these ECM proteins interact physically and physiologically with the polycystins and affect their localization and/or function.

Methods: I have tested this by constructing markers to visualize EMC protein location and proximity to LOV-1(PC1) and PKD-2(PC2) in C. elegans males to show direct or indirect interaction of polycystins and ECM proteins. Male mating assays are also being performed to determine the effect of ECM on *C. elegans'* polycystin protein activity.

Results: *mec-1, mec-5* and *mec-9* regulate PKD-2::GFP localization and polycystin-regulated male mating behaviors.

Conclusions: These studies, which link polycystins localization and activity with ECM proteins in *C. elegans,* will shed light on how sensory receptors like PKD-2 are targeted to cilia and may advance the understanding and treatment of ADPKD.

Funding: NIDDK Support

PUB409

Simvastatin Suppresses Synthesis of Fibronectin in Fibroblasts via WNT10A Expression Akihiro Kuma,[1] Hiromichi Ueno,[1] Yoko Fujimoto,[1] Nana Ishimatsu,[1] Tetsu Miyamoto,[1] Kenichiro Bando,[2] Ryota Serino,[2] Masahito Tamura.[2] *[1]Second Dept of Internal Medicine, Univ of Occupational and Environmental Health, Kitakyushu, Japan; [2]Kidney Center, Univ Hospital of Occupational and Environmental Health, Kitakyushu, Japan.*

Background: In vivo studies have shown that statins suppress kidney fibrosis, which if left untreated can lead to renal failure. We previously reported that WNT10A expression in the kidney tissue of acute interstitial nephritis patients is related to a lower estimated glomerular filtration rate and fibrosis. Here, we investigated the effects of the simvastatin against WNT10A-expressing fibroblasts.

Methods: COS1 cells (kidney fibroblasts from the African green monkey) were transfected with a WNT10A expression plasmid (COS1-10A). Western blot analysis confirmed the expression of extracellular matrix components and apoptosis-related proteins, and cells were counted by trypan blue staining. Cell cycle was comfirmed by flowcytometry.

Results: COS1-10A enhanced the expression of fibronectin about 3- to 5-fold compared with controls, but did not alter the expression of type I or type III collagen. In contrast to normal medium, supernatant from COS1-10A cells induced fibronectin expression in the controls, and WNT10A overexpression promoted cell growth. Conversely, simvastatin suppressed cell growth in COS1-10A cells ($p<0.005$). Western blot analysis revealed that simvastatin induced apoptosis of the controls and that COS1-10A cells expressed cleaved caspase-3 and poly ADP ribose polymerase. And, the population of sub-G1 in COS1-10A cells treated with simvastatin was 25%, but one of sub-G1 in COS1-10A cells without simvastatin was 7%. Furthermore, simvastatin also suppressed WNT10A-dependent expression of fibronectin.

Conclusions: As WNT10A enhanced the synthesis of fibronectin in COS1 cells, WNT10A expression in kidney tissue may promote kidney fibrosis. Conversely, simvastatin suppressed both fibronectin expression and cell growth. Although simvastatin did not show specific WNT10A expression in COS1 cells, it might have the ability to prevent kidney fibrosis.

PUB410

miR-146a Attenuates TGF-β_1 Induced Epithelial-Mesenchymal Transition in Renal Tubular Cells by Targeting smad4 Yoshiyuki Morishita, Shigeaki Muto, Daisuke Nagata. *Div of Nephrology, Dept of Internal Medicine, Jichi Medical Univ, Shimotsuke city, Tochugi, Japan.*

Background: The epithelial-mesenchymal transition (EMT) of renal epithelial cells plays pivotal roles in the development of renal fibrosis. microRNA (miR) has been recognized to interact with multiple mRNAs which are involved in the EMT process and as a potential target for the therapy of renal fibrosis; however, not all members of miR in EMT of renal epithelial cells have been identified. In the present study, we identified miR-146a attenuates TGFβ_1-induced EMT in renal tubular cells by targeting smad4.

Methods: The cultured HK-2 cells (human renal proximal tubular cells) were induced EMT by stimulating with 1ng/ ml TGF-β_1 for 24 hours. In some cases, miR-146a mimic or control oligo transfected HK-2 cells by lipofection were stimulated with 1ng/ ml TGF-β_1. Then, the changes of epithelial cell markers (E-cadherin and occludin) and mesenchymal cell markers (fibronectin-1 and vimentin) were investigated by qRT-PCR and/or western blotting analysis. The morphological change of cells was evaluated by phase contrast microscopy. Furthermore, the cell invasion ability was evaluated by wound healing assay.

Results: The epithelial cell markers were significantly decreased and mesenchymal cell markers were increased in HK-2 cells by TGF-β_1 stimulation. The morphology was changed from a cobblestone-like monolayer to spindle-shaped fibroblast-like, and the invasion ability was significantly increased in HK-2 cells by TGF-β_1 stimulation. Overexpression of miR-146a significantly attenuated these changes of cell phenotypic markers, morphology and invasion ability induced by TGF-β_1 stimulation whereas control oligo transfection did not show these effects. In addition, asmad4 expression was increased during EMT process induced by TGF-β_1 stimulation, and it was significantly inhibited by overexpression of miR-146a in HK-2 cells.

Conclusions: miR-146a attenuates TGFβ_1-induced EMT in renal tubular cells by targeting smad4. It may be a potential good therapeutic target of renal fibrosis.

Funding: Government Support - Non-U.S.

PUB411

Reversal of Epithelial to Mesenchymal Transition following Relief of Unilateral Ureteral Obstruction in the Rat Nan Shen. *Dept of Nephrology, The First Affiliated Hospital of Dalian Medical Univ, Dalian, Liaoning, China.*

Background: Renal fibrosis begins with renal tubular epithelial mesenchymal transition (EMT); the progression thereafter depends upon a number of fibrotic factors. Mesenchymal–epithelial transition (MET) is the reverse process of EMT.

Methods: Unilateral ureteral obstruction (UUO) is a well-described model of EMT. We used an improved reversible unilateral ureteral obstruction (RUUO) model to investigate whether a progressive renal injury model of EMT could be reversed to MET after relief of UUO in rats. Rats were subjected to UUO or sham operation and the obstruction was removed five days later. Rats developed EMT after reversal of 4, 8 and 12 weeks of ureteral obstruction as assessed by the expressions of fibrotic factors in this post-obstructive model.

Results: We found a significant decrease in the kidney weight and renal cortical thickness in the UUO group compared with the sham groups. This rise in the RUUO group was significantly reduced. In addition, UUO rats exhibited pronounced inflammatory and intrinsic proliferative cellular responses, and ultimately fibrosis. By comparison, RUUO mice had more controlled and measured extrinsic and intrinsic responses to EMT with a return to MET within several weeks after release of ureteral obstruction.

Conclusions: Our findings provide a model that allows investigation of the fibrotic factors during reversal of EMT that contribute to the development of fibrosis.

Funding: Private Foundation Support

PUB412

Automated Quantification in 2 Renal Interstitial Fibrosis Models with Sirius Red and Polarization Contrast Microscopy Jonathan Street, Ana C. Souza, Alejandro Alvarez-Prats, Taro Horino, Xuzhen Hu, Peter S.T. Yuen, Robert A. Star. *NIDDK, Bethesda, MD.*

Background: Kidney interstitial fibrosis is the final common pathway in chronic renal diseases. Improved methods for precisely quantifying the anatomic location of fibrosis are needed. Kidney fibrosis is commonly assessed by histology. The Masson trichrome stain is widely used, with semi-quantitative scores assigned by trained operators. Each operator may select and score fields differently, leading to subjective scoring. We have developed an objective technique combining Sirius Red staining, polarization contrast microscopy, and automated image segmentation and analysis.

Methods: Entire kidney cross-sections stained with Sirius Red were imaged using polarization contrast microscopy. Automated image segmentation identified the interstitial and perivascular regions and quantified the interstitial fibrosis. We compared the newly developed method with scoring of adjacent Masson trichrome stained sections in two kidney fibrosis models in CD-1 mice: 1) folic acid (FA, 250 mg/kg), and 2) unilateral ureteral obstruction (UUO).

Results: Repeated analysis of the same FA sections by the same operator ($r=0.99$) or by different operators ($r=0.98$) was highly consistent for Sirius Red, while Masson trichrome performed less consistently ($r=0.61$ and $r=0.72$ respectively). Both techniques performed equally well when comparing sections from left and right kidneys. Targeting interstitial fibrosis (via perivascular exclusion) improved Sirius Red reproducibility and agreement with Masson trichrome from $r=0.32$ to $r=0.41$ in the FA model. Agreement was similar in the UUO model ($r=0.49$). The greater specificity of Sirius Red, for collagen type I and III fibrils, may partly explain the differences between these techniques.

Conclusions: Combining whole section imaging and automated image segmentation and analysis with Sirius Red/polarization contrast is a rapid, reproducible, and precise technique that complements Masson trichrome. It also prevents biased selection of fields, as interstitial fibrosis is measured on the entire kidney cross-section. This new tool should enhance the search for novel therapeutics and non-invasive biomarkers for interstitial fibrosis.

Funding: NIDDK Support

PUB413

Establishment of a Novel Method for Longitudinal GFR Measurement in the Conscious 5/6 Nephrectomized Rat Damian C. Matera, Paul Harrison, Kathleen A. Lincoln, Hongxing Chen, Susan Goldrick, Kristina Gueneva-Boucheva, Nicholas F. Brown, Hu Sheng Qian, Carine Boustany, Glenn A. Reinhart. *Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT.*

Background: The subtotal nephrectomy (5/6 Nx) rat model exhibits many features of chronic kidney disease (CKD) including hypertension, decline in renal function, glomerulosclerosis (GS) and tubulo-interstitial fibrosis (TIF). We thus sought to characterize this model as a tool for screening novel CKD therapies. Furthermore, we sought to develop a method for assessing true glomerular filtration rate (GFR) over time in the conscious state.

Methods: Rats underwent a 5/6 Nx surgery (removal of the right kidney + occlusion of 2/3 of the renal arteries of the left kidney) and were implanted with telemetry transmitters for continuous recording of mean arterial pressure (MAP). 4 weeks post 5/6 Nx, rats received one of 3 doses of losartan (10, 30, 100 mg/kg in drinking water) or vehicle. 8 weeks post surgery, a subset of rats was implanted with subcutaneous mini-pumps linked to an IV jugular catheter to continuously deliver FITC-Inulin thereby allowing for longitudinal measurement of renal function in the conscious free-moving rat. In order to validate this method, inulin clearance was also measured in the anesthetized 5/6 Nx rats at the end of the study using the conventional method.

Results: Compared to sham operated, rats that underwent 5/6 Nx surgery demonstrated significant reductions in survival (50%), CrCl (55%), and GFR (both conscious and terminal) (94 and 73% respectively). At both 8 and 12 weeks post 5/6 Nx, GS and TIF were significantly increased in 5/6 Nx rats compared to sham. Losartan treatment resulted in significant improvement in survival ($p<0.01$), and reductions in MAP (≤26%), GS (46.9%), and TIF (53.6%). GFR measurement by the novel method yielded similar results to that generated in the anesthetized 5/6 Nx by the traditional method ($r^2=0.82$).

Conclusions: We have characterized over time the functional decline in GFR in conscious 5/6 Nx rats using a novel methodology of inulin clearance assessment. Our data highlight the utility of the 5/6 Nx rat as a model to investigate novel CKD therapies.

PUB414

A Novel Role of CCN3 in Renal Diseases Tarunkumar H. Madne,[1] Iain Macphee,[2] Mysore K. Phanish,[1] Mark E. Dockrell.[1] *[1]SWT Institute for Renal Research, London, United Kingdom; [2]St George's Univ of London, London, United Kingdom.*

Background: CCN3 (NOV) is a matricellular protein from the same family as the pro-fibrotic connective tissue growth factor CTGF (CCN2). CCN3 has been shown to have roles in: endothelial cell adhesion and migration; osteoblast differentiation; progression of cancer; and the possible regulation of renal fibrosis. In the last of these it is proposed that CCN3 may directly oppose the actions of CTGF, although the precise mechanism for this is undefined. We have previously demonstrated that TGFβ1 activates Smad 1/5/8 in human podocytes in a manner that was inhibited by the presence of CCN3. Here we investigate whether CCN3 can modulate extracellular matrix (ECM) production by human podocytes in culture.

Methods: Experiments were conducted on conditionally immortalised human podocytes incubated with TGFβ1 (2.5ng/mL) and CCN3 (360ng/mL). Real time PCR was performed to look for gene expression of Fibronectin, collagen IV and collagen I. Immuno fluorescence microscopy (IF) was performed to look for collagen IV protein expression.

Results: CCN3 in similar pattern as TGFβ1 induced the fibronectin, Collagen IV and Collagen I gene expression in human podocytes. But co-incubation of CCN3 with TGFβ1 did not modify any of above outcomes. Both TGFβ1 and CCN3 induced a similar pattern of perinuclear localisation of collagen IV as demonstrated by IF.

Conclusions: Our results suggest that CCN3 can inhibit the TGFβ1 induced Smad1/5/8 phosphorylation. CCN3 also can induce the ECM proteins gene expression: Fibronectin, Collagen IV and Collagen I. CCN3 induced collagen IV expression has been proposed in melanocytes but we believe this is the first report of it in podocytes. Thus our results suggest that CCN3 could play a key role in regulation of fibrosis. Further studies involving CCN3 are required to define its role in renal fibrosis.

PUB415

Changes in Expression Profile of microRNAs and Target Genes in the Development of Peritoneal Fibrosis in a Model of Progression Chronic Kidney Disease Teresa Renata Romero,[1] Carmen Josefina Mora,[1] Marcela Avila,[1] Carmen María del Prado,[1] Virgilia Soto,[2] Ramon Paniagua-Sierra.[1] *[1]Unidad de Investigacion Medica en Enfermedades Nefrologicas, IMSS, Mexico; [2]Dept de Patologia, Instituto Nacional de Cardiologia., Mexico.*

Background: Fibrosis is a problem of peritoneal dialysis (PD), the first involves a network of proteins whose translation is regulated by molecular factors such as microRNAs (miRNAs), which is unknown in the progression of Chronic Kidney Disease (CKD).The aim was analyze fibrosis development in parietal peritoneum, the expression of miRNAs, mRNAs of protein targets genes related to fibrosis in progression of CKD in a rat model.

Methods: Six experimental groups were developed; control group and CKD group (Nx 5/6) in 4, 8 and 10 progression weeks, seven miRNAs expression and its possible target mRNA in parietal peritoneum were analyzed by qT-PCR with Taq-Man probes.

Results: Quantification of serum creatinine and albumin confirmed the functionality of the IRC model. Analysis by ANOVA showed that the histological results of CKD group in 4 (72.52 px, $p \leq 0.01$), 8 (130.68 px, $p \leq 0.01$) 10 weeks (161.56 px, $p \leq 0.01$) by staining Trichrome Masson confirmed presence of progressive fibrosis in the IRC. A pattern of differential expression of seven miRNAs mir195_1, mir409-3p_1, mir376a_1, mir26a_1, mir206_1, mir29b-1_1* and mir30b_1, respect to time progression of CKD was observed. Direct correlation was found between COL4A5 mRNAs ($r = -.777$, $p \leq 0.01$), CTHRC1 ($r = -.647$, $p \leq 0.05$), Faim ($r = -.619$, $p \leq 0.05$) and TGFß ($r = -.796$, $p \leq 0.01$) in presence of fibrosis.

Conclusions: Our results suggest that fibrosis may result from the combined effects of multiple miRNAs, mRNAs and progression time in parietal peritoneum able to uremia and proceeding of any renal replacement therapy in CKD.

Funding: Government Support - Non-U.S.

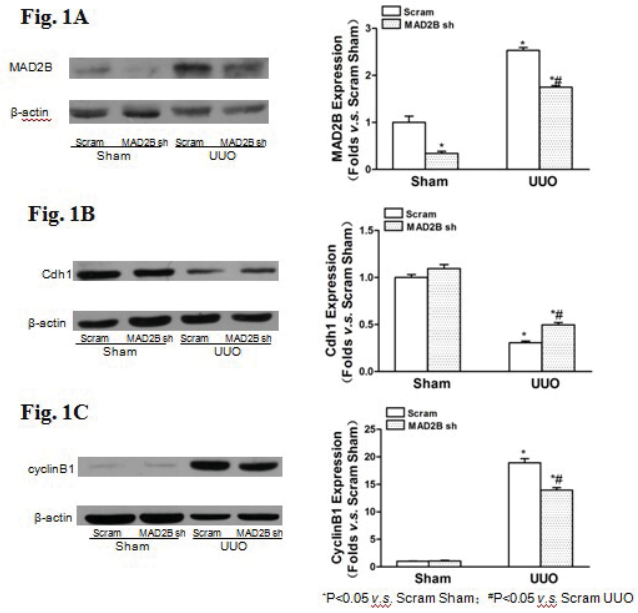
PUB416

MAD2B, a Novel Participant of Renal Tubulointerstitial Fibrosis in a Mouse Unilateral Ureteral Obstruction Model Hui Tang, Di Fan, Hua Su, Chun Zhang. *Dept of Nephrology, Union Hospital, Tongji Medical College, Huazhong Univ of Science and Technology, Wuhan, Hubei Province, China.*

Background: MAD2B was known as an APC/C inhibitor which plays a critical role in maintaining mitotic fidelity. Our previous study has detected expression of MAD2B in both human renal tissue and renal cell lines, but it's particular role in renal disease remains unexplored. In this study, we aimed to explore the potential role of MAD2B in the pathogenesis of renal tubulointerstitial fibrosis in a mouse model of UUO.

Methods: Twenty C57 mice were randomly divided into four groups (n=5): sham, UUO-1d, UUO-3d, UUO-7d. Expressions of MAD2B, APC/C complex regulatory molecules Cdh1, as well as its substrates cyclinB1, skp2 and snon in each group were detected by Western blot and immunohistochemistry. Afterwards, in order to stably knockdown MAD2B, we developed shRNA Lentiviral directed against MAD2B and injected it into the renal cortex two days before UUO operation. Renal tissues were collected at 7-day after surgery. The expressions of APC/C regulatory molecules and substrates were detected by Western blot. Immunohistochemistry was performed to evaluate the expressions of a-SMA and FN.

Results: Comparing with sham groups, expressions of MAD2B were significantly upregulated in the UUO-7d group. Meanwhile, the obstructed kidney showed accumulation of cyclinB1 and skp2 but reduced Cdh1 and snon expressions. MAD2B deficiency attenuated the upregulation of cyclinB1 and increased the expression of Cdh1 in UUO groups, which was companyed with less severe renal interstitial fibrosis, reduced expression of α-SMA, less deposition of FN, compared to the UUO WT mice.



Conclusions: MAD2B participated in the development of renal interstitial fibrosis in UUO model through changing the expressions of Cdh1 and cyclinB1. Blockade of MAD2B pathway may be a novel approach for the prevention of renal interstitial fibrosis.

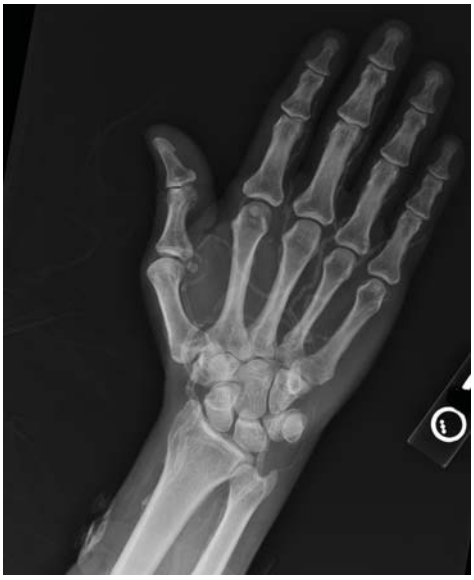
Funding: Government Support - Non-U.S.

PUB417

Calciphylaxis from Uremic and Nonuremic Etiologies Waqas Jehangir, Mohammed Amzad Hossain, Qiang Nai, Tarek Aly. *Internal Medicine, Raritan Bay Medical Center, Perth Amboy, NJ.*

Introduction: Calciphylaxis is a life-threatening syndrome of small vessel calcification and skin.

Case Description: A 55y/o male presented with dry gangrene to left ring finger and glans penis associated with pain and dysuria. PMH includes sarcoidosis, HTN, DM, CAD, IgA nephropathy and interstitial nephritis, nephrotic syndrome, ESRD, ILD, RF positive arthritis, hyperuricemia and anemia. Patient was taking prednisone for sarcoidosis. On physical exam, BP 180/87 with dry gangrene of left ring finger; black eschar to the tip of left middle finger and glans penis. Labs showed Ph 6.4, Ca 12.6, hep C antibody 2.5, RF 75, C3: 62, C4: 42, PTH 175, beta-2 microglobulin 6.3, d-dimer 1.03. CT showed diffuse sarcoidosis. Mediastinal lymph node biopsy revealed noncaseating granulomas. Xray showed extensive vascular calcification of medium and small blood vessels in hands, feet and penis.



Kidney biopsy showed diabetic nephropathy, IgA nephropathy and AIN. ECHO showed LV diastolic dysfunction. Upper extremities arterial Doppler showed bilateral peripheral arterial occlusive disease including small vessel disease in both hands. Carotid Doppler showed small amount of calcification to bilateral carotid bulbs. Amputation of left ring finger was performed. A suprapubic tube was placed.

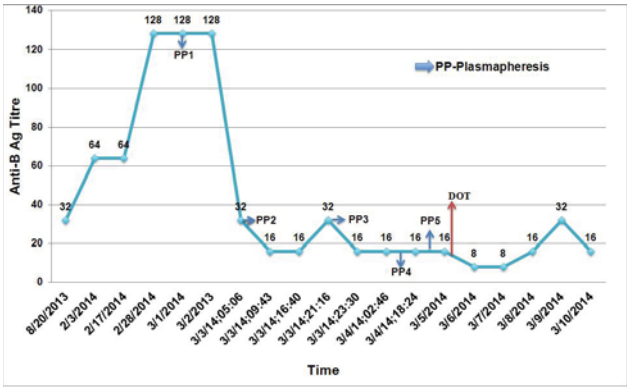
Discussion: Etiology of calciphylaxis remains elusive and may involve multiple co-morbid factors. We report a unique case where ESRD and multiple non-uremic conditions including sarcoidosis, cryoglobulinemia and DM may directly or indirectly contribute to the pathogenesis of calciphylaxis. Given the high mortality rate and increasing prevalence, emphasis should be on prevention, early diagnosis of vascular calcification and prophylaxis of secondary infection. Reduction of calcium phosphate product, avoiding prednisone usage, optimized glycemic control and effective wound care to prevent infection would enhance quality of life and prolong the life span.

PUB418

NxStage® System One™ Allows for Rapid, Cost-Effective Plasmapheresis for ABO Incompatible Kidney Transplantation Siddiq Anwar, Mohammed Abuzar Khan, Ravkiran Kaur Khurana, Ana Paula Rossi, Timothy A. Horwedel, Daniel C. Brennan. *Renal Div, Washington Univ School of Medicine, St Louis, MO.*

Introduction: ABO incompatible kidney transplantation (KT) requires removal of anti-A or B antibodies using expensive and time intensive selective adsorption columns or plasmapheresis. NxStage® System One™ is membrane-based technology that has been recently approved by the FDA for Therapeutic Plasma Exchange (TPE) and Plasmapheresis (PP). This allows for 4.0 liters of plasma exchange per hour with a low extracorporeal blood volume (110 ml), with a drop-in cartridge design for easy and quick set up plus a design feature which has 9 connections, which allows for concurrent set up of all the replacement fluid.

Case Description: 73yr female underwent a preemptive living related KT, blood group B into O from her son. 4 weeks prior to KT she received rituximab 200mg plus commenced on mycophenolic acid. Anti-B titers were 1:128 five days before planned surgery. PP was commenced using NxStage® System One™ aiming for a 1.5 plasma volume per exchange (4 liters). She received a total of 5 treatments with mean treatment duration of 95 minutes per treatment. Short treatment duration and easy tolerability allowed us to do 4 treatments in 2 days to deal with the rebound phenomenon associated with IgG which is predominantly extravascular. Anti-B titers went from 1:128 to 1:16 allowing for KT to proceed safely and on-schedule.



Discussion: Advantages of NxStage® System One™ for TPE and PP include ease of use and setup, ability to hang all the replacement fluid at once,and ability to run blood flows of 200mls/min with a large dialyzer cartridge 0.5m^2 that allows for 4L/hour of apheresis or exchange. These allow for shorter duration of hospitalization plus lower hospital and nursing costs. As demonstrated in our case, this technology is safe and effective at reducing ABO isoagglutinin titers rapidly.

PUB419

Catastrophic Lactic Acidosis with Post Embolization Syndrome after Trans Arterial Hepatic Embolization Ashwin Reddy Ganta. *Dept of Nephrology, Vanderbilt Univ Medical Center, Nashville, TN.*

Introduction: Chemoembolization is being increasingly used in the treatment of Hepatic metastasis, for palliative and definitive treatment purposes. Acute Kidney injury (AKI) is being increasingly recognized as a complication of this procedure.

Case Description: A 67 y/o WF was transferred to the ICU after undergoing hepatic artery embolization (HAE). A few weeks ago, she was diagnosed with Carcinoid tumor with liver mets. Her PMH was significant for CAD, DM, CKD-II with baseline Scr of 1.3. Given her extensive hepatic involvement and recurrent abdominal pain, she underwent HAE. CT abdomen with contrast was done and HAE was successful with after adequate IVF. A few hours later, she c/o RUQ abdominal pain and she was initiated on PCA morphine. After a few hours, she became increasingly somnolent and oliguric. She was transferred to the ICU and labs showed elevated AST, ALT (3 x), K of 6.0 and a Scr 3.0. IVF and anti-hyperkalemia measures were started. Her Scr continued to worsen with worsening AGMA, lactate 6.2, AST and ALT in the 2000's and CPK 25000. She became hypotensive and was initiated on pressors. She developed fulminant hepatic failure with anuric AKI and was started on CRRT (CVVHDF). Her uric acid and phosphate levels were normal. She was continued on CRRT for the next 36 hrs and she died within 48 hours of her HAE.

Discussion: Discussion: HAE is being increasingly used for the management of Liver metastasis. Embolization can be done using Chemotherapy (TACE Transarterial Chemo Embolization) or using embospheres. AKI occurs in approximately 9% of patients after HAE and is most commonly secondary to Contrast induced Nephropathy(CI) in the setting of volume depletion from severevomiting that occurs in 90% of patients and is termed as post-Embolization syndrome. AKI can also occur form TLS. In our patient, AKI was of rapid onset and we hypothesise that the HAE precipitated massive tissue necrosis with release of inflammatory mediators which precipitated MOF. Ours is the only case reported in literature to our knowledge, with rapid such rapid onset AKI post-HAE and given the increasing use of HAE, nephrologists need to be aware of this catastrophic complication.

PUB420

Trapped in a Relationship: An Unexpected Case of Encapsulating Peritoneal Sclerosis Yaakov Y. Liss,[1,3] Jaime Uribarri.[2] *[1]Nephrology, Hospital of the Univ of Pennsylvania, Philadelphia, PA; [2]Nephrology, Mount Sinai Hospital, NY, NY; [3]Medicine, Mount Sinai Hospital, NY, NY.*

Introduction: Encapsulating peritoneal sclerosis (EPS) is an uncommon, devastating complication of peritoneal dialysis (PD), with an incidence of 0.5 - 4.4% and a mortality as high as 50%. Diagnosis is based on clinical features of bowel obstruction and radiographic or histopathologic findings of bowel encapsulation. This is a case of EPS occurring in a patient with no known risk factors for this uncommon condition.

Case Description: A 72 year old woman on PD for 3 years presented to the hospital with intractable nausea and vomiting of 3 months duration. She reported vomiting minutes after eating and a recent 20 pound weight loss. She had been hospitalized twice during the last 3 months for similar issues. Physical examination was significant for dry mucous membranes only. Prior workup was unremarkable and included esophagram, barium swallow, and EGD. Upon admission, the patient had a CT abdomen which demonstrated peritoneal thickening, loculated ascites, small bowel fecalization, sigmoid colon collapse, and moderate right hydroureter, consistent with a presumptive diagnosis of EPS. The patient was started on prednisolone and was switched from PD to hemodialysis (HD). Over the course of hospitalization, the patient's symptoms improved, and the patient was discharged home able to tolerate oral intake. She is currently doing well on HD.

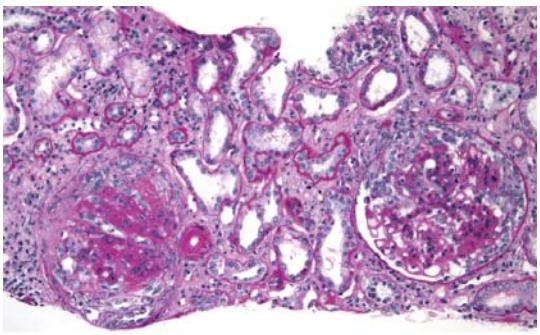
Discussion: This is a case of EPS occurring in a patient with no known risk factors for this condition. Risk factors for developing EPS include duration on PD, prior episodes of peritonitis, and use of high glucose containing PD solutions. For patients on PD for less than 3 years, the incidence of EPS is less than 1%. The patient described above was on PD for 2 years 11 months at diagnosis, had no prior episodes of peritonitis, and had minimal past use of high glucose containing PD solutions. Her lack of known risk factors for EPS undoubtedly contributed to a delay in diagnosis. This case highlights the need to determine additional risk factors for the development of EPS to allow for optimal prevention and early diagnosis.

PUB421

Recurrence of Lupus Nephritis in Transplanted Kidney Ardavan Mashhadian, Amir Abdi Pour. *Nephrology, Loma Linda Univ Medical Center, Loma Linda, CA.*

Introduction: The incidence of graft loss due to recurrent lupus nephritis (RLN) is extremely low, being less than 2-4% in most studies. Whereas the majority of patients with ESRD caused by lupus have had diffuse proliferative (class III or IV) disease, mesangial proliferative (Class II) disease is the most commonly described lesion after transplantation.

Case Description: 37 year-old African American male with lupus nephritis status post diseased donor allograft at age 20 was admitted for fever, weakness, and acute renal failure. He stated being compliant with immunosuppressive medications. Urinalysis showed numerous muddy brown casts suggestive of acute tubular necrosis. Serologic studies, including dsDNA antibody and complement levels were normal. Viral studies were negative. Doppler ultrasound was unremarkable. Renal biopsy was showed combined diffuse and membranous lupus nephritis with approximately 40% crescents, consistent with recurrent lupus (class IV + V) nephritis.



Despite pulse methylprednisolone and increased dose of immunosuppressive medications kidney function continued to worsen. He was discharged home on hemodialysis, and on follow up he continued to be hemodialysis dependent.

Discussion: Lupus nephritis patients who receive kidney transplant rarely develop clinically significant RLN. The excellent long-term outcome has been largely attributed to the use of chronic immunosuppressive therapy. The clinical consequences of RLN on allograft survival have ranged from no effect to a significant increase in the risk for graft loss and patient mortality. RLN significantly increases the risk for graft failure. However, it contributes far less than rejection to incidence of allograft loss. As such, diagnosis of lupus nephritis should not keep patients with advanced kidney disease from seeking a kidney transplant. In addition, successful transplantation has been reported after graft loss due to recurrence.

PUB422

"Pseudodiabetic" Glomerulosclerosis Cátia V.T. Cunha, Ana Marta Gomes, Daniela Lopes, Sónia Sousa, Susana Pereira, Joao Carlos Fernandes. *Nefrologia, Centro Hospitalar de Vila-Nova de Gaia, Vila-Nova de Gaia, Portugal.*

Introduction: The histological pattern of nodular glomerulosclerosis is typical of diabetic nephropathy (DN), but it is not exclusive of this entity.

Case Description: A 45-year-old man was studied at nephrology clinic to evaluate microscopic hematuria and proteinuria (protein-creatinine ratio of 2,44). He had a medical history of hypertension diagnosed 5 years early, dyslipidemia, active smoking (22 packs-year), an estimated alcohol consumption of 60 to 70 g per day and a body mass index of 27. The patient was taking an angiotensin II receptor antagonist and a statin. There was no history of diabetes. Laboratory values included a normal serum creatinine; high total cholesterol; mild elevation of gama glutamiltransferase; normal transaminases and alkaline phosphatase; normal serum glucose and glycosylated hemoglobin; and a normal hemogram. All serologies and immunology study were at normal range. No abnormalities were found on renal ultrasound. Renal biopsy was performed. The findings were a nodular glomerulosclerosis pattern and arteriolar hyalinosis. Congo red staining was negative. Immunofluorescence revealed 2+ intensity granular segmental focal capillary and tubular basement membrane positivity for C3. The podocyte foot processes were focally effaced on electron microscopy. There was also a mesangial and capillary membrane sclerosis. Rare subendothelial were found on mesangio-capilar transition. A diagnosis of idiopathic nodular glomerulosclerosis was established, probably in association with smoking and hypertension.

Discussion: The histological pattern of nodular glomerulosclerosis can be found in other situations besides DN, namely amyloidosis, thrombotic microangiopathy, chronic membranoproliferative glomerulonephritis, heavy chain deposition disease and fibrillary/immunotactoid glomerulopathy. The diagnosis of idiopathic nodular glomerulosclerosis is made by exclusion of the previous. It is a distinct clinicopathologic entity that should be considered in patient with active smoking, especially if there is concomitant hypertension. Some authors actually suggest the designation of "smoking-related nodular glomerulosclerosis" to this "pseudodiabetic" glomerulopathy.

PUB423

Acute Acetaminophen Toxicity Associated with Acute Kidney Injury without Hepatic Failure Sherif Y. Isshak, Bahar Bastani. *[1]Nephrology, Saint Louis Univ, St Louis, MO.*

Introduction: Acetaminophen toxicity commonly causes isolated acute hepatotoxicity,less commonly acute hepatotoxicity with acute kidney injury(AKI).The present case was unique in that AKI developed without hepatic failure.

Case Description: 63 year old Caucasian man committed suicide by taking 40 grams Acetaminophen, next day Acetaminophen level was 630 mg/dl. On admission, a baseline serum creatinine (Scr)1mg/dl, had increased to 1.9 mg/dl, FeNa was 2.7%,urinalysis showed multiple granular casts, ABG: PH 6.78,pCo2 76mmhg,pO2 173 mmhg, Hco3 10 mmol/l and 92% O2 saturation. Other labs: AST 142 u/l, ALT 150u/l, T.Bili 1mg/dl, lactic acid 11.3 mmol/l, phosphorus 10.1mmol/l, and blood glucose 277mg/dl with no ketones in urine. After mechanical ventilation and one session of Hemodialysis, ABG improved:PH 7.42,pCo2 40 mmhg,p02 100 mmhg,Hco3 25 mmol/l, and O2 saturation 95.5%.Next day patient was extubated, Acetaminophen level progressively improved to 341,130 and 3 mcg/ml;lactic acid 1.2 mmol/l and phosphorus 3.7 mg/dl .His Scr peaked at 4.3 mg/dl on the 5th day and declined to 2.5mg/dl by day 8 when he was transferred out of ICU.

Discussion: Patient developed AKI with mixed metabolic and acute respiratory acidosis with minimal liver damage. Patient never developed encephalopathy, Jaundice or coagulopathy.He had acidemia on admission, due to anion gap metabolic acidosis secondary to lactic acidosis and AKI, and acute respiratory acidosis. Patient was taking Metformin, which might have contributed to his Lactic acidosis.Hyperphosphatemia is thought to be due outward Transcellular shift and inhibited cellular glycolysis secondary to lactic acidosis. Hemodialysis is as an effective treatment for acute acetaminophen toxicity, however seldom used, because of the effectiveness of N-acetyl cysteine. The patient developed AKI despite receiving both N-acetyl cystiene and one session of hemodialysis.Our patient is the 37th case reported with the same presentation.It is Suggested that renal injury occurs when glutathione in tubular cells is overwhelmed with highly reactive metabolites inciting cellular damage, or reduction of PGE2 causing reduced renal blood flow.

PUB424

Chronic Granulomatous Interstitial Nephritis Recurring after Successful Glucocorticoid Therapy Namita Singh, Patricia L. Cantlin. *Div of Nephrology and Transplantation, Maine Medical Center, Portland, ME.*

Introduction: Granulomatous interstitial nephritis (GIN) is a rare histologic diagnosis that is present in 0.5 to 0.9% of native renal biopsies. GIN has been associated with medications, infections, sarcoidosis, crystal deposits, paraproteinemia, Wegener's granulomatosis and is also seen in an idiopathic form. We present a patient with chronic idiopathic GIN diagnosed by native renal biopsies 8 years apart.

Case Description: A 70 year-old woman presented with complaints of fatigue, poor oral intake, nausea and generalized weakness for one month. She had a history of hypertension, hyperlipidemia, chronic kidney disease stage 2, and of biopsy-proven acute interstitial nephritis with granulomata 8 years previous in the setting of exposure to nitrofurantoin. She had reported taking Naprosyn sodium consistently over at least one month period. Blood work was notable for peripheral eosinophilia and a serum creatinine of 10.4 mg/dL. Urine sediment revealed clumping white blood cells, 5-10 per high power field. Creatine kinase, complements, ANCA, hepatitis profile, serum protein electrophoresis and free light chains were within normal limits. A renal biopsy was performed which showed acute tubulointerstitial nephritis with poorly defined mixed necrotizing and non- necrotizing granulomata. Additional work up including renal tissue staining for mycobacteria and fungi, serum calcium, angiotensin converting enzyme and vitamin D 1,25-hydroxy levels, tuberculin skin test, and a chest x-ray, was all normal. The patient was started on prednisone 80 mg orally daily. Serum creatinine has trended down to 2.3 mg/dL, two months into therapy. Of note, the patient was treated with steroids 8 years ago and serum creatinine improved from 12 mg/dL to 1.1 mg/dL. We suspect that the patient had two episodes of acute interstitial nephritis caused by different agents, with a background of idiopathic chronic GIN.

Discussion: GIN is a rare finding; however, it should be considered in all patients with unexplained acute or chronic renal insufficiency and sterile pyuria. It can persist or recur after successful treatment with steroids.

PUB425

Acute Rejection after Prednisone Dose Reduction in a Failed Renal Transplant 4 Years after Return to Dialysis Ben Broome,[1] Anthony J. Langone,[1] David Shaffer,[2] Giovanna A. Giannico,[3] Rachel B. Fissell.[1] *[1]Div of Nephrology, Vanderbilt Univ, Nashville, TN; [2]Div of Kidney and Pancreas Transplantation, Vanderbilt Univ, Nashville, TN; [3]Dept of Pathology, Microbiology and Immunology, Vanderbilt Univ, Nashville, TN.*

Introduction: The optimal level of maintenance immunosuppression in patients with a failed renal transplant is not known. We present a case of acute rejection in a graft that had failed 3 years previously, after decrease of prednisone.

Case Description: A 47yo woman with ESRD secondary to FSGS, received a deceased donor kidney transplant on the left side in 1999, and a subsequent 1 antigen mismatched living related donor transplant on the right side in 2009. She had biopsy proven collapsing glomerulopathy indicative of secondary injury 2 months after the second transplant, and returned to dialysis 4 months later. She was maintained on peritoneal dialysis and prednisone 5mg po each day. After 3 years the patient's nephrologist reduced the prednisone dose to 5mg QOD because of concerns about side effects. One month after the prednisone decrease, the patient presented with gross hematuria and right-sided tenderness at the site of the most recent graft. The prednisone taper was maintained at that time. Review of her medical record showed the presence of persistent microscopic hematuria since the second transplant. The patient underwent cystoscopy and ureteroscopy which revealed bleeding from the right transplant ureter as well as adjacent to the upper pole calyx. Because of symptomatic pain, tenderness and hematuria a right transplant nephrectomy was performed. Pathology showed changes suspicious for acute/active ABMR, chronic/active ABMR and numerous calcium oxalate crystals, in a background of near-global scarring. The hematuria resolved, and postoperative course was uneventful.

Discussion: This patient had ABMR after a slight decrease in maintenance prednisone, in a graft that had failed several years prior. Additional research may be needed to develop consensus recommendations for immunosuppression management and indications for graft removal in the growing population of patients with non-functioning renal transplants.

PUB426

Immunotactoid Glomerulopathy with Membranous Pattern in Patient with Rheumatoid Arthritis and Monoclonal Gammopathy Ghayyath Sultan,[1] Gaurav Agarwal,[1] Brent Wagner,[3] Sherry L. Werner.[2] *[1]Dept of Nephrology, Univ of Texas, San Antonio, TX; [2]Dept of Pathology, Univ of Texas, San Antonio, TX; [3]Dept of Nephrology, South Texas Veterans Health Care System, San Antonio, TX.*

Introduction: Immunotactoid Glomerulopathy (IMTG) is a rare cause of glomerular renal disease and frequently leads to end stage renal disease within few years. The optimal treatment still uncertain although steroids and other immunospressive agents have been tried with variable success.

Case Description: Proteinuria and microscopic hematuria occurred in a 69 year old man with history of rheumatoid arthritis receiving infliximab and sulfasalazine. Past medical history was significant for ground glass changes of the lungs (by computed tomography imaging), hypertension and monoclonal gammopathy of undetermined significance. Serum chemistries were non–contributory. Urine sediment was notable for one dysmorphic red blood cell and no casts. A 24 hour urine collection revealed 600 mg of protein. His

serologic evaluation for proteinuria was negative for hepatitis B panel, hepatitis C, human immunodeficiency virus, rapid plasma reagin, cryoglobulins, anti–nuclear antibody, double–stranded DNA antibody and anti–neutrophil cytoplasmic antibody. Complement C3 and C4 were normal. His serum protein electrophoresis showed an increased beta and gamma fractions with a possible monoclonal spike in the beta region. Immunofixation electrophoresis showed monoclonal bands in IgA/kappa and free kappa chains. Kidney biopsy showed morphologic changes consistent with immunotactoid glomerulonephritis with a membranous pattern.

Discussion: Most cases of ITGN are reported as idiopathic but screening for occult malignancy, paraproteinemia and automimmune diseases should be always considered once the diagnosis is established, as the treatment of the underlying disease will have great impact on the renal prognosis.

PUB427

Levamisole Induced ANCA Associated Glomerulonephritis

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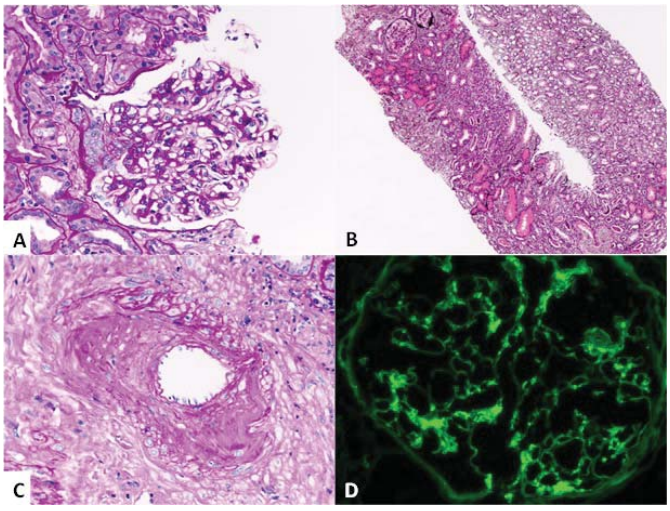
Introduction: 69% of the cocaine in the U.S. may be contaminated with Levamisole. Levamisole has been associated with cutaneous vasculitis and agranulocytosis, while renal involvement has rarely been described.

Case Description: 56 year old female referred for acute kidney injury, diffuse rash and anemia. History of hypertension, depression and cocaine use. The rash was pruritic and maculopapular involving her face, upper chest, back, and the extensor surfaces of the forearms with a necrotic lesion on the helix of her right ear. There was associated weight loss, weakness, fatigue, and diffuse joint pain. No daily medications were being taken. Laboratories revealed AKI (serum creatinine 5mg/dl), anemia, elevated ESR (130mm/hr) and CRP (8.4mg/dl); positive ANA (1:160), decreased C3 (69mg/dl), normal C4; positive ds-DNA, strongly positive MPO Ab (greater than 8AI), positive Histone Ab, and positive urine toxicology for cocaine. The patient was negative for PR-3 Ab, GBM Ab, RF, SCL-70, LAC, anticardiolipins, anti-Smith Ab, Hep B Ag, Hep C Ab, and HIV Ab.



Renal biopsy: (A) MPO-ANCA associated focal necrotizing and crescentic glomerulonephritis (B) moderate tubular atrophy and interstitial fibrosis (C) moderate to severe arterio and arteriolosclerosis (D) immunofluorescence with granular global mesangial positivity for IgG, C3, kappa, and lambda - a greater degree of immune complex deposition than is usually seen in pauci-immune ANCA-associated glomerulonephritis.

Discussion: Our patient is one of only two biopsy proven Levamisole induced ANCA glomerulonephritis cases in the literature. The patient was treated with high dose steroids, and although had an initial improvement in renal function remains with significant chronic kidney disease.

PUB428

Nephrotic Syndrome in a Patient with Autossomal Polycystic Kidney Disease

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Introduction: The kidney biopsy represents an important tool for the approach of a nephrotic syndrome. When this procedure is contraindicated, the nephrologist faces diagnostic and therapeutic challenges. The authors present a case report of a patient with a nephrotic syndrome who was diagnosed with Autossomal Polycystic Kidney Disease (APKD) and several renal cysts.

Case Description: A 31 years old caucasian female with family history of APKD presented with prostration, edema of the inferior limbs and foamy urine. No history of infections, cutaneous lesions, joint pains or drug intake. Blood test results showed a creatinine of 0,7mg/dl and an albumin of 2,8 mg/dl. On urine test results she had proteinuria of 7340mg/24h, with erythrocytes and leucocytes. She was put on antiproteinuric measures. Investigation proceeded: C3 of 22mg/dl, C4 of 2mg/dl, CH50 of 5mg/dl, she was positive for ANA, with a DNAse of 920mg/dl and negative for ANCAs, TASO, e RA. She was negative for HIV, HCV, HBV and had normal electrophoresis. No Bence-Jones proteinuria was detected. Renal ultrasound showed two kidneys with 12,5 and 13 cm with good parenchymal-sinusal and corticomedullary differentiation, three centimetric cortical cysts on each kidney, parapielic cyst of 2 cm on the left kidney and microcysts on both kidneys. A 7mm cyst was visible on the right lobe of the liver. From the ultrasound imaging and laboratorial findings, the most probable diagnose was a lupic nephritis on a patient with APRD. There were no safety conditions for a percutaneous renal biopsy. The patient refused a laparoscopic renal biopsy. She was put on Prednisolone 0,5mg/kg/day and Micofenolate 2gr/day. After three months DNAse was not detectable, C3, C4 e Ch50 were normal, albumin was 3,9 mg/dl and proteinuria of 223 mg/24h.

Discussion: APKD is associated with several renal manifestations as microalbuminuria, hematuria, isosthenuria. Still, when nephrotic syndrome superimposes it is mandatory to exclude other causes. Unfortunately, renal cysts are often an obstacle to renal biopsy making impossible the confirmation and staging of many causes. It is the nephrologist's responsibility to handle the approach and therapeutics.

PUB429

Hemodialysis Characteristics for Treatment of Hypermagnesemia

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Introduction: Magnesium sulfate infusion is commonly used to treat eclampsia.

Case Description: A 22-year-old African American female presented at 28 weeks gestation with eclampsia, HELLP syndrome, seizures, and hypertensive urgency. Initial blood pressure was 200/130 mm Hg, after which she had a seizure. She received intravenous (IV) magensium sulphate (6 gm loading dose followed by maintenance doses at 2 gm/hour), and underwent an emergent cesarean section. The patient then developed progressive somnolence, hyporeflexia, and anuria. Her serial labs revealed hypermagnesemia (8 mg/dl), profound hypocalcemia (ionized calcium 1.77 mmol/L), acute kidney injury (creatinine 2.8mg/dl), and hyperkalemia (8.7 meq/L). IV calcium gluconate was administered to antagonize the effects of elevated magnesium (hypermagnesemia induces hypocalcemia by binding to Ca-SR on parathyroid gland and in the renal tubules). Emergent hemodialysis was performed, which reduced the serum magnesium level by 3.6 mg/dl. The patient was dialyzed for 5 hours using a high calcium (3 meq/L) and normal magnesium (1.8 mg/dL) dialysate concentration, across high flux membrane, with high blood flows.

Discussion: Therapeutic serum magnesium concentration recommended for the treatment of eclamptic seizures is 4.8-8.4mg/dL. In patients with normal renal function, the induced hypermagnesemia resolves with renal clearance. However individuals with a GFR< 30ml/min are at risk of persistent hypermagnesemia, and it's fatal neurological and cardiovascular complications including ventricular arrythmias and sudden cardiac death. Hence obstetric patients with impaired renal function, who are treated with large parentral doses of magnesium are at increased risk of accidental overdose. Hemodialysis is efficient in rapid removal of magnesium, and can achieve a magnesium clearance of 100 ml/min, due to the small size and large, ionized fraction (70%) of magnesium in the serum. There is no need to lower dialysate magnesium concentration (average 1.8 mg/ dL), as low magnesium hemodialysis sessions are not well tolerated due to increased intradialytic hypotension and leg cramping.

PUB430

Dense Deposit Disease: A Case Study

Andrew R. Sacks, Jennifer A. Fillaus, Troy J. Plumb. *Nephrology, Univ of Nebraska Medical Center, Omaha, NE.*

Introduction: Dense deposit disease (DDD) is a rare form of glomerulonephritis, affecting 1 to 3 in one million people, typically children and young adults. Historically considered a type of MPGN (type II), it is now recognized as a pathologically distinct and one of the so-called C3 glomerulopathies. Progression to ESRD is common despite treatment, and recurrence after transplant is nearly universal.We discuss a case of DDD and its management.

Case Description: A 21 year old female with no past medical history presented to the ED after several days of worsening dyspnea, edema, and cough. Further review was negative, and her only medication was a rare NSAID. Physical exam was significant for blood pressure of 219/145, tachycardia, tachypnea, chest rales, and moderate leg edema. Labs showed elevated creatinine, low serum albumin, and high ESR. Urine studies showed red blood cell casts and profound proteinuria. Serologic workup for glomerular disease was negative. Kidney biopsy showed presence of C3 with electron dense subendothelial deposits and chronic change. She became dialysis dependent and began treatment within days of presentation. Several months later, she received a transplant from a living donor with excellent graft function and no sign of recurrence.

Discussion: DDD is a rare condition mediated by C3 and the alternative complement system. C3 activation is left unchecked by a stabilized C3 convertase, which results from C3 nephritic factor, reduced complement factor H activity, or both.This leads to deposition of C3 and terminal complement components within the capillary membrane.Patients present with varying degrees of proteinuria and hematuria. Serum C3 is often depressed. C3NeF and factor H deficiency may be present. Incidence and degree of hypertension and reduced kidney function are variable. Biopsy with electron microscopy is required for diagnosis. With no randomized trials, there is no clear best treatment. Options include plasma infusion or exchange, rituximab, and eculizumab. RPGN may warrant additional immune suppression. Proteinuria, hypertension, and hyperlipidemia should always be treated. Transplant is an option. Recurrence can be up to 100 percent, but this leads to graft loss in less than 30 percent of patients.

PUB431

Contrast-Induced Nephropathy in Enlarged Kidneys: Utility of Renal Ultrasound in Diagnosis of Multiple Myeloma Ekamol Tantisattamo, W. Charles O'Neill. *Renal Div, Dept of Medicine, Emory Univ School of Medicine, Atlanta, GA.*

Introduction: Broad range of renal manifestations makes multiple myeloma (MM) sometimes difficult to be diagnosed. However, classic presentations of MM could be a clue to further workup even in unsuspected cases. Renal ultrasound (RUS) is commonly used to diagnose an obstruction as a cause of acute kidney injury (AKI); however, kidney size is also one of the most important pieces of information to narrow differential diagnosis of underlying renal diseases.

Case Description: A 69 year-old woman presented with NSTEMI and underwent PCI. Two days later, she was discharged but had not been feeling well. Ten days after discharged, she came to follow-up and was found to have elevated serum creatinine (SCr) of 19 mg/dL from baseline SCr of 1.1 mg/dL. BUN was 119 mg/dL. Urinalysis showed protein 50 mg/dL, 26 RBC/hpf. RUS revealed enlarged echogenic kidneys with the right and left kidney length of 14 and 11.9 cm, respectively(figure 1). Further workup revealed urine protein/Cr ratio of 2.98 and urine albumin/Cr of 0.09 g/g of Cr, respectively. She was uremic; therefore, intermittent hemodialysis (HD) was initiated. κ and λ free light chain were 38.1 and 39,124 mg/L, respectively. Urine immunofixation demonstrates a free λ light chain paraprotein. Bone marrow biopsy revealed 40-50% plasma cells. She was diagnosed with MM. Chemotherapy was started as an outpatient. Her renal function had been improved and HD was discontinued 1 month later with stable SCr of 2.3 mg/dL.

Discussion: MM is one of the risk factors of CIN. CIN and enlarged kidneys could be an initial manifestation of MM and quantified proteinuria should be tested for possible paraproteinemic renal disease. Our case underscores the utility of RUS in measuring a kidney length as a clue to diagnose underlying renal diseases causing enlarged kidney such MM.

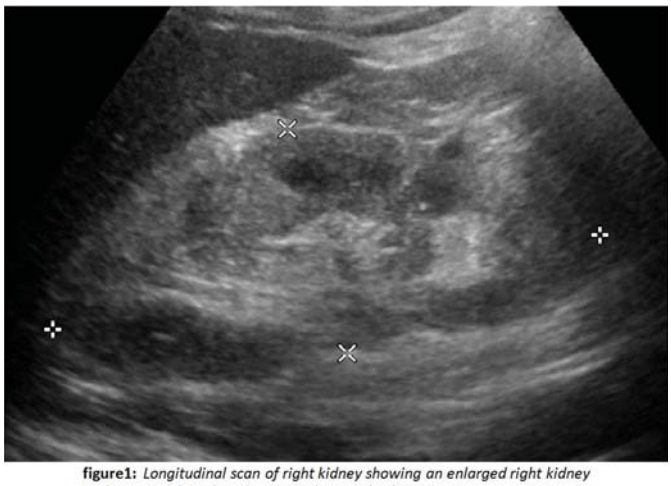

figure1: *Longitudinal scan of right kidney showing an enlarged right kidney with 14 cm in length and increased cortical echogenicity.*

PUB432

Light Chain Deposition Disease: The Importance of Early Diagnosis Paul Bradley Brasher,[2] Arif Asif.[1] *[1]Nephrology, Albany Medical College, Albany, NY; [2]Internal Medicine, Albany Medical Center, Albany, NY.*

Introduction: In this report, we present two unique cases of light chain deposition disease (LCDD) and the challenges facing diagnosis and treatment.

Case Description: *Case#1* is a 37-year-old female with a previous diagnosis of pulmonary hypertension and cryptogenic organizing pneumonia was on increasing dosages of steroids without improvement of her exertional dyspnea. She presented in moderate respiratory distress with significant lower extremity edema. Initial laboratory studies revealed a hemoglobin of 8.7 g/dL and a BUN/Cr of 108/3.2 mg/dL. Later she was found to have an elevated serum kappa light chain level of 5360 mg/L. Renal biopsy was consistent with LCDD. A right heart catheterization suggested a restrictive cardiomyopathy and endomyocardial biopsy revealed deposition disease, interstitial widening and negative amyloid. Bone marrow biopsy was consistent with lymphoplasmacytic lymphoma as the source of her kappa light chain deposition. She required hemodialysis for ESRD and was treated with bortezomib, rituximab and bendamustine with a resultant decrease in her serum light chains and a recent echocardiogram with no functional abnormalities. *Case#2* is a 31-year-old female with no prior history who presented with anasarca and hypertension after a one-year history of progressive numbness and tingling in her hands. She was found to have a BUN/Cr of 56/4.8 mg/dL. A renal biopsy demonstrated light chain deposition. Bone marrow biopsy revealed cyclin D1 kappa restricted plasma cells. The patient progressed to ESRD requiring hemodialysis. Treatment was initiated with bortezomib. She is currently undergoing evaluation for autologous stem cell transplantation (ASCT).

Discussion: There is no standard treatment for patients with LCDD. Treatment with high-dose chemotherapy with or without ASCT may result in disease stabilization and/or improvement in end organ damage. These two cases highlight the difficulties in diagnosing LCDD early in its course, demonstrate the importance of renal biopsy and the challenges of treatment thereafter. Each of these patients was symptomatic for greater than a year yet a diagnosis was only made as the patients were approaching renal failure.

PUB433

Acute Renal Infarction Secondary to Membranous Glomerulopathy Frederick Elises Ogbac, Cherisse Ann P. Panlilio, Kristine Tan Gapuz, Alicia Baldonado. *St. Luke's Medical Center-Quezon City, Philippines.*

Introduction: Acute flank pain is a common complaint of patients in the emergency department (ED). A frequently misdiagnosed differential for acute flank pain is acute renal infarction. It has an estimated incidence of 0.007% and clinically diagnosed in 0.014% of patients. We present a case of a young female diagnosed to have membranous glomerulopathy with acute renal infarction presented as sudden onset flank pain.

Case Description: A 19-year-old female diagnosed with membranous glomerulopathy presented in the ED with an acute onset, severe, right sided, flank pain associated with nausea and vomiting. No fever, dysuria, hematuria, or history of trauma. There was no family history of connective tissue disease or vascular diseases. Her BP was 110/70mmHg, heart rate of 82bpm with regular rhythm and afebrile. Pertinent examination showed non-tender abdomen, positive for shifting dullness, fluid wave, right sided costovertebral angle tenderness and grade 1 pitting bipedal edema. Initial diagnostics showed leukocytosis with neutrophilic predominance, serum creatinine of 0.77mg/dL, and proteinuria of >600mg/dL. Abdominal ultrasound showed normal sized kidneys with isoechoic right kidney raising the possibility of non-specific parenchymal disease and ascites. Contrast-enhanced CT-Scan of the abdomen was requested revealing areas of non-enhancement in the upper to middle portions of the right kidney which may relate to areas of ischemia and/or infarction, likely due to thrombosis involving the more distal portion of the right renal artery and massive ascites. Result was confirmed by CT-Angiography of the kidneys showing right renal artery thrombosis. Evaluations for the cause of renal artery thrombosis were done and were negative. Anti-coagulation therapy was initiated using LMWH and maintained on warfarin.

Discussion: A high index of clinical suspicion is needed to diagnose acute renal infarction because of its non-specific symptoms. Early diagnosis and prompt initiation of anti-coagulation therapy is important to avoid irreversible kidney damage. Acute renal infarction should be considered as a cause of acute onset flank pain in patients with risk factors and normal initial screening test.

PUB434

Ceftriaxone Induced Acute Encephalopathy in a Peritoneal Dialysis (PD) Patient Sami Safadi, Michael A. Mao, John J. Dillon. *Nephrology and Hypertension, Mayo Clinic, Rochester, MN.*

Introduction: Encephalopathy is a rare side effect of 3rd and 4th generation cephalosporins. Renal failure and preexisting neurological disease are notable risk factors. Recognition is important as discontinuation of the offending agent usually resolves symptoms. We present a case of acute encephalopathy in a patient with ESRD on PD who received IV ceftriaxone for peritonitis. This case raises awareness of the potential severe neurologic effects of cephalosporins, which are recommended by international guidelines as first-line antimicrobial therapy for spontaneous bacterial peritonitis.

Case Description: A 37 y/o female with ESRD due to lupus nephritis, on PD presented with fever and watery diarrhea. Medications included mycophenolate mofetil and prednisone. Her PD catheter exit site was clean. Initial lab workup showed no leukopenia/leukocytosis. The patient initially received IP ceftazidime(125 mg/L) for yersinia enterocolitica peritonitis. Two days later, she was admitted for possible sepsis, and switched to IV ceftriaxone(2 grams/day). PD was continued. After 3 days of IV ceftriaxone, she developed agitation and visual hallucinations. Her exam was non-focal. An EEG showed background moderate diffuse nonspecific slowing without seizure activity. An MRI showed cerebral volume loss but no focal findings to account for the symptoms. Since these symptoms occurred a few hours after ceftriaxone infusion, it was switched to ciprofloxacin with complete symptom resolution after 36 hours.

Discussion: Neurotoxicity has been reported in both 3rd and 4th generation cephalosporins, though more frequently with cefepime. Possible mechanisms are inhibition of GABA release leading to neurological excitation and endotoxin release from dead bacteria leading to cytokine generation. Dialysis patients are vulnerable due to altered drug pharmacokinetics, and dosing changes. In conclusion, encephalopathy is a rare but severe side effect of cephalosporins. Renal failure is a risk factor. Timely diagnosis can be delayed due to the broad differential diagnoses of encephalopathy in dialysis patients. However, recognition is important as cessation of the medication typically leads to resolution of symptoms, decreased morbidity and costs.

PUB435

Immediate-Peritoneal Dialysis Post-Kidney Transplantation: An Approach to Avoid Catheter-Related Vascular Complications Ekamol Tantisattamo. *Renal Div, Emory Univ School of Medicine, Atlanta, GA.*

Introduction: Delayed graft function (DGF) post-kidney transplantation may require dialysis and peritoneal dialysis (PD) is an option for PD patient. However, concerning for intraabdominal complications may lead to hesitation in performing PD. We report a case of a PD patient who developed DGF. Hemodialysis (HD) was planned despite PD catheter was still in place. Common carotid artery was accidentally punctured and dilated requiring insertion with a large-bored non-tunnel hemodialysis catheter to stop bleeding. The catheter was emergently surgical removed.

Case Description: A 50 year-old man with history of ESRD on PD was admitted for deceased donor kidney transplantation (DDRT). The preoperative induction immunosuppressive medications included basilliximab, methylprednisone, and belatacept. The operation was uneventful. Cold and warm ischemic times were 18 hours 41 minutes and 0.5 hours, respectively. Eighteen hours after operation, he developed DGF with

serum creatinine (SCr) of 10 mg/dL from preoperative SCr of 11 mg/dL. Urine output was 25-30 ml/hour. Serum K was 5.6 mmol/L. Tacrolimus trough level was 21 ng/mL. HD was planned via the right IJ vein despite PD catheter was not removed during DDRT. Unfortunately, the right common carotid artery was accidentally punctured and dilated. Due to rapidly bleeding from the dilated artery even direct pressure, the 14-Fr dialysis catheter was inserted. Emergent catheter removal and common carotid artery repair were performed in the operating room. His vital signs had been stable and neurological signs were intact. Postoperatively, he required HD once via the left femoral dialysis catheter and DGF was improved. He was discharged on postoperative day6 with SCr of 4.2 mg/dL and Hb of 8 mg/dL.

Discussion: Given extraperitonial approach during DDRT, PD is a feasible option for dialysis. The possible complications of immediate-PD post-kidney transplant including peritoneal leak, infectious complications such as peritonitis could be avoided by individualized PD prescription. This approach may prevent unnecessary invasive procedure with potential fatal complications such vascular HD catheter placement-related complications.

PUB436

Acute Tacrolimus Nephrotoxicity in Graft-versus-Host Disease Patient: Overdose and Interaction

Ekamol Tantisattamo, Harold A. Franch. *Renal Div, Emory Univ, Atlanta, GA.*

Introduction: Drug-drug interactions lead to calcineurin inhibitor (CNI) dosing much lower than expected by medical personnel creating potential for medical errors.

Case Description: A 60-year-old woman with history of diffuse large B-cell lymphoma status post allogeneic bone marrow transplantation, GVHD on tacrolimus 0.5 mg oral 3 times a week and boosted-voriconazole 200 mg twice a day, was admitted for an elective colectomy for colonic adenocarcinoma. The operation went well and pain was controlled with morphine PCA. However, she received tacrolimus 5 mg oral once post operative day 1. Twenty nine hours later she was unresponsive and did not respond to naloxone. Shortly, she awoke confused complaining of tinnitus, tingling, and tremors. She developed non-oliguric acute kidney injury with elevated serum creatinine of 2.02 mg/dL from the baseline of 1.10 mg/dL. Urine microscopy showed bland urinary sediment and FE_{Na} was 0.11%. Tacrolimus was held and the tacrolimus level was 18.5 ng/mL 31 hours after the dose. Renal function and neurological symptoms improved to baseline and tacrolimus level went down to therapeutic level (Figure1). The physician who wrote for the wrong dose of tacrolimus admitted that he was unfamiliar with extremely low doses of tacrolimus for drug-drug interactions.

Discussion: Even though tacrolimus absorption post-intestinal surgery may be decreased, our patient developed nephro- and neurotoxicity from a single 10 fold higher dose of tacrolimus, due to her concomitant voriconazole. Her small home tacrolimus dose contributed to the error. Carefully monitoring CNI level especially in perioperative period and with concomitant medications causing potential drug-drug interaction is crucial to achieve adequate dose and avoid CNI toxicity. In addition, automated flagging in the electronic medical record of large changes in CNI dosing could have prevented this outcome.

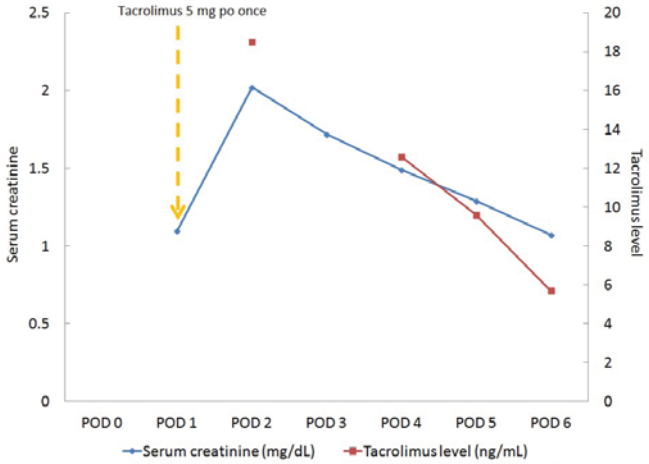

Figure1: *Postoperative course, serum creatinine and tacrolimus level*

PUB437

Lacosamide Intoxication Induced Acute Kidney Injury in a Suicide Attempt by an 18 Year Old Woman

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Introduction: Lacosamide (LCM) is approved by the U.S. Food and Drug Administration for the management of partial-seizures. LCM acts on sodium channels. Suicide risk and cardiac arrhythmias are reported with its use. Little is known about its kidney toxicity at higher doses.

Case Description: We report the clinical course of an 18-year-old woman with refractory partial-seizures, being treated with LCM, who overdosed herself with 12 g of LCM in a suicide attempt. Her course was complicated by coma, respiratory failure requiring mechanical ventilation, shock requiring IV pressors, and acute kidney injury (AKI) with anuria and severe acidemia managed initially with a session of hemodialysis (HD) then continuous renal replacement therapy (CRRT) for 60 h followed by a session of HD. On presentation, the pH, bicarbonate, lactic acid were 6.9, undetectable, 19.2 mmol/L, respectively which corrected to 7.44, 24 mg/dL and 7.05 mmol/L respectively. The serum BUN, Creatinine, potassium levels were 7 mg/dL, 1.09 mg/dL and 2.8 mmol/L respectively. Urine demonstrated 3-5 coarsely pigmented granular casts consistent with acute tubular necrosis (ATN). Complete clinical recovery occurred after several days of supportive care. The serum creatinine level on presentation was 1.09 mg/dL which peaked at 19 mg/dL before returning to 1.2 mg/dL in about 45 days post ingestion.

Discussion: This case highlights how LCM intoxication can result in AKI requiring CRRT and HD. After oral intake of LCM, it peaks in 1-4 h and has a 13 h elimination half-life. A recent study showed that severe renal impairment reduces total body clearance of LCM from 30% to 11% and that a 4 h HD session clears 50% of LCM. In our patient LCM intoxication may have induced cardiac dysfunction by its direct action on cardiac sodium channels, as a result, reduced cardiac output, leads to hypotension and ATN. Thus, patients initiated on LCM therapy should be alerted of the potential complication of AKI as a result of intoxication with LCM. The case also underscores how the clinical course of AKI after LCM intoxication and its management is still unclear and warrants further research.

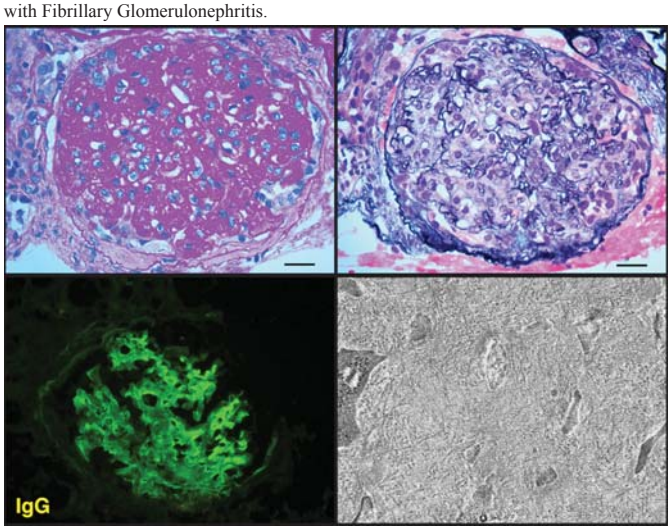
PUB438

An Unusual Renal Biopsy Finding in a Patient with Type 2 Diabetes Mellitus

Kerri A. McGreal, Elizabeth W. Dehmer, Timothy A. Fields. *Nephrology, Univ of Kansas Medical Center, Kansas City, KS.*

Introduction: Diabetes is the most common cause of ESRD in the United States, and many nephrology referrals are for patients who, without biopsy, are presumed to have diabetic nephropathy (DN). However, case series in the literature indicate that a significant number of diabetic patients who undergo renal biopsy have disease other than or in addition to DN. We present a case that highlights clinical features in diabetics that should prompt renal biopsy.

Case Description: A 55 year-old morbidly obese male with well controlled hypertension, type 2 diabetes mellitus of <10 years duration without retinopathy, and baseline CKD 3 was referred to nephrology clinic for worsening renal function. His renal function had been stable with a serum creatinine (SCr) of 1.9 g/dl that had worsened to 2.7 over the course of 4 months with an increase in urine protein/creatinine ratio (UP/C) from 1.8 to 4. Urinalysis showed 3+ protein without blood; urine microscopy was unremarkable. Serologic work up for glomerular disease was negative. Six months later his SCr was shown to be 3.67 with a UP/C of 8. Renal biopsy was performed, which revealed IgG-containing, non-congophilic, fibrillar deposits (15-25 nm in diameter) in mesangial areas, consistent with Fibrillary Glomerulonephritis.



Discussion: DN is an unfortunately common complication of type 2 diabetes. The prevalence of non-diabetic renal disease in diabetics is difficult to assess, since most patients with presumed DN are not biopsied. Clinical features in diabetics that should prompt renal biopsy to rule out non-diabetic disease include shorter duration of diabetes, absence of retinopathy, microscopic hematuria, abrupt onset of proteinuria, and active urine sediment. This case, with rapid progression of renal insufficiency and proteinuria, illustrates the utility of applying these criteria for selecting diabetic patients for biopsy.

PUB439

A Case of Plavix Induced Thrombotic Thrombocytopenic Purpura (TTP)

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Introduction: Ticlopidine has been associated with the development of TTP, estimated incidence 1: 1600 to 5000. Clopidogrel widely replaced it due to more favorable safety profile. Approved by FDA in early 1998, > 3 million people received the drug with few reported cases of induced TTP.

Case Description: Case: 82 year old male with CAD s/p CABG, Type 2 DM, HBP, CKD, baseline creatinine (Cr) of 1 presents to ER with SOB and CP. The diagnosis was

NSTEMI, treated (Tx) with ASA, atorvastatin, and Plavix 300mg load followed by 75mg daily. On day 7, hemoglobin dropped to 8.0, platelet (Plt) to 69 from 280 thou/mm^3 and Cr to 2.0 mg/dl. He became confused and required intubation. LDH was 521 U/l with haptoglobin level 126. HIT panel was negative. Peripheral smear demonstrated > 50% schistiocytes. Thinking of Plavix induced TTP, Plavix was substituted with Ticagrelor. ADAMTS13 was sent and daily plasmapheresis (Plex) initiated with cryo poor FFP. After 5th session Plt counts were 150 thou/mm3, Cr stabilized between 1.2 – 1.3 mg/dl and LDH improved to 188. With this improvement sessions were decreased to alternate day Tx. ADAMTS13 returned normal at 52%. Plex was then tapered to twice weekly for 1 week and then stopped. Plt counts stabilized at 190-220 thou/mm^3 and Cr improved to 1.0mg/dl. He was still intubated more alert and responding by head nodding.

Discussion: TTP is a life-threatening multisystem disease. Drugs associated are Quinine, Mitomycin C, Gemcitabine, Cisplatin, Tamoxifen, Bleomycin, Cytosine, Arabinoside, Daunomycin, Cyclosporine A, OCP, Penicillin, Rifampin and Ticlopidine. Historically, TTP in patients receiving clopidogrel have some interesting features. Firstly, TTP usually occurs within 2 weeks after the initiation of treatment. Secondly they require more plasmapheresis, sometimes >30, and have frequent relapses. Our patient was interesting as improvement was noted after 5th treatment and did not have any relapse, perhaps related to rapidity of diagnosis and onset of treatment. Early consideration of TTP with this presentation among patients receiving clopidogrel may impact outcomes.

PUB440

Hypertensive Emergency after AV Fistula Balloon Angioplasty in a Patient with Non-Obstructive Hypertrophic Cardiomyopathy Jose F. Lizcano Perez,[1] Ammar Almakkee,[1] Craig S. Courville,[2] Alfredo M. Peguero.[2] *[1]Nephrology, Univ of South Florida, FL; [2]JAHVA Medical Center, Tampa, FL.*

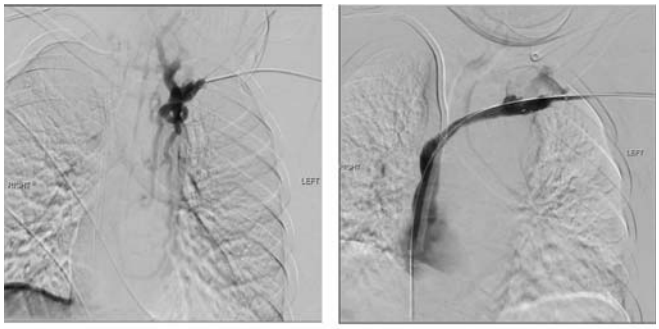
Introduction: 69 year-old patient with ESRD secondary to hypertensive nephrosclerosis and non-obstructive hypertrophic cardiomyopathy without valvulopathy and preserved LV function by echocardiography underwent balloon angioplasty of subclavian vein for stenosis. Followed successful intervention patient experienced severe pulmonary edema in association with arterial hypertension without evidence of cardiac arrhythmias or acute coronary event. On clinical examination he was found to be in acute respiratory distress with hypoxemia requiring supplemental oxygen by non-rebreather mask with FiO2 100%. BP 210/96, HR 112 (sinus tachycardia), and bibasilar wheezes with crackles on auscultation but no significant peripheral edema or any other clinical evidence of volume overload. Post procedure CXR significant for pulmonary edema. Patient's hypertensive emergency was treated medically with appropriate BP response and improvement of symptoms prior to initiation of dialysis. Patients with HCM usually have normal to supra-normal LV systolic function; however, they can experience diastolic dysfunction due to decreased ventricular compliance and impaired relaxation. Impaired relaxation in HCM results in a reduced rate and volume of filling during the rapid filling period of diastole, with a resultant compensatory increase in atrial systolic filling. Late in the evolution of diastolic dysfunction, a restrictive type of diastolic filling defect may become evident in which a high atrial pressure results in an increased rate and volume of filling during the rapid filling period with reduced filling during atrial systole. Our case illustrates the pathophysiological changes experienced by a patient with HCM having pulmonary edema secondary to hemodynamic changes post angioplasty which leads to rapid increased flow and venous return to the right heart in someone with decreased ventricular compliance and impaired relaxation who cannot maintain an adequate stroke volume despite having a good LV systolic function in the face of increased sympathetic response leading to an increase in afterload.

PUB441

Recannulation of Central Vein Stenosis—Maintain Lifeline Patent Jie Cui,[1,2] Zubin Irani.[2] *[1]Nephrology Dept, Massachusetts General Hospital, Boston, MA; [2]Interventional Radiology Dept, Massachusetts General Hospital, Boston, MA.*

Introduction: Central vein stenosis (CVS) is one of the most common complications for dialysis access. Failure to recannulate central veins is detrimental, and usually leads to loss of dialysis access in upper extremities. Successfully recannulate stenotic lesions can not only save patient's upper extremity for future access creations but sometimes can also salvage nonfunctional arteriovenous fistula (AVF).

Case Description: A 44-year-old women with a history of SLE induced end-stage renal disease (ESRD) was initiated on dialysis therapy 12 years ago. She was initially received peritoneal dialysis, which was failed due to frequent peritonitis. In 02/2013, hemodialysis was initiated via right tunneled internal jugular dialysis catheter and right brachiocephalic AVF was created in the meantime. One month later, fistulogram showed occluded right cephalic vein, which failed angioplasty. She then had a left brachiobasilic AVF creation in 04/2013. However, the left side fistula failed to mature, and patient remained catheter dependent. In 08/2013, patient developed facial swelling and was diagnosed with superior vena cava syndrome. Fistulogram showed occluded bilateral brachiocephalic veins, and left brachiocephalic vein was successfully recannulated. A drug-eluting stent was placed in the left brachiocephalic vein.



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Patient's left AVF was matured in 02/2014. Patient received hemodialysis through left AVF since then and fistulogram in 04/2014 showed widely patent AVF.

Discussion: Bilateral CVS is detrimental for ESRD patient, which leads to no access options in upper extremities. Great attention and best effort should be given to treat CVS. Successful recannulation of CVS can salvage dysfunctional AVF. Dialysis access strategy should be carefully planned prior to dialysis initiation, and catheter should be avoided if possible.

PUB442

Use of High-Flux Hemodialysis for Vancomycin-Induced Acute Kidney Injury (AKI): A Case Report Snigdha Reddy, Jerry Yee, Vivek Soi. *Div of Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Introduction: Vancomycin is commonly selected as therapy for presumed serious bacterial infections. Serum trough concentrations of 15–20 mcg/mL are recommended for treatment of pneumonia in adults. Vancomycin-associated nephrotoxicity (VAN) has occurred, despite adherence to these recommended levels. A standardized outpatient vancomycin dosing protocol may not apply to all patients. There is paucity of literature on the use of hemodialysis (HD) to treat VAN. We report a case of a patient treated by high-flux HD for VAN to prevent multiorgan failure.

Case Description: A 45 y.o. male with untreated AIDS and esophageal candidiasis was discharged on a 3-week course of vancomycin and ertapenem for necrotizing pneumonia. His outpatient vancomycin trough level was 141.6 mcg/mL and further evaluation revealed oliguric AKI with a SCr level of 5.71 mg/dL (repeat 154.9 mcg/mL) and eosinophilia 11%. Urinalysis revealed trace protein, and the sediment exam revealed 0–2 granular casts/LPF. Labs pre-discharge showed a SCr 0.54 mg/dL and vancomycin trough level of 18.9 mcg/mL. To avoid extrarenal toxicities (ototoxicity, agranulocytosis, etc) that may occur with high levels, hemodialysis (HD) was initiated for vancomycin clearance. An initial 5-h treatment was conducted with a 1.6 m^2, high-flux membrane and followed by 2 additional HD treatments using a 2.5 m^2, high-flux membrane (Rexeed 25S) to mitigate vancomycin "rebound". Notably, 71% of vancomycin was removed in 36 h, without any adverse effects. HD was discontinued when the vancomycin level was <50 mcg/mL and urine output exceeded 1 L/24-h. After conclusion of 3 HD sessions, the SCr was 1.29 mg/dL on hospital day 10. A kidney biopsy performed on the fourth hospital day demonstrated acute tubular necrosis and severe allergic interstitial nephritis. At follow-up, 4 months post-discharge, the SCr was 0.8 mg/dL.

Discussion: Frequent monitoring of vancomycin trough levels is recommended in patients treated with long-term regimens. Serial, high-flux HD should be considered in patients who are at risk for potentially irreversible vancomycin toxicity.

PUB443

Intraoperative Extracorporeal Membrane Oxygenation Rescue for Pulmonary Hemorrhage during Orthotopic Liver Transplantation Muhammad R. Syed, Gary S. Xiao, Stephen Guy, Talal A. Khan, Purna Bindu Nandigam, Dhaval Sureja, Shabnum Haleem, Amit A. Deshpande, Disha Narula, Karthik M. Ranganna, Gregory Malat, PharmD, Alden Michael Doyle. *Div of Nephrology, Drexel Univ College of Medicine, Philadelphia, PA.*

Introduction: Herein we report the first case of the use of intraoperative extracorporeal membrane oxygenation (ECMO) for a transplantation procedure. Data from the international extracorporeal life support organization (ELSO) reports nearly 51,000 patients have received extracorporeal life support (ECLS) through July 2012. Of these, 50% (>25,000) were for neonatal respiratory failure. Previously stable at about 100 cases a year for a decade, adult respiratory failure ECLS cases saw a dramatic increase in 2009 with the H1N1 influenza pandemic.

Case Description: A 54 year old white female with alcoholic cirrhosis underwent an orthotopic liver transplant procedure at our facility. Soon after the liver was reperfused, profuse bleeding started through the endotracheal tube. Despite multiple blood product transfusions and an intraoperative bronchoscopy, the bleeding continued. Blood gases revealed a PO2 of 48 mmHg and a PCO2 of 180 mmHg. The transplantation procedure could not proceed further so a quick decision was made in the OR to initiate high flow ECMO through the existing venovenous bypass circuit to reverse the severe hypoxemia. After initiating ECMO, the congestion around the liver reduced enough to allow for the biliary system anastomosis. Patient remained on ECMO for 7 days post-op until the pulmonary

hemorrhage was controlled with embolization of the bronchial arteries. Our patient made a remarkable recovery and is now doing well at home with excellent allograft function.

Discussion: Our case demonstrates that ECMO can be successfully employed emergently during a complicated transplantation procedure to overcome acute respiratory failure, especially when an existing venovenous bypass circuit is in place.

PUB444

Myositis Ossificans – A Case Report Bo Broberg,[1] Ewa Lewin,[1] James G. Heaf,[1] Henrik Groenborg,[2] Henrik Post Hansen.[1] *[1]Dept of Nephrology, Copenhagen Univ Hospital at Herlev, Copenhagen, Denmark; [2]Dept of Orthopedic Surgery, Univ Hospital Copenhagen at Herlev, Copenhagen, Denmark.*

Introduction: Extraskeletal calcifications (EC) are common manifestations of Chronic Kidney Disease - Mineral and Bone Disorder (CKD-MBD) in patients with advanced CKD. Myositis Ossificans is a rare example of EC with severe calcification related to muscle tissue, often due to trauma or surgery. The condition is often related to a high calcium-phosphate product.

Case Description: We present a 67-year old woman with ESRD, who had been on peritoneal dialysis for 6 years. She previously had severe hyperparathyroidism (HPT), treated with parathyroidectomy and later had a second relapse treated with alfacalcidol, calcium carbonate, and cinacalcet. Due to Budd-Chiari Syndrome the patient was treated with Warfarin. The patient had undergone a total hip arthroplasty in 2009. Over several months from 2012 she developed progressive, invalidating pain at the right gluteal region. Clinical examination and X-ray revealed a large solid tumor mass (20x15 cm) at the right gluteal region and the diagnosis of Myositis Ossificans was made. This was confirmed by MR/PET-CT and DEXA scan. A biopsy showed changes compatible with chondrocalcinosis and no signs of malignancy. Due to a high risk of recurrence of the tumor mass, surgical removal was not chosen. The patient was subjected to intensive treatment, focusing upon reducing the calcium and phosphate product. Alfacalcidol and calcium carbonate was discontinued and the patient was switched to intensive HD (4 hours 5 times per week), using ultralow calcium (Ca^{++} 1.00 mM) and low bicarbonate dialysate (25 mM). Treatment with cinacalcet was intensified. Initially an increase in blood levels of calcium and phosphate was observed and interpreted as a sign of mobilization from the tumor mass. The symptoms gradually diminished and clinical examination and radiographic investigation 3 months later showed significant reduction of the tumor mass.

Discussion: The present case demonstrates an impressive and rapid reversal of large extraskeletal calcification, induced by intensive treatment of CKD-MBD.

PUB445

A Rare Case of Hyperkalemia in a Patient with ESRD Amir Abdi Pour. *Internal Medicine/Nephrology, Loma Linda Univ Medical Center, Loma Linda, CA.*

Introduction: Hyperkalemia is common in ESRD patients it could be be fatal. It should not be observed in adequately dialyzed patients, especially post dialysis. We report a case of hyperkalemia in a patient with ESRD undergoing kidney transplant.

Case Description: A 55 year old female with ESRD due to hypertension was called in for deceased donor kidney transplant. She was dialyzed adequately prior to admission. Her exam was unremarkable. She was not on a beta blocker or NSAIDs. She was anuric. Her serum potassium (K) was 4.4 mg/dl on admission, which was drawn 6 hours after dialysis. An hour after induction of anesthesia with Recuronium, Propofol, versed, and fentanyl while on Sevoflurane for maintenance, the anesthesiologist noted a serum K of 6.7 mg/dl and pH of 7.53 on ABG. Stat basic metabolic panel showed blood sugar of 124 mg/dl and K of 6.8 mg/dl. She did not have any electrocardiogram changes. She was given calcium gluconate, insulin, and glucose. Her serum K gradually decreased to 4.4 mg/dl post surgery. She remained anuric during the operation. She was oliguric over the next day and was taken back to the operating room for exploration of possible arterial kinking. Her serum K was 4.5 mg/dl prior to the second operation. She was exposed to same anesthesia medications except that cisatracurium was used this time. Immediate post operative serum K was 6.4 mg/dl.No labs were drawn during the second operation. She was dialyzed for 2 hours. Her serum K postdialysis was 3.7 mg/dl.

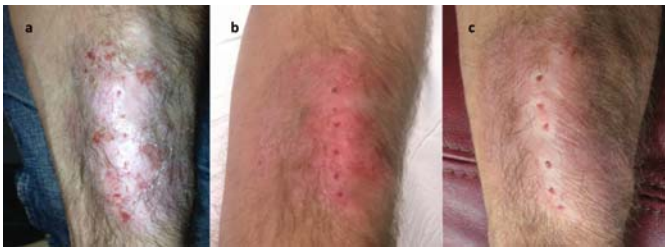
Discussion: Sevoflurane is a nonflammable volatile liquid and is administered via inhalation of the vaporized liquid. Sevoflurane is defluorinated via cytochrome p450 (CYP) 2E1 resulting in the production of hexafluoroisopropanol (HFIP) with release of inorganic fluoride (F) and carbon dioxide (1). Studies have shown that F^- increases intracellular Ca^{2+}, which is thought to trigger Ca^{2+}-dependent K^+ channels and produce a K^+ efflux. (2) We believe hyperkalemia was due to K shift form being exposed to sevoflurane. To the best of our knowledge, this has not been reported in the literature. References 1. medicines.org.uk 2. Fluoride-induced hyperkalemia: The role of Ca^{2+}-dependent K^+ channels Charles C. Cummings, MD, Michael E. McIvor, MD AJEM, January 1988.

PUB446

Dogs Are Not Always Our Best Friend Karlien Francois, Christopher T. Chan. *Div of Nephrology, Univ Health Network Toronto General Hospital, Toronto, ON, Canada.*

Introduction: We present a case of Staphylococcus pseudintermedius bacteremia highlighting the unique interaction of risk factors between environment and host in home hemodialysis.

Case Description: Mr. ME is a 49-year-old man with IgA nephropathy undergoing home nocturnal hemodialysis (HHD). During a regular clinic visit, we diagnosed severe allergic contact dermatitis at the level of his left radial cephalic arteriovenous fistula (AVF) [figure a]. We suspected chlorhexidine to be the allergen. Cleansing regimen was changed to water and soap wash and saline. Three months later, the skin lesions improved although dermatitis persisted [figure b]. A trial of topical 0.5% hydrocortisone cream was used and IV3000 dressing was switched to silk tape. Given the suspicion of skin superinfection, blood culture (BC) was drawn from the AVF site. One day later (D1), the gram stain of the BC showed gram positive cocci in clusters and BC from AVF site as well as from the other arm were repeated. Antibiotics were deferred given the patient was asymptomatic. On D4, both BC drawn from the AVF arm but not the peripheral grew coagulase-negative staphylococci and oral cefazolin was initiated. On D6, the results of both positive BC were corrected to Staphylococcus pseudintermedius. S. pseudintermedius isolates are not reliably susceptible to beta-lactam antibiotics. Intravenous Vancomycin was prescribed for a total of 5 doses. A favourable evolution of the skin lesions was noted when ending the Vancomycin treatment [figure c].



Discussion: Human colonization and bacteremia with S. pseudintermedius, a coagulase-positive staphylococcus occurring in domestic animals, is rare and has never been reported in patients treated with dialysis. Contact with dogs in the setting of an inflamed vascular access site resulted in the infection in our case. HHD programs need to have a continuous awareness for vascular access care and the interaction with environmental risks.

PUB447

Differentiating Cerebral Salt Wasting (CSW) from SIADH in Critically Ill Patients Muzzaffar Hussain, Jiuming Ye, Gregory Lee Braden, Benjamin J. Freda, Michael H. O'Shea, Jay Steingurb. *Dept of Medicine, Baystate Medical Center, Springfield, MA.*

Introduction: Differentiation of CSW from SIADH can be difficult and proper assessment of extracellular fluid volume (ECFV) status is key in differentiation. We studied a case of CSW characterized by continued elevation of Nt-proBNP levels and increased fractional excretion of urate(FEUrate) after correction of hyponatremia which differentiated CSW from SIADH.

Case Description: A 57 year old woman presented to the ICU with intractable seizures and respiratory failure. Brain MRI showed hyper-intensity lesions. Brain biopsy revealed lymphoma. She received intravenous dexamethasone and radiation therapy. 3 weeks later, she developed increased urine output > 3 liters/day. She was clinically volume depleted, marked by hypotension(SBP 88mmHg), tachycardia(HR 108),low CVP(<4mmHg). She had a serum sodium of 126 mEq/L, uric acid 1.3 mg/dL, a urine sodium of 190 mEq/L and urine osmolality of 508 mOsm/kg water. FEUrate was 33% (normal <12%); Nt Pro-BNP was 3618 pg/ml. Kidney, thyroid and endocrine tests were normal; echocardiogram was normal. The constellation of hypovolemia, marked natriuresis, hyponatremia and hypouricemia in the setting of cerebral lesions suggested CSW. With isotonic saline serum sodium increased to 133 mEq/L; urine osmolality was 120 mOsm/Kg water and urine sodium of 90 mEq/L. Though the serum sodium normalized at 72 hours, Nt-proBNP remained markedly elevated(6856pg/ml) and the patient continued to have significant renal salt wasting, low serum uric acid levels and increased FEUrate (29%). Serum ADH was undetectable at the time of diagnosis and at 72 hours. 1 week later, serum sodium remained normal but Nt-ProBNP and FEUrate were still significantly elevated.

Discussion: CSW can be differentiated from SIADH in critically ill patients by carefully assessing intravascular volume status and by monitoring for persistently high FEUrate and elevated levels of Nt-ProBNP after normalization of hyponatremia.

PUB448

A Unique Case of Falciparum Malaria-Induced Glomerulopathy Sumedha Dhar, Yashpal S. Kanwar, Eudora Eng. *Nephrology, Feinberg school of Medicine, Northwestern Univ, Chicago, IL.*

Introduction: Acute kidney injury in the setting of falciparum malaria occurs in 15-48% of non-immune cases with renal biopsy demonstrating acute tubular necrosis (ATN), tubulointerstitial nephritis and glomerular changes with mesangial proliferation and immune complex deposition. Falciparum-induced glomerulonephritis mainly occurs in children with autopsy studies reporting a rate of 18%. Falciparum glomerulopathy is usually mild (< 1 gm of proteinuria) and rarely causes nephrotic syndrome. The highest reported proteinuria is 15 gm in a single case study from 1967. We present an unusual case of falciparum-induced glomerulopathy with persistent nephrotic range proteinuria despite infection eradication.

After returning from Nigeria without antimalarial prophylaxis, a 29-year old African-American female presented with altered mental status, fever, and oliguria. Her serum creatinine was 1.2 mg/dl with urine granular casts and a spot urine protein: creatinine ratio of 83.5 gm. After prompt treatment with artesunate, parasitemia rappidly dropped from 20% to undetectable. Renal replacement therapy was initiated on day 1 for oliguric renal failure and she remained dialysis-dependent for a month. Following discontinuation of dialysis, her serum creatinine stabilized at 2.5 mg/dl with proteinuria of 25 gm. Renal biopsy was performed for the persistent proteinuria despite complete eradication of malaria

parasitemia, showed chronic tubulointerstitial disease, ATN, glomerular proliferation with thickening and splitting of the glomerular basement membrane and immunofluorescence with Ig M and C3 deposition. Even on angiotensin converting enzyme inhibition (ACE-I), she continues to have 7.2 gm proteinuria.

Discussion: Falciparum malaria-induced nephropathy has been shown to be reversible by 2-6 weeks after infection eradication with few case reports documenting persistent mild proteinuria. We add to the scant body of literature a case illustrating persistence of high grade proteinuria despite early, effective treatment and ACE-I. Our case highlights the importance of malaria prophylaxis when travelling to malaria-infested areas, especially in non-immune populations.

PUB449

A Case of Immune Complex-Mediated Necrotizing Glomerulonephritis due to Bartonella Infection Shan Shan Chen,[1] Ameet T. Karambelkar,[1] Sheldon Bastacky,[2] Roderick J. Tan.[1] *[1]Renal-Electrolyte Div, UPMC, Pittsburgh, PA; [2]Pathology, UPMC, Pittsburgh, PA.*

Introduction: Treatment of glomerulonephritis (GN) is challenging if the underlying etiology is unclear. Here we report a rare case of culture-negative, transesophageal echocardiogram (TEE)-negative Bartonella henselae infective endocarditis (IE) causing acute kidney injury (AKI) due to an immune complex-mediated necrotizing GN.

Case Description: A 20 year old caucasian male presented with weight loss for 2 months and was found to have AKI. Past medical history was significant for congenital heart disease with aortic and pulmonic valve replacements. He studied as a veterinary student. Physical examination was significant for a temperature of 38.4° C and a systolic murmur at the left upper sternal border. Serum creatinine was 3.1 mg/dl compared to a baseline of 1.1 mg/dl. Urinalysis showed microscopic hematuria with dysmorphic RBCs and mild proteinuria (870mg/gm creatinine). C3 and C4 were decreased at 77 and 12 mg/dl, respectively. Extensive serologic, hematologic and infectious work-up including blood cultures, TEE and white blood cell scan was negative. Renal biopsy showed a necrotizing and crescentic GN with mesangial and focal subendothelial immune complex-type deposits with immunofluorescence studies positive for IgM, IgA, fibrinogen and C3. As the patient continued to have deteriorating renal and cardiac function, cardiac catheterization with intracardiac echocardiography was performed and revealed vegetations on the pulmonic valve. This was treated with valve replacement and pathology revealed Bartonella infection. After antimicrobial treatment, the patient's creatinine improved to 1.4 mg/dl.

Discussion: GN associated with Bartonella IE is a rare condition and it can manifest as an immune complex-mediated necrotizing GN and rarely as an ANCA-positive pauci-immune necrotizing GN. As it is usually culture-negative, diagnosis is often delayed and in this case required invasive testing for definitive diagnosis. It should be considered in patients with risk factors in which the initial work-up for infection is unrevealing. A high index of suspicion is needed, as misdiagnosis may lead to suboptimal or inappropriate treatment.

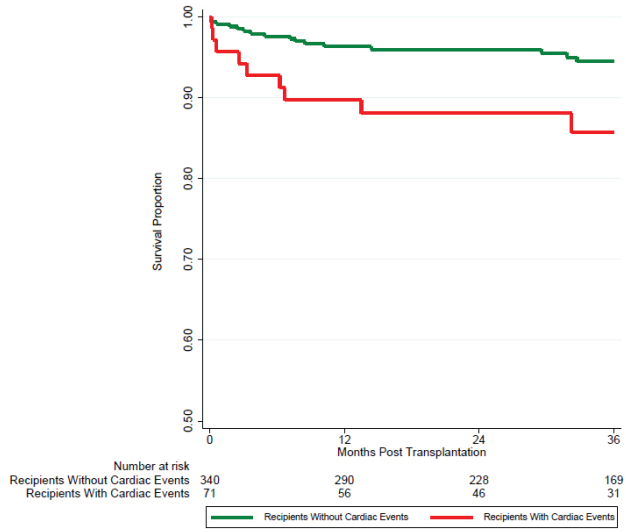
PUB450

Circulating Levels of Troponin of 0.5 ng/mL in the Immediate Post Transplantation Period: A Prognostic Biomarker of Mortality in the First 36 Months of Kidney Transplantation Guan Hua Xu, Darshana Dadhania, Thangamani Muthukumar, Manikkam Suthanthiran, John R. Lee. *Medicine, Weill Cornell Medical College, New York, NY.*

Introduction: Cardiovascular events are the leading cause of death in the first year of kidney transplantation. Early predictors for cardiac events as well as their outcomes on mortality have not been fully characterized.

Case Description: We performed a retrospective chart review of 411 renal allograft recipients who received their allografts from 2005 to 2010 at our center and who had a troponin evaluated within the first month of transplantation. We collected demographical characteristics and pre-transplant cardiac stress test evaluations and evaluated whether they were associated with development of cardiac events defined as a post-transplant circulating troponin level greater than 0.5 ng/mL. We also evaluated whether the cardiac events were associated with death using Kaplan Meier survival analysis.

71 of the 411 renal allograft recipients had a cardiac event (troponin greater than 0.5 ng/mL) within the first month of transplantation. Recipients with a cardiac event were more likely to be diabetic (55% versus 43%, P=0.09, Fisher's exact test) and older (61.5±11.3 versus 58.2±11.3, P=0.02, two sample t test). The most common cardiac stress test utilized among the recipients who developed a cardiac event was a nuclear perfusion test. 53% of the nuclear perfusion tests had no perfusion defects prior to the cardiac event. Recipients with a cardiac event during the first month of transplantation had inferior survival than those without a cardiac event (P=0.01, log rank test).



Discussion: Better pre-transplant screening tests are needed to identify recipients at risk for critical post-transplant cardiac events. Moreover, circulating troponin level of 0.5 may serve as a prognostic biomarker of mortality in the first 36 months following kidney transplantation.

PUB451

Renal Failure as an Isolated Presentation of Sarcoidosis Bhupesh Khadka, Thomas M. Kaneko, Clay A. Block. *Nephrology, Dartmouth Hitchcock Medical Center, Lebanon, NH.*

Introduction: Sarcoidosis is a multisystem disorder characterized by formation of non-caseating granulomas in organs, most commonly in lungs, but may also involve other organs including eyes, lymph nodes and kidneys. Renal failure as an isolated manifestation of sarcoidosis is uncommon. Here we present one such case.

Case Description: A 65 year old white male with no medical history who started having progressive dry mouth, anorexia, listlessness, insomnia and weight loss of 25 pounds over the last 4 months was referred to renal clinic. He had creatinine of 1.64 mg/dl and calcium of 9 mg/dl. His chest x-ray, serum/urine protein electrophoresis and renal ultrasound were normal. A week later his creatinine was down to 1.38 mg/dl. Proteinuria was absent and urine sediment bland. Anti-nuclear antibody and anti-ssA/ssB antibody were negative. After 10 weeks at scheduled follow up visit, creatinine had risen to 2.7 mg/dl and calcium to 15 mg/dl. He was hospitalized and treated with intravenous normal saline, calcitonin and a dose of zoledronic acid. Computed Tomography scan of the chest/abdomen/pelvis showed bilateral <5 mm solitary pulmonary nodules without lymphadenopathy and bilateral enlarged pelvic lymph nodes. Biopsy of a pelvic node revealed non-caseating granulomas. He had elevated angiotensin converting enzyme level and dihydroxy vitamin D. He was started on prednisone with slow taper. His symptoms resolved, calcium level normalized and renal function improved to normal.

Discussion: Renal involvement in patients with sarcoidosis is uncertain but may occur in up to 35-50% based on several small series, but renal failure as isolated presentation of sarcoidosis is very rare. Also, hypercalcemia may be present only in about 20% of these cases. Delay in diagnosis and treatment of renal sarcoidosis may lead to chronic calcium deposition and ESRD in some cases. We should consider sarcoidosis in the differential diagnosis of unexplained renal failure and be aware that calcium level may be normal in such patients.

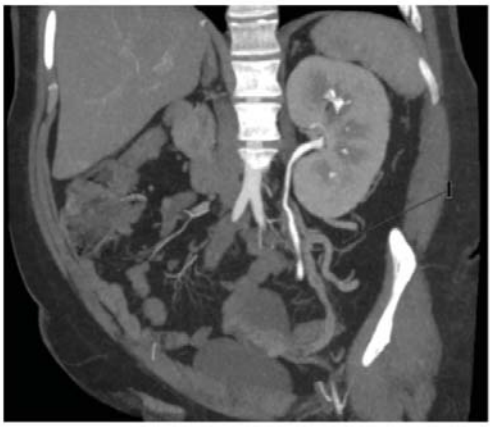
PUB452

Case Report: An Unusual Cause of Hematuria in Lupus Patient Jonatas Dantas, Alcino Pires Gama, Cristiane B. Dias, Luis Yu, Lectícia Jorge, Viktoria Woronik. *Nephrology, Univ of Sao Paulo, Sao Paulo, Brazil.*

Introduction: Lupus nephritis (LN) is the major cause of kidney injury in SLE patients and it demands prompt recognition and treatment. However, physicians must be prepared to recognize other causes of hematuria to avoid unnecessary immunosuppression (IS).

Case Description: A 43-year-old woman was referred to nephrologist due to "refractory hematuria". Six years ago received diagnoses of SLE and APS (with multiple episodes of venous thrombosis). IS and anticoagulation (AC) were started. Two years later, her rheumatologist diagnosed proliferative LN (based on increase serum creatinine plus hematuria and hypocomplementemia) and started prednisone plus mycophenolate mofetil. Kidney biopsy was not performed due to AC. After 6 months, both serum creatinine and complement levels normalized but hematuria persisted (despite INR levels always on therapeutic range). IS was not stopped nor reduced. Before nephrology consultation, she was previously investigated by an urologist, who performed an urinary tract ultrasound and cystoscopy (both unremarkable for hematuria causes). At presentation, except for persistent hematuria, there was no feature of lupus activity and the INR was on normal

range. Since the isolated hematuria did not seem to be due to glomerular or urologic disorders, we performed a Computed Angiotomography, then we identified the cause of persistent hematuria. The patient has an atrophic right kidney(probably secondary to renal artery thrombosis) and a vicarious left kidney into a venous hypertension regimen (due to multiple chronic thrombosis sites). Her left gonadal vein was remarkably thickened and in intimate contact with collecting system.



Discussion: Physicians who treat LN must be prepared to recognize other causes of hematuria and avoid unnecessary IS. Vascular disorders must always be remembered as a cause of isolated hematuria.

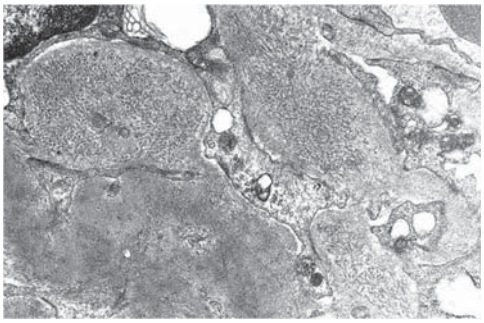
Funding: Government Support - Non-U.S.

PUB453

Fibrillary Glomerulonephritis in a Morbidy Obese Patient Presenting with Nephrotic Range Proteinuria and Elevated Creatinine Gaurang P. Mavani, Jordan L. Rosenstock. *Div of Nephrology, Lenox Hill Hospital, New York, NY.*

Introduction: 43 year old morbidly obese white male presented with elevated serum creatinine and proteinuria. Kidney biopsy revealed fibrillary glomerulonephritis. This case supports the recommendation that kidney biopsy should be considered in morbidly obese patients presenting with nephrotic range proteinuria.

Case Description: 43 year old male with past history significant for morbid obesity, hypertension and hypothyroidism was referred to a nephrologist for CKD. Creatinine was 1.5 mg/dl in 2012 associated with proteinuria. In October 2013 creatinine increased to 1.95 mg/dl associated with persistent proteinuria. Home medications include amlodipine, synthyroid and simvastatin. Physical examination revealed morbid obesity with BMI 42, blood pressure 150/100 mmHg; pulse 70/min. Examination did not reveal edema. Hb 12.7 g/dL, BUN 27 mg/dL, creatinine 2.27 mg/dL, eGFR 34. Urine analysis revealed 4+ proteinuria, RBC 4-10. 24 hour urinary protein excretion was 8902 mg. Sonogram of the kidneys revealed normal size. Serum immunofixation and hepatitis panel was negative. CT guided kidney biopsy revealed mesangial proliferative and sclerosing glomerulonephritis with segmental membranoproliferative features. Electron microscopy revealed fibrillary glomerulonephritis.



He was started on losartan and is scheduled for follow up visit and may be treated with rituximab for his disease.

Discussion: This case is an example of an unexpected diagnosis in a patient whose kidney disorder was thought to be related to his morbid obesity. Kidney biopsy should be considered in morbidly obese patients who have features atypical for obesity related secondary FSGS such as high grade proteinuria.

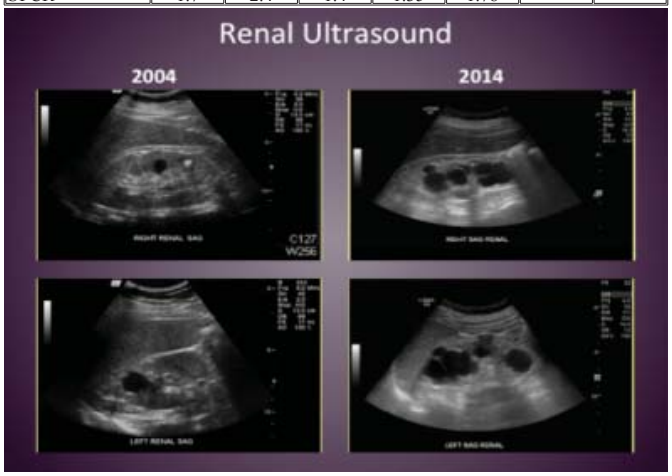
PUB454

Natural History of Renal Involvement of Fabry Disease in a Female Carrier Basma Omar Merhi, Reginald Y. Gohh. *Medicine, Brown Univ, Providence, RI.*

Introduction: Fabry disease(FD) is an X-linked genetic disease caused by mutation in GLA gene resulting in α-galactosidase A (GalA) deficiency, characterized by globotriaosylceramide accumulation in vessel walls and cortical and parapelvic renal cysts, progressing to end-stage renal disease(ESRD) in males and some females. We report a case of FD in a female carrier where normal GalA resulted in misdiagnosis and progression to ESRD.

Case Description: Case presentation: A 41-year old female presented in 1997 for 3-year history of subnephrotic proteinuria and creatinine(Cr) 0.8mg/dl. She had a normal renal ultrasound(US), negative ANA, C3/C4, lupus anticoagulant and hepatitis serologies. In 2000, ophthalmologic evaluation revealed corneal whorl-like opacities suspicious for FD, but normal GalA. She declined kidney biopsy and managed with lisinopril. In 2004, follow-up US showed thin lobulated renal cortices with cysts and stable Cr. She developed stroke-like symptoms in 2007 and atrial fibrillation in 2009. 2010,routine labs revealed Cr up to 1.8mg/dl. She was lost to follow-up until 2013, when she was referred for Cr 4.5mg/dL (Table 1). US showed cortical thinning and cystic parenchymal replacement(figure 1). Symptoms and exam are notable for leg pain, anhidrosis and angiokerotomas. Genetic testing demonstrated heterozygous mutation of Y207X variant of GLA gene, confirming FD. She got referred for enzyme replacement therapy (ERT).

Year	2000	2003	2007	2009	2010	2013	2014
Creatinine	0.9	1.2	1.2	1.79	1.83	4.5	5.36
UPCR	1.7	2.4	1.4	1.35	1.78		



Discussion: Unlike in males, α-GalA activity is not a reliable marker of FD in carrier females. Genetic testing represents a more reliable diagnostic assay. Despite having normal α-GalA level, the renal involvement in females can be as severe as in males progressing to ESRD. Early diagnosis and ERT may alter the natural history of kidney disease in affected patients.

PUB455

Case Report of a Patient with Crescentic Necrotizing Lupus Nephritis (LN) with Positive Anti-Neutrophil Cytoplasmic Antibody (ANCA) and False Positive Human Immunodeficiency Virus (HIV) Test Maybel M. Tan, Aiyu Zhao, Suchita J. Mehta, Mary C. Mallappallil, Moro O. Salifu. *Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.*

Introduction: Patients with systemic lupus erythematosus (SLE) may present with a variety of autoantibodies and some false positive results such as HIV. In addition, ANCA have also been detected in the serum of some patients with LN. We present an unusual case with both HIV and ANCA positive in newly diagnosed SLE. Verification of HIV was negative by repeat western blot testing. Positive myeloperoxidase (MPO) and proteinase-3 (PR-3) verified to positive ANCA.

Case Description: 50 year-old-woman with history of chronic sinusitis, hypertension, pancytopenia presented with intractable nausea, vomiting and weakness for 6 weeks. Physical exam was significant for mild lower extremities edema. She was found to have acute kidney injury with peak serum creatinine of 6.7mg/dl, low serum albumin, nephrotic range proteinuria and microscopic hematuria with no cast. HIV ELISA test was positive, however which was negative with western blot testing. She was also noted positive antinuclear antibodies, ANCA, MPO, PR-3 and Anti-Smith. A kidney biopsy showed grade IV-G LN with necrotizing crescents. Hemodialysis was initiated because of worsening of kidney function. Her condition was noted to be progressive despite steroids, cyclophosphamide, IV gammaglobulin and she developed spontaneous pulmonary hemorrhage resulting in death after an attempt at bronchoscopic embolization.

Discussion: ANCA can be seen in 37.3% of LN, predominantly perinuclear pattern (p-ANCA). The titers of ANCA were more in LN as compared to SLE without nephritis. LN cases with crescents had higher titer p-ANCA positivity with corresponding anti-MPO antibodies. A common problem in SLE is that nearly all the patients are ANA positive by indirect immuno-fluoresce (IIF) and this might vitiate the IIF readings for ANCA, therefore the ELISA test is used to detect the presence of specific antibodies to individual

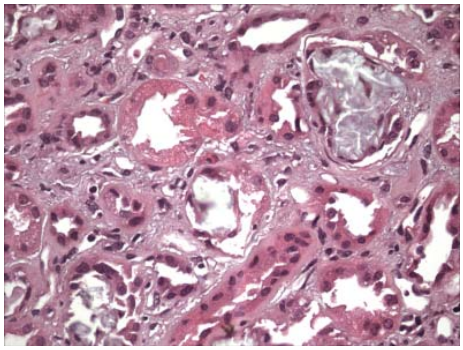
ANCA specificities. In this case HIV testing looking for antibodies to the p 24 gag protein of HIV-1 was a false positive due to the molecular mimicry to homologous host proteins.

PUB456

Acute Kidney Injury as a Complication of Total Gastrectomy: An Interesting Case of Oxalate Nephropathy Mamta Shah,[1] Rahul Mutneja,[1] Scott M. Benson.[2] *[1]Internal Medicine, Univ of Connecticut, Farmington, CT; [2]Nephrology, Hartford Hospital, Hartford, CT.*

Introduction: Roux-en-Y esophagojejunostomy is the preferred mode of reconstruction after total gastrectomy for gastric carcinoma. Oxalate nephropathy, although uncommon, has been seen as a complication of Roux-en-Y gastric bypass (RYGB). Prognosis is poor, and efforts towards prevention should be implemented early. In patients with acute renal functional deterioration and a history of RYGB, the differential diagnosis should include oxalate nephropathy, and renal biopsy should be considered.

Case Description: A 70 year-old male with newly diagnosed metastatic gastric adenocarcinoma underwent a total gastrectomy with Roux-en-Y esophagojejunostomy. Serum creatinine (Cr) at the time was at his baseline of 1.2 mg/dL. Four months later he was referred to nephrology for rapidly worsening renal function (Cr-4.8 mg/dL) with remarkable decline compared to 2 months prior (Cr-2.1 mg/dL). One month later he had kidney biopsy for worsening creatinine (Cr-8.1 mg/dL) and an otherwise negative work up. Biopsy revealed acute and chronic tubulointerstitial nephropathy with abundant calcium oxalate deposits consistent with oxalate nephropathy.



Hemodialysis was initiated.

Discussion: Oxalate nephropathy as a complication of RYGB procedure is thought likely from fat malabsorption with increased free fatty acid load in the intestine which binds calcium. This leads to increased free oxalate which is absorbed causing hyperoxaluria. Recurrent kidney stones and progressive medullary nephrocalcinosis lead to relatively rapid loss of kidney function, requiring dialysis or transplantation. Diagnosis is made by biopsy. It may be prevented by low-fat, low-oxalate diet along with calcium supplementation; but once the disease develops prognosis is rather poor with majority of patients eventually requiring permanent dialysis.

PUB457

Pericardial Effusion Contributing to Decrease Renal Function even without Tamponade Aiyu Zhao, Moro O. Salifu, Aung Kyaw S. Maung, Maybel M. Tan, Mary C. Mallappallil. *State Univ of New York at Downstate, Brooklyn, NY.*

Introduction: Pericardial effusion (PE) is usually not associated with hemodynamic compromise unless it leads to cardiac tamponade. Acute renal injury (AKI) resulting from PE is rare, with only six cases reported to date. We are reporting a patient with chronic kidney disease (CKD) who presented with AKI secondary to PE without cardiac tamponade and with improvement of kidney function after pericardial window placement.

Case Description: A 56 year woman with history of hypertension, diabetes, CKD 4 and hypothyroidism presented with shortness of breath (SOB) and bilateral leg swelling for 2 months. Physical exam was significant for mild lower extremities edema. There was no pericardial rub, pulsus paradoxus or jugular venous distension. Hemoglobin was 9 g/dl, serum creatinine was 4.5 mg/dL which was 2.2 mg/dl one year ago. Thyroid function tests were normal. Echocardiogram showed large PE with ejection fraction of 55%. Workup for AKI was unrevealing. Her symptoms were significantly improved after pericardial window with drainage of 1 liter serosanguineous fluid. Her Creatinine went down to 3.9 mg/dl. Patient refused initiation of dialysis. One week later, she presented again with SOB, repeat echocardiogram showed a moderate PE. This time, she refused further drainage. The amount of PE decreased with high dose diuretics; however, kidney function got worse and dialysis was initiated.

Discussion: Several mechanisms have been proposed to explain the pathophysiology of AKI caused by PE. Hemodynamic derangement lead to decreased renal blood flow and ultimately decreased GFR. PE decreases transmural pressure which is associated with multiple hormone changes, including decreased secretion of atrial natriuretic peptide and increased release of renin and arginine vasopressin. Increase of renal nerve activity may occur before hemodynamic collapse. PE with or without tamponade should be included in the differential diagnosis of AKI. Two-dimensional echocardiogram is the "gold standard" for diagnosis and remains the most common and sensitive method of detecting PE. When a CKD patient presents with unexplained AKI, echocardiogram should be obtained to rule out PE.

PUB458

Nephrotic Syndrome Associated with Renovascular Hypertension Worsening after Renal Angioplasty and Successfully Treated with Renin:Angiotensin System Inhibitors Yuri Kasagi, Tatemitsu Rai, Keita Kusaka, Soichiro Iimori, Shotaro Naito, Eisei Sohara, Tomokazu Okado, Sei Sasaki, Shinichi Uchida. *Dept pf Nephrology, Tokyo Medical and Dental Univ., Tokyo, Japan.*

Introduction: Nephrotic syndrome (NS) is not a common complication of renovascular hypertension (RVH). However, there are a few reports demonstrating simultaneously occurring RVH and NS in certain clinical settings, which have often been in cases of unilateral occlusion of the renal artery. Although the mechanism is not fully understood, it has been postulated that activation of the renin-angiotensin system(RAS) by unilateral renal artery occlusion may produce hyperfiltration injury to the contralateral kidney, leading to profound proteinuria. Here we report a patient presenting with NS and hypertension with severe bilateral renal artery stenosis / occlusion.

Case Description: A nineteen-year-old man consulted our hospital for evaluation of hypertension (200/135 mmHg). He also had concomitant NS with a proteinuria of 4.7g/day. He had multiple stenosis of major arteries and a diagnosis of aortitis syndrome was made. Total occlusion of the left renal artery and severe stenosis of the right renal artery were seen. RVH was considered as the cause of hypertension, and percutaneous transluminal renal angioplasty (PTRA) was performed to the stenotic right renal artery. Massive increase of proteinuria (40g/day) was seen immediately after the procedure. With the left renal artery still occluded, RAS-mediated hyperfiltration injury to the recanalized right kidney was speculated to be the cause of the acute exacerbation of NS. Intensive treatment aimed at blockade of the RAS system was initiated. Combination therapy by angiotensin-II-receptor blocker and direct renin inhibitor succeeded in controlling both hypertension and proteinuria satisfactorily.

Discussion: This is a unique report of NS complicating RVH. Paradoxical exacerbation of NS was seen after unilateral angioplasty whereas resolution of NS was achieved by intense blockade of the RAS system. This case illustrates the importance to understand the effects of RAS-mediated renal hemodynamics and hyperfiltration injury in the treatment of NS complicating RVH with renal artery stenosis.

PUB459

Membranoproliferative Glomerulonephritis following Cardiac Transplantation Phillip Madonia,[1] Agnes B. Fogo,[1,2] Anna Marie Burgner.[1] *[1]Nephrology, Vanderbilt Univ, Nashville, TN; [2]Pathology, Vanderbilt Univ, Nashville, TN.*

Introduction: Successful solid organ donations in the United States have been increasing since the 1980s with the advent of calcineurin inhibitors (CNI) like cyclosporine and tacrolimus. With this success comes the burden of increasing chronic kidney disease following transplantation due to CNI toxicity. Membranoproliferative glomerulonephritis (MPGN) following heart transplant has only been described in the post-infectious setting.

Case Description: A 44-year-old Caucasian woman with congenital heart defects of dextrocardia and single ventricle underwent an orthotopic heart transplant 11 years prior to presentation to nephrology clinic for evaluation of worsening renal function and nephrotic syndrome. Since transplant, she had been maintained on cyclosporine and mycophenolate, and her baseline creatinine was 1.4 mg/dL. Creatinine was increased to 2.3 mg/dL, with 15 grams/24 hours proteinuria, and albumin of 1.9 gm/dL. Complements were low. Rheumatoid factor, ds-DNA, ANA, hepatitis panel, cryoglobulins, HIV, SPEP, and UPEP were negative. She had no history of recent infection or rash. Renal biopsy had characteristic features of MPGN, with full-house staining of deposits. With no apparent infectious cause, she was treated with prednisone and lisinopril with stabilization in creatinine and decrease in proteinuria. She subsequently developed disseminated opportunistic infections. After minimizing her immunosuppression, her renal disease worsened and she was started on dialysis.

Discussion: Chronic kidney disease is common following cardiac transplantation. Traditional risk factors play a major role, but the incidence and progression of CKD is complicated by use of nephrotoxic agents like immunosuppressants, contrast dye, and antimicrobials, and an increase in unresolved AKI. GN following heart transplant is rare, much lower than the general population. The cause of this discrepancy is unknown but could be because transplant patients are maintained on immunosuppressants. This case illustrates that transplant patients are not completely protected from these diseases, and that de novo GN can be considered in the differential of renal dysfunction in these patients.

PUB460

A Case of Syphilitic Nephropathy Complicated by Nephrotic Syndrome and Acute Kidney Injury Presenting with Liver Dysfunction Kyoko Watanabe,[1] Akihiro Shimizu,[1] Kentaro Koike,[1] Nobuo Tsuboi,[1] Masahiro Suyama,[1] Keita Hirano,[2] Masato Ikeda,[1] Yoichi Miyazaki,[1] Takashi Yokoo.[1] *[1]Dept of Medicine,Div of Nephrology and Hypertension, The Jikei Univ Hospital Tokyo, Japan; [2]Div of Nephrology, Ashikaga Red Cross Hospital, Japan.*

Introduction: In Japan,syphilis is now rarely seen in routine clinical practice,and regarded as a disease of the past.Most common presentations of syphilitic nephropathy(SN) are isolated proteinuria and nephrotic syndrome(NS).To our knowledge,there have been only few reports of SN requiring dialysis.

Case Description: A 46-year-old male with no past medical history presented with acute dyspnea after drinking alcohol.Blood tests showed liver dysfunction with AST

150IU/l,ALP 2183IU/l and γGTP 602IU/l.Liver biopsy demonstrated granulomatous hepatitis.On day 6 of hospital admission, he developed gross proteinuria and AKI with a serum creatinine(sCr) level of 4.5mg/dl,and anuria.His renal function since admission had been normal until this day with no proteinuria.Nephrologists were consulted,and he was commenced on hemodialysis(HD).Renal biopsy was also performed during that time,which showed 10% glomerular sclerosis and 30% tubulointerstitial damage.In addition,there was endocapillary proliferation in some glomeruli,but this was an insignificant finding on light microscopy.On electron microscopy,there was a loss of podocyte foot processes with endothelial deposits.Morphological diagnosis was stage I membranous nephropathy. Syphilis was diagnosed on the basis of positive Treponema Pallidum andtibody and Rapid Plasma Reagin(RPR) with an RPR titer of 166.4 T.U.Taken together,the diagnosis of SN was confirmed.For treatment,oral benzylpenicillin was started with a good response.HD was then discontinued,and proteinuria ceased with recovery of his renal function to sCr 1.10 mg/dl.

Discussion: SN is a condition,which can be treated with penicillin.The condition can present in various ways.Hence,it is important to always bear in mind the possibility,and test for syphilis and other infections at an early stage in management of patients with AKI or NS.

PUB461

Case Series of Dialysis Patients Receiving Lanthanum Carbonate as Treatment for Calciphylaxis Quratulain Shabbir,[1] Narayana S. Murali,[2] Brad C. Astor,[3] Micah R. Chan.[4] *[1]Div of Nephrology, Dept of Medicine, Univ of Wisconsin, Madison, WI; [2]Div of Nephrology, Marshfield Clinic, Marshfield, WI; [3]Div of Nephrology, Dept of Medicine, Univ of Wisconsin, Madison, WI; [4]Div of Nephrology, Dept of Medicine, Univ of Wisconsin, Madison, WI.*

Introduction: There are limited effective treatment options for patients with calciphylaxis. There is anecdotal evidence that non-calcium based phosphorus binders may offer some benefit. Our goal was to determine if lanthanum carbonate would be of benefit as a non-calcium based phosphorus binder in a prospective study.

Case Description: We identified four dialysis patients who had biopsy proven diagnosis of calciphylaxis with phosphorus levels >4.5mg/dL. We wanted to determine if treatment with lanthanum would be helpful for inducing remission of calciphylaxis. Also we assessed if there was improvement in the quality of life assessed with a validated Dermatology Life Quality Index (DLQI) survey as a result of treatment of these lesions. As secondary outcome measures we assessed PTH levels, calcium and albumin levels. All the patients received lanthanum carbonate titrated to achieve phosphorus level 3.5-5.5mg/dL. We assessed them over 12 week period and obtained photograph of the skin lesions at the end of this period as well as DLQI responses at the end of this period.

All four patients were assessed at the end of 12 weeks with photographs that demonstrated remission in skin lesions. In addition there was a statistically significant improvement in the serum albumin. There was also significant improvement in DLQI score at end of this period with subjective improvement in symptoms and feelings (p=0.01), personal relationships (p=0.03) treatment effect (0.03) and overall score (p=0.02).

Discussion: Lanthanum carbonate as a non-calcium containing phosphorus binder appears to be efficacious in terms of improving the lesions of calciphylaxis and can be helpful in improving quality of life associated with the burden of these lesions along with some beneficial impact on serum albumin level.

PUB462

An Unexpected Cause of Hyponatremia in a Clinically Euvolemic Patient Sandipani Sandilya, Deep Sharma. *Nephrology, Montefiore Medical Center, Bronx, NY.*

Introduction: We typically evaluate hyponatremia by assessing a patient's volume status. Cardiac etiologies of hyponatremia are usually hypervolemic states with reduced effective arterial blood volume. We describe a case of a clinically euvolemic patient whose evaluation led to the diagnosis of a cardiac etiology of her hyponatremia.

Case Description: An 80 year old female with a history of hypertension, idiopathic pulmonary fibrosis, Sjogren's syndrome, esophageal dysmotility, Hashimoto's thyroiditis, 2:1 AV block with a dual chamber pacemaker inserted three weeks prior to admission was admitted with cough and asymptomatic hyponatremia (serum sodium 118 mEq/L). She had a serum Na+ of 135 mEq/L twenty days prior to admission. Three days prior, the serum Na+ was 128 mEq/L. Physical examination was significant for clear lungs and no lower extremity edema. Significant serum labs were: potassium 5.3 mEq/L, creatinine was 0.7 mg/dL, uric acid was 2.3 mg/dL, osmolality was 249 mOsm/kg. Morning cortisol and thyroid function were within normal limits. Other significant labs were: urine Na+ below 20 mEq/L, urine osmolality 737 mOsm/kg. The patient was clinically euvolemic and thought to have SIADH from pulmonary and thyroid disease, with a "tea and toast" diet contributing to hyponatremia. She was started on sodium chloride tablets with minimal improvement. Because the low urine Na and borderline hyperkalemia suggested a low flow state, we obtained an echocardiogram to evaluate her ejection fraction. The echo showed a large hemopericardium due to perforation by the right atrial pacemaker lead. The patient underwent pericardiocentesis and her serum sodium subsequently gradually improved to baseline (130-135 mEq/L).

Discussion: We present this as a case of a clinically euvolemic patient with potential causes of SIADH (pulmonary and thyroid disease) and a low solute diet. In this case, the low urine sodium led us to investigate for a cardiac etiology which revealed hemopericardium as the cause of hyponatremia.

PUB463

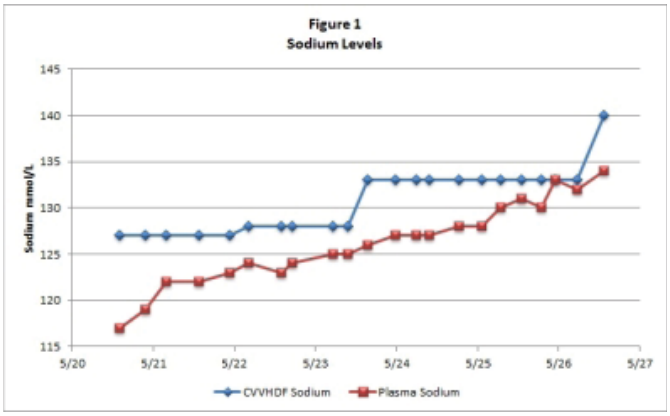
A Simple Solution for Hyponatremia in Patients Needing Renal Replacement Therapy Jiten Patel,[1,2] Julio C. Chirinos,[1,2] Susan Hedayati.[1,2] *[1]Nephrology, Univ of Texas Southwestern, Dallas, TX; [2]Nephrology, VA North Texas Healthcare System, Dallas, TX.*

Introduction: Hyponatremia in patients needing renal replacement therapy is challenging. This case illustrates a simple solution.

Case Description: A 55 year old male with hypertension, diabetes mellitus, heart failure, IgA nephropathy, and chronic kidney disease stage 3a was admitted for a right foot infection. He developed acute kidney injury, hyperkalemia, hyponatremia, and volume overload. Despite aggresive treatment his renal function worsened. We decided to start dialysis, but his serum sodium was 122meq/L. There was concern that potential large sodium shifts with conventional hemodialysis with a reduced dialysate sodium of 130meq/L or continuous venovenous hemodiafiltration (CVVHDF) with standard PrismaSol® (sodium of 140meq/L) may lead to central pontine myelinolysis. We chose CVVHDF and added 500mL of sterile water to each 5L bag of PrismaSol® to decrease the sodium concentration (Table 1). Lesser amounts of sterile water were serially added to PrismaSol® as the serum sodium slowly rose. The patient's sodium gradually improved without adverse events associated with over-correction of hyponatremia, and we were able to switch to standard PrismaSol® (Figure 1).

Table 1
Fluid Concentration

	PrismaSol® $4K^+/2.5Ca^{2+}$	Plus 500 mL Sterile Water	Plus 450 mL Sterile Water	Plus 250 mL Sterile Water
Na^+(meq/L)	140	127	128	133
Ca^{2+}(meq/L)	2.5	2.3	2.3	2.4
Mg^{2+}(meq/L)	1.5	1.4	1.4	1.4
Cl^-(meq/L)	113	103	104	108
HCO_3^-(meq/L)	32	29	29	30
Glucose(mg/dL)	100	91	92	95
Osmolarity (mOsm/L)	300	273	275	286



Discussion: Addition of sterile water to standardized dialysate to lower the sodium concentration has been previously described in hyponatremic patients with hepatorenal syndrome for intraoperative CVVHDF during liver transplantation. We demonstrate that this approach can also be safely used in hyponatremic patients with AKI in the ICU setting.

PUB464

Case of Transplant Renal Artery Stenosis Treated with Intervention Using Carbon Dioxide Angiography Joon-Seok Kim, Chung Hee Baek, Hyosang Kim, Su-Kil Park. *Div of Nephrology, Dept of Internal Medicine, Asan Medical Center, Seoul, Republic of Korea.*

Introduction: Transplant renal artery stenosis (TRAS) is a relatively common, treatable cause of graft dysfunction and refractory hypertension after transplantation. TRAS can also cause sodium retention and recurrent episodes of pulmonary edema (flash pulmonary edema). We are reporting a case of TRAS in elderly patient that was managed with endovascular intervention using carbon dioxide (CO2) as a contrast agent.

Case Description: A 78-year-old Korean man with a history of ESRD due to diabetic nephropathy received an expanded criteria donor allograft in 2006 and presented with a history of generalized edema. He had chronic allograft dysfunction after transplantation. One month ago, he suffered a traffic accident in the U.S.A. and entered a community hospital. Severe anasarca and worsening of graft function were developed during hospitalization. So, thrice-weekly hemodialysis was begun 3 weeks ago. He wanted to undergo treatment in homeland and transferred to our hospital. Physical examination revealed a generalized edema and crackles at the bases of both lungs. Chest radiogram showed a pulmonary edema and bilateral pleural effusions. Hemodialysis was continued and body weight was reduced about 13 %. During the hospital course, however, episodes of severe hypertension and bouts

of pulmonary edema were repeated. CO2 angiography was performed to rule out TRAS. Angiography revealed a tight stenosis at the anastomosis site, so balloon angioplasty was done. Following intervention, an angiogram showed improvement of stenosis and graft perfusion. Since then hemodialysis was stopped and graft function and clinical status were closely monitored. Notwithstanding some weight gain, pulmonary edema did not recur and serum BNP level was decreased markedly from 1376 pg/ml to 249 pg/ml. His graft function and urine output is maintained stably.

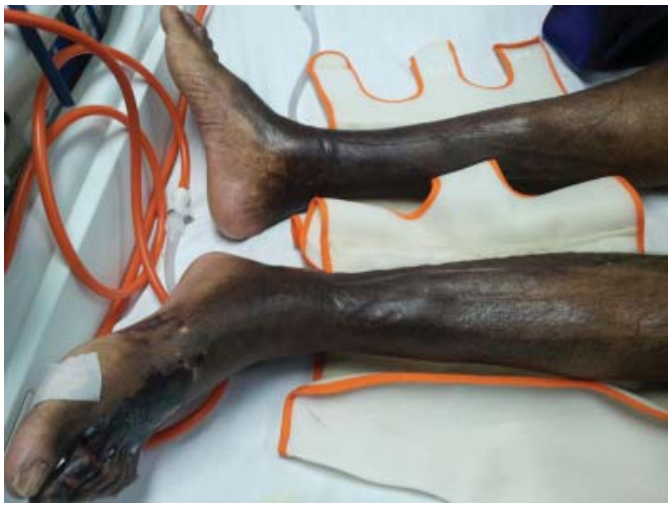
Discussion: TRAS should be considered as a cause of unexplained edema, flash pulmonary edema or graft dysfunction. If graft function is marginal and iodinated contrast material use is concerned, CO2 angiography may be used for both diagnosis and intervention of TRAS.

PUB465

Muckle Wells Syndrome - A Case Report Jhoomar R. Makhija, Hemant J. Mehta, Anup Chaudhari. *Nephrology, Lilavati Hospital and Research Center, Mumbai, India.*

Introduction: Muckle-Wells syndrome, a rare disorder, has been reported in many regions of the world, but its prevalence is unknown. It is characterized by periodic episodes of skin rash, fever, and joint pain. Progressive hearing loss and kidney damage also occur. It is uncommon in India, and this is probably first report of such case from India.

Case Description: 62 yrs old dentist, presented with loose motions for 1 month. He had hearing impairment since early childhood, and progressively increasing blackening of both legs for past several years.



(sclerodermoid lesions with hyperpigmentation and sclerosis).

He had normal S. Creatinine to begin with but 1 week back, it was 9mg/dl and 17mg/dl on admission. He was admitted with lethargy, loss of appetite, breathlessness and decreased urine output. He had high anion gap metabolic acidosis, hyponatremia, hyperkalemia and TLC of 32,000. He was initiated on dialysis. Audiogram showed bilateral sensory neural deafness. Ophthalmic examination did not show spherophakia, lenticonus or retinal changes. His skin biopsy and colonic biopsy showed evidence of amyloidosis. there was a family history of deafness in younger son who had developed meningitis leading to quadriparesis but cause of meningitis was unknown. Analysis of all coding exons with flanking intronic regions of MEFV gene by bidirectional sequencing showed compound heterozygote (including heterozygous missense variants c.443G>C/p.E148Q and c.605G>A/p.R202Q in exon2). Heterozygous mutations p.E148Q and p.R202Q are individually reported as pathological variants, consistent with the diagnosis of familial Mediterranean fever. This case has clinical features of Muckle Wells syndrome.

Discussion: Patient was started on colchicine without response. We were trying to get kineret (anakinra), recombinant, non-glycosylated synthetic form of human Interleukin-1ß receptor antagonist. But the patient succumbed to septicaemia.

PUB466

Oxalate Nephropathy: An Unexpected Cause of Acute Kidney Injury Shivani Shah, Derek M. Fine, Srinivas Ramakrishna Gottipati. *Nephrology, Johns Hopkins Univ, Baltimore, MD.*

Introduction: Oxalate nephropathy with Roux-en-Y gastric bypass is well-described, but it is rare in the current era. Nevertheless, the diagnosis should be considered in otherwise unexplained acute kidney injury.

Case Description: A 69 year old woman with a history of hypertension, Roux-en-Y gastric bypass in 2003, Crohn's disease, and a creatinine of 1.9 mg/dL two months prior to admission, presented to a hospital with pneumonia. Her creatinine was 3.56 mg/dL five days prior to admission and increased to 9 mg/dL on presentation. She was transferred to our institution the following day where she was afebrile, had a heart rate of 88 bpm, and blood pressure of 130/57 mm Hg. Her physical exam was notable for altered mental status, normal heart sounds, 1+ lower extremity edema, and asterixis. Her chemistry panel was significant for BUN 107 mg/dL, creatinine of 9.1 mg/dL, and phosphate level of 15 mg/dL. Urinalysis revealed 1+ protein and 13 RBC/hpf. Serologic testing was negative for C-ANCA, P-ANCA, SPEP, UPEP, HCV, and HBV antigens. Complement levels were normal. Renal ultrasound showed no obstruction. The patient became anuric and initiated dialysis. Her acute kidney injury was attributed to acute phosphate nephropathy, although no inciting etiology was discerned historically. A renal biopsy was performed which showed focally severe tubular injury with oxalate crystals in the setting of hypertensive nephrosclerosis. The patient ultimately had some improvement in renal function and was discharged off dialysis with a creatinine of 4.9 mg/dL.

Discussion: This patient appears to have had undiagnosed hyperoxaluria secondary to increased gastrointestinal absorption of oxalate in the setting of gastric bypass surgery and Crohn's disease. Though oxalate nephropathy with Roux-en-Y gastric bypass is well-described, it is unusual to occur so long after the procedure. Her concomitant Crohn's disease, chronic kidney disease, and acute illness likely exacerbated her hyperoxaluria to necessitate dialysis. In patients with acute kidney injury and a history of gastric bypass, the differential diagnosis should include oxalate nephropathy; kidney biopsy should be considered to determine the diagnosis.

PUB467

A Case of Mistaken Identity: 2, 8-Dihydroxyadenine Misdiagnosed as Uric Acid Nephrolithiasis Akinwande A. Akinfolarin, Julian L. Seifter. *Renal Div, Brigham and Womens Hospital, Boston, MA.*

Introduction: Adenine phosphoribosyl transferase (APRT) deficiency is a rare autosomal recessive disorder with a higher prevalence among Japanese. Normally adenine is converted to adenine monophosphate by APRT. Known gene mutations result in APRT deficiency in which adenine is alternatively oxidized to 2, 8-dihydroxyadenine (2, 8-DHA) by xanthine oxidase. 2, 8-DHA is an insoluble purine that may result in nephrolithiasis or in some cases renal failure. We present a patient with recurrent nephrolithiasis initially thought to be uric acid in origin.

Case Description: We consulted on a 44 year old Japanese-American woman with a history of hypertension and recurrent kidney stones for 20 years. The stones were radiolucent on abdominal X-ray but CT scan revealed numerous calculi of varying sizes. Colorimetric analysis previously revealed the stones to be 100% uric acid. She was placed on sodium citrate, achieving a urine pH of 6.5. However she continued to develop new stones. 24-hour urine collection showed a normal uric acid super saturation of 0.628. Stone analysis using infrared spectrophotometry revealed the stone was 2, 8-DHA and not uric acid. Maltese crosses, typical of 2, 8-DHA crystals were seen under polarized light microscopy. Red blood cell APRT activity was normal. Gene sequencing showed a mutation of methionine to threonine (Met136Thr) confirming type II APRT deficiency. Allopurinol, a xanthine oxidase inhibitor was initiated with a low purine diet and high fluid intake. Stones have not recurred and repeat CT imaging demonstrated disappearance of her previous calculi.

Discussion: Colorimetric stone analysis cannot distinguish adenine from uric acid and is not recommended. Infrared spectrophotometry or x-ray crystallography is preferred. Alkalinization of urine is not effective for 2, 8-DHA as it is for uric acid nephrolithiasis. Allopurinol decreases formation of 2, 8-DHA in favor of the soluble 8-hydroxyadenine. In this case, recognition of 2, 8-DHA and therapy with allopurinol prevented stone growth and may have led to dissolution of existing stones.

PUB468

Antiphospholipid Syndrome Nephropathy - From Migraine to the Kidney Ana Teresa Nunes,[1] Inês Castro Ferreira,[1] Eva Borka Mariz.[3] *[1]Nephrology, Centro Hospitalar de São João, Oporto, Portugal; [2]Internal Medicine, Centro Hospitalar de São João, Oporto, Portugal; [3]Rheumatology, Centro Hospitalar de São João, Oporto, Portugal.*

Introduction: Antiphospholipidic syndrome (APS) can affect any organ with a wide range of clinical features, requiring a high index of diagnosis suspicion. We describe a case of APS nephropathy preceded by frequent migraine and an episode of amaurosis fugax.

Case Description: A 21 years old woman without any medical/obstetric history started frequent migraines followed by an episode of amaurosis fugax. Brain MR showed bilateral ischemic lesions. Hypertension was detected along with renal impairment, proteinuria and intermittent hematuria. She presented a slightly increased activated partial thromboplastin time with no abnormalities in immunological evaluation. Kidney biopsy revealed segmental glomerulosclerosis, mesangial proliferation and arteriosclerosis with recanalized thrombosis. Immunofluorescence was negative for all sera but ultrastructural evaluation detected mesangial immune deposits. An antiphospholipid syndrome was suspected. Triple positivity for phospholipid antibodies (aPL) was detected and anticoagulation was started (INR 2-3) with a remarkable improvement of neurological complains. Currently she is at 3rd year of follow-up without any major thrombotic event or migraine recurrence. Renal function and proteinuria improved and hematuria resolved. Immune evaluation persists negative.

Discussion: APS is characterized by vascular thrombosis and/or pregnancy morbidity along with presence of aPL. The kidney is a major target organ with a broad spectrum of histological findings. Our patient started with mild neurological manifestations followed with an ischemic event and renal involvement. A secondary APS cannot be excluded since renal histology identified immune deposits beyond the thrombotic lesions. Anticoagulation is the main therapy for APS providing a dramatic improvement of clinical complaints as well preventing new events. This case highlights the complexity of the diagnosis. Non-criteria manifestations should alert the nephrologist to this entity so that morbidity and mortality can be tackled at the earliest.

PUB469

Elevated Creatinine, Tacrolimus Toxicity and Ketonuria in a Kidney Transplant Recipient following Dietary Preparation for Gastric Sleeve Bariatric Surgery Maybel M. Tan, Laurel W. Yap, Mariana S. Markell. *Renal Diseases, SUNY Downstate Medical Center, Brooklyn, NY.*

Introduction: Following successful kidney transplantation, weight gain can result in morbid obesity. Very little has been written about post-transplant bariatric surgery, and there have been no reports of adverse effects of pre-op preparation, which can be very restrictive.

Case Description: A 44 year old woman with history of systemic lupus erythematosis, received a deceased donor kidney in 2010. Post-transplant, the patient gained 60 pounds, weighing 240 pounds (BMI 40). She was accepted for gastric sleeve procedure and was prescribed 1000 calorie, very low carbohydrate, liquid diet for two weeks pre-operation. At start of the diet, creatinine was 1.66 mg/dl, tacrolimus level 4.5 and urine analysis negative. Diet consisted of "Muscle Milk", chopped carrots, diet pudding and jello. She complained of diarrhea, but continued the diet. One week later, creatinine was 2.1 mg/dl. She was told to increase fluid intake. After two weeks, BUN was 42, creatinine 2.44 mg/dl, tacrolimus 11.9, glucose 110, bicarbonate 17, chloride 113, urine analysis small acetone, SSA 1+, specific gravity 1.030, CBC with WBC 11K, 4 Bands, and multiple abnormal RBC's. After overnight hydration, creatinine was 2.5 and a kidney biopsy was performed which revealed no lymphocytic infiltrate, no tubulitis or intimitis and minimal tubular vacuolization. She continued hydration and tacrolimus dose was decreased. One week later, the creatinine was 1.55, tacrolimus level was 4.1 and other laboratory studies normalized.

Discussion: This previously stable patient developed a rapid rise in creatinine and ketonuria coincident with a diet of "Muscle Milk", a protein supplement that contains creatine in unknown amounts; large quantities of ground carrots and artificially sweetened pudding. We believe that she developed ketosis secondary to carbohydrate restriction, and severe dehydration secondary to diarrhea caused by artificial sweeteners and high carrot intake. An interaction with tacrolimus from an ingredient in the "Muscle Milk" is also possible. It is unclear whether the presence of creatine in the supplement contributed to her rise in creatinine and dehydration.

PUB470

Previously Unreported GLA Gene Mutation as a Cause of Kidney Dysfunction and Classic Fabry Disease Phenotype A Case Report Ruth Ixel Rivas Bucio, Odette D. Avendano, Elvira González, Juvenal Torres Pastrana. *Nefrologia, Centro Medico Nacional 20 de Noviembre Issste, Mexico DF, Mexico.*

Introduction: Fabry Disease (FD) is an X-linked disease which affects glycosphingolipid metabolism due to a deficiency in the production of the lysosomal enzyme alpha-galactosidase A (α-gal A).

Case Description: Case presentation: A 30 year old male patient with only medical information was a 6 year history of medically controlled hypertension. He refers his first symptoms 5 years prior, with acroparesthesias, intermittent pain crises and foamy urine. 4 months before being admitted he had sudden hearing loss, increase in pain, intermittent episodes of dyspnea. He was admitted with lab serum creatinine 3.8 mg/dL, eGFR (MDRD) 19.7 mL/min and albuminuria 1.2 g/24 hr urine sample. α-gal A activity in dried blood spot revealed extremely low activity 0.1 μmol/L/hr. Diagnosis of Fabry Disease was confirmed with molecular analysis, which revealed a previously unreported mutation in exon 1 of the GLA gene (c.107T>Gp.L36W). Plasma lyso GL-3 levels were 31.7 ng/mL. Echocardiography established left ventricle diastolic dysfunction. Ophthalmology confirmed the presence of bilateral *cornea verticillata*. He began therapy was started on ACEI/ARB therapy. ERT was initiated with agalsidase beta on standard dose of 1 mg/kg every other week; so far, he has received 4 infusions. Since the initiation of ERT pain perception and pain crises have diminished.

Discussion: We report the finding of a previously unreported mutation in the GLA gene, presenting as classic Fabry disease. Enzyme replacement therapy seems to have stanched kidney disease progression.

PUB471

Air Embolism: Alarming but Clinically Insignificant and Common in Hemodialysis Jose F. Lizcano Perez,[1] Panagiotis Zervogiannis,[1] Craig S. Courville,[2] Alfredo M. Peguero,[2] Ammar Almakkee.[1] *[1]Nephrology, Univ of South Florida, FL; [2]JAHVA, Tampa, FL.*

In one day, an 81 year old male with ESRD and LVEF of 10-15 % had a thoracentesis, fistulagram with angioplasty of upper arm AV fistula, and immediately afterwards was started on hemodialysis. Five minutes after the initiation of hemodialysis treatment, his blood pressure decreased with dyspnea and mental changes. An evaluation for possible pulmonary embolism or pneumothorax revealed free air in the right ventricle. Discontinuation of dialysis and supportive care with 100% oxygen per non-rebreathing mask and placement in left lateral decubitus positionat 30 degrees trendelenburg was initiated. CT of the head did not reveal acute changes or air embolism. Serial troponins were negative. Intermittent episodes of non-sustained ventricular tachycardia with severe stunned myocardium on echocardiography prompted the use of amiodarone, pressors, inotropic agents and CVVHDF. Later evaluation of the initial chest CT showed right ventricular air embolism was a bystander as is seen in 20-43% of cases after contrast procedures. The most likely cause of the decompensation was the acute increase in the venous return after angioplasty and thrombectomy of the AV fistula, taxing the left ventricle's severely limited function. A complete investigation of the dialysis circuit and air trap mechanism ruled out hemodialysis procedure as source of air entry into blood vessels. Hemodialysis immediately after fistulogram is common in the hospital setting. Air in the right ventricle is also common after fistulagrams and the discrimination of its magnitude and location through CT scan and analysis of the circuit lines easily rules out air embolism. As hypotension and dyspnea are not rare events during dialysis, it is concluded that hypotension, dyspnea and air in the right ventricle may coexist without causality. Analgorithm for the diagnosis and treatment of clinically relevant pulmonary air embolism during hemodialysis was designed.

PUB472

A Case Report with Acute Renal Failure due to Plasmodium Falciparum Sook Hyeon Park, Jason Cobb. *Renal Divison, Emory Univ School of Medicine, Atlanta, GA.*

Introduction: Malaria has a significant health impact in some parts of the world, especially Asia and Africa. Many Americans are at risk of infection while traveling in endemic areas. In 2011, 1925 cases of malaria were reported to the Centers for Disease Control and Prevention and 16% of the patients had acute renal failure. Malaria associated renal disease is caused primarily by *P. falciparum* and *P. malariae.*

Case Description: A 49 year old white female with no significant past medical history presented with fever and generalized malaise. The patient went on a mission trip to Haiti from 6/9/2013 until 6/16/13. On 6/25/13, she developed fever, abdominal pain, dark urine. Seven days after the first symptoms developed, the patient presented to our academic medical center with positive malaria test at a local clinic. On admission day, her creatinine was 2.51mg/dL, BUN 34mg/dL, bicarbonate 19 mmol/L, sodium 128mmol/L, hemoglobin 10.1gm/dL, platelets 28000 10E3/mcL, elevated liver function tests and total bilirubin 2.5mg/dL. Peripheral blood smear showed 2.1 % *P. falciparum*. She was admitted to the ICU for close monitoring and started on Quinine and doxycycline. A urine microscopic exam showed innumerable muddy brown casts. Her cr peaked at 3.26mg/dL and started trending down with conservative treatment. On discharge date, her cr went down to 1.56mg/dL. She finished her malaria treatment during hospital stay and discharged home. The patient was seen at nephrology clinic 1 week and 4 months after discharge. Her creatinine has remained at 1.08mg/dL.

Discussion: Malaria incidence is approximately 1,500 travelers per year in the Unites States. Malaria related acute renal failure occurs 1% to 4.5% among native population of endemic areas. However, acute renal failure can occur up to 25-30% in non-immune European adults. *P. falciparum* is mainly associated with acute renal failure and patients infected with *P. malariae* can progress to chronic progressive glomerulopathy and nephrotic syndrome. It is important to recognize that malaria can be the cause of acute or chronic kidney disease in febrile patients who have returned from abroad.

PUB473

Acute Kidney Injury and Hypercalcemia due to Sarcoid-Lymphoma Syndrome Vanya Grover,[1] Adebayo Shakir Adewale,[1] Kristine H. Jang,[2] Tahmeena Ahmed,[3] Nand K. Wadhwa.[1] *[1]Nephrology, Stony Brook Medicine; [2]Medicine, Stony Brook Medicine; [3]Pathology, Stony Brook Medicine, Stony Brook, NY.*

Introduction: Sarcoidosis and lymphoma can occurs simultaneously or lymphoma mainly Hodgkin's lymphoma can precede sarcoidosis. We report a case with acute kidney injury and hypercalcemia due to sarcoidosis associated with incidental marginal zone lymphoma.

Case Description: An 81 year old man with hypertension, anemia, COPD, and coronary artery disease, presented with altered mental status, dragging of left foot and worsening lethargy. Physical examination revealed BP 157/70 mmHg, HR 76/minute, RR 20/minute. Neurological examination was normal. He had no palpable lymphadenopathy but had mild splenomegaly. Laboratory data showed WBC 6.2 k/mcL, hemoglobin 10.1 mg/dL, platelets 139 k/mcL, serum sodium 137 mEq/L, potassium 4.4 mEq/L, chloride 104 mEq/L, and bicarbonate 30 mEq/L, BUN 34 mg/dL, creatinine 2.4 mg/dL, calcium 13.3 mg/dL, angiotensin converting enzyme (ACE) 107 U/l and vitamin D 1,25 Dihydroxy 103 ng/ml. Bone marrow clot section showed non-caseating granulomas and a small lymphocytic infiltrate composed of clonal B cells consistent with immunophenotype of marginal zone

lymphoma by flow cytometry (which was seen on both blood and bone marrow). He was treated with IV hydration and prednisone. His serum calcium normalized to 9.8 mg/dl and creatinine decreased to 1.67 mg/dl over a period of one week. He was discharged home on tapering doses of prednisone. He did not receive treatment for marginal zone lymphoma. His serum calcium, ACE, vitamin D 1,25 Dihydroxy remained normal over a 2- year follow up period.

Discussion: Sarcoidosis and lymphoma can co-exist. Both can have common presenting features which pose a diagnostic challenge. Bone marrow granulomas may be seen in patients with lymphoma but are nonspecific and do not usually cause elevated ACE and hypercalcemia Our patient had acute kidney injury and hypercalcemia likely due to sarcoidosis as he responded to steroids and remained stable. He may have incidental marginal zone lymphoma or it may be sarcoid-lymphoma syndrome.

PUB474

Hanging Diagnosis of Acute Thrombocytopenia and Hemolytic Anemia in Sickle Cell Disease Patient with End Stage Renal Disease

Akshita Narra, Gaurav Alreja, Ruchir D. Trivedi. *Div of Nephrology, Univ of Connecticut, CT.*

Introduction: Microangiopathic hemolytic anemia and thrombocytopenia without other apparent etiology can be manifestations of thrombotic thrombocytopenic purpura (TTP), a potentially life threatening disorder. In a sickle cell disease (HbSS) patient with ESRD, malignant hypertension, systemic infections, bone marrow necrosis, or delayed transfusion reaction (DHTR) can mimic TTP creating a diagnostic dilemma.

Case Description: A 39 year-old female with HbSS disease and ESRD on peritoneal dialysis presented with acute onset bilateral hip and shoulder pain. Her only medication was folic acid. The patient was febrile, with mild scleral icterus and elevated BP(220/110mmHg) but with no evidence of papilledema. Lab data revealed hemoglobin(Hb) 8.6mg/dL, wbc 7.7×10^9/l, platelets 118,000/cm^2, reticulocyte count 4.6%, LDH 1495 IU/L, haptoglobin <15mg/dL, indirect bilirubin 2.5mg/dL, alkaline phosphatase 450 IU/L and negative direct and indirect coomb's test. Peripheral smear revealed 2+ schistocytes, 1+ sickle cells and no platelet clumping. Prothrombin time and fibrinogen were normal. There was no evidence of infection. In 72hrs, Hb dropped to 3.4mg/dL and platelet count to 14,000/cm^2 despite PRBC and platelet transfusions. Lab data was consistent with intravascular hemolysis with elevated LDH, indirect bilirubin, and low haptoglobin. Plasmapheresis (TPE) and systemic steroids were initiated for a presumptive diagnosis of TTP. After 7 consecutive sessions of TPE the platelet count improved to 76,000/cm^2 on day-10. A bone marrow aspiration biopsy in the interim revealed marrow necrosis. A repeat indirect coomb's test was positive. ADAMTS13 activity returned as normal (32%). Patient had significant clinical improvement with stabilization of Hb and platelet count.

Discussion: The renal manifestations, one of the pentad diagnostic features of TTP, are irrelevant in ESRD patients. In the acute setting when the diagnosis is uncertain, the high fatality of untreated TTP cases favors the urgent initiation of TPE while awaiting ADAMTS levels. Our patient initially had HbSS related bone marrow necrosis later complicated by DHTR that improved with steroids and TPE.

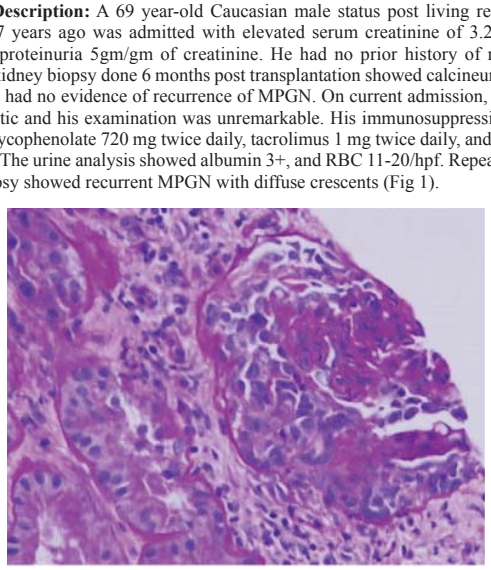
PUB475

Recurrent Membranoproliferative Glomerulonephritis Type Presenting as Crescentric Glomerulonephritis in Living Related Donor Renal Transplant Recipient

Sandeep M. Patil, Denisse E. Menendez, Neeraj Singh. *Div of Nephrology, Louisiana State Univ Health Sciences Center, Shreveport, LA.*

Introduction: Recurrent glomerulonephritis is a significant cause of allograft failure post-kidney transplantation. We present a patient with recurrent crescentic membranoproliferative glomerulonephritis type 1(MPGN-1) leading to rapid graft loss despite intensive treatment.

Case Description: A 69 year-old Caucasian male status post living related donor transplant 7 years ago was admitted with elevated serum creatinine of 3.2 mg/dl and increasing proteinuria 5gm/gm of creatinine. He had no prior history of rejection. A transplant kidney biopsy done 6 months post transplantation showed calcineurin inhibitor toxicity but had no evidence of recurrence of MPGN. On current admission, patient was asymptomatic and his examination was unremarkable. His immunosuppressive regimen included mycophenolate 720 mg twice daily, tacrolimus 1 mg twice daily, and prednisone 5 mg daily. The urine analysis showed albumin 3+, and RBC 11-20/hpf. Repeat transplant kidney biopsy showed recurrent MPGN with diffuse crescents (Fig 1).



Electron microscopy revealed large irregular electron dense mesangial deposits. Serum BK and CMV virus PCR, serum and urine protein electrophoresis, complements levels, hepatitis B and C serology, c-ANCA and P-ANCA were negative. The serum creatinine and proteinuria worsened despite treatment with oral cyclophosphamide, pulse steroids, plasmapheresis, rituximab, intravenous immunoglobulin and patient was initiated on hemodialysis 5 weeks after admission.

Discussion: Although, MPGN type -1 is a rare cause of ESRD, the disease recurs in a significant proportion of patients after kidney transplantation. Unlike the disease in native kidneys, recurrent MPGN may present with a cresentic form of glomerulonephritis with a rapid fulminant clinical course unresponsive to treatment.

PUB476

Hyponatremia, a Constant Clinical Challenge- Euvolemic Hyponatremia Secondary to Solute Loss

Ramya Vejella, Sarah Khan, Neeraj Singh, Zulqarnain Abro, Stacey Wells. *Nephrology, LSUHSC Shreveport, Shreveport, LA.*

Introduction: Poor solute intake has been reported to cause euvolemic hyponatremia typically in the setting of chronic alcoholism or malnutrition when patients present with low serum Na,low urine osmolality, and normal urine output. We present a patient who developed euvolemic hyponatremia secondary to solute loss from diarrhea while maintaining adequate free water intake.

Case Description: A 42 year old Hispanic female with a history of kidney transplant thirteen years ago was evaluated for diarrhea for 4 weeks. Her immunosuppression consisted of mycophenolate 720 mg oral twice daily, tacrolimus 2 mg oral twice daily, and prednisone 5 mg oral daily. Patient had her mycophenolate decreased and subsequently stopped 2 weeks prior to presentation due to continuous diarrhea. The stool studies ordered at that visit were unremarkable. She was advised to maintain adequate hydration. At her current visit, she was afebrile with BP of 128/68 mmHg, HR of 61/minute, RR of 18/min, weight 122.6 lbs, and BMI 21.7. Her weight had been unchanged over the past 6 months. She was euvolemic and non-orthostatic and rest of the examination was unremarkable. Laboratory data revealed: Serum Na-116mmol/l, K-4.0mmol/l, Cl-83mmol/l, CO2-24mmol/l, Glucose-80mg/dl, BUN-8mg/dl, Creatinine-0.9mg/dl, Calcium-8.2mmol/l, Albumin-2.5g/dl, Phosphorus-2.7mmol/dl, and Magnesium-1.2mmol/dl. Patient denied excessive water intake. Further work up revealed measured serum osmolality of 239, TSH- 0.208, Free T4- 1.4, random cortisol- 7.5, urine osmolarity- 62, Urine Na < 19, Urine K < 7.5, Urine Cl < 33 and 24 hour urine volume of 2000ml. SIADH which was initially suspected, was ruled out due to remarkably low urine osmolarity. Instead, a diagnosis of low solute hyponatremia was confirmed. Patient responded well to IV hydration with normal saline and to high salt diet with normalization of serum Na at a desired rate.

Discussion: Diarrhea is known to cause hypovolemic hyponatremia. However, if the adequate hydration is maintained despite chronic diarrhea, the patients may instead present with euvolemic hyponatremia. This presentation should be considered in the discussion of the causes of euvolemic hyponatremia.

PUB477

Severe Tubulo-Interstitial Nephritis (TIN) Accompanying Mesangio-Capillar Glomerulonephritis in Patient with Chronic Lymphocytic Leukemia (CLL)

Hanna Bartosik,[1] Krzysztof Letachowicz,[1] Magdalena Krajewska,[1] Agnieszka Halon,[2] Katarzyna Madziarska,[1] Waclaw Weyde,[1] Marian Klinger.[1] *[1]Dept of Nephrology and Transplantation Medicine, Wroclaw Medical Univ, Wroclaw, Poland; [2]Dept of Pathomorphology and Oncological Cytology, Wroclaw Medical Univ, Wroclaw, Poland.*

Introduction: Glomerulonephritis in B-chronic lymphocytic leukemia is rarely reported. The association between nephrotic syndrome and hematological malignancy should be revealed in the future.

Case Description: 62-year old female with diagnosed in 2010 chronic lymphocytic leukemia was admitted to Nephrology Department due to severe nephrotic syndrome [NS] (total serum protein level 4.4g/dl serum albumin level 1.8 g/dl) with significant renal insufficiency (serum creatinine level 4.0 mg/dl; eGRF 12ml/min/1.73m^2). Nephrotic syndrome occurred 4 months along with renal impairment (serum creatinine level 1.7 mg/dl). For 4 weeks the patient was treated with steroids (prednisone 20 mg per day) and 3 pulses of cyclophosphamide were administered (500 mg/m^2). This treatment was abandoned due to infectious complications. After admission to Nephrology Department pulses with methylprednisolone were introduced (40 mg per day) and intravenous diuretics were applied (160 mg of Furosemide per day). 3 weeks later kidney biopsy was done showing mesangio-capillar glomerulonephritis with severe TIN (III0). This intensive tubulo-interstitial nephritis differs our case from primary mesangio-capillar glomerulonephritis. Such a histological pattern, especially with concomitant TIN, is very rarely seen in a patient with chronic leukemia. Due to progressive renal impairment and exhaustive, non-responding to described treatment NS dialysis with ultrafiltration were started. After 2 weeks the patient was referred to Hematology Department in stable, good clinical shape in order to start chemotherapy (rituximab, cyclophosphamide).

Discussion: This atypical histological finding might be a feature of hematological malignancy-associated nephrotic syndrome.

PUB478

Methotrexate Induced Acute Symptomatic Hyponatremia Priyanka Govindan, Arjun V. Sharma. *Nephrology, Univ of Washington, Seattle, WA.*

Introduction: 61 year old female with a history of CNS lymphoma treated with intrathecal methotrexate infusion who developed acute symptomatic hyponatremia. This case demonstrates salt wasting as the mechanism of methotrexate induced hyponatremia.

Case Description: This is a 61 year old female with CNS lymphoma who underwent her 8th out of 10 intrathecal methotrexate infusions with hyperhydration with sodium bicarbonate and subsequently developed hyponatremia with altered mental status. She was initially treated with normal saline then 2% saline which caused her sodium to drop from 120 to 116. Lasix 40 mg was given and she was started on 3% saline. With this as well as discontinuation of her methotrexate, she corrected to a sodium of 128 mEq/L and ultimately sustained at 135 mEq/L. During this time her urine osmolality remained high. The serum sodium correction correlated with decreasing levels of methotrexate. Mental status subsequently improved and became normal in 24 hours.

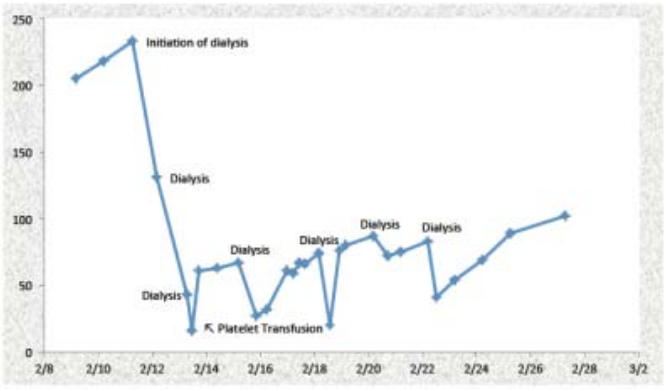
Discussion: The diagnosis of drug induced salt wasting was reached due to the fact that hyponatremia occurred as the patient received 600 mEq of sodium (as sodium bicarbonate) and urinated 600mEq of sodium with a net even fluid balance (rather than retaining free water as in SIADH) in spite of severe hyponatremia. There are three previously described cases of methotrexate induced salt wasting. This is the first instance of methotrexate induced salt wasting in the presence of SIADH as seen by the patient's continued high urine osmolality. This case is also unusual as the salt wasting occurred on her eighth dose of methotrexate rather than on her first.

PUB479

Dialysis Induced Thrombocytopenia Adil R. Ali, Biruh Workeneh. *Div of Nephrology, Baylor College of Medicine, Houston, TX.*

Introduction: Thrombocytopenia (TCP) associated with HD has been attributed to a number of causes including complement activation from membrane surface interaction, exposure to filter preservative, direct activation of cellular components and so on. Cuprophane and cellulosic membranes, which activate the complement system, are often implicated but these membranes are no longer in common use. In this case report, we described a patient who developed profound membrane-associated TCP after exposure to modern biocompatible polysulfone membranes.

Case Description: A 28 yo woman with progressive CKD secondary to lupus nephritis, antiphospholipid antibody syndrome (h/o pulmonary embolus) presented with uremic symptoms. She was initiated on HD with 3 sessions using a electron-beam sterilized Fresenius Optiflux 160. On the morning prior to initiation her plt count was 233 x10^3/uL; on the second day 131 x10^3/uL; and after the third fell to 12 x10^3/uL. She received no heparin with HD treatments, and evaluation for DIC, HIT and thrombotic microangiopathy were negative. TCP continued to occur after HD despite flushing the priming solution with 1 liter of saline prior to HD (see figure).



The dialyzer was switched to steam sterilized Gambro Revaclear, and her plt count again decreased from 87 x10^3/uL to to 41 x10^3/uL. She was subsequently transitioned to peritoneal dialysis, her plt count recovered and remained stable while on PD.

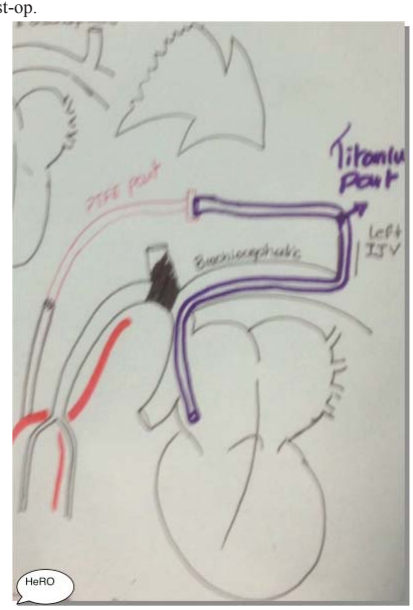
Discussion: This case report shows persistent TCP associated with hemodialysis with two different biocompatible membranes. There are reported cases of resolution of dialysis-induced TCP after switching dialyzers, but this patient's TCP did not improve. This report suggests studies to examine risk factors and pre-existing antibody or induced antibody that activate plt are sorely needed in order to able to predict occurrence of this potentially devastating complication.

PUB480

The Use of the HeRO Graft in Haemodialysis Patients with End Stage Vascular Access: A Case Series Frank J. O Brien, Mohamed Shantier, David Lappin. *Dept of Nephrology, Galway Univ Hospital, Galway, Ireland.*

Introduction: The Hemodialysis Reliable Outflow graft (HeRO) is a subcutaneous implant consisting of a silicone venous outflow component and an arterial PTFE component. It traverses central venous stenoses and allows for long-term vascular access in haemodialysis patients who have exhausted venous access sites. We describe the first three cases of HeRO graft use in Ireland.

Case Description: A 59 year old with hypertensive ESRD who commenced RRT via left brachiocephalic AVF. She developed left subclavian stenosis which failed multiple angioplasties. She underwent a HeRO procedure to relieve the proximal obstruction as well as restoring her AVF as source of vascular access. She was dialysed successfully via the HeRO day 1 post-op. A 71 year old with hypertensive ESRD who commenced HD via left brachio-cephalic fistula. Following its failure, a R AVF was created. She subsequently developed SVCO syndrome. She underwent a HeRO procedure to relieve the SVCO and provide permanent dialysis access. She was dialysed successfully via the HeRO day 1 post-op.



83 year old with ESRD secondary renovascular disease commenced haemodialysis via RIJ permcath 2007. Subsequently had R Brachiocephalic AVF created in 2008. Developed SVC obstruction secondary to R brachiocephalic vein stenosis. Required multiple angioplasties and had high venous pressures resulting in recurrent bleeding from AVF cannulation site. Underwent HeRO procedure to relieve this stenosis, and provide permanent access. He successfully dialysed 2 weeks post op.

Discussion: These cases highlight the potential benefit of the HeRO graft in patients with difficult vascular access. We also describe the use of the HeRO in restoring previously failing AVFs.

PUB481

Hypernatremia in a Patient with Paranoid Adipsia Laith Farah Al-Rabadi, Jean M. Francis. *Nephrology, Boston Univ Medical Center.*

Introduction: Hypernatremia is associated with high mortality. Antidiuretic hormone (ADH) secretion and thirst mechanism are tightly regulated within hypothalamus circuits to prevent hypernatremia. Adipsia is an extremely rare entity with fewer than 100 reported cases worldwide. However, more unreported cases are likely present. Psychogenic adipsia, a syndrome extremely opposite to the frequently reported psychogenic polydipsia associated with hyponatremia, is even rarer and represents a diagnosis of exclusion in a patient presenting with hypernatremic dehydration. It is marked by normal ADH activity, intact urinary concentrating ability with maximally concentrated urine. Herein, we describe a case of severe hypernatremia caused by paranoid adipsia.

Case Description: A 32 year old white male incarcerated patient with history of schizophrenia had paranoid thoughts of water carrying chemicals that may poison his body. He indulged in drinking his own urine and avoiding any sources of water despite his severe thirst. He was obtunded and confused on presentation. Sodium level reported as higher than 176 mmol/l as lab assay could not accurately measure such high levels, Cr 2.8 mg/dl, Glucose of 143 mg/dl. Serum osmolality of 414 mosm/kg and urine osmolality of 960 mosm/kg with urine Na less than 20 mmol/l. Isotonic fluid was chosen in the absence of definite Na to guide treatment. We asked the lab to dilute the sample to have a better estimate of Na level. Level was measured at 177 mmol/l after 12 hours of hydration with 0.9%NS. Urine osmolality of 382 mosm/kg and urine Na of 57mmol/l following resolution of hypernatremia revealed suppressed ADH and reflecting intact regulatory mechanisms. Psychiatric management of his disease helped resuming normal drinking habits.

Discussion: Hypodipsia–adipsia related hypernatremia can occur from psychiatric illness which may directly disturb thirst mechanism or induces psychiatric conflicts with

voluntary self-restriction of water intake. However, this does very rarely occur and exclusion of other disorders and structural diseases is mandatory as thirst mechanism is a very powerful sensation and very difficult to thwart. Reinstituting normal drinking behavior will be most beneficial in preventing further episodes of hypernatremia.

PUB482

Idiopathic Pulmonary Hemorrhage in a Peritoneal Dialysis Patient

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Introduction: Infants on peritoneal dialysis (PD) are at risk for complications. Tachypnea and tachycardia frequently occur but are non-specific. Rarely they are a precursor of pulmonary hemorrhage (PH). Idiopathic pulmonary hemorrhage (IPH) is rare but well described in infants and prompt recognition and management is vital.

Case Description: Two month-old term female with bilateral renal dysplasia, pulmonary hypoplasia and moderate pulmonary hypertension (pHTN) on PD. On the floor she had intermittent tachypnea and tachycardia. On day three she developed an oxygen requirement. There was no issue with PD or fluid status. Electrolytes were stable and a chest x-ray (CXR) and blood gas were normal. She received a fluid bolus with no improvement. She acutely decompensated and was intubated. Ventilation was difficult with gross blood on laryngoscopy and suctioning. Conventional mechanical ventilation required high settings. CXR showed diffuse consolidation, hematocrit dropped from 25 to 20 and she received packed red blood cells, platelets, fresh frozen plasma and desmopressin. There was no evidence of infection. Echocardiogram showed improved pHTN and coagulopathy workup and CT angiogram were negative. Five days later she had cardiorespiratory arrest with an acute drop in hematocrit due to PH associated with Klebsiella pneumonia requiring blood products. Bronchoscopy and cardiac catheterization three weeks later were negative. A rheumatologic workup of the patient and her mother was negative. She was transferred to the floor at age four months. PD was stable with no oxygen requirement. No etiology was found and she was diagnosed with IPH. Over the next year she had multiple cardiorespiratory arrests without PH and she eventually passed away during an arrest.

Discussion: Infections and congenital heart disease most commonly cause pediatric PH. Pediatric IPH rarely occurs but prompt diagnosis and treatment is essential. Infant IPH is defined as abrupt airway bleeding with diffuse pulmonary infiltrates leading to severe respiratory illness requiring mechanical ventilation. Our patient met these criteria, though she was not previously healthy and had neonatal medical problems.

PUB483

A Case of Spontaneous Subcapsular Renal Hematoma and Severe Thrombocytopenia in a Patient with Antiphospholipid Syndrome

Alexander Osei-Bonsu, Jabulani Sidile, Nand K. Wadhwa, Edward P. Nord, Patrick Gerard Lynch. *Nephrology, Stony Brook Univ Hospital, Stony Brook, NY.*

Introduction: Anti-Phosphosholipid syndrome (APS) is characterized by thrombotic events with antiphospholipid antibodies. However bleeding diathesis can occur. We report a case of spontaneous renal subcapsular hematoma with primary APS and severe thrombocytopenia.

Case Description: A 71 year old woman with deep vein thrombosis and pulmonary embolism on coumadin underwent aorto-femoral bypass and endarterectomy for an occlusive clot of the left common iliac artery. This was complicated by acute kidney injury, anemia and thrombocytopenia associated with a large right subcapsular renal hematoma requiring embolization of the right renal artery to achieve hemostasis. She was started on emergent hemodialysis. Physical examination revealed right CVA tenderness, and areas of ecchymosis. Laboratory data showed a WBC19.0 k/mcl, Hgb 9.1 g/dl, platelet 33k/mcl, BUN 83mg/dl, creatinine 7.58mg/dl, HIT Ab negative, C3 normal and C4 low. ADAMTS13 was 64% with positive lupus anticoagulants, anti-cardiolipin IgM antibodies were 14 mpl units and beta 2 glycoprotein-1 IgM antibodies were 27mpl units. Right kidney biopsy showed thrombotic microangiopathy. A diagnosis of catastrophic APS was made. She was treated with plasmaphersis for 7days; IVIG daily and methylprednisolone 1 g IV daily for 3 days followed by oral prednisone. Her platelets increased to 82 k/mcl. Heparin was restarted for anticoagulation. Her platelets again decreased to 25k /mcl. She received a second 3 day course of IVIG and 4 doses of Rituximab. Her kidney function did not recover and she remained on chronic hemodialysis. She was discharged home on coumadin and prednisone taper. Her Platelets were 268 k/mcl at 8 week outpatient follow up.

Discussion: Severe thrombocytopenia in APS appears to be an immune mediated process. Our case demonstrated resolution of thrombocytopenia with a combination therapy of plasmaphersis, IVIG and Rituximab. A subcapsular renal hematoma is rare in APS and was stabilized with an improvement in thrombocytopenia while receiving coumadin.

PUB484

An Unexpected Presentation: Minimal Change Disease in an Adult with Hepatitis C Virus-Related Cryoglobulinemia

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Introduction: The hepatitis C virus (HCV) infection is associated with several renal diseases including mixed essential cryoglobulinemia, membranoproliferative glomerulonephritis (MPGN) and less frequently membranous nephropathy and crescentic glomerulonephritis.

Case Description: A 43-year-old male was referred due to progressive edema in the lower limbs and nephrotic range proteinuria, His past medical history included being an intravenous drug abuser. Biochemistry test showed raised serum creatinine of 163μmol/l. He had nephrotic range proteinuria of 12g/day and a serum albumin of 19 g/l. Viral serology for hepatitis B and HIV was negative but confirmed evidence of HCV infection with genotype 3A and viral load of 659000 copies. Laboratory parameters showed presence of cryoglobulin.He had a renal biopsy and histology demonstrated features of minimal change disease. We commenced treatment with intravenous methylprednisolone 24 mg once daily for 10 days, followed by oral Medrol 24 mg Q.d. Concurrently, Mycophenolic acid 0.5g Bid was commenced. After a 2-week treatment, proteinuria had remitted, his renal function showed remarkable recovery with creatinine reduced to 81 μmol/l. The patient didn't receive antiviral therapy. At 4 months follow-up clinic, his renal function was normal with serum creatinine of 68 μmol/l, 24-h urinary protein had dropped to 0.12 g/day, serum albumin increased to 42 g/l and HCV RNA didn't increase and Alanine transaminase and aspartate aminotransferase were normal.

Discussion: This is the first case report that a patient who presented as nephrotic syndrome with renal pathology minimal change disease associated with hepatitis C virus-related cryoglobulinemia was treated successfully with steroid and Mycophenolic acid. Our case highlights an important treatment strategy and may be beneficial to nephrologists facing this clinical scenario. However, a randomized controlled trial is required to evaluate the efficacy of this treatment combination before it can be a standard treatment.

Funding: Government Support - Non-U.S.

PUB485

Acute Kidney Injury in the Post Cardiac Surgery Setting Is Not Always Acute Tubular Necrosis

Haris Farooq Murad,[1] Hernan Rincon-Choles,[2] Fahad Saeed.[2] *[1]Internal Medicine, Cleveland Clinic, Cleveland, OH; [2]Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.*

Introduction: Post cardiac surgery acute kidney injury due to an overlap of thrombotic thrombocytopenia purpura and disseminated intravascular coagulation is rarely reported.

Case Description: We report a case of a 69 year old gentleman with coronary artery disease, cardiac surgery and solitary kidney who underwent aortic and mitral valve replacement surgery. His post-operative course was complicated by hypotension and creatinine peaked to around 2.4mg/dL but stabilized around 1.9 mg/dl. Eleven days after the surgery, his Creatinine again started rising (3.3 mg/dL) without any obvious insult. Platelets dropped to 72,000, he had peripheral schistocytosis, and hemoglobin was 8.8g/dL. Urine studies showed microscopic hematuria, 30mg/dL protein and muddy brown casts. ADAMTS 13 level was low (7) and ADAMTS 13 inhibitor was detectable (1.4). He was treated with a total of 11 sessions of plasma exchange (PLEX) resulting in improvement of anemia, thrombocytopenia and creatinine. ADAMTS 13 activity also improved to 44 and ADAMTS 13 inhibitor assay became negative. However, a few days later he relapsed with recurrent anemia and thrombocytopenia, along with a rise in Creatinine to 2.4 mg/dL. He was restarted on PLEX for an additional 14 sessions leading to improvement of hematological parameters and renal function. During the next week, he had episodes of hypotension resulting in anuric renal failure requiring renal replacement therapy. He continued to have thrombocytopenia with high LDH, low haptoglobin, normal prothrombin time, decreased fibrinogen, increased d-dimer and fibrinogen split products. This time ADAMTS13 level was normal. Bone marrow biopsy was unrevealing. He had treatment with broad spectrum antibiotics for presumed underlying infection and his thrombocytopenia eventually resolved 2 weeks later. However, he remained dialysis dependent.

Discussion: Our case highlights that TTP/DIC overlap is an important cause of AKI in the post cardiac surgery setting. Physicians should consider sending ADAMTS13 level and the inhibitor activity to entertain this diagnosis, which has high morbidity and mortality.

PUB486

Focal Segmental Glomerulosclerosis in a Patient with Mantle Cell Leukemia

Sumeet Hindocha, Seerapani Gopaluni. *Renal Unit, Churchill Hospital, Oxford, United Kingdom.*

Introduction: Focal segmental glomerulosclerosis (FSGS) can either be a primary disease or secondary to an underlying condition. FSGS is rarely reported in association with haematological malignancies.

Case Description: A 67 year old gentleman presented with a 1 month history of progressive lower limb swelling and shortness of breath. He was previously fit and well. He was severely nephrotic with marked peripheral oedema and nephrotic range proteinuria. Urinalysis showed 3+blood and 4+ protein. Investigations revealed high urea 27.8mmol/L, high creatinine 272 umol/L and decreased serum albumin. Haemoglobin was 13.7g/dL, WCC $8.19x10^9$/L. Immunoglobulins, complement, ANA and protein electrophoresis were all normal. He underwent a renal biopsy and the histology was consistent with early FSGS, showing segmental sclerosis, epithelial hypertrophy and hyperplasia, endocapillary foam cells and tip lesions, with positivity for IgM. He had good response to prednisolone 60mg OD. Within 6 weeks his creatinine had normalised and proteinuria improved. Ciclosporin was added following a 2nd relapse 9 months after finishing his first treatment. This was again initially met with good response. Further follow up showed a persistent, marked lymphocytosis and nephrotic syndrome began to relapse. Peripheral blood immunophenotyping revealed a significant increase in the number of CD19+ve B-cells. CT imaging showed no significant lymphadenopathy. Fluorescence In-situ Hybridization analysis was positive for t(11:14) consistent with mantle cell leukaemia. He was commenced on 3 cycles of R-CHOP chemotherapy, to which he responded well. His renal function normalised and proteinuria resolved. He remained in remission for more than one year.

Discussion: Although nephrotic syndrome rarely occurs in association with acute leukemia, they have often been described in chronic lymphocytic leukemia (CLL).

Membranoproliferative glomerulonephropathy and membranous glomerulonephropathy are the most common lesions observed in CLL. Nephrotic syndrome has been well documented among patients with lymphomas, in particular, Hodgkin's lymphoma. However FSGS remains a rare association with mantle cell leukemia.

PUB487

Pseudo Colonic Obstruction Induced Hypokalemia in End Stage Renal Disease Patient Ramya Vejella, Cherinet S. Adgeh, Deepa Gali. *Nephrology, LSUHSC Shreveport, Shreveport, LA.*

Introduction: Hyperkalemia is one of the most common complications we encounter in CKD and ESRD patients, since excretion of potassium is mainly renal. Here we present a rare case where an ESRD patient who was on long term dialysis who presented with chronic hypokalemia.

Case Description: This is a 69 year old male with history of ESRD requiring HD since October 2011 who had multiple admissions since April 2012 for severe watery diarrhea, abdominal distention. During the same time frame, patient was noted to have persistent hypokalemia(highest potassium level being 3.5 mmol/l) despite being on a high potassium bath prescription on his outpatient hemodialysis. The current admission, abdominal x-ray showed colonic distention up to 11 cm in diameter. Obstruction secondary to volvulus, which was initially suspected was ruled out after a colonoscopy. Subsequently, a diagnosis of pseudo-colonic obstruction secondary to Ogilvie Syndrome was made, which was confirmed by return of colonic peristaltic movement after administration of IV neostigmine. The patient was managed conservatively, continued on a high potassium hemodialysate bath as an outpatient and discharged on oral potassium supplements.

Discussion: Persistent hypokalemia in a setting of End Stage Renal Disease is not a common presentation. This is a rare presentation of an ESRD patient presenting with chronic hypokalemia secondary to colonic pseudo obstruction. The mechanism involved in this patient is most likely massive up regulation of colonic BK channels, which is known to happen rarely in ESRD patients.

PUB488

Polymyxin B in Septic Shock: Two Case Reports Luigi Vernaglione. *Nephrology and Dialysis Unit, "A. Perrino" Hospital, Brindisi, Italy.*

Introduction: Nephrologist are involved in the management of septic shock by performing hemoperfusion with Polymyxin B (HPB) which consists in 2 hours sessions repeated over 3 consecutive days. HBP is indicated in case of sepsis coming from post-surgical, intra-abdominal, triggers and plasma endotoxin levels (PEL) higher than 0.6 EAA™ units. We present 2 cases in which HBP has been performed in our unit.

Case Description: CASE #1: G.M., a 37 y.o. male admitted with fever (41 °C) resistent to paracetamol and steroids. He was hypotensive (73/40 mmHg) and oliguric (200 ml/day). Lab values: hb 9.3 g/dl, WBC 79.100/ml, neutrophils 90%, INR 2.92, ATIII 43%, s.sodium 127 mEq/l. Renal function was normal and blood cultures were positives for Bacteroides ureolyticus. He was prescribed vancomycin (1 g x 2/day) and meropenem (1g x 3/day) without effects. Due to PEL=0.77 EAA™ units HPB was prescribed. After 3 days diuresis rose to 3000 ml/day, PEL fell to 0.4 EAA™ units, body temperature was normalized, WBC became 20.000/ml. After 24 hours from the third HPB session AKI occurred (s. creatinine 4 mg/dl) coupled to hypernatremia (156 mEq/l) and hypotension. Infusion of steroids and vasopressors was started but 36 hours later the patient died.

CASE #2: V.M., a 55 y.o. female admitted with headache, hypertension, hypoesthesia and hypotonia to the left side of body. For the last 5 days she had been complaining for fever. The CT scan revealed subarachnoid bleeding due to aneurysm rupture. She undervent surgical intervention. After 1 month she experienced hypotension (80/60 mmHg), high PEL (1.02 EAA™ units) and blood culture positive for klebsiella pneumoniae. After tigecycline (1 g x 2/ day) and meropenem (1g x 3/day) without improvement she started HPB. After this treatment the clinical picture improved with remission of fever, normalization of blood pressure and lab values, even if PEL remained high (0.8 EAA™ units).

Discussion: In conclusion, HPB in sepsis lowers the PEL but this is not sufficient if the septic trigger remains. It is the right combination between timing of HPB intervention, surgical treatment and antibiotic therapy the key of clinical success that can be appreciated, also without a significant reduction of PEL, even in case of extra-abdominal trigger of sepsis.

Funding: Government Support - Non-U.S.

PUB489

Dilemma of Diagnosing Partial Central Diabetes Insipidus and Partial Nephrogenic Diabetes Insipidus Mayurkumar Gohel, Evan Norfolk. *Nephrology, Geisinger Medical Center, Danville, PA.*

Introduction: Partial central diabetes insipidus and partial nephrogenic diabetes insipidus are very difficult to differentiate with each other. Clinical history, blood tests and response to desmopressin (dDAVP) are helpful to make accurate diagnosis.

Case Description: 60 years old male with significant history of schizophrenia on chronic lithium, mental retardation and autism admitted due to fever, cough and hypernatremia. As per his caregiver, he was having polyuria, polydipsia and nocturia since last 4 yrs. Chest x-ray was within normal limits. Water restriction test was without change in urine osmolality so we gave dDAVP 4 microgram intravenous and urine osmolality went up 307mOsmol/kg and urine output was drop to 10 ml in first hour and 60 ml in second hour. The diagnosis of partial central diabetes insipidus was made and started on dDAVP 0.2 mg oral twice a day. Serum cortisol, growth hormone, adrenocorticotrophic hormone and insulin like growth factor 1 were within normal range. Magnetic resonance imaging of brain showed 1.7 mm pituitary microademona. Over the next 2 days, requirement of D5W went down, urine osmolality was in 200s and urine output was 100-150 ml/hr. Then patient allow to have more water and then his urine osmolality went down <200 and urine output was increase so increase dDAVP dose to 0.4 mg orally twice a day however urine output remain high and urine osmolality remained low. So with history of chronic lithium use and was not responding to high dose oral dDAVP, patient was treated for nephrogenic diabetes insipidus with hydrochlorothiazide 25 mg orally daily and patient urine output decrease and serum sodium was remain stable. Antidiuretic hormone level came later which was normal.

In our case patient has partial response with intravenous dDAVP and pituitary microadenoma suggest he has some partial central diabetes insipidus, however he was not responded well with oral dDAVP. Patient was on chronic lithium use, normal antidiuretic hormone level and was not adequately responded with dDAVP are favoring diagnosis of partial nephrogenic diabetes insipidus.

Discussion: Clinical history and response to dDAVP are very important factors to make accurate diagnosis of diabetes insipidus.

PUB490

Flank Pain and Shortness of Breath: Think outside the Box Mohamed Hassanein, Shalini Barlapudi. *Capital Health - Regional Medical Center, Trenton, NJ.*

Introduction: It is well documented in the literature that venous thromboembolic events such as DVT, PE and RVT are highly associated with nephrotic syndrome. This risk is particularly high in patients with idiopathic membranous nephropathy.

Case Description: A 24 year old Hispanic man with no past medical history presented to the emergency room with 3 months of left sided flank and back pain. He also reported progressing left sided chest pain, aggravated by inspiration and associated with dry cough and shortness of breath. Vital signs were Temperature: 98.4 °F, HR: 78bpm, BP: 147/69 mmHg, RR: 16/min, and SO2: 98% on room air. Physical exam was unremarkable. Laboratory findings showed serum creatinine: 0.76mg/dl, BUN: 16mg/dl, electrolytes were within normal range, total protein 4.7g/dl, albumin 2.1g/dl and cholesterol 358 mg/dl. Urinalysis showed moderate occult blood, hyaline and granular cast, protein > 300mg/dl which was confirmed by a 24 hour urine protein of 11gm. Computed Tomography showed a left lower lobe pulmonary artery embolism, associated with a moderate to large left pleural effusion and thrombus in the left renal vein and associated IVC. Left thoracocentesis was performed for diagnostic and therapeutic purposes which yielded 800 ml of transudative fluid. Serological studies including antinuclear Ab, anti-GBM Ab, p-ANCA and c-ANCA titers, CRP, complement levels, hepatitis panel, HIV-1 RNA PCR and RPR screen were negative; hypercoagubility workup was negative as well. Renal biopsy showed stage 2 membranous nephropathy and focal acute tubular injury and interstitial edema associated with renal vein thrombosis. Therapeutic anticoagulation was initiated, the patient was started on lisinopril and atorvastatin as well as prednisone and cyclosporine.

Discussion: Pleuritic chest pain with shortness of breath or flank pain in a patient with findings of nephrotic range proteinuria may be an alarming symptom of underlying serious thromboembolic complications of nephrotic syndrome. Close attention should be paid to patients with albumin levels < 2.8 g/dl. It is well documented in the literature that the risk of VTE increases 5.8-fold when serum albumin level is < 2.2 g/dl.

PUB491

Renal Infarction Associated with Oral Contraceptives: Third Case Report in Literature Suchita J. Mehta, Maybel M. Tan, Moro O. Salifu, Mary C. Mallappallil. *Suny Downstate.*

Introduction: Renal infarct is an uncommon condition that is reported to have an incidence of as low as 0.007% to 1.6%. This could partly be attributed to non-specific clinical presentation that can be misleading resulting in a missed/delayed diagnosis. The common causes are atrial fibrillation causing thromboembolism, renal artery injury/dissection, hypercoagulable conditions and occasionally polyarteritis nodosa, cocaine. Here below, we report the third case of renal infarction associated with oral contraceptives, based on our review of the literature.

Case Description: 28 year-old woman non-smoker with no past medical history presented to the emergency room on 14th September 2013 for 2 days duration of worsening right-sided flank pain without any fever. She was menstruating at that time but denied hematuria or dysuria. She was on oral contraceptive pills since 2009. Her BP was 118/70, normal sinus rhythm 79/min, and normal renal function. Urinalysis showed trace proteinuria with 182 RBCs, 6 WBCs with trace leucocyte esterase. Lactate dehydrogenase (LDH) was elevated at 434 IU/L. On C.T. abdomen she had a focal wedge shaped right renal infarct. Extensive work up for hypercoagulable conditions including protein C, protein S, antithrombin deficiencies, lupus anticoagulant, anticardiolipin antibody, beta2 gylcoprotein, Factor V Leiden mutation; were all negative. Her contraceptives were discontinued and symptoms of flank pain resolved in a week.

Discussion: Renal infarction, a relatively uncommon condition can often be missed and thus possibly under-reported. Patients present with pain in 96.8% cases, 46% uncontrolled hypertension, 90% have increased LDH, 40% with abnormal renal function Mean age of presentation is 53 years. We report a patient who was diagnosed with renal infarct at a relatively young age and was noted to have only two of the common clinical presenting signs and more importantly the only possible etiology, after ruling out atrial fibrillation and hypercoagulable conditions, is likely oral contraceptives. Based on our literature review, this is only the third case of renal infarct associated with oral contraceptives without any other risk factors for thromboembolism or hypercoagulable conditions.

PUB492

Normotensive with Proteinuria During Pregnancy: Is This Preeclampsia? Purna Bindu Nandigam, Talal A. Khan, Muhammad Awais Arif, Akshay Sharma, Ziauddin Ahmed. *Div of Nephrology, Drexel Univ, Philadelphia, PA.*

Introduction: Preeclampsia is defined as new onset of hypertension (HTN) and either proteinuria or end organ dysfunction after 20 weeks (wks.) of gestation in a previously normotensive woman. In 2013, ACOG removed proteinuria as an essential component for diagnosing preeclampsia. In the United States, preeclampsia/eclampsia is one of 4 leading causes of maternal death. For this reason, most healthcare providers tend to make a diagnosis of preeclampsia based on both HTN and proteinuria being present. However over diagnosis can result in premature delivery. Here in we present a case of isolated proteinuria in a normotensive parturient.

Case Description: 41 years old south Asian G3P3 female presented at 28 wks. gestation for evaluation of proteinuria. She had no complaints and denied any headache, vision problems, or abdominal pain. Her blood pressure was 110/60 and physical examination was unremarkable except for 2+ lower extremity edema. UA showed 4+ proteinuria. Urine tests done at the 24th week showed micro albumin to creatinine ratio of 2713 mg, serum BUN of 7mg/dl and creatinine of 0.6 mg/dl. Her platelets, hepatic profile, ANA, and complements were all within normal limits. She had no known history of proteinuria or kidney disease. Preeclampsia was a strong differential diagnosis with discussion of the need for possible early delivery. Since the past medical history of proteinuria was unknown and the patient remained normotensive without any signs or symptoms of hypoperfusion, the decision was made to monitor the patient's BP and proteinuria every 2 wks. for any signs of disease progression. The patient's proteinuria fluctuated during subsequent visits but BP remained normal. The patient was induced at 38 wks. secondary to possible cholestasis, and delivered a full term baby without complications.

Discussion: Our case demonstrates that careful monitoring of a pregnant patient with normal BP and proteinuria especially diagnosed after 20 wks. can be managed to a term delivery. As per new ACOG guidelines new onset of HTN after 20 weeks of gestation is the most important factor in the diagnosis of preeclampsia. Isolated proteinuria should not be a trigger for encouraging early delivery.

PUB493

Case Report : Acute Kidney Injury and Hemolytic Anemia in Falciparum Malaria Anila P. Sankar,[1] Sudheer K. Sankar.[2] *[1]Medicine, Baylor College of Medicine, Houston, TX; [2]Medicine/Nephrology, Memorial Hermann Southwest Hospital, Houston, TX.*

Introduction: Falciparum malaria (FM) is a mosquito vector borne disease caused by a parasite plasmodium malariae. Though not endemic to U.S., incidence of FM is our country is around 1500 per year mainly from travelers and immigrants. Common renal manifestation of acute FM are acute interstitial nephritis, and acute tubular necrosis from hemolysis. Post infectious glomerulonephritis and membranoproliferative glomerulonephritis are less common.

Case Description: 46 year old AAM with no past medical history but a recent travel to Africa presented to the ER with fevers and altered mental status. Physical examination: BP 93/44 mmHg, temperature 103.3, abdominal examination with splenomegaly, and no pedal edema. Hemoglobin was 7.7 gm/dl. Total bilirubin was 2.8. Creatinine was 3.1mg/dl, BUN 45 mg/dl, sodium 126,with albumin of 2.3. Urine analysis with specific gravity of 1.005,large blood, 1+protein, and RBCs. LDH was 351 with haptoglobin of 26.Peripheral smear showed plasmodium falciparum with parasitemia of 6 %. Serological workup was negative except for a new diagnosis of HIV. Ultrasonography showed splenomegaly and enlarged kidneys .Renal biopsy for FM glomerulonephritis shows widening of mesangial area, hypertrophy and hyperplasia of endothelial cells. EM with electron dense deposits in subendothelial and paramesangial areas. Patient was treated with aggressive volume resuscitation, quinine and supportive care with plans for exchange transfusion and renal replacement therapy. He subsequently became polyuric. As parasitemia improved renal function returned to baseline creatinine of 1.1.

Discussion: Early detection and aggressive treatment prevents worsening of renal function in FM from acute tubular necrosis and acute interstitial nephritis. Hyponatremia is observed in 6% of patients with FM secondary to increase in antidiuretic hormone. Acute kidney injury is common secondary to acute intravascular hemolysis or heavy parasitic infection. Patients diagnosed with FM with parasitemia greater than 10% have complications of renal failure requiring renal replacement therapy. Quinine still remains the treatment of choice.

PUB494

A Mysterious Case of Hypomagnesaemia Patricia Nasr, Marc M. Saad, Ninad D. Parekh, Rabih Nasr. *Internal Medicine, Staten Island Univ Hospital, Staten Island, NY.*

Introduction: Since there is no major hormone regulating serum magnesium level, the etiology of hypomagnesemia includes poor intake or gastrointestinal loss or renal magnesium wasting. Here we present an interesting case of hypomagnesemia.

Case Description: A 24 year old male presented for repeated episodes of acute muscle twitching and numbness. He has no significant past medical history and takes no medications. His vitals including blood pressure were within the normal range. Neurological exam was normal. Laboratory values showed low magnesium (1.3 mg/dL) with a fractional excretion of magnesium of 18.3%, and hypocalciuria with a urine calcium creatinine ratio of 0.03. He also had metabolic alkalosis with bicarbonate of 31, and potassium of 3.9 mmol/L. An AST was found to be 42 IU/L and ALT 67 IU/L and were persistently elevated with negative hepatitis B and C serology and no history of alcohol abuse. Renal ultrasound showed renal cysts. He was treated with IV magnesium and switched to oral magnesium oxide upon discharge.

Discussion: Causes of renal magnesium wasting include genetic renal tubular defects or drugs. Gitelman syndrome, characterized by renal magnesium wasting, metabolic alkalosis, hypokalemia and hypocalciuria, is caused by mutation of the SCL12A3 gene that encodes the sodium-chloride cotransporter (NCCT). However, our patient had normokalemia. Isolated dominant hypomagnesemia (IDH) with hypocalciuria occurs due to mutation of FXYD2 gene that encodes a gamma subunit of the DCT basolateral Na+/K+ ATPase. Hepatocyte nuclear factor 1β (HNF1β) is a well-known transcription factor that plays a role in the activation of FXYD2 gene and mutations in HNF1β is reported to cause renal magnesium wasting, renal cysts, elevated liver function tests. To the best of our knowledge, Gitelman syndrome does not present with normal serum potassium and HNF1-B mutation spectrum is not characterized by metabolic alkalosis and hypocalciuria. Our case is unique in that it represents a hybrid of two well-defined renal magnesium disorders. Our patient was referred for further genetic testing, which are pending at this time.

PUB495

Bilateral Renal Vein Thrombosis: A Rare, Life-Threatening and Reversible Cause of Acute Kidney Injury Dearbhla Kelly, Mark N. Canney, Brenda B. Griffin. *Dept of Renal Medicine, Cork Univ Hospital, Ireland.*

Introduction: Renal vein thrombosis (RVT) is a rare thrombotic entity outside of the context of nephrotic syndrome or malignancy. It may occur with hypercoagulable states, trauma, infection or with use of oral contraceptive pill. Its prevalence within the nephrotic syndrome ranges from 5-60%. It is particularly common with membranous nephropathy or in those with protein excretion >10g/day. It can be diagnosed by Doppler ultrasound, computed tomography (CT) or magnetic resonance imaging. The gold standard test is a selective renal venogram. Chronic RVT is often asymptomatic but acute RVT can present dramatically as outlined in this case.

Case Description: A 47 year old male presented with a 10 day history of abdominal pain, distension and diarrhoea. On examination, he had ascites and significant bilateral lower limb oedema. He had developed an oliguric acute kidney injury (AKI) with a rapidly rising creatinine from 163μmol/l. to 684μmol/l. He had microscopic haematuria without significant proteinuria. He had a persistent thrombocytosis (platelet levels of $1059X10^9$/L). A contrast-enhanced abdominal CT scan was performed. This revealed an extensive thrombus in his inferior vena cava with bilateral RVT and a right segmental pulmonary embolism. In addition, he had acute appendicitis. He was commenced on unfractionated heparin and intravenous antibiotics. He required intermittent haemodialysis for 2 months and then made an excellent renal recovery. The patient was subsequently diagnosed with a JAK2 positive myeloproliferative disorder and is now maintained on lifelong aspirin, warfarin and hydroxyurea.

Discussion: This gentleman illustrates classic symptoms of RVT including flank pain and haematuria. He had multiple risk factors including a previously undiagnosed thrombophilia and abdominal sepsis. Bilateral disease can cause a rapidly progressive AKI as in this case. Treatment of symptomatic RVT involves anti-coagulation with either unfractionated or low molecular weight heparin followed by warfarin. There is limited evidence for local thrombolytic therapy with or without thrombectomy. Early diagnosis and prompt management is key as untreated acute RVT has a mortality of greater than 50%.

PUB496

Henoch-Schonlein Purpua Presenting as a Paraneoplastic Syndrome with Pancreatic Cancer Smita Mahendrakar, Shiba Khorsandi, Suneet Verma, Arshia Abbasi, Nitin Behl, Reza Amerinasab, Michael Yudd. *Renal Section, Dept of Veterans Affairs NJ Healthcare System, East Orange, NJ.*

Introduction: Adult-onset Henoch –Schonlein Purpura (HSP) rarely has been described as a paraneoplastic syndrome with solid organ malignancies, and to our knowledge, this is the first description with pancreatic cancer.

Case Description: 54 year-old AA man was found to have purpura, microscopic hematuria and intermittent abdominal pain during an admission for alcohol rehab. No heroin or cocaine use. The rash involved legs, torso and arms. Serum creatinine was 1.2 mg/dl. Urinalysis: 3+ protein, 30 RBC/HPF, many were dysmorphic, and RBC casts. Urine protein/creatinine ratio was 5.3. Negative or normal: ANA, ANCA, anti-dsDNA, complement, hepatitis B, C, HIV, toxicology screen, serum and urine electrophoresis. A skin biopsy showed leukocytoclastic vasculitis. Renal biopsy light microscopy showed mild to moderate endocapillary proliferation and increased mesangial matrix, no crescents. EM : small mesangial and rare subendothelial deposits; IF: granular mesangial deposits of IgA (2+), C3 (2+), and IgM(1+)in all glomeruli. These findings of purpura, glomerulonephritis with predominant IgA deposits, and abdominal pain with negative serologies favored the HSP diagnosis. He was given steroids, improved, and was transferred for prolonged rehab. Seven months later, CT of abdomen showed a mass in the pancreatic tail. A month later, a repeat CT showed new liver lesions. Liver biopsy showed metastatic high grade carcinoma, consistent with pancreatic primary. In retrospect, the pancreatic mass was present on CT interventional images done at renal biopsy.

Discussion: HSP is a small vessel leukocytoclastic vasculitis with deposition of IgA which commonly involves the skin, kidney and GI tract. In adults, paraneoplastic HSP has infrequently been described in lung, prostate and renal carcinomas.This is the first case of HSP that is associated with pancreatic cancer. Unfortunately, the pancreatic cancer, although present at the time of the renal biopsy (see above), was diagnosed too late for our patient. It may be prudent to screen older patients presenting with HSP for occult malignancies.

PUB497

Minimal Change Disease in Adults: Challenges Posed; a Series of 3 Cases Roveena N. Goveas,[1,2] Inder Patel,[1,2] James H. Sondheimer.[1,2] *[1]Wayne State Univ School of Medicine, Detroit, MI; [2]Detroit Medical Center, Detroit, MI.*

Introduction: We present a series of 3 cases of minimal change disease referred to adult nephrology clinic and the challenges faced in their management.

Case Description: All 3 patients, previously healthy, presented with nephrotic range proteinuria, and renal biopsy demonstrated minimal change disease. All had normal renal function and no precipitating factors identified such as non -steroidal use, atopy or lymphoma. The first patient, 45 yr old African American (AA) male received prednisone for 16 weeks, but remained steroid dependent. Proteinuria persisted on low dose maintenance prednisone, and addition of MMF led to only a transient improvement. He is being started on cyclosporine which was previously declined. The second patient, a 40 year old AA female achieved complete remission with prednisone, then relapsed 4 months later. ACE inhibitor was added to low dose prednisone and patient is maintained in remission. The third patient, a 25 year old AA female was initiated on prednisone, but failed to achieve remission. She was offered but declined cyclosporine or cyclophosphamide; a trial of MMF achieved a partial remission. None of these 3 patients had complications related to nephrotic syndrome at 1 year of follow-up.

Discussion: These cases had in common; normal renal function at presentation, AA race, age group of 25-45, normotension at presentation and during treatment and absence of underlying disorders; however, each of these patients responded quite differently. The partial response and frequently relapsing proteinuria may raise the suspicion of a underlying focal segmental glomerulosclerosis, however, renal function remained normal for over 1 year of follow up. Adults presenting with minimal change disease may respond differently to therapy. None of these patients had repeat renal biopsy yet as its role in treatment failure is not well established. Unlike in children, our adult minimal change disease patients appear to follow a more chronic course. As minimal change disease is rare in adults compared to children, there are limited randomized and prospective clinical studies, making the treatment and prognosis assessment challenging.

PUB498

Boric Acid Poisoning in the Very Elderly: A Case Report Selena Longhi, Monica Limardo, Lucia Del Vecchio, Maria Carla Bigi, Donatella Casartelli, Mauro Maria Corti, Flavia Tentori, Giuseppe Pontoriero, Francesco Locatelli. *Nephrology and Dialysis, A. Manzoni Hospital, Lecco, Italy.*

Introduction: Boric acid is a weak acid, which is often used as an antiseptic, insecticide, fungicide and as eye wash in dilute solution. In nature it exists in the form of colorless crystal or as white powder. 90% of oral dose is excreted through kidneys. Letal acid boric poisoning is defined as ingestion of 15-20 g or serum concentration of 1000 µg/ml. The clinical manifestations are hypotension, fever, gastrointestinal, cutaneous, central nervous system symptoms and acute renal failure. There is no agreement on therapy following acid boric intoxication. Hemodialysis removes 60% of acid boric and it is combined with forced diuresis. Forced diuresis may be used alone; peritoneal dialysis is an option mainly in children.

Case Description: 95 year-old woman ingested 20 g of boric acid by mistake. On admission she had fever, vomit, diarrhea. Laboratory data showed: s-creatinine 1.79 mg/dl (basal s-creatinine 1.3 mg/dl), leukocytosis, anemia. Boric acid serum concentration was not available. Rehydration therapy and forced diuresis were decided given mild acute renal failure and comorbidities. After few hours severe hypotension, oliguria and increased s-creatinine level (2.52 mg/dl) occurred. Two hemodialysis sessions were performed followed by rehydration and high dose of diuretic therapy. Overall patient conditions improved showing stable blood pressure levels (130/70 mmHg) and diuresis recovery. After few days renal function was completely restored.

We described a theoretical lethal dose of acid boric poisoning with renal involvement in an elderly patient. Despite high dose, advanced age and comorbidity, the patient had a complete recovery of acute renal failure thanks to combined therapy (hemodialysis and forced diuresis). This case confirms the efficacy of hemodialysis in the treatment of acid boric poisoning and it suggests its early start.

PUB499

A Case of Nephrotic Syndrome due to Antiphospholipid Syndrome Nephropathy Miho Karube, Shinya Kaname, Kazuhito Fukuoka, Yoshinori Komagata, Yoshihiro Arimura. *First Dept of Internal Medicine, Kyorin Univ School of Medicine, Tokyo, Mitaka, Japan.*

Introduction: Although renal complications are common in patients with antiphospholipid syndrome (APS), nephrotic syndrome is rarely seen.

Case Description: [Case] A 71-year-old woman with a history of hypertension was referred to our hospital because of leg edema that had appeared six months before and the laboratory findings including elevated serum creatinine, nephrotic range proteinuria and pancytopenia. The anticardiolipin antibody titers were repeatedly positive, but serum cryoglobulin was negative. Renal biopsy revealed five global sclerosis among 9 glomeruli, while diffuse hypercellularity in the mesangium, double contour of the capillary walls, and foam cells were found in the rest of the glomeruli. Chronic lesions such as focal cortical atrophy and fibrous intimal hyperplasia of the arterioles were also observed. Immunofluorescence study revealed granular deposits of IgM in the mesangial areas. On the other hand, IgG, IgA, C1q, and C3 were all negative. Electron microscopy revealed mesangial interposition and subendothelial deposits with endothelial swelling and widening of subendothelial spaces all of which suggested thrombotic microangiopathy (TMA). During the course of disease, she presented with autoimmune hemolytic anemia and thrombocytopenia, but did not show findings suggesting SLE such as fever, oral aphtha, skin rash, arthritis, serositis, neurological sign, and antinuclear and anti-DNA antibodies. Based on the pathological findings and the presence of anti-phospholipid antibodies, we diagnosed her as APS nephropathy. She was treated with intravenous cyclophosphamide and steroid pulse therapy, and proteinuria decreased two months later.

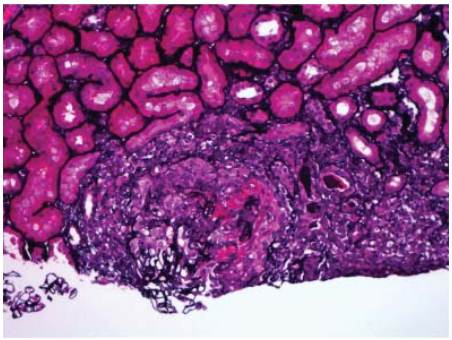
We report a rare case of APS nephropathy.presenting with nephrotic syndrome. We should consider the possibility of APS nephropathy when we see the patient with proteinuria and renal dysfunction showing chronic glomerular and vascular lesions with TMA but no immune deposits.

PUB500

Fortunate Enough to Pick Up Very Early - Double Positive ANCA and Anti-GBM Disease Girish Singhania, Jogiraju V. Tantravahi. *Nephrology, Univ of Florida, Gainesville, FL.*

Introduction: Double positive serologies for both ANCA and Anti-GBM antibodies is common in patients with either antibody. They usually present with severe disease and have a poor prognosis. We report a case of double positive for both ANCA and Anti-GBM antibodies picked up very early and got appropriate treatment in time.

Case Description: 71 y/o male with history of idiopathic pulmonary fibrosis was referred for microscopic hematuria and positive P-ANCA (ordered by pulmonologist due to worsening dyspnea). Exam was remarkable for minimal lung crackles. Labs showed normal sodium, potassium and bicarbonate. BUN was 17 mg/dL and S. creatinine was 1.1 mg/dL (Baseline 0.8-1.0). CT was negative for kidney mass. Urine showed 6 RBCs and proteinuria of 0.63 gm/gm of creatinine (negative in past). His SPEP, UPEP, hepatitis panel, ANA and complements were negative. To explain microscopic hematuria and positive P-ANCA, kidney biopsy was performed which showed necrotizing and crescentric glomerulonephritis (GN) with no immune complex deposition in EM consistent with pauci-immune GN.



Anti-GBM came back positive also. Treatment was started with cyclophosphamide and prednisone. In the last clinic followup, he was feeling better and his kidney function was stable.

Discussion: Pauci-immune GN correlates closely with ANCA disease due to circulating antibodies. There are cases of coexistence of Anti-GBM Antibodies and MPO-ANCAs in Crescentic GN. Although etiology is unknown, some believe that it is related to activation of matrix metalloproteinase-9 (MMP-9) by MPO and cleavage of goodpasture epitope by MMP-9. Studies have shown that histological characteristics in double-positive patients contain elements of MPO-ANCA disease (ie, granulomatous periglomerular inflammation) and renal survival in these patients is not better than that in anti-GBM-positive patients and is worse compared with patients with MPO-ANCAs only.

PUB501

An Atypical Case of Light Chain Deposition Disease Presenting as Acute Interstitial Nephritis Sadaf S. Khan,[1] Yashpal S. Kanwar,[2] Cybele Ghossein.[1] *[1]Div of Nephrology, Northwestern Univ Feinberg School of Medicine, Chicago, IL; [2]Dept of Pathology, Northwestern Univ Feinberg School of Medicine, Chicago, IL.*

Introduction: Light chain deposition disease (LCDD) typically presents with asymptomatic proteinuria or nephrotic syndrome due to glomerular infiltration of monoclonal light chains. We report a rare case of LCCD in a patient who presented without proteinuria and tubulo-interstitial nephritis on renal biopsy.

Case Description: A 59 year old female was noted to have an elevated serum creatinine 2mg/dl on routine lab testing. Initial renal biopsy demonstrated acute tubulo-interstitial disease with granulomatous reaction. Serum protein electrophoresis (SPEP) and urine protein electrophoresis (UPEP) were initially negative. Prednisone was initiated for presumed allergic interstitial nephritis without improvement. Five months later, serum creatinine increased to 3.11 mg/dl and repeat SPEP and UPEP demonstrated monoclonal free kappa light chains. Repeat renal biopsy demonstrated chronic tubulo-interstitial disease, acute interstitial nephritis with granulomatous reaction, and 3+ linear kappa light chain deposition along the tubular basement membrane on immunofluorescence. Further diagnostic workup was consistent with a diagnosis of multiple myeloma.

Discussion: In conclusion, LCDD usually presents as a glomerular disease with nephrotic range proteinuria. A primary tubulo-interstitial pattern, however, can also be seen. A high index of suspicion and low threshold for renal biopsy is important to identify this group of patients.

PUB502

Successful Pregnancy in End Stage Renal Disease Secondary to Lupus Nephritis: A Case Report Nicole G. Reyes. *Internal Medicine-Section of Nephrology, Univ of the Philippines Manila-Philippine General Hospital, Manila, NCR, Philippines.*

Introduction: Chronic kidney disease (CKD) secondary to Lupus Nephritis in a pregnant patient have always been a challenge among nephrologists. There is paucity of clinical guidelines in the management of hemodialysis (HD) requiring pregnant patients, more so in patients with Systemic Lupus Erythematosus (SLE).

Case Description: We are presented with a 25-year old female with SLE, with baseline creatinine of 238 umol/L (2.69 mg/dL), 24-hour urine protein of 2.76g, maintained on Azathioprine, Prednisone, and Calcium Carbonate+Vitamin D, admitted due to decreasing urine output, swelling of lower extremities, and vomiting on her 27th week of gestation. On physical exam, blood pressure was 150/80, afebrile, with anasarca. Laboratory results showed hemoglobin 112 g/L, WBC 15.4 x10^3g/L, Platelet 232 x 10^3g/L, Creatinine of 670 umol/L, BUN 38.2 mmol/L, TCO2 15.7 mmol/L, 24-h urine protein 2.2g, normal C3, routine urinalysis showed pyuria, no hematuria and no cast, and urine culture showed >100,000 colonies of Hafnia alvei. She was given Piperacillin-tazobactam, Prednisone, Methyldopa, Hydroxychloroquine, Ferrous Sulfate, and Unfractionated Heparin. HD was initiated, targeting a BUN of <15 mmol/L, TCO2 of ~20mmol/L, and hemoglobin maintained at 100-110g/L; average HD was 20 hours per week. Ultrafiltration adjusted in accordance to the expected weight gain of the patient, blood pressure controlled to maintain a MAP of 106, and serial fetal monitoring performed. However, fetal distress was noted at the 28th week age of gestation and consensus was to deliver the baby the soonest time possible, hence, cesarian delivery was done delivering a baby boy with an APGAR score of 9,9 and weighed 850grams. On follow up, patient's creatinine was ranging between 200 and 300 umol/L (2.26-3.39 mg/dL), on maintenance HD 2x/week. The baby was discharged after a month of staying in the neonatal ICU.

Discussion: This case illustrates the potential benefit of intensified, tailored, early initiation of HD regimen in a pregnant patient, as well as BP control, aggressive infection containment, with the goal of carrying the fetus into term as much as possible.

PUB503

Steal Syndrome Causing Delayed Graft Function Shukri Abdullah, Warren L. Kupin.[1,2,3] *[1]Nephrology Dept, Univ of Miami Hospital, Miami, FL; [2]Transplant Nephrology, Jackson Memorial Hospital, Miami, FL.*

Introduction: The presence of AVG or AVF in lower extremities can lead to DGF of the transplanted kidney if placed on ipsilateral side. If untreated, Steel syndrome from lower extremity AV Graft or AVF can lead to DGF or even allograft loss. The importance is to increase awareness of the effect of lower extremities AV graft or fistula and make plan to prevent DGF. In this case even diagnosis was a challenging and took two months following transplant surgery while patient was on dialysis three times a week.

Case Description: 24 year old born with right ventricle dysplasia, had heart transplant at age 13. At age 20, she, being non adherent to medications, suffered rejection, cardiac arrest, had heart transplant again, was hypotensive before and during surgery so she developed acute kidney injury, needed hemodialysis permanently. At age 24, she received a living related kidney transplant from her sister but suffered DGF and started on hemodialysis again. Her kidney biopsy showed ATN, no rejection. After two months of being on hemodialysis, an extensive radiologic and vascular workup reveal steel syndrome of her right femoral AV graft from her transplanted kidney which was also placed on the right. (poor diastolic flow and pressure to allograft was detected). Her vasculature was exhausted following cardiac arrest.

Discussion: Steal syndrome: (the arterial flow is diverted to the low-resistance system of the AV graft rather than allograft) can lead to DGF, graft loss and hemodialysis following surgery. Options: Ligation of femoral AV grafts, contralateral placement of the allograft, performing a detailed preoperative workup, ultrasound, venography, pressure measurements, or conducting intraoperative ultrasonography can prevent it and regain allograft function. It is important to consider vascular access availability in case dialysis needed. Steal syndrome can represent a diagnostic challenge as in this case report.

Funding: Private Foundation Support

PUB504

Profound Catheter Related MRSA Bacteremia Causing Cardiac Vegetations and Septic Emboli From the New Catheter in Just Three Days Lisa Aimee Hechanova, Seyed-Ali Sadjadi. *Jerry L Pettis VA Medical Center, Loma Linda, CA.*

Introduction: Hemodialysis catheter related bacteremia is an unfortunate but relatively common complication of hemodialysis in patients using either temporary or tunneled hemodialysis catheters, at a 10-fold higher rate than that of AVF or AVG. Unfortunately when there is no other functional access, catheters become a necessity. When a patient develops a catheter related infection, current IDSA guidelines recommend empiric antimicrobial therapy and immediate catheter removal if the infectious agent is Staphylococcus aureus, Pseudomonas, or Candida. They also recommend temporary catheter insertion at another anatomic site. Although these measures are usually adequate for management of a catheter related infection when initiated promptly, occasionally one can see further complications. Herein we present such a case.

Case Description: A 65-year-old male with longstanding hypertension, diabetes mellitus, and recent ESRD due to hypertension and diabetes, was admitted for a one day history of diarrhea. He was noted to have marked leukocytosis, as well as fever and chills. Blood cultures and stool studies were obtained. Two days later, blood cultures revealed MRSA (MIC for vancomycin of <0.5). IV vancomycin was initiated, and his right IJ tunneled hemodialysis catheter was immediately removed. Therapeutic vancomycin levels were achieved, and on hospital day 5, a new right IJ temporary hemodialysis catheter was inserted by IR for continuation of hemodialysis. TTE done on the same day did not show any obvious cardiac vegetations. Patient had persistent MRSA bacteremia, so on hospital day 8, patient underwent CT chest with contrast and TEE. CT chest revealed evidence of septic pulmonary emboli. TEE showed normal EF, patent foramen ovale, 0.5cm mitral valve vegetation, and a 0.5cm vegetation attached to the tip of the temporary hemodialysis catheter. The new hemodialysis catheter was immediately removed, blood cultures eventually cleared, and patient continued for a 6 week course of antibiotics for MRSA endocarditis.

Discussion: One must continually reevaluate for potential complications of MRSA bacteremia. In addition, prompt catheter removal and echocardiographic evaluation are also mandatory.

PUB505

A Case of Nephrotic Syndrome with Biopsy Evidence of Both IgA and Membranous Glomerulonephritis Gilsemar E.C. Boavida, Eduardo Jorge Duque de Sa Carneiro Filho, Lilian Cordeiro, Epitácio Rafael da Luz Neto, Lectícia Jorge, Leonardo Abreu Testagrossa, Roberto Zatz, Cristiane B. Dias, Viktoria Woronik. *Univ of São Paulo, Brazil.*

Introduction: We present an uncommon association between membranous glomerulonephritis and IgA nephropaty.

Case Description: A 45-year-old Caucasian woman sought medical attention due to edema of the lower limbs. She had been healthy until about one year earlier, when she noticed progressive leg swelling. Her past medical history was unremarkable. Urinalysis prior to admission showed proteinuria in excess of 1 g/L, without hematuria. She was treated with hydrochlorothiazide, 25 mg/day, referred to Nephrology and admitted for investigation. She was in good general condition. Blood pressure was 140/70 mmHg and BMI was 26 kg/m^2. Leg edema, 1+, was also noted. New tests showed: Hemoglobin 12 g/dL, BUN 10.3 mg/dL and Cr 0.62 mg/dL. Serologic tests for ANF and ANA were negative, whereas C3 (180 mg/dL) and C4 (37 mg/dL) were normal. Serum albumin was 3.5 g/dL, and total cholesterol was 266 mg/dL. Albumin/creatinine was 3.0. Ultrasonography showed kidneys with normal size and echogenicity. Renal biopsy revealed thickening of the glomerular basement membrane, with spikes seen in silver stained sections, and an occasional double contour. Diffuse mesangial expansion was also observed, along with slight focal endocapillary proliferation. Immunofluorescence showed diffuse mesangial deposition of IgA (2+), as well as diffuse and intense (3+) granular loop deposits of IgG and less intense deposits of IgM (1+), C1q (1+), C3 (1+) and lambda chains (1+). These findings prompted the diagnosis of IgA Nephropathy combined with Membranous Nephropathy. The patient was treated with enalapril and diuretics, with amelioration of proteinuria and preservation of renal function. She is presently being followed in an outpatient clinic.

Discussion: Combined IgA nephropathy and membranous GN is a rare condition, with relatively mild clinical manifestations, good prognosis, stable renal function and no specific therapeutic requirement. The mechanisms underlying this combination are unknown, although fortuitous association cannot be excluded.

PUB506

Vancomycin Nephrotoxicity: A Case Report of Biopsy Proven Vancomycin-Associated Acute Tubular Necrosis Coral Parikh,[1] Jacob Esquenazi,[3] James M. Pullman,[2] Deep Sharma.[1] *[1]Nephrology, Montefiore-Einstein Medical Center, Bronx, NY; [2]Pathology, Montefiore-Einstein Medical Center, Bronx, NY; [3]Medicine, Montefiore-Einstein Medical Center, Bronx, NY.*

Introduction: Vancomycin toxicity is commonly thought to be an acute interstitial nephritis. There are few reported cases of adult vancomycin-associated acute tubular necrosis (VAATN). The purpose of this report is to highlight the biopsy findings of a patient thought to have VAATN.

Case Description: The patient received vancomycin and ceftriaxone with no other known nephrotoxic agents for osteomyelitis/discitis on hospital day 1. Her baseline serum creatinine of 0.9 mg/dL rose to 4.5 mg/dL on day 9 of therapy. Peak vanco random level was 43.9 ug/mL. Renal biopsy revealed acute tubular necrosis with only mild interstitial inflammation. In addition, despite the lack of clinical findings suggestive of an intrarenal infection, the biopsy showed the extensive presence of neutrophils and dead cells in the tubular space resembling "pus casts".

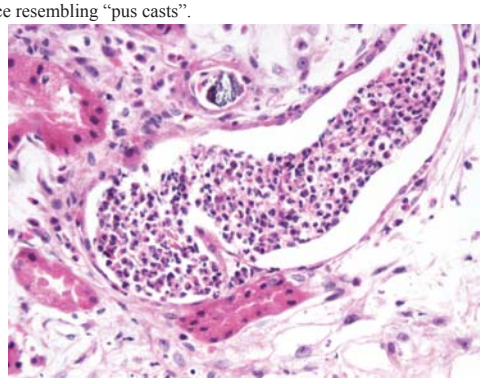


Figure 1 demonstrates renal tubule with neutrophils and surrounding interstitial edema.

Discussion: The presence of neutrophil casts may be a unique biopsy findings in VAATN.We present this case of adult VAATN as there is insufficient biopsy proven evidence of the pathological changes associated with vancomycin in kidney injury available to clinicians or pathologists.

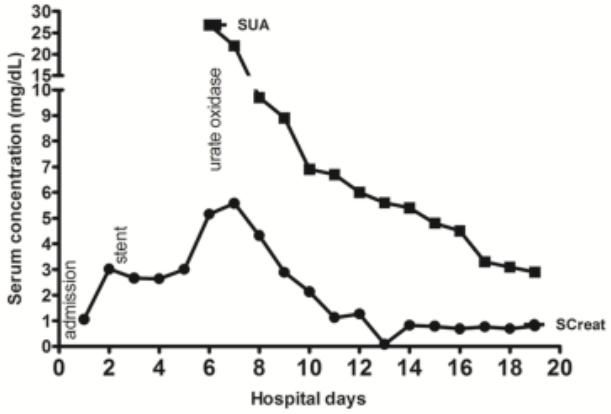
PUB507

Acute Uric Acid Nephropathy- Reversible If Recognized and Treated Early with Uric Acid Lowering Agents Hem P. Chataut, Imtiaz M. Ather, Taha Ayach, Yuvaraj Thangaraj, Keerti K. Bhanushali, A. Ahsan Ejaz. *Medicine, Div of Nephrology, Univ of Florida, Gainesville, FL.*

Introduction: Serum uric acid may have a role in acute kidney injury. We present a case to illustrate that crystal-independent mechanisms maybe involved in this phenomenon.

Case Description: We present a 67-year-old cardiac transplant recipient admitted with newly diagnosed lymphoma. Patient developed AKI and investigations revealed left hydronephrosis secondary to ureteral obstruction from retroperitoneal lymphadenopathy. Left ureteral stent placement did not result in improvement in renal function. Nephrology was consulted due to progressive worsening of AKI. Physical exam was unremarkable. Labs showed worsening SCreat of 5.6mg/dl. BUN 76 mg/dl, Na 134mEq/L, K 4.5mEq/L, Cl 108mEq/L, HCO3 23 mEq/L, Phos 5.8 mg/dL, calcium 8.8 mg/dl, UA: 100 RBC/hpf, WBC 7/hpf, trace protein and occasional calcium oxalate crystals. Serum uric acid (SUA) was found to be 26 mg/dL and the diagnosis of clinical tumor lysis syndrome (TLS) established. Patient received two doses of rasburicase (3 mg IV 12 hours apart), a recombinant urate oxidase and SUA normalized within 48 hours and was associated with prompt improvement in renal function.

Discussion: SUA causes renal vasoconstriction and has pro-inflammatory and anti-angiogenic properties. While crystal-dependent mechanisms of AKI are frequently observed in TLS, our case demonstrates the presence of crystal-independent mechanisms, which if recognized early and treated with uric acid lowering agents can lead to resolution of AKI and avoid need for renal replacement therapies.



PUB508

A Rare Cause of Renal AA Amyloidosis Bita Fakhri,[1] Lauren D. Stern.[1] *[1]Internal Medicine Residency Program, Boston Univ School of Medicine, Boston, MA; [2]Renal Section, Boston Univ School of Medicine, Boston, MA.*

Introduction: Serum Amyloid A (AA or secondary) amyloidosis is an unfortunate and rare complication of chronic inflammatory disease. This condition develops when proteolytic fragments of serum amyloid A (SAA), are deposited into tissue as amyloid fibrils and then lead to progressive deterioration in organ function. A number of conditions have been well known to be associated with secondary amyloidosis, but systemic sarcoidosis has rarely been mentioned in the literature in association with this condition. We present a case in which AA amyloidosis was found to be secondary to systemic sarcoidosis.

Case Description: A 64 year old African-American woman with past medical history significant for diabetes mellitus, hypertension, and systemic sarcoidosis was referred to nephrology clinic for evaluation of proteinuria. A kidney biopsy was performed and revealed AA amyloidosis as the etiology of her nephrotic syndrome. A workup was performed to identify the etiology of secondary amyloidosis due to its very rare association with sarcoidosis. After other common causes were ruled out, sarcoidosis was determined to be the cause of AA amyloidosis.

Discussion: A number of conditions are well known to be associated with secondary amyloidosis. These include chronic inflammatory conditions, malignancies, and chronic infections; as well as a number of cases which have no identifiable cause of secondary amyloidosis. The mainstay of treatment in AA amyloidosis is controlling the underlying disease, and thus reducing the production of serum Amyloid A protein. Antibiotics and colchicine are effective at treating and preventing infection related and familial Mediterranean fever-related AA amyloidosis, respectively. Recently, TNF-alpha blockers have emerged as effective agents in AA amyloidosis associated with some autoimmune disease. Eprodisate, a new agent, binds to the glycosaminoglycan binding site on amyloid fibrils, thus targeting amyloid fibril polymerization and tissue deposition. The results of a recent randomized trial showed that eprodisate may slow the decline of renal function in AA amyloidosis and confirmatory studies are underway.

PUB509

Acute Glomerular Disease Masked by Severe Rhabdomyolysis Juan M. Quevedo, Farhang Ebrahimi. *Medicine/Nephrology, Richmond Univ Medical Center, Staten Island, NY.*

Introduction: Severe rhabdomyolysis is a known cause of renal failure. We however present a case of underlying acute interstitial nephritis (AIN) and IgA nephropathy (IgAN) which could have been overlooked as concomitant causes of our patient's severe renal failure in the context of rhabdomyolysis had we not followed the leads of proteinuria, hematuria and the presence of active urine sediment.

Case Description: A 38 year old male with no past medical history was brought to the emergency room after he was found lying on the floor for 12 hours due to worsening leg swelling and muscle pain. The muscle pain started after shoveling snow for which he took ibuprofen. Physical exam revealed ill looking male, BP 145/83 mmHg, nauseas, localized epigastric tenderness and bilateral leg swelling. Laboratory findings were significant for BUN 166 mg/dL; creatinine 17.3 mg/dL; Potassium: 6.6 mmol/L, CO2: 8 mmol/L; Lipase 640 U/L, creatinine phosphokinase (CPK): 190,000 U/L; Hgb: 12.1 g/dL; WBC: 11.4 x 10^3; ethanol level < 3 mg/dl. Urinalysis: cloudy; SG: 1.019; protein > 300 mg/dL; glucose: 100; blood: large; leukocyte esterase: trace; RBC 10-15; WBC 10-15/ HPF. Urine total protein: 536.9 mg/dL; urine creatinine: 158.6 mg/dL; urine protein-to-creatinine ratio (UPCR): 3.4 g/day which peaked to 6.6 g/day. Patient underwent emergent dialysis which continued for the next 12 days. Repeat urinalysis showed dysmorphic red blood cells which in addition to nephrotic range proteinuria led to further workup with renal biopsy as serology results have been negative. Interestingly, drug induced acute interstitial nephritis and IgAN was found. Patient markedly responded to steroid treatment and was discharged one week after initiation of therapy with creatinine of 1.4mg/dl and UPCR less than 500 mg per day.

Discussion: It is well established that there is a spectrum of IgAN presentations however, to our knowledge there have been no reports of IgAN with simultaneous complications like rhabdomyolysis and drug induced AIN. Though myoglobin tubular toxicity can cause severe renal failure the degree of proteinuria and hematuria observed is usually in the subnephrotic range and typically not characterized by active urine sediment.

PUB510

Severe Hypokalemia as First Clinical Manifestation of Lung Cancer Rohini Prashar, Ginius Pradhan, Deepak K. Malhotra. *Univ of Toledo Medical Center, Toledo, OH.*

Introduction: Ectopic ACTH Production by malignant neoplasms is well known to cause severe metabolic derangements. We report an interesting case in which the workup for hypokalemia helped uncover underlying lung cancer.

Case Description: A 67 year old male without any past medical history presented with fatigue, confusion, and dyspnea. He had a blood pressure of 220/100 mm Hg. Physical examination was unremarkable except muscle weakness and confusion. Initial tests revealed hypokalemia(1.9 mmol/l), metabolic alkalosis (40 mmol/l) and normal renal function. The patient was intubated due to respiratory failure. His serum potassium did not improve beyond 2.7 mmol/l, despite aggressive replacements up to 150 meq KCl for 10 days. At that juncture, nephrology was consulted. Further workup for hypokalemia revealed a TTKG of 12 and a Aldosterone to Renin Ratio(ARR) of 16. In the setting of hypokalemia, metabolic alkalosis, high TTKG, uncontrolled hypertension, new onset diabetes mellitus and normal ARR the diagnosis of hypercortisolism was entertained. Hypercortisolism was confirmed with a positive dexamethasone suppression test and an elevated ACTH level (215 pg/ml). No abnormality was detected on CT scan of the brain; therefore, an ACTH producing paraneoplastic syndrome was suspected. Further imaging revealed a lung mass with extensive metastatic disease. Biopsy of the mass confirmed the diagnosis of small cell lung carcinoma. The patient was started on potassium sparing diuretics along with potassium supplements and his levels improved. Unfortunately his respiratory failure did not improve. Due to the extensive nature of his disease, the family decided to withdraw care.

Discussion: Ectopic ACTH secretion(EAS) occurs in about 10% of all cases of Cushing's syndrome, and about 25% of cases of ACTH-dependent Cushing's syndrome. EAS due to malignant neoplasms has been reported to have earlier and more aggressive metabolic effects. Our case emphasizes the importance of suspecting EAS in a patient with severe hypokalemia, metabolic alkalosis and rapid clinical deterioration. The diagnosis may easily be overlooked unless the clinical suspicion is high. Failure to diagnose EAS may result in increased morbidity and even death.

PUB511

A Case of the Primary Membranous Nephropathy with the Necrotizing Cellular Crescent Yukinao Sakai,[1] Yuichiro Sumi,[1] Shizuka Yui,[1] Masami Sukegawa,[1] Anna Suzuki,[1] Koji Mugishima,[1] Yusuke Otsuka,[1] Tomoyuki Otsuka,[1] Akira Shimizu,[3] Shuichi Tsuruoka.[2] *[1]Dept of Nephrology, Nippon Medical School Musashikosugi Hospital, Kawasaki, Kanagawa, Japan; [2]Dept of Nephrology, Nippon Medical School, Tokyo, Japan; [3]Dept of Analytic Human Pathology, Nippon Medical School, Tokyo, Japan.*

Introduction: The patient was a 66-year-old Japanese man. He was referred to our hospital, because of BUN 81.4 mg/dL, Cr 4.45 mg/dL and proteinuria. Autoimmune and viral serological tests showed no significant abnormalities. Neither monoclonal gammopathy nor Bence Jones proteinuria was detected by immunoelectrophoresis. Serum complement levels were normal. Urinalysis showed proteinuria (0.54 g/day) but no hematuria.

Case Description: Several glomeruli exhibited diffuse thickening of the peripheral capillary walls. PAM staining revealed variable basement abnormalities such as diffuse spike formation. Some glomerulus shows partial necrosis of the capillary tuft overlaid by

the cellular crescent and disruption of the basement membranes. Also, the endocapillary proliferation was found. No tubulointerstitial lesions were evident. IF staining produced strong glomerular staining of IgG and C3 globally in the peripheral capillary loops. EM analysis revealed discrete subepithelial deposits with spike formation of basement membrane material and electron-lucent areas.

Discussion: Based on the above histological and immunohistological findings, we diagnosed this case as MN (Ehrenreich-Churg Stage II - III). The oral administration of PSL was initiated at a dosage of 45 mg/day. After 4 weeks, CR of proteinuria was achieved. The PSL dosage was reduced to 40 mg/day; urinalysis remained negative for the next 4 weeks and the patient was discharged. In addition, we screened the malignant tumor, but there were no significant findings. Primary MN was diagnosed. The membranous change by immune complexes deposition to glomerular basement membrane is the main characteristic of the MN, and it is usually said that crescent formation and endocapillary proliferation are not associated. We experienced a rare case of the primary MN.

PUB512

Recovery of Residual Renal Function in a Patient That Restarted Peritoneal Dialysis after Temporary Inpatient Intermitent Hemodialysis Alejandra Mena- Gutierrez, Dumitru Rotaru. *Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

Introduction: Residual renal function (RRF) is an indicator of remaining glomerular filtration rate, endocrine functions, including erythropoetin production and bone metabolism, and in different series has shown association with mortality and quality of life. Prospective clinical trials have come to the conclusion that patients on peritoneal dialysis (PD) have 65% lower risk of RRF loss compared to those on hemodialysis (HD). Hemodialysis can alter hemodynamics and solute concentrations affecting RRF which is inversely correlated with frequency of treatments and number of years on this modality.

Case Description: 44 year old male with history smoldering multiple myeloma (SMM) and end-satge renal disease (ESRD) secondary to focal and segmental glomeruloesclerosis (FSGS). Renal biopsy revealed collasing glomerulopathy, chronic interstitial nephritis, mild to moderate arterionephrosclerosis and was negative for amyloidosis, light chain deposition disease or cast nephropathy. He had been on peritoneal dialysis with no complications. PD adequacy was monitored periodically and he had good RRF with urine output of more than 1 L/day. During his 2nd year on PD the SMM progressed to overt disease. At the request and insistance of his myeloma team he was transitioned to intermittent hemodialysis (IHD), while inpatient treatment with chemotherapeutic agents and autologous stem cell transplant was undertaken. During his 31days long hospitalization patient became oligo-anuric without evidence of significant hypovolemia, tumor lysis syndrome or nephrotoxin use as a possible cause. After hospital discharge patient had increase in urine output up to 800 ml/day. At his PD clinic follow up his RRF was 1.06.

Discussion: We presented a PD patient that developed anuria while receiving temporary IHD and then unexpectdly had recovery of RRF upon restarting PD as outpatient. While rapid loss of RRF has been described in patients on IHD and SDHD, this regain of RRF has not been described in the literature. Such clinical outcome should be sought in other settings where PD patients receive temporary HD for different medical reasons.

PUB513

Membranoproliferative Glomerulonephritis and Renal Cell Cancer: Case Report Epitácio Rafael da Luz Neto, Suellen Klein, Alcino Pires Gama, Lectícia Jorge, Cristiane B. Dias, Luis Yu, Viktoria Woronik. *Div of Nephrology, Univ of Sao Paulo, Sao Paulo, SP, Brazil.*

Introduction: Glomerular diseases can occur in context of neoplasms. Several histopathological patterns have been described in association with different types of cancer. Membranoproliferative glomerulonephritis (MPGN) is a clinical-pathological entity due to infections, autoimmune diseases, plasma cells dyscrasias, tumors or idiopathic. We report a case of a patient with nephrotic syndrome, as result of kidney cancer-associated MPGN.

Case Description: A 40 year-old man presented to outpatient of a tertiary hospital complaining of generalized edema and hypertension for 4 months. The laboratory evaluation revealed creatinine 2.8 mg/dl; urea 86 mg/dl; hemoglobin 10.8 g/dl; serum albumin 1.9 g/dl; total cholesterol 362 mg/dl; C3 21 mg/dl (90-180); C4 4 mg/dl (10-40); FAN, Anti-DNA and cryoglobulins negatives; rheumatoid factor 80 IU/ml (<20). The urinalysis shows +100 erythrocytes/field. The 24-hour proteinuria was 21.6 g. The HBV, HCV and HIV serologies were negative. Abdominal CT scans showed solid nodule in lower pole of left kidney. Partial nephrectomy was performed. The pathological study shows clear cell renal cell carcinoma stage T1aN0M0 with adjacent MPGN. Persistent hematuria, nephrotic syndrome, and complement consumption were still present 6 months after the tumor resection and patient underwent to dialysis.

Discussion: Paraneoplastic glomerulopathy pathogenesis involves immune activation mediated by tumor associated antigens, re-expressed fetal antigens, or viral antigens. Glomerular injury may be due to circulating immune complexes or formed *in situ*. Nephrotic syndrome improvement can be associated with the successful tumor treatment and resection. Immunosuppression is not indicated due to risk of neoplasia progression. In contrast to showed case, in literature there was a case-report in which there was laboratory and clinical improvement after 7 months of neoplasm resection. Two possible reasons for the differences are persistent disease since surgery was not curative or the diseases are independent and complement consumption would be a MPGN idiopathic marker. The patients follow-up may help to elucidate this question.

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PUB514

Salisylate Induced Pulmonary Edema - Dialysis or No Dialysis? Girish Singhania, Gurjit Dhatt, Charles S. Wingo. *Nephrology, Univ of Florida, Gainesville, FL.*

Introduction: Salicylate ingestion is a common cause of poisoning in children and adolescents. It can be acute or chronic. Salicylate induced pulmonary edema (SIPE) is a rare complication from salicylate intoxication. Management of it is always a question. We present a severe case of salicylate intoxication presented with SIPE.

Case Description: 28 y/o female was complaining of headache, dyspnea and fevers. There were multiple BC powder wrappers around her per EMS. Exam showed BP: 128/67 mmHg, Pulse: 141/min, RR: 19/min went to 35-40 in next hour, afebrile, 84% saturation on room air. There were crackles in lungs. Labs showed sodium: 140 mmol/L, potassium: 3.3 mmol/L, Chloride: 107 mmol/L, Bicarbonate: 17 mmol/L, S. Creatinine: 1.0 mg/dL, Glucose 156 mg/dL, Alb: 2.9 g/dL, Salicylate: 48 mg%, Serum pH: 7.18, Urine pH: 6.0. Chest X-ray showed pulmonary edema. CT showed early changes of cerebral edema. She was intubated. We were consulted for further management of salicylate poisoning. In the next 2 hours, she was aggressively hydrated with normal saline and isotonic bicarbonate. She made more than one litre of urine. ICU team asked us for hemodialysis due to SIPE and early cerebral edema but due to good renal function, we didn't dialyse her. We recommended to continue aggressive hydration and alkalanization of urine. Over the next 48 hours, patient was clinically improved.

Discussion: Salicylates are absorbed within 15-30 minutes and is metabolized quickly but in overdose, the half life increases due to hepatic enzyme saturation. In Acidosis, there is increased penetration in tissues. SIPE is a serious complication which can occur in both acute and chronic users and should be managed aggressively. Absolute indications for dialysis are renal, cardiac or hepatic compromise. Relative indications are salicylate level >120mg%, pulmonary edema, refractory acidosis, severe CNS manifestations. Misdiagnosis can lead to a significant increase in morbidity and mortality. In the recent review, there were no clear guidelines exist on when to use dialysis in salicylate intoxication or SIPE. However, they suggest the use of dialysis in patients with organ damage, such as pulmonary edema, CNS disturbances, and renal impairment.

PUB515

Drug Induced Acute Interstitial Nephritis Coupled with Crescenteric Glomerulonephritis in a HIV Positive Patient Purna Bindu Nandigam, Muhammad R. Syed, Talal A. Khan, Kenneth Lau, Azka Arif, Muhammad Awais Arif, Arif Jan, Hasan Arif, Sandeep Aggarwal. *Div of Nephrology, Drexel Univ, Philadelphia, PA.*

Introduction: Acute kidney injury is common in human immunodeficiency virus (HIV) infected patients, and has been associated with increased morbidity and mortality. AKI remains a significant problem in the antiretroviral era, and is still commonly attributed to infection or nephrotoxic medications.

Case Description: A 50-year-old African American male with history of HIV, diabetes mellitus type 2 and chronic kidney disease stage III was admitted with complaints of hematuria. He was started on Bactrim and Doxycycline for an infected back wound 5 days ago. On initial evaluation he was found to have acute kidney injury with a creatinine of 3.46 mg/dL up from a baseline of 1.6 mg/dL. Urine microscopy revealed many RBCs, but did not reveal any casts or eosinophils. Serological tests revealed a positive ANA titer (1:160). Anti GBM Ab, c-ANCA, p-ANCA, ASO Ab, serum cryoglobulins and HCV PCR were all negative. Serum complements were within normal limits. Spot protein to creatinine ratio was 8.4 grams per day. A Peripheral blood smear showed no evidence of schistocytes. A kidney biopsy showed crescents in 8/23 glomeruli with 70-80% podocyte effacement and scattered sub-endothelial, sub-epithelial and intramembranous fine granular electron dense deposits. Immunofluoresence was positive for granular staining in the mesangium with C3 and IgM. There was also a dense tubulo-interstitial inflammatory infiltrate consistent with acute interstitial nephritis (AIN). These findings suggested multiple injury patterns with crescenteric Glomerulonephritis (GN) secondary to post infectious GN coupled with AIN.

Discussion: Our case illustrates an uncommon presentation of rapidly progressive GN with hematuria in an HIV positive patient. With multiple patterns of injury in an immunocompromised host the dilemma was in choosing the treatment modality. We stopped the offending antibiotics and elected to treat with high dose pulse steroids followed by a short taper. This resulted in improvement in renal function to near baseline without the need for hemodialysis.

PUB516

A Case of Primary Hyperaldosteronism with Unusual Serum Bicarbonate Disha Narula, Amit A. Deshpande, Monalisa Joseph, Hasan Arif. *Div of Nephrology, Drexel Univ.*

Introduction: Primary hyperaldosteronism is a well known cause for uncontrolled HTN coupled with hypokalemic metabolic alkalosis. However, we present a case in which a patient was found to have primary hyperaldosteronism with an unusually low serum HCO3. This case is an example of diagnostic challenges with complicated acid-base and electrolyte disturbances in patients with concomitant medical problems.

Case Description: 55-year-old AA male with history of HTN and CVA with residual left sided weakness presented to the ER for nausea and vomiting for 1 day. He was found to have a BP of 230/115. Labs showed potassium of 1.9, bicarbonate of 19, Cr 0.65. His arterial blood gas showed a pH of 7.7, a PCO2 of 18, a PO2 99, HCO3 of 22 and O2 sat 99%. He was admitted to medical ICU and started on a nicardipine drip for his blood pressure. His potassium was aggressively repleted, but he had persistent hypokalemia. For his respiratory

alkalosis, a CT scan for PE was negative. A secondary HTN workup revealed a plasma aldosterone of 50 ng/L, plasma renin 0.39 ng/L, urine metanephrines 199 ug/24hours and plasma metanephrines 290ug/24hours. Renal US with dopplers showed normal sized kidneys with no detectable renal artery stenosis. Urine K+ was found to be 60meq/L. TTKG was calculated to be 14. Plasma aldosterone/renin ratio was 128. A CT scan of the abdomen revealed bilateral adrenal hyperplasia. Patient was tapered off the nicardipine drip and started on spironolactone. His hypertension and hypokalemia significantly improved.

Discussion: In view of high plasma aldosterone coupled with uncontrolled hypertension and hypokalemia, a diagnosis of primary hyperaldosteronism was made. On first glance, it appears that the patient's serum bicarb is not consistent with metabolic alkalosis and makes the diagnosis difficult to make. However, given that the patient had primary respiratory alkalosis the expected compensation for his chronic respiratory alkalosis would lead to a serum bicarb of 11meq/l. However in the presence of primary hyperaldosteronism his serum bicarbonate was higher than expected. Our case represents an unusual presentation of Primary Hyperaldosteronism with unusual serum bicarbonate due to concomitant medical problems.

PUB517

Successful Patient and Renal Outcome in p-ANCA Vasculitis Inspite of Worst Prognostic Factors Ruchira Sengupta,[1] Egbert C. Liquete,[2] Sarika Deshmukh.[2] *[1]Internal Medicine, Henry Ford Hospital; [2]Nephrology and Hypertension, Henry Ford Hospital, Detroit, MI.*

Introduction: ANCA associated vasculitis is a rare disease. It is associated with high morbidity and mortality due to systemic inflammation as well as treatment side effects especially in elderly population. We present a case with features of P-ANCA associated vasculitis in an elderly patient. Its successful outcome was dependent on early recognition, immediate initiation of immunosuppressant therapy and continuous renal replacement therapy using regional citrate anticoagulation method.

Case Description: A 68-year-old lady, with no significant medical history, presented with progressive worsening of shortness of breath over the course of 3 weeks. Patient had acute respiratory decompensation requiring mechanical ventilation. Bronchoscopy showed active diffuse alveolar hemorrhage. She had auric AKI and *p-ANCA* titers of 1:80. Kidney biopsy showed focal segmental necrotizing glomerulonephritis, 56% global sclerosis, 16% segmental sclerosis, 3% cellular crescent, with moderate tubular atrophy and interstitial fibrosis. She had pulse dose intravenous methylprednisolone 1 gm daily for 3 days then maintained on oral maintenance dose; 10 sessions of plasmapheresis, and oral cyclophosphamide (induction and maintenance) carefully dosed based on her age, AKI requiring dialysis status. She had full recovery of kidney function after requiring dialysis for 7 weeks. Her serum creatinine is 1.5 mg/dL with proteinuria less than 1 gm.

Discussion: Our patient with microscopic polyangitis presented with risk factors that have been associated with the highest mortality, including age, active pulmonary hemorrhage with respiratory failure, and acute kidney injury requiring daily dialysis. Despite the predicted poor prognosis, she had significant recovery in renal function and overall functional ability. Our case illustrates successful use of oral cyclophosphamide, with no adverse complications, or relapse in first year. We believe that along with high index of suspicion, prompt initiation of immunosuppression, unique availability of continuous renal replacement therapy with regional citrate anticoagulation helped to decrease morbidity and rapid recovery.

PUB518

Unexplained Hypomagnesemia in an Adult Roshan A. Patel, Viral G. Gandhi, Salwa Rhazouani, Maritza Brown. *Nephrology, Elmhurst Hospital Center, Icahn School of Medicine, Elmhurst, NY.*

Introduction: Case of a patient presenting to renal clinic with Magnesium (Mg) level < 1 mg/dl in spite of daily intravenous Mg repletion.

Case Description: A 61 year old Tibetan woman with a history of hypertension was referred for profound hypomagnesemia. She had been admitted to an outside hospital for weakness and paresthesias, which were attributed to severe hypomagnesemia. Omeprazole was discontinued and she was discharged on daily intravenous Mg infusion. On evaluation, the patient's fractional excretion of Mg was 21.6 % with a serum Mg level of 1 mg/dl and 24 hour Urine Mg of 738 mg. On follow up, her Mg had dropped to 0.9 mg/dl and she was admitted to the hospital. Bicarbonate and Potassium level were not consistent with Gitelman's Sydrome. Intravenous repletion was changed to oral Mg; three days later serum Mg remain unchanged at 1 mg/dl but urine Mg declined to 110 mg. Though the urine Mg improved, it remained relatively high considering the patient's low serum Mg.

	Clinic Visit	Hospital Admission	Hospital Discharge	2 months after Discharge
Serum Mg	1.6	1	1	1.1
Urine Mg	42.3	-	4.4	-
Serum Potassium	3.9	3.7	4.3	4
Serum Calcium	9.4	9.1	9	8.6

Differential Diagnosis
Gitelman's Syndrome
Bartter's Syndrome
Medication (PPI, Diuretic, Aminoglycoside)
Hypercalcemia
Uncontrolled Diabetes Mellitus
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis
Epidermal growth factor gene mutation
Cyclin M2 mutation

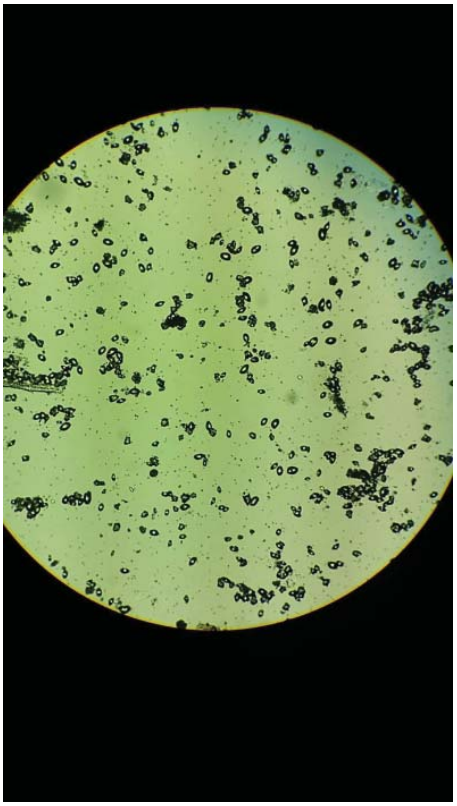
Discussion: Severe Hypomagnesemia is a serious clinical condition associated with life threatening arrhythmias and neurologic manifestations. Few adult cases with persistent isolated severe hypomagnesemia despite treatment have been described in the literature. In Mg deficiency, the kidneys can normally reduce the 24-hour urinary Mg excretion to less than 10 to 30 mg or a Fractional excretion of Mg < 2%. Moreover, acquired renal Mg wasting can result from many known medications. Several inherited renal tubular disorders such as Gitelman's and Bartter's syndrome are associated with loss of Mg but have other electrolyte disturbances. A rare hereditary tubulopathy manifested solely by isolated hypomagnesemia has been described in the literature.

PUB519

Acute Kidney Injury Secondary to Sulfamethoxazole Crystals in an AIDS Patient Sandeep M. Patil, Karina Sulaiman. *Div of Nephrology,Dept of Medicine, Louisiana State Univ Health Sciences Center-Shreveport, Shreveport, LA.*

Introduction: Pneumocystis jiroveci pneumonia (PJP) commonly causes opportunistic infections in patients with Acquired Immune-Deficiency Syndrome (AIDS) and the standard therapy consists of Trimethoprim-sulfamethoxazole (TMP-SMX). Acute kidney injury (AKI) secondary to obstructive uropathy from TMP-SMX crystalluria has not been reported. We report a case of TMP-SMX crystal induced AKI in an AIDS patient with PJP.

Case Description: A 51 year old African American man presented with fatigue, malaise, chest congestion and shortness of breath for a 2 months. He was vitally stable and physical exam was remarkable for lower lung field crackles. Chest x-ray showed bilateral patchy infiltrates and he tested positive for HIV.His renal function was normal and urine analysis (UA) was unremarkable. Blood and fungal cultures were drawn. He was started on TMP-SMX 320mg-1600 mg orally every 8 hours for empiric treatment of PJP.Over the next four days he developed AKI and oliguria. His serum creatinine increased to 2.5mg/dl, peaking at 6.5mg/dl and repeat UA was notable for sulfa crystals as depicted in fig 1.



Adding acetone to the urine dissolved the sulfa crystals.Urine Wright's stain was negative for eosinophils. He became anuric and required dialysis once. TMP-SMX was stopped and intravenous fluid administered. Renal function and urine output subsequently improved. No crystals were seen in a repeat UA.

Discussion: Sulfa-containing medications can cause crystal-induced AKI from intratubular deposition in addition to allergic interstitial nephritis. Crystalluria is rarely reported with newer sulfonamides. Sulfamethoxazole can also precipitate in the kidneys and cause AKI from tubular obstruction. Treatment includes drug cessation, aggressive fluid resuscitation and alkalinization of urine to a target pH of 7.5.

PUB520

A Case Report of Valproic Acid Toxicity and the Role of Renal Replacement Therapy Amit A. Deshpande, Disha Narula, Hasan Arif. *Drexel Univ.*

Introduction: Valproic acid is a branched-chain carboxylic acid first introduced in 1978 for the treatment of seizure disorders and various psychiatric conditions. Occasionally patients prescribed the medication will present with signs of toxicity.

Case Description: 15 y.o. F. with pmh of bipolar disorder, multiple suicide attempts, and hypothyroidism presented to ER by paramedics following a suicide attempt after ingesting 8 valproic acid tabs (500mg tabs). On initial evaluation in ER, patient was noted to be lethargic and falling asleep during encounter. Labwork revealed mixed acid base disorder on ABG: pH 7.30, pCO2 41.7, bicarb of 23, valproic acid level of 161 and ammonia level of 53 in setting of normal LFTs. Vitals remained stable except for a heart rate of 113, BMP and CBC were within normal limits. Pt. was intubated in ER for airway protection as her mental status began to decline. A stat CT head was performed revealing possible cerebral edema. Pt. was transferred to medical ICU. Gut decontamination was performed with activated charcoal, and the pt. was started on carnintie. Nephro was consulted for possible RRT. Initial plan was to start hemodialysis, but after much discussion, the decision was made to hold treatment given the lack of strong evidence based data supporting RRT in the setting of such low valproic acid levels. The patient was observed in ICU setting. LFTs, ammonia levels, valproic acid levels were trended every 4-12 hours and neuro checks were performed routinely until valproic acid levels returned to therapeutic range. Patient was extubated successfully 3 days after presenting to the ER and transferred to a telemetry floor.

Discussion: The role of RRT in the setting of valproic acid toxicity is grossly under investigated. Available data has shown RRT to be beneficial in such toxicity, especially at levels greater than 850mg/L. Unfortunately no significant trials have been conducted to establish appropriate drug levels at which to initiate treatment. Most evidence available is based on a minute number of case series and review articles. A review of available literature can help clinicians have a clearer sense of when to utilize renal replacement therapy as a potentially life-saving treatment modality.

PUB521

Oral Acyclovir, Nephrotoxicity, and Herpes Simplex Virus Prophylaxis in a Patient with Multiple Myeloma Judy K. Tan, Girish N. Nadkarni, Ioannis Konstantinidis, Achint Patel, Vijay Lapsia. *Icahn School of Medicine at Mount Sinai, New York, NY.*

Introduction: Acyclovir is commonly used as prophylaxis for herpes simplex virus (HSV) during chemotherapy for multiple myeloma. Intravenous acyclovir is well known to cause crystal induced nephrotoxicity leading to acute kidney injury (AKI). About a third of patients may also experience a transient increase in serum creatinine levels during therapy, thought to be secondary to interference in renal handling of creatinine. It is often difficult to distinguish between these two entities early on and may impact clinical decision making for infection prophylaxis.

Case Description: A 61-year man with no previous medical history presented with anorexia, back pain and weight loss. He was found to have anemia (hemoglobin 8.3 gm/dL) and hypercalcemia (10.6 gm/dL) with elevated creatinine to 1.7 mg/dL. A bone marrow biopsy revealed 15% plasma cells with binucleated forms and serum electrophoresis showed 10gm/dL of IgG lambda. He was admitted to the hospital for initiation of chemotherapy with bortezomib, cyclophosphamide and dexamethasone. In addition, acyclovir was started at 400 mg twice daily for HSV prophylaxis. After receiving 2 doses of acyclovir, his creatinine acutely trended up to 2.7 from 1.7 mg/dL; however, other markers of kidney function including urea nitrogen improved (from 27 to 23 mg/dL). Initial workup for other causes of acute kidney injury (AKI) was negative. After discontinuation of acyclovir, creatinine trended down almost immediately to baseline value of 1.6 mg/dL. The patient was safely switched to valacyclovir for prophylaxis.

Discussion: Drug induced nephrotoxicity is a serious adverse reaction that can be deleterious. AKI induced by oral acyclovir is uncommon and early identification and discontinuation may prevent further morbidity.

PUB522

A Case of Metformin-Induced Lactic Acidosis Complicated by Ketoacidosis Sharad D. Patel,[1] Leslie F. Thomas.[2] *[1]Nephrology Fellowship, Univ of California Los Angeles; [2]Div of Nephrology and Hypertension, Mayo Clinic Arizona, Phoenix, AZ.*

Introduction: Uncommonly, metformin use may promote the development of lactic acidosis. Mortality is high (83%) in those individuals in whom severe acidemia ensues (pH < 6.9). Known risk factors for the development of lactic acidosis in metformin users include end stage liver disease, congestive heart failure, and renal insufficiency. Heretofore, associated ketoacidosis has not been described as an associated feature of metformin-induced acidemia. Here, we describe the successful treatment of a patient in whom metformin-induced lactic acidosis and associated ketoacidosis produced a severe metabolic acidemia (pH <6.671).

Case Description: A 65-year-old woman with a history of type 2 diabetes mellitus without known history of kidney disease presented with four days of progressive nausea, vomiting, generalized weakness, and one day of anuria. Her only medication was metformin 1000 mg by mouth twice daily. Initial examination revealed significant tachypnea but was otherwise normal. Initial arterial blood gas revealed a pH < 6.671 (rechecked twice). Other labs at presentation included a serum bicarbonate < 5 (22-29 mmol/L), anion gap (AG) 49, creatinine 8.3 (0.6-1.1 mg/dL), lactate 19.1 (0.6-2.3 mmol/L), and metformin 55.1 mcg/mL (therapeutic range 1-2 mcg/mL). Shortly after her arrival, the patient became hemodynamically unstable, and she was placed on a norepinephrine infusion. Emergent hemodialysis was performed, and she subsequently demonstrated improved hemodynamics and blood pH (7.224). Within 24 hours, the patient's AG persisted at 31 despite normalization of lactate (1.0 mmol/L). At that point, a beta-hydroxybutyrate level was measured and found to be 53.3 (< 0.4 mmol/L). The patient was subsequently treated for ketoacidosis with normalization of the AG. The patient subsequently recovered renal function and was discharged without serious sequelae after five days.

Discussion: Significant accumulation of beta-hydroxybutyrate may occur in patients with acute renal failure and metformin-induced lactic acidosis. This complicating factor may potentially lead to deleterious outcomes if not recognized swiftly. Proper treatment for patients with metformin-induced lactic acidosis should include prompt hemodialysis and recognition and treatment of ketoacidosis.

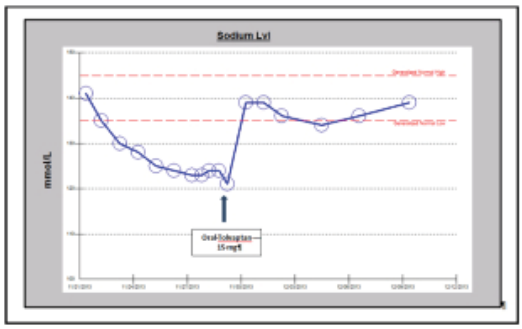
PUB523

Rapid Normalization of Post-Traumatic Severe Symptomatic Acute Hyponatremia after a Lone 15 mg Tolvaptan Dose: A Mayo Clinic Health System Trauma Unit Experience – A Call for Caution with Tolvaptan in Younger Patients with Preserved Renal Function Macaulay A. Onuigbo,[1,2] Nneoma Agbasi.[3] *[1]Medicine, Mayo Clinic College of Medicine, Rochester, MN; [2]Nephrology, Mayo Clinic Health System, Eau Claire, WI; [3]Psychiatry Nursing, North East London NHS Foundation Trust, London, United Kingdom.*

Background: Tolvaptan is established treatment for hyponatremia from SIADH. Over rapid correction can cause fatal pontine demyelination. We report the dramatic rapid correction of hyponatremia after one 15 mg dose in a young man with normal kidney function (eGFR of 126).

Methods: Case report.

Results: A 32-year old Caucasian male was admitted after a MVA with altered consciousness, rib fractures, a large scalp hematoma, and subarachnoid hemorrhage. Na fell from 141 to 121 mEq/dL despite fluid restriction (800 ml/day), oral NaCl tablets and repeated IV 3% NaCl. Symptoms were blurred vision, poor memory and cognitive impairment. Following a single dose of Tolvaptan, 15 mg, urine volume quintupled with increased thirst. Serum Na swiftly increased to 139 mEq/dL over 18 hours. AVP was 1.4 pg/mL consistent with SIADH. After 2 days, symptoms resolved. He was discharged. A week later, asymptomatic but for persistent left gaze diplopia, Na was 139 mEq/dL.



Conclusions: Earlier studies on oral Tolvaptan for symptomatic hyponatremia (SALT-1, SALT-2, EVEREST, SALTWATER) recruited older patients, age 64-67 years, with baseline serum creatinine ~1.3-1.4 mg/dL and utilized doses of 30 mg/d or higher. The very rapid correction of hyponatremia in our young patient after a single oral dose of 15 mg of oral Tolvaptan is the most recorded. Kidney function was normal an eGFR of 126 ml/min/1.73 sq. m BSA. We recommend lower doses of Tolvaptan (≤15 mg/d) in younger patients with preserved kidney function to prevent over-rapid correction of hyponatremia and risk potentially fatal central pontine myelinolysis.

PUB524

Electrocardiogram (EKG) Changes of Hyperkalemia in Patients with Normal Renal Function and with Chronic Kidney Disease (CKD) Sairah Sharif,[1] Leonel Carrasco,[2] Amin Paulino,[2] Jose M. Sosa Popoteur,[2] Mei-An Ty-Arias,[2] Rose Calixte,[1] Sudhanshu Jain,[2] Damian Kurian,[2] Jeffrey D. Wallach.[2] *[1]Winthrop Hospital, Mineola, NY; [2]Harlem Hospital, New York, NY.*

Background: Potassium (K+) is the major intracellular cation and its ratio to the extracellular K+ determines resting cell membrane potential. Hyperkalemia can lead to serious cardiac arrhythmias. It increases rate of repolarization and slows conduction velocity manifested as peaked T waves and prolonged PR interval. It has been suggested that patients with advanced CKD tolerate hyperkalemia better than patients with normal renal function. EKG is a clinical tool to assess potential toxicity of hyperkalemia. We tested if patients with advanced CKD demonstrate lesser EKG abnormalities in the setting of hyperkalemia compared to patients with normal renal function.

Methods: We reviewed records of patients ≥18 years seen in the emergency or inpatient setting from 2008 to 2011 with hyperkalemia (5.5 to 8.8 meq/l) and normokalemia (3.5 to 5.1 meq/l) who had an EKG done within 4 hours of the determination. We excluded patients who received treatment to reduce K+, had hemolyzed samples, had pacemakers, or unreadable EKG deemed by cardiologist. 71 patients were selected and demographics, serum K+, serum creatinine, EKG parameters were measured. The association of serum K+ with changes in EKG parameters was examined and comparisons made among populations with normal renal function (NRf), chronic kidney disease stage 4 and 5 (CKD4/5), and hemodialysis patients (CKD5D).

Results: Using Wilcoxan rank sums we found that hyperkalemic patients had significantly elevated mean T/R ratios and mean PR intervals compared to normokalemic patients (p values 0.049 and 0.034). However there was no difference in mean T/R ratios or PR intervals when comparing patients with NRf, CKD4/5 and CKD5D.

Conclusions: Hyperkalemic patients with CKD4/5 and CKD5D did not have significantly different T/R ratios and PR intervals compared to patients with NRf. Thus CKD may not provide protection from the EKG manifestations of hyperkalemia.

PUB525

Amiodarone-Induced Hyponatremia- A Rare but Life Threatening Adverse Effect Ujwala Koduru, Nishkarsh Saxena, Vaishali Thudi, Radhika Kakarala, Arvind R. Kunadi. *Internal Medicine, McLaren Regional Medical Center/ MSU, Flint, MI.*

Background: We describe a case of chronic hyponatremia that was misdiagnosed for years as cerebral salt wasting syndrome (CSW), which was later diagnosed as SIADH secondary to amiodarone. Our literature search found a recent review article highlighting only 12 previous cases.

Methods: Our patient is a 93-year-old female who was admitted for weakness and debility, and was found to have acute hyponatremia with a sodium level of 119 mEq/L. She had known chronic hyponatremia with baseline sodium of 128 mEq/L. Her medications included amiodarone (for 10yrs), metoprolol, miralax, omeprazole, clopidogrel and acetaminophen. On admission, She did not have any orthostatic blood pressure changes. She was started on normal saline at 75 ml/hr for 24hrs. Her serum sodium level did not improve even after 24 hours. Lab studies revealed serum and urine osmolality levels (248 mOsm/kg and 351 mOsm/kg), urine sodium 60 mmol/L, uric acid 1.5 mg/dL, TSH 3.50 uIU/mL and random serum cortisol 18 ug/dL. She underwent echocardiogram (echo) to evaluate the inferior venacava (IVC), which was not collapsible. Other echo findings include ejection fraction 60%, sclerotic aortic valve with trivial aortic insufficiency, mild to moderate mitral regurgitation and trivial tricuspid regurgitation. Her hyponatremia was thought to be secondary to amiodarone, which was stopped. As fluid restriction failed, she was put on tolvaptan 15 mg orally once a day for three days. Her serum sodium level gradually increased to 138 meq/L by third day. Outpatient follow-up after one month revealed that she was doing well with sodium levels maintained above 135 mEq/L.

Conclusions: Amiodarone causing SIADH has previously been reported to occur, few days to few months after starting treatment. Elderly men are especially prone to this side effect. The management requires stopping amiodarone and symptomatic treatment with restriction of fluids, hypertonic saline or tolvaptan. In conclusion, our case demonstrates the importance of vigilance for commonly used medication's adverse effects, especially in elderly patients.

PUB526

Lactic Acidosis due to Lymphoma Tumor Burden Jack Rubin, Milan Rohit Sheth, Ricky Mac, Heena M. Contractor.

Background: Lactic acidosis (LA) and hypoglycemia (HG) related to lymphoma tumor burden portends a grave prognosis.

Methods: Ms. X, a 141 kg, 241 cm, presented 1 yr before her death with a presacral mass compressing the thecal sac. Biopsy was c/w low grade follicular lymphoma treated by XRT and 3 cycles of CHOP-R. After 5 months a pathologic right elbow fracture and pancytopenia evolved and the bone marrow (BM) was now c/w high grade B cell lymphoma with c-myc and bcl-2 rearrangements. She was treated with 3c HyperCVAD but repeat BM showed progression. After 8 months a mild LA resolved after a cycle of chemotherapy (4.4>2.2 mEq/L). During the terminal 4 weeks (12th month) there was persistent LA and HG. Hepatomegaly (on CT) and elevated liver enzymes were noted. Another ALL protocol (ECOG 2993) was tried. Her LA level initially improved but then increased stabilizing around 20 meq/L. She required D5 1/2 NS and 50 meq NaHCO3/L @ 150 ml/hr to maintain glucose over 80 mg%. Steroids and diazoxide were eventually started and HG was controlled. Due to edema from the infusion +/- diazoxide she was tried on combinations of diuretics, oral Bicitra and NaHCO3 plus IV replacement. She started refusing these interventions as need for care became more then she wanted to tolerate (fluid + Lasix > bathroom and refused Foley). She had a fatal cardiac arrest.

Results: The IV bicarbonate infusion may have precipitated edema but decreased at rest tachypnea of 30/min. D-lactic acid was negative; C-peptide and insulin level were appropriately suppressed. Cortisol level confirmed adequate adrenal function. Growth hormone was normal. IGF-2 was 431 ng/ml and Igf2/Igf1 = 8 (both in normal range). Boh-butyrate was not elevated. The anion gap remained 25 to 29 with Lactate accounting for the increase in the gap (6.3 and 5.8 at Day 1 and 2 to 14 - 21 meq/L after the 9th day of hospitalization). Arterial ph was measured twice with a ph 7.21 and 7.24, pCO2 35, 38 and pO2 84, 63 Torr respectively.

Conclusions: Base replacement was used to keep the ph over 7.2 in a patient who had inadequate respiratory compensation due to morbid obesity. Although IGF-2 and IGF1 levels were normal the ratio approached 10 suggesting IGF-2 had a role in the HG. As reported LA and HG related to tumor burden portends a grave prognosis.

PUB527

Acute Psychosis in Two Patients with Bartter Syndrome Kathryn K. Ridout,[1] Matthew D. Willis,[2] M. Khurram Faizan.[3] *[1]Dept of Psychiatry, Butler Hospital; [2]Dept of Psychiatry, Bradley Hospital; [3]Dept of Pediatrics, Hasbro Children's Hospital, Providence, RI.*

Background: Bartter syndrome is an inherited condition affecting NaCl reabsorption in the loop of Henle. Loss of Na^+ causes volume depletion, increased renin and aldosterone release and K^+ and H^+ losses leading to hypokalemia, metabolic alkalosis, and hypotension. We report 2 patients with Bartter syndrome presenting with psychosis requiring inpatient psychiatric hospitalization.

Methods: Retrospective case series, 2013-14.

Results: Case 1: 18 y.o. male with a PMH of Bartter syndrome, depression, and previous episode of delusional ideation and grandiosity presents with a 1 wk history of disorganized thought process, auditory hallucinations, hyper-religious delusions, suspicions, and decreased sleep. On presentation, Na^+ 128 mEq/L, K^+ 4.7 mEq/L, CO_2 15 mEq/L, BUN 25 mg/dl, creatinine 1.35 mg/dl. With increase in his olanzapine and initiation of lorazepam his catatonic and psychotic symptoms improved and his BUN and creatinine normalized. He remained hyponatremic during the entire stay, (126-133 mEq/L). He was discharged on day 15 with notably improved mental status. **Case 2:** 15 y.o. male with a PMH of Bartter syndrome and no prior psychiatric history presented with hypokalemia (K^+ 2.6 mEq/L) after medication refusal. After hypokalemia resolution, on hospital day 4, pt reported auditory and visual hallucinations, paranoid delusions and hyper-religiosity. His mental status exam was notable for a guarded attitude, psychomotor retardation mixed with agitation, tangential thought process, and auditory and visual hallucinations. His psychosis resolved with initiation of risperidone. During his hospital stay, his creatinine and Na^+ remained normal.

Conclusions: We describe 2 cases of Bartter syndrome presenting with acute psychosis. Psychotic symptoms did not specifically correlate with their metabolic or electrolyte disturbances. To our knowledge, these are some of the first reports of Bartter syndrome associated with psychosis. Abnormal neuronal ion transport or aberrant acid-base status may underlie shared pathophysiology between these disorders. Further investigation is needed to elucidate potential mechanisms relating these two rare conditions.

PUB528

Controlled Correction of Severe Hyponatremia due to Heart Failure in an ICU Setting Birinder S. Singh, Panupong Lisawat. *Internal Medicine, Danbury Hospital, Danbury, CT.*

Background: The most important factor in the treatment of severe hyponatremia in a patient with CHF, receiving a diuretic, is the emergence of rapid water diuresis. This causes a sudden correction of sodium and may lead to osmotic demyelination. We illustrate a case of severe hyponatremia in a patient with CHF and successful use of DDAVP to prevent this sudden rise of sodium.

Methods: A 61 y/o female with history of hypertension was brought in the hospital for ischemic CVA and received tPA. She was found to have dilated cardiomyopathy of unclear etiology with E.F. of less than 15%. After 24 hours, she showed significant improvement in her mental status and was transferred out of ICU. Five days after the primary event, she suddenly developed confusion and was increasingly SOB. She was on a heparin drip with D5W as rider and water as her only oral intake for the last 3 days. P/E revealed crackles and bilateral 2+ pitting edema. A chest x-ray confirmed her volume overload state. Lab work uncovered a sodium level of 99. The patient's last sodium level 4 days ago was 132. At this point, she was given furosemide and her sodium increased to 108. As this rate of correction was above the goal correction rate of 6-8 mEq in the first 24 hours, the patient was started on D5W; which was problematic due to her CHF. DDAVP was used intermittently to help prevent water diuresis, and thereby control the rate of correction. Furosemide was given based on her urinary output and the sodium level was checked every 1-2 hours. On the 2nd day after stopping DDAVP, her sodium level corrected rapidly above goal rate; DDAVP was subsequently restarted. After 4 days of slow correction, the patient's sodium level reached 132 and DDAVP was stopped.

Conclusions: Various modalities have been designed to correct chronic hyponatremia as a result of CHF; however acute management of hyponatremia in CHF remains an area of research. One of the challenges in treatment of hyponatremia in CHF is the emergence of water diuresis. To overcome this, we used DDAVP with a loop diuretic, and were successful in controlled correction of sodium at a goal rate without side effects. Hence, we propose using this treatment modality in a similar setting.

PUB529

Evaluating Hypokalemia and Renal Potassium Handling: Time to Recycle the TTKG? Sajeet S. Sawhney,[1,2] Richard M. Treger.[1,3] *[1]Nephrology, Greater Los Angeles VA Healthcare System, Los Angeles, CA; [2]Nephrology, Cedars-Sinai Medical Center, Los Angeles, CA; [3]Nephrology, David Geffen School of Medicine, UCLA, Los Angeles, CA.*

Background: TTKG has been used as a clinical estimate of the tubular fluid potassium (K^+) concentration in the cortical collecting duct; although more recently its reliability has been questioned. The assumption underlying the TTKG, that the majority of osmoles delivered to the medullary collecting duct are not reabsorbed, is not valid in light of intrarenal urea recycling (IUR). The IUR has been described primarily in rat and mouse models and the clinical significance of IUR and its effect on TTKG in humans are unknown.

Methods: A case series was constructed of patients with hypokalemia.

Results: 6 patients were included (Table 1). All patients had urine osmolality that was greater than serum osmolality and normal serum magnesium levels. 5 patients had clinically apparent causes of renal potassium wasting. All 5 patients had TTKGs significantly > 3 and urine K^+-to-creatinine (Cr) ratios > 13 mEq/gm [even patients B and C despite low urine sodium (Na+)]. Patient F had gastrointestinal K^+ loss due to diarrhea. The TTKG was < 3 and urine K^+-to-Cr ratio was < 13 mEq/gm, consistent with known extrarenal K^+ loss.

Patient	Serum K^+	Ur Na^+	Ur K^+	Ur K^+/Cr	TTKG	Diagnosis
A	3.0	58	40.7	60.0	11.1	Diuretic-induced
B	*2.6-->3.9	23	90.7	35.3	11.49	Primary Hyperaldosteronism
C	3.4	<10	26.9	17.0	8.4	Vomiting, Gastroparesis
D	3.3	115	49.9	66.2	8.9	Renal Artery Stenosis
E	3.5	79	59.7	49.7	10.6	Primary Hyperaldosteronism
F	3.3	33	10.5	8.1	2.6	Diarrhea, Crohn's disease

*Prior to treatment

Conclusions: Our case series suggests that TTKG may be a reliable and clinically useful test. Although IUR plays a role in the excretion of K^+, it may not contribute a clinically significant effect in humans. Rats and mice excrete a substantially more concentrated urine compared to humans. Therefore in humans IUR may only play a minor role in K^+ excretion. More robust data is necessary to definitively evaluate the clinical utility of the TTKG. Regardless, the urine K^+-to-Cr ratio may be the preferred test in the evaluation of hypokalemia given its simplicity and reliability.

PUB530

A Severe Case of Alkalemia in Advanced Kidney Disease

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Background: A 44 year old Caucasian gentleman with type 1 diabetes mellitus, chronic kidney disease with a baseline creatinine of 4.0 to 4.95, and gastroparesis presented with a two day history of nausea and vomiting. On admission, vital signs showed relative hypotension, tachycardia, absence of fever with a normal respiratory rate and oxygenation. Initial investigation showed acute renal failure with sodium 125, potassium 2.8, chloride 65, carbon dioxide 40, anion gap 20, calcium 10, albumin 4.7. An ABG showed severe alkalemia with a pH of 7.72, a pCO2 of 27.3, and a bicarbonate of 34.7. Other laboratory data showed WBC 19.5, lactic acid 2.9.

Methods: The patient had been on torsemide, pantoprazole, metoprolol, diltiazem, metoclopramide and insulin. At presentation, he had not yet been started on dialysis. Due to severe alkalemia and uremia, the patient was dialyzed emergently in an ICU setting with subsequent improvement in the patient's respiratory and metabolic parameters.

Dialysis parameters	Admission	2nd cycle	3rd cycle
Sodium	130	137	140
Potassium	4	3	3
HCO 3	33	33	30
Blood flow	180-200	250	300

Our patient presented with severe alkalemia due to a combined metabolic and respiratory alkalosis on top of a metabolic acidosis. The anion gap metabolic acidosis was likely due to advanced kidney disease. We suspect the metabolic alkalosis was generated by vomiting and maintained by hypokalemia, volume depletion and diuretic use. His serum bicarbonate was markedly elevated but his pCO2 was unexpectedly low. The etiology of the respiratory alkalosis remained unclear. He was not hypoxic. Salicylate toxicity and sepsis were ruled out and his chest x-ray showed no acute process. He did report anxiety.

Conclusions: There are few, if any, articles in the literature on acute alkalemia in patients with advanced chronic kidney disease, wherein dialysis is required urgently. Our case highlights the importance of recognizing the underlying acid base disturbance in order to afford appropriate treatment in a timely fashion. To our knowledge, this is the first time that such a combined respiratory and metabolic alkalosis has been reported in a patient with advanced kidney disease requiring acute dialysis.

PUB531

Hypermagnesemia-Induced Hypothermia in a Pregnant Patient

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Background: Hypermagnesemia is a rare entity seen mostly in the setting of renal disease. Symptomatic hypermagenesemia is even rarer. Serious side effects include neuromuscular blockage and cardiac conduction defect. Herein,we present a unique case of patient with idiopathic membranous nephropathy who was admitted for induction of labor and received Magnesium sulfate for seizure prophylaxis in the setting of dramatically elevated BP and concern about preeclampsia.

Methods: Case report.

Results: Patient was given a bolus dose of 4 gram then started on continuous infusion of 2 gram per hour. She developed hypothermia, bradycardia and relative hypotension within 24 hours of initiating infusion. Magnesium level was more than 7 mg/dl. Creatinine continues to be stable at 1.6 mg/dl. Other electrolytes were within normal range. Holding Mg Sulfate resulted in reversal of bradycardia and hypothermia. Workup for hypothermia including septic workup came back negative. LFT were normal. Peripheral blood smear, LDH and haptoglobin were not suggestive of hemolysis. Cortisol and TSH were normal. Decision was made for her to undergo caesarian section based on fetus bradycardia. Prior to going to operation room, patient received calcium Gluconate to reverse magnesium toxicity and for cardio-protection effect.

Conclusions: Magnesium hypothetically can cause hypothermia by blocking Calcium channel blocker and causing muscle paralysis in toxic levels. Vasodilator effect is another suggested mechanism for inducing hypothermia. Holding the causative agent may be sufficient to correct hypothermia. However, dialysis may be warranted if severe symptoms persist and failed to respond to conservative management. Therapeutic hypothermia is becoming more common following MI and cerebrovascular event. Treatment of shivering while avoiding the negative consequences of sedative medications is challenging. Magnesium has found therapeutic application in the promising hypothermic protocols as an appealing anti-shivering medication. Hypothermia as a side effect of hypermagnesemia was very rarely reported. Hypermagnesemia should be considered as a potential precipitant of hypothermia.

PUB532

A Greater Anion Gap (AG) Is Present in Mild to Moderate CKD

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Background: Previous studies have demonstrated an increase in the AG only in relatively advanced kidney disease. Elevated AG is associated with HTN and decreased renal function. Whether a higher anion gap is present even in mild to moderate CKD is not known.

Methods: We conducted a cross-sectional study of 15,133 adults aged≥20 yrs, eGFR≥15 enrolled in the National Health and Nutrition Examination Survey III, 1988-1994. AG was determined from laboratory tests using the traditional AG (serum Na-(serum Cl +serum bicarbonate)), albumin-adjusted AG, and full AG reflecting other electrolytes of serum albumin,Ca,P,and K. We defined moderately increased CKD risk progression as eGFR 45-59, 60-89 or ≥90 ml/min/1.73m^2 and albumin-to-creatinine ratio (ACR)>30mg/g and low CKD risk groups eGFR 60-89 or ≥90 ml/min/1.73m^2 and ACR<30 mg/g. We used ordinal logistic regression to examine moderately increased CKD risk progression groups is associated with a greater AG than the low CKD risk groups adjusting for demographics,education history,income,DM,and HTN. We repeated our analysis with eGFR categories of 15-29, 30-44, 45-59, and ≥60 ml/min/1.73m^2.

Results: 11.8% of the participants had moderately increased CKD risk; the mean age was 62 yrs. A greater traditional AG was evident in this moderate risk group compared to the low CKD risk group (OR[95% CI])=1.5 [1.3-1. 8]) and remained associated after adjustment for the several confounders (1.5[1.2-1.8]). We observed similar associations with the elevated levels of albumin-adjusted (1.2[1.03-1.6]) and full AG (1.5[1.1-1.9]). Results were similar when using eGFR categories as the predictor; an eGFR 45-59 ml/min/1.73 m^2 compared to an eGFR≥60 was associated with a greater traditional AG (1.6[1.2-2.1]); albumin-adjusted AG (1.7[1.03-2.7]); and full AG (1.5[1.1-2.1), adjusted for the covariates.

Conclusions: A greater AG is present even among persons with moderately increased CKD risk. Further research is required to identify the unmeasured anions accounting for the higher levels of AG and to determine their physiological significance.

PUB533

Kidney Biopsy for Renal Tubular Acidosis: A New Paradigm?

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Background: Renal Tubular Acidosis (RTA) is a well-defined entity. Prevailing diagnostic strategies focus on serum and urine studies but percutaneous kidney biopsy (PKB) is routinely not part of the diagnostic evaluation. We describe a case of unexplained distal (d) dRTA presenting as acute interstitial nephritis (AIN) due to a proton pump inhibitor (PPI).

Methods: A 33 year old male with history of hypertension, GERD, and low testosterone was admitted with complaints of fatigue, weight loss, and unexplained acidosis for 2 months. He had no exposure to lead, gasoline, paints, or adhesives/glues. Prior medications included losartan,omeprazole, potassium chloride, sildenafil, and testosterone propionate injections. Physical exam was unremarkable with a blood pressure of 120/80 mmHg. Initial lab work showed a non-anion gap metabolic acidosis, serum bicarbonate level 16 mM/L, potassium 3 mM/L, Urine pH 6.5, and a positive urine anion gap. Serum creatinine was 1.2 mg/dL and no eosinophilia/eosinophyluria was detected. Renal ultrasound was unrevealing. Renal biopsy results showed findings consistent with AIN and mild (30%) interstitial fibrosis.

Conclusions: dRTA is well described as a chronic tubulointerstitial disease, however it is rarely seen as sole pathologic finding of AIN. In this case, dRTA remained elusive despite extensive blood, urine and imaging testing. Given his normal baseline, we were suspicious for an anatomical process to explain the marked biochemical abnormalities. AIN is often defined as an acute decline in renal function with an inflammation of the kidney interstitium. It can have more subtle presentations as well. It is not uncommon for PPI's to cause an AIN, but it is exceedingly rare for it to present solely as dRTA. Our case underscores the importance of PKB during work-up of otherwise unexplained RTA. RTA is an entity that can have a variable and subtle presentation and traditional laboratory work up may not suffice to fully evaluate the underlying etiology. PKB may be warranted to rule out discrete histopathologic injury such as AIN.

PUB534

A Novel SLC5A2 Mutation in a Patient with Severe Renal Glucosuria and Aminoaciduria Eric E. Gheuens,[1] Karin Dahan,[3] Tania Daems,[2] Ronald Daelemans.[1] *[1]Nephrology, Ziekenhuis Netwerk Antwerpen, Antwerpen, Belgium; [2]Endocrinology, Ziekenhuis Netwerk Antwerpen, Antwerpen, Belgium; [3]Institut de Pathologie et de Génétique, Gosselies, Belgium.*

Background: In the kidney the bulk of glucose is reabsorbed at the S1 segment of the proximal tubule by the high-capacity sodium/glucose co-transporter SGLT2. The protein is encoded by the SLC5A2 gene in the chromosomal region 16p11.2. Several different mutations, scattered throughout the SCL5A2 gene, have been associated with familial renal glucosuria (FRG). Here we describe a case report of a young female (aged 26 y) who was referred for glucosuria without signs or symptoms of diabetes mellitus. She migrated from Iraq to Belgium 4 years earlier and had no medical history. She did state that her mother and sisters experienced renal problems, but she could not define the nature of these problems. She noted frequent urination and an occasional urinary tract infection. Physical exam was unremarkable, and blood pressure was normal (108/74 mmHg). An oral glucose tolerance test was normal, as was the glycosylated hemoglobin. The kidney function was normal, with a normal urinary sediment and absence of proteinuria. There was no metabolic acidosis or electrolyte disturbance.

Methods: Mutational analysis of the SLC5A2 was performed by sequencing of the entire coding region.

Results: Two 24-hour urinary collections (> 3500 ml/day) showed severe glucosuria (84-97 g/ 1.73m²/day). Sodium excretion was slightly elevated (334.3 mmol/day) and aminoaciduria was present for taurine, threonine, serine, asparagine, glutamine, glycine, alanine, tyrosine, phenylalanine and proline. A novel heterozygous mutation **c.12del (p.His4Glnfs*16)** was identified, in the SLC5A2 gene. This deletion is a truncating mutation probably leading to a dysfunctional SLGT2-proteïn. There is also another heterozygous variation, an intronic unclassified variant **c.655+6G>C**.

Conclusions: This report adds to the number of reports of FRG caused by a large number of different mutations. As seen in our patient, severe renal glucosuria reflects the characteristics of autosomal recessive inheritance and has not been shown to have severe clinical consequences.

PUB535

Plasma Homocysteine Levels as a Predictor of Pre-Eclampsia in Pregnancy Bhavna Pandya,[1,2] Martin Myers,[3] Sohan Shah,[1] Noveen Awan.[2] *[1]Faculty of Health and Life Sciences, Univ of Liverpool, Liverpool, United Kingdom; [2]Nephrology Dept, Aintree Univ Hospitals, Liverpool, United Kingdom; [3]Clinical Biochemistry, Lancashire Teaching Hospitals, Preston, United Kingdom.*

Background: Plasma homocysteine levels have been adversely associated with several obstetric complications, including pre-eclampsia. We aimed to compare plasma homocysteine levels in normal pregnancy and pre-eclampsia to assess whether quantification of homocysteine levels can be used as a predictor of disease.

Methods: We conducted a prospective cohort study with 300 pregnant women. Homocysteine levels were measured on 3 occasions during pregnancy: enrolment, 28 weeks and delivery. We also analysed genotype amongst other factors such as folate levels, vitamin B12 levels and smoking.

Results: A total of 203 patients with available outcome of pregnancy records were included in the study. The proportion of pre-eclampsia cases was 4.4% (95%CI: 2.3-8.3). The optimal cut-off point for homocysteine level on ROC curve analysis was 9.3 µmol/L, where sensitivity = 33.33% (95%CI: 7.49-70.07) and specificity = 94.85% (95%CI: 90.72-7.50). The area under the curve was 51% and indicated that the accuracy of homocysteine plasma levels as a diagnostic test is extremely low. However, for every unit (umol/l) increase in homocysteine levels, the odds of pre-eclampsia presence are increased by 21% (95%CI:1–44) (p=0.034). We found that there was a statistically significant difference (p=0.020) in the mean homocysteine level among the three genotypes analysed. Statistically significant relationships were also found between homocysteine levels and the following variables: vitamin B12 levels (rho = -0.2131, p = 0.0008); folate levels (rho = -0.3855, p < 0.001); smoking (z = -4.472, p = 0.001).

Conclusions: With data available from our study, there is no evidence to suggest that homocysteine levels can be accurately used as a screening tool for pre-eclampsia. Several different factors affect the level of homocysteine during pregnancy.

PUB536

Apolipoprotein L1 Is Expressed in the Renal Microvasculature and the Risk Genotype Is Associated with More Severe Arteriolar Hyalinosis Preeti Chandra,[1] Avi Z. Rosenberg,[2] Cinthia Drachenberg,[3] Xiongce Zhao,[4] Cheryl Ann Winkler,[5] Stephen M. Hewitt,[2] Jeffrey B. Kopp.[4] *[1]Int Med, Univ of MD, Baltimore, MD; [2]NCI, NIH, Bethesda, MD; [3]Pathology, Univ of MD, Baltimore, MD; [4]NIDDK, NIH, Bethesda, MD; [5]NCI, NIH, Frederick, MD.*

Background: Variants in *APOL1*, encoding apolipoprotein L1, termed G1 and G2 risk alleles (RA), are strongly associated with kidney disease in African Americans (AA). APOL1 has been reported to localize to the media in arteries and arterioles in focal segmental glomerulosclerosis (FSGS) and HIV-associated nephropathy but not in normal kidney. Our aim was to study, in native kidney biopsies with diagnosis of FSGS, arterionephrosclerosis (ANS) and diabetic nephropathy, associations between *APOL1* genotype and APOL1 expression in microvessels.

Methods: This IRB-approved retrospective study involved 73 native renal biopsy samples, obtained from 18 Whites and 55 AA and included FSGS (35), diabetic nephropathy (25), and ANS lacking another glomerular diagnosis (13). Glomerulosclerosis score was determined as the fraction of glomeruli with obsolescence or solidification. Interstitial fibrosis/tubular atrophy (IFTA), arteriolar hyalinosis and APOL1 staining were semi-quantitatively scored. Paraffin sections were genotyped for G1 and G2 RA, and stained for APOL1 using a rabbit monoclonal antibody (Epitomics).

Results: 54 cases had an *APOL1* low risk genotype (0 or 1 RA) and 19 (26%) had a high-risk genotype (2 RA). Cases with 2 RA had similar scores for glomerulosclerosis, arteriosclerosis and IFTA compared with the low risk group (with and without adjustment for the disease diagnosis.) APOL1 vascular expression (in arterial/arteriolar endothelia/walls) was similar in high-risk and low-risk subjects, in the entire study group and in the disease subgroups.Individuals with 2 RA manifested similar degrees of arteriosclerosis but more severe arteriolar hyalinosis ($p<0.05$).

Conclusions: APOL1 expression in the renal microvasculature is a typical feature of common renal diseases, including those associated and unassociated with the risk genotype, and is not influenced by genotype. The *APOL1* risk genotype is associated with more severe arteriolar hyalinosis.

Funding: NIDDK Support, Other NIH Support - NIDDK and NCI provided support via their respective Intramural Research Programs

PUB537

Renal Replacement Treatment Effects on Global DNA Methylation Pattern in End-Stage Renal Disease Patients Karin Luttropp,[1] Peter F. Barany,[2] Olof Heimburger,[2] Peter Stenvinkel,[2] Louise Nordfors.[2] *[1]Dept of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [2]Dept of Clinical Science, Intervention and Technology, Karolinska Univ Hospital, Stockholm, Sweden.*

Background: It has been found that chronic kidney disease (CKD) patients display differences in DNA methylation compared to healthy controls. However, the effect of renal replacement therapy (RRT) on methylation status is still a quite novel field of research. In this study, we investigated the effect of RRT on global DNA methylation.

Methods: 12 kidney recipients (RTx) and 12 patients starting dialysis (8 on peritoneal dialysis, 4 on hemodialysis) were included. Baseline samples were collected before therapy initiation and a second sample was obtained after 12 months on RRT. 24 age-matched healthy controls were also included. DNA was prepared from whole blood and analysed by the Illumina HumanMethylation450 BeadChip, which measures DNA methylation at 450 000 CpG sites. The patients were compared to the controls using the lumi package in R.

Results: When comparing pre-RTx patients to controls, we found 39085 significantly (adjusted p-value < 0,05) differentially methylated CpG sites (see Table 1); post-RTx patients versus controls 12708 sites; pre-dialysis patients versus controls 34321 sites; and post-dialysis patients versus controls 11428 sites. For RTx patients, 10854 of the statistically significantly differentially methylated CpG sites remained significant over time; for the dialysis patients, this number was 9479 sites.

Significant CpG sites	Baseline	12 months
RTx vs controls	39085	12708
Dialysis vs controls	34321	11428

Table 1

Conclusions: Following start of dialysis as well as after RTx, the difference in number of differentially methylated CpG sites between patients and controls was markedly reduced. This could indicate a normalisation process, where the DNA methylation profile of CKD patients gradually approaches the one seen in healthy subjects. Closer investigation regarding the genes and regions displaying differences in methylation after initiation of dialysis and transplantation is currently being performed.

Funding: Government Support - Non-U.S.

PUB538

APOL1 Genotype Is Associated with Glomerular But Not Tubular Injury in HIV Infection Vasantha Jotwani,[1] Michael Shlipak,[1] Rebecca Scherzer,[1] Rulan S. Parekh,[2] Wen Hong Linda Kao,[3] Michael R. Bennett,[4] Mardge H. Cohen,[5,6] Anjali Sharma,[7] Phyllis Tien,[1] Chirag R. Parikh,[8] Mary A. Young,[9] Michelle M. Estrella.[3] *[1]UCSF; [2]Univ of Toronto; [3]Johns Hopkins; [4]Cincinnati Children's Hospital; [5]Rush Univ; [6]Stroger Hospital; [7]Albert Einstein; [8]Yale; [9]Georgetown.*

Background: *APOL1* risk alleles are associated with advanced kidney disease in African Americans, but their specific effects on glomerular and tubular health are unknown.

Methods: This cross-sectional study of 431 HIV-infected African American women from the Women's Interagency HIV Study evaluated associations of *APOL1* risk alleles with albumin-creatinine ratio (ACR) and urine biomarkers of tubular damage: interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and a1-microglobulin (a1m).

Results: Women with 2 risk alleles had higher ACR levels (median 24 versus 11 mg/g, p=0.0002) and higher prevalence of ACR>30mg/g (47% versus 21%, p=0.0011) compared to women with 0/1 risk allele. These differences persisted after multivariable adjustment (**Figure 1**). *APOL1* genotype showed little association with IL-18/cr, NGAL/cr, or detectable a1m in adjusted analyses. The presence of 2 *APOL1* risk alleles was associated with lower KIM-1/cr, but showed weaker association with unstandardized KIM-1 levels (-15%, 95%CI: -35,13).

Figure 1: Multivariate-adjusted[1] associations of 2 vs 0/1 *APOL1* risk alleles with biomarker levels

	% Estimate (95% CI)	P-value
Continuous outcomes		
ACR (mg/g)	104 (29,223)	0.0023
IL-18/Cr (pg/mg)	-5 (-24,18)	0.64
KIM-1/Cr (pg/mg)	-20 (-36,-1)	0.044
NGAL/Cr (ng/mg)	10 (-26,64)	0.64
	Prevalence Ratio (95% CI)	**P-value**
Dichotomous outcomes		
ACR >30mg/g	2.00 (1.17,3.44)	0.012
Detectable α1m	1.13 (0.65,1.97)	0.66

[1]Adjusted for age, hypertension, diabetes, BMI, HCV, HIV viral load, eGFR, and ancestry

Conclusions: Among HIV-infected African American women, *APOL1*-associated kidney injury appears to localize to the glomerulus, rather than the tubules.

Funding: NIDDK Support, Other NIH Support - NIA, NIAID, NCI, NHLBI, NIDCD

PUB539

Exome Sequencing Identifies a Novel Mutation in APOL1 as the Cause Gene of Focal and Segmental Glomerulosclerosis in a Chinese Pedigree

Guisen Li, Li Wang. *Renal Dept, Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: Studies of familial FSGS have identified the variation of genes related to podocytes. These results were not confirmed in Chinese Han population. Therefore, the purpose of this study was to identify the causal mutations of FSGS in Chinese pedigree.

Methods: Sequence of the exon 8 of *ACTN4*, exon 2,5,12,and 13 of *TRPC6*, as well as exon 2,3,and 4 of *INF2 was performed in the proband of one family. Then, exome sequencing in probands of a familial FSGS was done. By PCR sequencing methods, we detected candidate causal mutaions in all members of this family. And then, we tested the variations in other 97 sporadic FSGS patients and 96 normal controls.*

Results: This pedigree was an identical twins pedigree, no mutation was detected from those exons of *INF2*, *ACTN4* and *TRPC6 in* the proband. We identified the mutations of *APOL1*(c.C761T) in the proband and her sister, their parents did not have this mutation.

Conclusions: The strategy that we use the exome sequencing for FSGS pedigree was demonstrated successfully for the first time in the Chinese Han population. We identified a new missense mutation of *APOL1* from a Chinese FSGS pedigree by using exome sequencing.

Funding: Clinical Revenue Support

PUB540

Exome Sequencing Identified MYH9 as a Candidate Gene for a Familial Focal and Segmental Glomerulosclerosis

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Background: Studies identified that the variation of genes related to podocytes were associated with the pathogenesis of FSGS. These results were not confirmed in Chinese Han population. Therefore, the purpose of this study was to identify the causal mutations of FSGS in Chinese Han population.

Methods: Sequence of the exon 8 of *ACTN4*, exon 2,5,12,and 13 of *TRPC6*, as well as exon 2,3,and 4 of *INF2* was performed in the proband of this family. Causal mutaions were detected by exome sequencing in the probands of this family. By PCR sequencing methods, we detected candidate causal mutaions in this proband. And then, we tested the variations in other 97 sporadic FSGS patients and 96 normal controls.

Results: This pedigree was with autosomal dominant inheritance, no mutation was detected from those exons of *INF2*, *ACTN4* or *TRPC6 in* the proband. We identified the mutations of *MYH9*(c.G3282C) in the proband, while the mutation effect on protein function remains unknown.

Conclusions: The strategy that we use the exome sequencing for FSGS pedigree was demonstrated successfully for the first time in the Chinese Han population.We identified a new missense mutation of *MYH9* from a FSGS pedigree by using exome sequencing.

PUB541

Predictive Value of Genome Copy Number Variation to ShenQi MoShen Recipe in the Treatment of Idiopathic Membranous Nephropathy

Lin Wang,[1] Shihui Li,[2] Xianwen Zhang,[1] Chunsong Zhang,[1] Jiao Li,[1] Siyu Zhao,[3] Yueyi Deng,[1] Jianhui Tian,[1] Minping Qian,[2] Lin Wan,[4] Yiping Chen.[1] *[1]Longhua Hospital, China; [2]Peking Univ, China; [3]Shanghai Univ of TCM, China; [4]Chinese Academy of Sciences, China.*

Background: Idiopathic Membranous Nephropathy (IMN) is one of the leading causes of nephrotic syndrome (NS) in adults. Current treatment of IMN is far from satisfactory. ShenQi MoShen Recipe (SQMSR) is a Chinese herbal medicine prescription used to treat IMN in Long Hua Hospital for decades. To explore the correlation between Genome Copy Number Variation (CNV) of the patients of IMN and the clinical response of SQMSR, we compared the differences of gene background among the IMN patients with different clinical outcomes.

Methods: Eighty patients were divided into four groups according to the treatment and outcomes: the complete remission (CR) group of SQMSRwas indicated as the TCR group (36 cases); the no remission (NR) group of SQMSR as the TNR group (11 cases); the CR group of the immunosuppressive agent (IA) as the ICR group (18 cases); the NR group of IA as the INR group (15 cases). Genomic DNA from peripheral blood was extracted and genotyped with the Affymetrix Genenome-Wide Human SNP Array 6.0. CNV analysis was performed on the raw data by the software of CNV hac.

Results: The most significant CNVs regions among groups were located on chromosome 5, 6 and 8, on which patients of TCR group showed no loss while patients of TNR group showed loss (P<0.05). The CNV of HLA gene family which located on chromosome 6 showed amplification on patients of TCR group, but loss on patients of TNR group. However, there were no significant CNVs between ICR and INR group.

Conclusions: The differences in genetic background of IMN patients might be the cause of different clinical response of SQMSR. The CNVs of HLA gene family could become the predictive factor of the treatment of SQMSR and be worthy of further study.

PUB542

Lower eGFR Is Associated with Worse Muscle Fatigue and Muscle Quality among Older Adults

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Background: CKD promotes malnutrition and inflammation leading to sarcopenia. The association of CKD with objective muscle fatigue and dynamic muscle impairment is unknown. Here we investigate the association of renal function with muscle function by isokinetic fatigue testing and assess the potential contribution of body composition in older adults.

Methods: We studied 1993 adults in the Health, Aging and Body Composition Study who had isokinetic muscle fatigue testing and Cystatin C collected at the year 3 visit. DXA scans were performed to assess body composition. Fatigue index ([final torque/initial peak torque]*100) represented fatigue resistance over 30 knee extensions. Total knee extension work was measured. We estimated associations of GFRcysc with total work and fatigue index using multivariable linear regression adjusting for demographics, BMI, smoking, cognitive function, diabetes, CAD, cerebrovascular disease, and physical activity. We used linear regression to estimate the association of CKD (eGFRcysc<60) with each isokinetic index and the potential contribution of body composition.

Results: Average participant age was 75.5 ±2.8 with 50% females, 37% African Americans and average eGFRcysc of 85.6 ±19. CKD was associated with lower fatigue index (greater fatigue) and lower muscle work compared to non-CKD.

	Fatigue Index (%)		Total work(Joules)	
	Mean (SD)	β (95% CI)	Mean (SD)	β (95% CI)
non-CKD (n=1782)	73.8 (17.4)	Reference	1070 (454)	Reference
CKD (n=211)	70.9 (17.6)	-3.0 (-5.48, -0.53)	922 (387)	-106.6 (-154.7, -58.5)
Per -10ml/min/1.73m^2		-0.44 (-0.87, -0.01)		-20.1 (-28.9, -111.3)
P- value		0.045		<0.001

Adjustment for leg lean mass, but not fat mass, modestly attenuated the association of CKD with fatigue index and total work, but associations remained statistically significant.

Conclusions: Lower eGFRcysc is associated with greater lower extremity fatigue and diminished muscle quality. The association of CKD with fatigue and total work is independent of lean mass.

Funding: NIDDK Support, Other NIH Support - NIA

PUB543

Nursing Home Status Among Older Adults with Incident End Stage Renal Disease C. Barrett Bowling,[1,2] Rebecca H. Zhang,[2] Harold A. Franch,[1,2] Yijian Huang,[2] Anna Mirk,[1,2] William M. McClellan,[2] Theodore M. Johnson Ii,[1,2] Nancy G. Kutner.[2] *[1]Atlanta VA Medical Center; [2]Emory Univ.*

Background: End-stage renal disease (ESRD) patients who require nursing home (NH) care may be at risk for adverse health outcomes. The Centers for Medicare and Medicaid Services (CMS) revised the Medical Evidence Report Form CMS-2728 in 2005 to include data collection on NH institutionalization. While these data provide an opportunity to describe the utilization and outcomes associated with NH care, the validity of NH institutionalization status on CMS-2728 has not been reported.

Methods: There were 27,913 patients ≥ 75 years of age with incident ESRD in 2006, which constituted our analysis cohort. We determined the accuracy of the CMS-2728 using a matched cohort that included the CMS nursing home Minimum Data Set (MDS), a previously employed "gold standard" metric for identifying patients receiving NH care. We calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the CMS-2728 nursing home item. Additionally, among those with MDS data indicating NH institutionalization, we compared characteristics and mortality risk for those with (true positive) versus without (false negative) a CMS-2728 NH indicator.

Results: The sensitivity, specificity, PPV and NPV of the CMS-2728 for nursing home status were 33%, 97%, 80% and 79%, respectively. Among 7,801 patients with MDS data indicating NH institutionalization, the CMS-2728 NH indicator was associated with an inability to ambulate (41% versus 8%), inability to transfer (25% versus 3%), and need for assistance with activities of daily living (57% versus 12%). Among those with MDS data, multivariable adjusted hazard ratios (95% CI) for mortality for those with and without the CMS-2728 NH indicator were 1.54 (1.46–1.63) and 1.47 (1.41–1.53), respectively.

Conclusions: The CMS-2728 may underestimate NH institutionalization among older adults with incident ESRD, but identifies a group likely to have received NH care. Despite a higher prevalence of functional impairment among those identified as requiring NH care by both the MDS and CMS-2728, regardless of source, NH institutionalization was associated with an increased risk for mortality.

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

PUB544

Effective Control of Hypertension in Elderly with Chronic Kidney Disease Eleni Chelioti,[1] Dimitrios Athanasopoulos,[2] Evdokia Efthimiou,[1] Alexia Papalexandrou,[1] Maria Sotiraki,[1] Maria Tsilivigou.[1] *[1]Dept of Nephrology, General Hospital of Piraeus, Athens, Greece; [2]Health Centre of Dimitsana, General Hospital of Tripoli, Tripoli, Greece.*

Background: The new guidelines of JNC 8 emphasize control of systolic blood pressure (SBP) and diastolic blood pressure (DBP) with age and comorbidity. In patients 18 to 59 years of age without major comorbidities, and in patients 60 years or older who have diabetes, chronic kidney disease (CKD), or both conditions, the new goal blood pressure level is <140/90 mmHg. ***Aim*** *of the study was to* examine the effective control of hypertensionin elderly with CKD according the new guidelines of JNC8.

Methods: A cross-sectional observational study was done. The study included elderly with CKDin the outpatient nephrology clinic during one year. Adequate blood pressure control (ABPC) was defined as SBP<140 and DBP<90mmHg on time 0 and 12months. Estimated glomerular filtration rate (eGFR ml/min/1.73m^2) was calculated using CKD-EPI formula. All patients were receiving three or more antihypertensive drugs.

Results: *130 patients were studied (52%men, mean age77±7years-old, mean serum creatinine 1,9±0,8mg/dl).*There are not statistically differences between mean eGFR on time 0 and 12 months (31,9±10,7 and 32,6±11,6 ml/min/1.73m^2 respectively). Statistically differences found between mean SBP on time 0 and 12 months (135±14,7 and 130±14,2mmHg respectively) whileno found statistically differences between mean DBP on time 0 and 12 months (73,5±13 and 72±13 mmHg respectively).*Among elderly with CKD, 63,7% had ABPC on time 0 and 82,9% on 12months and we found that there is a* statistically significant difference between ABPC on time 0 and 12.

	Time 0	Time 12	p
eGFR Mean(SD)	**31,9(10,7)**	**32,6(11,6)**	**NS**
SBP Mean(SD)	**135(14,7)**	**130(14,2)**	**<0,001**
DBP Mean(SD)	**73,5(13)**	**72(13)**	**0,33**
ABPC (%)	**63,7**	**82,9**	**<0,001**

Conclusions: Our study suggests that new guidelines for hypertension control in elderly with stable CKD is possible, but it requires close follow-up and antihypertensive drugs.

PUB545

Effective Control of Anemia in Elderly and Very Elderly with Chronic Kidney Disease? Eleni Chelioti, Evdokia Efthimiou, Alexia Papalexandrou, Maria Sotiraki, Maria Tsilivigou. *Dept of Nephrology, General Hospital of Piraeus, Athens, Greece.*

Background: Chronic kidney disease (CKD) is a growing problem due to our aging population, many of who have increased comorbidities. Anemia emerges as a main independent modifiable risk factor of cardiovascular and renal damage. Knowledge of anemia medication patterns use allows identifying opportunities for improving care.The **aim** of the study was to evaluate the effective control of anemia in elderly and very elderly patients with CKD.

Methods: A prospective study was carried out. The study included elderly ang very elderly patients with CKD and eGFR<60ml/min/1.72m^2, who follow-up in the outpatient nephrology clinic over 12 months. The anemia management evaluated in two visits: at baseline and 12-month visit. Anemia was defined as severe (Hb <11g/dL) or mild (Hb: 11–12 g/dL). The patients divided in 2 age groups (AG): AG1 65-79, and AG2 >80 years old. Estimated GFR was calculated using MDRD formula.

Results: A total of 125(85 AG1/40AG2) patients were studied (52,3 %men, mean eGFR 31±18ml/min/1.73m^2). The percentage of severe and mild anemia at baseline for the AG1 was: 41,2% and 58,8% respectively, while for the AG2 was 45% and 55% respectively. At 12 months, the percentage was 32,6% and 67,4% for the AG1 while for the AG2 was 43,6% and 56,4% respectively. The correlation between severe and mild anemia in both of age groups at baseline and at 12months was not statistically significant and not statistically significant with the age.

Conclusions: Our results suggest that the effective control of anemia in the elderly and very elderly populations with CKD, seems to be unexpectedly difficult and likely is a result of poor management.

PUB546

Hemoglobin Stability in Elderly with Chronic Kidney Disease Eleni Chelioti,[1] Dimitrios Athanasopoulos,[2] Alexia Papalexandrou,[1] Evdokia Efthimiou,[1] Maria Sotiraki,[1] Maria Tsilivigou.[1] *[1]Dept of Nephrology, General Hospital of Piraeus, Athens, Greece; [2]Health Centre of Dimitsana, General Hospital of Tripoli, Tripoli, Greece.*

Background: Hemoglobin(Hb) variability is common not only in patients with end-stage kidney disease on dialysis, but also in chronic kidney disease (CKD) patients who are not yet on dialysis (non-dialysis CKD). The reported prevalence of hemoglobin variability in non-dialysis CKD patients varies between 61 to 86%. Hb levels in individuals with CKD fluctuate frequently above or below the recommended target levels within short periods of time even though the calculated mean hemoglobin remains within the target range of 11 to 12 g/dl. **The purpose** of the study was to assess the prevalence of Hb stability in CKD elderly patients.

Methods: The prospective study was carried out in a outpatient nephrology clinic over a period of 12 months. The study included elderly patients with CKD and eGFR<60ml/min/1.72m^2. The stability of Hb levels (10-12g/dl**)** with administration of erythropoiesis-stimulating agents, evaluated in two visits: at baseline and 12-month visit.Estimated GFR was calculated using CKD-EPI formula.

Results: A total of 170 patients were studied (53,5% men, mean age 77±11 years-old). The mean differences of eGFR and Hb levels between baseline and 12-months was not statistically significant (33±12 versus 33±12,4ml/min/1.72m^2 and 12,1±1,7 versus 12,1±1,4g/dl, respectively). The correlation between hemoglobin stability and gender and age was not statistically significant. The percentage of patients who had stable Hb levels was only 27,6% (47/170).

[table 1]	***Baseline***		***12-months***		***P value***
Stages of CKD	Stable Hb levels (n)	Percentage (%)	stable Hb levels (n)	Percentage (%)	
Stage 3	*20*	*19,6*	*20*	*19,6*	*NS*
Stage 4	*25*	*40,9*	*23*	*41,8*	*NS*
Stage 5 (non-dialysis)	*2*	*28,5*	*4*	*30,7*	*NS*

Conclusions: *Our results suggest that the* maintaining stable hemoglobin levels *in elderly with CKD* is unexpectedly difficult in the setting of tertiary nephrology care.

PUB547

Clinical Outcomes of Geriatric Kidney Transplantation in Korea Kyung Don Yoo,[1] Su-Kil Park,[2] Dong-Wan Chae,[1] Yun Kyu Oh,[1] Yon Su Kim,[1] Chun Soo Lim,[1] Jung Pyo Lee.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine; [2]Internal Medicine, Asan Medical Center and Univ of Ulsan College of Medicine.*

Background: Elderly individuals who underwent kidney transplantation are growing rapidly. In Korea, proportion of kidney transplant recipients older than 60 years increased from 2.3% to 11.4% between 2000 and 2013. However, clinical outcomes in elderly patients were not well evaluated in Asian population.

Methods: This is a multicenter cohort study including adult patients underwent kidney transplant between 1997 and 2011 in 5 tertiary hospitals in Korea. A total of 2642 kidney

transplant patients were enrolled. Patient survival, allograft survival, and biopsy-proven acute rejection were monitored as end-points in elderly transplant patients comparing with younger population.

Results: Participants older than 60 years old was 8.5%(N=223). Their mean age was 63.2±2.7 years old. Total patients were divided into 5 groups according to transplant age by 10 years. The male patients(69.5%),diabetes mellitus(39.0%), and history of pretransplant ischemic heart disease(16.9%) were more in oldest age group. Deceased donor transplantation was more frequent. The over-all mortality rate was significantly higher in the oldest group(1yr survival 94.6%, 5yr survival 91.9%). However, age did not affect allograft survival(1yr survival 94.1%, 5yr survival 91.9%). In addition, the prevalence of BPAR conversely decreased as the age increased(*p<0.001). In recipients older than 60 years, BPAR was an independent risk factors for allograft loss after adjusting for age, gender, donor age, donor relation, and HLA mismatches (P=0.032, hazard ratio 3.10, 95% confidence interval 1.11-8.74).*

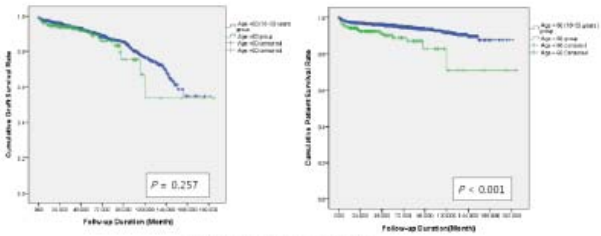


Figure 1. Comparision of Graft Survival and Patient Survival between Adult Patients (18~59 years) and Geriatric Patients (Over 60 years) by Kaplan-Meier curve

Conclusions: Kindey transplantation in the elderly was comparable to younger age in Korean. Episode of acute rejection decreased as the age increased and could significantly affect allograft survival.

PUB548

Rethinking and Reevaluating the Biomedicalization of the Very Oldest: Should We Recommend Automatically Dialysis Treatment for Octogerian Patients? Time for Improving Dialysis-Decision Making Capacity and Implementing Succesfully Advance Care Planning. Experience of a French Region in an Aging France Achour Laradi,[1] Francois Babinet,[1] Alain Cremault,[1] Stephanie Lanoiselee,[1] Maryvonne Hourmant,[2] Cécile Couchoud.[3] *[1]Nephrologie- Dialyse, ECHO-CMCM, Le Mans, France; [2]Nephrologie -Transplantation, CHU Hôtel Dieu, Nantes, France; [3]Coordination Nationale du Rein, Agence de Biomedecine, Saint Denis-Paris, France.*

Background: The societal changes that have taken place over two decades are a major medical issue and an important societal challenge with the fastest growing of Chronic Kidney Disease (CKD)in elderly patients (Epts) over 75 years old.The medical community is concerned by the appropriateness of dialysis therapy(DT) for these pts at high risk of cognitive impairment or dementia with societal pressures influencing medical practices.

Methods: Epts represents 40 % of incidents pts in France.We report here the clinical characteristics and the outcomes of 101 incidents pts treated by DT in our institution between 2007 and 2011 and using data of the Pays de Loire region from the french dialysis network REIN.

Results: The median age was 71.7 years and 56.6 % of these incidents pts were over 75 years old.Diabetes accounts for 30.8 % in 2009 and 41.7 % in 2012. Hypertension was present in 85 % of pts and 31.6 %. In 92 prevalent pts in 2012, 58.7 % were over 75 yeays old, 2 or more chronic heath conditions were present and 15.2 % suffer from disability.Visual impairments were affecting 8.7 % of pts.15 % were on the kidney transplant waiting list with 2 pts experiencing a combined transplantation.The death rate one year after initiation of DT is high and 15.7 % dying less than three months after initiation of DT.Survival rate after 5 years is 15 %.

Conclusions: The older dialysis population has a high burden of comorbidities and a high risk of death particulary at the start of dialysis. There is a delicate balance between benefits and risks in these pts.Clinical judgement,improving dialysis decision making to avoid routinization of automatically recommending dialysis to pts with poor outcome resulting from appropriate prognosis tools n favor or against initiation of DT.

PUB549

A Novel Method of Six-Day Intensive Locking with Urokinase Combined with Heparin for Treatment of Long-Term Catheter Dysfunction: Efficacy and Safety Data from Sixteen Patients Tian Xu, Xiaoliang Zhang, Rining Tang, Hong Liu, Yinfeng Guo. *Nephroloy, Zhongda Hospital, Southeast Univ, Nanjing, Jiangsu, China.*

Background: Long-term central venous catheter dysfunction seriously affects dialysis adequacy. In this study, we established a novel method to prevent and treat catheter dysfunction.

Methods: Inclusion criteria: Regular hemodialysis patients with long-term central venous catheter were hospitalized for catheter dysfunction, and have received thrombolytic therapy with urokinase (Single thrombolytic treatment) before. Exclusion criteria: Patients with coagulation disorder or vein stenosis. Catheter dysfunction is defined as failure to attain a sufficient extracorporeal blood flow less than 200mL/ min. Six-day Intensive treatment : urokinase (100,00U/ml) used in daily morning(8:00) and heparin (4000U/ml) used in daily afternoon (16:00), lasted six days. Research programme: The time from patients firstly received thrombolysis treatment to their enrollment is considered as observation time. We observe the incidence of catheter dysfunction in the observation time as well as the equal observation time after the intensive therapy by a self-controlled method.

Results: A total of 16 patients were enrolled, aged 60~89 years old, male 7 cases, female 9 cases; The observation time of prior treatment and post treatment was 170.4 ± 92.7 days respectively. The rate of catheter dysfunction after intensive therapy is 0.98%, which is significant lower than single therapy 51.9% (P<0.01). The first time to recurrence of catheter dysfunction was significantly shorter after single therapy compared with Intensive therapy (3.0 days versus 167.6 days, P<0.001). The successful declotting rates for catheter at three time points (the second, forth, and sixth injection urokinase/heparin) were 25%, 50%, and 25% respectively. Besides, the study showed no significant difference on PT and APTT after intensive therapy.

Conclusions: Six-Day Intensive Locking with Urokinase Combined with Heparin can treat catheter dysfunction and prevent recurrence of clotting episodes effectively, which is safe to application in clinic.

Funding: Government Support - Non-U.S.

PUB550

Lack of Utility of Indium Tagged Leukocyte Scintigraphy in Clinically Suspected Arteriovenous Graft Infections Jonathan T. Lin, Ioannis Konstantinidis, Francis Scott Nowakowski, Girish N. Nadkarni, Rabi Yacoub, Victoria J. Teodorescu, Joseph A. Vassalotti. *Dept of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY.*

Background: Vascular access infections are a common cause of morbidity in hemodialysis patients, but the role of indium-tagged leukocyte scintigraphy in evaluating arteriovenous graft (AVG) infections is unclear. We assessed the utility of indium scans in evaluating clinically suspected AVG infection.

Methods: All charts with indium scans and AVGs from this single tertiary referral hospital during the years 2000-2013 were reviewed for demographic, blood culture, clinical, and outcome data. Scans performed for suspected AVG infections were compared to those performed for non-access infections (controls).

Results: Of the 30 scans with complete data, 14 were for suspected AVG infection (cases) and 16 were controls. Demographic and clinical characteristics in the two groups were similar. There was a trend for a greater proportion of positive scans (defined by AVG uptake) in cases than in controls (8/14[57%] versus 3/16[19%], respectively; p=0.07). Comparing ongoing clinical evidence of infection as a standard, the sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were 57%, 81%, 73%, and 68%, respectively (Table 1). In 24/30 patients with bacteremia, the results were Sn 29% and Sp 86%. A positive scan did not significantly affect AVG revision rates (22% versus 25%; p=0.71).

Table 1: 2x2 contingency table showing positive scans in clinically suspected AVG infections with summary statistics (n=30)

		Clinically Suspected AVG Infection		
		Positive	**Negative**	**Total**
Indium Scan AVG Uptake	**Positive**	8	3	11
	Negative	6	13	19
	Total	14	16	30

Conclusions: Although the proportion of positive scans was higher in patients suspected of having AVG infection compared to controls, a positive scan was of moderate Sn and Sp. The lack of correlation between treatment decisions and scan results suggests limited utility of costly indium scans in evaluation of AVG infections.

PUB551

Why Are Catheters Still Used to Initiate Hemodialysis Treatment of ESRD? Tanya Tocharoen Tang, Sumedha Dhar, Sadaf S. Khan, Antonio Alvarado, Shubhada N. Ahya, James J. Paparello, Murray L. Levin. *Nephrology, Feinburg School of Medicine, Northwestern Univ, Chicago, IL.*

Background: Complications of long-term use of tunneled catheters for hemodialysis involve a high risk of infection, thrombosis and high risk of mortality. This study aims to identify why an unacceptably large number of chronic hemodialysis patients in the United States are initiated on dialysis using tunneled hemodialysis catheters despite increased mortality, hospitalizations and expense.

Methods: We conducted a 13-question survey of 20 CKD patients who had been newly initiated on dialysis through a tunneled central vein dialysis catheter during their hospital admission in a university hospital from December 2013-June 2014.

Results: Of 20 subjects, 12 were male and 8 female. Subjects' mean age was 60 years old. Demographics included 12/20 (60%) African Americans, 5/20 (25%) Hispanics, 2/20 (10%) Caucasians and 1/20 (5%) Asian. Ninety-five% of patients were seen by a physician within the past year, had labs obtained and had been told their kidney function was poor. Fifteen% (3/20) of patients were not referred to a nephrologist. Eighty% of patients (16/20) were referred to a nephrologist and fifteen% (3/20) did not schedule an appointment due to noncompliance. Only 13 patients saw a nephrologist, 7 of whom were not referred to a

surgeon for access. Two of the latter were being evaluated for transplant. Of the 6 patients referred to a surgeon for access, 2 patients were non-compliant, and 4 had non-functioning access placed. Thus, of 20 patients starting hemodialysis with a catheter, only 4 had seen a surgeon for access placement.

Conclusions: Based on our study, there are numerous points in the path to arteriovenous access where attrition occurs. Patients and primary doctors need to be aware of the importance and causes of delay to access placement. Primary care physicians or nephrologists should oversee proper follow up and follow through to successful access placement prior to dialysis initiation of patients. Nephrologists need to recognize who is likely to progress, and how fast. Surgical fistula success rate is another important determinant of whether patients require catheters for their first dialysis.

PUB552

Usefulness of the Ultrasound Scan in the Implantation of Temporary Catheters in Femoral Vein Maite Rivera, Nuria Rodriguez Mendiola, Victor Burguera, Viviana Raoch Michaels, Rodrigo Hernandez Loyola, Sandra Elias, Fernando Liano, Carlos Quereda, Maria Teresa Tenorio. *Nephrology, Hospital Univ Ramón y Cajal.UAH.Irycis, Madrid, Spain.*

Background: The clinical guides recommend, with high level of evidence that ultrasound-guided canalization is mandatory for jugular venous catheters .Nevertheless, it is not well established for the implantation of femoral venous catheters.

Methods: We performed a prospective study that included all the temporary catheters implanted in the femoral vein (FC) during one year. 146 FC were implanted for renal replacement therapy. Patients were divided into two groups,Group 1: ultrasound-guided FC and iGroup 2: FC was implanted with conventional landmark localization. Nephrologists chose using ultrasound or not according the clinical situation of the patient and their own experience with FC implantation. Data regarding the number of attempts, failures, and complications were recorded.

Results: 95 patients in Group 1, 51 in Group 2. Mean age was lower in Group 1 (66 years versus 72 years (p = 0.007). There were no differences with respect to: number of platelets, INR or patients with double antiaggregation and anticoagulation.The number of patients with severe hepatic disease was higher in Group 1 (18 % versus 6 % (p=0.038)). Complications derived from the catheter insertion are showed in Table 1.

	Ultrasound-guided (n=95)	Anatomical landmark(n=51)	p
Catheter Side: *Right *Left	 63 (66 %) 32 (34%)	 38 (74.5 %) 13 (25.5%)	 ns
Nº of attempts * one * more than one	 66 (69.5 %) 29 (30.5%)	 29 (57 %) 22 (43 %)	 ns ns
Complications No : Complications Yes:	82 (86 %) 13 (14 %)	42 (82 %) 9 (18 %)	ns ns
Catheter disfunction	2 (2.1 %)	3 (5.9%)	ns
Cannulation failure	2 (2.1 %)	2 (3.9 %)	ns

Conclusions: In our work, ultrasound-guided femoral vein cannulation for HD was not superior to conventional technique. The success of implantation with the first puncture and complications were similar in both groups. The great proportion of catheters in femoral location with respect to jugular in our centre and the experience of the nephrologists in this area might explain this fact. Our work adds to the lack of evidence about the advantage of channeling FC with ultrasound.

Funding: Other NIH Support - Public Health Service (Spain)

PUB553

Retrospective Observational Study of Fistulograms Conducted in a Single UK Renal Unit on End Stage Renal Failure Patients with Arterio-Venous Fistula Sourjya Kar, Suresh Mathavakkannan. *[1]Renal Medicine, Lister Hospital, Stevenage, Hertfordshire, United Kingdom.*

Background: Preparation and creation of arterio-venous fistula is an integral part of haemodialysis patients. In certain situations there is malfunctioning of fistula and to ascertain the cause a radiological investigation named fistulogram is undertaken. Depending on the findings different approaches are undertaken – surgery, angioplasty or even conservative management. We hereby present a single UK renal centre study of all the fistulograms of either predialysis or haemodialysis patients conducted over a period of 2 ½ years analysing the indications, findings of fistulograms and the eventual outcomes in relation to demographics.

Methods: Retrospective data collection for 2 ½ yrs of all the fistulograms performed in haemodialysis and predialysis patients in Lister Hospital. Data collected from medical notes and renal software - analysed by exel sheet.

Results: 94 patients had undergone fistulograms during the period specified of which 24 had repeat procedures. The average time duration from fistula creation to fistulogram performed is 24 months which differs in various demographics. The most common indication for fistulogram is poor maturation (30%) and swollen arm (27%). The most common findings are no stenosis (29%), draining vein stenosis (26%) and central venous stenosis (24%). Intervention was not required in most (31%). The fistula was working in 60% of cases after undergoing procedure. On subanalysis it was noted that 67% of fistulae were working after radiological intervention (versus 79% post surgery). Finally demographics of patients with solely non-functioning fistula were analysed in comparison to baseline demographics. Only slight increase of failure rate more in diabetics (30% versus 25.2%).

Conclusions: Most common indication for fistulogram was poor maturation and the most common finding was no stenosis. Surgery had slightly better success rate than radiological intervention if required. Most fistulae failed as intervention was not possible. Except slight prevelance with diabetics there was no demographic difference related to malfunctioning fistulae.

PUB554

Influence of Calcium-Phosphate Product on Ateriovenous Fistula (AVF) Maturity: A Pilot Study Azreen Syazril Adnan,[1] Azlima Ali,[1] Tauqeer Hussain Mallhi,[2] Fauziah Jummaat,[3] Azhar Amir Hamzah,[1] Amer Hayat Khan,[1,2] Nurul Jannah Ambak,[1] Yusra Habib Khan,[2] Muhammad Salman.[1,2] *[1]Chronic Kidney Disease Resource Center, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia; [2]Dept of Clinical Pharmacy, Univ Sains Malaysia, Penang, Malaysia; [3]Dept of Obstetrics and Gynecology, Univ Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.*

Background: An Arteriovenous fistula (AVF) is considered as the best long-term vascular access for hemodialysis patients. Adequate blood flow and lower complications rate made it quite durable vascular access. However, the maturity of AVF access is influenced by various mechanical and medical factors.

Methods: A prospective cohort study was conducted at Chronic Kidney Disease Resource Centre, Hospital USM from March to June, 2013. After applying inclusion and exclusion criteria, agreed patients were recruited in this study. Consent forms were signed by participants. Blood levels of calcium, phosphate and urea were recorded before surgery. Postoperative follow-up was done at 2nd, 4th and 6th week. Maturity of AVF was assessed by rule of 6s.

Results: Fifteen patients with mean age 45±20 years underwent surgery for AVF creation. Majority of patients were 60 years or above. The prevalence of early AVF maturity was 23.1% (95% CI 0.2, 46.0). Percentage of patients with diabetes mellitus in early AVF maturity was 17.71% whereas in late AVF maturity was 82.29%. Hyperlipidemia was present in all late AVF maturity. The mean value of calcium - phosphate product was 37.07±11.78 mg^2/dl^2. Calcium - phosphate product was higher in late mature AVF than early mature AVF group (46.37 mg^2/dl^2 versus 36.82 mg^2/dl^2).

Conclusions: Calcium - phosphate product may influence AVF maturity and low levels may predict early maturity of AVF. Optimization of these parameters might result in successful outcome of vascular access.

PUB555

Survival in Tunnelized Catheters Installed in the Superior and Lower Segment in Maintenance Hemodialysis Patients Gerardo Guillermo Corpus, Manolo Ramos Gordillo, Marco Antonio Carmona-Escamilla, Daniel Diaz, Ivanna Rocha, Laura Elisa Caballero Castellanos, Jose C. Pena. *Nefrologia, CEDIASA Grupo Angeles, Mexico, DF, Mexico.*

Background: Tunneled hemodialysis catheters is widely utilized in patients requiring long-term hemodialysis. The vascular access located in the upper segment (jugular and subclavian) generally are preferred over the lower segment (femoral). However, the femoral approach may offer some advantages, similar or superior to the upper segment (Left jugular and subclavian).

Methods: A retrospective study of all tunneled hemodialysis catheters placed between January 2012 and April 2014 (27 months) was done. During this study period 733 in cuffed catheters were placed. All procedures were performed by a single interventional nephrologist. Catheters in the upper segment were compared with the vascular access in the lower segment. The functional survival of the catheters and the presence of infection were estimated overall.

Results: The average age of the whole population was 53.5 years±15.33. 379 Men (52%), 354 women 48.2%. Sixty percent of patients had diabetes. The catheters placed in the upper segment were 615 (83.7%), right internal jugular 549 (89.2%), left internal jugular 43 (6.9%), subclavian left and right 23 (3.7%) and 118 (16.0%) in the lower segment. Overall survival of the catheters was 95.7% at 27 months. The survival rate of the upper segment catheters was 96.3% and 93.2% in the lower segment at 27 months; Log-rank p< 0.03. Mean value of survival time in months was 26 CI (26.04 - 26.43). In 29 percent of all patients an AVF was performed Catheter related infections occurred in 25 cases of the 733 catheters (3.4%) during the study period; culture-proved bacteremia was demonstrated in 16 patients (2.2%).

Conclusions: The survival of tunneled catheters placed in the upper right jugular vein is superior compared with tunneled catheters placed in the lower segment. However after the failure of the right jugular access the lower segment was a second option instead of the other upper veins as is the usual procedure. This approach allows the vascular surgeons to use the left arm more freely to built an AVF and reduces the risk of upper vein obstruction.

PUB556

A MWPNC Physician Survey of Physical Restrictions in Dialysis Patients Deepa H. Chand,[1] Susan F. Massengill.[2] *[1]Pediatrics, Rush Children's Hospital, Chicago, IL; [2]Pediatrics, Levine Chidren's Hospital, Charlotte, NC.*

Background: One goal in the treatment of pediatric hemodialysis (pHD) patients is to allow patients to engage in "regular activities". Also, regular exercise has cardiovascular and psychosocial benefits in dialysis patients. However, the data supporting limitation

of activities is scarce. We would like to better illicit provider practices when advising pHD patients on limitation of exercise and activity. **Objective:** We aimed to determine if variability and evidence-based rationale exist in regards to physical restrictions placed on pediatric pHD patients.

Methods: This was a cross-sectional survey of pediatric nephrology attending physicians within the MWPNC, who were asked what physical restrictions they placed on pHD patients.

Results: A total of 12 respondents completed the survey. For patients with an AVF/AVG for pHD access: 50% stated they allow patients to lift weights; all respondents restrict patients from football, wrestling, and martial arts; all respondents stated no patient had lost an access after engaging in sports. Respondents stated they did not have evidence for practice, but rather practice was based on "experience", "institutional practice", or "how I was taught". In patients with a CVL for pHD access: all allow the patient to shower, but only 3 allow a "bath"; no providers allow swimming. Six episodes of CVL infection were reported in the past 6 months.

Conclusions: Pediatric HD providers rely on experience or personal bias to advise patients regarding restrictions in the care of their HD access. This study highlights the need for longitudinal data collection for the creation of evidence based clinical practices.

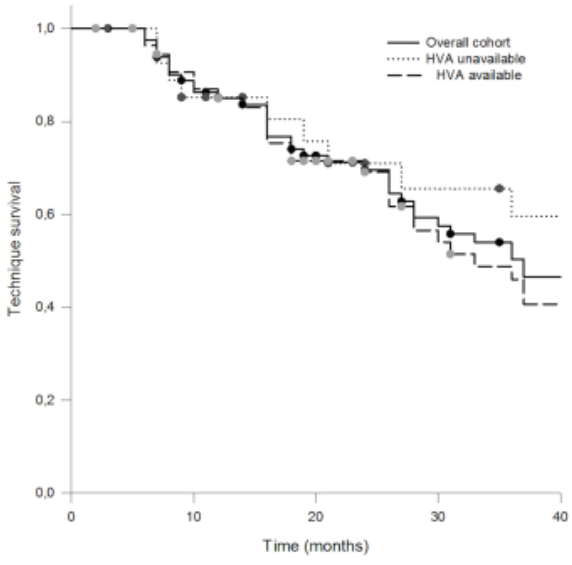
PUB557

Utility of Home Visit Audits in Home Hemodialysis Karlien Francois, Christopher T. Chan. *Div of Nephrology, Univ Health Network Toronto General Hospital, Toronto, ON, Canada.*

Background: Home visits (HV) and HV audits (HVA) are standard practice in home dialysis programs however with limited clinical evidence.

Methods: We performed a single-centre retrospective cohort study in patients starting home hemodialysis (HHD) at University Health Network to ascertain the impact of HVA on technique survival. The HVA is a questionnaire addressing the practice of home hemodialysis and patient well-being. All incident HHD patients starting dialysis at home between July 18, 2008 and June 30, 2013 were included in the cohort and were followed until death, kidney transplantation, transfer to another center or study end, December 31, 2013. Demographic data and information on HVA recordings during the HV when the patient started HHD, were collected. Data are presented as mean±SD or median with IQR. Technique survival is assessed by Kaplan Meier analysis.

Results: 84 patients initiated HHD during the study period. All patients had a baseline HV at the start of HHD and a HVA was surveyed in 56 patients. Overall, patients were 45.8±14.1 years old and 51.2% were male. ESRD was secondary to glomerulonephritis, diabetic and ischemic nephropathy in 33.3, 14.3 and 9.5% of subjects respectively. 67.9% of patients started HHD with a central venous catheter as vascular access. Age, gender, training duration, follow-up time, ESRD cause, vascular access and co-morbidities were similar in the patient group with surveyed HVA (HVA+) compared to those without (HVA-). Technique survival, censored for death, kidney transplantation and transfer to another center, was similar for HVA+ and HVA- groups (p=0.91) [figure 1]. The ascertainment of deficiencies through HVA did not influence technique survival (p=0.84).



Conclusions: HV aim to assure quality of care in patients undergoing HHD. Our results raise questions in establishing the appropriate metrics to ensure patient safety with optimal use of resources.

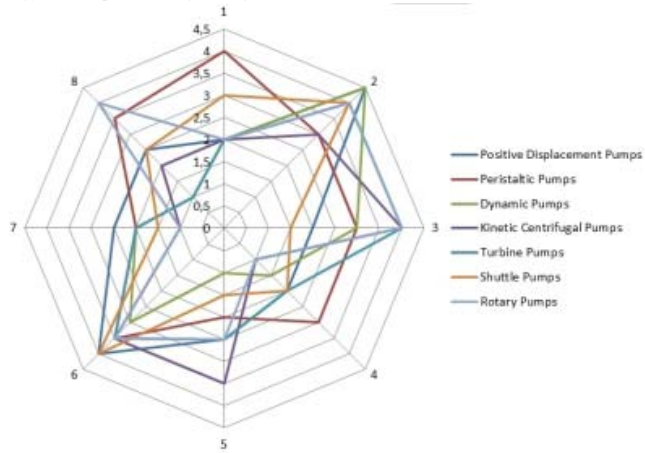
PUB558

Pumps in Wearable Ultrafiltration Devices Paolo Armignacco, Francesco Garzotto, Mauro Neri, Claudio Ronco. *IRRIV (International Renal Research Institute Vicenza), Italy.*

Background: The Wearable Artificial Kidney(WAK) is a device that allows prolonged and frequent dialysis treatments for ESRD patients;its functioning is strictly related to its pumping system,that is responsible for propelling blood and dialysate through the tubing of extracorporeal circuits.The aim of this poster is to analyze in detail the WAK pumping system,with the production of a radar graph comparable of different pumps'performances.

Methods: The WAK consists of a battery,an external flowmeter,a dialyzer and three basic systems:one for regenerating the dialysate,an alarm/shutoff one and a pumping one.A pump is a device that moves fluids by mechanical action;in the case of WAK,the pumping system is responsible for propelling blood and dialysate through the tubing of extracorporeal circuits.So,many of the WAK most important characteristics are strictly related to its pumping system.Many different pumps designs have been suggested for WAK.In the actual prototype,the pumping system is mainly constituted by blood and infusion pumps.Blood pumps can be distinguished,according to the way they work,in positive displacement,peristaltic,dynamic,kinetic centrifugal,turbine,shuttle and rotary pumps;they are all characterized by different values of the most important parameters defining their quality:hemolysis,biocompatibility,precision,flow rate,energy consumption,weight,ergonomics,size and cost.

Results: The following graph,comparative of blood pumps performances,considers eight parameters,(1-8),that respectively are:1Hemolysis;2Biocompatibility;3Precision;4Energy consumption;5Weight;6Ergonomics;7Size;8Cost



Conclusions: This pumps' performances comparison permits to define the characteristics of a perfect pump.It would allow:high safety rates,elevated biocompatibility,infinite MTBF,mean valued flux speeds,fluxes compatible with the subject's well-being,low hemolysis,small dimensions and cheap production costs.

PUB559

Is Self-Cannulation a Weak Link in the Uptake of Home Haemodialysis? Anuradha Jayanti, The Basic-Hhd, Sandip Mitra. *Dept of Nephrology, Manchester Royal Infirmary, Manchester, United Kingdom.*

Background: Self-cannulation(SC) empowers patients and brings together the possibility of home haemodialysis (HD) and haemodialysis commencement with native AVF. It ensures consistent technique for needling, once expert skills are attained. Published literature in SC of vascular access is limited to cannulation technique and related complications. This study provides the a comprehensive evaluation of the subject from a patient perspective.

Methods: In a cross-sectional, multicentre study (BASIC-HHD study) 3 cannulation-related questions were posed to patients (n=535). Demographic and clinical data were also obtained. SCQ1How well do you tolerate needle insertion for blood tests? SCQ2Could you do the same if required, for dialysis treatment? SCQ3What aspect of needling one's self for dialysis bothers you most? 3 study cohorts included 212 predialysis, 200 hospital HD and 97 home HD patients, with complete responses. Participants also completed the depression (BDI), anxiety inventory (STAI) and Autonomy Preference Index.

Results: There is a statistically significant difference between the cohorts in their responses to the SCQ1, with 13% of hospital HD cohort 'fearful' of routine phlebotomy (p=0.002). 60% of the predialysis cohort (A) and 43% of hospital HD cohort (B) feels able to consider SC (SCQ2). Over 20% of concerns from A, were modifiable and were related to skill and fear of potential procedural complications. Pain as the sole cause for concern was perceived by 10% in ALL cohorts. 5 significant variables from HD cohorts were identified from multivariate analysis- response to SCQ1, education, employment, care-giver type, 3MS score with a cut-off of 80 in home and hospital cohorts. Negative response to SCQ1 and trait anxiety score >39 were predictors of a negative disposition to SC in predialysis cohort. In a logistic regression model, the predictive accuracy of the response to SCQ2 dichotomised into Yes or No, selected from hospital and home cohorts and validated in the predialysis cohort was 63.2%.

Conclusions: Self-cannulation fears are a surmountable barrier to uptake of home HD, in a significant number of patients. Targeted interventions in the predialysis phase will help promote self-care HD.

Funding: Pharmaceutical Company Support - Baxter Clinical Evidence Council supports this investigator initiated and investigator led research

PUB560

Nephron+ Wearable Artificial Kidney for Continuous Dialysis Frank Simonis,[1] Karin G. Gerritsen,[2] Maarten Wester,[2] Jaap A. Joles.[2] *[1]Nanodialysis, Oirschot, Noord Brabant, Netherlands; [2]Nephrology and Hypertension, Univ Medical Center Utrecht, Utrecht, Netherlands.*

Background: The Nephron+ wearable dialysis system enables a slow and continuous 24/7 dialysis treatment resembling the functioning of healthy kidneys. Hereby variations of concentration levels in the blood can be kept to a minimum and blood clearance can be improved with obvious health benefits. Patients will benefit in quality of life while being mobile during dialysis.

Methods: Nephron+ makes use of a new technology requiring only 100 ml of dialysate being continuously refreshed with sorbents (150 grams) in combination with catalytic oxidation of toxins. This allows for a wearable device of about 2 kg. Hereby the system can dialyze 150 hours per week, a factor 10 slower then in-center hemodialysis. Blood tubings and dialyzer filter can be downsized to pediatric size. Excess water is removed in ultrafiltration mode. A range of sensors is used to control the blood clearance and to secure a safe operation. Examples are sensors for K, Ca and pH, conductivity, pressure, temperature sensors and redox state. Sensor data, messages and alarms generated by the operating system are being sent and logged to a smartphone and webportal. Both doctor and patient have access to the history and actual status of the device and the running treatment.

Results: Prototype versions have been submitted to extensive invitro efficacy tests and invivo tests using a goat as animal model. Adequate clearance of electrolytes, urea and other organic toxins was obtained. Biocompatibility studies using endothelial cell lines show no side efects sofar but more proof is needed.

Conclusions: The efficacy of the Nephron+ concept has been proven both by invitro and invivo tests. In order to prepare the system for clinical trials, research effort is now directed to assess the biocompatibility aspects in depth.

Funding: Government Support - Non-U.S.

PUB561

Dialysate Regeneration in a Wearable Artificial Kidney by Electro-Oxidation and Activated Carbon Does Not Induce Oxidative Stress or Endothelial Cytotoxicity Hendrik Gremmels,[1] Diënty Hazenbrink,[1] Frank Simonis,[2] Maarten Wester,[1] Walther H. Boer,[1] Jaap A. Joles,[1] Karin G. Gerritsen.[1] *[1]Nephrology, UMC Utrecht, Utrecht, Netherlands; [2]Nanodialysis, Oirschot, Netherlands.*

Background: We are developing a wearable artificial kidney (EU FP7 project NEPHRON+), based on continuous regeneration of a small volume of dialysate by adsorption and electro-oxidation (EO). Clinically relevant degradation of urea and creatinine was achieved using EO in series with activated carbon (AC) for removal of chlorine by-products. Major concern of EO is that toxic, in particular oxidative, by-products are generated that are not removed by AC. Therefore we investigated the effect of EO combined with AC on oxidative status and endothelial cytotoxicity of uremic plasma and dialysate.

Methods: Human plasmapheresis plasma (1L, urea 24-43mM) was dialyzed for 1h and dialysate was recirculated in countercurrent direction through an EO-unit in series with AC. Oxygen radical adsorbance capacity was assessed using fluorescein. Oxidation reduction potential (ORP) was measured using a platinum electrode and Ag/AgCl reference electrode. Endothelial colony forming cells isolated from umbilical cord blood were exposed to EO- and AC-treated fluids. Induction of intracellular reactive oxygen species was evaluated using CM-H2DCFDA. Resazurin, neutral red and MTT were used to assess cell viability. Electric cell-substrate impedance sensing was used to monitor real-time cell proliferation. Data (n=3 plasma's x 3 cell donors) were normalized to PBS- or medium-treated controls.

Results: EO plus AC had no influence on total anti-oxidant capacity of dialysate and plasma as compared to treatment by AC alone. Remarkably, while ORP remained stable during treatment by AC, EO-plus-AC-treated fluids changed from an oxidative into a reductive state (plasma: 133±59 to -131±43mV; dialysate: 113±59 to -165±47mV; $p<0.05$). No effects of EO and/or AC treated dialysate and plasma were observed on intracellular generation of ROS, cell viability and cell proliferation.

Conclusions: Our data suggest that dialysate regeneration by EO combined with AC does not increase oxidative status or endothelial cytotoxicity of dialysate and uremic plasma as compared to AC without EO.

PUB562

Three Hour Daily Dialysis Is Associated with Long Term (4 Year) Control of Mineral Metabolism Steven Achinger,[1] Nora Angelica Fuentes,[2] Juan Carlos Ayus.[2,3] *[1]Wason Clinic, LLP, Lakeland, FL; [2]Hospital Italiano, Buenos Aires, Argentina; [3]Renal Consultants of Houston, Houston, TX.*

Background: Abnormal mineral metabolism increases the risk of death in persons receiving hemodialysis. Three hour daily dialysis in one year studies has been shown to improve mineral metabolism goals (*JASN* 2005; 16:2778-88). However, it is not known to what extent three hour daily dialysis will lead to long term mineral metabolism control.

Methods: We conducted a non-randomized, controlled trial of the effect of 3 hour daily dialysis (DHD) (six sessions/week of three hours each) versus conventional hemodialysis (CHD) (three sessions/week of four hours each) hemodialysis on mineral metabolism. We enrolled 77 patients, 26 DHD and 51 CHD matched conventional hemodialysis patients. We collected baseline, yearly and up to 48 month measures of phosphorus, calcium, calcium x phosphorus product and intact parathyroid hormone. Baseline characteristics were similar between groups.

Results: At 48-month follow-up, daily hemodialysis was associated with improved attainment of mineral metabolism goals for phosphorus (adjusted HR 3.6, P = 0.002) and phosphorus product (adjusted HR 3.66, P = 0.001) **(Table 1).** Moreover, a sensitivity analysis was performed in which the goal phosphorus was changed to < 5.0 mg/dL of phosphorus and the crude hazard ratio was 1.5 (0.6 to 3.8) and adjusted HR was 2.7 (1.06 to 6.9). There were no differences between the two groups in terms of attainment of calcium and parathyroid hormone goals **(Table 1).**

Mineral metabolism goal	Crude HR (95% CI)	p	Adjusted HR (95% CI)	p
Phosphorus (3.5 - 5.5 mg/dl)	2.5 (1.6 - 5.4)	0.019	3.6 (1.6 - 8.2)	0.002
Calcium (8.4 - 9.5 mg/dl)	2.1 (1.02-4.3)	0.045	2.15 (0.95 - 4.9)	0.064
Calcium phosphorus product (< 55 mg2/dl2)	3.08 (1.7 - 5.6)	< 0.001	3.66 (1.9 - 6.9)	< 0.001
Parathyroid hormone (150-300 pg/ml)	1.57 (0.54- 4.5)	0.4	2.44 (0.77 - 7.8)	0.13

Conclusions: Three hour daily hemodialysis, compared with conventional hemodialysis leads to maintenance long-term improvements in phosphorus and calcium-phosphorus product which are known surrogates of poor outcomes among hemodialysis patients.

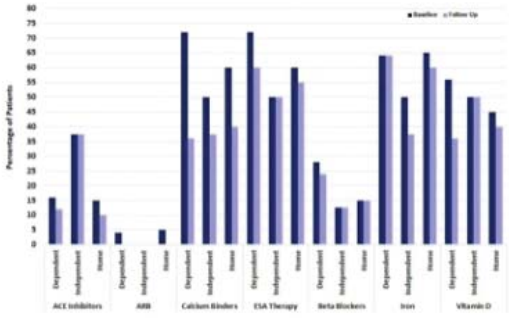
PUB563

Unique System of Nocturnal Dialysis Sunita Nair, Michael A. Copland, Lee Er, Mercedeh Kiaii, Adeera Levin. *[1]Nephrology, Vancouver General Hospital, Vancouver, BC, Canada; [2]Nephrology, St Pauls Hospital, Vancouver, BC, Canada; [3]Statistics, B C Renal Agency, Vancouver, BC, Canada.*

Background: Description of a Nocturnal Hemodialysis (ND)programe.

Methods: Home Hemodialysis (HHD) was established as a dialysis option in 2004 in BC. 50% of patients were on Home ND (HND). Barriers to uptake were prohibitive home situations, or clinical instability. To overcome this, two facility based (FB) options were evaluated. In 2009, a facility based independent nocturnal hemodialysis (FBIND) program started, and in 2011 a FB dependent ND (FBDND)was started. This cohort study describes the outcomes of those patients who commenced in one of these programmes between 1st Jan 2011 and 31st October 2012. Follow up was a year after start, or until the termination of program. Outcomes included change in biochemical parameters and medications pre/post study period. For continuous variables, mean with SD or median with inter-quartile were presented. Frequencies and percentages were reported for categorical variables. All tests were 2-sided and performed in SAS software version 9.3 (SAS Institute,NC).

Results: Of 71 patients on NHD, 46% were in the FBDND, 14%in FBIND and 40% in HND. Biochemical parameters did not change substantially over time, nor was it statistically significant between the three groups. All groups had reduction in medication:median number of medications in FBDND was 4 to 3 at the end of follow up, in FBIND 2.5 to 1.5 and in HHD from 4 to 3



Conclusions: Our study shows that irrespective of co-morbidities and dialysis location,biochemical outcomes are similar. Medication reduction is possible in those who underwent ND. The uniqueness of our system allows patients to undergo ND in center before they move to home. This allows patients to undergo ND independently within the facility, who otherwise cannot dialyse at home. This allows room for respite care and helps patients sustain their chosen modality.

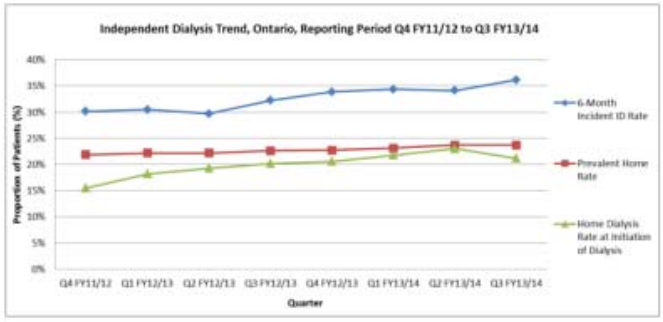
PUB564

Increasing Uptake of Independent Dialysis in Ontario, a Strategic Initiative of the Ontario Renal Network Andreas Pierratos,[1,2,4] Gokulan Kandasamy,[4] Graham Woodward,[4] Gihad E. Nesrallah,[1,3,4] Camila Iraheta,[4] Kiren Handa.[4] *[1]Nephrology, Humber River Hospital, Toronto, ON, Canada; [2]Medicine, Univ of Toronto, Toronto, ON, Canada; [3]Medicine, Univ of Western Ontario, London, ON, Canada; [4]Ontario Renal Network, Toronto, ON, Canada.*

Background: Uptake of Independent Dialysis (ID) is a key strategic priority in Ontario. **ID is defined** by Ontario Renal Network (ORN) as dialysis independent of in-centre facilities. The **key metric** of success is the **6-month ORN incident ID rate**. It is **defined** as the patients on ID within 6 months of starting dialysis, over the patients who stayed on dialysis long enough (6 months) to be given such an opportunity. The Ontario Renal Plan **goal** for the 6-month ORN incident ID rate is **40%** by March 31, 2015. Some of the **supportive initiatives** launched to date are: patient-based funding (including frequent home hemodialysis); promotion of patient education; creation of a 'collaborative' network for newly-funded ID coordinators; establishment of a yearly ID conference.

Methods: The objective was to **report the interim results of the ID strategic initiative** including the **6-month ORN incident ID rate** as well as conventional home dialysis incident and prevalent rates in Ontario. Data reported to the Ontario Renal Reporting System (**ORRS**) starting in Q3 2011/12, were analyzed on a quarterly basis and reported.

Results: Figure 1 depicts the ID trends in Ontario.



Attrition rate from home dialysis was 25% per year. Patient follow up in CKD clinic >1 year associates with higher ID incident rate.

Conclusions: ORN initiatives led to **the 6-month ORN ID incident rate increase from 30 to 36%.** Similar trends were observed in home dialysis rate at initiation of dialysis. The improvement of the prevalent home dialysis rate is smaller partially related to the high attrition rate.

Funding: Government Support - Non-U.S.

PUB565

"The Self Dialysis Ladder". An Educational Program in Self Hemodialysis Willy Aasebo, Liliana Monica Bivol, Carl Erik Halvorsen. *Dept of Nephrology, Akershus Univ Hospital, Lorenskog, Norway.*

Background: Home hemodialysis may improve the quality of dialysis, reduce costs and increase the patient's well-being. To become independent of help may in itself be desirable to many patients, even when treated in hospital.

Methods: From October 2012 through 2013 hemodialysis patients were considered for the program. During 2013 all suitable patients started directly in the program. The patients had to be physically fit to handle the machine, be mentally prepared to learn and be able to take charge of their own treatment. "The Self Dialysis Program" is a stepwise educational tool describing how to perform hemodialysis and consists of a practical section, "the Self Dialysis Ladder", and a theoretical section with information and procedures, "the Self Dialysis Teaching Document". Procedures of every aspect of the training are described in great details in an everyday language in the teaching document. "The Self Dialysis Ladder" provides an overview of the requirements the patients should learn. Color codes give a quick overview of where the patient is in the training process. The code "Red light" means that the patient has started the teaching process by being showed and explained the procedure. "Yellow light" means that the patient is doing the procedure with guidance of a nurse. When the nurses have observed that the patient knows and does the procedure correctly without guidance, the patient has become independent in this topic and reached "Green light", after which the patients perform these tasks independently.

Results: A total of 31 patients were included in the program. During 2013 34% of all new hemodialysis patients were included. Seventeen patients completed the education, of whom 16 % reached full independence. Fourteen did not finish the education, of whom 4 converted to peritoneal dialysis, 5 received a transplant, 1 regained kidney function and 4 were still training.

Conclusions: "The Self Dialysis Ladder" is a useful tool in teaching patients to become independent of help in hemodialysis. The patients soon required less help compared to ordinary patients and express more self confidence and well-being compared to the others.

PUB566

Analyses of Urate-Lowering Effects by Calcium Channel Blockers Nobuyuki Onizawa,[1,2] Go Tsuchiya,[1,3] Takayuki Hori,[1,3] Promsuk Jutabha,[1] Motoshi Ouchi,[1] Hajime Hasegawa,[2] Hirotsugu Fukuda,[3] Naohiko Anzai.[1] *[1]Dept of Pharmacology and Toxicology, Dokkyo Medical Univ School of Medicine, Mibu, Tochigi, Japan; [2]Dept of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical Univ, Kawagoe, Saitama, Japan; [3]Dept of Cardiovascular Surgery, Dokkyo Medical Univ School of Medicine, Mibu, Tochigi, Japan.*

Background: Hyperuricemia is associated with hypertension and is now recognized as a risk factors for cardiovascular diseases. Among anti-hypertensive drugs, ARB losartan is known to have urate-lowering effects in addition to its class effect. However, some calcium channel blockers (CCBs) has been reported to decrease serum uric acid level. Here, we sought to elucidate the molecular mechanism of the urate-lowering effects found in CCBs.

Methods: We measured [^{14}C] urate uptake using stable cells expressing human urate transporter 1 (HEK-URAT1) together with mock cells (HEK-mock) to evaluate the uricosuric action of CCBs. Next, we measured the activity of human xanthine oxidase (XO) to clarify whether CCBs have inhibitory effects on urate production. Nifedipine, amlodipine, nitrendipine, benidipine hydrochloride, verapamil, diltiazem hydrochloride, nilvadipine, nisoldipine, nicardipine hydrochloride, and efonidipine hydrochloride were used in this study.

Results: Among these, nifedipine, nitrendipine, verapamil, nisoldipine and efonidipine hydrochloride demonstrated the inhibitory effect on URAT1-mediated urate uptake. On the contrary, urate production mediated by XO was not inhibited by the CCBs tested here.

Conclusions: URAT1, a major contributor of renal urate reabsorption and a major target of uricosuric drugs such as losartan and benzbromarone, interacted with several CCBs, whereas XO, a major enzyme involved in urate production in the body, did not interact with CCBs. These results suggested that the urate-lowering effects of CCBs are associated primarily with the inhibition of renal urate reabsorption via renal urate transporters such as URAT1.

Funding: Government Support - Non-U.S.

PUB567

The Sunny Side of Blood Pressure Regulation: Skin UV Exposure and NO Release Richard Beresford Weller.[1,2] *[1]Centre for Inflammation Research, Univ of Edinburgh, Edinburgh, United Kingdom; [2]Dept of Dermatology, Univ of Edinburgh, Edinburgh, United Kingdom.*

Background: High blood pressure (BP) is the leading risk factor for disability adjusted life years lost globally, and underlies 12% of all deaths. Population BP correlates directly with latitude and is lower in summer than winter. The ultraviolet (UV) B component of sunlight is necessary for vitamin D synthesis. Individuals with serum Vitamin D levels in the lowest quartile are twice as likely to have hypertension as those in the upper quartile, yet meta-analyses of oral vitamin D supplementation studies show no effect on BP. UV is the major environmental risk factor for skin cancer, yet there are no data showing that sunlight increases all-cause mortality. Individuals with non-melanoma skin cancer have half the relative risk of all-cause death as the general population. Increasing sun seeking behaviour dose dependently correlates with reduced all-cause mortality (and increased skin cancer) after correcting for major confounders. Human skin contains large stores of nitrogen oxides, particularly nitrate (NO_3).

Methods: 24 healthy volunteers were irradiated for 20 minutes with 2 Standard Erythemal Doses (SED) of UVA or a temperature matched sham irradiation. Active/sham irradiation of the forearm was performed in 12 volunteers while measuring blood flow by plethysmography and intra-brachially infusing the NOS antagonist L-NMMA.

Results: UVA reduced BP and raised HR during and for 1 hour after irradiation. Contemporaneously circulating NO_3 fell and nitrite rose. Sham irradiation reduced BP during the irradiation period only and did not induce nitrate reduction. Vitamin D levels were unaltered. Active, but not sham irradiation vasodilated NOS antagonised arteries.

Conclusions: We have identified a vitamin D independent mechanism by which UV radiation lowers BP. We suspect that photoreduction of cutaneous NO_3 with export of NO activity to the systemic vasculature is responsible. This mechanism may account for the

reduced cardiovascular and all-cause mortality associated with sun-seeking behaviour. Public health advice on sun-avoidance needs to be revised and based on reduction of all-cause mortality.

PUB568

Effects of the SGLT2 Inhibitor, Empagliflozin, on Blood Pressure and Urinary Excretion of Sodium in Salt-Treated Obese Rats Yui Takeshige,[1,2] Hiroaki Ogata,[1] Akira Nishiyama.[2] *[1]Internal Medicine, Showa Univ Northern Yokohama Hospital, Yokohama, Kanagawa, Japan; [2]Pharmacology, Faculty of Medicine, Kita-gun, Kagawa, Japan.*

Background: Studies were performed to examine the effects of the sodium-glucose co-transporter 2 (SGLT2) inhibitor, empagliflozin, on blood pressure and urinary excretion of sodium in salt-treated obese Otsuka Long Evans Tokushima Fatty (OLETF) rats.

Methods: Sixteen-week-old obese OLETF rats were treated with 1% NaCl (in drinking water, n = 10) and vehicle (0.5% carboxymethylcellulose, n = 10) or empagliflozin (10 mg/kg/day, p.o., n = 10) for 5 weeks. Blood pressure was continuously measured by telemetry. Glucose metabolism and urinary sodium excretion were evaluated by oral glucose tolerance test and high salt challenge test, respectively.

Results: High salt plus vehicle-treated obese OLETF rats developed non-dipper type hypertension (136+/-2 mmHg in waking time and 132+/-2 mmHg in sleeping time). Compared with high salt plus vehicle-treated animals, high salt plus empagliflozin-treated OLETF rats showed an approximately 1,000-fold increase in urinary glucose excretion and improved glucose metabolism. Furthermore, empagliflozin significantly decreased blood pressure and improved blood pressure circadian rhythm to a dipper profile, which were associated with increases in urinary sodium excretion of 30%.

Conclusions: These data suggest that empagliflozin elicits beneficial effects on both glucose metabolism and hypertension in salt-treated obese rats.

PUB569

Aggravated Renal Damage by Disturbed Circadian Rhythm of the Intrarenal Angiotensinogen and Angiotensin II Type 1 Receptor in Anti-Thymocyte Serum Nephritis Rats Shinsuke Isobe,[1] Naro Ohashi,[1] Hideo Yasuda,[1] Yoshihide Fujigaki.[2] *[1]1st internal medicine, Hamamatsu Univ School of Medicine, Hamamatsu, Japan; [2]Medicine, Teikyo Univ School of Medicine, Itabashi-ku.*

Background: We previously reported the disturbed circadian rhythm of intrarenal renin-angiotensin system (RAS) activation that is evaluated bythe urinary angiotensinogen (AGT) may lead to renal damage and hypertension, which are associated with diurnal blood pressure (BP) variation. Our purpose is to clarify the contribution by disturbed circadian rhythm of the intrarenal RAS components to renal damage and BP.

Methods: Anti-thymocyte serum (ATS) nephritis rats were made as chronic progressive glomerulonephritis models (A group) and compared with control rat (C group). Rats with ATS nephritis in AO group or AH group received olmesartan or hydralazine, respectively to lower BP equally to that in C group. Rats were sacrificed every 6 hour (7:00 am, 1:00 pm, 7:00pm and 1:00 am, respectively) after urine collection and BP measurement. The levels of the intrarenal RAS components were evaluated.

Results: The degrees of fibrotic renal lesions were significantly increased in A group compared with C group, and attenuated in AO group but not AH group compared with A group. The results were shown at 1:00 pm when the circadian rhythm of each parameter was disturbed in A group compared with C group. The levels of BP and urinary protein and AGT excretion in A group were significantly higher than those in C group and those in AO group were significantly lower than those in A group. Although AH group showed significantly lower BP than A group, the levels of urinary protein and AGT excretion in AH group were not significantly different from those in A group. The levels of intrarenal AGT and angiotensin II type 1 receptor (AT1R) protein expression in A groups were significantly increased 3.9- and 3.2-fold compared with those in C group, and those in AO group were significantly decreased to 51% and 35% of those in A group, respectively. However, AH group did not change the parameters compared with A group.

Conclusions: Enhanced AGT and AT1R proteins at 1:00 pm may aggravate the renal damage independent of BP in ATS nephritis rats.

PUB570

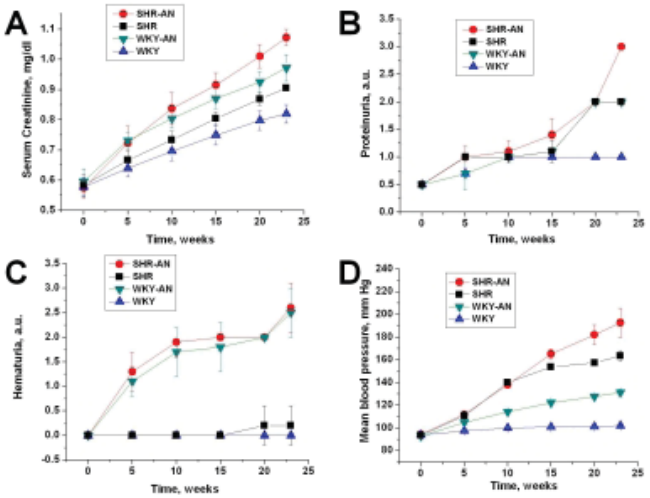
Hypertension Increases Chronic Kidney Injury in 5/6 Nephrectomy Rats Sergey V. Brodsky, Kyle M. Ware, Navin S. Muni, Lee A. Hebert, Metin Gurcan, Jessica Hemminger, Iouri Ivanov, Gyongyi Nadasdy, Anjali A. Satoskar, Tibor Nadasdy, Brad H. Rovin. *The Ohio State Univ, Columbus, OH.*

Background: Hypertension (HTN) is a leading cause of end stage kidney disease worldwide. With the pandemic of obesity, chronic kidney disease (CKD) with progressive nephron loss, a form ablative nephropathy (AN) is becoming more common. Early studies demonstrated the short-term effects of HTN on kidney function and morphology in AN rats. The aim of this study was to investigate the long-term consequences of HTN in the AN model.

Methods: AN was created by 5/6 nephrectomy in spontaneous hypertensive (SHR) and genetically similar normotensive Wistar Kyoto (WKY) rats. Blood pressure (BP), serum creatinine (SCr), hematuria and proteinuria were monitored weekly for 23 weeks. Kidney morphology was assessed at the end of the study. Sham-operated rats from both strains were used as controls.

Results: AN resulted in increases of SCr, hematuria and proteinuria in both SHR and WKY. The increase in SCr was accelerated in SHR-AN as compared to WKY-AN (Fig 1A). Similarly, SHR-AN had earlier onset and higher proteinuria than WKY-AN (Fig 1B). Hematuria was similar in SHR-AN and WKY-AN rats (Fig 1C). Mean BP was higher in SHR-AN than in SHR, as well as in WKY-AN than in WKY, but not that high as in SHR-AN (Fig 1D). Histologically, SHR-AN had enlarged glomeruli and increased interstitial fibrosis as compared to WKY-AN.

Conclusions: HTN increases long-term kidney injury in AN. On the other hand, AN increases BP in SHR. We propose that a vicious cycle occurs in which HTN accelerates kidney injury, which consequently increases HTN.



PUB571

SIRT1 Activation Protects the Endothelial Dysfunction By Inhibiting FGF and TNF-α Generation Hideyuki Negoro. *Medicine, Harvard Medical School, Boston, MA.*

Background: SIRT1 is a conserved NAD(+)-dependent deacetylase and possesses beneficial effects against aging-related diseases, but little information is available on a putative role of SIRT1 in endothelial and vascular homeostasis. Endothelial senescence causes endothelial dysfunction to promote atherosclerotic change and contribute to age-related vascular diseases. Growth factors, such as fibroblast growth factor (FGF) or tumor necrosis factor (TNF)-α can be produced by additional cells involved in the pathogenesis of arteriosclerosis and play an important part in the progression of age-related vascular diseases.

Methods: We established an in vitro senescence model by prolonged culture of primary endothelial cells isolated from bovine aorta. The freshly isolated young endothelial cells gradually underwent senescence during 1 month of repetitive passages. We knocked down SIRT1 to evaluate the protein levels of LKB1, phosphorylated AMPK, FGF and TNF-α in the knocked down cells.

Results: It was observed that protein expressions of SIRT1 were decreased time dependently in the senescent endothelial cells. In contrast, the protein levels of LKB1, a serine/threonine kinase, the phosphorylation of its downstream target AMP-activated protein kinase (AMPK), FGF and TNF-α generation were dramatically increased in the senescent cells. On the other hand, resveratrol activated SIRT1 in the endothelial cells. SIRT1 activation with resveratrol inhibited the increase of LKB1, AMPK. At the same time, SIRT1 activation with resveratrol reduced FGF and TNF-α generation in the endothelial cells significantly. We knocked down SIRT1 and the protein levels of LKB1, phosphorylated AMPK, FGF and TNF-α elevated in the knocked down cells. The protein levels of LKB1, phosphorylated AMPK, FGF and TNF-α generation did not changed in the SIRT1 knocked down cells even if they were stimulated with resveratrol.

Conclusions: These findings indicate that activation of SIRT1 provides beneficial effects against the endothelial dysfunction to promote atherogenesis by inhibiting FGF and TNF-α generation.

Funding: Government Support - Non-U.S.

PUB572

Role of the Renal Nerves and Angiotensin II in a Model of Postmenopausal Hypertension Rodrigo Maranon, Chetan N. Patil, Carolina Dalmasso, Huimin Zhang, Jane F. Reckelhoff. *Physiology and Biophysics, Univ of Mississppi Medical Center, Jackson, MS.*

Background: Hypertension in postmenopausal women is not as well controlled in men regardless of ethnicity of the cohort. In our model of postmenopausal hypertension, the aging female spontaneously hypertensive rats (PMR), we found that blood pressure remains 110 mm Hg despite concomitant treatment antagonists of angiotensin AT1 receptors, endothelin ETA receptors and 20-HETE synthesis inhibitors. We have also shown that the sympathetic nervous system and the renal nerves contribute to the hypertension in PMR. In the present study, we determined whether renal denervation in combination with AT1 receptor antagonists would reduce BP below that found with triple therapy.

Methods: PMR (aged 18 mos, n=5-6/grp) underwent uninephrectomy, and two weeks later, unilateral renal denervation (RD) or sham (S) surgery and telemetry transmitter

implantation. After two weeks recovery, mean arterial pressure (MAP) and heart rate (HR) were recorded for 5 days. Then PMR were treated with losartan for 5 days, and kidneys were removed for measurement of norepinephrine (NE) content (D. Mattson, MCW).

Results: Renal NE content was higher in S-PMR than RD-PMR (S: 137.9±12.6 versus RD: 33.4±10.5). Renal denervation attenuated the hypertension in PMR by approximately 11% compared with shams (MAP: S: 188±6 versus RD: 167±6 mmHg; p<0.01). Losartan reduced MAP in both groups by similar percentages (9-11%) (S: 166±6 versus RD: 151±7 mmHg, p<0.001), but failed to normalize the BP.

Conclusions: These results suggest that while renal denervation and AT1 receptor antagonism attenuation the hypertension in PMR, other mechanisms, likely endothelin and 20-HETE also contribute to their hypertension. These data also suggest that multiple interventions including pharmacotherapy may be required to control BP in postmenopausal women. Supported by NIH R01HL66072, P01HL05971 and AHA 14POST18640015.

Funding: Other NIH Support - NIH RO1 HL66072

PUB573

New Histone Modification, H4 Lysine 20 Acetylation Is Associated with Gene Repression and Induced in Cardiac Hypertrophy Jun-Ya Kaimori,[1] Masaki Hatanaka,[3] Yoshitsugu Obi,[3] Satoko Yamamoto,[3] Shiro Takahara,[1] Hiromi Rakugi,[3] Yoshitaka Isaka.[3] *[1]Advanced Technology of Transplantation, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan; [2]Geriatrics and Nephrology, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.*

Background: Hypertension is known to cause cardiac hypertrophy, whose mechanistic insights were largely unknown. We focused uncharacterized histone modification, H4 lysine 20 acetylation (H4K20ac). The existence of H4K20ac in mammalian cell was confirmed by mass spec study with Hela-S3 cells. Making use of a feasible H4K20ac specific antibody, we performed ChIP-seq analysis using HeLa-S3 cells. Interestingly, H4K20ac was accumulated around the transcription start sites (TSSs) of low expressed and silent genes, in contrast to most histone acetylation that occur around TSSs of expressed genes, which is totally opposite to conventional view of histone acetylation as a transcriptional activator. A correlation analysis with known histone modification (H3K9me3, H3K27me3, H3K9ac, and H3K4me3) revealed that H4K20ac is a new class of suppressive mark. The motif search in H4K20ac-enriched sequence and transcription factor binding based on ENCODE ChIP-seq data revealed that most transcription activator is excluded from H4K20ac-enriched genes and only a transcription suppressor NRSF/REST was co-localized with H4K20ac. At the same time, the gene ontology study using H4K20ac-enriched genes reviled that H4K20ac is related to cardiac hypertrophy signaling pathways. Coincide with these data, H4K20ac was increased in angiotensin II stimulated rat smooth muscle cells and mouse transverse aortic constriction induced cardiac muscle cells. H4K20ac may regulate cardiac hypertrophy by repressing proliferation-associated genes. In conclusion, this new histone modification may be a key marker to change the gene expressions associated cardiac hypertrophy.

Funding: Government Support - Non-U.S.

PUB574

Differences of Antihypertensive Treatment in Older Adults Are Related to Gender Status and Kidney Function, the Berlin Initiative Study (BIS) Antonios Douros,[1] Elke Schaeffner,[2] Olga Jakob,[3] Reinhold Kreutz,[1] Natalie Ebert.[2] *[1]Clinical Pharmacology, Charité; [2]Nephrology, Charité; [3]Clinical Epidemiology, Charité.*

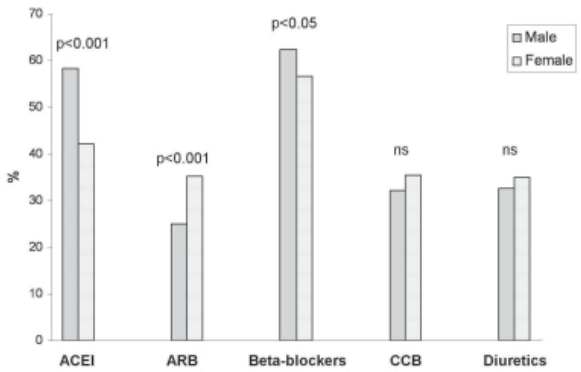
Background: Arterial hypertension (AHT) is a well-known risk factor for cardiovascular and renal diseases and has a high prevalence in older adults. Here, we examined the antihypertensive medication of elderly participants of BIS with regard to gender, cardiovascular risk factors, and kidney function (KF).

Methods: BIS is a population-based cohort study initiated in 2009 in order to evaluate KF in individuals ≥ 70 years. Medication was assessed through personal interviews. Treated AHT was defined as intake of antihypertensive medication. Cockcroft-Gault equation was used for creatinine clearance (eCrCl) estimation.

Results: Prevalence of treated AHT was 79% (1627 of 2069 participants). Table 1shows main characteristics of patients.

	Patients with treated AHT (n = 1627)
Age (y) - Mean (± SD)	81 (± 7)
Female sex, n (%)	851 (52)
eCrCl (ml/min/1.73m²) - Mean (± SD)	57 (± 18)
Diabetes mellitus, n (%)	478 (29)
Albuminuria, n (%)	470 (29)
Myocardial infarction, n (%)	272 (17)
Stroke, n (%)	159 (10)

Gender-specific intake of antihypertensive drugs is displayed in Figure 1.



Individuals with eCrCl < 60 ml/min/1.73m² received more often beta-blockers (BB) and diuretics than individuals with eCrCl ≥ 60, while use of angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB) or calcium channel blockers (CCB) was similar in both groups.

Conclusions: The different frequency of ACEI and ARB use between men and women cannot be interpreted based on current guidelines and may be associated with gender-specific differences in drug selection by physicians or tolerability. The increased intake of BB and diuretics among individuals with poorer KF may reflect the lower anticipated risk of adverse effects, e.g. hyperkalemia, of these agents in this cohort.

Funding: Private Foundation Support

PUB575

Side Effects and Adverse Events Associated with Intravenous Angiotensin II in Humans - A Systematic Review and Meta-Analysis Divya M. Chalikonda,[1] Laurence W. Busse,[2] David H. Yoo,[1] Lakhmir S. Chawla.[3] *[1]GWU; [2]Inova Fairfax Hospital; [3]Veterans Affairs Medical Center, DC.*

Background: Angiotensin II (ATII) is a naturally occurring hormone with important endocrine and hormonal effects. It has recently been evaluated in a prospective randomized controlled trial for the treatment of high output shock. ATII has been administered to patients with a variety of medical conditions, and has only been shown to exacerbate one underlying disease, asthma. To date, however, there has been no effort to systematically review the side effects and adverse effects associated with exogenous ATII administration.

Methods: We performed a comprehensive literature search of the administration of ATII to humans. Keywords included "intravenous administration, intravenous Injection, intravenous infusion, and angiotensin II." We identified all studies which reported physiologic effects associated with the administration of ATII, as well as associated doses. Side effects were defined as physiological effects that were unintended or incongruous with the known effects of ATII. Adverse events were defined as deaths or serious morbidities that were attributable to ATII.

Results: 1193 studies were screened. 137 studies (2653 participants) reported physiologic effects associated with ATII. The most commonly cited side effects and adverse events included altered renal function (23 studies),hyperaldosteronism and other disturbances of the renin-angiotensin-aldosterone axis (21 studies), other endocrine abnormalities (13 studies), and electrolyte disturbances (9 studies). The most serious side effect was bronchoconstriction, which was reported in 2 studies. The dose range for ATII across all studies was 3.56 ± 6.62 ng/kg/min to 12.16 ± 16.84 ng/kg/min. No deaths or serious adverse events were reported. Significant heterogeneity in study design prevented meta-analysis of results.

Conclusions: The incidence of side effects associated with ATII is uncommon, and side effects are generally mild, with exacerbation of asthma being the most serious. In aggregate, ATII appears to be safe in humans. Further studies of the therapeutic use of ATII at a dose range of 1ng/kg/min to 20ng/kg/min are warranted.

PUB576

Periodic Limb Movements of Sleep and Nocturnal Hypertension Ivan E. Porter,[1] William E. Haley,[1] Nabeel Aslam,[1] Bhupendra Rawal,[4] Vichaya Arunthari,[2] Paul A. Fredrickson.[3] *[1]Nephrology and Hypertension, Mayo Clinic, Jacksonville, FL; [2]Allergy and Pulmonary Medicine, Mayo Clinic, Jacksonville, FL; [3]Psychiatry, Mayo Clinic, Jacksonville, FL; [4]Health Sciences Research, Mayo Clinic, Jacksonville, FL.*

Background: Studies have demonstrated the association between obstructive sleep apnea and nocturnal hypertension (NH) where activation of the sympathetic nervous system in a repetitive sequence contributes to increased cardiovascular risk. Ambulatory blood pressure monitoring (ABPM) can detect the presence of NH, missed on office visit blood pressure monitoring. NH and a non-dipping pattern (<10%) increases the risk of adverse renal and cardiovascular outcomes. A growing body of evidence suggests that periodic limb movements of sleep (PLMS) influence vascular morbidity. The present study investigates the relationship between PLMS and NH.

Methods: A retrospective review from January 1, 2010 to December 31, 2012 of subjects at our institution with both ABPM and polysomnography performed within a six month timeframe was performed. This criterion was met by 190 individuals: 56% male and 91% white. Charts were reviewed for demographic data, results of 24 hour ABPM and polysomnography, concurrent medication use by class, comorbid conditions and serum/urine lab data. 8 patients were excluded due to incomplete measurements on ABPM.

Results: Patients with NH were more likely to be diabetic, have worse sleep efficiency and, as expected, have an elevated apnea-hypopnea index (AHI, number of events per hour of sleep). There was not an association of NH with limb movement index (LMI) (OR=1, p-value=0.88) though there was a trend with the total number of arousals per hour of sleep (p-value=0.06). Controlling for age, the LMI was associated with all type arousals (OR=1.07, p=0.019). Patients with a LMI>25 were, however, more likely to be older, have a fib and DM. Analysis of this data suggests a possible correlation between NH and all type arousals.

Conclusions: Elevated arousal indices were more prevalent in those with NH. This analysis does not confirm an association between NH and PLMS however; further investigation is warranted in light of evidence suggesting a relationship with vascular morbidity.

PUB577

Strikingly High Prevalence of Blunted Circadian Rhythm of Interdialytic Blood Pressure in Chinese Dialysis Patients Wenjin Liu, Junwei Yang. *Center for Kidney Disease, Second Affiliated Hospital, Nanjing Medical Univ, Nanjing, Jiangsu, China.*

Background: Management of hypertension is one of the fundamental interventions in dialysis patients. However, the profile of interdialytic blood pressure (BP) in Chinese dialysis patients remains elusive. We aim to investigate this issue as well as the effect of antihypertensive medication in this population.

Methods: 44-hour ambulatory blood pressure was monitored in ninety patients on maintenance hemodialysis. Patients were classified as "Dipping", "Non-Dipping" or "Reverse-Dipping" based on night/day ratio(N/D) of systolic blood pressure on nondialysis day.

Results: The prevalence of blunted circadian BP pattern was strikingly high (92.2%), with more than half of the patients (55.6%) were classified as reverse-dipping. There was a closely association between high erythropoietin dose used and deteriorated circadian rhythm. Patients in the Dipping group also displayed a dipping state for heart rate (HR) compared with the other two groups (N/D of HR: 81.5±6.6 versus 92.1±6.0 and 91.3±10.7, p=0.02). Only 26.7% patients have a controlled nocturnal BP. Patients with bedtime dosing had lower N/D of SBP compared with patients without (100.1±7.0 versus 105.2±7.1, p=0.01).

Conclusions: Nondipping and reverse-dipping are highly prevalent in Chinese patients. Erythropoietin use and autonomic dysfunction may contribute to the blunted circadian rhythm. More tightly control of night-time BP is an urgent need and bedtime dosing may be beneficial.

PUB578

Moderate Atherosclerotic Renal Artery Stenosis Is also Associated with Hypertension Arend Jan Woittiez,[1] Sicco Braak,[2] Myrthe Vestering,[2] Huib J. Van den Hout.[2] *[1]Internal Medicine, Hospital Group Twente, Almelo, Netherlands; [2]Radiology, Hospital group Twente, Almelo, Netherlands.*

Background: Recently it has been suggested to reconsider the role of so-called moderate (30-70% lumen reduction) atherosclerotic renal artery stenosis (ARAS) in the development of renovascular hypertension, given the negative results of angioplasty trials, using a cut-of point of 50-70% stenosis. This prompted us to undertake a study, comparing three degree of stenosis: mild (<30%), moderate (30-70%) and severe (>70%) ARAS and the incidence of hypertension.

Methods: We reviewed 418 consecutive CT-scans (Siemens Somatom 128 slice) of renal arteries, made for several clinical reasons. Degree of stenosis was calculated using the Siemens MPR system. Hypertension was defined as either an office blood pressure of >140/90, and/or the use of antihypertensive drugs. Clinical data for cardiovascular morbidity and renal function were collected.

Results: Clinical data of 22 patients were not available. On 50 CT-scans renal arteries were not sufficient visible, so 344 CT-scans remained for analysis. In the 208 patients with mild ARAS 68 %, in the 92 moderate stenosis 86%, and in 44 with severe stenosis 98% had hypertension (p<0.001, moderate versus mild stenosis). The median eGFR in the severe stenosis was 47 versus 65 ml/min in the moderate stenosis group (p=0.01); other clinical data were not different. The moderately and severely stenotic groups had more cardiovascular diseases (stroke, coronary heart disease and heart failure) than the group with mild stenosis (p<0.01).

Conclusions: In this study we found evidence that also moderate (30-70%) ARAS is associated with increased incidence of hypertension, whereas mild stenosis (<30%) shows a background-incidence of hypertension. Whether ARAS is a cause of renovascular hypertension, or a consequence of advanced cardiovascular disease in the moderate and severe groups could not be determined. Our data provide evidence to re-establish the threshold for so-called significant ARAS, and to consider also patients with moderate ARAS suitable for angioplasty.

PUB579

Five Year Review of Health Status - Health and Kidney Clinics for World Kidney Day Events in Liverpool, UK Sohan Shah,[1] Bhavna Pandya.[1,2] *[1]Faculty of Health and Life Sciences, Univ of Liverpool, Liverpool, United Kingdom; [2]Nephrology Dept, Aintree Univ Hospitals, Liverpool, United Kingdom.*

Background: World Kidney Day (WKD) is an annual kidney awareness event. Since 2009, we have held health and kidney awareness clinics for the public in Liverpool, UK to identify any risk factors and to determine their general health. the events were organised in the hospital for hospital staff and visitors, and in the shopping mall for the public.

Methods: Participants attending various World Kidney Day events over the last 5 years volunteered for various health tests: blood pressure (electronic blood pressure monitor), random capillary blood glucose (CBG) (blood glucose meter), random blood cholesterol (blood cholesterol meter), urinalysis (automated urinalysis machine) and manual heart rate assessment.

Results: There were a total of 903 records of visits from 2009-2013 with a F:M ratio of 3:1; of these, 43 returned to give data on numerous occasions. The median age was 54.2 (IQR:42.8-68.3). Hypertension (>140/90) was present in 372 (47.8%) patients with 4 cases of systolic BP >200 mmHg. The median systolic and diastolic BP was 137 (IQR:123-152) and 80 (IQR:73-89) mmHg respectively. Hypertesion was most prevalent (68%) in 70-79 year old patients. Median heart rate was 76 bpm (IQR:68-86). CBG was normal in 73% of patients whilst 8 individuals had glucose >10 mmol/L (median 5.2; IQR:4.6-5.9). Data from urinalysis showed that 18.5% had some degree of haematuria and 27% some degree of proteinuria. Only 1.5% of patients had severe haematuria (+++) and some form of proteinuria. There were 2.2% of patients with haematuria, proteinuria and leucocyturia. Fifteen patients showed positive (+) nitrate results suggestive of infection. Median cholesterol was 5.03 mmol/L (IQR:4.5-5.7) with a level >6.5 found in 8.7% of patients.

Conclusions: The above analysis shows that hypertension, urine abnormalities and hypercholesterolaemia are prevalent in the community. Regular events like this help to discover undiagnosed patients and increase confidence thus improving health check-up attendances. It also helps identify and prevent severe consequences of undiagnosed and prevalent risk factors in the community.

PUB580

Incorporating *APOL1* Genetic Testing in Primary Care: A Randomized Controlled Trial of the IGNITE Network (Implementing GeNomIc medicine in pracTicE) Girish N. Nadkarni,[1,2] Rennie Negron,[1] Kadija Ferryman,[3] Noura S. Abul-Husn,[2] Stephen B. Ellis,[2] Saskia Sanderson,[1] Ebony Madden,[4] Heather A. Junkins,[4] Emilia Bagiella,[1] Mayra Rodriguez,[1] Diane Hauser,[5] Neil S. Calman,[5] Carol Horowitz,[3] Erwin P. Bottinger.[1,2] *[1]Div of Nephrology, Icahn School of Medicine at Mount Sinai, New York, NY; [2]Charles Bronfman Institute of Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, NY; [3]Dept of Health Evidence and Policy, Icahn School of Medicine at Mount Sinai, New York, NY; [4]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; [5]The Institute for Family Health, New York, NY.*

Background: Two alleles(G1/G2) in the *APOL1* gene confer five-fold increased risk for development/progression of kidney disease in hypertensive, non-diabetic African Americans(AA's).Since 14% of AA's carry 2 *APOL1* risk alleles, hypertension management in AA's with high-risk *APOL1* genotypes is an important genomic medicine opportunity. This study is a randomized controlled trial incorporating *APOL1* risk information into primary care of hypertensive AA's.

Methods: We will recruit hypertensive,non-diabetic adults with no kidney disease and self-reported AA ancestry from 10 primary care practices and randomize them to *APOL1* testing at baseline(intervention) or 1 year(control). We will educate providers about genomic medicine/*APOL1* risk alleles through training sessions,electronic medical records-clinical decision support (CDS) and educational materials. Genetic counselor-guided trained staff will return results to patients with educational materials.During encounters, providers will receive point-of-care CDS to alert them of patients' *APOL1* risk status and provide recommendations.

Results: At 3 and 12 months, we will assess primary outcomes(blood pressure reduction to recommended goals and urine albumin/creatinine) and secondary psycho-behavioral outcomes, comparing 3 patient groups:increased genetic risk, no increased genetic risk, and genetic risk not yet assessed.

Conclusions: This study constitutes a novel approach to implement genomic medicine in practice and will help to establish the incorporation of *APOL1* risk status in the management of hypertensive AA's at high risk of kidney disease.

Funding: Other NIH Support - 1U01HG007278 NHGRI/NIH (PIs; E.P.B and C.H.); Genomic Medicine Pilot For Hypertension And Kidney Disease In Primary Care

PUB581

Renin Angiotensin Aldosterone System Antagonists as First Line Therapy in Malignant Hypertension Neetika Garg, Kambiz Zandi-Nejad. *Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.*

Background: Prognosis of malignant hypertension (MH) remains poor despite significant advances in hypertension (HTN) management. MH and scleroderma renal crisis (SRC) share many characteristics and are associated with massive overproduction of renin and aldosterone. Unlike SRC where angiotensin converting enzyme inhibitors (ACEI) are the mainstay of therapy and are associated with improved renal and patient survival, current guidelines do not recommend use of renin angiotensin aldosterone system (RAAS)

antagonists in MH. Furthermore, presence of acute kidney injury (AKI) may further deter physicians from using them. We examined current management practices regarding use of ACEIs/ angiotensin receptor blockers (ARBs) in MH.

Methods: Retrospective chart review of 453 patients from Harvard affiliated hospitals since 2000 identified 15 patients admitted for MH, defined as severe HTN (BP ≥ 180/120 mm Hg), AKI, thrombocytopenia and hemolytic anemia.

Results:

Age (Mean ± SD, years)	36.6 ± 11.4
Known diagnosis of Hypertension (n; %)	13; 86.6%
Known diagnosis of chronic kidney disease (n; %)	4; 26.6%
Admission serum creatinine (Mean ± SD, mg/dL)	6.7 ± 3.9
Admission serum potassium (Mean ± SD, mEq/L)	3.5 ± 0.6
Plasma renin activity (n=7) (Median (IQR), ng/mL/h)	10.0 (3.13-16)
Serum aldosterone (n=7) (Median (IQR), ng/dL)	21.9 (17.2-72)

Aldosterone levels > 15 ng/dL and aldosterone renin ratio < 20 suggesting renin dependent aldosterone hypersecretion was seen in 6 (85.7%) of 7 cases where these measurements were available. ACEIs/ ARBs were discontinued in 100% of the patients taking them prior to admission (4; 26.6%). Lisinopril was used for management of MH in only 1 patient. 5 (33.3%) patients were discharged on lisinopril, and 2 (13.3%) on spironolactone due to hypokalemia.

Conclusions: Progressive decline of renal function remains a major problem in patients with MH. RAAS overactivation provides a pathophysiologic basis for use of ACEIs/ ARBs as the mainstay of treatment; yet, they remain highly under-utilized. Early administration of these drugs, even in the presence of AKI, provides early and better blood pressure control, and potentially improved long term renal outcomes.

PUB582

Steroidogensis and the Role of Spironolactone in Hypertensive Young Male Adults: A Pilot Randomized Open Label Head to Head Study of Spironolactone versus Calcium Channel Blocker Byeong Yun Yang,[1] Jangwon Lee,[1] Ihm Soo Kwak,[1] Eun Young Seong,[1] Soo Bong Lee,[2] Dong Won Lee,[2] Il Young Kim,[2] Sang Heon Song.[1] *[1]Internal Medicine, Pusan National Univ Hospital, Busan, Seogu, Republic of Korea; [2]Internal Medicine, Pusan National Univ Yangsan Hospital, Yangsan, Gyeongsangnamdo, Republic of Korea.*

Background: In clinical practice, many hypertensive young male adults (HYMA) hardly reach a recommended level of blood pressure (BP) with commonly used anti-hypertensives. While spironolactone is used as an add-on agent to resistant hypertension, there are few reports describing its value as a beginner therapy in hypertension (HT). We designed a head to head study comparing the effect of spironolactone in HYMA with that of barnidipine, a frequently used calcium channel blocker (CCB) and checked the blood level of peptide and steroid hormones.

Methods: We included HYMA aged 19 to 40 years. They were randomly assigned into spironolactone group (SG; 50mg maximally) and barnidipine group (BG; 15mg maximally). To identify the effect in BP reduction, 24-hour ambulatory BP monitoring (24-ABPM) was done at the initiation and the completion (at 16th week) of the study. Before entering run-in, we measured hormone levels such as aldosterone, renin (plasma renin activity), cortisol, total testosterone, sex hormone binding globulin (SHBG), leptin, and insulin in hypertension group (HG: SG and BG) to compare with those in age and sex-matched healthy control group (HCG).

Results: Forty five patients were enrolled and 22 of them went through the randomization. The initial mean systolic BP (SBP) by 24-ABPM was not different between both groups. At the end of the study, the mean SBP in SG was slightly more reduced than that of BG (SBP reduction: 14.6 ± 10.8 (SG) versus 12.3 ± 13.8 mmHg (BG), p=0.09). In the analysis of hormones, leptin, insulin, and SHBG showed differences between HG and HCG. But only the level of SHBG was statistically significant (29.2±13.1 (HG) versus 58.8±30.2 nmol/L (HCG), p=0.008).

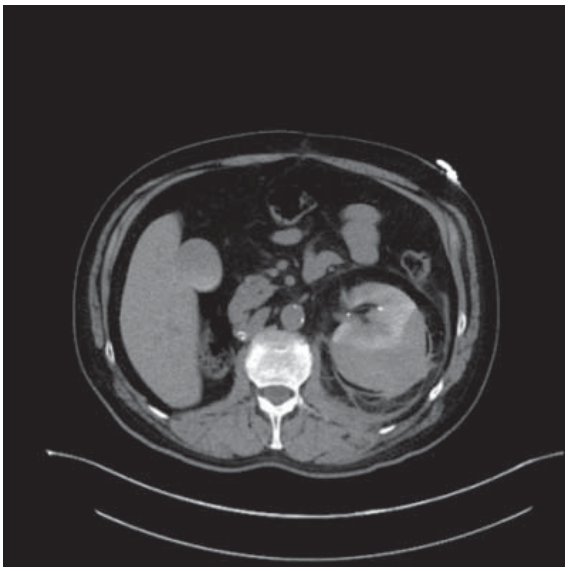
Conclusions: As a single agent, the short-term effect of spironolactone might not be inferior to that of barnidipine in HYMA. The role of steroidogenesis in HYMA should be more studied for specific treatment in this population.

PUB583

Page Kidney following Peripheral Intervention in a Patient with a Solitary Kidney Jordan Bohinc, Halle Elizabeth Field, Madhu Kandarpa. *Internal Medicine, Kettering Medical Center, Kettering, OH.*

Background: Page kidney is a rare form of secondary hypertension due to renal parenchyma compression, typically by a subcapsular hematoma. We present a patient with a solitary kidney and Page kidney physiology.

Methods: A 61 yo male presented with left flank pain after a peripheral vascular procedure. Past history includes renal cell carcinoma treated with total right nephrectomy 2 years prior. Blood pressure (BP) was 177/89mmHg, hemoglobin (Hb) was 10.4g/dL (baseline 13g/dL) and serum creatinine (SCr) 1.77mg/dL (baseline 1.1mg/dL). Non-contrast CT revealed a 3.5 x 8.1cm left renal subcapsular hematoma. Repeat Hb was 7.3g/dL and he was transfused. SCr remained elevated, however urine output was adequate. BP persisted despite Metoprolol and analgesia. Due to a solitary kidney and fear of renal injury from intervention, he was treated conservatively. Hb and SCr returned to baseline. Lisinopril was started with improvement in BP.



CT showing left renal hematoma.

Conclusions: The pathophysiology of Page kidney is likely from compression of renal parenchyma causing microvascular ischemia and activation of renin-angiotensin-aldosterone system (RAAS). Causes include trauma, complications of procedures, extracorporal shock wave lithotripsy or as an incidental finding. There are no guidelines for managing Page kidney. Treatment focuses on improving BP with RAAS blockade, preservation of renal function, and improving anemia. Intervention is indicated for hemodynamic instability, worsening renal function, and ongoing bleeding or pain. Options include nephrectomy/ capsulectomy, percutaneous drainage, or coil embolization. With a solitary kidney, nephrectomy was not desirable, renal insufficiency prevented starting ACE/ARB therapy and stopping anticoagulation ran the risk of in-stent thrombosis. Page kidney is rare but should be considered in patients with refractory hypertension.

PUB584

Flash Pulmonary Edema and Renal Failure in Previously Stented Renal Artery Stenosis/What Is the Best Approach? Syed S. Haqqie, Paul Bradley Brasher, Krishnakumar D. Hongalgi, Arif Asif. *Medicine, Albany Medical College, Albany, NY.*

Background: While major clinical trials have emphasized that medical management should be preferred over angioplasty and stenting for the treatment of RAS, clinical scenarios continue to raise doubts about the optimal management strategy.

Methods: Herein, we present three cases (age:89,85,65Y) that were admitted with flash pulmonary edema (blood pressure [BP]:180-220/100-120 mmHg) and renal dysfunction. All had undergone angioplasty and stent insertion for RAS in the past. Intravenous antihypertensive therapy reduced BP, however, renal deterioration necessitated dialysis in two patients. Both patients underwent angioplasty (for >75% in-stent stenosis) and stent insertion with successful resolution of stenosis. Postoperatively, BP gradually decreased with improvement in serum creatinine. Dialysis therapy was discontinued after a week of its initiation. The third patient became anuric with creatinine increasing to 8.2 mg/dl. Renal ultrasound revealed atrophic left kidney and normal right kidney with plump cortex. An angiogram revealed total occlusion of bilateral renal arteries. Right renal artery angioplasty was performed and flow to the kidney was successfully restored. This was followed by increased in urine out and a gradual decline in serum creatinine to 2.8 mg/dl at discharge and outpatient creatinine of 1.7 mg/dl. At a mean follow-up of 10.6 months, all patients continue be off hemodialysis and do well with BP readings in the 138-154/70-94 mmHg range (on three meds).

Results: Three patients underwent renal revascularization, 2 patients by angioplasty; 1 bypass surgery. BP became easier to control and all came off hemodialysis. Serum creatinine is currently between 1.4 and 1.7mg/dl respectively.

Conclusions: This investigation highlights the importance of percutaneous interventions not only in the management of blood pressure but also in restoring flow, ameliorating ischemic nephropathy and avoiding dialysis therapy in the context of previously treated RAS. The report also calls for increased awareness and appropriate strategies to follow patients who had previously undergone renal artery interventions.

PUB585

Cinacalcet Lowers Serum Magnesium Levels John Sy,[1] P.M. T. Pham,[2] Rohit H. Godbole,[1] P.T. T. Pham,[3] P.C. Pham.[4] *[1]Dept of Medicine, Olive View-UCLA Medical Center, Sylmar, CA; [2]Dept of Medicine, Greater Los Angeles Veterans Administration, Sepulveda, CA; [3]Kidney and Pancreas Transplant Program, David Geffen School of Medicine, UCLA, Los Angeles, CA; [4]Div of Nephrology and Hypertension, Olive View-UCLA Medical Center, Sylmar, CA.*

Background: The calcium sensing receptor agonist (CaSR) cinacalcet provides effective treatment for primary and secondary hyperparathyroidism and parathyroid carcinoma. Congenital gain-of-function mutations of CaSR have been reported to inhibit intraluminal K+ recyling at the renal outer medullary K+ channel in the thick ascending limb of Henle loop thus leading to reduced divalent cation (Ca^{2+}/Mg^{2+}) paracellular reabsorption and Bartter type V syndrome. We aimed to evaluate electrolyte changes, particularly serum magnesium, with cinacalcet use.

Methods: This is a retrospective study performed at Olive-View-UCLA Medical Center involving non-dialysis patients who were prescribed cinacalcet for any reason from April 2006 to December 2013. Patient baseline characteristics, medical history, medications, and laboratory findings including serum potassium, total CO_2, blood urea nitrogen, creatinine, calcium, magnesium, and phosphate were collected and analyzed for any changes following cinacalcet therapy.

Results: There were 5 non-dialysis all-female patients, mean age of 60.0+8.3 years old. A statistically significant decrease in serum calcium (10.79+0.3 to 10.05+0.55 mg/dL, p=0.026) and magnesium (1.92+0.23 to 1.74+0.25 mg/dL, p=0.005) were observed. No differences were noted for any other biochemical factors.

Conclusions: The current case series suggests that cinacalcet significantly reduces both serum calcium and magnesium. Prospective studies to confirm cinacalcet-induced hypomagnesemic effect are warranted.

PUB586

The Association of Demographics and Clinical Variables with iPTH Levels in Hemodialysis Patients: Based on Japanese Society for Dialysis Therapy Clinical Practice J. B. Chen. *Nephrology, Div of Nephrology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung Univ College of Medicine, Kaohsiung, Taiwan.*

Background: The purpose of study was to investigate the association of demographics and clinical variables in a cohort of hyperparathyroidism (HPT) hemodialysis (HD) patients.

Methods: A cohort of regular HD patients in one medical center were enrolled for study. The demographics and a longitudinal 5-year laboratory data including dialysis adequacy indices and cardiothoracic ratio were analyzed. A total of 567 patients were fulfilled the inclusion criteria. The mean of blood iPTH levels in five years was assigned a value for iPTH stratification. The optimal serum Ca, P and iPTH levels in HD patients based on Japanese Society for Dialysis Therapy clinical practice guideline 2012. The recommendations for serum Ca level: 8.4-10.0 mg/dL, P level: 3.5-6.0 mg/dL, iPTH level: 60-240 pg/ml.

Results: The patients were divided into three groups according to iPTH levels, group 1: 122 subjects, iPTH < 60 pg/ml, group 2: 186 subjects, iPTH 60-240 pg/ml, group 3: 259 subjects, iPTH > 240 pg/ml. There were significant comparison between three groups: HD duration (11.7 versus 8.5 versus 9.2 yrs), alkaline phosphatase (68.7 versus 85.0 versus 106.9 IU/L), BUN (73.8 versus 69.4 versus 71.7 mg/dL), Ca (9.1 versus 9.1 versus 9.6 mg/dL), P (4.9 versus 4.9 versus 5.3 mg/dL), CaxP (45 versus 45 versus 51). The ratio of patients' number with recommended Ca,P levels in guidelines in three groups were 91% (group 1), 81% (group 2), 62% (group 3) in Ca levels, 77% (group 1), 79% (group2), 76% (group 3) in P levels. Further analysis in HPT group (group 3), 7% subjects had Ca >10 and P > 6 mg/dL, 13% subjects had Ca 8.4-10.0 mg/dL and P > 6.0 mg/dL, 47% subjects had Ca 8.4-10.0 mg/dL and P 3.6-6.0 mg/dL, 1.93% subjects had Ca 8.4-10.0 mg/dL and P < 3.5 mg/dL.

Conclusions: There were heterogenous distribution of serum Ca and P levels in various iPTH levels. Although the serum Ca and P levels within recommended values, there were patients reaching a criteria of HPT in HD patients.

Funding: Private Foundation Support

PUB587

Phosphate Binders: Less Is More Kelly P. Moore, Mary M. Bridgeman, Eileen F. Duffy, Amay Parikh. *Pharmacy/Nephrology, Robert Wood Johnson Univ Hospital, New Brunswick, NJ.*

Background: Along with dietary restriction, phosphate binders are used to inhibit the intestinal absorption of phosphate for patients with hyperphosphatemia and CKD stages 3, 4, and 5 targeting serum phosphate levels 2.5-4.5 mg/dL. Clinical guidelines recommend the addition of other phosphate-lowering agents to help achieve phosphate goals. Dual treatment with phosphate lowering agents is often employed without any known benefit in phosphate control within the inpatient setting.

Methods: 549 patient charts were reviewed in a retrospective study design. Patients were selected between January 1, 2012 and December 31, 2012 with CKD stages 3, 4, 5 who have hyperphosphatemia and have been treated with a single calcium-based or non-calcium-based phosphate binder or a combination of two or more phosphate binding agents during the inpatient hospitalization. 165 patients met inclusion criteria. Patients were determined to be admitted with either one phosphate binder prescribed (SING) or multiple phosphate binders (MULT). The associations between potential confounding factors, such as serum calcium levels, parathyroid hormone levels, use of vitamin D analog, and use of cinacalcet were analysed. Primary outcomes were 1) the number of patients with a phosphate level less than 4 mg/dL and 2) the change in phosphate levels from admission to discharge. Patients were followed through the course of the hospitalization.

Results: 133 patients patients were placed on a single phosphate binder; 32 patients were on combination phosphate binders. No observable differences were noted in patient's baseline characteristics. No differences in achieving phosphate levels less than 4 mg/dl were noted between SING and MULT (p=NS). No difference in the change in phosphate levels from admission to discharge between SING and MULT (p=NS). Significantly higher proportion of MULT patients also were placed on cinacalcet (p=0.007).

Conclusions: When single phosphate binders do not adequately control phosphate levels, other modalities should be considered instead of adding another binder. Dual treatment is often utilized resulting in therapeutic duplication without additional benefit in phosphate control within the inpatient setting.

PUB588

Phosphorus Social Networks Depend on the Dialysis Shift: A Validation Study Avrum Gillespie,[1] Athanasia Polychronopoulou,[2] David Conway,[3] Klemens B. Meyer,[4] Teri Browne,[5] Zoran Obradovic.[2] *[1]Temple Univ School of Medicine; [2]Bioinformatics, Temple Univ; [3]DCI Philadelphia; [4]Tufts Univ School of Medicine; [5]Univ of South Carolina College of Social Work.*

Background: Health behaviors have been shown to diffuse across social networks in chronic disease. In a previous study we modeled inferred social networks within an urban hemodialysis clinic, using simultaneous changes in serum phosphorus as marker of adherence, and found differences in densities among shifts. In this current study, we validate our methodology in another urban clinic and compare the densities of networks between shifts.

Methods: Retrospective data were obtained from 121 active patients at DCI Philadelphia between July 2009 and March 2014. The clinic runs 3 shifts daily. Phosphorus values that were different from the patient's mean and variance were considered significant changes in the patient's phosphorus. Simultaneous significant changes (p<0.0001) in serum phosphorus among patients were considered to be a phosphorus social network link. The number of links per shift was used to calculate the network density. Computer generated random network modeling was used to validate the discovered social links.

Results: The average patient serum phosphorus was 5.5mg/dl and the variance was 1.7 mg/dl. Patients demonstrated on average of 5.7 links (1-14), only 3 patients (2.5%) showed no links. All six of the shifts demonstrated phosphorus social networks with significant link densities when compared to the average density (0.16) of the randomly generated network, 0.16. The highest network density were on the second shifts Monday, Wednesday, Friday (MWF) 0.37 (p <0.0001) and Tuesday, Thursday, Saturday (TTS) 0.31 (p <0.0001). The lowest density was on the newest shift TTS 3rd shift 0.1944.

Conclusions: Once again we were able to demonstrate serum phosphorus networks among patients on the same shift and validate our methodology. Furthermore, as shown in our previous study, the midday shifts have a stronger network than the early shifts. Further research is needed to understand how to positively affect patient interactions and phosphorus social networks to improve adherence and outcomes.

Funding: Private Foundation Support

PUB589

Effect of Cholecalciferol on Concentrations of Oxidized PTH in a One-Year Randomized Study in Hemodialysis Patients Pierre Delanaye, Laurent E. Weekers, Xavier Warling, Martial Moonen, Nicole Simone Smelten, Jean-Marie H. Krzesinski, Etienne Cavalier. *Univ of Liège.*

Background: Native vitamin D is recommended in hemodialysis by the K-DIGO. In a one-year randomized study, we showed that cholecalciferol could have a benefit effect on parathormone (PTH) concentrations. Indeed, PTH variations were significantly different in the placebo and treated groups (cholecalciferol, 25.000U every two week). However, we did not observe any significant difference between the two groups at 1-year. Non-oxidized PTH (noxPTH) is the biological active form of PTH. We tested if the conclusions of our study were the same if noxPTH was considered.

Methods: We retrospectively analyzed the samples of the randomized study. The results of 30 patients who completed the study at 1-year were considered. PTH concentrations were measured at baseline and at 1-year with a intact assay (iPTH) (Roche, Mannheim, Germany). The same samples were also measured with the same assay but after treatment with anti-oxidized antibodies that separate the oxidized, inactive form from the non oxidized (noxPTH), active PTH (Immundiagnostik, Bensheim, Germany).

Results: In the placebo group (n=14), the median concentration of iPTH was not different from the treated group at baseline: 240 [195;410] and 285 [185;462] pg/mL, respectively. Similarly, the concentrations of noxPTH did not differ: 27 [18;37] and 30 [20;35] pg/mL. In the placebo group, iPTH increased of 80 [-21;162] pg/mL over the one-year period. In the treated group, the iPTH decreased of -73 [-230;43] pg/mL. The difference between these variations was significant (p=0.0261). The variations of noxPTH results were however not significant : variation of 4 [-1 ;8] and -7 [-15;2] pg/mL (p=0,088) for the placebo and active group, respectively. At one-year, PTH concentrations were not different in the placebo or the treated group. This conclusion was similar considering either iPTH (367[122;542] and 221[154;322], respectively) or noxPTH (32[19;41] and 25[15;44] pg/mL, respectively).

Conclusions: In our study, the measurement of noxPTH has no adding value to describe the effect of cholecalciferol on PTH changes over 1-year. These results must be confirmed in a larger sample.

PUB590

Osteoporosis in Dialysis Patients – Results of Pilot Cross-Sectional Study
Ivan Rychlik,[1,3] Jana Veresova,[1] Petra Ronova,[1] Petra Beranova,[1] Ludmila Brunerova.[2,3] *[1]Dialysis Centre Vinohrady, Fresenius Medical Care, Prague, Czech Republic; [2]Bone Metabolism Unit, Mediscan Euromedic, Prague, Czech Republic; [3]3rd Faculty Med., Charles Univ., Prague, Czech Republic.*

Background: Metabolic bone disease (CKD-MBD) is frequent entity in patients (pts) with advanced CKD, particularly as a result of secondary hyperparathyroidism (SHPT). However, only a little attention is paid to other types of bone disease. The aim of our pilot study was to assess the occurrence and severity of osteoporosis among pts on maintenance dialysis treatment in one dialysis centre.

Methods: 26 pts, mean age 66.7±13.4 years, 73% men, 37% diabetics, mean dialysis vintage 41±15 months, treated with high-volume online hemodiafiltration were enrolled in the study. All pts underwent densitometry (Lunar Prodigy) and laboratory examination of selected bone biomarkers (cross laps, PINP /collagen-1 propeptide/, 25-OH vitamin D, serum calcium and phosphate, and PTH).

Results: Normal bone mineral densitometry (BMD) was found in 5 pts, whereas the decrease of BMD to osteoporotic range (T-score ≤-2.5) was observed in 10 pts (38.5%; mean age 66.7±11.0 years; 60% men). Remaining pts (11) were proofed to have osteopenia /decreased BMD (in men). Among pts with T-score ≤-2.5, high bone turnover (average cross laps 2.9±0.2 ug/l) was observed in 40% out of them, however, severe SHPT was observed only in 1 patient (PTH 1064 ng/l), whereas PTH levels in the others were within recommended range for dialysis pts with the average 299 ±15 ng/l. "Adynamic bone" with markedly decreased bone turnover did not occur in any pts. Only 2 pts were deficient in vitamin D (less than 50 nmol/l, average 40 nmol/l), however, the rest of the pts were either insufficient in vitamin D of normal. All the pts were treated with active analogue of vitamin D. After exclusion of other types of bone disease 9/10 patients with low T-score were diagnosed suffering from osteoporosis although not bioptically proven.

Conclusions: We showed surprisingly high prevalence of osteoporosis mostly in men with ESRD treated with hemodiafiltration. Osteoporosis seems to be an underestimated bone pathology beside CKD-MBD in ESRD. Thus, densitometry and further specific therapy of those pts should be considered.

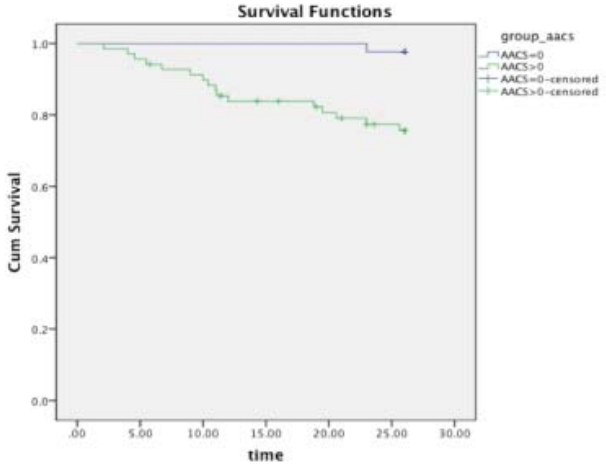
PUB591

The Association Between Aortic Artery Calcification and Cardiovascular Events in Chinese Maintenance Hemodialysis Patients
Xiaonong Chen,[1] Zijin Chen,[1] Xiaobo Ma,[1] Liang Hou,[2] Huawei Ling,[2] Hong Ren,[1] Nan Chen.[1] *[1]Nephrology, Ruijin Hospital,Shanghai Jiao Tong Univ, School of Medicine, SHANGHAI, China; [2]Radiology, Ruijin Hospital,Shanghai Jiao Tong Univ, School of Medicine, Shanghai, China.*

Background: To investigate the relationship between aortic artery calcification(AAC) and mortality in Chinese maintenance hemodialysis(MHD) patients.

Methods: 133 MHD patients from Ruijin Hospital were enrolled in this study in July, 2011. All patients clinical characteristics, baseline biochemical parameters and cardiovascular and death events were collected. AAC was detected by lateral lumbar X-ray plain and evaluated by aortic artery calcification score(AACS).

Results: Among 113 MHD patients, 67 were male and 46 were female. The mean age of MHD patients was 54.9±15.0 years old, the mean dialysis duration 40.6±38.2 months, the mean FGF23 level 27691.42±55646.41 RU/ml. The mean follow-up time was 23.2±6.1 months. 69 MHD patients (61.1%) had AAC from lateral lumbar X-ray, and the mean AACS was 4.1±5.3. During the follow-up, total 16 MHD patients had adverse events, which 9 patients with cardiovascular events(including 2 cases of cerebral hemorrhage, 3 cases of acute myocardial infarction, 2 cases of heart failure and 1 case of lower extremity deep venous thrombosis). Logistic regression analysis showed that plasma albumin and FGF23 were independent risk factor of AAC(Background Logistic, R^2=0.71,P<0.001). Kaplan-Meier survival curves suggested that all-cause and cardiovascular mortality of MHD patients with AAC was significantly higher than those MHD patients without AAC(Log Rank,P<0.05). Cox regression analysis showed that dialysis duration and AAC were independent risk factor of cardiovascular death in MHD patients.



Conclusions: The incidence of cardiovascular event in MHD patients was high. AAC is associated with cardiovascular and all-cause mortality in Chinese MHD patients.

PUB592

Bone Mineral Disorders in Patients on Dialysis and Its Relationship to Death
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Background: Several studies have shown that chronic kidney disease-mineral bone disorder (CKD-MBD) is closely associated with cardiovascular events, hospitalization and death.

Methods: We conducted a prospective study for CKD-MBD in ESRD patients in a dialysis single-center in order to measure their impact on mortality. We included 70 patients, over 18 years old, both incident and prevalent at November 1, 2009. We studied biochemical markers and measured vascular calcifications with X-rays calculating the Adragao and Kaupilla scores.

Results: The average age was 58.0±14.9 years old, 62.0% male. The main causes of CKD were diabetes and nephroangioesclerosis (28.2 and 23.5% respectively). 81.7% were on hemodialysis, with an average time from onset therapy of 41.6 months. 54.7% had secondary hyperparathyroidism, with an average iPTH level of 536±481 pg/mL. The serum calcium and phosphorus were 8.4±0.8 mg/dL and 5.2±.8 mg/dL respectively. FGF23 values showed wide variations with a mean of 166±196 pg/dL, whereas fetuin A show lower values, of 0.5±0.3 mg/dL. The Adragao and Kaupilla scores were 3.4 and 8.1 respectively. No association between vascular calcification and FGF23 or Fetuin A were found. The follow-up time was 36 months. 27.5% of patients were transplanted, 34.8% continuing on dialysis and 37.7% died. The main cause of death was cardiovascular (42.3%). Those who died showed a higher phosphatemia and iPTH than those who stayed alive (6.1±2.2 versus 5.2±1.6 mg/dL, p=0.05 and 808.7±662.6 versus 481.1±426.2 pg/dL, p=0.06, respectively). Patients who died showed lower values of FGF23 (60.8±86.9 versus 203.6±210.7 pg/dL, P=0.05) and a greater tendency to vascular calcifications quantified by Adragao score (4.54±2.96 versus 2.94±3.19, p=0.14). In multivariate analysis, the increase of one unit of the calcium-phosphorus product resulted in an increase of 3% on risk of death with statistical significance (p=0.02).

Conclusions: Our data shows a high prevalence of secondary hyperparathyroidism, with an association between serum phosphorus, PTH levels and calcium-phosphorus product and death. The deceased patient showed a tendency for lower levels of FGF23 and major vascular calcifications.

Funding: Clinical Revenue Support

PUB593

Interaction of PAT1 and Dicarboxylate (Citrate) Transport in Proximal Tubule Cells
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Background: Recently the Cl^-/ oxalate exchanger PAT1 (SLC26A6) has been shown to inhibit the major kidney dicarboxylate and citrate transporter NaDC1, while NaDC1 was shown to stimulate PAT1. (Ohana 2013) This mutual regulation was demonstrated in heterologous expression models and in animals with knockout of PAT1. The mutual regulation of these 2 apical transporters may have significant implications for calcium stone formation.

Methods: To further characterize and to determine whether this mutual regulation could be exploited to modify citrate transport, we used established cell lines that constitutively express citrate transport.

Results: In each of the cell lines studied, we found that PAT1 mRNA and protein were expressed. We tested the effect of DIDS, which has been shown to inhibit PAT1, hypothesizing that citrate transport would be stimulated. Addition of 100 μM DIDS inhibited ^{14}C-Citrate transport in OK cells (43±5 % inhibition), and immortalized mouse S1 and S2 cells (39±4 and 28±9 % inhibition, respectively). However, DIDS had no effect on HRPE cells with overexpressed NaDC1. We also tested succinate, the usual model substrate for NaDC1, a dicarboxylate transporter; DIDS had similar inhibition of succinate transport as with citrate. In OK cells, and in other proximal tubule cells, we find calcium-sensitive citrate and succinate transport which we have hypothesized represents a novel apical process, not NaDC1 transport. Interestingly, DIDS did lessen or eliminate the calcium-sensitivity. Oxalate, which should stimulate PAT1 transport, was hypothesized to inhibit NaDC1. With 100 μM oxalate, no significant inhibition of citrate or succinate transport occurred in OK, S1, or S2 cells, and in fact citrate uptake was stimulated in S1 and S2 cells.

Conclusions: In sum, DIDS and oxalate did not alter citrate transport in the direction anticipated based on the reported mutual interaction/regulation of PAT1 and NaDC1. This interaction needs to be further explored to understand the full nature and possible utility for manipulation of citrate excretion to prevent calcium stones.

Funding: NIDDK Support

PUB594

Predictors of Urinary Supersaturation of Calcium Phosphate in Stone Formers Jason M. Scovell,[1] Adam B. Hollander,[1] Jatinder K. Hothi,[2] Wesley Mayer,[3] Charles G. Minard,[4] Richard E. Link,[1] Sreedhar A. Mandayam.[2] *[1]Urology, Baylor College of Medicine, Houston, TX; [2]Nephrology, Baylor College of Medicine, Houston, TX; [3]Urology, Houston Methodist Hospital, Houston, TX; [4]Statistics, Baylor College of Medicine, Houston, TX.*

Background: 24-hour urine collection is a cornerstone of the metabolic evaluation of stone formers. There is an incomplete understanding of how urine components associate with supersaturation of calcium phosphate (SSCaP) and calcium phosphate stones.

Methods: To determine the relationship between saturation of calcium phosphate and the urine metabolic profile in recurrent stone formers we performed a retrospective analysis of our 24-hour urine database of stone formers ≥ 18 years of age. We included only the initial valid metabolic stone work-up. Data was normalized by log transformation. Associations between SSCaP and urine parameters were evaluated using univariable and multivariable linear regression. Significant covariates (0.05) on univariable analysis or clinically significant covariates were included in final multivariable analysis.

Results: 363 metabolic stone workups (male n=186, female n=178) were included in analysis. Multivariable analysis found a positive association between supersaturation of calcium phosphate and calcium, pH, phosphorus and a negative relationship to 24-hour volume, ammonium, citrate, and age. There was no correlation between SSCaP and urine oxalate, sodium, weight, or BMI (p>0.05).

Variable	β	P
logCa	0.40	<.0001
logVol	-0.65	<.0001
pH	0.54	<.0001
logP	0.40	<.0001
logNh4	-0.15	<.0001
logCit	-0.08	0.03
Age	-0.002	0.007

Conclusions: Contrary to the usual wisdom that calcium phosphate supersaturation and calcium phosphate stones occurs in obese middle aged women with impaired glucose tolerance, we found no association between SSCaP and BMI or gender. Not surprisingly there is a significant correlation between urine calcium, phosphorus, volume, alkaline pH and SSCaP. Interestingly we found a negative correlation with urine ammonium, urine citrate which could be due to the co-existence of distal renal tubular acidosis in these stone formers. Minor changes in urine volume have dramatic impact on urine SSCaP.

PUB595

Predictors of Urinary pH in Stone Formers Jason M. Scovell,[1] Adam B. Hollander,[1] Jatinder K. Hothi,[2] Wesley Mayer,[3] Charles G. Minard,[4] Richard E. Link,[1] Sreedhar A. Mandayam.[2] *[1]Urology, Baylor College of Medicine, Houston, TX; [2]Nephrology, Baylor College of Medicine, Houston, TX; [3]Urology, Houston Methodist Hospital, Houston, TX; [4]Statistics, Baylor College of Medicine, Houston, TX.*

Background: 24-hour urine collection is a cornerstone of the metabolic evaluation of stone formers. There is an incomplete understanding of how many urine components associate with urinary pH in stone forming patients.

Methods: To determine the relationship between urine pH and the urine metabolic profile in recurrent stone formers we performed a retrospective analysis of our metabolic stone database of stone formers ≥ 18 years of age. We included only the initial valid metabolic stone work-up. Data was normalized by log transformation. Associations between pH and urine parameters were evaluated using univariable and multivariable linear regression. Significant covariates (p<0.05) on univariable analysis or clinically significant covariates were included in final multivariable analysis.

Results: 363 metabolic stone workups (51% male) were included in the analysis. Multivariable analysis found a positive relationship between urine pH and volume, uric acid, sodium,calcium, and oxalate. pH was negatively associated with age, weight, phosphorus, and ammonium. There was no statistically significant association between urinary pH and protein catabolic rate or sulfur (p≥0.16).

Variable	β	P
logVol	0.35	<.0001
logUA	0.24	0.028
logNa	0.18	0.008
logCa	0.14	0.002
logOx	0.18	0.008
Age	-0.005	0.017
Weight	-0.56	0.004
logP	-0.46	<0.001
logNh4	-0.14	0.035

Conclusions: The urinary pH increases with increasing volume, UA, Na, Ca, and Ox, and decreases with increasing age, weight, phosphorus and ammonium. We found a trend towards a higher pH as volume increased. Surprisingly, there was a trend towards basic pH with increasing uric acid, and an acidic pH with increasing ammonium. It is possible that these constituents are markers of other biological processes that affect urinary pH rather than major drivers themselves. Clinically, this could suggest that in treating urate nephrolithiasis; it may be more clinically relevant to increase pH with alkalinizing agents or to increase urinary volume.

PUB596

Evidence of Early Atherosclerosis in Pediatric Nephrolithiasis Patients Kirsten A. Kusumi, Evan Barr-Beare, Vijay Saxena, Sally Smith, Andrew L. Schwaderer. *Nationwide Children's Hospital, Columbus, OH.*

Background: Kidney stones are a common affliction for adults, and pediatric nephrolithiasis is increasing with approximate incidence of 3% annually. Adults with nephrolithiasis have 60% increased risk for carotid atherosclerosis. The objectives of this study: 1) determine if nephrolithiasis associated atherosclerosis has pediatric origins 2) determine if kidney stones induce expression of genes responsible for atherosclerosis.

Methods: Case control study of 9 controls and 5 experimental patients aged 12-17 years. Experimentals were diagnosed by ultrasound or computed topography within the last two years. Carotid ultrasounds were done with Phillips iU22 Ultrasound (Philips Healthcare, Andover, MA) and measurements of carotid intimal thickness (cIMT) were made with Q lab software. Kidney stones were induced in mice by intraperitoneal oxalate. Normal saline was used for controls. Renal tissue mRNA expression was evaluated with the mouse Atherosclerosis RT² *Profiler*™ PCR Array (Qiagen) which profiles 84 genes related to atherosclerosis. Statistical analysis of the cIMT data was evaluated by paired t test. PCR data was evaluated by RT2 Profiler PCR Array Data Analysis.

Results: Left carotid bulb measurements' mean 0.43±0.029mm in controls and 0.48±0.25mm in experimental group (95%CI -0.04-0.13); right carotid bulb 0.44±0.02mm in controls and 0.45±0.03mm in experimental (95% CI -0.07 -0.03). Neither side showed significance; there is a trend consistent with our hypothesis. Per our initial sample size calculations a total of 20 participants are needed to reach significance. The mouse atherosclerosis arrays revealed 19 atherosclerosis genes unregulated by greater than 4 fold in experimental versus controls (p< 0.01). These genes included secreted phosphoprotein 1, tumor necrosis factor and vascular cell adhesion protein 1.

Conclusions: Evidence of early signs of atherosclerotic disease is present in pediatric nephrolithiasis patients. Kidney stones induce expression of factors associated with atherosclerosis in an animal model. Expansion of the cIMT samples size and evaluation for the presence of the of up regulated atherosclerosis factors in pediatric nephrolithiasis patients is ongoing.

PUB597

Optimal Dietary Recommendation in Calcium Oxalate Stone Formers with Idiopathic Hyperoxaluria Maryam Taheri,[1] Elani Streja,[1] Fatemeh Taheri,[2] Kamyar Kalantar-Zadeh.[1] *[1]Harold Simmons UC Irvine MC, Orange, CA; [2]Tarbiat Modares Univ, Tehran, Iran.*

Background: Calcium oxalate stones (COS) typically occur in healthy people whose risk factors are attributed to diet and life styles. There are currently no established dietary guidelines to decrease hyperoxaluria in calcium oxalate stone formers (CSFs). We sought to synthesize results from major randomized controlled trials (RCTs) of dietary interventions in CSFs.

Methods: We searched MEDLINE using the terms "low oxalate diet" and "diet and hyperoxaluria" up to April 2014 and included all RCTs investigating dietary interventions in CSFs and those with idiopathic hyperoxaluria with at least one 24-hr urinalysis.

Results: We examined dietary interventions in 13 identified RCTs with 1637 participants examining specific metabolic interventions. In 4 RCTs, dietary recommendations were made according to urine metabolic abnormalities and main nutrients included high fluid intake (urine output>2L/day), low oxalate (80-100 mg/day), low salt (<2400 mg/day or<140 mEq/day), adequate calcium (1000-1200 mg/day), low protein intake (15% of energy) and low fat (≤25% of energy), resulting in a significant decrease in urinary oxalate or calcium oxalate supersaturation index. Another study examining protein, fluid, sodium, calcium, and oxalate also showed in a significant decrease in urinary oxalate. 5 trials assessing the relation between oxalate absorption and calcium availability in the intestine showed dietary calcium to be an important component in oxalate absorption and urine excretion. In another study, recurrent CSFs with mild hyperoxaluria were found to have higher fasting urinary oxalate and an exaggerated urinary response to an oral oxalate load. In the last study we examined, dietary oxalate restriction did not yield substantial benefit in all of the hyperoxaluric patients.

Conclusions: The most common dietary intervention in CSFs in RCTs is low dietary oxalate, low salt and normal calcium intake without intervention on other main nutrients of food content which largely impact urinary metabolic abnormalities. Further studies are needed to determine if a low oxalate diet versus controlled metabolic diet or balanced diet more effectively improve outcomes in CSFs.

Funding: NIDDK Support

PUB598

Serum Calcium Is a Leading Determinant of Fibroblast Growth Factor-23 Levels in Young Dialysis Patients Michael Freundlich,[1] Marta A. Azocar,[2] Iris Delgado,[2] Maria F. Ugarte,[2] Francisco Cano.[2] *[1]Pediatric Nephrology, Univ of Miami School of Medicine, Miami, FL; [2]Pediatric Nephrology/Calvo Mackenna Children's Hospital, Univ of Chile Faculty of Medicine, Santiago, Chile.*

Background: Elevated FGF23 levels, a known cardiovascular risk factor, are further increased following calcitriol (Ct) treatment. In animals, FGF23 levels are mainly determined by serum calcium (SCa) but in dialysis patients (Dp) data are conflicting. We hypothesized that SCa may be an important determinant of FGF23 levels in Dp and that low-dose Ct may prevent marked elevations of FGF23.

Methods: In 31 (age 8.3±4.6 yr) peritoneal Dp, intact FGF23 (Immutopics), Klotho (Ko), SCa and other analytes were measured at various timepoints throughout 12 months. Correlation and logistic regressions analyzed associations of multiple variables with FGF23 levels.

Results: All Dp required Ca-carbonate and n=21 received Ct (0.05±0.07 mcg/kg/week). Baseline FGF23 and Ko levels (215±304 pg/ml, >20-fold normal; 132±58 pg/ml, 40%<normal, respectively) remained stable without significant changes after 1 year (195±301; 133±29 pg/ml, respectively). Baseline and final SCa (9.9±1.1; 9.4±0.9 mg/dl), P (5.4±1.2; 5.3±1.3 mg/dl), PTH (331±273; 321±205 pg/ml), 25-OHD (34±6.8; 25±6 ng/ml) and 1,25$(OH)_2$D (27±22; 28±21 pg/ml) were similar. Daily Ca and P intake, normal at baseline, remained >70%normal by month 12. logFGF23 correlated only with SCa and the CaxP product at months 1, 6 and 12 (all p<0.05), and not with P or any other analyte. By multiple regression analysis, SCa emerged as the strongest variable determining FGF23 levels at months 1, 6 and 12. Potential interactions with P revealed robust positive associations of SCa with logFGF23 (p<0.02 at all 3 timepoints) when P was < 6mg/dl but weaker associations when P>6 mg/dl (p=NS at same 3 timepoints).

Conclusions: We demonstrate that in Dp: (1) SCa was the strongest determinant of FGF23 levels, particularly when P remains <6mg/dl, (2) low-dose Ct helps prevent further FGF23 elevations, and (3) calcitriol treatment is associated with stable circulating Ko levels. The impact of these findings on improved clinical outcomes requires further studies.

Funding: Clinical Revenue Support

PUB599

Parathyroid Hormone Response to Cholecalciferol Treatment in Hemodialysis Patients Laura Sola, Susana B. Gonzalez, Juan Carlos Diaz, Maria C. Sehabiague. *HD Center, CASMU, Montevideo, Uruguay.*

Background: Vitamin D deficiency is frequent in hemodialysis (HD) patients (Pts). Its treatment has not shown consistently a reduction in PTH levels. We aimed to determine if cholecalciferol supplementation (Chol Sup) in HD Pts allows obtaining normal serum 25-hydroxyvitamin D (25VD) status and reduce serum Parathyroid Hormone (PTH).

Methods: Cohort study of Pts on HD for ≥ 3 months and 25VD < 30 ng/mL. Before and after treatment calcium (Ca) phosphorus (P), 25VD, PTH, alkaline phosphatase (AP) Hb, Ferritin, C Reactive Protein (CRP), Erythropoietin (epo) dose and resistance index (epo IU/wk/kg/Hb) were recorded. PTH >300 risk was assessed by logistic regression. 25VD severe deficiency (25VD-SD) defined as <10ng/ml. Pts were given Chol Sup for 8 weeks (15000 IU/wk). Protocol aproved by Ethics Committee. Pts signed inform consent. Response was analyzed according basal PTH <150 or ≥ 150 ng/ml. Data were compared by t test or chi square as needed. Was considered significant p <0.05.

Results: Were 91 Pts, 55 men (60.4%), 69.5 ± 13.2 years, 31 (34.1%) diabetics, on dialysis for 35.1 ± 40.9 months. PTH>300 risk increased significantly with 25VD-SD (OR 5.71, 1.28-25.50) adjusted to gender, body mass index (BMI), diabetes, AP and CRP. After Chol Sup, 25VD increased (12.2 ± 5.9 to 31.7 ± 10.7 ng/ml) and 48 (52.7%) normalized. PTH significantly decreased (470±356 to 426±351ng/ml) with 20% reduction in 38.5%. Ca increased (8.88 ± 0.61 to 9.45 ± 0.79 mg/dL), with no differences in P, epo/Hb index or CRP. Pts with PTH<150 had lower BMI (23.6 versus 27.0), P (4.32 versus 5.36) and higher CRP (17.5 versus 8.4), ferritin (709 versus 477), epo/Hb index (12.1 versus 5.8). In Pts with PTH<150, PTH significantly increased (87±36 to 124±62) as Ca and 25VD, with no difference in P. In Pts with PTH≥150, 25VD (12.1 ±5.0 to 31.7 ± 10.7) and Ca (8.88 ± 0.6 to 9.45±0.8) increased, and PTH significantly decreased (470± 356 to 426± 351 ng/ml) with no change on P, Hb or epo/Hb index.

Conclusions: Chol Sup for a short period of time significantly increase 25VD and frequently normalize 25VD levels. Its effect differ according PTH level and benefits both low and high basal PTH levels, with a significant decrease only in the latter. Ca levels needs to be followed-up.

Funding: Pharmaceutical Company Support - Celsius

PUB600

Determinants of [FGF23] in Stages 3-4 CKD Kenneth R. Phelps,[1] Darius Mason,[2] Kim Stote.[3] *[1]Stratton VAMC, Albany, NY; [2]Albany College of Pharmacy and Health Sciences, Albany, NY; [3]SUNY Empire State College, Saratoga Springs, NY.*

Background: The serum phosphorus concentration ($[P]_s$) equals E_P/C_{cr} + TR_P/C_{cr}, where E_P = urinary excretion rate and TR_P = tubular reabsorption rate of P. Influx of P (I_P) determines E_P, and FGF23 reduces TR_P/C_{cr}. [FGF23] varies directly with I_P even if $[P]_s$ does not change, but [FGF23] also correlates with $[P]_s$ in CKD. We analyzed this paradox with data from a study of secondary hyperparathyroidism (Clin Nephrol, in press).

Methods: Control subjects (C; n = 28) were studied once. Patients with stages 3 and 4 CKD (n = 29) were seen 5 times at 4-wk intervals. Consent was obtained at visit 1. A 4-wk course of vitamin D was provided at visit 2. Dietary P restriction was prescribed at visit 3 and continued through visit 5. At visit 4, patients were randomized to placebo or sevelamer carbonate (2400 mg) with meals. At all visits, intact [FGF23] (Immutopics) and serum and urine [cr] and [P] were measured in fasting specimens. E_P/C_{cr} was calculated as $[P]_u[cr]_s/[cr]_u$ and TR_P/C_{cr} as $[P]_s - E_P/C_{cr}$. Pertinent linear regressions were examined.

Results: In C, $[P]_s$ correlated with TR_P/C_{cr} but not E_P/C_{cr}; determinants of [FGF23] were not identified, and TR_P/C_{cr} did not vary with [FGF23]. In CKD, at each visit, $[P]_s$ correlated with E_P/C_{cr} and TR_P/C_{cr}, and [FGF23] correlated with $[P]_s$ and E_P/C_{cr} ($P < 0.05$ for all regressions). TR_P/C_{cr} varied with [FGF23] at visit 4 only ($P = 0.007$).

Visit	2	3	4	5	C
Regression	**R^2**	**R^2**	**R^2**	**R^2**	**R^2**
$[P]_s$ on E_P/C_{cr}	0.42	0.56	0.52	0.39	0.05
$[P]_s$ on TR_P/C_{cr}	0.28	0.50	0.37	0.38	0.95
[FGF23] on E_P/C_{cr}	0.26	0.16	0.14	0.35	0.09
[FGF23] on $[P]_s$	0.15	0.24	0.42	0.20	0.03
TR_P/C_{cr} on [FGF23]	0.001	0.09	0.24	8×10^{-4}	0.01

Conclusions: In CKD, [FGF23] correlated with E_P/C_{cr} and $[P]_s$ whether TR_P/C_{cr} varied with [FGF23] or not. Because $[P]_s$ was affected similarly by E_P/C_{cr} and TR_P/C_{cr}, [FGF23] should have been associated with both ratios consistently if $[P]_s$ was the sole determinant of [FGF23]. We speculate that P influx, as measured by E_P/C_{cr}, influenced [FGF23] and $[P]_s$ independently; [FGF23] then correlated with E_P/C_{cr} and $[P]_s$ while remaining dissociated from TR_P/C_{cr}.

Funding: Veterans Administration Support, Pharmaceutical Company Support - Genzyme Corporation

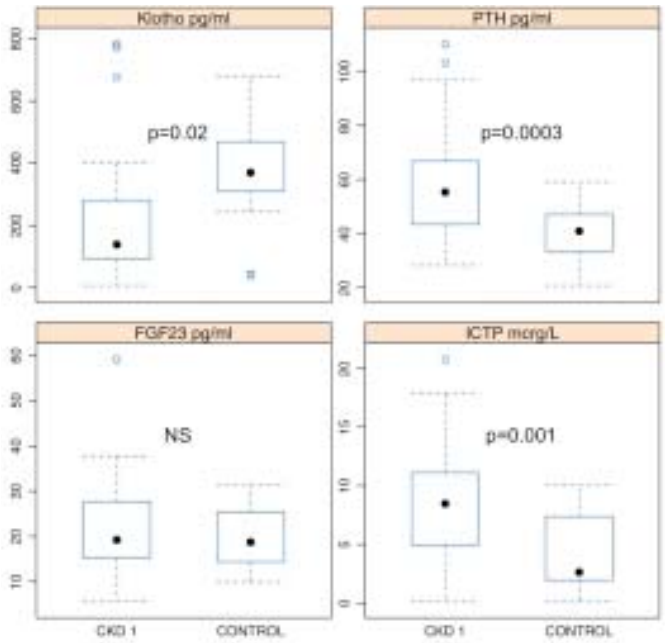
PUB601

A Reduction in Klotho May Be the Key in the Earliest Development of Secondary Hyperparathyroidism Isabel Martinez,[1] Ramon M. Saracho,[2] Yolanda Almaden Peña,[5] Adriana S. Dusso,[3] Mariano Rodriguez.[4] *[1]Nephrology, Hospital de Galdakao, Usansolo, Vizcaya, Spain; [2]Nephrology, Hospital Univ Araba, Vitoria, Alava, Spain; [3]IRBLleida, Lleida, Alava, Spain; [4]IMIBIC, Córdoba, Spain; [5]IMIBIC, Córdoba, Spain.*

Background: In the course of CKD, the onset of SH precedes the elevations in calcium and phosphate levels. Our prior demonstration that in CKD 1, the elevations in serum PTH also precede those of FGF23 led us to examine the contribution of klotho loss to the onset of abnormalities in the tight reciprocal control of the PTH/calcitriol/calcium and FGF23/calcitriol/phosphate axis in early CKD.

Methods: Thirty three patients with CKD stage 1 of different etiology who were not receiving calcium, vitamin D, calcimimetic or biphosphonate were compared with 17 normal controls. Chemistries included serum levels of calcium, phosphate, 25OHvitaminD, calcitriol, PTH, FGF23, soluble klotho, and serum carboxy-terminal telopeptide of type I collagen (IRCP) as a marker of bone resorption, and 24h urinary calcium, phosphate and creatinine. GFR was estimated by CrCl adjusted for 1.73 m2 body surface.

Results: Both PTH and ICRP were higher in CKD1 patients than in normal controls (PTH: 58.1 versus 41.4 pg/ml, p=.0003; ICRP:8.8 versus 4.4µg/L, p=0.001). These differences in serum PTH persisted after adjustment for age and gender. In contrast, serum levels of soluble klotho were lower in CKD1 patients (218.8 versus 374.8 pg/ml, p=0.02), while there were similar serum levels of FGF23, total and ionized calcium, phosphate, 25OHvitamin D and calcitriol, and urinary calcium and phosphate excretion. As expected, fractional excretion of phosphate was higher in CKD1 patients.



Conclusions: The lower levels in serum soluble klotho in CKD 1 suggest that decreases in renal klotho that precede actual reductions in GFR could limit FGF23 suppression of PTH secretion thereby increasing bone resorption and PTH-driven phosphaturia.

Funding: Government Support - Non-U.S.

PUB602

Transfer from Oral Alfacalcidol to Oral Calcitriol in the Treatment of Secondary Hyperparathyroidism in Chronic Hemodialysis Patients

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Background: There is no clear evidence in the literature regarding the optimal vitamin D(Vit-D) therapy for the treatment of secondary hyperparathyroidism(SHPT) in chronic hemodialysis patients. Recent animal studies suggest that uremia is associated with hepatic enzymatic dysfunction altering Vit-D 25-hydroxylation. Therefore, in uremia, calcitriol, the fully hydroxylated active form of Vit-D, could be more effective than alfacalcidol, a precursor that is lacking 25-hydroxylation. The goal of this study was to compare the efficacy of calcitriol and alfacalcidol for the treatment of SHPT in chronic hemodialysis patients.

Methods: We switched the treatment of SHPT of 36 chronic hemodialysis patients. The initial Vit-D analog, alfacalcidol, was switch for calcitriol at a dose about 20% lower. We then compared the different components of the mineral metabolism at baseline and at 2,4,6 and 8 weeks post conversion.

Results: We observed a significant increase of 1,25-(OH)2Vit-D level of 23.4 pmol/L (p=0.0002), with a mean value of 57.2 at baseline and 81.1 pmol/L at 8 weeks post switch. The level of 25-(OH)Vit-D also increased by 4.0 nmol/L (p=0.0014) at 8 weeks. There was no significant statistical reduction of PTH, with a mean difference of -0,89 pmol/L (p=0.49). The mean corrected calcium for albumin increased by 0.07 mmol/L (p=0.045). There was no significant change in phosphorus levels.

Conclusions: Despite a 20% decrease in dosage of calcitriol compared to alfacalcidol, calcitriol was as effective at maintaining serum PTH levels and was associated with a significant increase of serum 1,25(OH)2 Vit-D and total corrected calcium. Bypassing hepatic 25-hydroxylation appears to be associated with better efficacy of calcitriol at increasing serum 1,25(OH)2 Vit-D, intestinal calcium absorption, serum calcium and inhibiting PTH secretion. These observations must be taken into consideration before choosing a vitamin D analogue or switching from one to the other.

Funding: Private Foundation Support

PUB603

Prescription Habit Changes in Spain following the KDIGO Recommendations on Vitamin D (Cholecalciferol or Calcifediol) Repletion in Dialysis Patients: Two-Year Follow-Up

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Background: To describe clinical practice regarding 25(OH)D (C25) level repletion over 2 years in patients with CKD stage 5D-HD, 3 years after the publication of the KDIGO Guideline for CKD-MBD.

Methods: Treatment prescription was reviewed for active vitamin D (A-VD) (alfacalcidol, calcitriol or paricalcitol), and non-active VD (NA-VD) (cholecalciferol or calcidiol), as well as its impact on biochemical MBD parameters in 1134 patients at 54 Fresenius Medical Care clinics in Spain. Subjects were on dialysis for at least 24 months (April 2012 to March 2014). Three groups were defined: group 1) patients treated with NA-VD only; group 2) patients treated with A-VD only; and group 3) patients not treated with VD.

Results: 349 patients (37.64 % females), 71 ± 14 y.o. (on dialysis for 87.41 ± 83.60 months) were reviewed. In patients who were not treated with NA-VD (groups 2 and 3), serum C25 levels distributed as follows: 27.05 % had < 10 ng/mL; 67.97 % had 10-30 ng/mL; and 4.98% had > 30 ng/mL.

Table. Patient MOM biochemical data

Groups	n	Ca mg/dl	P mg/dl	iPTH ng/l	25(OH) VD ng/ml
1	68	9.0±0.3	4.3±0.87	350±254**	21.4±7.7***
2	172	9.0±0.4	4.2±0.8	400±238	15.6±9.3
3	109	8.8±0.4*	4.3±1.3	305±231	16.0±8.4
Total	349	9.0±0.4	4.35±1.0	361±242	16.8±9.0

mean±standard deviation *significative vs 1 and 2 **significative vs 3 ***significative vs 2 and 3

Over the study period, prescription of cholecalciferol and calcidiol increased (0.4 to 4.2%, and 4.9 to 14.4% respectively).

Conclusions: The KDIGO recommendations to "normalize" C25 levels have been adhered to rapidly by Spanish nephrologists. Patients who do not receive VD have lower serum calcium levels. Treatment with NA-VD is associated with higher levels of C25. Normal and target C25 levels in dialysis patients remain to be defined. Spanish nephrologists appear to prefer calcifediol over cholecalciferol.

PUB604

Alkaline Phosphatase, iPTH and Bone Turnover Markers in Advanced CKD Patients

Yueming Liu, Qiang He. *Nephrology, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China.*

Background: Some bone turnover markers are available in clinical care for CKD patients for many years. The aim of this study was to describe any changes of these biochemical markers of bone remodeling and evaluate the correlation among them in CKD patients.

Methods: Total 317 advanced CKD patients(58 CKD3, 38 CKD4, 221 CKD5) were enrolled in the study. We measured serum intact-PTH(iPTH), N-terminal midfragment (N-MID) osteocalcin, P1NP, β-CTx, total alkaline phosphatase (ALP), 25-Hydroxyvitamin D in accordance with manufacturers' instructions.

Results: Levels of iPTH, N-MID osteocalcin, P1NP, and β-CTx and serum phosphorus all reached statistical significance between different stages of CKD. ALP and vitamin D (25-OH) serum levels were higher in HD patients than PD patients. ALP, osteocalcin and P1NP were significantly higher in dialysis patients than conservative treatment patients. The relation between iPTH, ALP and levels of N-MID osteocalcin, P1NP, and β-CTx were significant but weak. No correlation was observed between vitamin D and iPTH or ALP.

Conclusions: Measuring N-MID osteocalcin, P1NP, and β-CTx with iPTH may be useful in assessing CKD-MBD.

PUB605

Treatment of Secondary Hyperparatiriroidism (shpt) with Paricalcitol in Patients on Hemodialysis (hd)

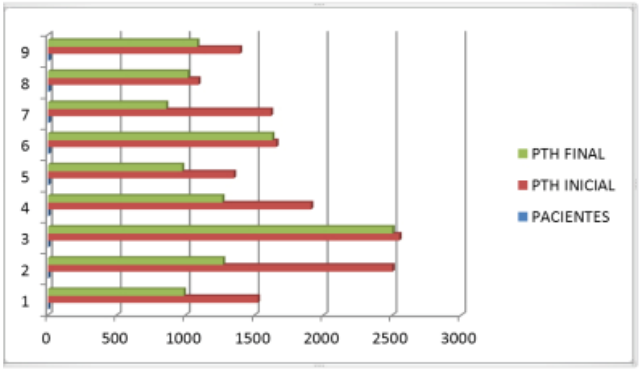
Cristobal Santacruz. *Clinica de los Rinones Menydial, Quito, Ecuador.*

Background: We know about the alterations in the balance of calcium, fosfate and PTH metabolism in patients (CRD) starting with clearance < 60ml/min, until stage 5D when the manifestations are evident in the skeleton and other organs.All start with a decrease in the production of the calcitriol and hipocalcemia, the phosphate retention and resistance to the calcemic effect of the PTH; however a close normal free blood calcium because of an secondary HPTS compensation. This metabolic disorder chronically maintained in the patients generate some abnormalities named renal osteodistrophy. The treatment and control of the bone disease, hyperfosfatemia and the HPTS is mandatory because if not done it increases the morbility and mortality.

Methods: A prospective interventional clinical assay not controlled, not randomized was done with nine patients 6 men and 3 women who were selected to use the drug, the medium age was 45,7 years (from 27 to 61 years), average HD time 9 years (from 5 to 15 years), PTH levels above of 300 UI and seric calcium and fosfate normal; all of them receive an average dosis of 0.08 mcg/kg after hemodialysis treatment, nobody of the patients did respond to previous oral calcitriol more phosphate binders treatment.

Results: 66% of the patients respond very well to the paricalcitol IV and decrease like group 38% of the initial level of PTH and it was statistical significant (p=0.009) applying the t for paired samples means matched.

Figura 3. Evolución en los niveles de PTH en todos los pacientes.



Fuente: Clinica de los riñones Menydial

PATIENTS	INITIAL PTH	FINAL PTH	PARICALCITOL DOSIS
1	1523	985	0.08
2	2500	1270	0.07
3	2550	2500*	0.07
4	1909	1265	0.08
5	1349	973	0.09
6	1657	1628*	0.1
7	1618	857	0.1
8	1092	1015*	0.07
9	1393	1083	0.07
AVERAGE	1732.33	1286.22	0.081111111

Conclusions: The paricalcitol IV showed to be an option in treating uncontrolled patients with long time on HD and high PTH levels, who developed a severe HPTS and had failed treatment with oral calcitriol more phosphate binders previously.

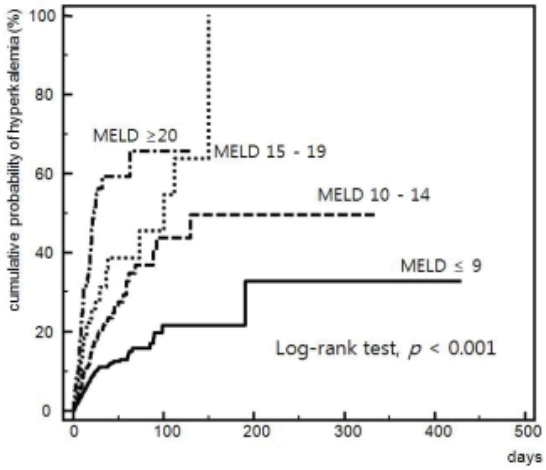
PUB606

The Model for End-Stage Liver Disease Score Is Potentially a Useful Predictor of Hyperkalemia Occurrence Among Hospitalized Angiotensin Receptor Blocker Users Inwhee Park, Eunjung Kang. *Dept of Nephrology, Ajou Univ School of Medicine, Suwon, Gyeonggi-do, Korea.*

Background: Angiotensin receptor blockers (ARBs) are medications commonly used for treating conditions such as hypertension. However, ARBs are frequently associated with hyperkalemia, a potentially critical adverse event, in high-risk patients. Although both the liver and the kidney are major elimination routes of ARBs, the relationship between hepatorenal function and ARB-related hyperkalemia has not yet been investigated. The purpose of this study was to evaluate the risk of hyperkalemia, in terms of various hepatorenal functions, for hospitalized patients newly initiated on ARB treatment.

Methods: We evaluated ARB-related hyperkalemia in a cohort of 5530 hospitalized patients, who had not previously used ARBs, between April 12, 2004 and May 31, 2012. Hepatorenal function was assessed by the Model for End-Stage Liver Disease (MELD) score. Hyperkalemia risk was assessed by hepatorenal function, risks were categorized into the four MELD scoring groups, and the groups were compared with one another.

Results: The MELD score was significantly different between the hyperkalemic and non-hyperkalemic groups (independent t-test, $p < 0.001$). The MELD score 10-14, 15-19, and ≥20 groups showed higher risks of hyperkalemia than the lowest MELD score group {log-rank test, $p<0.001$; multiple Cox proportional hazard model, hazard ratios 1.478 ($p=0.003$), 2.285 ($p<0.001$), and 3.024 ($p<0.001$), respectively}.



Conclusions: The MELD score showed a stronger predictive performance for hyperkalemia than either serum creatinine or estimated glomerular filtration rate alone. Furthermore, the MELD score showed good predictive performance for ARB-related hyperkalemia among hospitalized patients. The clinical implications and reasons for these findings merit future investigation.

Funding: Government Support - Non-U.S.

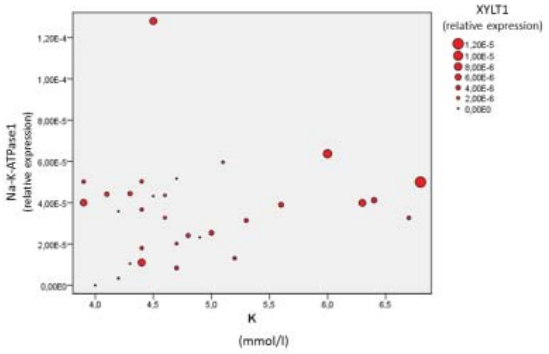
PUB607

Expression of XYLT1 in Human Muscles Correlates with Serum Potassium and Na-K-ATPase1 Expression Michael Fischereder,[1] Franz Xaver Beck,[2] Antje Habicht,[3] Ulf Schoenermarck,[1] Peter J. Nelson,[1] Manfred J. Stangl.[4] *[1]Renal Div, Medizinische Klinik IV, Klinikum der LMU, Munich, Germany; [2]Dept of Cellular Physiology, Physiologisches Institut, LMU, Munich, Germany; [3]Transplant Center, Klinikum der LMU, Munich, Germany; [4]Dept of Surgery - Grosshadern, Klinikum der LMU, Munich, Germany.*

Background: We have previously shown that inter-individual and highly variable tissue concentrations of sodium can be found in human muscle. This high variability may be due to osmotically inactive sodium, bound to glycosaminoglycans (GAG). GAG synthesis is initiated by XYLT1 which correlates with tissue sodium concentrations. We now sought to better characterize this regulation of XYLT1 expression in human muscle.

Methods: We studied 27 dialysis patients. 21 live kidney donors served as healthy controls. There was no clinically detectable edema. During transplant surgery, abdominal muscle and arteries were biopsied. Expression of XYLT1, Na-K-ATPase1 and Na-K-ATPase2 were determined by real-time PCR (n= 31), normalized to s18 RNA and correlated with clinical data. Intracellular sodium concentration was measured by electron microprobe analysis (n=16).

Results: Na-K-ATPase1 expression was significantly higher in men than women (4.4 x 10E5 versus 2.4 x 10E5; p = 0.017). On univariate analysis, XYLT1 expression was significantly correlated with serum potassium (r = 0.508; p = 0.004), serum urea (r = 0.305, p = 0.095) and muscular expression of Na-K-ATPase1 (r = 0.392; p = 0.029) and Na-K-ATPase2 (r = 0.359, p = 0.047). On multivariate analysis, XYLT1 expression was significantly correlated with serum potassium (p = 0.005) and Na-K-ATPase1 expression (p = 0.041).



Conclusions: XYLT1 expression in human muscles is correlated with serum potassium concentrations and Na-K-ATPase1 expression. This may form a pathophysiologic basis for the understanding of myopathy in patients with hyperkalemia, e.g. in advanced renal failure.

PUB608

A Case Report of Ondansetron Induced Bartter Like Syndrome in Pregnancy Ashish Gummadi,[1] Wajid M. Choudhry.[2] *[1]Dept of Internal Medicine, Unity Health System, Rochester, NY; [2]Nephrology, Univ Of Rochester, Rochester, NY.*

We report a case of severe hypokalemia in 30 year old pregnant Caucasian woman. She developed hyperemesis gravidarum during first trimester of pregnancy. She received ondansetron 4 to 8 mg 2-3 times a day. At 29 weeks gestation, she was admitted to the hospital because of severe lower extremities weakness. She had normal Physical exam with heart rate of 80 beats/min and blood pressure of 92/50mmHg.Labs: serum sodium of 139mEq/L, potassium 2.1mEq/L, chloride 99mEq/L, carbon dioxide of 26 mmol/L, Bun 7 mg/dl, Creatinine of 0.66 mg/dl, calcium 8.2mg/dl, albumin 3.7gm/dl, magnesium 1.7 mg/dl, phosphorus of 3.1 mg/dl, uric acid 2.9mg/dl, CK 3501 U/L and TSH 1.29uIU/ml. She was treated with IV hydration, IV potassium and magnesium supplements. Her serum potassium remained low at 2.8mEq/L. 24-hour urine with a total volume of 2700ml and 1.58 grams of excereted creatinine showed sodium 392mEq, potassium of 86 mEq, chloride 440 mEq and calcium 502mg. Inappropriate hyperkaliureia and hypercalciuria in the setting of hypokalemia and hypocalcemia is consistent with Bartter like syndrome. We stopped her ondansetron and she was discharged home on 40 mEq of potassium chloride TID. After a few days of ondansetron cessation she did not require any further potassium and magnesium supplements. Her serum potassium remained stable at around 4.1 mEq/L. Cases of polyvalent cations like aminoglycosides causing Bartter like syndrome have been reported in literature. Ondansetron is also a bivalent cation with two methyl groups. We hypothesize that ondansetron may also have calcimimetic action which could have caused bartter like syndrome in our patient.

PUB609

Dynamic, Not Static Diagnostic Approach Can Predict Overcorrection of Hyponatremia Sae Aratani,[1] Masahiko Nagahama,[1] Takuya Fujimaru,[1] Yuki Heath,[1] Fumika Taki,[1] Osamu Takahashi,[2] Yasuhiro Komatsu.[1] *[1]Nephrology, St. Luke's International Hospital, Chuo-ku, Tokyo, Japan; [2]Clinical Epidemiology, St. Luke's International Hospital, Chuo-ku, Tokyo, Japan.*

Background: Treatment of hyponatremia becomes difficult when spontaneous water diuresis emerges during therapy, which may lead to rapid increase of serum sodium (SNa) and to serious neurological sequelae. There is a paucity of information regarding the prevalence and risk factors for overcorrection. The aim of the present study is to clarify the prevalence and the predictive factors of overcorrection to establish effective preventive strategies.

Methods: We analyzed clinical and biochemical parameters of 58 patients whose SNa was below120 mEq/L on admission at St. Luke's International Hospital in Tokyo from March 2012 to April 2014. Standardized management protocol, which was reported previously (ASN 2001), were applied. All patients were monitored closely in ICU, with serial measurement of urine volume, electrolyte and osmolality and serum electrolyte.As per our protocol, we used parenteral 5% dextrose in water (D5W) or DDAVP to avoid overcorrection which is defined as the rate of correction of SNa over 10 mEq/L in 24 hours and 18 mEq/L in 48 hours.

Results: Among 58 patients, 23 patients (39.7 %) required management of overcorrection prevention with DDAVP or D5W. The mean age of the patients was 70±17.4 (mean+/-S.D.) yr, the male/female ratio was 29/29. The mean initial SNa was 117.2±5.6 mEq/L, sum of urinary sodium and potassium (UNa + UK) was 90±65.1 mEq/L and urinary osmolality (UOosm) was 352±172 mEq/kg. On univariate analysis, patients who required overcorrection prevention are more likely to have large urine volume in the first 2 hours (P=0.028).Multivariate logistic regression analysis identified urine volume for the first 2 hours was significantly larger in patients with overcorrection (odds ratio =1.003, P=0.025). Neither UOosm nor urinary UNa + UK are predictive for overcorrection.

Conclusions: The present study shows that overcorrection of hyponatremia was found more frequently than we expect. Our study underscores the importance of assessment of urine volume (dynamic approach) rather than UOsm or UNa+UK (static approach) to predict overcorrection.

PUB610

Hyponatremia Is a Risk Factor for Increased Cardiovascular and Non-Cardiovascular Mortality in Incident Hemo- and Peritoneal Dialysis Patients Hakan Nacak,[1] Merel Van Diepen,[1] Marit M. Suttorp,[1] Ewout J. Hoorn,[3] Joris I. Rotmans,[2] Friedo W. Dekker.[1] *[1]Clinical Epidemiology, Leiden Univ Medical Center, Leiden, Zuid-Holland, Netherlands; [2]Nephrology, Leiden Univ Medical Center, Leidne, Zuid-Holland, Netherlands; [3]Internal Medicine - Nephrology, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands.*

Background: Hyponatremia is associated with increased mortality in the general population as well as in dialysis patients. It's unknown whether this increased mortality in dialysis patients is mainly cardiovascular (CV) or non-cardiovascular (non-CV) related. Therefore, the aim of this study is to assess the effect of hyponatremia on cardiovascular and non-cardiovascular mortality in incident dialysis patients.

Methods: The NECOSAD-study is a Dutch prospective multi-center cohort study of incident dialysis patients. All patients with measured plasma sodium 90 days after the start of dialysis were investigated (n=1719, mean plasma sodium [SD]=138.6 [3.5]). Multivariable Cox models were used to estimate the effect of hyponatremia and plasma sodium (per mmol/L) on CV and non-CV mortality in HD and PD patients. In addition, time-dependent (TD) Cox models were used to estimate short-term effects of hyponatremia and plasma sodium.

Results: Hyponatremia leads to a 2.12-fold increased risk (TDadj) for CV mortality and 2.05-fold increased risk for non-CV mortality in HD patients. In PD patients, hyponatremia leads to 1.53 and 1.61-fold increased risk for CV and non-CV mortality, respectively. See table for more detailed results.

	Hemodialysis patients		Peritoneal dialysis patients	
	Hyponatremia (< 135 mmol/L)	Plasma Sodium (per mmol/L)	Hyponatremia (< 135 mmol/L)	Plasma Sodium (per mmol/L)
HR (95% CI)	CV mortality		CV mortality	
Crude	1.28 (0.89-1.83)	0.98 (0.95-1.02)	2.77 (1.60-4.81)	0.90 (0.85-0.95)
Adjusted	1.30 (0.90-1.87)	0.98 (0.95-1.02)	2.35 (1.30-4.24)	0.91 (0.85-0.97)
TD Crude	1.99 (1.48-2.68)	0.93 (0.90-0.96)	1.93 (1.20-3.11)	0.91 (0.86-0.96)
TD Adjusted	2.12 (1.57-2.87)	0.92 (0.87-0.95)	1.53 (0.93-2.50)	0.92 (0.87-0.98)
HR (95% CI)	Non-CV mortality		Non-CV mortality	
Crude	1.49 (1.10-2.03)	0.95 (0.92-0.98)	2.21 (1.16-4.22)	0.97 (0.90-1.03)
Adjusted	1.46 (1.06-1.99)	0.96 (0.93-0.99)	2.11 (1.06-4.19)	0.98 (0.91-1.05)
TD Crude	1.96 (1.50-2.57)	0.91 (0.88-0.93)	2.04 (1.26-3.30)	0.92 (0.87-0.98)
TD Adjusted	2.05 (1.56-2.71)	0.90 (0.87-0.93)	1.61 (0.97-2.66)	0.93 (0.88-0.99)

* Adjusted for age, sex, primary renal disease, cardiovascular disease, diabetes mellitus, and anuria.

Conclusions: Hyponatremia is a strong and independent risk factor for both CV and non-CV mortality in incident HD and PD patients.

PUB611

Urinary Sodium Excretion One Year Post Transplantation Does Not Predict Subsequent Transplant Failure Hasnain Raza, Harsha Wodeyar Kagodu Surendranath, Matthew L.P. Howse. *Nephrology, Royal Liverpool Univ Hospital, Liverpool, United Kingdom.*

Background: There is evidence in patients with CKD that high sodium intake limits the antihypertensive and antiproteinuric effects of angiotensin converting enzyme inhibitor (ACE-I) and hence leads to progression of CKD independent of blood pressure (BP) control. (J Am Soc Nephrol 23: 165-173, 2012).However there has not been any study in patients with renal transplant and the effect of high sodium intake on renal transplant survival.

Methods: Data was retrospectively collected from the 385 patients who had renal transplant from 2000 to 2005. All patients performed 24 hour urine collections. To allow for incomplete collections Na excretion was corrected for urinary creatinine excretion. Death with function graft was counted as graft failure.

Results: Out of all (385) 303 transplant recipient were included in analysis due to completeness of data. Some patients were excluded due to early graft failure within one year. (Male 182 Female 121) Mean age was 46 years Mean BP at 1 year was 138/80 mm Hg with Median BP of 140/80mm Hg Mean serum creatinine at 1 year was 163umol/L with median creatinine of 146umol/L Mean Na/creatinine at 1 year was 19meq/day with median 18meq/day Mean Total Na excretion at 1 year was 201 with median 192 Mean 24 hour urine protein excretion was 0.46 gm/L with median 0.2gm/L Mean HLA-A, B, DR 0.8-0.8-0.4 with median of 1-1-0 Follow up was until 1st April 2013. Statistical tests (T-Test) were done to determine if the one year urinary sodium had any effect on renal graft survival .There was no statistically significant difference in 1 year urinary Na/creatinine with subsequent transplant failure until last follow up.

Conclusions: We have shown that dietary sodium intake measured as urinary sodium excretion one year post renal transplantation does not predict subsequent graft failure. This was an unexpected result.We have shown that factors other than sodium intake, such as serum creatinine at one year and urinary protein excretion are far more powerful predictors of Graft survival.

Funding: Government Support - Non-U.S.

PUB612

The Relevance of Significant Discrepancy between Brain Extracellular and Serum Potassium Mohammad Ali Shafiee, Masih Rikhtehgar. *Medicine, Toronto General Hospital, Univ of Toronto, Toronto, ON, Canada.*

Background: Brain function comprises a series of action potentials (AP) which are influenced by membrane voltage and extracellular fluid potassium concentration (ECF [K]). Nevertheless, each AP releases a significant amount of K, which could interfere with ongoing brain function. **Hypothesis:** Since optimum brain function is crucial, we hypothesized that even with significant K release in each AP; ECF [K] should be relatively constant to allow optimal brain function. **Objective:** To propose a physiology-based model of K buffering system in the brain.

Methods: Seventy-two English-language articles (cross-sectional, book chapters, and case-control studies) were reviewed in which Serum [K] and Central Nervous System (CNS) ECF [K] were simultaneously measured in 511 normal human subjects, 52 uremic, and 291 CNS-diseased patients. Hypo/Hyperkalemic symptomatology was reviewed in articles as well as reference texts. The conventional mechanisms of maintaining CNS ECF [K] were critically appraised. Considering significant flaws in the explanation, a new model is proposed.

Results: No study identified significant CNS signs and symptoms in hyper/hypokalemic states. CSF [K] is maintained in a remarkable tighter and lower range (2.9±0.22) regardless of substantial variations of serum [K] (4.49±0.43) in healthysubjects of different ages (1 week-80 years). The same result was observed in uremic patients with extreme serum K fluctuations (range 2.2-8.1). However, CSF [K] altered in some neurological disorders such as Alzheimer, seizure, and coma. CNS potassium is maintained by a supporting system including spatial K buffering, siphoning, net uptake, and blood brain barrier.

Conclusions: A new physiology-based model in conjunction with conventional mechanismsis offered, which demonstrates K buffering yet availability to neurons in an economically efficient manner. In such model released K by each AP will be buffered locally via exchange with sodium and protons in addition to reversible binding with organic anions. Furthermore, the CNS ECF [K] change in neurological disorders makes the proposed model a new avenue for better understanding of CNS disorders like Alzheimer disease.

PUB613

Correction Rate of Severe Hypernatremia and Morbidity and Mortality in Hospitalized Patients Ameer Abdulrazzak, Mina El-Kateb, Minas Melidonian, Heather L. Henderson, Joel Topf. *Internal Medicine, St.John Hospital and Medical Center, Detroit, MI.*

Background: Hypernatremia is common among hospitalized patients and has been associated with hospital mortality. Guidelines for the rate of correction in adult patients are based on studies in children. Current recommendations state that the rate of correction should not exceed 10-12 mEq/L/day. The study objective is to determine whether the rate of serum sodium correction in hospitalized adult patients with severe hypernatremia affects morbidity or mortality.

Methods: This is a retrospective, observational, chart review, to look at neurologic and patient outcomes according to the rate of correction of hypernatremia in hospitalized adult patients admitted with sodium more than 160 mEq/L. Data collected included demographics, comorbidities, neurologic status at admission and discharge, and fluids given during the first 72 hours of admission or until normalization of serum sodium. We evaluated the correction rate at the first 24, 48, and 72 hours. The primary outcomes were mortality during hospitalization and change in neurologic status between admission and discharge.

Results: Fifty nine patients met the inclusion criteria (mean age 78.3±16.3 yrs, 35% male, 33% White). There were no significant differences in mortality by demographic factors or comorbidities, except that patients who survived had a significantly higher diastolic blood pressure on admission (69.2±16.0 mm Hg^2 versus 55.7±20.5mm Hg^2, p=0.01 respectively). There was no worsening in the neurologic status between admission and discharge in any of the patients. The mean correction rate of hypernatremia at 24 and 48 hours was significantly faster in patients who survived than those who died (24 hrs: 8.13±7.2 mEq/L/day versus 4.6±2.9, respectively, p=0.01; 48 hrs: 13.6±8.2 mEq/L/day versus 9.5±4.7, respectively p=0.03). No difference was found at 72 hours. In ten patients, the sodium was corrected faster than 12 mEq/L in 24 hours. Mortality in this sub-group was zero compared to 34.8% in patients whose sodium was corrected within the guidelines.

Conclusions: Correction of hypernatremia faster than current guidelines was not associated with increased mortality or poor neurologic outcomes.

PUB614

Prevalence of Uromodulin-Associated Kidney Disease May Be Under-Estimated - Renal Biopsy Based Investigation Tamehito Onoe,[1] Kazunori Yamada,[1] Ichiro Mizushima,[1] Kiyoaki Ito,[1] Shoichiro Daimon,[2] Mitsuhiro Kawano.[1] *[1]Dept of Internal Medicine, Kanazawa Univ Graduate School of Medicine, Kanazawa, Ishiikawa, Japan; [2]Dept of Nephrology, Daimon Clinic for Internal Medicine, Nephrology and Dialysis, Nonoichi, Ishikawa, Japan.*

Background: Uromodulin-associated kidney disease (UAKD) is characterized by urinary concentration defect, hyperuricemia, gout and slowly progressive renal failure. Affected patients usually reach end-stage kidney disease (ESKD) between the fourth and seventh decade. UAKD is caused by Uromodulin (UMOD) gene encoding Tamm-Horsfall protein (THP) which is the most abundant protein in human urine. UAKD is reported to be a rare inherited disease whose prevalence is about one case per million population. However it is suggested that this disease is under-recognized because hyperuricemia is common in patients with chronic kidney diseases.

Methods: To clarify the incidence of UAKD in patients undergoing renal biopsy, immunostaining of THP was performed for patients with renal insufficiency and hyperuricemia under 50 years old without overt urinalysis abnormality from 1992 to 2013 in Kanazawa University and related facilities.

Results: 15 of total 3787 patients were selected for immunostaining. Pathological diagnosis of all these patients was interstitial nephritis or nephrosclerosis. In four independent patients of them, abnormal THP accumulation in renal tubular cells was detected. Only one of the four patients had a strong family history of kidney disease. A novel missense A247P mutation was detected in his affected family members including him. Other three patients had no family history of kidney disease.

Conclusions: Renal biopsy and immunostaining for THP may be a useful tool to diagnose UAKD patients, because abnormal THP accumulation was detected in 4/15 patients who met all the above criteria, despite the little chance for renal biopsy due to the paucity of proteinuria in UAKD patients. It is suggested that a considerable number of UAKD patients may exist and reach ESKD without an exact diagnosis, with more sporadic cases of UAKD existing than reported before.

PUB615

The Differential Diagnosis between Inherited Salt-Losing Tubulopathy and Pseudo-Disorder from Clinical Characteristics Natsuki Matsunoshita,[1] Kandai Nozu,[1] Naohiro Kamiyoshi,[1] Koichi Nakanishi,[2] Norishige Yoshikawa,[2] Kazumoto Iijima.[1] *[1]Dept of Pediatrics, Kobe Univ Graduate School of Medicine, Kobe, Hyogo, Japan; [2]Dept of Pediatrics, Wakayama Medical Univ, Wakayama, Wakayama, Japan.*

Background: Bartter's syndrome (BS) and Gitelman's syndrome (GS) are autosomal recessive inherited salt-losing tubulopathy characterized by hypokalemic metabolic alkalosis. Phenotype overlapping frequently occurs in type III BS and GS patients, which are difficult to diagnose from their clinical pictures. Moreover, some unique acquired or inherited cases can occasionally cause BS/GS-like disorder, or pseudo-BS/GS (p-BS/GS) which clinical symptoms are identical to BS/GS. Although it is very important to make the accurate diagnosis for these three diseases, the analysis for clarifying differences in clinical characteristics is rarely performed.

Methods: We retrospectively studied the clinical data from 161 Japanese patients (type III BS:30, GS:88, p-BS/GS:43). All type III BS and GS patients had homozygous or compound heterozygous mutations in *CLCNKB* and *SLC12A3*,respectively, whereas none of p-BS/GS had disease-causing mutations in these two genes.

Results: P-BS/GS patients had significantly higher mean age at diagnosis than type III BS and GS (36.7±2.5 versus 4.2±2.6 versus 18.3±1.8 yr ; $p < 0.05$). When p-BS/GS patients were compared with GS, p-BS/GS patients had significantly higher percentage of female (76.3 versus 44.8 %; $p < 0.05$), lower BMI (17.9±0.7 versus 21.2±0.7 kg/m^2; $p < 0.05$) and lower eGFR (65.1±6.0 versus 99.8±5.8 ml/min/1.73m^2; $p < 0.05$) than GS patients. Furthermore, p-BS/GS patients had significantly higher serum magnesium level (1.95±0.12 versus 1.57±0.07 mg/dL; $p<0.05$) and urinary excretion of calcium (Ca/Cr ratio of 0.07±0.01 versus 0.03±0.01 mg/mg; $p<0.05$) than GS patients.

Conclusions: We could demonstrate clinical distinction between genetically-proven type III BS, GS and p-BS/GS patients. Our results indicated p-BS/GS patients were found mostly in adult female with lower BMI and eGFR. It is helpful in the differential diagnosis of these three diseases to check ages at diagnosis, gender, BMI and renal function in addition to serum magnesium level and urinary excretion of calcium.

PUB616

Application of Whole Exome Sequencing to Detect New Causal Variants in Autosomal-Dominant Steroid Resistant Nephrotic Syndrome Katrina Soderquest,[1,2] Alka Saxena,[3] Efterpi Papouli,[3] Graham M. Lord,[1,3] Andrey S. Shaw,[4] Michael A. Simpson,[2] Ania B. Koziell.[1] *[1]Experimental Immunobiology, King's College London, United Kingdom; [2]Medical and Molecular Genetics, King's College London, United Kingdom; [3]NIHR Biomedical Research Centre, Guy's and St Thomas' Hospitals, United Kingdom; [4]Pathology and Immunology, Washington Univ.*

Background: Whole Exome Sequencing (WES) is now a popular choice for finding uncommon genetic variants of high effect in rare disease. The technique hypothesizes that these variants are primarily found in the exons of the genome but is hypothesis free in terms of gene candidates. Although studies of SRNS have, to date, found causal variants in > 37 genes, it is clear that not all causal mutations are known. Initial analysis of 3 Caucasian families with Autosomal-Dominant Steroid Resistant Nephrotic Syndrome (AD-SRNS) did not detect mutations in previously published genes. We therefore investigated a role for other candidates using WES.

Methods: Three generations were available for study. DNA was exome-enriched using Agilent SureSelect V5 and sequenced on Illumina HiSeq2000, including donor and acceptor consensus sites. Sequence was aligned to build hg19 of the human genome by Novoalign. Variants were called by SAMtools. Exome sequences were investigated to confirm absence of mutations in known candidate genes. Data were analysed, at first, assuming one dominant variant with complete penetrance.

Results: WES confirmed the absence of mutations in genes previously associated with SRNS. Eight potential new candidates in one family include genes participating in calcium-mediated signaling and encoding DNA binding proteins. Further analysis of samples is underway to allow sufficient refinement for functional confirmation.

Conclusions: Our data indicates that even well-defined cases of AD-SRNS may not have a known podocyte gene mutation, or the mutation may reside outside the exome. Of particular interest is that some patients responded to the Calcineurin inhibitor, Tacrolimus. One patient had a recurrence post-transplant. Analyses of families such as this with respect to phenotypic qualities, including drug response, broadens our understanding of molecular pathways involved in SRNS.

Funding: Government Support - Non-U.S.

PUB617

Identification of Novel Risk Contributing Loci within Steroid Resistant Nephrotic Syndrome Using Exome Sequencing on a Large National Pediatric Cohort Denis Andrew Baird,[1] Agnieszka Bierzynska,[2] Ania Koziell,[3] Gavin Iain Welsh,[2] Ian N. Day,[1] Moin Saleem.[2] *[1]School of Social and Community Medicine, Univ of Bristol, Bristol, United Kingdom; [2]Academic Renal Unit, Univ of Bristol, Bristol, United Kingdom; [3]Dept of Immunobiology, King's College London, London, United Kingdom.*

Background: Steroid Resistant Nephrotic Syndrome (SRNS) is genetically heterogeneous, with highly penetrant single disease causing mutations having been discovered in over 20 genes. Although, some genes have been discovered to contain variants which contribute risk towards SRNS, the non-Mendelian component of the disease remains largely unexplored. Genome Wide Association Studies (GWAS) have been successful in uncovering associated variants from large genotyped cohorts for many common, complex diseases. The development of Next Generation Sequencing (NGS) has made feasible the direct sequencing of large cohorts, which enables the search for risk contributing mutations in rare diseases using a similar statistical methodology as GWAS. An exome-wide association study was run on 64 individuals with childhood SRNS, collected from the UK RADAR Registry, in order to elucidate potential risk contributing variants in novel SRNS genes.

Methods: Patients with truncating or missense mutations within the SRNS candidate genes were removed from the analysis. Principal Component Analysis (PCA) was performed in Plink and non-European patients removed. Fisher Exact tests were performed on rare point mutations and uni-variate chi-squared tests for common variants. Multiple controls obtained from UK10K were used. Pathogenicity was assessed by Combined Annotation Dependent Depletion CADD tool. Haplotype estimation was carried out in BEAGLE.

Results: 35 loci were detected with significant associations across the non-candidate genes, 10 of which were predicted to be potentially damaging (C-score > 10). The analysis is currently being repeated with 97 further patients to investigate if further associations exist and to confirm the already found ones. This will be followed up with haplotype analysis to determine associated variants with a common point of origin.

Conclusions: The method revealed evidence of associated variants in novel SRNS genes.

PUB618

Diagnostic/Prognostic Tests for All Hereditary Kidney Diseases Lisbeth Silva,[1] Olaya Lamas-Gonzalez,[1] Beatriz Sobrino,[2] M. Lara Besada-Cerecedo,[2] Helena Covelo-Molares,[1] Patricia Regueiro Casuso,[1] Ana Barcia de la Iglesia,[1] Marina García,[1] Jorge Amigo,[1] Francisco Barros Angueira,[2] Angel Carracedo,[2] Candido Diaz Rodriguez,[1] Miguel A. Garcia-Gonzalez.[1] *[1]Group of Genetics and Developmental Biology of Renal Diseases, Health Research Institute of Santiago de Compostela (IDIS). Clinical Univ Hospital (CHUS), Santiago de Compostela, Spain; [2]Galician Public Foundation of Genomic Medicine, Clinical Univ Hospital (CHUS)., Santiago de Compostela, Spain.*

Background: The combination of NGS and Sanger sequencing is a traditional and reliable method of sequencing pools of genes for a particular disease, and thechnology is not a limitation anymore to diagnose hereditary renal disorders. Only level of efficiency, time and costs are the main objectives to develop and efficient strategy for genetic diagnosis and prognosis of patients.

Methods: We have developed a strategy to be used by nephrologists for the diagnosis of genetic renal disorders in an easy, cheap and fast way. Nephrologists tend to classify renal diseases in three main groups: cystic diseases, tubulopathies and glomerulopathies. For this reasons we developed 4 NGS panels considering precious criterias: 1) Panel for common hereditary cystic dieases (8 genes), 2) Panel for Rare and ultra-rare cystic disease (72 genes, for the diagnosis and possible prognosis); 3) Panel for tubular diseases (36 genes), and 4) Panel for glomerular diseases (26 genes).

Results: These panels have the following coverage of the target regions (exons and flanking regions): Panel 1 (common cystic diseases) 98.5%, Panel 2 (rare and ultra-rare cystic disease) 99.6%, Panel 3 (glomerular diseases) 99.97% and Panel 4 (tubular diseases) 99. 7%. We have applied these panels to a cohort of 187 patients with different hereditary renal pathologies, showing impressive results. We also have developed bioinformatic algorithms crossing every single known database of reference, in order to determine in a confident fashion the level of variant pathogenicity. These help us to provide new tools for clinical decision.

Conclusions: Here, we first describe a quick and cost effective strategy for genetic diagnosis of every single known hereditary renal disorder.

Funding: Government Support - Non-U.S.

PUB619

Estimation of Daily Protein Intake Based on Urea Nitrogen Concentration in Spot Urine in Chronic Kidney Disease Patients Hiroko Kanno,[1] Eiichiro Kanda,[3] Asako Sato,[1] Kaori Sakamoto,[4] Sanae Watanabe,[4] Yoshihiko Kanno.[2] *[1]Clinical Laboratory, Tokyo Women's Medical Univ, Japan; [2]Dept of Nephrology, Tokyo Medical Univ, Japan; [3]Dept of Nephrology, Tokyo Kyosai Hospital, Japan; [4]Dept of Clinical Nutrition and Dietetics, Kagawa Nutrition Univ, Japan.*

Background: It is not easy to determine the daily protein intake (DPI) in chronic kidney disease (CKD) management. Recording dietary intake is inaccurate, and estimation from total urea excretion is also not easy because it is difficult for patients to collect whole urine for 24 hours (h). Spot urine has been used in the measurement of daily sodium intake and urinary protein excretion. In this cross-sectional study, we investigated whether urea nitrogen (UN) concentration (C) in spot urine could be used to predict DPI instead of that obtained by 24h urine collection in CKD patients and compared it with UNC in healthy adults we reported previously.

Methods: One hundred forty-eight Japanese CKD patients (male 46.3%) provided their urine samples collected over 24h. After the 24h urine collection, they provided one spot urine sample. DPI was estimated using the Maroni formula and data from 24h urine collection. Multivariate linear models adjusted for patients' characteristics were used to predict protein intake on the basis of UNC. Variable selection was examined by backward elimination procedure.

Results: The following results were obtained: mean estimated glomerular filtration rate (±standard deviation), 52.1±29.5 ml/min; UN excretion, 6.08±2.19 g/day; and estimated DPI, 47.7±13.95 g/day. Diabetes mellitus (DM) was observed in 54.4%. A univariate liner model showed that the UNC in spot urine was associated with DPI [β=0.027±0.0070, p=0.0001]. The following final model showed that UNC in spot urine could predict the amount of 24h UN excretion: DPI = 36.69 + 8.66×gender (male=1, female=0) - 10.91×DM (yes=1, no=0) + 0.22 × UNC in spot urine, adjusted R^2=0.21, p=0.0001.

Conclusions: The findings of our present study suggest that UNC determined by the spot urine test can be used to estimate DPI and that the prediction formula would be useful for nutritional control in CKD patients.

PUB620

A 7 Year Analysis of Inflammatory and Nutrition Markers Predicting Cardiovascular (CV) Events in Predominantly African-American (AA) Patients with End Stage Renal Disease (ESRD) on Hemodialysis (HD) Sairah Sharif,[1] Srijan Shrestha,[2] Mallika Pradhan,[2] Sudhanshu Jain,[2] Rose Calixte,[1] Jeffrey D. Wallach.[2] *[1]Winthrop Univ Hospital, Mineola, NY; [2]Harlem Hospital, New York, NY.*

Background: CV disease is the leading cause of death in ESRD. ESRD patients have higher levels of oxidation, inflammation, and malnutrition leading to CV morbidity and mortality. It is suggested that that AA patients may be protected from the effects of inflammation relative to Caucasians. Studies show that higher CRP, higher phosphate (P), lower bicarbonate (B), lower albumin (A), lower prealbumin (PA) correlate with adverse CV outcomes in ESRD patients. We studied monthly trends of these markers and tested for associations with adverse CV outcomes.

Methods: The electronic records of 116 ESRD patients, >90% AA were reviewed from 2006 to 2012. Monthly levels of CRP, PA, A, P and B were recorded. We used group-based trajectory model to understand the changing course of each marker over 84 months. Patient demographics, comorbids and adverse CV events (myocardial infarction, stroke, amputation, congestive heart failure (CHF), cardiac arrest and death) were noted. Pearson's exact chi-square was used to test for an association between clinical outcomes and each marker. Covariate analysis by logistic regression was performed.

Results: Of the 116 patients, 53 patients had one or more adverse outcomes as outlined above. A was associated with the presence of any adverse outcome (p-value = 0.036). Patients with low A were 7.33 times more likely to have an adverse event as compared ones with normal A (p-value = 0.019). PA was associated with combined adverse events (p-value = 0.031). Patients having low PA were 5.85 times more likely to experience an adverse event than ones with normal PA (p-value = 0.009). The cardiovascular event most strongly associated with these two markers was CHF. CRP was strongly associated with future amputation. No other associations were found between CRP, P, or B and any CV event or death.

Conclusions: In a largely AA ESRD population on HD low A and PA are strongly associated with CHF whereas CRP, high P and low B are less predictive of CV events or death.

PUB621

Protein Intake and Metabolic Acidosis in Stages 3 and 4 of Chronic Kidney Disease Larissa Rodrigues Neto Angeloco, Miguel Moyses-Neto, Elen Almeida Romao, Paula Garcia Chiarello. *Internal Medicine, Ribeirao Preto Medical School – Univ of São Paulo, Ribeirao Preto, Sao Paulo, Brazil.*

Background: Metabolic acidosis is a common complication in Chronic Kidney Disease (CKD) and a factor that can produce negative effects on the energetic-proteic nutritional status. The aim of this study was to investigate whether protein intake interfere with acid-base balance in stages 3 and 4 CKD.

Methods: Anthropometric measures (weight, height), laboratory analysis (creatinine, serum bicarbonate, urine pH) and estimated protein intake using protein nitrogen appearance (PNA) were evaluated in patients with CKD stages 3-4 in a High Complexity Hospital. The glomerular filtration rate (GFRe) was estimated using the Modification of Diet in Renal Disease formula. Metabolic acidosis was defined as serum bicarbonate < 22 mEq/L. Data are reported as means+SD and Spearman's correlation was used to measure associations between variables, considering p <0.05.

Results: The sample consisted of 48 patients, where 60% were male. Most patients (74%) were in stage 3 CKD. A median of 36.3 ml/min/1,73m^2 for GFRe was observed (ranging 15.8-59.5 ml/min/1,73m^2). The mean age of patients was 56.8±8.6 years. We found an average of 29.1±5.9 kg/m^2 for the Body Mass Index and 66% were overweight. The average PNA was 0.98±0.3 g protein/kg/day (ranging from 0.45 and 1.93g protein/kg/day), indicating that 65% of patients had a protein intake above the KDOQI recommendation for this population. Metabolic acidosis was present in 17% of cases. Serum bicarbonate levels were not correlated with PNA or GFRe and there was a positive correlation between serum bicarbonate levels and pH urine (r = 0.46, p = 0.001).

Conclusions: Most patients were overweight, especially patients in stage 3 of CKD, and although no correlation was observed between protein intake and acid-base balance, the total protein intake was above recommendation for this group. Further details about quality of this protein, regarding vegetable source or the acid load of the diet, and its relations with acid-base balance will certainly be useful for guiding diet interventions for a better metabolic control and nutritional status for these patients.

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PUB622

Malnutrition, Inflammation, and Frailty in Hemodialysis Patients Masataka Tsunoda,[1] Ryota Ikee,[2] Naomi Sasaki,[3] Nobuo Hashimoto.[3] *[1]Dept of Nephrology and Dialysis, H.N.Medic Sapporohigashi, Sapporo, Hokkaido, Japan; [2]Dept of Nephrology and Dialysis, H.N.Medic Kitahiroshima, Kitahiroshima, Hokkaido, Japan; [3]Dept of Nephrology and Dialysis, H.N.Medic, Sapporo, Hokkaido, Japan.*

Background: Frailty is a condition in which the individual is vulnerable to develop increased dependency and/or mortality when exposed to a stressor. Sarcopenia is a major cause of frailty and its prevalence is high in hemodialysis (HD) patients. The association between sarcopenia and malnutrition prompted us to examine the relationship between frailty and nutritional markers in HD patients.

Methods: We enrolled 80 patients with HD duration ≥ 1 year in the present study. We made a diagnosis of frailty based on FRAIL scale, which includes simple 5 physical components, and Tilburg Frailty Indicator (TFI), which includes 15 physical, social, and psychological components.

Results: The diagnosis of frailty was made in 18 patients (22.5%) by FRAIL scale and in 50 (62.5%) by TFI. FRAIL scale-based frailty was associated with higher age, female sex, and lower Barthel index. On the other hand, TFI based-frailty was associated with a lower serum albumin level and lower creatinine generation rate. The number of positive components of FRAIL scale and TFI positively correlated (FRAIL scale: r = 0.469, p < 0.001; TFI: r = 0.335, p = 0.002) with malnutrition-inflammation score (MIS). Receiver-operating characteristic analysis showed that when the cut-off value of MIS was set at 4.5, the sensitivity and specificity for detecting FRAIL scale-based frailty were 72.4% and 60.8%, respectively (AUC 0.699). If the cut-off value of MIS was set at 3.5, the sensitivity and specificity for detecting TFI-based frailty were 75.0% and 69.7%, respectively (AUC 0.817).

Conclusions: Frailty was significantly associated with nutritional markers including MIS, although the proportion of the patients who were diagnosed with frailty markedly differed between the 2 sets of frailty criteria.

PUB623

Inflammatory Stress Exacerbates the Progression of Non-Alcoholic Fatty Liver Disease via the Activation of CXCL16/CXCR6 Pathway Kun Ling Ma, Wu Yu, Jing Liu, Yang Zhang, Zebo Hu, Bi-Cheng Liu. *Institute of Nephrology, Southeast Univ, Nanjing City, Jiangsu Province, China.*

Background: CXC chemokine ligand 16(CXCL16) and its receptor CXC chemokine receptor 6 (CXCR6) were recently found to participate in the process of recruiting inflammatory cells and mediating intracellular uptake of oxidative low density lipoprotein (oxLDL). This study aimed to investigate the effects of inflammation on the activation of CXCL16/CXCR6 pathway in non-alcoholic fatty liver disease (NAFLD).

Methods: Sixteen apolipoprotein E knockout mice were fed with the Western diet for 8 weeks in the absence or presence of 10% casein injection (casein) to induce an inflamed NAFLD model *in vivo* and *HepG2* cells were treated by IL-1β or with CXCL16 siRNA *in vitro*. The effects of inflammatory stress on lipid accumulation in liver were evaluated by hematoxylin-eosin staining and Filipin staining and in HepG2 cells were examined by Oil red O staining and a quantitative assay of intracellular cholesterol. The protein expression of CXCL16, CXCR6, human a disintegrin and metalloprotease 10 (ADAM10), fibronectin, and collagen type I in tissues and cells was checked by immunohistochemical staining, Real-time polymerase chain reaction (PCR), and Western Blot.

Results: Serum levels of lipid profile (total cholesterol and triglyceride, LDL and high density lipoprotein) in flamed mice were significantly decreased compared to the controls. However, inflammatory stress increased lipid accumulation in liver and HepG2 cells. Furthermore, inflammatory stress increased the protein expression of CXCL16, CXCR6, and ADAM10 in livers, accompanied with increased the expression of fibronectin, collagen type I, α-SMA, and decreased E-cadherin. These effects were further confirmed by *in vitro* studies.Further analysis showed that the activation of CXCL16/CXCR6 pathway was correlated with lipid accumulation and the production of extracellular matrix.

Conclusions: Inflammatory stress induced lipid accumulation and extracellular matrix excretion and exacerbated the progression of NAFLD via activation of CXCL16/CXCR6 pathway.

PUB624

Nutritional Status Assessment in Patients on Maintenance Hemodialysis at a Tertiary Care Dialysis Center Syed Rizwan Bokhari,[1] Mirza Faizan Ali,[1] Hafiz I. Ahmad,[1] Javeria Ikram,[1] Muhammad Zaman Khan Assir,[1] Arif Asif.[2] *[1]Dept of Nephrology, Allama Iqbal Medical College/ Jinnah Hospital, Lahore, Pakistan; [2]Div of Nephrology and Hypertension, Albany Medical College, Albany, NY.*

Background: Malnutrition is commonly seen in dialysis population and is associated with significant morbidity and mortality. Nutritional assessment is a neglected area in patients undergoing hemodialysis in developing countries. We studied the frequency of malnutrition in our patients and its associations with several factors.

Methods: All 58 ESRD patients on maintenance hemodialysis (HD) in our dialysis unit were considered for this cross sectional study, 9 patients refused to participate. The nutritional status was assessed by a predesigned questionnaire including subjective global assessment (SGA), a semi-quantitative scale for estimating nutritional status. Anthropometric measurements, peripheral neuropathy and pertinent laboratory parameters were checked. The duration on HD of the study patients ranged between 3 months to 10 years (mean 4 ± 1.5 years).

Results: Of these 49 patients, 26 (53%) were males with a median age 45 (25-76) years. Fifteen patients (31%) were well-nourished and 34 (69%) were under-nourished including 9 (19%) patients classified as severely malnourished according to SGA. Malnutrition appeared more prevalent in males 80% versus 56%., however, this did not reach statistical significance (p-value 0.063). On univariate and multivariate analysis, no significant between-group differences were found across well-nourished and malnourished patients in terms of age, BMI, calorie count, duration and frequency of dialysis, dry weight, inter-dialytic weight loss or gain in last 6 months, body fat percentage, serum albumin, blood pressure, intra-dialytic hypotension,URR, Kt/V, peripheral neuropathy and co-morbidities. Psychosocial factors were identified in 24 (49%) patients with 19 (79%) having some degree of malnutrition but the finding did not reach the statistical significance.

Conclusions: In our cohort, 2/3rd of the participants had malnutrition. Surprisingly, the traditional factors studied in previous trials have not shown any significant association to malnutrition in our study.

PUB625

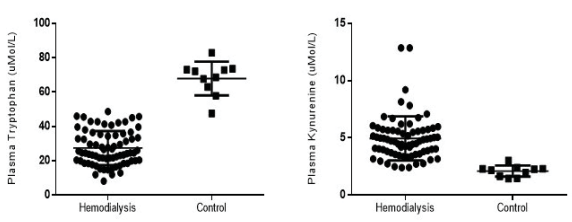
A Pilot Study on the Relationship between Tryptophan, Its Metabolites, Sleep, Depression and Fatigue in Hemodialysis Patients Rakesh Malhotra,[1,5] Vanja Persic,[5] Weifang Zhang,[2] Garry J. Handelman,[2] Laura Rosales,[5] Mark L. Unruh,[3] Fredric O. Finkelstein,[4] George A. Kaysen,[5] Peter Kotanko.[5] *[1]VUMC; [2]U Mass; [3]U New Mexico; [4]Yale; [5]RRI.*

Background: Sleep and mood disorders are common in hemodialysis (HD) patients. Tryptophan (TRP) and its metabolites play prominent roles in neural pathways related to sleep, fatigue, and depression. Here we report levels of TRP and its metabolite kynurenine (KYN) and their association with sleep, non-physical fatigue and depression in HD patients.

Methods: In this prospective study we measured pre-dialysis levels of TRP and kynurenine (KYN) by high-performance liquid chromatography (HPLC). We performed a concurrent survey which included 3 self-reported questionnaires: Medical Outcomes Study Sleep Scale (MOS-Sleep); PROMIS Short form Fatigue 8a questionnaire (PROMIS-F); and Patient Health Questionnaire (PHQ-9). Spearman rank correlation coefficient (r_s) was used to assess correlations.

Results: We enrolled 72 HD patients (age 60.0±15.6 yrs, 62% male). Compared to healthy controls, TRP levels were decreased (27.3±10.0 µmol/L) and KYN levels were elevated (4.9±1.9 µmol/L) in HD patients. MOS-sleep scores confirmed poor sleep quality. PROMIS-F T score was 54.0±12.2, indicating a trend towards moderate fatigue. PHQ-9 score was 6.3±5.6, revealing the presence of mild depression. We found negative correlation between TRP levels and sleep disturbances. A positive correlation was found between CRP, KYN and depression severity. TRP and KYN levels were not correlated with indicators of fatigue (r_s=.101 and 0.184, respectively).

A



B

	Correlation Coefficient	TRP	Kyn	CRP
Sleep Problem Index		-.307**	-.199	.332*
PROMIS Fatigue T score		.101	.184	.102
PHQ-9 score		.248***	.232***	.238***
* P<.005; ** P <.01, *** P<.05				

Conclusions: Our study indicates disturbed metabolism of the essential amino acid TRP, poor sleep health, fatigue, and depressive symptoms in HD patients. Low TRP levels relate with sleep disturbances. A larger sample size is necessary to explore the potential spectrum of interactions between TRP status and patient reported outcomes.

PUB626

Gut Microbiota Lactbacillus Protects against the Progression of Renal Impairment through the Modulation of Gut Environments in Rats Ayumi Yoshifuji, Shu Wakino, Junichiro Irie, Kazuhiro Hasegawa, Koichi Hayashi, Hiroshi Itoh. *Internal Medicine, Keio Univ, Tokyo, Japan.*

Background: Gut microbiota has been shown to play some roles in the pathogenesis of various diseases and was altered in CKD condition. We previously reported that Lactbacillus (Lact) decreased in CKD rats and oral activated charcoal adsorbent AST-120 (AST) improved renal function with the concomitant recovery of Lact. In this study, we elucidated the significance of Lact in the pathogenesis of CKD.

Methods: Six-week-old spontaneously hypertensive rats (SHR) were rendered CKD by 5/6th nephrectomy (Nx). The rats were divided into five groups and kept for 8 weeks; sham-operated SHR (SHR), SHR with Nx (Nx), Nx given AST(Nx+AST), Nx given Lact (Nx+Lact,), and Nx given AST plus Lact (Nx+AST+Lact). AST was orally given at a dose of 4g/kg/day and Lact at $1*10^{10}$ CFU/kg/day. The gut flora population was analyzed by T-RFLP and RT-PCR. Next, we administered antibiotics for the eradication of microbiota to Nx rats and kept for 8 weeks with or without the supplementation of Lact. Gut permeability was assessed by serum Diamine oxidase (DAO) levels. Human colon cell line, Caco-2 cells were treated with uremic toxin precursor, indole in the presence or absence of Lact or OxPAPC, an inhibitor of toll-like receptor 2 (TLR2) and TRL4 recognizing Lact.

Results: The decrease in Lact in Nx was restored in Nx+AST, Nx+Lact, and Nx+Lact+AST. Both serum indoxylsulfate and serum IL-6 increased in Nx. These increases were ameliorated in Nx+AST, Nx+Lact, and Nx+AST+Lact, although the additive effects by the combination therapy were not observed. The decrease in the tight junction proteins Occludin and Zo-1 in Nx were mitigated by AST and/or Lact. The elevation of serum DAO in Nx was mitigated by Lact. In the germ free condition, Lact supplementation decreased the urinary protein excretion of Nx rats. In Caco-2 cells, the downregulations of Occludin and ZO-1 by indole were ameliorated by Lact, which effects were attenuated by OxPAPC.

Conclusions: The recovery of Lact in the gut of CKD rats improved the gut environment and systemic uremic condition. This probiotic therapy can provide novel therapeutic strategy against the progression of CKD.

Funding: Government Support - Non-U.S.

PUB627

Inflammation and Quality of Life in Hemodialysis: A Cross Sectional Study Marcelo Rodrigues Bacci, Daniela Gimenes Grilli, Andson Jesus Santos, Renato R. Bertagna, Fernando R. Adami, Winter R. Figueiredo, Victor Couto Rosa Jordão, Felipe R. Bruniera, Felipe Moreira Ferreira, Fernando Luiz Affonso Fonseca. *General Practice, Faculdade de Medicina do ABC, Santo Andre, Sao Paulo, Brazil.*

Background: CKD is highly prevalent worldwide.Pacients with CKD on hemodialysis are more likely to present behavioral changes and worse quality of life as a result of their routine and complications.They have also higher levels of cytokines.The aim of this study is evaluate the inflammatory profile in relation to their quality of life measured by the KDOQL-SF36 in hemodialysis out-patients.

Methods: We included patients on hemodialysis for at least 6 months,aged 21 years with a week regular basis of treatment.The exclusion criteria was existence of previous depression,cancer,infection or antibiotic usage at the time of inclusion and hospitalization for the previous 30 days.It was evaluated anthropometric parameters and serum inflammatory markers:interleukin6(IL6),high sensitive C-reactive protein,tumour necrosis factor alpha(TNF-@)and homocystein.Quality of life was acesses with the KDOQL-SF36 questionnary by the same interviewer for every patient.Samples were collected in the first session of the month before the initiation of dialysis.Analysis of data distribution was made with Shapiro-Wilk test.The alpha level was 5%.Pearson correlation was used to analyze the relationship between inflammatory parameters and quality of life.

Results: Thirty patients consented to answer the KDOQL-SF36 and participate in the study.Demographic data are shown in table 1.Homocystein levels were correlated with worse GFR and creatinine(p=0.003;p=0.002).IL6 was not correlated with worse nutritional status taking into account BMI(p=0.83)in contrast to TNF-@ which was positively correlated with albumin(p=0.008), nutritional status by BMI(p=0.04)and nutritional status taking into account the circumference area of the arm(p=0.04).IL6 was correlated with activity limitation(p=0.02) and homocystein with working condition(p=0.04).

Conclusions: Homocystein is related with nutritional status and inflammatory markers. In this population the majority of sections in the KDOQL-SF36 were not correlated with the levels of cytokines.

PUB628

Uric Acid and Carotid Intimal Media Thickness Associated with Kidney Dysfunction in Rheumatoid Patients Suad Ma Hannawi,[1] Issa A.L. Salmi.[2] *[1]Dept of Medicine, MOH Hospital, Dubai, Dubai, United Arab Emirates; [2]Dept of Medicine, MOH Hospital, Muscat, Oman.*

Background: Rheumatoid arthritis(RA) patients develop kidney dysfunction(KD) over time. Cardiovascular disease(CVD) and associated factors play a major role. In addition, RA with KD may lead to an increase in morbidity from CVD progress. Uric acid(UA), is a metabolic product of purine metabolism, that may function as an antioxidant, linked to CVD in general population and in RA, even after adjustment for traditional CVD risk factors. Also, UA is an independent predictor of KD in RA. The association of UA and cIMT(an independent predictor of CVD in RA) had never been investigated in RA. We recorded UA and studied cIMT to determine whether UA is correlated with CVD in RA.

Methods: 36 patients, aged 44.7 yrs(min 24, max 73) satisfying 1978 ACR criteria for RA diagnosis were included. UA was related to demographic factors, disease activity variables, established cardiovascular risk factors [i.e., cholesterol, high density lipoproteins, low density lipoproteins, triglycerides---etc] and common cIMT; measured by carotid duplex scanning.

Results: A simple linear regression shows that UA is positively correlated with age(p=0.02,CI 0.00,0.02). The correlation remained significant after adjustment for sex(p=0.033,CI 0.001,0.024). UA was positively correlated in linear relationship with cIMT(p=0.031,CI 0.113, 2.26), and negatively with high density lipoprotein(p=0.017,CI -0.569, -0.060). Creatinine and glomerular filtration rate, were correlated significantly with the level of UA(p=0.000 for both). Urine microalbumine and urine albumin creatinine ratio, another marker of KD, showed a significant positive linear relationship(p=0.037 and 0.024, respectively). Multiple linear regression model confirmed the significant positive relation with age(p=0.018),cIMT(0.024) and GFR(p=0.000), with R-squared =0.59.

Conclusions: This study shows for the first time that atherosclerotic burden as manifested by cIMT is positively associated with increase in UA in RA patients. Data suggest that increase in cIMT predicts presence of underlying KD in RA and its value is a good non-invasive tool to screen for predicting KD.

Funding: Government Support - Non-U.S.

PUB629

Cross-Sectional Examination of Metabolites and Metabolic Phenotypes in Uremia Sahir Kalim,[1] Clary B. Clish,[2] Joseph James DeFerio,[1] Guillermo Ortiz-Sanjuan,[1] Ravi I. Thadhani,[1] Eugene P. Rhee.[1,2] *[1]Nephrology Div, Massachusetts General Hospital, Boston, MA; [2]Broad Institute, Cambrdige, MA; [3]Cardiology Div, Massachusetts General Hospital, Boston, MA.*

Background: Although metabolomic approaches have begun to document numerous changes that arise in end stage renal disease (ESRD), how these alterations relate to established metabolic phenotypes in uremia is unknown.

Methods: We used partial least squares discriminant analysis to identify metabolites that best discriminate individuals with or without diabetes, and across tertiles of body mass index, serum albumin, total cholesterol, and systolic blood pressure among 200 incident hemodialysis patients.

Results: Our data do not recapitulate metabolomic signatures of diabetes and obesity identified among individuals with normal renal function (e.g. elevations in branched chain and aromatic amino acids) and highlight several potential markers of diabetes status specific to ESRD, including xanthosine-5-phosphate and vanillylmandelic acid. Further, our data identify significant associations between elevated tryptophan and long-chain acylcarnitine levels and both decreased total cholesterol and systolic blood pressure in ESRD. Higher tryptophan levels were also associated with higher serum albumin levels, but this may reflect tryptophan's significant albumin binding. Finally, an examination of uremic retention solutes captured by our platform in relation to 24 clinical phenotypes provides a framework for investigating mechanisms of uremic toxicity.

Conclusions: In sum, these studies leveraging metabolomic and metabolic phenotype data acquired in a well-characterized ESRD cohort demonstrate striking differences from metabolomics studies in the general population, and may provide clues to novel functional pathways in the ESRD population.

Funding: NIDDK Support, Private Foundation Support

PUB630

Diet Record Could Not Predict the Real Protein Intake in Chinese CKD Patients with Low Protein Diet Xiaoyan Peng,[1] Limeng Chen,[1] Hailong Li,[2] Yan Qin,[1] Wei Zhang,[1] Wei Chen,[2] Xue-Mei Li.[1] *[1]Dept of Nephrology, Peking Union Medical College Hospital, Beijing, China; [2]Dept of Parenteral and Enteral Nutrition, Peking Union Medical College Hospital, Beijing, China.*

Background: Dietary protein restriction acts as a basic therapy for patients with chronic kidney disease (CKD). The purpose of this study is to identify the compliance of low protein diet education by nutritionist. And to figure out whether daily diet record could present the amount of protein intake.

Methods: The CKD (stages 3b to 4) patients(n=50) were enrolled in this prospective study in PUMCH, Beijing, China. They were recommended and educated to take a low protein diet(0.4-0.6g/kg BW) supplemented with α-keto acid. Diet protein intake was calculated from 24h-urinary urea using Maroni's formula and according a 3-day diet record by the nutritionist. Clinical data, blood and 24h urine samples were taken at baseline, 2wks, 6wks, 10wks and 14wks.

Results: The mean age was 49±13 years old, 46% were male. After 14-weeks study, the overall protein intake didn't change (0.77±0.2 versus 0.77±0.17 g/kg/d), but during the following-up period at different time the level of protein intake fluctuated slightly. At 6 weeks, the intervention of dietary protein reached the lowest, but then slowly began to pick up. There were no statistically significant changes in eGFR (30.2±13.4 versus 31.5±14.3), serum creatinine(221.5±96.9 versus 218.5±97.5), 24h proteinuria(median 0.8 versus1.0), Mean arterial blood pressure (92.8±10.8 versus 91.6±11.9), Hemoglobin(125.7±18.0 versus 127.8±17.3) and serum albumin(44.2±3.0 versus 44.7±2.6). Diet record surely correlated with actual protein intake(r=0.376,*P*<0.001), but is much less than the latter (0.46 ± 0.15 versus 0.77±0.21, P<0.001). Significant correlation between the changes of diet protein and proteinuria were observerd (r=0.334, P<0.001), as well as the changes of serum urea (0.404,P<0.001) and phosphorus(r=0.224,P<0.003).

Conclusions: Despite the regular intervention of nutritionists, compliance of low protein diet varies, and the diet record couldn't correctly reflect protein intake. Daily protein intake correlated with the change of proteinuria, serum urea and phosphorus.

Funding: Government Support - Non-U.S.

PUB631

The Relationship between Protein Energy Wasting and Fluid Status in Hemodialysis Patients under Invasion Phase Tomofumi Fushima,[2] Mariko Miyazaki,[1] Tae Yamamoto,[1] Takefumi Mori,[1] Nobuyuki Takahashi,[2] Hiroshi Sato,[2] Sadayoshi Ito.[1] *[1]Blood Purification, Tohoku Univ Hospital, Sendai, Japan; [2]Graduate School of Pharmaceutical Sciences, Tohoku Univ, Sendai, Japan.*

Background: The protein energy wasting(PEW) is well documented during maintenance hemodialysis(HD), however, little is known about the management strategy under the critical complication.

Methods: We conducted cross-sectional observational study for 72dialysis inpatients to treat their complications (M/F=49/23, 63±13 years old). We analysed the physical measurement, subjective global assessment(SGA), bioimpedance spectroscopy (InBody10), and laboratory markers for uremia, anemia, nutrition, and inflammation.

Results: Pre-HD ALB were 2.8 ± 0.4 g/dL. The balance of post versus pre-HD ALB(ΔALB) was positive in 53 patients. In 30 of them showed over 40% of post-HD Extra Cellular Water(ECW)/Total Body Water(TBW). Totally, patients' fluid status were classified to 4 groups using cut-off ΔALB and 40% of ECW/TBW.

	A (N=23)	B (N=7)	C (N=12)	D (N=30)
ECW/TBW, Post-pre HD Alb	**<40, ≥0**	**<40, <0**	**≥40, <0**	**≥40, ≥0**
CVD	13(52.0%)	1(14.3%)	5(35.7%)	17(68.0%)
SGA=2	1(5.0%)	0(0.0%)	3(28.6%)*	10(37.5%)*
GNRI	93.2±1.9	90.5±3.5	84.9±2.4*	81.1±1.8*
Grip,kg	25.5±1.5	22.0±2.9	18.5±1.9*	15.0±1.5*
Cr mg/dL	9.9±0.6	8.2±1.0	6.4±0.7*	6.9±0.6*
BUN/Cr	5.1±0.4	5.4±0.9	6.7±0.6	7.8±0.4*
Hb g/dL	11.2±0.3	10.1±0.6	9.5±0.4*	9.7±0.3*
TIBC μg/dL	248.4±10.8	247.1±20.5	210.5±14.5	199.9±11.1*
Ferritin ng/mL	94.5±75.9	149±1 4 0	343.8±96.0	399.2±75.9*

Table 1. Fluid status and clinical marker of nutrition, muscle, and anemia

*P<0.05 vs A ANCOVA, adjusted by gender, age, dialysis vintage

The decreased plasma vollume but overhydration (group D) showed malnutrition, muscle weakness, and anemia with disorder of iron utilization.

Conclusions: The fluid status difined by bioimpedance spectroscopy and ALB balancet crossly linked PEW under the critical complecation for dialysis patients. They requires intensive nutrition and fluid management to better outcome.

PUB632

Transient Late Onset Neutropenia (LON) in an Adult Patient on Peritoneal Dialysis (PD) following Treatment of Resistant Nephrotic Syndrome with Rituximab Fabrizio Grosjean, Maria Lucia Scaramuzzi, Giuseppe Sileno, Alessandra Manini, Gianluca Marchi, Pasquale Esposito, Filippo Mangione, Gianluca Fasoli, Antonio Dal Canton. *Nephrology,Dialysis,Transplantation, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.*

Background: LON has been described after Rituximab treatment for hematologic tumors and rheumatologic diseases. There are only anecdotal cases reported, mainly paediatrics, following Rituximab treatment for resistant nephrotic syndrome, none of them described in patients with ESRD.

Methods: Case description: A 30 year old female patient with steroid resistant nephrotic syndrome secondary to FSGS was treated with Rituximab 375 mg/1,73 m^2 administered weekly for 4 weeks. Because of progressive worsening of kidney function associated with refractory nephrotic syndrome and oliguria she started hemodialytic treatment, subsequently she was shifted to PD without any major complications. Leucocyte count and formula were normal for 11 weeks after the last Rituximab administration (WBC 4420/μl, NEU 2100/μl). Of note haemoglobin levels were kept above 11 g/dl with ESA and the platelet count was constantly normal. Blood count exam performed to evaluate ESA adjustment surprisingly showed severe neutropenia (WBC 3170/μl, NEU 100/μl)at 13 weeks after the last Rituximab administration. EBV and CMV infection were excluded. Neutrophils progressively and spontaneously increased to normal values after 1 week (WBC 7100/μl, 3500/μl). Our patient had no major infectious complications, she underwent successfully two short cicles of oral fluorochinolon treatment (5 days) for a submandibular localized suppurative infection of the skin with palpable reactive regional swollen lymph nodes.

Conclusions: Because Anti-CD20 therapy is increasingly used in the treatment of various patterns of nephrotic syndrome in adults and children, Nephrologists should be aware of the possible onset of LON. Because of its late onset patients must be carefully monitored especially in case of evolution of the kidney disease toward ESRD requiring dialysis, a condition known to induce immunodepression.

PUB633

Thrombotic Microangiopathy Associated with Intravenous Reformulation of Opana Ruth Schreiber, Ryan Hunt, Tal Schiller, Andrew Wu, Chava Kimchi-Sarfaty. *Laboratory of Hemostasis, Div of Hematology, FDA, Bethesda, MD.*

Background: Thrombotic microangiopathy (TMA) have been reported in association with the intravenous abuse of Opana,an opioid which was reformulated to extend the duration of response and prevent adulteration and non-oral administration.

Methods: 1. A Pubmed search was performed to identify reports describing Opana abuse complicated by TMA. Key words: Opana, TTP,TMA. Demographic and clinical characteristics were recorded.**2.** PEO, the excipient of Opana was introduced at various concentrations and time periods to ADAMTS13 expressing HEK293T cells. Expression levels were analyzed using Western blotting and ADAMTS13 specific activity was calculated.

Results: Demographic and clinical characteristics Among the 37 patients described in 7 reports, 15(40.5%) were women. All reported cases were young white adults, age ranging from 22-52 years, 36 patients admitted abusing intravenous Opana. 32(86.5%) patients presented with acute kidney injury, 3(8.1%) with CNS symptoms, 3(8.1%) with respiratory distress and 15(48.6%) with infection. The serum creatinine ranged from 0.5-14.4mg/dL and pltelates counts ranged from 9,000-128,000/mm^3. Kidney biopsies of 3 patients showed evidence for TMA. 16(43.2%) patients underwent plasmapheresis, 2(5.4%) underwent plasma exchange, 2(5.4%) underwent hemodialysis and 2(5.4%) were treated with the combination of both. The short-term kidney outcomes ranged from CKD1 to 5, with no correlation to treatment modality and no mortality. 15(40.5%) patients were treated for infection (sepsis, pneumonia, cellulitis and endocarditis). 20/28 (71.4%) patients demonstrated normal or above 70% ADAMTS13 activity levels, but the timing of this measurements in relation to therapeutic interventions is not clear.**ADAMTS13 expression and activity levels** at 10 and 24 hours were shown to be unaffected at 0.3, 3 and 30µg/mL of PEO in HEK293T cells.

Conclusions: A correlation between Opana abuse and TMA has been established by repeated reports.The short-term kidney outcome in intravenous Opana abusers appears unclear despite treatment, ranging from CKD1 to 5 .PEO does not affect in vitro expression nor activity of ADAMTS13, in a system of HEK293T cells.

PUB634

Survey to Assess the Use of Smartphones in Dialysis Patients Manisha Singh, Channoah Mckindra Williams, Shree G. Sharma, Michelle W. Krause. *Nephrology, Univ of Arkansas for Medical Sciences, Little Rock, AR.*

Background: Adverse events related to errors in medications and missed dialysis prescriptions during transition of care are frequent .The resulting burden of error on patient welfare and healthcare is significant. We hope to enable the patient to carry with him a tool that can be updated easily and reflects his/her current prescription. As a quality improvement measure and as a patient safety tool, we hoped to introduce an appropriate measure to help our patients. Proposed tools are apps for smart phones, and/or hard copies on the patients (wallets or pocket diaries).

Methods: In order to identify the most effective tool, we undertook the present study of verbal survey of patients on dialysis.We attempt to identify the fraction of patients that are comfortable using smart phones and mobile apps. We conducted a verbal survey of the patients on outpatient dialysis. We asked the patients 1. if they had a smart phone and 2. if they had one, how comfortable were they in using apps for medications.

Results: Out of a total of 56 patients asked, 40 had phones, of which 16 had smart phones and most were comfortable using apps. Our preliminary data shows that approximately 71% (40/56) of patients had phones and approximately 30 %(16/56) of patients on dialysis are currently using smart phones/apps. Of the smart phone users, 14 out of 16 (87%) were comfortable using the apps.

Conclusions: Our preliminary data shows that only approximately 30% of dialysis patients in our outpatient center are currently using smart phones/apps. Therefore, we plan to implement pocket diary /wallet (per patient preference) for our patients in the next phase. We recognize the caveat that our study population is small, so we are expanding the pool to include all patients that we meet on dialysis inpatient, outpatient and at different centers. This simple preliminary study helped us recognize the importance of choosing an intervention which will be most helpful and more relevant to the target population. The study also helped us to focus our resources on developing the pocket diary /wallet. After analyzing the data from the second phase of our study we will focus on developing an app.

PUB635

A Measurement Framework for Driving Performance in Renal Care in Ontario Gihad E. Nesrallah,[1,2] Kelly Woltman,[1] Kiren Handa,[1] Camila Iraheta,[1] Jane Ip,[1] Mary Ann Murray.[1,3] *[1]Ontario Renal Network, Cancer Care Ontario, Toronto, ON, Canada; [2]Keenan Research Cenre, The Li Ka Shing Knowledge Institute, Toronto, ON, Canada; [3]Nephrology, The Ottawa Hospital, Ottawa, ON, Canada.*

Background: The Ontario Renal Plan (ORP), developed through extensive stakeholder engagement, provides a roadmap through which the Ontario Renal Network promotes optimal patient outcomes, experience of care, and sound stewardship of public dollars. An effective and evidence-based measurement framework is central to these efforts, and must be developed *de novo*. The second ORP will launch in early 2015.

Methods: Goals of this methodological framework are: 1) To improve outcomes for Ontario residents with CKD by developing and applying a novel provincial measurement strategy based on clinically meaningful, feasible, scientifically sound, and actionable measures. 2) To create a culture of quality improvement in the Ontario renal community. 3) To minimize data collection and reporting burden for renal programs. 4) To align measurement with quality improvement practices using the knowledge-to-action (KTA) cycle.

Results: We developed a project plan comprised of 8 workstreams: 1) knowledge syntheses to identify outcome, process, and structure variables in each of the clinical domains outlined in the ORP-II; 2) development of a prioritization strategy for selecting the most useful measures; 3) construction of conceptual and statistical models to empirically validate candidate measures using provincial data-sets; 4) finalize measurement sets drawing on the best validated measures; 5) develop primary data collection tools including facility level surveys; 6) alignment of data needs with the The Ontario Renal Reporting System (provincial renal database) development cycle; 7) application of the KTA framework at the regional program and facility level (including KTA gap and barrier management); and 8) a formative and summative evaluation of the measurement framework.

Conclusions: We describe a novel framework for identifying, validating, and measuring quality and performance measures that align with stake-holder identified priorities in renal care in Ontario, and a strategy for incorporating measurement into renal health systems improvement.

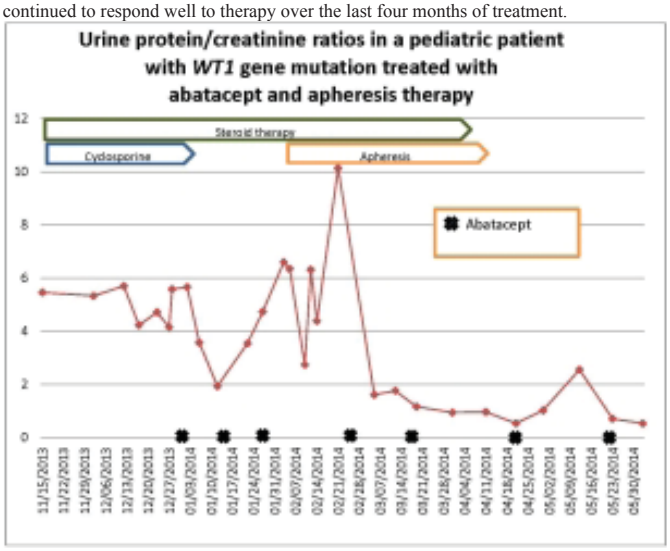
PUB636

Successful Treatment of Collapsing Focal Segmental Glomerulosclerosis Associated with WT1 Mutation in a Pediatric Patient with Combined Abatacept and Apheresis Therapy Robert M. Haws,[1] Deepa Bassi,[2] Syed M. Sajjad.[2] *[1]Pediatric Nephrology, Marshfield Clinic, Marshfield, WI; [2]Pathology, Marshfield Clinic, Marshfield, WI.*

Background: Wilms Tumor 1 (WT1) gene mutations are associated with a variety of childhood glomerulopathies. WT1-associated renal diseases respond poorly to immunosuppressive therapy and usually culminate in end stage renal disease. Cytotoxic T lymphocyte-associated antigen 4-immunoglobulin fusion protein (Abatacept), a B7-1 (CD80) inhibitor is posited to stabilize ß1-integrin activation in podocytes and reduce proteinuria in patients with glomerular disease. We report the first successful treatment of WT1-associated steroid resistent nephrotic syndrome (SRNS) with combined abatacept and apheresis therapy.

Methods: Focal segmental glomerulosclerosis (FSGS) with collapsing features on renal biopsy was identified in a 12 year old female with SRNS. Genetic testing revealed a WT1 gene mutation in exon 8 unique to the patient and not present in either parent. Severe combined hyperlipidemia (TC 703 mg/dl and TG 1238 mg/dl) despite pharmacologic therapy was treated with apheresis. Abatacept administered at a dose of 500 mg intravenously on day 0, 15, 30 and monthly thereafter combined with 19 apheresis treatments resulted in resolution of nephrotic range proteinuria and severe hyperlipidemia.

Results: Abatacept treatment over a six month window in conjunction with apheresis was associated with a clinically significant decline in proteinuria in the patient. The patient continued to respond well to therapy over the last four months of treatment.



Conclusions: This is the first report of response to combined abatacept and apheresis therapy in a patient with a WT1 gene mutation presenting with FSGS. The treatment holds promise for patients with SRNS, potentially delaying or negating the need for renal transplantation.

PUB637

The Pediatric Renal Replacement Therapy: The Fitted Pumps and Catheters Francesco Garzotto, Paolo Armignacco, Anna Lorenzin, Mauro Neri, Claudio Ronco. *Nephrology-IRRIV, St. Bortolo Hospital, Italy.*

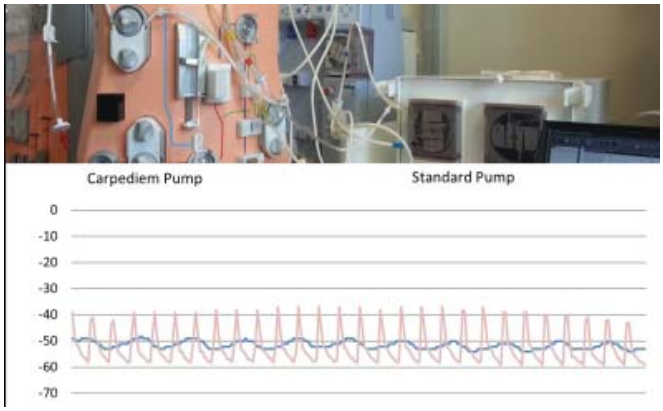
Background: Provision of CRRT to small children require particular procedures and prone patients to additional risks.Catheterization with larger catheters may impair venous drainage and thrombosis;in addition circuit survival is shorter.Functional survival of the circuit increased significantly using larger catheters.The aim of our study was to evaluate

the different caractheristics of the arterial pressure when different pumping system, carpe diem(CArdioRenalPEdiatricDIalysisEmergencyMachine)and adults pumps,are conected to a small catheter.

Methods: To evaluate the trend of the arterial pressure induced by the 2 pumps connected to small catheters(4FR 5cm and 5FR 6cm)we utilized a dedicated instrument with a sample frequency of 1s.

Results: The fluid trapped between two rollers form a pillow of fluid.Alternating pillows and voids cause fluid flow to be pulsated,rather than smooth and continuous.As show in figure 1 for the 5 Fr catheter,the amplitude of the oscillations for 15 ml/min of Blood Flow where 22 mmHg(-50.2±6.6)(-5 to-36) for the Standard pump and 4mmHg(-51.4±0.7)(-53to-49)for the carpediem pumps.Table 1 sumarize the results for the 4 FR catheter at different flows.

Conclusions: Circuits connected to small catheters and standard adults pumps show a wide spike of pressure, not present with small pumps.This phenomena increase capillary shear forces favoring the reduction on protein layering,decreasing membrane clotting with a prolongation of filter life.Per contro a worst negative effect is the increased stress on the proximal portion of the dialyzer causing clotting.Concluding the small pump is adequate to drive blood into a thin catheter avoiding dangerous spike and,in consequence,favor the circuit survival



Qb ml/min	Standard av (min - max) mmHg	Carpediem av (min - max) mmHg
10	-18.9 (-26 -6)	-17.1 (-20 -15)
15	-50.6 (-52 -49)	-63.4 (-71 -52)
30	-178.3 (-184 -169)	-159.5 (-161 -152)

Funding: Private Foundation Support

PUB638

Eculizumab Use in the Treatment of Atypical Hemolytic-Uremic Syndrome: A Pediatric Patient Case Series from the United Arab Emirates

Gurinder Kumar, Omar Nihad Al Masri. *Pediatric Nephrology, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates.*

Background: Atypical hemolytic-uremic syndrome (aHUS) is a rare and life threatening disease. It results from uncontrolled alternative complement pathway activation resulting in thrombotic microangiopathy(TMA). Prognosis had been poor using plasma as the majority of patients (pts) ended up with significant renal morbidity or death. Eculizumab (Ecu) has changed the course of aHUS.

Methods: We report our experience of 7pts with aHUS in our centre.

Results: Age at diagnosis and time from diagnosis to initiation of Ecu are shown in Table.

	Patient						
	1	2	3	4	5	6	7
Age at aHUS onset (months)	5	24	3	3	8	35	22
Time from diagnosis to Ecu initiation (months)	35	2.5	72	72	2	8.1	0.25
Number of TMA manifestation pre Ecu/post Ecu	2/0	1/0	2/0	6/2	1/0	1/0	1/0
Dialysis pre Ecu	No	Yes	Yes	No	Yes	No	No
Dialysis post Ecu	No	No	No	No	Yes	No	No
Antihypertensives	2	3	3	4	1	1	0
Lab parameters(at initiation of Ecu and after 12 and 24 months Ecu)							
Protein /creatinine ratio	3.0 6.95 6.68	0.19 0.6 0.57	3.6 1.56 0.57	1.73 2.78 2.91	- - -	0.66 0.68 0.80	3.3
eGFR (mL/min/1.73m²)	2/0 182 258	35 62 78	93 71 73	63 71 73	5 7 -	11.35 88.64 122	43.63

On initial referral to our clinic, all pts were managed with plasma. 4/7 had serious renal dysfunction. # 4 had received Ecu while resident abroad, but discontinued upon arriving in UAE. Ecu was reinstated after a pause of 5 months. Duration of Ecu administration ranges from 1–46 months. All pts except #5 had significant renal recovery. Dialysis was discontinued in #2 and #3. There were no TMA manifestations during Ecu treatment except for # 4 who had TMA manifestations after initiating Ecu, first after interruption of therapy and a second episode due to inadequate dosing probably due to significant proteinuria. No pts experienced adverse events during Ecu treatment. We were able to discontinue treatment successfully in #6 after 1 yr (with no identified genetic mutation).

Conclusions: The long-term efficacy of Ecu in treating aHUS was confirmed in our cohort and treatment was well tolerated. Despite recovery in eGFR, most pts continue to require antihypertensive medication and have variable degree of proteinuria. Outcomes were found to be dependent on factors like early initiation of Ecu and proper dosing schedule.

PUB639

Successful Treatment with Eculizumab in a Plasma Exchange-Dependent Child with Persistent Complement Factor H Autoantibodies

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Background: Atypical hemolytic-uremic syndrome (aHUS) is a rare, life-threatening disease characterized by systemic thrombotic microangiopathy (TMA). Prognosis is poor; despite intensive plasma-exchange (PE), >60% of patients die, require dialysis or sustain permanent renal damage. aHUS is caused by chronic complement activation, caused by complement protein mutations or autoantibodies. We report long-term follow-up of a 9 year-old girl who presented in 2009 with aHUS due to CFH autoantibodies.

Methods: Initial investigations revealed low hemoglobin (Hb; 8.2 g/dL) and platelet (plt) count ($30x10^9$/L), and high serum creatinine (sCr; 250 µmol/L) and lactate dehydrogenase (LDH; 36.9 µkat/L, range 1.2-3.5 ukat/L). aHUS was diagnosed and initial clinical improvement was seen with PE. Several attempts to withdraw or reduce frequency of PE led to severe manifestations of TMA. Weekly PE for 12 months controlled symptoms but progressive renal decline (eGFR; 70 mL/min/1.73m^2), proteinuria and hypertension (multiple antihypertensive drugs necessary) with left ventricular hypertrophy was evident. Persistently low serum C3 (0.4 g/L) was noted and immunogenetic analysis revealed a homozygous CFHR1 deletion and CFH autoantibodies (788 AU/mL).

Results: In May 2012 eculizumab (Ecu) was initiated (900 mg qw x4, 1200 mg q2w) and PE was discontinued 1 month later. After ~4 months on Ecu, Hb (12.6 g/dL) and plt count ($287x10^9$/L) were normal and eGFR increased to 85 mL/min/1.73m^2. CFH autoantibodies were 408 AU/mL. At last follow-up, March 2014 (22 months of Ecu), Hb was 13.3 g/dL, eGFR 88 mL/min/1.73m^{2}LDH 2.83 µkat/L and plt count $254x10^9$/L, and C3 levels were normal. Blood pressure was 115/70 and controlled with ramipril and amlodipine (5 mg each). No adverse events have been noted, and the child has normal growth and development.

Conclusions: Ecu inhibited TMA in a pediatric patient with aHUS due to CFH autoantibodies and allowed the discontinuation of chronic PE and improved quality of life. Hematological and renal parameters were normalized and maintained long-term.

Funding: Government Support - Non-U.S.

PUB640

Clinicopathological Analysis and Genotypic Features of X-Linked Alport Syndrome in 12 Children

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Background: To analysis the clinicopathological features and genotypic features of children with X-linked alport syndrome(XLAS).

Methods: The clinical features, laboratory and pathological findings, and type IV collagen expression in the kidney were retrospectively analyzed in 12 children, including 11 boys and 1 girls, with XLAS in our hospital between 2008 and 2013.Genetic mutation of COL4A3, COL4A4, COL4A5 were detected using exon trapping-the second generation sequencing technology in 3 families.

Results: In this study, all patients had microscopic hematuria, 6 in 12 patients (50%) presented with nephrotic syndrome, 2 in 12 patients (16.7%) had the intermittent attacks of macroscopic hematuria.Among 12 patients, the small amount of crescents were observed by light microscopy in 3 cases, the weak positive staining for IgG and IgA in mesangial region respectively was showed by Immunofluorescence in 2 cases, and the small amount of dense deposition in mesangial region was presented by electronic microscopy in 2 cases. Renal immunofluorescence examination showed that 2 in 11 male patients had capsular expression of COL4α5, and 1 female patients had segmental expression of COL4α3 and COL4α5 in glomerular basement membrane (GBM). Two glycine replacement missense mutations were identified in COL4A5 gene in 2 families, including Gly 515 Arg and Gly 539 Val, which were not reported previously. One nonsense mutation of Arg373Ter was found in COL4A5 in one girl with NS for four years, who have renal failure family history, showing segmental staining of COL4α3 and COL4α5 in GBM.

Conclusions: XLAS patients may partly accompany with the pathological immuno-inflammatory injury. Heterozygous XLAS females in the nonsense mutation shoud not be considered to be benign carriers.

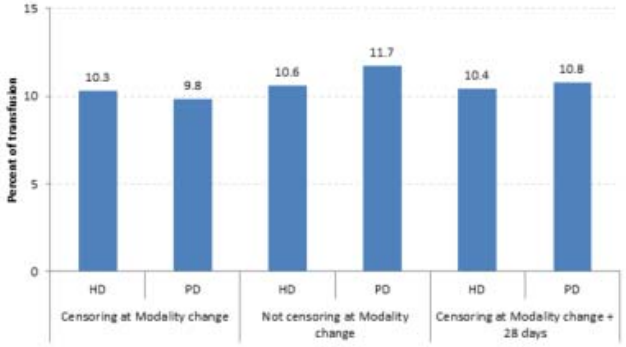
PUB641

Calculation of Transfusion Rates in Peritoneal Dialysis and Hemodialysis Patients Yi Peng,[1] David T. Gilbertson,[1] Deborah Kim,[2] Keri Monda,[2] Brian D. Bradbury,[2] Allan J. Collins.[1] *[1]Chronic Disease Research Group, Minneapolis Medical Research Foundation, Minneapolis, MN; [2]Amgen Inc, Thousand Oaks, CA.*

Background: Recent changes in the U.S. dialysis PPS have created incentives for home therapy, particularly PD. Little is known about transfusion use in PD pts, who have little or no blood loss exposure compared with HD pts. We studied transfusion use in PD and HD pts, and compared different methods of calculation.

Methods: Pts receiving dialysis on 1/1/2010 were selected from the Medicare ESRD SAF and followed for up to 6 months. Dialysis modality (HD/PD) was defined as of 1/1/2010. All analyses were censored at the end of 6 months, death, loss to follow-up, or kidney transplant. Three additional censoring schemes were compared: (1) censor or (2) not censor at modality change; (3) censor at 28 days after modality change. Transfusion events were assessed during the follow-up periods.

Results: When follow-up was censored at modality change, a lower percentage of PD than HD pts received a transfusion (9.8% versus 10.3%). However, when follow-up was not censored at modality change, a higher percentage of PD than HD pts received a transfusion (11.7% versus 10.6%). Similarly, with censoring at 28 days post-modality change, a slightly higher percentage of PD pts received transfusions (10.8% versus 10.4%).



Conclusions: Transfusion rates for PD versus HD pts are sensitive to censoring criteria. Censoring at modality change attributes events to the subsequent modality, when, in fact, precipitating events may have begun under the prior modality. A short post-modality change window more appropriately allows attribution of these events, and suggests that transfusion rates in PD pts may be similar to or higher than in HD pts.

Funding: Pharmaceutical Company Support - Amgen

PUB642

Clinical and Physiologic Parameters in Silent "De Novo" Ascitis Developed in PD Patients Javier De Arteaga,[1,2] Fabian Ledesma.[1,2] *[1]Nephrology, Hospital Privado, Cordoba, Argentina; [2]Nephrology, Fundacion Nefrologica de Cordoba, Cordoba, Argentina.*

Background: Silent ascitis developed "de novo" on PD may sometimes be tricky to diagnose, turning physiologic parameters of importance: 4 pts from our historic PD cohort exhibited a sudden and unusually high UF despite a high transport state. Further studies confirmed a previous nondiagnosed hepatic disease. 2 pts entered PD with ascitis. **Objective** to compare fluid and solute transport parameters between cirrhotic-ascitic and non cirrhotic High Transport (HT) pts. **Patients:**We evaluated all of our HT patients (n = 18) followed since 2001. 6 pts are cirrhotic.

Methods: PETS, Minipets and lately UNIPETS are performed yearly and when required extra (UFF) filling an Adequest database.

Results: Cirrhotic (Gr 1) versus non cirrhotic(Gr 2) parameters. Demographic and clinical data.

	Gr1	Gr2	P
N	6	12	-
Age (ys)	58,17 ± 8,26	46,83 ± 15,74	0,12
HVC (+)	33.33 %	16 %	-
KTV (wk)	2,27 ± 0,82	1,90 ± 0,46	0,25
Prot loss in dialysate (gr/vol)	5,99 ± 2,58	9,79 ± 3,34	**0,036**
Serum alb.	3,11 ± 0.55	3,46 ± 0,49	**0.03**
Time PD (ms)	38,40 ± 23,51	25,08 ± 20,60	0,20
D/P Creat 4 hs	0,80 ± 0,03	0,84 ± 0,03	**0,03**
D/P Creat 1 hs	0,60 ± 0,05	0,59 ± 0,12	0.79
DNa T 0 (meq/lt)	131,33 ± 5,57	129,54 ± 2,23	0,33
DNa 60 min (meq/lt)	127,81 ± 5,50	124,54 ± 3,5	**0,14**
Plasma Na (meq/lt)	140 ± 2,86	137,61 ± 3,81	**0,10**
Sodium removal (NAR) 1 hs	75.59 ± 18.06	48,98 ± 27,63	**0.0012**
% FWT	16,83 ± 6,55	35,67 ± 21,24	**0.048**
UF 1 hs (ml)	645 ± 120,29	373,33 ± 172,56	**0,003**
UF 4 hs (ml)	950 ± 265,59	665 ± 139,62	0,089

Conclusions: Cirrhotics have a higher UF and sodium removal (P=0.02). According to a HT state, NA sieving is blunted in both groups (Na difussion correction not done) and this reflects a lower free water transport mostly for cirrhotics (p= 0.04). Daily loss of protein and serum albumin is higher in non cirrhotics (0.03). Sudden UF augmentation, NAR, hypoalbuminemia and lower FWT in HT pts, HVC (+), raises suspicion of "de novo" ascitis.

PUB643

The Association of Preservation Residual Renal Function and Left Ventricular Hypertrophy in Continuous Ambulatory Peritoneal Dialysis Damir Rebic,[1] Senija Rasic,[1] Mirjana Sabljar-Matovinovic.[2] *[1]Clinic for Nephrology, 1Univ Clinical Centre of Sarajevo, Sarajevo, Bosnia and Herzegowina; [2]School of Medicine, Zagreb, Croatia.*

Background: Cardiovascular disease is the leading cause of morbidity and mortality in end-stage renal disease (ESRD) patients on maintenance dialysis. Residual renal function (RRF) has been shown to influence survival of peritoneal dialysis (PD) patients. This study examined the relations between RRF and left ventricular hypertrophy (LVH) before switching on dialysis treatment and observed during 18 months on CAPD treatment.

Methods: A prospective longitudinal study was performed in 50 non-anuric (defined as >200 mL urine output in a 24-hour period) PD patients. Echocardiography, RRF, daily collection of urine and other known risk factors for LVH were determined at study baseline and end of follow-up.Adequacy of dialysis (K_r/V_{urea}) was calculated from weekly total removed urea mass by daily volume of dialysate and urine (K_r) and divided with urea distribution volume (V). RRF was estimated as the mean of renal creatinine clearance (mL/min).

Results: Baseline, in ESRD patients 75 % had LVH, while at the end of follow-up 68%. RRF at the start of the study showed no significant difference between patients with and without LVH, as well as indailycollectionof urine. After 18 months, patients without LVH had better RRF, lower CRP and better Kt/V compared to patients with LVH ($p < 0.001$). Patients with better-preserved RRF not only had significantly higher total Kt/V, but were less anemic and hypoproteinemic, lesser presence of LVH and had a trend towards normal blood pressure ($p<0.05$). Logistic regression analysis showed that the RRF is a negative independent risk factor for the development of LVH. Daily urine collection was independently associated with LVEDD and LA diameter.

Conclusions: This study demonstrated that RRF and LVH are interrelated; they combine adversely to enhance the cardiovascular risk of morbidity in PD patients. Preservation of RRF may result to reduce LVH, subsequently translating to decreased cardiac disease. CAPD in non-anuric ESRD patients the first 18 months has a positive effect on the preservation of RRF and partial regression of left ventricular remodeling.

PUB644

Under (Selective) Pressure: Atypical Mycobacterial Exit Site Infections Associated with Prophylactic Topical Antibiotic Use Bashir El-Khoury, Shubha Ananthakrishnan. *UC Davis Medical Center, Sacramento, CA.*

Background: Peritoneal dialysis is an effective means of renal replacement therapy for many patients with end stage renal disease. Use of prophylactic topical antibiotics at the exit site is often used to decrease peritoneal dialysis related infections. However, its widespread use may select out other less common pathogens. We present 3 cases of rapidly-growing atypical mycobacterial exit site infection (ESI) associated with use of prophylactic topical antibiotics.

Methods: Mr. P is a 52 yo M with DM, HTN, and ESRD on CCPD who presented with erythema and purulent drainage at his catheter exit site. Wound culture was + for AFB, patient started on clarithromycin 500 mg po BID and completed a 6-month course. Mrs. M is a 57 yo F with DM, HTN, and ESRD on CCPD who presented with the same complaints. Culture revealed rapidly growing Mycobacterium, and she was treated with

moxifloxacin 400 mg po QD, azithromycin 500 mg po QD, and linezolid 600 mg po BID for 4 months. Mrs. L is a 57 yo F with DM, HTN, and ESRD on CCPD who presented with similar complaints. Culture demonstrated 4+ AFB. Patient started on moxifloxacin 400 mg po daily and azithromycin 500 mg po daily, continued for total of two week course. All three wound cultures were further identified as Mycobacterium chelonae with clinical resolution and preservation of PD catheters in all cases after treatment.

Conclusions: ESI remains a major cause of morbidity in patients undergoing PD. The routine application of prophylactic topical antibiotics to PD catheter exit sites may select out rare atypical organisms that lead to clinical infection. Recently, there have been numerous reports of atypical mycobacterial ESIs in PD patients who had used gentamicin cream prophylaxis. Here we report a series of three cases associated with use of mupirocin cream. In all 3 cases, clinical resolution was noted and PD catheters could be preserved. There is no uniform guideline on the management of ESI caused by rapidly growing atypical mycobacterium. Early recognition and the development of a uniform treatment strategy may improve morbidity associated with rapidly growing atypical Mycobacterial ESIs.

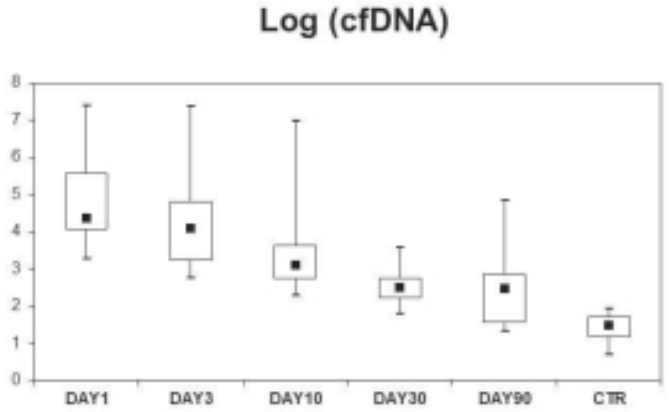
PUB645

Cell-free DNA: An Inverse Index of Peritoneal Membrane Repair Process after Peritonitis Sabrina Milan Manani, Grazia Maria Virzì, Alessandra Brocca, Massimo de Cal, Alessandra Dalla Costa, Ilaria Tantillo, Carlo Crepaldi, Claudio Ronco. *Nephrology-IRRIV, S Bortolo Hosp, Italy.*

Background: In Peritoneal dialysis (PD), peritonitis cause loss of mesothelium. A better understanding of the molecular peritoneal injury may minimize cell damage. Cell-free DNA (cfDNA) is a circulating extracellular DNA and is released from cells as a consequence of inflammation, cell injury and death. There are no data on the role of cfDNA after peritonitis. The aim of this study was to evaluate cfDNA during peritonitis in PD patients and investigate the repair process of the membrane.

Methods: We enrolled 23 PD(14 M, mean age 68±16 yrs) with an acute peritonitis and without history of systemic inflammation. We collected peritoneal effluents at day1, day3, day10, day30 from the start of peritonitis for WBC counts and cfDNA evaluation. We enrolled 30 PD patients without any history of systemic inflammmation and peritonitis in the last 3 months (CTR). CfDNA was extracted from peritoneal effluents and quantified, in genome equivalents (GE)/ml, by Real time PCR for β-globin gene.

Results: A significant positive correlation was observed between cfDNA concentration and WBC at day1 (rho=.89) and day3 (rho=.5)(both, p<.05). No statistically significant correlation was observed between cfDNA levels and WBC at day10 and day30. Quantitative analysis of cfDNA showed significantly higher levels in PD patients with peritonitis compared with CTR at day1, day 3, day 10 and day30.



Conclusions: Our data suggest that cfDNA correlates with WBC at the beginning of peritonitis. Furthermore, cfDNA is increased in peritoneal effluent of PD patients with peritonitis during the first 30 days from the start of peritonitis progressively decreasing. cfDNA could potentially serve as a specific marker for noninvasive monitoring of tissue damage after peritonitis. cfDNA could be an inverse index of peritoneal membrane repair process.

Funding: Private Foundation Support

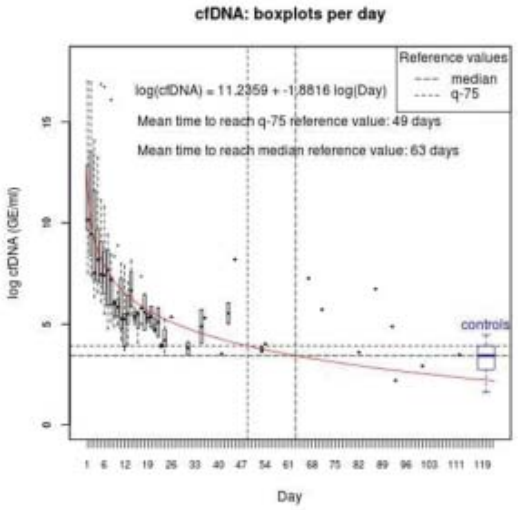
PUB646

cfDNA in PD-Related Peritonitis: A Noninvasive Marker for Monitoring the Recovery Process of the Peritoneal Membrane Grazia Maria Virzì, Sabrina Milan Manani, Massimo de Cal, Alessandra Brocca, Sonia Berti, Ilaria Tantillo, Carlo Crepaldi, Claudio Ronco, Silvia Pastori. *Nephrology-IRRIV, St Bortolo.*

Background: Peritoneal dialysis (PD) is complicated by peritonitis that cause loss of mesothelium by cell death. Cell-free DNA (cfDNA) is clinically used as a diagnostic and prognostic marker of cell injury. cfDNA is present in the peritoneal effluent of stable PD patients (pts). The biological role and the levels of cfDNA in PD-related peritonitis have not been yet investigated. The aim of this study was to evaluate cfDNA trend in the peritoneal effluent in PD pts whit peritonitis from the first day and during follow up period.

Methods: We enrolled 23 PD pts whit diagnosis of peritonitis (14 male, mean age 68±16 years). We collected peritoneal effluent samples from the first day of peritonitis until the 120th day. CfDNA was extracted from all peritoneal effluents and was quantified by Real time PCR in Genome Equivalent (GE)/ml for β-globin gene. We enrolled also 30 PD pts without any history of systemic inflammmation and peritonitis in the last 3 months, as control group (cfDNA: 31 GE/ml; IQR:16-51).

Results: All 23 PD pts were treated and clinically recovered from peritonitis after 13.5 days±5.4. The median concentration of cfDNA in the peritoneal effluent at the first day of peritonitis was very high (25523 GE/ml; IQR 18831-459541); subsequently cfDNA level tends to progressively decrease during follow up period.



	1	2	3	4	5	10	15	20	25	30	60	90	120
1	100.0	27.1	12.7	7.4	4.8	1.3	0.6	0.4	0.2	0.2	0.0	0.0	0.0

From this decreasing curve, we estimated that serve 49days to reach the value of 51 GE/ml (75percentile in controls) and 63days to reach 31 GE/ml (median in controls).

Conclusions: Our data demonstrated that cfDNA is increased in peritoneal effluent of PD pts with acute peritonitis and decrease progressively. cfDNA in peritoneal effluent could be a potential good biomarker to monitoring the recovery process of the membrane in PD-related peritonitis.

Funding: Private Foundation Support

PUB647

The Effects of Icodextrin on Metabolic Indices and Patient Survival in Non Diabetic CAPD Patients Jiyun Park, Yang Gyun Kim, Kyung-Hwan Jeong, Chun-Gyoo Ihm, Ju-Young Moon, Sang Ho Lee, Tae Won Lee. *Internal Medicine, Kyung Hee Univ School of Medicine, Seoul, Korea.*

Background: Icodextrin based peritoneal dialysis has shown favorable metabolic effects over conventional glucose based peritoneal dialysis in CAPD patients. However, no studies have been conducted only in non-diabetic CAPD patients on whether icodextrin based peritoneal dialysis improves metabolic control and survival.

Methods: We reviewed 84 cases of non-diabetic CAPD patients who were on CAPD for 3 years retrospectively. Sixty four were treated with conventional glucose based peritoneal dialysis (glucose group : mean age 54.5 ± 15.5years) and the other 20 were treated with 3 glucose based solutions during the day and 1 icodextrin dwell during the night (icodextrin group : mean age 57.0 ± 17.3 years). We measured variables related to lipid status, HbA1c, and left ventricular ejection fraction and compared all-cause mortality and cardiovascular mortality.

Results: There were no significant differences between age, gender, BMI, blood pressure, history of dyslipidemia, total cholesterol, LDL, HDL, triglyceride, HbA1c, and left ventricular ejection fraction of the two groups. Serum LDL (p<0.001) and HbA1c (p=0.005) improved in icodextrin group after 3 years but total cholesterol, HDL, triglyceride, and left ventricular ejection fraction of the groups were not different. Seven patients of glucose group developed non-insulin dependent diabetes mellitus during peritoneal dialysis (p=0.182). There were no significant differences in all-cause mortality rate (p=0.131) and cardiovascular mortality rate (p=0.290) between two groups.

Conclusions: These results provide that icodextrin improves metabolic indices in non-diabetic CAPD patients, which is similar to earlier findings. However, mortality rates were not significantly different between two groups and these results may be related to better glycemic control in non-diabetic patients. To confirm this data, large randomized prospective studies are needed.

PUB648

Take on Characteristics of Incident PD Patients in Poland, Romania, and Hungary at Integrated Care Clinics for Renal Services Belen Marron Ochoa,[1] Janusz Ostrowski,[4] Delia Timofte,[2] Marietta Torok,[3] Jose C. Divino-Filho.[1] *[1]Home Therapies Medical Office, Diaverum, Munich, Germany; [2]Diaverum, Bucharest, Romania; [3]Diaverum, Budapest, Hungary; [4]Diaverum, Wloclawek, Poland.*

Background: Our institution as a Renal Service provider is committed to integrated care, offering all types of RRT and focusing in patient´s choice. **Objectives:** To analyze HD and PD take on and its relationship with the type of previous referral and provided care.

Methods: Retrospective analysis of 547 incident patients starting dialysis in 23 HD/PD Diaverum clinics in 2012. Early referral (ER) considered if patient known ≥ 3 months in Nephrology, and scheduled initiation of dialysis with a permanent access was considered planned (P).

Results: Population: 30% diabetes, mean age 64 years, 84% with previous medical care of renal disease, 49 % late referral, 80% modality informed, 58 % unplanned start, 11% PD (3% early switch from urgent HD). PD therapy in non-planned start applied in 5/59 PD patients. No differences in HD/PD take on were observed for gender, diabetes, initial renal and predialysis follow up, at structured units or in elapsed time between early follow up and dialysis start. PD patients (p=0.02) received more modality information than HD (92% versus 78%) and were mainly under 50 years (p <0.001). PD incidence varied according with different studied groups.

Studied group	All patients (N=547)	HD (N=488)	PD (N=59)	p value
ER + P	168 (31)	132 (27)	35 (59)	<0,001
Late referral + P	63 (12)	58 (12)	5 (9)	
ER + Unplanned start	113 (20)	104 (21)	9 (15)	
Late referral + Unplanned start	203 (37)	193 (40)	10 (17)	
Optimal care: ER + modality informed + P	121 (22)	96 (20)	25 (42)	

Conclusions: Despite commitment to offer PD/HD as complementary treatments, PD incidence is still low. Optimal care provision is important to improve outcomes but also to involve patients in their therapy choice.

PUB649

Risk Factors for Death From Peritonitis in Hospitalized Peritoneal Dialysis Patients From the USRDS Noble Iwuagwu,[1] Irene Nicolette Falk,[1] Rhonda E. Colombo,[1] Stephanie L. Baer,[1,2] Lulu Huber,[1] Mufaddal F. Kheda,[1] Thomas Ryan Gallaher,[1] Jordan T. Powner,[1,2] N. Stanley Nahman,[1,2] Kristina Weis Kintziger.[1] *[1]Dept of Medicine, Georgia Regents Univ, Augusta, GA; [2]Dept of Medicine, Charlie Norwood VAMC, Augusta, GA.*

Background: Peritonitis (PTN) in peritoneal dialysis (PD) patients with septic symptoms or severe abdominal pain may be admitted for management. We have shown that PTN in PD in-patients is associated with DM, bacteremia (BAC) and MRSA infection (J Invest Med 62:554, 2014). In the present work we queried the United States Renal Data System (USRDS) to assess risk factors for mortality in PD patients hospitalized for PTN.

Methods: All incident PD cases from the years 2005-2008 were included and defined according to dialysis modality on the most recent form 2728. PTN and all comorbidities were defined by ICD9 diagnosis codes. Only PD at PTN diagnosis was included in this study. Survival analysis was performed using Cox regression.

Results: There were 24,522 patients and PTN occurred in 3575 (14.6%). Demographics: 54% male, 72% Caucasian and 64% < 65 yr. 27.9% of patients on PD died (39.4% of PTN, 25.9% of non-PTN). Co-variates conferring the greatest risk of death (hazard ratio (HR), 95% confidence intervals (CI)) included decubitus ulcer (HR 3.74, CI 3.41, 4.09) and age > 65 years (HR 3.36, CI 3.20, 3.52). Those with HR > 2.0: cirrhosis, BAC, PVD, endocarditis, C. difficile colitis, BAC with SIRS, candidemia, and pancytopenia. DM and MRSA infection showed HR of 1.76 and 1.79, respectively. The most common covariates with significant HR for death included age > 65 years (N=8,679, 35.4%), DM (N=7,550, 30.8%), PTN, BAC (N=2,197, 9.0%), PVD (N=1,993, 8.1%), and BAC w/SIRS (N=1,788, 7.3%).

Conclusions: PTN leading to a hospital admission occurred in nearly 15% of incident PD patients. Older age, DM and infectious complications are common morbidities with an increased risk of death. Less common conditions also confer an increased risk of death, but we would speculate they may represent surrogate markers for disease severity. Aggressive treatment of PTN in older diabetics, with any suggestion of systemic infectious complications, is supported by this study.

Funding: Clinical Revenue Support

PUB650

The Role of Peritoneal Dialysis in Acute on Chronic Liver Failure Maria Jimena Mucino-Bermejo, Renhua Lu, Carla Estremadoyro, Sara Samoni, Aashish Sharma, Carlo Crepaldi, Claudio Ronco. *IRRIV Vicenza, Vicenza.*

Background: The ability of peritoneal dialysis to achieve ultrafiltration and middle-molecular weight solute clearance at low haemodynamic cost makes it an attractive therapy in situations of hemodinamyc instability, needance of multiple high flux intravenous devices, or hemorrhagic diathesis, allof them may be present on cirrhotic patients.

Methods: Review of current literature: 100 articles; 55 reviews, 32 case reports, 1 experimental study and 12 case series.

Results: In cirrhotics AKI is related to circulatory disturbances that include diminished peripheral vascular resistances because of splachnic vasodilation triggered by portal hypertension and increased endogenous vasodilators. Also there is vasoconstriction via the renin-angiotensin-aldosterone axis, sympathetic nervous system activation and arginine hypersecretion. Dilutional hyponatremia, ascites and hepatorenal syndrome are manifestations of the same pathogenic axis. A second renal injury can trigger AKI, the fifth leading cause of hospitalization and the third leading cause of ICU admission among cirrotics. In patients with liver and renal failure, PD represents a reasonable treatment since it avoids anticoagulation, allows removal of ammonia, bilirubin, and free fatty acids, and even allows for partial and progressive draining of the ascitic fluid. Its use has been limited because of fear of excessive bleeding during catheter placement, inadequate ultrafiltration, removal of solutes in the presence of ascites, increased risk of bacterial peritonitis and hypoalbuminaemia. Peritoneal dialysis has been described to be a safe and efficient treatment in hepatorenal syndrome type 1, refractory ascites due to portal hypertension in autosomal dominant polycystic kidney disease, and as renal replacement therapy in end-stage renal disease patients with chronic liver disease and ascites. Reported benefits include amelioration of quality of life, diminished hospitalization days and increase in hemoglobin, total serum protein and albumin levels.

Conclusions: On cirrhotic patients, PD may be a reasonable treatment option in some clinical setting. Randomized large clinical studies are still needed to asses its safety and efficacy.

PUB651

Proteomic Analysis of Peritoneal Fluid of Patients Treated with Continuous Ambulatory Peritoneal Dialysis and Automated Peritoneal Dialysis Magdalena Luczak,[1] Lukasz Marczak,[1] Dorota Formanowicz,[2] Elzbieta Pawliczak,[2] Lidia Koziol,[2] Maria Wanic-Kossowska.[2] *[1]Polish Academy of Science, Poznan, Poland; [2]Poznan Univ of Medical Sciences, Poznan, Poland.*

Background: Long-term peritoneal dialysis (PD) is associated with progression of peritoneal fibrosis and lead to functional deterioration of the peritoneum. The development of new prognostic biomarkers assessing changes in peritoneum during long-term PD seems to be essential. The major goal of this study was proteomic analysis of peritoneal fluid (PF) in order to identify of differential proteins between CAPD and APD dialysate. Additionally, we sought to determine whether there are any protein biomarkers characteristic for patients treated for a longer time with CAPD.

Methods: Our studies involved patients treated with APD (11 cases) and CAPD (21 cases) - cured with distinct period of time, ie. for 24 and 48 months. Label-free proteomic approach using LC-MS/MS to analysis of PF proteome was used. Qualitative and quantitative differences in the accumulation of the individual proteins were determined.

Results: As a result, PF proteomes of CAPD and APD patients were demonstrated. 235 (CAPD) and 198 (APD) unique proteins were identified. Quantitative differences in accumulation of albumin, lysozyme C, antithrombin-III, apolipoprotein III, chondroadherin-like protein, Ras-GEF protein, transcription initiation factor, apolipoprotein A-IV, AI and hemopexin were detected. Increased accumulation of transthyretin, apolipoprotein CIII, peroxidasin homolog, beta-2-glycoprotein 1, kininogen 1, basigin and serotransferrin were revealed in CAPD patients treated for 48 months. Interestingly PCA test revealed that this group of patients clearly differed from CAPD (24 months) and also APD patients. Moreover, also PCA indicated that these differences were not related with an inflammatory process (CRP level).

Conclusions: Obtained data indicate that treatment modality (CAPD versus APD) and treatment period strongly influence on PF proteome. Increased kininogen level may indicate the enhanced neoangiogenesis process in CAPD patients with longer treatment time. Identified proteins may serve as potential markers of PD modality and represent potential therapeutic targets.

Funding: Government Support - Non-U.S.

PUB652

Peritoneal Dialysis Patients Preserve Residual Renal Function and Reduce Oxidative Stress during the Initial Period of Dialysis Therapy Atsushi Ueda,[1] Kei Nagai,[3] Aki Hirayama,[2] Chie Saito,[3] Kunihiro Yamagata.[3] *[1]Nephrology, Hitachi General Hospital, Hitachi, Japan; [2]Integrated Medicine, Tsukuba Univ of Technology, Tsukuba, Japan; [3]Nephrology, Univ of Tsukuba, Tsukuba, Japan.*

Background: In 2012 ASN meeting, we reported the dialysis patients with preserved the residual renal function (RRF) showed lower oxidative stress at 24 months after the initiation of renal replacement therapy (RRT). However, the modification of oxidative stress remains unclear. To answer this issue, we investigated the changes of RRF and oxidative stress markers of hemodialysis (HD) and peritoneal dialysis (PD) patients.

Methods: We employed retrospectively 54 HD and 23 PD patients. HD patients treated regularly 4 to 5 hours and 3/week. PD patients treated 4 bag changes a day or automated PD

with low glucose dialysate. Daily urinary volume, serum asymmmetric dimethylarginine (ADMA), pentosidine and indoxyl sulfulic acid (IS) as oxidative stress markers from the start of RRT until 36 months in every 6 month (0, 6, 12, 18, 24, 30, 36 months) were measured. And survival rate were examined.

Results: Urinary volume at the start of RRT in HD group was 974±499 mL/day, and decreased rapidly from 6 months (598±442) to 36 months (250±667). In PD group, urinary volume at the start of RRT was 1424±579mL/day and decreased at 12 months (961±523), however, maintained till 36 months (1173±629). Urinary volume in PD group was larger than that in HD group at every observed point. ADMA level in PD group at 0, 6 and 12 month were decreased significantly compared to HD group. Pentosidine level in HD group was increased gradually from the start of RRT (0.22±0.09μg/mL) until 36 months (0.45±0.19), and was continuously higher than that of PD patients during the whole observation period. IS level in PD group was also declined at 6 and 12 months significantly. However, there was no difference in survival rate between HD and PD groups.

Conclusions: Our study demonstrated that PD patients showed an advantage to preserve RRF and to reduce the oxidative stress in the initial period of RRT, howeverfor the small number of cases this could not revealed the beneficial effect for the prognosis,but may expect to have a good prognosis.

PUB653

Dialysis Quality Analysis of Single-Center Peritoneal Dialysis Patients

Tu Qiudi, Qiang He, Yueming Liu. *Nephrology, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China.*

Background: Analyze the data of all peritoneal dialysis (PD) patients in a single PD center and search for the counter measures of continuous quality improvement.

Methods: All 113 patients undergoing PD were retrospectively studied from 1st January 2007 to 31th December 2012. We investigated causes of PD withdrawal and death, analyzed demographic characteristics, laboratory data according to their medical record. Incidence of peritonitis, drop out rate (DOR) and length of the time of PD therapy (TOT) were calculated.

Results: Total of 113 patients were enrolled, 12 patients have died. The causes of death were cancer (1 Patient), pulmonary infection (2 patients), cerebrovascular disease (1 patient), cardiovascular disease (1 patient) and other reasons (4 patients). 16 patients were transfered to hemodialysis, and peritonitis (7 patients) and volume overload (6 patients) were two main reasons. There were 22 episodes of peritoitis among 10 patients in 2012. The major cause was non-standard operating (13 episodes in 6 patients). TOT and DOR were 19.59m and 23.61% respectively. There are 71 patients still been followed, include 41 men and 24 women. In these patients, 27.6% had dissatisfied KT/V(<1.7), 31.3% had a hemoglobin blood-level lower than 90g/L, 25.3% had an albumin blood-level lower than 30g/L, 59.7% were hyperphosphatemia (P>1.45mmol/L), and 45.2% PTH concentrations>300 pg/ml.

Conclusions: In our PD center, causes of death were various. PD associated infection was the first reason for transfering to hemodialysis. DOR is related to many factors, including age structure, education and so on, many aspects remain to be researched and improved in our center.

PUB654

Percutaneous and Conventional Surgical Insertion of Peritoneal Dialysis Catheter: A Comparative Study of Outcome and Technique Survival

Raj K. Sharma, Narayan Prasad, Amit Gupta, Anupama Kaul. *Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, UP, India.*

Background: The study was planned to compare the outcome of percutaneous peritoneal dialysis catheter placement which can be performed by the Nephrologists at bed side and permits rapid initiation of CAPD. We prospectively compared 230 percutaneous insertions and 1200 surgical dissection PD catheter insertions for technique survival and immediate and delayed complications.

Methods: The mean age of the patients was 48.75+16.8 years (3-74 yrs), Male:Female 70: 20. There were more diabetic patients in percutanoeus group 79% versus 40% than in surgical group. Mean break in period was 13.39+4.38 days (1 to 21 days). In percutaneous group peritoneal dialysis was started with in 24 hrs of insertion. Immediate surgical complications were similar in both group except slightly more outflow problems (6.45%) and hemorrhagic outflow (45.2%) in surgical group as compared to percutaneous (2%) and (5.5%).

Results: Exit site and peritonitis rates were similar in both groups. Technique survival was also similar in the two group. There was slightly increased incidence of surgical complications and early catheter failure in surgical insertion as compared to the percutaneous insertion of PD catheter.

Conclusions: Catheter removal was higher in surgical group (46.4%) as compared to percutaneous group (15%) ($p<0.001$). 1 and 2 yr catheter survival was 90% and 80% in percutaneous gr versus 85% and 70% in surgical group. Nephroligsts can safely practice the percutaneous split sheath (guide wire) technique for catheter insertion with similar outcomes like surgical insertion of PD catheter.

PUB655

Comparative Incidence of Peritonitis Between Peritoneal Dialysis Patients Receiving Icodextrin versus Dextrose Based Solutions

Ramanath B. Dukkipati, Anuja P. Shah. *Medicine, Harbor-UCLA Medical Center, Torrance, CA.*

Background: Patients who receive Icodextrin based peritoneal dialysis (PD) solutions may be a high-risk group susceptible to peritonitis. A significant proportion of these patients may have been switched to Icodextrin after they have had peritonitis, which rendered them susceptible to be high transporters or to ultra filtration failure.

Methods: We conducted retrospective analyses among 86 prevalent PD patients originating from one dialysis center during January 2013 to May 2014. We first examined the number of patients who are receiving Icodextrin and evaluated the number of episodes of peritonitis and compared them with the number of episodes of peritonitis in patients who are receiving Dextrose based PD solutions. We then examined associations between various demographic data and type of PD solution using logistic regression adjusted for case-mix and laboratory covariates.

Results: There were 86 PD patients out of which 16 were on Icodextrin and 70 patients were on Dextrose based PD solutions. Patients were receiving Icodextrin for at least 6 months. There were 7 episodes of peritonitis in Icodextrin group and 44 episodes in Dextrose based PD solutions group. There were 2 episodes of fungal peritonitis in the Icodextrin group and none in the Dextrose based group. There was 1 patient in the Dextrose group who had 2 episodes. There was no statistical difference in peritonitis between Icodextrin group versus Dextrose based group.

Conclusions: In PD patients there was no statistically significant difference in incidence of peritonitis and the type of PD solution used. The difference in incidence of fungal peritonitis did not achieve statistical significance. There was no correlation between the number of vintage months on PD and incidence of peritonitis.

PUB656

The Analysis of Outcome and the Risk Factors for Mortality in Peritoneal Dialysis Patients

Nanmei Liu. *Dept of Nephrology, Jimin Hospital of Shanghai, Shanghai, China.*

Background: To analyze the outcome and the risk factors for mortality in peritoneal dialysis (PD) patients in a single peritoneal dialysis center in a period of 27 years, and to discuss the approaches to obtain a better prognosis.

Methods: We retrospectively analyzed patients on PD for more than one month during the period from January 1985 to December 2011 in this hospital. We collected and analyzed their outcomes, and the causes of dropout from PD, demographic characteristics, laboratory data, dialysis, residual renal function, peritoneal transport characteristics, and nutritional status.

Results: A total of 841 patients were enrolled, of whom 431 were females (51.2%). The mean age was 58.53± 16.67 years. The primary diseases were glomerulonephritis (52.3%), hypertension (16.6%), and diabetes (15.3%). The mean peritoneal dialysis duration was 18.63 ± 20.35 months. Up to December 2011, the patients remained on PD in 246 cases (29.2%), died in 296 cases (35.2%), changed to hemodialysis in 152 cases (18.1%), treated with kidney transplant in 64 cases (7.6%), and lost for follow-up in 83 cases (9.9%). The main cause of death was cardiovascular events (41.6%), followed by infection (33.0%) and the cerebral vascular accident (9.5%). The major reason (59.2%) changing from PD to hemodialysis was the PD associated infection. Cox proportional hazards model analysis indicated that the risk factors for mortality were age, diabetes, change of hemodialysis to PD, low serum albumin leve, high C-reaction protein, low creatinine clearance rate and high peritoneal transport status.

Conclusions: In our peritoneal dialysis center, the major cause of dropout from PD was death, followed by the change to hemodialysis. Cardiovascular events was the most important cause of death, and PD-associated infection was the major reason for the change from PD to hemodialysis. Older age, diabetes, malnutrition, inflammation, insufficient peritoneal dialysis, high peritoneal transport status and change of dialysis from hemodialysis to PD predict the higher risk for all cause-death.

Funding: Government Support - Non-U.S.

PUB657

Salvage of Accidentally Sectioned Peritoneal Dialysis Catheters: Single Center Experience

Arzu Velioglu, Ebru Asicioglu, Derya Guler, Gurdal Birdal, Izzet Hakki Arikan, Serhan Tuglular, Cetin Ozener. *Nephrolgy, Marmara Univ, School of Medicine, Istanbul, Turkey.*

Background: In clinical practice, the accidental cutting of the peritoneal catheters is seen in peritoneal dialysis (PD) units. Although mechanical problems of the PD catheter remain a significant cause of removal of the catheter, several salvaging methods have been also introduced. With the following study, we present our experience on accidentally cut peritoneal dialysis catheters.

Methods: Accidentally sectioned catheters were salvaged using a new transfer set. The catheter was clamped with tweezers under sterile condition. The indwelling catheter was transected with sterile scissors below the cut. A new transfer set was inserted into the catheter. To prevent peritonitis, prophylactic antibiotics were administrated for at least three days. Outcome was measured by the successful return to PD and prevention of peritonitis.

Results: Between 1994 and 2014,a total of 551 peritoneal dialysis patients are followed. Eighteen of them were admitted to the hospital due to a cut of the catheter with leakage of dialysis fluid (mean age: 48.15±16.7; range [21-82 years]; F/M: 14/6; range PD time

[1-125 months]. All of the patients had accidentally cut the catheter with scissors while they were applying the dressing to the exit site. Five of the catheters were totally cut while the others were partially. All of the sectioned catheters were cut minimally 3 cm above the exit site. A salvage method was used in all of these patients. All patients resumed PD in 24 hours. Two patients presented with culture negative peritonitis after the procedure. Both were treated successfully with intraperitoneal antibiotics.

Conclusions: The salvaging of accidentally sectioned PD catheters is a safe and effective method. Nephrologists should be eager and enthusiastic for salvaging of the catheter in this setting.

PUB658

Bioimpedance Spectroscopy before and after Introducing a Daily NUTRINEAL ® Exchange in Peritoneal Dialysis Mario Prieto Velasco1, Benjamin De Leon Gomez, Jose Edgardo Gonzalez Arregoces, Elena Astudillo, Igor Romaniouk Jakovler, Jorge Estifan Kasabji, Aranzazu Sastre, Cristina Lucas. *Nephrology, Complejo Asistencial Univ León, León, Spain.*

Background: To compare the nutrition and hydration status of a group of CAPD patients before and after the introduction of one Nutrineal ® Baxter (PD4 1.1% amino acids) bag in daily exchanges.

Methods: we obtained parameters of body composition and hydration using the BCM ® (Body Composition Monitor, Fresenius Medical Care, Bad Homburg, Germany) before and 6 months after the introduction of Nutrineal in one daily peritoneal exchange. t -student test for comparison of means was performed. Values were calculated as the mean of three consecutives monthly measures.

Results: 16 CAPD patients with an average age of 66.41 years, 56.25% men. The difference in the various parameters before and after the introduction of Nutrineal are shown in the figure 1

PARAMETERS	BEFORE NUTRINEAL	ST DE	AFTER NUTRINEAL	ST DE	p
WEIGHT (Kg)	67,04	6,47159	66,06	6,45388	0,821
BMI (Kg/m2)	25,86	2,22662	25,78	2,36129	0,95
OH (L)	0,94	0,518935	0,61	0,6865	0,41
TBW (L)	33,61	3,60016	32,31	3,42096	0,58
ECW (L)	15,7	1,62848	15,07	1,54582	0,55
ICW (L)	17,9	2,11091	17,23	2,01513	0,62
LTI (Kg/m2)	13,86	1,37965	13,28	1,25308	0,51
FTI (Kg/m2)	11,41	2,62402	12,01	2,75074	0,74
LTM (Kg)	36,55	5,29115	34,6	4,85783	0,56
rel LTM [%]	55,2	7,23963	53,15	6,78209	0,66
Fat (Kg)	21,28	4,51475	22,17	4,76145	0,77
rel Fat (%)	31,3	5,2491	32,96	5,21391	0,63
ATM (Kg)	28,96	6,14446	30,16	6,48151	0,77
BCM (Kg)	20,5	3,43885	19,17	3,14015	0,54
Phi 50	4,94	0,480944	4,66	0,354377	0,33

BMI (Body Mass Index). OH L (Overhydration) TBW (Total Body Water). ECW (Extracellular Water). ICW (Intracellular water). LTI (Lean Tissue Index). FTI (Fat Tissue Index). LTM (Lean Tissue Mass). rel LTM (LTM in relation to body weight). Fat (Fat Tissue Mass). relFat (Fat Tissue Mass in relation to body weight). ATM (Adipose Tissue Mass). BCM (Body Cell Mass) Phi 50 (Phase angle 50 kHz)

Conclusions: Following the introduction of Nutrineal in patients on CAPD, we have not observed any significant difference in body composition or hydration status analyzed by BIA.

PUB659

Preserved Effectiveness of Potassium-Sparing Diuretics in Peritoneal Dialysis Patients to Maintain Normal Serum Potassium Jamie Davidson,[1] Lindsey Norris,[1] Divya Monga,[1] Zsolt Lengvarszky,[2] Mihaly B. Tapolyai,[3] Tibor Fulop.[1] *[1]Nephrology, Univ of Mississippi Medical Center, Jackson, MS; [2]Mathematics, Louisiana State Univ, Shreveport, LA; [3]Dialysis, Semmelweis Univ, Budapest, Hungary.*

Background: Hypokalemia is a vexing problem in end-stage renal disease patients on peritoneal dialysis (PD) and oral potassium supplements (OPS) have limited palatability. Potassium-sparing diuretics (KSD) [spironolactone, amiloride] may have a preserved effectiveness in these subjects.

Methods: We have performed a cross-sectional review of 29 prevalent (vintage >3 months) PD patients with regard to serum potassium (K+), OPS and KSD utilization. We reviewed chart for multiple clinical and laboratory variable, including dialysis adequacy and co-existing medical therapy. Data analysis was performed with SPSS v19. Results were expressed with percent (%) and means (SD).

Results: The cohort was middle-aged: 46.2 (13.9) years and overweight: BMI 28.3 (8.4) kg/m2; 69% female, 79% African-American and 38% diabetic with PD vintage 28 (8.4) months. Weekly Kt/V was 2.14(0.49), creatinine clearance 68.3 (28.5) L/week with total exchanged volume 10.4 (2.8) L. Residual urine output (RUO) measured 453 (525) mL. Three-month average serum K+ measured 3.9 (0.5) mEq/L and 48% of participants was taking K+-supplements (mean dose of K+: 22 (10) mEq/day) and 72% taking KSD (spironolactone dose: 25-200 mg/day; amiloride dose: 5-10 mg/day). Potassium correlated significantly with weekly Kt/V (p=0.03), creatinine clearance (p=0.05) but not with PD modality/vintage, RUO or KSD use. However, PSD use was associated with decrease use of OPS (r: -0.639; p<0.001).

Conclusions: KSD was tolerated in this cohort of PD patients and decreased the need for OPS utilization.

PUB660

Successfull Treatment of *Acinetobacter baumannii* Peritonitis Necmi Eren, Mehmet Tuncay, Sibel Bek, Mustafa Tosun, Betul Kalender, Itir Yegenaga. *Nephrology, Kocaeli Univ, Faculty of Medicine, Kocaeli, Turkey.*

Background: Peritonitis is the major complication in peritoneal dialysis (PD) patients with the risk of technique failure. *Acinetobacter* species are rare gram negative agents of usually nosocomial origin and have remarkable risk of catheter loss in PD patients. Here we present two PD patients with *Acinetobacter baumannii* (*A. baumannii)* peritonitis successfully treated with antibiotics.

Methods: Case 1: A 49-year-old woman on PD for 60 months was admitted to our hospital with acute abdominal pain and cloudy dialysate. She was hypertensive and had an abdominal surgery for gastric cancer 6 weeks ago. Physical examination was unremarkable other than diffuse abdominal tenderness. Her effluent white cell count was 226/mm^3 with 78% neutrophils. Empirical intraperitoneal (IP) cefazolin and gentamicin was started. On the 3rd day, *A. baumannii* sensitive to gentamicin and resistant to carbapenems was identified in effluent culture. Then cefazolin was stopped. Catheter exit-site and blood cultures were negative. On the 4th day of gentamicin treatment, dialysate leukocyte count was less than 100/mm^3 and treatment was continued for 3 weeks. **Case 2:** A 70 year old woman on PD for 72 months admitted to our hospital with abdominal pain, nausea and cloudy dialysate of one day. Physical examination was normal except abdominal tenderness. Effluent leukocyte count was too many to count. Empirical IP cefazolin and gentamicin was started. On the 3rd day dialysate culture revealed *A. baumannii* sensitive to gentamicin and carbapenems and thus we stopped cefazolin. Catheter exit-site and blood cultures were negative. On the 5th day of gentamicin treatment due to persistence of cloudiness in effluent we replaced gentamicin with intravenous meropenem. On the 3rd day of meropenem treatment, the dialysate leukocyte count was less than 100/mm^3. The patient was discharged after 3 weeks of treatment.

Conclusions: Acinetobacter peritonitis is a rare condition, *A. baumannii* is the cause in nearly 90% of the cases with high potential of antibiotic resistance. Our cases demonstrate that the importance of *A. baumannii* in not only nosocomial but also community-acquired infections.

PUB661

Serum Biomarkes of Mineral Metabolism and Peritoneal Dialisys Modalities: Automated versus Continous Ambulatory Rafael Weissheimer, Fellype C. Barreto, Roberto Pecoits-Filho, Daniela Veit Barreto. *School of Medicine, Pontifícia Univ Católica do Paraná (PUCPR), Curitiba, Parana, Brazil.*

Background: Mortality rates in ESRD are unacceptably high. Disorders of mineral metabolism (hyperphosphatemia, hypercalcemia, and secondary hyperparathyroidism) are potentially modifiable. To evaluate the effect of peritoneal dialysis (PD) modalities, APD versus CAPD, over serum alkaline phosphate (AP), calcium (Ca) and phosphate (P) in incident adult patients over one year.

Methods: analysis of a multicenter prospective observational study. Patient who had three first and last months, P, Ca and AP measurements and had been treated exclusively by APD or CAPD during follow-up were included. T-student test, Fisher´s exact or Qui-square test were used for comparisons. ANOVA models were used to evaluate differences between repeated measurements. ANCOVA models were used to evaluate change over time (end-of-study versus baseline) for each parameter, adjusted for previous hemodialysis, baseline serum creatinine, diabetes, BMI, gender and age.

Results: 237 APD versus 228 CAPD patients were evaluated. APD patients were older (61.5±15.6 versus 57.8±149y, p=0.009), had higher baseline creatinine (7.1±3.9 versus 6.3±3.8mg/dL, p=0.027) and were less likely to have had previous hemodialysis (38.8% versus 50.4%, p=0.027). Baseline and end-of-study phosphate was significantly higher in APD patients (5.10±1.28 versus 4.86±1.20, p=0.04 and 5.10±1.53 versus 4.62±1.16mg/dL, p<0.001). Moreover, P reduced significantly over time in CAPD, but not in APD patients (-0.238±1.20 versus 0.004±1.40, p=0.01). Ca was significantly higher but there were no changes over time (0.08±0.87 versus 0.07±0.81, p=0.81). AP did not change over time (-2.15±91.3 versus 4.18±89.5, p=0.83).

Conclusions: It seems that the peritoneal dialysis modalities interferes in control of the match. Randomized controlled studies are needed to better ratings and causality with these outcomes. PD modality seems to have an effect on p levels in incident patients. Further studies warranted to evaluate if the worse P control in APD is associated with negative outcomes.

PUB662

Intraperitoneal Vancomycin Induced Liver Injury Fahad Y. Edrees, Robert Mark Black. *Internal Medicine, Saint Vincent Hospital, Worcester, MA.*

Background: With its efficacious, infrequent dosing and safety profile, intraperitoneal vancomycin (IPV) has long been used to treat peritonitis in peritoneal dialysis patients. Liver toxicity has been reported via intravenous (IV) and oral (PO) routes; however, IPV-associated toxicity has not been reported. We describe a case of IPV-induced liver injury in a patient with peritonitis.

Methods: A 29-year old man with a history of ESRD on peritoneal dialysis, psoriasis, hypertension and hypothyroidism was admitted with one day of abdominal pain, fever, nausea and vomiting. Home medications were limited to levothyroxine, omeprazole,

amlodipine, valsartan, procrit and sevelamer. Initial examination showed a pulse of 120 b/m, BP 124/86 mmHg, and temperature of 98.3°F. Abdominal examination revealed diffuse tenderness, voluntary guarding and sluggish bowel sounds. The remainder of the examination was unremarkable. Significant initial work-up showed a WBC of 20.2 μ/l with 88% neutrophils, BUN 43 mg/dl, creatinine 11.8 mg/dl, and a normal lactate level. The peritoneal fluid was cloudy in appearance and showed 3403 WBC/μl with 85% neutrophils. He was diagnosed with peritonitis and started on 2g IPV once weekly. The pre-treatment levels of ALT/AST were normal; during and post- levels are shown below, along with levels in two similar episodes of peritonitis 1 and 3 years ago.

	1st episode		2nd episode		3rd episode	
	during Rx	after stopping	during Rx	after stopping	during Rx	after stopping
ALT	72	21	133	29	83	35
AST	37	25	62	24	35	23

Conclusions: IV, oral and IP administration of vancomycin have been used. Liver injury has been reported with IV and oral administrations; however, we did not find any published reports of liver injury with IPV. In our case the liver injury was most likely due to vancomycin because there were no other identifiable causes. Furthermore, we observed similar patterns of liver enzyme elevations with institution and discontinuation of vancomycin on three separate occasions. Use of the Naranjo probability score indicated that it was a probable adverse drug associated event. To our knowledge this is the first reported case of IPV-induced liver injury.

PUB663

Providing Care to Peritoneal Dialysis Patients within an Existing Day Care Center Program Ying Ying Terina Seow, Zhenli Yu, Lee Ying Yeoh. *Gen Med, Khoo Teck Puat Hospital, Singapore, Singapore.*

Background: Lack of expertise and knowledge is a major barrier for accepting peritoneal dialysis (PD) patients into an established day care center program. This project was conducted to explore how to empower a day care center to care for PD patients.

Methods: PD patients were referred by different sectors of healthcare professionals. Care staff from St Luke's Eldercare (SLEC) were trained to assist with PD exchanges and adapt the existing rehabilitation program for PD patients. Outcome measures included patient length of stay, peritonitis rate; change of quality of life (SF-12) and depression score (HADS-D) over time; as well ascare center staff knowledge and confidence caring for PD patients.

Results: A total of 64patients were referred, out of which 16 were enrolled. Inclusion criteria included age over 50, no infectious diseases with rehabilitation potential.Participant median age was 66.0 (range 54-85) and 37.5% were in wheelchairs.Median length of stay was 44.5 days (range 1-194). There were no peritonitis episodes while patients were enrolled in the program. Physical functioning was maintained though not improved. Depression scores improved within first few months and then remained relatively stable. Sixprocesses were implemented in the centers: standard operating procedures for PD-related tasks, training manual, problem-solving guide, infection control instructions, staff competency and certification, and emergency contact person. Although initially anxious, care staff built up knowledge and confidence caring for PD patients. A simplified referral process was vital to expedite the use of day care services.

Conclusions: Utilizing a day care service for elderly PD patients is safe and can maintain patients' functionality, improve mental status and alleviate depression.A structured training program is required to build care staff knowledge and confidence.

Funding: Government Support - Non-U.S.

PUB664

Elevated Soluble Receptor for Advanced Glycation End Products (sRAGE) Level Is Associated with Indicators of Inflammation and Malnutrition in Peritoneal Dialysis Patients Hui Peng, Tang Li, Yan-Ru Chen, Jun Zhang, Xun Liu, Cailian Cheng, Tan-Qi Lou. *Nephrology Div, Dept of Medicine, the Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: The advanced glycation end-products (AGE) and receptors for AGE (RAGE) interaction may contribute to the inflammatory. It is already known that soluble RAGE (sRAGE) is the inhibitor of AGE-RAGE interaction and it is considered as a protect marker of coronary artery disease, hypertension, metabolic syndrome, arthritis, amongst others. Some studies have showed sRAGE is elevated in the circulation of peritoneal dialysis patients, which correlates with the concentration of sRAGE in spent peritoneal dialysate solution. We conduct this study to investigate whether the level of sRAGE in spent dialysate of PD patients predicts inflammation of PD patients.

Methods: Forty one patients undergoing continual ambulatory peritoneal dialysis (CAPD) from the peritoneal dialysis center of the Third Affiliated Hospital of Sun Yat-sen University were enrolled in the cross-section observational study. sRAGE level in spent dialysate was assessed by enzyme-linked immunosorbent assay(ELISA). Plasma fibrinogen, CRP, Zinc and Cu as the indicators of malnutrition and systemic inflammation were measured as well as other clinical biochemistry parameters.

Results: we found sRAGE level in spent dialysate was inversely correlated with serum hemoglobin (r=-0.320, p=0.042), serum albumin (r=-0.313, p=0.047), serum zinc (r=-0.385, p=0.014) and plasma fibrinogen (r=-0.521, p=0.015). sRAGE showed positive relationship with Cu/Zn ratio(r=0.341, p=0.031). However, we did not found there was correlation between sRAGE and serum CRP.

Conclusions: Our finding shows that sRAGE level in spent dialysate inversely correlates with indicators of malnutrition and inflammation, which suggests that it might become a potential predictor of malnutrition and inflammation state of PD patients.

Funding: Government Support - Non-U.S.

PUB665

Late Peritoneo-Scrotal Leak in Type 1 Diabetic Patient: A Case Report Katarzyna Madziarska, Jan Penar, Slawomir C. Zmonarski, Maria Magott, Magdalena Krajewska, Hanna Bartosik, Tomasz Golebiowski, Miroslaw Banasik, Maciej Szymczak, Krzysztof Letachowicz, Marian Klinger. *Nephrology and Transplantation Medicine, Medical Univ, Wroclaw, Poland.*

Background: Dialysate leakage represents a major noninfectious complication of peritoneal dialysis (PD) and occurs between 5-10% of PD patients (pts). Early leaks occur within 30 days, late leaks up-to first year. Treatment includes surgical repair, transfer to hemodialysis (HD), low PD dialysate volume or cycled-PD.

Methods: Case Report A 53-year old diabetic patient type 1; 36 years of diabetes; cigarettes smoker and active businessman, was accepted for PD in 2013. He had been under the care of our center since 1990 due to diabetic kidney disease. The Tenckhoff catheter was implanted with laparoscopy in Oct-2013 when urea reached 123 mg/dL, eGFR 12 ml/min. CAPD was started in Jan-2014 using four 2L dwells daily;3x1.5% Glucose, 1xExtraneal at night. He was stable and active for 2,5 months with well-controlled blood pressure, HbA1c 7%, satisfactory peritoneal Kt/V and no peripheral edema. In March 2014, 7 days before admission, he noticed slowly progressing bilateral, painless swelling of scrotum and penis with no signs of inflammation but causing walking problems. Initially he didn't attribute edema to PD. He linked edema with PD after sudden dramatic drop of fluid outflow down to 500 mL together with scrotum enlargement to the size of 2 grapefruits. There was no evidence of inflammation on admission. Inguinal hernia and abdominal swelling were excluded. An immediate HD with Perm-Cath catheter was initiated. The patient disagreed any diagnostics and surgical treatment.Standard treatment (bed rest, scrotal elevation) and HD caused disappearance of scrotal edema within 10 days. The patient decided to continue HD. Now he awaits renal–pancreas transplantation.

Conclusions: In scrotal edema accompanying PD therapy an inguinal hernia needs to be excluded. The presence of edema limited to the scrotum and penis with fast resorption after transfer from PD to HD suggests that the cause of the leakage may be an open processus vaginalis. Any chronic disease causing periodic increase of abdominal pressure, i.e.: chronic cough may contribute to leak.

PUB666

Pathogens and Drug Sensitivity in Peritoneal Dialysis Related Peritonitis in a Single Peritoneal Dialysis Center Chenggang Shi, Mei Li, Qiong-Li Yin, Weizhao Mo, Cailian Cheng. *Dept of Nephrology, the Third Affiliated Hospital of Sun-Yat Sen Univ, Guangzhou, Guangdong, China.*

Background: To investigate the pathogens and their drug sensitivity in peritoneal dialysis-related peritonitis in a single peritoneal dialysis center.

Methods: Pathogens and drug susceptibility test results of 62 cases in 35 peritoneal dialysis(PD) related peritonitis were analyzed retrospectively from January 2008 to May 2014.

Results: Among 62 cases of PD drainage fluid culture, cases were cultured with a positive rate of 61.3%, including 21 cases of gram- positive cocci (51.2%), 19 cases of gram- negative bacilli (46.3%), and 1 cases of fungi (2.4%), mixed bacteria in one episode. Staphylococcus aureus was the most common Gram-positive pathogen, and escherichia coli was the most common Gram-negative pathogen. Drug sensitivity test results showed that Gram- positive cocci showed a high resistance rate of 44.4% (4/9) to cefazolin and 33.3% (5/15) to rifampin and a low resistance rate of 6.3%(1/16) to vancomycin. 78.6% (11/14) of Gram- negative bacilli were resistant to ciprofloxacin and 28.6% (4/14) to gentamycin. There was a low resistance rate of 11.1% (1/9) to imipenem. The total PD withdrawal rate in peritonitis was 17.7%.

Conclusions: Gram- positive cocciis was still the main pathogen in PD related-peritonitis.There is a high resistance rate to cefazolin, rifampin, ciprofloxacin gentamycin and a low resistance to vancomycin, imipenem. Therefore, vancomycin and imipenem can be used as empiric therapy for PD related peritonitis.

Funding: Government Support - Non-U.S.

PUB667

Long-Term Safety and Efficacy of Darbepoetin Alfa in Peritoneal Dialysis Patients Yukinao Sakai,[1] Shizuka Yui,[1] Masami Sukegawa,[1] Yusuke Otsuka,[1] Yuichiro Sumi,[1] Anna Suzuki,[1] Koji Mugishima,[1] Tomoyuki Otsuka,[1] Shuichi Tsuruoka.[2] *[1]Dept of Nephrology, Nippon Medical School Musashikosugi Hospital, Kawasaki, Kanagawa, Japan; [2]Dept of Nephrology, Nippon Medical School, Tokyo, Japan.*

Background: Darbepoetin alfa (DA) was introduced in Japan in 2007. Its effect persists for a longer duration, compared with erythropoietin (EPO), and it has helped improve the quality of life of PD patients. In the present study, we examined the long-term safety and efficacy of DA in PD patients.

Methods: We retrospectively reviewed 17 cases wherein the treatment was switched from a conventional EPO preparation to DA. The mean age of the patients was 60.5 years, whereas the mean PD duration was 55.5 months. We assessed the changes in the

Hb, Ht, Ret, Fe, TIBC, ferritin, iPTH, HANP, BNP, and CRP levels before and after DA administration. The study period was 60 months, and the Dunnett multiple comparison assay was used for analysis.

Results: The average DA dosage was 183.9 μg/month following the switch to DA treatment and 217.5 μg/month 5 years after the switch (ns). Following the switch to DA treatment, the Hb level almost remained unchanged, and remained at approximately 11 g/dL, which was consistent with the target value of the KDOQI guidelines. Moreover, although the Ht level did not show a significant change, the reticulocyte count significantly decreased. With regard to ferrokinetics, the Fe level decreased and the TIBC level increased, although the changes were not significant; furthermore, the ferritin levels significantly decreased. In addition, the iPTH levels significantly decreased, although the HANP, BNP, and CRP levels did not show any significant change. I understand that you mean "not significant" here, but it is unclear what is not significant. Please clarify this point. Please check whether this is what you mean.

Conclusions: Through DA administration, the anemia in PD patients could be well managed for a long duration. As the ferritin levels tended to decrease after switching to DA treatment, we believe that DA is superior in terms of iron mobilization and is more effective for hematopoiesis compared with EPO.

PUB668

Simple Method to Estimate Daily Sodium Intake during Measurement of Dialysis Adequacy in Chronic Peritoneal Dialysis Patients So Mi Kim,[1] Eun Kyoung Lee.[2] *[1]Div of Nephrology, Dept of Internal Medicine, Jeju National Univ School of Medicine, Jejushi, Jejudo, Korea; [2]Div of Nephrology, Dept of Internal Medicine, Dankook Univ Hospital, Cheonan, Chungnam, Korea.*

Background: Restriction of dietary sodium intake for peritoneal dialysis (PD) patients is recommended, but there is limited information on the measurement and monitoring of sodium intake. We have developed a simple method to estimate daily sodium intake during the measurement of dialysis adequacy in PD patients.

Methods: A total of 83 PD patients from the Asan Medical Center were enrolled into the study. The patients were divided into two groups based on residual renal function (RRF). We measured total sodium removal and estimated daily sodium intake using dietary recall for 1 day, during the assessment of dialysis adequacy.

Results: There were 39 patients in the RRF(-) group and 44 in the RRF(+) group.In both groups, and all patients, there were significant positive correlations between sodium intake and total sodium removal: RRF(-) group, r = 0.598; RRF(+) group, r = 0.577; total patients, r = 0.595. There were linear relationships between dietary sodium intake and total sodium removal in both groups: RRF(-) group, sodium intake (mg/d) = 19.28 × peritoneal sodium removal (mEq/d) + 211; RRF(+) group, sodium intake (mg/d) = 15.40 × total sodium removal (mEq/d) + 609. All PD patients, sodium intake (mg/d) = 15.64 × total sodium removal (mEq/d) + 646.

Conclusions: Themeasurement of total sodium removal during the assessment of dialysis adequacy could be an effective and simple method to estimate dietary sodium intake in PD patients. A dietary intake of 2,000 mg of sodium corresponds to a total sodium removal of approximately 87 mEq/d.

PUB669

Urgent Peritoneal Dialysis Start through Percutaneous Catheter Insertion: Technique Survival and Overall Outcome Ramon Medina, Maria Luz Alcantar, Karina Renoirte, Guillermo Garcia-Garcia. *Nephrology, Hospital Civil Fray Antonio Alcalde, Guadalajara, Jalisco, Mexico.*

Background: In Mexico, Peritoneal Dialysis (PD) accounts for 60% of patients (pts) in dialysis. Hospital Civil is a tertiary care facility where >95% of End Stage Renal Disease (ESRD) pts are started urgently on dialysis due to late referral and diagnosis. We aim to evaluate survival of percutaneous urgent PD catheter insertion and early and late complications related to immediate peritoneal dialysis start with a 2-L dialysate volume.

Methods: Prospective, observational study including all pts with first time ESRD diagnosis from April 2013 to May 2014, who underwent PD percutaneous catheter insertion and initiated PD without break-in period. All catheters were evaluated for 90 days (d) survival, integration to CAPD program and complications. All pts started PD with 2-L dialysate volume right after PD catheter insertion.

Results: We enrolled 122 pts (75.4% men), with a follow up period (FUP) of 7-12 months, 60 pts (49.1%) had ESRD of unknown etiology. Catheter survival at 90d was 79.5%, but only 51 pts (41.5%) were integrated to CAPD (average time to integration 105d + 47d). At the end of the FUP 40 pts (39%) were alive and in CAPD; 36 pts (30%) alive and not integrated to CAPD; 30 pts (19%) switch to hemodialysis (HD) and 16 pts (13%) died. The early complication included 1 (0.81%) bowel perforation, 7 pts (5.73%) presented dialysate leakage, 5 pts (4.09%) with infection including 2 tunnelitis and 3 peritonitis, 17 pts (13.9%) with mechanical malfunction of which 5 (29.41%) were rescued. Late peritonitis was the main cause to switch to HD.

Conclusions: Our technique without break.in period is safe for unplanned dialysis patients and the catheter related complications at 90d were similar to other published data. Age and low serum albumin level were predictors of death and PD failure. Late peritonitis was the mayor cause of PD failure.

PUB670

Variances Between Peritoneal Dialysis Catheter Care Patterns and Infection Rates: A Study from the MWPNC Kera E. Luckritz,[1] Ibrahim F. Shatat,[2] Hiren P. Patel,[3] Deepa H. Chand.[4] *[1]Pediatrics, Univ of Michigan, Ann Arbor, MI; [2]Pediatrics, Medical Univ of South Carolina, Charleston, SC; [3]Pediatrics, Nationwide Children's Hospital, Columbus, OH; [4]Pediatrics, Rush Children's Hospital, Chicago, IL.*

Background: Peritoneal Dialysis (PD) is the most common form of RRT for children with end stage renal disease (ESRD). Peritonitis and membrane failure are the most common reasons to transition to hemodialysis (HD). Effective catheter care to prevent peritonitis are essential to successful PD. Objective: Our aim is to investigate whether catheter care practices correlate with infections.

Methods: This study was conducted through the MWPNC, after obtaining IRB approval. Participating dialysis centers were provided electronic copies of Patient, Provider, and Event Questionnaires, which were completed in a de-identified manner. Event forms were completed since catheter placement for all prevalent peritoneal dialysis patients. Peritonitis was defined per ISPD guidelines.

Results: A total of 4 centers (23 patients) participated. There were 17 infectious events among 11 patients (5 peritonitis, 3 tunnel infections, 11 exit site infections). Cefazolin was the post commonly prescribed antibiotic. The primary mode of antibiotic administration was intra-peritoneal with the exception of isolated exit site infections which were predominately treated with oral medications. Time from catheter placement to first infectious event averaged 295 days (1-494). All providers prescribed cleansing site with soap and water. All patients followed these instructions at a minimum, and using some type of dressing. Providers recommended showers and discouraged baths. However, 50% patients stated they took baths v. showers. Swimming restrictions were varied ranging from swimming in private pools only to swimming in the ocean. Using student's T-test there was no difference in infection rates between patients who did and did not follow instructions on swimming or bathing.

Conclusions: Many patients did not adhere to PD catheter care practices as prescribed. Better educational tools and adoption of best practices are recommended.

PUB671

Pharmacokinetics of Polymyxin B in Two Patients on Continuous Venovenous Hemodialysis Eugene K. Essandoh, Serge Cremers, Jai Radhakrishnan, Michael T. Yin, Christine J. Kubin, Maya K. Rao, Zhengxiang Zhu, Sumit Mohan. *Nephrology, Infectious Disease, Pharmacy, Columbia Medical Univ Hospital, New York, NY.*

Background: Polymyxin B is an old antibiotic with increasing clinical relevance in the treatment of multidrug-resistant gram-negative bacterial infections. There is limited data on the pharmacokinetics and the potential need to dose adjust polymyxin B with renal replacement therapies. Objective of this study was to evaluate the pharmacokinetics of polymyxin B in patients on continuous venovenous hemodialysis (CVVHD).

Methods: Two critically ill patients with oliguric renal failure undergoing polyethersulfone filter based CVVHD (Nxstage machine) received intravenous polymyxin B 80mg q12 and 90mg q24 h, respectively. Pre and post-filter plasma and hemofiltrate samples were collected at regular time points at steady state. Plasma and hemofiltrate concentrations of polymyxin B1+B2 were determined by a previously described liquid chromatography-mass spectrometry assay. Non-compartmental pharmacokinetics were calculated using Phoenix Winnonlin pharmacokinetics software.

Results: Polymyxin B concentrations at the end of infusion were 3.2 and 4.1 mg/L respectively, and decreased bi-exponentially with a terminal half-life of 29 and 37 h, to 2.0 and 2.1 mg/L, resulting in area under the plasma concentration time curves of 26.2 and 59.1 h*mg/L. Post-filter plasma concentrations were lower than pre-filter, and dialysate concentrations were even lower resulting in an average sieving coefficient of 0.3-0.4 over time. Over a single dosing interval, 15 and 42 mg were recovered in the dialysate for q12h and q24h, respectively. The rate of appearance in dialysate ranged from 0.9 to 3.5 mg/h, and showed a clear relationship with the plasma concentration of the drug.

Conclusions: Our preliminary data show that polymyxin B is cleared by CVVHD with a Sieving coefficient between 0.3 and 0.4. Investigation of additional subjects and comparison with non-CVVHD patients will allow further characterization of the PK in these patients and will determine the need for dose adjustments in CVVHD.

PUB672

Glycopeptides Pharmacokinetic Comparison during Direct Hemoperfusion by Lixelle Adsorption Column Marco Sartori,[1,2] Sonya Day,[1] Maria Grazia Ricatti,[1] Federico Nalesso,[1] Mirella Zancato,[2] Claudio Ronco.[1] *[1]Nephrology and Renal Research Institute of Vicenza, St. Bortolo Hospital, Vicenza, Italy; [2]Pharmaceutical and Pharmacological Sciences, Univ of Padua, Padua, Italy.*

Background: Different pharmacokinetic properties characterize Vancomycin (VAN) and Teicoplanin (TEC). Extracorporeal therapy is additional kinetic parameter which can extent this disparity. Thus we set up an in-vitro study in order to investigate VAN and TEC adsorption removal by direct hemoperfusion (DHP) with Lixelle S-35 cartridge.

Methods: Mock DHP was performed using VAN and TEC solutions (46.08±0.81 and 74.79±1.24 mg/L per N=6). Solutions were circulated in a closed DHP circuit using Lixelle S-35 (Qb 250 ml/min). Samples were taken at 10, 60 and 120 min from inlet and outlet the cartridge. Drug levels were measured with particle enhanced turbidimetric inhibition immunoassay and fluorescence polarization immunoassay for VAN and TEC, respectively. VAN and TEC mass balance were calculated.

Results: As shown in table, VAN baseline levels decreased rapidly during the first ten minutes of DHP than reached plateau at sixtieth experiment minute: the total mass adsorbed onto Lixelle polymer was 45.68±2.26 mg. The observed reduction of TEC levels greatly exceeded than VAN. The total mass of TEC introduced into the system was completely adsorbed after 10 minutes by Lixelle cartridge (126.86±0.91 mg).

Table I. Vancomycin and Teicoplanin Levels during DHP.

Time [min]	Arterial Concetration [mg/L]	Venous Concentration [mg/L]
Vancomycin		
baseline time [0]	46.08±0.81	—
time [10]	25.67±0.51	22.17±0.68
time [60]	21.93±0.72	20.47±0.62
time [120]	21.90±0.77	21.37±0.83
Teicoplanin		
baseline time [0]	74.79±1.24	—
time [10]	11.98±0.78	LOD
time [60]	LOD	LOD
time [120]	LOD	LOD

LOD indicates Low Limit Of Detection.

Conclusions: Different adsorption kinetics was occured between VAN and TEC. This should be considered when Glycopeptides are used in patients receiving DHP with Lixelle S-35 to avoid potential underdosing: loading dose may be required for both antibiotics. Furthermore, the high Lixelle S-35 sorbent capacity against TEC may represent an option as rescue therapy in accidental overdose.

Funding: Private Foundation Support

PUB673

Impact of Rifampicin on Antihypertensive Drug Levels and Their Requirement in CKD5D

Akansha Agrawal,[1] Sanjay K. Agarwal,[1] Thomas Kaleekal.[2] *[1]Nephrology, AIIMS, New Delhi, India; [2]Pharmacology, AIIMS, New Delhi, India.*

Background: Patients with CKD on dialysis (CKD5D) have many fold higher incidence of tuberculosis (TB) than the general population and >95% of them are also hypertensive. Rifampicin, a first-line antitubercular (ATT) drug, is a potent inducer of hepatic cytochrome P450. Hypertensive patients receiving rifampicin-based ATT (RBATT) often have worsening of hypertension. There is potential for pharmacokinetic interaction between rifampicin and antihypertensives that are CYP substrates: amlodipine and metoprolol. However, this hypothesis has not been systematically studied.

Methods: In a prospective cohort study, hypertensive CKD5D patients with TB were followed after RBATT initiation. BP was <140/90mmHg with stable antihypertensives at inclusion. Serum amlodipine, metoprolol and prazosin levels were estimated at baseline and 3, 7, 10 and 14 days after RBATT initiation. Measurement was by HPLC after standardization. BP and antihypertensive requirement were also monitored for 2 weeks or until stabilization. For comparison, numbers and dosage of antihypertensives were computed into units.

Results: 24 CKD5D patients were enrolled. After RBATT initiation, serum amlodipine, metoprolol and prazosin levels declined in all patients (Table).

Antihypertensive	% Patients with decline in level	% Decline in level from baseline		Time to first decline in level (days)		% Patients with decline to undetectable level
		Mean	Range	Mean	Range	
Amlodipine (n=17)	100	82.8±20.4	52-100	3.9±3.2	2-15	47
Metoprolol (n=4)	100	91.9±16.2	67.6-100	5.3±3.9	2-10	75
Prazosin (n=4)	100	98.1±2.7	94.3-100	4.3±3.9	2-10	50

After RBATT, 83.3% patients required increase in drugs to maintain BP <140/90mmHg. Drug requirement increased from 4.5+3.6 (1-14) to 8.5+6.4 (2-29) units (*p<0.0001*). Mean time to first increase in dose was 6.5+3.6 (2-14) days. 46% patients had a hypertensive crisis at a mean of 9.1+3.8 (3-14) days. In 2 patients, rifampicin had to be stopped to control BP.

Conclusions: Rifampicin causes significant decrease in serum levels of commonly used antihypertensives. This decrease in levels correlates well with worsening of BP. Thus, we suggest close monitoring for worsening hypertension after rifampicin initiation.

Funding: Government Support - Non-U.S.

PUB674

Tobramycin Kinetics Removal on Renal Replacement Therapy by Carpediem Machine *In Vitro* Model

Marco Sartori,[1,2] Elena Faggiana,[1,2] Elena Barzon,[1] Leopolda Zampieri,[1] Federico Nalesso,[1] Mirella Zancato,[2] Claudio Ronco.[1] *[1]Nephrology and Renal Research Institute of Vicenza (IRRIV), Pharmacology Section, St. Bortolo Hospital, Vicenza, Italy; [2]Pharmaceutical and Pharmacological Sciences, Univ of Padua, Padua, Italy.*

Background: Tobramycin(TOB)is an antibiotic active against Gram(-) frequently used in pediatric patients with sepsis. These patients can develop Acute renal failure requiring renal replacement therapy by the newcomer Carpediem Machine(CM). Therefore,we designed an *in vitro* study to assess TOB extracorporeal kinetics removal by CM.

Methods: Mock hemofiltration was performed in sham mode for 240min using saline solutions and blood(N=6)spiked with TOB(9.36±0.11 and 11.30±0.37µg/mL,respectively).After 5min of priming,each solution(blood;saline)was circulated in the closed system(Qb30mL/min;Quf2mL/min)using 0.25m^2 polysulfone membrane(PS). Samples were taken from arterial,venous,ultrafiltrate ports at 10,30,60,120 and 240 min. Sample levels were measured by TOBR Flex method (Siemens Healthcare,Newark,NJ.,U.S.A.).TOB Sieving coefficient(Sc)and mass balance were calculated.

Results: As shown in table I Sc rose rapidly during first 60 then decreased and increased over 1 indicating a complex drug-membrane interaction in saline solution.On blood, Sc rose till 30min, thereafter it reached plateau likely due to protein cake was formed on PS.Mass balance analysis has shown a different amount of adsorption between saline solution and blood at 240min:744.59±94.23 and 350.35±42.35µg,respectively.This indicate probably TOB adsorption was not concluded in blood.

Table I. Tobramycin Sieving Coefficient and Percentage of Mass Adsorbed.

Time [min]	Sc	%Mass adsorbed
saline		
10	0.50±0.04	10.12%
30	0.92±0.02	8.75%
60	1.04±0.06	8.93%
120	0.91±0.02	4.30%
240	1.10±0.05	21.83%
blood		
10	0.44±0.05	5.63%
30	0.51±0.04	2.13%
60	0.53±0.04	7.90%
120	0.53±0.01	8.88%
240	0.52±0.03	8.50%

Conclusions: Our preliminary evaluation demonstrates that TOB could be adsorbed on PS when CM is used.This finding should be taken in consideration when CM is used in pediatric patients treated with TOB.In this setting,the rate of TOB administration should be modified in order to avoid potential underdosing.

Funding: Private Foundation Support

PUB675

In Vitro Extracorporeal Removal of Linezolid by Miniaturized System

Marco Sartori,[1,2] Arianna Loregian,[2] Silvana Pagni,[2] Giorgio Palù,[2] Claudio Ronco.[1] *[1]Nephrology and Renal Research Institute of Vicenza, St. Bortolo Hospital, Vicenza, Italy; [2]Molecular Medicine, Univ of Padua, Padua, Italy.*

Background: Linezolid(LZD) is an alternative antibiotic for patients with gram(+) glycopeptides resistant infections.Septic patients frequently develop acute kidney injury then they require renal replacement therapy(RRT).It is therefore fundamental to understand drug removal by RRT.We performed an *in vitro* study to estimate kinetic removal of LZD.

Methods: We performed sham hemofiltration(SHF)for 240min using blood and saline solutions(N=6)spiked with LZD(11.75±0.08 and 17.24±0.54mg/L,respectively).After 5 min of priming,each solution was circulating in a closed circuit simulating SHF(Qb 30mL/min;Quf 2mL/min)using a 0.25 m^2 polysulfone membrane(PS)and Carpediem Machine. Samples were taken from arterial,venous and ultrafiltrate lines at 10,30,60,120 and 240 min. LZD levels were measured by high performance liquid chromatography.LZD adsorption/release from PS and Sieving Coefficient(Sc)were calculated.

Results: As shown in table Sc values gradually rose during experiments in both conditions(blood;saline).Results subsequent to SHF,primary assessment interval(10 min) mass was 1.93±0.25mg in blood and 3.78±1.89mg in saline indicating a significant reduction to starting mass (4.57±0.04 and 6.70±0.21mg in blood and saline,respectively).After 10 min,in the closed system LZD mass gradually increased till 2.64±0.09mg in blood and 5.91±0.34mg in saline at 240min.

Table I. Linezolid Sieving Coefficient and Percentage of Mass Adsorbed.

Time [min]	Sc	%Mass adsorbed
LZD-Blood		
0	—	—
10	0.66±0.13	57.80%
30	0.77±0.14	53.17%
60	0.81±0.11	47.67%
120	0.82±0.13	45.67%
240	0.91±0.12	42.96%
LZD-Saline		
0	—	—
10	0.35±0.20	43.61%
30	0.69±0.09	44.64%
60	0.81±0.12	40.71%
120	0.91±0.05	29.83%
240	0.95±0.07	18.00%

Conclusions: We observed a great LZD adsorption at the beginning of SHF,later LZD was slowly released by the PS until the end of experiments.This findings suggest that rebound phenomena occurred indeed,after rapid adsorption,LZD was released to the circulating pool.A redistribution should be considered when LZD is administered in patients receiving RRT.

Funding: Private Foundation Support

PUB676

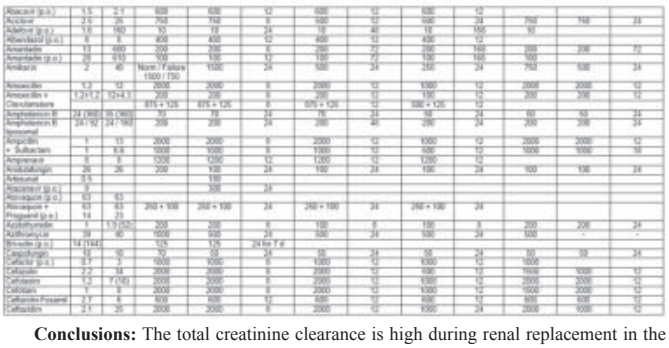
Antibiotic Drug Dosing in Patients with Renal Replacement Therapy: Comparison between Anesthesiology and Medical Intensive Care

Jasmin Frick, Ferrah Okur, Frieder Keller. *Nephrology, Internal Medicine, Univ Hospital, Ulm, Germany.*

Background: Kidney failure increases the risk of drug over dosage with toxic adverse events while renal replacement might increase the risk of under dosage with insufficient efficacy. This makes antibiotic therapy a delicate act of balancing between too much or too less dosing in the intensive care patients.

Methods: In this prospective non-interventional cohort study, we compared the antibiotic dosing in the anesthesiology and the medical intensive care unit (ICU) at our hospital. All patients were included who needed renal replacement therapy during the 10 months study period. We have taken the total creatinine clearance as a combined measure of both, residual kidney function and artificial filtration.

Results: Overall, 57 patients were recorded, 43 men and 14 women. The mean age was 61 years (SD 16). Continuous hemofiltration was preferred as compared to intermittent dialysis (89 % versus 11 %). We recorded 37 different anti-infective drugs with 4 on average per patient. The mean duration of anti-infective therapy was 8 days (SD 5). The average creatinine clearance was 44 ml/min in the patients at anesthesiology ICU and 42 ml/min at medical ICU. The mean dose was 2297 mg/day (SD 3751) in the anesthesiology ICU (632 days) and significantly higher ($p < 0.001$) than the 1664 mg/day (SD 2297) in the medical ICU (1242 days). With regard to the guidelines in our hospital, a significantly higher proportion of cases was not receiving the proposed dose adjustment in the anesthesiology ICU as compared to the medical ICU (51 % versus 41 %, $p = 0.001$).



Conclusions: The total creatinine clearance is high during renal replacement in the intensive care setting and on average > 33 % of normal kidney function. We observed a significantly higher trend to refrain from renal dose adjustment in the anesthesiology ICU as compared to the medical ICU.

PUB677

Triferic via Dialysate Reliably Delivers Iron: Clinical Pharmacology in CKD- HD Patients

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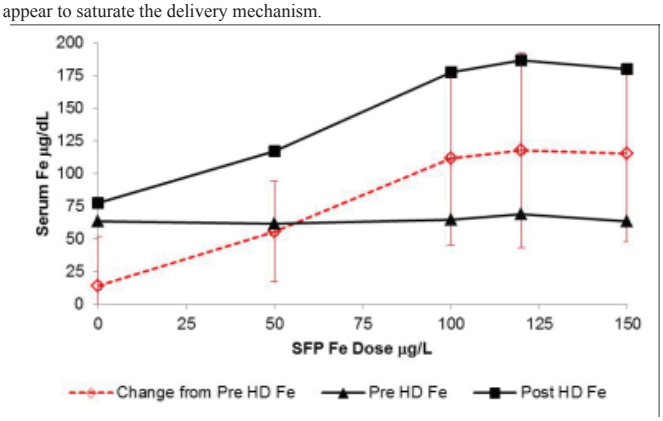
Background: Triferic™ is an iron salt, MW ~900, administered via dialysate to replace iron losses and maintain hemoglobin levels in CKD-HD. Triferic directly donates iron and pyrophosphate to human apotransferrin, thereby bypassing the reticuloendothelial system.

Methods: Serum iron, transferrin saturation (TSAT) and unsaturated iron binding capacity (UIBC) data were obtained pre and post HD at intervals in controlled clinical trials of Triferic.

Results: Triferic at doses of 20 to 110 µg Fe/L added to dialysate demonstrated a dose-proportional increase in incremental iron delivery. Doses between 110 and 150 µg/L appear to saturate the delivery mechanism.



The dose of 110 µg/L dialysate was chosen for patients, as it maximized iron delivery, reduced ESA dose, and maintained Hgb. Controlled studies demonstrated similar incremental pre-HD to post-HD increases in serum iron levels of approximately 85.9 to 139.2 µg/dL Fe over basal values. Triferic iron was rapidly cleared from the circulation and serum iron parameters returned to baseline prior to each HD treatment. Dialysis parameters including vascular access type, dialysate flow, membrane type and dialyzer reuse did not affect the delivery of Triferic iron. UIBC values decreased as serum total iron increased indicating donation of iron to transferrin. Pre-dialysis serum iron, UIBC and TSAT values remained at baseline levels throughout the study indicating no accumulation of iron in serum.

Conclusions: Triferic behaves like a middle molecule that traverses the dialyzer membrane and reliably delivers iron with each HD. Clinical trials have demonstrated that sufficient iron is delivered via dialysate to maintain Hgb by compensating for ongoing iron losses due to retained blood in the dialyzer circuit, GI losses and routine phlebotomy. Triferic is a novel iron replacement product delivered via the hemodialysate to replace dialytic iron losses.

PUB678

Development of an RNAi Therapeutic for the Treatment of Primary Hyperoxaluria Type 1

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Background: Primary Hyperoxaluria Type 1 (PH1) is an autosomal recessive disorder of glyoxylate metabolism. Hepatic glyoxylate detoxification is impaired due to mutation of the AGXT gene, which encodes for the liver peroxisomal alanine-glyoxylate aminotransferase (AGT) enzyme. Loss of AGT function to convert the intermediate metabolite glyoxylate to glycine causes accumulation and reduction of glyoxylate to glycolate which is oxidized to oxalate by the enzyme glycolate oxidase (GO). Excess oxalate in PH1 patients is unable to be fully excreted by the kidneys leading to the formation and deposition of calcium oxalate crystals in the kidneys and urinary tract. Renal damage is caused by a combination of tubular toxicity from oxalate, nephrocalcinosis and renal obstruction by stones. Greater than 30% of patients advance to end stage renal disease (ESRD).

Methods: RNA therapeutics are a new class of medicines that work by degrading a specific target mRNA to reduce the production of the disease causing protein. Here we explore the development of an RNAi therapeutic targeting GO using our clinically validated GalNac-siRNA conjugate platform. Conjugation of siRNA to the sugar N-Acetylgalactosamine (GalNAc) mediates targeted delivery to the Asialoglycoprotein receptor (ASGR) on the surface of hepatocytes, the major site of oxalate production. Using bioinformatics we designed a panel of GalNAc-conjugates that were screened *in vitro* and *in vivo* for their ability to silence the GO transcript.

Results: We identified several GalNAc-siRNA compounds from *in vitro* screening in liver cells that potently down regulated the GO transcript. The best group were evaluated *in vivo* with subcutaneous dosing to identify the conjugate demonstrating the most potent, dose responsive silencing of liver GO mRNA. Finally, we tested a lead candidate GO GalNAc-siRNA conjugate in a rodent model of PH1 to characterize the effects on oxalate production.

Conclusions: We conclude that liver silencing of GO with an RNAi therapeutic may have potential in the treatment of PH1.

Funding: Pharmaceutical Company Support - Alnylam Pharmaceuticals

PUB679

Variability in Response to Albuminuria Lowering Drugs: True or Random? Sergei Petrykiv,[1] Dick de Zeeuw,[1] Frederik I. Persson,[2] Peter Rossing,[2] Hans-Henrik Parving,[3] Ron T. Gansevoort,[1] Hiddo Jan Lambers Heerspink.[1] *[1]Clinical Pharmacy and Pharmacology, UMC, Groningen, Netherlands; [2]Steno Diabetes Center, Gentofte, Denmark; [3]Rigshospitalet, Copenhagen, Denmark.*

Background: Changes in albuminuria are monitored to estimate the response of an individual to albuminuria lowering treatment. However, albuminuria varies from day-to-day in the absence of drug treatment. The change in albuminuria after drug administration may thus reflect a random change or a true response to treatment. If the change represents a true drug response, the initial albuminuria response to treatment should correlate with the change after treatment cessation. We tested this in a series of (published) prospective clinical trials lasting 2 to 6 months.

Methods: We first analyzed the intra-individual coefficient of variation (CV) over 6 months follow-up of a first morning void albumin:creatinine ratio (ACR) in the placebo arm of 7 clinical trials of patients with normo- (n=2), micro- (n=1), and macroalbuminuria (n=4) who were on stable medication. Four of these trials included a wash-out period. In these trials we correlated the change in albuminuria during placebo or active treatment with the change in albuminuria during wash-out (off-treatment). Clinical trials included patients with macroalbuminuria and involved the following drugs: enalapril, losartan, aliskiren, atrasentan, and paricalcitol.

Results: The intra-individual CV was 19 to 25% in the normoalbuminuria range, 41% in the microalbuminuria range, and 21 to 33% in the macroalbuminuria range. In the placebo arm of the clinical trials no correlation between the on- and off-treatment albuminuria change was observed (all R^2<0.01). However, with all drugs, except paricalcitol, we observed a significant correlation between the on- and off-treatment albuminuria change (R^2 0.14 to 0.57). Correlations were stronger for trials with 24-hr albuminuria compared to trials with first morning void ACR.

Conclusions: Despite the natural day-to-day variability in albuminuria, the variability in drug response can be adequately quantified in a clinical trial setting. These data support research to identify which factors determine drug response in order to optimize use of current and new interventions.

PUB680

Quality Analysis following Adoption of Heparin-Free Dialysis with Streamline® Airless Bloodlines (SL) in Large Tertiary Inpatient Hemodialysis Practice Sami Safadi,[1] Mary Ann Ryan,[2] Amanda L. Severson,[2] Fares Alahdab,[3] Marie C. Hogan.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Nursing, Mayo Clinic, Rochester, MN; [3]KER Unit, Mayo Clinic, Rochester, MN.*

Background: Extracorporeal circuit (EC) anticoagulation (AC) with heparin was a key advancement in routine hemodialysis (HD). However, AC in patients at risk of bleeding remains a frequently encountered problem in practice. SL bloodlines eliminate blood-air contact, and reduce turbulence in dialysis circuit. Small outpatient studies suggest that SL reduces heparin use and improves dialysis efficiency. We prospectively evaluated EC clotting event rates, impact on dialysis efficiency, and associated risk factors.

Methods: Patients requiring inpatient HD were prospectively followed during their hospitalization. HD protocols were performed without routine EC heparin. Patients could be on AC, including heparin for other indications. All sessions were performed using SL bloodlines and Fresenius 2008K equipment. HD sessions were observed for clotting events defined as an interruption of dialysis session, loss of dialysis circuit, or inability to return blood to the patient.

Results: In this ongoing study, 145 adult patients received 514 inpatient HD runs (Jan-April 2014). 61% were males, 86% were white, median age 66 yrs. 46% were on antiplatelet agents, and 10% received therapeutic systemic AC with heparin. The main indication for HD was ESRD (76%). Median HD session time was 210 min (IQR 180-220), delivered BFR was 350 ml/min (IQR 350-350), UF 2 liters (IQR 1-2.5), and KTV 1.4 (IQR 1.2-1.7). The clotting rate was 6%. Clotting episodes were associated with a lower delivered KTV (est -0.4, p<0.001), and BFR (est -20, p=0.02). Major risk factors for clotting (table 1) were transfusion of blood products, temporary HD lines, and AKI.

	OR	p
Transfusions	5.4	<0.001
Temporary lines	4.6	0.002
AKI	2.6	0.01

Conclusions: We report new data on inpatient EC clotting rates and prevalent anticoagulant use in hospitalized HD patients. Our data shows that heparin-free HD using SL tubing is feasible, safe, and effective. Clotting rates are comparable to previously reported data with heparin. Further studies are needed to identify best techniques for heparin-free dialysis.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

PUB681

Intradialytic Hypotension: Prevalence and Significance in Practice Patterns as Quality Improvement Initiative Marco Formica. *Nephrology and Dialysis Dept, ASLCN1, Cuneo, Italy.*

Background: Intradialytic hypotension (IH) severely affects patients' (pts) tolerance, inducing reduced session's efficiency, high risk of vascular access failure, increasing cardiovascular events and mortality. It is reported in 15 to 50% of the dialysis sessions.

Methods: We prospectively studied 1985 sessions consecutively performed in 110 pts for about 2 months. Dialysate temperature was constant at 36°. Dialysate sodium ranges from 138 to 144 mEq/l. All pts had been on a blood volume control system. IH was defined as arterial blood pressure lower than 90 mmHg (with a drop >20 mmHg from the first measurement) and/or request of a therapeutical intervention (reduction of programmed ultrafiltration rate (UFR), infusion of saline, hypertonic NaCl, mannitol, colloids).

Results: 184 sessions were reported as complicated by IH (9.3%). They regarded 33/110 pts (30%); among these 33 pts, IH episodes ranged from 5.5% to 83% of their sessions. In all the pts but one, <33% of the sessions were complicated by IH.Mean age was 64±13 years and mean dialysis vintage 79±7 months. 26 pts were on bicarbonate dialysis (BHD) with high-flux membrane, 5 in on-line post hemodiafiltration (HDF), 1 in acetate-free biofiltration and 1 in hemodiafiltration with endogenous reinfusion. Mean UFR was 605±198 ml/min. In 59 cases it was sufficient to reduce UFR; in 111 cases infusion of saline, hypertonic NaCl or 18% mannitol was performed; in 2 cases anti-hypertensive therapy was modified and in 11 dry body weight was increased. BHD pts showed a mean of 6.5 IH sessions versus 2 IH sessions for the HDF regimens. None of the session had been stopped before prescribed time.

Conclusions: IH incidence was lower than reported: in this regard may be the regular use of blood control devices could play a role in preventing hemodynamic disturbances. It seems crucial the dry body weight estimate by constant pts surveillance as well as repeated evaluation of home pharmacological therapy. Some pts seem more prone than the mean: in this case shift from BDH to HDF could further improve IH. IH could be a usefull surrogate marker to improve practice patterns, ameliorating dialysis pts comfort and possibly reducing long term complications.

PUB682

Social Work Staffing in For-Profit and Not-for-Profit Dialysis Facilities: Implications for Patient Outcomes Teri Browne,[1] Joseph R. Merighi.[2] *[1]College of Social Work, Univ of South Carolina, Columbia, SC; [2]School of Social Work, Univ of Minnesota, Minneapolis, MN.*

Background: In accordance with a Medicare mandate, a Master's level social worker is required in every U.S. dialysis facility because of the many psychosocial barriers to optimal dialysis outcomes. Studies in the literature indicate that high dialysis social worker caseloads are associated with lower patient satisfaction, less successful patient rehabilitation outcomes, and a decreased ability to provide clinical social work interventions The aim of this study is to assess variations in social workers' practice experiences between for-profit and not-for-profit dialysis corporations.

Methods: A cross-sectional online survey contained 70, multicomponent questions and took 25 minutes to complete. Data were gathered between January 2 and March 1, 2014 using the Council of Nephrology Social Worker listserv. A sample of 617 full-time (32 hrs/wk or more) social workers was obtained from all 50 states and the Northern Mariana Islands.

Results: Overall, full-time social workers in for-profit settings (n=495) experienced greater burdens than their colleagues in not-for-profit settings (n=122). Specifically, significant differences were found between for-profit and not-for-profit providers with regard to workload demands, emotional exhaustion, and caseloads. In each case, employees in for-profit corporations reported higher workload demands (19.1 versus 17.1, p<.001), higher emotional exhaustion (19.2 versus 14.8, p<.001), and higher caseloads (108.8 versus 95.0, p<.01) than employees in not-for-profit settings. Hourly rate did not differ significantly between work settings.

Conclusions: This study represents an important national effort to quantify disparities between for-profit and not-for-profit social work practice settings. The majority of nephrology social workers in the United States are employed by large dialysis chains, and nearly 80% of dialysis patients receive their care from for-profit providers. Unfortunately, the corporate ethos of many for-profit dialysis providers has social workers performing tasks that support the financial goals of the organization and may negatively impact patient outcomes.

PUB683

Direct Hemoperfusion with Lixelle S-35: Can Be a New Option of Hyperbilirubinemia Treatment? Marco Sartori,[1,2] Aashish Sharma,[1,2,3] Mauro Neri,[1] Francesco Garzotto,[1] Davide Giavarina,[1] Claudio Ronco.[1] *[1]Nephrology and Renal Research Institute of Vicenza (IRRIV), Pharmacology Section, St. Bortolo Hospital, Vicenza, Italy; [2]Pharmaceutical and Pharmacological Sciences, Univ of Padua, Padua, Italy; [3]Nephrology, Indraprastha Apollo Hospitals, New Delhi, India.*

Background: Critically ill patients with acute liver failure have limited, complex and expensive options of treatment, therefore we decided to explore the bilirubin removal capacity of Lixelle S-35 cartridge through an in vitro test by direct hemoperfusion (DHP).

Methods: We tested two separate hyperbilirubinic bags containing plasma and blood obtained from a plasmapheresis and exchange transfusion,respectively.The total bilirubin(TBIL)and direct bilirubin(DBIL)were assessed with Jendrassik-Grof method.All tests were performed in triplicate.TBIL and DBIL baseline concentrations were 17.57±0.53, 12.57±0.23 mg/dL for plasma 23.10±0.47, 15.37±0.24 mg/dL for blood. Mock DHP was

performed for 120 min for each bag(Qb100 mL/min).TBIL and DBIL levels were measured at 10, 30, 60,120 min for samples drawing from inlet and outlet the cartridge.Mass balance analysis were calculated for TBIL and DBIL.

Results: Mass balance analysis is shown in table. TBIL and DBIL levels reached plateau subsequently to DHP. At the end of tests TBIL and DBIL levels were 7.67±0.47, 5.17±0.17 in plasma and 8.37±0.58, 5.37±0.39 mg/dL in blood, respectively.The total removal was seen as TBIL in plasma 55.60%, TBIL in blood 62.16 %, DBIL in plasma 58.87%, DBIL in blood 64.41% at the end of DHP.

Time [min]	Total Bilirubin Adsorbed mg	Direct Bilirubin Adsorbed mg
plasma bag		
10	707.70±15.85	487.20±3.64
30	863.87±5.51	606.73±10.19
60	924.38±3.39	654.79±10.49
120	958.20±5.72	680.70±10.68
blood bag		
10	819.00±16.70	522.90±6.30
30	1059.53±6.57	694.27±3.22
60	1175.86±11.72	780.36±6.15
120	1233.60±10.22	818.10±4.68

Conclusions: In vitro bilirubin adsorption capacity of Lixelle S-35 was found to be apparently superior than in vivo available techniques, less complex and cheaper.This bench to bedside approach has shown that Lexille S-35 should represent an option for the management of hyperbilirubinemia.

Funding: Private Foundation Support

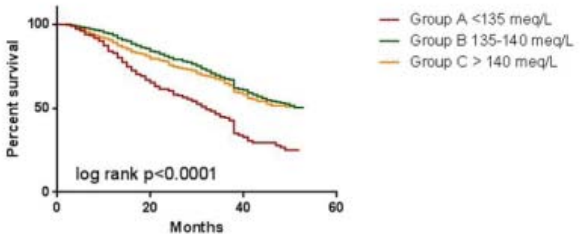
PUB684

Mortality Associated with Low Serum Sodium Concentration in Chronic Hemodialysis Patients Manolo Ramos Gordillo, Marco Antonio Carmona-Escamilla, Jose C. Pena, Ivanna Rocha, Israel Jeronimo Roque. *CEDIASA, México, D.F., Mexico.*

Background: Hyponatremia is associated with increased mortality in the general population as well as in hemodialysis patients. However the normal extracellular fluid volume balance and the osmo-regulation responsible for this complication is lacking in dialysis patients; so the mechanisms for this low serum sodium are of different etiology than the ones found, in the general population.

Methods: A retrospective study was conducted from January 2010 to March 2014 in nine of our Hemodialysis Units in Mexico City. Our study included 4128 patients, 2078 men (51%) and 2050 women (49%). All patients were treated with high flux hemodialysis during 3 to 4 hours, 3 times per week. Monthly predialysis serum sodium [Na^+] was obtained in all patients. The patients[Na^+] in mEq/L was stratified in three groups: (A:<135; B: 135-140; C: >140). Other variables studied were: BUN, creatinine, hemoglobin, hematocrit, albumin, calcium and phosphorus Data were analyzed for absolute and relative frequencies as well as survival curves (Kaplan- Meier and Cox Regression).

Results: Mean[Na^+] of all patients was 137.9 mEq/L. Groups: A 133.2 ± 1.97; B 137.8 ± 1.34; C 141.2 ± 1.10 ($p< 0.05$). Percent survival analysis at 4 years showed: A: 57% ;B 72% and C 69% (long rank t test 0.001).



Mean value of survival time in months. Groups: A 31 (CI 29.2-33);B 41 (CI 40-41.5) C 39 (CI 38-40).A higher risk of death was found in group A (Fig1). Multivariable adjustment for demographic, clinical and laboratory data, did not appreciably change the results (Hazard ratio 0.772 CI 95% 0.68-.88). However serum albumin in Group C improved survival.

Conclusions: Low serum sodium concentration was associated with a higher risk of death. It is possible that hyponatremia per se is a causal of mortality in this large cohort but with a different etiology than the general population.

PUB685

Migration to Heparin-Free Hemodialysis (HD) with Streamline® Airless Bloodlines (SL) in a Large Medical Center Inpatient Practice Sami Safadi,[1] Mary Ann Ryan,[2] Amanda L. Severson,[2] Marie C. Hogan.[1] *[1]Nephrology and Hypertension, Mayo Clinic, Rochester, MN; [2]Nursing, Mayo Clinic, Rochester, MN.*

Background: The development of the routine HD practice was facilitated by the introduction of extracorporeal circuit (EC) anticoagulation (AC) with heparin. However, EC-AC carries an increased risk of bleeding, a frequent concern in the hospitalized patients with multiple medical comorbidities including coagulopathies. Conventional HD tubing also involves blood-air contact, typically in the arterial and venous chambers which increases the likelihood of clotting. Streamline® (SL) bloodlines eliminate blood-air contact, and reduce turbulence in dialysis circuit, which decreases the likelihood of clotting. Small outpatient studies suggest that SL reduces heparin use and improve dialysis efficiency. Our inpatient HD units moved to heparin-free dialysis with SL tubing with subsequent reductions in heparin use. Here we report our experience.

Methods: All dialysis staff were trained and assessed in competence for correct setup-up of the air free tubing, spanning one month. Procedural guidelines for set up and critical assessment of the dialysis circuit were implemented across the inpatient practice. Staff underwent training in the assessment of circuit clotting. Fresenius 2008K HD machines were calibrated to meet tubing specifications the night prior, and on the specified date we migrated to the new system. Clotting during HD is now routinely assessed in all inpatient HD sessions through a post rinse-back visual guide. We validated the method showing good reproducibility across all dialysis nursing staff.

Results: Our heparin prescription rates during hemodialysis dropped from an average of 15-25% before the introduction of SL tubing to <1% afterwards. There was no change in the clotting rate (around 2-8%) with the change to heparin-free dialysis with SL.

Conclusions: We have successfully migrated from intermittent extracorporeal AC with heparin to heparin-free airless with SL tubing in a busy inpatient safely. Routine monitoring for extracorporeal circuit clotting should be a standard quality procedure in inpatient dialysis units.

Funding: Pharmaceutical Company Support - NxStage Medical, Inc.

PUB686

Measurement of Polyvinylpyrrolidone from Un-Primed and Primed Fresenius Dialyzers Subjected to Exaggerated Extraction and Simulated Use Conditions Betsy J. Endrizzi,[1] Candice Moon,[1] Daniel E. Mclain,[2] Sherry Parker,[3] James P. Kennedy,[1] Chih-Hu Ho.[1] *[1]Global Research and Development, Fresenius Medical Care, Ogden, UT; [2]Walker Downey & Associates, Inc., Verona, WI; [3]WuXi AppTec, St. Paul, MN.*

Background: A study was conducted to gain thorough understanding of the polyvinylpyrrolidone (PVP) profile in Fresenius dialyzers. The dialyzer models chosen for this study included electron beam (E-beam) sterilized Optiflux® F200NR, F250NR and Optiflux® Ultra F230.

Methods: Exaggerated extractions of un-primed and primed dialyzers involved a complete filling of the dialyzer with ethanol, water or saline for 24 hours at 50-70°C. PVP was quantified in the extracts using a validated NMR method.

Results: Results indicate that exaggerated extraction of un-primed dialyzers with ethanol, water and saline removed an average of 63, 104 and 91 mg of PVP, respectively. Exaggerated extractions of primed dialyzers removed 52, 17 and 16 mg of PVP with ethanol, water and saline, respectively. Notably, priming the dialyzers as instructed for clinical use significantly ($p<0.0005$) reduced the amount of PVP extracted by exaggerated methods in aqueous solvents, but not with ethanol. Two independent toxicologists calculated the margin of safety (MOS) for PVP in these exaggerated extracts to range from 2-22 for primed dialyzers, with a likely 10-100 fold increase in MOS during actual clinical use conditions.

Conclusions: Even considering the mean values of the exaggerated extraction data plus two standard deviations, the MOS values were still greater than 1 for all extraction solvents and dialyzer models, indicating the likelihood of an adverse event occurring in an exposed patient population would be negligible.

Funding: Pharmaceutical Company Support - Fresenius Medical Care

PUB687

Serum Sodium Concentration in Haemodialysis: Is There a Set Point? Eduardo Baamonde,[1] Elvira Bosch,[1] German Perez Suarez,[1] Fatima Batista,[1] Gloria Anton,[1] Mar Lago,[2] Agustin Toledo,[2] Cesar Garcia-Canton.[2] *[1]Nephrology, Avericum, Telde, Las Palmas, Spain; [2]Nephrology, Hospital Univ Insular de Gran Canaria, Las Palmas, Spain.*

Background: In patients on haemodialysis (HD) Serum sodium concentration (SSC) is maintained to a constant individualized level called the "sodium set point". Some observations question this approach **Objective.** To analyze the SSC and variability of a standard HD population, with constant dialysate (139 mEq/l).

Methods: Retrospective study including 261 HD prevalent patients (60.9% males; 51.7% diabetic; mean age 60.04 ± 14.09 years; time in HD 69.64 ± 50.53 months; follow-up time 48.78 ± 19.09 months). SSC was corrected for glucose. 24 measurements were used to calculate the mean and to analyze variability. Demographic, clinical parameters, laboratory test results and mortality rates were analyzed.

Results: 6221 measurements of natremia was made. The mean SSC was 138.26 mEq/l (range: 120.86-151.65 mEq/l); 917 (14.74%) were lower than 135mEq/l. Natremia was negatively correlated with the sodium variation coefficient (VC) (r: -0.243; p<0.000), the inter-dialysis weigth gain (IDWG)(r:-0.254; p<0.000) and the ultrafiltration rate (r:-0.279; p<0.000) and positively with serum albumin (r: 0.169; p:0.006).

Sodium tertiles	<137.49 (n: 87)	137.5-139.11 (n:87)	>139.11 (n: 87)	*p*
Natremia (mEq/l)	135.83 ± 1.54	138.34 ± 0.47	140.49 ± 1.01	*0.000*
DM (%)	52.9	46	46	*n.s*
VC Sodium (%)	2.34	1.74	1.66	*0.014*
DST Sodium	3.17	2.41	2.33	0.024
Glucose (mg/dl)	150.84 ± 60.85	148.14 ± 54.49	145.11 ± 47.46	*n.s*
IDWG (%)	3.08 ± 0.69	2.98 ± 0.70	2.68 ± 0.71	*0.001*
Uf rate (ml/kg/mn)	8.06 ± 1.89	7.67 ± 1.94	6.92 ± 1.75	*0.000*
Albumin (g/dl)	3.62 ± 0.24	3.72 ± 0.27	3.74 ± 0.24	*0.006*

No difference was found in mortality rates or hospitalization days during follow-up.

Conclusions: Pre-haemodialysis SSC is inversely correlated with inter-dialysis hydration. The variability of natremia increases in the lowest tertile, coincidently with more severe overhydration. A positive diffusive sodium gradient between dialysate and plasma could explain these observations.

PUB688

Evaluation of Antihypertensive and Nitrate Use in Patients with Intradialytic Hypotension in the Inpatient Setting Andrew Nishimoto,[1] Benjamin Duhart,[2] David Shoop,[1,2] Robert B. Canada,[3] Joanna Hudson.[1,2] *[1]Methodist Le Bonheur Healthcare; [2]Univ of Tennessee College of Pharmacy; [3]Univ of Tennessee College of Medicine, Memphis, TN.*

Background: Intradialytic hypotension (IDH) is the most common complication of hemodialysis (HD). Administration of antihypertensive medications (AHTs) and nitrates in the inpatient setting often occurs prior to dialysis; however, the influence on the rate of IDH is unclear. The purpose of this study was to evaluate the association of AHT and nitrate therapy on development of IDH and to identify other factors associated with IDH.

Methods: A retrospective chart review was performed to identify patients admitted to Methodist University Hospital with ESRD requiring HD from August 2011 to August 2013 who met inclusion criteria. The study population was divided into IDH and non-IDH cohorts. We evaluated a maximum of five dialysis sessions per patient. AHTs and nitrate use within 12 hours prior to each HD session was compared between groups. The association between development of IDH and serum albumin, pre-HD blood pressure, serum sodium and the ultrafiltration rate during HD was also evaluated. Univariate analyses were used to detect significant differences between groups and multivariate logistic regression identified predictors of IDH.

Results: In 104 patients studied (50 IDH, 54 non-IDH), the proportion of patients receiving AHTs was higher in the IDH group compared to the non-IDH group (82% and 65%, respectively). Additionally, a greater percentage of the IDH cohort had an arteriovenous graft as their HD access (26% versus 5%) and had a higher mean pre-dialysis systolic blood pressure (SBP) (141 versus 134 mmHg). IDH was also associated with higher admission hemoglobin, higher pre-dialysis SBP, and graft access. Subanalysis of only dialysis sessions when IDH occurred demonstrated a negative correlation between total AHT/nitrate doses received and IDH. Graft access was no longer significant in this prediction model.

Conclusions: This study suggests that in addition to the timing of administration of AHTs, other factors including pre-dialysis SBP and hemoglobin are important to consider when evaluating risk of developing IDH.

PUB689

Pre-Haemodialysis Natremia and Body Composition Eduardo Baamonde,[1] Elvira Bosch,[1] German Perez Suarez,[1] Gloria Anton,[1] Fatima Batista,[1] Mar Lago,[2] Agustin Toledo,[2] Cesar Garcia-Canton.[2] *[1]Nephology, Avericum, Telde, Las Palmas, Spain; [2]Nephrology, Hospital Univ Insular de Gran Canaria, Las Palmas, Spain.*

Background: In patients on haemodialysis, sodium serum concentration (SSC) remains stable although with large interindividual variability. Some results have suggested that lower SSC are associated with more severe overhydration. **Objective:** to analyze the relationship between pre dialysis SSC and body composition, especially between SSC and body water.

Methods: Retrospective study included 129 conventional HD prevalent patients (65.9% males, mean age 61.12 ± 13.83 years, 49.31% diabetic). Body composition was analyzed immediately after mid-week dialysis session with a bioimpedance monofrequency monitor (BIA) (AKERN). SSC was calculated by using the last 12 monthly readings before BIA and corrected for glucose.Demographic and clinical parameters, laboratory test results and body composition were analyzed.

Results: The mean SSC was 137.86 ± 2.49 mEq/l. Patiens were grouped into tertles according SSC.

Sodium tertiles	<137.29 (n: 42)	137.3-138.93 (n:44)	>138.93 (n: 43)	*p*
Natremia (mEq/L)	135.24 ± 2.38	138.16 ± 0.51	140.13 ± 0.90	*0.000*
DM (%)	48.4	44.2	43	*n.s*
IDWG (%)	3.45 ± 0.84	3.10 ± 0.74	2.69 ± 0.90	*0.000*
Uf rate (ml/kg/mn)	9.06 ± 2.49	8.08 ± 1.93	7.34 ± 2.72	*0.005*
Albumin (g/dl)	3.63 ± 0.18	3.71 ± 0.16	3.69 ± 0.25	*n.s*
TBW (%)	67.20 ± 13.7	67.36 ± 10.4	68.48 ± 10.33	*n.s*
ECW (%)	58.66 ± 48.86	51.67 ± 7.27	53.31 ± 8.68	*n.s*
ICW (%)	49.39 ± 9.96	46.47 ± 8.57	46.12 ± 9.06	*n.s*
FFM (%)	30.09 ± 7.77	36.72 ± 11.4	36.87 ± 13.79	*0.008*

Pre-dialysis sodium was negatively correlated with: inter-dialysis weight gain (IDWG) (r -0.353; p 0.000), ultrafiltration rate (UR) (r -0.338; p 0.000), and extracellular water (%) (ECW)(r -0.230; p 0.009) and positively correlated with faty free mass (%) (FFM) (r 0.207; p 0.018).

Conclusions: Patients with the lowest sodium levels presented the most severe interdialysis overhydration. No differences were found in the distribution of body water between patients in different tertiles of natremia. Natremia and body water are negatively correlated.

PUB690

Proteomic Evaluation of Low- and High-Flux Hemodialysis Carlo Donadio,[1] Danika Tognotti.[2] *[1]Clinical and Experimental Medicine, Univ of Pisa, Pisa, Italy; [2]Istituto di Biofisica, CNR, Pisa, Italy.*

Background: The principal mechanisms of blood depuration by hemodialysis are diffusion, convection and adsorption. Hemodialysis may be performed using low-flux (LF) membranes which remove only small molecules, or high-flux (HF) membranes with remove also bigger molecules. Aim of the this study was to assess, by means of proteomic techniques, the relevance of convection and adsorption of proteins with different membranes.

Methods: Seventeen patients treated with LF polysulphone and with HF membranes (Triacetate; Helixone; Polyamid). Proteomic analysis.30 min after the beginning of the dialysis a sample of ultrafiltrate fluid was analyzed by means of biochemical and proteomic techniques. At the end of the dialysis we assessed the adsorption of proteins on the different dialytic membranes. The proteins in the ultrafiltrates and those eluted from the membranes were analyzed through SDS PAGE, 2DE, and MALDI-TOF.

Results: Biochemical analysis.A very high removal of small molecules was demonstrated, which was similar with all the membranes. The removal of B2M was high with HF membranes and insignificant with LF membrane. The removal of bigger molecules (myoglobin and BNP) was higher with triacetate than with the other HF membranes. **Proteomic analysis.** No convection of small proteins (LMWP) in the ultrafiltrate fluid was found through LF polysulfone. Polyamid and helixone allowed the convection of LMWP. In the ultrafiltrate of triacetate a higher amount of different LMWP was demonstrated. Proteins with different MW were found in the eluates from the different membranes. In particular, albumin and LMWP were demonstrated on the inner part and inside the triacetate membrane. Much more than with the other membranes.

Conclusions: Proteomic technology allows a better understanding of mechanisms of blood depuration of the different membranes.

Funding: Government Support - Non-U.S.

PUB691

Fluid Status and Impact on Lab Parameters for Adequacy of Hemodialysis Care Kotagal Shashi Kant,[1,2] Heather Duncan,[1,2] Mahmoud T. El-Khatib.[1,2] *[1]Internal Medicine, Div Nephrology and Hypertension, Univ of Cincinnati College of Medicine, Cincinnati, OH; [2]Dialysis Clinic, Inc, Cincinnati, OH.*

Background: Most clinical measures in a dialysis population are targets for medical intervention, and clinic performance is evaluated by the clinic's performance measures for hemoglobin, phosphorous and albumin. As part of CMS associated Quality Improvement Program (QIP) projects, hemodialysis units are monitored for outcome based reimbursement. However, these measures are likely to vary for a number of reasons, not least because of measurement related artifacts. It is likely that fluid status will affect lab parameters via a dilution effect and/or the effects on inflammation and cardiac/pulmonary performance. We examined lab parameters affected by fluid status by comparing several measures collected from hemodialysis patients after the long interdialytic interval versus a short one.

Methods: A retrospective chart review identified instances where the same lab parameter was collected after a short and a long interdialytic interval. Hemoglobin, phosphorous, calcium, albumin, total protein, CO2, creatinine, potassium and weight gains were then compared with paired t-tests.

Results: Measures obtained after both a short and long interval in the same month (n=38) were examined. When measured after a short interdialytic interval (mean weight gain 2.17 kg) versus a long interval (mean weight gain 3.41 kg) hemoglobin, albumin and total protein were higher, while creatinine was lower. These changes are important both for anemia management, ESA dosing, as well as reported quality measures. Phosphorous and calcium were also higher, but still within DOQI guidelines. Neither CO2 nor potassium varied by interdialytic interval.

measure mean	long interval	short interval
hemoglobin g/dL	10.80	11.05*
albumin g/dL	3.82	3.94*
total protein g/dL	6.90	7.00*
creatinine mg/dL	10.62	9.36*
phosphorous mg/dL	4.92	5.20**
calcium mg/dL	8.92	9.23**
potassium meq/L	4.68	4.59ns
CO2 meq/L	22.16	22.66 ns
interdialytic weight gain kg	3.41	2.17*
* P<0.001	** P<0.05	

Conclusions: We propose that outcomes measures and reporting, as well as computer generated algorithms for dose adjustments, should account for the interdialytic interval.

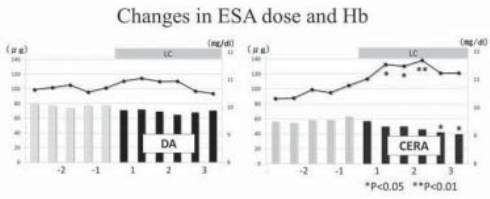
PUB692

Intravenous L-Carnitine Administration Demonstrates Superior Cost-Effectiveness when Combined with CERA Compared to Darbepoietin (DA) for Treatment of Anemia in Hemodialysis (HD) Patients Jyunichiro Hashiguchi,[1] Yoshiaki Lee,[1] Kenji Sawase,[1] Hiroshi Ichinose,[1] Osamu Sasaki,[1] Rica Etoh,[1] Masatoshi Hayashida,[1] Yoko Obata,[2] Tomoya Nishino,[2] Takashi Harada,[1] Satoshi Funakoshi.[1] *[1]Nagasaki Kidney Center, Nagasaki, Japan; [2]Nagasaki Univ, Nagasaki, Japan.*

Background: A number of clinical studies have suggested that LC, a naturally occurring compound involved in bioenergetic processes, may reduce the dose of erythropoiesis stimulating agents (ESA) in the treatment of the anemia of HD patients. In this study, our aim was to evaluate the effect of intravenous administration of LC on hematological parameters and also on monthly requiring dose of DA or CERA in HD patients.

Methods: Seventy-four HD patients receiving various dose of either DA (n=48) or CERA (n=26) biweekly were enrolled in this study after appropriate IC. Intravenous L-carnitine (1000 mg) was administered after each HD session for 24 weeks. The doses of each ESA were adjusted in each patient to keep Hb levels in the range 10-12 g/dL, and other treatment procedures including iron supplementation stayed the same. The weekly requiring doses of DA or CERA and hematological parameters of patients were recorded every other week.

Results: As shown in Figure 1 significant reduction of monthly ESA dose was observed in CERA-treated group after 6 weeks. In DA-treated group, monthly ESA dose tended to decrease, but not significant.



Conclusions: In our study the combination of intravenous LC with CERA was superior to the combination of LC with DA in dose-minimizing effect of ESA, and potentially be cost-effective.

Funding: Private Foundation Support

PUB693

Pre-Dilutional Hemodiafiltration Using Xevonta Dialyser Seems to Generate Less Pro-Inflammatory Reaction Carlos Frangié, Rosier Emmanuelle, Thierry Baranger, Frank Berge. *Néphrologie et Dialyse, Polyclinique Bordeaux Nord, Bordeaux, France.*

Background: Chronic inflammation in dialysis patients is correlated with an increase in mortality rate from cardiovascular disease. It is secondary to factors related to the patient and to the dialysis technique, especially with the biocompatibility of the membranes. Cytokines play a crucial role in the development and maintenance of the inflammatory state. We compare in this study the effect on cytokine production of two polysulfone membranes.

Methods: This is a prospective study carried out in two phases. We included ten patients treated by pre-dilutional hemodiafiltration, using biocompatible polysulfone membranes with very high permeability. This is for Phase 1 (P1), a new generation membrane (Xevonta®,BBraun) and for Phase 2 (P2) an older membrane (PF210H®,Gambro). Serum assays of IL-6 (pro-inflammatory), IL-10 (anti-inflammatory), and CRP were performed before and after at the last dialysis of the P1, and after 6 weeks wash-out, at the last dialysis of the P2. The IL6 and IL10 assays were performed by Elisa.

Results: The assay of interleukin 6 and 10 proved to be generally low. The median of IL-6 before dialysis is 6.655 pg/ml (P1) and 6.075 pg/ml (P2), and after dialysis is 6.275 pg/ml (P1) and 5.23 pg/ml (P2). There is no significant difference between the two phases, or within each phase. The median of IL-10 before dialysis is 4.19 pg/ml (P1) and 1.29 pg/ml (P2), with a statistically difference (p=0.0055) and after dialysis to 5.65 pg/ml (P1) and 2.42 pg/ml (P2) with a statistically difference (p = 0.0329). There was a statistically significant difference between before / after dialysis for P2 (p = 0.0329), but not for P1 (0.229). The median PCR is 4.5mg/l, without any correlation with interleukins rates. The results showed that the immunological balance is leaning pro-inflammatory side at P2 while the balance is maintained in the P1.

Conclusions: The rate of interleukins 6 and 10 in pre-dilutional hemodiafiltration is low. The new generation of biocompatible membranes appears to generate less pro-inflammatory reaction. A study involving a larger number of patient is however necessary to confirm these results.

Funding: Pharmaceutical Company Support - BBraun-Avitum

PUB694

Effects of Shensongyangxin Capsule on Heart Rates Variability and Insomnia in Maintenance Hemodialysis Patients Zhenda Zheng,[1] Cailian Cheng,[2] Chenggang Shi,[2] Xun Liu,[2] Tan-Qi Lou.[2] *[1]Dept of Cardiology, the Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China; [2]Dept of Nephrology, the Third Affiliated Hospital of Sum Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: The decrease of heart rate variability is very common and is related to the risk of death in maintenance hemodialysis patients. Shensongyangxin capsule(SSYX) is an Chinese patent medicine.To investigate the effects of SSYX on heart rate variability and insomnia in maintenance hemodialysis patients.

Methods: Sixty-three maintenance hemodialysis patients in the third affiliated hospital of Sun Yat-Sen University from 2013 June to December were divided into two group, control group(n=30) and experimental group(n=33).The experimental group were received SSYX 4capsules QID for eight weeks, all patients received holter test and Pittsburgh sleep quality index were measured before and after the study.

Results: SSYX can improve heart rate variability[SDNN (93.2±1.4) *versus*(82.4±13.1) ms, SDNNi (41.2±12.8) *versus*(28.4±12.2)ms, SDANNi (81.3±21.1) *versus* (73.2±20.7) ms, RMSSD (28.3±13.2) *versus*(21.8±11.9)ms, PNN50 (9.6±7.1) *versus*(7.1±5.8)%, $P<0.05$], the incidence of premature atrial contraction, atrial tachycardial, premature ventricular contraction, ventricular tachycardial and atrial- ventricular blocker decreased significantly($P<0.05$), SSYX also improved sleep quality significantly[PSQI(6.28±2.12) *versus*(14.39±2.84), $P<0.05$].

Conclusions: SSYX is an effective and safe agents to improve heart rates variability and insomnia in hemodialysis patients.

Funding: Government Support - Non-U.S.

PUB695

Observation of Clearance Effects of Hemodialysis and Hemodiafiltration on Uremic Toxins in DKD-Caused Uremia Patients Chenggang Shi, Mei Li, Xun Liu, Cailian Cheng, Qiong-Li Yin, Weizhao Mo. *Dept of Nephrology, the Third Affiliated Hospital of Sun Yat-sen Univ, Guangzhou, Guangdong, China.*

Background: Uremic Toxins are thought to be involved in many complications of diabetic kidney disease (DKD) caused Uremia Patients.The aim of the current study was to investigate the different blood purification techniques on removal uremic toxins in DKD caused uremia patients with hemodialysis (HD) and hemodiafiltration (HDF). Survey the clearing effects of blood urea nitrogen (BUN), middle molecular substances (MMS),pentosidine and indoxyl sulfate (IS).

Methods: 36 cases DKD caused uremia hemodialysis patients were divided into HD group (n = 24), HDF group (n = 12). Before and after each conventional HD and HDF therapy, blood was sampled for BUN with automatic biochemical analyzer(Olympus company, Japan), MMS with Ultraviolet spectrophotometry, pentosidine with enzyme-linked immunosorbent assay method (from ADL Company, U.S.A.), IS with high performance liquid chromatography method. Single dialysis adequacy (KT/V) was calculated by the formula Daugirdas.

Results: For HDF versus HD, the RR was significantly higher for MMS (29.10± 5.54 versus 22.06 ±6.27%, $p < 0.05$), pentosidine (28.97 ± 6.37 versus 21.45 ± 9.82%, $p < 0.05$). There were no significant differences in the RR for IS (20.09 ±6.60 versus 24.36 ± 8.74%, $p > 0.05$) and BUN (67.70± 8.98 versus 68.05 ±5.52%, $p>0.05$). KT / V were more than 1.3, and KT / V difference was not statistically significant.

Conclusions: HDF may provide an improved form of treatment that achieves significantly better MMS, pentosidine level reduction than conventional HD for DKD-caused uremia patients.

Funding: Government Support - Non-U.S.

PUB696

Do Proton Pump Inhibitors Affect Serum Acidity in the Patient on Maintenance Hemodialysis? Youssef El Douaihy, Ninad D. Parekh, Chetana Rondla, Emad Gobran, Mazen Zaarour, Iskandar Barakat, Najamus Sehar, Vera Zaraket, Suzanne E. El Sayegh, Osama Souied, Marc M. Saad. *Nephrology, Staten Island Univ Hospital, Staten Island, NY.*

Background: A few case reports mention the effects of proton pump inhibitors on pH. The objective was to investigate associations between PPIs and metabolic acidosis in patients on hemodialysis (HD).

Methods: This is a retrospective analysis conducted at a single HD facility in 2013. The primary outcome was metabolic acidosis (bicarb ≤24mEq/L). The independent variables are shown in table1. T-test and Chi^2(χ^2) analyses were used to compare means and proportions respectively between controls and acidotics. Nominal regression was used to test for predictors and account for confounders.

Results: 154 patients on HD were included in this study.

Variable	Control distribution (%) or *mean	Metabolic acidosis distribution (%) or *mean	P value χ^2 or *t-test	Entire cohort distribution (%) or *mean
Sex				
F	32.1%	37.6%	>0.05	35.7%
M	67.9%	62.4%		64.3%
Ethnicity				
African	20.8%	20.8%		20.8%
American	45.3%	39.6%		41.6%
Caucasian	18.9%	21.8%	>0.05	20.8%
Hispanic	3.8%	6.9%		5.8%
Asian	11.3%	10.9%		11%
Other				
PPI				
No	54.7%	58.4%	>0.05	57.1%
Yes	45.3%	41.6%		42.9%
H2 Blocker				
No	88.7%	91.1%	>0.05	90.3%
Yes	11.3%	8.9%		9.7%
Calcium Acetate				
No	64.2%	69.3%	>0.05	67.5%
Yes	35.8%	30.7%		32.5%
Sevelamer bicarb				
No	69.8%	59.4%	>0.05	63.0%
Yes	30.2%	40.6%		37%
Lanthanum				
No	98.1%	98.0%	>0.05	98.1%
Yes	1.9%	2%		1.9%
URR				
<65	1.9%	8.9%	>0.05	6.5%
>65	98.1%	91.1%		93.5%
Acidosis				
No	-	-	-	34.4%
Yes				65.6%
Kt/V				
<1.2	2%	6.9%	>0.05	5.2%
>1.2	98%	93.1%		94.8%
Age	*63	*62	>0.05	*61.9
Bicarbonate mEq/L	-	-	-	*23.2
Albumin g/dL	*3.7	*4	.002	*3.8

The t-test comparison of independent means showed a higher mean albumin 4 for the acidotic patients versus 3.7 with statistical significance. χ^2 showed no differences between the comparison groups. The nominal regression analysis confirmed the relationship between albumin and acidosis with a p=.008.

Conclusions: In the sample of randomly selected patients the use of PPI had no correlation to acidosis. However high levels of albumin proved to carry a predictive power of acidosis contrary to what's established. This relationship held its validity even after accounting for cofounders making it worthwhile to investigate its etiology in the future.

PUB697

Association between Serum Bicarbonate and Mortality in Haemodialysis Population. A Single Centre 5-Year Experience Asmaa Y.M. Al- Chidadi,[1] Abdelgalil Abdelrahman Ali.[2] *[1]Faculty Of Epidemiology, London School of Hygiene and Tropical Medicine, London, London, United Kingdom; [2]Nephrology Dept, Midessex Hospitals Trust, Chelmsford, Essex, United Kingdom.*

Background: Many observational studies showed that uncorrected metabolic acidosis can worsen dialysis outcomes. However Dialysis Outcome Practice Pattern Study and other observational studies showed that moderate acidosis was associated with a better nutritional and survival outcomes. We set off to investigate the association of metabolic acidosis with the risk of death in our cohort.

Methods: Data were obtained from electronic records at the Broomfield Centre. 288 patients' dialysis records from Jan 2007 till December 2012 were reviewed. Multivariate analysis of the association between Serum bicarbonate level and all-cause mortality was analysed using Poisson regression model. Serum bicarbonate was divided into 4 categories ranging from >19 to 22, 23-24, 25-26 and >27mmol/l.

Results: 288 participants were studied, of whom 59 were lost to follow up. Over the follow up period, 149 deaths were recorded with a total time at risk of 13619.6 months. The crude rate of mortality in the lowest S bicarbonate group (19-22 mmol/l) was 0.01 with similar crude rates seen in the remaining 3 S bicarbonate groups. Rate ratios were adjusted for age, sex, ethnicity, BMI, dialysis vintage, Kt/V, Haemoglobin level, predialysis systolic and dialstolic BP, WBC and lymphocytes count, and inflammatory markers (CRP, S.Alb, S. Ferritin) Comorbidities (Ischaemic heart disease, CVA, cancer, DM, COPD and smoking). The rate ratios, adjusted for the above variables were 0.61 (95% CI 0.26-1.40), 1 (baseline group which was 23-24 mmol/l), 1.19 (95% CI 0.59-2.38) and 1.82 (95% CI 0.82- 4.05) for the above 4 bicarbonate groups respectively. All rates were not statistically different from each other. The rate of death increased with the higher levels of S bicarbonate. However the sample size was not large enough to detect this statistically.

Conclusions: Mild acidosis did show some protective effect with a trend towards increasing mortality rate after adjustment for various confounders but this did not reach a significant level. A larger sample size is required for future studies.

PUB698

eEOC-Mediated Modulation of Endothelial Autophagy, Senescence, and EnMT in Murine Diabetic Nephropathy Daniel Patschan, Susann Patschan, Gerhard A. Mueller. *Nephrology and Rheumatology, Univ Hospital Göttingen, Germany.*

Background: *Early Endothelial Outgrowth Cells* (eEOCs) protect mice from ischemic AKI. Autophagy acts as endogenous cellular defense mechanism. Nevertheless, defective autophagy has been shown to promote Stress-Induced Premature Senescence (SIPS) of endothelial cells. Endothelial-to-Mesenchymal Transition contributes to renal fibrosis in CKD and it possibly reflects a state of aggravated endothelial senescence. Aim of the study was to analyze endothelial autophagy, senescence, and EnMT in eEOC-treated murine diabetic nephropathy.

Methods: Diabetes was induced in male, 8-12 weeks old C57/Bl6N mice by intraperitoneal injection of streptocotozin (STZ). Untreated and BMP-5 pretreated syngeneic murine eEOCs were systemically administered at day 2 after the last STZ injection. Animals were analyzed 8 weeks later for renal function, proteinuria, morphology, endothelial autophagy, senescence, and EnMT, respectively.

Results: Renal function and protein excretion were significantly affected / increased at week 8. Intrarenal collagen deposition was increased although not to a significant extent. Endothelial cells diaplayed higher expression of aSMA, indicating EnMT. Administration of native and BMP-5 treated eEOCs protected animals from CKD and proteinuria, the latter was even more diminished in mice receiving BMP-5 treated eEOCs. EnMT was reduced by the cells as well. Both cell populations reduced endothelial SIPS and BMP-5 treated eEOCs diminished endothelial autophagy.

Conclusions: Particularly BMP-5 treated eEOCs act renoprotective in murine diabetic nephropathy. The data suggest that by eEOC-mediated modulation of intrarenal endothelial autophagy and senescence, EnMT is reduced and progression of diabetic nephropathy is inhibited.

PUB699

Bone Marrow-Derived Mesenchymal Stem Cells and its Conditioned Medium Attenuate Fibrosis in an Irreversible Model of Unilateral Ureteral Obstruction Andrei Furlan Silva, Kleiton Augusto Santos Silva, Nestor Schor. *Nephrology, Univ Federal de São Paulo, São Paulo, Brazil.*

Background: Recently the therapeutic potential of Mesenchymal Stem Cells (MSCs) and its Conditioned Medium (MSC-CM) has been extensively studied. It is known about their ability to repair tissue, reduce local inflammation and its immunomodulation. Renal tubular interstitial inflammation induces chronic damage, resulting in fibrosis and progress to CKD. A establish fibrosis model is Unilateral Ureteral Obstruction (UUO). Here, it was evaluated factors influenced by MSCs or MSC-CM in UUO.

Methods: MSCs extracted from rat's bone marrow were cultivated *in vitro* and characterized by flow cytometry and cellular differentiation. Four groups of female rats were used in *in vivo* experiments (n=7): SHAM, UUO, UUO+MSC and UUO+MSC-CM. The MSCs or MSC-CM were administered via abdominal vena cava after left ureter total ligation. After 7 or 14 days rats were killed and serum and obstructed kidney were collected.

Results: It was observed reduction in the amount of fibrosis assessed by deposition of collagen in obstructed animals treated with MSCs or MSC-CM. By Immunohistochemical assays it was observed reductions in the amount of Caspase-3, α-SMA and PCNA in treated animals.

	Sirius Red (7 d)	Sirius Red (14 d)	alpha-SMA (7 d)	alpha-SMA (14 d)	PCNA (7 d)	PCNA (14 d)	Casp-3 (7 d)	Casp-3 (14 d)
UUO	1.2±0.2	1.8±0.2	7.2±1.2	10.8±1.2	3.0±1.1	2.0±1.2	1.8±1.0	7.7±1.0
UUO+ MSC	0.3±0.1*	0.6±0.1*	1.3±0.1 *	2.5±0.3 *	0.5±0.3 *	5.1±0.5 *	0.8±01	3.9±0.4 *
UUO+ CM	0.4±0.1*	0.3±0.1*	1.0±0.0*	1.7±0.2 *	1.0±0.5 *	3.7±0.8 *	1.1±0.2	6.5±1.0

Table 1. Picro Sirius Red staining and immunohistochemistry for alpha-SMA, PCNA and Caspase 3. Values are expressed in % of staining area (mean±SD). * vs UUO group.

Conclusions: Results suggested that administration of MSCs or MSC-CM improves fibrosis progression and change factors involved in apoptosis, inflammation, cell proliferation and epithelial-mesenchymal transition in Wistar rats subjected to unilateral ureteral obstruction.

Funding: Government Support - Non-U.S.

PUB700

Effect of Bone Marrow Mesenchymal Stem Cells (MSC) or Conditioned Medium (MSC-CM) in Rats with Acute Kidney Injury (AKI) due to Sepsis
Joelma Santina Christo, Nestor Schor. *[1]Nefrologia, Unifesp, São Paulo, Brazil.*

Background: Sepsis is one of the most commonly related causes of death in ICUs, especialy when associated with AKI.Recent studies suggested that AKI can be prevented or repaired by the MSC therapy. This study aims to evaluate this effect.

Methods: MSC were characterized for FACS analysis and were cultured and used at 4th passages.The female *Wistar* rats received E.coli (10^9) by renal one punctures(E.coli group) or PBS (CTL) in a single intrarenal dose (N=06).After 72 hours, the rats received i.v MSC ($1X10^6$cells) or saline or cultured medium from MSC,(MSC-CM,500µl) injection, in one dose.Then after 72 hours blood were collected for creatinine or urea.

Results: After 24 hours it was observed higher values in creatinine and urea in E.coli when compared to CTL (1.3 ± 0,1 versus 0.9±0,1mg/dlp<0.05) and urea (92.1± 0.2versus40.6±0.1 mg/dlp<0.05) characterizing this AKI model.The E.coli+MSC presented lower serum creatinine and urea after 72 hours (1.0±0.2 and 58.0±0.1 mg/dl,p<0.05),respectively.The E.coli+ MSC-CM also had lower values of creatinine (0,5±01 mg/dL) and urea (39.9±1)p<0.05 versusE.coli.Also it was observed an increase in pro-inflammatory cytokines and a decrease in anti-inflammatory in E.coli alone see table 1.These effects were much lower in the animals that received simultaneous E.coli with MSC or CM (p<0.05).

Serum cytokines (pg/mL)	CTL	E.coli 24 hours	E.coli+PBS 72 hours	E.coli+MSC 72 hours	E.coli+MSC-CM
IL-1α	12±04	49.±4	43±2.8	25±2.0*	23±5 #
IL-1β	40±14	290±11	268±12	86±9*	122±12#
IL-10	22±3	35.±5	32.±5.0	84±8*	83±5.7#
TNF α	7±1.2	53.±9	49±8	17±5*	21±4#
IFNγ	29±5	136±2	129.1±7	48±6*	64±3.2#
IL-6	61±9	496±8	457±10	192±9*	246±13#

Values are expressed as the mean±SEM *P<0.05 CTL vc E.coli+MSC 72 hours (n = 5), vs E.coli+ CM

Conclusions: Cell therapy with MSC or its MSC-CM are promising treatment for AKI due to sepsis, particularly in minimizing effects when administered soon after sepsis detection, opening a potential adjuvant therapy for this important disease.

PUB701

Effect of Mesenchymal Stem Cell and Steroid Pulse Therapy in Intractable IgA Nephropathy
Byoung-Soo Cho,[1] Yumi Choi,[1] Hyun Soon Lee,[2] Jin-Soon Suh.[3] *[1]TheAll Medical Hub Kidney Center, TheAll Medibio Research Institute, Republic of Korea; [2]Hankook Kidney and Diabetes Institute, Republic of Korea; [3]Dept of Pediatrics, Bucheon St. Mary's Hospital, The Catholic Univ of Korea, Republic of Korea.*

Background: There is no remedy to cure severe IgA nephropathy (IgAN) with irreversible histologic changes. Cell-based therapy, especially adipose derived stem cells (ASCs) is an emerging treatment modality in the nephrology field. We report the effects of steroid pulse therapy followed by autologous ASCs in patients with IgAN with severe histologic changes and decreased renal function.

Methods: The first case was for a female patient with 44 years old. Before treatment, serum creatinine was 1.6 mg/dL with GFR of 35 ml/min (stage 3 CKD). Renal biopsy showed IgAN (grade IV in H.S.Lee's classification) with sclerotic chanes in 61% of glomeruli. She received six cycles of MP pulse therapy and four cycles of ASCs intravenously with 3 weeks interval. After treatments, her serum creatinine declined to 1.28 mg/dL with GFR 45 ml/min. Furthermore, in follow-up biopsy, the sclerotic changes decreased to 37% of total glomeruli and the immune deposits disappeared. The second case was for a female patient with 31 years old. She had also stage 3 CKD. Before treatment, serum creatinine was 1.37 mg/dL with GFR of 43 ml/min. Her renal biopsy finding showed grade V IgAN with sclerotic changes in 80% of total glomeruli. She received five cycles of MP pulse therapy and 4 cycles of autologous ASCs. After treatments, serum creatinine declined to 1.26 mg/dL with GFR of 43 ml/min. And in follow-up renal biopsy, the sclerotic changes decreased to 55%.

Results: We found that the histopathological findings dramatically improved after MP pulse therapy and autologous ASCs Tx during last 6 months. The serum creatinine and GFR was a little improved in milder CKD, however serum creatinine was stationary in severe case.

Conclusions: In conclusion, MP pulse therapy with autologous ASCs treatment in intractable IgA nephropathy might be a promising therapeutic modality, especially in early stage CKD, although the efficacy and safety of stem cell infusion therapy need long-term follow-up.

PUB702

Gas Gangrene and Acute Respiratory Distress Syndrome in a Renal Transplant Patient with Middle East Respiratory Syndrome Corona Virus Infection
Ebadur Rahman, Muddassar Mahboob, Ramesh Nair, Seddeg Younis, Naveed Aslam. *Nephrology, Prince Sultan Military Medical City(PSMMC), Riyadh, Saudi Arabia.*

Background: Middle East respiratory syndrome corona virus (MERS-CoV) infection is rare and potentially fatal infection and here we report a case of corona virus infection in a renal transplant patient managed in Nephrology department of Prince Sultan Military Medical City.

Methods: We report a 42 years old male diabetic, hypertensive, morbidly obese, renal transplant patient with an average baseline creatinine of 1.5mg/dl since transplant .The patient was admitted under care of surgical team for toe amputation because of gas gangrene. The patient developed chest symptoms a days after uneventful toe amputation. At the same time the Patient became febrile and chest x-ray revealed right middle zone infiltrate .He had a drop of the room air saturation to 80% from base line 96%. The immunosuppressive medications were reduced and intravenous immunoglobulin (IV IG) was administered at a dose of 0.5mg/kg,for five days with the suspicion of MERS-CoV infection which was later on confirmed from the nasopharyngeal swab PCR. All the other viral markers were negative. The patient had mild reversible deterioration of renal function and although he was shifted to I.C.U but he was not ventilated. The patient was discharged in good general condition after 46 days.

Conclusions: MERS-CoV infection can cause reversible acute graft dysfunction and although it has a high mortality, early use of immunoglobulin and reduction of immunosuppressant might be beneficial even with multiple co morbidities and co infections.

Funding: Government Support - Non-U.S.

PUB703

Towards a Model of Ischemia/Reperfusion Induced Renal Fibrosis
Nathalie Le Clef, Anja Verhulst, Patrick C. D'Haese, Benjamin Arthur Vervaet. *Laboratory of Pathophysiology, Univ of Antwerp, Antwerp, Belgium.*

Background: Acute kidney injury (AKI) is an important risk factor for development of chronic kidney disease (CKD), hence renal fibrosis, in humans. Ischemia-reperfusion (I/R) is a widely used model of AKI, the severity of which is dependent on body temperature during, and duration of, ischemia. With the objective of optimizing a I/R induced model of renal fibrosis, we quantified the expression of 4 fibrosis-related genes 12-weeks after I/R of varying severity.

Methods: Male C57BL/6J mice (n=6/condition) underwent unilateral renal I/R with different conditions of ischemic temperature and duration. On the one hand, core temperature was kept stable at 34°C, 35°C, 36°C and 37°C during 30 min of ischemia. On the other hand, ischemic duration was varied from short (15 and 18 min) to long (25 and 30 min) at a fixed temperature of 36°C. Temperature control was achieved using a heating pad with rectal probe feedback. After reperfusion, animals were kept on a hot water pad (37°C) until awakening. Sham operated mice (n=6) were included as controls. Expression of Collagen I, TGFβ, CCN2 (CTGF) and CCN3 (NOV) was assessed by qPCR.

Results: Thirty minutes of ischemia at 37°C causes the most severe renal injury, leading to a 46.0±4.3 (Col I), 18.5±3.8 (TGFβ), 22.4±5.2 (CCN2) and 22.8±5.8 (CCN3) fold upregulation. Lower temperatures during ischemia result in gradually less upregulation of these fibrosis-related genes. The lowest expression is seen with 30 min at 34°C resulting in a 29.0±7.4 (Col I), 12.1±2.4 (TGFβ), 10.4±4.0 (CCN2) and 10.4±4.4 (CCN3) fold upregulation ($p < 0.05$ versus 37°C and Sham). Shorter duration of ischemia (18 min) results in far lower upregulations only of Col I (4.5±4.1), TGFβ (1.5±0.3) and CCN3 (3.4±2.0 ($p < 0.05$ versus 37°C and Sham)). The mildest condition tested, i.e. 15 min at 36°C, did not result in persistent upregulation of fibrosis-related genes.

Conclusions: Unilateral ischemia/reperfusion can induce persistent renal fibrosis. The extent of renal fibrosis, in terms of Col I, TGFβ, CCN2 and CCN3 expression, increases with increasing ischemic temperature and time. These data demonstrate I/R to be a useful model to investigate renal fibrosis.

Funding: Government Support - Non-U.S.

PUB704

Inflammatory Cell Types of Kidney Allograft Glomerulitis and Peritubular Capillaritis Are Association with Peritubular Capillaries C4d Deposition
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Background: The aim of our study was to determine the amount and composition of immune cells within glomeruli and peritubular capillaries (PTCs) and its relationship with PTC C4d deposition.

Methods: Immunohistochemistry staining for C4d, CD3, CD68, granzyme B and Foxp3 were used for phenotyping and enumerating immune cells within intracapillaries. C4d staining was present in 26 biopsy specimens (C4d+ group) and negative in 25 specimens (C4d- group).

Results: The total number of infiltrating cells in glomerulus and PTC in C4d+ group significantly higher than in C4d- group (P<0.001; P<0.0001). Although the C4d+ group showed a significantly higher mean number of macrophages per glomerulus and PTC than in C4d- group (P<0.0001; p=0.001), the C4d- group showed a higher mean number of T cells per glomerulus and PTC than in C4d+ group (P=0.023; P=0.031). Comparing cell counts in diffuse C4d+ and focal C4d+ groups, a significant difference of absolute numbers

of intracapillary cells could be observed in glomeruli and PTCs (P=0.001; P<0.0001). The mean number of macrophages per glomerulus and PTC in diffuse C4d+ were significantly greater than that of the focal C4d+ (P<0.0001;p=0.001). While mean T cells per glomerulus and PTC were less in cases of diffuse C4d+ than in focal C4d+.The differences however, did not achieve statistical significance (p=0.74; p=0.53). Not only glomerular T cells but also PTC's are granzyme B positive T cells totally.

Conclusions: The total number of infiltrating cells in glomeruli and PTCs have association with PTC C4d depositon, the infiltrating cells were predominantly macrophages in C4d+ group, especially in diffuse C4d+. whereas, the infiltrating cells were predominantly T cells in C4d- group. Glomerular and PTC T cells were cytotoxic phenotype completely.

PUB705

To Stent or Not? Management of Transplant Renal Artery Stenosis from an Intimal Flap Helen M. Wijeweera, Angelina Edwards, Ana Paula Rossi, Daniel C. Brennan. *Nephrology, Washington Univ, St. Louis, MO.*

Background: Transplant Renal Artery Stenosis (TRAS) is the most common vascular complication post transplant. Early anastomotic stenosis is often due to trauma to donor or recipient vessels. Less commonly, small intimal flaps or sub-intimal dissections of the vascular wall may lead to scarring and result in narrowing or occlusion of the artery. Rarely, thin, unstable flaps result in variable symptoms. Diagnosis is often delayed due to insidious onset of allograft dysfunction and absence of classic findings on ultrasonography. We present a successfully treated case of ARF attributed to a transplant renal artery intimal flap.

Methods: A 40 year old Caucasian male with ESRD secondary to Alports, underwent a living unrelated renal transplant with post operative nadir creatinine of 1.93 mg/dL. Seventeen days post-transplant, creatinine rose to 2.72 mg/dL and tacrolimus level was 15.3. Renal transplant ultrasound showed resistive indices (RI) at upper limits of normal, with peak systolic velocities highly variable, ranging from 308 to 500 cm/sec. Interestingly, blood pressure was well controlled. Allograft biopsy was consistent with interstitial nephritis. TMP/SMZ, famotidine were discontinued and Tacrolimus was reduced. Serum creatinine gradually decreased to 1.4 mg/dL. However, four days later, the patient developed diarrhea, dehydration and anuric AKI with creatinine of 5.98 mg/dL. Despite aggressive IV fluid resuscitation, the patient remained anuric and creatinine rose to 9 mg/dL. A renal angiography was performed which revealed a 50-60% eccentric, elliptical narrowing at the renal transplant artery ostium with 20 mm dynamic gradient across an intimal flap. A Herculink Elite® stent was deployed with prompt recovery of renal function, return of urine production, and improvement of creatinine to 1.7mg/dL.

Conclusions: TRAS remains an important reversible cause of acute kidney injury. However, when clinical criteria, including blood pressure, resistive indices, velocities and serum creatinine, are inconsistent, a renal artery intimal flap should be considered. Early diagnosis with angiography and intervention for such lesions can result in salvage of graft function.

PUB706

Histopathological Findings in the Early Diagnosis of Obstructive Uropathy Amaya Caviedes Aramburu, Virgilia Soto, Magdalena Madero. *Nephrology, National Institute of Cardiology, Mexico City, Mexico.*

Background: Obstructive uropathy is a common entity at all ages varying frequency depending on age and gender that is analyzed. In the case of transplant patients it is one of the most common complications with an incidence of 7-14%. In the case of a patient with use of JJ catheters with a lower incidence of 3.6%, but not absent; having urinary fistulas and strictures as the most common causes represented as a whole by 95%.

Methods: 2 cases were selected in the pathology service of post- transplant patients with acute graft dysfunction who despite rejection of discarded data evolution had not been adequate and podoplanin staining was performed and it was positive in lymph ducts.

Results: Two clinical cases with inicial creatinine 4.2 and 9.5 the first presented as an anuric pacient and the second with normal urinary volume. Both had biopsy and where positive for D 241 podoplanin. After biopsy both pacients had JJcatheter nda de final Cr was 1.2 for de fist case and 1.9 for the second.

Conclusions: The use of podoplanin could represent one more tool in the early diagnosis of patients with acute obstruction related to graft dysfunction.

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

PUB707

Diagnosing Transplant Tolerance By In Vitro Assay of CD4+CD25+T Cells Bruce M. Hall, Catherine Robinson, Karren Plain, Giang Tran, Nirupama Verma, Rochelle Boyd, Masaru Nomura, Suzanne Jean Hodgkinson. *Immune Tolerance Laboratory, UNSW Australia, Sydney, NSW, Australia.*

Background: Transplant tolerance in adults is usually mediated by $CD4^+CD25^+Foxp3^+$Treg, but there is no diagnostic test of the tolerant state. This study compared the reactivity of tolerant and naïve $CD4^+$, $CD4^+CD25^+$ and $CD4^+CD25^-$T cells. As antigen(Ag) specific Treg require Ag stimulation and cytokines to survive, we examined which cytokines enhanced proliferation of Ag specific Treg.

Methods: Specific immune tolerance was induced by a short course of immunosuppression after grafting fully allogeneic PVG heterotopic hearts in DA hosts. Cells from rats with grafts surviving >100 days were assayed. Different Th1 or Th2 cytokines were added to cultures. Cytokine receptor expression was assayed by RT-PCR.

Results: T cells subsets were similar in naïve and tolerant: $CD4^+$(49v42.5%), $CD8^+$(16v15.5%), $CD4^+CD25^+$(6.1v5.6%) and $CD25^+Foxp3^+$(10.9v9.1%). Serial dilution showed proliferation of naïve and tolerant $CD4^+$T cells to PVG was the same as to third party Lewis, and less to self. The response of tolerant $CD4^+CD25^-$T cells was less to PVG than to third party. Tolerant $CD4^+CD25^+$T cells response to PVG was at background and similar to self. Tolerant $CD4^+CD25^+$ cells proliferated to Lewis similar to naïve $CD4^+CD25^+$T cells proliferation to PVG and Lewis but not to self. Proliferation of tolerant $CD4^+CD25^+$ cells was enhanced to PVG, but not Lewis or self, by addition of rIL-5 or interferon-gamma (IFN-g). Tolerant $CD4^+CD25^+$T cells had more expression of the receptor for IFN-gamma (ifng) and IL-5 (il5ra) compared to naïve $CD4^+CD25^+$T cells. After culture with rIL-5, il5ra expression was retained but not ifngr expression. After culture with IFN-g, ifngr expression was retained and il5ra lost.

Conclusions: These findings show the alloAg specific tolerant state was mediated by activated $CD4^+CD25^+$Treg similar to the Ts1 and Ts2 cells that develop after nTreg are cultured with specific alloAg respectively with IL-2 or IL-4. They support our hypothesis that the long-term activation to Ag specific Treg is dependent on late Th1 or Th2 cytokines, respectively IFN-g or IL-5. These in vitro assays may diagnose transplant tolerance.

Funding: Government Support - Non-U.S.

PUB708

Allograft Oxalate Nephropathy Sandesh Parajuli,[1] Megan L. Troxell,[1] Donald C. Houghton,[1] Thomas D. Batiuk,[2] William M. Bennett,[2] Eric D. Langewisch,[1] Douglas J. Norman.[1] *[1]Oregon Health and Science Univ, Portland, OR; [2]Legacy Good Samaritan Medical Center, Portland, OR.*

Background: Oxalate nephropathy is a known cause of end stage renal disease. There are relatively limited data about the implications of oxalate nephropathy in kidney transplant recipients except in primary hyperoxaluria. We sought to retrospectively review our local experience with allograft oxalate nephropathy (AON).

Methods: Cases of AON were identified from the OHSU pathology archive, and correlated with clinical data.

Results: Seven cases were identified (3 nephrectomy, 4 biopsy; 2 cases were previously reported: Troxell et al. Am J Transplant. 2013;13:501-9). All patients were adults (age 35-70). Native kidney failure was attributed to diabetes (2), interstitial nephritis (1), hepatorenal syndrome (1), and was undetermined in 3; none had primary hyperoxaluria. Retrospective review identified presumptive gastrointestinal etiologies of oxalosis in 6: gastric bypass or bowel surgery (4, many in distant past), chronic pancreatitis (1), Crohn's (1); at least 5 also had heavy antibiotic exposure. Five patients experienced allograft failure at post transplant intervals of 0 to 36 months (mean 25 months). Oxalate crystals were identified on urinalysis in 3 patients; serum oxalate levels were elevated in 1, normal in the patient with primary non-function, and unavailable in 5. Patient 1 had improved renal function with discontinuation of vitamin C, low oxalate diet and calcium carbonate. Interestingly, patient 3 had delayed graft function and was dialysis dependent for 7 months; while graft function slowly recovered, oxalate was an unanticipated finding on renal biopsy and clinical correlation revealed chronic pancreatitis, leading to dietary modification and increased fluid intake; current glomerular filtration rate is 52.

Conclusions: In our experience, patients with AON historically have poor outcomes; however, early clinicopathologic recognition and intervention to reduce oxaluria may lead to improvement of graft function and prevention of graft failure. Screening and prophylaxis of high risk patients (bariatric surgery, inflammatory bowel disease), with treatment of abnormal urine chemistries, has been instituted at one of the centers.

PUB709

Prolonged DGF Post Transplant with Excellent Long Term Outcome-Patience Is a Virtue Raghavesh Pullalarevu, Keith R. Superdock, Robert L. Benz. *Nephrology, Lankenau Medical Center, LIMR, Wynnewood, PA.*

Background: Delayed graft function (DGF) is defined as the need for dialysis in the first posttransplant week. 5% of kidneys with DGF never function. Risk of DGF is high with extended criteria donor kidneys, cadaveric transplants, and inherent donor disease. The most common cause of DGF are postischemic ATN and acute antibody mediated rejection.

Methods: 71 yo. white male had elective DDKT after being on dialysis for 5 years with ESRD due to HTN. Past medical history included hypothyroidism, Anemia, SHPT. Donor met ECD criteria, having a terminal creatinine of 1.3 and HTN. Warm and cold ischemic times were 11 hr 10 min and 30 min respectively. Time zero biopsy revealed 6 glomeruli with no significant pathologic changes. Patient was euvolemic during surgery and had no apparent intraoperative complications. Kidney function did not recover and the patient remained oliguric. Hemodialysis was continued. Induction therapy was 500mg IV solumedrol intraoperatively and 250mg on POD1. 100mg of IV Dacluzimab was given intraoperatively followed by a second dose on POD 14. Immunosuppression was maintained with tacrolimus, mycophenolate mofetil and prednisone, tapered and maintained at 10mg, po daily. Transplant kidney ultrasound (US) on POD2 and POD7 revealed normal resistive indices. Tacrolimus levels were consistently within the target range of 10-14 ng/ml. Kidney biopsy for nonfunction per protocol performed on POD 17 revealed 17 glomeruli with evidence of mild focal acute cellular rejection and mild acute vascular rejection, however C4d staining was negative. The patient was treated with IV steroids for 3 days and IV ATG for 7 days with documented suppression of CD3 counts.

Results: With time and continued immunosuppressive therapy, urine output improved and dialysis was stopped on POD 96. He is now over 7 and ½ years post transplant with excellent kidney function and a baseline creatinine of 1.5-1.6 mg/dl.

Conclusions: We believe this case represents the longest reported case of DGF with excellent long term kidney function. This case underscores the importance of patience in cases of prolonged DGF and justification for continued immunosuppression while awaiting recovery of renal function.

PUB710

The Effect of Steroid Pulse Therapy in BK Viremia Who Might Have Acute Rejection after Kidney Transplantation Hyosang Kim,[1] Su-Kil Park.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea; [2]Dept of Surgery, Asan Medical Center, Univ of Ulsan College of Medicine, Seoul, Republic of Korea.*

Background: Steroid pulse therapy would be necessary for BK viremia patients whose pathologic report argued acute rejection. We investigated the effect of high dose steroid pulse therapy on renal function and BK viral load in renal transplant recipients with BK viremia who might have acute rejection by pathology.

Methods: Study population consisted of 98 kidney transplant recipients with BK viremia (defined as serum BK viral load > $1x10^4$ copies/mL) consecutively detected at least two times from January 1, 2004 to December 31, 2011 at Asan Medical Center.

Results: There are 67 patients (68.3%) who did not receive steroid pulse therapy (G1), 13 (13.2%) with less than 2 grams of methylprednisolone (mPD)(G2), and 18 (18.4%) with more than 2 grams of mPD (G3). Mean follow-up duration was 37.6±19.3 months. The amounts of BK viremia were not different between groups (G1; 6.3±0.9 log copies/mL, G2; 5.6±1.4, G3; 6.6±1.0) at initial diagnosis. After 1 month, BK viral load was still persistent in G3 (6.7±0.8 log copies/mL), while it declined to 5.3±2.6 and 5.1±1.9 in G1 and G2, respectively. The initial renal function (eGFR) were 47.9±26.9 ml/min/1.73m^2 (G1), 35.2±19.4 (G2), and 29.3±8.9 (G3) at diagnosis (p<0.05), and after 1 year, no improvement in steroid therapy group (G1; 52.8±34.9 ml/min/1.73m^2, G2; 39.6±28.2, G3; 27.9±11.4). In Kaplan-Meier analysis for 50% decline of eGFR from baseline, renal function in G3 rapidly deteriorated as compared with others. (p=0.051). There are 67 patients (68.3%) who did not receive steroid pulse therapy, 13 (13.2%) with less than 2 grams of methylprednisolone (mPd), and 18 (18.4%) with more than 2 grams of mPd. Mean follow-up duration was 37.6±19.3 months. The amounts of BK viremia were not different between groups (≥2g; 6.6±1.0 log copies/mL, <2g; 5.6±1.4, No mPd; 6.3±0.9). After 1 month, BK viral load was maintained in mPd≥2g group (6.7±0.8 log copies/mL), while levels of viremia in other groups were declined to 5.1±1.9 and 5.3±2.6, respectively. This difference gradually decreased and disappeared after 3 months persisting for 1 years. Estimated GFRs (eGFR) were 29.3±8.9 ml/min/1.73m^2 (mPd≥2g), 35.2±19.4 (mPd<2g), and 47.9±26.9 (no mPd) when BK viremia was detected. After 1 year, differences of eGFR were sustained (≥2g; 27.9±11.4 ml/min/1.73m^2, <2g; 39.6±28.2, No mPd; 52.8±34.9). However, in Kaplan-Meier analysis for 50% decline of eGFR from baseline, renal function in mPd≥2g group rapidly deteriorated as compared with others. (p=0.051).

Conclusions: In BK viremia patients, high dose of steroid pulse therapy would not be necessary to preserve the long term graft function in spite of argued acute rejection pathologically.

PUB711

Early Acute Graft Pyelonephritis Is an Independent Predictor of Long-Term Kidney Allograft Outcome Eun Jung Kim,[1] Jung-Woo Noh,[1] Ja-Ryong Koo.[2] *[1]Dept of Internal Medicine, Hallym Univ College of Medicine Sacred Heart Hospital, Seoul, Republic of Korea; [2]Dept of Internal Medicine, Hallym Univ College of Medicine Sacred Heart Hospital, Gyeonggi Province, Republic of Korea.*

Background: Urinary tract infection (UTI) is the most common form of bacterial infection encountered in a renal transplant. Although UTI was benign nature, a UTI complicated by acute graft pyelonephritis (AGPN) is associated with acute kidney injury and allograft scarring. However, the influence of AGPN on graft outcome in renal transplant recipients still remains controversial.

Methods: One hundred and thirty two patients with renal transplantations were investigated to evaluate the impact of early-AGPN on graft function between January 2001 and December 2011. Early AGPN was defined as occurring within 6 months after renal transplantation. The changes in eGFR over time were compared between patients with and without early AGPN using a linear mixed model. Moreover, Kaplan-Meier plot and Cox analysis were conducted to evaluate the influence of early-AGPN on renal outcome, a reduction of estimated glomerular filtration rate (eGFR) by 50%.

Results: Among the 132 patients, 18 (13.6%) patients diagnosed with early-AGPN. During the mean follow-up of 70.7 ± 27.5 months, 28 (21.1%) patients reached to renal outcome and renal outcome was significantly higher in early-AGPN group (44.4% versus 17.5%, P = 0.025). Moreover, a liner mixed model revealed that there was a significant difference in the rate of eGFR decline over time between two groups (P < 0.001). Kaplan-Meier analysis also showed that renal event-free survival was significantly lower in the early-AGPN group (P = 0.001). In multivariate Cox regression analysis, early-AGPN was found to be an independent predictor of renal outcome (hazard ratio, 5.18; 95% CI, 1.78 - 15.11; P = 0.003).

Conclusions: This retrospective cohort study showed that early-AGPN on patients with renal transplantation was independently associated with deterioration of kidney allograft. So, this study demonstrates that early-AGPN could be predictor for long-term kidney allograft outcome.

PUB712

The Safety of Lower Doses of Immunosuppressants in Rituximab-Treated Kidney Transplantation Chung Hee Baek, Joon-Seok Kim, Hyosang Kim, Su-Kil Park. *Dept of Internal Medicine, Div of Nephrology, Asan Medical Center, Seoul, Republic of Korea.*

Background: Rituximab, an anti-CD20 antibody, effectively depletes B lymphocytes and is used in ABO-incompatible or HLA-sensitized kidney transplantation(KT). However, it is not clear whether the conventional doses of maintenance immunosuppressants in rituximab-treated KT are appropriate. We studied the safety of lower doses of immunosuppressants in rituximab-treated KT.

Methods: We previously reported the results of retrospective study that showed serious infectious complications were increased in rituximab-treated kidney transplant recipients. In this study, we prospectively evaluated 72 patients who used lower doses of maintenance immunosuppressants (tacrolimus, mycophenolate mofetil (MMF) and methylprednisolone) compared to previous study as a new protocol (group1). Sixty-seven patients of study group in the previous report served as control group (group2).

Results: The doses of MMF (in grams/day) at the following times postoperatively were lower in group1 than in group2: 1month: 0.95±0.241versus 1.26±0.42, p=0.000; 3 months: 0.93±0.30 versus 1.14±0.51, p=0.007; 6 months: 0.94±0.26 versus 1.07±0.50, p=0.095; 1 year: 0.93±0.28 versus 0.88±0.52, p=0.637; 2 years: 1.08±0.20 versus 0.69±0.55, p=0.252). The doses of tacrolimus and methylprednisolone were also significantly lower in group1. Mean follow up time (months) showed no difference (14.89±6.01 versus 12.63±7.59, p=0.053). Total infection occurred more often in group2 (29.2% versus 52.2%, p=0.006). The incidence of CMV infection was also higher in group 2 (2.8% versus 16.4%, p=0.007). One patient of group1 and 2 patients in group2 died of infection. The reduction of maintenance immunosuppressants did not increase the incidence of acute rejection in group1 (4.2% in group1 versus 4.5% in group2, p=1.000). If patients who died with functioning graft were excluded, graft survival was 100% in group 1 and 98.5% in group2 (p=0.482).

Conclusions: Lower doses of maintenance immunosuppressants reduced the incidence of infection without increasing rejection or graft loss and it might be adequate to reduce the doses of maintenance immunosuppressants in rituximab-treated KT.

PUB713

Asymptomatic Caecal Perforation in a Renal Transplant Recipient: Should Sodium Polystyrene Sulfonate (SPS) Be Used for Treating Hyperkalemia? Montish Singla, Sunggeun Lee, Donald I. Baumstein, Roger F. Carbajal Mendoza, Ashok P. Chaudhari. *Dept of Nephrology, Metropolitan Hospital Center, New York, NY.*

Background: We present a case of renal transplant recipient who suffered caecal perforation after single oral dose of SPS (15 grams).

Methods: 50 year old female with history of living related renal transplant in 1997, now CKD stage 4 due to chronic allograft nephropathy, on immunosuppression with cyclosporine and prednisone was admitted for right hip arthroplasty. On post-op day three she had a drop in hematocrit. The etiology of drop in hematocrit was unclear with stable vital signs, mild diffuse abdominal tenderness, absent bowel sounds, which were attributed to paralytic ileus. Laboratory examination was negative for any hemolysis and fecal occult blood testing was negative. So, abdominal CT was obtained which surprisingly revealed caecal perforation. Patient was operated emergently but the cause of intestinal perforation was unclear. A careful review of the chart showed that patient was given one dose of SPS for potassium of 5.8 mg/dL on post-op day one. Subsequently, the colonic pathology confirmed colonic necrosis and presence of SPS crystals in necrotic colonic mucosa. In the ensuing months she developed multiple episodes of acute kidney injury (AKI) due to volume depletion from excessive ileostomy loses. The patient was eventually discharged home four months after the surgery with in situ ileostomy and small bore tunneled internal jugular catheter for fluid administration at home to prevent AKI.

Conclusions: The case underlines the need for physicians and nephrologists to be cognizant about the potential side effects of SPS in CKD and especially renal transplant recipients, even at small doses. The immunosuppressive agents can mask the usual signs and symptoms of intestinal perforation so subtle symptoms like abdominal pain should rise high index of suspicion. Intestinal perforation itself has high morbidity and mortality but with concomitant immunosuppression the morbidity increases several fold as illustrated by our case. SPS should be used with extreme caution in post-op period especially in renal transplant recipients on immunosuppression.

PUB714

Sirolimus Based Dual Immunosuppressive Therapy in Renal Transplant Recipients with Malignancies Joon-Seok Kim, Chung Hee Baek, Hyosang Kim, Su-Kil Park. *Div of Nephrology, Dept of Internal Medicine, Asan Medical Center, Seoul, Republic of Korea.*

Background: Sirolimus exhibits both antineoplastic and immunosuppressive effects. Conversion to sirolimus based regimen is considered as a common practice when de novo posttransplant malignancy (dNPTM) is newly diagnosed. We evaluated the effect and safety of low dose sirolimus based dual immunosuppressive regimen in renal transplant recipients with dNPTMs.

Methods: From January 2003 to October 2013, calcineurin inhibitor based triple immunosuppressive regimen was converted to dual regimen consisted of low dose sirolimus and corticosteroid in 11 patients among renal transplant recipients who diagnosed as

dNPTM. The initial dose of sirolimus was 2mg daily, without a loading dose at converson. Subsequently, sirolimus dose was adjusted to maintain whole blood level between 4 and 6 ng/ml.

Results: The median time from transplantation to the diagnosis of dNPTM was 66 (13-151) months with median follow-up period of 25 (3-47) months. At the end of follow-up, median doses of sirolimus and prednisolone were 1 mg/day and 5 mg/day (or equivalent doses of other corticosteroids), respectively. Estimated GFRs by CKD-EPI equation were increased from 68.36±6.75 ml/min/1.73m² to 78.2±6.99 ml/min/1.73m² at last follow-up (p=0.02). Total cholesterol level was significantly increased (Δ 40.36±14.08 mg/dl, p=0.017). When compared as an ordinal scale, there was a tendency to increase proteinuria determined by a semi-quantitative dipstick test without statistical significance (p=0.084). Hemoglobin level, WBC and platelet counts showed no significant difference. Six patients (55%) experienced adverse events, presumably related to sirolimus. One patient needed to stop sirolimus due to nephrotic ranged proteinuria and 3 patients required its dose reduction due to pneumonitis. There was no episode of rejection. At the end of follow-up, 8 patients including 7 patients stayed in remission of cancer were alive, and 3 patients died of cancer related events.

Conclusions: Low dose sirolimus with corticosteroid was effective to maintain renal graft function and relatively well tolerated in renal transplant patients with dNPTMs.

PUB715

Ongoing Atypical Hemolytic-Uremic Syndrome after Second Kidney Transplantation on Eculizumab Therapy: Complete Recovery after Plasma Exchange and Intense Eculizumab Therapy Jorge Alexandre Fares, Carlos Eiji Koga, Andre F.G. Larrubia. *Nephrology, REDE D´ÓR - Hospital Sao Luiz Morumbi, Sao Paulo, Brazil.*

Background: CSM, 42 yrs-old, female, was submitted to a 2nd kidney tx(Ktx), unrelated donor, spouse, 6MM, on last feb. 15th. Genetic analysis revealed C3 and Factor I mutations.

Methods: Patient received meningococcal vaccine, Cipro prophylaxis, and Eculizumab infusions started 30 days before second Ktx: induction phase(900mg-weekly)with the forth dose at the day before Ktx, and a extra dose 24h after and at day 8, and 15, when relapse occurred(day15). Plasma Exchange started on day 20,and Eculizumab 600mg was infused after each session(15 sessions). Imx protocol: Basiliximab 2 doses induction and maintenance, Sodium Mycophenolate, Tac., and St. Daily Hemogram, Haptoglobin, LDH and Reticulocytes count were obtained to monitor Thrombotic Microangiopathy activity.

Results: Serum creatinine was 1.97mg-dl on day3 and 1.69m-dl on day 17. Two Kidney bxs were performed on day 3 and 8, both showing ATN. Pneumonia occurred on day 9, and a Subcutaneous Hematoma after 2nd bx(on day8) occurred before relapse. Labs results when patient relapsed: haptoglobin <36mg-dl[22]; Hb;7.9g-dl;Reticulocytes:19% on day15, and hapto undetectable on day 18,and 19;LDH:602 U-l; Hb;7,4g-dl;tacrolimus level on day16:4,6 ng-ml; Plat.:190.000 mm3. Labs at discharge on day 41: Hapto:81mg-dl;Hb:9,5g-dl; Plat:150,000mm3; LDH:303U-l; WBC:7,060mm3; BUN;15mg-dl; C:0.95mg-dl; TAC:11,1ng-ml. Current labs results on day108: Hb:11,4 g-dl; Hapto:131mg-dl;Plat;269.000mm3; LDH:314;C;1,08; BUN:29.

Conclusions: Despite prophylactic use of Eculizumab, this patient presented overt TMA post-Ktx probably related to surgery, ischemia-reperfusion and infection. The ongoing aHUS after transplant was successfully treated with PE and extra doses of Eculizumab. We therefore recommend that hemolysis biomarkers be monitored in the immediate post-Ktx period in order to detect early activity and prompt intervention be done to achive hematological and kidney function recovery.

Funding: Private Foundation Support

PUB716

Clinical Outcome of BK Virus Associated Nephropathy in Renal Transplant Recipients: Single Center Experience In-Ae Jang,[1] Bum Soon Choi,[1] Cheol Whee Park,[1] Yeong Jin Choi,[2] Chul Woo Yang,[1] Yong-Soo Kim,[1] Byung Ha Chung.[1] *[1]Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Korea; [2]Dept of Hospital Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic Univ of Korea, Seoul, Korea.*

Background: BK virus-associated nephropathy (BKVAN) is an important cause of allograft dysfunction in kidney transplant recipients. It has an unfavorable clinical course, and no definite treatment guidelines have yet been established. Here, we report our center's experience with biopsy-proven BKVAN cases and investigate factors associated with its progression.

Methods: Between January 2004 and April 2013, 25 patients with BKVAN were diagnosed by biopsy at Seoul St. Mary's Hospital. Of the 25 patients, 10 were deceased-donor transplant recipients and the remaining 15 were living-donor transplant recipients. Three of the patients underwent retransplantation. The primary immunosuppressant used was cyclosporine in 8 patients and tacrolimus in 17 patients.

Results: BKVAN was observed at a mean duration of 22.8 ± 29.1 months after transplantation. The mean serum creatinine level at biopsy was 2.2 ± 0.7 mg/dL. BKVAN occurred with acute rejection in 8 patients (28%). Immunosuppression modification was performed in 21 patients (84%). In addition, leflunomide and intravenous immunoglobulin were administered to 13 (52%) and 2 patients (8%), respectively. Allograft loss occurred in 5 cases (27.8%) during the follow-up periods of 0.7, 17.1, 21.8, 39.8, and 41.5 months from the BKVAN diagnosis. Advanced stages of BKVAN, increased creatinine levels, and accompanied acute rejection at BKVAN diagnosis increased the risk of allograft failure.

Conclusions: In the present study, the clinical outcomes in patients with biopsy-proven BKVAN were unfavorable, especially in those with advanced-stage BKVAN, poor renal function, and acute allograft rejection.

PUB717

Immunologic and Non-Immunologic Complication of Third Kidney Transplantation Hyunseon Kim, Chul Woo Yang, Byung Ha Chung, Yong-Soo Kim, Cheol Whee Park, Bum Soon Choi. *Transplant research center,Div of Nephrology, Dept of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, Seoul, Korea.*

Background: Patients who undergo repeat kidney transplantations (KT) are considered at high risk of experiencing immunologic and non-immunologic complications. In this study, we aimed to investigate the clinical outcomes including medical and surgical complications in patients who underwent a third KT in our center.

Methods: Between March 1969 and December 2012, 2,110 KT were performed at the Seoul St. Mary's Hospital. Of them, we examined 11 patients who underwent a third KT, and investigated the allograft outcomes and complication rates.

Results: The mean follow-up duration after KT was 151.8 ± 143.2 months. The mean age at KT was 38.2 ± 8.0 years, and 7 patients (63.6%) were men. Nine patients (81.8%) underwent living donor KT (LDKT). A cross-match test yielded positive results in 4 of the 9 patients, and all underwent pre-transplant desensitization therapy. After KT, 3 patients (27.2%) showed delayed graft function. Acute rejection developed in 4 patients (36.4%), and surgical complications required surgical correction developed in 4 patients. Allograft failure developed due to acute rejection (n = 3) or chronic rejection (n = 1) in 4 patients.Allograft survival rates at 1, 5, and 10 years were 81.8%, 42.9%, and 42.9%, respectively; however, allograft survival rate at 5 years was >80% when examining patients who underwent KT only after results of the panel reactive antibody test became available.

Conclusions: Thus, a third KT procedure may be acceptable, although aggressive pretransplant immune monitoring and patient selection may be required to reduce the risks of acute rejection and surgical complications.

PUB718

Incidence and Clinical Significance of De Novo Donor Specific Antibodies Post Kidney Transplantation: The Role of the Primary Disease Sophia Lionaki,[1] Apostolos Pappas,[1] Helen Kapsia,[1] Nikolaos Altanis,[1] Georgios Liapis,[1] Aliki G. Iniotaki,[2] George Zavos,[1] Ioannis Boletis.[1] *[1]Nephrology and Transplantation, Laiko General Hopsital, Athens, Greece; [2]National Tissue Typing Centre, Gennimatas General Hospital, Athens, Greece.*

Background: To explore the impact of the Primary Disease (PD) nature in the incidence of de novo Donor Specific Antibodies (DSA) after Kidney Transplantation (KTx), and their correlation with subsequent Acute Rejection (AR).

Methods: We studied patients who were transplanted between 2005 and 2011. Patients with unknown PD, a history of non-compliance, ABO incompatible KTx and a history of DSA prior to KTx were excluded. Patients were categorized by the PD as following: Group A included patients with glomerulonephritis in the native kidneys, primary or in the setting of autoimmune disease, and group B patients with hypertension, obstructive uropathy, polycystic kidney disease, or congenital hypoplastic kidneys. We estimated the frequency of de novo DSA post KTx in the two groups, and the incidence of AR.

Results: Of the 269 patients with known PD, 38 were excluded. Group A and group B were consisted of 92 and 142 patients respectively.

	Group A	Group B	P value
Recipient age(years)	42.7	43.8	0.537
Donor (deceased)	50.7	57.5	0.366
Donor age (years)	52.2	51.25	0.663
Hemodialysis time (months)	46.25	54.4	0.219
PRA >30%	11.4%	15%	0.486

The frequency of de novo DSA was 10.9% for group A, and 11.8% for group B (p=0.835). The mean time to detection was 20.9 months from KTx. Biopsy proven AR (BPAR) was recorded in 13.45% of the total cohort, during a follow up time of 56.5 months. In group B, detection of de novo DSA was associated with higher rates of BPAR (37.5% versus 8.3%, p=0.002, RR=6.6), compared to the patients of the same group without de novo DSA, while in group A there was no difference in BPAR between the patients with and without de novo DSA.

Conclusions: The incidence of de novo DSA was not different between patients with a PD of autoimmune origin, or not. However, detection of de novo DSA was associated with higher rates of BPAR among patients with a non-autoimmune PD, while in patients with an autoimmune PD the rate of BPAR was not influenced by the development of de novo DSA.

PUB719

A "First-in-Human Study" of Implantation of Neo-Kidney Augment®, an Autologous Selected Renal Cell Population, in Type-2 Diabetic CKD 3-4 Patients Torbjörn Lundgren,[1] Jonas Wadstrom,[1] Pontus Blomberg,[2] Torkel Brismar,[3] Peter Stenvinkel.[4] *[1]Dept of Transplantation Surgery, Karolinska Hospital, Stockholm, Sweden; [2]Vecura at Clinical Research Center, Karolinska Hospital, Stockholm, Sweden; [3]Radiology, Karolinska Univ Hospital, Stockholm, Sweden; [4]Renal Medicine, Karolinska Hospital, Stockholm, Sweden.*

Background: Multiple animal models of CKD have demonstrated that a selected population of therapeutically bioactive renal cells (Selected Renal Cells, SRC) can be effectively delivered to the kidney through intra-parenchymal injection resulting in a decrease in disease progression. Direct injection of SRC has been shown to reduce chronic

infiltration by monocytes/macrophages and T-lymphocytes and attenuate the NFkB response known to drive tissue inflammation and promote tubular cell expansion. We present preliminary results from the first-in-human (FIH) clinical study with NKA.

Methods: 5 male type-2 diabetic pts (64±7 yrs) with CKD stage 3-4 were selected. After evaluation of iohexol clearance, MRI, renal scintigraphy and albumin-creatinine ratio (ACR) pts underwent regular ultrasound guided renal biopsy. Two cores of renal tissue were shipped to Tengion manufacturing plant (NC, U.S.A.) for cell isolation, culture and product preparation. Formulated NKA was shipped back to Karolinska Hospital (range 59-87 days after biopsy) for intracortical injection using a laparoscopic technique.

Results: Implantation of NKA was uneventful in all pts. A postoperative complication was observed in one pt (ileocecal volvulus). Infectious complications were observed in 3 pts during follow-up. Antihypertensive medication has been reduced in 2 pts during the first 6 months post-implantation period. Kidney volume, obtained using 1.5 T MRI, was unchanged on the side of injection, 221±62 mL before and 219±54 mL after 3 months. On the contralateral side the kidney volume was 242±75 mL before and 243±59 mL at 3 months.

Conclusions: Using laparoscopic techniques, NKA was safely implanted in 5 CKD pts in this FIH trial. Complications after implantation seem unrelated to the product. Longer follow-up and larger number of pts is needed to reveal if this novel technique arrest progression of CKD and delay the start of renal replacement therapy.

PUB720

Renal Transplantation in Elderly Patients - Polish Multicentre Study

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Background: The elderly are the fastest growing population among dialysis patients and on waiting lists for kidney transplantation. We analyzed the results of the renal transplantation in recipients elder than 60 years and their younger pairs receiving graft from the same donor between Jan 1994-Dec 2012 in 3 centres.

Methods: The older renal transplantation (ORT) group included 164(102m, 62f) patients aged from 60 to 81(mean 65±4) years. Their pairs created a younger renal transplantation (YRT) group consisting of 164(104m, 60f) patients aged from 14 to 59 (mean 45±12)years. The groups wew similar in respect to dialysis modality, initial immunosuppression. ORT was on dialysis longer duration of dialysis significantly longer, more ORT suffered from coronary heart disease (40% versus 15%) and DM (18 versus 3%).

Results: ORT and *YRT* did not differ significantly with respect to: graft function estimated 1 year after transplantation (CKD-EPI) (50.7 ml/min versus 54.0 ml/min), but the decline in eGRF was significantly faster in YRT (Δ 12-3; p<0.01). One year patient survival (93.9% *versus* 97.0%),one-year graft survival (90.4% versus 82.3%),one-year death- censored graft survival(93.9% *versus* 85.3%; p=0.0222)and the incidences of delayed graft function and acute rejection were similar in OTR and YTR, respectively. During the observation period patient, graft and death –censored graft survivals were as follow: 85.3% versus 92.6% (p=0.0122); 78% versus 72.6% and 89.6% versus 76.9% (p=0.014) in ORT and YRT, respectively. Significantly more cardiovascular complications and post transplant DM were noticed in ORT (p<0.01) and the leading cause of death during 1 year after transplantation were infection and cardiovascular events, followed by neoplasia in the next years.

Conclusions: Our multicentre results confirm that renal transplantation is a good option of renal replacement therapy in patients older than 60 years.

Funding: Government Support - Non-U.S.

PUB721

Can Mg Blood Levels in Renal Transplant Recipients Predict Allograft Outcome?

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Background: Hypomagnesemia has been found to be correlated with higher CNI levels, new onset diabetes after transplantation and also hyperparathyroidism post renal transplantation, a known risk factor for vascular calcifications and nephrocalcinosis. Little, however, is known about the relationship between magnesium (Mg) levels and allograft outcome.

Methods: We conducted a retrospective single-center pilot analysis in order to assess Mg blood levels posttransplant association with allograft outcome.

Results: Subjects had a mean age of 49±15 with 57.5% being male. Patients were divided into 2 groups based on the median Mg level measured bewteen 1-12 months posttransplant. Surprisingly those with median Mg level< 1.7 mg/dL (N = 39) versus median Mg level≥ 1.7 mg/dL (N=37) had lower serum creatinine (Scr) values 3 years posttransplant (mean Scr±SD of 1.35±0.51 and 1.78±0.87 respectively, p = 0.018) and a higher GFR (mean GFR±SD of 54.5±15.86 and 46.5±17.8, p=0.04). In 479 patients the odds of a GFR < 60 ml/min three years after transplant was two-fold higher (OR 2.01 95%CI 1.26- 3.20, p=0.004) in patients with Mg level ≥ 1.7 mg/dL relative to those < 1.7 mg/dL when adjusted for age, gender and race.

Conclusions: Lower Mg levels posttransplant are associated with better long term renal graft outcome despite the known association of hypomagnesemia with diabetes and CNI toxicity. Mg levels may serve as a marker of better renal tubular function.

Table 1: Baseline characteristics of the low Mg group versus the high Mg group

	Mg<1.7 (N=39)	Mg≥1.7 (N=37)	P value
Age	48 (14)	52 (16)	0.303
Males	21 (53.8%)	23 (62.2%)	0.469
Females	18 (46.2%)	14 (37.8%)	
White	28 (72%)	23 (62%)	0.6
Black	6 (15%)	8 (22%)	
Asian	2 (5%)	4 (11%)	
Hispanic	3 (8%)	2 (5%)	
ESRD d/t diabetes	8 (21%)	12 (32%)	0.2
LRD/LURD	18 (46%)	20 (54%)	0.5
DDRT	21 (54%)	17 (46%)	
DCD/ECD	10 (25.6%)	11 (29.7%)	0.7
Thymoglobulin	25 (64%)	24 (65%)	0.96
Simulect	11 (28%)	11 (30%)	
None	3 (8%)	2 (5%)	
CNI	39 (100%)	36 (97%)	0.3
SGF	8 (20.5%)	10 (27%)	0.48
Scr 1 year post Tx	1.33 (0.54)	1.66 (0.6)	0.03
Scr 3 years post Tx	1.35 (0.51)	1.78 (0.87)	0.018
GFR 3 years post Tx	54.5 (15.86)	46.5 (17.8)	0.04

PUB722

Bortezomib Based Regimen in the Treatment of Antibody Mediated Rejection in Kidney Transplant Recipients

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Background: Bortezomib is an emerging therapy for antibody mediated rejection (AMR). We describe our experience with bortezomib in conjunction with intravenous immunoglobulin (IVIG) and plasmapheresis (PP) in 6 patients with AMR.

Methods: A retrospective review was performed on 6 renal transplant recipients with AMR. Therapy for AMR included serial PP, IVIG (500mg/kg) and bortezomib (1.3mg/m2) on days 1, 4, 8 and 11. DSA were detected at the time of rejection and were monitored serially after treatment.

Results: Between Jan 2011 to Apr 2012, AMR was diagnosed in 6 recipients of DDKTx. Four patients were highly sensitized with PRA of > 90%. All episodes of AMR occurred within 6 months posttransplant. 4 patients received induction therapy using ATG (n=3) and alemtuzumab (n=1). At the time of rejection, 3 patients had de novo class I, 2 had class II and 1 had both class I and class II DSA. The mean pretreatment mean fluorescence intensity (MFI) for DSA class I and class II were 5890 and 9733 respectively. Posttreatment, all patients had a significant reduction in MFI (class I - 1700, class II 1688). This response was sustained up to a mean ff up of 9 months posttreatment with mean MFI for both class I and class II DSA remaining low at 1214 and 671 respectively. Four patients underwent follow up biopsies posttreatment and in all cases, C4d staining converted from positive to negative. Patient and graft survival at 1 year was 100%. Mean creatinine at the time of rejection was 5.7mg/dl. At 12 months, the mean creatinine was significantly lower at 1.14 mg/dl

DSA	Patients					
	1	2	3	4	5	6
Pretreatment MFI	A30-5750	A3 - 3180	DR53 - 17939	A24 - 4303	B45-6441	A24 - 2588
	A31 - 6072	CW5 - 12902				DR4 - 1527
Posttreatment MFI		A3 - 1086	DR53 - 3359	A24 - 261	B45-NEG	A24 - 41
		CW5 - 7115				DR4 - 17
MFI at last follow up (9mo)	A30-1031	A3 - 1000	DR53 - 1308	A24 - 99	B45 - 60	A24 - NEG
	A31- 1374	Cw5 - 5000				DR4 - 35

Conclusions: Bortezombi in addition to PP and IVIG is an effective treatment combination for AMR and achieves a sustained reduction in DSA.

PUB723

Vitamin D Deficiency Is Significantly Associated with Anemia in Patients with End-Stage Renal Disease Chang-Yun Yoon,[1] Hyunwook Kim,[3] Yungly Kim,[1] Eunyoung Lee,[1] Seung Gyu Han,[1] Young Su Joo,[1] Shin-Wook Kang.[1,2] *[1]Dept of Internal Medicine, College of Medicine, Yonsei Univ, Seoul, Korea; [2]Brain Korea 21 PLUS, Severance Biomedical Science Institute, Yonsei Univ, Seoul, Korea; [3]Dept of Internal Medicine, College of Medicine, Wonkwang Univ, Gyoeonggi-do, Korea.*

Background: Recent studies have indicated that vitamin D may affect the efficacy of erythropoiesis through its anti-inflammatory action. Even though both vitamin D deficiency and anemia are prevalent in patients with end-stage renal disease (ESRD), the association between these two factors remains poorly understood. In this study, therefore, we investigated the impact of vitamin D deficiency on anemia in ESRD patients.

Methods: This study included 106 ESRD patients who underwent kidney transplantation at Yonsei University Health System between April 2002 and May 2004. Patients were divided into tertiles according to the levels of 25-hydroxyvitamin D [25(OH) D] (Group 1, <9.35; Group 2, 9.35 to 15.50; Group 3, >15.50 ng/mL). Independent association between vitamin D and hemoglobin (Hb) concentrations was evaluated by multivariate linear regression analysis.

Results: The mean serum 25(OH)D and Hb levels were 13.06±6.31 ng/mL and 9.45±1.76 g/dL, respectively. Hb concentrations showed a significant trend of increase across the 25(OH)D groups (Group 1, 9.0±1.8; Group 2, 9.4±1.7; Group 3, 9.9±1.7 g/dL; P for linear trend=0.047). Multivariate linear regression analysis revealed that serum 25(OH) D levels were independently associated with Hb concentrations after adjustment for sex, age, serum calcium and phosphorus levels, intact parathyroid hormone concentrations, and the use of erythrocyte stimulating agents (per 1 ng/mL increase, β=1.168, 95% confidence interval=0.434 to 1.901, P=0.02).

Conclusions: Vitamin D deficiency was significantly associated with anemia in ESRD patients. Further studies are needed to verify whether vitamin D supplement can improve anemia in this population.

PUB724

Change in Body Mass Index (BMI) After Kidney Transplantation and Long-Term Outcomes Tushar Malavade, Olusegun Famure, Yanhong Li, Joseph Kim. *Div of Nephrology and the Multi-Organ Transplant Program, Toronto General Hospital, Univ Health Network, Toronto, ON, Canada.*

Background: The long-term consequences of weight change over the first year after kidney transplantation remains unclear. We studied the relationship between change (Δ) in BMI over the first year after kidney transplantation with graft and patient outcomes.

Methods: We performed a cohort study of all adult recipients of kidney transplants at our center from 1 Jan 2000 to 31 Dec 2011 to determine if Δ BMI over the first year is associated with total graft failure, death-censored graft failure, and death with graft function. Recipient Δ BMI was categorized as ≤ -5%, -4.9 to - 0.1%, 0 to 4.9% (reference), 5 to 9.9% and ≥ 10%. The association of Δ BMI and graft/patient outcomes was assessed in multivariable Cox proportional hazards models.

Results: A total of 1186 patients were studied during which 91 graft losses and 86 deaths occurred over 5346.4 patient-years of follow-up. Patients with a reduction in their BMI greater than 5% over the first year post-transplant was linked to a significantly elevated relative hazard of total graft failure when compared to the referent group of Δ BMI 0 to 4.9%

Outcomes	Δ BMI at 1 year post-transplantHazard Ratio (95% CI)				
	< -5% (n=175)	-5 to -0.1% (n = 205)	0 to 4.9% (n=300)	5 to 9.9 % (n=227)	> 10 % (n=279)
Total Graft Failure	1.65 (1.06, 2.55)	1.02 (0.64, 1.62)	Reference	0.95 (0.59, 1.53)	0.66 (0.41, 1.08)
Graft Failure	1.21 (0.61, 2.37)	0.95 (0.50, 1.81)	Reference	1.00 (0.52, 1.92)	0.76 (0.41, 1.42)
Death	2.00 (1.09, 3.69)	1.00 (0.51, 1.95)	Reference	0.87 (0.42, 1.79	0.45 (0.20, 1.04)

Table 1: Hazard ratios for graft failure and/or death by Δ BMI categories

An increase in weight over the range studied by 1-year was not associated with the risk of graft loss or death beyond 1-year.

Conclusions: A reduction in BMI more than 5% in the first year after kidney transplant is associated with an increased risk of death and total graft failure whereas weight gain during the same time was not detrimental to long-term outcomes.

PUB725

Daily Fluid Intake and Outcomes in Kidney Recipients: Post-Hoc Analysis from the Randomized ABCAN Trial Marc L. Weber,[1] Danielle M. Berglund,[2] Scott Jackson,[1] Arthur J. Matas,[2] Hassan N. Ibrahim.[1] *[1]Medicine, Univ of Minnesota, Minneapolis, Minnesota; [2]Surgery, Univ of Minnesota, Minneapolis, MN.*

Background: Generous and even excessive fluid intake is routinely recommended to kidney transplant recipients despite minimal evidence to support this practice. We hypothesized that increased fluid intake, ascertained by 24-hour urine volume output, may adversely affect graft outcomes as it would impose an extra work load on a limited number of nephrons.

Methods: Kidney transplant recipients who were randomized to losartan versus placebo in the ABCAN trial (n=153) underwent baseline, five year biopsies and annual iothalamate GFR. Participants were divided into tertiles based on 24-hour urine volume (<1.73 L/day, 1.73-2.56 L/day, and >2.56 L/day) at time of randomization. A multivariate logistic regression model was constructed to evaluate the association between baseline urine volume as a predictor of the primary and secondary outcomes.

Results: Recipients with higher urine volume at randomization had higher urinary sodium and also higher urinary protein. The distribution of diuretic use or CNI based regimens were similar across urinary volume tertiles. Highest urinary volume tertile (>2.56 L/day) did not predict the development of interstitial volume doubling or ESRD from IF/TA (OR=3.21, 95% CI 0.31, 33.40, p=0.33), was modestly associated with interstitial volume doubling or all-cause ESRD (OR=11.34, 95% CI 0.79, 161.96, p=0.07), but was not associated with the conventional endpoint of doubling serum creatinine, all cause ESRD, or death (OR=0.53, 95% CI 0.12, 2.40, p=0.41).

Urinary Volume >2.56 L/day* as a predictor of the primary and secondary endpoints:

Outcome	OR (95% CI)	*p* value
Doubling of Interstitium or ESRD from IF/TA	3.21 (0.31, 33.40)	0.33
Doubling of Interstitium or any ESRD	11.34 (0.79, 161.96)	0.07
Doubling of Serum Creatinine, All Cause ESRD or Death	0.53 (0.12, 2.40)	0.41

*Urine volume compared to reference of UV <1.73 L/day

Conclusions: These results suggest that the current practice of liberal fluid intake may not only be not beneficial but maybe associated with more allograft fibrosis.

Funding: NIDDK Support, Pharmaceutical Company Support - Merck Inc

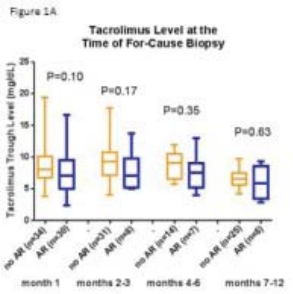
PUB726

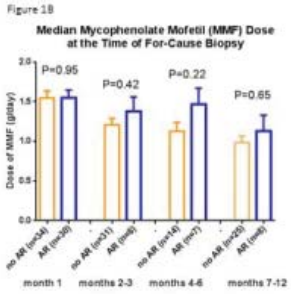
Why Do Allograft Recipients Develop an Episode of Acute Rejection while on Immunosuppressive Therapy? A Single Center Study of 543-Kidney Graft Recipients Sean R. Campbell, John R. Lee, Thangamani Muthukumar, Manikkam Suthanthiran, Darshana Dadhania. *Medicine, New York Presbyterian Hospital, New York, NY.*

Background: Acute rejection (AR) of a kidney transplant, whether it is T cell mediated acute cellular rejection (ACR) or antibody mediated rejection (AMR), is a major cause of graft dysfunction and loss. Why patients on immunosuppressive (IS) therapy develop AR is an unresolved puzzle. Insufficient immunosuppression due to physicians prescribing inadequate amounts of IS drugs and/or patient medication non-adherence is a plausible hypothesis.

Methods: We investigated whether AR diagnosis is associated with: (i) lower tacrolimus trough levels, (ii) lower mycophenolate mofetil (MMF) dosage or (iii) non-adherence. Among 543 patients transplanted between 1/2008 and 12/2010, 157 patients underwent a for-cause allograft biopsy during the first year post-transplantation: 53 patients with AR biopsies, 44 with Tubular Necrosis/Toxicity, 31 with Other findings and 29 with Non-Specific findings.

Results: Figures 1A and 1B illustrates tacrolimus trough levels and MMF dosages were not significantly different between patients with and without AR during the first 12 months. Two of 157 patients had documented non-adherence. Serum anti-HLA donor specific antibodies (DSA) were tested in 135 patients, of which 52 tested positive and 83 tested negative. Mean±SD tacrolimus trough levels were 7.8±3.1 ng/ml and 8.1±2.9 ng/ml (P= 0.67) in the DSA positive and negative groups respectively and mean±SD daily MMF dosage were 1.5±0.51 grams 1.2±0.47 grams (P<0.0001) in the DSA positive and negative groups respectively.





Conclusions: Our data demonstrates that medication non-adherence and IS therapy at the time of a for-cause biopsy fail to explain the development of AR. These results suggest the need for more research on better methods for ascertaining the in-vivo immune status of the graft recipient.

PUB727

Invasive Aspergillosis after Kidney Transplantation: A Case-Control Study

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Background: Transplant recipients are among the most significant subgroups of immunosuppressed hosts at risk for invasive aspergillosis (IA). Renal transplantation-specific risk factors for IA have not been defined.

Methods: A retrospective case-control study was performed, including 41 renal transplant recipients with a diagnosis of proven (44%) and probable (56%) IA from 1995 through 2013. The two patients that underwent renal transplantation immediately before and after each case and did not develop IA, were used as control population (n = 82). Variables included were age, gender, duration of hemodialysis before transplantation, smoking habits, chronic lung disease, diabetes mellitus, respiratory function, HLA mismatch, use of basiliximab or antithymocyte globulin, donor and recipient CMV seropositivity and its prophylaxis, and leukopenia. Cox proportional hazard analysis was used to analyze week 12 mortality of the cases with the same covariates, as well as the serum and bronchoalveolar lavage (BAL) galactomannan (GM) index.

Results: The occurrence of leukopenia after kidney transplantation increased the risk of IA among all patients (OR 2.345 (95% CI 1.084-5.071)). 37% had early-onset IA (i.e., occurred during the first 3 months after transplantation). A longer duration of hemodialysis before transplantation and the occurrence of leukopenia after transplantation were risk factors for early-onset IA (OR 1.192 (95% CI 1.006-1.413) and OR 3.346 (95% CI 1.063-10.527), respectively), while donor CMV seropositivity was a risk factor for late-onset IA (i.e., occurred >3 months after transplantation) (OR 3.677 (95% CI 1.388-9.743)). Mortality rate at week 12 after diagnosis was 39%. Disseminated IA, leukopenia at presentation and the height of the serum GM index were associated with an increased risk of death (HR 5.080 (95% CI 1.740-14.830), HR 3.198 (95% CI 1.183-8.649), HR 1.371 (95% CI 1.123-1.674), respectively).

Conclusions: We described for the first time an association between duration of hemodialysis before kidney transplantation and early-onset IA. The serum GM index could predict mortality, whereas the BAL GM index did not.

PUB728

Deflazacort versus Prednisolone: A Study in Renal Transplant Recipients

Richard Savio Fernandes Almeida, Amar Kulkarni, Alan F. Almeida, Jatin P. Kothari, Rasika A. Sirsat. *P.D. Hinduja National Hospital and Medical Research Centre, Mumbai.*

Background: Deflazacort (DEF), a derivative of the old molecule prednisolone(PRD), reported to have a similar efficacy and a more favourable adverse effect profile, has not been evaluated adequately in Indian kidney transplant recipients.The objective of the present study was to compare DEF with PRD with respect to efficacy and safety in kidney transplant recipients.

Methods: Out of 103 archived medical records that were available for review, 60 patients (30 on DEF, 30 on PRD) fulfilled the inclusion criteria. Patients included in the study had been transplanted at least a year before, had received a CNI based immunosuppression (tacrolimus or cyclosporine) along with an anti-proliferative agent (mycophenolic acid or azathioprine) with either DEF or PRD. Retrospective analysis of clinical, biochemical, histological and radiological data was performed. Patients were started on DEF (1.2 mg/kg/day) or PRD (1 mg/kg/day) and were tapered to a maintenance dose of 6 – 12 mg (DEF) and 5 – 10 mg (PRD) by the end of 1 month. For efficacy, interval serum creatinine, graft and patient survival, presence of acute and chronic rejection, were examined. For safety, BMI gain, new-onset diabetes, cataract, cosmetic appearance, dyslipidemia, osteoporosis and post transplantat infections were analysed. Statistical analysis was conducted using Student's t-tests and Fisher's exact test.

Results: The groups were similar in age, sex, pretransplant BMI and HLA mismatches. The patients on DEF were followed up for a period of 3.13±0.93 and those on PRD for 3.46±0.97 years. There was no significant difference between the 2 groups in parameters related to efficacy. However, there was a significant difference in the incidence of steroid facies (p=0.025), new onset diabetes (p=0.036), cataract (p=0.010), osteoporosis (p=0.0001), chest infections (p=0.025) and a percentage increase in BMI over baseline at 180 days (p=0.03) and 1 yr (p=0.02), with those on DEF demonstrating higher levels of safety.

Conclusions: As DEF has a more favourable adverse effect profile, it could be the preferred option in the Indian set-up. However, further research is required to substantiate these findings.

PUB729

Subclinical Cardiac Damage and Its Determinants in a Cohort of Kidney Transplanted Patients

Carlo M. Alfieri,[1] Riccardo Floreani,[1] Maria Meneghini,[1] Anna Regalia,[1] Francesca Zanoni,[1] Simone Vettoretti,[1] Maria Teresa Gandolfo,[1] Maria Daniela Croci,[1] Maria Pia Rastaldi,[2] Piergiorgio Messa.[1] *[1]Nephrology, Dialysis and Kidney Transplantation, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Milan, Italy; [2]Renal research laboratory, Fondazione IRCCS Ca' Granda Ospedale Policlinico, Milan, Italy.*

Background: Left ventricular hypertrophy (LVH) is mostly responsible for the relevant cardiovascular risk in kidney transplanted patients (KTx). The aim of our study was to evaluate: 1) the prevalence of LVH 2) the factors related with left ventricular mass index (LVMI) and 3) those implicated in the increase of LVMI during the 1st year of KTx.

Methods: In 121 pts(M=69) over the 470 transplanted between 2004 and 2013 in our Unit, clinical parameters, blood and urinary samples were collected at 1st(T0) and 12th(T12) month after KTx. In addition, plasmatic levels of FGF-23, Fetuin-A, 25OH-Vitamin D, and Osteoprotegerin (OPG) were assessed. Cardiac parameters were determined by echocardiography: LVMI was indexed at the height$^{2.7}$ and LVH was defined as LVMI>51g/m$^{2.7}$.

Results: At T0,LVMI correlated with age, albumin, FGF-23, SBP, U-Prot-24h and BMI, whereas at T12 it was directly correlated with age, albumin, OPG, FGF-23. In multivariate regression, FGF23 at T0 and albumin at T12 resulted the only parameters associated with LVMI at T0 and at T12 resp. (both p=0.04). LVH was present in 49% of pts at T0 and in 50% at T12, and during the 1styear of KTx, 15% of patients developed LVH,whereas 19% had its regression. Considering the percentage of increase of LVMI in the two time points, in multiple regression LVMI at T0 resulted the only modifiable risk factor associated with LVMI worsening(p=0.001). No influence for immunosuppressive and anti-hypertensive therapy both in general and class-specifically was found as like as for Hb and mineral metabolism parameters.

Conclusions: The prevalence of LVH is relatively high in KTx patients. Nevertheless, in a small portion of them, we observed an improvement of cardiac abnormalities. FGF-23 and albumin seem to influence LVMI. In any case, LVMI at baseline is the only modifiable risk factor able in predicting LVMI increase in the 1st year of KTx.

PUB730

Cinacalcet Use in the Treatment of Hyperparathyroidism in Patients after Renal Transplantation

Nelomi Anandagoda, Tanuja Yalamarti, Mona Wahba. *SW Thames Renal Unit, St. Helier Hospital, Carshalton, Surrey, United Kingdom.*

Background: There are no current guidelines on the use of Cinacalcet in the treatment of hyperparathyroidism in patients following renal transplantation. Thus, evaluation of the effect of Cinacalcet in treatment of hyperparathyroidism after renal transplantation and assessment of potential adverse effects is needed.

Methods: This observational, retrospective, single-centre study collected data from 15 patients with stable renal transplant function and persistent hypercalcaemia. Cinacalcet was commenced at a dose of 30mg/day and up titrated according to corrected calcium levels and serum parathyroid hormone. Serum calcium, phosphate, parathyroid hormone(PTH) and estimated GFR were monitored throughout the study period. All patients were radiologically assessed for parathyroid adenoma with parathyroid SESTAMIBI CT and ultrasound neck.

Results: 26.6% Male, 73.3% Female; Mean Age 59.2years (Range 31y to 75y); Median follow up time 12 months (range 3 months to 48 months); Mean initial serum corrected calcium was 2.83 ±0.13 mmol/L; the mean calcium with treatment was 2.52 ±0.18 mmol/L (p<0.001). Mean initial serum phosphate was 0.75 ±0.24 mmol/L; the mean phosphate with treatment was 0.86 ±0.19 mmol/L (p=0.026). Mean initial serum parathyroid hormone was 51.36 ±53.66 pmol/L; the average PTH with treatment was 36.68 ±26.46 pmol/L (p=0.08). There was no significant change in eGFR pre and post treatment with Cinacalcet. 93.3% of patients had confirmed parathyroid adenoma. Two patients underwent parathyroidectomy with no post-operative complications. The remaining patients were referred for surgery but did not proceed in accordance with patient's wishes. There were no recorded cardiac events, fractures sustained or renal stone disease during the study period.

Conclusions: In this study group, Cinacalcet was safe and effective in treating hypercalcaemia secondary to hyperparathyroidism. Further studies are required with a larger sample size in order to further evaluate the efficacy of this drug in the renal transplant population.

PUB731

Superior Outcomes of Kidney Transplantation Compared with Dialysis in Korea: A Propensity–Matched Analysis of a National Population-Based Cohort Study between 2005 and 2008 Kyung Don Yoo,[1] Clara Tammy Kim,[2] Jeonghwan Lee,[3] Myoung-Hee Kim,[4] Hyunwook Kim,[5] Dong-Ryeol Ryu,[6] Dong Ki Kim,[1] Chun Soo Lim,[1] Yon Su Kim,[1] Jung Pyo Lee.[1] *[1]Internal Medicine, Seoul National Univ College of Medicine, Korea; [2]School of Public Health, Seoul National Univ, Korea; [3]Internal Medicine, Hallym Univ Hangang Sacred Heart Hospital, Korea; [4]Dental Hygiene, College of Health Science, Eulji Univ, Korea; [5]Internal Medicine, Wonkwang Univ College of Medicine Sanbon Hospital, Korea; [6]Internal Medicine, Ewha Womans Univ, Korea.*

Background: Kidney transplantation (KT) can decrease all-cause and cardiovascular mortality. However, the data of KT outcome compared with dialysis was scarce in Asian population. Here, overall survival and cardiovascular outcomes of KT in Korean were compared with those of dialysis using the claim data of the Korea Health Insurance Review and Assessment Service (HIRA).

Methods: Among 35,418 all the incident dialysis patients between 2005 and 2008 in Korea, 1,563 patients who underwent KT were compared with patients maintaining dialysis without before and after 1:1 matching of propensity score. Over-all survival rate and post-transplant Major Adverse Cardiovascular Event (MACE) were traced.

Results: Before matching, dialysis group was older and had more comorbidities. After matching, there were no difference in age, sex, dialysis modalities, and comorbidity. Patient's survival in transplant group was significantly better than in matched-dialysis group by log-rank test (P <0.001). Multivariate analysis also showed that KT significantly decreased risk of death after adjusting for age, sex, and comorbidities (P <0.001, hazard ratio [HR] 0.16, 95% confidence interval [CI] 0.13-0.22). In addition, transplant group showed a better MACE free survival compared with dialysis-maintaining group (P <0.001, HR 0.16, 95% CI 0.13-0.22).

Conclusions: Long-term dialysis had significantly higher cardiovascular mortality and all-cause mortality compared with kidney transplantation in Korean incident dialysis patients. Kidney transplant could be recommended more actively in Korean population.

PUB732

Determinants of Vascular Stiffness in Pediatric Renal Transplant Recipients Michael E. Seifert,[1,2] Lisa de las Fuentes,[2] Nazia Kulsum-Mecci,[2] Victor G. Davila-Roman,[2] Keith A. Hruska.[2] *[1]Southern Illinois Univ; [2]Washington Univ in St. Louis.*

Background: CKD imparts enormous cardiovascular (CV) risk that is only partially improved by renal transplantation. We sought to understand determinants of pulse wave velocity (PWV), a surrogate for vascular stiffness and a biomarker associated with CV risk, in pediatric renal transplant recipients with excellent graft function.

Methods: This prospective cohort of 16 pediatric renal transplant recipients underwent carotid-femoral PWV and pulse wave analysis using applanation tonometry. Circulating biomarkers associated with CV risk were measured at the time of PWV. PWV was transformed to a z-score (PWV-Z) using the LMS method to normalize PWV for gender, age and height. The primary outcome was vascular stiffness, defined *a priori* as a PWV-Z > 1. Secondary outcomes included PWV, peripheral blood pressure (BP), central BP, body mass index (BMI), estimated GFR (Schwartz eGFR), transplant vintage, plasma fibroblast growth factor-23 (FGF23) and plasma phosphorus.

Results: The cohort had a mean age of 15 ± 3.1 years, mean BMI of 23.5 ± 7.8 kg/m^2, mean eGFR of 100 ± 24 ml/min/1.73 m^2, mean PWV of 5.9 ± 1.2 m/s and mean PWV-Z of 1.2 ± 0.9. All had BP < 95th percentile for age/height but 4/16 (25%) were prehypertensive (BP between 90th and 95th percentiles). Vascular stiffness was observed in 9/16 (56%) subjects. Those with vascular stiffness were older and had higher peripheral and central BP within the normal range. Prehypertensive subjects had particularly high PWV-Z (mean 2.1 ± 0.9). BMI, eGFR, transplant vintage, FGF23 and phosphorus were similar in those with versus without vascular stiffness. Using Pearson's correlation PWV-Z was significantly and positively correlated with age (r^2 = 0.610), peripheral SBP and DBP (r^2 = 0.717, 0.662), and central SBP and DBP (r^2 = 0.743, 0.673). PWV-Z did not correlate with other secondary outcomes.

Conclusions: In this modest-sized cohort of pediatric renal transplant recipients, the majority of children had vascular stiffness despite excellent graft function, normal BP and normal BMI. As in the general population, increasing age, peripheral BP and central BP correlated with increasing PWV.

Funding: NIDDK Support, Other NIH Support - NCATS KL2 Career Development Award via Washington University's CTSA

PUB733

Is Polyoma Virus Common Among Middle East Kidney Transplant Recipients? Yousef Boobes, Bassam O. Bernieh, Qutaiba Hussain Daoud, Mohamed Raafat Al Hakim, Hanan Eljack, Mohamed E.O. Ahmed. *Nephrology/Medicine, Tawam Hospital, Al Ain, Abu Dhabi, United Arab Emirates.*

Background: BK viremia and nephritis is an increasing problem in renal transplant recipients. Because there is no safe and effective anti-viral therapy available presently, screening-based prevention and pre-emptive strategy are recommended. Data about its prevalence in Middle East renal transplant recipients are lacking.

Methods: All renal transplant followed in our clinic between 2011 and 2012 (ns116) were screened for the BK virus (BKV) and JC virus (JCV). Quantitative real-time polymerase chain reaction (PCR) for both blood and urine was used. Renal biopsy was performed only in patients with deteriorating renal function associated with positive urine/blood PCR. Patients who showed positive PCR assay were followed-up for at least six months by monitoring serum creatinine level and viral load.

Results: Total number of kidney transplant recipients was 116, 65 (56%) were male, age 51±15 years. Average time on transplantation was 131±61 months. JCV was found in 8% of urine and 1% of plasma specimens (weak positive). While BKV was found in 15% of urine and 2.7% (3 patients) of plasma specimens, two of them had deterioration of kidney function. Renal biopsy confirmed the diagnosis of BK nephropathy in both cases. The three cases were managed by reducing the immunosuppressive treatment with relative stabilization of their kidney function. Cases with stable renal function and positive urine PCR cleared the virus spontaneously during follow-up after minor reduction of the immunosuppressive treatment or without any intervention. None of our patients lost the graft due to BK NP. The immunosuppressive therapy was similar in both PCR positive and negative patients.

Conclusions: These results suggest that polyoma virus is not uncommon in our kidney transplant recipients. Routine screening suggested by KDIGO Guidelines could help minimizing its detrimental effect on graft survival.

PUB734

Characteristics of Immunosuppressant Noncompliance: Experience of One Urban Transplant Center Cheng Chu, Varun Malik, Christopher Bosh Zakhary, Jack Jacob, Mujtaba A. Hasnain, Thet N. Zaw, Heather Rush Lefkowitz. *Nephrology, Newark Beth Israel Medical Center, Newark, NJ.*

Background: Immunosuppressant noncompliance is a major cause of rejection and graft loss. While studies have indentified risk factors for noncompliance, there is a paucity of literature focusing on minorities and indigent populations, the group which historically has the highest rates medication non-adherence.

Methods: A total of 60 questionnaire surveys were distributed and collected at our transplant clinic to all patients post-renal allograft on chronic immunosuppression.

Results: The demographics of our respondents were 65% African American, 68.33% unemployed, and 56.67% Medicaid only patients. 5 out of 60 patients who responded to the survey self-reported some level of immunosuppressant noncompliance. The noncompliant group was associated with African American race(80%), unemployment(80%), and high school education(80%). In addition, a mann-whitney test showed that the depression score is higher in the noncompliant group than the compliant group(U=22.5, p = 0.001, r= 0.47).

Conclusions: The main limitation of our study is the small sample size of the noncompliant group, which can partly be explained by reporting bias, as the percentage of noncompliance nationally is higher. Larger studies are needed to identify and target independent risk factors relevant to our patient population.

PUB735

Role of Cytomegalovirus (CMV) Viremia Monitoring in Kidney Transplant Recepients (KTR) Receiving Basiliximab Induction Dinesh Kumar, Reetesh Sharma, Vijay K. Kher. *Nephrology, Medanta Hospita;, Gurgaon, Haryana, India.*

Background: Prophylactic and preemptive treatment strategies have been employed for Cytomegalovirus (CMV) disease prevention. While these strategies have been well tested in patients receiving thymoglobin, there is paucity of such data in those receiving basiliximab induction.

Methods: This was an observational study of consecutive 19 live donor KTRs (5 females and 14 males) at our center with positive CMV IgG serology in both donor and recipients (D+R+) to evaluate role of preemptive treatment for CMV in patients receiving basiliximab induction (20 mg day 0 and 4). They receivedIV methylprednisolone 500mg on day 0 and maintenance triple immunosuppression with tacrolimus(0.1 mg/kg/day), MMF(1gm twice daily) and prednisolone (40 mg on day 1 and tapered to 5 mg/day at end of 3 to 6 months). None of the patients received CMV prophylaxis. CMV monitoring was done using quantitative nucleic acid PCR test on day 0, week 1, 2, 3, 4, 6, 8, and month 3, 4, 5, 6. CMV infection was defined as persistent viremia with CMV DNA PCR ≥ 2000 copies while CMV disease was defined as fever, leukopenia or organ involvement in addition. Patients with CMV infection were treated with oral valgancyclovir in standard dosage.

Results: Mean recipient and donor age were 45.32 ± 11 and 50.94 ± 9.11 years. CMV infection developed in 1 patient(5.25%) and responded well to valgancyclovir. None of the patients developed CMV disease. At end of follow up, patient and graft survival were 100% and 94.74% respectively and mean serum creatinine was 1.44±1.04 mg/dl. One patient (5.26%) had biopsy proven acute rejection.

Conclusions: Preemptive treatment is an effective and economical strategy as compared to universal prophylaxis for CMV infection in moderate risk (D+R+) KTRs receiving basiliximab induction with maintenance triple immunosuppression of tacrolimus, MMF and prednisolone.

PUB736

High Grade Urothelial Neoplasms, Associated with BK Polyomavirus, Arising in Renal Allografts Daniel J. Kenan, Akanksha Gupta, Harsharan Kaur Singh, Volker Nickeleit. *Pathology and Laboratory Medicine, Univ of North Carolina, Chapel Hill, NC.*

Background: Tumors arising in renal allografts are rare; however, the incidence of urothelial carcinoma is known to be increased at least 3-4 fold in renal transplant patients. Although some renal allograft tumors have been shown to express polyomavirus large T antigen, a causal association between polyomaviruses and human malignancy has never been demonstrated. Here we discuss molecular details of the virus-host interaction that may be related to oncogenesis.

Methods: DNA was isolated from formalin fixed paraffin embedded and frozen tissue sections and submitted for deep sequencing to a read density averaging 10 reads per genomic nucleotide position. The sequences were mapped onto both the human genome as well as a canonical BK polyomavirus sequence. The mapped sequences were used to identify genomic insertion sites, viral break points, and point mutations, insertions and deletions within the viral sequences. These results were compared with related findings published in the literature. Findings were confirmed by PCR analysis. Expression of certain gene products was further analyzed by immunohistochemistry.

Results: A common feature of polyomavirus-related high grade urothelial malignancies is integration of the virus into the genome of the neoplastic cells. This is accompanied by high levels of expression of large T-antigen, without replication of the virus or expression of late viral gene products. There is no evidence of significant amplification of the virus. Upregulation of large T-antigen expression is likely related to rearrangements of the noncoding control region (NCCR), which result in deletion of sequences that down-regulate early gene expression.

Conclusions: BK polyomavirus is associated with high grade malignancies in a variety of clinical settings, including renal transplantation. This may occur in the absence of polyomavirus nephropathy, implying direct involvement of BK polyomavirus in oncogenesis that does not require latent or active infection. Such oncogenesis likely requires upregulation of large T-antigen and may also involve insertional inactivation of human genes at sites of viral integration.

PUB737

The Protective Effect of Cryotherapy Over Surgery in Post-Allograft De Novo Prostate Malignancy Cheng Chu, Varun Malik, Thet N. Zaw. *Nephrology, Newark Beth Israel Medical Center, Newark, NJ.*

Background: Chronic immunosuppression in post-transplant patients have contributed to increasing incidence of de novo malignancy. Immunosuppression reduction have shown to benefit tumor regression in some cancers, however immunosuppression reduction in conjunction with cryotherapy over standard surgical resection in prostate adenocarcinoma have not been well studied.

Methods: Data of 501 renal allograft recipients who developed post-transplant prostate de novo solid tumor was reported for transplants performed between 01/01/2000 and 06/30/2012 by United Network for Organ Sharing. Patients were followed over time for outcomes(i.e. alive with tumor, alive free of tumor, death) after undergoing different sets of treatments(i.e. Observation, surgery, cryotherapy, immunotherapy, radiation) with reduction of immunosuppressant treated as an exposure.

Results: The mean recipient age of renal allograft was 59.8 years and the time to diagnose prostate cancer post-transplantation was 4.2 years. Treatment sets were compared using odds ratio only if they had similar follow-up times. In those patients with immunosuppression reduction, Cryotherapy over surgery showed regression of tumor(OR = 0.21, 95% CI = -3.32 – 0.6, p<.05) while other treatment sets showed maintenance of tumor progression: Immunotherapy over chemotherapy(OR = 7.5; 95% CI = 7.17 – 11.86, p<.05); Radiation over chemotherapy(OR = 3.57; 95% CI = 3.39 – 6.3, p< .05); Radiation over Immunotherapy(OR = 3.57; 95% CI = 3.39 – 6.3; p<.05).

Conclusions: Our results show that in patients who had their immunosuppression regimen reduced, cryotherapy had an protective effect over surgical resection in demonstrating tumor regression. Further studies in this area are needed to elucidate this phenomenon.

PUB738

Long Term Outcomes of Renal Transplant Recipients with prior Plasmapheresis Sapna M. Jairath, Parvathy Madhavan, Amit Basu, Nicole M. Ali, Aditya Kadiyala, Vivek Jayaschandran, Neenu Sukumaran, Navya Voleti, Madhu C. Bhaskaran, Ernesto P. Molmenti. *Renal Transplant Center, NSUH, Manhasset, NY.*

Background: Plasmapheresis (PP) is a treatment modality instituted in patients to reduce risk of and treat antibody mediated rejection. Compared to other immunosuppressive agents, PP may allow for a more intense immunosuppression for a shorter time span, in the initial, crucial period when acute rejection is more likely. While the incidence for acute rejection in Renal Transplant recipients (RTR) remains low, there is limited data on long term outcomes of renal transplant "Recipients who received Pre-transplant Plasmapheresis" (RPTP).

Methods: We reviewed clinical and lab data of RTR who received their transplants from 1/1/2010 to 1/1/2013 at a single center and followed their clinical course till 6/1/2014. We compared the outcomes of RPTP for Donor Specific Antibodies, to that of all RTR in the same period. There were a total of 94 RTR, out of which 17 received PP preceding the transplant. The outcomes, including allograft failure due to recipient death or rejection, in both groups, were considered. Lab parameters including serum creatinine, proteinuria, and acute or chronic rejection on biopsy were recorded. Other variables influencing outcomes such as changes in immunosuppression, infections (BK virus, other major infections like pneumonia and sepsis), noncompliance, and lymphoma were also recorded.

Results: After a period of 18-60 months, RPTP had allograft and recipient survival comparable to overall recipient population. Moreover, BKV infections and acute and chronic rejection were also comparable in both groups. The tabulated representation of the data is shown:

	Total Recipients of Transplant	Pre-transplant Plasmapheresis Recipients(RPTP)
Total Transplant	94	17
Total Allograft Loss (Mortality + Allograft Failure)	16	3
Mortality	11	2
Alive with Failed Allograft	5	1
Acute Rejection	5	3
Chronic Rejection	13	2

Conclusions: Pre-transplant Plasmapheresis may allow comparable allograft and recipient survival in renal transplant candidates with DSA. This may be achieved without unacceptable increase in the rate of opportunistic or severe infections and allograft rejection.

PUB739

Parathyroidectomy for Tertiary Hyperparathyroidism in Kidney Transplant Recipients Zahra Deen,[1] Emad H. Asham,[2] A. Osama Gaber,[2] Dana M. Hong,[2] Luan D. Truong,[2] Sreedhar A. Mandayam,[1] Venkataraman Ramanathan.[1] *[1]Baylor College Of Medicine; [2]Methodist Hospital.*

Background: After kidney transplant, tertiary hyperparathyroidism (ThPT) can cause hypercalcemia and worsen bone disease. While calcimimetic drugs can be potentially useful, their use after transplant is not covered. We present our prevalence data, indications for parathyroidectomy (PTx), and accuracy of sestamibi scan in predicting parathyroid pathology.

Methods: In this retrospective study, we identified transplant recipients who underwent kidney transplant between 2008 and 2013 and who underwent PTx for ThPT at Houston Methodist Hospital. In addition to demographic and clinical information, data collected included serum calcium, phosphorus, Vitamin D and intact PTH (iPTH) levels obtained pre transplant, and at months 1,3,6 and 12 months post-transplantation and also bone density measurement and parathyroid scan.

Results: We identified 26 (2.5%) patients who underwent PTx, among the 1001 patients who underwent kidney transplant at our center, during the 5-year study period. Mean age was 52±12 yrs and 58% were women. The majority (88%) underwent deceased donor transplantation and all patients were on Tacrolimus, Mycophenolate and Prednisone. Prior to surgery, mean calcium and iPTH levels were 10.4±1.3 mg/dl and 508±404 pg/ml, respectively. Median time duration between transplantation and PTx was 482 days (Q1,Q3: 225,909). Indications for PTx included persistent hypercalcemia (42%), significant osteoporosis or worsening osteopenia (35%) or both (15%). Few patients (8%) underwent surgery after failing medical therapy, including calcimimetics. Sestamibi scan, obtained in all patients, showed glandular hyperplasia in 50% and adenoma in 34% of patients. However, surgical pathology showed hypercellular parathyroid tissue in 25 specimens (96%). Only one biopsy showed parathyroid adenoma with thick, fibrous trabecula and calcifications.

Conclusions: In kidney transplant recipients, ThPT is common and in small proportion of cases, it warrants PTx for hypercalcemia and worsening bone disease. Parathyroid hyperplasia is the commonest pathology in these patients. Sestamibi scan is inaccurate in making the distinction between hyperplasia and adenoma.

PUB740

Comorbidity Is Less Prevalent on the Active Kidney Transplantation Waiting List Relative to Inactive Ones Jacek S. Malyszko,[1] Jolanta Malyszko,[1] Andrzej Milkowski,[2] Tomasz Rafal Prystacki,[2] Teresa Rydzynska.[2] *[1]Nephrology, Medical Univ, Bialystok, Poland; [2]Fresenius Medical Care, Poznan, Poland.*

Background: Renal transplant is the best form of treatment for most patients with end-stage renal disease (ESRD). The aim of this study was to examine demographic and comorbidity factors of patients with end-stage renal disease on the kidney transplantation waiting list in regard to their status (active versus temporary disqualified).

Methods: The cross-sectional study was conducted on 270 prevalent patients on the waiting list from 26 dialysis centers in Poland (population of 5500 dialysis patients). They were also 15 peritoneally dialyzed patients.

Results: Patients who had been registered in the cadaver kidney waiting list were aged 49±13 years, median dialysis duration 34 months, median residual renal function was 550 ml, prevalence of hepatitis B and C was below 5%. The primary cause of end-stage renal failure was hypertensie glomerulopathy in 41%, chronic glomerulonephritis in 29% and, diabetic nephropathy in 24%. There were no statistically significant differences except total cholesterol, LDL cholesterol, aspartate aminotransferase and weakly dialysis dose between patients on the active list and temporary disqualified. Clinical status, including cardiovascular status (echocardiography parameters, coronary angiography results) were similar in both groups. Similarly peritoneally dialyzed patients did not differ from those

on hemodialyses, except lower level of protein, albumin, higher fibrinogen and platelet count. When all these parameters were compared with those on dialyses no on the waiting list from a single dialysis unit, dialyzed patients not candidates for transplantation were almost 20 years older, with more comorbidities with diabetes being the most prevalent followed by liver viral infections.

Conclusions: Potential kidney transplant recipients are not typical dialyses patients: they are relatively young, with rather short dialysis vintage, without significant comorbidities. Preemptive transplantation is offered to seldom in this population. Kidney transplantation seems the best option of renal replacement therapy however, minority of dialyzed patients seem suitable for this therapeutic approach.

PUB741

The Role of Systemic Monitoring of Calcineurin Inhibitor (ICN) Concentrations on the Excretory Function of the Transplanted Kidney and Liver Function Marek Kuzniewski, Magdalena Barbara Kaziuk, Wladyslaw Sulowicz. *[1]Dept of Nephrology, Jagiellonian Univ Medical College, Krakow.*

Background: In uncontrolled high concentrations of ICN, acute nephrotoxicity of the transplanted kidney occurs. Such a state presents as rapid decrease of glomerular filtration and liver damage which can be diagnosed by evaluating serum total bilirubin levels and alanine aminotransferase (ALT) concentrations.

Methods: The study included 205 patients with end stage renal disease (ESRD) post kidney transplant (Ktx), 86 females and 120 males between the ages of 18-72 years (mean age- 47 years), treated with ICN. Patients were divided into 4 groups depending upon time post kidney transplant: up to 3 months post transplant, 4-12 months post transplant, 1-5 years post transplant and >5 years after kidney transplant. The patients were under control for a period of 2 years. The hemolytic- uremic syndrome was excluded in the study group. Glomerular filtration rate was evaluated by estimating GFR (eGFR) using the Modification of Diet in Renal Disease fomula (MDRD). Liver function was assessed by measuring serum total bilirubin and ALT levels.

Results: A significant correlation was noted between values of eGFR and ICN concentrations in the study group (p<0,05). Correlations between cyclosporine as well as tacrolimus concentrations with total serum bilirubin are statistically significant (p<0,05). This is essential proof that upholds the existence of an association between high concentractions of cyclosporine and tacrolimus with high levels of total serum bilirubin. Increased levels of ALT significantly correlated with concentractions of cyclosporine A and tacrolimus (p<0,05).

Conclusions: It is essential to systematically monitor ICN concentrations due to their possible future chronic nephrotoxic effect on the function of the transplanted kidney (arteriolopathology). Increased serum total bilirubin and ALAT levels not only represent damage of liver cells but are indirectly indicative of high non- therapeutic concentrations of ICN.

PUB742

Low Dose of Sirolimus Shows No Advantage over Tacrolimus as Immunosuppressive Regimens after Renal Transplantation: A Meta-Analysis Jingyi Zhou, Yan Jiang, Jianghua Chen. *Kidney Center, the First Affiliated Hospital, College of Medicine, Zhejiang Univ, Hangzhou, Zhejiang, China.*

Background: Tacrolimus as a representative of calcineurin inhibitors (CNIs) has been proved nephrotoxic with long-term use. Replacing tacrolimus with sirolimus after renal transplantation is quite attractive for its potential possibility to avoid side effects and gain better outcomes.

Methods: PubMed, Web of knowledge, Medline and the Cochrane Library were searched. Patient and graft survival, acute rejection and adverse events were evaluated as primary outcomes and glomerular filtration rate (GFR) was an additional surrogate for renal function.

Results: Fifteen studies were included with 2480 patients. Patients in the sirolimus group showed an increased rate of acute rejection within one-year's follow-up 2.02 (95% CI 1.37~2.99, $P < 0.05$) and also a higher risk of adverse events 1.31 (95% CI 1.02~1.68, $P < 0.05$). The incidence of hyperlipidaemia was significantly higher with RR=1.75 (95% CI 1.17~2.61, $P < 0.01$) in the sirolimus group, as well as a significantly higher serum level of triglyceride (MD=74.03, 95% CI 53.89~94.16, $P < 0.01$) and cholesterol (MD = 35.13, 95% CI 21.18~49.08, $P < 0.01$). The other outcomes were insignificantly different between two groups.

Conclusions: This meta-analysis concluded that sirolimus showed no advantage over tacrolimus when used early after transplantation. When used with higher concentrations, the disadvantages may be avoided.

PUB743

Rare Post Transplant Lymphoproliferative Disorder; Long Term Remission with Chemotherapy and Bone Marrow Transplant Madan Bahadur,[1] Keyur K. Dave.[1] *[1]Consultant, Dept of Nephrology, Jaslok Hospital and Research Centre, Mumbai, Maharashtra, India; [2]Fellow in Nephrology, Jaslok Hospital and Research, Mumbai, Maharashtra, India.*

Background: Multifocal Extramedullary Plasmacytoma in post renal transplant recipient is a rare form of post transplant lymphoproliferative disorder (PTLD). Conventionally treatment of PTLDs includes significant reduction in immunosuppression but this can cause rejection or even graft loss. For this refractory rapidly progressive malignancy an aggressive treatment plan was followed.

Methods: A 42 year old, 14 year post transplant patient on Cyclosporine, Azathioprine and Prednisolone, developed rapidly progressive plasmacytoma lesions in pancreas, gums, subcutaneous tissue and near transplant kidney. His lesions responded only partially to immunosuppression reduction and 6 Bortezomib and Dexamethasone chemotherapy cycles. To attain long term remission or cure he was treated with high dose chemotherapy including Melphalan followed by rescue autologous bone marrow stem cell transplant.

Results: Post engraftment, by Day 90 patient achieved complete remission as all PTLD lesions completely disappeared. Patient has remained in complete remission even after 5 years of rigorous follow up with a stable kidney function. His whole body PET CT does not show any malignancy and his creatinine is a stable 1.6 mg % on triple immunosuppression.

Conclusions: We report the first case of long term remission, possible cure, of a rare form of PTLD, multi focal plasmacytoma with high dose chemotherapy and autologous bone marrow transplant. The icing on the cake is a stable renal function 19 years post transplant as immunosuppression was continued. In the review of literature we could find only 4 cases of unifocal post transplant plasma cell dycrasia treated conventionally with varied results. We propose that the same protocol as for multiple myeloma in non transplant setting can be used for this PTLD with rewarding results.

PUB744

Long Term Outcome in a Patient with Post Transplant Epstein Barr Virus Associated Smooth Muscle Tumor Naveed Aslam, Dildar Ahmed Laghari, Seddeg Younis, Nader Mohamed Omran, Muddassar Mahboob, Ramesh Nair, Ebadur Rahman. *Nephrology, Prince Sultan Military Medical CITY, Riyadh, Saudi Arabia.*

Background: Epstein Barr virus associated smooth muscle tumors (EBV+SMT) following solid organ transplantation are rare. This case report discusses use of Valacyclovir and Sirolimus as a successful treatment post surgical removal of multicentric tumors.

Methods: A 40 years old male underwent cadaveric kidney transplantation in 1996. The EBV serostatus was negative both in the donor and the recipient. The patient's immunosuppressive regimen included cyclosporine, mycophenolate mofetil, and prednisolone and induction with anti-thymoglobulin. The baseline creatinine was 80 umol/l (range 70-110). In 2002, due to gradual deterioration of graft function (creatinine 120-130 umol/l), a graft biopsy was performed that showed transplant glomerulopathy. Subsequently, in July 2004, abdominal and chest computed tomography (CT) scan examination was performed following complaint of intermittent epigastric pain of several weeks duration. CT scan showed well-defined masses in mesentery, right lung, and liver. CT scan guided Tru-Cut biopsy taken from the right hepatic mass showed hepatic leiomyoma; a tumor usually associated with EBV infection. Serology for EBV came positive with a high titer of PCR.

Results: Patient underwent surgical removal of the tumors. Post surgically the immunosopressive treatment was changed, from Cyclosporine to Sirolimus with Prednisolone and Valacyclovir; Mycofenolate Mofetil was discontinued. Sirolimus levels were maintained in the range of 4-8 ng/ml. Currently, the patient maintains a stable graft function (creatinine 116 umol/l, proteinuria 0,6 g/day), EBV PCR is still positive with very low titer and follow-up CT did not show any signs of recurrence.

Conclusions: The mTOR pathway plays an important role in regulating cell growth and proliferation and its deregulation is associated with many human cancers. Sirolimus inhibits mTOR and hence could be a potential therapeutic alternative for cancers for which the mTOR pathway is activated. It has been successfully used for the treatment of Kaposi's sarcoma and, in our experience, with EBV+SMT.

PUB745

Renal Graft Survival in Patients with Thymoglobulin Induction Therapy, at the Hospital Centro Medico Nacional De Occidente, Jalisco, Mexico Paloma Arleth Zavalza Camberos, Virginia Ibarra Pedroza, Benjamin Gomez-Navarro, Adriana Banda Lopez. *[1]Nefrologia Y Trasplante, Centro Medico Nacional De Occidente, Imss, Guadalajara, Jalisco, Mexico.*

Background: Induction with Thymoglobulin offers advantages such as reducing the frequency of acute rejection and improved graft function. The objective was to evaluate renal graft function and survival in patients receiving Thymoglobulin induction.

Methods: Retrospective cohort, in the period Jun 2010 to Dec 2013 included patients who has over 15 years of age, who received induction therapy with Thymoglobulin. Excluded patients with graft loss in the 1st week of renal transplantation. Was recorded the dose cumulative mean of Thymoglobulin, and serum creatinine to assess graft function. Kaplan-Meier estimates of freedom from death and graft loss. The frequency of acute rejection-free period it was obtained, and secondarily, freedom from infection- related death and posttrasplant frequency of infections and lymphoproliferative disorders.

Results: 250 patients were included. A 70% received living donor transplants and 30% of deceased donor.

Cumulative dose induction, mean (SD), mg/kg	**4.3 (± 1.5)**
Graft function	**Serum creatinine (mg/dl)**
Basal (at discharge)	0.97 ± 0.46
12 months	1.20 ± 0.90
2 years	1.13 ± 0.82
3 years	1.30 ± 1.10
Acute Rejection at 12 months, n(%)	**24 (9.6)**
3 years of transplant, n.	**98**
Freedom from Acute Rejection	97%
Freedom from infection-related death	96%
Freedom from posttransplant lymphoproliferative disorders	100%

Actual patient and graft survival were 98% (n=250) at 12 months. Kaplan-Meier estimates of freedom from death and graft loss, through 2 years were 98.3% and 97.8% (n=185), respectively, and 96% and 93.9% (n=98), through 3 years.

Conclusions: Induction with Thymoglobulin allowed good graft function and survival. The use of Thymoglobulin as induction agent in our patients at high immunological risk, is safe and associate with excellent patient survival and low rates of acute rejection renal graft survival.

PUB746

Homeostatic Repopulation of CD8$^+$ T-Cells After T-Cell Depletion in a Kidney Allograft Recipient Hajeong Lee, Seung Hee Yang, Yon Su Kim. *Internal Medicine, Seoul National Univ College of Medicine.*

Background: The T-cell depletion for induction therapy leads to clinical tolerance strategy in autoimmune disease and transplantation (TPL), however, that effect of high dose-rejection treatment remains uncertain.

Methods: A 42-year men visited our institution for his delayed graft function after living donor kidney TPL. Although ATG induction therapy was done before TPL, however, he developed acute T-cell mediated rejection type IB. After pulse steroid treatment, he received ATG therapy with a total dose of 13.5 mg/kg. We collected his peripheral blood monocyte before, 2-week, and 6-month after completion of ATG treatment. We used flow cytometry to measure the expression and frequency of T-cell repertoire and compared with those of healthy control.

Results: After ATG treatment, there was greater depletion of CD4$^+$ subsets than CD8$^+$ T-cells within the total T-lympocytes. CD8$^+$ T-cells recovered faster than CD4$^+$ subsets. Among CD8$^+$ T-cells, central memory and naïve T-cells remained depleted until 6months, T_{EM}s (CCR7$^-$CD45RA$^{+/-}$) showed slight drop immediately after ATG, with a return to growth by 6 months. Two-weeks after ATG treatment, homeostatic proliferation was found, leading to the higher expression of Ki-67 and it returned to be stable in 6-months after ATG. Among T_{EM}s, CD45RA$^+$ T_{EM}s more steeply increased than CD45RA$^-$ T_{EM}s. In addition, IL7Rα^{low} T_{EM}s were increased, whereas IL7Rα^{high} T_{EM}s were decreased, which suggested the presence of repetitive antigenic stimulation. Cytotoxic perforin and granzyme B productions were stable persistently in IL7Rα^{low} T_{EM}s. In addition, suppressor T-cells (CD8$^+$CD28$^+$), especially CD27$^+$CD28$^-$ subsets were increased. In case of regulatory T-cells (Tregs), both allogenic Tregs (CD8$^+$CD28$^-$CD56$^+$ and CD8$^+$CD103$^+$ T-cells) were increased after 2-weeks, and only CD8$^+$CD28$^-$CD56$^+$ Tregs remained increased state even after 6-months after ATG. On the contrary, natural Tregs (CD8$^+$CD25$^+$Foxp3$^+$) remained stable.

Conclusions: In this interesting case, we found that T-cell depletion treatment in kidney TPL recipient induced homeostatic repopulation, mainly with expansion of IL7Rα^{low} T_{EM}s, suppressor T-cells and allogenic Tregs, under persistent antigenic stimulation.

PUB747

Thymoglobulin Induction Therapy in Kidney Transplant: Standardizing Dose. Use of 1mg/kg as Initial Dose at the Hospital Centro Medico Nacional de Occidente, Jalisco, Mexico Virginia Ibarra Pedroza, Paloma Arleth Zavalza Camberos, Adriana Banda Lopez, Benjamin Gomez-Navarro. *[1]Nephrology and Transplantation, IMSS, Gdl, Jalisco, Mexico.*

Background: Induction dose of Thymoglobulin modified constantly. In recent years, this has tended to decrease in order to avoid side effects. The purpose was to compare the initial dose in patients receiving thymoglobulin.

Methods: An ambispective cohort of patients with renal transplant, included in the period Jun 2010 to Dec 2013, which has received thymoglobulin induction an initial dose of 1mg/kg and those who received doses above 1.1mg/kg, for 4 days. Evaluating haematological tolerance, dose adjustments, and frequency of acute rejection and rejection-free period. Secondarily, the frequency of infections in the first year after transplantation and the presence of lymphoproliferative disease.

Results: 209 patients were included, divided into 2 groups, those who received initial doses of 1mg/kg (n = 119 patients: 84 received a live donor graft and 36 deceased donor) and those who received doses ≥1.1mg/kg (n= 90; 58 received a live donor graft and 32 deceased donor).

	1mg/kg (n=119)	≥1.1mg/kg (n=90)
Cumulative dose induction, mean (SD), mg/kg	3.5± 0.5	5.1± 0.75
Serum creatinine, mg/dl	0.95 ± 0.31	1.01 ± 0.36
Patients receiving full intended induction dose, n(%)	108 (90.7)	69 (76.6)
Acute Rejection at 12 months, n(%)	10 (8.3)	11 (12.2)
Reported frecuency of infections at 12 months, n(%)	43 (36)	44(49)
Freedom from AR after trasplantation, %	96	94
Freedom from posttransplant lymphoproliferative disorders, %	100	100

Conclusions: The induction dose of Thymoglobulin is different in each hospital, without unifying the standard dose used worldwide. Patients those receiving 1mg/kg, had adequate drug tolerance and no need for adjustments to the starting dose, observed a frequency similar to international registrations acute rejection, a reduction of 13% from infection diseases and no posttransplant lymphoproliferative disorders. An initial dose of 1mg/kg administered for 4 days (cumulative dose of 4mg/kg) is an appropriate dose for our patients.

PUB748

ABO Incompatible Transplantation: A Novel Risk Factor for Post Transplant Erythrocytosis Madan Bahadur, Chandan Laxman Chaudhari, Amjadkhan Mahebubkhan Pathan. *Dept of Nephrology, Jaslok Hospital and Research Centre, Mumbai, Maharashtra, India.*

Background: The incidence of Post transplant Erythrocytosis (PTE) varies from 8% to 22% among published reports.The mechanisms of PTE is unclear and is likely to be multifactorial. We explored this phenomenon in the ABO incompatiable transplant setting.

Methods: This study was carried out to evaluate incidence of PTE in patients undergoing renal transplant at our centre between march 2011 to July 2013. Patients with three consecutive value one week apart of hematocrit above 51% were classified as PTE cases. Patients with graft lost within three months of transplant, those on EPO, with chronic airway disease or a pre transplant hematocrit more than 51% were excluded. Rest of the patients who did not have post transplant erythrocytosis were taken as control. Association with different risk factors, native kidney diseases, immunosuppressives was studied.

Results: During study period out of 150 transplants performed 17 were ABO incompatible and 133 were blood group compatible. PTE was seen in 5/17 in ABO incompatible and 17/133 controls. Total 22 cases of PTE were noted. incidence was 29.41% in incompatible group compared to 12.78% in ABO compatiable transplants. All cases were males. No particular association could be found with any native kidney disease. Out of 5 cases in ABO incompatible group 4 received thymoglobulin as induction and one basiliximab. All cases received rituximab and plasmapheresis prior to surgery.

Conclusions: ABO incompatible transplants differ mainly from routine transplants in more aggressive immunosuppression. In different indications, Rituximab, Basiliximab and Thymogobulin have been used in treatment of autoimmune haemolytic anemias and aplastic anemias. Whether the increased incidence of PTE in ABO incompatible transplants is related to their use or some other mechanisms exist should be matter for future research. Incidentally all PTE cases occurred in patients with stable graft function and in those who had no episodes of rejection.

PUB749

Long Term Outcome of Renal Transplantation in Williams-Beuren Syndrome Parvathy Madhavan, Neenu Sukumaran, Sapna M. Jairath, Aditya Kadiyala, Praveen Kolumam Parameswaran, Nicole M. Ali, Amit Basu, Madhu C. Bhaskaran, Ernesto P. Molmenti. *Renal Transplant Center, North Shore Univ Hospital, Manhasset, NY.*

Background: Williams-Beuren Syndrome (WS) is a genetic disorder caused by mutation in chromosome 7q11.23. Several kidney and urinary tract abnormalities have been reported in WS including bladder diverticula, ectopic or horseshoe kidney, and renal aplasia or hypoplasia. Features of WS like hypertension, hypercalcemia, renal artery stenosis and stenotic abnormalities of abdominal aorta can have adverse effects on the outcome of renal transplant and literature on long term implications of this treatment in WS is scarce.

Methods: A 22 year old, Asian male patient with WS, underwent a deceased donor renal transplant at 5yrs of age, after 5 months on peritoneal dialysis for ESRD. He has been on chronic immunosuppressant therapy with CellCept, cyclosporine and prednisone. His other clinical comorbidities include mild supra valvular aortic stenosis, pulmonary valve stenosis, Wolf Parkinson White Syndrome post ablation and medically controlled hypertension. Physical examination was unremarkable, with the exception of characteristic facial dysmorphia. He has remained normotensive and latest lab values have been within reference ranges. Over the past 8 years, serum creatinine values have ranged from 1.03-1.4mg/dL with insignificant proteinuria and a serum calcium range from 8-10.1mg/dL.

Conclusions: In this patient with WS, the post transplantation clinical course has remained uneventful with normal renal function. His quality of life, post transplant improved and there was no incidence of major complications such as infections due to immunosuppression. In spite of a number of adverse factors that could potentially affect the outcome of the allograft in WS, the successful long term outcome in this transplant recipient, demonstrates that with careful selection and excellent follow up, renal transplant can be considered in this population.

PUB750

Acute Vitamin D2 Administration Does Not Alter Ischemia Tolerance in a Rodent Model of Reversible Myocardial Ischemia Kieran Mccafferty, Conor J. Byrne, Magdi Yaqoob. *William Harvey Research Institute, Queen Mary Univ, London, United Kingdom.*

Background: Vitamin D receptor activation has been associated with many effects beyond bone and mineral metabolism. Vitamin D deficiency is common in patients with CKD and is associated with cardiovascular mortality. Indeed there is evidence to support the role of vitamin D in many of the characteristics of the uremic cardiovascular phenotype: LVH, endothelial dysfunction, arterial stiffness, inflammation and vascular calcification. Chronic administration of vitamin D has been shown to be protective in animal models of both renal and cerebral ischaemia reperfusion injury. It is unknown if acute administration of vitamin D has an effect on subsequent ischemia tolerance.

Methods: Male Wistar rats were given either IP ergocalciferol 100,000IU/kg (n=10) or vehicle (n=10) 24hours before undergoing a reversible LAD ligation model of acute myocardial infarction with 25minutes ischemia and 2h reperfusion. Area at risk was measured using Evans blue and infarct size was measured using NBT and expressed as a % of the AAR.

Results: Acute administration of ergocalciferol lead to a 50% rise in vitamin D2 levels, but had no effects on other biochemical or cardiovascular parameters. Following myocardial IR injury, acute vitamin D2 treatment was not associated with alteration in myocardial infarct size (49.8% v 50.9% p=0.97).

	Vehicle	Ergocalciferol	p Value
Mean Arterial Pressure (mm/Hg)	140 (133-154)	130 (115-151)	0.35
Heart rate (BPM)	430 (420-49)	434 (427-463)	0.46
Calcium (mmol/l)	2.36 (2.33-2.56)	2.38 (2.23-2.57)	0.86
Phosphate (mmol/l)	2.3 (1.78-2.37)	2.32 (1.8-2.39)	0.72
Vitamin D2 (nmol/l)	8 (2.5-9)	12 (7-18)	**0.002**
Total Vitamin D (nmol/l)	28.6 (8.2)	37.7 (10.6)	**0.009**
Area at risk (%)	50.4 (42.9-55.5)	52.1 (45.8-57.1)	0.71
Infarct size (%)	49.8 (30.4-61.2)	50.9 (26.7-62.9)	0.96

Conclusions: Acute administration of vitamin D does not alter myocardial ischemia tolerance. Future studies should examine the effect of chronic vitamin D supplementation on myocardial ischemia tolerance.

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PUB751

Decreased Locomotion Is Associated with an Increased Incidence or Worsening of Peripheral Artery Disease in Hemodialysis Patients Manae Harada,[1] Atsuhiko Matsunaga,[1] Yoshifumi Abe,[1] Kei Yoneki,[1] Ryoma Ishikawa,[1] Takaaki Watanabe,[1] Toshiki Kutsuna,[1] Ryota Matsuzawa,[1] Atsushi Yoshida,[2] Naoyoshi Aoyama,[1] Kouju Kamata.[1] *[1]Kitasato Univ, Kanagawa, Sagamihara, Japan; [2]Sagami Junkanki Clinic, Kanagawa, Sagamihara, Japan.*

Background: Hemodialysis (HD) patients are at a higher risk of developing peripheral artery disease (PAD) than the general population. However, factors associated with the incidence or worsening of PAD in HD patients have not been fully elucidated. We performed a prospective and observational study to examine factors associated with changes in Ankle-Brachial Index (ABI) in HD patients.

Methods: A total of 159 Japanese outpatients (age, 64 ± 11.6; male, 59.1%) undergoing maintenance HD three times a week were followed for one year. Age, sex, body mass index, dialysis vintage, history of smoking, comorbid conditions, use of medication (sarpogrelate hydrochloride, cilostazol, clopidogrel and lipid lowering drugs), blood biochemical data (hemoglobin, serum albumin, creatinine, lipid profile, and calcium-phosphate products), and locomotion ability (ambulation and wheelchair use) were obtained from clinical records at baseline. Our analysis divided groups of patients with newly diagnosed PAD (ABI ≥ 1.00 at baseline and ABI ≤ 0.99 at the endpoint) and those without, and patients with progressive PAD (ABI ≤ 0.99 at baseline and a decrease in subsequent ABI ≥ 0.10) and those without, according to changes in ABI during the study period. Multivariable logistic regression analysis, adjusted for baseline clinical variables, was performed to identify factors that predict the incidence or progression of PAD after one year.

Results: During the follow-up period, there were 15 HD patients (9.5%) with newly diagnosed and progressive PAD. Adjusted multivariable logistic regression analysis revealed that the incidence or progression of PAD was significantly associated with locomotion ability (odds ratio [OR], 5.0; 95% confidence interval [CI], 1.0 – 23.7; P = 0.042) and use of medication (OR, 3.0; 95% CI, 1.0 – 9.1; P = 0.049).

Conclusions: These results suggest that decreased locomotion is closely associated with an increased incidence or worsening of PAD in HD patients.

PUB752

End-Stage Renal Disease Associated with Metabolic Syndrome: A Study of Biomarkers Utilizing BioChip Array Profiling Methods Vinod K. Bansal,[1] Debra Hoppensteadt,[2] Daneyal Syed,[2] Schuharazad Abro,[2] Syed Mustafa Ahmed.[2] *[1]Nephrology, Loyola Univ Medical Center; [2]Pathology, Loyola Univ Medical Center.*

Background: Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors which contribute to myocardial infarction and stroke in end stage renal disease (ESRD) patients. Several metabolic biomarkers have been identified and their circulating levels provide a better understanding of the pathogenesis of MS/ESRD. The purpose of this investigation is to profile biomarkers of MS in ESRD patients.

Methods: Plasma samples from 87 patients with ESRD undergoing maintenance hemodialysis and 50 normals were collected prior to a routine session. These samples were profiled for metabolic biomarkers using multiple protein chip bioarray technology. The protein chip array was comprised of several biomarkers of MS. All results were compiled in terms of group means +/- 1 SD (SEM) and statistically analyzed.

Results: In comparison to the normal samples, the ESRD group showed marked increase in circulating levels of all biomarkers. TNFa (47.4 +/- 18.3 *versus* 4.1 +/- 1.1 ng/mL) and IL-6 (7.8 +/- 9.1 *versus* 0.8 +/- 0.4 ng/mL) showed the most pronounced increase. C-peptide (14.5 +/- 4.1 *versus* 2.8 +/- 1.6 ng/mL), leptin (29.7 +/- 29.2 *versus* 5.2 +/- 7.9 ng/mL), resistan (16.1 +/- 6.0 *versus* 2.3 +/- 0.7 ng/mL) and ferritin (274 +/- 57 *versus* 57 +/- 63 ng/mL) showed a 5-fold increase in the ESRD group compared to normal. PAI-1 (5.4 +/- 5.2 *versus* 2.9 +/- 2.5 ng/mL), IL-1a (1.3 +/- 1.7 *versus* 0.35 +/- 0.07 ng/mL) and insulin (32.1 +/- 17.3 *versus* 17.1 +/- 1.7 ng/mL) showed modest increase in the ESRD patients. The increase in all of the MS biomarkers in the ESRD patients were highly significantly elevated, $p<0.05$.

Conclusions: The specific biochip array for MS allows the selective determination of various biomarkers associated with this syndrome in the ESRD patients and normals. Parallel increase in resistan, insulin, C-peptide, and leptin points to the derangement of glucose metabolism in these patients. Increase IL-1a, TNFa, and ferritin suggests the upregulation of an inflammatory process. PAI-1 increase suggests a fibrinolytic deficit. All of these biomarkers stimulate multiple pathways through signal transduction processes.

PUB753

Mechanisms Linking Atherosclerosis and Chronic Kidney Disease: Inflammation and Plaque Microenvironment in an Autopsy Study Miguel Hueso,[1] Juan Torras,[1] Estanis Navarro,[2] Laura De Ramon,[3] Alberto M. Martinez-Castelao,[1] Josep M. Grinyó.[1] *[1]Nephrology, Bellvitge Hospital-IDIBELL, L'Hospitalet, Barcelona, Spain; [2]Laboratori d'Oncología Molecular, IDIBELL, L'Hospitalet, Barcelona, Spain; [3]Laboratori de Nefrología Experimental, IDIBELL, L'Hospitalet, Barcelona, Spain.*

Background: Chronic Kidney Disease (CKD) increases the incidence of atherosclerosis (ATS), an inflammatory disease with a focal distribution in which pathogenesis seem involve the activity of microRNAs (miRNAs). The goal of our study was to detect differences in the CKD-related miRNAome, to gain insights into local factors involved in the onset and progression of atherosclerosis.

Methods: In a case-control studywe compared ATS plaques with normal areas, both obtained from the abdominal aorta of patients with CKD. Lesions were evaluated after hematoxylin-eosin staining, and were classified according to AHA criteria. Inflammation was evaluated by immunohistochemistry as S100, CD3 and NF-kB stainings. miRNA expression in ATS and normal samples was quantified by using Taqman Low Density Arrays (TLDA).

Results: We analyzed 12 paired samples (6 atherosclerotic plaques obtained from abdominal aortas and 6 normal areas from the same patient and vessel) from 6 patients (4 patients with CKD). NF-kB activation was correlated with the severity of the lesions (r=0.53, p=0.004) and with the number of lymphocytes in the intima (r=0.49, p=0.009) and adventitia (r=0.58, p=0.001). ATS plaques showed reduced levels of miR15b (4.86-fold change, p=0.014), miR17 (4.2-fold, p=0.041) and miR106a (4.5-fold, p=0.048). Furthermore, patients with CKD displayed a downregulation of miR125a-5p (3.8-fold, p=0.006).

Conclusions: Aterosclerotic plaques were associated with inflammation and with the dowregulation of miR15b and of the miR17 and miR106a. Interestingly, miR15b suppress the expression of VEGF and angiotensin-2, and promote VSMC proliferation and differentiation in a synthetic phenotype. miR17/106a modulate endothelial function and angiogenesis, and miR125a-5p regulates the differential activation of macrophages promoting alternative activation (M2). Thus, changes in the expression of miR15b, miR17/106a and miR125a-5p may contribute to cardiovascular disease in patients with CKD.

Funding: Government Support - Non-U.S.

PUB754

Far-Infrared Therapy Inhibits Renin-Induced Vascular Smooth Muscle Cell Proliferation and Hypertrophy By Blocking the Interaction Betweenpromyelocytic Leukaemia Zinc Finger Protein and (Pro)renin Receptor Yung-Ho Hsu,[1] Tso Hsiao Chen,[2] Cheng-Hsien Chen.[3] *[1]Div of Nephrology, Dept of Internal Medicine, Shuang Ho Hospital,Taipei Medical Univ, New Taipei City, Taiwan; [2]Div of Nephrology, Dept of Internal Medicine, Wan Fang Hospital, Taipei Medical Univ, Taipei, Taiwan; [3]Div of Nephrology, Dept of Internal Medicine, Shuang Ho Hospital,Taipei Medical Univ, New Taipei City, Taiwan.*

Background: High plasma (pro)renin activity is highly associated with chronic hypertension, and indicative of future cardiovascular disease and death. It is widely acknowledged that the (pro)renin receptor (RER) mediates angiotensin (Ang) II-dependent and Ang II-independent effects of (pro)renin. Some studies indicated that far-infrared (FIR) therapy is beneficial for cardiovascular system. In this study, we examined the effect of FIR on renin-induced proliferation and hypertrophy in rat vascular smooth muscle cell (A7r5). Recombinant rat renin significantly increased cell proliferation, and [H]-leucine incorporation, which were attenuated by FIR irradiation. Renin increased extracellular signal-regulated kinase (ERK) 1/2 phosphorylation and the expression of renin receptor (RER) at an early stage in A7r5 cells. FIR irradiation significantly reduced renin-induced ERK1/2 activation and the expression of renin receptor. We found that FIR therapy induced the nuclear translocation of promyelocytic leukaemia zinc finger protein (PLZF) in A7r5 cells, which is independent from thermal effect. There was a physic interaction between PLZF and RER, which was promoted by renin as revealed by immunoprecipitation. FIR irradiation blocked the interaction between PLZF and RER. Fluorescent staining showed that FIR irradiation blocked renin-induced nuclear translocation of RER in smooth muscle cells. PLZF siRNA transfection significantly inhibited renin-induced proliferation and hypertrophy. Renin-induced ERK1/2 activation was also reduced by PLZF siRNA transfection. Our results reveal that FIR irradiation inhibits renin-stimulated smooth muscle cell proliferative and hypertrophic changes by blocking the interaction between PLZF and RER.

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PUB755

MCP-1 and VCAM-1 Overexpression in Response to AGES via RAGE and PKC-β Pathway in a Co-Culture of Monocytes and Endothelial Cells Lisienny C.T. Rempel,[1] Alessandra Becker Finco,[1] Rayana Ariane Pereira Maciel,[1] Bruna Bosquetti,[1] Maíra Barbosa e Reis,[1] Larissa Magalhaes Alvarenga,[1] Roberto Pecoits-Filho,[2] Andréa Marques Stinghen.[1] *[1]Experimental Nephrology Laboratory, UFPR; [2]School of Medicine, PUCPR, Curitiba, Brazil.*

Background: Advanced glycation end products (AGES) induce endothelial dysfunction, a major risk factor for cardiovascular disease in chronic kidney disease (CKD) patients. To explore the underlying mechanisms in the AGES/endothelium interaction via RAGE and PKC-β pathway, we evaluated the expression of monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1), in a co-culture of endothelial cells and monocytes.

Methods: AGES were prepared by albumin glycation and characterized by absorbance and electrophoresis. Cell viability was assessed by MTT. Endothelial cells derived from human umbilical vein (HUVEC), monocytes (U937 cells) and HUVEC + monocytes were treated with AGES (0.2 mg/mL) with or without PKC-β inhibitor in a kinetic of 0, 3 and 6 or 0, 12 and 24h. MCP-1 and VCAM-1 expression was investigated on cell supernatant by ELISA. RAGE expression was evaluated by immunocytochemistry.

Results: There was no significant differences in cell viability after AGES exposure. When HUVEC were exposed to AGES, there was a significant increase in MCP-1 expression after 3 and 6h ($P<0.05$) and VCAM-1 after 24h ($P<0.05$) when compared to control. Moreover, when HUVEC were incubated with AGES and PKC-β inhibitor, there was a significant decrease in MCP-1 ($P<0.005$) and VCAM-1 ($P<0.05$) expression. The same effect was observed when monocytes were exposed to AGES after 6h ($P<0.05$) and PKC-β inhibitor ($P<0.001$). When HUVEC were co-cultured with monocytes, no significant effect after AGES exposure and PKC-β inhibitor treatment was observed. Finally, AGES treatment was able to induce RAGE expression by HUVEC.

Conclusions: This study is the first to demonstrate in three cellular models a regulatory mechanism involved in MCP-1 and VCAM-1 production, mimicking the endothelial dysfunction caused by AGES in the early events of atherosclerosis. We believe that such mechanism could serve as a therapeutic target to reduce the harmful effects of uremic toxicity in CKD patients.

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PUB756

The Effect of Sex on Humanin Levels in Healthy Adults and Patients with Uncomplicated Type 1 Diabetes Mellitus Yuliya Lytvyn,[1] Pinchas Cohen,[2] Junxiang Wan,[2] David Cherney.[1] *[1]Div of Nephrology, Univ Health Network, Univ of Toronto, Toronto, ON, Canada; [2]Univ of Southern California School of Medicine, Los Angeles, CA.*

Background: Diabetes mellitus (DM) is associated with a loss of renal and vascular protection observed in women compared with men in a non-diabetic renal disease setting, but the responsible mechanisms are unclear. Recent experimental work in animals has implicated a novel hormone called humanin (HN) as a vasodilatory factor that protects against vascular complications, but the role of HN in humans is unclear. The aim of the present study was to measure sex-related differences in HN levels in men and women with uncomplicated type 1 DM (T1DM) versus healthy controls (HC), as well as the interaction between HN, circulating neurohormones and vascular function.

Methods: Plasma HN, cGMP and aldosterone, blood pressure (BP), glomerular filtration rate and effective renal plasma flow (inulin and paraaminohippurate) were measured in HC (11 men, 10 women) and T1DM (23 men and 18 women) during clamped euglycemia (glucose 4-6 mmol/L).

Results: Plasma HN levels were higher in the T1D group versus HC (969±154 versus 834±214 pg/ml, p=0.006). HN levels were generally lower in HC men versus women but differences (762±154 versus 913±249 pg/ml) were not significant. In contrast, levels in T1DM men were significantly higher compared with T1DM women (1016±139 versus 909±154 pg/ml, p=0.026) and HC men (1016±139 versus 762±154, p<0.0001). In HC men but not women, HN correlated with systolic BP (r=-0.63, p=0.037), but not with renal function, cGMP or aldosterone. HN correlated with plasma cGMP in T1DM men (r=-0.31, p=0.048), but failed to reach significance in T1DM women (r=-0.46, p=0.06). In men and women with T1DM, HN did not correlate with BP, renal function or plasma aldosterone.

Conclusions: HN is higher in T1DM men versus T1DM women and healthy men, and is associated with lower levels of vasodilatory cGMP. Elevated humanin levels in this context likely represent an adaptive response to decreased insulin sensitivity and endothelial dysfunction.Future work should determine the role of HN in the pathogenesis of sex-related vascular function differences in DM.

Funding: Government Support - Non-U.S.

PUB757

Relationship of Pulse Wave Velocity to Blood-Based Biomarkers in Mexican-Americans Harold M. Szerlip, Raul Vintimilla, Tori Conger, Sid O'bryant. *Internal Medicine, UNTHSC, Fort Worth, TX.*

Background: Pulse wave velocity (PWV), a reflection of "arterial stiffness" is a well-established marker of cardiovascular risk. Aging, metabolic factors, and inflammation lead to the loss of elastin and increase in collagen in the large arteries resulting in loss of aortic elasticity and increased PWV. Being able to identify biomarkers associated the increase in PWV will enable the development of therapeutic strategies to counter this increase.

Methods: The link between PWV and various biomarkers was assessed among 23 participants of the Health and Aging Brain among Latino Elders study. Aorto-femoral PWV was measured using the SphygmoCor® XCEL system. Serum levels of CRP, SAA, sICAM-1 and sVCAM-1 were assayed via electrochemiluminescence using the Meso Scale Discovery multiplex platform. Correlations were assessed via Pearson bivariate correlation coefficients.

Results: There were 12 males and 11 females in the study with an average age of 62.0 (sd=5.6). A total of 44% of the sample had diagnoses of diabetes and 65% hypertension. In the total sample, PWV was not significantly correlated with any biomarkers. However, when split by gender PWV was significantly related to sVCAM-1 among men (r2=0.70, p=0.02), but not other important clinical variables or serum biomarkers: CRP r2=0.36 (p=0.3), SAA r2=0.12 (p=0.7), sICAM-1 r2=0.41 (p=0.2), HDL (r2=-0.40, p=0.2), SBP r2=0.44 (p=0.2), DBP r2=0.41, (p=0.2), HbA1c r2=0.03 (p=0.9), total cholesterol r2=0.1 (p=0.8) or LDL r2=0.14 (p=0.7). Among women, PWV was not significantly related to any variable.

Conclusions: This pilot project supports the link between serum biomarkers of vascular functioning among Mexican American men. sVCAM-1 has been implicated in endothelial cell signaling and atherosclerosis. Although possibly due to small sample size the lack of association of PWV with several markers of inflammation in this cohort is of note. These findings support the notion of the importance of gender in the PWV – biomarker link, which is consistent with other areas of study. This is the first-ever study of the link between PWV and blood-based biomarkers among Mexican Americans and additional work is greatly needed.

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PUB758

Acute Inhibition of Oxidative Stress Does Not Overcome Vascular Endothelial Dysfunction and Increased Arterial Stiffness in Patients with Stage 3-4 Chronic Kidney Disease Kristen L. Jablonski, Heather Farmer, Emily Decker, Michel Chonchol. *Univ of Colorado Denver, Aurora, CO.*

Background: Chronic kidney disease (CKD) is characterized by vascular endothelial dysfunction and stiffening of the large elastic arteries. Patients with CKD also exhibit increased systemic oxidative stress; however, oxidative stress at the level of the vasculature is not well described in this population. An acute supraphysiological intravenous infusion of ascorbic acid scavenges superoxide and has been previously used as a physiological tool to acutely reduce vascular dysfunction in various conditions including aging, coronary artery disease, and the metabolic syndrome. We hypothesized that acute inhibition of oxidative stress using this methodology would improve vascular endothelial function and reduce arterial stiffness in patients with moderate-to-severe CKD.

Methods: We assessed vascular endothelial function (using brachial artery flow-mediated dilation [FMD_{BA}]) and arterial stiffness (using aortic pulse-wave velocity [aPWV] and the carotid artery β-stiffness index) in 21 patients (16M/5F; 64±2 years; mean±s.e.) with stage 3-4 CKD (MDRD estimated glomerular filtration rate [eGFR] 35±3 ml/min/1.73 m^2) following an acute infusion of ascorbic acid (priming bolus of 0.06 g kg^{-1} fat free mass [FFM] dissolved in 100 ml of saline infused at 5 ml min^{-1} for 20 min followed by 0.02 g kg^{-1} FFM dissolved in 30 ml of saline administered over 60 min at 0.5 ml min^{-1}) or isovolumic saline (non-randomized cross-over design).

Results: Acute infusion of ascorbic acid failed to improve FMD_{BA} (saline: 3.75±0.65 %, ascorbic acid: 3.76±0.83 %, p=0.99), aPWV (saline: 1017±66 cm/sec, ascorbic acid: 1020±48 cm/sec, p=0.95), or the carotid artery β-stiffness index (saline: 12.7±1.8 AU, ascorbic acid: 13.0±1.1 AU, p=0.75).

Conclusions: Acute inhibition of oxidative stress did not improve vascular endothelial function or decrease arterial stiffness in patients with stage 3-4 CKD. Either vascular oxidative stress does not explain these vascular impairments, or perhaps vascular oxidative stress in moderate-to-severe CKD is too great to be overcome by the scavenging of superoxide.

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PUB759

Epoetin Beta Pegol Prevents Vascular Endothelial Dysfunction in Type-2 Diabetic Rats Ken-Ichi Serizawa, Kenji Yogo, Yoshihito Tashiro, Ryohei Kawasaki, Michinori Hirata, Koichi Endo. *Product Research Dept, Chugai Pharmaceutical Co., Ltd., Gotemba, Japan.*

Background: Patients with diabetic nephropathy have a high cardiovascular morbidity and mortality, and diabetes is regarded as a major independent risk factor for cardiovascular events, which are mainly caused by endothelial dysfunction. Epoetin beta pegol (continuous erythropoietin receptor activator, C.E.R.A.) is widely used in the treatment of renal anemia. In this study, we examined the effect of C.E.R.A. on endothelial dysfunction in type-2 diabetic rats.

Methods: Male Spontaneously Diabetic Torii (SDT) rats (22 wks old) were used as type-2 diabetic rats. Male Sprague-Dawley (SD) rats were used as age-matched controls. C.E.R.A. (Low, 0.6 μg/kg; High, 1.2 μg/kg) was administered subcutaneously once every 2 wks. After 8 wks, we measured blood pressure by tail-cuff method, collected urine from individual metabolic cages, and sampled blood from the jugular vein. Endothelial function was assessed by flow-mediated dilation (FMD) measured in the femoral arteries of anesthetized rats with an ultrasound system.

Results: In SDT rats, FMD was significantly decreased as compared to SD rats. C.E.R.A. dose-dependently prevented the reduction of FMD in SDT rats (SD, 19.5±1.6%; SDT, 11.3±0.6%; SDT+C.E.R.A. Low, 14.6±2.0%; SDT+C.E.R.A. High, 21.2±3.7%; mean ± S.E., *n*=4-9). C.E.R.A. did not inhibit urinary protein excretion in SDT rats (SD, 7.2±0.6 mg/day; SDT, 67.9±12.0 mg/day; SDT+C.E.R.A. Low, 85.0±27.3 mg/day; SDT+C.E.R.A. High, 87.9±28.4 mg/day). C.E.R.A. increased hemoglobin levels (SD, 14.8±0.4 g/dL; SDT, 15.4±0.4 g/dL; SDT+C.E.R.A. Low, 16.9±0.5 g/dL; SDT+C.E.R.A. High, 17.4±0.3 g/dL). Systolic blood pressure was higher in SDT rats than in SD rats. In addition, SDT rats had lower heart rate and body weight than did SD rats. C.E.R.A. did not influence these parameters.

Conclusions: These results demonstrated that C.E.R.A. prevented endothelial dysfunction as evaluated by FMD in type-2 diabetic rats, and these results are expected to clarify how C.E.R.A. affects endothelial function in patients with diabetic nephropathy.

PUB760

Assessment of Renal Vascular Alterations in eNOS -/- Mice by Advanced Contrast-Enhanced microCT Technique Daniel S. Perrien,[1,2] Keiko Takahashi,[3] Mohamed A. Saleh,[4] David G. Harrison,[4] Raymond C. Harris,[3] Takamune Takahashi.[3] *[1]Orthopaedic Surgery and Rehabilitation, Vanderbilt Univ, TN; [2]Tennessee Valley Healthcare System, Dept of Veterans Affairs, TN; [3]Nephrology, Vanderbilt Univ, TN; [4]Clinical Pharmacology, Vanderbilt Univ, TN.*

Background: Proper perfusion is critical for kidney function and many renal diseases result from disruption of blood flow. The imaging technique that enables detailed analysis of renal vasculature undoubtedly facilitates our understanding of renal disease. This study explored a novel rigorous method for 3D microCT-based analysis of total and cortical renal vasculature and assessed vascular alterations in eNOS -/- kidneys.

Methods: Wildtype and eNOS -/- mice (8 wks, n=15) were perfused with radiopaque Microfil™ and excised kidneys were imaged with μCT50 (Scanco Medical) at an isotropic voxel-size of 5.0μm. The entire kidney was selected as the initial volume of interest (VOI). A custom algorithm was created to define the cortical VOI as the entire volume within 600 μm of the renal surface. 3D vascular renderings were created using a grey-scale threshold and noise filter. Vessel thickness was analyzed by plotting the distribution of vascular volume at each thickness.

Results: Fractional vascular volume (vascular volume/kidney volume; VV/KV) and vessel number/mm (V.N) were significantly lower in eNOS-/- kidneys ($p<0.05$). Vessel thickness analysis displayed significant reduction in perfusable vessels in eNOS -/- kidneys in the range of 20-40μm, corresponding to arterioles. The cortical analysis of eNOS -/- kidneys showed similar results, significantly lower VV, VV/cortical V, and V.N, with an increase in the distance between vessels, and lower volume of vessels in the range of 20-30 μm (all $p<0.05$, versus WT), though total cortical V was not different. Further, the volume of vessels (40-90 μm) whose size corresponds to glomerulus was also obviously reduced in eNOS -/- kidneys and cortex.

Conclusions: eNOS -/- kidneys have severe defects in vascular perfusion/structure in the fraction of arterioles and glomeruli, which may contribute to their renal pathology. This study demonstrates the power of 3D microCT analysis to assess vascular phenotyping of murine kidney.

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

PUB761

N-Acetylcysteine as a Potential Strategy to Attenuate the Oxidative Stress Induced by Uremic Serum in the Vascular System Lia S. Nakao,[1] Silvia Daniéle Rodrigues,[1] Clarice K. Fujihara,[3] Roberto Pecoits-Filho.[2] *[1]Basic Pathology, Univ Federal do Parana, Curitiba, PR, Brazil; [2]School of Medicine, Pontificia Univ Catolica do Parana, Curitiba, PR, Brazil; [3]Faculdade de Medicina, Univ de Sao Paulo, Sao Paulo, SP, Brazil.*

Background: Chronic kidney disease (CKD) progression is accompanied by systemic oxidative stress, which contributes to an increase in the risk of cardiovascular diseases. N-acetylcysteine (NAC) is among the most studied antioxidants, but its effects remain controversial.

Methods: Endothelial and smooth muscle cells were challenged with human uremic or nonuremic sera, and the effects of an acute pretreatment (24 h) with 2 mM NAC were evaluated. ROS production, protein oxidation and total GSH/GSSG ratios were measured. Five-sixths nephrectomized or sham-operated rats were orally treated (in the drinking water) with 60 mg/kg/day NAC or not treated for 53 days. Systemic oxidative stress ($E_{h(Cyss/2Cys)}$) was quantified by measuring the plasma cysteine and cystine levels by HPLC and coulometric detection 7 and 60 days after the surgery.

Results: Uremic serum induced increased oxidation of DHE after 3 h incubation, and $DCFH_2$-DA and MitoSOX Red oxidation after 24 h incubation with both cell lines. NADPH oxidase, but not mitochondria, was determined to be the main source of ROS. An increased amount of protein carbonylation was measured after 3 and 24 h incubation. The total GSH/GSSG ratios were decreased along 24 h of treatment with uremic sera. NAC pre-treatment inhibited all the oxidative stress parameters above, likely by supplying cells with GSH. Indeed, total GSH/GSSG ratios increased after NAC incubation. The beneficial effect of NAC was also observed *in vivo*. CKD rats presented a more intense systemic oxidative stress than sham controls. Orally administered NAC attenuated the $E_{h(Cyss/2Cys)}$ in uremic rats by increasing plasma cysteine levels.

Conclusions: The present results indicate that NAC, by preventing GSH depletion in vascular cells exposed to uremic sera, emerges as a facile strategy to prevent uremic toxicity. Early administration of NAC induces a systemic adaptive redox response to buffer the oxidative stress that is associated with uremia.

Funding: Government Support - Non-U.S.

PUB762

Association of Vascular Injury Markers with Progressive Chronic Kidney Disease in Type 2 Diabetes Shayan Shirazian, Robert Diep, Ronak Patel, Monika Wadhwani, Candace D. Grant, Joseph Mattana. *Medicine, Winthrop Univ Hospital.*

Background: Albuminuria, the most commonly used biomarker for progressive diabetic renal damage, is in the normal range in up to 50% of patients with type 2 diabetes (T2DM) and stage 3 chronic kidney disease (CKD). The objective of this ongoing pilot study is to determine which vascular injury markers are associated with eGFR decline in patients with T2DM and stage 3 CKD.

Methods: In this cross-sectional study, adults patients were included if they had: 1) T2DM for at least 8-years, 2) stage 3 CKD, 3) normal to moderately increased albuminuria, 4) three prior creatinine values over 4 years. Patients were excluded if they had 1) non-diabetic kidney disease, 2) renal transplant, 3) renal artery stenosis, 4) active malignancy. Circulating endothelial cells (CECs), von willebrand factor, high-sensitivity C-reactive protein (hsCRP), uric acid and morning spot urine-albumin to creatinine ratio (UACR) were measured. Levels of CECs were measured using the CellSearch System (ImmuniconCorp., HuntingdonValley, PA). Average yearly eGFR decline from 2011-2014 was calculated. Subjects were separated into slow and fast eGFR decline groups (cut-off 2 mL/min/1.73m^2/year). Groups were compared using unpaired two-tailed t-tests.

Results: 16 patients were studied. The average age was 75 years, 88% were male, 88% were White and average T2DM duration was 16 years. The average eGFR was 40 mL/min/1.73m^2, UACR was 82 mcg/mg, and the average eGFR decline was 2.6 mL/min/1.73m^2/year. There were 9 patients in the slow and 7 patients in the fast eGFR decline group. There were no statistically significant differences between groups however UACR levels (48 +/- 81 ug/mg versus 120 +/- 109 ug/mg, p 0.18) and hsCRP levels (2.2 +/- 1.8 mg/L versus 6.01 +/- 6.2 mg/L, p= 0.16) were higher in the fast eGFR decline group.

Conclusions: In this ongoing pilot study, hsCRP levels were higher in normal to moderately albuminuric subjects with T2DM and fast eGFR decline. This finding did not reach statistical significance likely because of our small sample size. Future research is needed to determine whether hsCRP can predict progressive eGFR decline in non-albuminuric patients with T2DM and early CKD.

PUB763

The Role of Hypoxia-Inducible Transcription Factors in Determination of Macrophage Phenotypes Enda Patrick Masterson, Catherine Godson, Debra F. Higgins. *Diabetic Complications Research Centre, Conway Institute of Biomedical and Biomolecular Research and School of Medicine, Univ College Dublin, Dublin, Ireland.*

Background: Versatility is a characteristic of macrophages mediating both tissue damage and repair, exemplified by M1 and M2 phenotypes respectively. Understanding how versatility is regulated is important in the context of inflammation. Monocyte-Macrophage recruitment is a feature of responses to tissue hypoxia resulting in accumulation of hypoxia-inducible transcription factors [HIFs]. Different forms of HIF exist (e.g. HIF-1α and HIF-

2α) regulating expression of overlapping and unique target genes. The role of HIF-1α in inflammation is well described, while the role of HIF-2α is relatively unexplored. We hypothesise that HIF-1α drives an M1 pro-inflammatory phenotype and HIF-2α encourages M2 pro-resolution phenotypes.

Methods: Endogenous HIF-1α and HIF-2α protein expression in THP-1 monocytes and macrophages subjected to hypoxia (1% O2) or normoxia (21% O2) was examined using immunoblotting. To determine the effect of over-expressing HIF isoforms on macrophage phenotype, THP-1 cells were transfected with HIF-1α or HIF-2α and subsequently differentiated into macrophages, after which expression of M1 and M2 markers was analysed. The effect of polarization on HIF isoform expression was examined in macrophages polarized into M1 and M2 states by treatment with LPS/ IFNY and IL-4/ IL-13 respectively and subjecting macrophages to either hypoxia or normoxia.

Results: HIF-1α expression was detected in both hypoxic monocytes and macrophages. HIF-2α levels were lost on differentiation of monocytes to macrophages under hypoxia and normoxia. ELISA analysis revealed HIF-1α transfected macrophages had increased levels of MCP-1, an M1 marker and reduced levels of IL-10, an M2 marker. HIF-2α transfected macrophages had significantly decreased levels of MCP-1 ($P < 0.05$). M1 polarized macrophages had increased expression of HIF-1α without detectable HIF-2α expression while M2 polarized macrophages had decreased expression of HIF-1α and regained expression of HIF-2α.

Conclusions: These findings support our hypothesis and highlight the divergent roles of HIF-1α and HIF-2α in inflammation and resolution.

Funding: Private Foundation Support

PUB764

Impairment of Post-Ischemic Angiogenesis in Chronic Kidney Disease in Rats: Underlying Changes in Ischemia-Induced Early Regulation of Gene Expression

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Background: Ischemia-induced angiogenesis in skeletal muscle of rats with chronic kidney disease (CKD) is impaired which may aggravate peripheral vascular disease. We investigated underlying mechanisms by a systematic comparison of early gene expression in response to ischemia in rats with or without CKD.

Methods: CKD rats underwent 5/6 nephrectomy; controls sham operation. Eight weeks later, ischemia of the right limb was induced by ligation and resection of the femoral artery. Rats were sacrificed 24 h after the onset of ischemia. RNA from Musculus soleus of 4 control and 5 CKD rats (ischemic and non-ischemic sides, respectively, from each rat) was analyzed using 18 Affymetrix GeneChip Rat Genome 230 2.0 Arrays. A 2fold change of gene expression ($p<0.05$) was set as a cutoff. The Ingenuity Pathways Knowledge Base was employed for analysis of functional groups and networks. RT-PCR analysis of selected genes was performed to validate observed changes.

Results: Ischemia up-regulated 266 genes in CKD and 322 genes in control rats (65% overlap) whereas few genes were down-regulated, compared with the respective non-ischemic side. Comparison between the ischemic sides of CKD and control animals revealed down-regulation of 69 genes in CKD; 39 of these genes were also among the ischemia-induced genes in controls. Among the 11 known angiogenesis-related genes which were induced by ischemia but reduced in CKD were VEGF-A, c-MYC, TIMP-1, IL-6 and the chemokines CCL2, CCL7 and CXCL9. Other affected functional groups of genes were leukocyte recruitment and fatty acid metabolism. The differential gene expression of VEGF-A, CCL2 and CCL7 was confirmed by RT-PCR.

Conclusions: Gene expression profiling points to a relatively small number of differentially expressed genes which may underlie impaired post-ischemic angiogenesis in CKD.

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PUB765

Abdominal Aortic Calcification of Hemodialysis Patients and Its Association with Traditional Therapeutic Targets of Bone Disease

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Background: Vascular calcification (VC) has been implicated in high mortality of dialysis patients. We studied the association of abdominal aorta VC with traditional parameters-targets of diagnosis and treatment of CKD-MBD.

Methods: We studied 79 hemodialysis patients, 51 men, 28 women, 64.2±14.9 years. Diabetes, hypertension, cardiovascular disease and smoking were present at 23, 18, 40.5 and 25.5% respectively. Dialysis vintage was 36.8 months (range 1-228). VC of the abdominal aorta was assessed by Leena Kauppilla score (range 0-24) in lateral abdominal radiographs. Patients were stratified according to the calcification score: Group 1 (grade 0), Group 2 (grade 1-4) and Group 3 (grade 5-22). One-way ANOVA with SPSS was used.

Results: Abdominal aorta calcification was 5.7 (range 0-22). VC score was statistically associated only with dialysis vintage and not with age, sex, smoking, blood Ca^{2+}, P^{+}, iPTH, cholesterol, triglycerides, Ca×P product or use of calcium or non-calcium phosphate binders, calcimimetics or paricalcitol

Patient Groups	Group 1	Group 2	Group 3
Calcification Score	**0**	**1-4**	**5-22**
Patients %	28	25.3	46.7
Age (years)	59±3.8	64.2±4.3	66.2±1.8 NS
Dialysis Vintage (months)	2±0.3	32.5±9	58±12.9 **p<0.05**
Ca^{2+}mg/dl	8.94±1.08	8.86±0.96	9.1±0.14 NS
P^{+}mg/dl	5.78±0.36	6.66±0.29	6.31±0.26 NS
Ca×P	52.63±4.10	59.39±3.94	57.61±2.61 NS
iPTH pg/ml	427.97±102	447±140.29	476.6±82.98 NS

Positive history of cardiovascular events was significantly associated with higher VC score: 7.8±1.33 versus 4.29±0.8, $p<0.05$. When grouping patients according to achievement of KDIGO targets, VC scores were significantly different:PTH<150, 150<PTH<500 and PTH>500pg/ml patient groups had VC scores 3.22±0.54, 5.93±1.41 and 7.87±1.47 respectively, $p<0.05$.

Conclusions: Radiological evaluation of VC was positively associated with dialysis vintage and cardiovascular disease. In our study, achieving PTH was the only CKD-MBD treatment target presenting a difference in terms of VC.

PUB766

A Comparison of Pulse Wave Analysis Results Using Vicorder and SphygmoCor Devices in a Chronic Kidney Disease Cohort

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Background: Pulse wave analysis (PWA) is a noninvasive measure of vascular stiffness. The Vicorder and SphygmoCor are two devices used to measure PWA, by oscillometry and applanation tonometry respectively. This study compared measurements from both devices undertaken in the same patients to determine whether results from the two devices could be used interchangeably.

Methods: 91 patients with CKD stages 3 to 5 seen in outpatient clinic underwent PWA using both devices. The augmentation pressure (AP) and augmentation index (AIx) from each device were compared using the Bland Altman method.

Results: The mean age was 63.8±12.2 years, eGFR 33.1±15.9mL/min/1.73m², and 57 patients (63%) were male. For AP, mean difference was 1.85mmHg and 95% limits of agreement (LoA) 14.21to17.91 mmHg. The Bland–Altman plot (fig 1A) shows with higher AP values, the Vicorder gives lower measurements. This is formally seen from the regression of the differences in measurements on the means of the measurements (slope=-0.47, $p<0.001$, fig 1B). For AIx, the mean difference was 0.02%, and 95% LoA -19.0%to19.0% (fig 2A). The Bland-Altman plot (fig 2A) shows the Vicorder gives higher measurements for lower values. The expected disagreement is 12.31-0.44a, (fig 2B). These limits are preferred to those in figure 2A, as the latter do not accommodate that the extent to which the Vicorder may over or underestimate AIx compared to SphygmoCor depends on the size of the measurement.

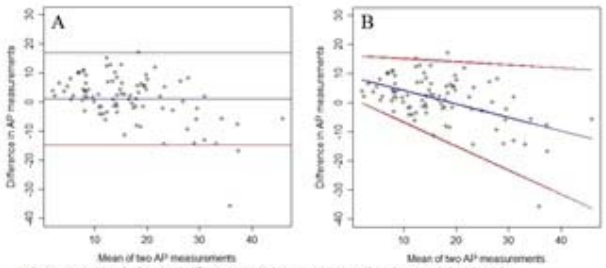


Figure 1. A: Bland-Altman plot for Augmented Pressure (mmHg) and B: regression-based 95% limits of agreement.

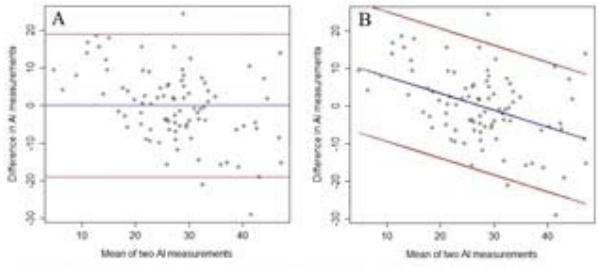


Figure 2. A: Bland-Altman plot for augmented index(%) and B: regression-based 95% limits of agreement.

Conclusions: There is disagreement between the two methods for both AP and AIx. Although the mean difference between AIx was small, the LoA were large, at extremes of measurement. This means that results from the two devices cannot be considered interchangeable.

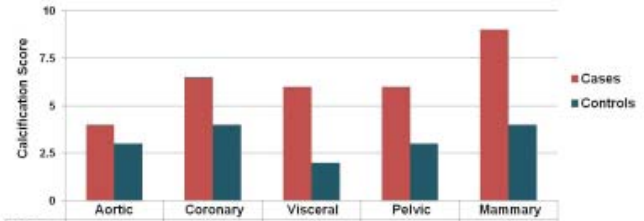
PUB767

Calcific Uremic Arteriolopathy: A Systemic Vascular Calcification Disorder? Sagar U. Nigwekar,[1] Sean H. Novak,[2] Rosalynn Nazarian,[1] Rajeev Malhotra,[1] Yanchen Zhang,[1] Julia Beth Wenger,[1] Mukesh Harisinghani,[1] Ravi I. Thadhani.[1] *[1]MGH; [2]Mount Auburn Hospital.*

Background: Calcific uremic arteriolopathy (CUA) is considered to be a dermal arteriolar calcification disorder but the extent of calcification involving other vascular beds is unknown. Understanding the overall burden of vascular calcification in CUA may offer mechanistic and therapeutic insights into this devastating disease with unclear pathobiology.

Methods: We conducted a matched case-control study comparing the extent of vascular calcification on CT and mammography in chronic dialysis patients with (cases; n=10) and without CUA (controls; n=10). Skin-biopsy confirmed CUA cases were identified from review of pathology department records and included if they had undergone CT and mammography. Controls were randomly selected from our institutional research database and matched to cases by age, gender, dialysis modality, and dialysis vintage. Calcification on CT and mammography was scored in 5 anatomic vascular distributions (aortic, coronary, visceral, pelvic, and mammary circulations) from 0 (no visible calcification) to 10 (heavy calcification). We also systematically reviewed autopsy records of 8 CUA patients to identify calcification of vascular beds.

Results: The mean age (± standard deviation) was 61 ± 3 years (cases) and 62 ± 2 years (controls). 50% of patients were males, 70% on hemodialysis, and median dialysis vintage was 4.1 years in both cases and controls. Median vascular calcification scores for cases and controls are shown below.



	Aortic	Coronary	Visceral	Pelvic	Mammary
Cases	4	6.5	6	6	9
Controls	3	4	2	3	4

Review of autopsy records identified diffuse mesenteric, pelvic, aortic, coronary, and valvular calcifications in CUA patients.

Conclusions: Dialysis patients with CUA may have a higher calcification burden across multiple vascular beds compared to dialysis patients without CUA suggesting that CUA is a systemic vascular calcification disorder.

Funding: Pharmaceutical Company Support - Sanofi-Aventis, Private Foundation Support

PUB768

Relationship of Fetuin-A and Coronary Artery Calcification Score in Patients with Chronic Renal Failure on Maintenance Hemodialysis Ali Abdulmajid Allawi,[1] Mohammed Younus Naji,[2] Jawad Ibrahim Rasheed.[3] *[1]Medicine, Baghdad College of Medicine, Baghdad, Iraq; [2]Medicine, Baghdad Medical City, Baghdad, Iraq; [3]Medicine, Baghdad Teaching Hospital, Baghdad, Iraq.*

Background: In patients on maintenance haemodialysis, serum Fetuin-A levels inversely correlate with the extent and grade of coronary calcification, measured by Coronary artery calcium scoring (CACS) were performed by cardiac CT scan.

Methods: The study was conducted in dialysis department/ Baghdad teaching hospital from February 2013 to the end of March 2014. A total numbers of 60 patients with end stage renal disease already on maintenance hemodialysis and control 30 cases, were examined from both sexes, who already on maintenance hemodialysis, were examined for serum Fetuin A and Coronary artery calcium scoring (CACS) were performed by a 64-slice CT scan. Fetuin A has been tested by quantitative solid phase enzyme immunometric assay (ELISA) designed for the determination of Fetuin A in human serum, using DiaMetra kit.

Results: This case control study that enrolled 60 patients with end stage renal disease already on maintenance hemodialysis were included in the study, 25 males and 35 females and their age range from 15 to 70 years old, with male to female ratio 0.7 :1. There was low serum Fetuin A level in studies patients with chronic renal failure on maintenance hemodialysis with higher coronary artery calcium score 11-400 and >400 as compared to control group, the difference were statistically significant, (p=0.000)

Calcium calicification score	low S. Fetuin A	%	normal S. Fetuin A	%	total No.	%	p value
0-10	6	13	24	54.5	30	33.3	
11-400	25	54.3	7	15.9	32	35.6	
>400	15	32.6	13	29.5	28	31.1	
Total	46	100	44	100	90	100	0.000

Conclusions: Fetuin-A levels is robust marker of vascular calcification in patients with end stage renal failure. Negative relationship between Fetuin-A levels and total coronary artery calcification scores. Fetuin A level is decreased in patients with chronic renal failure with cardiovascular risk factors as male, age older than 55 years, hypertension, diabetes and anemia.

Funding: Private Foundation Support

PUB769

Determinants of Therapeutic Success in Sodium Thiosulfate Treated Chronic Kidney Disease Patients with Calciphylaxis Kateri Bourbeau,[2] Chloé Lajoie,[2] Simon Desmeules,[1] Fabrice Mac-Way.[1] *[1]Pharmacy, Hôtel-Dieu de Quebec, QC, Canada; [2]Medicine, Hôtel-Dieu de Quebec, QC, Canada.*

Background: Calciphylaxis is a severe medical condition that affects patients with end-stage renal disease. One-year mortality has been reported to as high as 70% in hemodialysis patients. In the literature, treatment based on a multi-modality approach has been proposed but the optimal therapy remains poorly unknown. In this study, we aimed to evaluate the incidence of calciphylaxis in our center and determine the effects of different treatments approach on the evolution and mortality.

Methods: All patients with chronic kidney disease that were diagnosed with calciphylaxis either clinically or with skin biopsy were included in this retrospective study from l'Hôtel-Dieu de Québec hospital (Québec, Canada). Complete response to treatment was defined as complete lesion healing and partial response as lesion or pain improvement. Univariate analysis was performed to evaluate the role of each different intervention on therapeutic response.

Results: Twelve patients were included in this study. The majority of the lesions were located on the lower limbs. The patients associated comorbidities are consistent with previously reported risk factors for calciphylaxis: diabete (n=11), obesity (n=7), warfarin (n=9) and calcium-based chelators (n=10) treatments. A multi-modality approach was the base of the treatment and included cessation of calcium-based chelators and warfarin, lowering of calcium dialysate, intensification of hemodialysis treatments, variable use of cinacalcet and bisphosphonate. All patients were treated with intravenous sodium thiosulfate. There was a complete lesion healing in 42% of patients, 50% if we add the patients with improved lesion and 100% of our cases had pain improvement. One-year mortality was 25%. No single factor has been found to be associated with positive therapeutic response.

Conclusions: Calciphylaxis is a highly morbid condition if not optimally treated. A multi-modality approach should be encouraged including the use of sodium thiosulfate. The combination of these interventions probably explained the low mortality rate as compared to literature.

PUB770

Osteoprotegerin Is Associated with Vascular Calcification in Peritoneal Dialysis Juan Carlos Ramirez-Sandoval,[1] I. E. Casanova,[1] Jorge A. Villar-Tapia,[1] Enrique Gómez,[2] Cristinoc Cruz,[1] Ricardo Correa-Rotter.[1] *[1]Nephrology and Mineral Metabolism, Inst Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico; [2]Physiology of Nutrition, Inst Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico.*

Background: Vascular calcification (VC) is associated with cardiovascular mortality. In peritoneal dialysis(PD), VC has been scarcely studied and biomarkers associated to calcification not well characterized. This study aimed to determine the association of osteoprotegerin(OPG), osteopontin(OPN), osteocalcin(OCN), fibroblast growth factor 23(FGF-23), and P clearance with VC.

Methods: The Adragao simple vascular calcification score(SVCS) from pelvis and hand X-rays and Kauppila index(KI), from lateral lumbar X-ray were assessed in 76 adults on PD (43 women, median age 39 yrs, median time on DP 1.4 yrs). OPG, OPN, OCN, and FGF-23 were determined by luminometry.

Results: OPG levels were higher in subjects with increased VC in both indexes (n=22 with SVCS>3; n=19 with KI>7;$p<0.001$). Correlation coefficient between OPG and VC scores were: r=0.49 for SVCS and r=0.51 for KI, (both $p<0.001$). Subjects with VC had significantly lower renal P clearance. In a multiple regression analysis, VC assessed by SVCS was significantly associated with age(r:0.2), DM(r:2.4), BMI(r:0.09), and OPG/100^2(r:0.22); VC assessed by KI was significantly associated with age(r:0.16), time on PD(r:0.54), and OPG/100^2(r:0.08). OCN, OPN, and FGF23 were not associated with VC.

Table. Relations between osteogenic biomarkers and P Clearance(Cl)*,SVCS and KI						
	VSCS ≤3 (n=54)	VSCS >3 (n=22)	p	KI≤7 (n=57)	KI>7 (n=19)	P
OPG, pg/mL	641 (477-998)	1110 (709-1569)	**0.04**	896 (604-1341)	1656 (1118-1850)	**<0.001**
OPN, ng/mL	18 (5-36)	12 (5-29)	0.52	15(5.0-34)	26 (9-40)	0.14
OCN, ng/mL	54 (29-131)	55 (25-91)	0.67	55 (29-116)	42 (21-127)	0.58
FGF-23, ng/mL	1.1 (0.2-9.1)	3.2 (0.4-8.7)	0.22	1.3 (0.3-10.4)	1.7 (0.2-7.0)	0.37
Perit. P Cl, L/wk per 1.73m^2	34 (25-39)	34 (29-43)	0.39	35 (24-42)	33 (26-39)	0.058
Renal P Cl, L/wk per 1.73m^2	12 (0-27)	4 (0-14)	**0.02**	11 (2-35)	6 (0-11)	**0.045**

*Median (interquartile range)

Conclusions: Higher OPG levels showed a consistent association with VC in PD patients.

PUB771

Calcium Level Is an Independent Predictor of Vascular Calcification in Hemodialysis Patients Fellype C. Barreto,[1,2] Andréa Marques Stinghen,[3] Tammy Vernalha Rocha Almeida,[2] Rodrigo Leite Silva,[2] Daniela Veit Barreto.[1,2] *[1]School of Medicine, Pontificia Univ Catolica do Parana, Curitiba, Parana, Brazil; [2]Nephrology, Hospital Santa Casa de Curitiba, Curitiba, Parana, Brazil; [3]Univ Federal do Parana, Curitiba, Parana.*

Background: Vascular calcification (VC) is a common manifestation of chronic kidney disease-mineral bone disorder (CKD-MBD) associated to adverse outcomes in hemodialysis (HD) patients. In this study, we sought to investigate the prevalence and risk factors associated with VC in a cohort of HD patients.

Methods: 81 HD patients (age: 57±14 yrs; 61% males; 40% diabetics; length on HD: 18±8 months) underwent plain X-rays of hands and hips (Adragao score) and laboratory measurements (calcium, phosphate, alkaline phosphatase, 25(OH)vitamin D, iPTH, albumin and lipid profile).

Results: VC was present in 45% of the patients. Patients with VC were older (62±10 versus 53±19 yrs; p=0.002), most of them were diabetics (64%; p=0.01) and had higher calcium levels (9.2±0.9 versus 8.7±0.6 mg/dL; p=0.005) when compared to patients without VC. Interestingly, there was a greater proportion of VC (58%;p=0.04) among patients with calcium levels above the median (8.9 mg/dL). Calcification score was directly associated with age (r=0.27;p=0.01) and calcium levels (r=0.27;p=0.01), and inversely associated with iPTH (r=-0.22;p=0.04), total cholesterol (r=-0.22;p=0.04) and albumin (r=-0.21;p=0.05) levels. Multivariate logistic regression analysis showed that VC was independently associated with calcium (β coefficient= 2.36; p=0.03; 95% CI= 0.393 - 4.771) and diabetes (β coefficient=3.06; p=0.05; 95% CI= 0.566 - 3.903).

Conclusions: VC was highly prevalent in the study population. In addition to traditional risk factors, such as age and diabetes, mineral metabolism derangement, inflammation and altered lipid metabolism may also play a role on VC development. The independent association between VC and calcium gives further support to the recommendation that calcium overload should be avoided and suggests that calcium levels should be kept below the upper limit of the reference range in HD patients.

PUB772

Pelvic Radiograph May Be an Alternative for Coronary Artery CT in Assessing Vascular Calcification in Hemodialysis Patients Daqing Hong, Li Wang. *Renal Dept, Sichuan Provincial People's Hospital, Chengdu, Sichuan, China.*

Background: KDIGO (Kidney Disease: Improving Global Outcomes) guidelines recommend that a lateral abdominal radiograph should be performed to assess vascular calcification (VC) in dialysis patients. However, our previous study of 36 hemodialysis patients found that the vascular calcification (VC) in the pelvic radiograph was superior over VC in abdominal radiograph in association with coronary artery calcification (CAC). The objective of this study is to further unravel the story of this finding in a larger sample.

Methods: A total of 117 maintenance hemodialysis patients were enrolled at the Sichuan Provincial People's Hospital between June 2013 and December 2013. Multi-slice spiral computed tomography was taken to detect the CAC. Radiographs of the abdomen, pelvis and hands were evaluated by a radiologist to assess the presence of VC. Predict value of VC in different areas was analyzed using receiver operating characteristic (ROC) curve.

Results: The prevalence of VC in abdominal, pelvic and hands' X-rays was 67.52%, 55.56% and 15.38%, respectively. Area under the ROC curve (AUC) ofabdominal aortic calcification was 0.765 when predicting the presence of CAC, while the AUC of VC observed on pelvis X-ray was 0.711, the AUC of VC observed in all radiograghs (abdomen, pelvis and hands) was 0.771, while the AUC of VC observed on hands X-ray was less than 0.6.When predicting for CACs>100, AUC of abdominal aortic calcification was less than 0.7, while pelvic X-ray, hands' X-ray, total X-rays was 0.735,0.722,0.722, respectively. Predicting for CACs>400, AUC of pelvis X-ray, the total X-rays was 0.719, 0.708, and the AUC of abdominal radiograph was less than 0.7.

Conclusions: Our study demonstrated that the prevalence of VC in different arteries is different. VC presented in pelvic X-rays seemed to be superior over other vascular calcification in predicting coronary artery calcification in hemodialysis patients.

PUB773

Contribution of Hyponatremia (HN) to Hip Fractures (HF): The Under-Reporting of HN and Need to Increase Awareness of Symptomatic HN Sairah Sharif, Mary Joelanny Dominguez, Louis J. Imbriano, Joseph Mattana, John K. Maesaka. *Winthrop Univ Hosptial, Mineola, NY.*

Background: HN is the most common electrolyte abnormality that contributes independently to increased length of stay and mortality. Chronic HN induces significant osteoporosis and even mild HN is associated with impaired cognition, four-fold increase in falls and in the elderly a four-fold increase in bone fractures. These complications of HN are not generally appreciated and HN is thus left untreated. HF are associated with a 25% mortality. We therefore performed a retrospective study on the association between HN and HF.

Methods: We reviewed the medical records of 148 patients admitted to Winthrop-University Hospital with HF between July and Sept, 2013, focusing on demographic information, serum sodium at time of admission and during hospital course, comorbid conditions, falls associated with HF, and inclusion of HN in ICD-9 coding at discharge.

Results: The mean age was 77±SD 16 years, 116 females and 32 males. 16 patients had HN on admission, 15 of whom were females and fell. 15 patients developed HN after admission, of which 5 developed HN post-operatively. The most common co-morbid conditions were hypertension, diabetes and congestive cardiac failure. Out of the 31 HN patients 29 patients were discharged to skilled nursing and rehabilitation facilities and 29 of 31 patients with HN were not included in ICD-9 coding at discharge.

Conclusions: There are enough data to associate HN with significant adverse outcomes and increased morbidity and mortality. HN occurs with equal frequency at admission and as inpatient and contributes to HF which occur more commonly in females. HN is a significant problem that not only contributes to but is possibly induced by HF. However, it's contribution to morbidity and mortality is unappreciated as evidenced by under-reporting in ICD-9 coding in discharge summaries. Efforts are needed to raise awareness of and treat HN to prevent potentially serious complications.

PUB774

Clinical Evaluation of Patients with Apparently Idiopathic SIADH Alain Soupart,[1,2] Guy Decaux,[2] Michel Coffernils,[1] Irène Nyenyeli,[1] Fabrice Gankam Kengne,[4] Bruno Couturier,[2] Frédéric Vandergheynst,[2] Didier Francois.[3] *[1]Dept of Internal Medicine, Jolimont-Tubize Hospital, Jolimont, Belgium; [2]Dept of Internal Medicine, Erasme Univ Hospital, Bruxelles, Belgium; [3]Dept of Radiology, Jolimont-Tubize Hospital, Belgium; [4]Dept of Nephrology, Erasme Univ Hospital, Belgium.*

Background: Idiopathic SIADH refers to hyponatremia of no obvious cause (± 30% of SIADH). It can be associated with numerous diseases and predate their diagnosis for months and it's incidence increases with aging. The diagnosis workup of idiopathic SIADH is unknown.

Methods: Between 2008 and 2014, 84 successive patients with SIADH lasting for at least 1 month and no obvious cause were enrolled in a prospective study. Inclusion criteria were no specific symptoms/signs, no lab abnormalities pointing toward diagnosis and a normal CXR. All the patients had a brain CT and a FDG-PET.

Results: Underlying disease known to be associated with SIADH was found in 31% of the cases and was intrathoracic in 73% (10 cancer, 7 infections, 3 bronchiectasis), cerebral in 19% (chronic subdural hematoma, normotensive hydrocephaly, intracranial cavernoma, empty sella syndrome, olfactive neuroblastoma), abdominal in 3%, ENT cancer in 3% and 1 NSIAD. Neoplasms were found in 50% of the patients with a high mortality rate (70%). Final diagnosis was made by chest CT in the majority (73%) of the cases. Old ischemic brain lesions were also found in 37% of the patients.

Mean±SD	Total	Negative cases	Positive cases
N	84	58 (69%)	26 (31%)
Age (Range)	70±11 (28-99)	70±14 (51-99)	66±12 (28-84)
Male	53 (63%)	32 (55%)	21 (80%)
SNa (mEq/l)	122±6	120 ±15	122± 6
Duration of Hyponatremia (Months)	37±38	41±35	30±44
Hyponatremia < 12 months	30(35%)	16(28%)	14(53%)
Mortality	20(23%)	9(15%)	11(42%)

Conclusions: In asymptomatic patient with apparently idiopathic SIADH additional investigations can lead to diagnosis of treatable diseases in a significant number of cases. This study shows also that extensive diagnostic procedures are unnecessary and the diagnostic work-up should include only a chest CT scan. In young patients with negative chest CT, brain CT scan can be done. The prognosis of positive cases is poor despite early care.

PUB775

Can Tolvaptan Be Used Safely in the Management of Acute Hyponatremia due to Syndrome of Inappropriate Secretion of Antidiuretic Hormone? David Cucchiari, Manuel Alfredo Podesta', Silvia Santostasi, Paola Arosio, Simona Verdesca, Rosa Pedale, Claudio Angelini, Salvatore Badalamenti. *Nephrology, Humanitas Clinical and Research Center, Rozzano, Milano, Italy.*

Background: Tolvaptan is a selective oral vasopressin V2-receptors antagonist that has great efficacy in the management of the Syndrome of Inappropriate Secretion of AntiDiuretic Hormone (SIADH). However, it is not indicated by international guidelines because of concerns about too rapid increases of serum sodium levels. For this reason, Hypertonic Saline (HS) administration is the treatment of choice, although the quality of evidence supporting this indication is low.

Methods: We present 5 cases of SIADH in which the HS infusion had only partial benefit and led us to consider the off-label administration of Tolvaptan to achieve symptoms remission. The patients were euvolemic. Hypothyroidism and hypocorticism were ruled out while natriuresis was elevated. All these criteria were consistent with SIADH diagnosis.

Results: The treatment was started in every patient at least 12 hours after the beginning of 3% HS infusion. The dose of Tolvaptan was 15 mg for the first three patients and 7.5 mg for the last two ones. We assisted to an immediate increase of sodium levels in all patients, which led to symptoms remission.

Patient #	SIADH Cause	TSH (mUI /L)	ACTH 8am (pg /ml)	Cortisol 8am (mcg /dl)	Glycemia (mg /dl)	Urine Na^+ (meq /L)	Tolvaptan Dose (mg)	Na^+ Basal (meq /L)	Na^+ after HS (meq /L)	Na^+ 24h after Tolvaptan (meq/l)
1	Neoplasm	1.1	29	11	194	111	15	116	123	128
2	Meningitis	0.6	14	13	121	105	15	119	124	136
3	Psychosis	1.3	23	13	155	112	15	114	123	129
4	Neurosurgical	4.1	15	14	181	101	7.5	114	124	131
5	Traumatic	0.1	18	12	147	175	7.5	116	120	135

None of the patients experienced signs of myelinolysis due to rapid correction. However, in patient #2 and #5 we used glucose 5% solution to slow the sodium increase.

Conclusions: In our series, Tolvaptan had great efficacy in increasing sodium levels and achieving symptoms remission in SIADH patients who had an incomplete response to HS infusion. Strict monitoring of serum sodium is essential in order to avoid a too rapid correction that may lead to myelinolysis.

PUB776

Analysis on Body Composition and Related Factors in Maintenance Hemodialysis Patients Yi Yu. *Dept of Blood Purification, Dongfang Hospital of Fujian Province, Fuzhou, Fujian, China.*

Background: To measure body composition by body composition monitor (BCM) and analyze related factors in maintenance hemodialysis (MHD) patients.

Methods: We selected 57 MHD patients and measured their body composition and overhydration (OH) by BCM and collected their blood samples. We detected their serum cholesterol, triglyceride, albumin, hemoglobin and creatinine. Then we divided into 2 groups according to the mean value of BMI, fat tissue of unit surface area and muscle tissue of unit surface area, and compared blood biochemical parameters between the 2 groups. According to the difference value between OH measured by BCM and actual ultrafiltration volume, we divided into 2 groups: group A>300ml and group B≤300ml. We recorded the occurrence of adverse reactions on dialysis.

Results: Compared the high and low BMI groups (BMI≥21.68kg/m^2 versus BMI<21.68 kg/m^2), the former's fat tissue of unit body surface area was higher than the latter's (P<0.05). There was a positive correlation between BMI and fat tissue unit surface area (r=0.588, P<0.05), but there was no correlation between BMI and muscle tissue of unit surface area. Compared the groups of high and low fat tissue of unit surface area (≥12.29 kg/m^2 versus <12.29 kg/m^2), the former's cholesterol, triglyceride and hemoglobin were higher than the latter's (P<0.05). There were a positive correlation between fat tissue of unit surface area and cholesterol, triglyceride and hemoglobin (r=0.370, 0.403, 0.405, P<0.05). Compared the groups of high and low muscle tissue of unit surface area (≥22.80 kg/m^2 versus <22.80 kg/m^2), the former's serum creatinine and albumin were higher than the latter's (P<0.05). There were a positive correlation between muscle tissue of unit surface and serum creatinine and albumin (r=0.416, 0.315, P<0.05). We recorded the incidence of adverse reactions of hypotension, nausea and vomiting and convulsion between group A and B on dialysis. The adverse reaction occurrence rate was apparently higher in group A than that in group B. (χ^2=7.402, P<0.05).

Conclusions: In MHD patients, body composition by BCM is an accurate, non-invasive and quick way for assessment of nutritional and overhydration status of MHD patients.

Funding: Government Support - Non-U.S.

PUB777

Abstract Withdrawn

PUB778

Losartan Improves Renal Function and Pathology in a Rat Model of Type II Diabetic Nephropathy Deborah Widomski, Laura Leys, Kelly Larson, Steve McGaraughty, Zhi Su, Arthur L. Nikkel. *Early Renal Discovery, Abbvie, IL.*

Background: Losartan, a blocker of angiotensin II type I receptor, is an important part of the standard of care for diabetic nephropathy (DN). Obese ZSF-1 rats (bred by crossing female Zucker diabetic fatty rat with male spontaneously hypertensive heart failure rat) display many aspects of the clinical features of human type II DN. The current study was designed to examine the treatment effects of Losartan in ZSF-1 rats as part of the model validation.

Methods: All rats (at 7-10 weeks old) underwent a right uninephrectomy (Unx) surgery and were maintained on a high carbohydrate diet throughout the study. Losartan was dosed orally once a day at 3, 10, and 30 mg/kg, starting 3 weeks post Unx and lasting for 3 months. Blood, 24-hr urine samples, and metabolic parameters were collected once every 2-4 weeks. Blood pressure (BP) was measured by tail cuff. Glomerular filtration rate (GFR) was measured by transdermal clearance of FITC-sinistrin. Kidney collagen content was estimated by Picro Sirius Red (PSR) stain and measuring hydroxyproline.

Results: Compared to lean control rats, ZSF-1 rats showed significant increases in urinary protein excretion rate (UPER), blood lipids and glucose, body weights, food and water intake, and urine output. Systolic BP was slightly decreased by Losartan at 10 and 30 mg/kg. UPER was significantly reduced by Losartan in a dose-dependent manner, with a maximal 41% reduction at week 12 (30 mg/kg). Losartan (30 mg/kg) also reduced blood lipids. Losartan at 3 and 10 mg/kg had little effect on any metabolic parameter, but 30 mg/kg increased blood glucose levels, water intake and urine output. GFR was significantly lower in ZSF-1 rats than lean rats, and all doses of Losartan significantly improved GFR in ZSF-1 rats. Kidney collagen content (PSR stain and hydroxyproline) was significantly increased in ZSF-1 rats (versus lean rats) and reduced by Losartan in a dose-dependent manner.

Conclusions: Those results indicate that Losartan (administered in a treatment/ intervention mode) provided significant renal protection in ZSF-1 rats.

AUTHOR INDEX

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KEYWORD INDEX

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HI-OR01

HALT Progression of Polycystic Kidney Disease Trials: Primary Results of a Randomized Trial in Moderately Advanced Stage CKD

Vicente E. Torres,[1] Kaleab Z. Abebe,[2] Arlene B. Chapman,[3] Robert W. Schrier,[4] William E. Braun,[5] Theodore I. Steinman,[6] Franz Winklhofer,[7] Godela M. Brosnahan,[4] Peter G. Czarnecki,[6] Marie C. Hogan,[1] D. Miskulin,[8] Frederic F. Rahbari-Oskoui,[3] Jared J. Grantham,[7] Peter C. Harris,[1] Michael F. Flessner,[9] Charity G. Moore,[2] Ronald D. Perrone.[8] *[1]Mayo, MN; [2]Univ of Pittsburgh, PA; [3]Emory Univ, GA; [4]UCHSC, CO; [5]Cleveland Clinic, OH; [6]BIDMC, MA; [7]KUMC, KS; [8]Tufts, MA; [9]NIH, MD.*

Background: Hypertension in ADPKD develops early and associates with disease progression. The renin-angiotensin-aldosterone system (RAAS) has been implicated in its pathogenesis. Dual RAAS blockade may circumvent compensatory mechanisms that limit the efficacy of ACE-I or ARB monotherapies.

Methods: In a double-blind, placebo-controlled, prospective 5-8 year trial, 486 ADPKD subjects (18-64 yrs-old, eGFR 25-60 ml/min/1.73 m^2)were randomized to an ACE-I (lisinopril) and placebo or to lisinopril and an ARB (telmisartan), titrated to achieve systolic and diastolic BP of 110-130 and 70-80 mmHg, respectively. The composite primary outcome was time to death, ESRD or 50% reduction of baseline eGFR. Secondary outcomes included rates of change of urine aldosterone and albumin excretions, frequency of all-cause and cardiovascular hospitalizations and ADPKD related symptoms, quality of life, and adverse study medication effects.

Results: Lisinopril/placebo or lisinopril/telmisartan lowered urine aldosterone excretion and adequately controlled BP. Estimated GFR declined by 3.91 (95% CI 3.65, 4.17) and 3.87 (95% CI 3.61, 4.14) ml/min/1.73m^2 per yr, respectively. The incidence of primary outcome events (hazard ratio = 1.08, 95% confidence interval [CI], 0.82 to 1.42), rate of eGFR decline and frequency of other secondary outcomes, hyperkalemia and AKI were similar in both groups.

Conclusions: ACE-I monotherapy is safe and by itself sufficient to achieve BP control in the majority of patients with ADPKD and CKD stage 3. Addition of ARB, although relatively safe, does not alter decline in eGFR or other renal outcomes at this stage of the disease.

Funding: NIDDK Support, Other NIH Support - This study was supported by cooperative agreements (grants DK62408, DK62401, DK62410, DK62402, and DK62411, DK082230) with the National Institute of Diabetes and Digestive and Kidney Diseases, NIH, the National Center for Research Resources General Clinical Research Centers (RR000039 Emory University, RR00585 Mayo Clinic, RR000054 Tufts University, RR000051 University of Colorado, RR23940 Kansas University, and RR024296 Beth Israel Deaconess Medical Center), and the Centers for Translational Science Activities at the participating institutions (RR025008 Emory University, RR024150 Mayo Clinic, RR025752 Tufts University, RR025780 University of Colorado, and RR024989 Cleveland Clinic)., Pharmaceutical Company Support - Study drugs were donated by Boehringer Ingelheim Pharmaceuticals Inc (telmisartan and matched placebo) and Merck & Co Inc (lisinopril)., Private Foundation Support

HI-OR02

HALT Progression of Polycystic Kidney Disease (HALT PKD) Trials: Primary Results of a 2x2 Factorial Trial in Early Stage CKD

Arlene B. Chapman,[1] Kaleab Z. Abebe,[2] Ronald D. Perrone,[3] Vicente E. Torres,[4] William E. Braun,[5] Theodore I. Steinman,[6] Franz Winklhofer,[7] Godela M. Brosnahan,[8] Peter G. Czarnecki,[6] Marie C. Hogan,[4] D. Miskulin,[3] Frederic F. Rahbari-Oskoui,[1] Jared J. Grantham,[7] Peter C. Harris,[4] Michael F. Flessner,[9] Kyongtae Ty Bae,[2] Charity G. Moore,[2] Robert W. Schrier.[8] *[1]Emory Univ; [2]Univ of Pittsburgh; [3]Tufts; [4]Mayo; [5]Cleveland Clinic; [6]Beth Israel Deaconess; [7]Univ of Kansas; [8]Univ of Colorado; [9]NIH, NIDDK.*

Background: In autosomal dominant polycystic kidney disease (ADPKD), hypertension is common, associates with greater total kidney volume (TKV), activation of the renin-angiotensin-aldosterone system (RAAS) and progression to ESRD. The optimal level of blood pressure control and the effects of inhibition of the RAAS on progressive renal disease in ADPKD are unclear.

Methods: In a double-blind, placebo-controlled trial, 558 hypertensive ADPKD subjects age 15-49 years with eGFR greater than 60 ml/min/1.73m^2 were randomized to compare 1) angiotensin converting enzyme inhibitor (lisinopril) versus lisinopril and an angiotensin receptor blocker (telmisartan); and 2) standard (120-130/70-80 mmHg) versus low BP (95-110/60-75 mmHg). The primary outcome was annual percent change in TKV.

Results: Annual percent increase in TKV was significantly less in low versus standard BP (5.6 versus 6.6%/year, P=0.006) with no differences between treatment groups (5.9 versus 6.2%/year, P=NS). The rate of change in eGFR was similar between BP groups with a negative acute (P<0.001) and marginally positive (P=0.050) chronic slope difference. Left ventricular mass index (LVMI) and urine albumin excretion (uAlb) decreased more in low versus standard BP (-1.17 vs. -0.57 gm/m/year, P=0.0002; -3.77 vs. 2.43%/yr, P<0.001). Dizziness and lightheadedness were more common in the low BP group (80.7% versus 69.4%, P<0.003).

Conclusions: In early ADPKD, treatment with the combination of lisinopril/telmisartan did not alter the rate of increase in TKV. However, rigorous BP control conferred a reduced rate of increase in TKV, no overall change in eGFR and greater declines in LVMI and uAlb excretion.

Funding: NIDDK Support

HI-OR03

ADVANCE-ON: Long Term Benefits of Intensive Glucose Control for End-Stage Kidney Disease

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Background: The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial suggested that intensive glucose control, targeting an HbA1c of < 6.5%, may prevent end-stage kidney disease (ESKD), but the small number of events limited the strength of the conclusions.

Methods: Survivors, previously randomized to intensive or standard glucose control and who completed the trial period, were invited to post-trial follow-up (ADVANCE-ON). Data on ESKD outcomes were collected, defined as the need for dialysis or kidney transplantation.

Results: 8494 of 10261 survivors (84%) at the end of ADVANCE entered post-trial follow-up for a median of 5.4 years and had similar characteristics to the original 11140 trial participants. In-trial differences in HbA1c disappeared by the first post-trial visit. The in-trial reductions in the risk of ESKD (7 vs 20 events, HR 0.35, 95% confidence interval [CI] 0.15- 0.83, p=0.02) persisted after 9.9 years of follow-up in total (29 vs 53 events, HR 0.54, CI 0.34-0.85, p<0.01). Greater benefits were observed in people with an eGFR above 60 mls/min/1.73m^2 at trial baseline (HR 0.29, CI 0.14-0.62) compared to lower levels (HR 0.89, CI 0.49-1.62, p heterogeneity = 0.02), as well as in those with BP levels below 140 mmHg (HR 0.19, CI 0.06-0.55) compared to higher levels (HR 0.77, CI 0.46-1.3, p heterogeneity = 0.01). Effects were similar across randomized BP lowering therapy, sex, age, and race as well as baseline albuminuria stage, BMI and HbA1c (all p heterogeneity >0.1).

Conclusions: Intensive glucose control leads to longer-term reductions in the risk of ESKD. The benefits are greater in people with preserved kidney function and better blood pressure control (ClinicalTrials.Gov ID: NCT00949286).

Funding: Pharmaceutical Company Support - Servier, Government Support - Non-U.S.

HI-OR04

Renal Efficacy and Safety of Anti-TGF-β1 Therapy in Patients with Diabetic Nephropathy

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Background: TGF-β over-activity has been implicated as a key pathogenic factor in diabetic nephropathy (DN). This multi-center, randomized, double-masked, Ph 2 dose-ranging study assessed whether modulating TGF-β1 with a -β1 specific humanized monoclonal neutralizing antibody (LY) was safe and more effective than placebo at slowing renal function loss in patients (Pts) with advanced DN on appropriate and stable renin angiotensin system inhibitor (RASi) treatment (Rx).

Methods: 416 eligible Pts ≥25 years of age with T1 (n= 44) or T2 (n=370) diabetes and a SCr of 1.3 to 3.3 mg/dL for women and 1.5 to 3.5 mg/dL for men (or eGFR 20 to 60 mL/min/1.73m^2), and a 24-hr urine PCR of ≥800 mg/g, were randomized to LY (2, 10 or 50 mg monthly subcutaneously dosing for 12 mo) or placebo with Rx stratified by diabetes type. The primary and secondary efficacy outcomes were mean changes from baseline to 12 mo for the mean of duplicate SCr and 1st AM urine PCR, respectively.

Results: Pt demographics were balanced across groups, including age 62.2±10.2 yrs (mean±SD), 77% men, SCr 2.14±0.61 mg/dL, eGFR 35.46±11.85 mL/min/1.73m^2, urine PCR 3.26±2.51 g/g, BMI 33.9±6.7, HbA1c 7.87±1.74 % and SBP 138.1±13.4 mmHg. The trial was terminated 4 mo early for efficacy futility. Results indicate the predicted SCr change (based on entry criteria) but no apparent LY Rx effect on mean change in SCr (table) or PCR (data not shown). LY appeared safe and well tolerated.

Mean SCr ± SD (mg/dL)	Placebo N=103	2 mg N=105	10 mg N=103	50 mg N=105
Baseline	2.22±0.66	2.15±0.63	2.12±0.71	2.15±0.67
12-mo Endpoint	2.48±0.96	2.49±0.84	2.49±1.12	2.50±1.01
LS* mean change from baseline (SE)	0.38±0.08	0.48±0.08	0.48±0.08	0.55±0.08

* No significant Rx effect demonstrated by repeated measures analysis including fixed effects for Rx, visit, and Rx by visit and baseline value as covariate.

Conclusions: LY added to background RASi Rx appeared safe and well tolerated but did not slow progression of DN.

Funding: Pharmaceutical Company Support - Pharmaceutical Company Support

Underline represents presenting author/disclosure.

HI-OR05

Randomized Clinical Trial of Ergocalciferol Supplementation in 25 Vitamin D Deficient Hemodialysis Patients

D. Miskulin,[1] Hocine Tighiouart,[1] Karen M. Majchrzak,[2] Richard S. Muther,[3] Toros Kapoian,[2] Doug Johnson,[2] Daniel E. Weiner.[1] *[1]Tufts Medical Center; [2]Dialysis Clinic Inc.; [3]Kidney Assoc of Kansas City.*

Background: Many hemodialysis (HD) patients are vitamin D deficient. The major circulating form of VitD is 25-hydroxyvitamin D (25-VitD), but HD patients are treated primarily with 1,25 $(OH)_2$ VitD (1,25-VitD). The VitD receptor and CYP27B1-hydroxylase are widely expressed suggesting local production and action of 1,25 VitD. Reports have extolled the health benefits of VitD administration.

Methods: In a 6-month, randomized, double-blind, placebo controlled trial, 276 HD patients with 25-VitD levels <30 ng/mL were treated with ergocalciferol or placebo. Therapy consisted of 50,000 IU ergocalciferol weekly X 6m for 25-VitD level <16 ng/mL and 50,000 IU weekly X 3m followed by 50,000 IU monthly X 3m for 25-VitD levels of 16-30 ng/mL. The change in prespecified outcomes of Epogen dose, PTH, Ca, P, CRP, 1,25-VitD dose, cinacalcet dose, and infection rate, between arms was tested using ANCOVA.

Results: Mean age was 61 years and median vintage 3.5 years; 60% were black, 45% women and 46% had diabetes as cause of ESRD. There were no differences in clinical factors between arms at baseline. Mean 25-Vit D levels in the ergocalciferol (n=137) vs. placebo (n=139) group at baseline, 3m and 6m, respectively, were 16.0 vs. 16.9, 41.0 vs. 17.3, and 39.2 vs. 17.5 ng/ml. There was no significant difference in EPO dose, Ca, P, 1,25-VitD dose, CRP level or cinacalect utilization between groups, while PTH levels were significantly higher in those receiving placebo. The rate of all-cause or infection-related hospitalization or in total infections did not differ across groups.

Parameter	Placebo (n=139)		Ergocalciferol (n=137)		p-value ^
	Baseline	Month 6	Baseline	Month 6	
EPO dose, units/week	5400 (2400, 11500)	6100 (2000, 12000)	5800 (2600, 12200)	7000 (2700, 15200)	0.22
PTH pg/mL	475 ± 295	450 ± 239	440 ± 257	505 ± 339	0.01
1,25 Vit D dose change, n (%)					0.70
<25% change		61 (43.9)		63 (46.0)	
25% increase		47 (33.8)		49 (35.8)	
25% decrease		31 (22.3)		25 (18.3)	
Taking Cinacalcet, n (%)	35 (25.2)	38 (27.3)	34 (24.8)	47 (34.3)	0.21
hs CRP (mg/dL)	3.8 (1.5, 12.0)	4.4 (1.7, 10.8)	5.1 (1.8, 10.3)	6.0 (2.0, 14.7)	0.10
All-cause hospitalization		140.5 (114.0, 173.1)		111.3 (87.8, 141.2)	0.15
Infectious hospitalization		14.4 (7.5, 27.6)		11.5 (5.5, 24.0)	0.65
Total Infections*		38.3 (25.7, 57.2)		62.2 (45.3, 85.5)	0.06

Results are mean ± standard deviation or median (25th, 75th percentile). Events are per 100 patient-year (95% confidence interval). ^Statistical test of the difference in the change from baseline to 6m between treatment arms. * Includes infectious hospitalization and bacteremia or tissue infection without hospitalization. Where missing, 6 month values are determined using last value carried forward.

Conclusions: Administering ergocalciferol to 25-Vit D deficient HD patients normalizes circulating 25-Vit D levels by 3 months but this did not reduce EPO dose, CRP, PTH, 1,25-VitD dose, cinacalcet use or infection rates.

HI-OR06

Effect of Perioperative Aspirin and Clonidine on Acute Kidney Injury

Amit X. Garg. *POISE-2 Investigators, Western Univ, London, Canada.*

Background: Acute kidney injury, a common complication of the approximate 200 million major non-cardiac surgeries performed each year, is associated with poor outcomes and high healthcare costs. Early promising animal, human, and randomized clinical trial data suggest aspirin (a common anti-platelet agent) or clonidine (a central acting α2-adrenergic agonist), administered in the perioperative period, may reduce the risk of acute kidney injury. However, these effects are uncertain and each intervention has the potential for harm (perioperative bleeding with aspirin and perioperative hypotension with clonidine), which could increase the risk of acute kidney injury. We designed and conducted a substudy of the PeriOperative ISchemia Evaluation-2 (POISE-2) trial to determine whether the risk of acute kidney injury after non-cardiac surgery is altered by aspirin compared to placebo, and clonidine compared to placebo [substudy protocol BMJ Open 2014 Feb 25;4(2):e004886].

Methods: A 2 x 2 factorial randomized, blinded, clinical trial in 6,905 patients undergoing non-cardiac surgery from 88 centres in 22 countries between January 2011 and December 2013. Patients were assigned to take aspirin (200 mg) or placebo 2-4 hours before surgery and then aspirin (100 mg) or placebo daily up to 30 days after surgery, and were assigned to take oral clonidine (0.2 mg) or placebo 2-4 hours before surgery, and then a transdermal clonidine patch (which provided clonidine at 0.2 mg/day) or placebo patch that remained until 72 hours after surgery. Acute kidney injury was primarily defined as an increase in serum creatinine concentration from the preoperative concentration by either of the following criteria: (1) an increase ≥ 26.5 µmol/L (≥ 0.3 mg/dL) within 48 hours of surgery, or (2) an increase ≥50% within 7 days of surgery.

Results: Aspirin (n=3,443) versus placebo (n=3,462) did not alter the risk of acute kidney injury (13.4% versus 12.3%, respectively; adjusted relative risk 1.10 [95% CI, 0.96 to 1.25]). Clonidine (n=3,453) versus placebo (n=3,452) did not alter the risk of acute kidney injury (13.0% versus 12.7%, respectively; adjusted relative risk 1.03 [95% CI, 0.90 to1.18]).Aspirin increased the risk of major bleeding which in post hoc analysis was associated with a greater risk of subsequent acute kidney injury (23.3% when bleeding was present versus 12.3% when bleeding was absent, hazard ratio 2.20 [95% CI, 1.72 to 2.83]).. Similarly, clonidine increased the risk of clinically important hypotension which in post hoc analysis was associated with a greater risk of subsequent acute kidney injury (14.3% when hypotension was present versus 11.8% when hypotension was absent; adjusted hazard ratio 1.34 [95% CI, 1.14 to 1.58]).

Conclusions: Among patients undergoing major non-cardiac surgery, neither aspirin nor clonidine administered perioperatively reduced the risk of acute kidney injury. Trial Registration: NCT01082874

Funding: Government Support - Non-U.S.

HI-OR07

Six-Month Results of the RESCUE Trial: Fluency® Plus Endovascular Stent Graft versus PTA for In-Stent Restenosis

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Background: The Fluency® Plus Endovascular Stent Graft for In-stent Restenosis (RESCUE) study is a multicenter, randomized, concurrently-controlled study designed to compare the safety and effectiveness of the stent graft to balloon angioplasty (PTA) for treatment of in-stent restenotic lesions in the venous outflow of the Arteriovenous (AV) access circuit.

Methods: 265 patients from 23 U.S. sites were randomized to PTA versus PTA plus placement of the stent graft. The primary study endpoints were superiority of access circuit primary patency (ACPP) through 6 months and safety through 30 days. The secondary endpoint of post-intervention lesion patency (PLP), defined as the interval after the index intervention until the next re-intervention at the original treatment site or until the extremity was abandoned for permanent access, was also hypothesis tested. A 90-day follow-up angiogram assessment of binary restenosis was completed by an independent Core Lab.

Results: The intention-to-treat (ITT) population included 265 randomized patients, of which 244 were evaluated for 30-day safety, and 220 for 6-month effectiveness (111 PTA alone and 109 stent graft). The overall ACPP rate at 6 months was significantly higher in the stent graft group (16.7%) than in the PTA group (3.0%; p<0.001). PLP at 6 months was 65.2% in the stent graft group, and 10.4% in the PTA group (p<0.001). The stent graft groupalso demonstrated a significantly higher PLP rate in the two strata (AV Graft and Fistula). In the AV Graft subgroup, the PLP rate was 57.7% vs. 5.2% (stent graft vs. PTA; p<0.001). In the AVF subgroup, the PLP rate was 72% vs.14.7% (stent graft vs. PTA; p<0.001). The freedom from binary restenosis rate at 90 days was 81% in the stent graft group vs. 25% with PTA alone. The percentage of subjects free from safety events through 30 days was comparable between groups (96.8% PTA; 96.6% stent graft; p = 0.007).

Conclusions: The Fluency® Plus Endovascular Stent Graft is superior to PTA alone for both ACPP and PLP rates through 6 months in the treatment of in-stent restenosis.

Funding: Pharmaceutical Company Support - Bard PV

HI-OR08

Impact of Extended Weekly Hemodialysis Hours on Quality of Life and Clinical Outcomes: The ACTIVE Dialysis Multinational Trial

Meg J. Jardine,[1] Li Zuo,[2] Nicholas A. Gray,[3] Janak Rashme de Zoysa,[4] Christopher T. Chan,[5] Martin P. Gallagher,[1] Alan Cass,[6] Vlado Perkovic.[1] *[1]The George Institute for Global Health, Australia; [2]Peking Univ People's Hospital, China; [3]Nambour General Hospital, Australia; [4]North Shore Hospital, Auckland, New Zealand; [5]Univ Health Network, Toronto, Canada; [6]The Menzies Institute, Darwin, Australia.*

Background: Observational studies suggest a relationship between increased dialysis hours and better clinical outcomes. We aimed to assess the impact of extended weekly dialysis hours on quality of life (QOL) and clinical outcomes.

Methods: Participants were randomised to extended (target ≥24 hrs weekly) or standard (target 12-15 hrs) dialysis hours for 12 months. The primary outcome was the difference in change in QOL between baseline and 12 months assessed using EQ-5D (potential score 0-1) in the two groups. Secondary outcomes included access events and other clinical and safety outcomes.

Results: Among 200 participants, mean age was 52 (standard deviation 1.2) years, baseline dialysis hours 13.9 (2.7) hrs/week and most were male (69.5%). At study end, achieved mean weekly hours were 22.1 (4.3) in the extended group compared with 14.2 (4.0) in the standard group. QOL was similar in both groups at study end adjusted for baseline (mean difference in EQ5D 0.038 [-0.03, 0.11] p=0.27). Systolic blood pressure at study end was not different, although those randomised to extended dialysis were receiving fewer blood pressure lowering agents (mean difference -0.35 agents [-0.62, -0.08] p=0.01). Randomisation to extended hours was associated with higher haemoglobin, lower potassium and lower phosphate levels during followup (respective differences 3.51g/l [0.21, 6.81] p=0.037; -0.28mmol/l [-0.43, -0.14] p=0.0001; -0.17mmol/l [-0.27, -0.06] p=0.002). There were 5 deaths in the extended arm, and 2 in the standard arm. The numbers of patients with adverse vascular access events were similar in the two arms.

Conclusions: Extending weekly dialysis hours for 12 months did not improve quality of life, but was associated with improvement in some laboratory parameters and reduced blood pressure medication requirement. (Trial registration: NCT00649298)

Funding: Pharmaceutical Company Support - Baxter CEC Investigator Initiated Research Grant

Baxter International, Government Support - Non-U.S.

Underline represents presenting author/disclosure.

HI-OR09

ACT-AKI: A Phase 2 Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial of AC607 for the Treatment of Acute Kidney Injury in Cardiac Surgery Subjects Madhav Swaminathan,[1] David Mazer,[2] Glenn M. Chertow,[3] David G. Warnock,[4] Viken Paragamian,[5] Robert M. Brenner.[5] *[1]Anesthesiology, Duke Univ, Durham, NC; [2]Anesthesiology, Univ of Toronto, Toronto, Canada; [3]Medicine, Stanford Univ, Stanford, CA; [4]Medicine, Univ of Alabama, Birmingham, AL; [5]AlloCure Inc., Burlington, MA.*

Background: Acute kidney injury (AKI) is common condition and there is no effective treatment. Pre-clinical studies have shown that mesenchymal stem cells (MSC) both prevent and facilitate recovery from established AKI. AlloCure has developed a proprietary process to isolate and expand allogeneic human MSC (AC607), which was well tolerated and associated with low rates of AKI in a phase 1 trial. A phase 2 trial was conducted to determine if AC607 reduced time to kidney recovery compared to placebo after cardiac surgery.

Methods: Subjects undergoing cardiac surgery with cardiopulmonary bypass (CPB) with AKI defined by a creatinine rise ≥ 0.5 mg/dL ≤ 48 hours after CPB were randomized 1:1 in a double-blind manner to receive an intra-arterial dose of 2 x 10E6 cells/kg AC607 or placebo. Endpoints were time to kidney recovery (return of creatinine to pre-surgical baseline – primary, assessed using a Cox proportional hazards model), and all-cause mortality and need for dialysis within 30 and 90 days (secondary).

Results: A total of156 subjects were randomized in 21 centers in North America to either AC607 (n=77) or placebo (n=79), while 10 in the treatment and 11 in the control arm were randomized but not dosed. Mean age (SD) was 66.6 (11.4) years, 26% were female, and 85% were Caucasian. Mean weight was 91kg (21.2), 44% had diabetes, 84% had hypertension, and 42% had congestive heart failure. Mean baseline creatinine was 1.4 (0.6) mg/dL and mean eGFR was 61 (24) mL/min. There were no differences between AC607 and placebo with regard to median time to kidney recovery (15 vs. 12 days; p=0.32); all-cause mortality (6 vs. 5) and dialysis (7 vs. 5) during the follow up period.

Conclusions: In this phase 2 trial of subjects with early AKI following cardiac surgery, treatment with AC607 did not improve the time to complete kidney recovery, mortality or the need for dialysis.

Funding: Pharmaceutical Company Support - AlloCure Inc.

SA-PO1091

Levofloxacin for BK Virus Prophylaxis in Kidney Transplantation John S. Gill,[1] Atul Humar,[2] Dean Fergusson,[3] Olwyn Johnston,[1] Andrew A. House,[4] Joseph Kim,[2] Tim Ramsay,[3] Michaël Chassé,[3] Xiaoli L Pang,[5] Jeffrey S. Zaltzman,[2] Sandra M. Cockfield,[5] Marcelo Cantarovich,[6] Martin Karpinski,[7] Louise Lebel,[3] Greg A. Knoll.[8] *[1]UBC, Canada; [2]U of Toronto, Canada; [3]OHRI, Canada; [4]Western, Canada; [5]U of Alberta, Canada; [6]McGill, Canada; [7]U of Mannitoba, Canada; [8]U of Ottawa, Canada.*

Background: Improvement in immunosuppression has led to a dramatic reduction in acute rejection after kidney transplantation. The trade-off of more potent immunosuppression has been the emergence of BK virus infection which can lead to allograft dysfunction with a very high risk of transplant failure. There are currently no therapies to prevent or treat BK virus infection. Quinolone antibiotics have been shown to have antiviral properties against BK virus but efficacy at preventing this infection has not been shown in prospective controlled studies.

Methods: We conducted a multicenter, double-blind, placebo-controlled randomized trial comparing a 3-month course of the quinolone antibiotic, levofloxacin, to placebo in kidney transplant recipients to reduce the risk of BK viruria in 11 Canadian kidney transplant centers. The primary outcome of the study was the time to occurrence of BK viruria within the first year post-transplantation.

Results: 154 patients were included in the study (76 in the levofloxacin group, 78 in the placebo group). The primary outcome of BK viruria occurred in 22 patients (29%) in the levofloxacin group and in 26 patients (33.3%) in the placebo group (Hazard Ratio: 0.91; 95% CI 0.51 to 1.63; P=0.58). There was no difference between the two groups in regards of other clinical events, including BK viral titers, occurrence of BK viremia, death, transplant failure or acute rejection. There was an increased risk of infections with isolates resistant to quinolones in the levofloxacin group versus placebo (58.3% versus 33.3%, RR 1.75; 95% CI 1.01 to 2.98) as well as an increased risk of tendonitis (RR 6.16; 95% CI 1.01 to 38.5).

Conclusions: A 3-month course of levofloxacin early post-transplantation did not prevent BK viruria in kidney transplant recipients. This intervention may however increase the risk of adverse events such as bacterial resistance and tendonitis.

Funding: Government Support - Non-U.S.

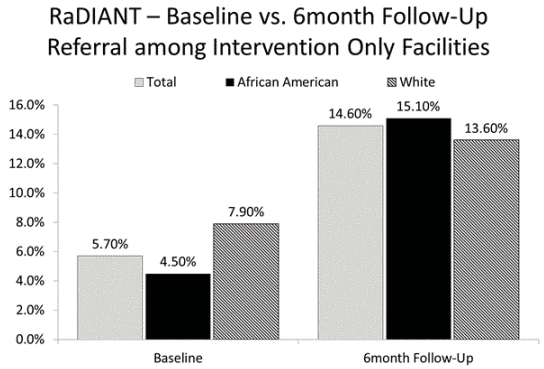
SA-PO1092

A Randomized Multicomponent Intervention to Reduce Disparities in Transplant Referral: Interim Results from the RaDIANT Community Study Rachel E. Patzer,[1] Leighann Sauls,[2] Jennifer C. Gander,[1] M. Ahinee Amamoo,[2] Laura Plantinga,[1] Debra D. Evans,[2] Eric M. Gibney,[3] Laura L. Mulloy,[4] Stephen O. Pastan.[1] *[1]Emory Univ, Atlanta, GA; [2]Southeastern Kidney Council, Raleigh, NC; [3]Piedmont Hospital, Atlanta, GA; [4]Georgia Regents Univ, Augusta, GA.*

Background: The Reducing Disparities In Access to kidNey Transplantation (RaDIANT) Community Study is a dialysis facility-level randomized clinical trial developed by the Southeastern Kidney Transplant Coalition to test the effectiveness of a multicomponent intervention to reduce racial disparities and improve kidney transplant (KTx) referral in Georgia, which has the lowest KTx rate in the U.S.

Methods: In 2013, 134 dialysis facilities were randomized to receive either "usual" KTx education (n=67) or a multicomponent intervention conducted by ESRD Network 6 and coalition partners consisting of KTx education and engagement activities for dialysis facility leadership, staff, and patients (n=67). We report 6-month pre- to post-data among intervention facilities only.

Results: At baseline (June-December 2012), the proportion of ESRD patients referred for KTx among intervention facilities was 5.5% (4.7% for AA vs. 7.9% for whites). After 6 months of intervention activities, KTx referral more than doubled (14.6%) among patients in the 67 intervention facilities and tripled (15.5%) among AA patients. A total of 56 (83.5%) intervention facilities improved KTx referral in the 6-month study period. Among the 26 dialysis facilities that had an AA vs. white racial disparity in KTx referral at baseline, 65.4% eliminated racial disparity in referral by 6 months.



Conclusions: Interim data from intervention facilities in the RaDIANT Study suggests that a large, randomized, quality improvement program among dialysis facilities in GA may improve equity in KTx access. Availability of control data at 12 months will allow assessment of the effectiveness of the intervention.

Funding: Other NIH Support - National Institute of Minority Health and Health Disparities

SA-PO1093

Envarsus XR (Once-Daily MeltDose Tacrolimus Tablets) Shows Continued Dose Reduction and Similar Efficacy and Safety vs. Prograf (Twice-Daily Tacrolimus Capsules) at Two-Years: Results of a Phase 3, Double-Blind, Randomized Study in *De Novo* Kidney Transplant Patients Lionel Rostaing,[1] Suphamai Bunnapradist.[2] *[1]Hôpital de Rangueil, Toulouse, France; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA.*

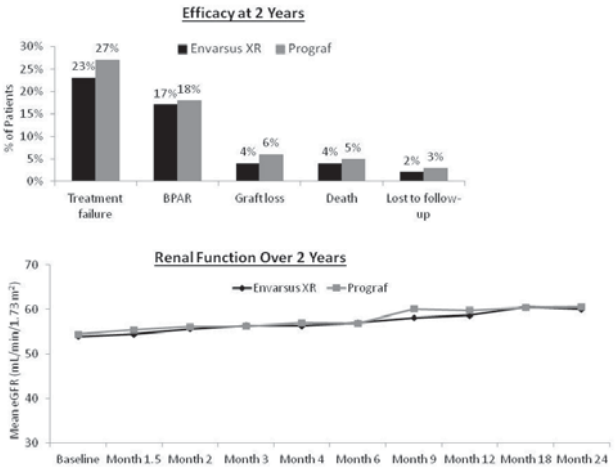
Background: Envarsus XR is a once-daily tacrolimus (Tac) tablet formulation that has increased bioavailability, lower C_{max}, less fluctuation between C_{max} and trough, and more rapid achievement of therapeutic Tac blood levels, with a ~30 lower dose, compared to twice-daily Tac capsules (Prograf).

Methods: Presented are 2-year results from a randomized trial of Envarsus XR vs. Prograf in 543 (Envarsus XR, n=268; Prograf, n=275) *de novo* kidney transplant recipients (KTR).

Results: Consistent with 1-year results, Envarsus XR was noninferior to Prograf, with the treatment failure (biopsy-proven acute rejection [BPAR], graft loss, death, or lost-to-follow-up] difference (95% CI) well below the 10% noninferiority margin (-4.14% [-11.38%, +3.17%]). The overall incidence of treatment failure within 24 months was, Envarsus XR: 23.1%; Prograf: 27.3%. In the immediate post-transplant period, target troughs were more rapidly attained for Envarsus XR vs. Prograf; consistent with 1-year results, at 2 years, the mean total daily dose (TDD) for Envarsus XR was 24% lower vs. Prograf, with similar troughs. Renal function was comparable between the groups over the 2 year study. The proportion of KTR with adverse events (AEs) was: Envarsus XR, 98.1%; Prograf, 97.8%; the proportion of KTR with serious AEs was: Envarsus XR, 61.9%; Prograf, 67.3%.

Conclusions: Envarsus XR showed noninferiority vs. Prograf with lower TDD of Envarsus XR. Renal function and AEs were similar between the 2 groups. With once-daily dosing and improved pharmacokinetics, Envarsus XR may offer a preferable option to twice-daily immediate-release Tac formulations.

Underline represents presenting author/disclosure.



Funding: Pharmaceutical Company Support - Veloxis Pharmaceuticals

SA-PO1094

Urgent-Start Peritoneal Dialysis versus Urgent-Start Hemodialysis: A Multicenter Clinical Trial Arshia Ghaffari,[1] Steven M. Brunelli,[2] Michelle Cassin,[2] Martin J. Schreiber.[3] *[1]Div of Nephrology, Univ of Southern California, Los Angeles, CA; [2]DaVita Clinical Research; [3]DaVita Healthcare Partners.*

Background: Peritoneal dialysis (PD) is rarely offered to patients presenting with advanced chronic kidney disease that require urgent initiation of dialysis. Several recent reports have described the feasibility of urgent-start peritoneal dialysis (USPD), a modality whereby patients are started on low-volume, recumbent PD soon after catheter placement. While clinical outcomes for USPD have been reported to be similar to urgent initiation of hemodialysis via a central venous catheter (HD-CVC), the reports are limited by small size and lack of comparable control groups.

Methods: This is a multi-center, propensity-matched, observational study comparing hospitalizations, infections and mortality in patients that started dialysis urgently either via a protocol-driven USPD program or traditional HD-CVC from multiple dialysis clinics between April 2012 and May 2014. Each USPD patient was 1:1 matched with an HD-CVC patient. Outcomes were compared by Poisson regression models and reported as incident rate ratios (IRR) with 95% confidence intervals (CI).

Results: 690 USPD patients met criteria to be included in the study. Of these 376 were successfully matched to HD-CVC controls. The median duration of followup was 179 days. Compared to matched patients who initiated HD via a CVC, USPD patients had 51% lower mortality (IRR 0.49; 95% CI, 0.29-0.84; $P = 0.009$), 39% lower rate of hospitalization (IRR 0.61; 95% CI, 0.49-0.77; $P < 0.001$) and 42% lower rate of all-cause infections (IRR 0.58: 95% CI, 0.39-0.87; $P = 0.008$). In the USPD group, majority of infections represented peritonitis with a rate of 1 infection every 60 months, whereas all infections in the HD-CVC group represented blood stream infections with a rate of 1 infection every 34 months.

Conclusions: This multi-center large cohort study demonstrates USPD provides superior clinical outcomes as compared to urgent HD with CVC with regards to mortality, hospitalizations and infectious complications. USPD should be more widely implemented to allow more of unplanned ESRD patients the option of starting PD while optimizing outcomes.

Funding: Pharmaceutical Company Support - DaVita Healthcare Partners

SA-PO1095

Standard versus Accelerated Initiation of Renal Replacement Therapy in Acute Kidney Injury Ron Wald,[1] Neill Adhikari,[2] Karen L. Pope,[1] Orla M Smith,[1] Matthew A Weir,[3] Sean M. Bagshaw.[4] *[1]St. Michael's Hospital; [2]Sunnybrook Health Sciences Centre; [3]London Health Sciences Centre; [4]Univ of Alberta Hospital.*

Background: In patients with severe AKI but no urgent indication for renal replacement therapy (RRT), the optimal time to commence RRT is controversial. While starting RRT pre-emptively may have benefits, this approach may also expose patients to unnecessary RRT.

Methods: We conducted a 12-centre open-label pilot RCT of critically ill adults with severe AKI (two of: doubling of creatinine; urine output <6 mL/kg over 12 hrs; blood neutrophil gelatinase-associated lipocalin ≥400 ng/mL), potassium ≤5.5 mmol/L, bicarbonate ≥15 mmol/L, and central venous pressure ≥8 mmHg. Exclusion criteria included eGFR <30 mL/min, presence of creatinine doubling for >48 hrs, or clinicians' belief that either immediate RRT initiation or deferral was mandated. Patients were randomized to **accelerated** (≤12 hrs from eligibility) or **standard** (if potassium >6 mmol/L, bicarbonate <10 mmol/L, CHF, or clinician judgment) RRT initiation. Outcomes were adherence to protocol-defined time limits for RRT initiation (primary), proportion of eligible patients enrolled and followed to 90 days, and safety (secondary).

Results: We enrolled 100/131 (76%) fully eligible patients.

Baseline characteristic	Accelerated RRT (n=48)	Standard RRT (n=52)
Age, y; mean (SD)	62.2 (11.9)	63.9 (13.6)
Sepsis; n (%)	26 (54)	30 (58)
SOFA score; mean (SD)	13.3 (2.5)	12.8 (3.0)
24-hr urine output, mL; median (IQR)	355 (196-528)	434 (214-735)
Ventilated; n (%)	45 (94)	48 (92)
Vasopressor; n (%)	42 (82)	43 (83)
Creatinine, μmol/L; mean (SD)	305 (111)	293 (214)

45/48 (94%) patients in the accelerated arm commenced RRT ≤12 hours after eligibility [median 7.4 (IQR 6.1-9.6) hrs]. In the standard RRT arm, 33 patients started RRT >12 hours after eligibility (median 31.6 (IQR 22.8-59.5) hrs); 19 patients did not receive RRT (6 died, 13 recovered kidney function). All patients were followed to Day 90. No safety signal was evident.

Conclusions: A RCT of accelerated vs standard timing of RRT initiation among critically ill patients with AKI is feasible. These results are informing the design of a large-scale definitive effectiveness RCT.

Funding: Pharmaceutical Company Support - Alere, Government Support - Non-U.S.

SA-PO1096

The Utility of Photoplethysmographic Measures of Cardiovascular Stress to Detect Intradialytic Hypotension D. Miskulin,[1] Klemens B. Meyer,[1] Mary kay Deck,[2] Anne M Brumfield,[2] Rajat Deo,[3] John E. Moran.[2,4] *[1]Tufts Medical Center; [2]Intelomed; [3]Univ Pennsylvania; [4]Stanford Univ.*

Background: The incidence of intradialytic hypotension (IDH) is high, despite the development of various monitoring technologies. This prospective trial evaluated the IDH detection accuracy of an algorithm that assesses cardiovascular (CV) stress via continuously measured pulse wave amplitude (PWA) and pulse rate (PR) (CVInsight, Intelomed, Wexford, PA).

Methods: 48 patients (12 controls; 36 with IDH in ≥5 HD treatments (tx) in past 2 months) were monitored with CVInsight (CVI) over 2 tx(1 long and 1 short interdialytic interval). Relative plasma volume (RPV) was also measured via Crit-Line III® (Fresenius, Kaysville, UT). Staff and patients were blinded to device parameters. BP, interventions, and symptoms were recorded by a study coordinator. Patients concurrently wore a Holter monitor. IDH was defined as 1) a drop in SBP to <90 mm Hg, 2) intervention for IDH, or 3) IDH symptoms. Crit-Line was considered positive if ΔRPV exceeded -8%/hour or -15%/tx. A positive test by CVI required defined changes in PWA and PR. Device accuracy for identifying IDH was assessed using ROC analysis.

Results: IDH occurred in 52 of the 96 monitored tx. CVI detected IDH with a sensitivity of 75% and specificity of 36%. Crit-Line yielded lower sensitivity but higher specificity (24%, 68%). False positive alerts that impacted CVI specificity occurred with cardiac arrhythmia and sleep.

	BP Event		INTERVENTION Event		SYMPTOM Event	
	CVI	CL	CVI	CL	CVI	CL
Sensitivity	83	29	73	16	71	21
Specificity	38	73	32	66	31	69

Conclusions: Photoplethysmographic assessment of CV stress is a sensitive indicator of IDH events. Many apparent false positive alerts are related to serious but otherwise unrecognized events, including cardiac arrhythmia and sleep disordered breathing. CVInsight allows better assessment of patient risk, identifies clinically important changes in CV status, and may assist titration of therapy to optimize fluid management.

Funding: Pharmaceutical Company Support - Intelomed, Wexford PA

SA-PO1097

The Beta-Blocker to LOwer CArdiovascular Dialysis Events (BLOCADE) Feasibility Study: A Randomized Controlled Trial of Carvedilol versus Placebo in Patients Receiving Dialysis Matthew Allan Roberts.[1,2] *[1]Eastern Health Clinical School, Monash Univ, Australia; [2]For the BLOCADE Feasibility Study Group and Australasian Kidney Trials Network.*

Background: Beta-blocking agents (BBA) reduce mortality from cardiovascular disease (CVD) in patients not requiring dialysis. The potential benefits and harm of BBA in dialysis patients are not known. We aimed to determine the feasibility of a large-scale randomized controlled trial (LSRCT) to address this uncertainty.

Methods: We recruited patients who had received peritoneal dialysis (PD) or hemodialysis (HD) for ≥3 months and were aged ≥50 years or aged ≥18 years with co-morbid diabetes or CVD, from 11 sites in Australia and New Zealand. After a 6-week run-in with the BBA carvedilol, we randomized participants 1:1 to carvedilol or placebo, titrated to the maximum tolerated dose and maintained for 12 months. The primary outcome was the proportion (95% confidence interval) of participants who tolerated carvedilol 6.25mg b.d. in the run-in. Other outcomes were proportions randomized who withdrew before 12 months and the incidence of intra-dialytic hypotension (IDH) measured at specific times.

Results: Of 1443 patients screened, 354 were eligible, 91 consented to participate and 72 (55 HD, 17 PD) entered run-in. The primary outcome was achieved by 49 [68% (57-79)] of 72 run-in participants; completers and non-completers did not differ by baseline characteristics. Current BBA use was the primary reason 13% of screened patients did not

Underline represents presenting author/disclosure.

participate; only 9 (13%) run-in participants had coronary disease and none had heart failure. Post randomization, 10 of 26 (38%) allocated carvedilol and 4 of 23 (17%) allocated placebo withdrew (p=0.10); median dose achieved was 25mg b.d. for both groups. The incidence of IDH was 0.07 events/session with carvedilol versus 0.02 events/session with placebo (P=0.14) in the 2 weeks following an increase in dose and 0.04 versus 0.03, respectively, on a stable dose (p=0.67).

Conclusions: Any LSRCT of BBA versus placebo in dialysis patients must allow that approximately 1/3 of patients will not tolerate the lowest effective BBA dose, a small proportion of screened patients will participate, and patients withdraw at all stages.

Funding: Pharmaceutical Company Support - Pfizer CVL Lipid Grant, Carvedilol supplied through commercial arrangement with Roche Australia Pty Ltd., Private Foundation Support, Government Support - Non-U.S.

SA-PO1098

Migalastat and Enzyme Replacement Therapy Have Comparable Effects on Renal Function in Fabry Disease: Phase 3 Study Results

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Background: To compare the efficacy and safety of oral migalastat with IV enzyme replacement therapies (ERTs) for Fabry disease (FD).

Methods: The co-primary endpoints were mean annualized changes in estimated glomerular filtration rate ($eGFR_{CKD\text{-}EPI}$) and measured GFR ($mGFR_{iohexol}$) assessed for migalastat and ERT over 18 mo. LS means and 95% CIs were calculated using an ANCOVA model.Median values were also calculated.Comparability of migalastat and ERT was pre-specified based on:≥50% overlap of 95% CIs for the annualized change in GFR, and mean changes within 2.2 mL/min/1.73 m^2/yr. Plasma lyso-Gb_3 (FD biomarker) was assessed over 18 mo.Patients were randomized 1.5:1 to switch to migalastat or remain on ERT.

Results: The study enrolled 60 patients (26m/34f), 56 with amenable mutations based on a validated *in vitro* assay, and 52 of whom also had ≥6 month GFR assessments.

	Overlap of 95% CI (means)	Difference between Migalastat and ERT (means)	Mean Values ± SEM		Median Values	
			Migalastat (n=34)	ERT (n = 18)	Migalastat (n=34)	ERT (n = 18)
$eGFR_{CKD\text{-}EPI}$	100%	+0.63	-0.40±0.93	-1.03±1.29	-1.29	-0.87
$mGFR_{iohexol}$	100%	-1.11	-4.35±1.64	-3.24±2.27	-3.23	-3.57

Levels of plasma lyso-Gb_3 remained low and stable in patients with amenable mutations who continued on ERT and those who switched from ERT to migalastat.In a composite endpoint (renal, cardiac, and cerebrovascular events), event frequency observed on migalastat was 29% compared to 44%on ERT. Migalastat was generally safe, well-tolerated.

Conclusions: Annualized changes in GFR were comparable for migalastat and ERT over 18 mo for amenable mutations. Plasma lyso-GB_3 levels remained stable following switch from ERT to migalastat. This study demonstrates that oral migalastat was comparable to ERT on renal function and an important biomarker of FD.

Funding: Pharmaceutical Company Support - Amicus Therapeutics Inc

SA-PO1099

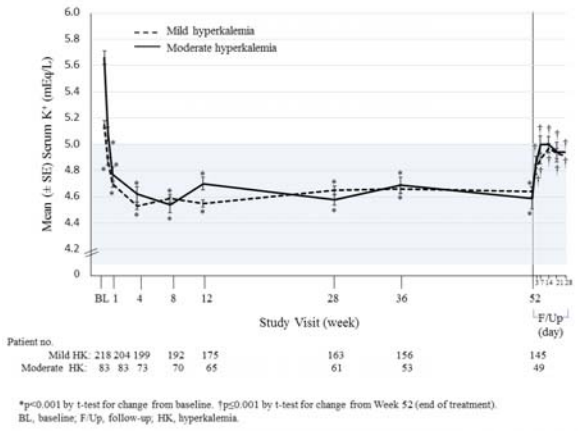
Effect of Patiromer on Hyperkalemia in Patients with Diabetic Nephropathy: Results of a 1-Year Randomized Trial

George L. Bakris,[1] Bertram Pitt,[2] Martha Mayo,[3] Dahlia Garza,[3] Yuri Stasiv,[3] Lance Berman,[3] David A. Bushinsky.[4] *[1]Univ of Chicago, Chicago, IL; [2]Univ of Michigan, Ann Arbor, MI; [3]Relypsa, Redwood City, CA; [4]Univ of Rochester, Rochester, NY.*

Background: Hyperkalemia (HK) is common in patients (pts) with CKD and often limits use of RAAS inhibitors (RAASi). Available K^+-binding resins are poorly tolerated and can cause colonic necrosis. We report results of a 52-week trial evaluating HK treatment in CKD pts on RAASi.

Methods: Multicenter, open-label trial of 306 pts with CKD, T2DM, HTN and serum K^+ (s-K^+) >5.0 mEq/L; pts randomized to patiromer starting doses by baseline s-K^+: K^+ >5.0-5.5 mEq/L (4.2, 8.4 or 12.6 g BID) mild HK (n=222); and K^+ >5.5-<6.0 mEq/L (8.4, 12.6 or 16.8 g BID) moderate HK (n=84). Patiromer was titrated to achieve and maintain normal s-K^+. All pts were on RAASi. A parallel lines ANCOVA model assessed s-K^+ changes from baseline.

Results: Reductions in s-K^+ (p<0.001) were observed at 48 hr in mild and moderate HK groups from respective baseline means (±SE) of 5.2±0.02 and 5.7±0.04 mEq/L, with similar effects across dose groups. Mean s-K^+ was controlled (≤5.0 mEq/L) at 48 hr (mild HK) and Wk 1 (moderate HK) and maintained for 52 wks.



*p<0.001 by t-test for change from baseline. †p≤0.001 by t-test for change from Week 52 (end of treatment).
BL, baseline; F/Up, follow-up; HK, hyperkalemia.

The % of pts with s-K^+ 3.8-5.0 mEq/L at visits between Wk 12-52 ranged from 77-95% for mild and moderate HK pts. Stopping drug at Wk 52 led to a rise in s-K^+. eGFR (baseline 42.5±17.7 mL/min/1.73m^2; Δ +1.8±16.3 mL/min/1.72m^2) and albuminuria (baseline 995±1641 mg/g; Δ -27±1422 mg/g) were unchanged over 52 wks. The most common GI side effect was constipation (6%, none severe); only 2 pts discontinued as a result. Thirteen (4%) pts had serum Mg <1.2 mg/dL (CTCAE grade 2; none <1.0 mg/dL).

Conclusions: Patiromer reduced and maintained control of s-K^+ for up to 1 yr in diabetic CKD pts on RAASi. Renal function was stable and patiromer was well tolerated.

Funding: Pharmaceutical Company Support - Relypsa, Inc.

SA-PO1100

ZS-9 Effectively Reduces Potassium Levels in Hyperkalemic Diabetic Patients Who Have Both Renal Impairment and a History of Heart Failure

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Background: Level 1A clinical evidence supports the routine use of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin-receptor blockers (ARB) in patients with heart failure (HF), diabetes mellitus (DM) and chronic kidney disease (CKD). Despite this, hyperkalemia (HK) remains a major hurdle for initiation and/or continuation of these cardiorenal protective agents. We previously reported the results of a randomized, placebo-controlled trial of sodium zirconium cyclosilicate (ZS-9), a nonabsorbed inorganic crystal, in a large (N=753) cohort of patients with HK (potassium [K^+] 5.0-6.5 mEq/L; Ash et al. ASN 2013).

Methods: Here we report the results in 98 patients from the initial trial who concurrently had DM, HF and CKD (eGFR<60 mL/min/1.73m^2) as probable indications for ACEi or ARB therapy. Patients were treated with ZS-9 5g or 10g (n=62) or placebo (n=36) TID for 48h, followed by QD (12 days) in those who attained normokalemia (K^+ 3.5-4.9 mEq/L).

Results: Results: Mean baseline K^+ was 5.3 mEq/L in the ZS-9 group and 5.4 mEq/L in the placebo group. After 48h, mean K^+ declined by 0.67 mEq/L in the 62 treated patients, compared with a mean of 0.24 mEq/L in patients on placebo (p<0.0001). In the 12-day extended phase, ZS-9-treated patients who continued treatment with ZS-9 remained normokalemic (mean K^+ 4.6 mEq/L), whereas those switched to placebo returned to hyperkalemia (mean K^+, 5.1 mEq/L; p<0.05). Importantly, 24% of the 98 participants were not receiving ACEi or ARB before or during the trial, despite having multiple clinical indications for their therapy and only 31%were receiving maximal recommended dose. Patients who were receiving ACEi or ARB and those who were not had a similar fall in serum K^+ on ZS-9.

Conclusions: ZS-9 at 5 and 10g doses facilitates use of ACEi or ARB in patients who are likely to benefit most from these cardiorenal protective agents. Our results suggest that ZS-9 may enable optimal treatment of these clinical conditions, leading to improved clinical outcomes.

Funding: Pharmaceutical Company Support - ZS Pharma, Inc.

SA-PO1101

Empagliflozin Reduces Blood Pressure and Markers of Arterial Stiffness and Vascular Resistance in Type 2 Diabetes

Robert Chilton,[1] Ilkka T. Tikkanen,[2] Christopher Paul Cannon,[3] Susanne Crowe,[4] Thomas Hach,[4] Hans-Juergen Woerle,[4] Uli Christian Broedl,[4] Odd Erik Johansen.[5] *[1]Univ of Texas Health Science Center, San Antonio, TX; [2]Helsinki Univ Central Hospital and Minerva Institute for Medical Research, Helsinki, Finland; [3]Harvard Clinical Research Institute, Boston, MA; [4]Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; [5]Boehringer Ingelheim Norway KS, Asker, Norway.*

Background: We aimed to assess the vascular effects of empagliflozin (EMPA) beyond reducing systolic blood pressure (SBP) and diastolic BP (DBP) in two cohorts of patients with type 2 diabetes.

Underline represents presenting author/disclosure.

Methods: Cohort 1 comprised 823 patients with hypertension from a 12-week ambulatory BP monitoring (ABPM) randomized trial (mean [SD] age 60.2 [9.0] yrs, HbA1c 7.9 [0.7] %, BMI 32.6 [5.1] kg/m^2). Cohort 2 comprised 2476 patients from four 24-week phase III randomized trials (age 55.6 [10.2] yrs, HbA1c 8.0 [0.9] %, BMI 28.7 [5.5] kg/m^2). Changes in HbA1c, SBP, DBP, pulse pressure (PP; SBP-DBP), a marker of conduit vessel stiffening, mean arterial pressure (MAP), reflecting cardiac output multiplied by vascular resistance (MAP = ([2 x DBP]+SBP)/3), and heart rate (HR) were assessed. Ambulatory arterial stiffness index (AASI), derived from 24h ABPM recordings, was assessed in cohort 1.

Results: In cohort 1/cohort 2, EMPA significantly reduced HbA1c relative to placebo (mean [SE] difference: -0.64% [0.04]/-0.65% [0.03], respectively; both p<0.001) and reduced SBP/DBP, PP and MAP without increases in mean HR (Table). AASI reduction was of borderline significance.

Table. Impact on BP, MAP, PP, AASI and HR in cohort 1 and 2 of treatment with empagliflozin (EMPA) and placebo (PBO).

	Cohort 1: 12-week 24h ABPM phase III trial (FAS[A], n=823) (24h ABPM recordings)		Cohort 2: Four pooled 24-week phase III trials (FAS[B], n=2476) (seated office recordings)	
	PBO (n=271)	EMPA 10/25 mg (n=552)	PBO (n=825)	EMPA 10/25 mg (n=1651)
SBP/DBP				
SBP BL (SE), mmHg	131.7 (0.7)	131.3 (0.5)	128.6 (0.5)	129.3 (0.4)
PBO-corrected change from BL (SE), mmHg	N/A	-3.9 (0.6)***	N/A	-3.6 (0.5)***
DBP BL (SE), mmHg	75.2 (0.5)	74.9 (0.3)	78.0 (0.3)	78.5 (0.2)
PBO-corrected change from BL (SE), mmHg	N/A	-1.5 (0.4)***	N/A	-1.3 (0.3)***
Markers of arterial stiffness and vascular resistance				
MAP BL (SE), mmHg	94.0 (0.5)	93.7 (0.4)	94.9 (0.3)	95.4 (0.2)
PBO-corrected change from BL (SE), mmHg	N/A	-2.3 (0.4)***	N/A	-2.1 (0.3)***
PP BL (SE), mmHg	56.6 (0.6)	56.4 (0.4)	50.5 (0.4)	50.8 (0.3)
PBO-corrected change from BL (SE), mmHg	N/A	-2.3 (0.4)***	N/A	-2.3 (0.4)***
AASI baseline (SE)	0.429 (0.008)	0.443 (0.006)	N/A	N/A
PBO-corrected change from BL (SE)	N/A	-0.016 (0.008) (p=0.059)	N/A	N/A
Heart rate				
HR baseline (SE), bpm	77.6 (0.6)	75.8 (0.4)	74.3 (0.3)	74.1 (0.2)
PBO-corrected change from BL (SE), mmHg	N/A	-0.6 (0.5) (p=0.208)	N/A	-0.8 (0.3)*

[A]: all randomized and treated patients who had a baseline HbA1c value and a baseline mean 24-h SBP value; [B]: all randomized and treated patients who had a baseline HbA1c value; all data mean (SE). P-values for adjusted means based on ANCOVA with last observation carried forward imputation. Values on rescue medication were excluded from analysis. *p<0.05; **p<0.01; ***p<0.001 (all vs PBO). FAS – full analysis set; BL – baseline, ABPM – ambulatory blood pressure monitoring.

Conclusions: EMPA had favorable effects on BP, arterial stiffness and vascular resistance which are intermediate markers of CV risk. The EMPA-REG OUTCOME™ trial (NCT01131676) will evaluate whether these benefits will translate into CV risk reduction.

Funding: Pharmaceutical Company Support - Boehringer Ingelheim and Eli Lilly

SA-PO1102

Ferric Citrate Safely Controls Phosphorus, Delivers Iron, and Reduces IV Iron/ESA in ESRD: A 48-Week Clinical Trial

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Background: Ferric citrate (FC) controls phosphorus (P),↑ iron stores, and ↓ IV Iron/ESA use while maintaining hgb over 52 wks with demonstrated safety. This trial NCT01554982 extends observations for 48 wks more.

Methods: Subjects from the pivotal FC RCT randomized to FC could enroll at 35 US sites. 1º outcome was safety (labs and AEs). 2º outcomes were P control, iron stores, IV Iron/ESA use, and Hgb. Labs were obtained at local dialysis units.

Results: 168 of 177 eligible subjects enrolled. 78.6% had a lag between the RCT and this trial. 166 received FC. 125 (75.3%) completed study. Commonest reason for study d/c was AE, incl kidney txpl and death. Age was 55.1±11.1y; 62.0% male; 62.0% black. Pill count was 7.9/day.

	BL	Wk 12	Wk 24	Wk 36	Wk 48
P, mg/dL	5.7±1.7	5.3±1.3*	5.2±1.4*	5.2±1.4*	5.2±1.3*
Ferritin, ng/mL	710±385	785±438*	848±445*	866±482*	821±412*
TSAT, %	32.4±13.5	37.7±16.8*	36.8±15.8*	39.5±18.4*	40.3±19.1*
IV Iron Use**, mg/d	NA	1.48±3.45	0.94±2.55	1.18±3.06	0.72±2.15
ESA Use**, U/d	NA	893±1247	837±1429	759±1054	699±936
Hgb, g/dL	11.1±1.4	11.3±1.4	11.1±1.4	11.1±1.3	11.3±1.4
Ca, mg/dL	9.1±1.5	9.1±0.8	9.1±0.9	8.9±0.9	9.0±0.8
PTH, pg/mL	466±352	507±320	523±344	468±231	455±330

*p<0.05, compared to baseline
**Mean use for each 12-wk period; Wk12 represents first 12wks (baseline) use

Both ferritin and TSAT↑ during 12-24 wks, then plateaued. 59.0% required *no* IV iron over 48wks; 85.1% required *no* IV Iron from Wk36-48. 85.5% had a TEAE. 6 died (3.6%). 44.0% had a GI TEAE, mostly N/V/D/stool color. 8 (4.8%) had GI TEAE that led to FC d/c. 45.2% had SAE. 10.8% had Cardiac, 5.4% GI, and 18.1% Infection SAEs, similar to prior results. No SAEs were FC related. No Δs in AST/ALT/Bili. No subject had LFT>3×ULN. Aluminum was similar over 48wks. No other significant Δs in labs.

Conclusions: FC continued to control P over 100 wks. In those reexposed to FC after a lag, FC↑ iron stores and ↓ IV iron/ESA use. Both TSAT and ferritin plateaued suggesting physiologic iron regulation. AE profile now over 100 wks was similar to the RCT's active control group. FC was associated with continued ↓ need for IV Iron/ESA while maintaining Hgb. Thus, FC is an effective P binder that↑ iron stores and ↓ IV iron/ESA use, with acceptable safety over 100 wks.

Funding: Other U.S. Government Support, Pharmaceutical Company Support - Keryx Biopharmaceuticals, Inc

SA-PO1103

Safety and Efficacy of Modified-Release Calcifediol for Secondary Hyperparathyroidism in Patients with Stage 3 or 4 CKD and Vitamin D Insufficiency

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Background: Vitamin D is often inadequate for controlling secondary hyperparathyroidism (SHPT) in patients with stage 3 or 4 CKD, probably due to inconsistent correction of vitamin D insufficiency (VDI). Orally administered modified-release calcifediol (MRC) was evaluated as a more effective alternative.

Methods: Two identical, randomized, double-blind, placebo-controlled trials were conducted in patients with SHPT (>85 pg/mL), stage 3 or 4 CKD and VDI (serum 25D of 10-29 ng/mL). The trials enrolled 429 subjects from 77 US sites, stratified by CKD stage and randomized 2:1 to receive MRC or placebo for 26 weeks. On completion, subjects could elect 26 more weeks of MRC treatment in an ongoing open-label extension trial. MRC dosing started at 30 µg/d and increased, as needed to lower iPTH, to 60 µg/d after 12 weeks. Efficacy endpoints were differences in response rates between treatment groups; the 1º response was a mean 30% decrease in plasma iPTH and a 2º response was a mean serum 25D of >30 ng/mL at end-of-treatment. Safety analyses included changes in serum calcium (Ca) and phosphorus, urine Ca/creatinine and adverse events.

Results: A total of 364 (85%) subjects completed the trials. Efficacy response rates for MRC vs. placebo treatment were 96 vs. 6% for serum 25D and 39 vs. 9% for plasma iPTH at 26 weeks, rising to 55% at 52 weeks (p<0.001). Response rates were similar in CKD stages 3 and 4. Safety and tolerability were comparable in both treatment groups. Mean serum Ca and P rose 0.1-0.2 mg/dL in both groups.

Conclusions: MRC effectively and safely manages SHPT in patients with stage 3 or 4 CKD by gradually correcting vitamin D insufficiency.

Funding: Pharmaceutical Company Support - OPKO Health

SA-PO1104

Phase III Study of ASP1585 (Bixalomer) - Randomized, Double-Blind, Placebo-Controlled Study in Chronic Kidney Disease Patients with Hyperphosphatemia Not on Dialysis

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Background: ASP1585 is a non-absorbable polymer that decreases serum phosphorus (Pi) levels by binding phosphate in the gastrointestinal (GI) tract. The efficacy and safety of ASP1585 versus placebo were compared for controlling hyperphosphatemia in chronic kidney disease (CKD) patients not on dialysis.

Methods: This was a phase III, randomized, double-blind, placebo-controlled multi-center study conducted in Japan. The primary endpoint was change from baseline in serum Pi level at the end of treatment (EOT). Secondary endpoints included the achievement rate of the target range of serum Pi level (2.5 ≤, < 4.6 mg/dL). Dosing of ASP1585 was started at 1500 mg/day and adjusted to 7500 mg/day maximum depending on the serum Pi level. Treatment continued up to 12 weeks.

Results: Overall 163 patients were randomized to either the ASP1585 group or placebo group (81 and 82 patients, respectively).In the primary endpoint, ASP1585 showed a statistically significant difference compared with placebo in adjusted mean +/- SE change from baseline in the serum Pi level (mg/dL) (-0.98 +/- 0.15, p < 0.001, Table1). The achievement rates of the target Pi range at the EOT were 57.5% in the ASP1585 group and 23.5% in the placebo group (p < 0.001). Incidences of adverse events (AEs) and GI AEs were comparable between ASP1585 and placebo groups.

Underline represents presenting author/disclosure.

	Placebo (N=81)	ASP1585 (N=80)
Adjusted Mean Change from Baseline (SE) [mg/dL]	0.20 (0.10)	-0.78 (0.10)
Difference with Placebo in Adjusted Mean Change from Baseline (SE) [mg/dL]	-	-0.98 (0.15)
95% CI	-	(-1.27, -0.69)
p-value	-	<0.001

Table1: Result for primary efficacy variable (FAS)

Conclusions: ASP1585 was effective in decreasing serum Pi level, while showing a well-tolerated safety profile compared to placebo. These findings indicated the clinical benefits of ASP1585 as a treatment for hyperphosphatemia in CKD patients not on dialysis.

Funding: Pharmaceutical Company Support - Astellas Pharma Inc.

SA-PO1105

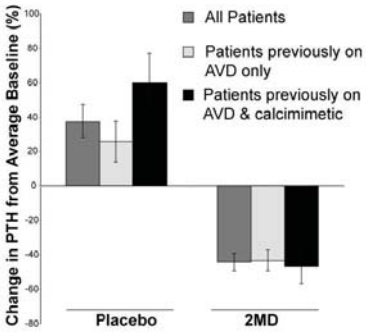
2MD, a New Treatment for Secondary Hyperparathyroidism

Hector F. Deluca. *On Behalf of Phase 2 Study Sponsor and PI's, Deltanoid Pharmaceuticals, Inc., Madison, WI.*

Background: Secondary hyperparathyroidism (SHPT) has been successfully managed with the use of active vitamin D analogs (AVDs) alone, or with the addition of a calcimimetic (CM). However, both classes of compounds have drawbacks. Hypercalcemia is of concern when AVDs are used, while CMs can cause hypocalcemia and have compliance issues because of nausea and vomiting. A novel, highly potent AVD (2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3, 2MD or DP001) specifically targets the parathyroid glands and bone and is being developed for the treatment of SHPT in patients on hemodialysis.

Methods: A Phase 2B, double-blind, placebo-controlled clinical trial was conducted in 62 Stage 5 patients previously maintained on a commercially available AVD or AVD plus a calcimimetic (NCT01922843). Following a washout period, patients were randomized to receive either placebo (n=28) or 2MD (n=34) softgel capsules orally 3X/week after dialysis for 12 weeks. Serum calcium, parathyroid hormone (PTH), and phosphorus levels were monitored weekly.

Results: 2MD, at doses ranging from 220-770 ng 3X/week, was effective at reducing serum PTH levels in 80% of patients, including those previously requiring an AVD and a calcimimetic. In patients previously treated with a calcimimetic, all but one on DP001 had calcium values in the normal range. Serum phosphorus levels were not affected by 2MD.



Conclusions: 2MD is safe and effective in the management of secondary hyperparathyroidism in renal failure patients on dialysis regardless of whether or not they previously required a calcimimetic together with an AVD.

Funding: Pharmaceutical Company Support - Deltanoid Pharmaceuticals, Inc.

SA-PO1106

Iron Isomaltoside 1000 (Monofer®) Compared to Oral Iron Sulphate in Patients with Non-Dialysis Dependent Chronic Kidney Disease (NDD-CKD)

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Background: Iron deficiency anemia is common in patients with CKD, and is often treated with iron. This study compared the efficacy and safety of intravenous (IV) iron isomaltoside 1000 and oral iron sulphate in patients with NDD-CKD.

Methods: The trial was a phase III open-label, comparative, multi-center, non-inferiority trial conducted in 351 NDD-CKD patients, randomized 2:1 to either IV iron isomaltoside 1000 (group A) or iron sulphate administered as 100 mg elemental oral iron b.i.d. (200 mg daily) for 8 weeks (group B). The patients in group A were equally divided into A1: IV infusion of max 1000 mg single doses over 15 min once weekly and A2: IV bolus injections of 500 mg administered over 2 min once weekly until full replacement dose is achieved (92% of patients in A1 gained full replacement in one visit). Calculation of iron need was according to a modified Ganzoni formula (target Hb13 g/dL, iron stores 500 mg). The primary objective was to demonstrate non-inferiority and the primary endpoint was change in Hb concentrations from baseline to week 4.

Results: Iron isomaltoside 1000 showed not only non-inferiority at week 4 ($p<0.0001$) compared to oral iron but a sustained superior increase in Hb from week 3 until the end of the study at week 8 ($p=0.008$, at week 3). This difference in effect increased week by week. The Hb response was more pronounced with IV replacement doses ≥1000 mg. Ferritin and transferrin saturation concentrations were also significantly increased. Adverse drug reactions (ADRs) were observed in 10.5% in group A and 10.3% in group B and no dose relationship was found. 0.85% of the subjects experienced serious ADRs in both groups. The frequency of a phosphate < 2 mg/dL was 2.2 % vs 1.7 % in groups A and B respectively. More subjects treated with iron sulphate withdrew from the study due to adverse events (4.2% B versus 1.3% A).

Conclusions: Iron isomaltoside 1000 was more efficacious than oral iron sulphate for increase in hemoglobin and proved to be well-tolerated at the tested dose levels in NDD-CKD patients.

Funding: Pharmaceutical Company Support - Pharmacosmos

SA-PO1107

In-Hospital, Electronic Alerts for Acute Kidney Injury: A Randomized, Controlled Trial

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Background: Small increases in creatinine are associated with adverse clinical outcomes, but may go unnoticed by clinicians. We evaluated the efficacy of an automated AKI alert system in a randomized trial.

Methods: Starting 7/2013, all patients at the Hospital of the Univ of Pennsylvania (HUP) with AKI as defined by the KDIGO creatinine criteria were identified by a computerized algorithm. Exclusion criteria were age <18, end-stage kidney disease, prior randomization, and presenting creatinine >4.0. Under a waiver of informed consent, patients were randomized 1:1 to usual care (UC) (no alert) or to an electronic alert. Randomization was stratified by medical versus surgical admission and ICU status. For patients randomized to the alerts, the primary provider and unit pharmacist received an automated text-page informing them of the AKI. The primary outcome, a rank-based composite of maximum relative change in serum creatinine, inpatient dialysis, and inpatient death, was assessed 7 days after randomization.

Results: 2393 consecutive patients with AKI were randomized. Baseline characteristics were similar in the two groups. The mean (SD) age was 60.4 (16.3) years, 27.0% of patients were Black, 42.3% were admitted to surgical services, and 30.2% were in intensive care at randomization. The mean (SD) baseline creatinine was 1.10 (0.73) mg/dl while the mean (SD) creatinine at randomization was 1.55 (0.84) mg/dl. At 7 days, the median (IQR) relative increase in creatinine was 0 (0–18%) in the alert group and 0.6 (0–17.5%) in the UC group ($p=0.88$). Seven-day mortality was 5.9% in the alert group vs 5.1% in the UC group ($p=0.40$). The seven-day dialysis incidence was 7.2% in the alert group and 5.9% in the UC group ($p=0.18$). Overall, the rank-based outcome was no different between the groups ($p=0.12$).

Conclusions: In this large trial, alerting providers to the presence of AKI did not significantly improve clinical endpoints. Further studies to identify higher-risk AKI patient subsets which might benefit from alerts paired with targeted interventions may be valuable.

Funding: NIDDK Support

SA-PO1108

A Randomized Trial of Home Video Monitoring by an Interprofessional Care Team on Outcomes in Patients with Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) is common and is associated with poor patient outcomes. Interprofessional case management has been demonstrated to improve surrogate outcomes in non-CKD populations. The purpose of our study was to determine if an interprofessional team using remote video monitoring of patients with CKD reduced a composite outcome of death, hospitalization, emergency department (ED) visits, or admission to a skilled nursing facility (SNF) compared with usual care.

Methods: We randomly assigned 601 veterans with an eGFR<60mL/min/1.73m^2 to an intervention (n=451) consisting of a remote video monitoring device (touch screen computer, BP cuff, scale, webcam, and glucometer) and care by an interprofessional team (nephrologist, nurse practitioner, nurses, pharmD, psychologist, social worker, and dietician) managing acute and chronic diseases, or to usual care (n=150).

Results: Baseline characteristics of the overall study group were: mean age 75.1±8.1 years, male 98.5%, Caucasian 97.3%, and mean eGFR 37±9 mL/min/1.73m^2. At 1 year, 70 (46.2%) patients in the usual care group and 208 (46.7%) patients in the intervention group experienced the primary composite outcome ($p=0.90$). Results were not altered in multivariable analysis (HR = 1.02, 95% CI=0.77, 1.34). There was also no difference between groups among the components of the primary outcome (Table 1).

Underline represents presenting author/disclosure.

Table 1: Study Group Outcome Rates and Hazard Ratios for Time to Event					
Outcome	Intervention	Usual Care	Hazard Ratio	95% CI	p-value[1]
Primary	208 (46.2%)	70 (46.7%)	0.98	(0.75, 1.29)	0.90
Mortality	13 (2.9%)	3 (2.0%)	1.46	(0.42, 5.11)	0.56
Hospitalization	134 (29.8%)	40 (26.7%)	1.15	(0.80, 1.63)	0.45
ED Visit	164 (36.4%)	58 (38.7%)	0.92	(0.68, 1.24)	0.58
SNF Admission	18 (4.0%)	2 (1.3%)	3.07	(0.71, 13.2)	0.13
[1] Wald Chi Square for Testing HR					

Conclusions: Our intervention of remote monitoring and care by an interprofessional team did not improve health outcomes in patients with moderate to severe CKD compared to usual care.

Funding: Veterans Affairs Support

SA-PO1109

Stent Treatment in Atherosclerotic Renal Artery Stenosis: Kidney Function and Clinical Events Katherine R. Tuttle, Lance D. Dworkin, Barbara A. Greco, William L. Henrich, Michael Steffes, Kenneth A. Jamerson, Joseph I. Shapiro, Karol M Pencina, Joseph Massaro, Donald Cutlip, Sheldon W. Tobe, Timothy P, Murphy, Christopher J. Cooper, Ralph B D'Agostino. *CORAL, Study Group.*

Background: The study purpose was to determine effects of revascularization by stenting on kidney function and clinical events in the Cardiovascular Outcomes in Renal Atherosclerotic Lesions (CORAL) clinical trial.

Methods: Participants with atherosclerotic renal artery stenosis (ARAS) >60% and systolic hypertension on ≥2 drugs or stage ≥3 chronic kidney disease (CKD) received medical therapy: renin-angiotensin system (RAS) inhibition; statin; target-driven control of blood pressure (BP), LDL cholesterol, and glycemia (diabetic subgroup); anti-platelet agents; smoking cessation education. They were randomized to treatment by stenting and medical therapy or medical therapy alone. The main outcome for the kidney function substudy was estimated glomerular filtration rate (eGFR, creatinine-cystatin C CKD-EPI). CKD events (death, end-stage renal disease, >30% eGFR loss) and cardiovascular disease (CVD) events (death, stroke, myocardial infarction, heart failure) were assessed. Variables were chosen stepwise in generalized estimating equation (eGFR) and pooled Cox proportional hazards (events) models.

Results: eGFR was 59±24 and 53±22ml/min/1.73m^2 (mean±SD) at baseline and 4 years (n=931). CKD and CVD events occurred in 19% (n=175) and 22% (n=207), respectively. No differences between treatment groups were detected for eGFR or events. Participants were followed for 43 (31-55) months (median, IQ range). Baseline predictors of eGFR over time were age, urine albumin-to-creatinine ratio (UACR), systolic BP, diabetes (all inverse); and male sex, total cholesterol, HDL cholesterol. For CKD events, baseline predictors included UACR, systolic BP, HDL cholesterol (inverse). For CVD events, baseline predictors included age, UACR, eGFR (inverse), bilateral ARAS, prior CVD.

Conclusions: Stent treatment did not improve eGFR or reduce CKD or CVD events in CORAL participants receiving RAS inhibition-based medical therapy. Predictors of kidney function and clinical events were traditional risk factors and characteristics.

Funding: Other NIH Support - National Heart, Lung, and Blood Institute, Pharmaceutical Company Support - Pfizer and Astra-Zeneca provided study medication; Cordis provided study devices

Underline represents presenting author/disclosure.